A STOCHASTIC MODEL TO PREDICT ANNUAL EGG PRODUCTION OF A FLOCK OF LAYING HENS

By

Shelley Anne Johnston

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ABSTRACT

Ovulation rate in laying hens is determined by the interaction of two biological systems; namely, a circadian rhythm that restricts the release of luteinising hormone to an eight- to ten-hour period of the day, and the process of follicle maturation. Etches and Schoch (1984) used a two-compartmental model to represent the circadian rhythm and a Gompertz equation for follicle maturation. In doing so, they were able to predict ovulation times for two- to nine-egg sequences. This model has been improved by replacing their table of values with continuous functions that predict the values for each parameter in the ovulatory model, for any ovulation rate. Consequently, ovulation times may be predicted for any sequence length.

A population model that simulates annual egg production has been developed in Visual Basic. Each parameter in the model is allocated a mean and standard deviation, so that variation is introduced into the flock. Mean age at first egg is predicted from the age at photostimulation and the lengths of the photoperiods applied during rearing.

Quadratic-by-linear functions are used to predict changes in the hen's internal cycle length over time, which in turn determine changes in the ovulation rate and rate of lay. Short egg sequences, frequently observed at onset of lay in experimental flocks, are simulated initially, followed by the prime (or longest) sequences, which are produced at the time of peak rate of lay, before gradual increases in the internal cycle length cause the egg sequences to become shorter once more.

In view of the fact that the interval between oviposition and the subsequent ovulation is about 30 minutes, time of lay may be predicted from ovulation time for all eggs other than the last egg of a sequence, because in this case there is no associated ovulation. A curvilinear function is used to predict the value of the last interval from the ovulation rate, because experimental data show that short sequences have longer intervals between the last two eggs than long sequences. The circadian rhythm of LH release is linked to the onset of darkness, so that mean time of lay occurs 13 to 14 hours after sunset. The distribution of oviposition times is unimodal for young flocks and bimodal for older flocks.

Yolk weight is predicted from hen age using a function appropriate for the genotype. Allometric functions are used to predict albumen weight from yolk weight and shell weight from the weight of the egg contents. Egg weight is given by the sum of the three components. With advancing hen age, the proportion of yolk in the egg increases at the expense of both albumen and shell.

Random events, such as internal ovulations, and the production of soft-shelled and double-yolked eggs, are accounted for in the model. Their incidence is linked to the genotype and to the age of the hens and their occurrence is restricted to a proportion of the flock. Internal ovulations cause interruptions to egg sequences, thereby reducing overall mean sequence length.

This model could be of benefit to a producer wanting to know how a change to the lighting programme would affect the laying performance of the strain, or to a nutritionist desiring to determine changes in voluntary feed intake and to the nutrient requirements of the birds over the laying period. It may also be used as a teaching aid, so that students gain a thorough understanding of the process of egg production and are able to test the response of layers to different environmental stimuli. The user has control over a number of inputs, thereby making it a generalised model that can be used for different strains. With a few modifications, the model may be used to simulate the erratic and variable laying behaviour of broiler breeders.

PREFACE

The experimental work described in this thesis was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from September 2002 to January 2004, under the supervision of Professor Rob Gous.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any university. Where use has been made of the work of others it is duly acknowledged in the text.

S. A. Johnston (candidate)

Professor Rob Gous (supervisor)

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LITERATURE REVIEW

1.1 Introduction

The domestic hen's ovulation and oviposition cycles continue to intrigue poultry scientists and to stimulate investigations. The complex timing relationships involved in the two events, which are subject to change via genetic selection or with the use of continuous or ahemeral light cycles, pose a tremendous challenge for the computer modeller trying to predict egg output for an individual hen and for the physiologist attempting to understand and integrate the hormonal and neurological involvement. Much controversy surrounds the issue of whether circadian rhythms are involved in timing the preovulatory surge of LH, since solid evidence is still lacking in poultry. Although tremendous progress has been made during the past 30 years in understanding hormonal involvement, due to the advent of radioimmunoassay techniques, it would be naive to assume that all the endocrine pathways have been established.

The differences between wild birds and domestic hens, in terms of laying patterns, have been well documented. Wild birds are inherently seasonal breeders, laying their eggs in clutches. The term 'clutch' refers to the set of eggs laid for one incubation (Romanoff and Romanoff, 1949). During the Middle Ages early farmers in Italy discovered that removing eggs from the nest encouraged the hen to continue laying and in so doing, delayed the onset of broodiness and increased clutch size (Etches, 1996). By exploiting this phenomenon over the centuries, along with genetic selection for high egg numbers, improved management practices and superior nutrition, the hen's natural clutch size of 11-14 eggs has been extended to enable modern hybrids to lay in excess of 300 eggs per annum.

Over the past ten years experimental focus has shifted from recording total egg numbers to measuring individual sequence lengths and time of lay. Although this approach necessitates keeping hens in single cages and is fairly labour-intensive, much useful information has been

gathered on the response of the individual bird to environmental stimuli, in particular for continued genetic progress. This suits the modeller, who chooses to start by predicting the rate of lay for a single bird before transforming the model into one suitable for a population. The process of recording data from individual hens enables the estimation of population means and standard deviations for the various traits.

The aim of this study is to produce a comprehensive stochastic model of flock egg production, based on sound biological principles. In order to be successful, a thorough understanding of the hen's pattern of sequential laying and of the temporal relationships between various events is essential. Detailed knowledge of the ovarian follicular dynamics is required in order to produce an acceptable model of the follicle hierarchy. An understanding of the physiology, in particular the hormonal control, of the process of ovulation will be of assistance in the numerous decision-making processes that arise during the development of the model. One of the ways in which the validity of the model output may be tested is by comparing the theoretical distribution of flock oviposition times with actual distributions recorded under experimental conditions. Another method is to test the effect of ahemeral light:dark cycles on ovulation rate and oviposition intervals, since much research of this nature has been reported in the literature. Finally, the model must include decay factors, so that over a period of time rates of ovulation and lay decline in a realistic fashion with age. To accomplish this, the process of reproductive senescence needs to be studied.

1.2 Sequence characteristics

Although the term clutch is still sometimes used, the modern domestic hen is considered to lay in sequences; a sequence being made up of a number of consecutive ovipositions followed by one or more pause days, when no egg is laid. Under normal lighting conditions, *i.e.* 24-hour daylengths with about 16 hours of light, the first egg of a sequence is laid early in the morning or about nine to ten hours after the onset of darkness. Each subsequent oviposition occurs progressively later in the day, at intervals slightly longer than 24 hours, on successive days. During long sequences, oviposition time advances by a similar increment each day (Reynard and Savory, 1999). The last egg of a sequence is normally laid during the afternoon (Gilbert

and Wood-Gush, 1971). Most ovulation sequences are formed by the absence of a mature follicle in the hierarchy on the day (Gilbert, 1972).

The difference in times of day at which oviposition occurs on consecutive days is known as the lag, as is the corresponding delay in the ovulation cycle (Fraps, 1955). Lag is caused by the deviation of a hen's internal circadian rhythm from the external daily rhythm (Koops and Grossman, 1992). An individual lag value may be anything from about 4½ hours in a two-egg sequence, to less than an hour in longer sequences and may even be negative in the middle of exceptionally long sequences. Within a sequence, the lag follows a reasonably well-defined pattern. It decreases initially, is at a minimum towards mid-sequence and then increases, with the greatest lag occurring at the terminal oviposition (Fraps, 1955; Romanoff and Romanoff, 1949; Etches and Schoch, 1984). Lag is positively related to the number of sequences produced during the laying cycle and negatively related to mean sequence length and total number of eggs laid (Koops and Grossman, 1992).

Total lag and mean lag vary for sequences of different lengths. On 24-hour light:dark cycles, total lag (the difference in times of day between the first and last ovipositions) tends to increase slightly as sequence length increases, but it reaches a maximum (Morris, 1973). This maximum is thought to be eight hours (Blake and Ringer, 1987) or seven to eight hours (Gilbert and Wood-Gush, 1971) for sequences of six eggs or more and less than eight hours for shorter sequences (Morris, 1973). It is possible that genetic selection for higher rates of lay has brought about a lengthening in the total lag. Total lag is a measure of the proportion of the day used for egg laying and can be regarded as an indication of the length of the open period for LH release and hence ovulation (Lillpers and Wilhelmson, 1993a). On ahemeral light:dark cycles, total lag increases as the length of the cycle increases (Morris, 1973). Mean lag (total lag divided by the number of places where lag occurs in a sequence) decreases as sequence length increases, since total lag does not increase indefinitely. When making a study of these relationships, it is important to remember that lag determines sequence length and not *vice versa*.

Table 1.1 summarises total and mean lag values calculated from data obtained from Morris

(1968-69, unpublished data). Oviposition times were recorded over a period of 94 days for laying hens on different lighting schedules. The results for the birds on 24-hour daylengths, with 14L:10D and with the lights turned on at 08:00 and off at 22:00, are used here. Total lag appears to reach a maximum of about eight hours for the range of sequence lengths analysed here, which concurs with the findings of Morris (1973), Blake and Ringer (1987) and Gilbert and Wood-Gush (1971) cited above. Figure 1.1 shows the negative relationship between mean lag and sequence length.

Table 1.1: Total lag and mean lag (± se mean) for sequences of two to nine ovipositions (from Morris, 1968-69; unpublished data).

sequence length	sample size	total lag (hrs)	mean lag (hrs)
2	233	4h 40m (± 0h 07m)	4h 40m (±0h 07m)
3	165	6h 56m (±0h 08m)	3h 28m (±0h 05m)
4	79	7h 20m (±0h 12m)	2h 27m (±0h 05m)
5	36	7h 58m (±0h 21m)	1h 59m (±0h 06m)
6	8	7h 41m (±1h 00m)	1h 32m (±0h 12m)
7	16	8h 14m (±0h 23m)	1h 22m (±0h 07m)
8	4	7h 44m (±0h 33m)	1h 06m (±0h 13m)
9	3	7h 54m (±0h 30m)	0h 59m (±0h 16m)

The oviposition (or intra-sequence) interval is calculated as the number of hours between successive ovipositions within a sequence. It is an indirect estimate of ovulation interval, since the time from ovulation to oviposition does not vary much within a sequence (Yoo *et al.*, 1988). The oviposition interval is commonly reported as being between 24 and 27 hours (Bednarczyk *et al.*, 2000). However, this measurement varies according to sequence length.

As sequence length increases, the mean interval decreases. Heywang (1938) calculated the mean intervals for White Leghorns, which ranged from 28.2 hours for a two-egg sequence down to 24.3 hours for a 20-egg sequence. For Rhode Island Red hens the range was 27.7 to 24.5 hours for the same sequence lengths. Within a sequence, the mean interval between the last two eggs in the cycle was greater than any other mean interval. The intervals were found to be shortest in the middle of sequences. This is hardly surprising; since oviposition interval is equal to 24 hours plus lag, the characteristic patterns must be the same as for lag. Clearly, it is the length of the intervals between ovipositions that determine sequence length, and not *vice versa*. Oviposition intervals are determined by the genotype, the age of the bird and the blocking effect of the light-dark cycle (Foster, 1972) under the assumption of adequate nutrition.

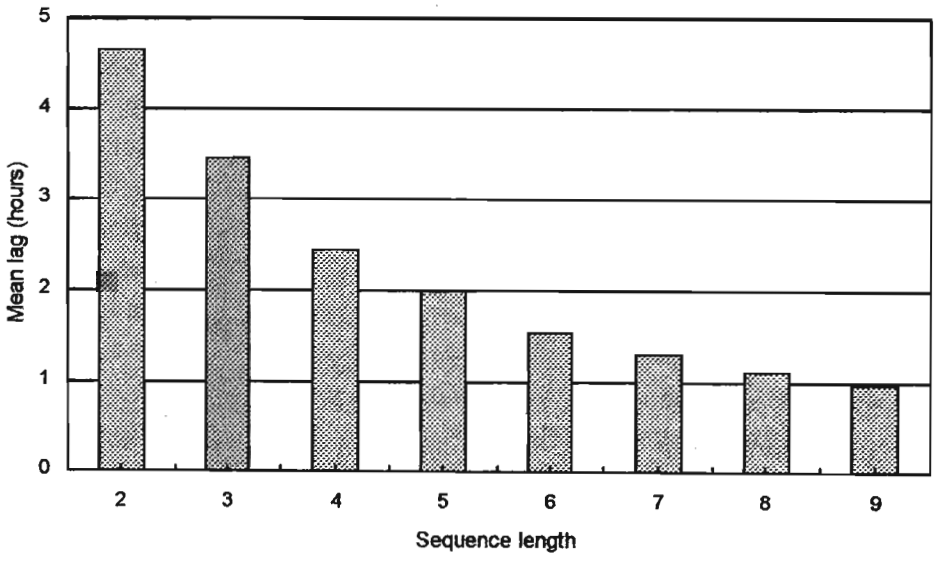


Figure 1.1: The negative relationship between mean lag and sequence length. (Data from Morris, 1968-1969).

Morris (1973) proposed a general equation for predicting rate of lay (p) from mean intra-clutch interval (i), cycle length (c) and total lag (L), provided that $i > c$:

$$p = \frac{24\,L + 24\,(i - c)}{cL + 2c\,(i - c)} \qquad \text{(Equation 1.1)}$$

However, the model was recognised as being unsatisfactory, because neither the total lag nor the intra-clutch (or intra-sequence) oviposition interval is constant; they change with age and sequence length and are modified by varying the light:dark cycle. Furthermore, the underlying distributions of rate of lay, intra-sequence interval and sequence length are not normal (Morris, 1973).

Two interesting observations by Lewis and Perry (1991) were made possible because oviposition times, as well as number of eggs in a sequence, were recorded for the 16 individually-caged hens in the trial. Some eggless days occurred within a sequence, presumably due to internal laying. Without the benefit of access to oviposition times, the single long sequence would have been recorded as two shorter sequences with a pause day in between. However, the times of lay of the two eggs before and after the eggless day clearly indicated that they were both part of the same sequence; the one event occurring later in the day than its predecessor and not early in the morning. Other sequences were not separated by an interceding eggless day, so under normal circumstances they would be counted as one long sequence instead of two shorter ones. A study of the trends in oviposition times enabled the authors to decide that of two successive eggs, the first laid late in the afternoon and the next one laid early in the morning of the following day, one was the terminal egg of a sequence and the other the first egg of a new sequence despite the fact that no pause day was observed. It is not known with what frequency these two events occur. It does raise questions on the reliability of sequence length data and on the accuracy of the method used, namely counting consecutive days of egg production.

Another possible source of error in experimental work is the automatic recording method for oviposition times, which relies on a device located in the egg cradle (Lillpers and Wilhelmson, 1993a). It sometimes happens that an egg is laid and for some reason it does not immediately roll down the cage floor into the cradle. This egg may never be recorded, in which case the sequence would incorrectly be terminated due to a false pause day, or it may (possibly with the assistance of the hen) roll down later and be given a false time of oviposition. In the former case, a study of the hen's oviposition pattern can help to estimate unregistered laying events (Lillpers, 1998). Reynard and Savory (1999) found it necessary to inspect the cages every ten

to fifteen minutes during peak laying times; however, this does somewhat defeat the purpose of an automatic recording system. Some poultry scientists have found, by a process of trial and error, that manual recording of oviposition times, although time-consuming and labour-intensive, is the more reliable method (P. Lewis, 2003, personal communication*).

In analysing experimental data where sequence length is one of the variables recorded, special attention needs to be given to the method of statistical analysis. Zuidhof *et al.* (1998) found that weekly analysis of sequence length with pause data included appeared to be the best statistical approach.

1.3 Yolk, albumen and shell weight

Yolk weight is influenced by the age and breed of the hen, sequence length and position in the sequence. Other environmental factors such as nutrition and ahemeral light cycles also play a role in modifying yolk size. Depending on the breed of the laying hen, the first eggs at sexual maturity usually contain yolks of about 9-12 g, whereas towards the end of the laying cycle yolk weights of 20-25g are normal (Gilbert, 1972). The weight of the follicle is negatively related to sequence length. Longer sequences are associated with smaller follicle size at ovulation, suggesting that high producers tend to lay smaller eggs.

Bastian and Zarrow (1955) found that yolk weight was related to the position of the egg in the clutch and tended to decrease as position advanced, especially for three-egg sequences. The absence of trends in longer sequences may have been due in part to the small sample size. These observations were confirmed by studies carried out by Zakaria *et al.* (1984a) and Zakaria (1999b). Gilbert (1972) found that the heaviest ovum occurred more frequently in the first two places of a sequence than expected.

The three components of the egg, namely yolk, albumen and shell, have intrigued researchers for most of the 20th century. There is an abundance of reports in the literature on the relationships between the weights of the individual components and total egg weight, on the

* Dr P.D.Lewis, Northcot, Cowdown Lane, Goodworth Clatford, Andover, Hants SP11 7HG, UK.

solids contents of yolk and albumen and on the factors influencing the proportions of the three components. An increased awareness of the dangers of high cholesterol in patients with heart or circulatory diseases led to interest in the yolk content of table eggs, as cholesterol is contained primarily in the egg yolk. It has been postulated that an egg with a comparatively smaller yolk may be more acceptable to the health-conscious consumer, thereby improving the image of eggs as a healthy protein source in the marketplace (Harms and Hussein, 1993; Yannakopoulos *et al.*, 1998). Apart from this aspect, the nature or yield of the three components has little relevance for table egg production. In contrast, it is of great importance to the egg processor, since yolk has a higher market value than albumen. In developed countries, the demand for liquid egg continues to grow (Harms and Hussein, 1993). An awareness of breed or strain differences in yolk yield and yolk solids content would enable the egg processor to optimise his profitability by selecting flocks laying eggs with a high yolk to albumen ratio. Renewed interest in the topic in more recent years has been prompted by the desire to accurately define the relationships between yolk, albumen and shell for simulation modelling purposes.

In a definitive work entitled *The Avian Egg*, Romanoff and Romanoff (1949) stated that an average 58 gram egg contains 55.8% albumen, 31.9% yolk and 12.3% shell. The lack of uniformity in hens' eggs was attributed to season, strain and age of hens, level of productivity, diet and environment (Romanoff and Romanoff, 1949; Fletcher *et al.*, 1981). Factors affecting the total weight of the egg also affect the proportions of the components. At a fixed hen age, larger eggs contain greater absolute amounts of the three components than smaller eggs, but relatively less yolk and more albumen. This is because albumen weight increases at a faster rate than yolk weight, as egg weight increases. Work done by Harms and Hussein (1993) and Hussein *et al.* (1993) confirms that albumen weight and yolk weight are positively associated with egg weight, although albumen weight is more closely associated with egg weight than yolk weight. The percentage of shell was thought by Romanoff and Romanoff (1949) to remain constant, but Fisher (1969) found an inverse relationship between egg size and percentage shell. Because hot weather depresses egg size, eggs laid during summer will have a larger percentage of yolk.

The position of an egg in the clutch also influences the proportions of the components. Egg weight of consecutive eggs within a clutch gradually decreases (Belyavin *et al.*, 1987). Miyoshi *et al.* (1997) reported a decrease in weight of 3 to 4 grams (about 6% of the first egg) in one line and 1.5 to 2 g (4%) in another line. The yolk weight of the second egg was found to be the heaviest, decreasing by 1.0 to 1.5g by the end of the clutch. Egg shell weight was heaviest in the terminal egg of the clutch, possibly because the lag in oviposition time was the longest for the last egg.

The age of the hen plays a role in that yolk output increases with age. Mean yolk weight will be determined by the rate of lay in a flock. In general, eggs from young hens will have a smaller percentage of yolk and a larger percentage of albumen than eggs from older hens. Pullet eggs tend to exhibit greater variability in the component yields (Romanoff and Romanoff, 1949). The occurrence of double-yolked eggs is more common in young birds coming into lay, as opposed to mature hens with established ovulation cycles (Harms and Abdallah, 1995).

The hen's diet can be used to manipulate the egg component yield, solids content of yolk and albumen and cholesterol level. Fisher (1969) fed protein-deficient diets to a flock of layers which reduced egg weight and component weight. He concluded that the increase in the percentage of yolk and shell and the decrease in the percentage of albumen could all be accounted for by the reduction in egg size. Keshavarz and Nakajima (1995) increased egg weight and albumen weight by increasing dietary protein content. Increasing levels of methionine were found to give increases in egg weight, albumen and yolk weight and total solids content (Shafer *et al.*, 1996; Shafer *et al.*, 1998). Prochaska *et al.* (1996) were also able to increase egg weight, albumen weight as well as protein and solids content of albumen, by feeding a range of lysine levels. By incorporating flax, a concentrated source of unsaturated fatty acids, in layer diets yolk lipid content could be manipulated (Scheideler and Froning, 1996; Scheideler *et al.*, 1998). Yannakopoulos *et al.* (1998) found that dietary natural zeolite, a supplement rich in calcium, increased egg weight and albumen weight, although yolk weight was not significantly affected. Hence the yolk:albumen ratio (Y:A ratio) was reduced by the inclusion of zeolite in the layer diet.

1.4 The oviducal term

Egg production is a reflection of ovarian activity. Unless internal laying or some unusual event (such as rupturing of the vitelline membrane) occurs, an ovulation is always followed by an oviposition some 24 or more hours later. If the likely time interval between the two events can be quantified, then it becomes possible to estimate the time ovulation occurred (an indiscernible event) from the time the egg is laid (a visible and recordable event). This requires knowledge of the time the ovum takes to travel through the different regions of the oviduct during egg formation. The period of time is known as the oviducal term.

Warren and Scott (1935) studied these time intervals in great depth, either by performing post mortems on hens at specific times during the ovulation and oviposition cycles, or by anaesthetising individual hens and opening the left side of the abdominal cavity. In twelve anaesthetised birds the process of ovulation was observed. In some cases the infundibulum picked up the ovum immediately, by design or by chance being near to the part of the ovary containing the mature follicle. In others, it took three to ten minutes following ovulation before the ovum was engulfed. In four of the hens the mechanism was seen to fail; there were two instances where the infundibulum failed to grasp the ovum and two cases of ruptured vitelline membranes. The authors were uncertain whether the anaesthetic and operation had an influence on the normal ovulation process. The mean time interval between ovulation and the start of engulfment was 2.9 minutes (range 0-10 minutes), with the mean time required to enclose the ovum recorded as 13.3 minutes (range 5-25 minutes).

Warren and Scott (1935) used five anaesthetised hens to study the rate of passage of the ovum through the entire oviduct. A shallow stitch was placed in the wall of the oviduct at 15-minute intervals, immediately behind the ovum. Once the egg had reached the uterus the hen was killed and the oviduct removed. Measuring the distance between the stitches enabled the authors to calculate the rate of passage of the ovum through the various parts of the oviduct, as well as to estimate the time spent by the ovum in each portion. Small round pieces of white paper were placed in the position of the stitches along the length of the oviduct, for purposes of photography. The result was a remarkable photograph of the five oviducts, which has often

been reproduced over the years and which clearly shows the rate of passage to vary from one region to another. Rate of passage was felt not to be influenced by the anaesthetic, since muscle movement in the oviduct is involuntary.

Table 1.2 shows estimates of the time spent by the egg in different regions of the oviduct, from Warren and Scott (1935), Romanoff and Romanoff (1949) and Fraps (1955). Since very little time lapses between ovulation and the start of engulfment (mean 2.9 minutes; see above), Fraps (1955) proposed that the interval from ovulation to oviposition be considered equal to the oviducal term.

Table 1.2: Duration of stay of egg in regions of the oviduct (from Warren and Scott, 1935; Romanoff and Romanoff, 1949 and Fraps, 1955).

	Warren & Scott		Romanoff		Fraps	
Region of oviduct	Hours	Minutes	Hours	Minutes	Hours	Minutes
Infundibulum		18		20		18
Magnum	2	54	3	0	< 3	
Isthmus	1	14	1	10	1	14
Uterus	20	40	19	0	18-20	
TOTAL	25	6	23	30	22-24	32

Warren and Scott (1935) were unable to find a consistent relationship between rate of egg production and length of the period between oviposition and subsequent ovulation, or time spent in the magnum and isthmus. They concluded that variations in the time spent in the uterus probably accounted for most of the differences in interval length. Gilbert and Wood-Gush (1971) thought that, for a particular hen, each egg of a sequence takes about the same time to travel down the oviduct; hence lag is not due to the time required to form an egg. The

last egg of a sequence spends more time in the shell gland, which results in a thicker shell. Bhatti *et al.* (1988) felt that the oviducal term is more variable than the interval from peak plasma LH concentration to ovulation (typically 5 hours) and that it is influenced by rate of lay, position in the sequence and ahemeral lighting patterns.

Ahemeral light:dark cycles longer than 24 hours allow the egg to spend more time in the oviduct, in particular in the uterus, where the additional hour spent in shell formation results in an increase in shell thickness (Morris, 1973). Since most eggs on long ahemeral cycles are laid in darkness, shell calcification takes place at the time of active absorption of calcium from the gut, which contributes to the increase in shell thickness. Egg size is also increased and this has been shown to be due to increases in yolk, albumen and shell weight. Melek *et al.* (1973) exposed hens to 24-hour and 27-hour light:dark cycles in order to measure the effect of an ahemeral daylength on the rate of passage of the ovum through the oviduct. The time spent in the upper oviduct and in the shell gland was prolonged by the ahemeral cycle. It was postulated that the time spent in the magnum depositing albumen may be a function of yolk size. The results of this work are summarised in Table 1.3. Birds kept on 23-hour light:dark cycles by Naito *et al.* (1989) were thought to have adapted by reducing the time spent in egg formation, especially in egg shell formation in the uterus.

Table 1.3: Duration of stay of the egg in regions of the oviduct, for hens on 24-hour and 27-hour light:dark cycles (from Melek *et al.*, 1973).

	24-hour cycle (14L:10D)		27-hour cycle (14L:13D)	
Region	Hours	Minutes	Hours	Minutes
Body cavity		7		19
Infundibulum, magnum, isthmus	5	9	5	25
Uterus	19	46	20	42
Total in oviduct	24	55	26	7

Periods of stress can delay oviposition in hens if occurring around the expected time of lay, although individuals respond differently. Reynard *et al.* (1996) found that relocating hens, from individual battery cages to group holding pens, delayed oviposition by a median of 104 minutes in 96% of the birds. The subsequent ovulation was also delayed by up to one hour in 85% of the hens. In further trials, Reynard and Savory (1999) provided environmental stress (by relocating hens from individual to group cages) for up to six hours, thereby inducing oviposition delays. A number of hens laid their eggs during the stress period, while others laid after its termination. The length of the delay in oviposition was influenced by the timing of the end of exposure to stress in relation to the expected time of lay. Short-term delays often resulted in eggs with a dusting of superficial calcium carbonate. Long-term delays, *i.e.* eggs laid seven to fifteen hours after the expected time, produced eggs that were white-banded and the subsequent eggs were slab-sided or soft-shelled. These shell abnormalities were described earlier by van Middelkoop (1971) following a study of egg shape and calcium deposition when two eggs occupied the uterus simultaneously.

Expulsion of the calcified egg from the reproductive tract is achieved by contractions of the uterus and vagina and by relaxation of the vaginal sphincter (Etches, 1996). As the egg is laid, the hen adopts a penguin-like stance. An event known as bearing down is characterised by a respiratory change and abdominal muscle contractions as the egg is pushed out of the vagina (Takahashi and Kawashima, 2003). Bearing down can occur when there is no egg in the vagina, which suggests that it may be caused either by hormonal stimulus of the arginine vasotocin receptors present in vaginal tissue or a nervous reflex induced by expansion of the vaginal lumen by the egg.

Prostaglandins play an integral role in both the timing and physical responses necessary for oviposition (Hargrove and Ottinger, 1992). Peak peripheral plasma concentrations of prostaglandins may be seen to coincide with oviposition. The smooth muscle contractions of the uterus are due to prostaglandin $F_{2\alpha}$, secreted by the granulosa layers of the follicles and arginine vasotocin, which is thought to be produced by the posterior pituitary gland. Prostaglandin $F_{2\alpha}$ also increases pressure in all segments of the oviduct. Prostaglandin E_2, also produced by the granulosa cells, is responsible for relaxation of the uterovaginal sphincter

muscle and an increase in luminal pressure of the magnum and isthmus. An increase in plasma prostaglandins is followed by an increase in circulating arginine vasotocin about ten minutes later.

The follicles responsible for the secretion of prostaglandins and hence the control of oviposition have been the subject of several studies. Typically, a surgical clamp is used to inhibit the blood supply to and from a follicle in order to determine whether its ligation blocks oviposition or prevents uterine contractions. Some of the results have been contradictory, but it may be that the effect of ligation depends on the time interval between ablation and the impending oviposition and on the position of the egg in the sequence (Kelly *et al.*, 1990). For the first and mid-sequence ovipositions, the secretion of prostaglandins appears to be associated with the preovulatory surge of LH (Etches, 1996). This would explain why uterine contractions are associated with the first ovulation of a sequence. The products of the largest preovulatory (F_1) follicle seem to induce oviposition for the first egg of the sequence without assistance from the second largest preovulatory (F_2) follicle or postovulatory follicles.

Kelly *et al.* (1990) found that, for the terminal oviposition of a sequence, both the F_1 and F_2 follicles played a role in inducing oviposition. Ligation of either follicle delayed the expulsion of the last egg by more than two hours. On the other hand, ligation of the fourth and fifth largest preovulatory follicles did not delay oviposition. Oviposition and uterine contractions are also eliminated when the largest postovulatory follicle is removed 18-24 hours before the terminal oviposition. Because the terminal oviposition of a sequence is not accompanied by an ovulation, these events occur in the absence of any endocrine changes associated with ovulation. In this case the signal for the release of prostaglandins is not known (Etches, 1996).

By injecting combinations of prostaglandins, Hargrove and Ottinger (1992) were able to induce premature expulsion of precalcified eggs, although it was not possible to induce oviposition earlier than five hours after the previous oviposition. At this stage the egg had a thin shell membrane and was in the distal portion of the isthmus or the proximal portion of the uterus. This suggests that prostaglandins may be involved in the premature expulsion of soft-shelled and shell-less eggs, although the cause is not understood.

1.5 Interval between oviposition and ovulation

Roughly half an hour after an egg is laid, the next ovulation takes place (Romanoff and Romanoff, 1949). Warren and Scott (1935) found the time lapse to vary from 14 to 75 minutes, with a mean of 30.7 minutes. This was achieved by anaesthetising hens immediately after laying, opening the body cavity, observing the process of ovulation and recording the time it occurred. Reviewing the literature, Fraps (1955) quoted estimates ranging from 7 to 74 minutes with a mean interval of 32.2 minutes. He used these times to calculate the oviducal term as 'the interval between lay of the designated egg and lay of the preceding egg, minus the interval between lay and associated ovulation'. In a discussion on temporal relationships, he assumed that the interval (lay to associated ovulation) decreased from 45 to 26 minutes as sequence length increased from two to six eggs. Melek *et al.* (1973) showed that the time interval between oviposition and ovulation may be altered by subjecting hens to long ahemeral light-dark cycles. The mean interval was 24 minutes for birds on 24-hour cycles and 36 minutes for birds on 27-hour ahemeral cycles. Etches (1996) used an interval of 30 to 45 minutes to estimate the time of ovulation from the time of the preceding oviposition.

Neal (1956) found that the retention of an egg in the uterus did not prevent the next ovulation from taking place. In several instances the oviposition of an egg that had been retained for 24 hours was followed within 2 hours by the laying of a thin-shelled egg. Under normal circumstances, ovulation did not precede oviposition. Hormonal interactions were thought at the time to inhibit ovulation during passage of the egg through the oviduct. Subsequently Morris (1973) assumed that under 21-hour cycles all eggs still spent about 24 hours in the oviduct, which meant that ovulation occurred about 4 hours before oviposition. Gow *et al.* (1985) observed that about 30% of their Australorp flock, selected under continuous lighting for reduced oviposition interval, ovulated before the associated oviposition took place. More recently, Naito *et al.* (1989) felt it likely that ovulation had occurred in some instances prior to oviposition, in their selection process for shorter oviposition intervals using 23-hour light:dark cycles. If not, then the time interval from oviposition to ovulation had certainly been shortened.

Ovulation is thus not dependent on oviposition; further evidence is provided by the fact that the

time of the next ovulation is not changed if an egg in the uterus is caused to be expelled early by manual crushing (Bastian and Zarrow, 1955). However, under normal circumstances oviposition is dependent on ovulation. If ovulation is induced prematurely by an injection of gonadotrophin, the time of oviposition of the egg in the uterus is also hastened. Since the terminal oviposition of a sequence is not followed immediately by an ovulation, both the F_2 follicle approaching maturation and the recently ruptured follicle play a role in timing egg laying. The integrity of the post-ovulatory follicle is known to be important in timing oviposition; its removal or damage can lead to a delay in laying and hence an increase in lag (Gilbert and Wood-Gush, 1971).

1.6 The phenomenon of internal laying

During the course of the ovulatory cycle, the infundibulum of the oviduct becomes extremely active just prior to ovulation. The fimbriae become engorged with blood and move in a waving motion over the preovulatory follicle, creating a massaging effect (Bahr and Palmer, 1989). The follicle is usually engulfed by the infundibulum before ovulation, so that ovulation occurs directly into the infundibulum. This process, although not fully understood, is under hormonal and neurological control and its effectiveness may therefore be impaired at puberty and with ageing. Internal laying occurs when, for some reason, an ovum is not grasped by the infundibulum and therefore remains in the body cavity, or it may be reversed up the oviduct and sent back into the abdomen.

One of the earliest recorded observations was made by Neal (1956) who described a hen that 'maintained the appearance of production' for six months, including nesting behaviour, without laying a single egg. Fully developed follicles were felt on palpation. The author surmised that the yolks were being absorbed from the body cavity. Sturkie (1955) studied the rate of resorption using blue dye that was intravenously injected to colour the yolk material. A laparotomy was performed to remove a mature ovum from the ovary and to place it in the body cavity. Subsequent autopsies revealed that the body absorbed the blue yolk within 24 hours, but only once the vitelline membrane had ruptured.

It is thought that internal laying is more common at the beginning (during sexual maturation) and end (due to reproductive senescence) of the laying period and may be partly attributable to asynchrony between the development of the ovary and oviduct (Gilbert, 1972; Koops and Grossman, 1992). This asynchrony occurs, for instance, if the oviduct development is retarded relative to the growth of the ovary, as seen in male line turkey hens (Melnychuk *et al.*, 1997). It is difficult to assess the prevalence of this defect in a flock, since susceptible birds tend to remain in perfect health, show normal pigmentation for their age and have normal pubic bone spread (Sunde, 1987). It has been suggested that as much as 45% of potential eggs may be lost to internal laying during the first and the twelfth month of the laying period and 5% during the remaining months (Gilbert, 1972). Wood-Gush and Gilbert (1970) estimated the amount of potential eggs lost to internal laying to be 11.6%. Regrettably the age of the birds in their trial was not specified. During the course of their experimental work, Lewis and Long (1992) found that 42% of non-layers between 30 and 40 weeks of age were internal layers; the rest suffered from neoplasia, diseased ovaries or oviducts, poor condition or sex reversals. In another study Sunde (1987) reported rates of internal laying of 7.2% and 15.3% by hens that had been in production for six and nine months respectively. On autopsy, internal layers were found to have enlarged abdominal areas, swollen kidneys, and small eggs in the body cavity or oviduct tears. All had active ovaries. In contrast, Sturkie (1955) found apparently normal ovaries and oviducts in hens known to be internal layers.

Turkey hens at initiation of egg laying were found by Bacon *et al.* (2000) to lay internally. Although all LH surges were associated with increases in progesterone, indicating the presence of a mature follicle, not all surges were associated with egg laying. An estimated 7% and 33% of ovulations occurred internally for hens given constant light and 14L:10D respectively.

On post mortem, Renema *et al.* (1999) found a number of unexplained post-ovulatory follicles in broiler breeder hens before the onset of lay. The incidence was higher in birds fed *ad libitum* during rearing and with earlier onset of sexual maturity, than in hens subjected to feed restriction during the same period. Follicles ovulated prior to the first oviposition were presumably lost internally. It was postulated that this may be due to the ovary reaching a mature state before the oviduct, resulting in a loss of potential eggs.

Because of this unobtrusive phenomenon, Etches (1996) urged caution in estimating ovulation rate from rate of oviposition. If internal laying is taking place, then rate of lay will be lower than rate of ovulation. Wood-Gush and Gilbert (1970) found nesting behaviour to be a better indication of ovulation rate than oviposition. This behaviour includes a pre-laying call, restlessness, peering into nest boxes or pacing about the cage floor in apparent frustration (Gilbert and Wood-Gush, 1971). Birds that exhibited nesting-without-laying were found by laparotomy to have ovulated about 24 hours previously. In one case, an atretic follicle caused nesting behaviour, suggesting that some event prior to rupture of the follicle arouses the nesting instinct (Wood-Gush and Gilbert, 1970).

It would be helpful to determine the extent of internal laying by examining the relationship between ovulation rate and oviposition rate throughout the laying year (Robinson *et al.*, 1990; Koops and Grossman, 1992). However, even if the pattern of internal laying over time were characterised in a sample of hens, it would be difficult to make broad assumptions for a population. It is presumed that individuals vary in their ability to synchronise ovary and oviduct function effectively. An oviduct tear may predispose a particular individual to internal laying, but there is also an element of uncertainty that makes predictions difficult. If, for example, the infundibulum happens to be far away from the surface of the ovary where ovulation takes place, that particular follicle may be lost within the body cavity; however, this may be an isolated incident and the hen may not be prone to internal laying. Factors such as nutritional status, ahemeral light:dark cycles and genetics also play a role in determining the frequency of internal laying in a hen. *Ad libitum* feeding during rearing has been observed to cause a higher incidence of internal laying in layers (Johnson *et al.*, 1984) and broiler breeder hens (Hocking *et al.*, 1987; Yu *et al.*, 1992a; Hocking, 1996) than in those birds subjected to feed restriction. This in turn causes a reduction in the production of marketable or settable eggs.

1.7 The incidence of double-yolked eggs

The occurrence of double-yolked eggs in young hens at onset of lay is a common phenomenon and has been attributed to some irregularity in the process of ovulation or in the regulation of the follicular hierarchy. The most likely cause is simultaneous development and ovulation of

two ova (Christmas and Harms, 1982). A smaller percentage result from two ova which were developing a day apart, being ovulated simultaneously, or from an ovum remaining in the body cavity for a day after ovulation and being picked up with the next ovum on the following day. In commercial layers, although double-yolked eggs may reduce the rate of lay, these extra large or jumbo eggs may be marketed at a premium price.

Strain and season influence the occurrence of double-yolked eggs (Christmas and Harms, 1982). Certain strains are more likely to lay eggs with double yolks at onset of lay. The seasonal influence is due to its effect on age at sexual maturity; the earlier the onset of maturity, the higher the incidence of double-yolked eggs, presumably due to asynchrony between ovary and oviduct.

Abplanalp *et al.* (1977) were able to show that the prevalence of double yolks was positively associated with body weight in layers. In their selection experiments for increased incidence of double yolks over eleven generations, body weight increased by 300g or 20%. The mean number per hen of eggs with double yolks to 40 weeks of age increased from about two in generation one to 30.6 in generation eleven. In addition, several eggs with triple or quadruple yolks began to appear. The authors felt that the ability to lay eggs with multiple yolks is an inherited trait and is not simply due to random occurrences.

Zakaria *et al.* (1983) found five double-yolked eggs, all produced during the first month of lay, in their studies of the follicular hierarchy. The mean duration of the rapid growth period for the five eggs was 8 days; not dissimilar to the period for normal single-yolked eggs.

In broiler breeder hens that are not subjected to feed restriction during rearing and are therefore overweight at point of lay, multiple hierarchies are found in the ovary, where two or more follicles are of a similar size. These follicles may ovulate simultaneously, resulting in double- or even multiple-yolked eggs. This is undesirable in broiler breeders because these eggs may not be set in the incubators, leading to a reduction in the potential number of settable eggs.

1.8 Calcium homeostasis and soft-shelled eggs

In growing pullets bone growth is regulated by osteoblasts, which form trabecular and cortical bone, and osteoclasts, which resorb bone (Whitehead, 2004). At the onset of sexual maturity, the large surge in estrogen changes the function of osteoblasts to form medullary bone rather than structural bone. Medullary bone is unique to birds and crocodiles, being a woven bone that acts as a labile source of calcium for eggshell formation. It lines structural bone and also occurs as spicules within the marrow cavity, especially in the leg bones. Medullary bone builds up rapidly during the early stages of lay and continues to accumulate slowly over the laying period. In the meantime the osteoclasts resorb both medullary bone and exposed structural bone with the result that the amount of structural bone declines while the hen is in lay. This leads to osteoporosis, which is thought to be widespread in laying flocks. Once the hen stops laying, estrogen levels fall and osteoblasts resume structural bone formation, thereby enabling skeletal regeneration to take place.

A high proportion of shell calcium comes from resorbed medullary bone. This is because the egg is normally in the uterus undergoing shell formation at night, when there is little calcium available from the digestive system. The medullary bone then accrues calcium during the day (Etches, 1987). Although medullary bone is important for shell formation, there is not a direct relationship between medullary bone content and shell quality (Whitehead, 2004). Shell quality is usually very good at the start of lay, when little medullary bone has been formed. Presumably the calcium for these early shells comes largely from structural bone.

The hormone calcitonin plays a role in the maintenance of calcium homeostasis (Ogawa *et al.*, 2003). It acts on the bone to resorb calcium into the blood and on the kidney to modify mineral excretion. Calcitonin receptor sites are found, amongst other places, on the endometrium of the uterus, so the hormone is presumably involved in regulating calcium uptake for shell formation. During the ovipository cycle, the binding affinity and capacity of the calcitonin receptors change apparently in relation to the stages of egg formation.

The deposition of calcium carbonate during shell formation is initially slow, increasing to a

maximum rate and finally slowing down about two hours before oviposition. Both parathyroid hormone and 1.25-dihydroxycholecalciferol are also believed to be involved in mobilisation of calcium from medullary bone (Etches, 1987). The form in which calcium is supplied in the diet plays a role in modifying calcium homeostasis. If included in fine powder form so that the hen is unable to select this nutrient, calcium will be consumed at a fairly even rate throughout the day. If, on the other hand, calcium is provided in large particles such as crushed oyster shell, it may be preferentially consumed at the end of the photoperiod. In a slow-release form, more calcium becomes available for shell formation at night, thereby reducing the need for osteoclastic activity.

Premature expulsion of eggs from the uterus for unknown reasons may lead to the production of soft-shelled eggs, if there was insufficient time for secretion of shell material in the uterus. Intravenous injections of arginine vasotocin have been seen to cause oviposition of soft shells in experimental hens (Takahashi and Kawashima , 2003). Soft-shelled eggs were found to be associated with short ovulation intervals (van Middelkoop, 1972). If two or more ovulations occurred within a 24-hour period, the resulting eggs were unlikely to have normal shells unless a double-yolked egg was produced.

Older hens have a higher incidence of soft shells and broken eggs, due to thinner shells, than young hens. This is due to their changing hormone profiles and reduced ability to transport calcium at the duodenum (Hansen *et al.*, 2003). There is a complex reciprocal relationship between calcium and estrogen. The failure of calcium regulating mechanisms with age is due to reduced populations of estrogen receptors in the kidney and uterus. The kidney is believed to be the primary site where estrogen sets off a cascade of events leading to the uptake of calcium from the gut. Older hens are therefore less efficient both in their absorption of dietary calcium and in their utilisation of calcium in the uterine fluid for shell formation.

1.9 Distribution of oviposition times

Oviposition time is affected by the position of the egg in a sequence (Wilson *et al.*, 1964). The initial egg of a sequence is usually laid early in the morning soon after the lights come on, whereas the terminal egg in the sequence is laid late in the afternoon (Foster, 1968). The

intermediate eggs fill the range between first and last in an orderly fashion, each oviposition time being slightly later in the day than its predecessor. Heywang (1938) found to the contrary; many sequences were observed where time of lay appeared to be almost erratic. Although he conceded that some errors in trap nesting may have occurred, he also claimed that highly irregular sequences were possible. It must be stated that the times of oviposition in his trials were recorded no more accurately than to the nearest hour.

The interval between successive ovipositions varies according to the length of the sequence, with longer sequences having shorter time intervals between ovipositions. Distributions of oviposition times are therefore influenced by sequence length. This is not apparent in most research reports, since distributions tend to be plotted as one curve for the entire flock, rather than grouping them per sequence length. As birds age (or the length of the light-dark cycle reduces) and sequence length shortens, there is a tendency for the distributions of oviposition times to change from a unimodal to a bimodal shape (Foster, 1968) with a corresponding reduction in the percentage of eggs laid in the modal eight hours (Lewis and Perry, 1991), *i.e.* an increase in variance about the mean. This is because in shorter sequences, the times of oviposition of the first and terminal eggs assume greater relative importance.

The distribution of oviposition times is modified by the characteristics of the light:dark cycle. Under continuous light, eggs are laid throughout the 24-hour period, with longer clutches being produced but at the expense of overall egg numbers, because the oviposition intervals are longer. Birds are capable of laying in continuous darkness, but their hen day production is low and may be readily improved by exposing the hens to light:dark cycles. Regular intermittent lighting is interpreted by the hen as continuous light, with the result that the distribution of oviposition times tends to be more or less uniform. On long ahemeral cycles, the gain in egg production due to the production of longer sequences is balanced by the loss due to longer oviposition intervals, although egg weight is increased significantly. The between bird variation in rate of lay is reduced. Light:dark cycles in excess of 30 hours may cause the distribution of oviposition times to change from unimodal to uniform (Foster, 1972).

Figure 1.2 shows the frequency distribution of oviposition times of the data from Morris (1968-69), over the entire period and in the form of a histogram. The mean time of oviposition was

13:31 with a standard deviation of 3h 05m. A total of 19 eggs (1.1%) were laid during the scotoperiod.

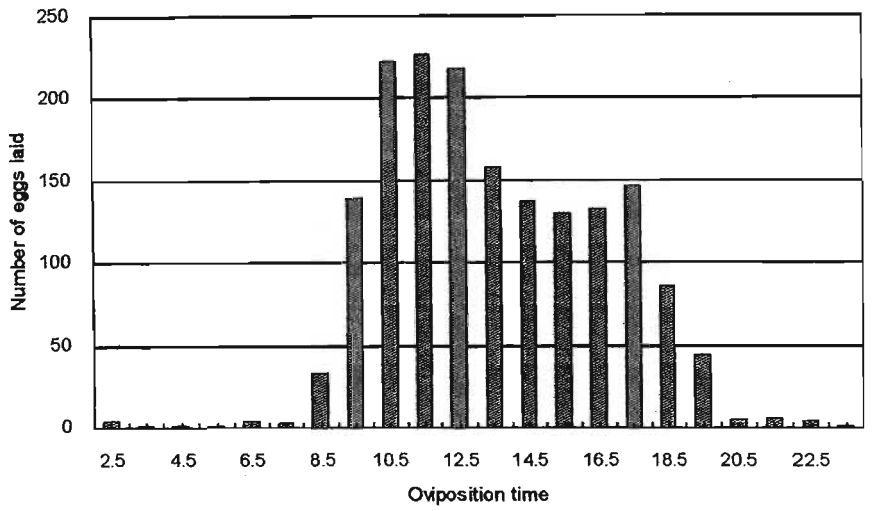


Figure 1.2: Histogram showing distribution of oviposition times for birds kept on 14L:10D, with lights turned on at 08:00 and off at 22:00 (Morris, 1968-69; unpublished data).

A histogram of the oviposition times for two- and three-egg sequences is shown in Figure 1.3. There are clearly two modes, one at 11:00 (three hours after lights on) and the other at 18:00. Figure 1.4 shows the histogram for 6-9-egg sequences, which is unimodal and slightly positively skew.

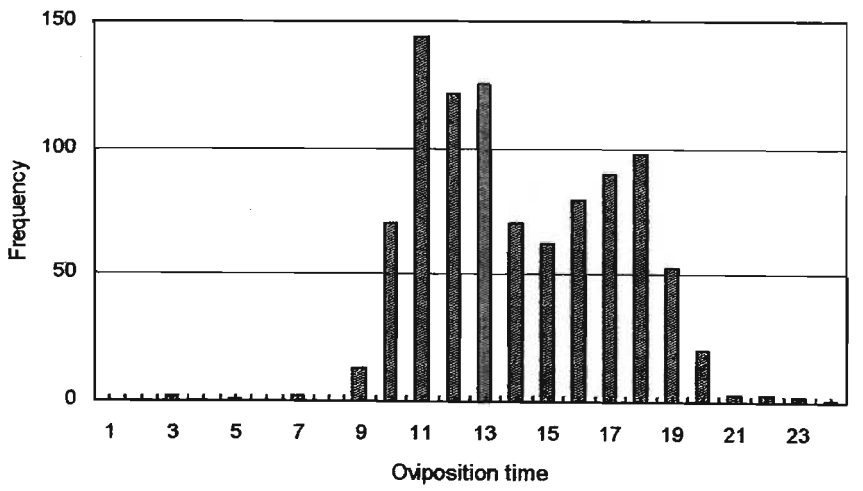


Figure 1.3: Histogram of oviposition times for two- and three-egg sequences, showing its bimodal nature (Morris, 1968-1969; unpublished data).

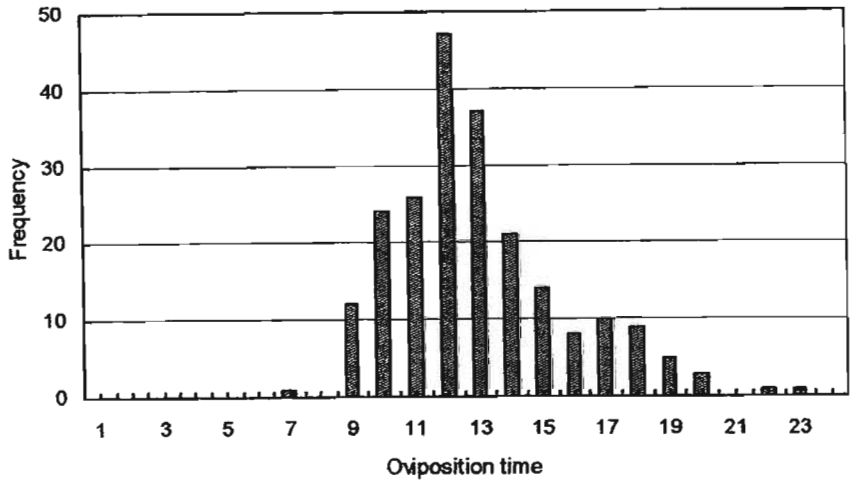


Figure 1.4: Histogram of oviposition times for six- to nine-egg sequences, showing its unimodal nature (Morris, 1968-1969; unpublished data).

The arithmetic mean oviposition time is commonly used by researchers to analyse variation in laying patterns. Bhatti and Morris (1988) used the time of lay associated with the minimum standard deviation as the best estimate of the mean. It has been suggested by Lillpers (1991) that the circular distribution method is more accurate if the range in oviposition times is more than six hours, which it normally is. This is accomplished by finding the mean of the polar angles for every oviposition time and transforming it back from degrees to hours and minutes. Their method does not appear to have found favour with other poultry scientists. If all oviposition times are concentrated within a limited period of the day and if the distribution is nearly symmetrical, then the arithmetic mean is reliable.

Arithmetic mean times of oviposition for the different sequence lengths are summarised in Table 1.4. It must be borne in mind that the sample sizes (shown in Table 1.1) for sequences of six, eight and nine eggs were relatively small. In general, as sequence length increased, the first oviposition of the sequence occurred earlier in the day and the last oviposition occurred later in the day. In all cases each subsequent oviposition occurred later in the day than its predecessor.

Table 1.4: Arithmetic mean oviposition times (± se) for laying hens kept on 14L:10D, with lights turned on at 08:00 and off at 22:00 (data from Morris, 1968-69; unpublished)

	Position of egg in sequence								
seq. length	1	2	3	4	5	6	7	8	9
1	13:47 (±2h 55m)								
2	11:26 (±1h 32m)	16:05 (±2h 00m)							
3	10:21 (±1h 19m)	13:06 (±1h 31m)	17:17 (±1h 38m)						
4	10:01 (±0h 55m)	11:55 (±0h 50m)	13:46 (±1h 23m)	17:21 (±1h 52m)					
5	09:22 (±1h 24m)	11:09 (±1h 00m)	12:20 (±0h 53m)	13:47 (±1h 16m)	17:20 (±1h 35m)				
6	09:09 (±1h 12m)	11:04 (±0h 39m)	11:52 (±1h 09m)	12:29 (±1h 04m)	14:16 (±1h 49m)	16:51 (±2h 45m)			
7	09:25 (±1h 04m)	10:43 (±0h 50m)	11:39 (±0h 57m)	12:15 (±1h 02m)	13:00 (±1h 14m)	14:24 (±1h 37m)	17:39 (±1h 49m)		
8	08:55 (±0h 29m)	10:01 (±0h 28m)	10:54 (±0h 58m)	11:08 (±1h 00m)	11:27 (±1h 10m)	12:02 (±1h 05m)	13:31 (±1h 39m)	16:38 (±1h 00m)	
9	09:55 (±0h 41m)	10:53 (±0h 50m)	10:57 (±1h 21m)	11:33 (±0h 59m)	11:38 (±1h 02m)	12:06 (±0h 54m)	12:50 (±0h 27m)	13:54 (±0h 19m)	17:49 (±0h 33m)

Mean time of oviposition is also influenced by sequence length and occurs about 14 hours after the onset of darkness (Wilson *et al.*, 1964). Long sequences tend to have an earlier mean than short sequences, since a greater proportion of the eggs are laid before noon. The mean times of oviposition in three selected lines of layers on 16L:8D were found by Lillpers (1991) to be 13.6, 14.5 and 13.3 hours after the onset of darkness. The strains were selected for egg number, egg mass and egg mass/food consumption respectively. Patterson (1997) gave estimates of the median time of oviposition as 13 hours for a young flock at peak on 15L:9D and 14 hours after lights out in an old flock on 15½L:8½D at the end of its laying cycle. This shift in oviposition time may be partly due to the change in hours of light, but it is also a manifestation of a reduction in sequence length with age. Mean time of oviposition is slightly later in the day for older flocks, because of the change in the distribution of times of lay that occurs with age (Lewis and Perry, 1991).

Bhatti and Morris (1988) proposed two general models to predict mean time of oviposition in relation to onset of darkness for all light:dark cycles that are capable of entraining oviposition. It must be noted that the model does not make allowances for the age of the hens. Both scotoperiod (S) and cycle length (C) influence mean time of lay. When the light:dark cycle is equal to or longer than 24 hours, mean time of lay (H) is predicted by the equation:

$$H = 64.62 - 2.161\ C + 0.268\ S \qquad \text{(Equation 1.2)}$$

For light:dark cycles shorter than 24 hours:

$$H = -4.97 + 0.740\ C + 4.482\ S - 0.175\ CS \qquad \text{(Equation 1.3)}$$

From the experimental data summarised by Bhatti and Morris (1988), birds on 24-hour daylengths and 16L:8D had a mean time of oviposition 13.77 hours after the onset of darkness, compared to the 14.9 hours predicted by the model. As the scotoperiod used in various trials increased up to 23 hours on 24-hour daylengths, the mean time of lay was delayed up to19 hours after dusk.

The range in oviposition times is usually limited to an eight- to nine-hour period of the day, corresponding to the length of the period when ovulation is induced (Lillpers, 1991). The length of the period varies depending on genetics, hen age and environmental factors, of which the light cycle is the most important. In the three strains used by Lillpers (1991), over 70% of the mean oviposition times for individual hens to 51 weeks of age fell within one standard deviation of the population mean. All three lines had a slight negative skewness for the frequency distributions of both the mean oviposition time of all eggs and the mean oviposition time of the first egg in a sequence. However, the line selected for egg numbers had an earlier mean oviposition time and more pronounced negative skewness than the line selected for egg mass, reflecting the longer sequence lengths.

Patterson (1997) found in two commercial flocks on 15L:9D that oviposition times were spread over a 14-hour period, although 75% were laid in a seven-hour period. All eggs were laid during the photoperiod and a small percentage was laid in the early evening. The distributions of oviposition times appeared to be positively skew, since a greater proportion of the eggs were laid in the morning than in the afternoon.

Brown-egg-laying strains lay their eggs earlier in the day than white-egg-laying strains, as evidenced by a significantly earlier mean oviposition time for the first egg of a sequence (Lillpers, 1991). Lewis *et al.* (1995) reported that the brown egg hybrid (ISA Brown) in their trial had a mean oviposition time 1.2 to 1.4 hours earlier than the white egg hybrid (Shaver 288), over a range of lighting treatments. They speculated that the genetic difference may be due to a number of reasons: a shorter egg formation time, a change in the phase setting of the open period, a reduction in the time interval between commencement of the LH surge and its peak plasma concentration, or a shorter period between peak plasma LH and ovulation. The most likely explanation was thought to be a phase shift in the timing of the open period for LH release.

1.10 Entrainment of oviposition

Under conventional light:dark cycles, hens normally lay their eggs during the light hours. This

is because the endogenous circadian rhythm, which determines the timing of the open period for LH release and hence the timing of ovulation, is synchronised with environmental signals. A small rise in the plasma concentration of LH immediately following the onset of darkness reflects a diurnal rhythm, which is present even in prepubertal pullets, so it is not caused by any positive feedback action of steroids (Williams and Sharp, 1978c). It also occurs in laying hens on nights when the preovulatory surge of LH does not occur. In the presence of a mature follicle, this small increase in the level of LH in the plasma at the onset of darkness causes the follicle to secrete progesterone, which in turn is responsible for the preovulatory surge of LH. Morris (1973) postulated that the open period for LH release commences about 7 hours after sunset on 24-hour cycles and 4 hours after sunset on 27-hour cycles. The LH surge occurs about 4-6 hours prior to ovulation.

In continuous light or continuous darkness and in the absence of other diurnal entraining factors, such as noise, temperature and human routines, the distribution of oviposition times should be even throughout the 24-hour period. This is because a constant environment would allow each bird to produce eggs at its own natural rhythm in free-running clutches (Foster, 1972). However, in practice it is difficult to provide a completely constant environment and the birds are not free-running but are affected by external rhythms (Morris, 1977; Bhatti and Morris, 1978a). The periodic factors of the environment by which free-running circadian oscillations can be synchronised are termed zeitgebers (Wilson *et al.*, 1964) or entraining factors. Morris (1961) attempted to rule out external influences by placing birds in a sealed chamber with continuous light and continuous noise (from a radio), although slight fluctuations in temperature were found to be unavoidable. Despite the measures taken, times of lay still showed significant diurnal periodicity.

Foster (1968) assumed that the hen has a constant minimum interval between ovipositions in a clutch, referred to as its natural rhythm, which will be blocked from expression by a light:dark cycle which exceeds it in length. Maximum egg production for an individual hen will occur when the length of the light-dark cycle is the same length as the hen's natural rhythm. In his research, he found the optimum daylength to be close to or exceeding 25 hours. In order to achieve maximum rate of lay in a flock, some compromise is necessary, since one light:dark

cycle is applied to all individuals (Foster, 1972). Prior to this, Byerly and Moore (1941) were able to increase clutch size by keeping birds on a 26-hour daylength with 14L:12D. They postulated that this was because the light:dark cycle was synchronised with the hen's natural ovulation cycle. It was presumed that a 26-hour daylength maintained pituitary and ovarian function and delayed the onset of refractoriness. Over the years, genetic selection for increased egg production and shorter oviposition intervals has reduced the length of this natural rhythm, so that today high producing hens at peak rate of lay show rhythms close to 24 hours.

Entrainment is the state in which the period of a rhythm regularly coincides with the period of an environmental cycle (Bhatti and Morris, 1978a). The degree of entrainment varies according to the strength of the signal, so some measure is required to enable evaluation of the relative success or failure of the signal in entraining the biological rhythm. The standard deviation about the mean time of lay is a useful measure of dispersion, but it is affected by cycle length. For instance, on 21-hour light:dark cycles the distribution of oviposition times tends to be bimodal with a large standard deviation (Bhatti and Morris, 1978a). These same authors found that the number of eggs laid in a modal eight-hour period is not affected by cycle length. The proportion of ovipositions occurring in the modal eight hours is therefore considered a suitable indicator of the degree of entrainment, with a proportion of 80% or higher considered to show satisfactory entrainment.

The time of ovulation, which determines the time of oviposition, is primarily controlled by the environmental pattern of light and darkness (Bhatti *et al.*, 1988). Although it is possible to alter oviposition times by sound and feeding time, light is the most powerful stimulus (Wilson *et al.*, 1964). The light:dark interface is the zeitgeber or synchroniser for hens. The onset of darkness, *i.e.* sunset, is the more important factor for phase setting the endogenous circadian rhythm (Bhatti and Morris, 1978b) although dawn has a subsidiary influence (Bhatti *et al.*, 1988). The best entrainment occurs if both sunset and sunrise are present and at times reinforcing other environmental cues (Bhatti and Morris, 1978b). For example, full entrainment may be achieved if the sunset signal is given at 4pm, 8pm or midnight but not at other times, suggesting that noise, temperature or human routines have a modifying influence.

Other characteristics of the light:dark cycle, such as light intensity, total daylength and the duration of the periods of light and darkness, play a role in entraining oviposition. Hens on ambiguous skeleton photoperiods, for example 2L:12D:2L:8D, interpret the light period after the longer dark period as dawn, or the start of the subjective day and ignore external signals such as the time of servicing of the birds (Mongin *et al.*, 1978). Morris and Bhatti (1978) reported that a bright:dim ratio of 10:1 is sufficient for full entrainment, but the more the light:dark cycle deviates from 24 hours, the greater the contrast between bright and dim needs to be. A 15-minute photoperiod is as satisfactory a timing cue as a 5-hour scotoperiod, indicating that photoperiod is the more potent signal for phase setting oviposition (Bhatti and Morris, 1978a). However, once again the more the light:dark cycle deviates from 24 hours, the stronger the signals need to be (*i.e.* longer photoperiods and scotoperiods) to achieve entrainment. Ultraviolet radiation has been shown to be ineffective in entraining oviposition in layers (Lewis *et al.*, 2000) although it acts as a zeitgeber in canaries, suppresses nocturnal melatonin secretions in rodents and penetrates the hypothalamus of Japanese quail. The authors suggested that a higher UV intensity than the one used in their experiments might be more effective.

Temperature usually has a significant but subsidiary effect to light on oviposition time. Under conditions of continuous light and with two 12-hour periods of high (30 °C) and low (20 °C) temperature, the period of high temperature mimics the light period in terms of the distribution of oviposition times (Bhatti and Morris, 1977). Mean time of lay is related to the onset of the cold period, which corresponds to sunset. With normal light:dark cycles and high temperatures during the dark hours, temperature has no effect on mean time of lay, indicating that the light:dark cycle is the dominant phase-setting signal. If the scotoperiod is gradually reduced, *e.g.* to one hour a day, temperature slowly becomes the more dominant factor in determining mean time of lay.

1.11 Circadian rhythms in reproduction

A circadian rhythm is one which occurs approximately every 24 hours in an oscillatory fashion. Similarly, a cycle which is described as circadian has an expected length of about 24 hours.

Birds are known to have a circadian rhythm in photosensitivity that is involved in their reproductive responses to light (Siopes and Wilson, 1980). If light falls within the daily photosensitive phase, photostimulation occurs. Male Japanese quail respond by increasing testicular weight, the amount of testicular stimulation being proportional to the amount of light provided within the photosensitive phase. In Japanese quail the photosensitive phase commences about twelve hours after the onset of light and lasts for four to six hours.

Non-visual light perception is known to be involved in entraining behavioural circadian rhythms and in photoperiodically-controlled reproduction (Binkley *et al.*, 1975). Birds possess sensitive extra-retinal photoreceptors, located in the pineal gland, which contribute photic information for the entrainment of circadian systems to light cycles (Menaker *et al.*, 1981). Recent trial results support the view that both the pineal and the suprachiasmatic nucleus, a structure in the hypothalamus, contain circadian oscillators. Damage to neural input to the pineal gland appears to have no effect on circadian rhythms, suggesting that the pineal is hormonally coupled to the rest of the system. The role of the pineal gland is to maintain the bird in harmony with its environment (Ralph *et al.*, 1974). Efforts to relate the pineal gland to photoperiodic control of LH have failed (Liou *et al.*, 1987) because no difference in plasma LH levels exists between normal and pinealectomised hens.

The major hormonal product of the pineal, melatonin, shows rhythmic release with a circadian oscillation and the enzyme regulating its synthesis, serotonin N-acetyltransferase (NAT), shows a circadian rhythm of activity. Both these rhythms entrain to the light cycle (Menaker *et al.*, 1981) although on ahemeral cycles, the entrainment of serotonin NAT is not entirely successful (Doi *et al.*, 1983). Plasma concentrations of melatonin increase rapidly with the onset of darkness, as does NAT activity, in both blinded and sighted birds. This coincides with a small rise in plasma LH (Liou *et al.*, 1987). Rapid decreases in the serum melatonin level and in NAT activity follow the start of the light period in both groups, although the rate of change in the enzyme is faster in sighted birds (Binkley *et al.*, 1975). Although the exact role of melatonin in avian reproductive cycles still remains uncertain (Noddegaard, 1998), Menaker *et al.* (1981) suggested that melatonin may couple the pineal with other components of the circadian system. Liou *et al.* (1987) thought that melatonin may be the primary coupler for

other hormones to participate in the entrainment of oviposition to the light:dark cycle. The duration of the melatonin elevation is likely to modify the time of oviposition under different light:dark cycles.

There seems to be a refractory period during the hours of light, when the birds are not responsive to a change from light to darkness, because hormone and enzyme levels remain low if a period of dark is given during the expected light time. The production of melatonin therefore appears to be controlled by the bird's subjective day and night, not by light and darkness *per se* (Lewis *et al.*, 1989). This is supported by earlier work done by Doi *et al.* (1983), where birds changed to either continuous light or continuous dark showed increases in NAT activity at the expected time of darkness, based on the previous light:dark cycle. Changes to the amplitude of the rhythms and phase shifts were observed. Similarly, in continuous darkness, the cyclic changes to serum melatonin content continue as free-running circadian rhythms although the oscillations are dampened (Ralph *et al.*, 1974). This research provides evidence of a self-sustaining circadian oscillator in the pineal. Daily injections of melatonin to hens kept on continuous light are capable of entraining time of oviposition (Liou *et al.*, 1987), although there is some debate about this as the doses used in these trials were well above physiological levels (Noddegaard, 1996). The rhythmic diurnal change in melatonin content in serum can be phase shifted by changes to the light:dark cycle; furthermore, the phases may be lengthened or shortened by changing the light-to-dark ratio (Ralph *et al.*, 1974). The shape of the oscillation of NAT activity is also influenced by the ratio of light to dark (Liou *et al.*, 1987). It would appear that the pineal gland is able to measure the length of the dark period and to convert this to a chemical signal, *i.e.* melatonin production (Binkley *et al.*, 1975).

The pineal itself may not be necessary for entrainment, because blind, pinealectomised sparrows can still be entrained to light:dark cycles (Menaker *et al.*, 1981). A second oscillator, possibly in the hypothalamus, may be entrained to external stimuli but it appears not to be self-sustaining; this suggests a role for the pineal as a pacemaker. Melatonin is also secreted by the retina, although in the rat retinal melatonin does not circulate in the plasma. This may also be the case in chickens, since melatonin levels are undetectable in nocturnal serum after pinealectomy (Ralph *et al.*, 1974).

Caution is needed when comparing species, because different neuroanatomical and neurochemical systems are involved and the role of the eyes is not the same (Binkley *et al.*, 1975). For instance, noradrenalin stimulates NAT activity in the rat but suppresses activity of the same enzyme in the chicken. In birds the onset of darkness is the signal for increased NAT activity; in rats and monkeys it is the onset of light (Doi *et al.*, 1983). Furthermore, the avian pineal gland has endocrine and photoreceptive functions, whereas the mammalian pineal has only endocrine functions.

In support of the above, Noddegaard (1998) found that plasma melatonin levels and oviposition patterns were synchronised with 24-hour and 28-hour light:dark cycles in hens. On the 24-hour daylength, almost all eggs were laid during the daylight hours whereas in the ahemeral cycle, ovipositions occurred primarily during the last 9 hours of the dark period. Both oviposition patterns and melatonin secretion could be entrained to phase shifts, *i.e.* changes in the onset of darkness. The melatonin response phase-led the oviposition response by two cycles. He concluded that the change in melatonin rhythm following phase shifts coincided with the change of the open period for LH release. Light immediately suppresses plasma melatonin while actual melatonin release during darkness is controlled by an endogenous clock.

Earlier work done by Tamarkin *et al.* (1976) indicated that the reproductive system in hamsters showed a diurnal rhythm in its sensitivity to melatonin. Injections of melatonin in the morning did not alter reproductive function. However, prolonged daily injections of the hormone in the afternoon caused decreased secretion of LH and FSH and testicular atrophy in male hamsters, and anoestrus in females. This treatment was considered to have the same effect on reproduction as short photoperiods.

In mammals, melatonin binds to both the pituitary and the suprachiasmatic nucleus of the hypothalamus. This may explain why their reproductive cycles are easily synchronised by exogenous melatonin. In avian species, however, the hormone binds only to the SCN of the hypothalamus. It has been proposed by Noddegaard (1996) that melatonin may exert its control of the preovulatory LH peak indirectly at the point of hypothalamic responsiveness to the progesterone feedback and subsequent LH-RH release.

Based on the evidence, melatonin is thought to be one of the hormones linking reproductive rhythms to the light cycle. Melatonin is somehow involved in the timing of ovipositions (Noddegaard, 1996) but is probably only one of several interacting systems.

1.12 The asynchronous ovulatory cycle

Bastian and Zarrow (1955) were amongst the first scientists to suggest that the pause in egg laying is caused by asynchronism between two cycles; a 24-hour day-night rhythm involving LH and a rhythmic maturation of ovarian follicles. A mechanical analogy was provided. A shoot containing balls represented the follicular hierarchy and the regular arrival of balls at the end of the shoot, rhythmic attainment of follicular maturation. A moving board with equally spaced slots represented the 24-hour day-night rhythm. The slots were comparable to an adequate stimulus for ovulation, such as a high concentration of LH in the plasma, which was present for a relatively long period (about 8 hours) every night. A diagram showed how a ball would fall into a slot if aligned, representing ovulation, or be held back if the stick and board were not in alignment. An empty slot was equivalent to a pause day. The number of balls falling into consecutive slots depended on the relative rates of movement of the balls and the board. This was analogous to clutch length being affected by the rate of follicular maturation and the duration of the open period. At the time this theory was put forward, limited information was available on the reproductive hormones and their functions as well as on the process of follicle maturation. Taken at face value, the hypothesis provided an easily understood explanation for the hen's sequential laying pattern. Much later, Williams and Sharp (1978c) objected to this hypothesis on the grounds that it failed to include a role for the positive feedback action of an ovarian steroid on the release of LH. The assumption that high concentrations of LH persist routinely every 24 hours even when a mature follicle is not present is inaccurate, since progesterone secreted by the maturing follicle is needed to initiate and maintain the ovulation-inducing surge of LH (Etches and Cunningham, 1976).

Fraps (1955) published his theory of the 'excitation cycle' which revolutionised thinking and provided a plausible explanation for the timing of events in a closed cycle (*i.e.* a sequence consisting of any number of consecutive ovulations followed by a pause of one day). The first

component was postulated to be an ovarian hormone (possibly progesterone), which excited the neural apparatus and was therefore known as the excitation hormone. The second component, the neural apparatus (possibly the hypothalamus), was presumed to be periodic in its sensitivity to the excitation hormone. When the excitation hormone levels reached the required threshold for neural stimulation, the event called excitation occurred, leading to ovulation-inducing hormone release (presumably LH-RH or LH). Because the sensitivity of the neural apparatus waxed and waned in a cyclical manner, excitation was restricted to an 8-hour open period. It was speculated that excitation occurred 8 hours before ovulation. Fraps (1955) assumed that the same lag observed between successive ovulations applied to successive excitations. The lag was created because slightly more than 24 hours was required for a follicle to produce threshold levels of the excitation hormone. A graphical representation showed the relationship between excitation hormone concentrations and thresholds of response for a 7-day cycle.

Etches and Schoch (1984) utilised and developed the theory of Fraps (1955), proposing a model of the hen's ovulatory cycle instead of the excitation cycle. The first component was a regulator substance (a hormone or neurotransmitter) subjected to a circadian rhythm that restricted the release of the preovulatory surge of LH to a limited portion of the day. The concentration of the regulator substance followed a similar curve to Fraps' sensitivity of the neural apparatus and was represented by a 3-compartmental model. The second component was the final phase of follicular maturation, which replaced Fraps' excitation hormone, and which was represented by a Gompertz equation. Where the two curves met, ovulation was considered to occur.

Gilbert (1972) was sceptical of the theory of Fraps (1955) on the basis that the excitation hormone had not been identified (although the most likely options were progesterone or oestrogen), neither was there any evidence of a cyclic sensitivity of the neural apparatus or in the threshold of response. Williams and Sharp (1978c) also refuted the existence of a diurnal rhythm in the sensitivity of the hypothalamus to the positive feedback action of progesterone, because Etches and Cunningham (1976) demonstrated that an injection of progesterone given during the proposed period of maximum insensitivity (at 16:00) caused increases in the plasma

concentrations of both LH and progesterone. Williams and Sharp (1978c) proposed an alternative hypothesis, similar to that of Bastian and Zarrow (1955); that the timing of the preovulatory release of LH could be due to a diurnal rhythm of basal LH secretion. Prior to this, Wilson *et al.* (1964) suggested that the observation that hens lay sequences of varying length could be explained by differences in the amount of LH synthesized for release and differences in the threshold of tissue sensitivity.

A number of researchers have queried the existence of a circadian rhythm in LH release, since no evidence has been put forward. Menaker *et al.* (1981) suggested that chicken pineals may contain an interval timer that synchronises with the light cycle. In support, Silver (1986) thought that the hen's ovulatory cycle appeared to provide an example of a reproductive system where circadian and interval-timing mechanisms are linked. He compared the hen's ovulatory cycle with that of the rat, where the LH surge system has a circadian basis, constraining the preovulatory LH discharges to occur only between 14:00 and 16:00. Consequently ovulation is confined to a limited portion of the day and occurs about 10 hours after the LH surge. In the rat the brain and pituitary monitor the condition of the ovary, so that the LH surge is generated only when a sufficient crop of follicles is mature enough to ovulate. The steroid hormone estradiol must be secreted to meet the requirements of the surge system. The system that times the critical estradiol-priming stimulus is an interval timer, *i.e.* it measures the duration of the estradiol stimulus at any time of day. The estradiol signal has a critical minimum duration of at least 7 hours. In the induction of the LH surge for ovulation, the excitatory hormone is an estradiol signal of adequate duration. In doing comparisons between the two species, Silver (1986) felt there was sufficient evidence that the chicken ovulatory cycle is similar to that of the rat in its reliance on two fundamentally different timing mechanisms, despite the fact that an oscillatory LH surge system has not been shown to exist, nor are the requirements of the steroid priming signal for LH release well understood. He felt that the excitation hormone is likely to be either estradiol alone or estradiol and progesterone acting synergistically. It is probable that the excitation hormone needs to be present for an adequate duration for ovulation to occur.

Despite these findings, Etches (1996) felt that another method of restricting LH release to an

eight-hour period needed to be found, unless or until the circadian oscillator is identified. Yet Lillpers (1998) stated that theories regarding the importance of a circadian rhythm in timing ovulation are still valid, because individuals with oviposition intervals shorter than 24 hours have as yet been identified only by using short ahemeral daylengths. This implies that the open period for LH release may be adjusted according to the length of the light:dark cycle.

In spite of the lack of conclusive evidence, it is still widely accepted that the interaction of two physiological systems is responsible for the asynchronous ovulation cycle in domestic hens (Etches, 1984; Lillpers and Wilhelmson, 1993a; Johnson, 1984). These are the maturation of the largest ovarian follicle and a circadian rhythm that times the preovulatory surge of LH. Ovulation occurs when the maturation of a follicle coincides with a certain phase of the circadian rhythm. It has been suggested by Naito *et al.* (1989) that follicular maturation works on a 24- to 27-hour cycle and the circadian oscillator (a biological rhythm entrained to environmental zeitgebers) shows 23- to 26-hour cycles. The difference in the lengths of the two cycles accounts for their being asynchronous. The eight to ten hours of the day coinciding with the release of LH is known as the open period and is entrained to the onset of darkness (Wilson and Cunningham, 1984).

1.13 Selection criteria and testing environments

Selection in layers has always been concerned with multiple objectives, so the selection intensity for any one trait has not been as high as in broilers (Hunton, 1984), although more recent breeding programmes in broiler breeders involve selecting for about ten traits simultaneously (Redpath, 2002). For many years one of the criteria for selecting for improved laying performance was total egg number up to a certain age (Yoo *et al.*, 1984; Bednarczyk *et al.*, 2000). This favoured those individuals with high ovulation rates, a low incidence of internal laying and mean oviposition intervals close to 24 hours. The outward manifestation would have been longer but fewer sequences with less pause days. Because the 24-hour light:dark cycle imposes a physiological barrier, masking the potential of birds capable of ovulating more frequently than once a day, genetic progress has slowed. As the mean oviposition interval approaches 24 hours, the variation between individuals decreases. A

frequency distribution of oviposition intervals may be seen to be truncated in the normal 24-hour environment (Lillpers, 1998). It has been suggested that most of the modern laying breeds will reach a plateau, where selection for egg number is no longer effective in improving rate of lay (Foster, 1981). The underlying cause is inadequate selection pressure, which is the case when there is a decrease in the variation between individuals in a particular trait. The low heritability estimates for egg numbers reported by Bednarczyk *et al.* (2000) and by Yoo *et al.* (1988), *i.e.* 0.05-0.14 and 0.18 respectively, suggest that only minute possibilities exist for further improvements to their strains using traditional methods of selection. As a consequence, both selection criteria and testing environment are being forced to change (Yoo *et al.*, 1984). Since strong relationships exist between total egg number and some clutch traits, such as mean sequence size and number of sequences, better results may be achieved by focusing on these as selection criteria (Bednarczyk *et al.*, 2000), although the heritability estimates for clutch traits were found to be moderate. Genetic and phenotypic correlations between mean oviposition interval and total egg number are negative and medium to high (Lillpers, 1998; Yoo *et al.*, 1988) and heritability estimates for oviposition interval appear to be high (Yoo *et al.*, 1988). This suggests that oviposition interval may be a good selection criterion for further improvements in egg production. Oviposition interval is after all an indirect measure of ovulation interval (Yoo *et al.*, 1986) and two decades ago there was still thought to be considerable variation amongst individuals for this trait (Hunton, 1984).

McClung *et al.* (1976) used oviposition interval as their only selection criterion and were successful in reducing the interval by 73 minutes (from 25.6 to 24.38 hours) over seven generations of selection in a SCWL line. A standard 24-hour light:dark cycle was used. Corresponding decreases in the standard deviations in successive generations were evidence of reduced variation amongst individuals about a mean interval. The average clutch length increased from 5.1 days in generation one to 12.8 days in generation seven. Most of the improvement occurred in the first three generations, suggesting that the population may have been approaching a genetic plateau for this trait. An unexplained increase in the mean number of pause days, from 1.5 to 3 days, was observed. Their estimate of heritability for oviposition interval was 0.35, compared to 0.54 for a White Leghorn population reported by Yoo *et al.* (1988). The distributions of oviposition intervals have been described by Yoo *et al.* (1986) as

having less variation, becoming more positively skew and increasing in kurtosis (peakedness) as selection under 24-hour light:dark cycles continually reduced the mean interval.

Hunton (1984) suggested that ahemeral lighting programmes could play a role in revealing the existence of genetic variation that had not been exploited. Truncation of the distribution of oviposition intervals at the length of the light:dark cycle is an indication that individuals with shorter natural rhythms exist. Subsequently, ahemeral light:dark cycles shorter than 24 hours have been used successfully in selection programmes, because it becomes possible to identify outstanding individuals with a high, but normally concealed, egg production capacity. Since it is unlikely that many birds exist with a natural rhythm much less than 22 hours (Foster, 1968), it is important to choose an adequate cycle length. An 18-hour light:dark cycle was found to be too short for successful selection (Naito *et al.*, 1989) and a 16-hour daylength resulted in a substantial drop in rate of lay (Woodard *et al.*, 1962).

Since ovulation is controlled physiologically by the light:dark cycle, the length of this cycle imposes a lower limit on ovulation interval (Yoo *et al.*, 1988). By plotting frequency distributions of mean intra-sequence intervals, Lillpers (1998) has shown that variation increases if the truncating effect of the 24-hour barrier is removed. In her work 8.4% of the hens, when kept on 23.5-hour daylengths, were found to have oviposition intervals between 23.5 and 24 hours. The adaptation to 23.5-hour cycles was found to be age-related, since no benefit to the short cycle was seen in older hens past their peak, whose mean oviposition interval was far from 24 hours.

Foster (1981) performed selection experiments over a number of years, initially using a 23-hour light:dark cycle, which was then reduced to 22 hours and subsequently to 21 hours. A control flock was kept on a normal 24-hour daylength. The selection criterion was increased ovulation frequency or rate, which was estimated from oviposition records. Internally laid eggs were deduced from studying times of oviposition for each sequence and were consequently included as ovulations. Interestingly, the incidence of internal laying was observed to be higher in the flocks kept on the ahemeral lighting regimes, compared to the control flocks. The heritability estimates were higher for ovulation frequency than oviposition frequency. The shorter

light:dark cycles allowed for continued improvement in ovulation frequency over the generations, whereas there was a diminishing response in the birds kept on normal daylengths. In a review of work done by others, Foster (1985) did express reservations about whether the selection technique of using short ahemeral cycles was justified by the results.

Naito *et al.* (1989) selected for improved rate of lay in two environments (23-hour and 24-hour daylengths) over five generations in a population approaching a plateau for improvement in laying performance. As a consequence, the mean intra-sequence interval was reduced by 74 minutes on the 23-hour cycle and by 29 minutes on the 24-hour cycle. In the base generation the greater variation in the distribution of mean oviposition intervals was observed in the hens on the 23-hour cycle, attributable to individual differences in adapting to the short cycle. This distribution gradually changed to one which appeared to be positively skewed with a much narrower range, indicating a reduction in individual variation over five generations. The distribution was eventually clearly truncated at 23 hours in generation 5, when 88% of the birds had mean intervals less than 24 hours. This suggests an opportunity for further genetic progress on even shorter light:dark cycles. The corresponding distribution for the hens given 24-hour daylengths was heavily truncated by generation 5, when 58% of individuals laid eggs with intervals of less than 24h10min. Following selection, the mean time of oviposition measured from the onset of light became earlier and the proportion of eggs laid in the modal 8 hours (a measure of the degree of entrainment) increased in both light:dark cycles. The distribution of oviposition times changes as a consequence of the reduction in intra-sequence intervals; more eggs are laid in the morning as sequences become longer, with the result that there are fewer terminal eggs.

Continuous lighting regimes have been used successfully for selecting for reduced oviposition intervals, although it is difficult to maintain a constant environment. Diurnal variations in temperature, humidity, outside noise, air movement and human routines are known timing cues for hens (Wilson *et al.*, 1964; Morris, 1977). Under normal light, as the mean oviposition interval of a population decreases due to selection, the distribution becomes increasingly positively skew with a more pronounced peak and a reduction in the standard deviation of the mean. These observations are less clear under continuous lighting regimes (Yoo *et al.*, 1986).

In continuous light, the ovulation cycle is presumably under the control of the ovarian cycle (rate of follicular maturation) and, in the absence of entraining factors, the free-running endogenous circadian rhythm; hence eggs would still be laid in sequences. The oviposition interval is determined by the longer of the two cycles (Yoo *et al.*, 1986).

Morris (1977) estimated the length of the free-running period by a sequential analysis of oviposition times. The cycle time that gave the highest number of eggs laid in the modal 8 hours was taken as the best estimate. About 25% of the hens were found to have free-running periods close to 24 hours on continuous light. The remainder of hens ranged from 24 to 28 hours. These figures represent an estimate of the length of the cycle of the endogenous circadian rhythm. The times of oviposition are expected to be uniformly distributed throughout the day in a perfectly constant environment. Morris (1977) reported that in hens which appeared to be free-running more eggs were laid between 08:00 and 19:00, suggesting some diurnal influence. Further evidence of an internal biological clock controlling the regular recurrence of open periods for LH release was given by the fact that the hens continued to lay in sequences and the first egg of a sequence was normally produced during the day, between 05:00 and 16:00. Other estimates of the length of the free-running period range from 21hours to 29 hours, with a mean of about 24.5 hours (Yoo *et al.*, 1986). These authors also found that some populations exhibited a clear diurnal distribution of oviposition times, indicating a degree of entrainment.

Gow *et al.* (1985) selected for reduced oviposition intervals on continuous light. The increase in rate of lay with successive generations was associated with shorter intervals, longer sequences and an advance in the mean time of oviposition. The distribution of oviposition times (on 15.25L:8.75D) for the selected Australorp birds was positively skew compared to that of the non-selected group, because of the longer sequences, but they occurred over a similar period of the day. In the opinion of the authors, neither the duration nor the timing of the open period was altered by selection.

It has been suggested by Naito *et al.* (1989) that the hen's circadian oscillator shows 23 to 26 hour cycles and is entrained to environmental zeitgebers, most notably light:dark cycles close to

24 hours. The cycle of follicle maturation is given as 24 to 27 hours by Naito *et al.* (1989) and as 24 to 26 hours by Yoo *et al.* (1986), the latter referring to it as the ovarian cycle. Hence, the oviposition interval normally reflects the length of the ovarian cycle, the longer of the two. The oscillator appears to adapt to a 23-hour cycle without undergoing a genetic change. The phase of the open period for LH release, which controls the timing of oviposition, appears to remain unaffected by selection for increased egg production (Naito *et al.*, 1989). It was established by Gow *et al.* (1985) that the plasma concentration of LH is not altered by selection. In their trials, more LH peaks were seen at the start of the open period, which accounted for the earlier mean time of lay. The reduction in oviposition interval was thought to result from two factors; firstly, a decrease in the oviducal term and secondly, an increase in the rate of follicle maturation, *i.e.* a decrease in the ovarian cycle (Yoo *et al.*, 1986). The short ahemeral light:dark cycles provide an environment for identifying individuals with the potential for increased rate of passage through the oviduct and faster rates of follicular maturation.

It must be noted that a consequence of selecting for reduced oviposition interval is a reduction in mean egg weight. Naito *et al.* (1989) reported a decrease in both the weight and the proportion of egg shell, which was more pronounced in birds on the 23-hour ahemeral cycle than those on a normal 24-hour cycle. This was thought to be due to a reduction in the time spent in egg formation; in particular in the uterus, which agrees with the findings of Yoo *et al.* (1986) cited above. McClung *et al.* (1976) found a 5% decrease in egg weight after selecting for reduced intervals for seven generations on 24-hour light:dark cycles.

The use of different selection criteria and altered selection environments may continue to yield significant genetic progress in egg production performance in flocks that were considered to be reaching a plateau for further improvement. The application of light:dark cycles of lengths shorter than 24 hours, and continuous light, are able to reveal concealed genetic variation for a particular trait, thus allowing for increased selection pressure (Naito *et al.*, 1989). Furthermore, the strong relationships that exist between total egg production and some clutch traits, such as oviposition interval, means that rapid assessments of genetic improvements may be made after a relatively short period in lay.

1.14 Follicular dynamics

The ovary contains millions of oocytes, all formed during embryogenesis, but only about one hundred of these have a diameter greater than 1mm (Gilbert and Wells, 1984). The smaller follicles are usually described as white, because the primordial yolk material is pale in colour. Palmer and Bahr (1992) reclassified the follicles in size groups according to their steroidogenic activity. Small white follicles (SWF) have a diameter less than 1mm, whereas large white follicles (LWF) range in size from 2-4 mm. The small yellow follicles (SYF) have some accumulation of yellow yolk material and vary from 4-10 mm in diameter. The significant differences in steroid production between SWF, LWF and SYF indicate that biochemical maturation accompanies follicular growth (Robinson and Etches, 1986). However, the granulosa cells from SYF with diameters of 6-8mm are steroidogenically incompetent; it is the theca cells that secrete progesterone, oestradial and androstenedione at this stage. The slow growth evident in these three groups is characterised by granulosa cell proliferation, which is associated with elevated mRNA levels for the nuclear transcription factor *c-myc* (Johnson, 1996). This *c-myc* is known to play a role in normal cell proliferation and to inhibit cell differentiation.

Large yellow follicles (LYF), also referred to as yellow growing follicles (YGF) since they are undergoing rapid growth, range from 7mm to about 40mm in diameter and make up the hierarchy. Since granulosa cells from the prehierarchical follicle are steroidogenically incompetent, the granulosa layer must undergo a considerable amount of differentiation after follicular recruitment. Once these follicles grow to reach a diameter of 9-12mm, progesterone is produced by the granulosa layer in response to FSH (Tilly *et al.*, 1991a). There appears to be a cooperative interaction between the granulosa and thecal layers to maximise steroid output in these follicles.

The number of smaller follicles varies greatly between hens. In general, as the follicle size increases, the number within the size group decreases. In the ovary of one particular hen, Gilbert and Wells (1984) counted 18 follicles between 1 and 2 mm in size, but only one follicle with a diameter between 6 and 8 mm. The number in the hierarchy remains fairly constant and

has been variously defined as four to eight (Zakaria, 1999a) or five to six, with an occasional ovary containing four or seven follicles (Gilbert *et al.*, 1983), or five to seven at the onset of lay (Hocking, 1996; Hocking and Robertson, 2000). These follicles are arranged in a hierarchy of increasing size, which is maintained by the daily recruitment of a single small follicle and the ovulation of the most mature one. It would appear, though, that there are no rigid time intervals of 24 hours between follicles (Gilbert, 1972).

The growth of follicles with diameters greater than 3mm occurs in an orderly, synchronous manner (Gilbert *et al.*, 1983). The smaller follicles are recruited into the hierarchy at diameters of about 7mm. Estimates of measurements of the size at transformation of 5-6mm and 6.7mm have been reported by Zakaria *et al.* (1983) and Zakaria *et al.* (1984b) respectively. The precise mechanism controlling recruitment is unknown, but it is thought to be a positive physiological process rather than a passive one (Hocking and Robertson, 2000). Why one follicle should remain viable and be selected for continued growth and differentiation, while many others are subjected to atresia, remains unclear. FSH and / or vasoactive intestinal peptide (VIP) may play a critical role in follicle selection and subsequent differentiation, due to the removal of some inhibitory influence in one follicle. It has been suggested by Rzasa and Paczoska-Eliasiewicz (2000) that noradrenalin may exert an influence on the destiny and growth of developing ovarian follicles. In addition, ovarian inhibin has recently been implicated in establishing and maintaining the hierarchy (Johnson, 1996). It is known that only one follicle enters the rapid growth phase at a time, after the largest follicle has ovulated (Zakaria *et al.*, 1984b). The transformation of a follicle from the resting stage to the rapid growth phase is restricted to the 10-hour period 6-16 hours after ovulation, although the highest occurrence rate is found 8-14 hours after ovulation (Zakaria *et al.*, 1984b). This phase is characterised by the rapid deposition of yellow yolk. Originally Bacon and Skala (1968) proposed that there is a rest period, between cessation of rapid growth and ovulation, of about one day, but yolk deposition is now thought to be a continuous process until ovulation (Gilbert *et al.*, 1983; Zakaria, 1999a).

There is considerable variation in the length of the rapid growth phase, with early reviews giving a length of seven to eleven days and an increase in mass from 0.5g to about 20g (Gilbert,

1972). The mean period of rapid development was found by Bacon and Skala (1968) to range from 7.47 to 8.78 days but this was influenced by both clutch length and position in the clutch. The trend was for a shorter rapid growth period with both longer sequences and advancing position in a sequence. Zakaria *et al.* (1984a) performed a detailed but similar study of the stage of rapid follicular growth, which occurs six to ten days prior to ovulation, in relation to clutch size and position of the egg in the sequence. Two dyes, Sudan black and Sudan red, were injected on alternate days into White Leghorn hens from 14 to 20 months of age, in order to determine follicular growth patterns. Eggs were collected and hard-boiled before cutting the yolk in half. The number of coloured rings found in the yolk gave the length of the rapid growth period. It was found that as sequences lengthened, the number of days of the rapid growth phase decreased but growth rate increased. For two-egg and six-egg sequences, the mean rapid growth periods were 8.52 and 7.36 days and the mean growth rates of follicles were estimated to be 0.252 and 0.310 respectively. The authors suggested that follicles with a longer growth period may develop the competency to ovulate at a slower rate. The number of growing follicles in the hierarchy and the total amount of daily yolk deposition was less in layers with short clutches. Zakaria (1999b) also found that within sequences, as the position advanced from the first towards the terminal ovulation, the period of rapid growth shortened. The first follicles tended to have the slowest growth rate. In this experiment there was no clear trend in follicular volume. It has been suggested by Bastian and Zarrow (1955) that successive follicles of a clutch are ovulated at progressively earlier stages of maturity. Zakaria (1999b) concluded that the terminal follicle acquired the competency to ovulate more quickly than the first follicle of a sequence. Sequence length is therefore largely determined by the rate of follicular maturation; hens that produce follicles which grow at a faster rate over a shorter period of time are likely to lay longer sequences.

Follicular atresia is a common process in the hen's ovary to prevent the further development of a large number of follicles. Atresia is in fact a normal alternative to growth, since the ovary contains millions of oocytes (Gilbert and Wells, 1984) far in excess of the hen's requirements. Gilbert *et al.* (1983) suggested that ovulation rate is a product of two complementary mechanisms, one for the initiation of growth of follicles and the other controlling the rate of atresia. The mechanism by which atresia takes place is known as apoptosis, or programmed

cell death (Tilly *et al.*, 1991b) and it is initiated in the granulosa layer (Johnson, 1996). Subsequently cells in both the theca and granulosa layers die in a controlled fashion due to endonuclease activity, which causes internucleosomal DNA fragmentation (Tilly *et al.*, 1991b). Initially atretic follicles may be seen to have surface haemorrhages and later they become collapsed, shrunken or deformed (Gilbert *et al.*, 1983). Within a period of about six days the yolk material is resorbed. Most of the atresia occurs amongst the smaller follicles in the 1-8 mm group, although Tilly *et al.* (1991b) found it to be confined to slowly growing white follicles with 4-6 mm diameters. It is rare to find a follicle undergoing atresia in the hierarchy of a bird at peak production; the usual fate of these follicles is ovulation (Gilbert *et al.*, 1983). However, calcium-deficient diets in laying hens have reportedly caused atresia in both the smaller follicle groups and amongst large yellow follicles in the hierarchy (Waddington *et al.*, 1985; Hocking *et al.*, 1987). Apoptosis is rapidly induced in postovulatory follicles (Tilly *et al.*, 1991b; Johnson, 1996). It is not precisely clear what physiological difference makes the smaller follicles prone to atresia in domestic hens, but mitotically active cells appear to be more susceptible to apoptosis (Johnson, 1996) and the prehierarchical follicles undergoing proliferation fit into this category. The rare instance of apoptosis in the hierarchy is attributed to the germinal disk region, which is an area of active cell proliferation. However, the germinal disk may also send paracrine signals to maintain follicle viability. In mammals, oestrogen is known to inhibit atresia (Hocking and Robertson, 2000). Recent research indicates that several genes may be implicated in both programmed cell death and follicle viability (Johnson, 1996).

The pituitary gonadotrophin FSH is generally regarded as being responsible for follicular recruitment and growth (Palmer and Bahr, 1992). The theca cells of the smaller follicles have FSH receptors, but their capacity to bind FSH is progressively lost during maturation (Etches, 1984). Similarly, the sensitivity of granulosa cells to LH increases and the sensitivity to FSH decreases as follicles mature (Yamamura *et al.*, 2000). This is due to a decrease in the number of FSH receptors in granulosa cells as the follicle in the hierarchy approaches ovulation, although the affinity of FSH receptors for FSH does not change (Ritzhaupt and Bahr, 1987). This decrease is associated with a decrease in FSH-stimulated steroidogenesis and adenylyl cyclase activity. Since the theca layer loses its ability to secrete oestrogen at the same time, it may be that oestrogen is involved in increasing the number of FSH receptors.

Hence FSH regulates the number of smaller growing follicles, their yolk deposition and atresia, but does not influence the growth of the large follicles in the hierarchy (Palmer and Bahr, 1992). As expected, the concentration of plasma FSH is lower in immature birds than in sexually maturing pullets undergoing development of the reproductive organs. An association exists between the time that FSH is elevated and maximum yolk deposition (Zakaria, 1999b), suggesting that follicular growth is due to an adequate stimulation of the ovary by FSH. It may be that young hens producing short sequences at onset of lay have inadequate amounts of FSH to maintain growth of the smaller follicles and to promote their subsequent recruitment into the hierarchy (Zakaria, 1999b).

Both small and large follicles produce steroids in response to an LH stimulus from the anterior pituitary. The smaller follicles are known to have LH receptor sites (Robinson and Etches, 1986) and are a major source of oestrogen, secreted from the theca cells. Estrogens are involved in yolk formation in the liver and in calcium deposition. No progesterone is produced by the smaller follicles (Robinson and Etches, 1986). Noradrenalin, the principle catecholamine in the ovary, affects steroid secretion by the follicles. The concentration of all catecholamines decreases as the follicles mature (Rzasa and Paczoska-Eliasiewicz, 2000). The follicles change from an FSH-dominated phase to an LH-mediated phase sometime during the transition from 12-15mm in diameter. Tilly *et al.* (1991a) suggested that there may be an increased coupling of LH receptors to the cAMP-generating system in these LYF. As the follicle approaches ovulation, there is an increase in LH-stimulated adenylyl cyclase activity (Ritzhaupt and Bahr, 1987). LH stimulates the production of progesterone from the granulosa layer of the large yellow follicles in the hierarchy. Normally progesterone that is produced by follicles other than the largest one is not released into the circulation system (Yu *et al.*, 1992b). As the follicle matures the aromatase activity is reduced, which results in an inability to convert progesterone to other steroids, including oestrogen. Consequently plasma levels of progesterone increase with advancing follicle maturation, so that the granulosa cells of the F_1 follicle are the primary source of progesterone (Etches, 1984). In support of this, Williams and Sharp (1978c) found the plasma levels of progesterone to be 30% higher when a mature follicle was present compared to an immature one. The positive feedback mechanism therefore largely involves a competent F_1 follicle, which is responsible for the initiation and maintenance of the

preovulatory surge of LH and which results in ovulation (Robinson *et al.*, 1990). Blood samples taken at regular intervals and subjected to radioimmunoassay show that a small rise in plasma LH immediately following dusk causes a gradual increase in progesterone levels and then a second steeper increase coinciding with the LH surge (Williams and Sharp, 1978c). A large amount of progesterone production is a prerequisite for the potentiation of the LH surge (Johnson, 1996) so LH surges are coupled with progesterone pre-ovulatory surges (Liu *et al.*, 2004). The physiological role of progesterone is therefore to maintain the ovulation-inducing surge of LH (Etches and Cunningham, 1976), although Nakada *et al.* (1994) found evidence in hypophysectomised hens that progesterone alone can induce ovulation. Estradiol levels, on the other hand, remain relatively constant during the ovulatory cycle (Liu *et al.*, 2004). The adrenal gland may also influence ovarian function, because injections of corticosterone, ACTH and deoxycorticosterone have the ability to induce ovulation (Etches, 1996).

Maturation of follicles is therefore associated with an increase in the mass and diameter of the ovum, due to the accumulation of yolk, and an increase in the size and number of granulosa and thecal cells (Etches, 1984). The final stages of maturation also involve development of endocrine capability (Etches, 1996) following cell differentiation. As follicles mature the number of gonadotrophin binding sites is altered, which enables them to change from an FSH-mediated phase to an LH-dominated phase (Zakaria, 1999a). The F_1 follicle appears to become sensitive to ovulation-inducing stimuli some 14-16 hours after the previous ovulation, which coincides with the development of LH receptors on granulosa cells (Etches, 1984). The readiness of the follicle to ovulate is determined by its ability to secrete progesterone in response to an LH surge and not by its size (Yu *et al.*, 1992b).

Ovarian follicle selection is thus a balance between growth and atresia (Gilbert *et al.*, 1983). The ability of the ovary to establish and maintain the hierarchy is crucial in determining the hen's ovulation rate and ultimately her rate of egg production. The ovaries of hens producing longer sequences tend to contain a greater number of smaller follicles and rapidly growing follicles (Gilbert, 1972). Variation in the number of follicles recruited into the hierarchy, in the rate of maturing of follicles and in the incidence of atresia within a population of hens are some of the factors causing individuals to produce ovulation sequences of different lengths and hence

to lay at different rates.

1.15 Reproductive changes at sexual maturity

Although laying pullets eventually reach sexual maturity spontaneously when reared on short daylengths, exposing them to longer photoperiods advances the age at first egg (Lewis *et al.*, 2001). Ovary and oviduct growth in the sexually maturing pullet are stimulated by the release of gonadotrophins from the pituitary, which in turn are secreted in response to gonadotrophin releasing hormone (GnRH) from the hypothalamus. Estradiol induces the production of yolk precursor lipoproteins by the liver as well as stimulating oviducal development and modifying calcium metabolism (Liu *et al.*, 2004). The pineal gland appears to play a role in the sexual maturation process. Pinealectomy has been found to delay yolk deposition in the follicles of juvenile hens, and to retard maturation of the ovary and oviduct, under stimulatory photoperiods, in juvenile Japanese quail (Brake *et al.*, 1985).

Although this neuroendocrine system mediating the photoperiodic control of reproduction is functional at about three weeks of age (Dunn, 1999), prepubertal birds that are stimulated by increasing daylengths do not show sustained gonadal development. As a consequence, age at first egg is not advanced by photostimulation prior to about 42 days of age. It has been suggested that gonadal steroids, most likely estradiol, are required for maturation of the neurones of the hypothalamus (Dunn, 1999). However, subsequent work done by Lewis *et al.* (2001) shows that estradiol on its own is not responsible for the lack of response to light stimulation before 42 days. Birds in the peri-pubertal stage show an increase in plasma LH and synchronised gonadal development following transfer to stimulatory daylengths. Plasma FSH concentration gradually increases from about 6 weeks of age, but a significant increase occurs following photostimulation (Lewis *et al.*, 1999).

In turkeys, the first ovulatory surge of LH occurs two to three weeks after photostimulation (Bacon *et al.*, 2000). Before photostimulation, LH secretion is pulsatile. As the turkey hens approach sexual maturity there is an increase in the baseline concentration of LH, which is thought to regulate initial ovary and oviduct growth, as well as to increase oestrogen secretion.

Unlike in some mammals, the first preovulatory surge of LH in turkeys is associated with the release of a follicle, indicating that no phantom LH surges occur at the onset of lay. The interval between the first and second surges appears to be longer than successive intervals. In their experimental work, Bacon *et al.* (2000) found that subjecting the turkey hens to either continuous light or 14L:10D did not affect the interval between the LH surges. Melnychuk *et al.* (1997) recorded the first ovipositions 26 and 27 days after photostimulation for female and male line turkey hens respectively, but the incidence of unexplained postovulatory follicles (and presumably internal laying) was high.

Prior to photostimulation, the ovary of the immature 15- to 16-week-old pullet contains both small and large white follicles up to 4mm in diameter (Rzasa and Paczoska-Eliasiewicz, 2000). These continue to grow so that a week later follicles with diameters up to 8mm may be observed. The deposition of yellow yolk ensues, resulting in the presence of large yellow hierarchical follicles one week before laying commences. During sexual maturation, as in the mature laying hen, the concentrations of the catecholamines in ovarian follicles decrease significantly as the follicles mature.

1.16 Reproductive senescence and sequence characteristics

Commercial egg producers are familiar with the flock egg production curve, where percent hen day production rises steeply after the onset of sexual maturity, reaches a peak about ten to twelve weeks into the laying cycle and then steadily declines with age. After a force moult, egg production is invariably lower in the second year than in the first, although Sykes (1986) suggested that previous laying and not age *per se*, reduces the potential for laying in the second period. The rate at which a flock approaches peak production is influenced by the distribution of ages at sexual maturity of the individuals in the flock, as well as by the mean early sequence length and its distribution. Persistency of lay is influenced by the changes to sequence characteristics with advancing age.

The rate of egg production in an individual hen is determined by the length of the oviposition sequences and by the number and duration of the pauses. A nine-egg sequence followed by a

single non-laying day constitutes a rate of lay of 90%, whereas a four-egg sequence followed by a single pause day is equivalent to a rate of lay of 80%. Oviposition sequence length is in turn determined by the length of the ovulation sequences; the two being equal unless internal laying takes place. The laying pattern of high-producing hens is therefore characterised by longer egg sequences with fewer inter-sequence pause days.

During a laying cycle of an individual hen, sequence length does not remain constant. Robinson *et al.* (1990) coined the phrase 'prime sequence' for the single characteristically long sequence of ovipositions that occurs around peak production in domestic poultry. The length of this prime sequence varies considerably amongst individuals but appears to be initiated at a similar chronological age, *i.e.* irrespective of whether the birds are early or late maturing. The length of the prime sequence was found to be correlated with total egg production (Robinson *et al.*, 1992; Robinson *et al.*, 1994) and so may be a good indicator of reproductive potential. Hens with long prime sequences are assumed to have finely-tuned ovulatory cycles.

Individual hens seem to have a high degree of consistency in their patterns of egg laying after peak (Lillpers and Wilhelmson, 1993a); a bird tending to lay in short sequences will continue to do so. However, within a population a considerable variation in sequence length exists between individuals. In one line the authors found a mean sequence length (from 31 to 51 weeks) of 8.3 days (±5.5). The large standard error is an indication that individuals do vary greatly from the flock mean, although it may also be a reflection of the different levels of persistency with age.

In a study of reproductive characteristics of broiler breeders, Robinson *et al.* (1994) found that the birds commenced their laying cycle with short sequences of three to five eggs, followed by a long prime sequence (mean 24.9 days) at 29 to 30 weeks. After 32 weeks of age, sequence length declined rapidly. The mean sequence length for the entire experimental period was 4.6 days. When sorted into four groups according to egg production it became obvious that sequence characteristics were significantly different. High producing hens had fewer sequences, with a longer prime sequence and a greater mean sequence length than low producers. In an earlier study on broiler breeders, Robinson *et al.* (1990) reported a mean

prime sequence length of 24.3 days, an overall mean sequence length of 4.1 days and a maximum inter-sequence pause length of 1.6 days.

As expected, laying hens have considerably longer sequences overall, as well as a longer prime sequence and shorter pauses than broiler breeders. Robinson *et al.* (1992) recorded the egg production data of 160 individually-caged SCWL hens, from 20 to 68 weeks. The mean number of laying sequences (± SEM) was 33.14 (± 0.9) with a mean sequence length of 9.67 (± 0.3) days and mean prime sequence length of 67.01 (± 2.4) days. The maximum recorded sequence length for one particular hen was 155 days. With advancing age, the incidence of sequences shorter than 5 days increased.

Lewis and Perry (1991) found a mean prime sequence length of 58.8 (± 7) days in sixteen individually-caged layers, monitored from 20 to 78 weeks of age. Mean sequence length for the first 4-week period (20-24 weeks) was 24.7 eggs, which increased to 52.7 and 58.8 eggs for the next two 4-week periods. Thereafter, sequence length gradually declined as the birds aged, so that by 76-78 weeks of age, the hens were laying in short sequences of mean 3.5 eggs (Figure 1.5). The shorter sequences at onset of lay were not due to the occurrence of internally-laid eggs; the correct sequence length being determined both by counting the number of eggs laid on consecutive days and by examining records of oviposition times. The trends observed here and shown in Figure 1.5 are similar to those reported by Robinson *et al.* (1998b) for broiler breeder hens. Over the entire experimental period, mean sequence length was 23.5 eggs or days. This differs substantially from the mean calculated by Robinson *et al.* (1992) above. Some of the discrepancy may be accounted for by differences in breed, photoperiod or other environmental factors. The small sample size used by Lewis and Perry (1991) may introduce some bias, in particular if the sixteen hens selected for the trial were all coincidentally high producers.

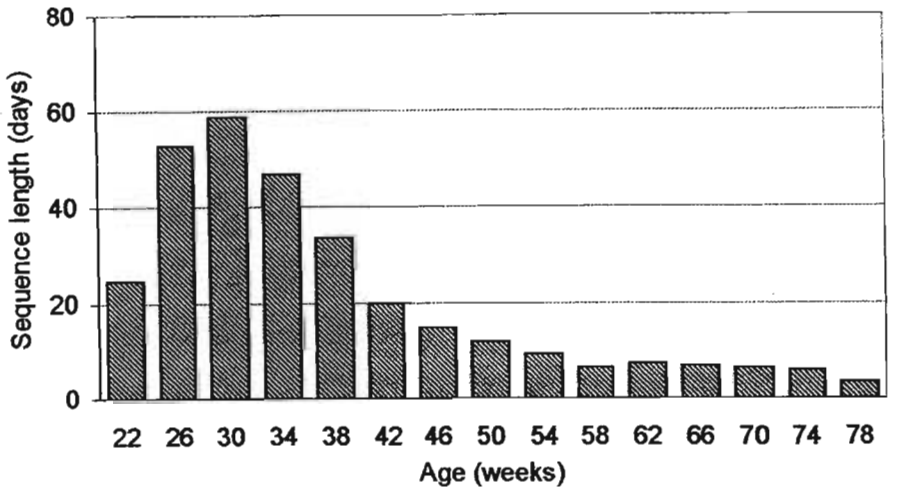


Figure 1.5: The change in sequence lengths in layers with advancing age (data from Lewis and Perry, 1991)

This decline in sequence length with advancing hen age is brought about by an increase in the intra-sequence oviposition interval (Lillpers and Wilhelmson, 1993b), which in turn is due to a lengthening of the ovulation interval. Young hens typically ovulate every 24 to 25 hours, whereas in older hens the ovulation interval increases to 26 to 27 hours or more (Bahr and Palmer, 1989). In one genetic line selected for egg numbers, mean interval lengthened from 25.1 hours at 44 weeks of age to 26.2 hours at 103 weeks (Lillpers, 1998). As a flock ages, mean sequence length decreases and mean number of pause days increases. The frequency of missing eggs within a sequence increases in older hens, presumably due to an increase in the incidence of internal laying. As the hen reaches the end of her laying year, pauses of more than one day become increasingly common.

1.17 Factors contributing to reproductive senescence

The measurable changes in sequence characteristics with advancing age are due to a large extent to a decline in the ovulation rate. Since the rate of ovulation is dependent on the asynchronous relationship between the rate of follicular maturation and the duration of the open period for LH release, reduced ovulation rate may be a consequence of either a reduction in the rate of follicle maturation, a shortening in the duration of the open period or both (Robinson *et*

al., 1990). Other factors contributing to a decrease in egg production with age are decreased recruitment of follicles into the hierarchy, increased atresia, increased incidence of soft-shelled and shell-less eggs and internal laying (Bahr and Palmer, 1989).

The first sign of advancing age is thought to be a reduction in the rate of recruitment of yellow follicles into the hierarchy (Williams and Sharp, 1978a). The process of recruitment appears to be influenced by the size of the pool of smaller follicles in the ovary available for selection and differentiation. In older hens, an increase in the incidence of atresia amongst small yellow follicles has been observed (Bahr and Palmer, 1989) and seems to be due to inadequate gonadotrophic support. A decrease in the rate of maturation of the smaller follicles with ageing may also reduce the size of the pool and hence the size of the hierarchy. It may be seen by opening the body cavity that older birds have larger but fewer rapidly growing follicles in the ovary. These are less closely ranked than in younger birds, so that the hierarchy is disrupted by gaps (Zakaria, 1999a). The absence of a follicle of a particular size in the hierarchy leads to the absence of a mature follicle on a particular day during the open period for LH release, which in turn results in a day when no ovulation takes place. Without the positive feedback stimulus of increased progesterone levels, normally secreted by the granulosa cells of the F_1 follicle, no LH surge can occur.

Palmer and Bahr (1992) found that ovaries from young hens commonly contained five to seven follicles in the hierarchy, whereas older hens had as few as four. Zakaria *et al.* (1983) reported a decrease in the mean number of growing follicles from 7.6 at the onset of lay to 6.1 at the end of a year. In a similar experiment conducted by the same authors, the mean number of large yellow follicles dropped from 7.8 at 7 months to 6.3 at 19 months of age. The number of follicles in the hierarchy reported by Hocking *et al.* (1987) for White Leghorns ranged from four to nine, with a mean of 6.3 at the onset of lay. By 60 weeks of age the mean was reduced to 4.4 with a range of five to six follicles; although one bird in the sample had no large yellow follicles at all and was excluded from the analysis.

A reduction in the rate of maturation of large yellow follicles with advancing age is apparent (Lillpers and Wilhelmson, 1993a; Zakaria, 1999a). This leads to an age-related increase in the

length of the rapid growth phase and corresponding increases in the size of each follicle at ovulation and in the total yolk volume of the hierarchy. It would appear that age affects the competency of the follicles to ovulate, by reducing their sensitivity to LH (Zakaria *et al.*, 1983; Bahr and Palmer, 1989). Johnson *et al.* (1986) reported that F_1 and F_2 follicles of young hens are more responsive to LH, as determined by the LH-stimulated adenylyl cyclase activity of the granulosa layer, than those of old hens. The adenylyl cyclase enzyme system is an accepted biological marker for determining the level of follicular maturity.

During the hen's laying cycle, atresia is mainly associated with smaller follicles. Under normal circumstances as many as ten to twenty atretic follicles of the smaller size group are visible to the naked eye. With advancing age an increase in follicular atresia occurs (Robinson *et al.*, 1990; Lillpers and Wilhelmson, 1993a; Palmer and Bahr, 1992), with larger follicles becoming atretic towards the end of the laying period (Gilbert and Wells, 1984). One of the consequences of atresia is the reduced number of large white follicles and small yellow follicles in the ovaries of older hens available for recruitment (Palmer and Bahr, 1992).

Both the large white and small yellow follicles secrete estradiol-17β (although the secretion is greatest in the SYF), which regulates yolk formation and calcium deposition (Palmer and Bahr, 1992). The higher incidence of soft-shelled eggs in older hens may be due to insufficient oestrogen secretion, because of the decreased numbers of SYF and LWF in the ovary. Furthermore, the estradiol output of the hierarchical follicles appears to be affected by age, since the concentration of estradiol in the F_4 and F_5 follicles, as measured by Johnson *et al.* (1986) was seen to be higher in young hens than in ageing hens.

Palmer and Bahr (1992) found ageing hens to be responsive to injections of FSH. The numbers of SYF and LWF in the ovaries increased following administration of FSH and the incidence of atresia in these two groups was diminished. Yolk deposition was increased, presumably because of the increase in serum estradiol-17β concentrations. It is possible that older hens produce inadequate amounts of FSH to maintain follicular growth and recruitment (Palmer and Bahr, 1992). This theory is supported by Williams and Sharp (1978b), who found a decrease in the baseline concentration of plasma LH in old laying hens, possibly due to a decrease in neural

activity controlling the secretion of LH-RH. These authors suggested that the reduced rate of follicular recruitment may partly be caused by the decrease in baseline levels of plasma gonadotrophins.

Age-related changes have been documented in endocrine, metabolic and behavioural circadian rhythms in a variety of animal species (Turek *et al.*, 1995). Most studies have been limited to measurements of the effect of age on the amplitude of circadian rhythms; for instance, the circadian rhythms in temperature, corticosterone, serum testosterone and melatonin are all dampened in old rats. The changes with advancing age could be upstream or downstream of the circadian clock, *eg.* a change in the sensory perception of light or an alteration in the mechanisms regulating the physiological processes between the clock and the overt rhythmic output. However, in rodents there is convincing evidence that the circadian clock itself is altered in senescence, because the free-running periods of the circadian rhythm of locomotor activity and sleep-wakefulness shorten with age in rats and hamsters on continuous light. Ageing is known to alter the circadian rhythms of glucose utilisation and α_1-adrenergic receptor levels in the suprachiasmatic nucleus. These changes in hypothalamic activity are correlated with changes in the circadian rhythm of LH release that are observed with ageing in female rats. Many of the effects of ageing on the circadian clock system can be simulated in young animals by depleting brain monoamine levels, suggesting that ageing alters monoaminergic inputs to the clock. In their work with hamsters, Turek *et al.* (1995) found several age-related changes in the circadian rhythm of locomotor activity. These were: alterations in the phase angle of entrainment to the light:dark cycle; an altered response to the phase-shifting effects of light pulses; changes in the time it takes to re-entrain to a new light:dark cycle; and a loss of responsiveness to the phase-shifting or entraining effects of stimuli which induce an acute increase in activity.

Williams and Sharp (1978b) thought that in domestic birds the decrease in reproductive activity with age was partly due to a functional change in the central nervous system. As hens age, the response of the LH-positive feedback mechanism to progesterone via the hypothalamus is decreased. The ovary, on the other hand, appears to remain potentially functional. This was determined by measuring the amount of progesterone released in response to an injection of

LH. No differences in progesterone output between young and old hens were observed. Similarly the response of the pituitary gland, in terms of increased levels of plasma LH following an injection of LH-RH, was not affected by age. It seems possible that the changes in hypothalamic activity, as reported by Turek *et al.* (1995) in ageing rodents, also occur with advancing age in poultry.

No evidence of a change in the duration of the open period for LH release with advancing hen age has been reported (Robinson *et al.*, 1990). Based on their observations, Lillpers et *al.* (1993a) suggested that a change in the circadian rhythm of the open period is a possible but unlikely cause of lower egg production in older hens. However, following the publication of the findings on rodents (Turek *et al.*, 1995) it is clear that further efforts need to be directed into research on circadian systems in domestic poultry. Such efforts may be rewarded with the recognition of similarities between species; in particular proof that changes in hypothalamic activity with age are correlated with changes in a circadian rhythm of LH release. Perhaps ahead of its time, the mathematical model of ovulation of Etches and Schoch (1984) uses both a reduction in the rate of follicle maturation and a change in the amplitude and duration of the circadian rhythm of the regulator substance to create shorter egg sequences, although in their paper these changes were not specifically related to hen ageing.

The increased incidence of pauses longer than one day in older hens is of interest, since this phenomenon is not accounted for in the theory of Fraps (1955). It may be that follicles mature at such a slow rate that more than one ovulation-inducing phase of the circadian rhythm is missed (Lillpers and Wilhelmson, 1993b). Alternatively two consecutive gaps in the hierarchy, from failure of the recruitment process in older hens, could result in two pause days in an ovulation sequence. Similarly the higher incidence of internal laying expected in older birds could provide an explanation for the longer pauses in oviposition sequences.

Advancing hen age is also responsible for changes to the efficiency of functioning of the oviduct, *i.e.* a decrease in the rate of passage of the ovum through the oviduct, retrograde transport in the oviduct, a greater incidence of internal laying and the production of defective eggs (Lillpers and Wilhelmson, 1993a; Robinson *et al.*, 1990). All these losses occur post-

ovulation and contribute to lower egg production in ageing hens.

1.18 Genetic and environmental manipulation of ovarian function

Potential rate of lay is determined genetically but it may be modified by the environment. A bird reared and maintained in ideal conditions should lay at her maximum potential rate, yet certain management decisions, such as the choice of a lighting programme, still play a role in fine tuning egg production. Because rate of lay is largely determined by ovulation rate, it is to be expected that breed and strain differences exist in both follicular efficiency and in the circadian control of ovulation by LH. Several trials reported in the literature confirm these observations.

Albino hens are known to have a higher rate of lay after peak and longer egg sequences than non-albino hens (Su *et al.*, 1999). This was found to be due to a faster rate of maturing of follicles in the hierarchy, a reduced growth period and a smaller mature size. Follicles also entered the rapid growth stage at a lower weight. After force-feeding with solutions of Sudan dye, albino hens had fewer dye rings in the yolks of their eggs than non-albinos (8.32 compared to 8.59). The total numbers of normal follicles and of atretic follicles did not differ between genotypes, although for some unexplained reason the albino hens were found to have a higher number of atretic follicles greater than 10mm. The output of progesterone from the F_1 follicle in response to LH was greater in albinos than in non-albinos. Because maturity is determined by the response to LH stimulation, this meant that follicles from albinos had earlier maturation, possibly owing to greater activity of the pituitary-ovarian axis.

The timing of onset of sexual maturity has been found to influence prime sequence length. SCWL pullets were reared on eight hours of light and then received an increase in photoperiod to 14L:10D at 16, 18 or 20 weeks of age (Robinson *et al.*, 1996b). Egg production data were recorded to 64 weeks of age. Prime sequence length was measured as 68.7, 81.7 and 82.4 days for the 16-, 18- and 20-week photostimulated groups respectively. Corresponding mean sequence lengths were 12.5, 12.7 and 16.6 days. Although definite trends existed, none of the differences in these treatment means was statistically significant, possibly owing to the large

variation in sequence length amongst individuals and despite significant differences in age at sexual maturity. As age at photostimulation increased, number of sequences and number of pause days decreased but total egg production was unaffected. This indicates that birds respond to a delay in onset of lay by increasing prime sequence length and decreasing the number of pause days (Robinson *et al.*, 1996b). It may be that the pullets stimulated at 16 weeks were physiologically immature and had insufficient body reserves to maintain persistency of lay. Based on the data reported, it appears that a curvilinear relationship exists between age at photostimulation and prime sequence length.

An inherent difference in prime sequence length between two strains of SCWL hens was established by Robinson *et al.* (2001). 150 birds of an early maturing strain and the same number of a late maturing strain, having been reared under identical conditions, were individually caged and monitored from 18 to 68 weeks of age. The late maturing group had a mean prime sequence length of 70.2 days and a mean overall sequence length of 12.8 days, compared to 52.6 days and 8.7 days respectively for the early maturing strain. The mean pause length was 1.16 days for both strains. Inter-sequence pause length increased towards the end of lay, although no details were reported. There was no difference in total egg production despite the 4.6-day difference in age at sexual maturity, because of the shorter sequences of the early maturing strain. The early maturing strain was found to produce a higher percentage of abnormal eggs (soft-shelled, shell-less and double-yolked eggs, and atypical shells), which may indicate erratic ovulation or aberrant movement through the oviduct. In their experimental work, Hocking *et al.* (1987) noted that the presence of two yolks in the oviduct disrupted shell formation. Higher numbers of large yellow follicles (LYF) were observed by Robinson *et al.* (2001) in the early maturing strain up to 23 weeks of age, beyond what would be expected from the 4.6 day advance in sexual maturity. In broiler breeders, defective egg syndrome is related to an excess of LYF (Yu *et al.*, 1992a). It was not clear whether these observations continued throughout the experimental period, or whether the abnormal eggs (in particular the double-yolked eggs) were confined to the initial stages of lay. The authors thought that a lower nutrient intake in the early maturing strain, due to reduced body weight at sexual maturity, may have contributed to the birds' inability to maintain long sequences. Alternatively, compromised control of follicular recruitment may have been due to the pullets allocating more energy to

oviduct development at a young age. This may be a similar phenomenon to the loss of reproductive control seen in overfed broiler breeders (Robinson *et al.*, 2001).

The nutritional status of hens is known to influence ovarian function. Waddington *et al.* (1985) fed low calcium diets to laying hens in order to induce poor egg production. Diets containing 3.5%, 1.0% and 0.1% calcium were fed from 42 to 70 weeks of age. Mean sequence lengths of young hens (47 to 49 weeks) were 2.7, 2.2 and 1.4 days respectively and for older hens (68-70 weeks) the corresponding figures were 1.4, 1.2 and 0.6 days. The growth rate of follicles larger than 3.5 mm in diameter appeared to remain unaffected by the calcium-deficient diets, but the incidence of atresia amongst the smaller follicles (less than 8 mm) was increased by feeding low calcium diets. The most striking feature of the birds on the calcium-restricted diets was the occurrence of atresia in the large yellow follicles, which went hand in hand with a reduction in the number of viable follicles in the hierarchy. Although the ovaries of these hens were still able to produce follicles of all sizes, the ovulation rate was modified by the loss of large follicles due to atresia. The difference between high and low producers was the number of atretic follicles in the hierarchy, which in turn affected sequence length.

On conventional pullet rearing programmes, laying-type pullets are fed *ad libitum* throughout the 18-week rearing period, with the aim of achieving a recommended target body mass at point of lay. Restricted feeding practices are not common in commercial enterprises, but have been used experimentally to delay the onset of sexual maturity. Johnson *et al.* (1984) found that *ad libitum* feeding during rearing caused more abnormal eggs in the first two months of lay than did restricted feeding in White Leghorn crosses. The majority of the abnormalities were in the form of shell defects and double yolks. The initial pattern of lay was erratic in these birds, suggesting a high incidence of internal laying. Very few double-yolked eggs were produced by the hens subjected to feed restriction during rearing. Since *ad libitum* feeding advanced the onset of sexual maturity relative to the restricted group, it was thought that the increased production of abnormal eggs was directly related to the earlier age at sexual maturity.

Broiler breeder body weight is routinely controlled from an early age to reduce reproductive problems associated with genetic selection for growth (Renema *et al.*, 1999). *Ad libitum*

feeding in broiler breeder replacement pullets and broiler breeder hens is known to affect reproductive performance via increased follicular recruitment, which leads to an excess of LYF, double hierarchies, multiple ovulations, internal ovulations and defective eggs (Yu *et al.*, 1992a; Hocking *et al.*, 1987; Hocking, 1996). Multiple ovulations are more pronounced during the initial few months of lay and sometimes give rise to triple-yolked eggs. These multiple ovulations are linked to the secretion of a significant amount of progesterone from the F_2 follicle, a circumstance that is not observed in restricted-fed birds (Yu *et al.*, 1992b). These authors found that feed allowance during breeding influenced steroidogenic capabilities of both small and large follicles. In *ad libitum* fed broiler breeders, the F_2 follicle had the endocrine profile of F_1, which allowed the LH surge to trigger the ovulation of both follicles simultaneously. Liu *et al.* (2004) found that restricted feeding did not influence the preovulatory surges of LH and progesterone in broiler breeders, when compared to those of hens fed *ad libitum*.

Ad libitum feeding is also linked to a higher incidence of atresia in LYF. Hocking *et al.* (1987) established a positive linear relationship between log body weight and the number of atretic yellow follicles. Heavier birds were also found to have higher numbers of both small white and large yellow follicles. This was confirmed by similar work done by Hocking (1996) later on, when a linear relationship was established between body weight and the number of LYF in the hierarchy. Consequently there is a reduction in the length of the prime sequence, an increase in the number of long inter-sequence pauses and an overall decrease in egg numbers. Robinson *et al.* (1991) measured mean prime sequence lengths of 24.4 and 14.9 days, for restricted and *ad libitum*-fed hens respectively. There was a high incidence of pauses longer than eleven days in the treatment fed *ad lib*. It has been suggested by Hocking *et al.* (1987) that the practice of feed restriction of broiler breeders during rearing could result in a rate of lay comparable to that found in laying flocks. Yu *et al.* (1992a) found that broiler breeders feed-restricted to 18 weeks of age and subsequently changed onto full feeding still had greater numbers of LYF in the hierarchy at sexual maturity, compared to birds left on restricted feeding. The work of Renema *et al.* (1999) confirmed these results, which indicates that the feed intake of broiler breeders needs to be controlled continuously. Restricted feeding during rearing is associated with a greater number of atretic white follicles (Hocking *et al.*, 1987; Renema *et al*, 1999),

which suggests that increased rates of small follicle atresia may be used to control the small follicle population and ultimately LYF numbers.

Sequence profiles in broiler breeders were also influenced by the magnitude of increases in feed allotment at 20 weeks, even though age at sexual maturity was not significantly different for the treatments (Robinson *et al.*, 1998b). Birds that were given small increases in their daily feed quota had slightly longer prime sequences and mean sequence lengths than those given larger increases. This was related to the presence of fewer large yellow follicles in the hierarchy and a smaller total LYF weight (Robinson *et al.*, 1998a). The method of increasing the photoperiod for the broiler breeders, *i.e.* abrupt or gradual increases in daylength, played a minor role in sequence characteristics. The treatments subjected to an abrupt increase in photoperiod had a longer mean sequence length only in early lay. Prime sequence length, number of laying sequences and overall mean sequence length were not affected (Robinson *et al.*, 1998b). The number and total weight of the LYF was less in the birds given the abrupt increase in photoperiod, indicating slower rates of recruitment and yolk deposition (Robinson *et al.*, 1998a). Earlier trials by Robinson *et al.* (1996a) showed that age at photostimulation did not significantly alter the number of rapidly growing follicles.

Hocking and Robertson (2000) established genetic differences in the number of LYF at the onset of lay from ovaries of selected (for high juvenile growth rate) and relaxed selection male- and female lines of broiler breeders. The number of positions in the hierarchy was greater in selected lines, indicating an increase in potential opportunities for ovulation. This was associated with an increase in the proportion of follicles developing as groups of similar size.

Ovarian activity in ducks has also been influenced by genetic selection. Hocking (1990) found evidence of multiple hierarchies in a line selected for improved juvenile feed efficiency and was able to establish a positive linear relationship between the number of LYF and body weight at sexual maturity. These ducks responded to feed restriction by decreasing the number of LYF in the ovary and the proportion in groups of similar weight. Selection for increased meat yield in turkeys has also resulted in multiple hierarchies (Buchanan *et al.*, 2000; Melnychuk *et al.*, 1997), where triple and quadruple hierarchies are not uncommon. However, neither feed

restriction nor delayed photostimulation has been successful in reducing the number of hierarchical follicles. It is not known why follicular control is possible in broiler breeders and ducks using these methods and not in turkey hens. Hormonal control of ovulation in large turkey hens appears to be compromised.

1.19 Discussion

This literature review confirms that the laying hen's reproductive system is extremely complex. It interacts with components of the skeletal, muscular, nervous, digestive and circulatory systems and it relies on numerous hormones to regulate the reproductive processes. Countless studies have been conducted on the subject over the past 50 years, with the result that our knowledge and understanding have expanded considerably. It is reasonably safe to assume, though, that there are still missing pieces to the puzzle that will be uncovered gradually by further research. The greater the understanding of the physiological processes involved in egg formation, the better the position the modeller is in to be able to make informed decisions.

It has long been recognised that the hen's ovulatory cycle is synchronised with the environmental pattern of light and darkness. This has enabled scientists to manipulate flock egg production. Short ahemeral cycles have been used in selection programmes to identify individuals with oviposition intervals shorter than 24 hours. Continuous lighting regimes have been used for the same purpose, but it has proved difficult to remove all environmental entraining factors. In the absence of entrainment, hens should achieve free-running cycles representative of their endogenous circadian rhythms. If this were the case, ovipositions would be distributed evenly throughout the day.

Trevor Morris was one of the first poultry scientists to study the relationship between the light:dark cycle and time of lay. He devoted a great deal of energy to researching the effects of different lighting programmes and light intensities applied during rearing on age at sexual maturity and subsequent egg production. Peter Lewis and Graham Perry continued with this line of research, measuring associated levels of gonadotrophic and steroid hormones. Their work, along with similar studies by others, has provoked interest in defining the physiological

mechanisms that link reproductive rhythms to light. The pineal gland contains photoreceptors which enable the bird to process information about the light:dark cycle. Both the pineal gland's hormone melatonin and the enzyme regulating its synthesis show circadian rhythms of activity and may be entrained to the light:dark cycle. There is circumstantial evidence therefore that melatonin is involved in the timing of ovipositions, although there is still no direct link between the pineal gland and LH release.

Many papers have been published on the structure of the hen's ovary, follicular dynamics, steroidogenesis and the hormones that are involved in regulating the growth and atresia of follicles and their recruitment into the hierarchy. It is still not clear why some follicles are selected for recruitment into the hierarchy while the majority undergo atresia. It is accepted that follicle maturation is due to an increase in size caused by yolk accumulation and to histological changes that are accompanied by the attainment of endocrine capability. The growth and recruitment of the smaller follicles is primarily regulated by FSH but as the follicles mature, their sensitivity to FSH declines and to LH increases. With advancing hen age, there is a reduction in the rate of recruitment of follicles into the hierarchy, a decrease in the rate of follicle maturation and reduced sensitivity of follicles to stimulation by LH. It is possible that ageing also brings about changes in the circadian rhythm of LH release.

The decline in the efficiency of the ovary with advancing age brings about corresponding changes in the length of ovulation and oviposition sequences. Laying hens usually start their reproductive cycle by producing a few short sequences, followed by the prime egg sequence that occurs around peak rate of lay for the flock. A gradual decline in sequence length follows, so that towards the end of the laying year sequences of two to three eggs are common. There is of course huge variation between individuals in terms of the length of the prime sequence, mean sequence length and mean pause length. Genetic and environmental factors also play a role in altering laying patterns. For instance, albino hens lay longer sequences than non-albino hens, because of faster rates of maturing of follicles. Early photostimulation advances the age at sexual maturity but also reduces both prime and mean sequence length. Low calcium diets cause atresia amongst follicles in the hierarchy, which increases the number of pause days and therefore decreases sequence length.

The objective of this study is to produce a simulation model capable of predicting rate of lay in a flock of hens. This model needs to be responsive to changes in photoperiod during rearing, to hen age and to strain of bird. Predicting the hen's ovulation rate is a good starting point because it largely determines rate of lay. In the perfect scenario, ovulation rate and rate of lay would be equal but in practice this is not always the case. A number of ova, upon release from the ovary, do not continue down the oviduct to form normal shelled eggs. If a double-yolked egg is responsible for an interruption in a sequence, or if an ovum fails to be grasped by the infundibulum immediately after ovulation and remains in the body cavity, the rate of lay will be lower than the rate of ovulation. In a commercial egg producer's view, soft-shelled eggs also reduce the rate of lay because they are not marketable as table eggs. As a result of these aberrations, oviposition sequences are likely to be shorter than ovulation sequences and the number of pause days will be greater. These issues need to be addressed during the development of a laying model.

Any mathematical model put forward to account for the asynchronous nature of the hen's ovulatory cycle should:

1. produce realistic ovulation times
2. restrict the cumulative lag to eight to ten hours
3. produce sequences of different lengths
4. allow the lag values within a sequence to vary; i.e. decrease towards the middle of a sequence and then increase towards the end of a sequence
5. allow sequence length to reduce with advancing hen age
6. have a sound biological basis

In the following chapter it will be seen that the model proposed by Etches and Schoch (1984) fulfils some of these requirements. The remainder will be satisfied by various modifications, to be discussed in subsequent chapters.

Chapter 2

AN IMPROVED MODEL OF THE HEN'S OVULATORY CYCLE

2.1 Introduction

Despite the uncertainty surrounding the physiological causes of the asynchronous ovulatory cycle, a mathematical model was put forward by Etches and Schoch (1984) to lend credence to the theory that two independent systems interact to produce the well-documented pattern of sequential laying. This was achieved by predicting times of ovulation close to actual times of oviposition recorded under experimental conditions for different sequence lengths, on the assumption that the ovulatory and ovipository cycles are displaced by the oviducal term. It must be mentioned that, owing to variation in the oviducal term, a model that attempts to compare calculated ovulation times to actual oviposition times may run into difficulties. Nevertheless, their model was able to demonstrate the characteristics of lag as described in detail by Fraps (1955). For instance, the calculated lag decreased initially between successive ovulations, was minimal for mid-sequence ovulations and increased towards the end of a sequence, so that the greatest lag was between the penultimate and last ovulations. The predicted total lag given by their model for the different sequence lengths fell within the prescribed eight- to ten-hour open period. Mean lag was negatively related to sequence length.

Certain criticisms remain, most notably that the parameter values seem to have been assigned arbitrarily, without much thought either to their biological significance or to any relationship between the parameter value and sequence length. The table of values used for the parameters in the model restricts the simulated sequences to two to nine ovulations. In reality the individuals in a flock of hens will show variation about a mean sequence length. One bird may lay an egg a day from onset of sexual maturity for more than 100 consecutive days, whereas another might lay smaller clutches with more frequent pauses from the start of her laying cycle. All hens exhibit a decline in rate of lay with advancing

age, with the prime sequence occurring around peak production (Robinson *et al.*, 2001). A hen that commences her laying cycle producing long egg sequences can be expected to lay only two or three eggs before pausing for a day towards the end of her cycle. The table of values as published by Etches and Schoch (1984) does not allow these longer sequences to be modelled. Another drawback is the model's inability to deal with an inter-sequence pause length greater than one day, although this aspect will not be dealt with here.

The objective of this chapter is to study in detail the existing mathematical representation of the ovulatory cycle before proposing a number of improvements. This may be accomplished by looking initially at the two equations that were used by the authors to represent the cyclical concentration of a regulator substance and follicle maturation. If each parameter in the equations is altered in turn, it is possible to observe the effect of the changes on the functions and in so doing, to understand the role of each parameter. Subsequently continuous functions will be created to estimate the value of all seven parameters in the model required to produce sequences of any length. This will remove the constraint of working with integers over the limited range of two to nine ovulations.

With these and a number of other additions and modifications, this mathematical model could prove to be useful in predicting rate of lay for a flock of hens over the entire laying period.

2.2 The regulator concentration function

A three-compartmental model is used to describe how the production of an hypothetical regulator substance could restrict the release of LH to a limited period of the day. The regulator is assumed to be produced in compartment one and instantaneously released into compartment two at regular intervals determined by a circadian oscillator. The regulator is then released into compartment three at a given rate and cleared from this compartment at a different rate. It is tempting to speculate that the oscillator may be the pineal gland or the suprachiasmatic nucleus of the hypothalamus. The regulator substance may contain, amongst other chemicals, the hormone melatonin. The concentration of the substance (Fraps' threshold of response of the neural component) in compartment three at time t depends on the rates at which it is entering and leaving the compartment and is given by:

$$R_3(t) = a_1 . e^{-\lambda 1 (t-S1)} - a_2 . e^{-\lambda 2 (t-S1)} \qquad \text{(Equation 2.1)}$$

where a_1 is a constant and S_1 is the time of day when the cycle is completed and the regulator function is reinitiated. In reality the events leading up to ovulation take place some hours beforehand, but the inclusion of the parameter S_1 shifts the curve so that $R_3(t)$ exceeds a threshold value from about 07:00 to early afternoon, not during the night. Thus the curve given by Equation 2.1 also represents the period of the day during which ovulation may take place. The values of the parameters λ_1, λ_2 and S_1 and of the constant a_1 used to predict ovulation sequences of varying lengths are given in Table 2.1.

The time frame for calculation of the concentration of regulator substance is not the time of day, but $S_1 \leq t \leq t_{max}$, where t_{max} = daylength + S_1. For a two-ovulation sequence and on a conventional 24-hour daylength, $8 \leq t \leq 32$ and for a nine-ovulation sequence, $6.5 \leq t \leq 30.5$. Thus in the early hours of the morning when the time of day is less than the value of S_1, time t ranges from 24 to t_{max}. However when $t = t_{max}$, t reverts to S_1. Thus the values allocated to t for the purpose of calculating R_3 are decimals, not conventional time format.

The value of the parameter a_2 is dependent on the values assigned to λ_1 and λ_2 and needs to be recalculated if either λ_1 or λ_2 changes. The concentration of the regulator must be the same at the time of day when the one cycle ends and the next one commences, *i.e.* when t = S_1 (the starting time) and t = S_1 + 24 (the end time), if the daylength is 24 hours. For a two-egg sequence, with S_1 = 8, $R_3(8)$ must equal $R_3(32)$. Thus substituting for t and S_1 in Equation 2.1:

$$a_1 . e^{-\lambda 1 (8-8)} - a_2 . e^{-\lambda 2 (8-8)} = a_1 . e^{-\lambda 1 (32-8)} - a_2 . e^{-\lambda 2 (32-8)} \qquad \text{(Equation 2.2)}$$

which may be reduced to:

$$a_2 = (a_1 - (a_1 . e^{(-\lambda 1 . 24)})) / (1 - e^{(-\lambda 2 . 24)}) \qquad \text{(Equation 2.3)}$$

Equation 2.3 may be used to determine the value of a_2 required to produce any sequence length, for 24-hour light:dark cycles. Table 2.2 shows the calculated values for a_2 for two- to nine-ovulation sequences, using corresponding parameter values from Table 2.1.

Table 2.1: The values of the parameters and constants used by Etches and Schoch (1984), University of Guelph data, to predict the times of ovulation and oviposition

Seq. length	a_1	λ_1	λ_2	S_1	b_1	b_2	b_3	S_2
2	2.175	0.14	0.25	8.0	0.75	4.5	0.22	17.0
3	2.175	0.14	0.25	7.5	0.75	5.1	0.27	17.0
4	2.175	0.14	0.25	6.75	0.75	5.2	0.30	17.0
5	2.175	0.15	0.285	6.5	0.75	5.15	0.315	17.5
6	2.175	0.15	0.285	6.5	0.76	5.05	0.32	17.5
7	2.175	0.15	0.285	6.5	0.78	5.2	0.325	17.5
8	2.175	0.15	0.285	6.5	0.78	5.15	0.33	17.5
9	2.175	0.15	0.285	6.5	0.785	5.17	0.334	17.5

Table 2.2: Calculated values of a_2 for sequences of different lengths, using Equation 2.3 and the parameter values of Table 2.1

Sequence length	a_2
2 – 4	2.104668
5 – 9	2.117837

Figure 2.1 shows the nature of the curve given by Equation 2.1 for a two-ovulation sequence, with one modification. In order to intersect with Equation 2.4 (refer to section 2.3), the function is plotted as $1\text{-}R_3(t)$ to reflect the curve as a mirror image. It may be seen that the function has a circadian rhythm, *i.e.* it repeats itself every 24 hours. The concentration of $R_3(t)$ is at a minimum in the early hours of the morning, starting to increase from 08:00 and reaching a maximum at 12:58. Thereafter the concentration gradually wanes. The range of values for $R_3(t)$ for the two-ovulation sequence is 0.070 to 0.477, or for $1\text{-}R_3(t)$, 0.930 to 0.523.

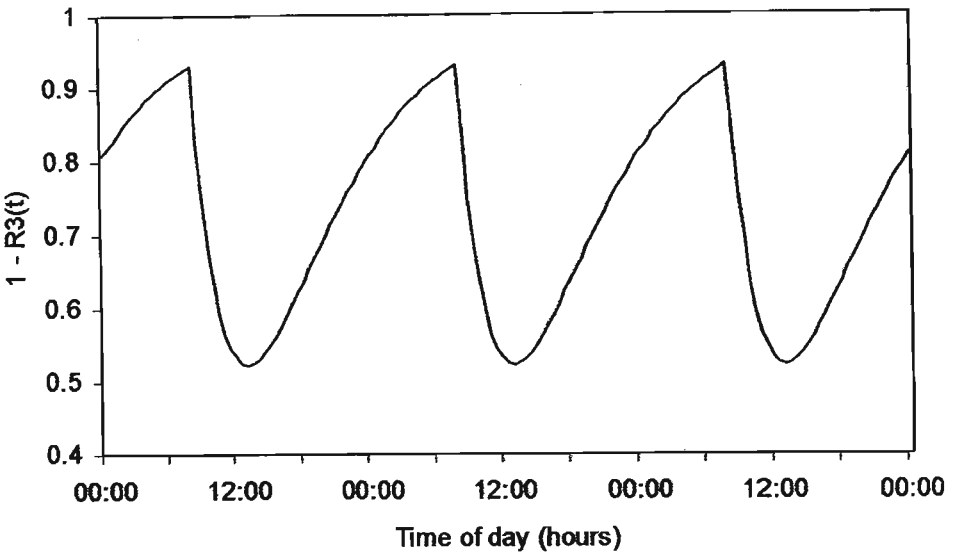


Figure 2.1: The regulator concentration curve, plotted as $1-R_3(t)$, for a two-ovulation sequence over a three-day period

Figure 2.2 shows how the output of regulator concentration differs when modelling sequences of two or nine ovulations, using the relevant parameter values from Table 2.1. The data used to plot the graph are shown in Appendix 2.1. For the longer sequence of nine consecutive ovulations, the regulator concentration starts to increase at 06:30, reaching a peak at 11:03 and then waning. The range of values for $R_3(t)$ is 0.057 to 0.520 or for $1-R_3(t)$, 0.943 to 0.480. The concentration of the regulator substance therefore starts its cycle earlier in the day, reaches a higher peak output at a faster rate but wanes slightly faster than for the two-ovulation sequence. The underlying implication here is that the circadian rhythm controlling LH release is not constant across birds; both the duration and the amplitude vary amongst individuals. High producing hens may have longer open periods for LH release and consequently longer periods during which ovulation may take place.

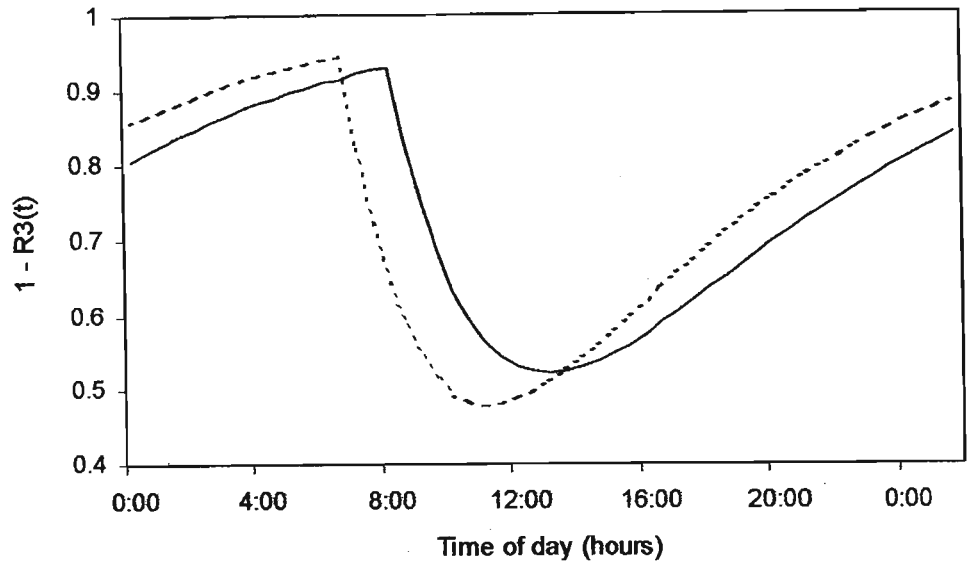


Figure 2.2: The regulator concentration curve for a two-ovulation sequence (solid line) and a nine-ovulation sequence (dotted line), over a 24-hour period

Interestingly, a change in either λ_1 or λ_2 (and recalculating a_2) alters the amplitude of the regulator concentration curve (Figures 2.3 and 2.4 respectively). Increasing λ_1 from 0.14 to 0.15, keeping all other parameters as for a two-ovulation sequence, decreases the peak output of R_3 (*i.e.* it increases 1-R_3) whereas an increase in λ_2, from 0.25 to 0.285, causes an increase in the peak output of R_3 (a decrease in 1-R_3). This opposite effect is due to the fact that λ_1 is inversely related to the volume of the hormone or neurotransmitter released from compartment two into compartment three (in the three-compartment model). If λ_1 increases, less volume of substance moves into compartment three so the concentration of R_3 is reduced. On the other hand, λ_2 is inversely related to the volume of substance moving out of the third compartment. If λ_2 increases, less volume of substance moves out of compartment three and so the concentration of R_3 is increased. It is not clear from the above why Etches and Schoch (1984) chose to increase the value of λ_1 for longer ovulation sequences. Intuition suggests that longer ovulation sequences should be a result of a greater volume of the substance moving into compartment three, which means λ_1 should decrease. However, their model does not work if λ_1 decreases as sequence length increases; the shape of the regulator concentration curve changes drastically. Extensive

alterations to the model may overcome the problem but this is felt to be unnecessary until the relevant physiological mechanisms are more fully understood.

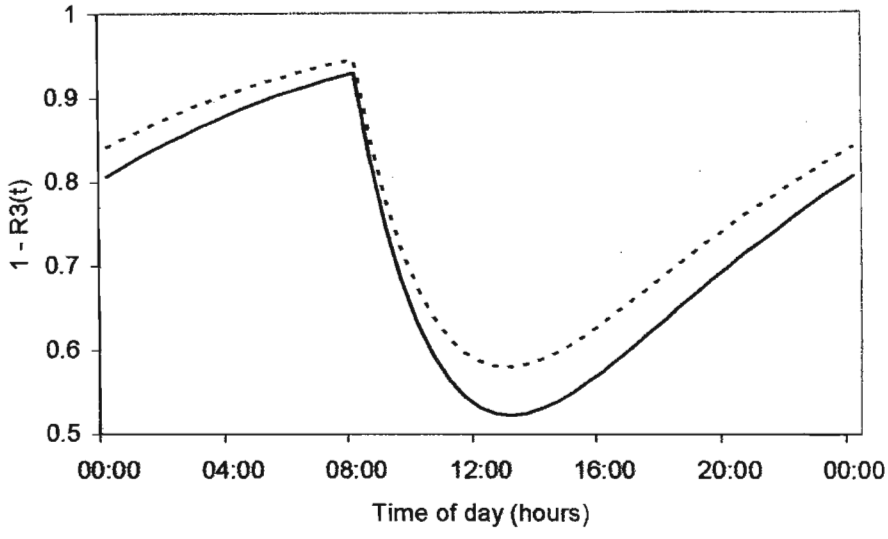


Figure 2.3: The effect of increasing λ_1 from 0.14 (solid line) to 0.15 (dotted line) on the regulator concentration curve for a two-ovulation sequence

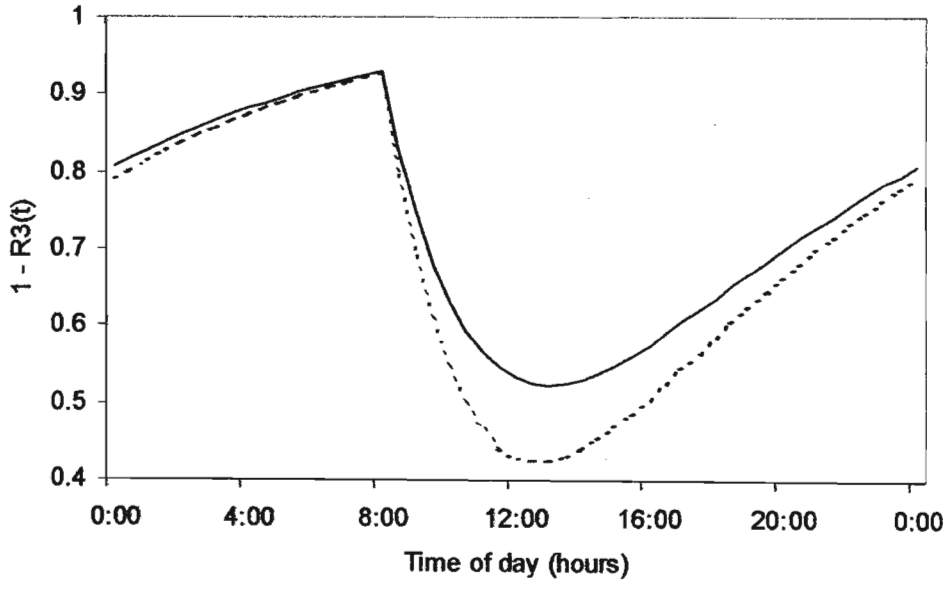


Figure 2.4: The effect of increasing λ_2 from 0.25 (solid line) to 0.285 (dotted line) on the regulator concentration curve for a two-ovulation sequence

Changes to S_1 result in a horizontal shift in the regulator concentration curve, without influencing the shape of the curve or the minimum or maximum levels (Figure 2.5). If S_1 is reduced from 8.0 to 6.5 (the values for two- and nine-ovulation sequences respectively, corresponding to times of day of 08:00 and 06:30) then the time of peak concentration changes from 12:58 to 11:28. The role of S_1 is therefore to manipulate the time from onset to peak, without affecting the levels of regulator concentration. Etches and Schoch (1984) wanted to match predicted times of ovulation with actual times of oviposition and the inclusion of S_1 enabled them to achieve their objective.

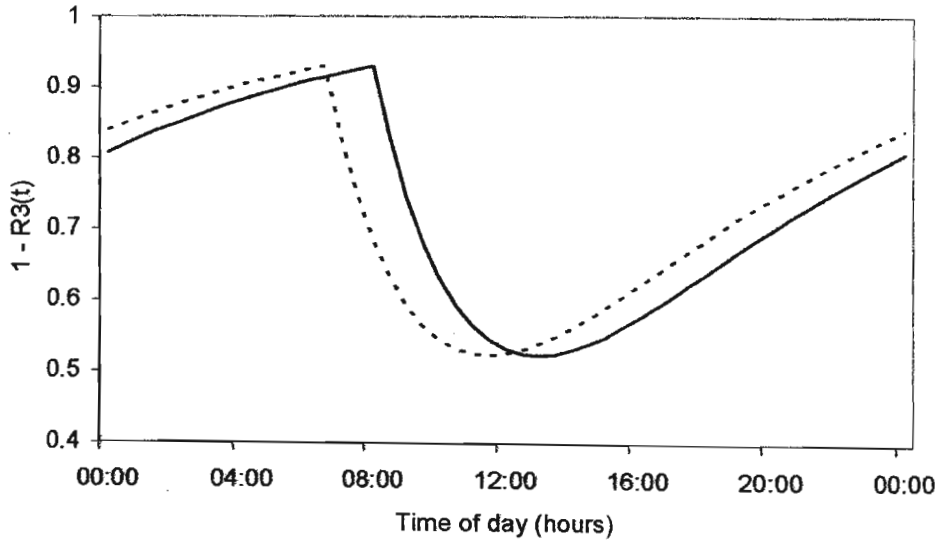


Figure 2.5: The effect of reducing S_1 from 8 (solid line) to 6.5 (dotted line) on the regulator concentration curve for a two-ovulation sequence

The three-compartmental model of Equation 2.1 would appear to be satisfactory for representing the circadian rhythm that presumably controls the release of LH. Longer sequences are modelled by shifting the curve to the left (*i.e.* starting earlier in the day), by a faster rate of increase and a greater peak output of the regulator substance. These changes are brought about by altering the parameters S_1, λ_1 and λ_2.

2.3 Follicle maturation

The second equation in the ovulation model of Etches and Schoch (1984) represents the maturation of the largest ovarian follicle (Fraps' excitation hormone concentration) and takes the form of a Gompertz function:

$$G(t) = b_1 . e^{-b2\, e^{-b3\,(t-S2)}} \qquad \text{(Equation 2.4)}$$

where S_2 represents the time taken after the previous ovulation to reinitiate G(t). The values for the parameters b_1, b_2, b_3 and S_2 to predict sequences of different length are shown in Table 2.1. An additional parameter T_5 represents the time at which the last egg of the previous sequence was laid, so that follicle maturation commences at time $T_5 + S_2$. For the pullet's first ovulation at sexual maturity, $T_5 = 0$.

The term follicle maturation does not refer to an increase in weight or diameter of the follicle itself, but rather to its competency to ovulate in response to an LH surge. This may, for example, be related to the differentiation of granulosa cells in hierarchical follicles to render them capable of secreting progesterone (Johnson, 1996), or to an increased coupling of LH receptors to the cAMP-generating system enabling the follicle to change from an FSH- to a LH-dominated phase (Tilly *et al.*, 1991a). Johnson *et al.* (1986) found that the sensitivity of the follicle to LH (as determined by the LH-stimulated adenylyl cyclase activity of the granulosa layer) and hence its ability to ovulate, declines with age. Although the precise physiological mechanisms need not be defined here, it is accepted that as the follicle enters its final stages of maturation, it is rendered sensitive to ovulation-inducing stimuli.

Figure 2.6 shows the maturation curves of two follicles using relevant parameter values from Table 2.1, one of which forms part of a two-ovulation sequence and the other, part of a nine-ovulation sequence. Appendix 2.2 summarises the data used to plot the chart. It is apparent that the follicle from the longer sequence matures at a faster rate and attains a greater mature state than the follicle from the shorter sequence.

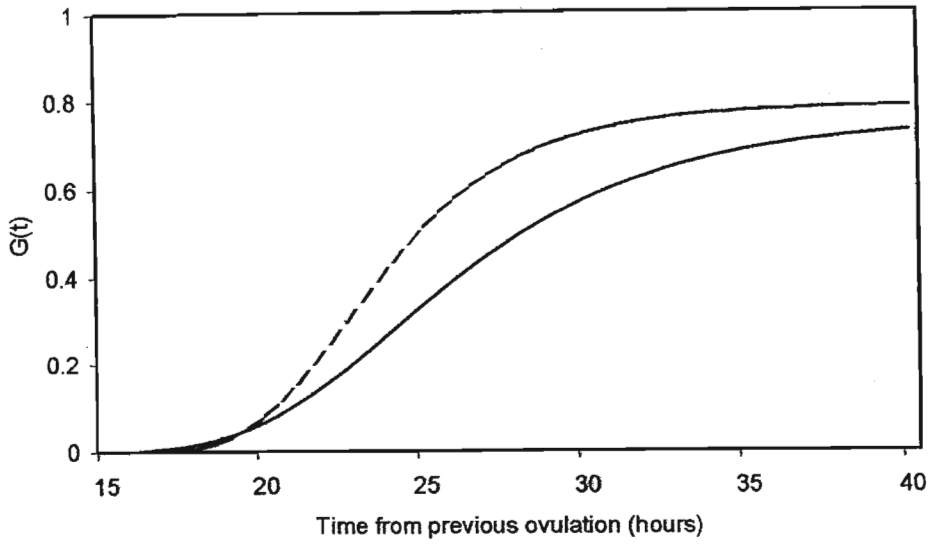


Figure 2.6: The maturation curves of two follicles, one from a two-ovulation sequence (solid line) and the other from a nine-ovulation sequence (dotted line)

The parameter b_1 is equivalent to the maximum value of G(t) and therefore represents the 'mature size' parameter in the conventional Gompertz equation. In this work b_1 will be referred to as the 'mature state' parameter; perhaps a measure of the follicle's competency to ovulate. Figure 2.7 shows how a change in the value of b_1 from 0.75 to 0.785 (the parameters for two- and nine-ovulation sequences respectively), with all other parameters kept constant, affects the follicular maturation curve. Although both follicles mature at the same rate, one follicle achieves a slightly larger mature state than the other.

The role of b_2, a parameter not present in the conventional Gompertz equation, is somewhat obscure but is linked to S_2. Increasing its value results in the curve being displaced to the right. Figure 2.8 shows the effect on the follicle maturation curve of increasing the value of b_2 from 4.5 to 5.17, *i.e.* the parameter values for a two-ovulation and a nine-ovulation sequence respectively.

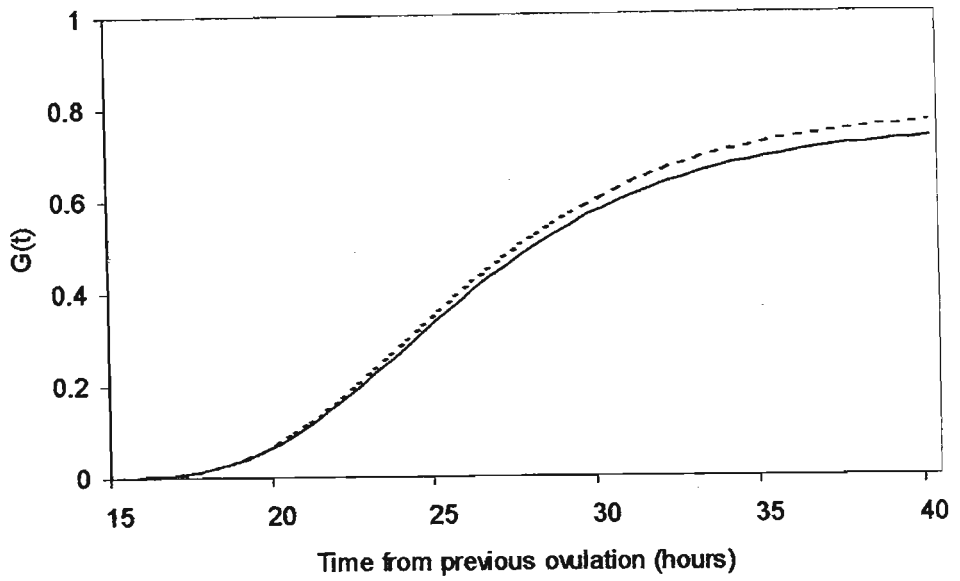


Figure 2.7: Effect of increasing b_1 from 0.75 (solid line) to 0.785 (dotted line) on the follicle maturation curve

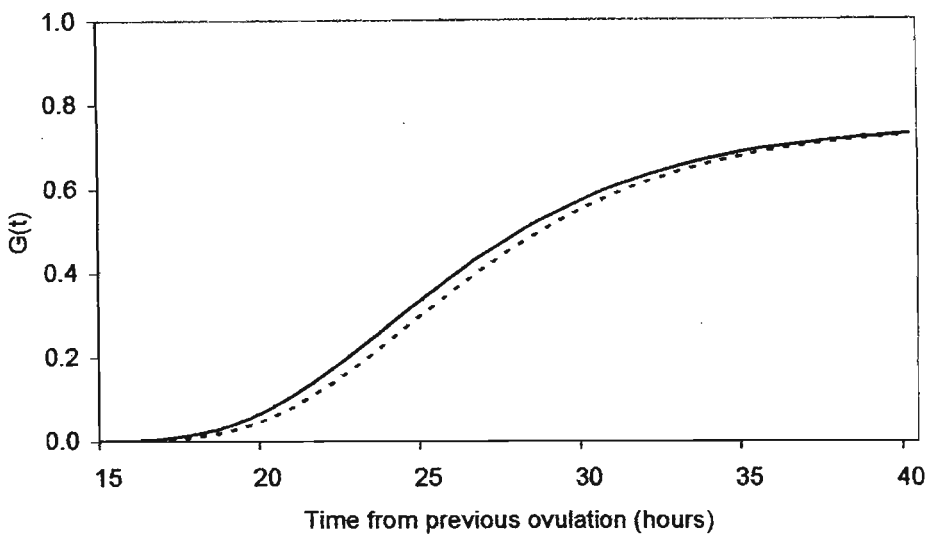


Figure 2.8: Effect of increasing b_2 from 4.5 (solid line) to 5.17 (dotted line) on the follicle maturation curve

The 'slope' of the curve is determined by b_3, which is therefore the rate of maturing in the Gompertz equation. The effect of altering b_3 from 0.22 to 0.334, while keeping the other parameters constant, is shown in Figure 2.9. It seems likely that the ability of follicles to mature at a faster rate makes a large contribution to longer ovulation sequences.

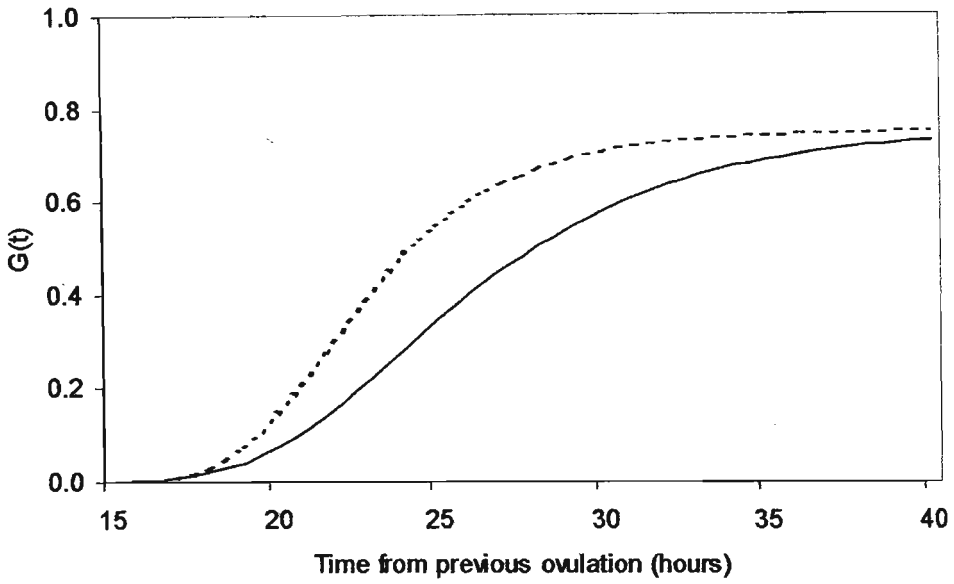


Figure 2.9: Effect of increasing b_3 from 0.22 (solid line) to 0.334 (dotted line) on the follicle maturation curve

Increasing S_2 from 17 to 17.5 (the difference between short and long ovulation sequences) simply displaces the curve to the right without altering its shape in any way (Figure 2.10). This is to be expected, since S_2 represents the time interval between one ovulation and the start of the next follicle's maturation process.

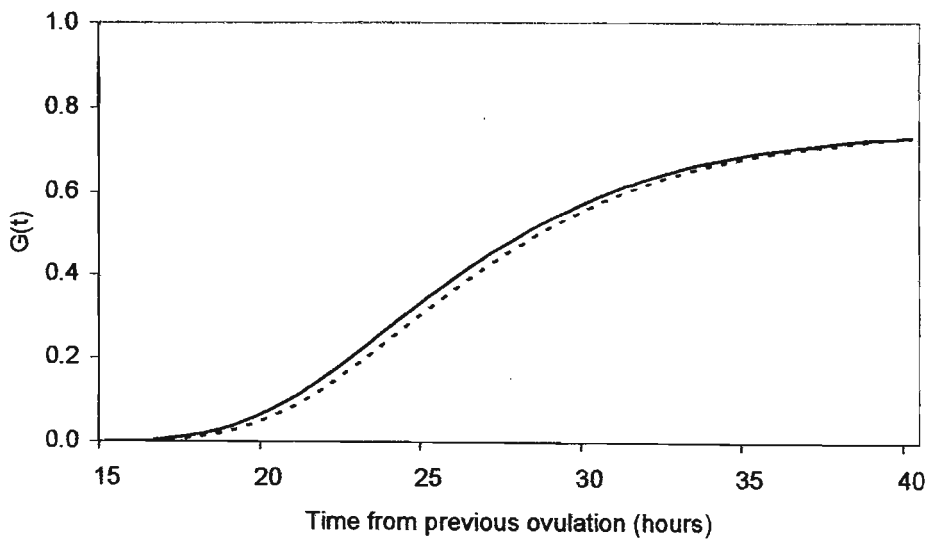


Figure 2.10: Effect of increasing S_2 from 17 (solid line) to 17.5 (dotted line) on the follicle maturation curve

2.4 The ovulation cycle

At the time of day when the two functions intersect, ovulation takes place. This is based on the premise that a sufficient concentration of the regulator coincides with a sufficient concentration of receptor to induce ovulation (Etches and Schoch, 1984). The intersection is brought about by using a mirror image of the curve produced by Equation 2.1, so that the moment of ovulation occurs when $G(t) - (1- R_3(t)) = 0$. The greater the output of regulator concentration and the more advanced the stage of follicle maturation, the greater is the likelihood of intersection of the two curves. The earlier in the day each consecutive ovulation occurs, the shorter the lag and hence the longer the sequence is likely to be.

In order to calculate the moment of intersection, the time frames for both the regulator concentration and follicle maturation need to be related to the time of day. The time used to calculate the concentration of regulator substance is the same as the time of day except from midnight to one minute before the time given by S_1, when it is equal to the time of day plus daylength (normally 24 hours). Thus for a two-ovulation sequence and working in half-hour intervals, the time for the regulator concentration runs from 8.0 to 31.5, corresponding to times of day running from 08:00 on day one to 07:30 on day two (see Appendix 2.1). The starting time for the calculation of follicle maturation is equal to the time of day, *i.e.* 0 (at midnight) + T_5, expressed as a decimal. Hence at the start of the calculations when time of day = 00:00, time t for follicle maturation = T_5. The value assigned to T_5 may be zero (as in the case of a pullet at sexual maturity) or an arbitrary number, *e.g.* 15.5, assuming the last egg of a previous sequence was laid at 15:30.

The initial model of the ovulatory cycle was developed as a simple spreadsheet in Lotus 1-2-3. This consists of eight columns containing: the day number, the time of day ('timeday') in half-hourly intervals, the time for the calculation of the regulator concentration ('timeRC'), the concentration of regulator ($R_3(t)$) from Equation 2.1, ($1-R_3(t)$), the time for the calculation of follicle maturation ('timeFM'), follicle maturation ('G(t)') from Equation 2.4 and a column containing the difference $G(t) - (1-R_3(t))$. The seven ovulatory cycle parameters are allocated values from Table 2.1 according to the desired sequence length, in a separate column. A formula for the calculation of a_2 is included, using Equation 2.3. Daylength ('DL') and T_5 need to be defined; the former is

fixed at 24 hours unless ahemeral cycles are being tested. T_5 is conventionally 13.5 for a two-ovulation sequence or 15.5 for longer sequences. These numbers are based on the mean of the observed times of oviposition of the ultimate egg in a sequence given by Etches and Schoch (1984) for the University of Guelph flock. The first point of intersection may be found by scanning the last column to find where $G(t) - (1 - R_3(t))$ becomes positive. The corresponding time of day constitutes the time (to the nearest half-hour) of the first ovulation. Here, it is taken as 09:00 on day two although in fact ovulation occurs somewhere just after 09:00. Before continuing to search for the second point of intersection, the time for follicle maturation needs to be reset to zero, or rather to 0.5 in the next row, *i.e.* at 09:30; half an hour after ovulation. The second ovulation takes place on day three fractionally after 13:30. The output of this model is summarised in Appendix 2.3.

A more complex model was subsequently developed in Lotus 1-2-3 using menus and macros. This enables the iterations to be performed in one-minute intervals, which greatly improves the accuracy of the estimation of ovulation time and dispenses with the need for the user to evaluate each point of intersection. Also, intersections are only looked for between the time given by S1 ('starttime') and a late afternoon time defined by the user ('endtime') after which ovulations are unlikely to occur. The number of days over which the predictions must be carried out are stipulated ('reps'), so that the model may run for a short period of *e.g.* ten days or an extended period of *e.g.* 200 days before returning to the main menu. The difference between G(t) and $1-R_3(t)$ ('diff') is evaluated at each stage. Two parameters ('mindiff' and 'mindiff1') determine the minimum difference allowed, as the model performs iterations in half-hourly and one-minute intervals. Appendix 2.4 shows the declaration of the constants and variables used in the model and Appendix 2.5 lists the macro commands that constitute the program.

A graphical representation of an ovulation sequence may be achieved by plotting the two functions given by Equations 2.1 (in the form $1 - R_3(t)$) and 2.4 on the same axes, with time of day along the x-axis. Where the two curves intersect, ovulation takes place. Figure 2.11 shows the pattern of events over a four-day period for a two-ovulation sequence, with T_5 set at 13.5. On day 2 the predicted ovulation time is 09:04. On day three ovulation

occurs at 13:37, resulting in a lag of 4 hours and 33 minutes. The two functions do not meet on day four; hence there is no ovulation, resulting in a pause day.

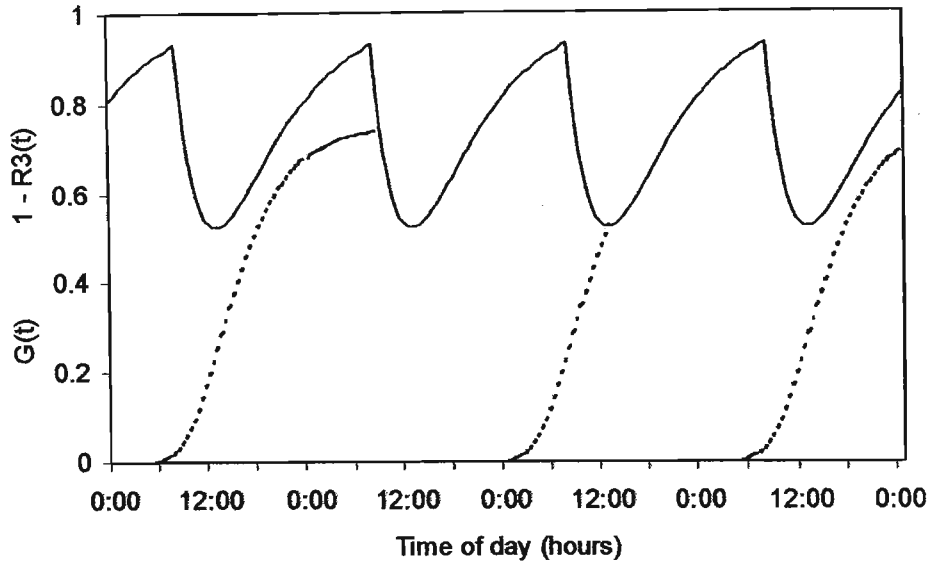


Figure 2.11: An illustration of the asynchronous cycle of ovulation for a two-egg sequence; ovulation occurs when the regulator concentration 1- $R_3(t)$ (solid line) intersects with follicle maturation G(t) (dotted line)

Figure 2.12 shows the point of intersection for the first of a two-ovulation sequence and also demonstrates how the time of ovulation may be influenced by the mature size of the follicle. For $b_1 = 0.75$, the two functions intersect at 09:04. If b_1 is increased to 0.785, ovulation is brought forward to 08:50. Similarly, a follicle with a faster rate of maturing ($b_3 = 0.334$) will intersect with the regulator concentration curve four minutes earlier than one with a slower rate ($b_3 = 0.22$) *i.e.* at 09:00 instead of 09:04 (Figure 2.13). It may also be shown that increasing λ_2 or decreasing S_1 results in an earlier ovulation whereas increasing λ_1 delays the time of ovulation.

The values for the parameters in the two equations (with the exception of S_1) not only influence the time of an ovulation, but also the time interval, or lag, between successive ovulations. If b_1, b_3 or λ_2 increase, the lag is reduced. On the other hand, increasing either b_2, S_2 or λ_1 increases the lag. For example, the first two consecutive ovulation times for a two-ovulation sequence (with $T_5 = 13.5$) are 09:04 and 13:37, giving a lag of 4h33. By

increasing b_3 from 0.22 to 0.334 the times change to 09:00 and 10:39 and the lag is reduced to 1h39 (Figure 2.13). Incidentally, the two-ovulation sequence becomes a nine-ovulation sequence. The shorter the lag, the longer the ovulation sequence becomes, because of the increased opportunity for further intersections within the eight- to ten-hour open period.

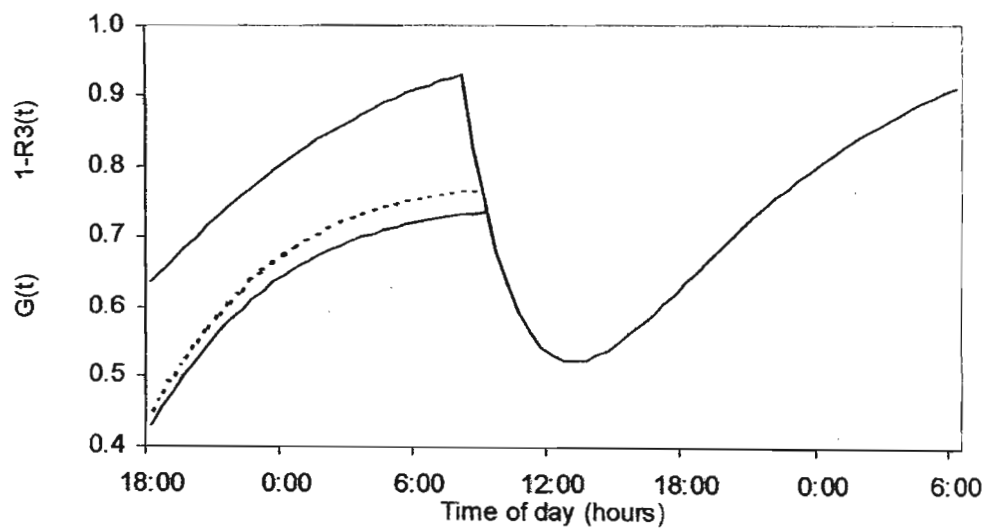


Figure 2.12: Influence of two mature states, *i.e.* $b_1 = 0.75$ (solid line) and $b_1 = 0.785$ (dotted line) on time of ovulation. Predicted ovulation times are 09:04 and 08:50 respectively

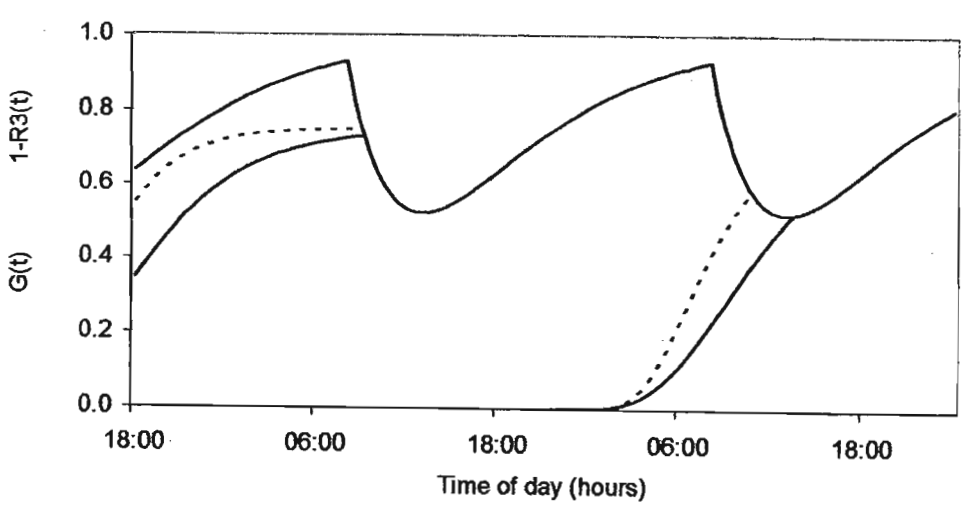


Figure 2.13: Influence of two rates of maturing, *i.e.* $b_3 = 0.22$ (solid line) and $b_3 = 0.334$ (dotted line) on both time of ovulation and interval between successive ovulations

Any factor influencing the time of the first ovulation in a sequence or the lag between successive ovulations will therefore influence the sequence length. Changes to the parameter values of Equations 2.1 and 2.4 lead to changes in the opportunities for the two functions to intersect and hence result in alterations to sequence length.

Table 2.3 summarises the predicted times of ovulation from the macro-driven model for two- to nine-egg sequences, using the values for the parameters given in Table 2.1 and the value of T_5 equal to 13.5 for two-ovulation sequence and 15.5 for all others. It may be seen that these predicted ovulation times are in reasonably close accord with those published by Etches and Schoch (1984) for the University of Guelph flock. Without knowing what values the authors used for T_5 for each sequence, it would be impossible to reproduce their ovulation times. The greatest discrepancies are found in the times of the ultimate ovulations of a sequence; these range from one to fourteen minutes. This is likely to be due to the limitation of calculating the point of intersection to the nearest minute in the model described here.

Table 2.3: Predicted ovulation times using the macro-driven model

	Sequence length							
Position	2	3	4	5	6	7	8	9
1	09:04	08:32	07:46	07:22	07:18	07:12	07:12	07:11
2	13:37	11:31	10:09	09:19	09:08	08:58	08:54	08:49
3		15:22	12:04	10:37	10:15	09:59	09:50	09:42
4			15:26	12:01	11:15	10:47	10:32	10:20
5				15:08	12:31	11:37	11:11	10:53
6					15:55	12:45	11:58	11:27
7						15:12	13:05	12:08
8							15:49	13:09
9								15:26

Table 2.4 gives the calculated lag values, the total lag and the mean lag for the different sequence lengths, as well as the predicted mean lag values from the data reported by Etches and Schoch (1984). In general, the mean lag values are in close accord.

Table 2.4: The calculated lag between successive ovulations, using the ovulation times summarised in Table 2.3

Sequence length

Position	2	3	4	5	6	7	8	9
1-2	04h33	02h59	02h23	01h57	01h50	01h46	01h42	01h38
2-3		03h51	01h55	01h18	01h07	01h01	00h56	00h53
3-4			03h22	01h24	01h00	00h48	00h42	00h38
4-5				03h07	01h16	00h50	00h40	00h33
5-6					03h24	01h08	00h46	00h34
6-7						02h27	01h07	00h41
7-8							02h44	01h01
8-9								02h17
total lag	04h33	06h50	07h40	07h46	08h37	08h00	08h37	08h15
mean lag	04h33	03h25	02h33	01h56	01h43	01h20	01h14	01h02
Etches *	04h32	03h25	02h32	01h55	01h41	01h19	01h11	01h00

*mean lag from the predicted ovulation times, Etches and Schoch (1984), Univ. Guelph flock.

Figure 2.14 shows the observed and predicted mean lag, for sequences of different lengths, from the University of Guelph data reported by Etches and Schoch (1984). As sequence length increases, the mean lag decreases but at a diminishing rate. Bearing in mind that changes to the parameter values of the ovulatory model affect ovulation rate by changing

the lag between successive ovulations, any function used to predict parameter values should perhaps reflect this trend. This suggests that the function should be asymptotic as sequence length increases indefinitely.

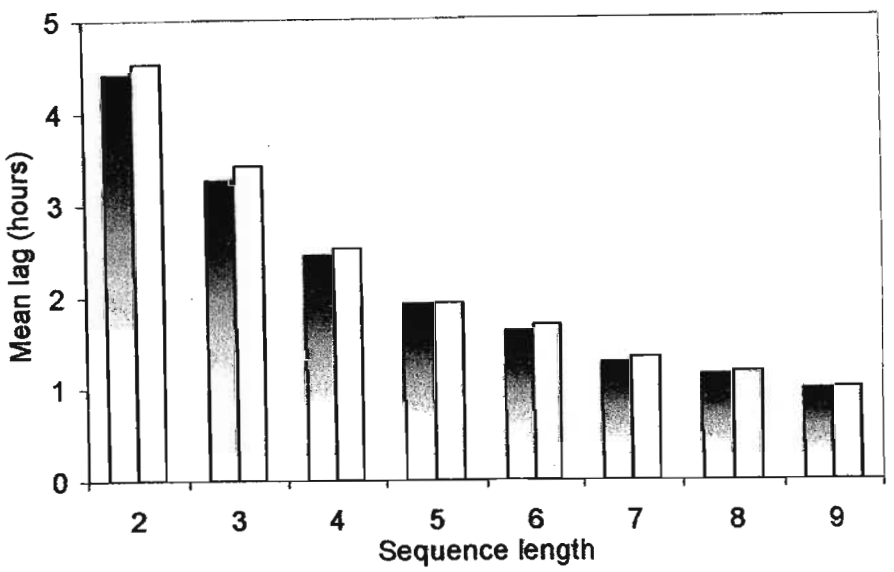


Figure 2.14: The observed (shaded columns) and predicted (blank columns) mean lag for eight sequence lengths. (Data from Etches and Schoch, 1984)

2.5 The parameters and sequence length

Figures 2.15 to 2.17 show the parameters λ_1, λ_2 and S_1 plotted against sequence length, using the numerical values from Table 2.1. The values for all three parameters remain constant for sequences of five eggs or longer. Interestingly, λ_1 and λ_2 only have two values each; one for sequences of two to four eggs and another for longer sequences. In the light of our understanding of the roles of these two parameters in determining the volume of regulator flowing from one compartment to another, it seems absurd that in a population of hens only two volumes should exist. It is intuitively felt that these parameters should change gradually as ovulation sequences lengthen.

The relationship between S_1 and sequence length is negative because the longer the sequence, the earlier in the morning the first ovulation takes place and consequently the

earlier in the morning of the following day the first egg is laid. The earliest time given for S_1 is 6.5 (06:30). S_1 does not play a role in determining sequence length; it merely adjusts the time frame so that the first ovulation occurs after sunrise. However, there is no need to restrict the values to the nearest quarter hour as was done by Etches and Schoch (1984).

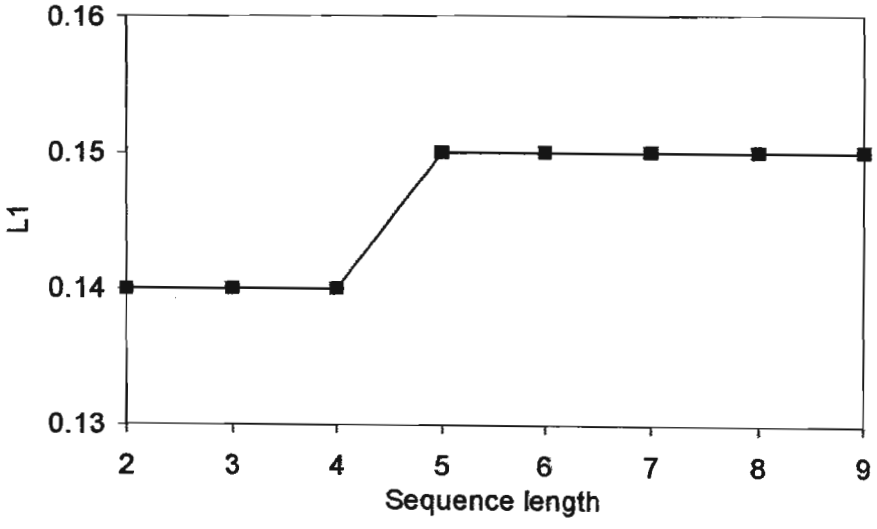


Figure 2.15: The relationship between λ_1 and sequence length (Etches and Schoch, 1984)

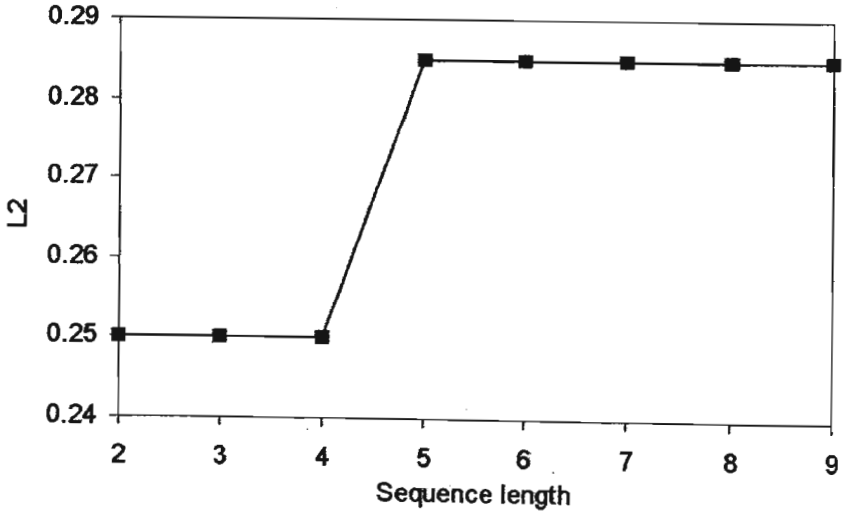


Figure 2.16: The relationship between λ_2 and sequence length (Etches and Schoch, 1984)

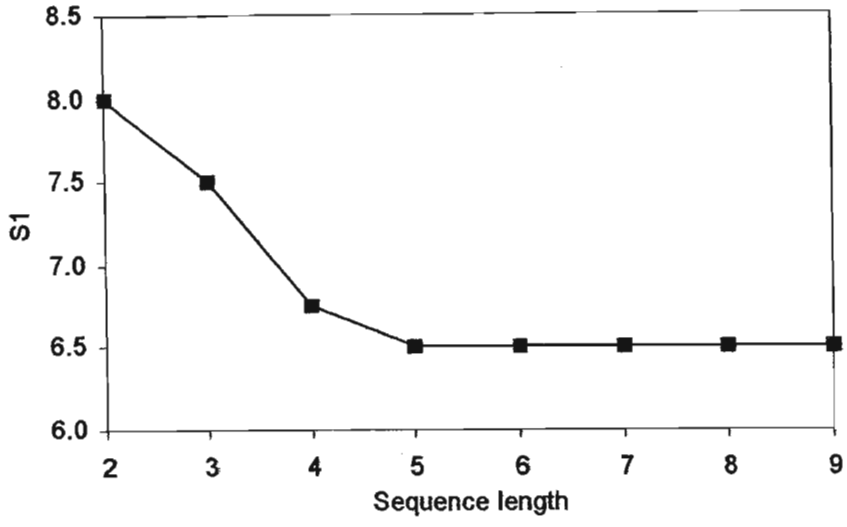


Figure 2.17: The relationship between S_1 and sequence length (Etches and Schoch, 1984)

Figures 2.18 to 2.21 show the relationships between b_1, b_2, b_3, and S_2 and sequence length. The rate parameter b_3 appears to be curvilinearly related to sequence length. It makes sense that longer ovulation sequences are largely the result of follicles that mature at a faster rate. As sequence length increases, b_3 increases but at a decreasing rate; a trend reminiscent of the relationship between mean lag and sequence length. Similarly b_1 is positively related to sequence length; longer sequences being due to follicles with a greater 'mature state'.

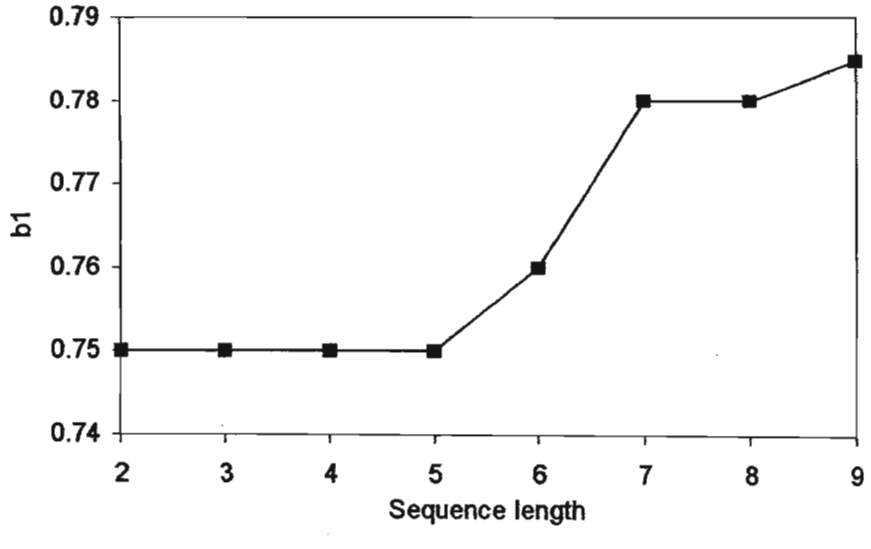


Figure 2.18: The relationship between b_1 and sequence length (Etches and Schoch, 1984)

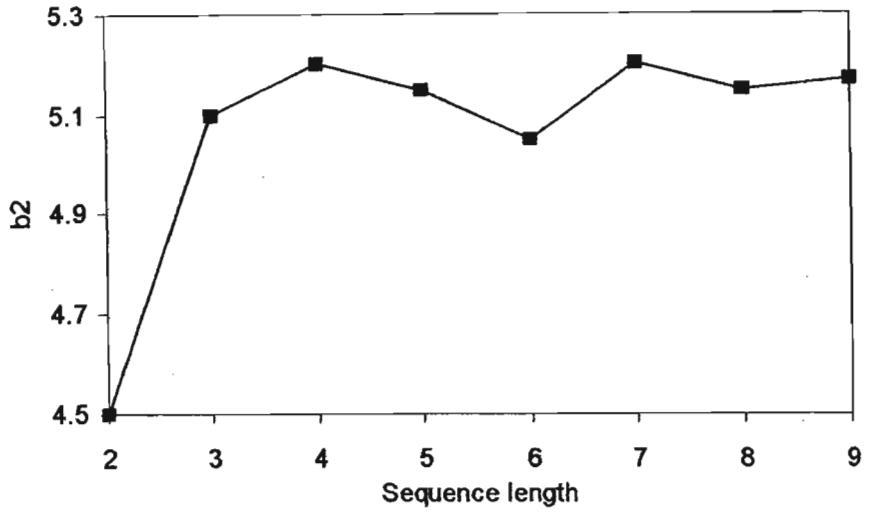


Figure 2.19: The relationship between b_2 and sequence length (Etches and Schoch, 1984)

It is apparent from Figure 2.19 that no obvious trend exists for the values of b_2, which suggests that it may have been manipulated more than the other parameters in order to force the intersection of G(t) and (1-R_3(t)) to occur at precise, predetermined times.

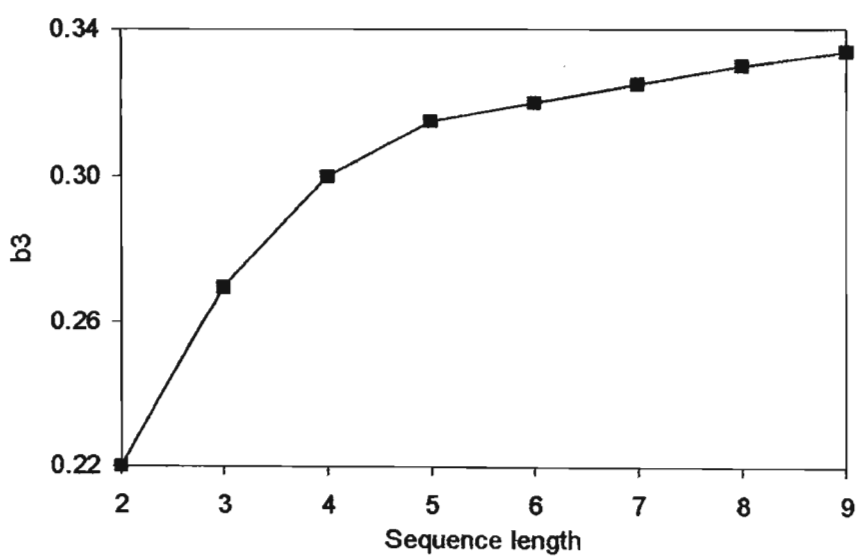


Figure 2.20: The relationship between b_3 and sequence length (Etches and Schoch, 1984)

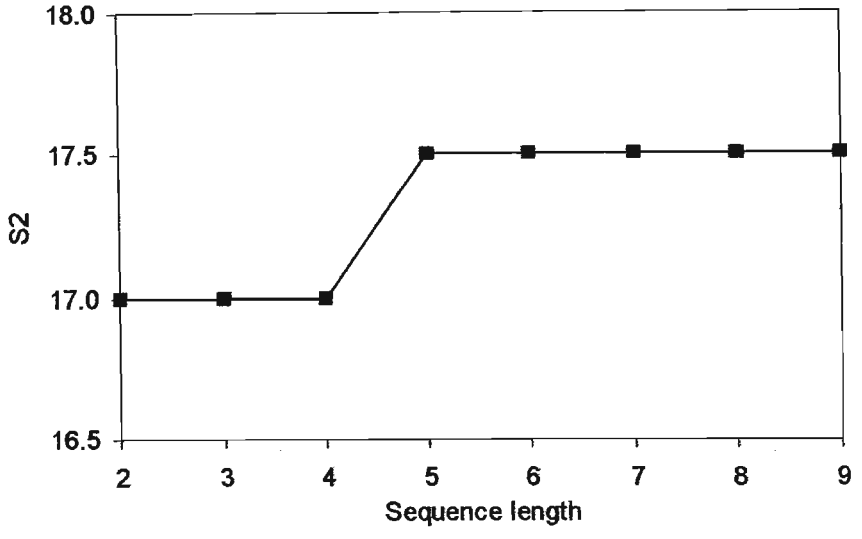


Figure 2.21: The relationship between S_2 and sequence length (Etches and Schoch, 1984)

The parameter S_2 only has two values; 17 for short sequences of two to four ovulations and 17.5 for longer sequences. It is felt that the relationship between S_2 and sequence length should be a negative one, not positive as given in Table 2.1. If S_2 represents the time after the previous ovulation that a follicle is not sensitive to stimulation, then it seems logical that in a high producing hen, this time interval is less than in a low producing hen. It has also been suggested that the period of non-sensitivity lies between 14 and 16 hours (Etches, 1984). As with λ_1 and λ_2, gradual changes in the value of S_2 with increasing sequence length would seem preferable. For this model it seemed reasonable to use a range of values between 17 (for a one-egg sequence) and 14 (for a nine-egg sequence).

2.6 The parameters and ovulation rate

Before attempting to find continuous functions to replace the values listed in Table 2.1, a continuous variate is needed. Each sequence length may be converted to an ovulation rate, which becomes the continuous independent variable. For example, a sequence of two ovulations followed by a single pause day gives an ovulation rate of 0.667 (two divided by three) and a three-ovulation sequence, 0.750 (three ovulations in four days). Table 2.5 shows the rates of ovulation for sequence lengths from two to nine ovulations.

Table 2.5: Ovulation sequence lengths converted to ovulation rates

Sequence length	Rate of ovulation
2	0.667
3	0.750
4	0.800
5	0.833
6	0.857
7	0.875
8	0.889
9	0.900

Figure 2.22 shows the rate parameter b_3 plotted against rate of ovulation. Because there appears to be a reasonable curvilinear relationship between the two variables, it was decided that this would be a good starting point for attempting to fit continuous functions to the parameter values suggested by Etches and Schoch (1984).

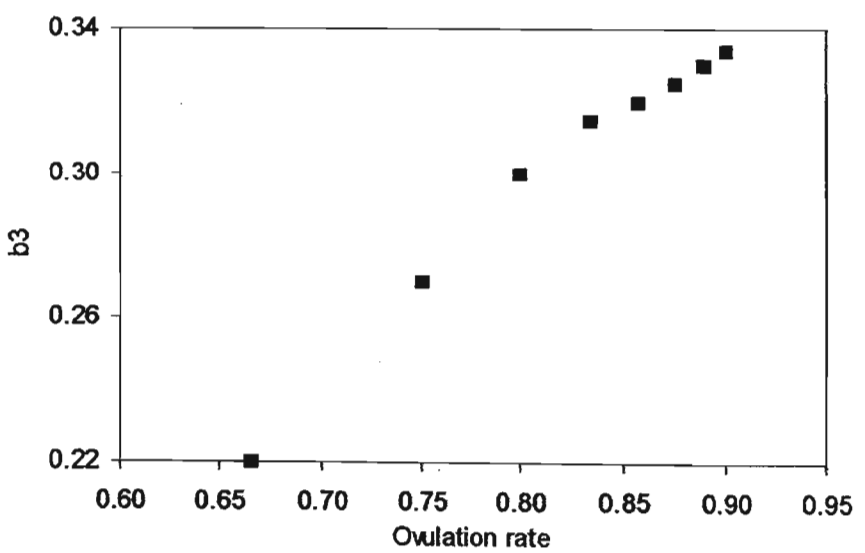


Figure 2.22: The relationship between b_3 and rate of ovulation

Using Genstat 5, different functions were fitted to the data and evaluated. A simple quadratic equation of the form $y = a + bx + cx^2$, with ovulation rate R as the predictor variable (x) and b_3 the response variable (y), fitted the data well ($r^2 = 0.997$):

$$b_3 = - 0.7377 + 2.135 \, . \, R - 1.050 \, . \, R^2 \qquad \text{(Equation 2.5)}$$

However, b_3 becomes negative below ovulation rates of 0.45. The follicle maturation function (Equation 2.4) requires positive parameter values, which renders the quadratic equation unsuitable. Furthermore, it has the disadvantage of allowing b_3 to decrease as the ovulation rate exceeds 1.02. On normal 24-hour daylengths rate of ovulation does not extend beyond 1, *i.e.* 100%, so this may seem irrelevant. However, with short ahemeral light:dark cycles, rates of ovulation and lay for high-producing hens exceed 100%. This needs to be taken into consideration if the ovulation model is to be adaptable to ahemeral cycles.

An exponential function of the form $y = A + B \, . \, R^x$ overcomes this problem at high ovulation rates because the curve is asymptotic. Genstat 5 fitted the following equation, which accounts for 99.6% of the variation in the data:

$$b_3 = 0.4023 - 3.08 * 0.0145^{\,R} \qquad \text{(Equation 2.6)}$$

where R = ovulation rate. The disadvantage of an exponential function is that the response variate decreases sharply as the predictor variable is reduced. In the case of Equation 2.6, negative values for b_3 were obtained for ovulation rates below 0.49. Hence the exponential equation must also be discarded.

The Gompertz equation

$$b_3 = 0.2030 + 0.1459 \, . \, \exp(-\exp(-12.36 \, . \, (R - 0.7289))) \qquad \text{(Equation 2.7)}$$

(where R = ovulation rate) accounted for 99.8% of the variation in the data but overcame the problems mentioned above. Gompertz equations in the form

$$y = A + C \cdot \exp(-\exp(-B \cdot (x - M))) \qquad \text{(Equation 2.8)}$$

are commonly used to model biological growth and are an extended form of the exponential equation. The equation has two asymptotes; one when y = A (the y-intercept when x = 0) and the other when y = A + C (the maximum). It is asymmetrical about the point of inflection at x = M and has a slope given by BC/e at the point of inflection. These features seem to fit with the requirements of the model. Because of its asymptotic nature, when the ovulation rate approaches zero the parameter values remain positive. Similarly, as the ovulation rate approaches 1.0, b_3 continues to increase but at a diminishing rate.

The calculated values for b_3 from the three equations are listed in Appendix 2.6. Appendices 2.7, 2.8 and 2.9 show the statistical summaries for the fitting of the quadratic, exponential and Gompertz equations respectively. Figure 2.23 shows the relationship between b_3 and ovulation rate, with the data points given by Etches and Schoch (1984) and the fitted Gompertz function (Equation 2.7).

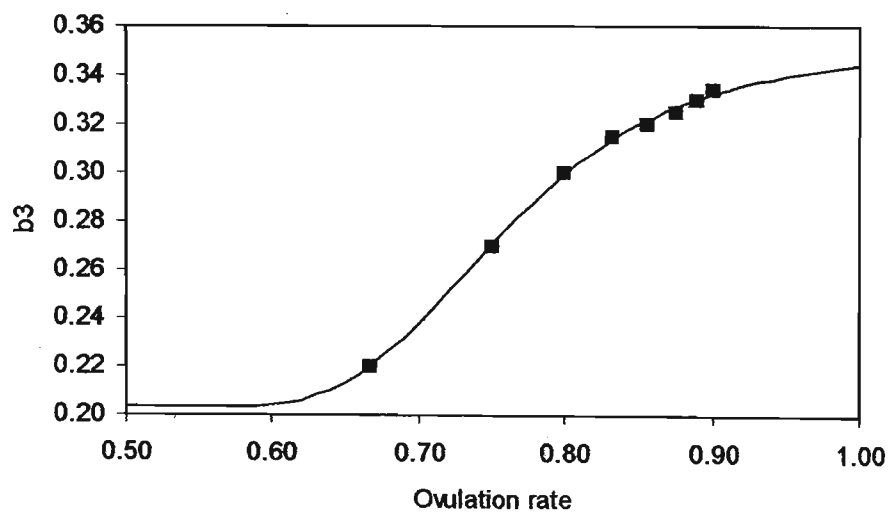


Figure 2.23: The curvilinear relationship between b_3 and ovulation rate, showing the fitted Gompertz function (—) and the given parameter values (■)

2.7 Creating Gompertz functions for the remaining ovulatory cycle parameters

Following the success in fitting a Gompertz function to the values of b_3 given by Etches and Schoch (1984), it was decided to use similar functions in the form given by Equation 2.8 for the six remaining parameters.

The process of defining the values for B and M (the point of inflection) for the six Gompertz equations, in order to model the longer sequences, was not straightforward. For ovulation rates over 0.9 and approaching 1.0, increasingly smaller changes to the values of the parameters caused large changes to the sequence lengths. An optimisation routine was needed to solve the equations simultaneously and to allow rapid reassessment of the end result of changes to B and the point of inflection on ovulation rate. For these reasons, AMPL software ('A Mathematical Programming Language', version 1.6, Bell Laboratories) was used. Gompertz equations were created for the six parameters λ_1, λ_2, S_1, b_1, b_3 and S_2. This was done by keeping within the same range of values given in Table 2.1 (because these values have been seen to work in the model); for example $\lambda_1 = 0.14$ for a two-egg sequence and $\lambda_1 = 0.15$ for a nine-egg sequence. The exception was S_2; the range specified was 14 for a nine-ovulation sequence and 17 for a one-ovulation sequence, as discussed in section 2.5 above. The values for B and M were kept constant in the six equations and the values of the other two parameters A and C were varied. Although there is no biological reason for doing this, the motivation was to simplify the starting point as much as possible. The initial values of B and M used in the model were the estimates given by Equation 2.7, *i.e.* 12.36 and 0.7289 respectively, but these were gradually modified to 8.0 and 0.667 (the point of inflection occurring at a two-ovulation sequence). This fine-tuning was necessary because B and M play critical roles in determining the success of the model, both in producing longer sequences with accuracy and in achieving an ovulation rate of 1.0. Figures 2.24 to 2.29 show the original data points given by Etches and Schoch (1984) for λ_1, λ_2, S_1, b_1, b_3 and S_2 and the curves produced by the Gompertz functions used to predict their values over the range of ovulation rates. Appendix 2.10 gives the values assigned by AMPL to A and C for each of the six ovulatory cycle parameters. By inserting these values into a Gompertz function of the form given by Equation 2.8, it becomes possible to predict the values of these parameters needed to

simulate any ovulation rate. This removes the restriction of working with a discontinuous variable over a limited range; *i.e.* sequences of two to nine ovulations only. Appendix 2.11 contains the AMPL programming procedure.

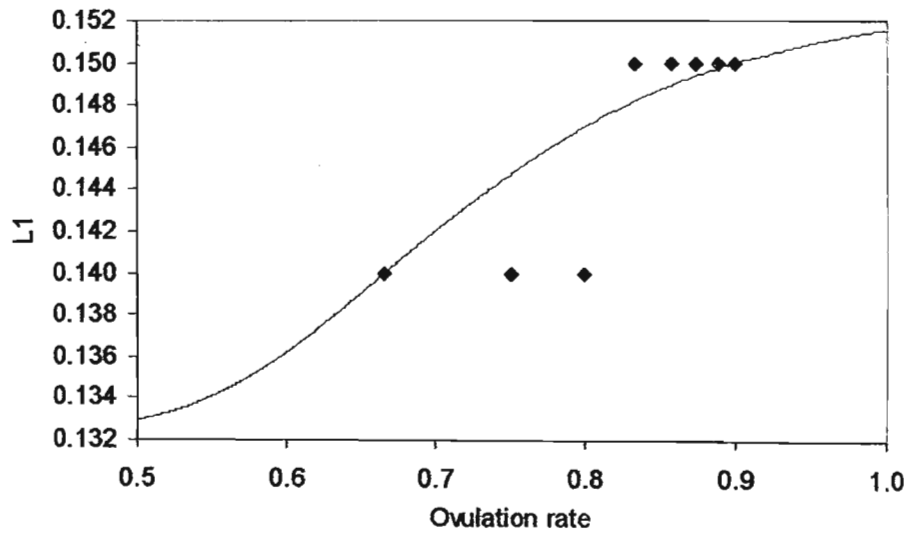


Figure 2.24: The values for λ_1 given by Etches and Schoch (1984) (◆) and the Gompertz function for predicting λ_1 from ovulation rate (—)

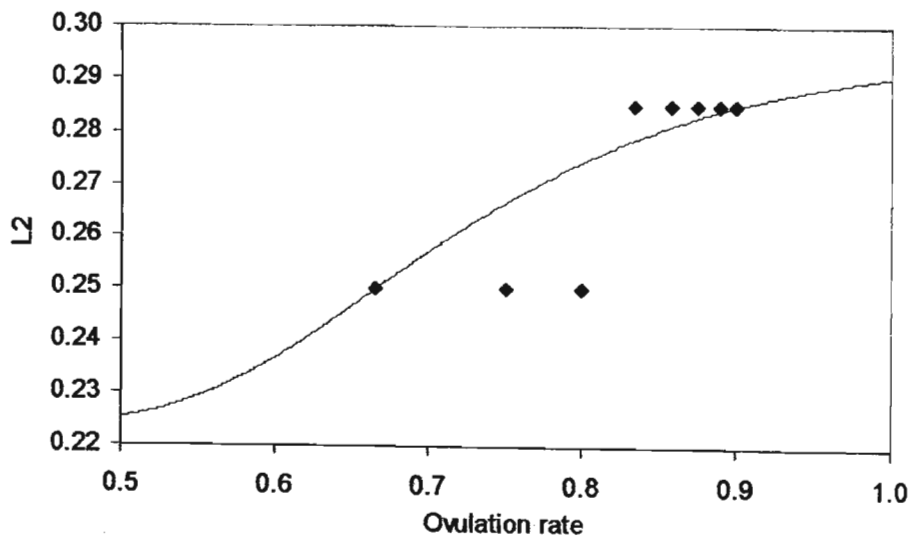


Figure 2.25: The values for λ_2 given by Etches and Schoch (1984) (◆) and the Gompertz function for predicting λ_2 from ovulation rate (—)

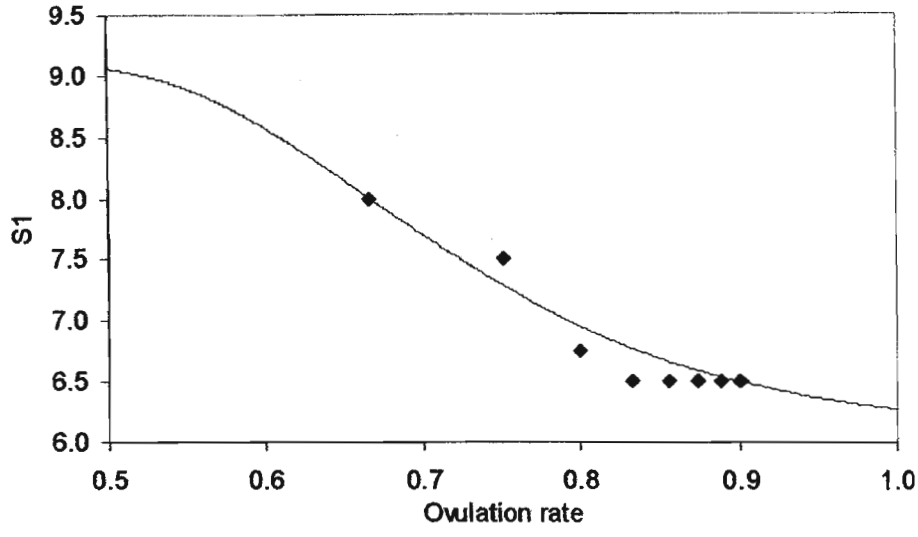


Figure 2.26: The values for S_1 given by Etches and Schoch (1984) (◆) and the Gompertz function for predicting S_1 from ovulation rate (—)

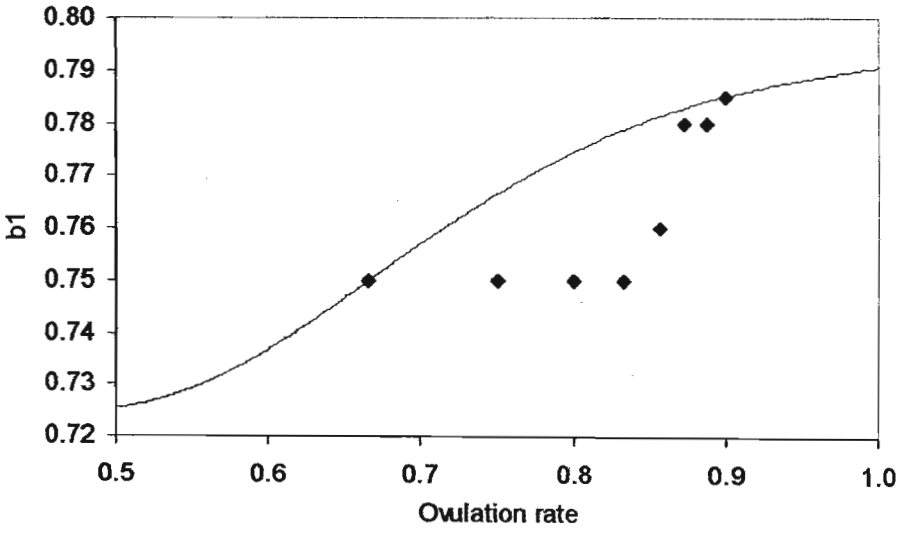


Figure 2.27: The values for b_1 given by Etches and Schoch (1984) (◆) and the Gompertz function for predicting b_1 from ovulation rate (—)

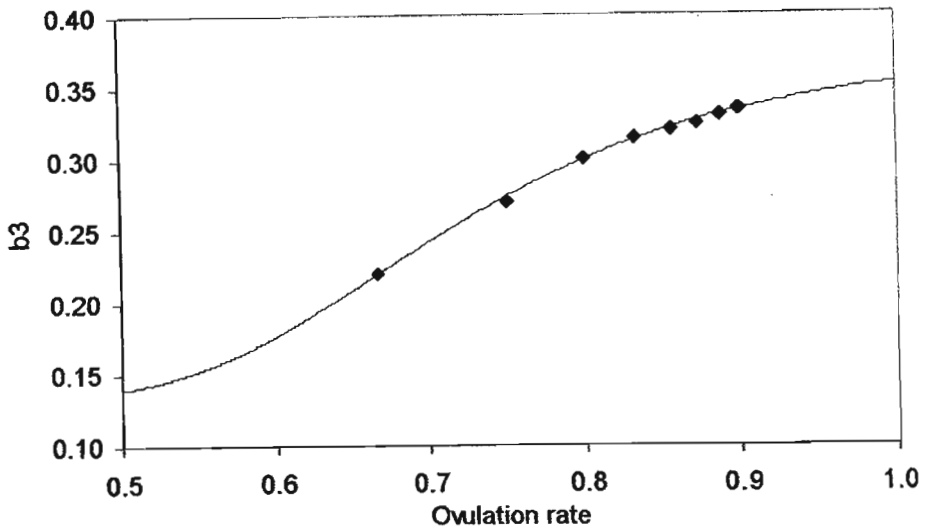


Figure 2.28: The values for b_3 given by Etches and Schoch (1984) (◆) and the revised Gompertz function for predicting b_3 from ovulation rate (—)

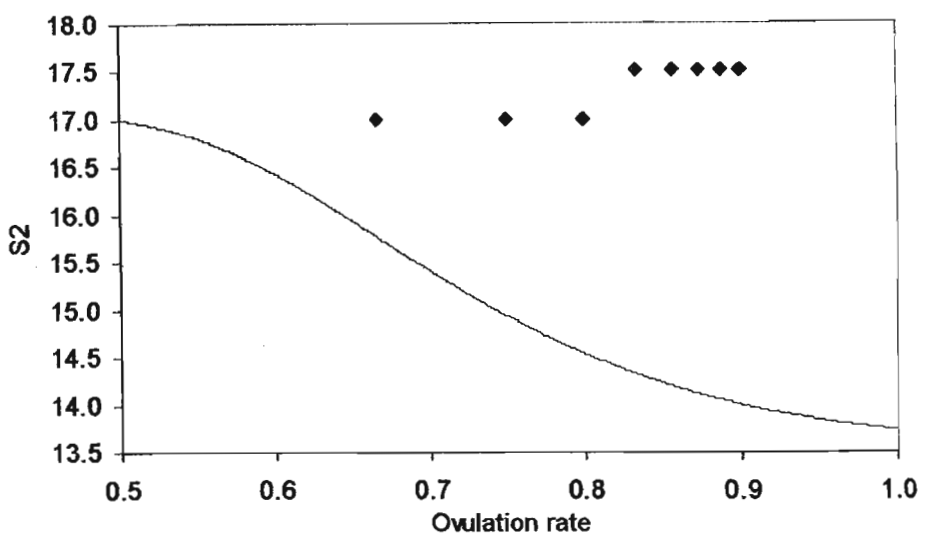


Figure 2.29: The values for S_2 given by Etches and Schoch (1984) (◆) and the Gompertz function for predicting S_2 from ovulation rate (—)

2.8 Solving for b_2

The problem remaining was to find an acceptable range of values for b_2 which would allow the model to create ovulation sequences of lengths from R = 0.5 to R = 1.0. Ovulation rates below 0.5 are not specifically dealt with here.

In the case of the minimum value for b_2 needed to give a two-ovulation sequence, AMPL was programmed to solve for b_2 and minimise the lag (or the intra-sequence ovulation interval) between successive ovulations on days one and two, without allowing the functions G(t) and $(1-R_3(t))$ to intersect on day three (see Appendix 2.12 for AMPL program files). A graphical representation of the method is shown in Figure 2.30. The constant T_5 ('2lastov') *i.e.* the time the last ovulation of the previous sequence occurred, was taken as 13.5. The values assigned to the variables by the model are listed in Table 2.6. In the case of the two- (and nine-) ovulation sequence, the parameters λ_1, λ_2, S_1, b_1 and b_3 may be seen to have the same values as given by Etches and Schoch (1984) and listed in Table 2.1; this is because of the decision made to keep within the range specified by those authors (see section 2.7).

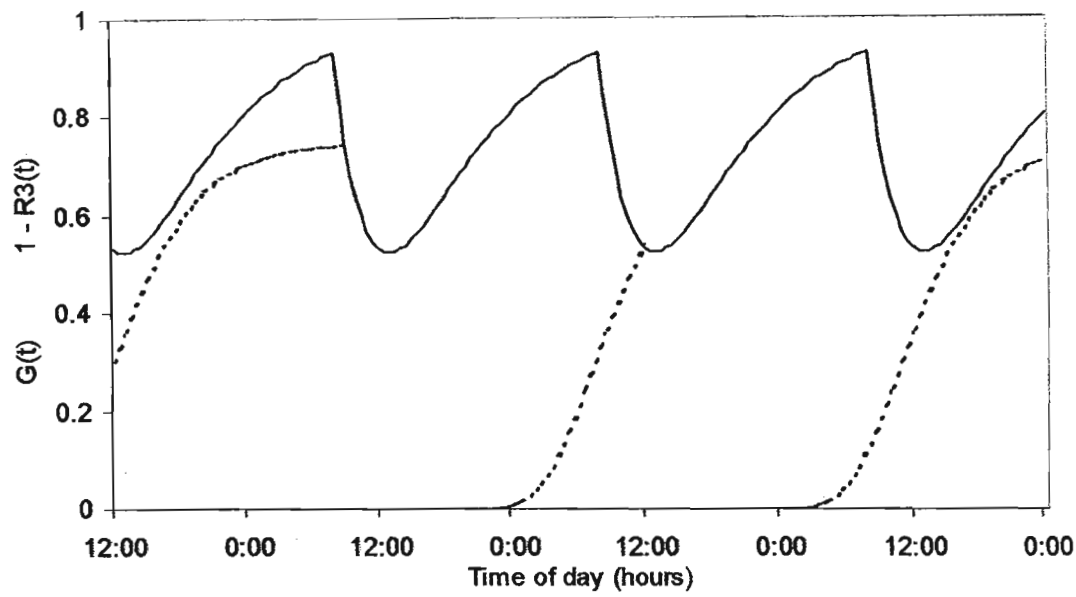


Figure 2.30: An illustration of how the interval between successive ovulations may be minimised without allowing the functions to intersect on the third day

Hence the minimum value of b_2 that will produce a two-ovulation sequence is 4.000933. Ovulations occur on days one and two at 09:02 and 11:58 respectively, so the minimum lag is 2h56m. The time of day given by the variable '2timeday3' represents the time at which the functions come within 0.00001 of each other without intersecting and the time when the tangents would normally meet, when $(1- R_3(t))$ is waning. Etches and Schoch (1984)

deemed that ovulation had occurred if the difference between the two functions was less than 10^{-5}. The regulator concentration times ('2timerc1' *etc.*) are the same as the times of day but are in decimal format. The follicle maturation times ('2timefm1' etc.) give the time that the particular follicle has been undergoing maturation, in decimal format; *e.g.* 2timefm1 = 43h32m.

Table 2.6: Values assigned to the variables by the AMPL model minimising the lag between successive ovulations in a two-egg sequence

Variable	Value	Variable**	Value
λ_1 ('2L1')*	0.14	2timeday1	09:02
λ_2 ('2L2')	0.25	2timeday2	11:58
S_1 ('2S1')	8	2timeday3	16:41
B_1 ('2b1')	0.75	2timerc1	9.03 (09:02)
b_3 ('2b3')	0.22	2timerc2	11.97 (11:58)
S_2 ('2S2')	15.76	2timerc3	16.69 (16:41)
A_2 ('2a2')	2.104668	2timefm1	43.53 (43h32m)
b_2 ('b2')	4.000933	2timefm2	26.93 (26h56m)
Minimum lag	2.932378 (2h56m)	2timefm3	28.72 (28h43m)

* denotes form of variable in AMPL model; the prefix '2' refers to a two-ovulation sequence

** the prefix '2' refers to a two-ovulation sequence; the suffix denotes the day number

The maximum possible value for b_2 to produce a two-ovulation sequence was found by allowing AMPL to solve for b_2, maximising the lag between the ovulations on days one and two. The last point of contact between the two curves on day two is given by the point at which the tangents coincide, *i.e.* where the derivatives of G(t) and $(1-R_3(t))$ are equal. The derivatives of equations 2.1 and 2.4 are as follows:

$$(1-R_3)'(t) = e^{(-(t-S1)\lambda 1)} . a_1 . \lambda_1 - e^{(-(t-S1)\lambda 2)} . a_2 . \lambda_2 \qquad \text{(Equation 2.9)}$$

$$G'(t) = e^{(-e^{(-b3\,(t-S2))}\,.\,b2\,-\,b3\,(t-S2))}.\ b_1\ b_2\ b_3 \qquad \text{(Equation 2.10)}$$

Appendix 2.13 shows the relevant AMPL program files, and the variable values are summarised in Table 2.7. Figure 2.31 depicts how the lag or intra-sequence interval was maximised. Thus the maximum value of b_2 that will produce a two-ovulation sequence is 7.558433. Ovulations occur on days one and two at 09:04 and 16:41 respectively, giving a maximum lag of 7h37m. The first follicle matures for 43h34m before rupturing. The final phase of maturation for the second follicle of the two-ovulation sequence lasts for 31h37m.

Table 2.7: Values assigned to the variables in the AMPL model maximising the lag between successive ovulations in a two-egg sequence

Variable	Value	Variable	Value
λ_1 ('2L1')	0.14	2timeday1	09:04
λ_2 ('2L2')	0.25	2timeday2	16:41
S_1 ('2S1')	8		
B_1 ('2b1')	0.75	2timerc1	9.07 (09:04)
B_3 ('2b3')	0.22	2timerc2	16.68 (16:41)
S_2 ('2S2')	15.76		
a_2 ('2a2')	2.104668	2timefm1	43.57 (43h34m)
b_2 ('2ub2')*	7.558433	2timefm2	31.61 (31h37m)
Maximum lag	7.610296 (7h37m)		

*upper (maximum) value of b_2 for a two-ovulation sequence

This procedure was repeated for one- and three- to nine-ovulation sequences, a fifteen- and a twenty-ovulation sequence. The value of T_5 was 15.5 for all sequence lengths except in the case of the one-ovulation sequence, where 13.5 was used. The student version of AMPL limits the number of constraints and variables that may be included, so it was not possible to use this procedure for longer sequences. The maximum value for b_2 which

gives R = 1.0 (there is no minimum) was found by allowing the lag between successive ovulations to approach zero as the time that G(t) and (1-R_3(t)) intersect approaches the time of day when R_3(t) is at a maximum (or (1-R_3(t)) is at a minimum). Appendix 2.14 summarises the minimum and maximum values allocated to b_2 and the associated lag values, for all the above-mentioned sequence lengths.

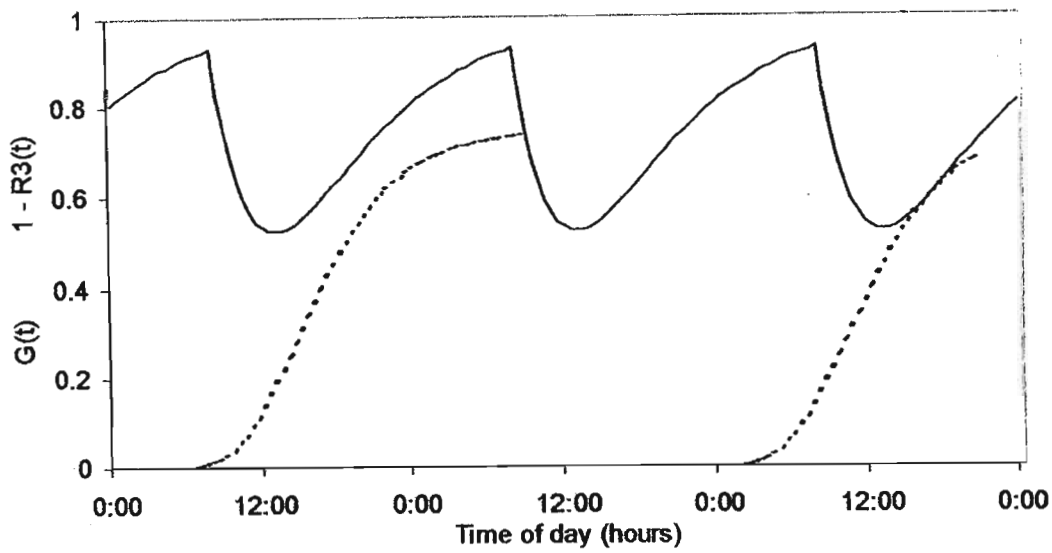


Figure 2.31: An illustration of how the interval between successive ovulations may be maximised; the second ovulation occurs where the tangents of G(t) and 1-R_3(t) coincide

Once the boundaries for b_2 were established, AMPL was programmed to find a Gompertz equation within the constraints set by the minimum and maximum values for b_2. However, this procedure was not as simple as expected. If all four variables A, C, B and M were simply declared as being greater than or equal to zero, the solution was aborted after about 20 iterations. The allowable ranges for B and M needed to be narrowed but the ranges themselves influenced the outcome of the model, so some process of trial and error ensued. In order to ensure that the longer ovulation sequences (more than 20 consecutive ovulations) were accurately produced, b_2 needed to be as close to the upper limit as possible; hence the objective function was to maximise b_2 at an ovulation rate of one. This resulted in a lag of six to seven hours in the two-ovulation sequence, which was felt to be abnormally long. According to the observed times (of oviposition) of Etches and Schoch (1984) the mean lag is more likely to be approximately 4h30m. The upper limit to b_2 for

this ovulation rate needed to be reduced. Next, the lower limit to b_2 for a one-ovulation sequence was increased slightly so that the Lotus model produced single ovulation sequences indefinitely. Otherwise a single ovulation would be followed by a two-ovulation sequence. Finally, the upper limits to b_2 for the nine-, fifteen- and twenty-ovulation sequences were reduced slightly so that the final Gompertz function did not actually come into contact with the upper limits. Without this modification, minor discrepancies between the Lotus model and the AMPL model resulted in, for instance, a twenty-ovulation sequence in AMPL being a nineteen-ovulation sequence in Lotus.

A Gompertz function that lies between the limits and that can be used to estimate b_2 is as follows:

$$b_2 = 0.760415 + 21.097792 \,.\, \exp(-\exp(-6.148166 \,.\, (R - 0.708292))) \qquad \text{(Equation 2.11)}$$

It must be stressed that there are a number of possible solutions. The outcome will depend largely on the constraints placed on the AMPL model by the user according to the desired result. Any function for b_2 which lies closer to the upper limits will produce ovulation sequences (of four or more ovulations) with a total lag of nine to ten hours, as the last ovulation will occur after 16:30. In contrast, a function that lies closer to the lower limits tends to reduce the total lag to five to seven hours; the terminal ovulation occurring before 14:00. Figure 2.32 shows the upper and lower limits to b_2 (excluding the upper limit for a one-ovulation sequence), as defined by AMPL, which will produce ovulation sequences of specific lengths, as well as the curve given by Equation 2.11. Appendix 2.15 documents the relevant AMPL program files.

With Equation 2.11 included in the menu-driven ovulatory model, it is now possible to calculate the precise value of b_2 required to produce any ovulation rate from 0.5 to 1.0. The user simply specifies a desired sequence length, which is converted by the model to an ovulation rate, and the number of days the model is to run. The predicted ovulation times are stored in the summary table.

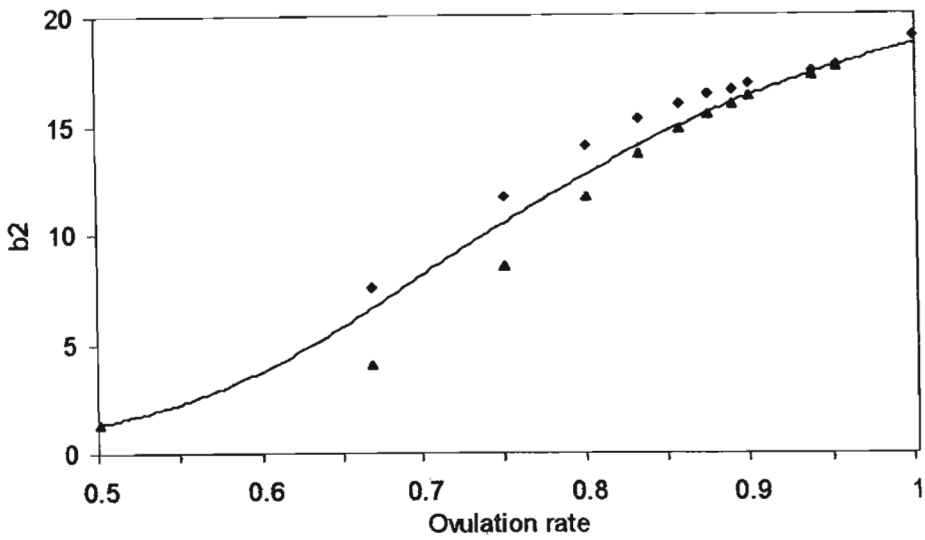


Figure 2.32: Upper (◆) and lower (▲) limits to b_2 for specific ovulation rates, and the Gompertz function (—) used to predict b_2 for any ovulation rate

At this stage it was found necessary to amend the Lotus 1-2-3 model to calculate the times of intersection to the nearest second, because there were discrepancies between the daily predicted times of ovulation given by the Lotus and AMPL models. The AMPL times tended to be slightly earlier, most noticeably in the afternoon when the functions $1\text{-}R_3(t)$ and G(t) are rapidly moving apart. During this period the addition of a whole minute to the three time scales in the Lotus model tended to take the calculation anything from a few seconds to a minute beyond the point of intersection. Once the necessary adjustments had been made to the Lotus model, the estimated times of ovulation predicted by AMPL were identical to those predicted by the Lotus model, except in the afternoon where variances of one or two seconds were still noticed. However, this is unavoidable since the spreadsheet program does not work in intervals smaller than one second. Because AMPL works in decimals it is unhampered by constraints of time and therefore locates the precise point of intersection of the two functions. The output produced in spreadsheet form by the modified Lotus model is shown in Appendix 2.16, and the revised model is detailed in Appendix 2.17. Tables 2.8 and 2.9 list the ovulation times given by the revised model for sequences from one to twelve, fifteen and twenty ovulations, as well as the total lag, mean lag and mean intra-sequence interval for each sequence length.

Table 2.8: Predicted ovulation times from the revised ovulatory model (one- to seven-ovulation sequences)

Sequence length

Position	1	2	3	4	5	6	7
1	10:37.17	09:03:48	08:08:42	07:43:09	07:29:46	07:21:46	07:16:33
2		14:19:15	11:00:28	09:57:10	09:27:14	09:10:17	08:59:33
3			14:18:09	11:36:40	10:41:48	10:13:40	09:56:44
4				13:51:34	11:53:30	11:06:21	10:40:28
5					13:36:46	12:03:53	11:22:17
6						13:29:45	12:11:39
7							13:27:26
totallag		05h15m27s	06h09m27s	06h08m25s	06h07m00s	06h07m59s	06h10m53s
mean lag		05h15m27s	03h04m44s	02h02m48s	01h31m45s	01h13m36s	01h01m49s
interval*		29h15m27s	27h04m44s	26h02m48s	25h31m45s	25h13m36s	25h01m49s

* Mean intra-sequence interval

Table 2.9: Predicted ovulation times from the revised ovulatory model (eight- to twenty-ovulation sequences)

Sequence length

Position	8	9	10	11	12	15	20
1	07:12:54	07:10:14	07:08:10	07:06:34	07:05:17	07:02:34	07:00:05
2	08:52:13	08:46:56	08:42:55	08:39:49	08:37:21	08:32:12	08:27:31
3	09:45:29	09:37:32	09:31:35	09:27:02	09:23:26	09:16:02	09:09:22
4	10:24:06	10:12:52	10:04:40	09:58:28	09:53:36	09:43:47	09:35:05
5	10:58:11	10:42:26	10:31:18	10:23:03	10:16:41	10:04:05	09:53:11
6	11:33:48	11:11:03	10:55:44	10:44:45	10:36:28	10:20:30	10:07:07
7	12:18:12	11:42:52	11:20:58	11:06:01	10:55:06	10:34:52	10:18:35
8	13:28:06	12:24:14	11:50:23	11:29:05	11:14:18	10:48:20	10:28:33
9		13:31:01	12:29:59	11:57:01	11:35:57	11:01:47	10:37:39
10			13:35:33	12:35:47	12:03:01	11:16:05	10:46:18
11				13:41:44	12:41:38	11:32:20	10:54:52
12					13:49:14	11:52:16	11:03:42
13						12:19:09	11:13:10
14						13:00:31	11:23:47
15						14:20:46	11:36:17
16							11:51:53
17							12:12:49
18							12:43:52
19							13:37:52
20							16:00:43
total lag	06h15m12s	06h20m47s	06h27m23s	06h35m10s	06h43m57s	07h18m12s	09h00m38s
mean lag	00h53m36s	00h47m36s	00h43m03s	00h39m31s	00h36m43s	00h31m18s	00h28m27s
interval*	24h53m36s	24h47m36s	24h43m03s	24h39m31s	24h36m43s	24h31m18s	24h28m27s

* Mean intra-sequence interval

2.9 Discussion

The mathematical model of Etches and Schoch (1984) demonstrates that two functions, representing two independent but interacting systems of the hen's asynchronous ovulatory cycle, are able to predict realistic ovulation times and intra-sequence ovulation intervals. However, a major disadvantage of their model is that predictions are restricted to sequences of between two and nine ovulations only. For each ovulation sequence, a different set of discrete values for the parameters is required, these values apparently having been somewhat arbitrarily chosen by the authors. The set of continuous functions, representing the changes required to the values of the different parameters, makes the prediction of any sequence length possible. This approach has considerably enhanced the value of the original ovulatory model.

Sequences from one to twenty ovulations may now be accurately simulated, by substituting R (rate of ovulation, between 0.5 and 0.952) in each of the Gompertz equations determining the different parameters. As the ovulation rate approaches one, and the mid-sequence lag approaches zero, such minute changes in the parameter values are required to create the longer sequences that the model becomes less accurate. For instance, when trying to create 40- and 50-ovulation sequences, this model produces 42 and 62 consecutive ovulations respectively. This may not be of great importance when modelling flock laying performance over a period of time, since there is always a huge amount of variation about a mean sequence length. Furthermore, a satisfactory population model will include a decay factor that will allow sequence length to decline with time from first egg, as will be discussed in Chapter 5. What is important here is that the functions are able to produce long prime sequences of 50 to 70 ovulations or more.

Mean rate of lay in a flock of hens at a particular age is determined by the individual patterns of sequential laying at that time. The slope of the initial rise in flock egg production and peak rate of lay are influenced by the distribution of ages at sexual maturity and by the ages at commencement of the individual prime sequences and the lengths thereof. The incidence of internal laying at onset of maturity plays a role in modifying rate of lay but not ovulation rate. The persistency of lay after peak will be determined by the

lengths of the prime sequences, the rate at which sequence lengths of individual hens shorten over time and by the number of pause days. Hence the prediction of sequence length is a logical step in predicting the performance of a flock of laying hens over an entire laying cycle.

One of the advantages of the improved model is that it lends itself to stochasticity. Within a population of birds, individuals of the same age show considerable variation about a mean sequence length, which may be due to variation in the length of the open period for LH release, or variation in follicular dynamics. This variance may be accounted for by using mean values and standard deviations for each of the parameters in the model. Such a population of birds would generate a range of ovulation times, the distribution of which should be unimodal and positively skew in young hens, becoming bimodal with age.

Reproductive senescence in hens manifests as an increase in the intra-sequence ovulation and oviposition intervals with time, as well as an increase in the number of pause days. Emmans and Fisher (1986) suggested that the hen's internal cycle length increased with time from first egg, resulting in a decline in the rates of ovulation and oviposition with age. They suggested that, at the start of the laying period, some hens had the capacity to lay at a rate greater than one egg in 24 hours, but that laying performance of these birds was constrained by the external cycle length. These hens would lay an egg a day for a period of time, until the internal cycle length became longer than the external cycle length, when ovulation rate and rate of lay would begin to decline. The model described in this chapter may reproduce this theory by allocating an initial internal cycle length and a rate of decay to each bird in the flock. This idea will be discussed more fully and utilised in the model in Chapter 5.

As discussed in Chapter 1, the decline in the ovulation rate with advancing age, characterised by shorter sequences, may be due to a change in the circadian rhythm controlling LH release or in the process of follicle maturation, or both. In spite of the lack of direct physiological evidence of such a circadian rhythm in poultry, the mathematical model of the ovulatory cycle satisfactorily accounts for the asynchronous cycles of ovulation and oviposition. Based on current knowledge it seems acceptable that the shorter

sequences commonly produced by older hens are modelled by reducing the amplitude of the curve given by Equation 2.1 (by modifying the parameters λ_1 and λ_2 accordingly) and by delaying the time at which the circadian rhythm is reinitiated (by increasing the value of S_1). Following the work of Turek *et al.* (1995) on rodents, it is plausible that ageing hens experience a loss of responsiveness to the entraining effects of the onset of darkness, which may alter the phase angle of the open period for LH release. Until proven otherwise, the theory of the involvement of a circadian rhythm remains attractive.

Sequence length is also an external indicator of the dynamics of the follicular hierarchy. With advancing age, the rate of follicle maturation is retarded, which results in a longer duration of the rapid growth phase and consequently shorter sequences (Zakaria, 1999a). In the ovulatory model, shorter sequences are produced by reducing b_1 (the mature state parameter) and b_3 (the rate of maturing) in Equation 2.4 and hence increasing the time taken for the follicle to reach an ovulable condition. These changes to the regulator concentration and follicle maturation curves alter the opportunities for intersection of the two functions.

The improved ovulatory model will be used as a starting point for predicting the performance of a flock of laying hens over an entire laying cycle. These developments will be dealt with in Chapter 5.

Chapter 3

MEASUREMENT OF SEQUENCE LENGTH AND TIME OF OVIPOSITION FROM ONSET OF LAY

3.1 Introduction

During the process of developing the ovulation model described in Chapter2, several questions arose. It is not known, for instance, whether the first egg produced by a point-of-lay pullet is always laid early in the morning, or whether it could appear at any time of day. The starting point for the final phase of follicle maturation, as predicted by Equation 2.4, will determine the time of the first ovulation at sexual maturity. The present ovulation model predicts that all first ovulations of the initial sequence occur early in the morning, but this may not be true, in which case the model needs to be adjusted. Since the process of follicle maturation is not linked to a circadian rhythm, it is unlikely that in a population of hens follicle maturation at sexual maturity will commence at roughly the same time of day in all individuals.

A second uncertainty is whether the assumption that a pullet commences her laying cycle with a long sequence is indeed correct. Emmans and Fisher (1986) published a theory which assumes that some hens have the ability to lay more than one egg in 24 hours at onset of lay, but that the laying performance of these birds is constrained by the external cycle length (the 24-hour light:dark cycle). These hens will continue to lay an egg a day, *i.e.* at a rate of lay of one, as long as their internal cycle length is less than or equal to 24 hours. Furthermore, the hen's internal cycle length increases with time from first egg. Once the internal cycle length exceeds the external cycle length, ovulation and oviposition rates will start to decline. Figure 3.1 shows the rates of lay for two individuals, one that lays an egg a day from first egg for 79 days and the other for 33 days. Since the intention is to incorporate this theory into the ovulation model so that both ovulation rate and rate of lay may decline with advancing age, the principles need to be validated; in particular since these contrast with the findings of Lewis and Perry (1991) (refer Chapter 1, section 1.16), *i.e.* that hens often start by producing short egg sequences.

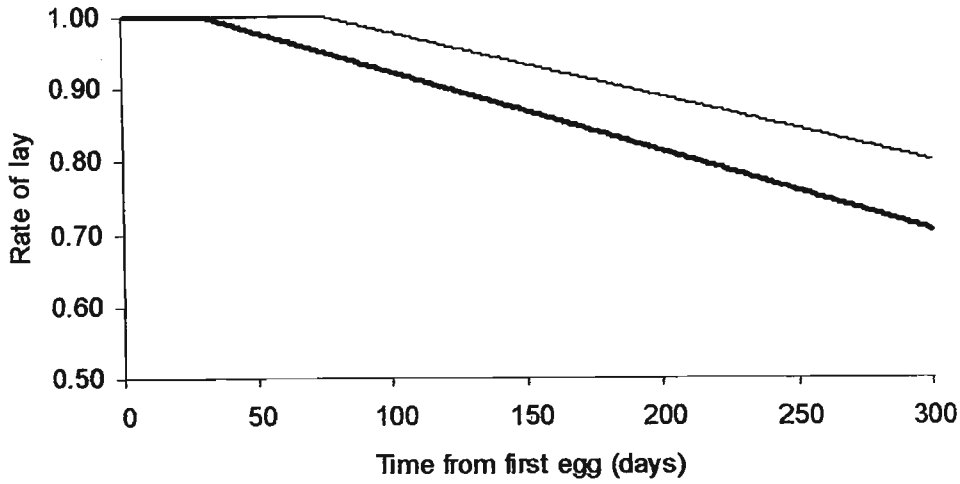


Figure 3.1: The rates of lay from first egg for two hens; one laying an egg a day for 79 days (solid line) and the other an egg a day for 33 consecutive days (bold line), before their rates of lay start to decline

It is of interest to study the relative frequency of internal ovulations amongst individuals in a flock of hens. Many poultry scientists have put forward estimates of the percentages of internal ovulations that occur during different stages of a laying cycle, but their distribution within the population remains unknown. An intensive investigation with a fairly large number of birds may provide some answers as to how many individuals are prone to lay internally and whether this occurs regularly or on an *ad hoc* basis. It seems likely that age at photostimulation plays a role if early onset of sexual maturity increases the probability that there will be some asynchrony between ovary and oviduct. In this case subjecting groups of pullets to different ages at photostimulation may provide some evidence of whether the incidence if internal laying may be influenced by rearing treatment.

Because double-yolked eggs are prevalent at the onset of lay and their production is thought to be influenced by the age at sexual maturity (refer Chapter 1, section 1.7), it is convenient to observe their frequency and distribution within a population in this trial. In addition, if the two yolk weights are recorded it may assist in understanding the functioning of the follicle hierarchy, *i.e.* whether the double yolks are due to the simultaneous ovulation of two follicles

developing together or a day apart. Similarly the frequency and distribution of soft-shelled eggs may be observed. The presence of a soft-shelled egg may be recorded on a routine basis without any extra effort or additional loss of income, as is the case when abnormally large eggs are broken open to confirm the existence of a double yolk.

Age at sexual maturity has been found by Robinson *et al.* (1996b) to influence both the mean sequence length and the length of the prime sequence (refer Chapter 1, section 1.18). Birds that are photostimulated earlier tend to lay shorter sequences. These findings may be confirmed or disproved by imposing different lighting regimes on experimental pullets.

A great deal of useful information may be gathered by housing laying hens one to a cage and recording daily egg production. Firstly, observations of sequence characteristics (such as the length of the prime sequence, the number of pause days and the number of sequences) for each hen and the changes therein over time are made possible. Secondly, the extent of the variation within a flock becomes apparent. In a commercial flock laying at a rate of lay of 80%, one is inclined to assume that all hens are producing four-egg sequences. On the other hand, it may be that most of the birds are laying an egg a day for weeks at a time and yet a small number have gone out of lay altogether. These factors are masked in large commercial enterprises where laying hens are housed four or more to a cage. However, in order to build a realistic population model the extent of the variation between individuals needs to be recognised.

Also of interest is the consistency of lay of an individual bird, both in terms of the time of oviposition and in the sequence length. It would be much easier to model the egg production of a hen laying at the same time every day because ovulation must then take place every 24 hours and the oviducal term can safely be assumed to be close to 24 hours. Similarly if the lengths of egg sequences decline in a systematic manner after peak, the application of the theory of the ovulatory cycle is straightforward. It would be far more difficult, although not impossible, to use the revised ovulation model to simulate egg production if the oviposition times and sequence lengths were erratic.

Much information is available on the weights of the three egg components - yolk, albumen and

shell - from 20 weeks of age. In contrast, little research appears to have been done on measuring the component weights and establishing their proportional relationships when pullets are stimulated at an early age and reach sexual maturity at about 15 weeks of age. Predicting egg weight from yolk weight forms part of this thesis and part of the simulation model; hence the need to record the weights of the three components during the current trial.

The objectives of this trial are therefore to establish:

- the distribution of times of lay of the first eggs produced at sexual maturity
- the mean sequence length in early lay
- the frequency of internal ovulations and their distribution in a flock
- the frequency and distribution within a flock of double-yolked and soft-shelled eggs
- the influence of age at photostimulation on mean sequence length and the length of the prime sequence
- the influence of age at photostimulation on mean age at first egg
- a database of sequence characteristics
- the extent of variation between individuals in laying performance
- the consistency in laying performance of individual hens
- a database of egg component weights at onset of lay

3.2 Materials and methods

3.2.1 Rearing procedures

1152 Hy-Line Brown and 1152 Hy-Line Silver Brown (referred to as Hy-Line Silver in this thesis for convenience) day-old pullets, hatched on Thursday, 19 September 2002, were housed in a light-tight, forced ventilation pullet rearing facility containing six rooms. The dimensions of each room were 4.9m x 3.7m. Each room was equipped with two separate three-tier cage systems; one to the left of the door (containing the Hy-Line Silver birds) and one to the right (containing the Hy-Line Brown birds). The cages measured approximately 1m in length and 0.54m in width. Each cage was supplied with four water nipples and a 1m feed trough. A ventilation sock carried hot air down the passage and the air was drawn into each room by a fan

above the door. During the initial brooding period only four of the rooms were used, because the remaining two rooms were occupied by hens from a prior trial. At five weeks of age the pullets were redistributed and housed sixteen to a cage, using 24 cages per room and all six rooms.

The pullets were weighed in their delivery boxes before housing on day one and again at four, six and eight weeks of age. For these early body weight measurements, all the birds from a cage were weighed together in crates. A random sample of six cages per room (three of each strain) was selected and marked (from six weeks onwards) for subsequent weighing. The mean body weight was calculated from the total weight (minus the weight of the crates) divided by the pullet number. At eleven weeks of age, the birds from the six cages (three per strain) in each of the rooms were weighed individually in order to assess uniformity. Following photostimulation of treatment one (rooms one and four), once the birds had been housed singly per cage, all the pullets in these two rooms were weighed individually on a weekly basis from thirteen weeks, until the treatment had reached sexual maturity (*i.e.* 50% production). Thereafter, only birds not in lay were weighed weekly until the week after they had produced their first egg.

At 15 weeks, the birds from two cages (one per strain) from treatments two and three (rooms two and five, and three and six respectively) were weighed individually. At 16 and 17 weeks of age, the weekly weight recordings included all 48 pullets from each of rooms two and five (following their photostimulation and separation into individual cages), as well as four cages (two per strain) from rooms three and six. From 18 to 21 weeks all 48 pullets per room were weighed individually until the treatment had reached sexual maturity. As with treatment one, birds not in lay were then weighed weekly until an egg had been produced.

The birds were fed a commercial Pullet Starter meal for the first six weeks, followed by a Pullet Grower ration from six to twelve weeks and a Pullet Developer feed thereafter. The composition of the diets is shown in Table 3.1. Two batches of the Starter and of the Developer were used during the trial and both are shown in the table. At all times the birds were given *ad libitum* access to feed.

On housing, 23 hours of continuous light was provided for two days. This was reduced to eighteen hours for days three and four and thereafter decreased daily to fourteen, twelve and ten hours. From day eight onwards, a constant eight-hour daylength was maintained until photostimulation.

A routine vaccination programme, recommended for the KwaZulu-Natal province, was followed (refer Appendix 3.1). The birds were beak trimmed at fourteen days of age.

3.2.2 Rearing treatments

The two varieties of Hy-Line constituted two treatments; therefore all measurements and recordings were done separately for the Brown and Silver birds.

Photostimulation was accomplished by an abrupt increase in daylength, from eight to fourteen hours, at three different ages (Figure 3.2). Each light treatment was replicated in two rooms. The birds from treatment one (rooms one and four) were photostimulated at twelve weeks of age; treatment two (rooms two and five) at fifteen weeks and treatment three (rooms three and six) at eighteen weeks (the usual age recommended for the Hy-Line strain). One week after an increase in the daylength, two birds per cage were randomly selected to remain behind for the layer trial and the surplus pullets were moved out of the pullet rearing facility into an open commercial laying house. At this stage the cages in the pullet house were divided in half by the insertion of a wire cage partition.

This method worked well for treatments one and two, but because one or two hens from room three (treatment three) came into lay spontaneously a few days prior to photostimulation, it was necessary to cage the experimental birds individually, by removing the surplus pullets, on the day that the lighting treatment was applied and not one week later. Careful monitoring was done during the subsequent days to ensure that none of the hens selected for the trial had already commenced laying.

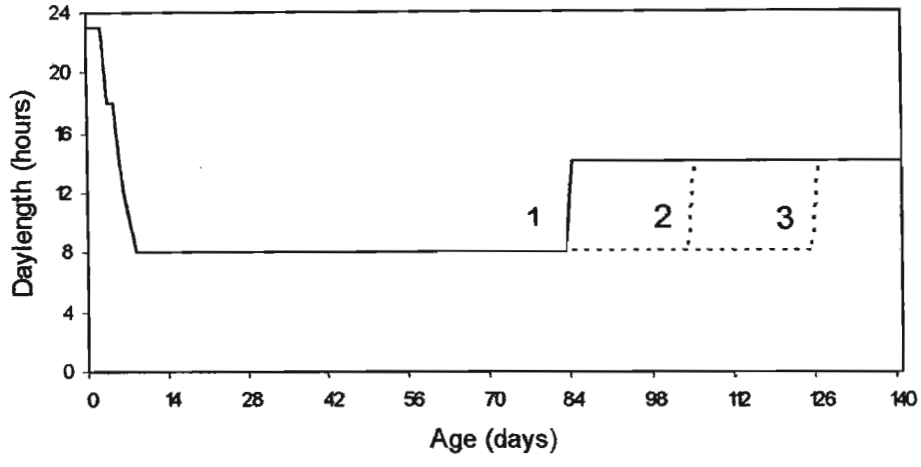


Figure 3.2: The lighting programme applied during rearing, showing the initial step-down period, the constant eight hours given during the growing phase and the step-up to 14 hours at either 12 weeks (treatment one), 15 weeks (treatment two) or 18 weeks (treatment three)

Table 3.1: The raw material compositions (%) and calculated analyses (%) of the commercial starter, grower and developer diets

Raw material	Starter	Starter	Grower	Developer	Developer
Yellow maize	46.653	46.631	46.937	43.113	27.588
Wheat middlings	7.867	7.033	20.000	20.000	20.000
Soybean oilcake	19.500	20.367	6.233		
Sunflower oilcake	10.000	10.000	12.000	11.567	12.622
Sorghum	10.000	10.000			15.000
Maize germ			9.033	19.733	18.810
Limestone	1.600	1.600	1.667	1.733	1.833
Mono dical. phosphate	1.027	1.027	0.667	0.493	0.547
Salt	0.300	0.300	0.333	0.300	0.334
Vitamin/mineral premix	0.150	0.150	0.150	0.150	0.150

Raw material	Starter	Starter	Grower	Developer	Developer
Sodium bicarbonate	0.100	0.100	0.100	0.100	0.100
DL methionine	0.100	0.103	0.077	0.037	0.041
Lysine HCL	0.113	0.100	0.210	0.183	0.185
Molasses	2.500	2.500	2.500	2.500	2.500
Choline chloride	0.040	0.040	0.040	0.040	0.040
Phytase	0.050	0.050	0.050	0.050	0.050
Specifications					
Dry matter	88.07	88.02	87.95	87.93	87.81
Crude protein	19.00	19.00	16.00	13.00	13.00
Available lysine	0.82	0.82	0.64	0.46	0.46
Available methionine	0.37	0.37	0.32	0.24	0.24
AME (MJ/kg)	11.75	11.75	11.40	11.30	11.30
Fat	3.02	3.01	3.41	3.54	3.95
Fibre	5.29	5.23	6.54	6.80	6.88
Calcium	0.90	0.90	0.85	0.85	0.85
Total phosphorus	0.73	0.73	0.71	0.70	0.70
Available phosphorus	0.40	0.40	0.37	0.35	0.35
Sodium	0.16	0.16	0.16	0.16	0.16

3.2.3 Laying protocol

The birds from each treatment were fed Pullet Developer until 5-10% of them had come into lay. Thereafter the hens were given *ad libitum* access to a commercial Layer Mash 96. The raw material composition and calculated analysis of the diet is shown in Table 3.2.

The lights in each of the six rooms came on at 07:30 and went off at 21:30, giving a 14L:10D cycle.

From Thursday, 2 January 2003, *i.e.* at 15 weeks of age, rooms one and four of Treatment one were closely monitored for daily egg production and time of lay, to the nearest half hour. This was accomplished by checking the two rooms every half hour, from 07:00 to 16:00 during the week or 07:00 to 15:00 at weekends. A torch was used to locate eggs at 07:00 and was also useful for collection from the bottom tier of cages, where the light intensity was low. During the two early collections eggs that were warm were noted as such, so that they could be recorded as having definitely been laid that day. A final check was done at about 21:00 each evening by the security personnel. Any eggs that had been produced were marked with a black permanent pen with the room number, the hen number, the date and time of collection. The eggs were then weighed and all the relevant information was recorded on data sheets. Soft-shelled or shell-less eggs, often broken in the cage by the hen's movements, were removed and recorded.

From Thursday, 9 January, when the pullets were 113 days old, rooms two and five (Treatment two) were also monitored every half an hour for egg production. This commenced one week after photostimulation and on the day that the surplus pullets were removed from the facility. The birds from Treatment three, *i.e.* rooms three and six, were included in the routine collections from Thursday, 23 January; the day of photostimulation and transfer of surplus pullets.

Table 3.2: The raw material compositions (%) and calculated analyses (%) of the commercial layer diets fed during the trial

Raw material	Layer 96	Layer 110
Yellow maize	58.660	47.00
Maize germ		5.30
Wheat middlings		16.75
Molasses	2.500	2.00
Full fat soya	4.772	7.50
Soya oilcake	14.352	1.45
Sunflower o/c	7.238	10.00
Limestone flour	10.777	8.65
Monocalcium Phosphate		0.57
Mono Dicalcium Phosphate	0.879	
Salt	0.322	0.43
dl Methionine	0.123	0.07
Lysine HCl	0.031	0.13
Vitamin/mineral premix	0.15	0.16
Sodium bicarbonate	0.100	
Phytase	0.050	
Choline chloride 75	0.046	

Specifications	Layer 96	Layer 110
Dry matter	88.50	88.00
Crude protein	16.00	14.55
Available lysine	0.66	0.626
Available methionine	0.34	0.306
ME (MJ/kg)	11.50	11.46
Fat	3.45	4.60
Fibre	3.75	5.15
Calcium	4.17	3.594
Total phosphorus	0.57	0.56
Available phosphorus	0.375	0.311
Sodium	0.16	0.191

During the first week of production for each of the three treatments, all eggs (apart from cracked, double-yolked and soft-shelled eggs) were subjected to measurements of the weights of the components yolk, albumen and shell. Thereafter, all eggs laid on a Thursday were used for egg component measurements. The procedure used to determine the weights is discussed in detail in Chapter 4. On the remaining days, for the first four weeks of lay for each treatment, all eggs were broken open daily to determine the number of double-yolked eggs.

On Wednesday, 26 February 2003, when the hens were 161 days of age (23 weeks), the first stage of the layer trial terminated. Of the 288 chicks housed at day-old, 285 survived. All birds had been wingbanded prior to this, so that they could be identified according to their room and pen number.

The 248 hens that were producing eggs at an acceptable rate were moved to an open laying house for the second stage of the trial, where they were housed in a two-tier system in

individual cages measuring 300 x 450 mm. There were eight rows of cages; four top and four bottom rows with 32 cages per row. The cage numbering in the layer house was done in a systematic fashion so that four Silver and four Brown hens from room one were followed by four hens of each strain from room two and so on. This meant that four of the pullet rooms were represented in each of the eight rows. One water nipple was shared between two birds in adjacent cages. Each cage had its own separate, removable feed trough with a vertical partition forming a hopper on one side, although feed intake was not monitored. The hens were provided with 14L:10D (with lights on at 05:00 and off at 19:00) and fed *ad libitum* on a commercial Layer 110 (see Table 3.2 for composition). Eggs were collected daily at about 09:30 and recorded for each hen, but time of lay and egg weight were no longer monitored. Stage two continued for six months until Friday, 29 August. Thereafter only 31 birds were monitored until each one had terminated a long sequence (Stage three). The trial stopped on 29 January 2004.

3.2.4 Post mortem procedures

Of the 37 birds identified as poor producers at the end of stage one, 23 were subjected to post mortems. They were transported in crates to the farm abattoir. Each bird was killed by stunning it with an electrical current applied to the head and the jugular vein was then cut, to allow the blood to drain from the carcass. Following this, the bird was immersed in hot water (50-60°C) for three minutes to loosen the feathers before being put into a plucking machine.

The clean carcass was placed on a counter and a vertical incision was made from the sternum to the pelvic area, as well as a horizontal cut along the bottom of the rib cage. The sternum and ribs were then broken and forced upwards to expose the abdominal cavity.

Liver colour and texture were assessed as an indication of the general health of the hen. The oviduct was examined for cysts or tears and for signs of immaturity (either overall or in particular regions), such as small size, reduced length or poor muscle development and vascularisation. Observations were made on the number and size of ripe yellow follicles contained in the ovary and the presence of cysts or post-ovulatory follicles. Any sign of yolk

material in the abdomen, indicating internal ovulation, as well as abnormal fluid retention, was recorded.

3.3 Statistical analyses

The analyses of variance were done using a split-plot design on Genstat Version 6, with the three light treatments as whole plots and the two strains as sub-plots. Blocks one and two were given by rooms one to three and four to six respectively. Initially the analysis for each variate looked for interactions between light and strain but where they were not significant at the 5% level, the analysis was redone determining main effects only.

Where the data were recorded as counts, *e.g.* the number of double-yolked or soft-shelled eggs produced by the birds in each treatment, these were calculated as the mean number per hen per treatment. This had the effect (by the central limit theorem) of transforming non-parametric data, *i.e.* data with no distribution, to parametric data. In this form normal analyses of variance could be performed on the relevant data.

On the advice of Morris (1999), results are reported in the form of tables of means without calculating least significant differences and adding subscripts to indicate significant differences. The magnitude of treatment differences is discussed rather than their statistical significance.

Although the experiment was not designed to be a dose response trial, the nature of the relationships between age at photostimulation and other variables was investigated. Linear regressions were done by fitting a simple linear model in Genstat 6 and using age at photostimulation as the explanatory variate and other variables, such as body weight at first egg or age at first egg, as the response variate.

3.4 Results

3.4.1 Body weights

The mean pullet weights per treatment at four, six and eight weeks of age are shown in Appendix 3.2, while the mean weights at 11, 15, 16, 17, 18 and 19 weeks are summarised in Appendices 3.5, 3.7, 3.9, 3.11, 3.13 and 3.15 . Although the commercial management guide supplied by the hatchery along with the day-old chicks indicated that no difference in body weights between the strains was expected, the Hy-Line Silver pullets were consistently heavier. The means for both strains were below target; most noticeably at six weeks of age. Appendices 3.3, 3.4 and 3.6 show the analyses of variance for mean body weight at six, eight and eleven weeks of age respectively. No statistical analysis was done on the four-week weights since the pullets had not occupied all six rooms during the brooding period. There were no significant interaction effects, nor were the differences in body weight between the three light treatments significant at any of these ages. This was as expected, since photostimulation had not yet commenced. However, the difference between the two strains was shown to be highly significant ($p < 0.01$) at six, eight and eleven weeks of age.

The analyses of variance for mean body weights at 15, 16, 17 and 18 weeks are summarised in Appendices 3.8, 3.10, 3.12 and 3.14 respectively. At no age were there significant interactions between light and strain, so the analyses were redone looking at main effects only. The differences in body weight between the two strains remained highly significant ($p < 0.01$) throughout, with the Hy-Line Silver birds being on average over time between 4.7 and 6.3% heavier than the Brown hens.

The light treatment had a significant ($p < 0.05$) effect on body weight at 15 and 18 weeks and a highly significant ($p < 0.01$) effect at 16 and 17 weeks of age. The pullets that were photostimulated at 12 weeks of age were consistently heavier than those photostimulated at 18 weeks, by an amount ranging from 110 to 132 grams. Initially the difference in mean body weight between the 12- and 15-week photostimulated pullets was about 90 grams, but this difference had disappeared altogether by 18 weeks of age (Figure 3.3). Although feed intake

was not measured during the course of this trial, it is known that increasing daylength stimulates appetite, leading to increased feed intake and faster growth (Lewis *et al.*, 1996). Part of the additional body weight may be attributed to the growth of the ovary and oviduct following photostimulation.

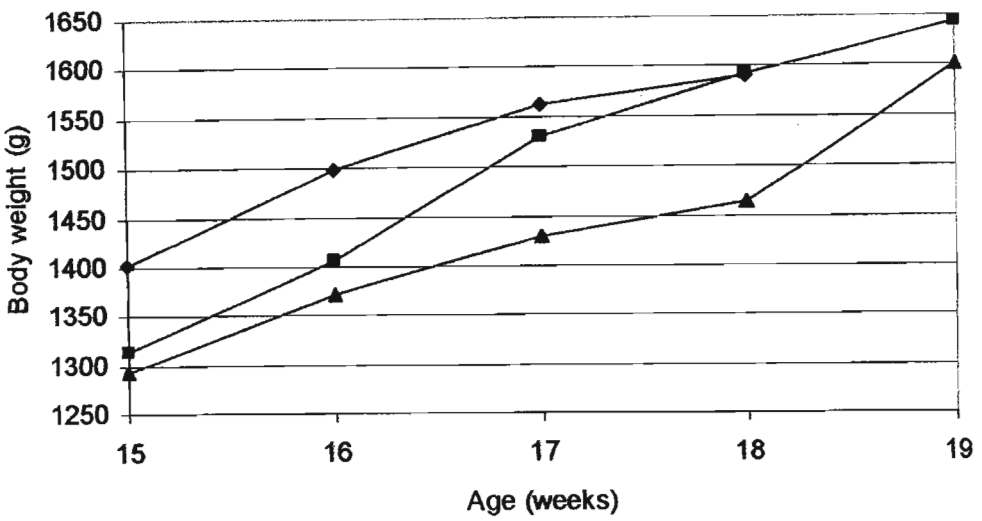


Figure 3.3: Differences in mean body weight for the three treatments photostimulated at 12 (♦), 15 (■) and 18 (▲) weeks

The mean body weights at first egg for the six treatments are shown in Table 3.3. As the age at photostimulation increased the variation appeared to decrease, as indicated by the smaller coefficients of variation.

Of interest is the relationship between age at photostimulation and body weight at first egg, as depicted in Figure 3.4. As the age at photostimulation increased, mean body weight at first egg also increased, although at a decreasing rate. This indicates that photostimulation is a more potent factor in bringing pullets into lay than the attainment of some threshold body weight. Regression analyses (Appendices 3.16 and 3.17) indicated that there were no significant linear relationships between the two variables, for either strain. There were too few levels of the predictor variable to test for curvilinear regressions. As a result, from the data presented here, mean body weight at first egg cannot be predicted from the age at photostimulation. However, the correlations between the variables were strong and positive, with correlation coefficients of 0.88 for the Hy-Line Silver and 0.94 for the Brown hens.

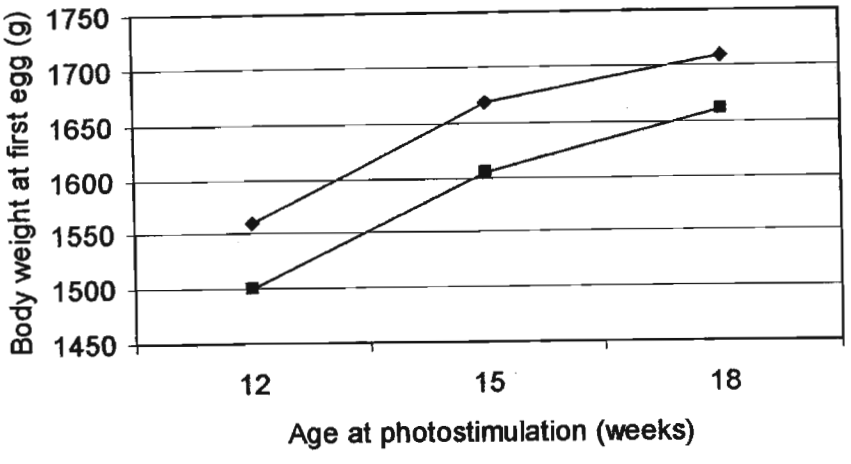


Figure 3.4: The relationship between age at photostimulation and mean body weight at first egg for the Hy-Line Silver (♦) and Hy-Line Brown (■) birds

Table 3.3: Mean body weights at first egg

		Age at photostimulation (weeks)			
		12	15	18	Strain mean
Hy-Line Silver	mean	1560.3	1667.7	1709.8	1647.1
	sd	138.8	144.9	128.3	150.2
	% cv	8.90	8.69	7.50	9.12
Hy-Line Brown	mean	1500.6	1604.2	1661.2	1589.0
	sd	112.4	109.9	104.7	126.7
	% cv	7.49	6.85	6.30	7.98
Light mean	mean	1530.8	1635.6	1686.3	1618.4
	sd	129.2	131.6	119.3	
	% cv	8.44	8.04	7.08	

3.4.2 Age at first egg

Table 3.4 summarises the mean ages at first egg for the treatments. It must be remembered that of the 37 hens excluded from stage two of the trial, 26 had yet to lay an egg. Had these hens been allowed to remain with the experimental birds, some or all of them may have come into lay eventually. Consequently the mean ages at first egg shown here may be slightly earlier than the true population means.

Table 3.4: Mean Ages at First Egg (days)

		Age at photostimulation (weeks)			
		12	15	18	Strain mean
Hy-Line Silver	mean	114.60	126.55	139.20	126.97
	sd	6.47	3.74	5.25	11.35
	% cv	5.6	3.0	3.8	8.94
Hy-Line Brown	mean	116.07	131.31	142.10	129.86
	sd	7.89	6.89	4.97	12.51
	% cv	6.8	5.2	3.5	9.63
Light mean	mean	115.33	128.96	140.60	128.5
	sd	7.20	6.03	5.29	
	% cv	6.24	4.67	3.76	
Interval (days)*		31.40	23.97	14.67	

*Interval between age at photostimulation and mean age at first egg

Appendix 3.18 summarises the analysis of variance for mean age at first egg for main effects only, since the interaction term was not significant. It may be seen that the difference between the strains was significant ($p < 0.05$), with the Hy-Line Silver pullets reaching sexual maturity on average 3.06 days earlier than the Brown birds. These results are not surprising, because the Hy-Line Silver are genetically earlier maturing birds, although not by a large margin. The Silver strain had a slightly lower standard deviation about the mean age at first egg than the Hy-Line Brown hens, which may indicate a better as well as a faster gonadotrophic response to the stimulatory effects of an increasing daylength.

As anticipated, earlier photostimulation advanced the age at sexual maturity and these treatment differences were found to be highly significant ($p < 0.01$). Birds photostimulated at the recommended 18 weeks achieved a mean age at first egg just past the expected 20 weeks of age. The groups given light stimulation three and six weeks earlier, *i.e.* at 15 and 12 weeks, achieved sexual maturity 11.7 and 25.3 days earlier. The interval between photostimulation and first egg decreased as age at photostimulation increased, in line with the findings of Gous *et al.* (2000). Furthermore, the flock uniformity appeared to improve as the age at photostimulation increased, as indicated by the reduced coefficients of variation. Lewis and Perry (1995) found that for the ISA Brown bird, the standard deviation of the mean age at first egg was not constant for all ages. Their equation predicted that the lowest standard deviation occurred when the mean age at first egg was 125 days.

The frequency distributions for age at first egg for the two strains are shown in Figures 3.5 and 3.6. Although there is some overlap between the three light treatments, it is clear that there are three separate distributions, each with their own mean and variance.

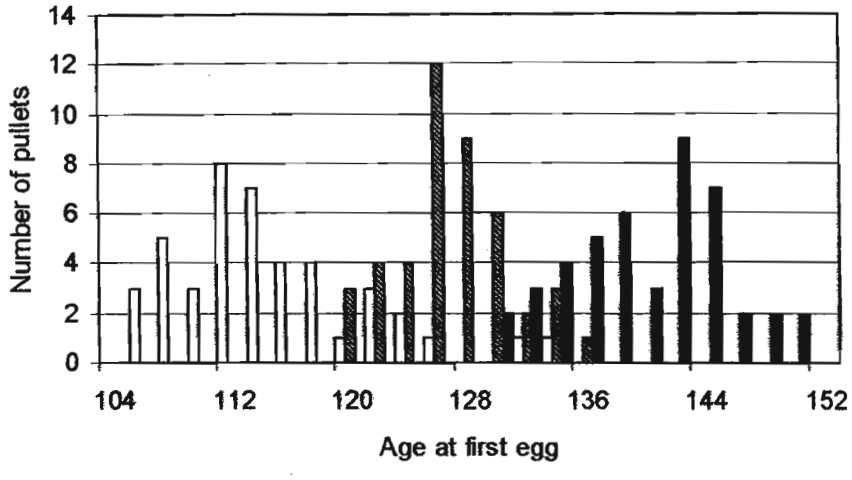


Figure 3.5: Histogram showing the ages at first egg for Hy-Line Silver pullets photostimulated at 12 weeks (blank columns), 15 weeks (hatched columns) and 18 weeks (solid columns)

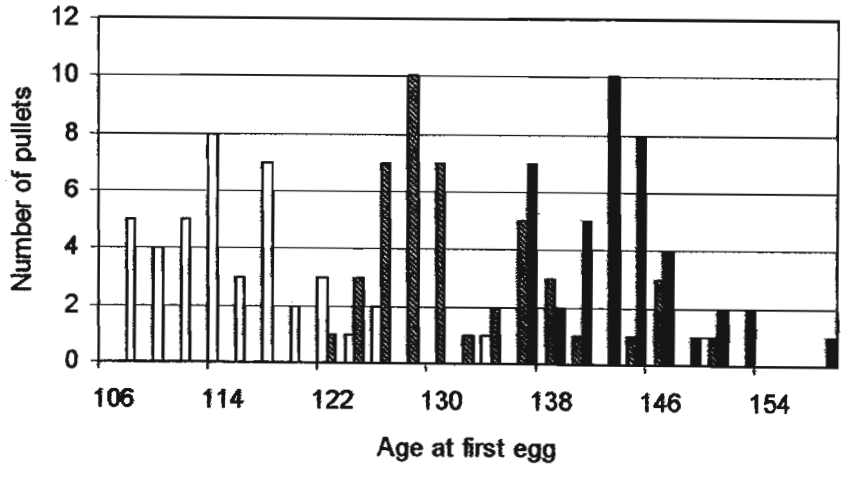


Figure 3.6: Histogram showing the ages at first egg for Hy-Line Brown pullets photostimulated at 12 weeks (blank columns), 15 weeks (hatched columns) and 18 weeks (solid columns)

There was a highly significant ($p < 0.01$) linear relationship between age at photostimulation (x) and mean age at first egg (y) for the Silver strain (Appendix 3.19), which is represented by the following equation that accounts for 99.9% of the variation:

$$y = 65.28 + 4.100\ x \qquad \text{(Equation 3.1)}$$

where y = age at first egg and x = age at photostimulation. This means that advancing light stimulation by one week will advance the age at first egg by 4.1 days, over the range tested. The same linear relationship for the Brown birds was just not significant at the 5% level (Appendix 3.20), although 98.1% of the variation in the data was accounted for. A quadratic function may well have given a better fit, but was not appropriate in this trial where only three predictor variables were used. These relationships are shown in Figure 3.7, along with the fitted linear regression given by the equation. Thus mean age at first egg for the Hy-Line Silver flock may be predicted using Equation 3.1, for ages at photostimulation from 12 to 18 weeks of age.

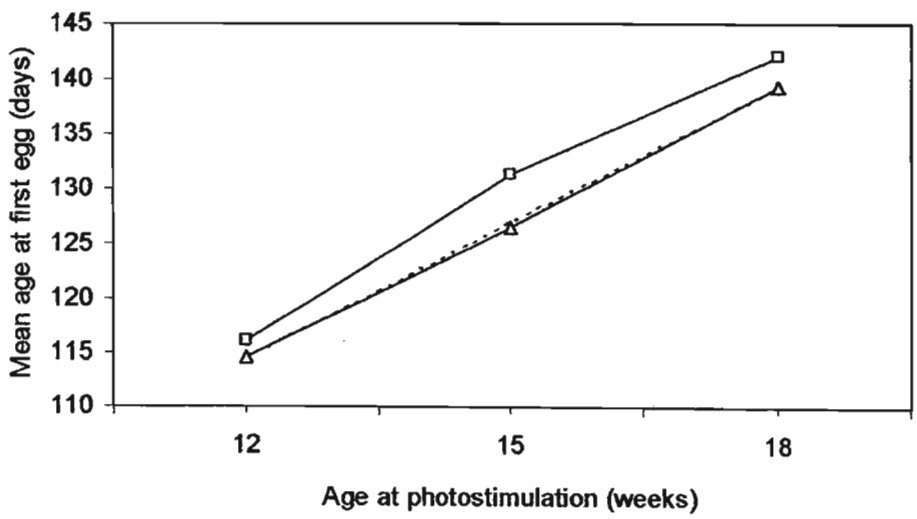


Figure 3.7: The relationship between mean age at first egg and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens. The fitted linear regression for the Silver strain is indicated by the dotted line

Having achieved the desired objective of bringing groups of hens into lay at different ages, it becomes possible to observe whether mean sequence length and the length of the prime

sequence are affected by the mean age at sexual maturity.

3.4.3 Time of lay of first egg

The frequency distribution for the time of lay of first eggs is shown in Figure 3.8. Appendix 3.21 lists the oviposition times of the first eggs for each of the 261 birds that came into lay. The four eggs found during the evenings were grouped together and allocated to 16:30, half an hour after the last routine collection of the day. Their collection times were after all not an accurate reflection of oviposition times. It was felt to be more likely that the ovipositions had taken place soon after the collections stopped, rather than four to five hours later.

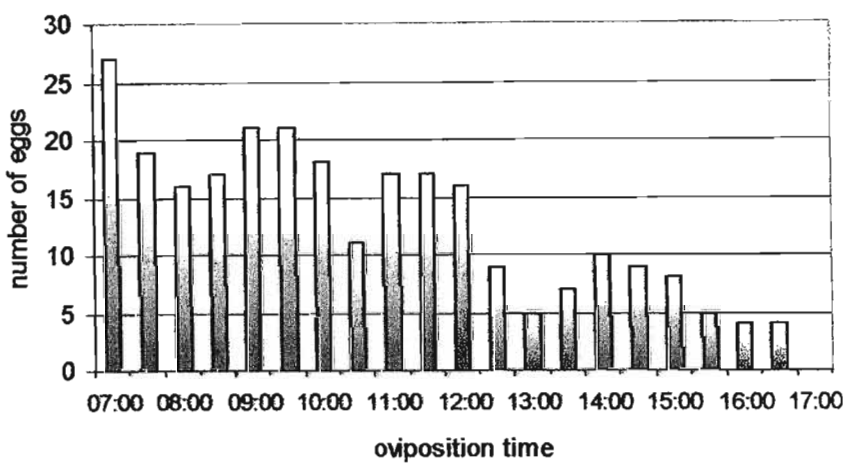


Figure 3.8: Histogram showing the time of lay of first eggs

It must also be borne in mind that the times of day on the x-axis are not indicative of actual times of lay, but rather the start of the various collection periods. In a conventional frequency distribution the class marks are the mid-points of the class intervals and it is these that are plotted along the x-axis. If the second class interval included all eggs collected between 07:30 and 08:00 then the class mark should be 07:45 and yet the eggs were laid during the preceding half hour, i.e. 07:00 to 07:30, in which case the class mark should be 07:15. A difficulty arises because firstly, eggs were collected from different rooms at different times during the half hour. If one person was doing the collections he or she would start in room one on the hour or half hour, working systematically through the rooms to finish in room six. If two people were

involved one would work through rooms one to three and the other, rooms four to six. The second irregularity occurred because, as more birds were photostimulated and as the rate of lay increased, more time was spent in each room. Therefore it was only in room one that egg collections started on the hour or half-hour throughout the trial. For these reasons it was decided to accept that the start of each collection would represent the so-called class mark.

The mean time of lay was 10:28, almost 13 hours after the onset of darkness. The median time of lay was 10:00. The modal frequency was in the first class, *i.e.* 07:00, although this would not have been the case if recordings had commenced at 06:30 instead of 07:00. The chart clearly shows an unnatural truncation at 7:00. Though this represents some loss of information about the true nature of the distribution and the population mean, for the purpose of modelling egg production at onset of lay, this loss is not critical. It is clear that the first egg produced by a hen at sexual maturity is more likely to be laid by midday, because 76.6% of the eggs were collected before 12:30, but that first eggs are also laid during the afternoon. Times of lay of first eggs are likely to be normally distributed. (T. Morris, personal communication [1])

The mean times of lay of the first eggs are shown in Table 3.5. No analysis of variance was done on the means because the times of lay were not recorded before 07:00. There does appear to be a slight trend, *i.e.* that the Silver strain laid their first eggs slightly earlier than the Brown birds and secondly, that the birds achieving earlier sexual maturity had an earlier mean time of lay of first eggs. However, this observation can only be confirmed by experiments where times of lay are recorded throughout the 24-hour period.

1 T.R.Morris, Dept. of Agriculture, University of Reading, Earley Gate, Reading, RG6 2AT, U.K.

Table 3.5: Mean times of lay (±sd) of first eggs at sexual maturity

	Age at photostimulation		
Strain	12 weeks	15 weeks	18 weeks
Hy-Line Silver	10:15 (±2h44m)	10:25 (±2h24m)	10:36 (±2h29m)
Hy-Line Brown	10:18 (±2h49m)	10:28 (±2h38m)	10:44 (±2h33m)

3.4.4 Exclusion of poor producers

The number of birds that could be transferred to the layer house at 23 weeks, in order to continue monitoring their pattern of lay, was limited by the fact that there are only 256 individual cages in that house. This necessitated omitting a number of hens, since of the original 288 pullets housed separately following photostimulation there were three mortalities and 285 survivors.

Thirty seven birds that had failed to come into lay by 23 weeks (161 days) of age, or that had laid fewer than ten eggs, were either sent to the farm abattoir for post mortems or placed with the commercial hens. Table 3.6 summarises the number of eggs laid by these hens per strain and light treatment. It may be seen that 18 of the Silver strain and 19 of the Brown strain were culled, indicating that both strains are equally likely to harbour poor producers. Similarly, fifteen hens belonged to the 12-week photostimulation treatment, ten to the 15-week and twelve to the 18-week photostimulation groups, which suggests that early light stimulation did not adversely affect attainment of sexual maturity or early egg production.

It is unlikely that any of the ten non-productive birds culled from the 12-week photostimulation treatment would have come into lay had they been given an extended life. Seventy-seven days had elapsed since the daylength was increased. Moreover, the mean age at first egg for the

productive hens was between 16 and 17 weeks. In contrast, it could be argued that some of the nine non-laying hens from the 18-week photostimulation treatment may have started laying eventually, since only 35 days separated the stimulatory increase in daylength and the end of stage one of the trial. It would have been interesting to continue monitoring these hens were it not for the constraints imposed by the available facilities.

The number of hens achieving sexual maturity but subsequently laying very poorly was surprising and difficult to understand. These may have been due to some internal condition, such as a torn oviduct or repeated internal ovulations. A study of the post mortem results may clarify the situation.

Table 3.6: The number of hens laying 0 to 9 eggs during stage one

				Number of hens producing:			
Age at photostim. (weeks)	Time interval *	Mean AFE	Strain	0 eggs	1-3 eggs	4-6 eggs	7-9 eggs
12	77	114.60	HS	4	3		
(84 days)		116.07	HB	6	0	2	
15	56	126.55	HS	4	2		
(105 days)		131.31	HB	3	1		
18	35	139.20	HS	3	1		1
(126 days)		142.10	HB	6	1		
Total				26	8	2	1

* Time interval (in days) from photostimulation to end of stage one, *i.e.* 161 days of age.

Both the hens not in lay and the poor producers exhibited normal wattle and comb

development. There were two hens that weighed over 2.5 kg at 21 weeks of age and had swollen abdomens, indicating some fluid retention. Apart from this, it would have been impossible to identify the unproductive hens based on their physical appearance and without looking at the production records.

3.4.5 Post mortem results

Appendix 3.22 shows the detailed post mortem results, while Table 3.7 gives a summary of the findings. Age at photostimulation did not appear to influence the growth and development of the reproductive tract, since abnormalities were found in the unproductive birds from all three light treatments. Although it would appear that the Hy-Line Brown birds were more likely to have abnormal or juvenile ovaries and oviducts, it must be remembered that of the 23 birds selected for post mortems, fourteen were Hy-Line Brown and nine were Hy-Line Silver.

Table 3.7: Summary of post mortem results

Number of hens					
Age at photostimulation	Strain	Normal organs	Abnormal ovary	Abnormal oviduct	Total
12	HS			3	3
	HB		1	3	4
15	HS	1	1	2	4
	HB			3	3
18	HS		2*	1	2
	HB		1	6	7
Total		1	5	18	23

* one hen had an abnormal ovary and oviduct

What is evident is that 78% of the poor performances were due to some problem with the oviduct; either reduced length, poor muscle development or poor vascularisation in specific regions, the presence of cysts or tears, or prolapses. In many instances ovulation was proceeding normally but the ova were ovulated internally, as indicated by the presence of yolk in the abdomen. The underdeveloped infundibulum appeared to be unable to grasp the ovum on its release from the follicle. Although the cause of this irregular oviduct development was not diagnosed, it is of interest to note that the Infectious Bronchitis virus can prevent the development of the oviduct in pullets, either partially or completely, so that the bird will lay few, if any, eggs during her adult life (Sainsbury, 2000). This leads to speculation about the role of the vaccines in challenging the immune system and in so doing, perhaps inadvertently affecting reproductive development. However, without any clinical signs of a disease outbreak, this is unlikely to happen. (Dr. R. Nixon, personal communication [2])

It is not known to what extent the poor performance of a proportion of hens is due to genetic factors, such as the sex-linked Restricted Ovulator gene. This is a recessive gene found on the Z chromosome (Stevens, 1991). Males (the homogametic sex, with two Z sex chromosomes) can thus be carriers without expressing the trait, but females (the heterogametic sex, with a Z and a W chromosome) that have the gene will give rise to a phenotype that lays few or no eggs. However, since both strains had these poor producers and since the breeding stock used to produce the commercial pullets is different, the cause is unlikely to be genetic. (O. Horstmann, Hy-Line, personal communication [3])

In order to make a proper diagnosis of the causes of liver disease, tissues would have to be submitted to a laboratory for histopathology or bacteriology. The changes in colour and texture merely indicate a liver in poor health.

3.4.6 Number and distribution of double-yolked eggs

As anticipated, the percentage of double-yolked eggs was influenced by the age at sexual maturity, as determined by the age at photostimulation (Table 3.8). Those birds that received

2 Dr R. Nixon, P.O.Box 117, Hilton, 3245, South Africa

an increase in daylength at 12 weeks laid more than double the number of double-yolked eggs than their counterparts. In a commercial environment these eggs would be marketed at a premium price in South Africa and there would be no loss of income unless a double-yolked egg was replacing the production of two single-yolked eggs. In practice pullets are given increasing daylength at the age recommended by the breeder and so the situation is avoided. An analysis of variance was done on the mean number of double yolks laid per hen (shown in Table 3.9). As expected, the difference between strains was not significant. However, the difference between light treatments was significant ($p < 0.05$). The statistical summary is given in Appendix 3.23.

Table 3.8: Total number of double-yolked eggs produced during the first four weeks of lay, also shown as a percentage of the total eggs laid by each treatment during this period

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Strain total
Hy-Line Silver	60 (7.7%)	17 (2.0%)	16 (1.7%)	93
Hy-Line Brown	53 (8.1%)	30 (4.4%)	11 (1.5%)	94
Light total	113	47	27	187

Table 3.9: Mean number of double-yolked eggs produced per hen during the first four weeks of lay

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Strain mean
Hy-Line Silver	1.250	0.354	0.333	0.646
Hy-Line Brown	1.104	0.625	0.229	0.653
Light mean	1.177	0.490	0.281	

3 O.Horstmann, Hy-Line, P.O.Box 101, Halfway House, 1685, South Africa

The linear regression of mean number of double yolks laid per hen on age at photostimulation was not significant for the Hy-Line Silver hens (Appendix 3.24). However, a significant linear regression was found for the Brown strain ($p < 0.05$), which is represented by the equation:

$$y = 2.840 - 0.14583\ x \qquad \text{(Equation 3.2)}$$

where y = number of double yolks and x = age at photostimulation, in weeks. A reduction in the mean number of double-yolked eggs of 0.14583 can be expected for each week photostimulation is delayed. The statistical summary is shown in Appendix 3.25. The trends are shown in Figure 3.9, along with the linear regression given by the equation. Over a wider range of ages at photostimulation, or with more levels of the predictor variable, the response is likely to be curvilinear so that the mean number of double yolks per hen declines at a decreasing rate as the age at photostimulation increases.

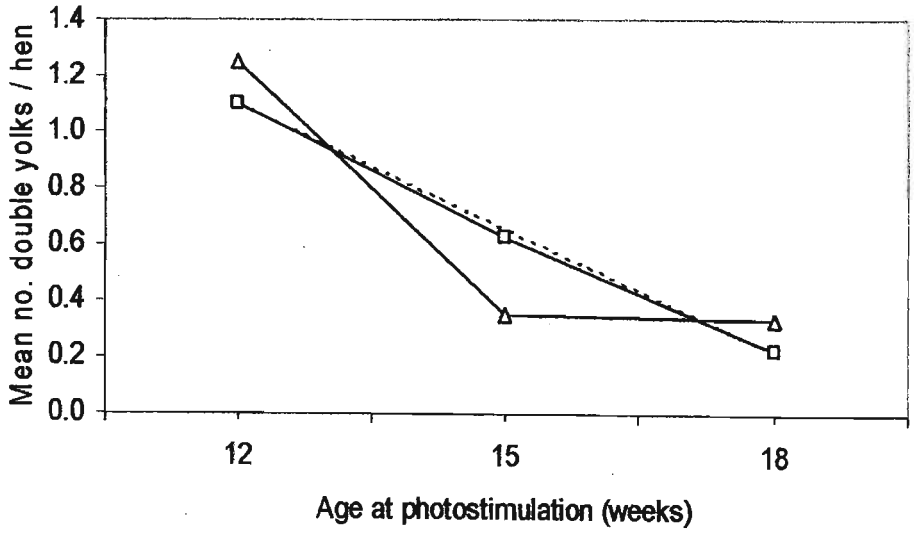


Figure 3.9: The relationship between the mean number of double-yolked eggs per hen and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens, with the fitted linear regression for the Brown strain (dotted line)

The production of multiple-yolked eggs per hen is shown in detail in Appendices 3.26 to 3.34, for the first four weeks of lay for each of the six rooms. The eggs were classified according to whether they were laid in the middle of a sequence without causing interruption (dy); as the

first egg laid by a hen at onset of sexual maturity (f); as the terminal egg of a sequence, including one-egg sequences (t); as a soft-shelled egg (s); or whether they were associated with a pause in mid-sequence, either before or after the double-yolked egg (p). Triple-yolked eggs were also noted (ty). The information is summarised per light treatment in Table 3.10.

Table 3.10: Analysis of the position in the sequence of double-yolked eggs

	Age at photostimulation			
Double yolks	12 weeks	15 weeks	18 weeks	Total
Uninterrupted (dy)	83 (73%)	32 (68%)	20 (74%)	135 (72.2%)
First egg (f)	3	4	1	8 (4.3%)
Terminal egg (t)	8	3	3	14 (7.5%)
Initial one-egg sequence (f,t)	6	4	1	11 (5.9%)
First egg associated with pause (f,p)	1			1
Associated with pause (p)	10	4	2	16 (8.6%)
Triple yolk, uninterrupted (ty)	1			1
Triple yolk at first egg (f,ty)	1			1
Total	113	47	27	187

72 % of double-yolked eggs occurred in the middle of normal sequences, without causing interruptions. This would indicate that two follicles of the same size were maturing simultaneously. Some hens appeared to have multiple hierarchies at the onset of lay; hen numbers 23 and 30 from room one, for example, laid four and six mid-sequence double-yolked eggs respectively. From room four, hen 21 produced six double-yolked eggs while hen 27 laid four eggs with double yolks and one triple-yolked egg. These all belonged to the 12-week photostimulation treatment. The weights of the yolks from multiple-yolked eggs, where egg component analyses were done, are summarised in Appendix 3.35. Two possibilities exist:

either two F_1 follicles ovulated simultaneously, in which case the two yolks may be expected to be of similar size, or an F_1 and one of a pair of F_2 follicles ovulated together, in which case the yolks would be noticeably different in weight. Examples of both options for uninterrupted sequences (dy) may be seen in Appendix 3.35.

9% of the eggs (including a first egg) were associated with a pause in the sequence, either on the day immediately before or after the double-yolked egg. From the times of oviposition it was obvious that these did not herald the end of a sequence, as the following example of the laying pattern of hen five (room one) shows:

Date	11/1	12/1	13/1	14/1	15/1	16/1	17/1	18/1...
Time of lay	07:00	07:30	07:00	07:00 (dy)	(pause)	07:30	07:00	07:00..

What probably happened here was two follicles, developing a day apart, for some reason ovulated simultaneously thereby leaving a gap in the hierarchy. In other words, the F_1 and F_2 follicles ovulated together on 13/1 so that there was no F_2 follicle to enter the final stage of follicular maturation for the subsequent day's ovulation and hence no egg for oviposition on 15/1. One would expect the two yolks to be noticeably different in size or weight. The interrupted sequence of hen 48 (room three),

Date	19/2	20/2	21/2	22/2	23/2	24/2	25/2	26/2
Time of lay	07:00	07:00	08:00	(pause)	09:00 (dy)	09:00	09:30	09:30

where the pause occurred before the production of the double-yolked egg, may be an example of a follicle ovulating internally (on 21/2) and the ovum being picked up on the following day along with the subsequent ovum. In this case the two yolks would probably be of similar size, both having come from F_1 follicles. Two good examples of this are presented by hens 15 and 38 (both from room four), where the yolk weights were 6.99 and 7.09, and 5.20 and 5.22

respectively (see Appendix 3.34). Their sequences are not shown here because their laying patterns at onset were erratic, with the production of soft shells and more than one pause day, although the double-yolked eggs were clearly preceded by pauses in the sequences.

Since a reasonably large number of double-yolked eggs were associated with pauses, it would be unwise to assume that internal ovulations had taken place on those pause days.

Nine of the double-yolked eggs and one triple-yolked egg were the first eggs produced by hens at sexual maturity. These were followed mostly by regular sequences, although in two cases the laying patterns were erratic, including soft shells and incidences of internal laying. Three of the hens involved laid only the one double-yolked egg, so a once-off double ovulation occurred at sexual maturity. In contrast, seven birds continued to produce a number of double yolks, which indicated imperfectly maintained hierarchies. Rate of lay was unaffected by the appearance of these multiple-yolked eggs.

There were a further eleven double-yolked eggs that formed one-egg sequences at the onset of lay, *i.e.* they were both the first egg to be laid by the point-of-lay pullet and the terminal egg of that sequence. These were without exception laid in the afternoon, between 13:30 to 15:30 and were followed after a pause day with an oviposition early in the morning. Three of these shown in Appendix 3.34 show large differences of over one gram in the yolk weights, which may mean that F_1 and F_2 follicles were involved.

Thirteen double-yolked eggs formed the terminal eggs of sequences, being laid either in the late morning (two eggs) or well after 12:00 and being followed after a pause day by an oviposition early in the morning. The possibility arises that some of these sequences were unnaturally terminated by a double ovulation. For instance, hen 26 (room five) laid a number of consecutive eggs, as follows:

Date	..23/1	24/1	25/1	26/1	27/1	28/1	29/1	30/1
Time of lay	..11:30	10:30	09:00	10:00 (dy)	10:30	14:30 (dy)	(pause)	07:00

If a double ovulation occurred at about 11:00 on 27/1, followed by the double-yolked egg and no subsequent ovulation on 28/1, hence the pause day on 29/1, this may be an example of an F_1 and F_2 follicle ovulating simultaneously. The F_3 follicle effectively becomes the F_1 follicle on 28/1, half a day earlier than expected and proceeds with its maturation process, to be ready to ovulate at the start of the open period for LH release on 29/1. The delayed oviposition time of the double-yolked egg may be explained by the lack of stimulus due to the absence of an F_1 follicle approaching ovulation on 28/1. The work of Kelly *et al.* (1990) confirms that oviposition may be delayed by two hours if both F_1 and F_2 follicles are not able to secrete prostaglandins. If this explanation is acceptable, then the measurement of sequence length can be affected negatively by double-yolked eggs that appear to terminate sequences prematurely. Without accompanying oviposition times, this would not be evident.

The distributions of multiple-yolked eggs for the first four weeks of lay for the Hy-Line Silver and Brown hens are shown in Figures 3.10 and 3.11 respectively. For both varieties, the individuals that produced four or more double yolks were all from the 12-week photostimulation group. Early photostimulation, leading to early onset of sexual maturity, may therefore be linked to poor control of the follicular hierarchy.

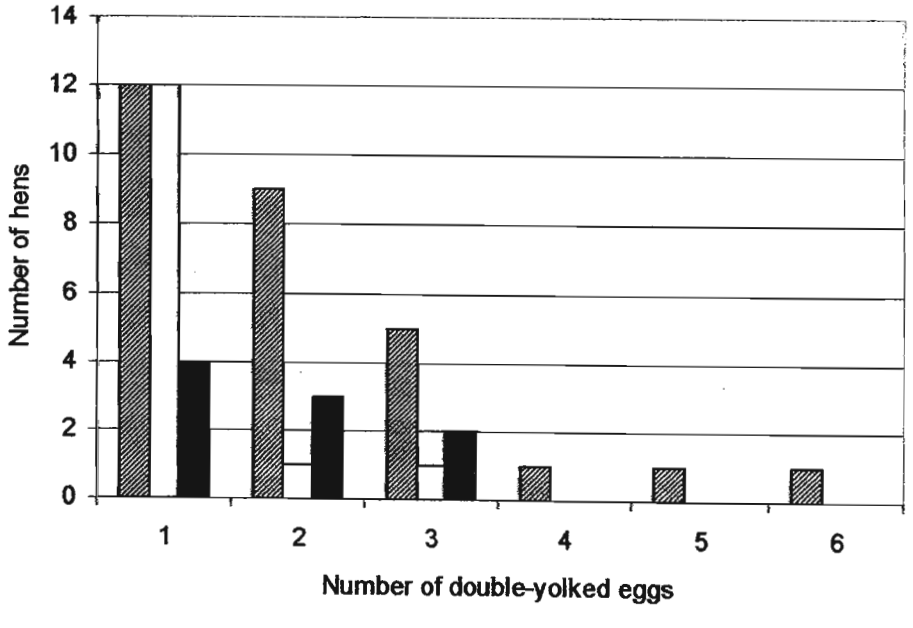


Figure 3.10: Histogram showing the number of double-yolked eggs produced during the first four weeks of lay by the Hy-Line Silver hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

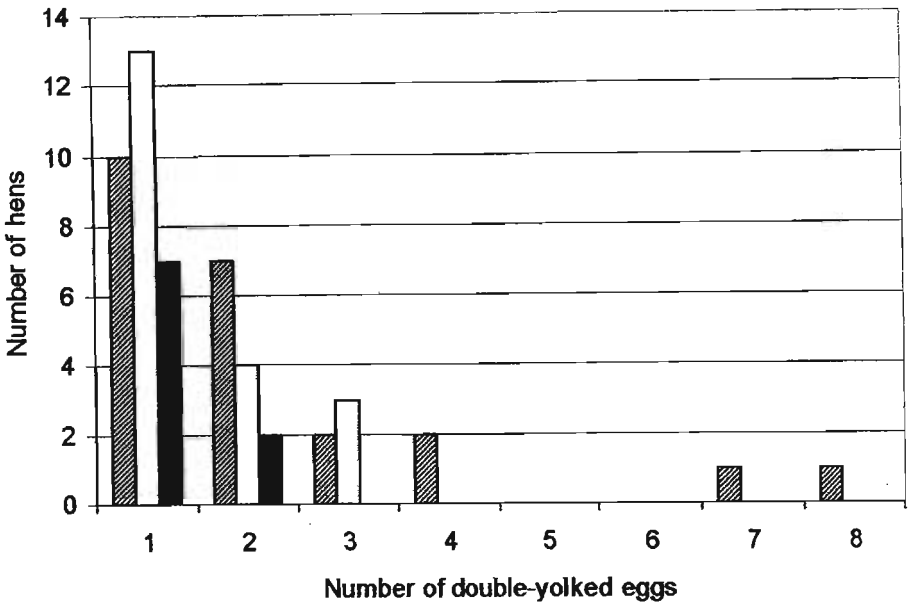


Figure 3.11: Histogram showing the number of double-yolked eggs produced during the first four weeks of lay by the Hy-Line Brown hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

The frequencies of multiple yolks per age at photostimulation treatment are summarised in Table 3.11. The double yolks were produced by 104 of the 288 birds housed, representing 36% of the flock, but 58 hens laid only one double-yolked egg during the first four weeks of lay. This represents 20.1% of the total number of hens housed, or 55.8% of the birds laying multiple-yolked eggs. Thus a large proportion of double-yolked eggs were simply due to random events; one hiccup in the usually smooth control of the follicular hierarchy which led to a single double ovulation. According to the information presented here, very few laying hens have multiple hierarchies; certainly less than one would expect to find in a broiler breeder flock. If any multiple hierarchies were present, they would be found in the early maturing group photostimulated at 12 weeks.

Table 3.11: Frequency table of the number of double yolks laid by the hens in each light treatment

Number of hens				
Number of double yolks	12-week photostim.	15-week photostim.	18-week photostim.	Total
1	22	25	11	58
2	16	5	5	26
3	7	4	2	13
4	3			3
5	1			1
6	1			1
7	1			1
8	1			1
Total	52	34	18	104

3.4.7 Number and distribution of soft-shelled eggs

The total number of soft-shelled eggs produced by the hens during the eight-week period of stage one is summarised by treatment in Table 3.12. The mean number of soft shells laid per hen for four weeks after each treatment reached 10% lay, is shown in Table 3.13. The relevant analysis of variance is summarised in Appendix 3.36. The production of soft shells was significantly ($p < 0.01$) influenced by the age at photostimulation. By far the greatest number of soft shells was laid by those hens photostimulated at 12 weeks. It is evident that there are problems in regulating calcium absorption and its deposition for shell formation associated with early sexual maturity. There was also a highly significant ($p < 0.0.1$) difference between strains;

the Hy-Line Silver birds producing more soft-shelled eggs than the Hy-Line Brown birds regardless of age at photostimulation. Furthermore, the interaction was highly significant ($p < 0.01$). The Hy-Line Brown hens photostimulated at 15 weeks laid considerably more soft shells than expected. This is unlikely to be a true indication of the response of the strain to the light treatment. Other factors may play a role, although the age at first egg relative to the age at which the birds were given the layer feed may be ruled out. The Hy-Line Silver and Brown hens photostimulated at 15 weeks went onto layer mash simultaneously, even though the mean age at first egg differed by 4.76 days in favour of the Silver birds. One Hy-Line Brown individual produced five soft-shelled eggs in the four weeks, which had a considerable influence on the mean.

Table 3.12: Number of soft-shelled eggs produced during the eight weeks of stage one, also shown as a percentage of the total eggs laid by each treatment during the same period

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Total
Hy-Line Silver	148 (8.3%)	37 (2.8%)	11 (1.2%)	196
Hy-Line Brown	96 (5.8%)	27 (2.2%)	5 (0.7%)	128
Total	244	64	16	324

Table 3.13: Mean number of soft-shelled eggs produced per hen during the first four weeks of lay for each treatment (total number shown in brackets)

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Strain mean
Hy-Line Silver	2.000 (96)	0.583 (28)	0.229 (11)	0.937
Hy-Line Brown	1.104 (53)	0.438 (21)	0.104 (5)	0.549
Mean	1.552	0.510	0.166	

Figure 3.12 shows the relationship between the mean number of soft-shelled eggs per hen for the two varieties and the age at photostimulation. Neither of the linear equations fitted to the soft shell data was significant (Appendices 3.37 and 3.38) although the trends are apparent. The Silver strain had a steeper decline in the number of soft shells than the Brown, as the age at sexual maturity was delayed. The true response is probably curvilinear.

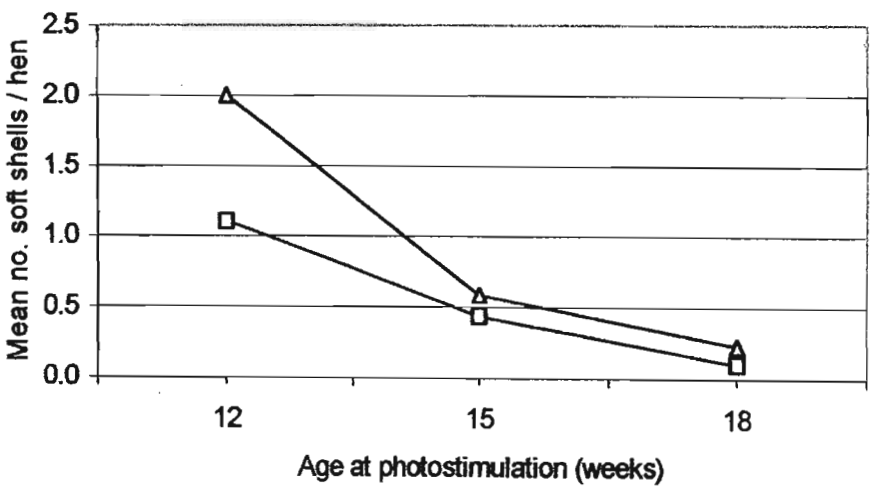


Figure 3.12: The relationship between the mean number of soft shells produced during the first four weeks of lay and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens

The distributions of soft-shelled eggs per hen for the two varieties are shown in Figures 3.13 and 3.14 and the relevant data for each strain is summarised in Tables 3.14 and 3.15.

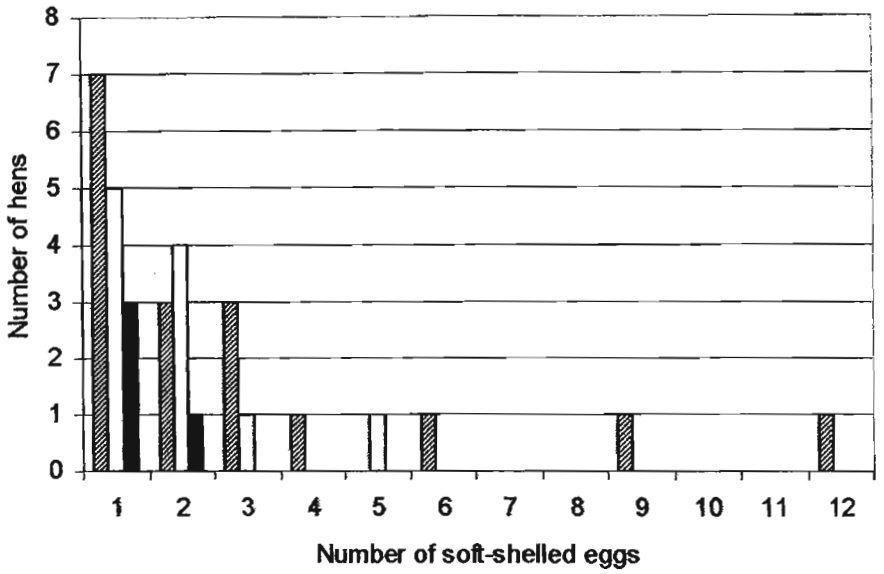


Figure 3.13: Histogram showing the number of soft-shelled eggs produced during the first four weeks of lay by the Hy-Line Silver hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

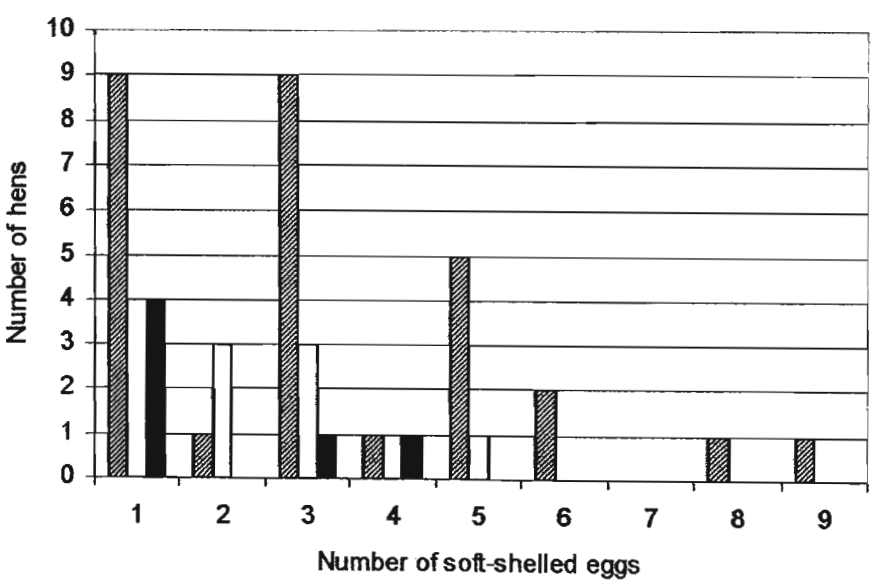


Figure 3.14: Histogram showing the number of soft-shelled eggs produced during the first four weeks of lay by the Hy-Line Brown hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

Table 3.14: Frequency table for the number of Hy-Line Silver hens laying soft-shelled eggs in each light treatment over the eight-week period

No. of soft shells	12-week photostim.	15-week photostim.	18-week photostim.	Total
1	11	3	4	18
2	1	2	0	3
3	6	7	1	14
4	2	1	1	4
5	5	1		6
7	4			4
10	1			1
11	1			1
13	1			1
22	1			1
Total	33	14	6	53

Table 3.15: Frequency table for the number of Hy-Line Brown hens laying soft-shelled eggs in each light treatment over the eight-week period

	Number of hens			
No. of soft shells	12-week photostim.	15-week photostim.	18-week photostim.	Total
1	7	5	3	15
2	4	5	1	10
3	6	1		7
4	0	1		1
5	0	1		1
6	1			1
9	1			1
10	1			1
15	1			1
23	1			1
Total	22	13	4	39

Table 3.16 summarises the appearance of soft-shelled eggs, either on their own or in combination with normal and double-yolked eggs. Appendices 3.39 to 3.46 show the detailed analysis per hen per room and per strain. The vast majority of soft-shelled eggs were the result of a single ovulation, presumably on the preceding day. A fair number also appeared as two soft shells laid on the same day or as one soft shell in combination with a normal shelled egg. On 123 occasions there were multiple ovulations associated with soft-shelled eggs, suggesting some association between poor control of the hierarchy or ovulatory cycle, insufficient calcium

deposition and premature ovipositions. The lack of proper hormonal control in physiologically immature pullets that have been forced into early lay may be responsible. Where a normal egg and one or more soft shells were produced on the same day, about 20% of them were laid simultaneously. The remaining 80% consisted of normal eggs followed by soft shells (or occasionally the other way round) after intervals ranging from half an hour to 14 hours; the mean interval being six hours.

Table 3.16: Combinations of normal and soft-shelled eggs produced per day

Combination	No. days	%
one soft shell	159	56.4
two soft shells	31	11.0
three soft shells	1	0.35
one normal, one soft	75	26.6
one normal, two soft	6	2.1
one normal, three soft	1	0.35
One normal, one double yolk, one soft	1	0.35
one double yolk, one soft	4	1.4
one double yolk, two soft	1	0.35
one broken egg, one soft	3	1.1
Total number of days	282	100.0

Tables 3.17 and 3.18 summarise detailed analyses of the locations of combinations of normal and soft-shelled eggs for each strain, either within uninterrupted sequences or preceding pause days in the sequences. For the Hy-Line Silver hens, on 54 occasions (31.6%) the combinations

of soft and normal eggs were associated with pauses in the sequences. The corresponding figure for the Hy-Line Brown birds was 34 (30.6%). In some instances, the soft shell (denoted by *) appeared to have been expelled prematurely; for example, hen four (room one):

Date	...22/1	23/1	24/1	25/1	26/1	27/1	28/1...
Time of lay	...07:00	07:00	07:30	08:00 16:30*	(pause)	07:00	07:00...

Assuming the relevant ovulation occurred at about 08:30 on 25/1, the ovum spent roughly eight hours in the oviduct, four of which would have been in the uterus, before being laid as a soft-shelled egg. It is presumed that an unexpected release of prostaglandins was responsible for the early expulsion (based on the findings of Hargrove and Ottinger, 1992). Hence the pause day on 26/1 should not be allocated to an internal ovulation. Less easy to interpret is the case of a single soft shell being followed by a pause day, as shown by a sequence from hen 40 (room one):

Date	...25/1	26/1	27/1	28/1	29/1	30/1	31/1...
Time of lay	...07:00	07:00	07:00*	(pause)	09:30	07:30	08:00...

For some reason the soft-shelled egg was not accompanied by an ovulation (or if it was, the ovum was not picked up by the infundibulum), which resulted in the apparent termination of the sequence. According to the history of oviposition times, the soft-shelled egg was produced at the expected time, *i.e.* 07:00, and was therefore unlikely to have been an egg due to be laid on 28/1. The egg appeared to spend the 24 hours in transit in the oviduct without much calcium deposition.

Table 3.17: Frequency table for the number of occasions soft shells were laid by Hy-Line Silver birds within normal sequences or associated with pauses in mid-sequence, for the three light treatments

	Number of occasions			
Combination	12-wk photo.	15-wk photo.	18-wk photo.	Total
Laid within a normal sequence:				
single soft shell	43	21	8	72
two soft shells	9	2		11
normal egg, soft shell	25	7	1	33
Normal egg, two soft shells	1			1
Followed by a pause day:				
Single soft shell	19	3	1	23
2-3 soft shells	10			10
normal egg, soft shell	14	2	1	17
normal egg, 2-3 soft shells	4			4
Total	125	35	11	171

Table 3.18: Frequency table for the number of occasions soft shells were laid by Hy-Line Brown birds within normal sequences or associated with pauses in mid-sequence, for the three light treatments

	Number of occasions			
Combination	12-wk photo.	15-wk photo.	18-wk photo.	Total
Laid within a normal sequence:				
single soft shell	31	9	2	42
two soft shells	6	4	1	11
normal egg, soft shell	20	2	1	23
normal egg, two soft shells	1			1
Followed by a pause day:				
Single soft shell	18	4		22
2-3 soft shells	1	1		2
normal egg, soft shell	6	2		8
normal egg, 2-3 soft shells	2			2
Total	85	22	4	111

There were 117 (68.4%) and 77 (69.4%) occasions for the Silver and Brown hens respectively where soft-shelled eggs were produced in mid-sequence in various combinations without causing interruptions. The most common occurrence was a single soft shell found in the middle of a normal sequence. Most of these were laid at the expected times, although several examples were seen of the soft-shelled egg being laid unusually early. The following sequence

of hen six (room five) demonstrates:

Date	22/1	23/1	24/1	25/1	26/1	27/1	28/1...
Time of lay	08:00	11:30	11:30	10:30	10:00	07:00*	10:30...

The soft-shelled egg may have been expelled three to four hours prematurely but the associated ovulation appears to have taken place at the expected time.

Given that almost one third of the days where soft-shelled eggs were laid were associated with mid-sequence pauses, this is further evidence that not all pauses in sequences can be attributed to internal ovulations.

3.4.8 Estimation of the incidence of internal ovulations

One of the objectives of recording time of lay during stage one of the trial was to make it possible to deduce where an internal ovulation had taken place. Since a large number of mid-sequence pauses seemed to be associated with the production of double-yolked or soft-shelled eggs, a meticulous approach was required in interpreting the data. A pause preceded by or followed by either a double-yolked egg or two or more soft-shelled eggs was not assumed to be due to an internal ovulation.

Several long sequences were seen to consist of ovipositions occurring at about the same time every day, both before and after a pause. In these cases there was no hesitation in interpreting the pause as being due to an internal ovulation on the preceding day. The following sequence is an example, produced by hen 20 from room one:

Date	..30/1	31/1	1/2	2/2	3/2	4/2	5/2	6/2	7/2	8/2..
Time	08:00	08:00	08:00	-	08:30	07:00	07:00	07:30	-	07:30

Quite clearly the first sequence did not end on 1 February, nor was it followed by a four-egg sequence that terminated on 6 February. Presumably there were two internal ovulations, occurring on 1 and 6 February, so that no eggs were laid on 2 or 7 February. There were also shorter sequences, where each subsequent egg was laid later every day, which were relatively easy to interpret. An example is provided by the records of hen 25 from room one:

Date	7/1	8/1	9/1	10/1	11/1	12/1	13/1	14/1	15/1	16/1
Time	09:30	14:00	-	07:30	-	09:00	11:30	15:30	-	07:30

In this case a two-egg sequence was followed by a five-ovulation sequence, with an internal ovulation on 10 January creating a pause on 11 January. Without the benefit of reviewing the times of oviposition, no interpretation of the accurate sequence length would be possible.

Once identified, the numbers of internal ovulations per treatment were counted. These are shown as the total number (Table 3.19) and as the number over a fixed period of time (Table 3.20). In the latter case, for each treatment the time period commenced from the onset of lay and terminated four weeks after 10% of the birds were in lay. An analysis of variance was not considered to be appropriate because there was no scientific measurement of the number of internal ovulations. However, judging by the numbers presented in Table 3.20, the Hy-Line Silver hens appear to be more prone to internal ovulations than the Hy-Line Brown birds, the differences between the strains being 31 over the eight-week period and 15 for the four weeks of lay. The number of internal ovulations also increased as the age at photostimulation was advanced.

Table 3.19: The estimated total number of internal ovulations occurring within each treatment, for an eight-week period from onset of lay to the end of stage one

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Total
Hy-Line Silver	54	42	15	111
Hy-Line Brown	45	24	11	80
Total	99	66	26	191

Table 3.20: The estimated total number of internal ovulations occurring within each treatment, for the first four weeks of lay

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Strain total
Hy-Line Silver	23	27	14	64
Hy-Line Brown	21	17	11	49
Light total	44	44	25	113

Figure 3.15 shows the presumed number of internal ovulations during the first four weeks of lay for both varieties plotted against the age at photostimulation. The relationship between the two variables was more predictable for the Brown strain, where the number of internal ovulations decreased in a regular fashion with an increase in age at photostimulation. One Silver hen from the 15-week photostimulation group is thought to have ovulated internally five times, which is why the total of 27 for that treatment is out of line with the expected trend. There was also an unexpectedly high number of hens producing single internal ovulations for that group.

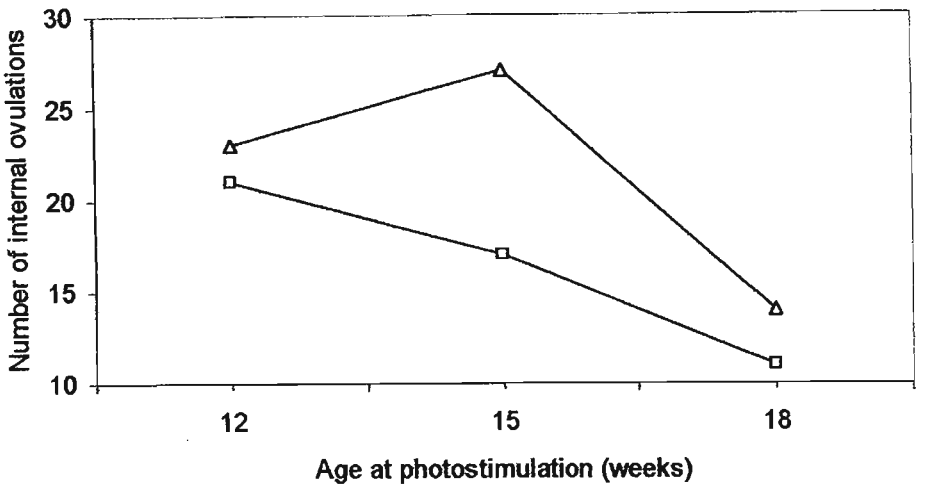


Figure 3.15: The relationship between the number of suspected internal ovulations and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens

There were 176 hens (61% of the flock) that did not appear to ovulate internally and 62 hens that had one internal ovulation. The distributions of internal ovulations within the flock for the two varieties per light treatment for the first four weeks of lay are shown in Figures 3.16 and 3.17. There appeared to be two hens that were prone to internal ovulations; these individuals produced four and five internal ovulations. Both of these birds were of the Silver strain, one from the 12-week and the other from the 15-week photostimulation groups. These birds may have had some anatomical abnormality, such as a poorly formed infundibulum, which caused repeated internal ovulations. Contractions of the oviduct or measurements of the hen's temperature rhythms, blood pressure or heart rate may have confirmed that internal ovulations were taking place.

In practice internal ovulations lower total egg production by disrupting sequences, with the result that the rate of lay is lower than the ovulation rate. Mean sequence length is reduced and both the number of pauses and number of sequences is increased. The extent of this may be seen in Section 3.4.11.

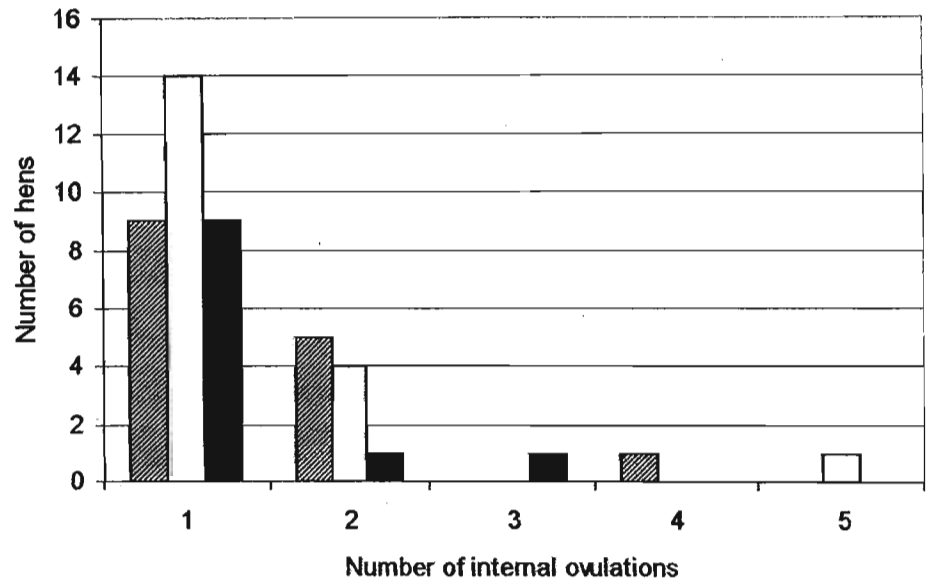


Figure 3.16: Histogram showing the number of internal ovulations during the first four weeks of lay for the Hy-Line Silver hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

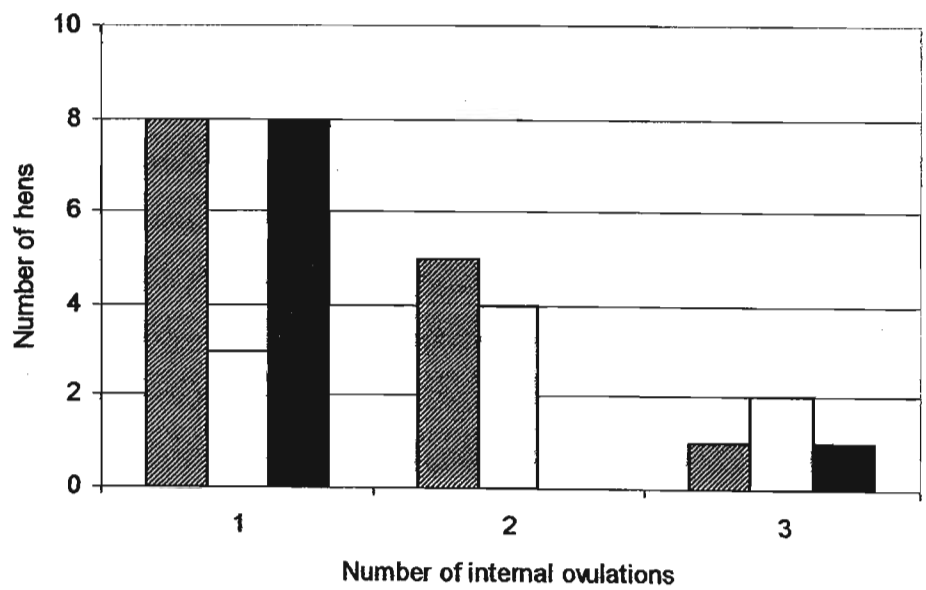


Figure 3.17: Histogram showing the number of internal ovulations during the first four weeks of lay for the Hy-Line Brown hens photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

3.4.9 Oviposition times for all eggs

The frequency distribution for time of lay of all eggs produced during the first stage of the trial is shown in Figure 3.18. The mean time of lay was 09:20 (11 hours and 50 minutes after the onset of darkness), the median time was 09:00 and the mode, 07:00. In a normal distribution the mean, median and mode coincide. The distribution is similar to that for the time of lay of first eggs (see Figure 3.8) in that the mode occurs at 07:00 because the recordings did not start early enough in the morning. Because of this, the true population mean is probably somewhat earlier than 09:20. In this case the oviposition times of the eggs collected late in the evening by the security personnel were not brought forward to 16:30, even though they do not reflect the actual times of lay.

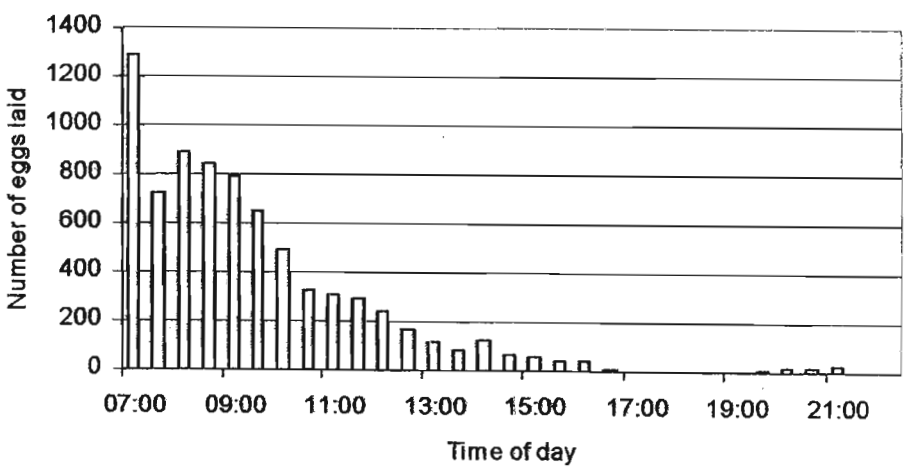


Figure 3.18: Histogram showing the time of lay of all eggs produced during the first stage of the trial

The mean oviposition times per treatment are listed in Table 3.21. It is worth commenting on the fact that there appears to be a trend in mean oviposition time for the light treatments; as the age at photostimulation increases from 12 to 15 and to 18 weeks, the mean time of lay is delayed by 20 and 29 minutes respectively. However, because the recorded oviposition times are not normally distributed, little emphasis may be placed on either the treatment means or their standard deviations.

Table 3.21: The mean times of lay (±sd) per treatment for all eggs produced during stage one of the trial

Age at photostimulation				
Strain	12 weeks	15 weeks	18 weeks	Strain totals
Hy-Line Silver	09:03 (±2h13m)	09:17 (±2h01m)	09:52 (±2h16m)	09:19 (±2h11m)
Hy-Line Brown	09:02 (±2h26m)	09:29 (±2h19m)	09:53 (±2h17m)	09:22 (±2h23m)
Light totals	09:03 (±2h19m)	09:23 (±2h10m)	09:52 (±2h16m)	09:20 (±2h17m)

3.4.10 Egg weights

Table 3.22 summarises the mean egg weights per treatment for stage one of the trial. Table 3.23 shows the mean egg weights for the first four weeks of lay, from 10% lay, for each treatment. For the latter data, the differences in mean egg weight between the strains was highly significant ($p < 0.001$); as expected, the Hy-Line Brown hens laid heavier eggs. For both periods the Brown strain had a mean egg weight of over 4g more than the Silver strain. The differences in egg weight between the light treatments were just not significant at the 5% level (see Appendix 3.47 for statistical summary). The number of double-yolked eggs laid by the various treatments presumably increased the variation about the means, thereby reducing the likelihood of attributing significance to treatments. Furthermore, most of the double yolks were laid by the 12-week photostimulation group, which would contribute to the reduction in the differences between treatment means. Under normal conditions one would expect birds that come into lay later to produce bigger eggs at onset of lay, provided that their body masses were heavier than those of the earlier photostimulation treatments. Despite the lack of statistical significance, there were definite trends in mean egg weight as the age at photostimulation increased. The birds subjected to increasing daylength at 18 weeks had a mean initial egg weight 3.86g and 8.16g heavier than the 15-week and 12-week treatments respectively.

Table 3.22: Mean egg weights (in grams) per treatment for the eight weeks of stage one

Age at photostimulation				
Strain	12 weeks	15 weeks	18 weeks	Strain means
Hy-Line Silver	43.35 (±6.39)	44.27 (±5.54)	47.54 (±5.22)	44.66 (±6.07)
Hy-Line Brown	46.51 (±6.79)	49.60 (±6.09)	51.87 (±5.39)	48.70 (±6.63)
Light means	44.89 (±6.78)	46.81 (±6.38)	49.45 (±5.71)	46.57 (±6.65)

Table 3.23: Mean egg weights (in grams) for the first four weeks of lay, from 10% lay, for each treatment

Age at photostimulation				
Strain	12 weeks	15 weeks	18 weeks	Strain means
Hy-Line Silver	39.79 (±6.21)	42.77 (±5.34)	47.32 (±5.24)	43.48 (±6.34)
Hy-Line Brown	42.82 (±6.39)	48.45 (±5.99)	51.87 (±5.39)	47.89 (±6.92)
Light means	41.26 (±6.47)	45.56 (±6.34)	49.42 (±5.77)	45.23 (±6.84)

Figures 3.19 and 3.20 show the mean egg weights for the Hy-Line Silver and Brown birds respectively, with the weights for the three light treatments plotted separately over the eight-week period of stage one. Removing the weights of the double-yolked eggs from the calculation of the treatment means should produce smoother trends and may also reduce the differences in mean egg weight for the Silver strain prior to 136 days of age between the 12- and 15-week photostimulation groups.

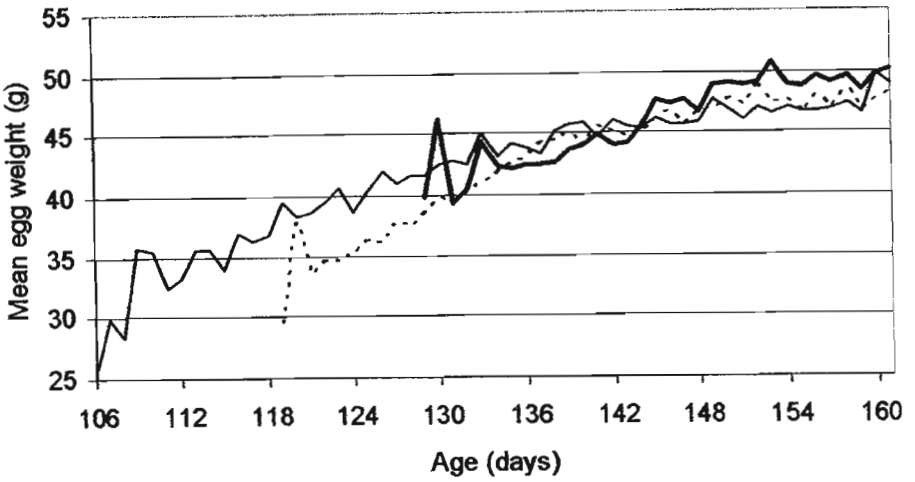


Figure 3.19: Mean egg weights for the Hy-Line Silver hens photostimulated at 12 weeks (solid line), 15 weeks (dotted line) and 18 weeks (bold line)

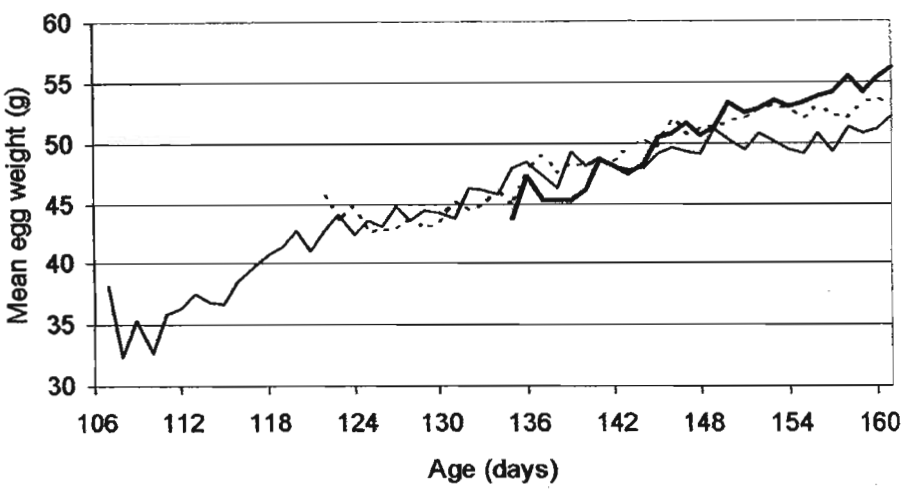


Figure 3.20: Mean egg weights for the Hy-Line Brown hens photostimulated at 12 weeks (solid line), 15 weeks (dotted line) and 18 weeks (bold line)

The revised mean egg weights, excluding double-yolked eggs, are summarised in Table 3.24. A factorial analysis looking at main effects (since the interactions were not significant) showed the differences between the strains to be highly significant ($p < 0.01$) and between the light treatments to be significant at the 5% level (Appendix 3.48). The revised graphs for the two

varieties are shown in Figures 3.21 and 3.22, the trends of which are noticeably less erratic. It needs to be mentioned that the high initial mean egg weight for the 15-week Hy-Line Brown treatment was due to a single large egg.

Table 3.24: Mean egg weights (in grams) of the single-yolked eggs for the first four weeks of lay, from 10% lay, for each treatment

	Age at photostimulation			
Strain	12 weeks	15 weeks	18 weeks	Strain means
Hy-Line Silver	38.58 (±4.60)	42.44 (±4.76)	46.96 (±4.51)	42.96 (±5.70)
Hy-Line Brown	41.94 (±5.46)	47.92 (±5.25)	51.59 (±4.84)	47.43 (±6.42)
Light means	40.21 (±5.31)	45.11 (±5.70)	49.10 (±5.21)	44.76 (±6.36)

Interestingly, the trends in the mean egg weights for the 12- and 15-week photostimulation treatments closely resemble those of the corresponding body weights (Figure 3.3). Clearly, the differences in egg weight between the two treatments during the first few weeks of lay are due to differences in body weight. At a fixed chronological age, *e.g.* 17 weeks, those pullets that were photostimulated at 12 weeks were able to lay heavier eggs because of the comparatively heavier body weights. Egg weight may be manipulated to some extent by lighting programmes and other environmental factors. For example, ahemeral light:dark cycles longer than 24 hours increase egg weight (Morris, 1973). However, the variable is essentially a function of the age of the hens. As the flock of hens ages, mean egg weight increases in a curvilinear fashion.

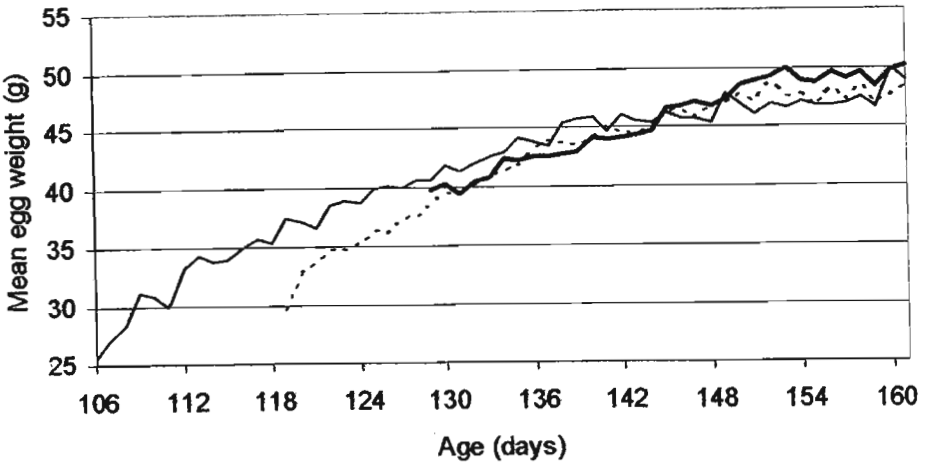


Figure 3.21: Mean egg weights (excluding double-yolked eggs) for the Hy-Line Silver hens photostimulated at 12 weeks (solid line), 15 weeks (dotted line) and 18 weeks (bold line)

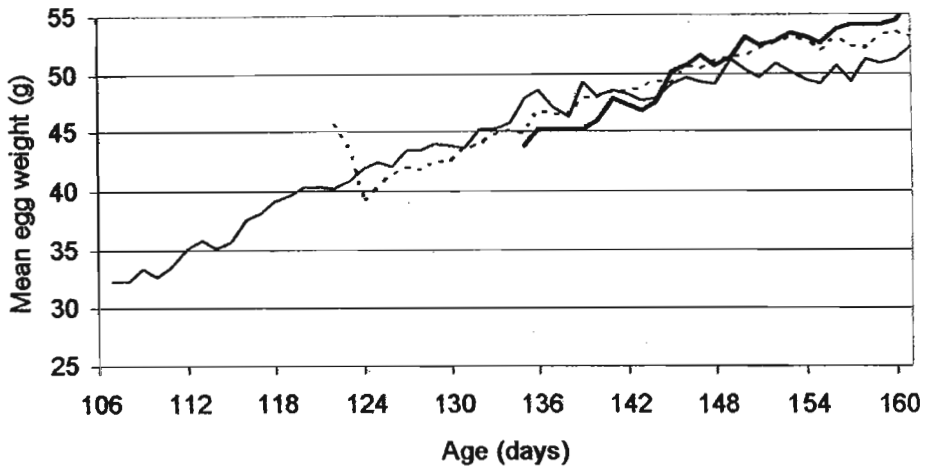


Figure 3.22: Mean egg weights (excluding double-yolked eggs) for the Hy-Line Brown hens photostimulated at 12 weeks (solid line), 15 weeks (dotted line) and 18 weeks (bold line)

The linear regression of mean egg weight (excluding double yolks) for the first four weeks of lay (y) on age at photostimulation, in weeks (x), was found to be significant ($p < 0.05$) for the Silver strain and is given by the equation:

$$y = 21.71 + 1.3967\,x \qquad \text{(Equation 3.3)}$$

The statistical summary is detailed in Appendix 3.49. The equivalent linear regression for the Brown strain was not significant (Appendix 3.50). The relationships between mean egg weight and age at photostimulation for the two varieties, along with the linear regression for the Silver birds, are shown in Figure 3.23. The increase in mean egg weight with delayed age at photostimulation is associated with heavier body weights at onset of lay.

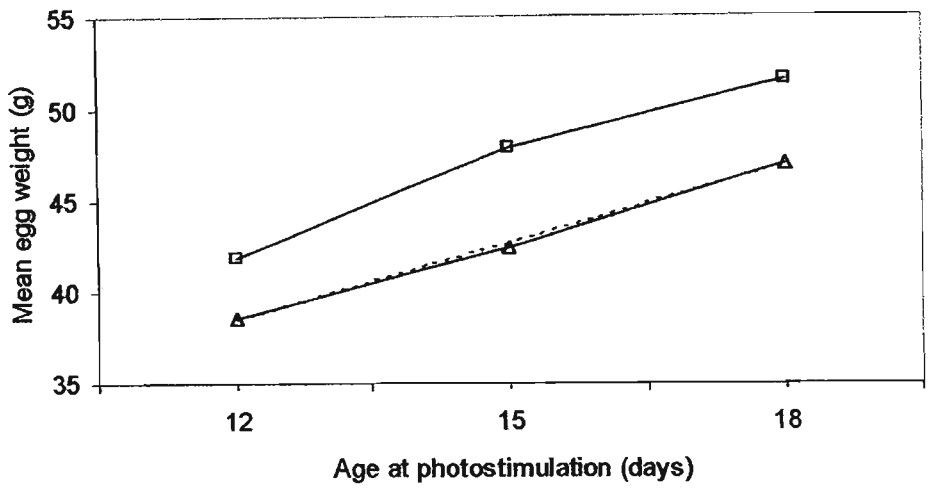


Figure 3.23: The relationship between mean egg weight for the first four weeks of lay and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens and the fitted linear regression for Hy-Line Silver (dotted line)

3.4.11 Egg component weights

The mean egg component weights for the Hy-Line Silver birds are summarised in Tables 3.25, 3.26 and 3.27, for pullets given increasing daylength at 12, 15 and 18 weeks respectively. Tables 3.28, 3.29 and 3.30 show corresponding values for the Brown strain. The most startling observation was that the yolk weights at onset of lay in the early maturing pullets ranged from 3.6 to 7.1g, which were much smaller than expected. In all three light treatments yolk weight increased steadily to 21 weeks of age (Figure 3.24). Initial yolk weight at onset of lay appears to be determined by the age of the birds and is presumably related to body size. Both shell weights and albumen weights increased over time in a curvilinear fashion for the three light treatments (Figures 3.25 and 3.26).

Table 3.25: Egg component weights (± sd) for Hy-Line Silver pullets photostimulated at 12 weeks of age

	Age of pullets (weeks)					
	15-16	17	18	19	20	21
sample size	44	18	26	33	23	16
egg weight (g)	31.08 (±3.33)	37.10 (±2.88)	39.82 (±3.04)	42.96 (±2.95)	44.24 (±1.55)	45.15 (±3.02)
yolk weight (g)	5.77 (±0.85)	7.37 (±0.61)	7.98 (±0.63)	9.05 (±0.64)	9.90 (±0.51)	10.64 (±0.39)
shell weight (g)	2.88 (±0.49)	3.67 (±0.45)	3.94 (±0.54)	4.18 (±0.36)	4.25 (±0.58)	4.29 (±0.36)
albumen weight (g)	22.43 (±2.51)	26.06 (±2.33)	27.91 (±2.51)	29.73 (±2.52)	30.09 (±1.54)	30.23 (±2.91)
% yolk	18.57	19.90	20.09	21.11	22.41	23.66
% shell	9.25	9.88	9.88	9.75	9.60	9.51
% albumen	72.19	70.22	70.03	69.14	67.99	66.83

Table 3.26: Egg component weights (± sd) for Hy-Line Silver pullets photostimulated at 15 weeks of age

	Age of pullets (weeks)			
	17-18	19	20	21
sample size	69	29	26	19
egg weight (g)	36.32 (±3.37)	41.49 (±3.07)	44.83 (±3.46)	45.75 (±3.11)
yolk weight (g)	7.61 (±0.86)	8.96 (±0.71)	9.95 (±0.37)	10.51 (±0.58)
shell weight (g)	3.38 (±0.46)	3.94 (±0.43)	4.30 (±0.46)	4.41 (±0.32)
albumen weight (g)	25.55 (±2.66)	28.59 (±2.51)	30.58 (±3.24)	30.83 (±2.74)
% yolk	20.94	21.60	22.19	22.98
% shell	9.31	9.49	9.60	9.63
% albumen	70.34	68.92	68.21	67.39

Table 3.27: Egg component weights for Hy-Line Silver pullets photostimulated at 18 weeks of age

	Age of pullets (weeks)		
	18-19	20	21
sample size	17	20	22
egg weight (g)	40.88 (±2.69)	43.92 (±4.11)	47.31 (±2.44)
yolk weight (g)	8.63 (±1.00)	9.35 (±0.91)	10.25 (±1.02)
shell weight (g)	3.31 (±0.27)	4.03 (±0.47)	4.41 (±0.39)
albumen weight (g)	28.94 (±2.05)	30.55 (±3.06)	32.65 (±1.81)
% yolk	21.10	21.29	21.67
% shell	8.10	9.17	9.33
% albumen	70.80	69.55	69.01

Table 3.28: Egg component weights for Hy-Line Brown pullets photostimulated at 12 weeks of age

	Age of pullets (weeks)					
	15-16	17	18	19	20	21
sample size	40	16	28	32	26	24
egg weight (g)	34.48 (±2.76)	40.61 (±2.91)	42.33 (±3.54)	45.59 (±3.99)	47.95 (±4.24)	48.32 (±5.14)
yolk weight (g)	5.81 (±0.63)	7.23 (±0.56)	7.83 (±0.76)	8.74 (±1.02)	9.84 (±0.63)	10.22 (±0.94)
shell weight (g)	3.13 (±0.45)	3.96 (±0.36)	4.19 (±0.40)	4.31 (±0.53)	4.65 (±0.43)	4.62 (±0.57)
albumen weight (g)	25.53 (±2.29)	29.42 (±2.28)	30.31 (±3.08)	32.54 (±3.07)	33.45 (±3.80)	33.48 (±4.31)
% yolk	16.85	17.79	18.50	19.18	20.53	21.15
% shell	9.09	9.75	9.90	9.45	9.70	9.56
% albumen	74.06	72.45	71.60	71.37	69.77	69.29

Table 3.29: Egg component weights for Hy-Line Brown pullets photostimulated at 15 weeks of age

	Age of pullets (weeks)			
	17-18	19	20	21
sample size	28	27	23	21
egg weight (g)	40.93 (±4.12)	45.48 (±2.69)	47.64 (±3.65)	51.37 (±4.79)
yolk weight (g)	7.34 (±0.84)	8.45 (±0.83)	9.27 (±0.77)	10.24 (±0.88)
shell weight (g)	3.65 (±0.37)	4.24 (±0.46)	4.53 (±0.54)	4.91 (±0.43)
albumen weight (g)	29.94 (±3.42)	32.78 (±2.62)	33.84 (±3.05)	36.21 (±4.17)
% yolk	17.93	18.58	19.47	19.94
% shell	8.93	9.33	9.50	9.56
% albumen	73.15	72.09	71.03	70.50

Table 3.30: Egg component weights for Hy-Line Brown pullets photostimulated at 18 weeks of age

	Age of pullets (weeks)	
	20	21
sample size	15	18
egg weight (g)	47.84 (±4.33)	52.05 (±3.06)
yolk weight (g)	9.34 (±0.90)	10.34 (±0.54)
shell weight (g)	4.24 (±0.36)	4.82 (±0.63)
albumen weight (g)	34.27 (±3.60)	36.89 (±2.52)
% yolk	19.51	19.87
% shell	8.86	9.26
% albumen	71.62	70.87

At a fixed age and for all three light treatments, shell weights of the eggs laid by Hy-Line Brown birds were heavier, but for nine of the twelve comparisons the percentages of shell were slightly lower. Albumen weights for the Hy-Line Brown eggs were consistently heavier than for the Hy-Line Silver eggs and the percentages of albumen were 1.57 to 3.18% higher over the range of ages for the three light treatments. In contrast, ten of the twelve comparisons showed that Hy-Line Brown eggs had slightly smaller yolks with the percentage differences ranging from 1.58 to 3.03. Thus the additional egg weight of the Hy-Line Brown eggs compared to the Hy-Line Silver eggs is largely due to the greater proportion of albumen.

The mean yolk weight data measured at 20 and 21 weeks of age were subjected to analyses of variance. Comparisons could not be done at earlier ages because all six treatments were not represented. The analyses showed that there were no significant differences between the breeds or the three light treatments; neither were the interactions significant. (Appendices 3.51 and 3.52). This suggests that, during the early stage of production, one function could be used to predict yolk weight from hen age, regardless of the strain or the age at photostimulation. However, the albumen and shell weights for the two varieties need to be predicted independently.

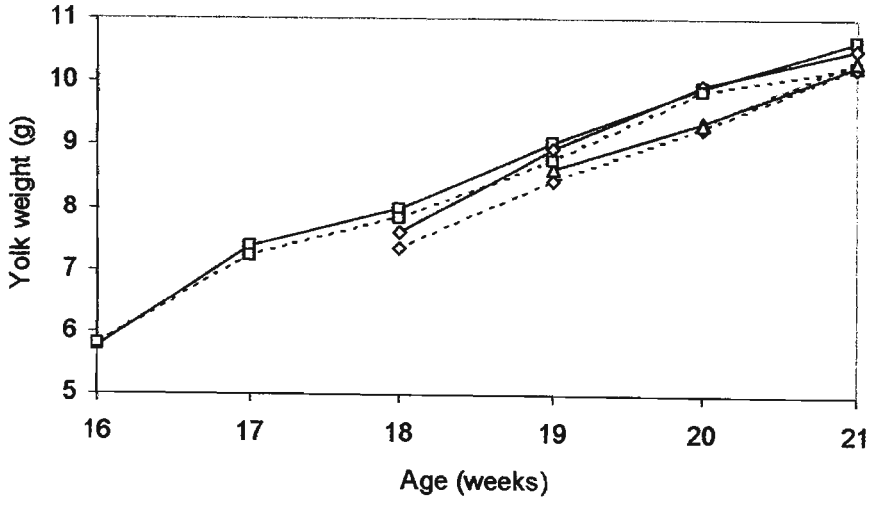


Figure 3.24: Increase in mean yolk weight as the birds age, for Hy-Line Silver (solid lines) and Hy-Line Brown (dotted lines) hens photostimulated at 12 weeks (□), 15 weeks (◊) and 18 weeks (Δ)

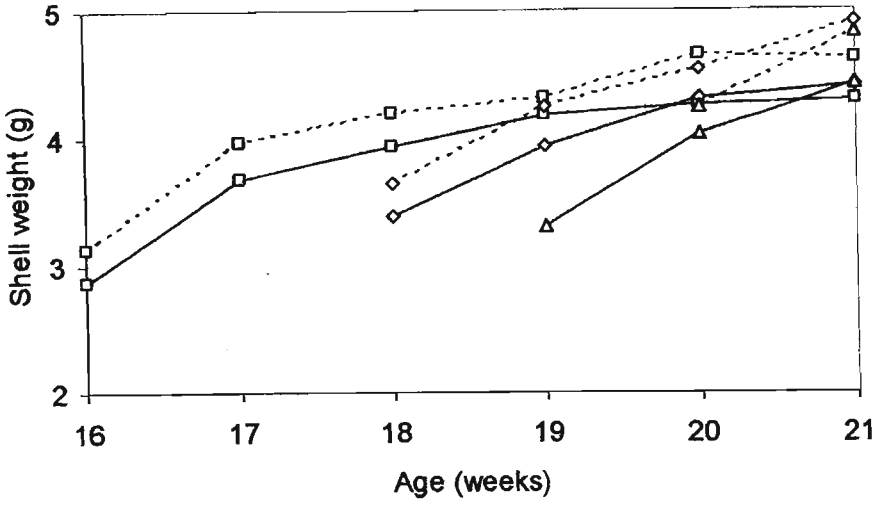


Figure 3.25: Increase in mean shell weight as the birds age, for Hy-Line Silver (solid lines) and Hy-Line Brown (dotted lines) hens photostimulated at 12 weeks (□), 15 weeks (◊) and 18 weeks (Δ)

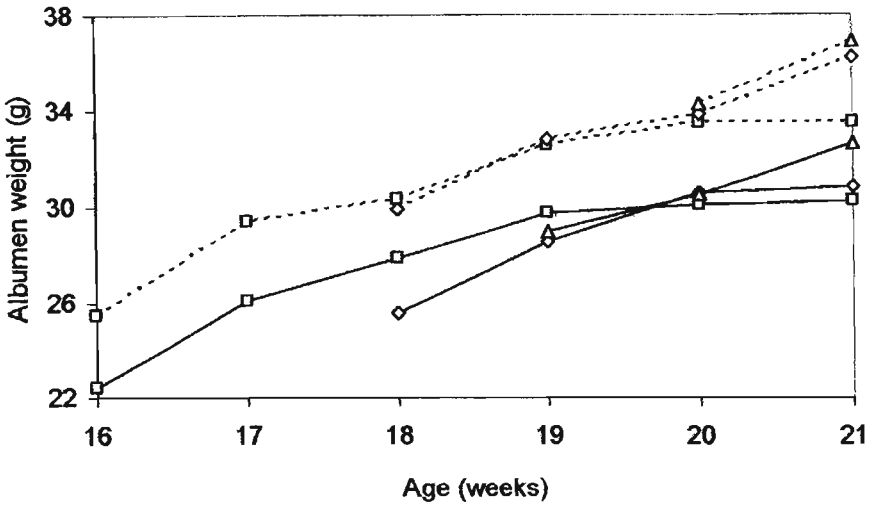


Figure 3.26: Increase in mean albumen weight as the birds age, for Hy-Line Silver (solid lines) and Hy-Line Brown (dotted lines) hens photostimulated at 12 weeks (□), 15 weeks (◊) and 18 weeks (Δ)

Some additional measurements of egg component weights were performed at a later date on eggs from 37- and 64-week-old Hy-Line Silver hens, and on eggs from 37-week-old Hy-Line Brown hens. This expanded database will increase the precision of any functions derived from

the data and used to predict component weights over the entire laying cycle. The means and standard deviations are summarised in Table 3.31

Table 3.31: Egg component weights for older Hy-Line Silver and Brown birds

	Hy-Line Silver (37 weeks)	Hy-Line Silver (64 weeks)	Hy-Line Brown (37 weeks)
sample size	30	29	30
Egg weight (g)	57.39 (±3.54)	59.44 (±3.81)	62.18 (±5.29)
yolk weight (g)	14.97 (±1.06)	16.07 (±1.09)	14.40 (±1.30)
shell weight (g)	5.18 (±0.46)	5.16 (±0.41)	5.62 (±0.49)
albumen weight (g)	37.24 (±3.08)	38.21 (±3.16)	42.15 (±4.53)
% yolk	26.08	27.03	23.16
% albumen	64.89	64.29	67.79
% shell	9.02	8.68	9.04

In order to find a suitable equation for predicting yolk weight from hen age, Genstat was used to fit various functions to the collective yolk weight data. A Gompertz equation of the form

$$y = -51107 + 51123 \cdot \exp(-\exp(-0.01771 \cdot (x + 370.1))) \qquad \text{(Equation 3.4)}$$

where y = yolk weight and x = hen age, in days, accounted for 99.6% of the variation in the data for the Hy-Line Silver. The statistical summary is given in Appendix 3.53. Similarly yolk weight (y) for the Hy-Line Brown birds may be predicted from the Gompertz equation (r^2 = 99.4%)

$$y = -101.1 + 116.0 \cdot \exp(-\exp(-0.01972 \cdot (x + 15.36))) \qquad \text{(Equation 3.5)}$$

where x = hen age, in days. Appendix 3.54 shows the statistical summary. Figure 3.27 shows the data points and the two fitted functions.

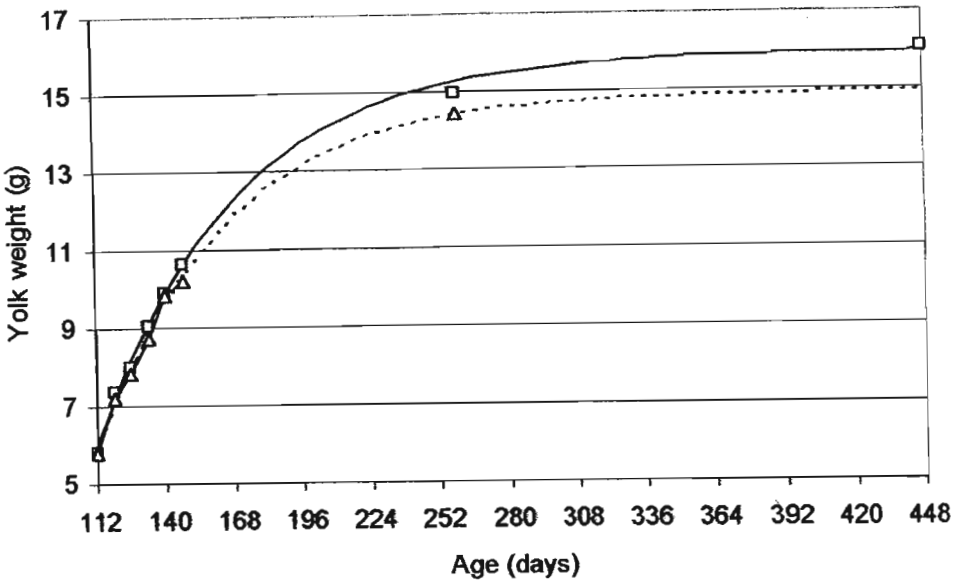


Figure 3.27: Observed and fitted yolk weights for the Silver (□) and Brown hens (Δ) and the fitted Gompertz functions for the Silver (solid line) and Brown (dotted line) varieties

Thus for modelling purposes and from the results presented here, the mean yolk weight produced by a flock of Hy-Line Silver or Hy-Line Brown hens may be predicted from the age of the birds, irrespective of the age at photostimulation. Individual yolk weights can be predicted using a coefficient of variation of about 9%. A method for predicting both albumen and shell weights will be discussed in depth in Chapter 4.

3.4.12 Analysis of sequence characteristics

An Excel spreadsheet was used to input the daily egg production data per hen over the 13-month laying period. Complete production records were used for the hens up to Friday, 29 August 2003. Because those birds in the middle of long sequences were monitored until each one paused, the individual production records stopped at different dates. However, the Sequence Analyzer program (Zuidhof *et al.*, 1999) is able to accommodate this; the subsequent days without production are not counted as pauses since by definition a pause is preceded by and followed by a sequence. Similarly the program allows for different start dates at sexual maturity. After the last hen had paused towards the end of January 2004, the file was imported into Sequence Analyzer. This program analyses the performance of each hen in terms of the

number of sequences and pauses, the number of soft shells and double yolks, mean sequence length and mean pause length and the length of the prime sequence. The number of ovulations is calculated from the number of single-yolked eggs plus twice the number of double-yolked eggs. Of little interest here, since they were not recorded, are variables such as the number of shell-less eggs, abnormally-shaped shells and eggs broken by either the bird or the handler.

The output files were imported back into Excel in order to manipulate the results. The 37 hens that were culled at 23 weeks of age due to poor performance were excluded from further analysis. Tables 3.32 and 3.33 summarise the important sequence characteristics per treatment for the two varieties. In each case the mean number of pauses closely follows the mean number of sequences, because for each bird there will always be one less pause than the number of sequences. An analysis of variance was not done on these means, because the huge amount of variation between individuals makes it unlikely that treatment differences will be found to be significant. Also, the experimental design changed from a split-plot to a randomised blocks design at 24 weeks of age. The observed trends in sequence characteristics are, however, of great interest.

Delaying photostimulation seemed to exert a positive influence on the sequence variables, with a few exceptions. For the Hy-Line Brown birds, the greater the age at photostimulation (and hence the age at first egg), the longer the mean prime sequence length and the overall mean sequence length and consequently, the lower the mean number of sequences and pauses. The mean prime sequence length and mean sequence length for the Hy-Line Silver hens did not follow any logical trend. The reason for this is not clear, although the large associated standard deviations may be an indication that a few individuals with exceptionally short or long prime sequences may have had a considerable influence on the mean. In line with expectations, however, the mean number of sequences and pauses did decrease with increasing age at photostimulation.

Table 3.32: Summary of mean sequence characteristics (±sd) for the Hy-Line Silver birds

	Age at photostimulation			
Sequence characteristic	12 weeks	15 weeks	18 weeks	Mean
Prime sequence length (days)	87.46 (±44.08)	84.71 (±50.04)	87.00 (±37.07)	86.38 (±43.60)
Number of sequences	13.18 (±7.03)	11.10 (±6.75)	10.14 (±5.39)	11.42 (±6.47)
Number of pauses	12.18 (±7.03)	10.10 (±6.75)	9.14 (±5.39)	10.42 (±6.47)
Sequence length (days)	20.91 (±13.33)	27.22 (±21.72)	26.47 (±19.25)	24.96 (±18.58)
Pause length (days)	1.69 (±1.14)	1.77 (±1.35)	1.32 (±0.80)	1.59 (±1.12)

Table 3.33: Summary of mean sequence characteristics (±sd) for the Hy-Line Brown birds

	Age at photostimulation			
Sequence characteristic	12 weeks	15 weeks	18 weeks	Mean
Prime sequence length (days)	69.70 (±31.82)	72.86 (±32.34)	83.44 (±41.89)	75.32 (±35.80)
Number of sequences	13.63 (±6.05)	12.48 (±7.71)	10.68 (±7.35)	12.26 (±7.14)
Number of pauses	12.63 (±6.05)	11.48 (±7.71)	9.68 (±7.35)	11.26 (±7.14)
Sequence length (days)	18.71 (±12.73)	20.18 (±12.67)	23.16 (±11.73)	20.70 (±12.42)
Pause length (days)	1.51 (±0.48)	1.55 (±0.95)	1.48 (±0.90)	1.51 (±0.81)

On average the Hy-Line Silver birds produced prime sequences that were 11.06 days longer than those of the Brown birds. In addition, they laid 0.84 fewer sequences over the period with 0.84 less pause days and consequently had a mean sequence length of 4.26 days longer than their counterparts. The mean pause length for the Silver strain was 0.08 days longer. These results are hardly surprising, because the Hy-Line Silver hen is expected to produce a few more eggs over a laying cycle than the Brown, although there is a sacrifice in egg weight.

Mean sequence lengths from 16 to 44 weeks of age for the two varieties for each light treatment are shown in Figures 3.28 and 3.29. From this it is evident that many of the hens commenced their laying cycles with short sequences, irrespective of the age at photostimulation. Mean sequence length reached a maximum between 28 and 34 weeks for the various treatments. Thereafter the flock mean sequence lengths started to decline. As discussed above, the effect of age at light stimulation on mean sequence length is far more apparent in the Hy-Line Brown birds than in the Silver. Advancing the age at sexual maturity by earlier light stimulation resulted in shorter egg sequences, as evidenced by a reduction in both the initial and overall mean sequence lengths.

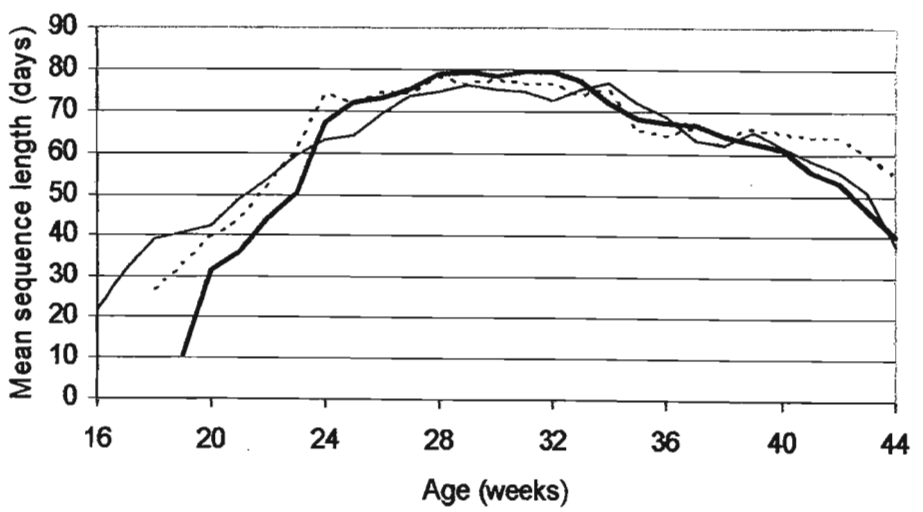


Figure 3.28: Mean sequence lengths for the Hy-Line Silver birds photostimulated at 12 weeks (solid line), 15 weeks (dotted line) or 18 weeks (bold line)

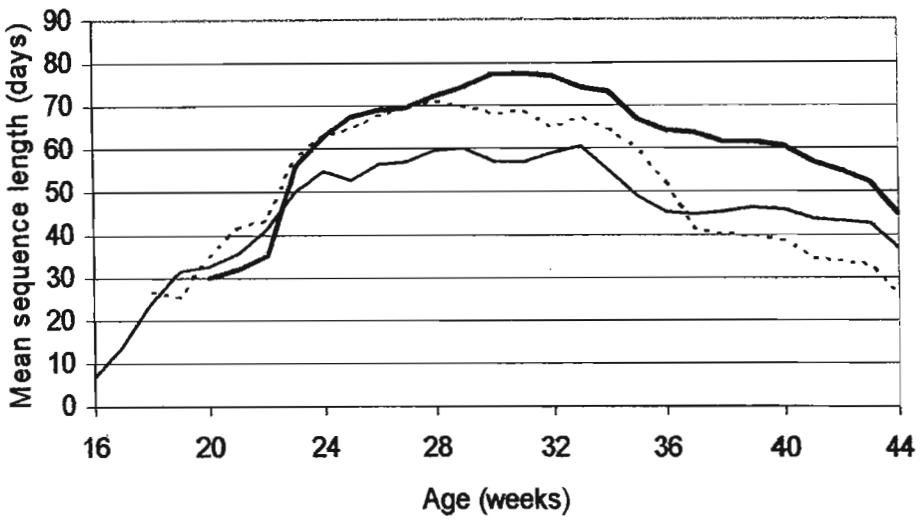


Figure 3.29: Mean sequence lengths for the Hy-Line Brown birds photostimulated at 12 weeks (solid line), 15 weeks (dotted line) or 18 weeks (bold line)

The relationships between mean sequence length and mean prime sequence length, and age at photostimulation are shown in Figures 3.30 and 3.31 respectively. The statistical summaries are shown in Appendices 3.55 to 3.58 for the two varieties. There were no significant linear regressions. These graphs clearly show that the Hy-Line Brown strain responded in a more predictable manner to delayed photostimulation than the Hy-Line Silver strain.

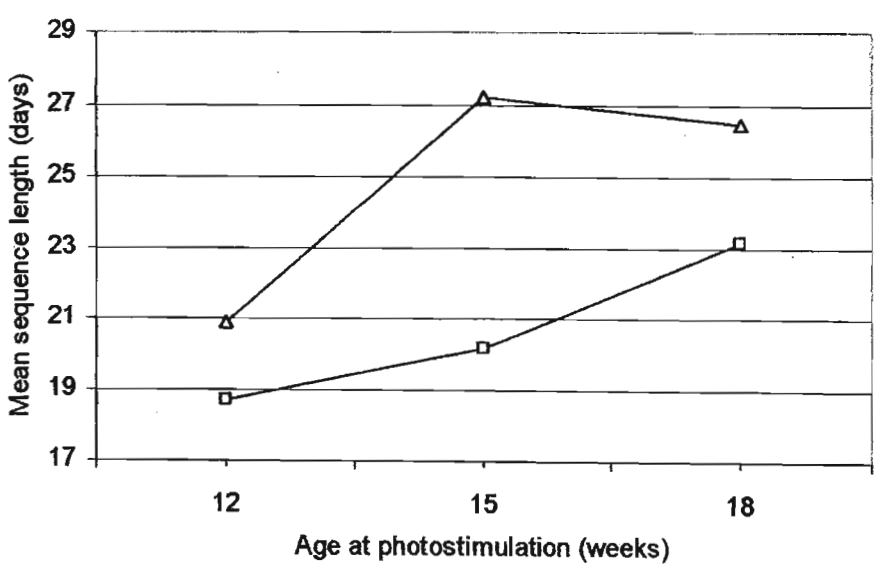


Figure 3.30: The relationship between mean sequence length and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens

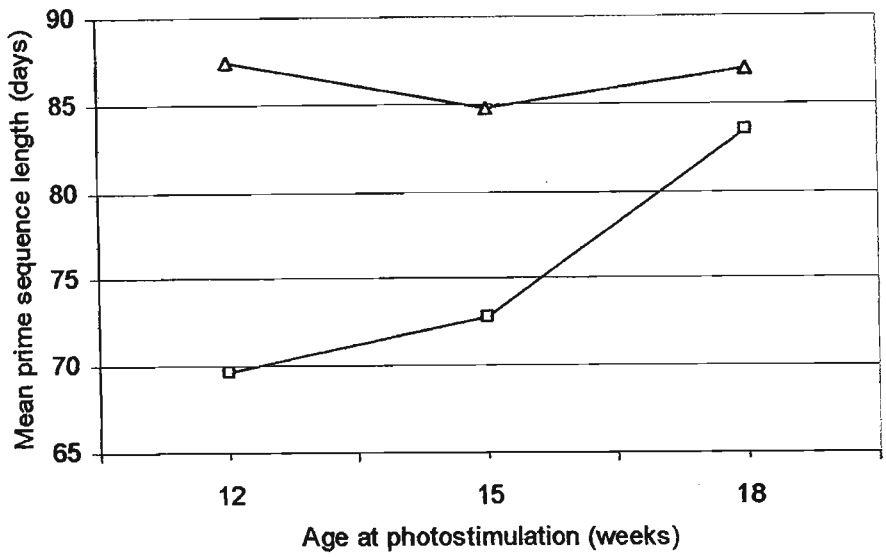


Figure 3.31: The relationship between mean prime sequence length and age at photostimulation for the Hy-Line Silver (Δ) and Hy-Line Brown (□) hens

During the running of stage two of the trial the huge variation between hens became evident. For example, hen number 12 from room two (Hy-Line Silver , 15-week photostimulation) laid twelve eggs in six months. At 30 weeks of age, when most hens reached their peak rate of lay, she produced a one-egg and a two-egg sequence followed by an 82-day pause. Her performance was erratic from the start; she laid 15 eggs during the first eight weeks of stage one with a prime sequence of four eggs. She exhibited a red and fleshy wattle and comb and her pubic bone spread was normal for a hen in lay. Perhaps the only indication of her poor productivity towards the end of the trial was her excellent feather cover. It may well be that this hen had a dysfunctional infundibulum and was an internal layer throughout because during stage one of the trial, when oviposition times were recorded, eight internal ovulations were suspected. In contrast hen 8 from room five (Hy-Line Silver, 15-week photostimulation) deserves special mention, because she produced 371 eggs in 375 days, with four single pauses, five sequences, a prime sequence length of 251 eggs and a mean sequence length of 74.2 eggs. She commenced her laying cycle with a three-egg sequence followed by an 18-egg sequence (interrupted by a possible internal ovulation) and a 98-egg sequence. There was an unexplained interruption at 35 weeks, when a one-egg sequence was recorded. Her prime sequence started

at 35 weeks of age and ended at 71 weeks. In a non-limiting environment this hen may well have laid an egg a day throughout the first laying year, after the initial short sequences.

The extent of the variation between individuals in the flock is highlighted by looking at the minimum and maximum values of some of the variables. Prime sequence length, for example, ranged from 4 to 251 days; mean sequence length from 1.42 to 97.0 days and mean pause length from 1.0 to 8.56 days in the Silver strain. Corresponding ranges in the Hy-Line Brown birds were 6 to 219 days, 1.92 to 74.0 days and 1.0 to 5.25 days. If those minimum values are a cause for concern, it must be remembered that the laying data for 12.8% of the very poor producers (37 hens) have been excluded from this analysis!

Because of these huge variations within the flock, the data for each strain per light treatment were then sorted in descending order according to mean sequence length and the flock was subsequently divided into thirds. For example, there were 39 Hy-Line Silver hens from the 12-week photostimulation group, which gave three groups of 13 birds. The third of the flock with the longest mean sequence lengths was referred to as the upper third. The next 13 hens made up the middle third. The lower third was derived from the bottom 13 hens, *i.e.* those with the shortest mean sequence lengths. The process of sorting and ranking was repeated on the data from the remaining two groups of Hy-Line Silver hens and on the Hy-Line Brown hens given increasing daylength at either 12, 15 or 18 weeks. Summaries are shown in Tables 3.34 to 3.36. From these it may be seen that within each treatment a considerable amount of variation between individual hens still exists.

Table 3.34: Mean sequence characteristics (±sd) for the birds photostimulated at 12 weeks, with the flock ranked according to mean sequence length and divided into thirds

	Hy-Line Silver			Hy-Line Brown		
Sequence characteristic	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Prime sequence length (days)	120.62 (±45.71)	82.77 (±38.61)	59.00 (±21.89)	95.69 (±31.31)	70.23 (±21.17)	45.07 (±19.73)
Number of sequences	7.23 (±2.65)	11.77 (±1.74)	20.54 (±6.86)	7.85 (±2.82)	13.00 (±2.48)	19.57 (±4.99)
Sequence length (days)	34.67 (±14.55)	17.60 (±3.08)	10.45 (±2.41)	31.05 (±15.55)	15.46 (±1.77)	9.17 (±2.41)
Pause length (days)	1.72 (±1.00)	1.76 (±1.61)	1.59 (±0.71)	1.50 (±0.56)	1.46 (±0.39)	1.56 (±0.51)
Sample size	13	13	13	13	13	14

Table 3.35: Mean sequence characteristics (±sd) for the birds photostimulated at 15 weeks, with the flock ranked according to mean sequence length and divided into thirds.

	Hy-Line Silver			Hy-Line Brown		
Sequence characteristic	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Prime sequence length (days)	136.46 (±48.57)	75.14 (±20.37)	46.21 (±27.20)	98.64 (±32.75)	68.67 (±21.15)	53.00 (±25.73)
Number of sequences	5.00 (±1.68)	9.07 (±1.59)	18.79 (±5.47)	6.79 (±2.08)	10.73 (±1.44)	19.53 (±9.35)
Sequence length (days)	51.80 (±22.57)	21.55 (±3.40)	10.07 (±3.83)	33.22 (±14.73)	17.75 (±1.96)	10.44 (±3.17)
Pause length (days)	1.59 (±1.03)	1.91 (±0.86)	1.79 (±1.97)	1.67 (±1.14)	1.36 (±0.71)	1.63 (±1.01)
Sample size	13	14	14	14	15	15

Table 3.36: Mean sequence characteristics (±sd) for the birds photostimulated at 18 weeks, with the flock ranked according to mean sequence length and divided into thirds

	Hy-Line Silver			Hy-Line Brown		
Sequence characteristic	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Prime sequence length (days)	114.86 (±34.68)	90.29 (±30.02)	57.93 (±21.84)	120.08 (±40.76)	85.50 (±27.22)	47.36 (±19.40)
Number of sequences	4.71 (±1.44)	9.00 (±1.47)	16.27 (±3.49)	5.62 (±1.19)	8.57 (±1.45)	17.50 (±9.07)
Sequence length (days)	47.27 (±20.79)	21.53 (±3.52)	11.67 (±2.71)	36.59 (±9.16)	22.06 (±3.19)	11.79 (±3.84)
Pause length (days)	1.56 (±1.35)	1.19 (±0.31)	1.23 (±0.25)	2.06 (±1.43)	1.20 (±0.24)	1.23 (±0.21)
Sample size	14	14	15	13	14	14

Figure 3.32 shows the mean sequence lengths each week to 44 weeks of age for the Hy-Line Silver hens photostimulated at 12 weeks of age, for the upper, middle and lower thirds of the flock. The high producing hens had longer sequences from onset of lay, commencing with a mean sequence length of 70.26 days compared to 4.90 and 13.01 days for the middle and lower thirds respectively. The longer mean prime sequence length meant that mean sequence length could be maintained at a higher level for a longer period of time.

Figures 3.33 to 3.37 show the changes in mean sequence length with advancing age for the Hy-Line Silver hens photostimulated at 15 and 18 weeks and for the Hy-Line Brown hens photostimulated at 12, 15 and 18 weeks respectively. Of interest is the remarkable difference in mean sequence length between the top third of the flock and the other two thirds brought to light by Figure 3.33. For about 17 weeks these birds maintained a mean sequence length above 120 days. In contrast, the middle and lower thirds of the flock had unexpectedly low mean sequence lengths over time. This observation helps to explain why the sequence characteristics of the Hy-Line Silver birds did not respond to changes in the age at photostimulation in a predictable fashion.

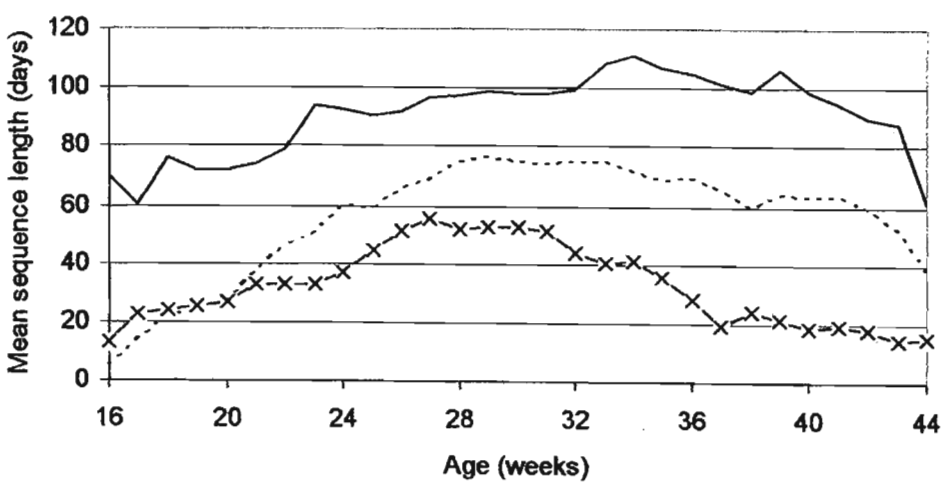


Figure 3.32: Mean sequence lengths for the Hy-Line Silver hens photostimulated at 12 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

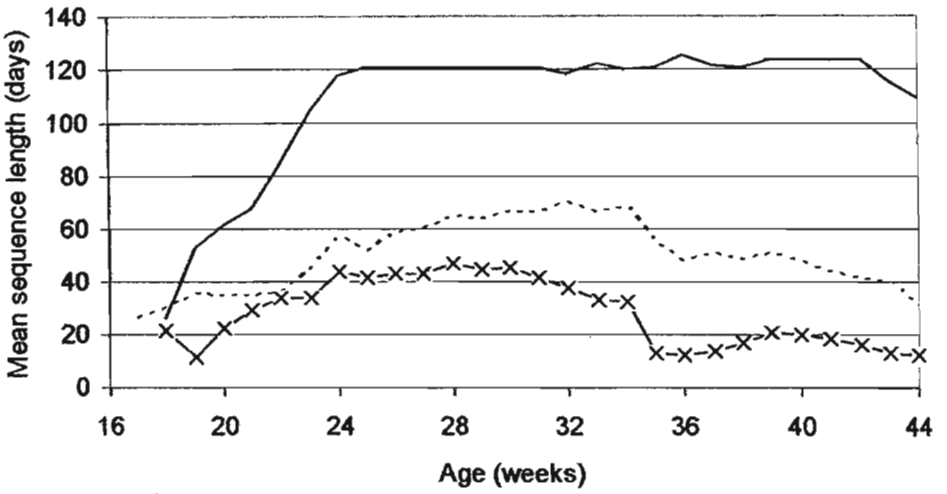


Figure 3.33: Mean sequence lengths for the Hy-Line Silver hens photostimulated at 15 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

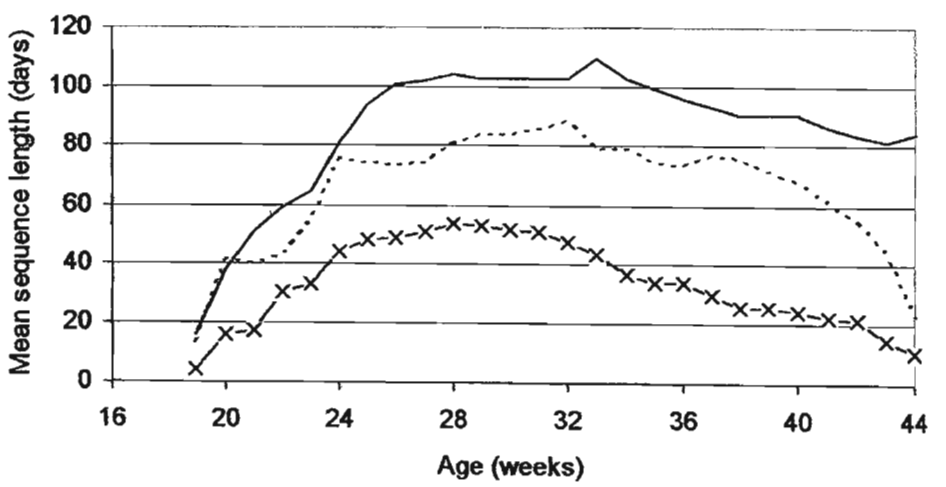


Figure 3.34: Mean sequence lengths for the Hy-Line Silver hens photostimulated at 18 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

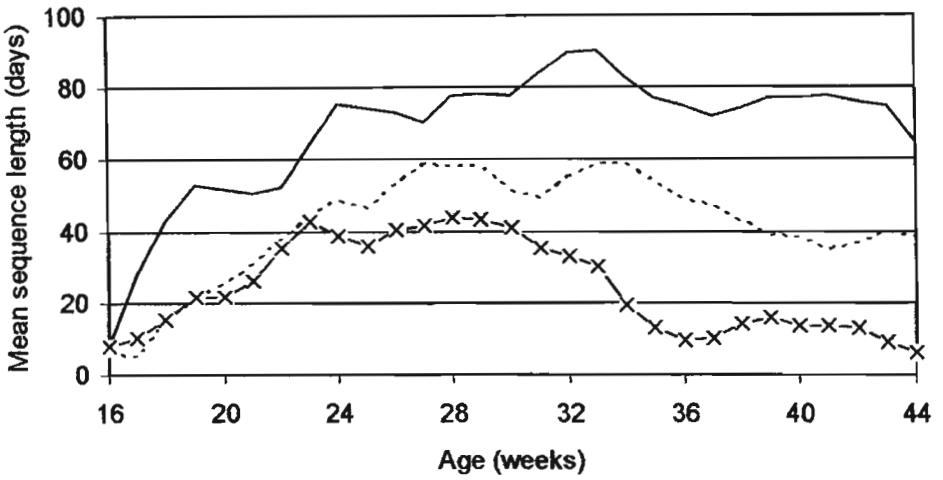


Figure 3.35: Mean sequence lengths for the Hy-Line Brown hens photostimulated at 12 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

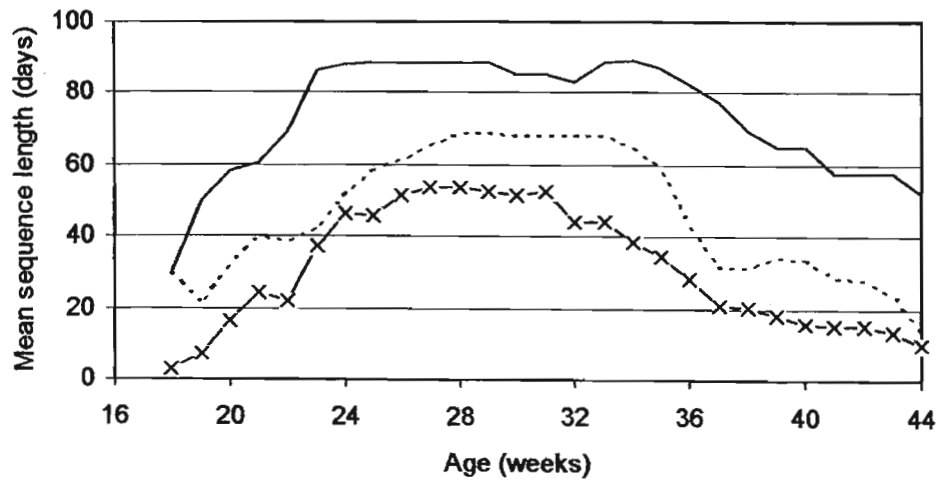


Figure 3.36: Mean sequence lengths for the Hy-Line Brown hens photostimulated at 15 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

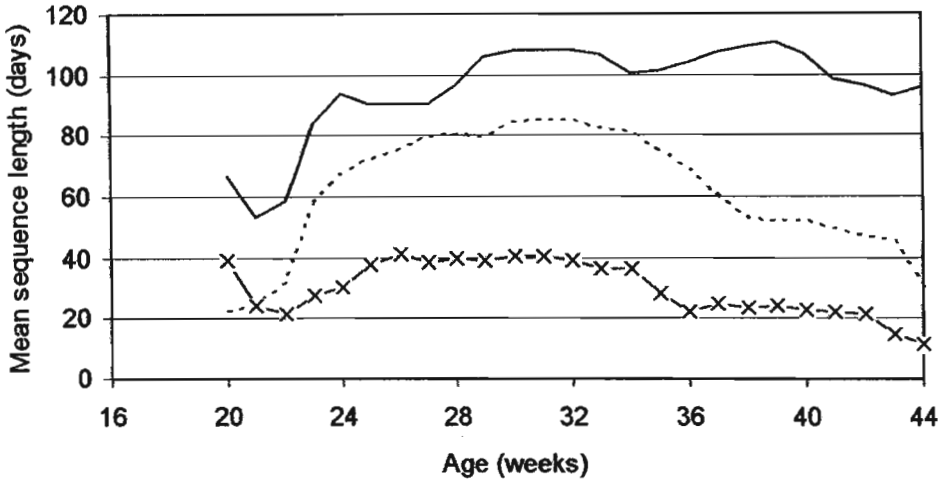


Figure 3.37: Mean sequence lengths for the Hy-Line Brown hens photostimulated at 18 weeks; top third (solid line), middle third (dotted line) and bottom third (x) of the flock

Figures 3.38 and 3.39 show the distribution of mean sequence lengths for the Hy-Line Silver and Hy-Line Brown hens respectively. For both varieties the largest groups of hens belonged to the class with an interval of 10 to 19.9 days, *i.e.* a class mark of 15.

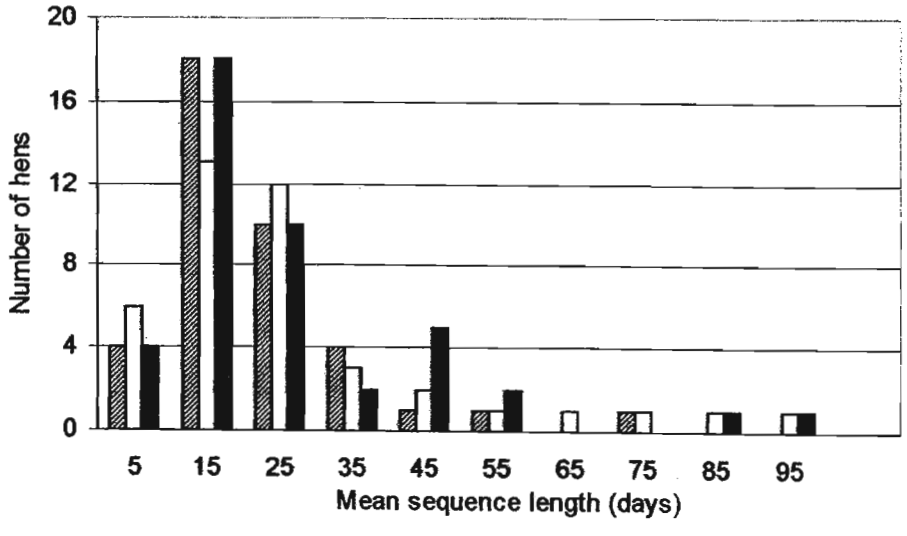


Figure 3.38: Histogram showing the distribution of mean sequence lengths for the Hy-Line Silver hens photostimulated at 12 weeks (hatched column), 15 weeks (blank column) and 18 weeks (solid column)

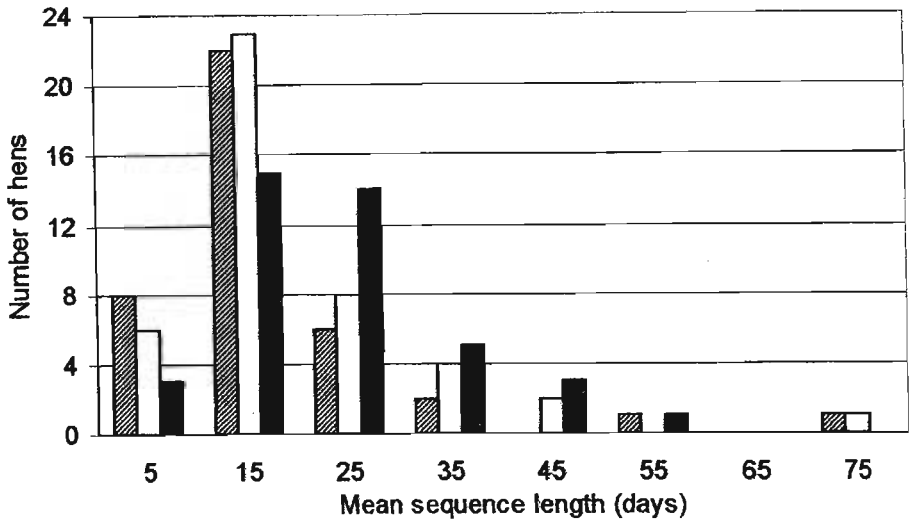


Figure 3.39: Histogram showing the distribution of mean sequence lengths for the Hy-Line Brown hens photostimulated at 12 weeks (hatched column), 15 weeks (blank column) and 18 weeks (solid column)

Four Silver birds from the 15-week photostimulation treatment had mean sequence lengths falling into the categories with class marks of 65 or longer, providing further evidence of why the trend in mean sequence length for the Hy-Line Silver hens photostimulated at different ages was not as expected. Obviously outliers such as these have a considerable influence on the means of variables that are recorded as counts. Conversely, it is surprising that none of the Hy-Line Brown hens photostimulated at 18 weeks fell into the classes with longer mean sequence lengths.

3.4.13 Inclusion of internal ovulations in sequence analysis

At this stage the Sequence Analyzer program was rerun, including the presumed internal ovulations in the laying data. The code C (normally reserved for shell-less eggs) was used to represent an internal ovulation. Tables 3.37 and 3.38 summarise the findings for the two strains. Although it would appear that the mean number of internal ovulations per hen is influenced by the age at photostimulation, it must be remembered that, owing to the different ages at sexual maturity for the three light treatments, the numbers of internal ovulations were counted over different periods of time.

If Table 3.37 is compared with Table 3.32 it may be seen that including the internal ovulations in the Hy-Line Silver data meant that the mean prime sequence length increased by 1.03 days. There was a decrease of 0.57 in the mean number of sequences. Mean sequence length increased by 1.04 days whereas the mean pause length increased by 0.13 days. This latter would be because in most cases internal ovulations replaced one-day pauses.

Similar results are obtained for the Hy-Line Brown data by comparing Tables 3.38 and 3.33. Mean prime sequence length increased by 0.28 days; the mean number of sequences decreased by 0.36; mean sequence length and mean pause length increased by 0.82 days and 0.04 days respectively.

Table 3.37: Summary of mean sequence characteristics for the Hy-Line Silver hens, including internal ovulations

Age at photostimulation				
Sequence characteristic	12 weeks	15 weeks	18 weeks	Mean
Prime sequence length (days)	87.97 (±43.04)	87.46 (±50.87)	86.86 (±37.20)	87.41 (±43.61)
Number of sequences	12.10 (±6.60)	10.58 (±6.36)	9.98 (±5.45)	10.85 (±6.15)
Number of internal ovulations	1.33 (±1.32)	1.02 (±1.41)	0.35 (±0.69)	0.89 (±1.24)
Sequence length (days)	23.13 (±15.64)	27.11 (±20.20)	27.53 (±20.70)	26.00 (±19.01)
Pause length (days)	1.86 (±1.40)	1.87 (±1.84)	1.45 (±1.53)	1.72 (±1.60)

Table 3.38: Summary of mean sequence characteristics for the Hy-Line Brown hens, including internal ovulations

Age at photostimulation				
Sequence characteristic	12 weeks	15 weeks	18 weeks	Mean
Prime sequence length (days)	71.33 (±32.05)	72.89 (±31.91)	82.68 (±41.77)	75.60 (±35.55)
Number of sequences	12.73 (±6.22)	12.32 (±7.38)	10.63 (±7.40)	11.90 (±7.04)
Number of internal ovulations	1.13 (±1.22)	0.55 (±0.87)	0.27 (±0.59)	0.64 (±0.99)
Sequence length (days)	20.81 (±13.50)	20.73 (±13.57)	23.06 (±11.43)	21.52 (±12.82)
Pause length (days)	1.57 (±0.56)	1.56 (±0.97)	1.51 (±0.93)	1.55 (±0.84)

These improvements in sequence characteristics need to be viewed in the context of the period during which they were estimated. Had it been feasible to record oviposition times for each hen over the entire trial period, thereby enabling the identification of birds that ovulated internally on an ongoing basis, the effect of these internal ovulations on sequence characteristics may have been more pronounced.

3.4.14 Consistency of lay

In order to determine whether sequence lengths of individual hens increased and then decreased in a regular manner in line with flock means, the sequence lengths for each Hy-Line Brown bird photostimulated at 18 weeks of age were studied. This treatment was chosen because the Hy-Line Brown strain produced fewer soft shells and supposed internal ovulations that would interfere with the pattern of lay. Furthermore, the sequence lengths would not have been influenced by premature photostimulation. Table 3.39 shows the individual sequence lengths for each hen, in the order that they were recorded over the trial period.

Table 3.39: Recorded sequence lengths for individual Hy-Line Brown hens photostimulated at 18 weeks of age

Hen no.	Room 3	Room 6
25	6 93 1 39 15 1 3 7 6 9 11	10 118 22 18
26		2 87 2 1 22 35 53
27	1 18 72 1 52 18 26	
28	52 4 30 2 4 1 1 3 20 4 3 5 9 12 12 2 7 8 8	1 9 6 1 1 3 1 2 1 3 5 2 5 1 20 19 1 1 10 1 8 1 5 1 10 4 6 1 6 12 3
29	40 69 40 3 2 5 7 8 11 7	11 1 1 2 9 13 45 1 57 17 13
30	10 1 1 87 1 144	13 7 101 17 15 23 8
31	1 1 2 2 14 21 20 21 15 17 11 13 10 3 7 8	118 24 19 3 4 4 5 4
32	57 78 24 2 26	1 3 39 128 4 4
33	1 20 49 19 18 11 9 14 6 4 7 8 4 2 6	29 12 8 8 12 2 7 2 5 2 4 3 1 1 4 3 5 1 3 2 3 2 3 2 2 1 2 2 1 1 3 2 1 1 1 2 1 1 1 1 1 1 2
34	3 9 148 4 4 4 4 8	3 3 14 4 33 5 101 24 4
35	3 168 9	27 30 13 22 8 1 4 1 37 18 7 5
36	1 6 77 2 2 23 45 18	3 3 2 2 13 5 14 12 35 12 4 6 15 18 10 12 18
37		16 30 24 26 16 32 27
38		84 1 9 1
39	98 53 15 10 8 10	12 4 81 8 6 12 5 12 32 18
40	5 5 1 27 34 37 7 22 19 12 1 6 5	20 47 35 6 3 21 8 10 12 4 5 5 2 3 3 5
41	1 1 1 1 2 219	2 3 41 131 5 1
42	5 12 80 14 8 14 7 8 7 5 7 6 5 5 1	85 70 26 3 9
43		1 20 6 9 66 42 26 2 2 3 10

Hen no.	Room 3	Room 6
44	10 5 76 55 14 11 15 2 5	1 49 31 9 1 11 46 11 6 4
45	1 31 79 24 12 12 7 6 7 3 4 4 6	
46	13 1 67 3 73 5 16 1	9 23 45 1 1 2 107
47	6 1 2 1 1 4 3 71 5 1 45 16 26	1 19 3 92 51 9 11
48	5 1 14 27 70 42 27	

Several interesting observations can be made. Some birds (hen 39 room three; hens 31 and 42, room six) did commence laying with a prime sequence, which was followed by gradually shortening sequences. Others laid a number of shorter sequences before the prime sequence started (hens 41 and 47 room three; hen 34 room six). Of great significance is the lack of consistency in the way that sequence lengths changed over time. There seems to be very little order in the manner in which maturing hens approach reproductive decline. Since the ovulatory model predicts a gradual change in the various parameters with advancing age, it is not well suited to dealing with irregular situations.

The mean age at commencement of the prime sequences was 177.9 days (25.4 weeks) with a range of 135 – 262 days (19.3 – 37.4 weeks). These findings do not concur with the theory of Robinson *et al.* (1990), who proposed that amongst individuals the prime sequence is always initiated at a similar chronological age. Moreover, the premise that individual hens have a high degree of consistency in their patterns of egg laying after peak (Lillpers and Wilhelmson, 1993a) also seems to be disproved. Several birds from this treatment laid both long and short sequences after the prime sequence (hen 36 room three; hen 26 room six). Erratic variables are of course much harder to model successfully, unless a reasonable explanation can be found for their behaviour as well as a method of dealing with them.

3.5 Discussion

Because of its very nature, sequence length is a difficult variable to monitor with precision. It relies on the meticulous recording of the presence or absence of a daily egg and as such, one

needs to be aware of the importance of properly gathering what appears to be simple data. A 'missing' egg will be interpreted as a pause day and depending on where it occurs, this may have huge implications for the calculation of sequence length. Sequence length is in essence a count, not a measurement and so unlike other variables, such as egg weight, there cannot be a statistical procedure to make allowances for missing data. If sequence lengths vary considerably over the laying period, the means for individual hens will also be vastly different. Treatment means will thus also vary although to a lesser degree; hence the likelihood of establishing any statistical significance in the differences between treatment means is diminished. The standard deviations about the mean sequence lengths are often very large, which means that the coefficients of variation are unlikely to be as low as 10%. In this trial the coefficients of variation ranged from 50.6 to 79.8%. For mean prime sequence length, the coefficients of variation ranged from 42.6 to 59.1%.

The experiment was not intended to be a dose response trial, simply because the available pullet rearing facility only contained six light-tight rooms. Accordingly the experimental design was restricted to three lighting treatments and two replicates and the statistical analyses were done using the split-plot method. For the purposes of modelling, it is always useful to quantify relationships between predictor and response variables. Attempts were made to fit linear regressions, some of which were successful, but it was not possible to perform non-linear regressions of the variables on age at photostimulation. There were significant linear regressions of mean age at first egg and mean egg weight on age at photostimulation for the Hy-Line Silver hens and of the mean number of double yolks on age at photostimulation for the Hy-Line Brown hens. Nevertheless, in most instances curvilinear functions would be a more logical choice for predicting the response of the variables to delayed age at photostimulation.

Age at sexual maturity can be predicted more accurately using the model published by Lewis *et al.* (2002). The authors used the results of twelve experiments sourced from the literature, as well as some unpublished data, and two further trials were conducted to provide specific information. Two coefficients are used to adjust for genotype, to allow for different responses to constant daylength and differences in the rate at which age affects the response to a single change in photoperiod. Age at first egg is expressed as a function of mean photoperiod and change in photoperiod during rearing. An attempt will be made to integrate their model into

the population ovulation model to be developed in a following chapter.

The most startling observation to come out of the trial was the significant number of hens that either failed to come into lay by 23 weeks of age or that had very poor egg production performances during the year. Although a large amount of variation is expected within a flock, the laying performance of some of the individuals was disturbing. It seems likely that this is due to genetic rather than environmental factors, in view of the fact that all birds were reared under similar conditions. Identification and subsequent research into the mutant restricted ovulator gene show that the hallmark phenotypic traits are extremely low egg production, an ovary containing an abundance of small yolk-filled follicles, the absence of a follicular hierarchy, hyperlipidemia and aortic atherosclerosis (Elkin *et al.*, 2003). This results from a mutation in the gene specifying the very low density lipoprotein receptor, whose protein product normally mediates massive oocyte uptake of egg yolk precursors from the circulation. Consequently hens carrying the restricted ovulator gene have yellow, lipemic plasma. Imbalances in estrogen and progesterone levels are also observed. Due to the absence of a large preovulatory follicle, whose granulosa cells possess receptor sites for LH, a progesterone surge is absent and ovulation does not occur. Elkin *et al.* (2003) found that their restricted ovulator hens had an average hen day production of 0.5% and not one of these hens laid more than five eggs in 294 days. There is no direct evidence to suggest that the Hy-Line birds used in this trial carried the restricted ovulator gene; this is merely put forward as a possible explanation for the exceptionally poor laying performances of some of the hens. The breeding company would be wise to carry out investigations in view of the fact that a method exists today for rapid identification of the restricted ovulator birds.

An alternative possibility is that these low-producing hens are common in laying flocks but that their presence is not acknowledged. It is probable that, for short-term experiments, researchers select a sample of hens that are laying at an acceptable rate, or that birds with unsatisfactory performances are removed during the course of the trial. It is understandable that with the large number of birds housed today, commercial poultry producers are not able to routinely inspect individuals. However, removing non-layers or sub-standard layers from a flock can

improve rate of lay by two to three percent (Murray Jackson, personal communication [4]). An indirect reference to poor laying performance was made by Robinson *et al.* (1990), when reporting inter-sequence pause lengths of 1.6 days in broiler breeder hens. It follows that there were a number of occasions when pauses of two or more consecutive days were recorded. In a study by Lewis and Long (1992), 0.21% of hens did not lay an egg between the ages of 30 and 34 weeks. The causes of non-laying were neoplasia, sex reversal, abnormal ovary or oviduct, internal laying or poor condition. The incidence of neoplasia was influenced by the genotype. A number of birds that had not achieved sexual maturity by 27 weeks of age, had come into lay by 34 weeks. The incidence of non-laying was thought to have been underestimated, owing to the short duration of the observation period.

The results of this trial confirm the findings of Lewis and Perry (1991) that hens often commence their laying cycles by producing short sequences. This is partially determined by the time of lay of the first egg at sexual maturity; a one-egg sequence will result from a first egg laid late in the afternoon. Many hens do produce a number of shorter egg sequences before launching into their prime sequence. Some of these may be prematurely terminated by soft-shelled or double-yolked eggs, since it has been established that a number of pause days in mid-sequence are associated with the production of eggs with either soft shells (31%) or double yolks (9%). In order to improve rate of lay at the onset of egg production, hens need to be photostimulated at the recommended age for the breed, because this will substantially reduce the incidence of both double yolks and soft-shelled eggs (Lewis *et al.*, 1997; Christmas and Harms, 1982). If birds are to be given increasing daylength at an early age, a pre-lay diet, with a higher calcium content than found in pullet developer diets, may help to reduce the incidence of soft shells.

Given that the lack of medullary bone in point-of-lay pullets is unlikely to affect shell quality because calcium can be resorbed from structural bone (Whitehead, 2004), the frequent occurrence of soft-shelled eggs from pullets that are brought into lay at a young age is probably due to hormonal incompetence. If the failure of calcium-regulating mechanisms in old hens is due to a reduction in estrogen receptors in various tissues (Hansen *et al.*, 2003), then it may be

4 M. Jackson, Sapuma Eggs, P.O.Box 67, Verulam, 4340, South Africa

that physiologically immature pullets given stimulatory daylengths also have insufficient estrogen receptors. Calcium homeostasis in the laying hen is known to be regulated by estrogen, calcitonin, parathyroid hormone and 1.25-dihydroxyvitamin D_3. Presumably normal shell formation relies on a finely-tuned mechanism that may be absent in young point-of-lay pullets and older hens reaching the end of their productive cycle.

The commencement of lay with shorter egg sequences is hard to understand in terms of the theory of Emmans and Fisher (1986). It seems unlikely that a hen's initial internal cycle length may be greater than 24 hours at onset of lay, then decrease to below 24 hours for a period before lengthening again. If a point-of-lay pullet has the ability to ovulate and lay an egg every day for a prolonged period, the two systems of LH release and follicle maturation must be well synchronised. Yet if a bird produces a three-egg sequence at onset of lay with oviposition times of 09:00, 13:00 and 14:30 (as did hen 8 from room five) before laying sequences of 18, 98, 1 and 251 eggs) this suggests that the follicles are initially taking longer than 24 hours to mature. There may be insufficient amounts of FSH to stimulate rapid growth of the large yellow follicles in the hierarchy at onset of sexual maturity, or the progesterone feedback mechanism to stimulate the release of the preovulatory surge of LH may not be properly established. Whatever the underlying cause, the easiest way to approach this problem in modelling would be to adapt the theory of Emmans and Fisher (1986) and use a curve for the hen's internal cycle length. This aspect will be covered in Chapter 5.

In general, poultry producers are not aware of internal ovulations taking place unless nesting behaviour of individuals is observed or times of lay are recorded. Internal ovulations disrupt egg sequences, reduce the mean sequence length and increase both the number of sequences and pauses. More important to the commercial producer, there is a loss of income when the ovulation rate is higher than the rate of lay. In this trial the number of internal ovulations may have been underestimated by assuming that some of the pauses in mid-sequence were due to the production of soft-shelled and double-yolked eggs. Also, for the greater part of the trial oviposition times were not recorded, so there was no way of knowing how many of the poor producers were continually ovulating internally. If the hens prone to internal ovulations could be identified and removed from the flock, the rate of lay would improve and feed cost per dozen eggs would be reduced. If there is a genetic component, where a particular strain is

seen to have a significant difference between ovulation rate and rate of lay, then the geneticists need to be aware of this. Although statistical analyses were not done, it would appear that birds photostimulated at the recommended age of 18 weeks are less likely to exhibit internal laying. Presumably this is because there is sufficient time for the infundibulum to mature and there is better synchrony between ovary and oviduct. The Hy-Line Brown hens appeared to have fewer internal ovulations than the Silver strain, so it may be that breeds or strains differ in their ability to synchronise the development of ovary and oviduct at the onset of lay. An ovulation model needs to account for genetic differences and different responses to environmental stimuli in the incidence of internal ovulations.

Rate of lay is further reduced by the appearance of double-yolked and soft-shelled eggs if the associated laying sequences are interrupted. These deviations from the production of normal, single-yolked, hard-shelled eggs are a common occurrence in commercial flocks and so need to be included in the simulation model. Three aspects need to be considered. Firstly, an estimate of the proportion of the flock that can be expected to ovulate internally or to lay a double-yolked or soft-shelled egg is required. This is because not all individuals are prone to these irregularities; many hens presumably have a rate of lay equal to their ovulation rate. Secondly, the distribution of each variable within that proportion of the flock needs to be described. For all three variables under discussion, a positive J-shaped distribution probably best describes the relationships. The majority of hens will have only a single occurrence of the deviation and a gradually decreasing number of birds will have an increasing number of multiple occurrences. Finally, the responses of the mean number of soft shells, double yolks or internal ovulations to changes in the age at photostimulation should be described by a curvilinear function. Linear equations (with negative slopes) are inadequate because they imply that at and beyond a certain age at photostimulation the incidence of the variable is zero. Similarly they predict that the incidence will continue to increase as age at photostimulation approaches zero. Since both of these predictions are nonsensical, linear equations are not satisfactory. Better predictions would be given by either generalised logistic or Gompertz ($b>0$) equations, which produce curves that tend towards asymptotes at the extreme values of age at photostimulation.

The production of sequences of varying lengths over the course of time may be common in a population of hens, or it may be influenced by the environment. Perhaps it is idealistic to

assume that sequence length gradually declines after peak, in the manner predicted by the ovulation model. In a non-limiting environment, the change in sequence lengths over time may well be more regular. If environmental stresses, such as relocation of birds, possible water or feed shortages, emptying and cleaning of feed troughs, manure removal, outside noise or temperature extremes, affect pituitary function then egg production may be disrupted unexpectedly. It is not known how sensitive the circadian rhythm of LH release is to external stress factors, as little research seems to have been done on the subject. If LH is not released on a particular day ovulation will not take place, even though a mature follicle is present, and the sequence will be terminated. Similarly, if FSH secretion is suppressed in anxious birds, follicle growth and recruitment into the hierarchy may be impaired for a period. Gaps in the hierarchy will result in the absence of a mature follicle on a particular day, even though there is a preovulatory LH surge, so once again there will be no ovulation. Long sequences with infrequent pauses depend on the optimum functioning of all facets of the reproductive system. A great deal of thought needs to be given to finding a method of simulating irregular sequence lengths over time for individual hens.

One of the shortcomings of the ovulation model is that it does not accommodate pauses longer than one day in length. The mean pause lengths were 1.59 and 1.51 for the Silver and Brown birds respectively. If the theory of a circadian rhythm interacting with follicle maturation is acceptable, then one needs to understand how these long pauses are brought about. One plausible method is that conditions of stress may interrupt the hypothetical circadian rhythm, thereby preventing the release of LH from the pituitary. If the bird has no preovulatory surge of LH for a number of days, ovulation would not occur during that period. Alternatively, there may be a disruption to the orderly recruitment into and maintenance of the follicular hierarchy, for reasons such as inadequate FSH secretion, atresia of large yellow follicles, double ovulations or dietary imbalances. Research done by Chowdhury and Yoshimura (2003) suggests that pituitary functions may be altered by feed regulation, because circulating levels of growth hormone and thyroid-stimulating hormone were seen to increase whereas levels of prolactin decreased during feed withdrawal. Furthermore, Sharp (2002) found that depression of plasma prolactin levels was associated with decreased LH secretion as well as a reduction in the rate of lay. It is therefore quite plausible that the secretion of gonadotropins from the anterior pituitary may be affected by environmental conditions, such as a stress factor. A

method of introducing longer pauses into the ovulatory model will need to be devised.

The two varieties of the Hy-Line strain used in the trial obviously differed in terms of their growth and laying performance. The Silver birds had a heavier body mass during rearing and achieved sexual maturity earlier than the Brown hens. Perhaps as a consequence of this, they were more likely to lay soft-shelled eggs or to ovulate internally. The Silver birds tended to lay longer prime sequences, to produce fewer but longer sequences and to have fewer pauses. Their mean egg weights were lower, but yolk weights tended to be heavier than those from the Hy-Line Brown hens. The responses of the two varieties to age at photostimulation also differed, although not always in a predictable manner. The increases in mean sequence length and mean prime sequence length in Hy-Line Brown hens with a delay in age at photostimulation were more in line with the published research findings of Robinson *et al.* (1996b). In most cases the trends in sequence characteristics need to be acknowledged, even though the treatment differences were not shown to be statistically significant.

A successful population model needs to take different genotypes into account and to be able to simulate the performance of a laying flock in a realistic fashion. A great deal of useful information has been gathered during the course of this trial and the challenge will be to utilise it to good effect. The means and distributions of the variables predicted by the model may be compared to means and distributions measured in the experimental hens as a method of endorsing the model assumptions.

Chapter 4

MODELLING THE CHANGES IN THE PROPORTIONS OF THE EGG COMPONENTS DURING THE LAYING CYCLE

4.1 Introduction

In a layer model egg weight may be predicted as a whole entity or as the sum of the weights of the three components - yolk, albumen and shell. It is far more challenging to model each component separately in view of the fact that the proportions change with hen age and egg weight (see Section 1.3). At a fixed hen age and over a range of egg weights, the weights of all three components increase as egg weight increases, with albumen increasing at the expense of yolk and shell. As the laying cycle progresses the component weights also increase, but the percentage of yolk increases at the expense of albumen and shell. The chemical compositions of yolk, albumen and shell differ substantially which means that the hen may have different nutritional requirements during the day, depending on the stage of egg formation, as well as over the laying period. A population model that predicts the weight of each component separately would be of benefit to the modeller making a study of the hen's changing nutrient requirements.

Much of the research conducted during the past 50 years confirms the findings of Romanoff and Romanoff (1949) in terms of the proportional changes in the components with increasing egg weight and hen age. More recently the emphasis has shifted towards quantifying the various relationships. Linear functions which predict the yolk:albumen ratio from egg weight (Yannakopoulos *et al.*, 1998; Hussein *et al.*, 1993; Harms and Hussein, 1993); albumen and yolk weight from egg weight (Hussein *et al.*, 1993); and % albumen and % yolk from egg weight (Ahn *et al.*, 1997) have been published. Fletcher *et al.* (1983) produced multiple linear regression equations to predict component weight from egg weight and flock age for Shaver hens. By using surface response techniques they were able to show that increased yolk yield was equally dependent on increased egg weight and increased flock age. Increased albumen weight and shell weight were found to be positive

functions of egg weight, but their proportions were negative functions of flock age. By using both egg weight and age of the hens as predictors, the model fit improved significantly for all three components.

Most of the previous studies have taken the whole egg weight as the predictor variable and component weights as the response variables. Emmans and Fisher (1986) adopted a different approach in their proposed biological model for egg production. Rate of production of yolk material may be predicted from the equation

$$y = a\, e^{-ct} \exp - (\exp (Go - bt)) \qquad \text{(Equation 4.1)}$$

and mean yolk weight (MYW) is given by yolk output (y) divided by the rate of lay (R):

$$MYW = y / R \qquad \text{(Equation 4.2)}$$

Albumen and shell weights are then predicted by the use of allometric functions of the form

$$y = a.\, x^{b} \qquad \text{(Equation 4.3)}$$

Allometry refers to non-isometric scaling, *i.e.* changes in size of irregular-shaped organisms. Biologists accept that many morphological and physiological variables are scaled relative to body size, according to allometric equations. A useful property of allometric functions is that when the two variables x and y are plotted on logarithmic coordinates, the result is a straight line:

$$ln\, y = ln\, a + b.\, ln\, x \qquad \text{(Equation 4.4)}$$

The exponent b from Equation 4.3 represents the slope of the straight line obtained in a logarithmic plot.

Emmans and Fisher (1986) proposed that albumen and shell weights may be predicted from these functions:

$$AW = a_1 . YW^{b1} \quad \text{(Equation 4.5)}$$

and

$$SW = a_2 . ECW^{b2} \quad \text{(Equation 4.6)}$$

where AW = albumen weight, YW = yolk weight, SW = shell weight and ECW = the weight of the egg contents, yolk plus albumen. Egg weight is given by YW + AW+ SW.

Possible values for the coefficients were given by Johnston (1993) although a relatively small sample of eggs was used and the samples were selected on the basis of egg weight rather than hen age. A more thorough investigation was felt to be necessary to provide a substantial database of egg component weights.

The aims of this chapter are:

1. to examine the relationships between yolk, albumen and shell weights at different egg weights and hen ages;
2. to derive suitable values for the coefficients a and b in the two allometric functions.

4.2 Method used to determine egg component weights

Two methods of determining the weights of the egg components are reported in the literature. In the first, whole eggs are weighed, broken open and the yolk separated from the albumen. The albumen is discarded and the yolk is then rolled on damp paper towel to remove traces of albumen and the chalazae, before being weighed. The shell is washed carefully to remove the remnants of the albumen, without damaging the shell membranes, and dried at 21°C for 48 hours prior to weighing. Albumen weight is determined by subtracting yolk plus dry shell weight from whole egg weight (Hussein *et al.*, 1993; Harms and Hussein, 1993). The second method involves separating yolk and albumen and weighing both components (Yannakopoulos *et al.*, 1998; Marion *et al.*, 1964). The shell is

weighed wet, washed and air dried at room temperature and then weighed dry. The difference between the wet and dry shell weights is assumed to be albumen and is therefore added to albumen weight (Fletcher *et al.*, 1981). A comparison of the two methods was conducted by Fletcher *et al.* (1981), who found that the amount of albumen adhering to the yolk varied and thus adversely affected the coefficient of variation for both mean yolk and mean albumen weights. Since it is relatively easy to remove traces of albumen, plus the chalazae, on damp paper towel, the first method seems to be more reliable. In addition it is quicker to perform in the laboratory and is therefore preferred.

Samples of 100-120 fresh eggs were collected from a flock of young Amber-Link hens, housed in an open house in two-tier cages. Shortly after these measurements were made the Amber-Link bird was withdrawn from the South African market, but it is hoped that the conclusions drawn from this database will be applicable to other genotypes. Initial collections were made fortnightly from 20 to 32 weeks of age, due to the rapid increase in egg weights of young hens and presumably corresponding increases in the component weights. Once the changes had diminished, eggs were collected every four weeks to 40 weeks of age and thereafter every ten weeks to 70 weeks of age. A point was chosen at random in the house and all eggs in the row were collected from top and bottom tier, except for obviously dirty or cracked eggs, to avoid possible sampling bias. Double-yolked eggs were collected, broken open and weighed, but did not form part of this database.

Eggs were numbered on both ends with a permanent marker, weighed individually and stored overnight in a fridge at ± 6°C. The following morning whole eggs were weighed again to determine weight loss. Any egg that had an above average weight loss was discarded, as it was usually found to have a hairline crack. Each egg was then carefully broken open using a knife, to minimise shell loss through fragmentation. The yolk was separated from the albumen using a domestic egg separator; the albumen was discarded and the yolk rolled on damp paper towel, according to the method found by Fletcher *et al.* (1981) to be superior. Any adhering chalazae were removed from the yolk with the aid of tweezers. A number of yolk membranes ruptured to a greater or lesser degree during the procedure and these eggs were immediately discarded. Intact yolks were weighed. The two halves of the shell were carefully washed to remove traces of albumen, left to dry at room

temperature for 48 hours and weighed. Attempts were made to retain any minute pieces of shell which broke away from the membrane, although in general they did not influence shell weight recorded to 2 decimal points.

4.3 Results

Table 4.1 summarises the means and standard errors of the component weights of eggs collected at intervals over a period of fifty weeks. The table also presents the percentages of each component, expressed as a percentage of the whole egg, and the yolk:albumen ratio.

At a constant age and over the range of egg weights produced, the absolute weights of all three components - yolk, albumen and shell - increased as egg weight increased. Figures 4.1 to 4.3 show the trends for the 20-week data, but these observations were consistent across all ages. There was a close relationship between albumen weight and egg weight, as evidenced by the relatively narrow spread of data points (Figure 4.2). At a fixed age the percentage of albumen increased at the expense of yolk and shell (Figures 4.4 to 4.6). This means that amongst the eggs collected from a flock of laying hens on a particular day (assuming all hens are the same age), the larger eggs are likely to have proportionally more albumen. As the hens aged, the yolk:albumen ratio increased steadily, indicating the greater proportion of yolk contained in the eggs from older hens.

Table 4.1: Summary of component weights of eggs collected from Amber-Link hens over an extended period (n=sample size)

Age (wks)		Egg weight (g)	Yolk weight (g)	Alb. weight (g)	Shell weight (g)	Yolk %	Alb. %	Shell %	Y:A ratio
20	mean	41.50	9.00	28.44	4.06	21.75	68.48	9.77	0.319
n=91	±se	3.44	0.76	2.74	0.55				
	cv%	8.30	8.39	9.65	13.57				
22	mean	47.63	11.04	31.98	4.61	23.20	67.11	9.68	0.347
n=92	±se	3.21	0.95	2.58	0.43				
	cv%	6.74	8.58	8.07	9.37				
24	mean	51.86	12.39	34.60	4.86	23.96	66.66	9.38	0.360
n=94	±se	3.46	0.78	2.99	0.42				
	cv%	6.67	6.31	8.64	8.66				
26	mean	54.88	13.46	36.38	5.04	24.61	66.18	9.20	0.373
n=90	±se	4.27	0.93	3.69	0.46				
	cv%	7.78	6.92	10.14	9.15				
28	mean	55.61	13.98	36.56	5.08	25.20	65.66	9.14	0.385
n=89	±se	3.82	0.94	3.28	0.49				
	cv%	6.87	6.71	8.97	9.59				
30	mean	56.66	14.62	36.98	5.06	25.86	65.20	8.95	0.398
n=90	±se	3.60	1.11	3.14	0.38				
	cv%	6.36	7.56	8.49	7.60				

Age (wks)		Egg weight (g)	Yolk weight (g)	Alb. weight (g)	Shell weight (g)	Yolk %	Alb. %	Shell %	Y:A ratio
32	mean	58.44	15.40	37.79	5.25	26.40	64.59	9.01	0.410
n=94	±se	3.85	1.05	3.19	0.45				
	cv%	6.58	6.81	8.44	8.55				
36	mean	60.21	15.94	38.73	5.53	26.51	64.29	9.20	0.414
n=102	±se	3.53	1.22	2.88	0.46				
	cv%	5.86	7.67	7.45	8.36				
40	mean	61.32	16.43	39.52	5.37	26.83	64.39	8.77	0.418
n=89	±se	4.08	1.10	3.25	0.43				
	cv%	6.66	6.72	8.22	8.05				
50	mean	62.28	17.83	38.84	5.61	28.64	62.34	9.01	0.460
n=98	±se	4.21	1.35	3.08	0.49				
	cv%	6.76	7.56	7.93	8.78				
60	mean	64.23	18.59	40.02	5.62	29.00	62.25	8.75	0.468
n=86	±se	4.11	1.43	3.41	0.60				
	cv%	6.40	7.69	8.52	10.73				
70	mean	65.12	18.44	41.11	5.57	28.37	63.07	8.56	0.452
n=93	±se	4.58	1.47	3.83	0.61				
	cv%	7.03	7.98	9.32	10.99				

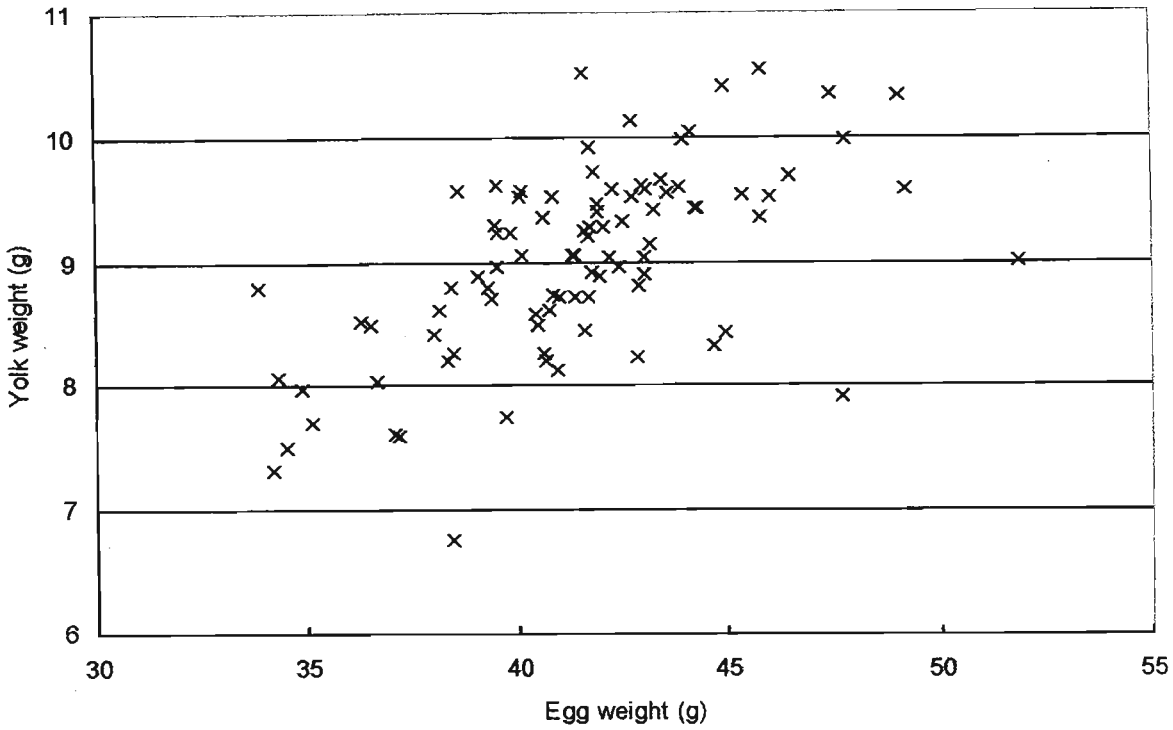


Figure 4.1: The positive relationship between yolk weight and egg weight at 20 weeks (r^2=0.60)

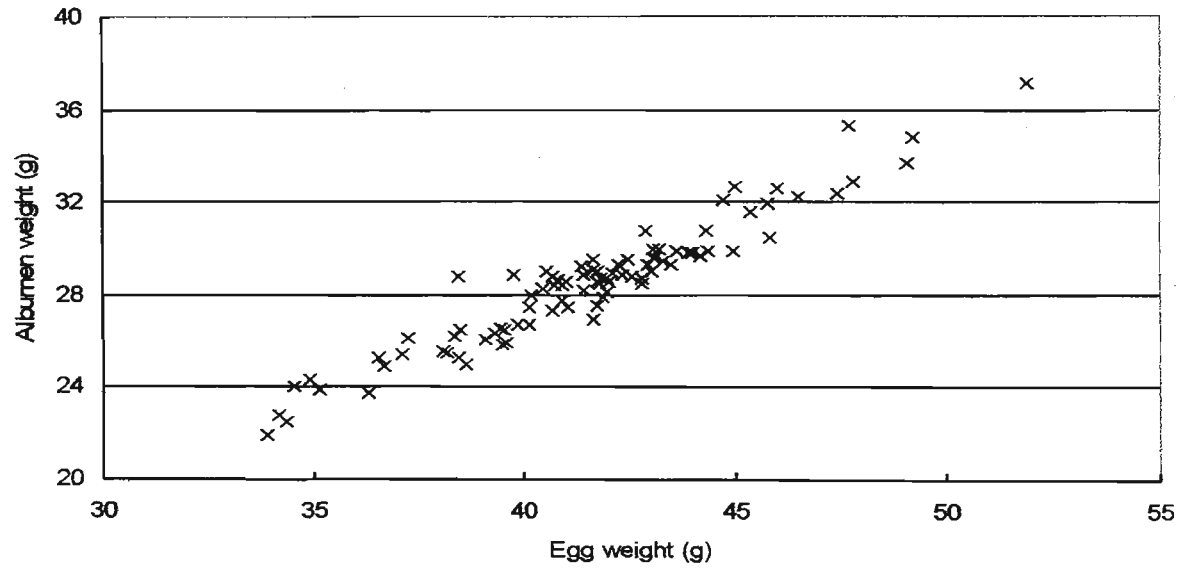


Figure 4.2: The positive relationship between albumen weight and egg weight at 20 wks (r^2=0.96)

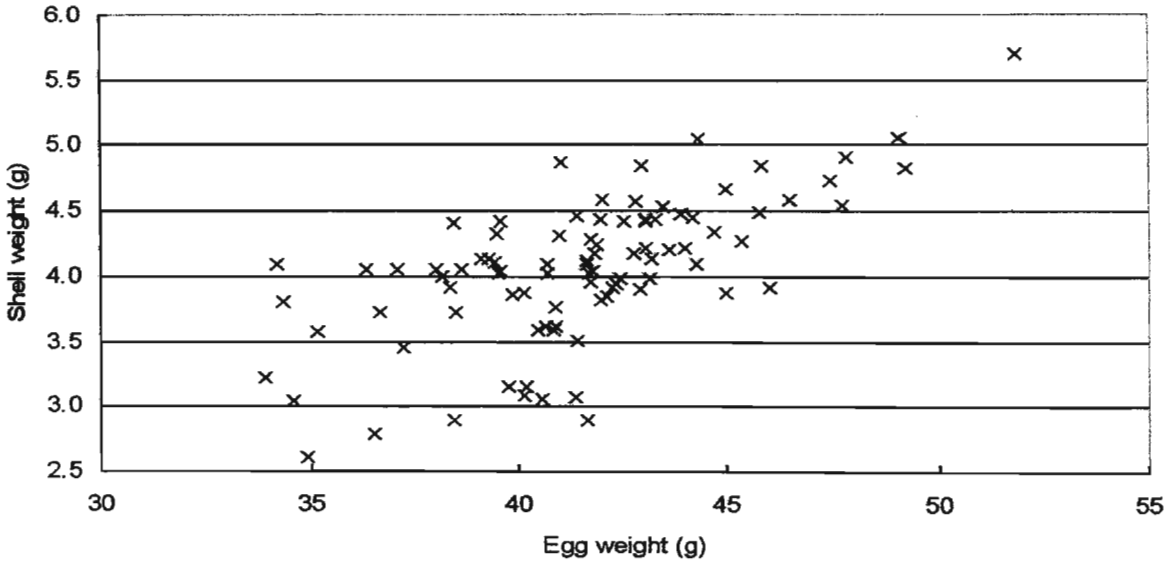


Figure 4.3: The positive relationship between shell weight and egg weight at 20 weeks (r^2=0.66)

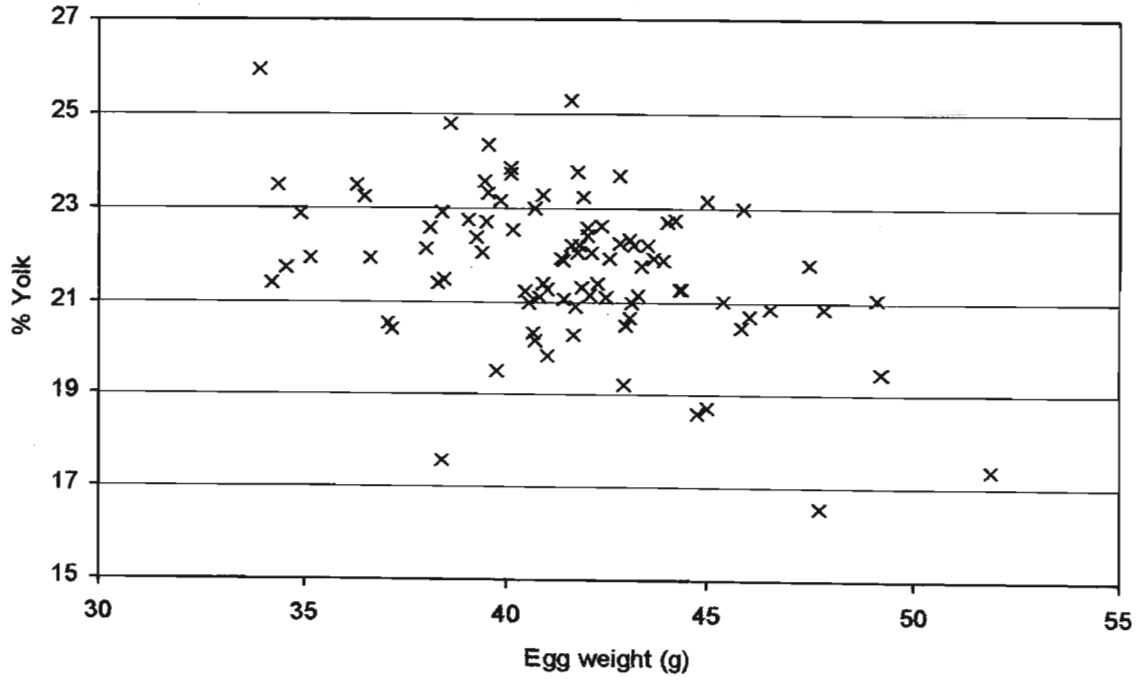


Figure 4.4: The negative relationship between % yolk and egg weight at 20 weeks (r^2=-0.43)

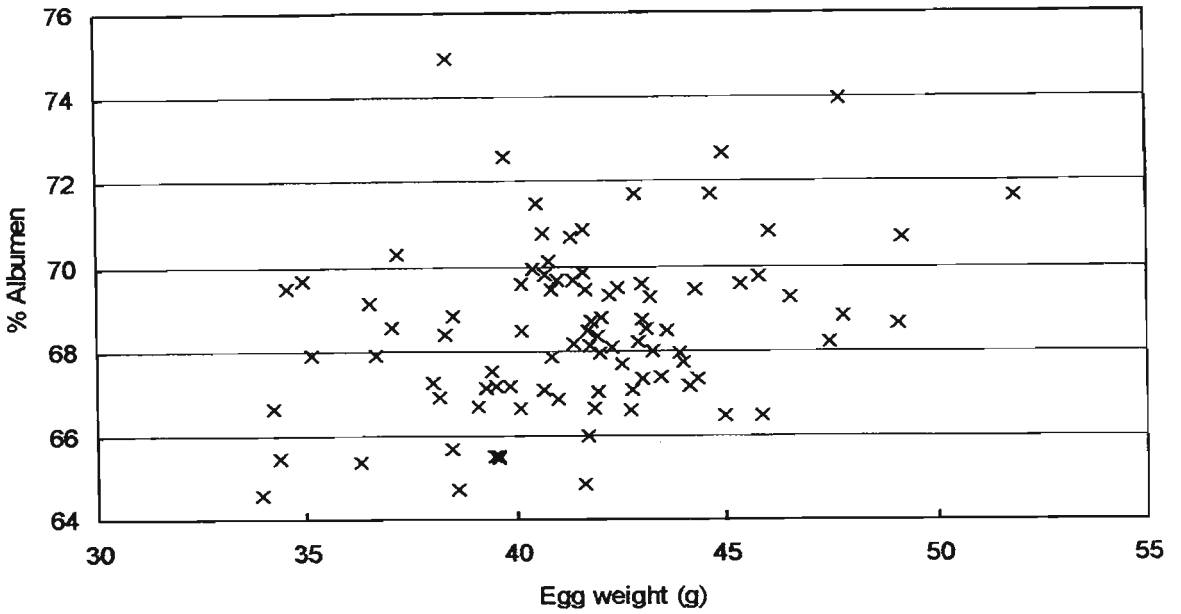


Figure 4.5: The positive relationship between % albumen and egg weight at 20 weeks (r^2=0.32)

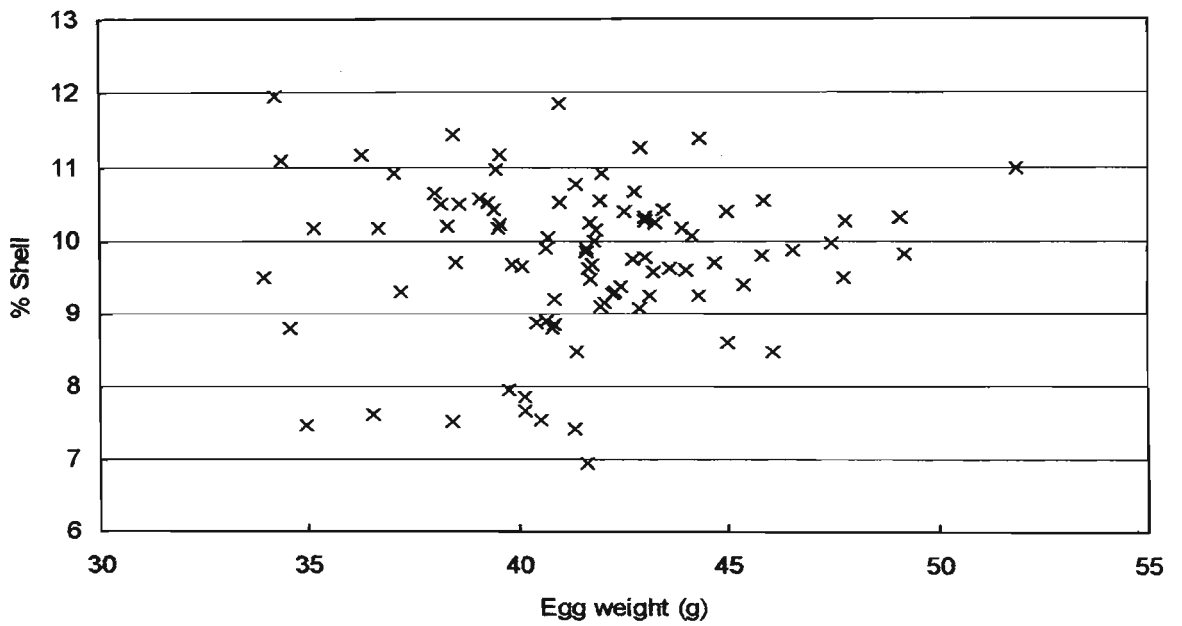


Figure 4.6: The relationship between % shell and egg weight at 20 weeks (r^2=0.04)

The changes in mean yolk, albumen and shell weight with advancing age are shown in Figures 4.7 to 4.9. All three variables increased in a curvilinear fashion in a manner similar to the egg weight curve. However, the proportion of yolk increased while the proportions of the other two components decreased. Figure 4.10 shows how the yolk percentage increased by 6.62% over the 50-week laying cycle. The albumen and shell contents of the eggs, on the other hand, decreased by 5.41% and 1.21% respectively (Figures 4.11 and 4.12).

The slight decreases in mean shell weight observed at 30 and at 40 weeks may have been in response to the unseasonably hot weather, which occurred at times during the laying cycle. The egg shell is perhaps the least consistent of the three components, as it is readily influenced by diet and environment. The panting behaviour exhibited by hens at high environmental temperatures results in the loss of bicarbonate ions, causing thinner shells. A deficiency of either vitamin D or calcium in the hen's diet will also reduce shell quality (Etches, 1996).

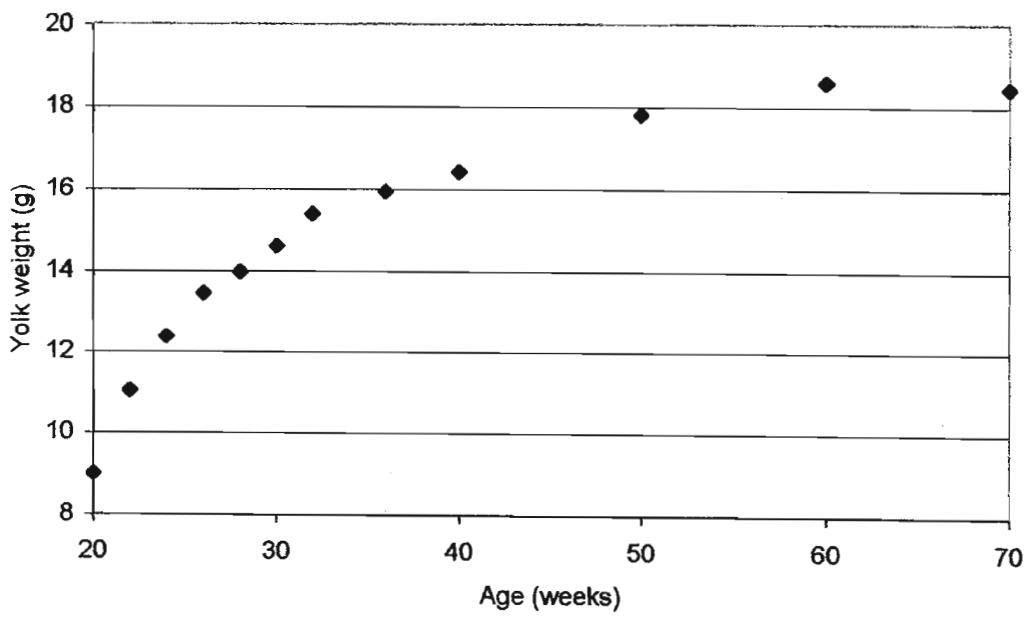


Figure 4.7: The relationship between mean yolk weight and age

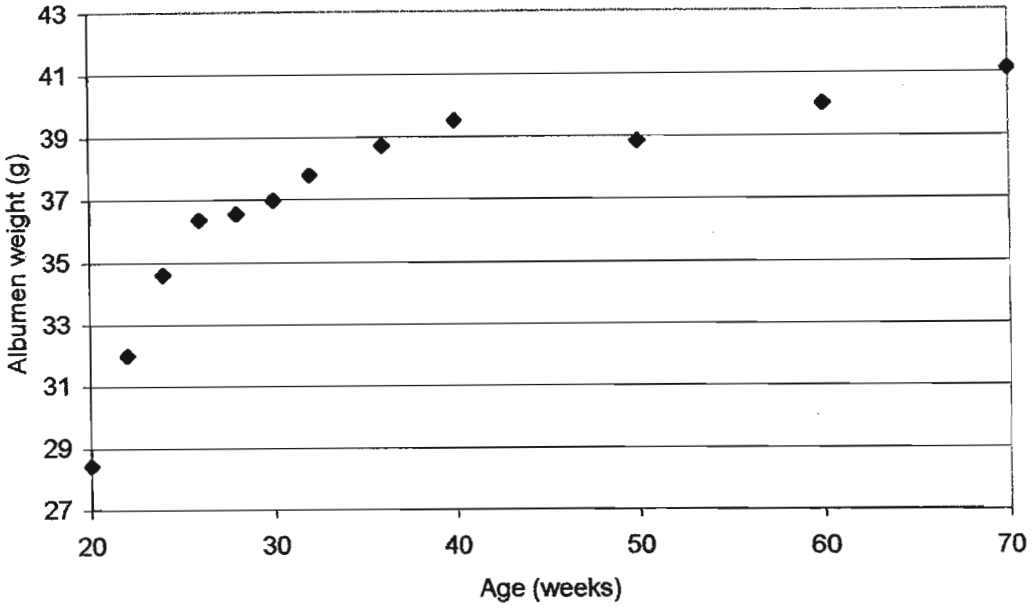


Figure 4.8: The relationship between mean albumen weight and age

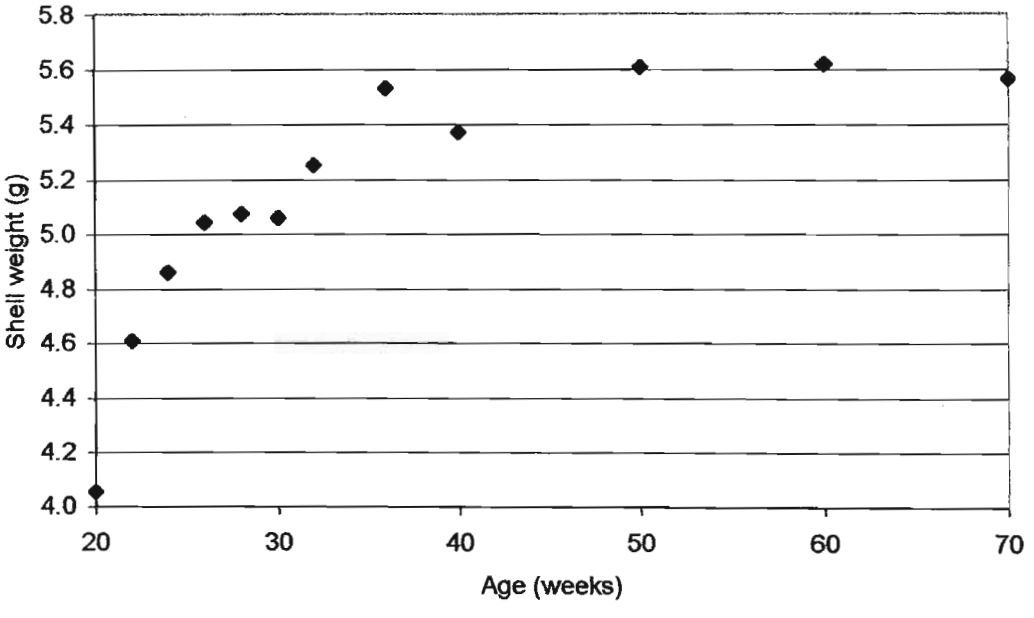


Figure 4.9: The relationship between mean shell weight and age

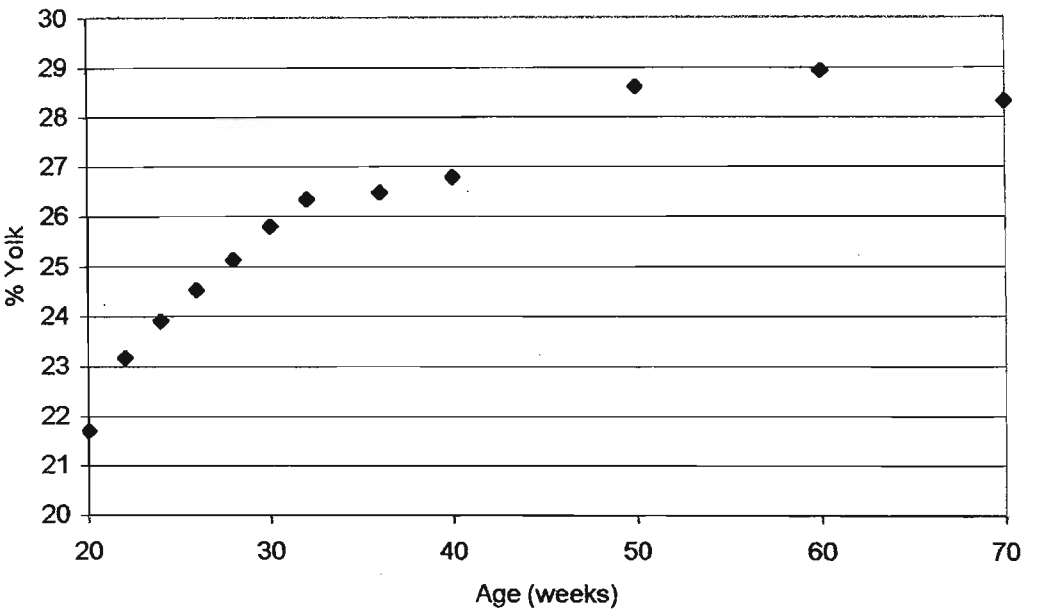


Figure 4.10: The rate of increase in the proportion of yolk with age

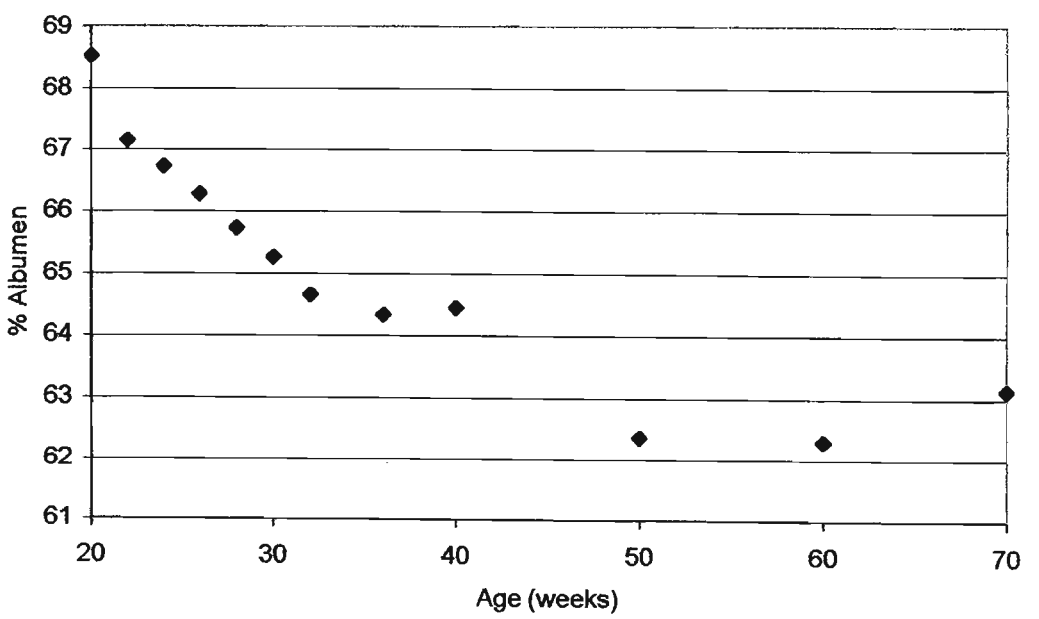


Figure 4.11: The rate of decrease in the proportion of albumen with age

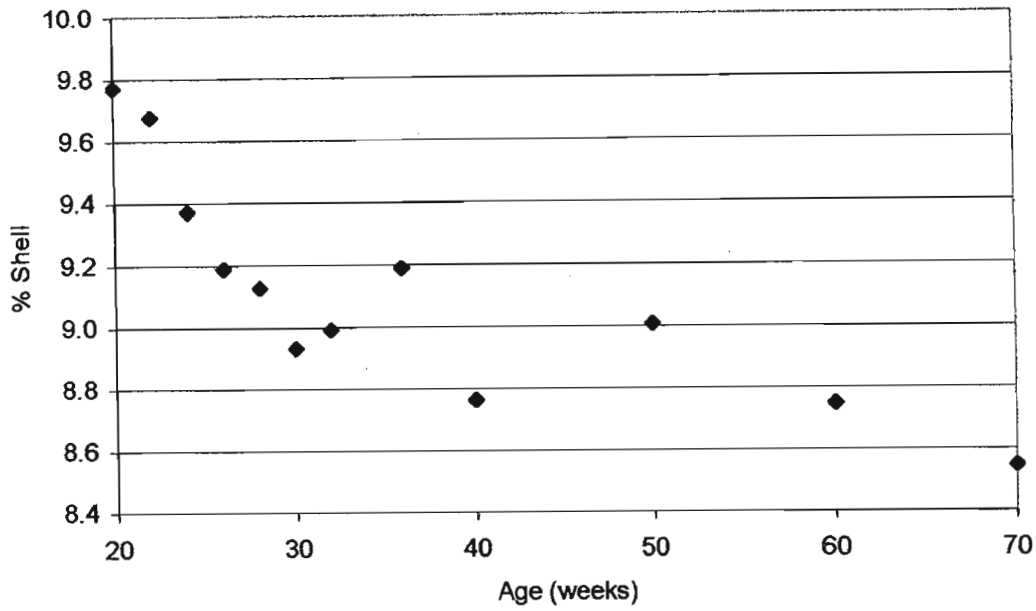


Figure 4.12: The rate of decrease in the proportion of shell with age

Two factors therefore play a role in determining the proportional contents of eggs; namely, egg weight and hen age.

Yolk weight may be predicted from hen age (in days) using the logistic function ($p<0.001$):

$$YW = -224.7 + 243.2 / (1 + \exp (-0.01268 . (\text{Hen age} + 116.4))) \quad \text{(Equation 4.7)}$$

which has an R^2 of 0.989, indicating the goodness of fit. The relationship is shown in Figure 4.13 and the statistical summary is given in Appendix 4.1. This equation may be useful in the population model to predict yolk weight from hen age, so that yolk weight increases as the hens mature. For interest, Figure 4.14 compares the curves given by Equation 4.7, representing the Amber-Link egg and by Equations 3.4 and 3.5, which predict yolk weight for the Hy-Line Silver and Brown varieties respectively. Although the Amber-Link function is likely to be more accurate, having been derived from a broader database, this clearly highlights the need for a simulation model to define constants and parameters for specific genotypes.

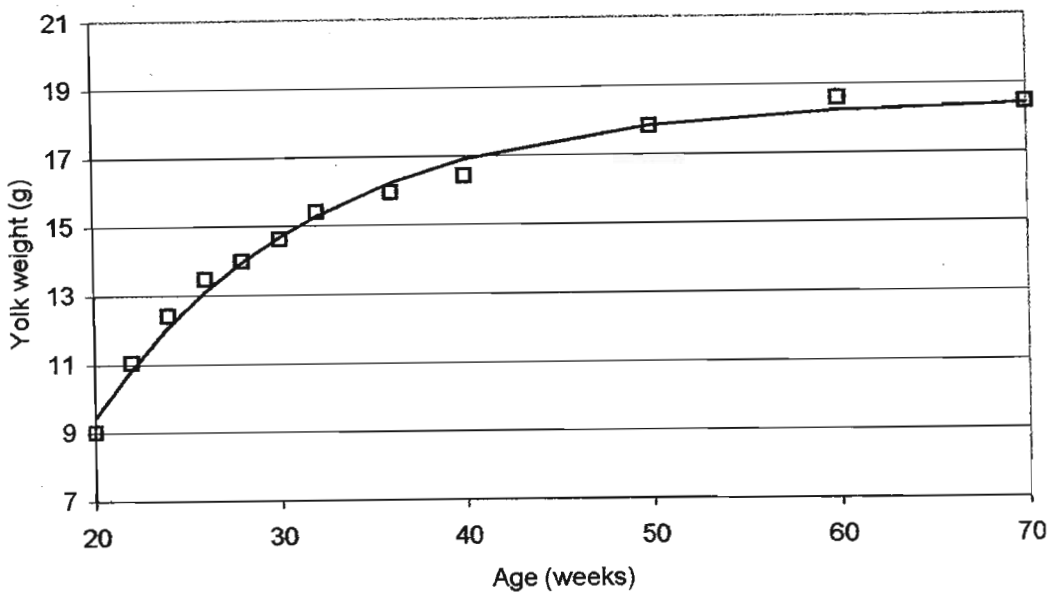


Figure 4.13: Observed mean yolk weights (□) and the fitted logistic equation (solid line) over time

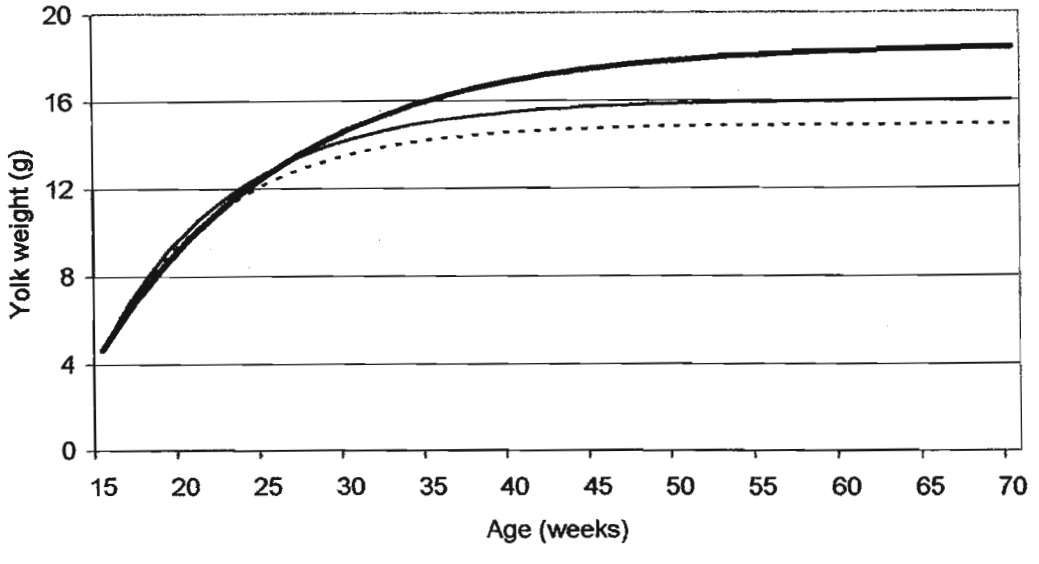


Figure 4.14: The prediction of yolk weight for Hy-Line Silver (solid line), Hy-Line Brown (dotted line) and Amber-Link (bold line) hens

The layer model needs to be capable of predicting both the weights of the three components and their relative proportions at each age as well as changes in the weights and proportions with advancing hen age. Figures 4.1 to 4.12 may be used to compare the

model output with trends derived from experimental data, when the allometric functions are used to predict albumen weight from yolk weight and shell weight from the weight of the egg contents.

4.4 The regression of albumen weight on yolk weight

Appendix 4.2 summarises the parameter estimates for the linear regressions of albumen weight on yolk weight, for eggs from hens at each age. The squared correlation coefficients ranged from 0% to 25.2% and several of the fitted equations were not significant. Fitting quadratic terms to the models did not at any stage improve the fit. Figure 4.15 depicts these linear equations fitted to the data for the different ages. In view of the fact that in many instances either the slopes or constants were not significant, t-tests could not be done to confirm whether there were significant differences in the responses of albumen weight to yolk weight between the various ages. In general the fit of the linear models was too poor to allow meaningful comparisons.

At a fixed age, therefore, there appears to be no consistent relationship between albumen weight and yolk weight. Yolk weight is known to vary according to the position of the egg in a sequence; the first or second eggs usually contain the largest yolks. The amount of albumen secreted around the yolk during egg formation presumably varies among individuals and from egg to egg. It is not surprising, therefore, that the proportions of the two components are inconsistent at a fixed age.

When all the component weights were pooled, over the range of ages from 20 to 70 weeks, a highly significant exponential equation ($p<0.001$) was found between albumen weight and yolk weight ($r^2= 54.8\%$):

$$AW = 45.37 - 50.97 \,.\, (0.8821^{\,YW}) \qquad \text{(Equation 4.8)}$$

Figure 4.16 shows the data points as well as the fitted function. The statistical summary is given in Appendix 4.3.

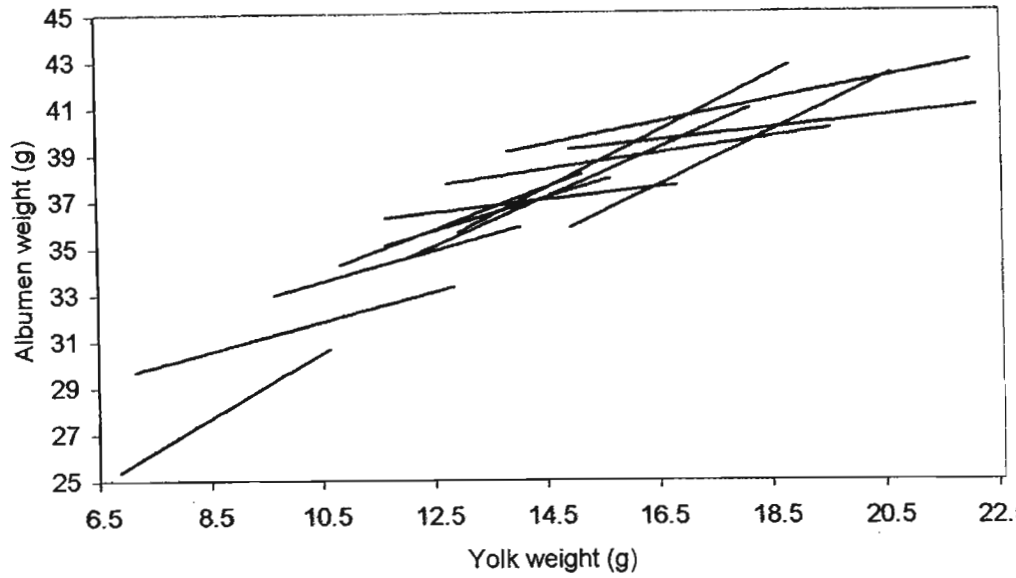


Figure 4.15: The effect of hen age on the linear regressions of albumen weight on yolk weight. Each line represents a different age

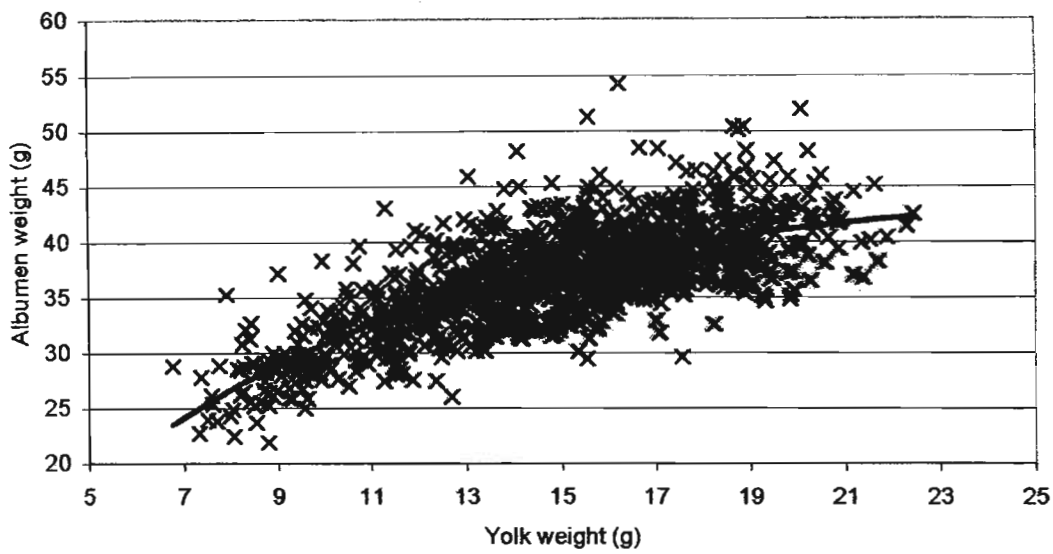


Figure 4.16: The relationship between albumen weight and yolk weight for the collective data, showing the data points (x) and the fitted exponential equation (bold line)

Regressing the *ln* albumen weight on *ln* yolk weight gave a highly significant ($p<0.001$) linear function ($r^2= 57.0\%$) of the form

$$ln \text{ albumen} = ln\ 2.397 + 0.4491.\ ln \text{ yolk} \qquad \text{(Equation 4.9)}$$

Appendix 4.4 summarises the parameter estimates.

Substituting the exponential of the constant a (2.397) and the value of the slope b (0.4491) in the allometric function of Equation 4.3, the relationship becomes

$$AW = 10.99.\ YW^{0.4491} \qquad \text{(Equation 4.10)}$$

Albumen weight may thus be predicted from yolk weight for the full laying cycle for Amber-Link hens using Equation 4.10.

4.5 The regression of shell weight on egg contents weight

In order to make further use of the allometric functions proposed by Emmans and Fisher (1986), shell weight needs to be predicted from the weight of the egg contents, yolk plus albumen. Appendix 4.5 summarises the parameter estimates for the linear regressions of shell weight (SW) on the weight of the egg contents (ECW) for each hen age. None of the relationships was found to be significantly curvilinear. Although the linear equations were all highly significant, the r^2 values ranged from 9.9% to 31.2%, indicating weak to moderate relationships between the variables. Figure 4.17 shows the linear trends between shell weight and egg contents weight at different hen ages. To establish whether the slopes or intercepts were influenced by hen age, t-tests were done using the function

$$t = \frac{p_1 - p_2}{\sqrt{(se_1^2 + se_2^2)}} \qquad \text{(Equation 4.11)}$$

where p = parameter; either the slope or the intercept and se = the associated standard error. None of the slopes or intercepts differed from each other from 24 to 70 weeks of age. There were some significant differences between the slopes or intercepts at 20 and at 22 weeks and the other ages. Taking into account the non-significance of the two intercepts

at 20 and 22 weeks, it would appear that age did not play a role in modifying the relationship between shell weight and egg contents weight.

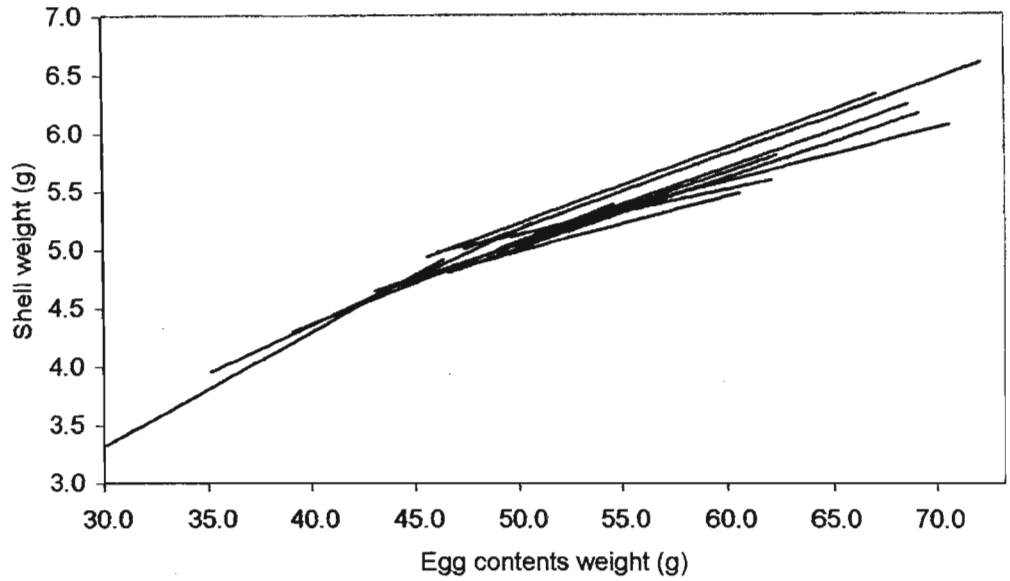


Figure 4.17: The effect of hen age on the linear regressions of shell weight on egg contents weight. Each line represents a different age

This assumption could be confirmed by pooling the data for egg components from 20 to 70 weeks of age. A highly significant ($p<0.001$) exponential equation relating shell weight to egg contents weight was found ($r^2= 56.0\%$):

$$SW = 7.564 - 9.599. (0.97326^{ECW}) \qquad \text{(Equation 4.12)}$$

Appendix 4.6 summarises the statistical analysis. Figure 4.18 shows the data points plus the fitted exponential function.

Transforming the data to natural logarithms, a highly significant ($p<0.001$) linear trend was established ($r^2 = 56.6\%$):

$$ln\ SW = -1.0825 + 0.6896\ ln\ ECW \qquad \text{(Equation 4.13)}$$

Appendix 4.7 summarises the parameter estimates. Substituting the exponential of the constant a (-1.0825) and the value of the slope b (0.6896) in the allometric function of Equation 4.3, the relationship becomes

$$SW = 0.33875 \,.\, ECW^{0.6896} \qquad \text{(Equation 4.14)}$$

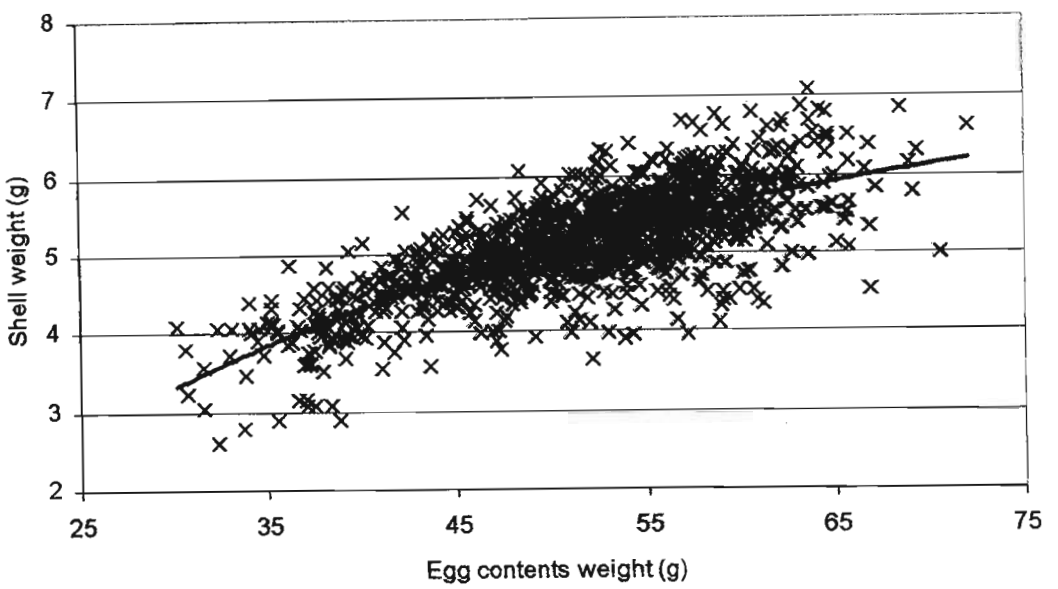


Figure 4.18: The relationship between shell weight and egg contents weight for the collective data, showing the data points (x) and the fitted exponential equation (bold line)

Equation 4.14 may therefore be used to predict shell weight from the weight of the egg contents for Amber-Link hens over the full laying cycle.

4.6 Comparison with Hy-Line egg component data

Although the component weights for the eggs from Hy-Line Silver and Hy-Line Brown trial birds were only measured up to 21 weeks of age (see Section 3.4.11), subsequent measurements were done on different flocks at 37 and at 64 weeks of age to broaden the database. It may be of interest to compare the coefficients of the linear regressions with those of the Amber-Link. As with the Amber-Link data, for both varieties of Hy-Line the component weights from all ages were pooled and Genstat was used to fit linear

regressions of *ln* albumen weight on *ln* yolk weight and *ln* shell weight on *ln* egg contents weight. Appendices 4.8 to 4.11 give the statistical summaries.

Figure 4.19 shows the relationship between *ln* albumen weight and *ln* yolk weight for the two varieties of Hy-Line and for the Amber-Link hens. The trends for the Hy-Line Silver and Amber-Link breeds are reasonably similar, but the higher proportion of albumen in Hy-Line Brown eggs (discussed in Section 3.4.11) is reflected in the larger y-intercept.

Figure 4.20 shows the relationship between *ln* shell weight and *ln* egg contents weight for the three genotypes. Interestingly, the slope and intercept of the regression for the Amber-Link eggs are very different to those of the two Hy-Line varieties, indicating much heavier shells initially. There appears to be a consistent relationship between shell weight and egg contents weight for the two Hy-Line varieties. Hy-Line Brown eggs are larger and have proportionally more albumen than Hy-Line Silver eggs, but eggs of the same size from each variety will have similar amounts of shell deposited around the egg contents.

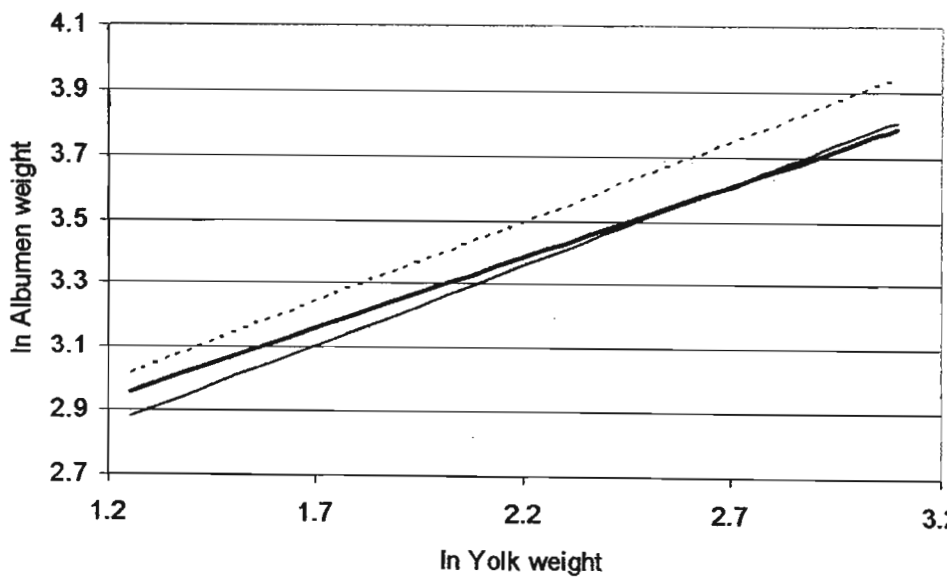


Figure 4.19: The linear regression of *ln* albumen weight on *ln* yolk weight for the Hy-Line Silver (solid line), Hy-Line Brown (dotted line) and Amber-Link (bold line) eggs

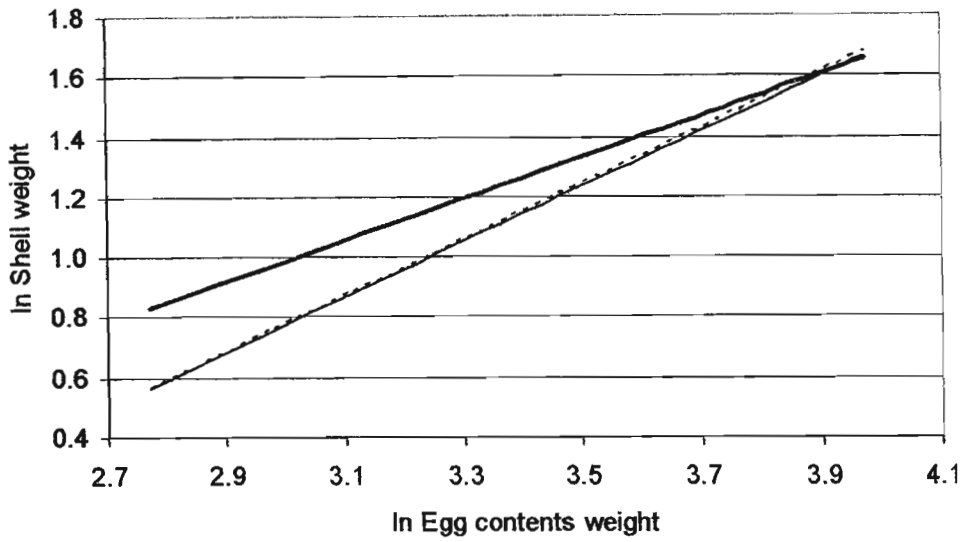


Figure 4.20: The linear regression of *ln* shell weight on *ln* egg contents weight for the Hy-Line Silver (solid line), Hy-Line Brown (dotted line) and Amber-Link (bold line) eggs

Table 4.2 summarises the values for the allometric function parameters for the three strains of birds under discussion. Albumen weight and shell weight may thus be predicted for the Hy-Line birds by inserting the relevant values into Equations 4.5 and 4.6.

Table 4.2: Estimates of the parameter values used in the allometric functions

	Parameter			
	a (albumen)	b (albumen)	a (shell)	b (shell)
Hy-Line Silver	9.473515	0.5044	0.138207	0.9180
Hy-Line Brown	10.8906	0.5020	0.133187	0.9310
Amber-Link	10.9900	0.4491	0.33875	0.6896

Although it is a time-consuming exercise, it would be beneficial to record the egg component weights over a more extensive range of ages for the Hy-Line varieties, as was

done for the Amber-Link. This would ensure that the allometric functions are able to accurately predict changes in the component weights over the full laying cycle. Furthermore, it is worth defining the allometric functions for all commercially available genotypes, in order to broaden the scope of the layer model. A sample size of 30 eggs gives adequate estimates of the means. Several more eggs should be collected and kept in reserve, because yolk membranes are inclined to rupture while being separated from the albumen. It is recommended that measurements are done at the same ages as for the Amber-Link eggs; *i.e.* more frequently during the early stages of lay when egg weight is increasing rapidly.

4.7 Discussion

The curvilinear relationship between albumen weight and yolk weight means that as the hens age and yolk weight increases, albumen weight increases but at a decreasing rate. Hence the proportion of albumen decreases. Similarly the curvilinear relationship between shell weight and the weight of the egg contents means that with advancing age and increasing yolk plus albumen weight, shells gradually become thinner because shell weight increases at a decreasing rate. Both albumen weight and shell weight may be predicted by the use of exponential functions (such as those given by Equations 4.8 and 4.12), but it is standard practice to use allometric functions when working with two non-isometric scaled variables.

Despite the success of fitting allometric functions to the component weight data, several issues need to be kept in mind. Given that the commercially available genotypes produce eggs of different weights, the component weights and their proportions presumably vary too. Indeed, differences have been shown between the three breeds discussed here. This requires the values of the parameters a and b to be established for each genotype to improve the accuracy of model predictions. It might also be worthwhile doing the same for broiler breeder, as the information would prove valuable when predicting the nutrient requirements of these birds. With continued genetic selection for greater egg numbers and heavier egg weight in layer-type hens, the proportions of egg components need to be continually reassessed (Hussein *et al.*, 1993). In addition, it may be helpful to quantify the

effect of the position of the egg in the sequence on the proportions. This may be one of the reasons why the relationship between yolk weight and albumen weight at a fixed age was inconsistent.

The variation in albumen weight in relation to yolk weight may be reproduced in a population model by generating a normal distribution of random numbers around the means for a and b and using the associated standard deviations. The same method can be used for creating inconsistency in the relationship between shell weight and egg contents weight, although the standard deviations would need to be smaller. The higher r^2 values shown in Appendix 4.5 (compared to Appendix 4.2) are evidence of the more consistent relationship between shell weight and egg contents weight at various ages.

In the model, any rearing treatment designed to manipulate age at first egg must be able to predict initial yolk weight and the corresponding component weights and proportions correctly. If initial yolk weight is predicted from hen age using Equation 4.7 for the Amber-Link, or Equations 3.4 and 3.5 for the Hy-Line Silver and Brown birds respectively, this should be feasible.

Finally, the simulation model needs to reflect the following:

1. At a given age, realistic yolk, albumen and shell weights for the strain and their standard deviations;
2. At a given age, larger eggs with a greater proportion of albumen;
3. With increasing age, an increase in the proportion of yolk;
4. Over the laying cycle, realistic egg weights and component weights for the strain.

The following chapter deals with the development of a population layer model. It is intended to compare the output of the model in terms of component weights and proportions, for the three genotypes under discussion, with the data presented here.

Chapter 5

DEVELOPMENT OF A STOCHASTIC POPULATION MODEL FOR PREDICTING EGG PRODUCTION OVER A FULL LAYING CYCLE

5.1 Introduction

The ovulatory model, as described in Chapter 2, predicts ovulation times for a single hen over the laying cycle. In order to model a population of hens, means and standard deviations need to be allocated to all the parameters. Furthermore, some modifications and additions need to be made. These include:

- predicting the age at onset of lay from the lighting programme applied during rearing
- amending the method of calculating the hens' internal cycle lengths
- linking the circadian rhythm to the time of 'sunset'
- predicting oviposition time from time of ovulation
- recording sequence length
- including internal ovulations
- including soft shells and double yolks
- predicting egg weight from yolk weight

Microsoft Excel 2003 was used to develop the model, with programming done in Visual Basic. Initially all the calculations were shown on a spreadsheet, but as the model advanced and each step had been verified, progressively more of the calculations were performed in Visual Basic without any output to Excel. This speeded up the procedure considerably. The Visual Basic code is given in Appendix 5.1.

One-dimensional Visual Basic arrays are used to store and process the variables. Each array represents a single variable and an index system (using numbers 1-100) allows a

value to be allocated to each element of the array. Arrays help to create shorter and simpler code because loops can be set up that deal efficiently with each element.

5.2 Selecting a strain

Four choices are presented to the user: Hy-Line Silver, Hy-Line Brown, Amber-Link and Other. The selection determines the values of the parameters used to predict internal cycle length, yolk weight, albumen and shell weights, mean age at first egg and the proportions of internal ovulations, soft-shelled and double-yolked eggs. Until specific information is available for more strains, selection of 'Other' results in the parameter values applicable to the Hy-Line Silver strain being used.

5.3 Predicting the age at first egg

Mean age at first egg is determined using the Bristol-Reading model (Lewis *et al.*, 2002), which predicts mean age at sexual maturity based on the genotype and the lighting programme applied during rearing. This model requires the following inputs: age at photostimulation, length of the initial constant photoperiod applied during the growing phase, length of the final photoperiod after photostimulation, age at first egg when reared on a constant photoperiod other than ten hours and given no stimulatory increase in hours of light, and the length of this constant photoperiod. The user is given an opportunity to enter values for all these variables. It is probable that the user does not know what values to use for the last two variables mentioned above. A recommendation is made to use 162 days for the Hy-Line Silver and 169 days for the Brown strain if reared on an eight-hour constant daylength, *i.e.* without any photostimulation whatsoever. These estimations are based on comparisons of output from the Bristol-Reading model with mean ages at first egg for the two strains reported in Chapter 3.

One of the analysis tools in Excel, random number generation, creates a normal distribution of 100 numbers using the predicted mean age at first egg and its standard deviation, in order to introduce variability in age at first egg within the population. This Excel array can then be passed to the Visual Basic module for further processing.

The minimum age at first egg within the distribution is found, because the model needs to start checking for ovulations on the preceding day, *i.e.* when age at first egg (rounded off to an integer) minus one is equal to the hen age. Table 5.1 shows the summary from the worksheet 'light program', which summarises some of the user input and calculations, the predicted mean age at first egg and its associated standard deviation. The distribution of ages at first egg for the population is shown in Figure 5.1.

Table 5.1: Predicted mean age at first egg

PREDICTING AGE AT SEXUAL MATURITY FOR BIRDS GIVEN A SINGLE CHANGE IN PHOTOPERIOD	
AFE (reared on constant phpd not 10h)	162 days
Length of other constant photoperiod	8 hours
AFE (reared on 10h constant)	158.54 days
Age at photostimulation (weeks)	17
Initial constant photoperiod	8 hours
Final photoperiod	14 hours
Age at photostimulation (days)	119
p (proportion sensitive to change)	1.000
m (proportion that will mature)	0.002
mean m	145.54
sd m	9.29
Mean age at first egg	**134.99 days**
Standard deviation	**7.98 days**

The assumptions about the strains were tested by first selecting Hy-Line Silver and running the model for three ages at photostimulation, namely 12, 15 and 18 weeks of age and subsequently doing the same after selecting Hy-Line Brown as the strain. The mean ages at first egg and standard deviations predicted by the model are summarised in Table 5.2. Figures 5.2 and 5.3 show the distributions for the two strains respectively, for the three ages at photostimulation. The model predictions are very similar to the recorded mean ages at first egg from the trial data (see Table 3.4) although the trend in the standard deviations differs. The Bristol-Reading model uses the function

$$\text{standard deviation} = -8.76 + 0.124 \,.\, \text{Mean AFE} \qquad \text{(Equation 5.1)}$$

to estimate the standard deviation for mean age at first egg. This equation predicts an increase in the standard deviation with delayed age at photostimulation, contrary to what was found in the trial. The larger variation about the mean for the population photostimulated at 18 weeks is clearly seen in Figures 5.2 and 5.3.

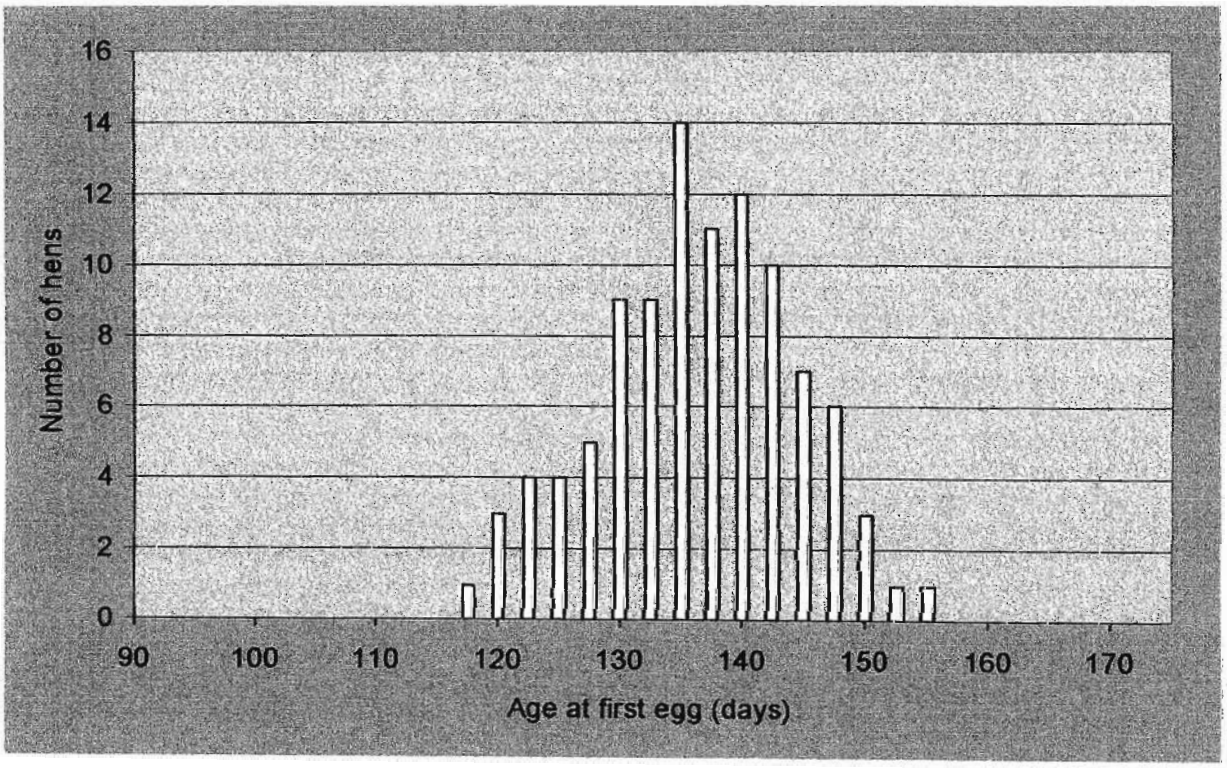


Figure 5.1: The distribution of ages at first egg for the theoretical population

Table 5.2: Predicted mean ages at first egg, in days, (± sd) for the two strains photostimulated at three different ages

Strain	12 weeks	15 weeks	18 weeks
Hy-Line Silver	114.20 (±5.40)	126.58 (±6.94)	139.46 (±8.53)
Hy-Line Brown	117.04 (±5.75)	129.40 (±7.29)	142.04 (±8.85)

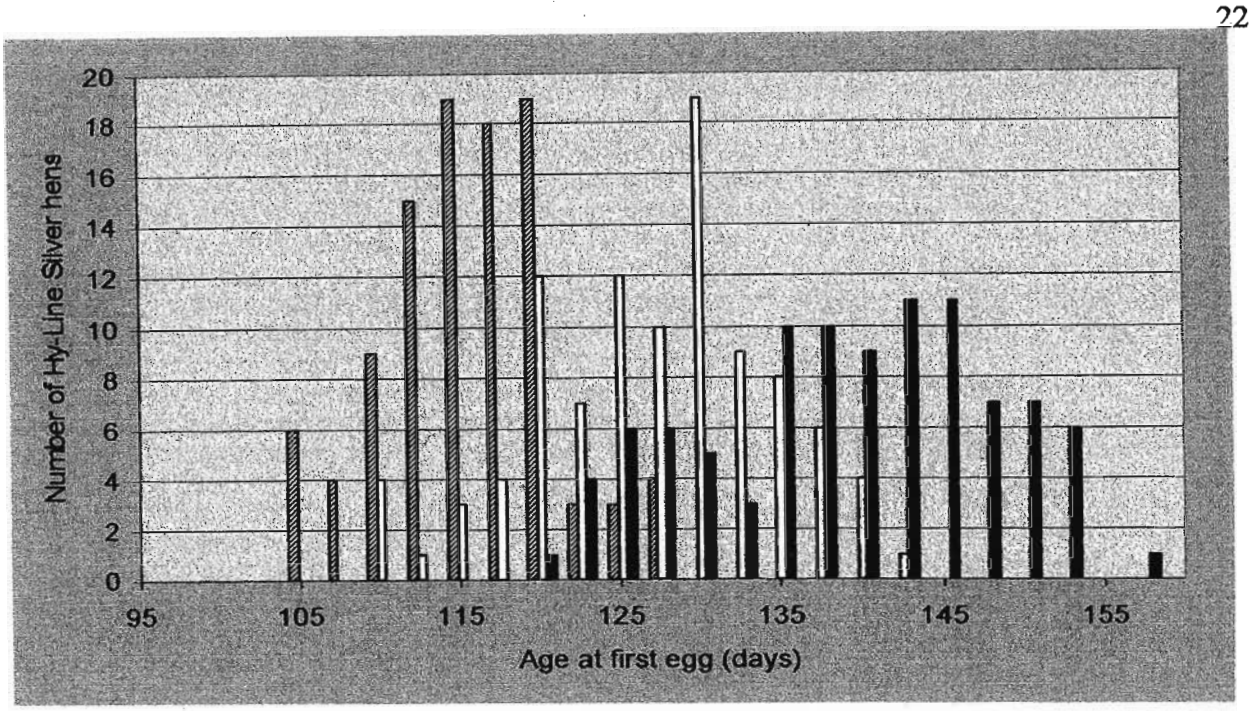


Figure 5.2: Theoretical distribution of ages at first egg for the Hy-Line Silver pullets photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

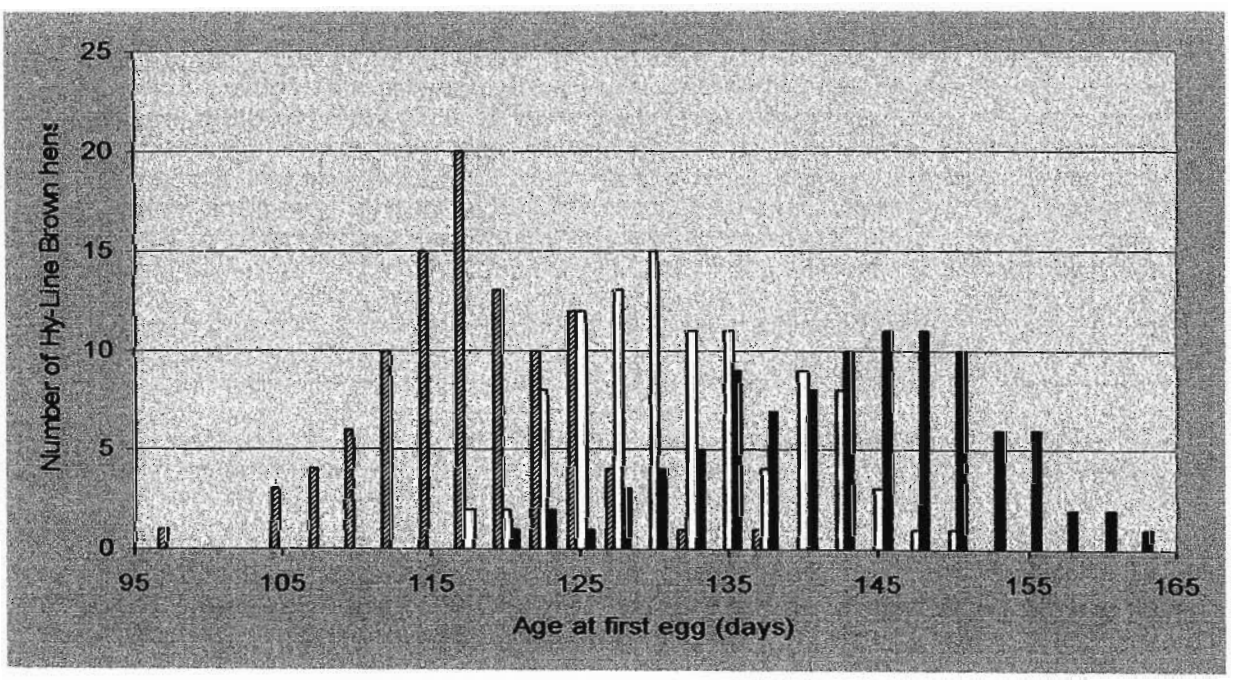


Figure 5.3: Theoretical distribution of ages at first egg for the Hy-Line Brown pullets photostimulated at 12 weeks (hatched columns), 15 weeks (blank columns) and 18 weeks (solid columns)

5.4 Creation of a theoretical flock

The flock size is fixed at 100 birds. The random number generation function is used to create normal distributions for two parameters from the ovulation model. 'Lag' represents the total lag between the first and last ovulations on successive days, or the portion of the day when ovulation may take place. In view of the fact that recent estimates of lag have been given as eight to nine hours (Lillpers, 1991), a normal distribution of random numbers is generated with a mean of 8.5 hours and a standard deviation of 0.17 hours, or 10.2 minutes; *i.e.* a coefficient of variation of 2%. The range in lag (six times the standard deviation) is therefore one hour and one minute. 'Start FM' (referred to as T_5 by Etches and Schoch, 1984) is the time the first follicle in each hen begins the final phase of maturation at sexual maturity. The array is filled with normally distributed random numbers. Because the initiation of the final phase of maturation is probably indicated by the ability of the follicle to secrete progesterone in response to LH, the mean 'Start FM' is calculated from the time the lights go off plus 2.5 hours. This allows the maturation of the first follicle to coincide with the open period for ovulation. A coefficient of variation of 5% is used to generate the distribution.

5.5 Internal cycle length

Internal cycle length represents the interval between successive ovulations. This interval is determined by the synchrony between the follicle hierarchy and the circadian rhythm of LH release. A hen that has a properly maintained hierarchy containing sufficient follicles, each capable of maturing every 24 hours, will have an internal cycle length of about 24 hours and will therefore lay an egg a day. With advancing age the follicles take longer to mature with the result that the internal cycle length increases. Emmans and Fisher (1986) proposed that the change in a hen's internal cycle length (ICL) over time may be calculated from the following:

$$ICL = ICLo - Lag + 1 / ((1/Lag) - kt) \qquad \text{(Equation 5.2)}$$

where ICLo = initial internal cycle length, k = a decay factor and t = time from first egg in days. If the internal cycle length is less than or equal to the 24-hour daylength or external cycle length (EXCL), rate of lay (R) is given by:

$$R = 24 / EXCL \qquad \text{(Equation 5.3)}$$

If the internal cycle length is greater than the external cycle length:

$$R = Lag / ((ICL - EXCL)(1 + (Lag / (ICL-EXCL)))) \qquad \text{(Equation 5.4)}$$

Figure 5.4 shows the internal cycle length as a function of time for two hens with different initial internal cycle lengths and values for k, but both with a lag of nine hours. The bird with an internal cycle length shorter than 24 hours (hen 1) is able to lay an egg a day for 180 days, *i.e.* at a rate of lay of one (Figure 5.5) but as soon as the internal cycle length moves above 24 hours, rate of lay starts to decline. This is because eggs are now laid in sequences interspersed with pause days, and sequence length decreases systematically over time. The second hen (hen 2) starts with an internal cycle length longer than the daylength and so never lays an egg a day. After 50 weeks, the first hen is producing four- or five-egg sequences whereas the second hen is laying one- or two-egg sequences.

These functions allow a decrease in egg production with advancing hen age, but they do not permit the simulation of the shorter egg sequences observed in the trial (reported in Chapter 3) produced by many hens at onset of lay. In order to reproduce these, the internal cycle length needs to be longer than 24 hours initially, before decreasing with advancing time from first egg to below the daylength and subsequently increasing above 24 hours. Quadratic-by-linear equations of the form:

$$y = A + B / (1 + D. x) + C. x \qquad \text{(Equation 5.5)}$$

where y = ICL and x = time from first egg, give the required curvilinear shape.

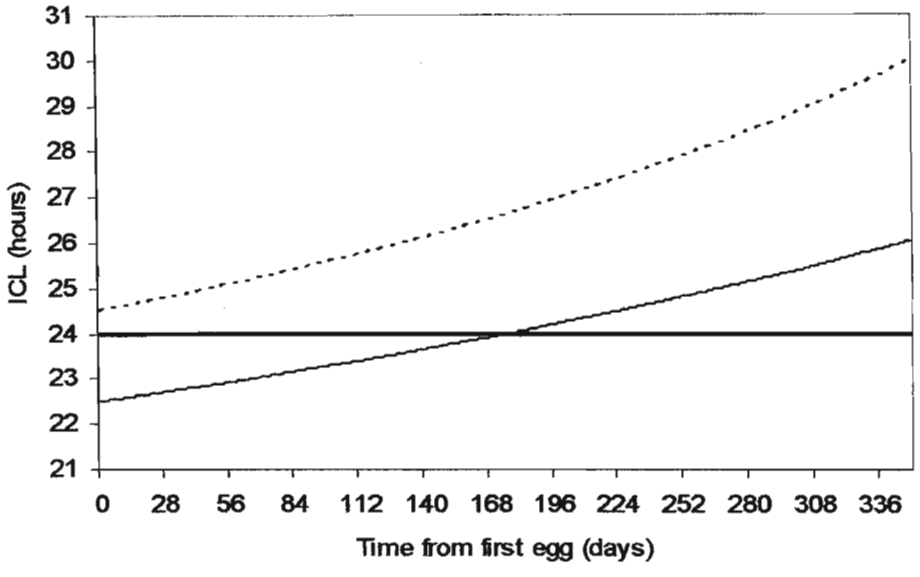


Figure 5.4: Change in ICL for two hens from first egg to the end of a laying year; hen 1 with ICLo = 22.5 and k = 0.00009 (solid line), and hen 2 with ICLo = 24.5 and k = 0.00012 (dotted line)

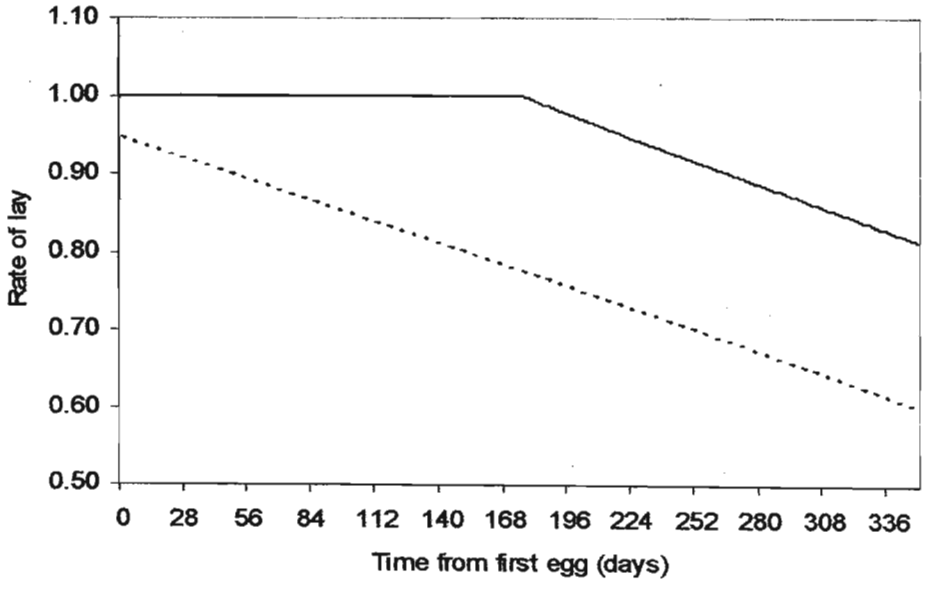


Figure 5.5: Rate of lay for the two hens in Fig. 5.4 over a laying year; hen 1, with the ICL shorter than 24 hours, initially has a rate of lay of one (solid line) whereas the rate of lay of hen 2 (dotted line) declines from day one

In Equation 5.5 A and B determine the y-intercept, D the rate of decrease and C the rate of increase of the curve. If each of the four parameters is assumed to be normally distributed and is given a mean and standard deviation then each hen in the flock may be allocated its own function to predict internal cycle length at a given time. It follows that sequence lengths will vary between individuals from onset of lay. Figure 5.6 illustrates three possible functions to predict internal cycle length, using Equation 5.5. The values of the parameters D, B, C and A are shown in Appendix 5.2. Hen 3 (solid line) starts with an internal cycle length longer than the 24-hour daylength, which means that her rate of lay is 0.9 (Figure 5.7). During the first three weeks of egg production, she would probably lay one 15-20-egg sequence before commencing her prime sequence. During the next twelve weeks, while the internal cycle length is less than 24 hours, her rate of lay will be 1.0 and so the prime sequence length will be about 84 days. Thereafter the internal cycle length increases beyond 24 hours, rate of lay declines and 301 days from first egg the bird produces two-egg sequences. In contrast hen 4 (bold line) commences her laying cycle with an internal cycle length shorter than 24 hours, which gives her a rate of lay of 1.0 from sexual maturity. This means that she is able to lay without pausing for 37 weeks, so her prime sequence length would be about 259 eggs; incidentally similar to the production of the experimental hen 8 from room five observed in the trial and discussed in Section 3.4.12. After 301 days in lay, this bird would lay a 16-egg sequence. Although the internal cycle length of hen 5 (dotted line) decreases initially with a corresponding increase in the rate of lay, it never goes below 24 hours and so the prime sequence would occur about 63 days from first egg, with a final sequence length of one or two eggs.

Thus it may be seen that, in contrast to the model of Emmans and Fisher (1986), these quadratic-by-linear functions produce the shorter sequences at onset of lay that were reported by Lewis and Perry (1991) and also recorded in the experimental work reported in this thesis. In view of the fact that rate of lay is determined by the ovulation rate, the internal cycle length is assumed to refer to the interval between successive ovulations, not ovipositions. Accordingly, in the population model, Equation 5.5 predicts the change in the interval between successive ovulations, with x = time from first ovulation. Equations 5.3 and 5.4 are used to calculate rate of ovulation.

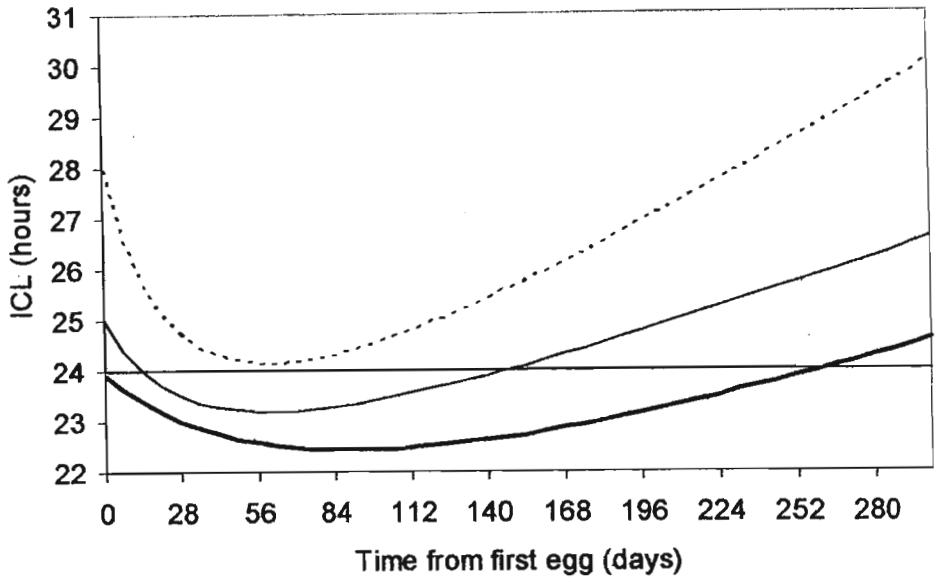


Figure 5.6: Curvilinear relationships between ICL and time from first egg for three hens; hen 3 (solid line), hen 4 (bold line) and hen 5 (dotted line) referred to in the text

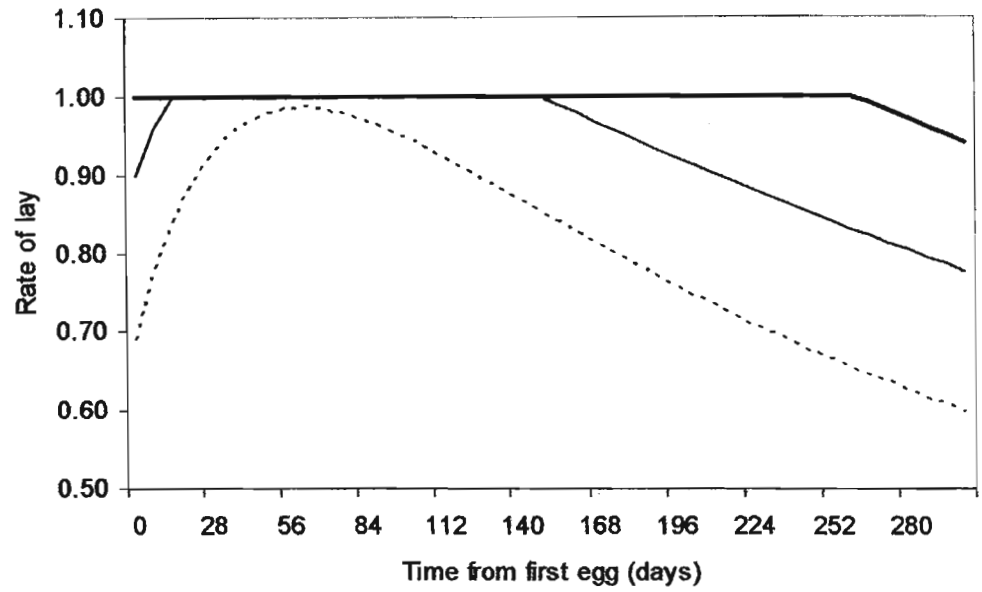


Figure 5.7: The rates of lay for hen 3 (solid line), hen 4 (bold line) and hen 5 (dotted line) referred to in the text, using the internal cycle lengths shown in Figure 5.6

In the model, the random number generator creates Visual Basic arrays of 100 elements each for the parameters D, C, B and A from the quadratic-by-linear function of Equation

5.5. The mean values for these parameters for the Hy-Line Silver and Hy-Line Brown birds (summarised in Appendix 5.3) were chosen so that a proportion of the flock will start laying short sequences and a proportion will commence laying with their prime sequence. A coefficient of variation of 1% is used to calculate the associated standard deviations. Figure 5.8 shows how the internal cycle lengths change with time from first egg for the Hy-Line Silver and Hy-Line Brown hens. It may be seen that neither curve, representing the population mean, drops below 24 hours. Nevertheless, owing to the variation introduced by the standard deviations for each parameter of Equation 5.5, a number of individuals will have internal cycle lengths shorter than 24 hours and will therefore commence laying at a rate of lay of one.

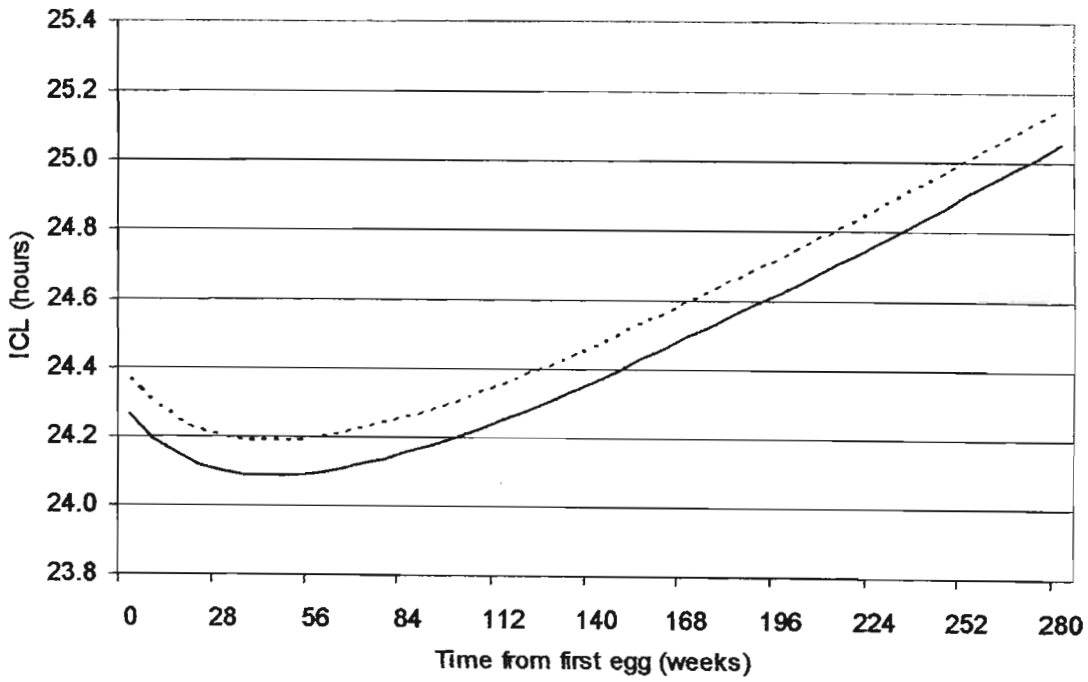


Figure 5.8: Illustration of the quadratic-by-linear functions used to predict internal cycle length from time from first egg for the Hy-Line Silver (solid line) and Hy-Line Brown (dotted line) hens

5.6 Linking ovulation time to sunset

Etches and Schoch (1984) used the parameter S_1 to determine the time that the circadian rhythm for the regulator concentration function reinitiated, so that ovulation cycles

commenced between the hours of 06:30 and 8:00. Longer sequences would start earlier in the morning. Bearing in mind that these authors were attempting to produce ovulation times similar to oviposition times observed under experimental conditions for sequences of varying lengths, the parameter fulfilled a specific role. However, because of the phase-setting effect of sunset, it would be more sensible to link S_1 to the time that the lights are turned off in the poultry house. This would enable the model to predict ovulation and oviposition times relative to the onset of darkness so that the mean time of lay could be about 13 hours after sunset, in accordance with the research reviews summarised in Section 1.9.

The open period for LH release is thought to commence about two hours after sunset and to last for about nine hours. The preovulatory surge of LH can take place at any time during the open period. Between four and six hours after the LH peak, ovulation occurs (see Section 1.10). There is thus an open period of about nine hours during which ovulation can take place. This means that the earliest ovulations at the start of a sequence take place about six to eight hours after the onset of darkness. Figure 5.9 shows the temporal relationships between the onset of darkness, LH release and ovulation.

Taking these relationships into account, the random number generation function is used to create a normal distribution with a mean of seven hours and a standard deviation of 0.35 hours, *i.e.* 21 minutes, or a coefficient of variation of 5%. The range in values is 126 minutes or just over two hours. Therefore the distribution should cover the required range from six to eight hours. This array represents the variable 'Open Period'; that is, the interval between the onset of darkness and the start of the open period for ovulation, which therefore determines the earliest opportunity for ovulation to occur. Individual hens within a population almost certainly vary in their hormonal responsiveness to the onset of darkness. The array containing different values for each element should allow this variation to be expressed. An additional variable 'minimum Open Period' finds and stores the minimum value in the array of random numbers, to be used in the initialisation of the starting time (described in the next section).

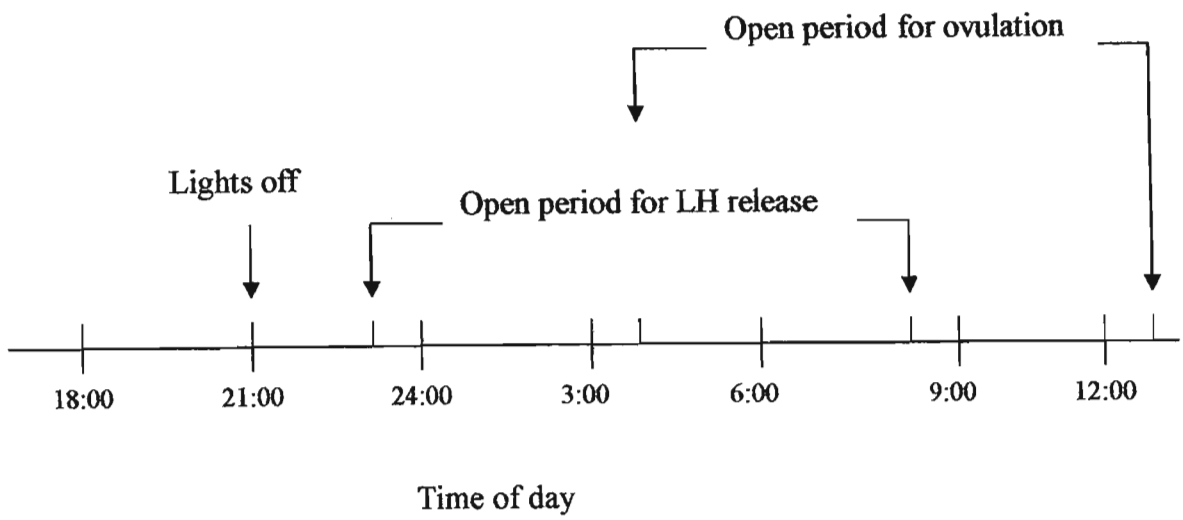


Figure 5.9: An illustration of the temporal relationships between lights off (21:00), the start of the open period for LH release (23:00) and the start of the open period for ovulation (04:00). (Open period for LH release = 9 hours; interval between onset of darkness and start of open period for LH release = 2 hours; interval between LH release and ovulation = 5 hours)

A Gompertz function of the form given by Equation 2.8, and with the values listed in Appendix 2.10 substituted for the parameters A, C, B and M, may be used to calculate S_1 for a given ovulation rate. As ovulation rate decreases the curve for S_1 tends towards an asymptote, the value of which is given by A (since C is negative). It is this parameter that needs to be modified in order to link S_1 to the onset of darkness. As may be seen in Figure 2.26, S_1 varies roughly between six and nine depending on the ovulation rate, with a range between the two asymptotes of about three. An array of one hundred numbers needs to be created for A, so that for each hen

A = lights off + sunset-ovulation interval – daylength + range (Equation 5.6)

where daylength = 24 hours and range between asymptotes = 3. If, for example, the lights are turned off at 21.5 (*i.e.* 21:30, as was the case in the experiment detailed in Chapter 3) and the sunset-ovulation interval for a given hen is 7.25 (7h15min), then A = 21.5 + 7.25 –

24 + 3 = 7.75 (07:45). This time determines the value of the asymptote below an ovulation rate of 0.5. The value for each element in the array S_1 is then calculated as a decimal from Equation 2.8 with C, B and M as constants and substituting the constant term A with the new Visual Basic array 'Parameter AS1'. These modifications still allow the value of S_1 to change with rate of ovulation, so that longer sequences are linked to an earlier initiation of the circadian rhythm, but S_1 is now also altered by the time the lights are turned off. As an illustration, Figure 5.10 shows two Gompertz curves for predicting S_1 from ovulation rate. In the first instance, sunset occurs at 21:30 and so the earliest the circadian rhythm of the regulator substance reinitiates itself is at about 04:30, which means that ovulation may take place shortly after this. In the second case sunset occurs at 20:00; in hens with high ovulation rates the circadian rhythm reinitiates at about 03:00 and ovulations can occur shortly thereafter.

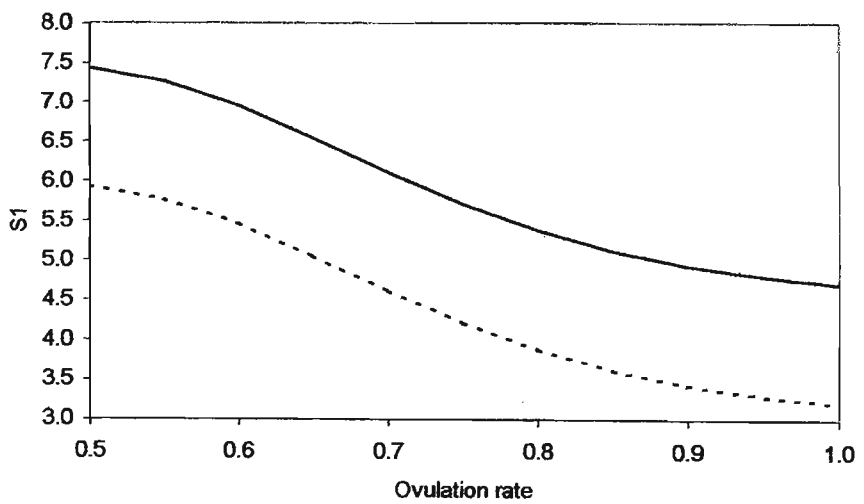


Figure 5.10: Gompertz functions used to calculate S_1 from ovulation rate for hens given sunset at 21:30 (solid line) or 20:00 (dotted line). (Sunset-ovulation interval = 7 hours; range between asymptotes = 3)

5.7 Predicting the time of ovulation

Visual Basic arrays are used to process the following variables: number of days of ovulation, internal cycle length, ovulation rate, the parameters L_1, L_2, S_1, b_1, b_2, b_3, S_2 and a_2, the time for the regulator concentration, the regulator concentration function, the time for follicle maturation, the follicle maturation function, intersection of the two functions,

ovulation and the ovulation time. The flow diagram is shown in Figure 5.11. The 'Start Time' is initialised at 'Sunset' + 'minimum Open Period', so that the model checks for ovulations at the earliest possible time. The variable 'Day Number' is set at one.

For each hen, the number of days of ovulation is zero until the age at first egg minus one is equal to the hen age. An ovulation needs to occur a day before the first egg is due to be laid. This number cumulates with each day that the model runs and is effectively the time from first egg. Once the number of days is equal to one or greater, the bird's internal cycle length is calculated from Equation 5.5 but using the values for the parameters for the specific breed. Ovulation rate is estimated from Equations 5.3 or 5.4, with the external cycle length set at 24 hours.

The values of the ovulatory cycle parameters λ_1, λ_2, b_1, b_2, b_3 and S_2 are governed by the ovulation rate and are calculated using the Gompertz equations discussed in Chapter 2. Equation 2.3 is used to calculate the value of a_2. The concentration of the regulator substance and the process of follicle maturation are computed by Equations 2.1 and 2.4 respectively. Two separate time scales are needed for these calculations and the method is explained in detail in Section 2.4. An intersection occurs if

$$G(t) - (1 - R_3(t)) \geq 0 \qquad \text{(Equation 5.7)}$$

The array 'Ovulation' stores the value one if the above condition is true or zero if it is false. If the condition is true and an ovulation has taken place, the time of day is temporarily stored in the array 'Ov time'. Once all hens in the flock have been processed, the ovulation times are stored in 'Store times', an array that retains the ovulation times until the end of the day.

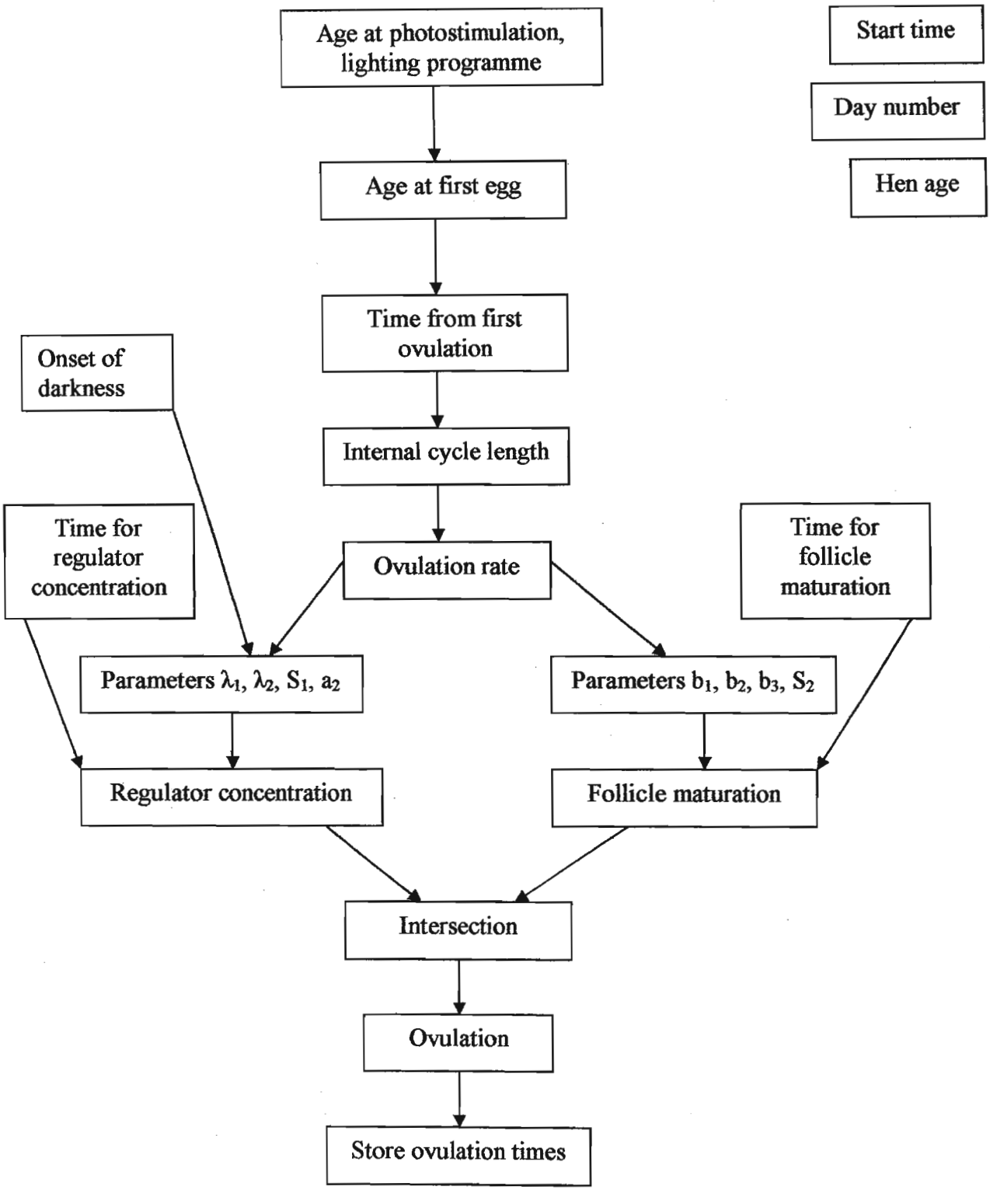


Figure 5.11: Flow diagram showing the initial setup of the population model

The time for follicle maturation is then set to zero for that particular hen, because a new follicle will shortly begin its maturation process. From the initial starting time to the end time of day (fixed at 18:00), the model adds one-minute increments and processes each array in turn, as illustrated in the flow diagram of Figure 5.12.

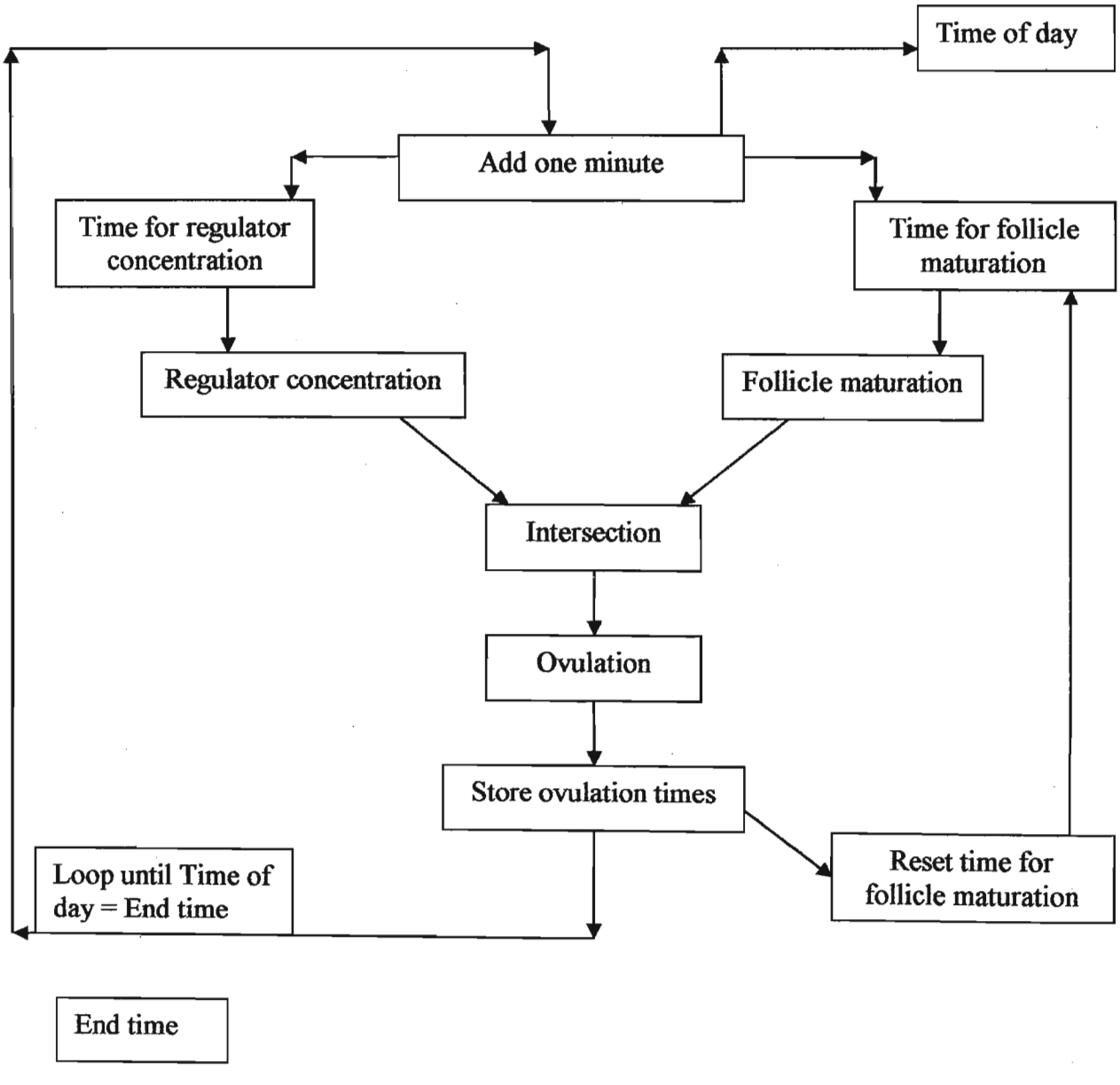


Figure 5.12: Flow diagram of the model showing the addition of time increments

Figure 5.13 shows the ovulation rate for the theoretical flock over the full laying period. The ovulation rate peaks over 90% and declines to about 78% at 69 weeks of age (51

weeks from first egg), displaying a trend similar to the rate of lay seen in commercial flocks today.

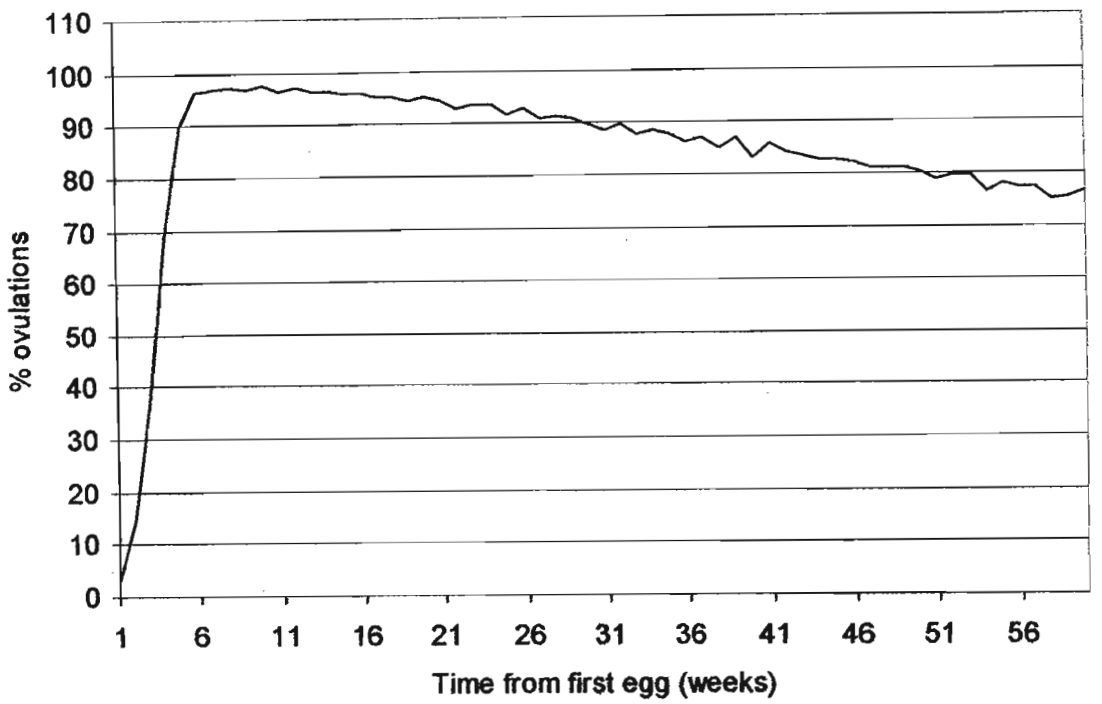


Figure 5.13: Predicted flock ovulation rate

Table 5.3 shows a section of the spreadsheet summarising the predicted ovulation times for the flock of 100 hens. A number of observations can be made. Ovulation times become progressively later in the day as the position in the sequence advances, with the terminal ovulation of a sequence occurring during the late morning or afternoon. The shortest lag values occur in mid-sequence, with the longest lag taking place between the last two ovulations. This confirms that the ovulatory model of Etches and Schoch (1984) is able to simulate ovulatory sequences for a population of hens. Sexual maturity is attained at different ages by the individuals and therefore the hens start ovulating at different times. This is brought about by using a mean age at first egg and its standard deviation. Initial sequence length varies, because some of the hens are producing short ovulation sequences at onset of lay while others are producing longer sequences. The quadratic-by-linear function that predicts internal cycle length is responsible for this significant achievement. The earliest ovulation shown in this section of the spreadsheet takes place at 04:31; 7h01m

after sunset. Had S_1 not been linked to sunset, ovulations would have commenced at about 06:00, irrespective of the applied lighting programme. Consequently it would appear that the adjustments, as described in the chapter thus far, have been implemented successfully.

5.8 Predicting oviposition time

The time that an egg is laid is determined by the time of the associated ovulation, which means that, except for the terminal egg of a sequence, the above ovulation times may be used to estimate oviposition time. Oviposition normally occurs about half an hour before the next ovulation with a range of seven to 75 minutes (see Section 1.5). An array is filled with 100 normally distributed random numbers around a mean of 30 minutes and a coefficient of variation of 30%, which gives a standard deviation of nine minutes and a range of about 54 minutes, *i.e.* from three to 57 minutes. This variable called 'Interval' allows each hen in the flock to have its own value for the interval between oviposition and ovulation. Furthermore, it is recalculated every day because the amount of time by which an oviposition precedes the associated ovulation is likely to fluctuate from day to day for each hen.

Table 5.3 shows that hen number one ovulated on day 19 at 11:56 and on day 20 at 12:39. The randomly-generated oviposition-ovulation interval (not shown) is 20m00s. From this the model predicts that the time of lay of the first egg on day 20 is equal to 12:39 – 00:20 = 12:19. Table 5.4 summarises the predicted oviposition times for the same section of the flock and over the same 35-day period as shown in Table 5.3.

Table 5.3: Predicted ovulation times for a theoretical flock photostimulated at 18 weeks

SUMMARY TABLE: OVULATION TIMES

day	hen no. 1	2	3	4	5	6	7	8
1								
2								
3								
4				08:47				
5				09:09				
6				09:33				
7				10:02				
8				10:44				
9				11:59				10:53
10					10:59			11:21
11				04:58	12:32			12:08
12				06:29				13:54
13				07:14	04:31			
14				07:42	05:55			05:38
15				08:03	06:33			07:03
16				08:20	06:54			07:42
17				08:35	07:08			08:04
18				08:49	07:17			08:19
19	11:56			09:03	07:23			08:29
20	12:39			09:18	07:27			08:36
21	14:18			09:35	07:30		11:21	08:41
22			10:01	09:57	07:32		12:15	08:45
23	06:00		10:28	10:28	07:33		14:52	08:48
24	07:22		11:01	11:19	07:34			08:50
25	07:59		11:50	13:17	07:35		05:21	08:52
26	08:19		13:27		07:35	09:25	06:44	08:53
27	08:32			04:56	07:35	09:22	07:22	08:54
28	08:41	09:54	05:52	06:25	07:35	09:18	07:43	08:55
29	08:47	10:08	07:23	07:08	07:35	09:13	07:56	08:55
30	08:51	10:24	08:08	07:35	07:35	09:08	08:05	08:55
31	08:54	10:43	08:37	07:54	07:35	09:03	08:11	08:55
32	08:57	11:08	08:58	08:09	07:35	08:58	08:16	08:55
33	08:59	11:45	09:15	08:22	07:35	08:53	08:19	08:55
34	09:01	12:54	09:30	08:34	07:35	08:48	08:21	08:55
35	09:02		09:44	08:45	07:35	08:43	08:23	08:55

Table 5.4: Predicted oviposition times for a theoretical flock photostimulated at 18 weeks

SUMMARY TABLE: OVIPOSITION TIMES

day	hen no. 1	2	3	4	5	6	7	8
1								
2								
3								
4								
5				08:48				
6				09:07				
7				09:29				
8				10:21				
9				11:28				
10				14:49				10:45
11					12:08			
12					15:15			13:12
13				06:49				16:38
14				06:47	05:17			
15					05:55			06:37
16				07:50	06:24			07:02
17				08:07	06:46			07:35
18				08:25	06:35			07:58
19				08:27	06:58			
20	12:19			08:46	06:54			08:17
21	13:42			09:01	06:55			08:18
22	16:59			09:19	06:55		11:50	08:15
23			09:50	09:45	06:50		14:24	08:04
24			10:33	11:04	06:59		17:34	08:23
25	07:37		11:23	12:36	07:15			08:25
26	07:53		12:54	16:05	06:56		06:01	08:40
27	07:54		16:17		07:11	08:56	07:03	08:24
28	08:03			05:38	07:05	09:04	07:18	08:20
29	08:19	09:25	07:00	06:31	07:07	08:40	07:12	08:41
30	08:07	09:56	07:44	07:25	07:04	08:31	07:53	08:36
31	08:15	10:00	08:03	07:13	06:58	08:28	07:22	08:10
32	08:35	10:19	08:28	07:35	07:06	08:30	07:31	08:30
33	08:22	11:04	08:33	07:59	07:03	08:25	07:59	08:32
34	08:36	12:16	09:12	07:48	07:17		07:59	08:27
35	08:46	15:40	09:34	08:11	06:58	08:02	07:41	08:18

Estimation of the oviposition time for the last egg of a sequence needs to be handled differently, because there is no associated ovulation. An analysis of the oviposition intervals between the penultimate and ultimate eggs of sequences of varying lengths was done on data provided by Morris (unpublished) and from the recorded experimental data. Figure 5.14 shows that this interval changes according to the length of the sequence; shorter sequences tend to have longer intervals between the last two ovipositions. The large variation between the intervals for the longer egg sequences is due to the fact that the sample sizes were relatively small.

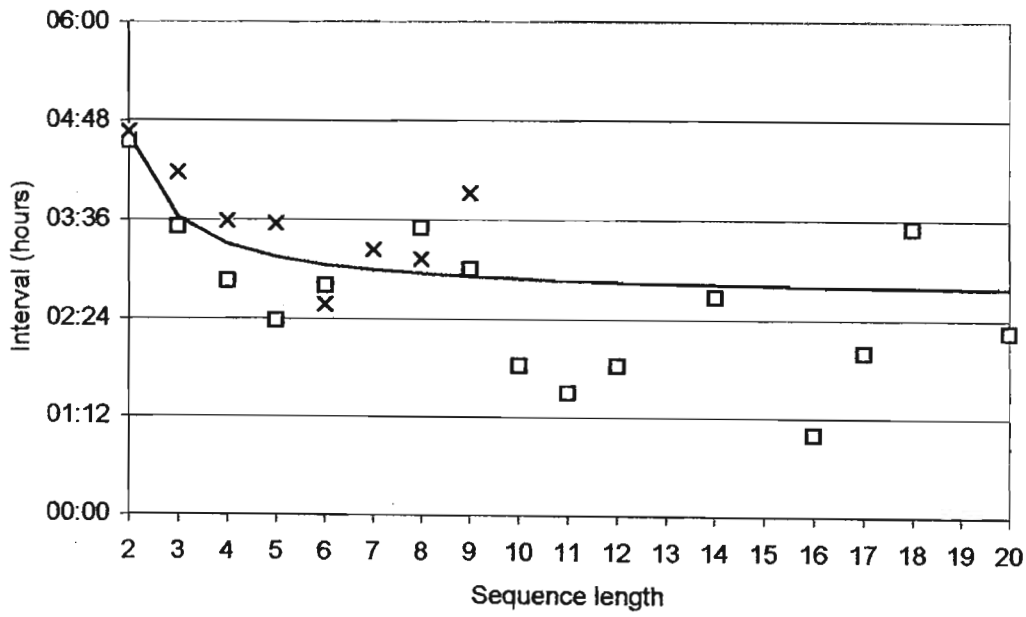


Figure 5.14: The relationship between the oviposition interval minus 24 hours between the last two eggs of a sequence and sequence length. Data from Morris (×) and Chapter 3 (□). The solid line shows the fitted linear-by-linear function

A fitted linear-by-linear equation of the form

$$y = A + B / (1 + D . x) \qquad \text{(Equation 5.8)}$$

makes it possible to predict the interval between the last two ovipositions of a sequence (y) from the ovulation rate (x) rather than from the sequence length, given that in the

population model ovulation rate determines sequence length. Appendix 5.4 lists the statistical summary as well as the values of the parameters used by Equation 5.8.

An array containing the variable 'Last Interval' stores the intervals, which are recalculated at the end of each day along with ovulation rate. Because the function gives the values in numeric form, the array is passed to Excel, converted to the correct time format and passed back to Visual Basic as 'Final Interval'. The oviposition time is calculated by adding the final interval to the previous day's ovulation time, which is stored in the array 'Yesterday ovtime'. Figure 5.15 presents a flow diagram of the procedure used to predict oviposition time.

Figure 5.16 illustrates the distribution of the predicted times of oviposition from 500 days of production. The distribution is clearly bimodal, the shorter sequences that come with advancing age lead to an increasing number of late afternoon ovipositions. The mean time of lay is 10:22, 12h52m after the sunset signal at 21:30. A 14-hour photoperiod was used in this exercise, which means that 9.4 % of the eggs are laid before the 07:30 sunrise.

Figure 5.17 illustrates the distribution of oviposition times for a similar flock also kept on a 14-hour photoperiod, but with sunset taking place at 19:30, *i.e.* two hours earlier. In this instance the predicted mean time of lay is 07:43, 12h13m after sunset. 11.7% of the eggs are laid during the scotoperiod in the hours before sunrise at 05:30.

A unimodal distribution of oviposition times is shown in Figure 5.18. This was created by simulating a population of hens to 40 weeks of age. The longer sequences produced at peak rate of lay are characterised by the majority of ovipositions occurring in the mornings.

The method used to link ovulation (and oviposition) time to the onset of darkness therefore seems to work satisfactorily. Mean oviposition time phase-shifts in accordance with changes to the start of the scotoperiod. Because the parameter S_1 is influenced by both the timing of the onset of darkness and the ovulation rate, the first egg of a long sequence is laid earlier in the morning than the first egg of a short sequence. Evidence of this may be seen in Table 5.4.

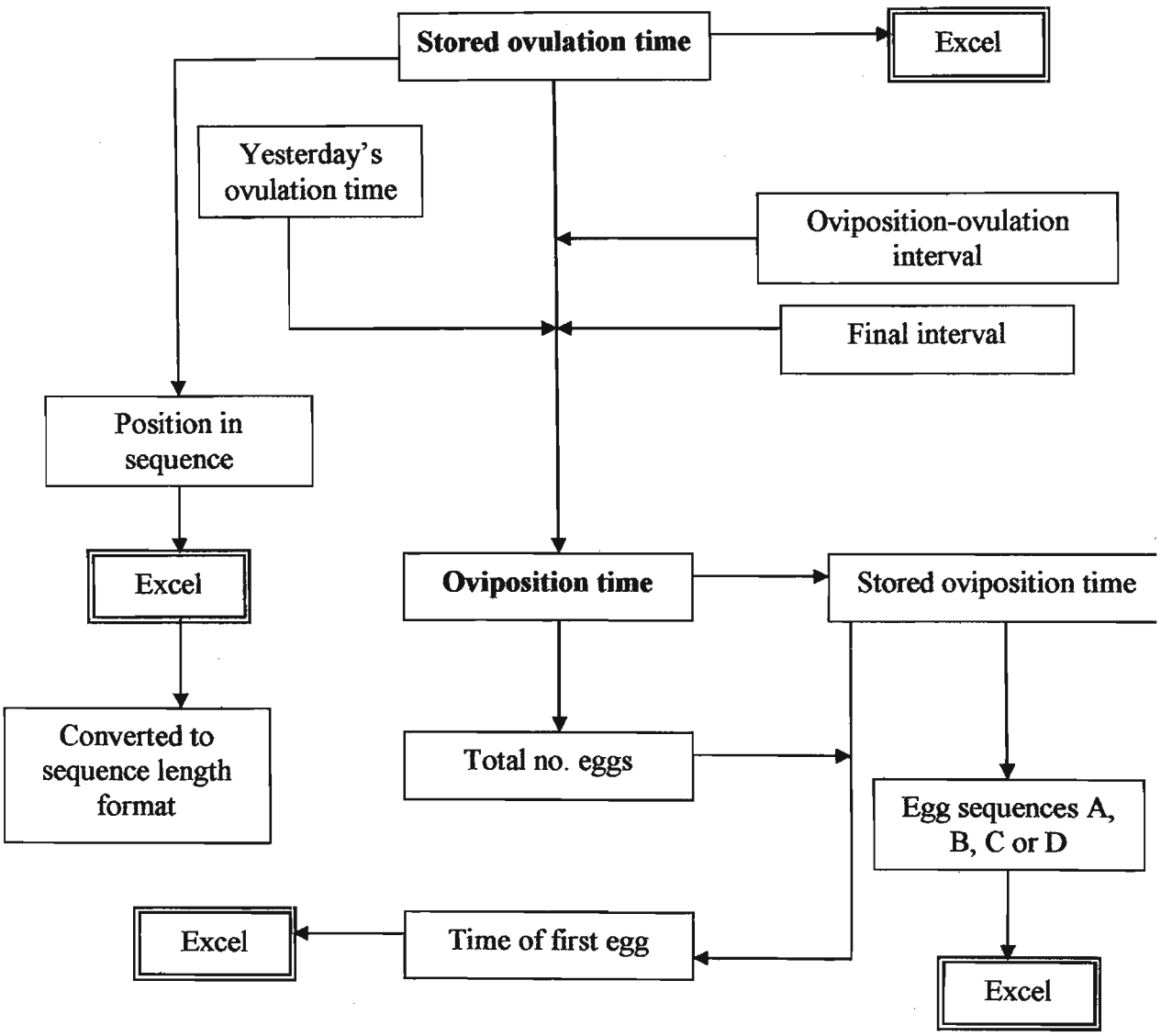


Figure 5.15: Flow diagram showing the prediction of oviposition times, the position of ovulations in a sequence and sequence length

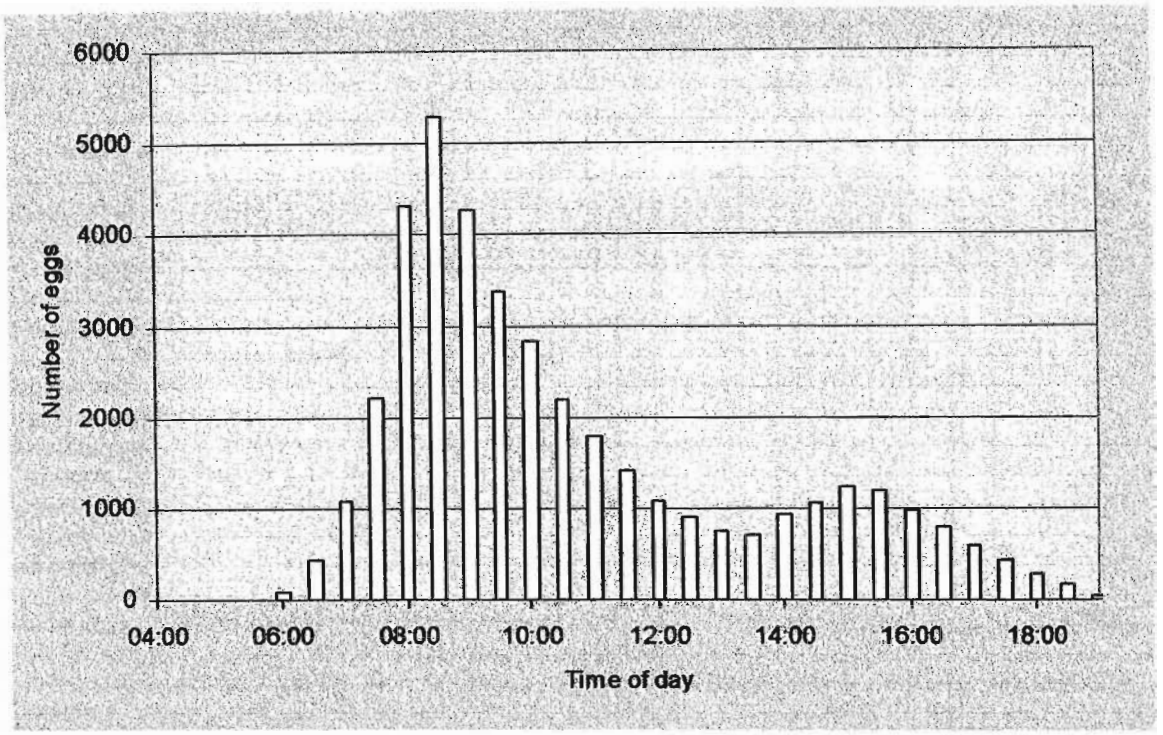


Figure 5.16: Frequency distribution of the predicted times of lay for a theoretical flock of 100 hens for 500 days, exposed to sunset at 21:30

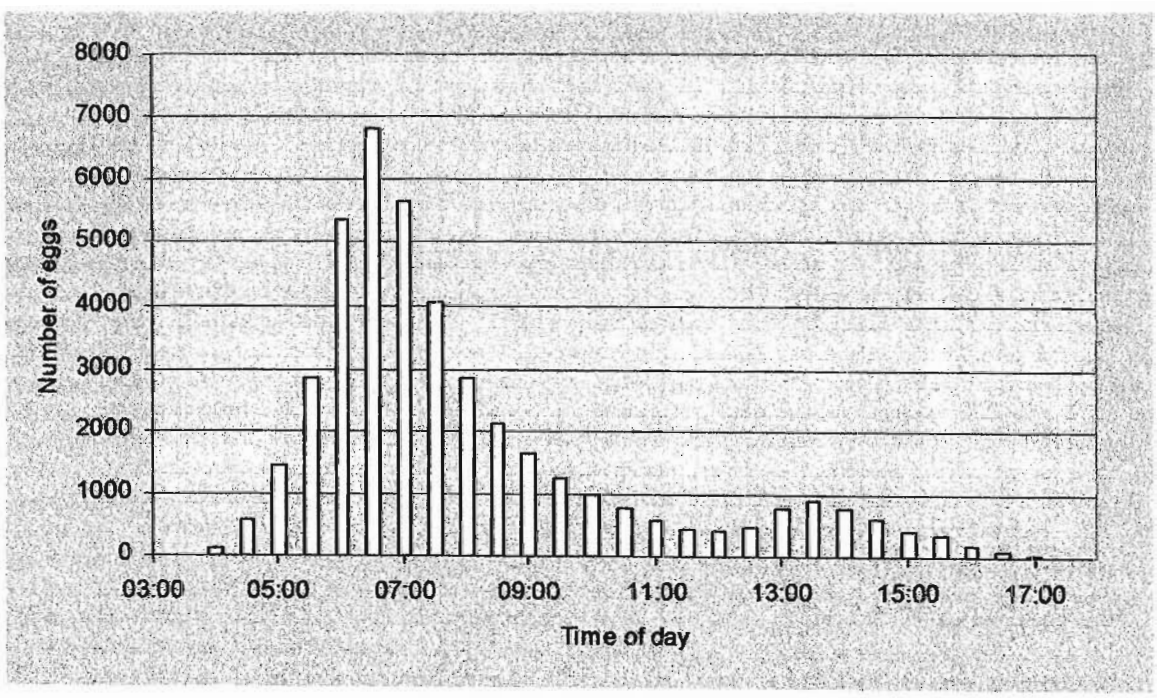


Figure 5.17: Frequency distribution of the predicted times of lay for a theoretical flock of 100 hens for 500 days, exposed to sunset at 19:30

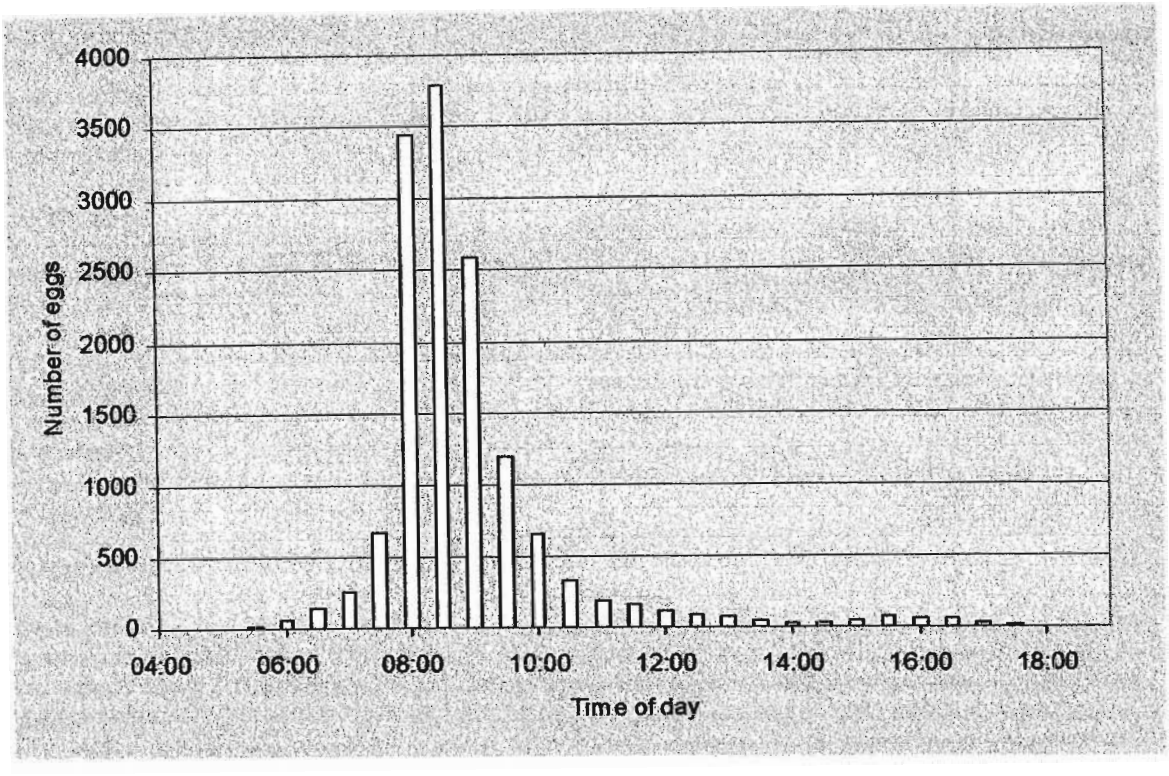


Figure 5.18: Frequency distribution of the predicted times of lay for a theoretical flock of 100 hens for 154 days, exposed to sunset at 21:30. The distribution is unimodal

5.9 Times of lay of first eggs

The oviposition time of the first egg laid by each hen at sexual maturity is stored in an array called 'Time First Egg'. The function refers to the array 'Total Eggs' so that a time is stored only if the total number of eggs for that hen is equal to one. Once the variable 'Count TFE' (which counts the number of times that are stored) is equal to 100, this sub-procedure is skipped.

This array is transferred to the Excel worksheet 'timefirstegg' so that a frequency analysis can be performed on the data and a histogram can be plotted. Figure 5.19 shows the distribution of times of lay of first eggs for a flock where the lights in the poultry house are turned off at 21:30. The mean time of lay is 10:53, which is 13h23m after sunset. In addition, 72% of the predicted first eggs are laid by noon, which compares favourably with the 76% reported in the trial (Section 3.4.3).

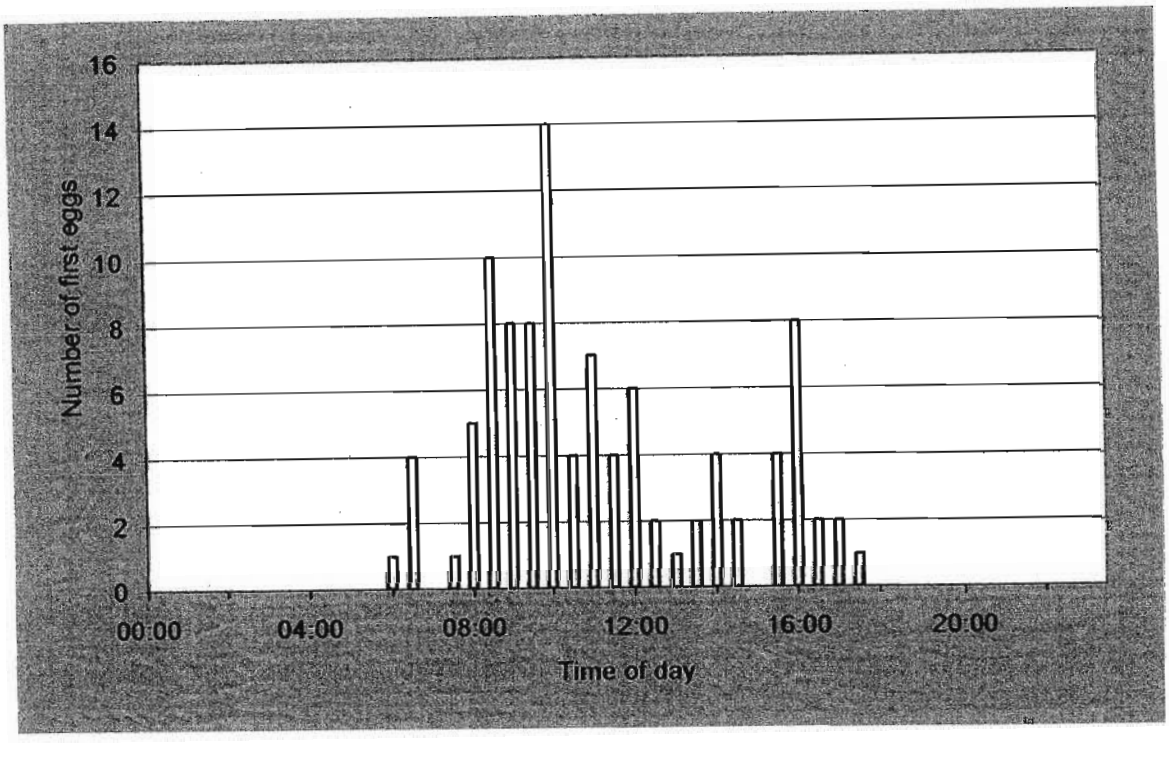


Figure 5.19: The distribution of oviposition times of first eggs at onset of sexual maturity for the theoretical flock of 100 hens

A first egg laid in the afternoon will more than likely form a one-egg sequence, which contributes to the shorter mean sequence length at onset of lay. Given that a substantial proportion of the theoretical flock laid their first eggs after noon, the method of determining a distribution of starting values for follicle maturation for the population (discussed in Section 5.4) may be accepted as successful.

5.10 Internal ovulations

The proportion of a flock expected to ovulate internally is dependent on the strain. For instance, according to the trial (see Section 3.4.8) about 39% of the experimental hens may have had one or more internal ovulations, but a greater proportion occurred among the Hy-Line Silver birds. Consequently, in a flock consisting of one strain it is estimated that about 48% of Silver and 28% of Brown birds are prone to internal ovulations. In order to restrict the incidence of internal ovulations to a given proportion of the flock, an array called 'Hen internal ovulations' is filled with uniformly distributed random numbers from zero to one. If an element in the array is less than or equal to 0.48 (for Hy-Line Silver) or

0.28 (for Hy-Line Brown) then the hen represented by this element is marked as a potential internal ovulator. In the theoretical flock, approximately 48 of the 100 Hy-Line Silver hens should have a number of 0.48 or less. Another array called 'Internal ovulations' is filled with random numbers from zero to one (generated by the Rnd function) for potential internal ovulators, but left empty for the remainder of the flock. This array is used to identify the birds that will produce an internal ovulation on a particular day and its sub-procedure is therefore recalculated daily, to ensure that each susceptible individual produces internal ovulations at random.

The percentage of internal ovulations is expected to decrease with age; pullets subjected to early photostimulation and hence coming into lay at a young age are more likely to exhibit asynchrony between ovary and oviduct. Older hens are also thought to be increasingly prone to internal ovulations (see Section 1.6). The quadratic-by-linear function

$$y = -58.08 + 67.17 / (1 + 0.001585 \,.\, \text{HenAge}) + 0.04426 \,.\, \text{HenAge} \qquad \text{(Equation 5.9)}$$

where y = % internal ovulations and HenAge is in days, was loosely derived from the experimental data for young hens and may be used to determine the percentage of internal ovulations expected at a particular age for Hy-Line Silver hens. For example, at 126 days of age, 3.49% of total ovulations are expected to occur internally. Similarly the percent internal ovulations for Hy-Line Brown birds may be predicted from the quadratic-by-linear function

$$y = -29.78 + 38.32 / (1 + 0.002352 \,.\, \text{HenAge}) + 0.02659 \,.\, \text{HenAge} \qquad \text{(Equation 5.10)}$$

No useful information is available on the precise frequency of internal ovulations for different breeds and at specific ages in older hens, so the predicted increase in percent internal ovulations towards the end of the laying cycle, used to derive these functions, is conjecture. The curves given by these functions are shown in Figure 5.20.

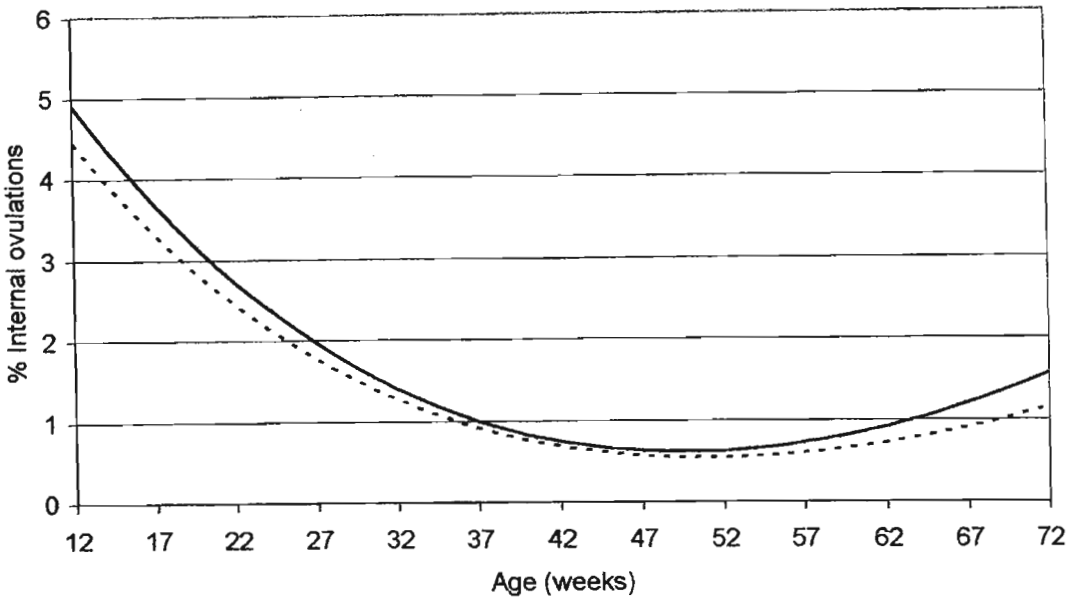


Figure 5.20: The relationship between expected percent internal ovulations and hen age for Hy-Line Silver (solid line) and Brown (dotted line) birds

The code in Visual Basic assesses the value of the random number in 'Internal ovulations' and allocates either zero or one to each element of a third array called 'Number IO', depending on whether each value is greater than or less than the percent given by Equation 5.9 or 5.10. For an internal ovulation to occur on a given day, two conditions must be met, *i.e.* the hen must be a potential internal ovulator and it must be one of the hens due to produce an internal ovulation on that day. Table 5.5 shows an example of the processes for predicting which birds will ovulate internally on a fixed day.

Table 5.5: Predicting the incidence of internal ovulations in Hy-Line Silver birds at 126 days of age (IO = internal ovulations)

Hen no.	Array: Hen IO	Potential internal ovulator	Array: IO	Array: Number IO	Internal ovulation
1	0.57	No			Never
2	0.23	Yes	0.01	1	Yes
3	0.02	Yes	0.07	0	No
4	0.47	Yes	0.21	0	No
5	0.13	Yes	0.03	1	Yes

With this method the number of predicted internal ovulations should decline with advancing hen age before increasing towards the end of the laying year, and should be restricted to a proportion of the laying flock. This latter is important because of the ability of an internal ovulation to disrupt egg sequences. Those hens that do not ovulate internally should have longer mean sequence lengths. In the model, internal ovulations will have associated ovulation times but no oviposition times or egg weights.

Figure 5.21 shows the distribution of internal ovulations within a theoretical flock of 70-week-old Hy-Line Silver hens photostimulated at 18 weeks. These internal ovulations were produced by 46 birds. One of these hens ovulated internally 14 times over the laying cycle. The histogram differs from those of the trial birds (Figures 3.16 and 3.17), which are J-shaped, but this is due to the substantially longer period of time during which the incidence of internal ovulations was predicted in the model. Figure 5.22 illustrates how the percent internal ovulations per week changes over time, with about 1% taking place in the middle of the laying cycle.

Table 5.6 summarises the predicted number of internal ovulations given by the model, for the Hy-Line Silver and Hy-Line Brown birds photostimulated at three ages. As expected, the Silver strain produced a higher number of internal ovulations than the Brown strain. Similarly, 12-week photostimulation increased the incidence of internal ovulations.

Table 5.6: The predicted number of internal ovulations to 70 weeks of age, and the percentage of total ovulations, for the two strains of Hy-Line birds photostimulated at different ages

		Age at photostimulation		
Strain		12 weeks	15 weeks	18 weeks
Hy-Line Silver	internal ov.	552 (1.62%)	384 (1.14%)	460 (1.42%)
	no. hens	52	40	51
Hy-Line Brown	internal ov.	354 (1.04%)	341 (1.03%)	341 (1.07%)
	no. hens	26	25	27

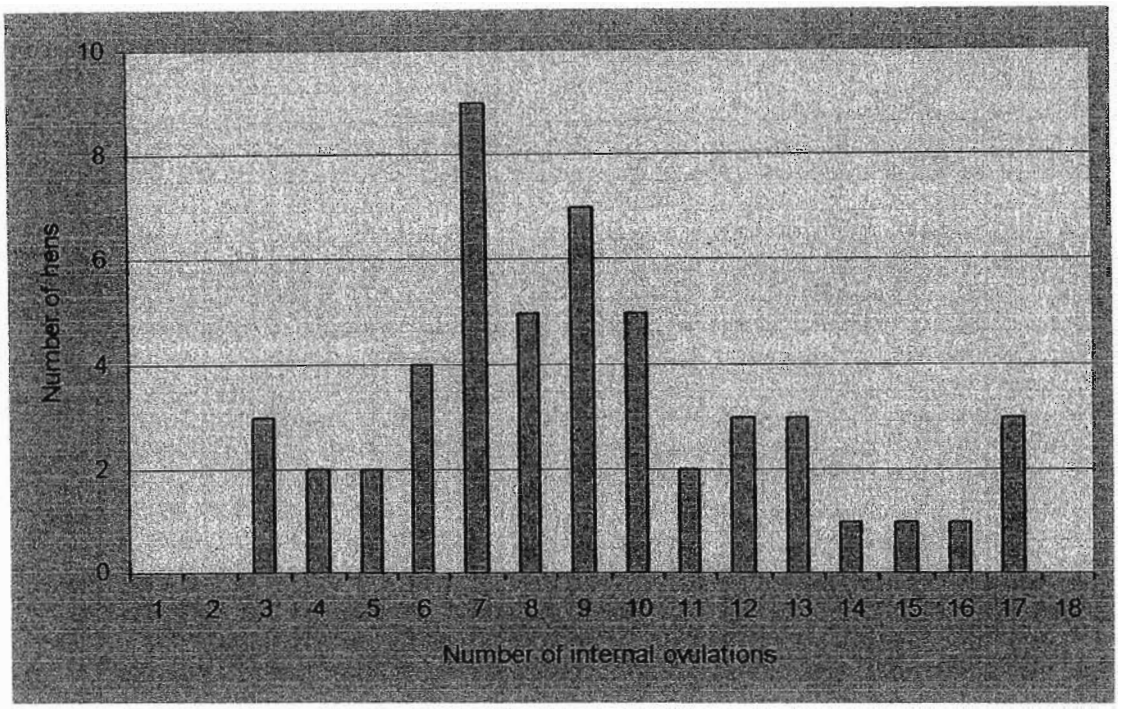


Figure 5.21: The distribution of internal ovulations in a theoretical flock of 100 Hy-Line Silver hens photostimulated at 18 weeks of age

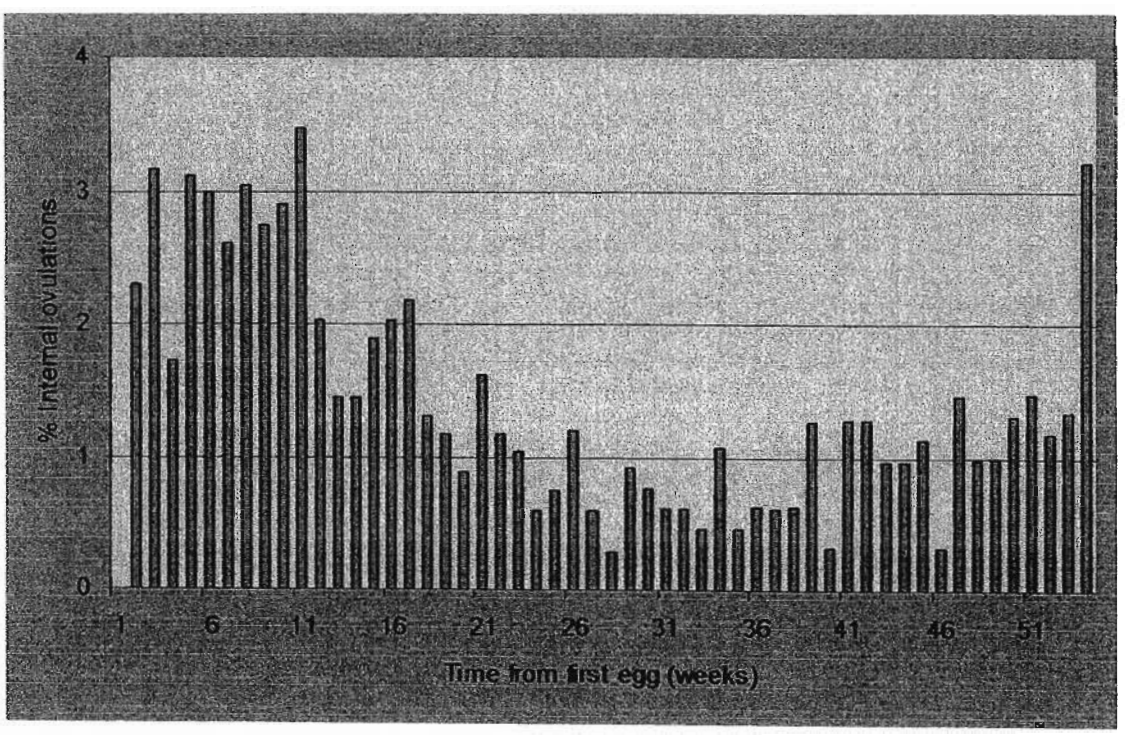


Figure 5.22: The percent internal ovulations produced over a full laying cycle, by a theoretical flock of 100 Hy-Line Silver hens photostimulated at 18 weeks

5.11 Soft-shelled eggs

A similar procedure determines the production of soft-shelled eggs on a daily basis. Thirty-two percent of the experimental hens laid one or more soft-shelled eggs. This proportion was made up of 38% of the Hy-Line Silver strain and 26% of the Brown strain. Although these numbers are derived from one trial and may be expected to vary according to environmental conditions and between populations, it is nevertheless useful to be able to demonstrate the capability of the model of responding to different genotypes.

The percentage of soft-shelled eggs produced by the flock varies according to hen age; both young hens at onset of lay and older hens nearing the end of their laying cycles are more likely to produce soft-shelled eggs. Two line-plus-exponential functions, again loosely derived from the experimental data, may be used to predict the percentage of soft shells for the Silver and Brown birds respectively:

$$y = -0.8006 + 1202.6 \,.\, (0.952622^{\,HenAge}) + 0.0036895 \,.\, HenAge \qquad \text{(Equation 5.11)}$$

$$y = -0.8134 + 714.6 \,.\, (0.953762^{\,HenAge}) + 0.0037948 \,.\, HenAge \qquad \text{(Equation 5.12)}$$

The curves given by these functions are shown in Figure 5.23. Hy-Line Silver birds that are photostimulated at 12 weeks and come into lay at about 15 weeks of age may be expected to produce 6.95% soft shells in the first week of lay.

As may be seen in Section 3.4.7, there are many instances when more than one soft-shelled egg is produced by a hen on a given day. Furthermore, about 31% of soft shells are thought to cause interruptions in sequences. An extremely complex model would be required to account satisfactorily for all possible permutations of soft-shelled and normal eggs. From a commercial point of view, the most important soft shells are those that replace normal eggs and therefore result in a loss of income to the producer. For this reason, it was decided to keep the model relatively simple and estimate the number of soft-shelled eggs that are laid singly in place of a normal shelled egg. A soft shell predicted in this manner therefore does not terminate an oviposition sequence but does reduce the number of marketable eggs laid.

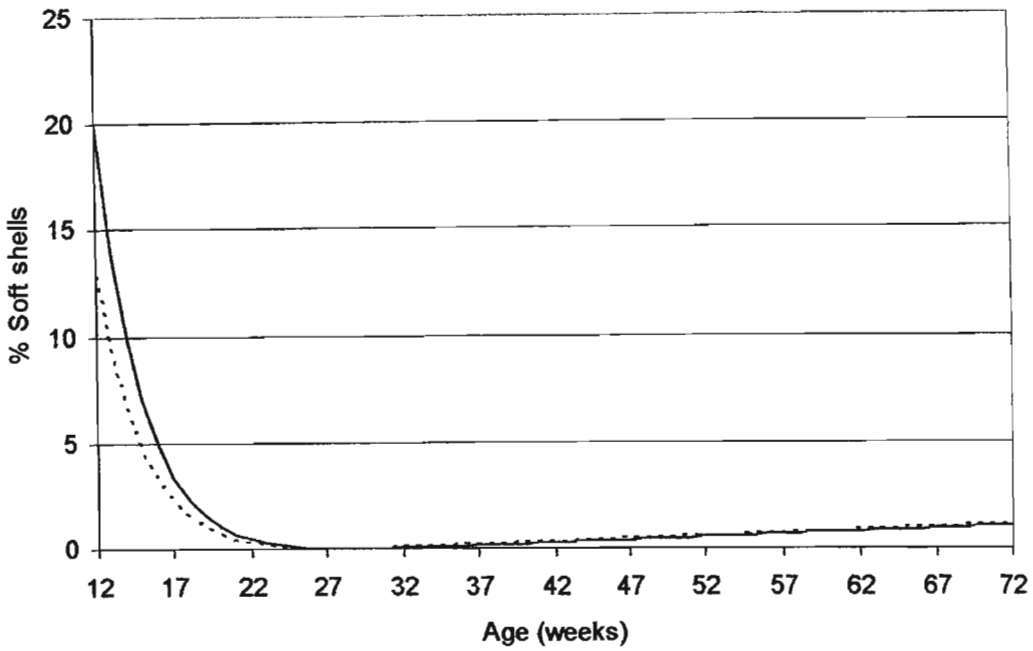


Figure 5.23: An illustration of the relationship between expected percent soft shells and hen age for Hy-Line Silver (solid line) and Brown (dotted line) birds

Figure 5.24 shows the distribution of soft shells within a population of 70-week-old Hy-Line Silver hens photostimulated at 18 weeks of age. Three birds laid one soft-shelled egg each and one bird laid eight. A total of 141 soft shells (0.43% of total eggs) was produced by 36% of the hens; 64 of the 100 hens did not lay any soft-shelled eggs. Figure 5.25 shows how the percent soft shells changed during the course of the laying year. Because the pullets were photostimulated at the recommended age, there were very few soft shells at onset of lay. An increasing number were produced towards the end of the productive period.

Table 5.7 summarises the predicted numbers of soft shells given by the model, for the Hy-Line Silver and Hy-Line Brown birds photostimulated at three ages. The Silver strain produced more soft-shelled eggs than the Brown strain. Earlier photostimulation also increased the incidence of soft shells. The method used to simulate the production of soft-shelled eggs in the model therefore appears to be successful.

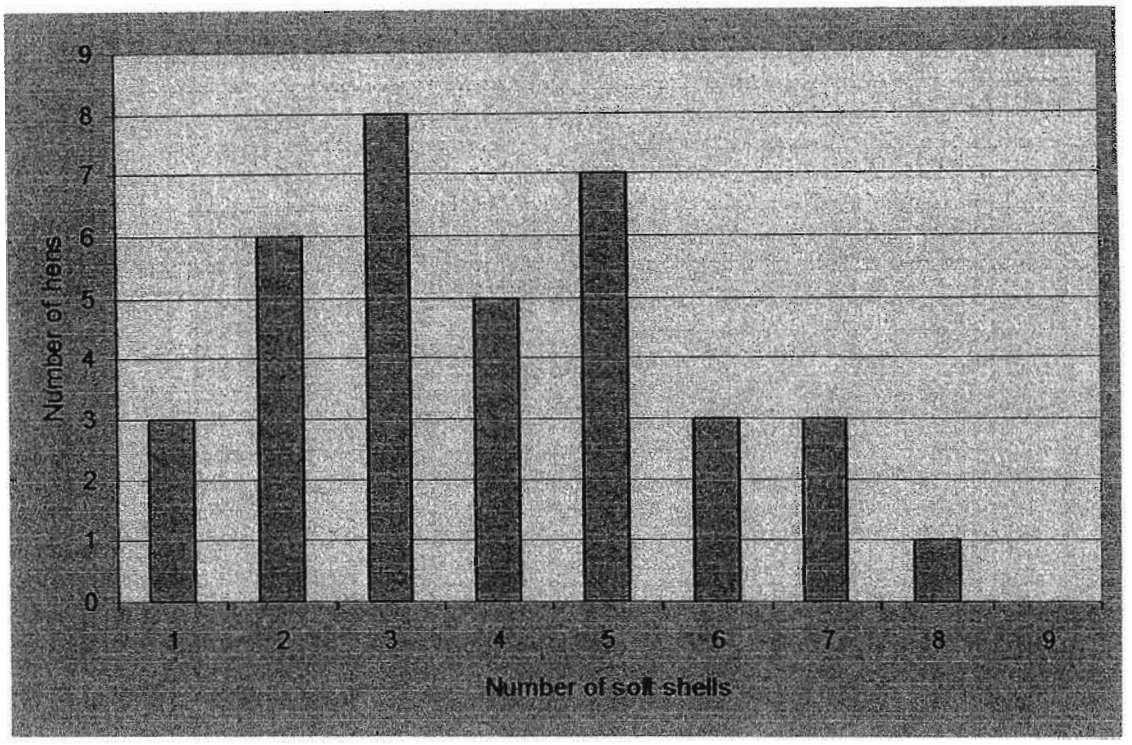


Figure 5.24: The distribution of soft shells in a theoretical flock of 100 Hy-Line Silver hens photostimulated at 18 weeks of age

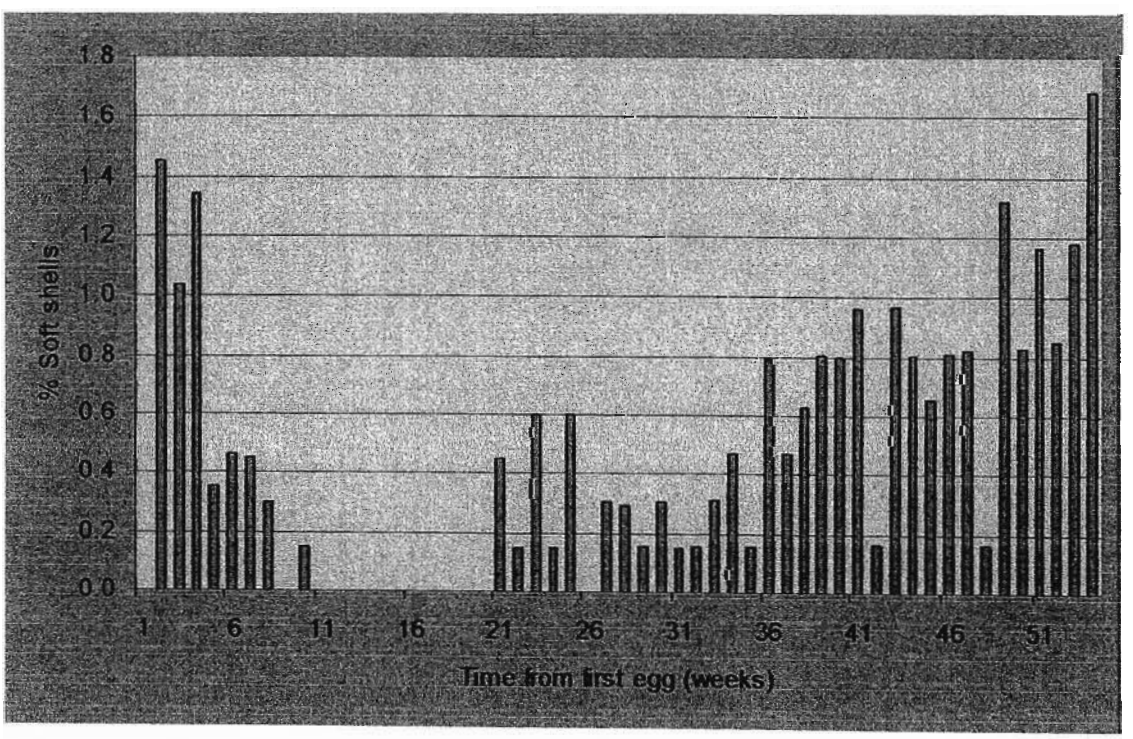


Figure 5.25: The percent soft shells produced over a full laying cycle, by a theoretical flock of 100 Hy-Line Silver hens photostimulated at 18 weeks

Table 5.7: The predicted number of soft shells to 70 weeks of age, and the percentage of total eggs, for the two strains of Hy-Line birds photostimulated at three ages

		Age at photostimulation		
Strain		12 weeks	15 weeks	18 weeks
Hy-Line Silver	soft shells	236 (0.69%)	159 (0.47%)	141 (0.43%)
	no. hens	48	36	36
Hy-Line Brown	soft shells	148 (0.44%)	141 (0.43%)	138 (0.43%)
	no. hens	21	24	22

5.12 Double-yolked eggs

A similar procedure is used in the population model to determine the production of double-yolked eggs on a daily basis. Thirty-six percent of the experimental hens laid one or more double yolks, the proportion being the same for both strains of Hy-Line. Double-yolked eggs are normally only laid at onset of sexual maturity and the earlier the pullets are brought into lay, the greater the incidence of double yolks.

An exponential function, based on the experimental data, may be used to predict the percent double yolks for both strains of Hy-Line birds:

$$y = -0.000475 + 8786.66 \,.\, (0.9403199^{\,HENAGE}) \qquad \text{(Equation 5.13)}$$

where y = % double-yolked eggs. The curve given by this equation is shown in Figure 5.26. Hy-Line pullets receiving increasing daylength at 12 weeks and coming into lay at about 15 weeks may be expected to produce 13.7% double yolks.

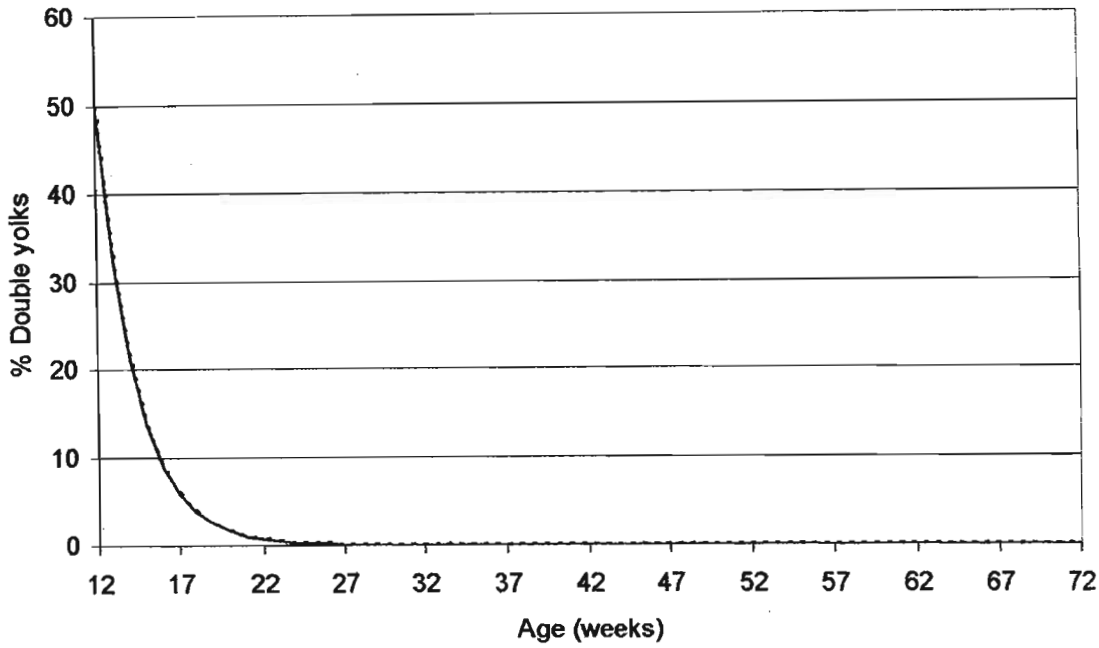


Figure 5.26: An illustration of the relationship between expected percent double yolks and hen age for Hy-Line birds

Figure 5.27 shows the distribution of double yolks within the theoretical flock of 70-week-old Hy-Line Silver hens photostimulated at 18 weeks. Twelve hens laid one double-yolked egg and nine hens laid two double-yolked eggs. Only 23% of the population produced double yolks. This proportion is substantially lower than anticipated, the model having identified 36 hens as potential multiple ovulators, but the figure is presumably influenced by the relatively short period allocated to the production of double yolks. These 37 double yolks accounted for 0.11% of the total eggs laid to 70 weeks of age.

Figure 5.28 provides evidence that all the double yolks produced by the model were laid during the initial eleven weeks of egg production. The distribution is J-shaped, as are the distributions from the experimental birds shown in Figures 3.10 and 3.11.

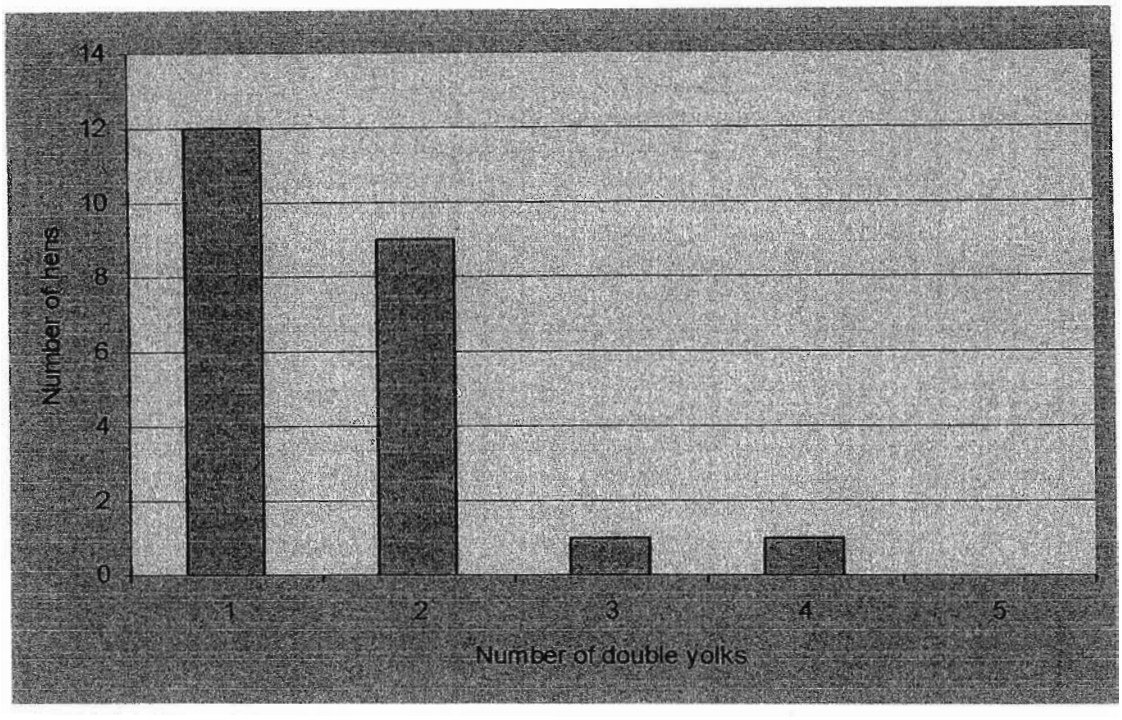


Figure 5.27: The distribution of double yolks in a theoretical flock of 100 Hy-Line Silver hens photostimulated at 18 weeks of age

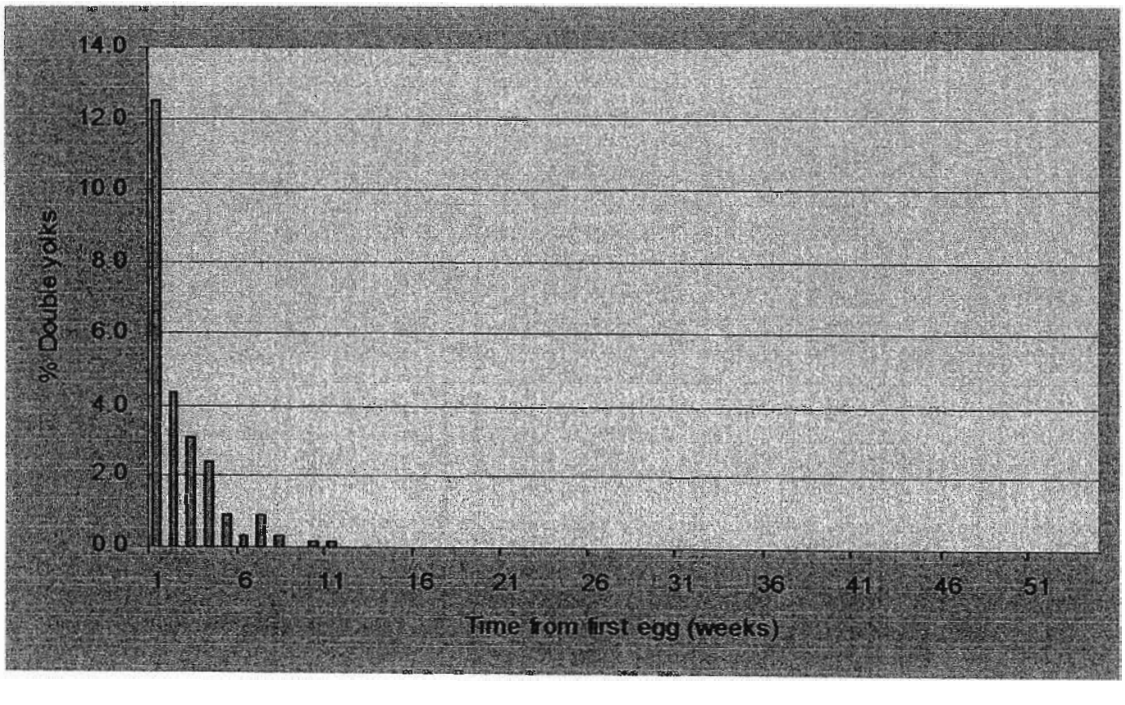


Figure 5.28: The percent double yolks produced over a full laying cycle, by a theoretical flock of 100 Hy-Line hens photostimulated at 18 weeks

Table 5.8 summarises the predicted number of double yolks given by the model, for the two strains photostimulated at three ages. In each case the proportion of the flock laying double-yolked eggs is indicated by the number of hens; for example, 37% of the Hy-Line Silver birds photostimulated at 12 weeks laid double-yolked eggs. In view of the fact that Equation 5.13 is used to predict the percent double yolks from hen age for both strains, the differences between the Silver and Brown hens are entirely due to chance. However, early photostimulation is seen to increase the incidence of double-yolked eggs.

Table 5.8: The predicted number of double yolks to 70 weeks of age, and the percentage of total eggs, for a flock of Hy-Line birds photostimulated at three ages

		Age at photostimulation		
Strain		12 weeks	15 weeks	18 weeks
Hy-Line Silver	double yolks	144 (0.42%)	45 (0.13%)	37 (0.11%)
	no. hens	37	24	23
Hy-Line Brown	double yolks	102 (0.30%)	54 (0.16%)	19 (0.06%)
	no. hens	38	28	16

5.13 Sequence lengths

In order to calculate the mean sequence length for the flock over time, the position of each ovulation in the sequence is stored in an array named 'Sequence' at the end of each day. This array is then transferred daily into the Excel worksheet called 'mean sl' into a row containing the data for the day after the current day. This effectively facilitates the calculation of the mean egg sequence length rather than the mean ovulation sequence length. Table 5.9 shows a section of the relevant spreadsheet from the model.

Table 5.9: Predicted egg sequences, showing the position of the egg in a sequence

SUMMARY TABLE: SEQUENCE LENGTHS

day	hen no. 1	2	3	4	5	6	7	8
1								
2								
3								
4								
5				1				
6				2				
7				3				
8				4				
9				5				
10				6				1
11					1			
12					2			1
13				1				2
14				2	1			
15					2			1
16				1	3			2
17				2	4			3
18				3	5			4
19				4	6			
20	1			5	7			1
21	2			6	8			2
22	3			7	9		1	3
23			1	8	10		2	4
24			2	9	11		3	5
25	1		3	10	12			6
26	2		4	11	13		1	7
27	3		5		14	1	2	8
28	4			1	15	2	3	9
29	5	1	1	2	16	3	4	10
30	6	2	2	3	17	4	5	11
31	7	3	3	4	18	5	6	12
32	8	4	4	5	19	6	7	13
33	9	5	5	6	20	7	8	14
34	10	6	6	7	21		9	15
35	11	7	7	8	22	1	10	16

Once the model has been run for the specified number of days, the data are converted into the required format for calculating mean sequence length. For example, a five-ovulation sequence recorded as 1-2-3-4-5 will be converted to 5-5-5-5-5 because on each of those five days, the hen is producing a five-egg sequence. It follows that the conversion can only be done at the end of the laying cycle and also that the procedure starts with the sequence data for the last day of the model and works backwards towards day one. A 187-ovulation sequence is not identifiable as such until it is terminated by a pause day. The summary sheet, after conversion, is shown in Table 5.10.

Figure 5.29 shows the mean sequence lengths for two theoretical flocks, both photostimulated at 18 weeks, one consisting of 100 Hy-Line Silver hens and the other of 100 Hy-Line Brown hens. It may be seen that mean sequence length is initially short. The maximum mean sequence lengths are 86.7 and 76.8 days for the Hy-Line Silver and Hy-Line Brown flocks respectively. The trends simulated by the model are similar to those observed in the trial (refer to Section 3.4.12, Figures 3.28 and 3.29). As anticipated, the Silver strain has slightly longer mean sequence lengths than the Brown strain for most of the laying cycle. Figure 5.30 illustrates the mean sequence lengths for the flock split into thirds, as described in Section 3.4.12. There is noticeably more variation in the simulated flock than in the experimental hens. The reason for this is not immediately apparent.

The sequence data are also stored in an array called 'Egg sequence' in a format required for a program that calculates sequence lengths, discussed in Section 3.4.12. For each hen, if an oviposition time is stored at the end of the day and the egg does not have a soft shell or double yolk, the letter 'A' is stored in 'Egg sequence' representing a normal-shelled egg. When a soft-shelled or double-yolked egg is produced, the letter 'B' or 'D' is stored. The letter 'C' represents an internal ovulation. This array is transferred daily to the Excel worksheet 'seq.length' and is available for importing into the Sequence Analyzer program, once the model has completed its run.

Table 5.10: Predicted sequence lengths, after conversion

SUMMARY TABLE: SEQUENCE LENGTHS								
	hen no.							
day	1	2	3	4	5	6	7	8
1								
2								
3								
4								
5				6				
6				6				
7				6				
8				6				
9				6				
10				6				1
11					2			
12					2			2
13				2				2
14				2	111			
15					111			4
16				11	111			4
17				11	111			4
18				11	111			4
19				11	111			
20	3			11	111			16
21	3			11	111			16
22	3			11	111		3	16
23			5	11	111		3	16
24			5	11	111		3	16
25	29		5	11	111			16
26	29		5	11	111		118	16
27	29		5		111	7	118	16
28	29			9	111	7	118	16
29	29	7	15	9	111	7	118	16
30	29	7	15	9	111	7	118	16
31	29	7	15	9	111	7	118	16
32	29	7	15	9	111	7	118	16
33	29	7	15	9	111	7	118	16
34	29	7	15	9	111		118	16
35	29	7	15	9	111	9	118	16

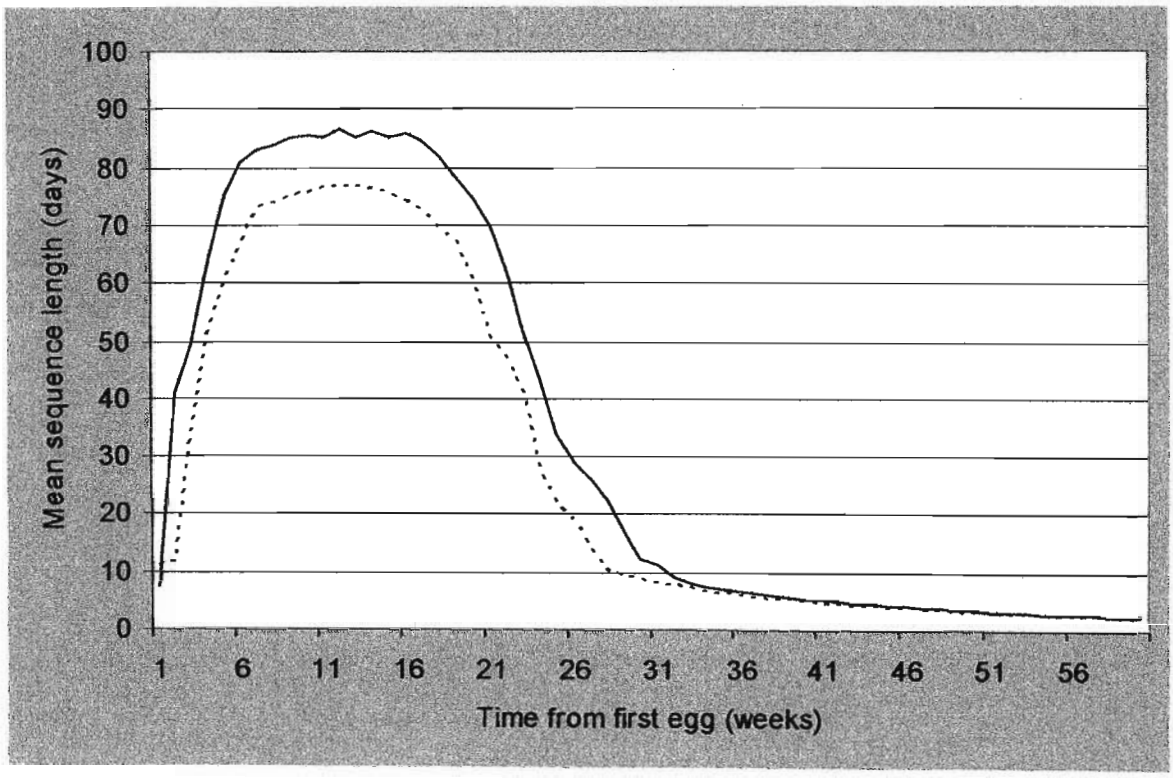


Figure 5.29: Predicted mean sequence lengths for 100 Hy-Line Silver (solid line) and 100 Hy-Line Brown (dotted line) hens

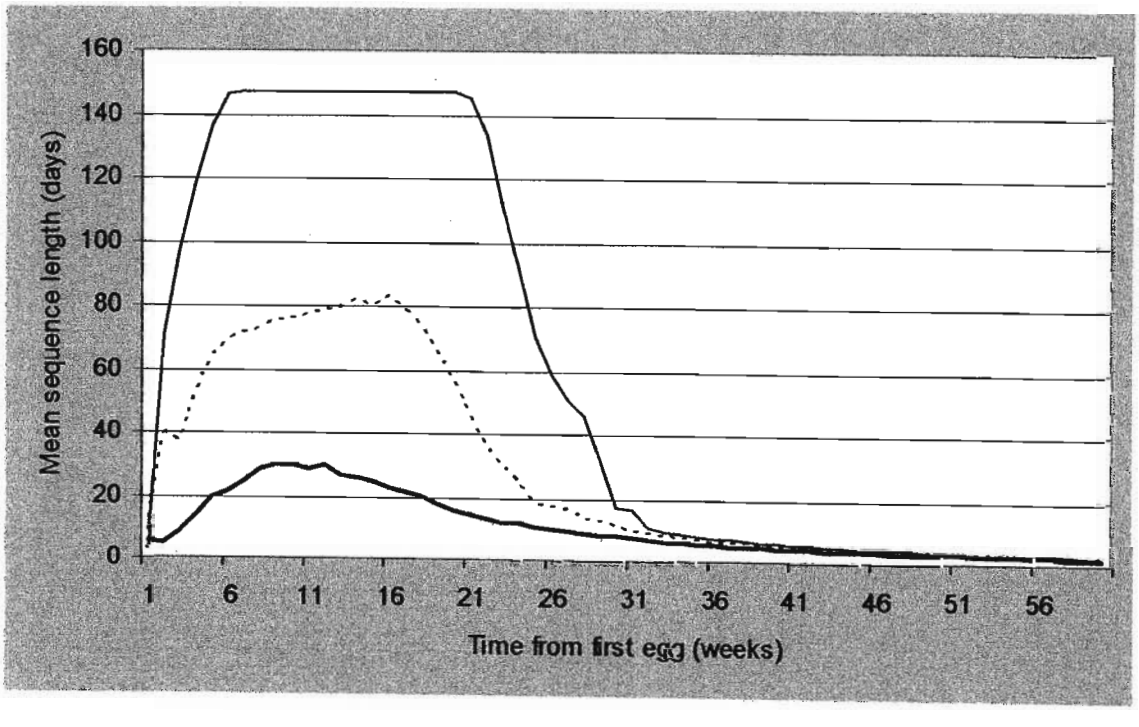
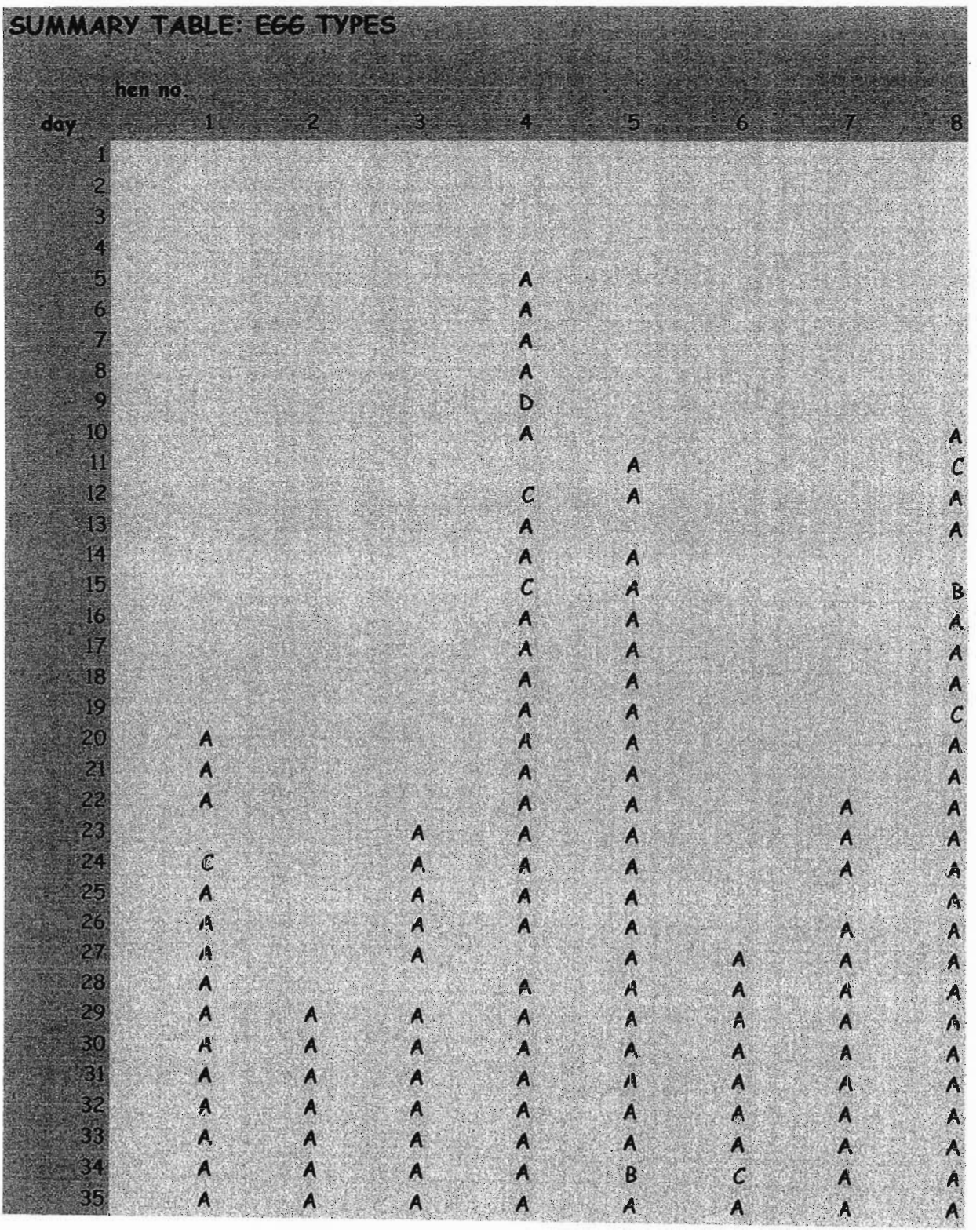


Figure 5.30: Predicted mean sequence lengths for the Hy-Line Silver flock split into thirds; top third (solid line), middle third (dotted line) and bottom third (bold line)

Table 5.11 contains a section of the spreadsheet, from which it may be seen that hen four laid a double-yolked egg on day nine, which did not interrupt the sequence (refer to Table 5.9). Soft-shelled eggs were produced by hen eight on day 15 and by hen five on day 34, and also formed part of normal egg sequences. In contrast, the six internal ovulations all resulted in pauses. For example, hen four had two internal ovulations; on days 12 (at the start of a sequence) and 15. This means that the 15-ovulation sequence (Table 5.3) is effectively reduced to a two-egg and an eleven-egg sequence (Table 5.10).

A spreadsheet in Excel called 'seq.analyzer' organises the sequence data in the correct format for importing into the Sequence Analyzer program. The layer model was run for theoretical flocks of Hy-Line Silver and Hy-Line Brown birds photostimulated at 12, 15 or 18 weeks of age. The Sequence Analyzer software was then used to analyse the performance of the flocks in terms of the sequence characteristics. These results are summarised in Table 5.12. The Hy-Line Silver hens exhibited longer mean prime sequences, fewer sequences and longer mean sequence lengths than the Hy-Line Brown hens. There is no difference in the mean pause lengths. The mean number of sequences is negatively related to age at photostimulation, whereas the mean sequence length is positively related to age at photostimulation. The mean prime sequence length did not respond as anticipated for either strain; early photostimulation is expected to reduce the lengths of the prime sequences. However, in the experimental hens there was also no apparent trend in mean prime sequence length for the Hy-Line Silver birds (Table 3.32). It may be that the large amount of variation between individuals, as indicated by the large standard deviations, plays a role in making the response to age at photostimulation unpredictable.

Table 5.11: Predicted egg types (A = normal; B = soft shell; C = internal ovulation; D = double yolk)



SUMMARY TABLE: EGG TYPES

day	hen no. 1	2	3	4	5	6	7	8
1								
2								
3								
4								
5				A				
6				A				
7				A				
8				A				
9				D				
10				A				A
11					A			C
12				C	A			A
13				A				A
14				A	A			
15				C	A			B
16				A	A			A
17				A	A			A
18				A	A			A
19				A	A			C
20	A			A	A			A
21	A			A	A			A
22	A			A	A		A	A
23			A	A	A		A	A
24	C		A	A	A		A	A
25	A		A	A	A			A
26	A		A	A	A		A	A
27	A		A		A	A	A	A
28	A			A	A	A	A	A
29	A	A	A	A	A	A	A	A
30	A	A	A	A	A	A	A	A
31	A	A	A	A	A	A	A	A
32	A	A	A	A	A	A	A	A
33	A	A	A	A	A	A	A	A
34	A	A	A	A	B	C	A	A
35	A	A	A	A	A	A	A	A

Table 5.12: Summary of mean sequence characteristics (±sd) for the theoretical flocks

Strain	APS* (weeks)	Prime seq.length	Number of sequences	Sequence length	Pause length
Hy-Line Silver	12	76.19 (±59.47)	30.73 (±9.20)	12.38 (±5.12)	1.03 (±0.04)
	15	84.21 (±62.13)	28.39 (±8.09)	13.38 (±5.97)	1.02 (±0.03)
	18	82.76 (±60.20)	27.41 (±9.15)	13.60 (±5.56)	1.02 (±0.03)
Strain mean		81.05	28.84	13.12	1.02
Hy-Line Brown	12	75.98 (±59.82)	31.42 (±9.59)	12.04 (±4.43)	1.02 (±0.04)
	15	75.85 (±63.67)	29.99 (±9.73)	12.60 (±5.14)	1.02 (±0.04)
	18	72.70 (±61.69)	28.47 (±9.82)	13.02 (±5.90)	1.02 (±0.03)
Strain mean		74.84	29.96	12.55	1.02

* age at photostimulation

The mean pause lengths of the experimental Hy-Line Silver and Hy-Line Brown hens were 1.59 and 1.51 respectively (Tables 3.32 and 3.33). The model of Etches and Schoch (1984), both in its original and in its revised form, does not create pauses longer than one day. In the population model a few longer pauses have been created by the inclusion of random internal ovulations during the laying cycles of certain individuals. In order to increase the mean pause length, either the incidence of internal ovulations needs to be increased or a proportion of double-yolked and soft-shelled eggs should be allowed to interrupt sequences. Alternatively, poor-producing hens, as observed amongst the trial birds, could be introduced into the flock by one of two methods. Continual interruptions to the circadian rhythm of LH release, perhaps due to environmental stress or genetic factors, would remove the preovulatory LH surge. This could be modelled by preventing the

initiation of the regulator concentration function for a number of days. Secondly, atresia of large yellow follicles in the hierarchy or inadequate recruitment would result in gaps in the hierarchy. The way to implement this in the model would be to prevent the follicle from commencing its final phase of maturation.

With either method, ovulation could be suspended for a number of consecutive days, therefore introducing longer pauses between the egg sequences. These would all be fairly easy to model, but further research is necessary in order to establish which option would be preferable. If oviposition time is recorded for an extended period in older hens that are laying infrequently, it should be possible to ascertain whether the pauses are due to internal ovulations, in which case the next oviposition will take place later in the day; or due to the absence of ovulation, in which case the next egg will probably be laid early in the morning.

5.14 Egg component weights

Mean yolk weight for the Amber-Link birds is predicted from hen age, using Equation 4.7. Similarly Equations 3.4 and 3.5 are used to predict mean yolk weight from hen age for the Hy-Line Silver and Hy-Line Brown hens. The random number generator creates 100 normally distributed yolk weights around the calculated mean using a coefficient of variation of 5%. This procedure is done daily so that yolk weight increases with advancing hen age.

At the end of each day, if an oviposition has occurred (*i.e.* there is no internal ovulation) and the egg is either a normal-shelled egg or a soft-shelled egg, the individual yolk weight is stored in an array called 'yolk weight'. For a double-yolked egg, the predicted yolk weight is multiplied by two and stored. The weights for the flock are transferred to the Excel worksheet 'yolk wt' so that the weekly mean yolk weight may be calculated and plotted on a chart.

For Amber-Link hens, albumen weight is predicted from yolk weight using the allometric function (Equation 4.10) described in Section 4.4. Equations 4.15 and 4.17 give the albumen weights for Hy-Line Silver and Hy-Line Brown hens. The array 'Albumen weight' is filled with the predicted albumen weight if there is a corresponding yolk weight

for that specific hen. The array is transferred to Excel and stored in the worksheet 'albumen wt'.

Shell weight is predicted from the weight of the egg contents (yolk plus albumen) using the allometric functions given by Equations 4.14 (Amber-Link), 4.16 (Hy-Line Silver) and 4.18 (Hy-Line Brown). Shell weight is stored in the array 'shell weight' (only if a corresponding yolk weight has been stored) and transferred to the Excel worksheet 'shell wt'.

The array 'Egg weight' is filled by adding the weights of the three components yolk, albumen and shell. Once again the array is transferred to Excel, into a worksheet called 'egg wt'. The procedure for calculating egg weight is shown in the flow diagram of Figure 5.31.

The increases in the weights of the three components with time from first egg and the corresponding changes in their proportions, for a theoretical flock of Amber-Link hens, are illustrated in Figures 5.32 to 5.37. The predicted mean egg weight is shown in Figure 5.38. The means of the weights and percentages from the Amber-Link data summarised in Chapter 4 (Table 4.1) are also plotted on the charts, so that comparisons may be made between predicted and actual values. The model realistically simulates the increases in yolk, albumen and shell weights with advancing age for the Amber-Link strain, as well as the increase in the proportion of yolk at the expense of albumen and shell. As a consequence, both the values of the parameters used in the allometric functions, and the procedures applied in the layer model to predict component weights, are shown to be acceptable.

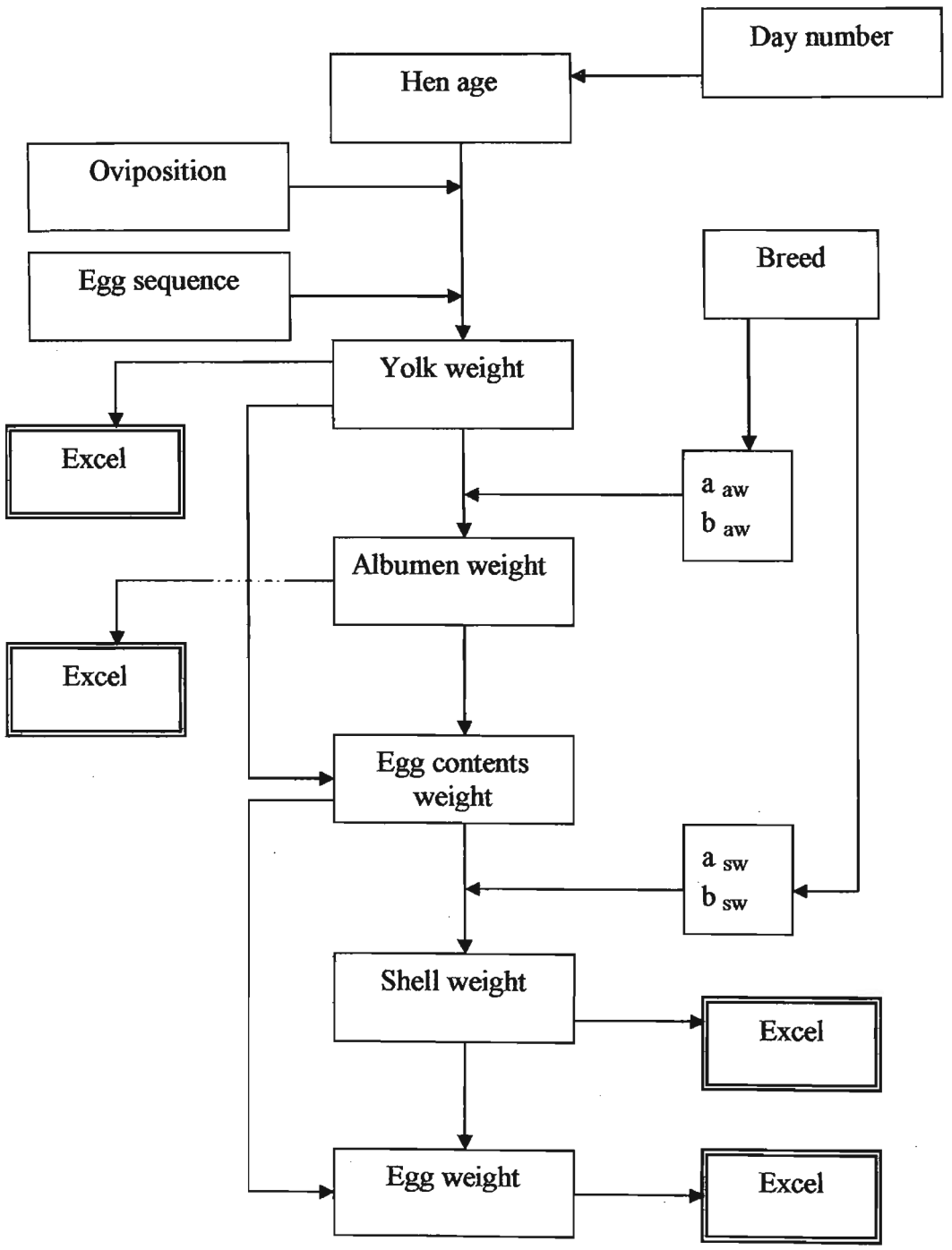


Figure 5.31: Flow diagram illustrating the procedure for calculating egg weight

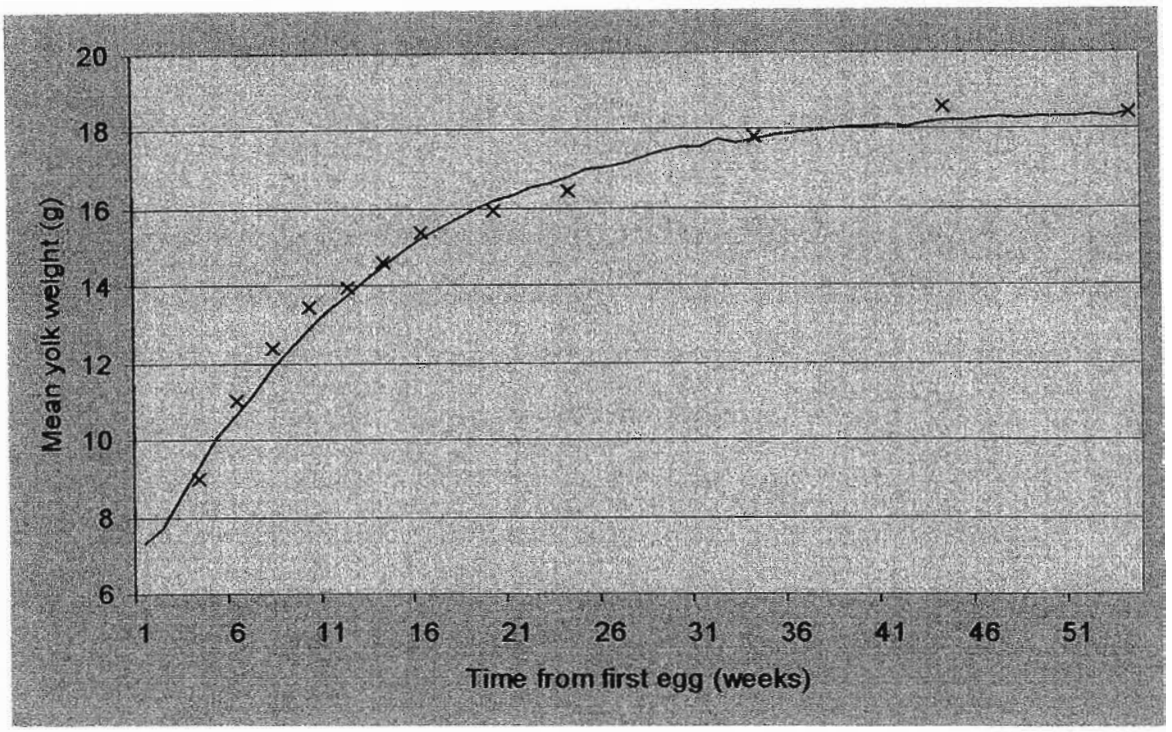


Figure 5.32: An illustration of how predicted mean yolk weight for 100 Amber-Link hens increases with age (solid line), and the actual data points from Chapter 4 (x)

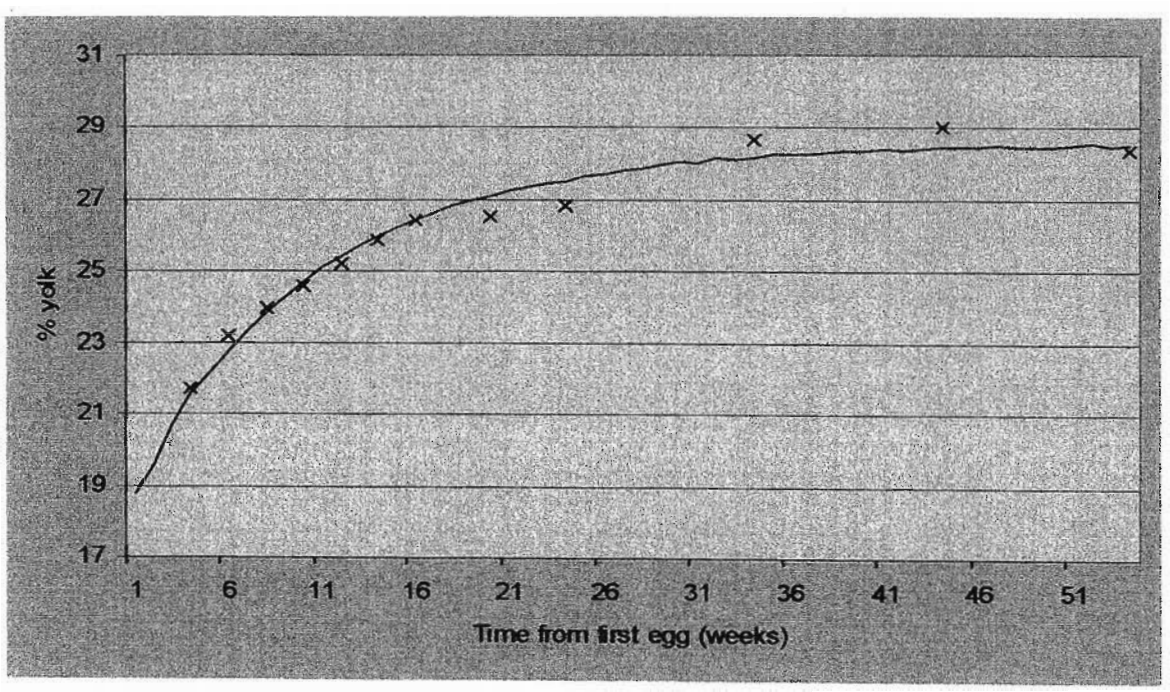


Figure 5.33: An illustration of how predicted mean yolk percentage for 100 Amber-Link hens increases with age (solid line), and the actual data points from Chapter 4 (x)

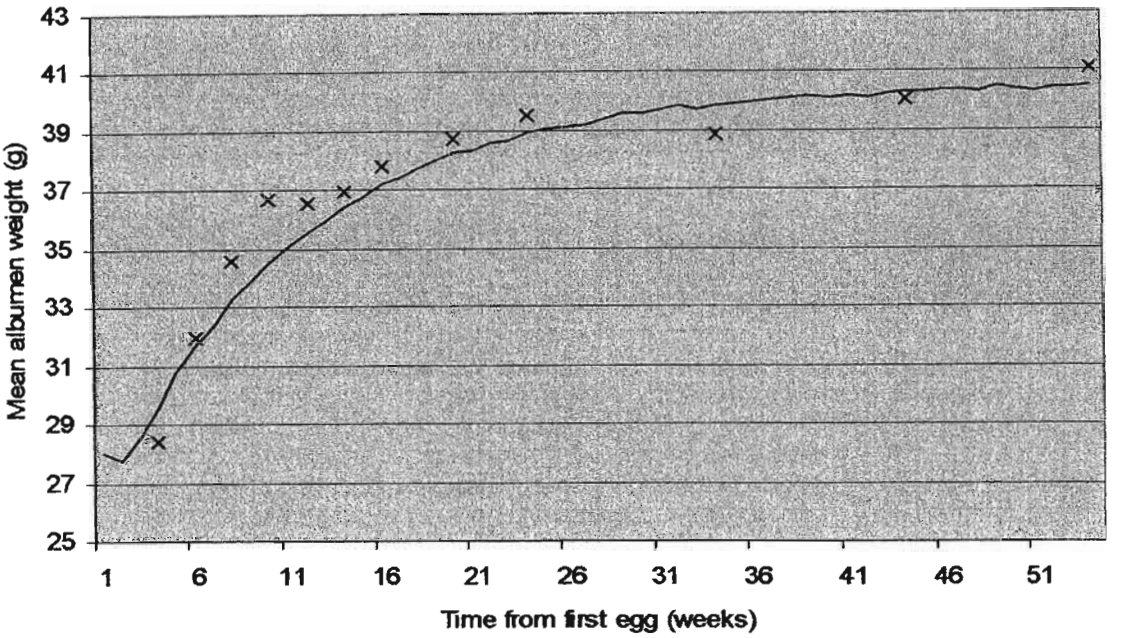


Figure 5.34: An illustration of how predicted mean albumen weight for 100 Amber-Link hens increases with age (solid line), and the actual data points from Chapter 4 (x)

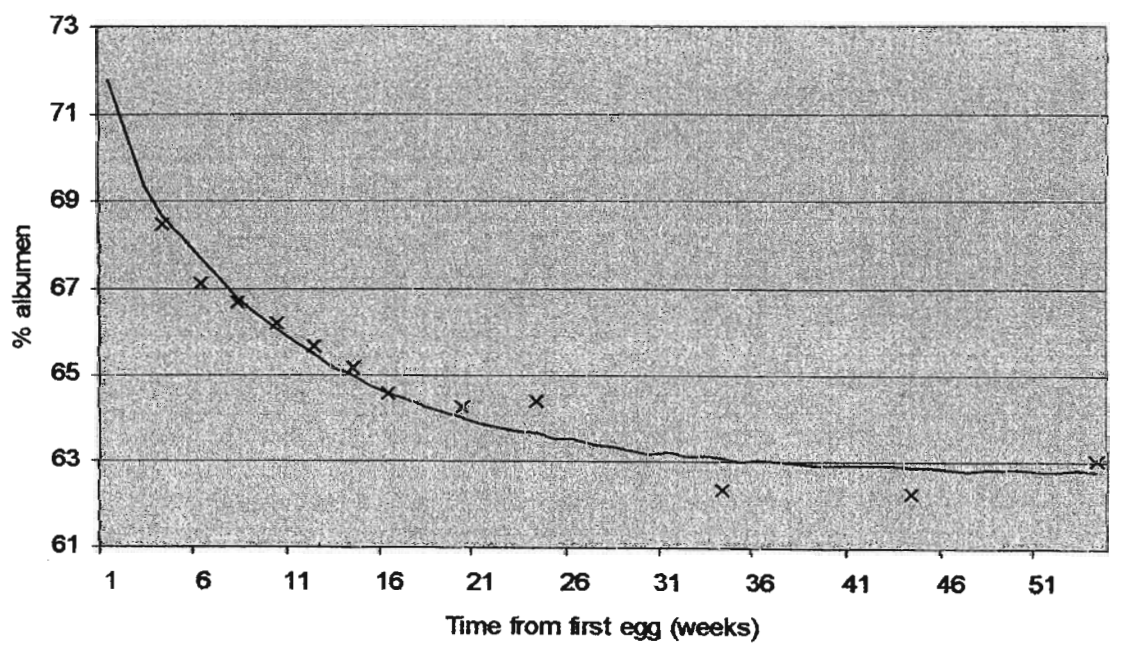


Figure 5.35: An illustration of how predicted mean albumen percentage for 100 Amber-Link hens decreases with age (solid line), and the actual data points from Chapter 4 (x)

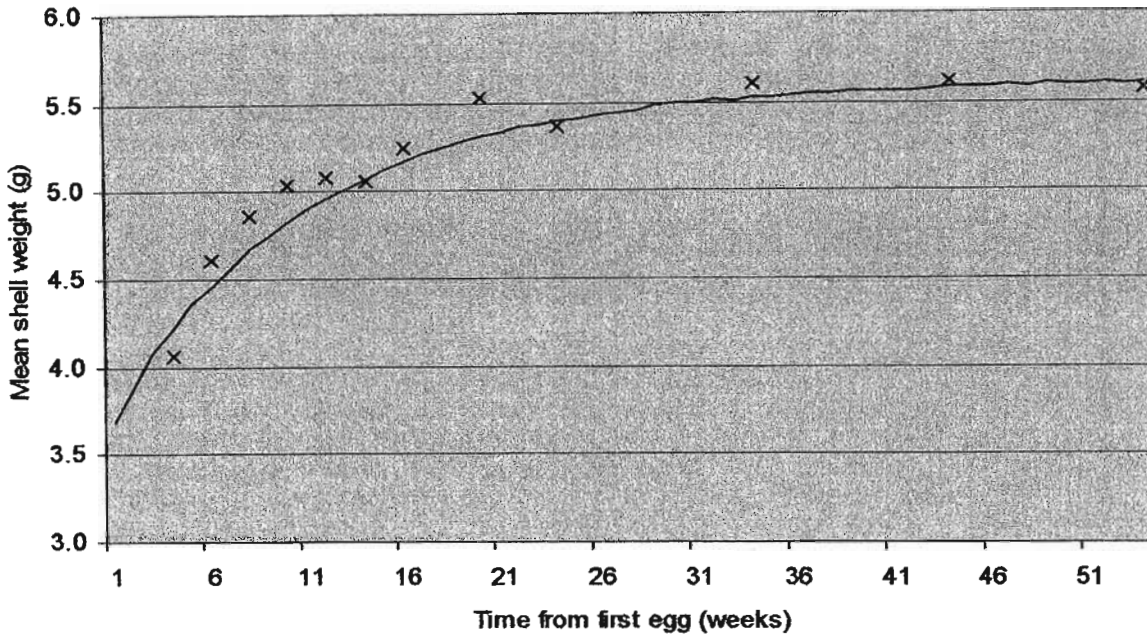


Figure 5.36: An illustration of how predicted mean shell weight for 100 Amber-Link hens increases with age (solid line), and the actual data points from Chapter 4 (x)

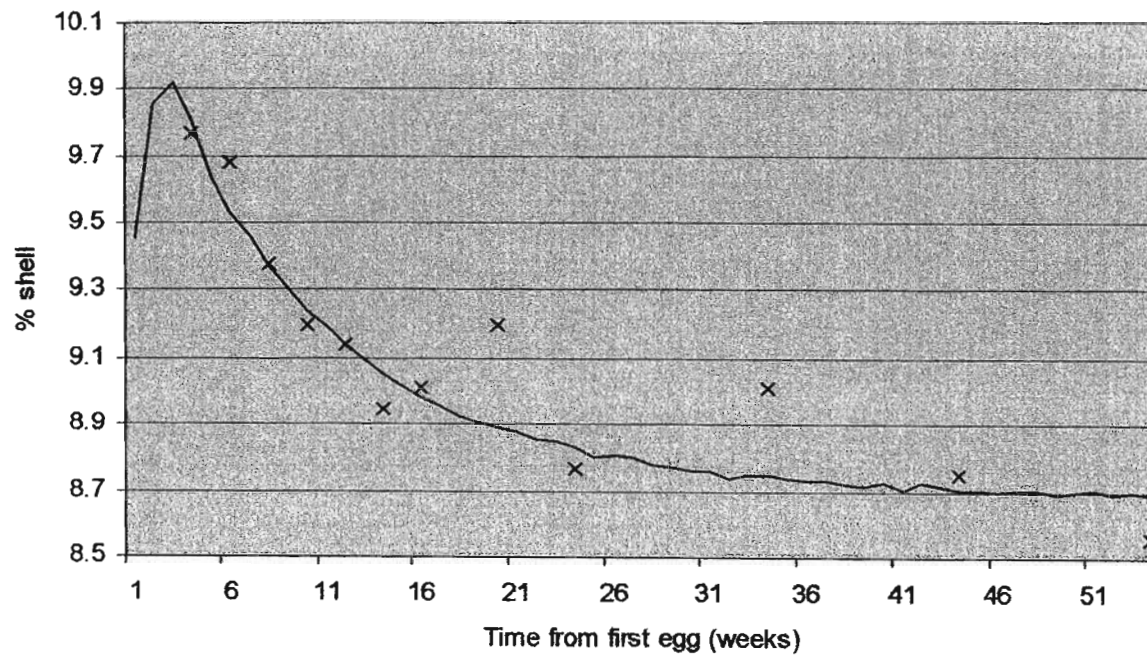


Figure 5.37: An illustration of how predicted mean shell percentage for 100 Amber-Link hens decreases with age (solid line), and the actual data points from Chapter 4 (x)

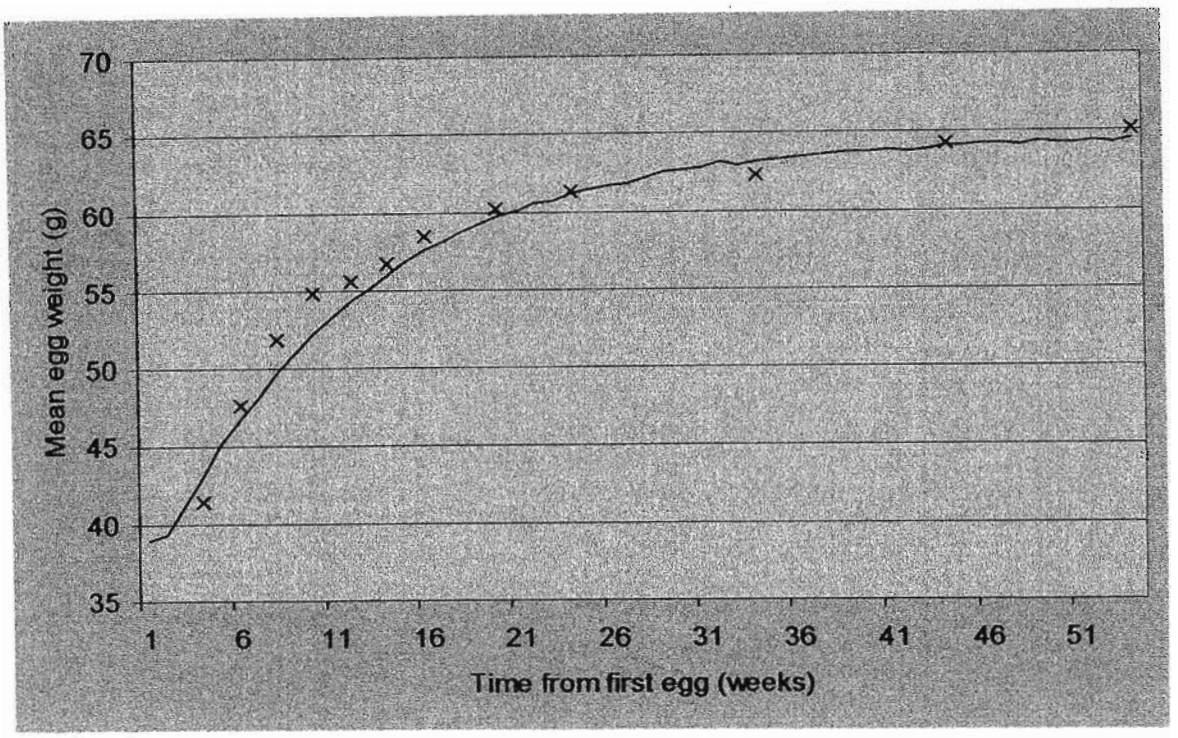


Figure 5.38: The predicted mean egg weight for 100 Amber-Link hens over the laying cycle (solid line), and the actual data points from Chapter 4 (x)

In order to assess the relationships between component weights and egg weight at a fixed hen age, scatter diagrams were plotted using the predicted weights at 163 days of age, *i.e.* when most of the flock had started laying. Figure 5.39 illustrates a weak positive relationship between yolk weight and egg weight ($r^2 = 0.22$). The correlation coefficient for the actual weights from 20-week-old Amber-Link hens (Figure 4.1) was 0.60, indicating a much stronger relationship. The reason for this difference is not clear. There is a very strong positive relationship between albumen weight and egg weight ($r^2 = 0.98$), as illustrated in Figure 5.40; this is not dissimilar to the relationship shown in Figure 4.2, where $r^2 = 0.96$. The relationship between shell weight and egg weight (Figure 5.41) is a reasonably strong positive one, with a correlation coefficient of 0.70. The relationship between the same two variables in Chapter 4 (Figure 4.3) had a correlation coefficient of 0.66. The population model is therefore able to show that increasing egg weight at a fixed age is due to increases in the weights of all three components.

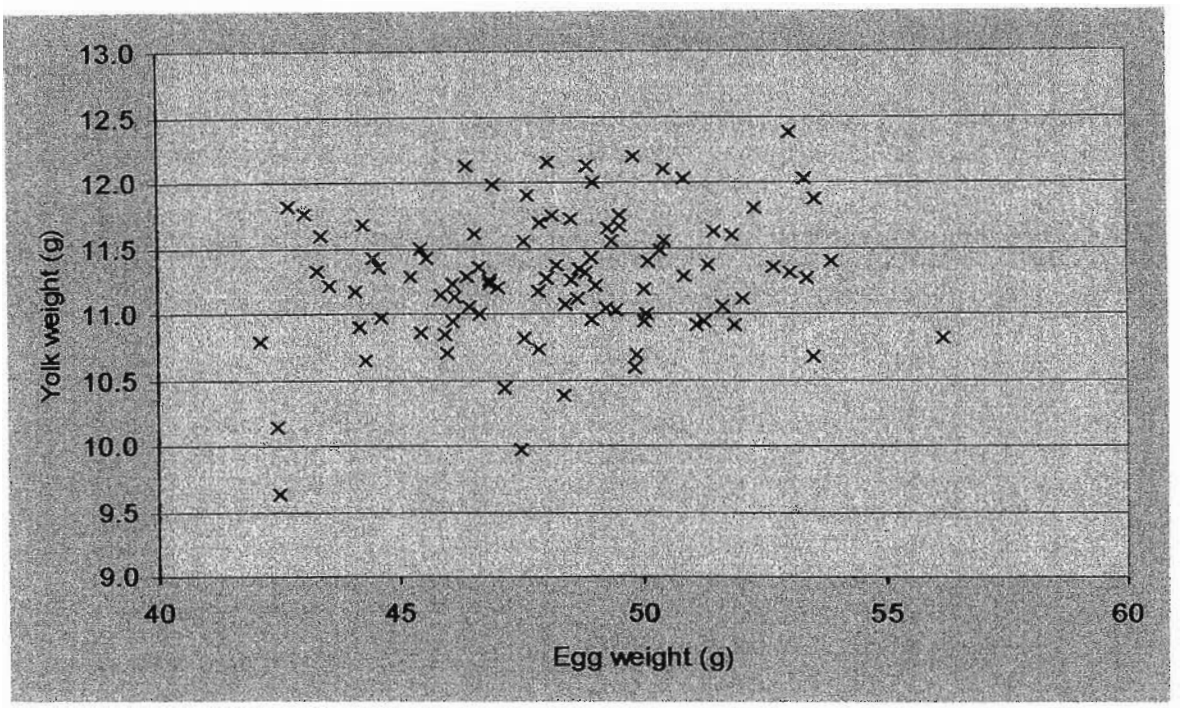


Figure 5.39: The positive relationship between predicted yolk weight and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = 0.22$)

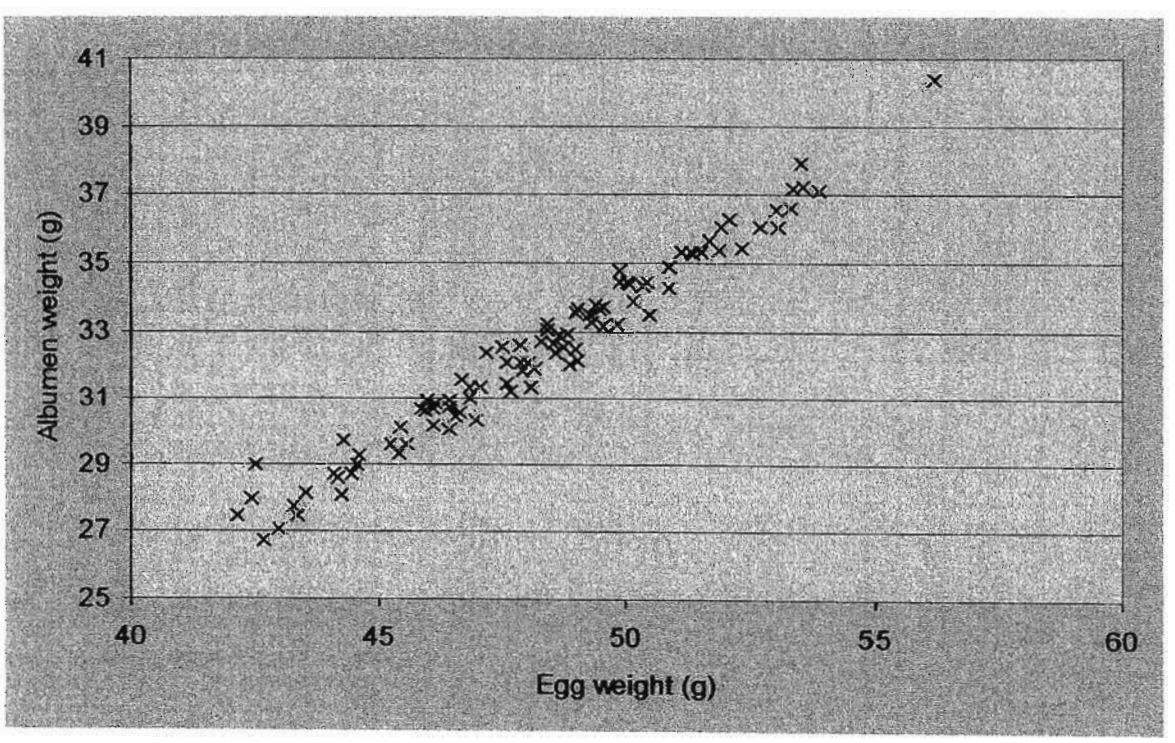


Figure 5.40: The positive relationship between predicted albumen weight and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = 0.98$)

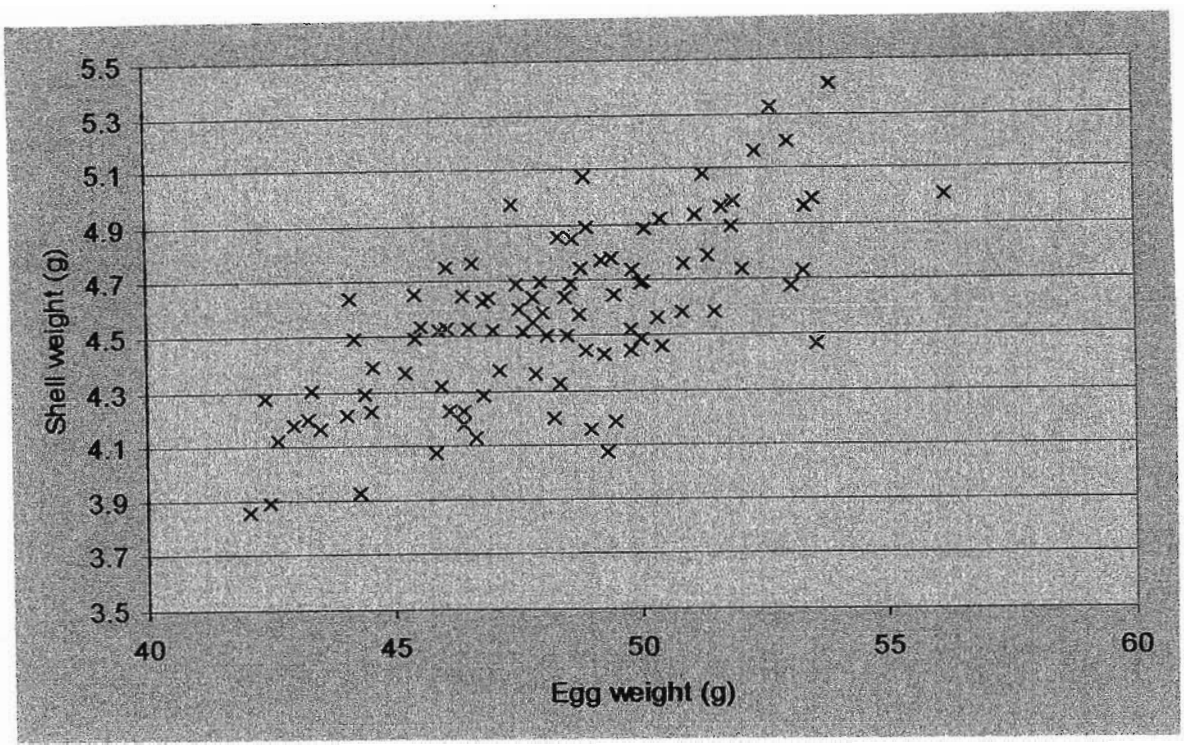


Figure 5.41: The positive relationship between predicted shell weight and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = 0.70$)

In line with trends in the actual egg component weight data for Amber-Link hens reported in Chapter 4, there is a strong negative relationship ($r^2 = -0.78$) between percent yolk and egg weight at a fixed age (Figure 5.42), although there was more variation in the actual data ($r^2 = -0.43$). Figure 5.43 illustrates the strong positive correlation between predicted percent albumen and egg weight ($r^2 = 0.80$). Again the actual data exhibited more variation, since the correlation coefficient was 0.32. The moderate negative correlation between percent shell and egg weight is shown in Figure 5.44 ($r^2 = -0.29$). There appeared to be no relationship between the variables for the actual data ($r^2 = 0.04$). These model predictions confirm that, at a fixed age, larger eggs have proportionally more albumen. It may be possible to change the correlation coefficients, so that they are more in line with those reported in Chapter 4, by modifying the standard deviations used to create normal distributions about the mean values for the allometric function parameters.

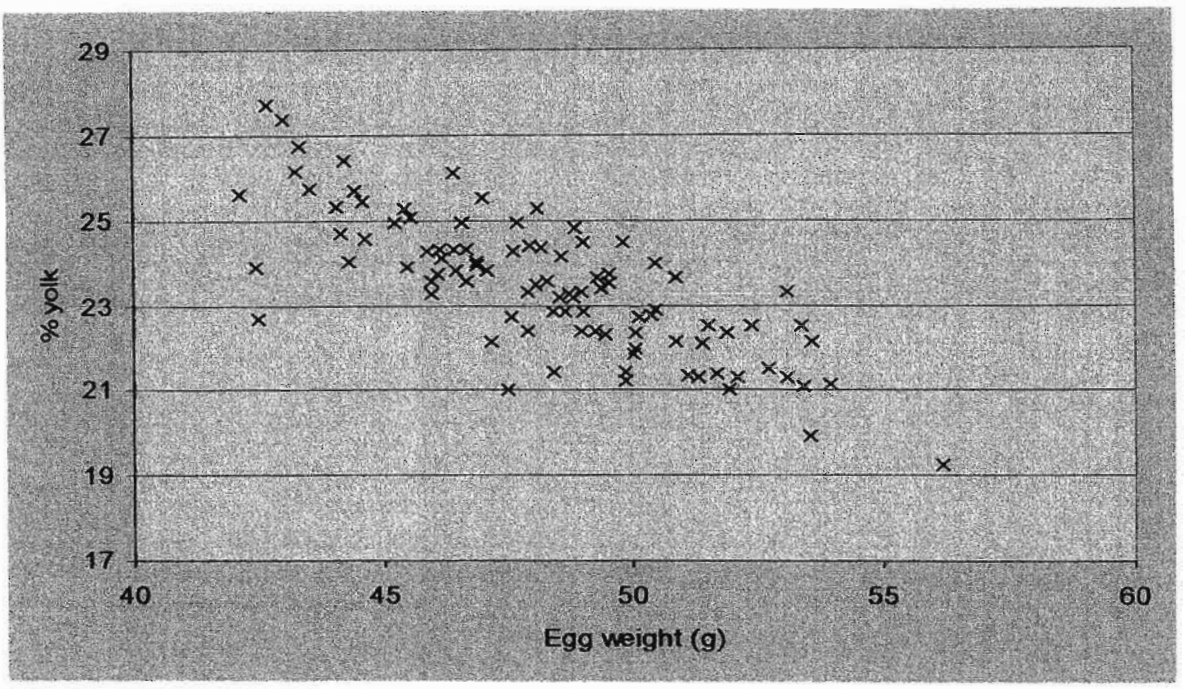


Figure 5.42: The negative relationship between percent yolk and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = -0.78$)

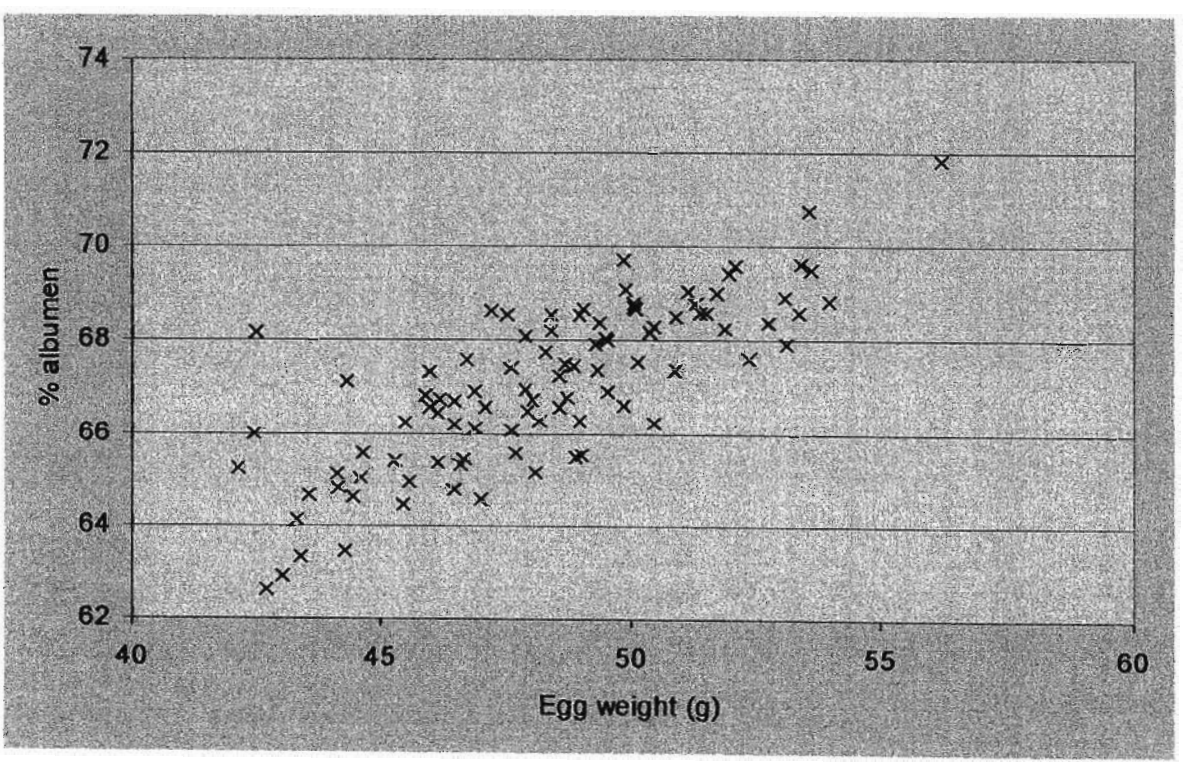


Figure 5.43: The positive relationship between percent albumen and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = 0.80$)

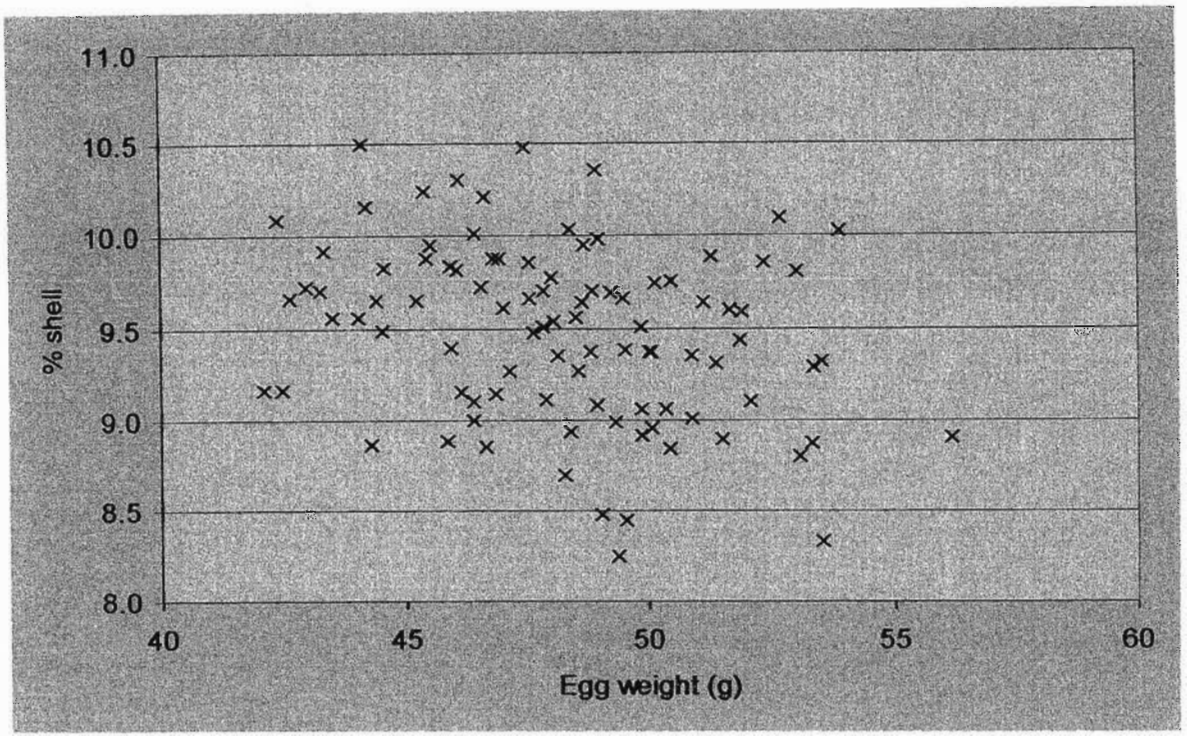


Figure 5.44: The negative relationship between percent shell and egg weight, for eggs from a simulated flock of 100 Amber-Link hens at 163 days of age ($r^2 = -0.29$)

Table 5.13 compares the predicted and actual weights and percentages for the two Hy-Line strains at selected ages. The model gives reasonably accurate predictions for the variables for both strains. This confirms the suitability of the allometric functions to predict albumen weight from yolk weight and shell weight from egg component weight for these genotypes.

Table 5.13: Predicted and actual egg component weights and proportions for Hy-Line Silver and Brown hens

	20 weeks		37 weeks		64 weeks	
Hy-Line Silver	Predicted	Actual	Predicted	Actual	Predicted	Actual
Egg weight	41.67	43.92	57.40	57.39	59.39	59.44
Yolk weight	9.38	9.35	15.13	14.97	15.99	16.07
Albumen weight	28.42	30.55	37.08	37.24	38.04	38.21
Shell weight	3.87	4.03	5.20	5.18	5.36	5.16
% yolk	22.50	21.29	26.36	26.08	26.92	27.03
% albumen	68.20	69.55	64.59	64.89	64.06	64.29
% shell	9.29	9.17	9.05	9.02	9.02	8.68
Hy-Line Brown						
Egg weight	47.23	47.84	62.01	62.18		
Yolk weight	9.23	9.34	14.37	14.40		
Albumen weight	33.61	34.27	41.91	42.15		
Shell weight	4.38	4.24	5.73	5.62		
% yolk	19.55	19.51	23.18	23.16		
% albumen	71.17	71.62	67.58	67.79		
% shell	9.28	8.86	9.24	9.04		

5.15 Discussion

In terms of the objectives set out at the beginning of this chapter, the development of a layer population model has been successful. All the required additions and modifications have been implemented and have given satisfactory results. As with any software,

however, there are always further enhancements that can be made. Developing and maintaining a model is an ongoing process.

The Bristol-Reading model (Lewis *et al.*, 2002) has, in part, been incorporated into the population model. This enables the estimation of mean age at first egg, based on the genotype and its response to a single change in photoperiod applied during rearing. In order to make adjustments for strain differences, the user is required to know the mean age at first egg for their strain when reared on a constant ten-hour daylength, or at the very least, on a constant daylength other than ten hours. It is unlikely that a commercial producer, or even a poultry scientist, would know this unless a specific trial had been conducted to provide the answer, in view of the fact that commercial pullets today are always given a stimulatory increase in daylength. The model calculates the proportion of the flock that is responsive to photostimulation, based on the age at transfer to longer days, and this is used to create a distribution around the mean age at first egg. For flocks that are photostimulated at or after twelve weeks, the distribution is normal. It is not clear whether the authors are correct in suggesting that as age at photostimulation is delayed, the standard deviation around the mean increases, because this is contrary to what was found in the trial reported in Chapter 3. Despite these reservations, the Bristol-Reading model is able to link age at first egg to the lighting programme, and in doing so becomes the starting point for simulating flock egg production.

The method of predicting the hen's internal cycle length, as proposed by Emmans and Fisher (1986), is attractive because of its simplicity and its biological significance. Lag is a well-defined variable that can be estimated by recording oviposition times. If successive eggs are laid on day one at 07:45 and on day two at 08:15, then the lag is 30 minutes. When the total lag exceeds eight to ten hours, the end of a sequence is signalled by a pause day. The value for the decay parameter 'k' may be estimated for a genotype by predicting the change in internal cycle length over time and comparing the resulting predicted rate of lay with the strain standard. The persistency in the rate of ovulation (or lay) is also influenced by lag; the greater the lag, the more rapid the increase in internal cycle length over time and consequently the more rapidly ovulation rate declines. If a hen is laying an egg every 24 hours at sexual maturity, then it is likely that her initial internal cycle length is less than the 24-hour daylength. All this makes sense to a poultry scientist, so the

application of Equation 5.2 is a logical process. Unfortunately the method does not allow hens to start their productive cycles by laying several short sequences before laying their prime sequence. It is important to be able to simulate these short sequences because they are a fairly common occurrence at onset of lay. The accompanying oviposition times recorded in Chapter 3 prove that these short sequences are by no means all due to internal ovulations, nor are they all caused by the first egg at onset of lay being laid in the afternoon. Presumably the rate of follicle growth in a proportion of immature hens is slower and these follicles may take longer to acquire the competency to ovulate (Zakaria *et al.*, 1984a). It was suggested by Zakaria (1999b) that young hens producing short sequences at onset of lay may have inadequate amounts of FSH to maintain growth of the smaller follicles and to promote their subsequent recruitment into the hierarchy.

The quadratic-by-linear functions, used in the model to predict changes in internal cycle length with advancing hen age for the two strains of Hy-Line birds, are able to reproduce shorter sequences at onset of lay. This is because the functions allow the internal cycle length to decrease towards 24 hours in the first few weeks of lay, before increasing. The corresponding ovulation rate therefore starts off at a value less than 1.0, before increasing to reach a peak and subsequently declining. However, the primary disadvantage of these quadratic-by-linear functions is that the parameters D, B, C and A have no biological significance. Moreover, it proved to be relatively difficult to find suitable values for the four parameters that gave a realistic mean sequence length for the genotype as well as a rate of lay comparable to the strain standard. It is felt that, while these quadratic-by-linear functions perform the job allocated to them, an improved or simplified method of creating shorter sequences needs to be found.

One possibility exists: the creation of a model of the follicular hierarchy. The internal cycle length is a measure of the interval between successive ovulations or ovipositions. A hen with an internal cycle length of 24 hours has two well-synchronised systems that enable ovulation to take place routinely once a day. In this case a model of the hierarchy is superfluous, the underlying assumption being that a mature follicle is present every 24 hours. It may be possible to adopt a completely different approach, by modelling the follicle hierarchy and ignoring the concept of internal cycle length altogether. The size of the hierarchy of large yellow follicles would determine ovulation rate. Because follicles

spend eight days rapidly accumulating yolk, at least eight follicles would be required for an ovulation rate of 1.0. A hierarchy from an older hen containing only four follicles would possibly mean that an egg would be laid every second day, *i.e.* an ovulation rate of 0.5, or that a four-egg sequence would be followed by four pause days. Ovulation would only occur if a mature follicle (one that had been growing for eight days) was present. With this approach, ovulation rate would depend on the size of the follicular hierarchy, which in turn would be influenced by the processes of recruitment and atresia. The erratic pattern of sequence lengths of some hens, as reported in Section 3.4.14, would be more readily modelled by allowing the absence or presence of a follicle in the hierarchy to dictate the ovulation rate.

Time of ovulation (and hence time of lay) is known to be entrained primarily to the onset of darkness although other factors, such as temperature, light intensity and the length of the photoperiod, also play a part (Bhatti and Morris, 1978b; Bhatti *et al.*, 1988; Lewis *et al.*, 2004). The parameter S_1 was used by Etches and Schoch (1984) to align predicted times of ovulation with observed times of oviposition, because it controls the time that the circadian rhythm of the regulator substance is reinitiated. The start of the open period for ovulation is therefore determined by the value of S_1. Linking S_1 to the onset of darkness, instead of using an arbitrary range such as 6.5 to 8.0 (see Table 2.1), allows the oviposition times to be entrained to sunset, so that mean time of lay shifts in accordance with the time of sunset decided upon by the user.

It is generally accepted that oviposition is dependent on ovulation, not *vice versa*. Secretions of prostaglandins from the pre-ovulatory and post-ovulatory follicles, along with arginine vasotocin from the pituitary, cause the expulsion of the egg roughly 30 minutes before ovulation (Hargrove and Ottinger, 1992; Etches, 1996) although Naito *et al.* (1989) suggested that ovulation could occur before oviposition. The population model makes use of this temporal relationship, predicting oviposition time from the time of the associated ovulation. The range in the randomly-generated intervals, *i.e.* from three to 57 minutes, is similar to oviposition-ovulation intervals reported in the literature (Warren and Scott, 1935; Fraps, 1955). Predicting the time of lay of the last egg of a sequence poses a slight difficulty, in that there is no associated ovulation. This is overcome by using a linear-by-linear equation to predict the value of the final interval from the ovulation rate.

The lower the ovulation rate, the shorter the ovulation sequence, the longer the interval between the ultimate ovulation of a sequence and the ultimate oviposition.

The proportional changes in the yolk, albumen and shell with increasing hen age have been observed and accepted by poultry scientists for more than half a century. It is important to be able to model these changes successfully, so that increases in egg weight with advancing hen age are brought about by increases in the three components occurring at different rates. The work done on measuring the component weights of three genotypes highlights the need for the parameters in the allometric functions to be carefully defined for each strain. It is probably not widely known that Hy-Line Brown eggs, although significantly heavier than Hy-Line Silver eggs, have slightly smaller yolks; the difference in egg weight being made up of albumen. This fact could be exploited in the market place if the cholesterol content of yolk continues to be an issue in certain consumer sectors. Although the Amber-Link hen no longer has a share of the South African market, the database of component weights proved extremely useful in verifying the ability of the model to predict with reasonable accuracy the proportional changes in yolk, albumen and shell with increasing age, as well as at a fixed age over a range of egg weights. It is advisable to gather similarly comprehensive databases for all currently available genotypes, to improve the accuracy of the egg weight predictions. Ideally a broader database needs to be collected for the two Hy-Line strains. Moreover, it is necessary to update the information every few years so that the allometric function parameters can keep pace with genetic advancements. The work reported in this thesis confirms the suitability of using allometric functions to predict proportional changes in the egg components.

In any population of laying hens, a percentage of the eggs will be soft-shelled or contain double yolks; the proportion being influenced both by strain and age at photostimulation, as well as other factors. Similarly, a number of hens will be prone to internal ovulations. The detailed analysis of soft-shelled eggs reported in Chapter 3 indicates that these occur singly or in groups, either on their own or in combination with normal-shelled eggs. Sometimes a normal and a soft-shelled egg are followed by a pause day, suggesting that the soft-shelled egg may have been laid prematurely. At other times, two or three soft shells are found in the middle of a normal sequence, indicating the presence of multiple hierarchies. It would be an extremely difficult exercise to model all the possible options

and the effort does not seem to be justified. That is why in its current form the population model only produces single soft shells in the middle of normal sequences. In addition, it is difficult to generalise about the influence of soft shells on oviposition time, because some seem to be laid at the expected time whereas others are laid hours earlier than anticipated. In this model the oviposition time for soft-shelled eggs is calculated in the same way as for normal eggs, but this may be an area for future improvement.

The production of double-yolked eggs could be accomplished via a model of the follicular hierarchy. In about 70% of the cases double yolks do not interrupt sequences, so a method of creating multiple hierarchies, or at least an occasional situation where two follicles of the same size exist in the ovary, would need to be formulated. In the remaining 30% of cases, double yolks are associated with pauses in egg sequences. It is assumed that this is because an F_1 and F_2 follicle ovulate simultaneously. In this case a double-yolked egg would cause a gap in the hierarchy, which in turn would terminate the sequence. The weights of the yolks at ovulation would be given by the weights of the follicles at maturity.

It has been rewarding to be able to generate theoretical flocks of Hy-Line Silver and Hy-Line Brown layers with mean sequence lengths (Figure 5.29) similar to those observed in the experimental flocks (Figures 3.28 and 3.29) and by Lewis and Perry (1991) (Figure 1.5). Such a large number of variables are used in the population model, each one having a mean value and an associated standard deviation, with many of the variables being generated by the random number function, that the output may not be as predictable as desired. Nevertheless, the model gives acceptable mean prime sequence lengths and mean sequence lengths for both strains. A method of introducing longer pauses needs to be found so that the predicted mean pause length would increase. It is felt that a few poor-producing hens, such as those observed and analysed in Chapter 3, need to be brought into the theoretical flock.

The layer population model predicts ovulation and oviposition times for 100 hens of a specified genotype; it calculates ovulation rate and rate of lay, as well as egg weight and egg component weights over the full laying cycle. Internal ovulations take place at random and a proportion of the eggs contain double yolks or soft shells. The mean sequence length is shorter at onset of lay, increasing to a maximum about ten weeks from first egg, before

gradually declining. Old hens at the end of the laying cycle produce two- to three-egg sequences. In spite of all the shortcomings of the model described here and the suggestions for further improvements, a great deal has been accomplished thus far.

Chapter 6

DISCUSSION

Since the advent of personal computers in the 1970's, scientists have been quick to recognise and harness their tremendous potential for aiding research and most notably for developing predictive models. As a consequence, computer models have become increasingly popular in all disciplines. Perhaps the most well-known example in agriculture is the advent of least cost feed formulation software. Sadly, there still remains a difference of opinion between academic and commercial organisations as to the usefulness of simulation models in industry. Many scientific personnel working in commerce are scathing about academics who they accuse of working in isolation and of conducting irrelevant research. What they fail to recognise is that most of the academic research findings do eventually filter down to industry in some form or another, and industry often benefits in an indirect way.

In poultry, simulation models have several advantages. Their development requires a sound and thorough knowledge and understanding of all aspects of poultry production. Before proceeding, the modeller therefore needs to do a comprehensive review of the available literature on the specific topic. During the development of the model, several missing pieces in the puzzle usually become evident. These may relate to the response of a particular breed to environmental stimuli, or to the values of variables or constants required by the model. In this way further research can be aimed at providing specific answers to pertinent questions. A certain amount of inventiveness is often required by the modeller, and these assumptions may be supported by experimental evidence or modified subsequently.

A number of publications have centred on attempts to use a single mathematical function to describe the egg production curve (Johnston, 1993). Although some have been relatively successful in approximating the shape of the curve, there is evidently no understanding of the underlying reasons for the shape. Furthermore, they all fail to take

into account the fact that hen day egg production for the flock is derived from individual performances. Rate of lay is determined by the ovulation rate, which varies amongst individuals and over time. The ovulation rate, in turn, is established by the interaction between the rate of follicle maturation and the circadian rhythm of LH release. It therefore makes sense that a model designed to predict the egg production of a flock of hens starts by predicting the ovulation rate for each hen. There are two main benefits to this approach. Firstly, the large number of variables that play a role in egg production are recognised by, and accounted for, in the model. In this manner the process of egg production is acknowledged as being an extremely complex one. Secondly, the amount of variation between individuals within the population is brought to light. Most poultry producers record performance indicators in terms of means (*e.g.* mean egg weight, rate of lay, egg output and feed intake) without having the vaguest idea of, or interest in, the extent of the variation within the flock. The danger is that unsatisfactory performances from some individuals may be masked by the population means, with the result that overall flock performance efficiency is reduced. In a simulation model, each variable has an associated standard deviation or coefficient of variation. These are either measured during experimentation, or educated guesses are made by the modeller.

It is unfortunate that scientists have yet to confirm the existence of a circadian rhythm in LH release, because such evidence would lend credence to the mathematical theory of the ovulatory cycle proposed by Etches and Schoch (1984). In their model, the concentration of the regulator substance is assumed to have a circadian rhythm, being reinitiated at the same time daily. It is generally accepted that certain behavioural patterns exist in birds that are circadian in nature, and that birds have a circadian rhythm in photosensitivity. There has been much interest in the pineal hormone, melatonin, in recent years, because it has a circadian rhythm of release and it entrains to the onset of darkness. Melatonin may indeed be one of the hormones linking the reproductive system to the light:dark cycle (Liou *et al.*, 1987), but the precise mechanism remains unknown. It is apparent that, despite the significant advances made over the past 50 years, there is still much that is not understood about the physiological processes involved in poultry egg production. In the meantime, and until proven otherwise, it is convenient to continue to assume that the restriction of LH

release to a limited portion of the day is brought about by a hormone or neurotransmitter that has a circadian rhythm.

The theory of ovulation control expounded by Fraps (1955) and modelled mathematically by Etches and Schoch (1984), with the amendments discussed in Chapter 2, provides the ideal starting point for a layer model. It has been shown to be capable of producing ovulation sequences of various lengths and of restricting the total lag between the first and last ovulations of a sequence to between eight and ten hours. The predicted ovulation times are therefore constrained to occur within an eight- to ten-hour open period. The model provides a sound mathematical explanation for the long-observed phenomenon of sequential laying in domestic hens and also provides insight into the reason for the progressively later times of lay on subsequent days. Furthermore, the manner in which the concentration of the regulator substance increases rapidly in the mornings and wanes more slowly after noon, intersecting with a succession of follicles, allows the individual lag values to change according to the position of the ovulation in the sequence; the shortest lag values occurring in the middle of sequences and the longest lag occurring between the last two ovulations of the sequence.

On its own, the ovulatory model is not able to show changes in ovulation rate or sequence length with advancing hen age. This is brought about by utilising the theory of Emmans and Fisher (1986), namely that a hen has an internal cycle length that increases with time from first egg, causing ovulation rate and rate of lay to decline. The interval between successive ovulations is brought about by the interaction of two biological systems; namely, the circadian rhythm of LH release and follicle maturation. If the two systems are well-synchronised, the intervals between successive ovulations will be close to 24 (being constrained by the 24-hour daylength). Both the quadratic-by-linear functions used in the model and the function given by Emmans and Fisher (1986), to predict internal cycle length from time from first egg, produce smooth curves. The rate of ovulation, calculated from internal cycle length, therefore declines in a linear fashion. This may be acceptable when calculating mean ovulation rate for a flock of hens, but individuals are unlikely to produce ovulation sequences that decline in length in a predictable way. A hen with an internal cycle length of 25 may be expected to ovulate every 25 hours, but this does not

allow for the fact that a gap in the hierarchy would result in the interval between successive ovulations being closer to 48 hours. The analysis of the pattern of sequence lengths for the trial birds (Table 3.42) shows that, while several hens lay in a consistent manner, many others produce long sequences that are interspersed with shorter sequences. The functions used by the model to predict changes in internal cycle length and ovulation rate from time from first egg are unable to reproduce these irregular patterns. The theory behind the concept of internal cycle length assumes that the circadian rhythm of LH release is uninterrupted and that a mature follicle is ready for ovulation on a daily basis, and this is obviously not always the case.

The way to resolve these issues is to develop a model of the follicular hierarchy. Since follicles take about eight days to mature (Section 1.14), a young hen approaching her peak rate of lay needs eight follicles in the hierarchy. This ensures the presence of a mature F_1 follicle daily. Follicle weight may be predicted from a Gompertz equation using an initial weight of about 0.5g, a rate parameter and a mature size parameter determined by the hen age, to enable yolk weights to increase with age. A second condition now needs to be met for ovulation to take place. Along with the intersection of the regulator concentration function and the Gompertz function for follicle maturation, a mature F_1 follicle (that has been growing for eight days) needs to be present. Occasional gaps in the hierarchy can be created by the production of double-yolked or soft-shelled eggs and this will result in more inconsistencies in sequence lengths. In view of the fact that one of the first signs of ageing in hens is a reduction in the rate of recruitment of follicles into the hierarchy (Williams and Sharp, 1978a), a model thereof will enable rate of ovulation to decline with age. The condition of the hierarchy at any point in time should primarily dictate the rates of ovulation and lay.

Additional benefits from developing a model of the follicular hierarchy can be expected. Opportunities may be created for introducing poor-producing hens into the population. For instance, a bird with a hierarchy size of three, either due to atresia of large yellow follicles or inadequate recruitment of new follicles into the hierarchy, will have short ovulation sequences and inter-sequence pauses of two or more days. If, for example, 5% of the flock are given a hierarchy size well below the mean for the population, a number of hens with

extremely low egg production will result. Although all commercial flocks may not have individuals with abnormally low rates of lay, it is possible that these birds exist as passengers in the flock; their poor performances being masked by acceptable flock means. Indeed, unless suspect birds are caged individually, there is no method of confirming or disproving their existence. It is doubtful whether the commercial suppliers of day-old- and point-of-lay-pullets will admit to, or even be aware of, the variation in laying potential that exists within their genotypes, but egg producers need to recognise that they may be able to improve flock efficiency and profit margins significantly if the low producers are identified and removed.

Another benefit to modelling the follicle hierarchy is that yolk weight can be predicted with a greater degree of accuracy. The weight of the F_1 follicle at ovulation determines the yolk weight. If the follicle weight for each hen is recalculated each minute during the model iterations, then a precise weight can be available at the time of ovulation. In this manner, both hen age and time of ovulation influence yolk weight. This means that the yolk of the first egg in a sequence will be heavier than that of subsequent eggs, having the longest period of rapid growth. The current model is not able to reproduce the changes in yolk weight according to position of the egg in a sequence; yolk weight being predicted from hen age alone. Given that hen age remains the same for the 24-hour period, for all ovulations occurring on a fixed day the same mean yolk weight (and a standard deviation) will be used to predict individual yolk weights.

It would be useful know why some birds have inter-sequence pauses longer than one day. These may well be due to inadequate maintenance of the follicular hierarchy, but there may also be times during the laying cycle when the preovulatory surge of LH does not proceed as expected. Stresses that are imposed on the hens over a number of days, such as the withdrawal of feed, cause apoptosis and subsequent cell proliferation in the tissues of the anterior pituitary, with corresponding changes to the LH secretions (Chowdhury and Yoshimura, 2002). Short-term stresses may also have the ability to prevent the secretion of LH. The current model presumes that the circadian rhythm of LH release continues without interruption. A method of blocking the rhythm of the regulator substance can be found, so that for one day or a number of days ovulation will not take place. This will

allow the model to respond to a limiting environment by altering rates of ovulation and lay. Accordingly, pauses longer than one day (that were frequently observed in the trial reported in Chapter 3), can be introduced into sequences.

A great number of factors are taken into account by the layer model presented in this thesis. Ovulation times are predicted for a theoretical flock of 100 birds over a laying year. The flock ovulation rate is seen to increase rapidly to reach a peak about eight weeks from onset of lay, before declining in a linear fashion. Oviposition times are predicted from the associated ovulation times. For all but the last egg of a sequence, oviposition occurs roughly half an hour before the associated ovulation. The oviposition-ovulation interval is recalculated daily so that the oviposition times vary slightly, even in the middle of long sequences where the lag is minimal or zero (when succeeding ovulation times remain constant). The interval between the penultimate and ultimate eggs of a sequence is calculated from an equation relating the length of the interval to ovulation rate; short sequences have a long interval between the last two ovipositions. The open period for LH release entrains to the onset of darkness, so that mean time of lay occurs 13 to 14 hours after sunset. The user is able to observe the effect of different times of sunset on the mean time of lay. The distribution of oviposition times is seen to be unimodal and positively skew in young flocks, when a greater proportion of the eggs are laid in the morning, and bimodal in older flocks, when due to the shorter sequences, comparatively more eggs are laid in the afternoon. Flock rate of lay follows a similar pattern to ovulation rate, and also follows the trend given in breed manuals. The mean time of lay of first eggs at sexual maturity is about 13 to 14 hours after sunset, but about 30% of the eggs are laid after noon. This is brought about by initiating the final phase of follicle maturation 2.5 hours after sunset, so that the maturation process is linked to the start of the open period for LH release. The positive feedback action of progesterone, secreted by the follicle, on the hypothalamus presumably plays a role in the timing of follicle maturation. Yolk weight increases with hen age. Allometric functions are used to predict albumen weight from yolk weight, and shell weight from the combined weight of yolk plus albumen, using the parameters appropriate for the genotype. Egg weight is calculated as the sum of the three component weights and may be seen to be in line with breed standards. The model correctly predicts the increasing proportion of yolk and the decreasing proportions of

albumen and shell with advancing hen age. Numerous charts illustrate the increases in the component weights with increasing age, as well as the changes in the proportions of the components. The weights and proportions of yolk, albumen and shell are also plotted against egg weight at a fixed age. Random occurrences of internal ovulations, restricted to a proportion of the flock, cause interruptions to egg sequences. The number of internal ovulations in the population is higher at the beginning and at the end of the laying cycle, when asynchrony between ovary and oviduct is more likely. Double-yolked eggs are produced by a proportion of the flock at the start of the laying period; the earlier the hens are brought into lay, the greater the incidence of double yolks. A number of hens are prone to laying soft-shelled eggs, with a greater frequency occurring at onset of lay and in older hens. The distributions of internal ovulations and abnormal eggs within the flock are plotted, as are the proportions of each with time from first egg. Mean sequence length is seen to start off at about 30-40, to increase to about 70-80 at peak rate of lay and then to decrease to less than 10 after a year of production. The extent of the variation in mean sequence length within the flock is highlighted by separating the flock into thirds and analysing the performances of the top, middle and lower thirds. A spreadsheet containing sequence information about the type of egg – normal, double yolk or soft shell – may be imported in the Sequence Analyzer program for further analysis. The mean prime sequence length, mean sequence length, mean pause length and mean number of sequences are the most useful figures obtained from the Sequence Analyzer. A summary table lists the main performance indicators.

Because specific values are allocated to the various constants and parameters in the model for each genotype, the theoretical flocks of diverse strains differ in terms of some of the production variables. For example, the Hy-Line Silver strain has an earlier predicted mean age at first egg, a higher ovulation rate and rate of lay, a smaller egg weight but a greater proportion of yolk within each egg, and a higher proportion of soft-shelled eggs and internal ovulations than the simulated Hy-Line Brown flock. As anticipated, the mean sequence length and mean prime sequence length are longer but the mean number of sequences is less.

To broaden the scope of the model, a number of parameters still need to be defined for a selection of genotypes. For instance, the Lohmann Silver and Brown strains possess a reasonably large share of the South African market for commercial pullets and layers and yet little academic research seems to have been conducted with these hens. They may respond to environmental stimuli in a similar manner to the Hy-Line birds, but this needs to be tested. No information appears to be available on the weights of the egg components for the Lohmann birds. In a slightly different vein, it is not known whether yolk, albumen and shell weights can be manipulated by the environment, although yolk weight is strongly influenced by hen age. Thus, while the model addresses many issues associated with egg production, it is hoped that it may be continually improved as new information comes to light.

Despite the fact that several improvements can still be made to the layer model, it is hoped that the model in its current form will be of benefit to sections of the poultry community. As a teaching aid to poultry science students it may be invaluable, given its ability to illustrate the complexities of egg production and to draw attention to the extent of variation within a population. By allowing the user to change various inputs, the model demonstrates the ability of hens to respond to their environment. The egg producer may be interested to see the response of the birds, in terms of laying performance, to changes to the lighting program applied during rearing. The importance of applying stimulatory daylengths to pullets at the recommended age for the breed is underlined by the effect of age at photostimulation on the incidence of internal ovulations, soft shells and double-yolked eggs. He or she would also gain a better understanding of the process of egg production and the effect that poor layers may have on flock efficiency. The producer may benefit from seeing how mean time of lay is influenced by hen age and the time the lights are switched off at night; daily egg collections could then be timed so that the majority of eggs are collected as soon as possible after being laid. The model may also be used to predict voluntary food intake on a daily basis and over the laying year; food intake being less on a day when no egg is laid. The nutritionist may be interested in estimating the nutrient requirements of laying hens according to the predicted weights of yolk, albumen and shell. Ultimately, this model may assist nutritionists to optimise the nutrient requirements for the various commercially available strains. With a few modifications, the

population model may be adapted for use with broiler breeder hens that have notoriously erratic egg production cycles, with long and frequent pauses between egg sequences.

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APPENDICES

Appendix 2.1: Values of $R_3(t)$ and $1-R_3(t)$ in half-hourly intervals for 2- and 9-ovulation sequences, from Equation 2.1

	2-ovulation sequence			9-ovulation sequence		
Time	Time R_3	$R_3(t)$	$1-R_3(t)$	Time R_3	$R_3(t)$	$1-R_3(t)$
0:00	24.0	0.193	0.807	24.0	0.143	0.857
0:30	24.5	0.182	0.818	24.5	0.134	0.866
1:00	25.0	0.171	0.829	25.0	0.125	0.875
1:30	25.5	0.161	0.839	25.5	0.116	0.884
2:00	26.0	0.152	0.848	26.0	0.109	0.891
2:30	26.5	0.143	0.857	26.5	0.101	0.899
3:00	27.0	0.134	0.866	27.0	0.094	0.906
3:30	27.5	0.126	0.874	27.5	0.088	0.912
4:00	28.0	0.118	0.882	28.0	0.082	0.918
4:30	28.5	0.111	0.889	28.5	0.076	0.924
5:00	29.0	0.104	0.896	29.0	0.071	0.929
5:30	29.5	0.097	0.903	29.5	0.066	0.934
6:00	30.0	0.091	0.909	30.0	0.061	0.939
6:30	30.5	0.086	0.914	6.5	0.057	0.943
7:00	31.0	0.080	0.920	7.0	0.181	0.819
7:30	31.5	0.075	0.925	7.5	0.279	0.721
8:00	8.0	0.070	0.930	8.0	0.356	0.644
8:30	8.5	0.171	0.829	8.5	0.414	0.586
9:00	9.0	0.252	0.748	9.0	0.456	0.544
9:30	9.5	0.317	0.683	9.5	0.486	0.514
10:00	10.0	0.367	0.633	10.0	0.506	0.494
10:30	10.5	0.406	0.594	10.5	0.516	0.484
11:00	11.0	0.435	0.565	11.0	0.520	0.480
11:30	11.5	0.455	0.545	11.5	0.518	0.482
12:00	12.0	0.468	0.532	12.0	0.511	0.489
12:30	12.5	0.475	0.525	12.5	0.501	0.499
13:00	13.0	0.477	0.523	13.0	0.488	0.512
13:30	13.5	0.475	0.525	13.5	0.473	0.527
14:00	14.0	0.469	0.531	14.0	0.456	0.544
14:30	14.5	0.461	0.539	14.5	0.438	0.562
15:00	15.0	0.451	0.549	15.0	0.420	0.580
15:30	15.5	0.438	0.562	15.5	0.401	0.599
16:00	16.0	0.425	0.575	16.0	0.382	0.618
16:30	16.5	0.410	0.590	16.5	0.363	0.637
17:00	17.0	0.395	0.605	17.0	0.344	0.656
17:30	17.5	0.379	0.621	17.5	0.326	0.674
18:00	18.0	0.364	0.636	18.0	0.308	0.692
18:30	18.5	0.348	0.652	18.5	0.290	0.710
19:00	19.0	0.332	0.668	19.0	0.273	0.727
19:30	19.5	0.316	0.684	19.5	0.257	0.743
20:00	20.0	0.301	0.699	20.0	0.242	0.758
20:30	20.5	0.285	0.715	20.5	0.227	0.773
21:00	21.0	0.271	0.729	21.0	0.213	0.787
21:30	21.5	0.257	0.743	21.5	0.200	0.800
22:00	22.0	0.243	0.757	22.0	0.187	0.813
22:30	22.5	0.230	0.770	22.5	0.175	0.825
23:00	23.0	0.217	0.783	23.0	0.164	0.836
23:30	23.5	0.205	0.795	23.5	0.153	0.847
0:00	24.0	0.193	0.807	24.0	0.143	0.857

Appendix 2.2: Values of G(t) in half-hourly intervals for 2- and 9-ovulation sequences, from Equation 2.4

	2-ovulation sequence	9-ovulation sequence
Time from first ovulation	G(t)	G(t)
15.0	0.00069	0.00001
15.5	0.00143	0.00003
16.0	0.00275	0.00015
16.5	0.00494	0.00057
17.0	0.00833	0.00174
17.5	0.01331	0.00446
18.0	0.02026	0.00988
18.5	0.02952	0.01937
19.0	0.04134	0.03423
19.5	0.05591	0.05542
20.0	0.07328	0.08331
20.5	0.09336	0.11763
21.0	0.11600	0.15751
21.5	0.14089	0.20165
22.0	0.16770	0.24853
22.5	0.19601	0.29662
23.0	0.22542	0.34451
23.5	0.25549	0.39103
24.0	0.28582	0.43527
24.5	0.31603	0.47660
25.0	0.34580	0.51462
25.5	0.37485	0.54915
26.0	0.40293	0.58017
26.5	0.42987	0.60779
27.0	0.45553	0.63218
27.5	0.47981	0.65359
28.0	0.50267	0.67226
28.5	0.52406	0.68849
29.0	0.54400	0.70252
29.5	0.56250	0.71462
30.0	0.57961	0.72502
30.5	0.59539	0.73393
31.0	0.60988	0.74157
31.5	0.62316	0.74809
32.0	0.63530	0.75365
32.5	0.64638	0.75839
33.0	0.65647	0.76242
33.5	0.66564	0.76585
34.0	0.67397	0.76877
34.5	0.68151	0.77124
35.0	0.68834	0.77334
35.5	0.69452	0.77512
36.0	0.70010	0.77663
36.5	0.70514	0.77791
37.0	0.70969	0.77900
37.5	0.71378	0.77992
38.0	0.71747	0.78070
38.5	0.72079	0.78136
39.0	0.72378	0.78192
39.5	0.72647	0.78239
40.0	0.72889	0.78279

Appendix 2.3: A simple spreadsheet model for calculating ovulation times for a 2-egg sequence; $T_5 = 13.5$. Ovulations occur just after 09:00 on day 2 and 13:30 on day 3

Day	Timeday	TimeRC	$R_3(t)$	$1-R_3(t)$	TimeFM	G(t)	G(t) – (1-R3(t))
1	00:00	24.0	0.193	0.807	10.5	0.000	-0.807
	00:30	24.5	0.182	0.818	11.0	0.000	-0.818
	...	...	...	...	...	...	...
2	00:00	24.0	0.193	0.807	34.5	0.682	-0.125
	00:30	24.5	0.182	0.818	35.0	0.688	-0.130
	01:00	25.0	0.171	0.829	35.5	0.695	-0.134
	01:30	25.5	0.161	0.839	36.0	0.700	-0.139
	02:00	26.0	0.152	0.848	36.5	0.705	-0.143
	02:30	26.5	0.143	0.857	37.0	0.710	-0.148
	03:00	27.0	0.134	0.866	37.5	0.714	-0.152
	03:30	27.5	0.126	0.874	38.0	0.717	-0.157
	04:00	28.0	0.118	0.882	38.5	0.721	-0.161
	04:30	28.5	0.111	0.889	39.0	0.724	-0.165
	05:00	29.0	0.104	0.896	39.5	0.726	-0.170
	05:30	29.5	0.097	0.903	40.0	0.729	-0.174
	06:00	30.0	0.091	0.909	40.5	0.731	-0.178
	06:30	30.5	0.086	0.914	41.0	0.733	-0.181
	07:00	31.0	0.080	0.920	41.5	0.735	-0.185
	07:30	31.5	0.075	0.925	42.0	0.736	-0.189
	08:00	8.0	0.070	0.930	42.5	0.738	-0.192
	08:30	8.5	0.171	0.829	43.0	0.739	-0.090
	09:00	9.0	0.252	0.748	43.5	0.740	**-0.008**
	09:30	9.5	0.317	0.683	0.5	0.000	-0.683
	10:00	10.0	0.367	0.633	1.0	0.000	-0.633
	10:30	10.5	0.406	0.594	1.5	0.000	-0.594
	11:00	11.0	0.435	0.565	2.0	0.000	-0.565
	11:30	11.5	0.455	0.545	2.5	0.000	-0.545
	12:00	12.0	0.468	0.532	3.0	0.000	-0.532
	...	...	...	...	...	...	...
3							
	10:00	10.0	0.367	0.633	25.0	0.346	-0.287
	10:30	10.5	0.406	0.594	25.5	0.375	-0.219
	11:00	11.0	0.435	0.565	26.0	0.403	-0.162
	11:30	11.5	0.455	0.545	26.5	0.430	-0.115
	12:00	12.0	0.468	0.532	27.0	0.456	-0.076
	12:30	12.5	0.475	0.525	27.5	0.480	-0.045
	13:00	13.0	0.477	0.523	28.0	0.503	-0.020
	13:30	13.5	0.475	0.525	28.5	0.524	**-0.001**
	14:00	14.0	0.469	0.531	0.5	0.000	-0.531
	14:30	14.5	0.461	0.539	1.0	0.000	-0.539
	15:00	15.0	0.451	0.549	0.5	0.000	-0.549
	15:30	15.5	0.438	0.562	1.0	0.000	-0.562
	16:00	16.0	0.425	0.575	1.5	0.000	-0.575
	16:30	16.5	0.410	0.590	2.0	0.000	-0.590
	17:00	17.0	0.395	0.605	2.5	0.000	-0.605
	17:30	17.5	0.379	0.621	3.0	0.000	-0.621
	18:00	18.0	0.364	0.636	3.5	0.000	-0.636

Appendix 2.4: Constants and parameters (used to produce a 2-ovulation sequence) for the macro- and menu-driven ovulation model in Lotus 1-2-3

Regulator concentration			Range names
a1 =	2.175	(from Table 2.1)	RCa1
L1 =	0.14	(from Table 2.1)	RCL1
L2 =	0.25	(from Table 2.1)	RCL2
S1 =	8.0	(from Table 2.1)	RCS1
a2 =	2.104668	(from Eqn. 2.3)	RCa2
Follicle maturation			
b1 =	0.75	(from Table 2.1)	FMb1
b2 =	4.5	(from Table 2.1)	FMb2
b3 =	0.22	(from Table 2.1)	FMb3
S2 =	17.0	(from Table 2.1)	FMS2

Cycle times		
Starttime =	08:00	(from S1)
TimeT5 =	13.5	time last egg of previous sequence laid
DL =	24	daylength
Endtime =	18.0	18:00; no ovulations expected after this time
Reps =	10	number of days model to run
tmax =	32	DL + S1; maximum time for reg.conc.
tint =	00:30	half-hour time interval; add to time of day
tint1=	0.5	half-hour interval; add to time reg.conc. and foll.mat.
tint2 =	00:01	one-minute interval; add to time of day
tint3 =	0.016667	one-minute interval; add to time reg.conc. and foll.mat.
mindiff =	-0.04	target for changing to one-minute intervals
mindiff1 =	-0.0005	minimum difference before ovulation deemed to occur

Appendix 2.5: Macro commands for the menu- and macro-driven ovulation model

PARAMETERS	allows user input of new parameters
{home}{getnumber CHANGE PARAMETERS? Yes=1 No=0,SELECT}	gives user the option of changing parameters, stores input in cell SELECT
{if SELECT=0}{menubranch mu}	if no, returns to main menu
{let DAY,0}	resets day counter
{get-number SEQUENCE LENGTH, SL}	allows input of sequence length
{get-number NUMBER OF DAYS,reps}	allows input of no.days model is to run;
{calc}	calculates
{menubranch MU}	returns to main menu

CALCULATE	resets time counters, erases old data, evaluates difference
{home}{let DAY,1}	sets day counter
{let TIMEDAY,STARTTIME}	resets time of day to equal starting time (from S1)
{let TIMERC,RCS1}	resets time of reg.conc. to equal S1
{let TIMEFM,DL-TIMET5+DL+RCS1}{calc}	resets time of foll.mat. ; aligns with time of day
{let SEQ,0}{blank SUMMARY}	resets sequence counter, erases old data in summary table
{if DIFF<MINDIFF}{branch OV}	evaluates G(t) - 1-R3(t), branches to macro OV
{if DIFF>=0}{SUBTRACTTIME}	subtracts the last half hour if intersection already
{branch OV}	exceeded, branches to macro OV
{menubranch MU}	returns to main menu

	OV	adds half-hour intervals, evaluates difference
	{let TIMEDAY,TIMEDAY+TINT}	adds half an hour to time of day
	{if TIMERC=TMAX-TINT1}{let TIMERC,RCS1}{branch OV1}	resets reg.conc. time to S1 if it equals maximum (DL + S1), moves to macro OV1
	{let TIMERC,TIMERC+TINT1}	adds half an hour (decimal) to reg.conc. time
ov1	{if TIMERC=DL+TINT1}{let DAY,DAY+1}{branch OV2}	advances to next day after midnight, moves to macro OV2
	{let DAY,DAY}	otherwise leaves day counter as it is
ov2	{let TIMEFM,TIMEFM+TINT1}	adds half an hour (decimal) to foll.mat. time,
	{calc}	calculates
	{if TIMERC>ENDTIME}	stops if reg.conc. time exceeds ENDTIME,
	{branch SKIP}	branches to SKIP macro
ov3	{if DIFF<MINDIFF}{branch OV}	repeats macro OV if G(t) - 1-R3(t) still large negative
	{if DIFF>=0}{SUBTRACTTIME}	performs subroutine SUBTRACTTIME if intersection has already occurred

```
{let TIMEDAY1,TIMEDAY}      prepares to add one minute intervals;
{let TIMERC1,TIMERC}            sets the three minute time counters to
{let TIMEFM1,TIMEFM}            equal the three half-hourly time counters,
{calc}                      calculates
{branch OVTIME}             branches to OVTIME macro
```

OVTIME — adds one minute intervals, evaluates difference

```
{let TIMEDAY1,TIMEDAY1+TINT2}   adds 1 minute to time of day
{let TIMERC1,TIMERC1+TINT3}     adds 1 minute (decimal) to reg.conc. time
{let TIMEFM1,TIMEFM1+TINT3}     adds 1 minute (decimal) to foll.mat. time;
{calc}                          calculates
{if TIMERC1>ENDTIME}            stops if reg.conc. time exceeds ENDTIME, branches
{branch SKIP}                   to SKIP macro
{if DIFF1<MINDIFF1}             repeats OVTIME macro if G(t) - 1-R3(t) still
{branch OVTIME}                 negative
{branch RECORD}                 branches to RECORD macro
```

SKIP — skips rest of day after late afternoon endtime

```
{if DAY<>0}{goto}SUMTABLE~{end}{d}{d}   records day number as value on summary table
+DAY~/rv~~{home}
{let TIMEDAY,STARTTIME}                 resets time of day to equal starting time
{let TIMEFM,TIMEFM+(DL-TIMERC)+RCS1}    continues follicle growth overnight
{let TIMERC,RCS1}{calc}                 resets time of reg.conc. to equal S1, calculates
{let SEQ,0}{let DAY,DAY+1}              resets sequence counter, adds one to day counter
{branch OV3}                            branches to macro OV3
```

ENDDAY — ends the day when ovulation time has been recorded

```
{let TIMEDAY,TIMEDAY1}      starts new time of day at yesterday's ovulation time
{let TIMEFM,DL}             follicle maturation progresses for 24 hours
{let TIMERC,TIMERC1}{calc}  starts reg.conc. time at ovulation time, calculates
{let DAY,DAY+1}             adds one to day counter
{branch OV3}                branches to macro OV3
```

SUBTRACTTIME — subtracts half an hour when intersection exceeded

```
    {let TIMEDAY,TIMEDAY-TINT}  subtracts half an hour from time of day
    {if TIMERC=RCS1}{let        if reg.conc. time already reset at S1, subtracts half an
    TIMERC,TMAX-TINT1}          hour from maximum reg.conc. time;
    {branch ST1}                moves to macro ST1
    {let TIMERC,TIMERC-TINT1}   subtracts half an hour (decimal) from reg.conc. time
ST1 {let TIMEFM,TIMEFM-TINT1}   subtracts half an hour (decimal) from foll.mat. time;
```

{calc}	calculates

RECORD	Records ovulation details on summary table
{let SEQ,SEQ+1}	adds one to sequence counter
{goto}SUMTABLE~{end}{d}{d}	finds empty row on summary table
+DAY~/rv~~{r}+TIMEDAY1~/rv~~{r}+ SEQ~/rv~~	records day, time and position in sequence as values
{if SEQ<>1}{r}+{l 2}-{l 2}{u}~	calculates time interval between ovulations
{home}{if DAY>=REPS}	returns to main menu if all days completed
{menubranch MU}	
{branch ENDDAY}	else branches to ENDDAY macro

Appendix 2.6: The calculated values for b_3 at different ovulation rates, using quadratic, exponential and Gompertz functions (Equations 2.5 - 2.7)

Ovulation rate	quadratic	exponential	Gompertz
0.40	-0.0517	-0.1641	0.2030
0.42	-0.0262	-0.1181	0.2030
0.44	-0.0016	-0.0758	0.2030
0.45	0.0104	-0.0560	0.2030
0.46	0.0222	-0.0370	0.2030
0.47	0.0338	-0.0188	0.2030
0.48	0.0452	-0.0014	0.2030
0.49	0.0563	0.0154	0.2030
0.50	0.0673	0.0314	0.2030
0.52	0.0886	0.0615	0.2030
0.54	0.1090	0.0892	0.2030
0.56	0.1286	0.1146	0.2030
0.58	0.1474	0.1380	0.2033
0.60	0.1653	0.1594	0.2041
0.62	0.1824	0.1791	0.2061
0.64	0.1986	0.1973	0.2103
0.66	0.2140	0.2139	0.2170
0.68	0.2286	0.2292	0.2264
0.70	0.2423	0.2433	0.2379
0.72	0.2552	0.2562	0.2508
0.74	0.2672	0.2680	0.2640
0.76	0.2784	0.2789	0.2769
0.78	0.2888	0.2890	0.2887
0.80	0.2983	0.2982	0.2993
0.82	0.3070	0.3066	0.3085
0.84	0.3148	0.3144	0.3163
0.86	0.3218	0.3215	0.3227
0.88	0.3280	0.3281	0.3280
0.90	0.3333	0.3341	0.3323
0.92	0.3378	0.3396	0.3358
0.94	0.3414	0.3447	0.3385
0.96	0.3442	0.3494	0.3408
0.98	0.3462	0.3537	0.3425
1.00	0.3473	0.3576	0.3439
1.02	0.3476	0.3613	0.3450
1.04	0.3470	0.3646	0.3458
1.06	0.3456	0.3677	0.3465
1.08	0.3434	0.3705	0.3470
1.10	0.3403	0.3731	0.3474

Appendix 2.7: Parameter estimates for the quadratic equation fitted to the values of b3

Response variate: b_3
Fitted terms: constant + R + R^2

Summary of analysis

	d.f.	s.s.	m.s.	v.r	F pr.
regression	2	0.01055774	0.005278870	1110.83	< 0.001
residual	5	0.00002376	0.000004752		
total	7	0.01058150	0.001511643		

% variance accounted for: 99.7
S.E. of observations estimated to be 0.00218

Estimate of parameters

	estimate	s.e.
constant	-0.7377	0.0934
R	2.135	0.239
R^2	-1.050	0.152

Appendix 2.8: Parameter estimates for the exponential equation fitted to the values of b3

Response variate: b_3
Explanatory: OR (ovulation rate)
Fitted curve: $A + B * R^{OR}$
Constraints: $R < 1$

Summary of analysis

	d.f.	s.s.	m.s.	v.r	F pr.
regression	2	0.01055102	0.005275510	865.42	< 0.001
residual	5	0.00003048	0.000006096		
total	7	0.01058150	0.001511643		

% variance accounted for: 99.6
S.E. of observations estimated to be 0.00247

Estimate of parameters

	estimate	s.e.
A	0.4023	±0.0197
B	-3.08	±1.20
R	0.0145	±0.0106

Appendix 2.9: Parameter estimates for the Gompertz equation fitted to the values of b3

Response variate: b_3
Explanatory: R
Fitted curve: A + C * exp(-exp(-B * (R - M)))
Constraints: C > 0
Direction: right

Summary of analysis

	d.f.	s.s.	m.s.	v.r	F pr.
regression	3	0.01056972	0.003523240	1196.24	< 0.001
residual	4	0.00001178	0.000002945		
total	7	0.01058150	0.001511643		

% variance accounted for: 99.8
S.E. of observations estimated to be 0.00172

Estimate of parameters

	estimate	s.e.
B	12.36	±2.14
M	0.7289	±0.0118
C	0.1459	±0.0186
A	0.2030	±0.0125

Appendix 2.10: Values assigned by AMPL to A and C for six of the ovulatory cycle parameters (B and M kept constant)

Parameter	B	M	C	A
λ_1	8	0.66667	0.020456	0.132475
λ_2	8	0.66667	0.071596	0.223662
S_1	8	0.66667	-3.068414	9.128776
b_1	8	0.66667	0.071596	0.723662
b_3	8	0.66667	0.233199	0.134213
S_2	8	0.66667	-3.596213	17.080953

Appendix 2.11: AMPL program files to create Gompertz equations for the six ovulatory cycle parameters λ_1, λ_2, S_1, b_1, b_3 and S_2

Key

param = parameter
var = variable
s.t. = constraint ('subject to')
*.mod = model files
*.dat = data files

b1.mod
(Creates a Gompertz curve for b1 within a defined range: b1=0.75 and 0.785 when ovulation rates are 0.667 and 0.9 respectively)

```
param B;
param M;
var Ab1 >= 0;
var Cb1 >= 0;
s.t. 2_eggb1: Ab1 + Cb1 * exp( -exp( -B * ((2/3) - M))) = 0.75;
s.t. 9_eggb1: Ab1 + Cb1 * exp( -exp( -B * ((9/10) - M))) = 0.785;
```

constants.dat
(Declares the values of the model constants)

```
param B := 8;
param M := 0.66667;
param a1 := 2.175;
param DL := 24;
```

b3.mod
(Creates a Gompertz curve for b3 within a defined range: b3=0.22 and 0.334 when ovulation rates are 0.667 and 0.9 respectively)

```
var Ab3 >= 0;
var Cb3 >= 0;
s.t. 2_eggb3: Ab3 + Cb3 * exp( -exp( -B * ((2/3) - M))) = 0.22;
s.t. 9_eggb3: Ab3 + Cb3 * exp( -exp( -B * (0.9 - M))) = 0.334;
```

S2.mod
(Creates a Gompertz curve for S2 within a defined range: S2=17.0 and 14.0 when ovulation rates are 0.5 and 0.9 respectively)

```
var AS2 >= 0;
var CS2 <= 0;
s.t. 1_eggS2: AS2 + CS2 * exp( -exp( -B * ((0.5) - M))) = 17.0;
s.t. 9_eggS2: AS2 + CS2 * exp( -exp( -B * (0.9 - M))) = 14.0;
```

L1.mod
(Creates a Gompertz curve for L1 within a defined range: L1=0.14 and 0.15 when ovulation rates are

0.667 and 0.9 respectively)

```
var AL1 >= 0;
var CL1 >= 0;
s.t. 2_eggL1: AL1 + CL1 * exp( -exp( -B * ((2/3) - M))) = 0.14;
s.t. 9_eggL1: AL1 + CL1 * exp( -exp( -B * (0.9 - M))) = 0.15;
```

L2.mod

(Creates a Gompertz curve for L2 within a defined range: L2=0.25 and 0.285 when ovulation rates are 0.667 and 0.9 respectively)

```
var AL2 >= 0;
var CL2 >= 0;
s.t. 2_eggL2: AL2 + CL2 * exp( -exp( -B * ((2/3) - M))) = 0.25;
s.t. 9_eggL2: AL2 + CL2 * exp( -exp( -B * (0.9 - M))) = 0.285;
```

S1.mod

(Creates a Gompertz curve for S1 within a defined range: S1=8 and 6.5 when ovulation rates are 0.667 and 0.9 respectively)

```
var AS1 >= 0;
var CS1 <= 0;
s.t. 2_eggS1: AS1 + CS1 * exp( -exp( -B * ((2/3) - M))) = 8;
s.t. 9_eggS1: AS1 + CS1 * exp( -exp( -B * (0.9 - M))) = 6.5;
```

Appendix 2.12: AMPL program files forming the project 2ovmin.amp; the objectives being to minimise the lag between two successive ovulations and to solve for b_2

b1.mod (From Appendix 2.11)
constants.dat
b3.mod
S2.mod
L1.mod
L2.mod
S1.mod

2pvalues.mod
(Calculates the values of the six ovulatory cycle parameters needed to produce a 2-ovulation sequence; calculates the value of a2.)

```
param DL;
param a1;
var 2b1 >= 0;
s.t. 2ovb1: Ab1 + Cb1 * exp( -exp( -B * ((2/3) - M))) = 2b1;
var 2b3 >= 0;
s.t. 2ovb3: Ab3 + Cb3 * exp( -exp( -B * ((2/3) - M))) = 2b3;
var 2S2 >= 0;
s.t. 2ovS2: AS2 + CS2 * exp( -exp( -B * ((2/3) - M))) = 2S2;
var 2S1 >= 0;
s.t. 2ovS1: AS1 + CS1 * exp( -exp( -B * ((2/3) - M))) = 2S1;
var 2L1 >= 0;
s.t. 2ovL1: AL1 + CL1 * exp( -exp( -B * ((2/3) - M))) = 2L1;
var 2L2 >= 0;
s.t. 2ovL2: AL2 + CL2 * exp( -exp( -B * ((2/3) - M))) = 2L2;
var 2a2 >= 0;
s.t. 2calca2: (a1 - (a1 * exp(-2L1 * 24)))/(1 - exp(-2L2 * 24)) = 2a2;
```

2regconc.mod
(Introduces the 3 time of day and 3 time of reg.conc. variables; relates time of reg.conc. to time of day)

```
var 2timeday1 >= 0, <= DL;
var 2timerc1 >= 0;
s.t. 2trc1: 2S1 <= 2timerc1;
s.t. 2tdrc1: 2timerc1 <= 2S1 + DL;
s.t. 2tdayrc1: 2timerc1 - (if 2timerc1 < DL then 0 else DL) = 2timeday1;
var 2timeday2 >= 0, <= DL;
s.t. 2td2td1: 2timeday2 >= 2timeday1;
var 2timerc2 >= 0;
s.t. 2trc2: 2S1 <= 2timerc2;
s.t. 2tdrc2: 2timerc2 <= 2S1 + DL;
s.t. 2tdayrc2: 2timerc2 - (if 2timerc2 < DL then 0 else DL) = 2timeday2;
var 2timeday3 >= 0, <= DL;
s.t. 2td3td2: 2timeday3 >= 2timeday2;
var 2timerc3 >= 0;
s.t. 2trc3: 2S1 <= 2timerc3;
s.t. 2tdrc3: 2timerc3 <= 2S1 + DL;
```

```
s.t. 2tdayrc3: 2timerc3 - (if 2timerc3 < DL then 0 else DL) = 2timeday3;
```

timegt.mod
(Introduces 3 time of foll.mat. variables; relates time of foll.mat. to time of day)

```
param 2lastov := 13.5;
var 2timefm1 >= (DL - 2lastov) + DL, <= (DL - 2lastov) + DL + DL;
subject to 2tdayfm1: 2timefm1 = 2timeday1 + (DL - 2lastov) + DL;
var 2timefm2 >= 0;
subject to 2timestart2: (DL - 2timeday1) <= 2timefm2;
subject to 2timeend2: 2timefm2 <= (DL - 2timeday1) + DL;
subject to 2tdayfm2: 2timefm2 = (DL - 2timeday1) + 2timeday2;
var 2timefm3 >= 0;
subject to 2timestart3: (DL - 2timeday2) <= 2timefm3;
subject to 2timeend3: 2timefm3 <= (DL - 2timeday2) + DL;
subject to 2tdayfm3: 2timefm3 = (DL - 2timeday2) + 2timeday3;
```

ovs.mod
(introduces objective function: minimises the lag between successive ovulations on days 1 and 2 while ensuring there is no intersection on day 3; solves for b2)

```
var b2 >= 0;
minimize lag: 2timeday2 - 2timeday1;
s.t. 2ov1: (2b1 * exp( -b2 * exp( -2b3 * (2timefm1 - 2S2))))
- (1 - (a1 * exp( -2L1 * (2timerc1 - 2S1)) - 2a2 * exp( -2L2 *
(2timerc1 - 2S1)))) = 0;
s.t. 2ov2: (2b1 * exp( -b2 * exp( -2b3 * (2timefm2 - 2S2))))
 - (1 - (a1 * exp( -2L1 * (2timerc2 - 2S1)) - 2a2 * exp( -2L2 *
(2timerc2 - 2S1)))) = 0;
s.t. intersection: (2b1 * exp( -b2 * exp( -2b3 * (2timefm3 - 2S2))))
 = ((1 - (a1 * exp( -2L1 * (2timerc3 - 2S1)) - 2a2
 * exp( -2L2 * (2timerc3 - 2S1)))) - 0.00001);
s.t. 2tangent: exp( -exp( -2b3 * (2timefm3 - 2S2))* b2 - 2b3
* (2timefm3 - 2S2)) * 2b1 * b2 * 2b3 = (exp( -(2timerc3 - 2S1) * 2L1)
 * a1 * 2L1 - exp( - (2timerc3 - 2S1) * 2L2) * 2a2 * 2L2);
```

solve.run

```
solve;
```

Appendix 2.13: AMPL program files forming the project 2ovmax.amp; the objectives being to maximise the lag between two successive ovulations and to solve for b_2.

b1.mod (From Appendix 2.11)
constants.dat
b3.mod
S2.mod
L1.mod
L2.mod
S1.mod

2pvalues.mod (From Appendix 2.12)

2maxrc.mod
(Introduces the 2 time of day and 2 time of reg.conc. variables; relates time of reg.conc. to time of day)

```
var 2timeday1 >= 0, <= DL;
var 2timerc1 >= 0;
s.t. 2trc1: 2S1 <= 2timerc1;
s.t. 2tdrc1: 2timerc1 <= 2S1 + DL;
s.t. 2tdayrc1: 2timerc1 - (if 2timerc1 < DL then 0 else DL) = 2timeday1;
var 2timeday2 >= 0, <= DL;
s.t. 2td2td1: 2timeday2 >= 2timeday1;
var 2timerc2 >= 0;
s.t. 2trc2: 2S1 <= 2timerc2;
s.t. 2tdrc2: 2timerc2 <= 2S1 + DL;
s.t. 2tdayrc2: 2timerc2 - (if 2timerc2 < DL then 0 else DL) = 2timeday2;
```

2maxgt.mod
(Introduces 2 time of foll.mat. variables; relates time of foll.mat. to time of day)

```
param 2lastov := 13.5;
var 2timefm1 >= (24 - 2lastov) + 24, <= (24 - 2lastov) + 48;
s.t. 2tdayfm1: 2timefm1 = 2timeday1 + (24 - 2lastov) + 24;
var 2timefm2 >= 0;
s.t. 2timestart2: (24 - 2timeday1) <= 2timefm2;
s.t. 2timeend2: 2timefm2 <= (24 - 2timeday1) + 24;
s.t. 2tdayfm2: 2timefm2 = (24 - 2timeday1) + 2timeday2;
```

2maxovs.mod
(introduces objective function: maximises the lag between successive ovulations on days 1 and 2; solves for b2)

```
var 2ub2 >= 0;
maximize 2lag: 2timeday2 - 2timeday1;
s.t. 2ov1: (2b1 * exp( -2ub2 * exp( -2b3 * (2timefm1 - 2S2)))) -
(1 - (a1 * exp( -2L1 * (2timerc1 - 2S1)) - 2a2 * exp( -2L2 *
(2timerc1 - 2S1)))) = 0;
s.t. 2ov2: (2b1 * exp( -2ub2 * exp( -2b3 * (2timefm2 - 2S2)))) -
(1 - (a1 * exp( -2L1 * (2timerc2 - 2S1)) - 2a2 * exp( -2L2 *
```

```
(2timerc2 - 2S1)))) = 0;
s.t. 2tangents: exp( -exp( -2b3 * (2timefm2 - 2S2))* 2ub2 - 2b3
* (2timefm2 - 2S2)) * 2b1 * 2ub2 * 2b3 = exp( -(2timeday2 - 2S1) * 2L1)
 * a1 * 2L1 - exp( - (2timeday2 - 2S1) * 2L2) * 2a2 * 2L2;

solve.run
solve;
```

Appendix 2.14: The upper and lower limits to b_2 and the minimum and maximum lag values given by AMPL, for sequences of different lengths

sequence length	minimum b_2	maximum b_2	minimum lag	maximum lag
1	1.317544	25.052215	-	-
2	4.000933	7.558433	2.93 (2h56m)	7.61 (7h37m)
3	8.523969	11.661940	4.30 (4h18m)	8.85 (8h51m)
4	11.718126	14.045908	5.07 (5h04m)	9.40 (9h24m)
5	13.602555	15.255627	5.54 (5h32m)	9.68 (9h41m)
6	14.730155	15.917356	5.85 (5h51m)	9.84 (9h50m)
7	15.444811	16.318413	6.07 (6h04m)	9.95 (9h57m)
8	15.926647	16.563950	6.22 (6h13m)	9.35 (9h21m)
9	16.270252	16.779481	6.34 (6h20m)	10.07 (10h04m)
15	17.232716	17.385434	6.69 (6h41m)	10.22 (10h13m)
20	17.577554	17.651497	6.80 (6h48m)	10.26 (10h16m)
R = 1*	-	18.981126		

* R = ovulation rate

Appendix 2.15: AMPL program files forming the project combined.amp; the objective being to find a Gompertz equation for b_2 between the defined minimum and maximum values

b2values.dat
(Declares the values of the model constants, i.e. lower and upper limits to b2 for specified sequence lengths; u = upper; rb2 = b2 for ovulation rate equal to 1)

```
param 1b2 := 1.317544;
param 2b2 := 4.000933;
param 3b2 := 8.523969;
param 4b2 := 11.718126;
param 5b2 := 13.602555;
param 6b2 := 14.730155;
param 7b2 := 15.444811;
param 8b2 := 15.926647;
param 9b2 := 16.270252;
param 15b2 := 17.232716;
param 20b2 := 17.5776938;
param 1ub2 := 25.052215;
param 2ub2 := 7.558433;
param 3ub2 := 11.661940;
param 4ub2 := 14.045908;
param 5ub2 := 15.255627;
param 6ub2 := 15.917356;
param 7ub2 := 16.318413;
param 8ub2 := 16.563950;
param 9ub2 := 16.779481;
param 15ub2 := 17.385434;
param 20ub2 := 17.651497;
param rb2 := 18.981126;
```

fitb2.mod
(finds a Gompertz equation for b2 which satisfies all constraints, while maximising b2 at R=1)

```
var M >= 0.6, <= 0.75;
var B >= 4;
var Ab2 >= 0;
var Cb2 >= 0;
s.t. 1_eggb2: 1b2 + 0.02 <= Ab2 + Cb2 * exp( -exp( -B * ((1/2) - M))) <= 1ub2;
s.t. 2_eggb2: 2b2 <= Ab2 + Cb2 * exp( -exp( -B * ((2/3) - M))) <= 2ub2 - 1;
s.t. 3_eggb2: 3b2 <= Ab2 + Cb2 * exp( -exp( -B * ((3/4) - M))) <= 3ub2;
s.t. 4_eggb2: 4b2 <= Ab2 + Cb2 * exp( -exp( -B * ((4/5) - M))) <= 4ub2;
s.t. 5_eggb2: 5b2 <= Ab2 + Cb2 * exp( -exp( -B * ((5/6) - M))) <= 5ub2;
s.t. 6_eggb2: 6b2 <= Ab2 + Cb2 * exp( -exp( -B * ((6/7) - M))) <= 6ub2;
s.t. 7_eggb2: 7b2 <= Ab2 + Cb2 * exp( -exp( -B * ((7/8) - M))) <= 7ub2;
s.t. 8_eggb2: 8b2 <= Ab2 + Cb2 * exp( -exp( -B * ((8/9) - M))) <= 8ub2;
s.t. 9_eggb2: 9b2 <= Ab2 + Cb2 * exp( -exp( -B * ((9/10) - M))) <= 9ub2 -0.1;
s.t. 15_eggb2: 15b2 <= Ab2 + Cb2 * exp( -exp( -B * ((15/16) - M))) <= 15ub2 - 0.01;
s.t. 20_eggb2: 20b2 <= Ab2 + Cb2 * exp( -exp( -B * ((20/21) - M))) <= 20ub2 - 0.01;
s.t. r_eggb2: Ab2 + Cb2 * exp( -exp( -B * (1 - M))) <= rb2;
```

```
var top;
s.t. xtop: Ab2 + Cb2 * exp( -exp( -B * (1 - M))) = top;
maximize top2: Ab2 + Cb2 * exp( -exp( -B * (1 - M)))

solve.run

solve;
```

Appendix 2.16: User input and model output for the macro- and menu-driven ovulation model calculating in half-hour, minute and second intervals

Additional constants

tint4 =	00:00:01	one-second interval; add to time of day
tint5 =	0.000278	one-second interval; add to time reg.conc. and foll.mat.
mindiff1 =	-0.0005	target for changing to one-second intervals
mindiff2 =	0.0000	minimum difference before ovulation deemed to occur

User input, counters and calculations

Day	10	day counter
Position in sequence	1	sequence counter
Sequence length	2	user input: 2-ovulation sequence
Rate of lay	0.6667	formula
Time T5	13.5	formula
Reps	10	user input: 10 days

Spreadsheet for calculating the time of intersection (figures as at the end of the 10th day)

variable	half-hour intervals	variable	minute intervals	variable	second intervals
timeday	09:00:00	timeday1	09:04:00	timeday2	09:04:43
timerc	9.0000	timerc1	9.0667	timerc2	9.0786
R3(t)	0.252	R3(t)	0.261	R3(t)	0.263
1-R3(t)	0.748	1-R3(t)	0.739	1-R3(t)	0.737
timefm	42.65	timefm1	42.72	timefm2	42.73
G(t)	0.7369	G(t)	0.7371	G(t)	0.7371
G(t)-(1-R3(t))	-0.011	G(t)-(1-R3(t))	-0.002	G(t)-(1-R3(t))	0.000

Summary table: Ovulation times

Day	Time	Sequence	Interval
1	09:03:48	1	
2	14:19:15	2	05:15:27
3			
4	09:04:41	1	
5	14:20:49	2	05:16:08
6			
7	09:04:43	1	
8	14:20:53	2	05:16:10
9			
10	09:04:43	1	

Appendix 2.17: Additional macro commands for the revised Lotus 1-2-3 ovulation model; includes commands to perform one-second iterations

OVTIME (modified from appendix 2.5)	adds one minute intervals, evaluates difference
{let TIMEDAY1,TIMEDAY1+TINT2}	adds 1 minute to time of day
{let TIMERC1,TIMERC1+TINT3}	adds 1 minute (decimal) to reg.conc. time
{let TIMEFM1,TIMEFM1+TINT3}	adds 1 minute (decimal) to foll.mat. time;
{calc}	calculates
{if TIMERC1>ENDTIME}	stops if reg.conc. time exceeds ENDTIME,
{branch SKIP}	branches to SKIP macro
{if DIFF1<MINDIFF1}	repeats OVTIME macro if G(t) - 1-R3(t) still negative
{branch OVTIME}	
{if DIFF1>=0}{SUBTRACTTIME1}	performs subroutine SUBTRACTTIME1 if intersection has already occurred
{branch OVTIMESEC}	branches to OVTIMESEC macro

	SUBTRACTTIME1	subtracts one minute when intersection exceeded
	{let TIMEDAY1,TIMEDAY1-TINT2}	subtracts one minute from time of day
	{if TIMERC1=RCS1}	if reg.conc. time already reset at S1, subtracts one minute
	{let TIMERC1,TMAX-TINT3}	from maximum reg.conc. time;
	{branch ST2}	moves to macro ST2
	{let TIMERC1,TIMERC1-TINT3}	subtracts one minute (decimal) from reg.conc. time
ST2	{let TIMEFM1,TIMEFM1-TINT3}	subtracts one minute (decimal) from foll.mat. time;
	{calc}	calculates

OVTIMESEC	prepares to add one second intervals;
{let TIMEDAY2,TIMEDAY1}	sets the three second time counters to equal the three
{let TIMERC2,TIMERC1}	minute time counters,
{let TIMEFM2,TIMEFM1}{calc}	calculates
{branch ADDSEC}	branches to ADDSEC macro

ADDSEC	adds one second intervals, evaluates difference
{let TIMEDAY2,TIMEDAY2+TINT4}	adds 1 second to time of day
{let TIMERC2,TIMERC2+TINT5}	adds 1 second (decimal) to reg.conc. time
{let TIMEFM2,TIMEFM2+TINT5}	adds 1 second (decimal) to foll.mat. time;
{calc}	calculates
{if TIMERC2>ENDTIME}	stops if reg.conc. time exceeds endtime,
{branch SKIP}	branches to SKIP macro
{if DIFF2>= MINDIFF2}{branch RECORD}	if G(t) - 1-R3(t) = zero or positive i.e. intersection occurred, branches to RECORD macro
{branch ADDSEC}	otherwise repeats ADDSEC macro

Appendix 3.1: Vaccination Program for Experimental Pullets

Age	Vaccination	Route
day-old	Marek	Subcutaneous
14 days	NCD / IBD (oil based)	Subcutaneous
21 days	IBD	drinking water
28 days	IB / NCD	Spray
7 weeks	IB / NCD (oil based)	Subcutaneous
	Pox	wing web
10 weeks	IB / NCD (La Sota)	Spray
12 weeks	AE	drinking water
14 weeks	Coryza 1	Subcutaneous
	EDS	Intramuscular
19 weeks	Coryza 2 (OPB 3 strain)	Subcutaneous
	IB /NCD / EDS	Intramuscular
34 weeks	NCD	Spray
38 weeks	IB	Spray

Appendix 3.2: Mean Body Weights at 4, 6 and 8 weeks of age

		4 weeks	6 weeks	8 weeks
Room 1	Hy-Line Silver	261.2	436.7	687.9
	Hy-Line Brown	246.6	420.4	667.1
Room 2	Hy-Line Silver	264.4	419.6	677.9
	Hy-Line Brown	248.7	417.9	659.6
Room 3	Hy-Line Silver	263.5	437.1	683.3
	Hy-Line Brown	253.9	412.1	650
Room 4	Hy-Line Silver		430.4	695
	Hy-Line Brown		404.6	651.4
Room 5	Hy-Line Silver		430.4	684.2
	Hy-Line Brown		395.8	640
Room 6	Hy-Line Silver	258.1	445.8	694.6
	Hy-Line Brown	250.7	406.7	638.3
Strain mean	Hy-Line Silver	261.71	433.33	687.15
	Hy-Line Brown	250	409.58	651.07
Strain standard		290	480	690

Appendix 3.3: Analysis of variance for mean body weights at 6-weeks, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	75.50	75.50	1.86	
Block.lights stratum					
lights	2	195.23	97.61	2.40	0.294
residual	2	81.25	40.62	0.45	
Block.lights.strain stratum					
strain	1	1692.19	1692.19	18.79	0.007
residual	5	450.41	90.08		
Total	11	2494.57			

Tables of means

Grand mean	421.5		
lights	12 wks	15 wks	18 wks
	423.0	415.9	425.4
strain	HS	HB	
	433.3	409.6	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	4.51	5.48

Appendix 3.4: Analysis of variance for 8-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	41.4	41.4	3.89	
Block.lights stratum					
lights	2	236.3	118.1	11.09	0.083
residual	2	21.3	10.7	0.10	
Block.lights.strain stratum					
strain	1	3906.0	3906.0	35.88	0.002
residual	5	544.3	108.9		
Total	11	4749.4			

Tables of means

Grand mean	669.1		
lights	12 wks	15 wks	18 wks
	675.4	665.4	666.5
strain	HS	HB	
	687.1	651.1	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	2.31	6.02

Appendix 3.5: Mean body weights at 11 weeks of age

		number of birds	mean weight (g) (±sd)	% cv
Room 1	Hy-Line Silver	46	1051.2 (±79.42)	7.6
	Hy-Line Brown	48	1009.3 (±58.02)	5.7
Room 2	Hy-Line Silver	47	1025.6 (±68.31)	6.7
	Hy-Line Brown	48	998.4 (±66.71)	6.7
Room 3	Hy-Line Silver	49	989.4 (±72.44)	7.3
	Hy-Line Brown	48	957.6 (±70.29)	7.3
Room 4	Hy-Line Silver	47	1030.6 (±66.95)	6.5
	Hy-Line Brown	49	988.7 (±84.96)	8.6
Room 5	Hy-Line Silver	47	1037.1 (±81.48)	7.9
	Hy-Line Brown	48	970.7 (±65.28)	6.7
Room 6	Hy-Line Silver	45	1048.2 (±72.20)	6.9
	Hy-Line Brown	47	968.0 (±77.09)	8
Strain mean	Hy-Line Silver	281	1029.8 (±75.8)	7.4
	Hy-Line Brown	288	982.2 (±72.6)	7.4
Strain standard			990	

Appendix 3.6: Analysis of variance for 11-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	11.6	11.6	0.01	
Block.lights stratum					
lights	2	1717.1	858.6	1.02	0.494
residual	2	1675.5	837.8	3.91	
Block.lights.strain stratum					
strain	1	6979.4	6979.4	32.54	0.002
residual	5	1072.3	214.5		
Total	11	11455.9			

Tables of means

Grand mean	1006.2		
lights	12 wks	15 wks	18 wks
	1020.0	1008.0	990.8
strain	HS	HB	
	1030.4	982.1	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	20.47	8.45

Appendix 3.7: Mean Body Weights at 15 Weeks of Age

		number of birds	mean weight (g) (±sd)	% cv
Room 1	Hy-Line Silver	23	1434.1 (±121.4)	8.5
	Hy-Line Brown	24	1350.8 (±115.0)	8.5
Room 2	Hy-Line Silver	15	1338.4 (±62.0)	4.6
	Hy-Line Brown	16	1306.6 (±59.6)	4.6
Room 3	Hy-Line Silver	16	1326.8 (±94.9)	7.2
	Hy-Line Brown	16	1275.4 (±73.5)	5.8
Room 4	Hy-Line Silver	24	1459.5 (±101.3)	6.9
	Hy-Line Brown	24	1366.7 (±109.1)	8
Room 5	Hy-Line Silver	16	1373.9 (±104.1)	7.6
	Hy-Line Brown	16	1237.4 (±61.0)	4.9
Room 6	Hy-Line Silver	17	1311.2 (±103.8)	7.9
	Hy-Line Brown	16	1255.8 (±64.0)	5.1
Strain mean	Hy-Line Silver	111	1383.7 (±115.0)	8.3
	Hy-Line Brown	112	1307.4 (±99.4)	7.6
Strain standard			1340	

Appendix 3.8: Analysis of variance for 15-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	63.5	63.5	0.13	
Block.lights stratum					
lights	2	27395.4	13697.7	28.64	0.034
residual	2	956.6	478.3	0.69	
Block.lights.strain stratum					
strain	1	16965.1	16965.1	24.32	0.004
residual	5	3487.5	697.5		
Total	11	48868.2			

Tables of means

Grand mean	1336.4		
lights	12 wks	15 wks	18 wks
	1402.8	1314.1	1292.3
strain	HS	HB	
	1374.0	1298.8	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	15.46	15.25

Appendix 3.9: Mean Body Weights at 16 Weeks of Age

		number of birds	mean weight (g) (±sd)	% cv
Room 1	Hy-Line Silver	23	1535.9 (±126.9)	8.3
	Hy-Line Brown	24	1464.5 (±110.7)	7.6
Room 2	Hy-Line Silver	24	1477.1 (±135.9)	9.2
	Hy-Line Brown	24	1356.0 (±94.8)	7
Room 3	Hy-Line Silver	31	1400.6 (±94.1)	6.7
	Hy-Line Brown	32	1344.4 (±83.9)	6.2
Room 4	Hy-Line Silver	24	1547.2 (±102.7)	6.6
	Hy-Line Brown	24	1450.0 (±95.1)	6.6
Room 5	Hy-Line Silver	24	1434.3 (±101.3)	7.1
	Hy-Line Brown	24	1357.7 (±92.5)	6.8
Room 6	Hy-Line Silver	31	1423.6 (±101.6)	7.1
	Hy-Line Brown	32	1320.5 (±88.5)	6.7
Strain mean	Hy-Line Silver	157	1464.2 (±121.9)	8.3
	Hy-Line Brown	160	1377.2 (±107.0)	7.8
Strain standard			1410	

Appendix 3.10: Analysis of variance for 16-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	170.3	170.3	1.34	
Block.lights stratum					
lights	2	34652.0	17326.0	135.99	0.007
residual	2	254.8	127.4	0.45	
Block.lights.strain stratum					
strain	1	23021.3	23021.3	81.52	<0.001
residual	5	1412.0	282.4		
Total	11	59510.4			

Tables of means

Grand mean	1426.0		
lights	12 wks	15 wks	18 wks
	1499.4	1406.3	1372.3
strain	HS	HB	
	1469.8	1382.2	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	7.98	9.70

Appendix 3.11: Mean Body Weights at 17 Weeks of Age

		number of birds	mean weight (g) (±sd)	% cv
Room 1	Hy-Line Silver	23	1600.7 (±146.0)	9.1
	Hy-Line Brown	24	1533.6 (±102.6)	6.7
Room 2	Hy-Line Silver	24	1615.1 (±166.3)	10.3
	Hy-Line Brown	24	1461.5 (±110.0)	7.5
Room 3	Hy-Line Silver	33	1460.6 (±90.1)	6.2
	Hy-Line Brown	30	1406.2 (±96.4)	6.9
Room 4	Hy-Line Silver	24	1611.7 (±134.0)	8.3
	Hy-Line Brown	24	1501.0 (±105.0)	7
Room 5	Hy-Line Silver	24	1567.8 (±123.0)	7.8
	Hy-Line Brown	24	1475.5 (±105.6)	7.2
Room 6	Hy-Line Silver	31	1473.4 (±121.9)	8.3
	Hy-Line Brown	32	1380.8 (±94.7)	6.9
Strain mean	Hy-Line Silver	159	1545.7 (±143.9)	9.3
	Hy-Line Brown	158	1453.7 (±113.9)	7.8
Strain standard			1480	

Appendix 3.12: Analysis of variance for 17-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	379.7	379.7	14.10	
Block.lights stratum					
lights	2	37662.6	18831.3	699.20	0.001
residual	2	53.9	26.9	0.04	
Block.lights.strain stratum					
strain	1	27141.5	27141.5	44.35	0.001
residual	5	3060.1	612.0		
Total	11	68297.8			

Tables of means

Grand mean	1507.3		
lights	12 wks	15 wks	18 wks
	1561.8	1530.0	1430.2
strain	HS	HB	
	1554.9	1459.8	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	3.67	14.28

Appendix 3.13: Mean Body Weights at 18 Weeks of Age

		number of birds	mean weight (g) (±sd)	% cv
Room 1	Hy-Line Silver	23	1646.3 (±181.5)	11
	Hy-Line Brown	24	1560.8 (±113.3)	7.3
Room 2	Hy-Line Silver	24	1664.8 (±170.8)	10.3
	Hy-Line Brown	24	1532.3 (±104.8)	6.8
Room 3	Hy-Line Silver	24	1501.6 (±121.7)	8.1
	Hy-Line Brown	24	1400.9 (±122.0)	8.7
Room 4	Hy-Line Silver	24	1627.6 (±127.8)	7.9
	Hy-Line Brown	24	1532.1 (±110.8)	7.2
Room 5	Hy-Line Silver	24	1618.8 (±126.5)	7.8
	Hy-Line Brown	24	1550.9 (±86.9)	5.6
Room 6	Hy-Line Silver	24	1515.3 (±159.0)	10.5
	Hy-Line Brown	24	1436.1 (±96.7)	6.7
Strain mean	Hy-Line Silver	143	1595.4 (±160.1)	10
	Hy-Line Brown	144	1502.2 (±121.0)	8.1
Strain standard			1550	

Appendix 3.14: Analysis of variance for 18-week body weights, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	55.9	55.9	0.09	
Block.lights stratum					
lights	2	43844.4	21922.2	33.95	0.029
residual	2	1291.3	645.6	2.58	
Block.lights.strain stratum					
strain	1	26254.8	26254.8	104.99	<0.001
residual	5	1250.3	250.1		
Total	11	72696.7			

Tables of means

Grand mean	1549.0		
lights	12 wks	15 wks	18 wks
	1591.7	1591.7	1463.5
strain	HS	HB	
	1595.7	1502.2	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d	17.97	9.13

Appendix 3.15: Mean Body Weights at 19 Weeks of Age

		number of birds	mean weight (g) (±sd)	% cv
Room 2	Hy-Line Silver	23	1711.4 (±187.9)	11
	Hy-Line Brown	24	1578.0 (±121.0)	7.7
Room 3	Hy-Line Silver	24	1658.3 (±117.0)	7.1
	Hy-Line Brown	24	1548.0 (±141.8)	9.2
Room 5	Hy-Line Silver	24	1677.3 (±108.5)	6.5
	Hy-Line Brown	24	1611.0 (±85.7)	5.3
Room 6	Hy-Line Silver	24	1627.9 (±181.6)	11.2
	Hy-Line Brown	24	1572.3 (±125.3)	8
Strain mean	Hy-Line Silver	95	1668.3 (±153.2)	9.2
	Hy-Line Brown	96	1577.3 (±120.4)	7.6
Strain standard	Hy-Line Silver		1630	
	Hy-Line Brown		1610	

Appendix 3.16: Linear regression of mean body weight at first egg for the Hy-Line Silver strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	11175.1	11175.1	15.72	0.157
Residual	1	710.7	710.7		
Total	2	11885.8	5942.9		

Percentage variance accounted for: 88.0
Standard error of observations is estimated to be 26.7

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	1272.2	95.5	13.32	0.048
Age at photostim.	24.92	6.28	3.97	0.157

Appendix 3.17: Linear regression of mean body weight at first egg for the Hy-Line Brown strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	12896.2	12896.2	35.63	0.106
Residual	1	361.9	361.9		
Total	2	13258.1	6629.1		

Percentage variance accounted for: 94.5
Standard error of observations is estimated to be 19.0

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	1187.2	68.2	17.42	0.037
Age at photostim.	26.77	4.48	5.97	0.106

Appendix 3.18: Analysis of variance for mean age at first egg, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.053	0.053	0.02	
Block.lights stratum					
lights	2	1279.995	639.998	216.89	0.005
residual	2	5.902	2.951	1.46	
Block.lights.strain stratum					
strain	1	28.213	28.213	13.93	0.014
residual	5	10.127	2.025		
Total	11	1324.290			

Tables of means

Grand mean	128.35		
lights	12 wks	15 wks	18 wks
	115.40	128.97	140.67
strain	HS	HB	
	126.82	129.88	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d.	1.215	0.822

Appendix 3.19: Linear regression of mean age at first egg for the Hy-Line Silver strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	302.58000	302.58000	3705.06	0.010
Residual	1	0.08167	0.08167		
Total	2	302.66167	151.33083		

Percentage variance accounted for: 99.9
Standard error of observations is estimated to be 0.286

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	65.28	1.02	63.77	0.010
Age at photostim.	4.1000	0.0674	60.87	0.010

Appendix 3.20: Linear regression of mean age at first egg for the Hy-Line Brown strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	338.780	338.780	102.65	0.063
Residual	1	3.300	3.300		
Total	2	342.081	171.040		

Percentage variance accounted for: 98.1
Standard error of observations is estimated to be 1.82

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	64.75	6.51	9.95	0.064
Age at photostim.	4.338	0.428	10.13	0.063

Appendix 3.21: Oviposition times for first eggs at sexual maturity

Hen no.	Room 1	Room 2	Room 3	Room 4	Room 5	Room 6
1	11:00	10:00	9:30	9:00		9:30
2	14:00	11:00	10:00	7:00	8:00	9:00
3	9:30	8:30	11:30	7:30	10:00	10:00
4	7:00	14:00	7:00	8:30	9:00	10:30
5	7:00	12:00	10:00	11:30	11:00	14:30
6	8:30	8:30		12:30	8:00	16:30
7	14:30		11:30	9:30	15:00	11:00
8			14:00	13:00	9:00	9:30
9		16:00	7:30	7:30	10:30	13:30
10	7:00	10:00	14:30	16:30	11:30	7:30
11	7:30	7:30	7:30	11:00	8:00	10:30
12	9:00	8:00	11:30	12:00	12:30	9:00
13	7:00	7:00	9:30	7:00	9:30	8:30
14	7:30	8:00	9:30	10:00	12:30	11:00
15	7:30	14:30	9:30	14:00	11:30	7:30
16	12:30	14:30	8:30	11:30	11:00	14:00
17	8:30	12:00	12:00	11:00	9:00	13:30
18		12:00	14:30	7:30		12:00
19	9:30	7:00	13:30	10:30	12:00	
20	13:00	8:00	8:00	11:00	12:00	
21	13:00	9:30	7:30	9:30	9:00	15:00
22	7:00	8:00	13:30		8:00	10:30
23	15:00	12:30	8:30		12:30	9:00
24	12:30	13:30	9:00	15:30	7:00	7:30
25	9:30	14:00	7:00	14:30	13:30	7:30
26	7:00	13:00		15:00	10:00	9:00
27	8:00	8:30	11:30	11:30	16:30	
28	11:00	10:30	11:00	10:00	10:30	9:00
29	12:00	11:30	10:00		12:00	8:00
30	10:00	7:00	8:00	15:00	7:00	9:00
31	16:00	10:30	15:00	14:30	9:00	10:00
32	8:30	11:00	12:00	7:00	16:00	12:00
33	11:00		10:30	7:30	7:00	12:00
34			16:30	9:30	13:00	12:30
35	11:00	11:30	11:30	7:00	10:30	9:00
36	10:00	11:00	7:00	7:30	9:30	9:30
37	14:30	7:00		15:30	15:30	8:30
38	8:30	12:00	14:00	8:00	11:30	10:00
39		11:00	10:00	7:00	8:30	9:30
40	15:00	15:30	9:00	14:00	10:30	8:00
41		7:00	15:30	7:30	8:30	9:30
42	9:00	7:00	9:00	8:00	10:00	8:30
43	9:30	7:00		7:00	9:30	11:00
44	9:00	8:30	11:30	7:00	10:00	14:00
45			15:00	11:30	11:30	
46	9:00	9:00	12:00	8:00	14:00	11:30
47	9:30	7:30	7:30	10:00	12:00	16:00
48		7:00	13:30	12:30	8:30	

Appendix 3.22: Post mortem results

hen	no. eggs	liver	abdomen	ovary	oviduct	possible diagnosis
room 1 hen 48 (12-wk; HB)	0	yellow, friable	presence of yolk	functional. 6 large yellow follicles in hierarchy	white (*i.e.* not vascular), small	immature oviduct, internal ovulation
room 1 hen 9 (12-wk; HS)	0	slightly yellow, friable	presence of yolk	functional. 6 large yellow follicles	infundibulum and magnum under-developed	immature oviduct, internal ovulation
room 3 hen 38 (18-wk; HB)	1	normal	large shell	functional. 8 large yellow follicles	normal size; hole in magnum; cloaca connection?	damaged oviduct
room 2 hen 8 (15-wk; HS)	0	normal		8 follicles	size normal	coming into lay?
room 5 hen 1 (15-wk; HS)	0	yellow, friable	presence of yolk	9 follicles	infundibulum aplasic, isthmus white short, thin	abnormal oviduct, internal ovulation
room 3 hen 43 (18-wk; HB)	0	yellow		7 follicles	infundibulum small; rest of tract ok.	immature oviduct
room 1 hen 45 (12-wk; HB)	0	yellow, friable	presence of yolk	6 follicles	infundibulum small	abnormal oviduct
room 1 hen 34 (12-wk; HB)	0	conges-ted with blood	presence of yolk, fluid	6 follicles	infundib.well developed; no muscle in magnum; persistent right oviduct	peritonitis, internal ovulation, abnormal oviduct

Appendix 3.22: Post mortem results (continued)

hen	no. eggs	liver	abdomen	ovary	oviduct	possible diagnosis
room 1 hen 8 (12-wk; HS)	0	brown, congested, friable	presence of fluid, yolk	9 follicles	short	internal ovulation, peritonitis, immature oviduct
room 2 hen 7 (15-wk; HS)	0	normal	presence of yolk	6 follicles	cysts on infundibulum	internal ovulation, abnormal oviduct
room 6 hen 27 (18-wk; HB)	0	yellow, congested	presence of fluid, yolk	8 follicles	infundibulum not developed	internal ovulation, abnormal oviduct
room 6 hen19 (18-wk; HS)	0	pale		under-developed; small follicles	few blood vessels; not active	immature ovary and oviduct; may have laid later
room 1 hen 39 (12-wk; HB)	0	slightly yellow		6 normal follicles; one pale yellow, wrinkled		ovary?
room 2 hen 34 (15-wk; HB)	0	yellow, congested	presence of yolk	8 follicles	small infundibulum	internal ovulation; poor oviduct
room 4 hen 23 (12-wk; HS)	0	slightly yellow	presence of yolk	9 follicles, one post-ovulatory follicle		juvenile oviduct, internal ovulation
room 5 hen 18 (15-wk; 0HS)	0	yellow, friable		under-developed; haemorrhagic follicle	normal	juvenile ovary

Appendix 3.22: Post mortem results (continued)

hen	no. eggs	liver	abdomen	ovary	oviduct	possible diagnosis
room 3 hen 26 (18-wk; HB)	0		fluid	post-ovulatory follicle	macerated, atrophied oviduct; vesicular formations, prolapsed vagina; right oviduct	ascites; abnormal oviduct
room 6 hen 20 (18-wk; HS)	0	yellow		under-developed ovary		juvenile ovary
room 3 hen 37 (18-wk; HB)	0	yellow	presence of yolk	7 follicles	small infundibulum	abnormal oviduct
room 2 hen 33 (15-wk; HB)	0	yellow, friable	presence of fluid, yolk	8 follicles, one post-ovulatory follicle	prolapsed oviduct; aplasic magnum; old yolks, blood spots in magnum	ascites, internal ovulation, abnormal oviduct, prolapse
room 6 hen 45 (18-wk; HB)	0	pale, friable		small, transparent follicles		juvenile ovary
room 6 hen 48 (18-wk; HB)	0	yellow, conges-ted		9 follicles	under-developed infundibulum	abnormal oviduct
room 2 hen 45 (15-wk; HB)	0	yellow, friable	presence of yolk	7 follicles, one post-ovulatory follicle	cyst near infundibulum; oviduct size normal	internal ovulation; abnormal oviduct

Appendix 3.23: Analysis of variance for mean number of double yolks per hen, using a split-plot design and testing for main effects

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.04189	0.04189	0.99	
Block.lights stratum					
lights	2	1.75827	0.87914	20.77	0.046
residual	2	0.08466	0.04233	1.30	
Block.lights.strain stratum					
strain	1	0.00014	0.00014	0.00	0.950
residual	5	0.16224	0.03245		
Total	11	2.04721			

Tables of means

Grand mean	0.649		
lights	12 wks	15 wks	18 wks
	1.177	0.490	0.281
strain	HS	HB	
	0.646	0.653	

Standard errors of differences of means

Table	Lights	strain
rep.	4	6
d.f.	2	5
s.e.d.	0.1455	0.1040

Appendix3.24: Linear regression of mean number of double-yolked eggs for the Hy-Line Silver strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	0.4204	0.4204	3.29	0.321
Residual	1	0.1276	0.1276		
Total	2	0.5480	0.2740		

Percentage variance accounted for: 53.4
Standard error of observations is estimated to be 0.357

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	2.94	1.28	2.30	0.261
Age at photostim.	-0.1528	0.0842	-1.82	0.321

Appendix3.25: Linear regression of mean number of double-yolked eggs for the Hy-Line Brown strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	0.382813	0.382813	333.41	0.035
Residual	1	0.001148	0.001148		
Total	2	0.383961	0.191980		

Percentage variance accounted for: 99.4
Standard error of observations is estimated to be 0.0339

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	2.840	0.121	23.40	0.027
Age at photostim.	-0.14583	0.00799	-18.26	0.035

Appendix 3.26: The production of double-yolked eggs by individual Hy-Line Silver hens in room 1 (12-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk

		Hen Number															
Date	Age (d)	4	5	6	7	11	12	13	14	15	16	17	19	21	22	23	24
1/1	105														f,p		
2/1	106																
3/1	107															f, t	
4/1	108																
5/1	109															dy	
6/1	110																
7/1	111															dy	
8/1	112																
9/1	113				f, t												
10/1	114																dy
11/1	115																
12/1	116					t											
13/1	117														dy		
14/1	118		p											f, t			
15/1	119															dy	
16/1	120																
17/1	121												dy				
18/1	122															dy	
19/1	123			dy		p											dy
20/1	124																
21/1	125					dy											
22/1	126						dy		dy		dy						
23/1	127	dy															
24/1	128		dy					dy									
25/1	129									dy							
26/1	130										dy			dy			
27/1	131						dy										dy
28/1	132																
29/1	133	dy										dy	dy				
(31)	Total	2	2	1	1	3	2	1	1	1	2	1	2	2	2	5	3

Appendix 3.27: The production of double-yolked eggs by individual Hy-Line Brown hens in room 1 (12-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	25	27	30	32	33	35	38	40	43	47					
1/1	105															
2/1	106															
3/1	107			f												
4/1	108															
5/1	109															
6/1	110															
7/1	111			dy												
8/1	112	t														
9/1	113															
10/1	114															
11/1	115															
12/1	116									s,p						
13/1	117								f, t							
14/1	118			dy		dy					dy					
15/1	119						dy			dy						
16/1	120									p						
17/1	121															
18/1	122			dy			dy				dy					
19/1	123							dy		dy						
20/1	124				t											
21/1	125		s	dy					dy							
22/1	126										dy					
23/1	127					dy										
24/1	128															
25/1	129															
26/1	130			dy												
27/1	131															
28/1	132			dy												
29/1	133	dy			dy											
(26)	Total	2	1	7	2	2	2	1	2	4	3					

Appendix 3.28: The production of double-yolked eggs by individual Hy-Line Silver and Brown hens in room 2 (15-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk

		Hen Number														
Date	Age (d)	2	6	9	14	23	24		25	29	35	37	40	47		
15/1	119															
16/1	120															
17/1	121															
18/1	122															
19/1	123															
20/1	124										dy					
21/1	125															
22/1	126															
23/1	127						f, t						f, t			
24/1	128									f						
25/1	129								f, t							
26/1	130						dy									
27/1	131															
28/1	132															
29/1	133															
30/1	134															
31/1	135															
1/2	136															
2/2	137		dy													
3/2	138				dy											
4/2	139	dy		dy												
5/2	140															
6/2	141															
7/2	142					dy										
8/2	143															
9/2	144								dy			dy				
10/2	145															
11/2	146									dy						
12/2	147													dy		
(7)	Total	1	1	1	1	1	2		2	2	1	1	1	1		(8)

Appendix 3.29: The production of double-yolked eggs by individual Hy-Line Silver and Brown hens in room 3 (18-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

Hen Number																
Date	Age (d)	1	15	19	20	23		34	44	45	48					
25/1	129															
26/1	130		t													
27/1	131															
28/1	132															
29/1	133					f										
30/1	134															
31/1	135															
1/2	136									f, t						
2/2	137															
3/2	138															
4/2	139															
5/2	140															
6/2	141					dy										
7/2	142							dy								
8/2	143							t								
9/2	144		dy													
10/2	145															
11/2	146															
12/2	147															
13/2	148															
14/2	149					dy										
15/2	150	t							dy							
16/2	151															
17/2	152															
18/2	153			dy	dy											
19/2	154															
20/2	155															
23/2	158										p					
(8)	Total	1	2	1	1	3		2	1	1	1		(5)			

Appendix 3.30: The production of double-yolked eggs by individual Hy-Line Silver hens in room 4 (12-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	1	3	4	7	8	10	12	14	15	17	18	20	21		
1/1	105															
2/1	106															
3/1	107															
4/1	108															
5/1	109															
6/1	110							f, ty						dy		
7/1	111															
8/1	112													dy		
9/1	113															
10/1	114							s, p		f, t						
11/1	115															
12/1	116													dy		
13/1	117															
14/1	118	dy														
15/1	119										t		t			
16/1	120									p				dy		
17/1	121		dy			dy				dy						
18/1	122					dy										
19/1	123			dy				dy								
20/1	124															
21/1	125															
22/1	126						dy							dy		
23/1	127										dy					
24/1	128				dy											
25/1	129	dy							dy							
26/1	130	dy									dy					
27/1	131											dy				
28/1	132											dy				
29/1	133										dy			dy		
(29)	Total	3	1	1	1	2	1	3	1	3	4	2	1	6		

Appendix 3.31: The production of double-yolked eggs by individual Hy-Line Brown hens in room 4 (12-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	26	27	28	31	33	34	35	37	38	39	40	41	46		
1/1	105															
2/1	106															
3/1	107															
4/1	108															
5/1	109		f													
6/1	110															
7/1	111		dy													
8/1	112		dy													
9/1	113									p						
10/1	114		ty						f, t							
11/1	115		dy											t		
12/1	116									p						
13/1	117											dy				
14/1	118			p												
15/1	119															
16/1	120					dy					dy	dy				
17/1	121											dy				
18/1	122	dy	dy													
19/1	123		p	dy			dy							t		
20/1	124															
21/1	125															
22/1	126				f											
23/1	127		s							dy						
24/1	128															
25/1	129												dy			
26/1	130									s, t						
27/1	131															
28/1	132							dy								
29/1	133															
(27)	Total	1	8	2	1	1	1	1	1	4	1	3	1	2		

Appendix 3.32: The production of double-yolked eggs by individual Hy-Line Silver hens in room 5 (15-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	2	8	10	11	12	22	23	24							
15/1	119															
16/1	120			f												
17/1	121															
18/1	122															
19/1	123															
20/1	124															
21/1	125															
22/1	126															
23/1	127															
24/1	128															
25/1	129															
26/1	130															
27/1	131															
28/1	132															
29/1	133						dy									
30/1	134							dy								
31/1	135					p	p									
1/2	136															
2/2	137															
3/2	138		dy													
4/2	139								dy							
5/2	140															
6/2	141	dy														
7/2	142															
8/2	143						p									
9/2	144															
10/2	145															
11/2	146				dy											
12/2	147															
(10)	Total	1	1	1	1	1	3	1	1							

Appendix 3.33: The production of double-yolked eggs by individual Hy-Line Brown hens in room 5 (15-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	25	26	28	30	31	32	35	36	37	38	39	42	43	45	
15/1	119															
16/1	120															
17/1	121								f							
18/1	122															
19/1	123															
20/1	124															
21/1	125	f, t														
22/1	126										f					
23/1	127															
24/1	128					t										
25/1	129															
26/1	130		dy													
27/1	131			dy					dy							
28/1	132		t													
29/1	133															
30/1	134											dy				
31/1	135															
1/2	136				p											
2/2	137					dy						dy		dy	t	
3/2	138									dy			dy			
4/2	139			dy												
5/2	140															
6/2	141															
7/2	142															
8/2	143						dy	dy								
9/2	144															
10/2	145											dy				
11/2	146			dy		dy										
12/2	147															
(22)	Total	1	2	3	1	3	1	1	2	1	1	3	1	1	1	

Appendix 3.34: The production of double-yolked eggs by individual Hy-Line Silver and Brown hens in room 6 (18-week photostimulation). Key: DY= within a normal sequence; F= first egg at onset of lay; P= associated with a pause in the sequence; T= terminal egg of a sequence; S= soft shell; TY= triple yolk.

		Hen Number														
Date	Age (d)	3	11	12	23		25	30	34	44	47					
25/1	129															
26/1	130															
27/1	131															
28/1	132															
29/1	133															
30/1	134															
31/1	135															
1/2	136															
2/2	137															
3/2	138															
4/2	139		dy													
5/2	140															
6/2	141								dy							
7/2	142															
8/2	143															
9/2	144		dy							dy						
10/2	145	dy	dy				dy									
11/2	146	dy														
12/2	147				dy											
13/2	148															
14/2	149			dy	dy											
15/2	150															
16/2	151															
17/2	152															
19/2	154															
20/2	155							p								
21/2	156															
23/2	157									dy						
25/2	158										dy					
(8)	Total	2	3	1	2		1	1	1	2	1		(6)			

Appendix 3.35: The weights of the yolks from multiple-yolked eggs

Date	Room	Hen	Classification	Yolk weights	Difference
3/1	1	30	f	5.1 4.4	0.7
3/1	1	23	f, t	4.8 4.0	0.8
5/1	1	23	dy	5.9 5.3	0.6
6/1	4	21	dy	6.3 6.0	0.3
6/1	4	12	f, t	5.7 5.5 5.3	0.2
7/1	1	23	dy	6.4 6.7	0.3
7/1	1	30	dy	6.4 6.3	0.1
7/1	4	27	dy	5.5 5.5	0.0
8/1	4	21	dy	6.5 6.2	0.3
8/1	4	27	dy	3.1 4.7	1.6
8/1	1	25	t	6.1 5.5	0.6
9/1	4	38	p	5.20 5.22	0.02
9/1	1	7	f, t	6.30 6.03	0.27
16/1	1	43	p	7.64 7.47	0.17
16/1	4	39	dy	7.80 6.82	0.02
16/1	4	21	dy	7.04 7.66	0.62
16/1	5	10	f	6.51 6.98	0.47
16/1	4	15	p	6.99 7.09	0.10
16/1	4	40	dy	7.33 7.41	0.08
16/1	4	33	dy	7.01 6.68	0.33
20/1	2	35	dy	6.81 6.92	0.11
21/1	5	25	f, t	6.37 7.52	1.15
23/1	4	27	s	7.95 7.23	0.72
23/1	4	38	dy	7.79 6.85	0.94
23/1	1	33	dy	9.30 8.66	0.64
23/1	4	17	dy	8.49 8.01	0.48
23/1	2	24	f, t	7.68 6.27	1.41
23/1	2	40	f, t	6.75 5.03	1.72
26/1	3	15	t	7.00 6.77	0.23
30/1	5	39	dy	9.10 8.77	0.33
6/2	3	23	dy	8.90 9.17	0.27

Appendix 3.36: Analysis of variance for mean number of soft-shelled eggs per hen, using a split-plot design and testing for interactions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.187750	0.187750	13.91	
Block.lights stratum					
lights	2	4.165176	2.082588	154.34	0.006
residual	2	0.026986	0.013493	2.23	
Block.lights.strain stratum					
strain	1	0.453574	0.453574	75.07	0.003
lights.strain	2	0.385287	0.192644	31.89	0.010
residual	3	0.018125	0.006042		
Total	11	5.236899			

Tables of means

Grand mean	0.743		
lights	12 wks	15 wks	18 wks
	1.552	0.510	0.166
strain	HS	HB	
	0.937	0.549	
lights.strain	12 wks	15 wks	18 wks
HS	2.000	0.583	0.229
HB	1.104	0.438	0.104

Standard errors of differences of means

Table	lights	strain	lights.strain
rep.	4	6	2
d.f.	2	3	3.70
s.e.d.	0.0821	0.0449	0.0988

Except when comparing means with the same level(s) of lights: s.e.d 0.0777 d.f. 3

Appendix 3.37: Linear regression of mean number of soft-shelled eggs for the Hy-Line Silver strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	1.5682	1.5682	8.33	0.212
Residual	1	0.1883	0.1883		
Total	2	1.7565	0.8783		

Percentage variance accounted for: 78.6
Standard error of observations is estimated to be 0.434

Estimates of parameters

	Estimate	s.e.	t(1)	t pr.
Constant	5.36	1.55	3.45	0.180
Age at photostim.	-0.295	0.102	-2.89	0.212

Appendix3.38: Linear regression of mean number of soft-shelled eggs for the Hy-Line Brown strain on age at photostimulation

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	0.50000	0.50000	27.22	0.121
Residual	1	0.01837	0.01837		
Total	2	0.51837	0.25919		

Percentage variance accounted for: 92.9
Standard error of observations is estimated to be 0.136

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	3.049	0.486	6.28	0.101
Age at photostim.	-0.1667	0.0319	-5.22	0.121

Appendix 3.39: The production of soft-shelled eggs by individual Hy-Line Silver hens in room 1 (12-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; E= earlier than expected; L= later than expected. Time interval shown to nearest half hour.

	Hen number																
Date	2	3	4	5	6	10	11	12	13	14	15	16	17	19	21	22	23
4/1		SP 2															
6/1										F (P)							
7/1		S															
9/1	F (P)						FF (P)										
10/1		SP 7.5									NP 0.5						
14/1	NT																
15/1											NS 0.5						NS 7.5
18/1						F (P)			FBr								
19/1	SN2 .5													S			
20/1									S	SS				SS P 9			
23/1								S			S					SP 8.5	NS
24/1																	L (P)
25/1			NP 8.5											LP 0.5			
26/1	NS 10.5																
27/1	S								NS 8.5					SS		NP 2.5	
28/1	S						NP		SP 9					S			
29/1				NP 4.5													
31/1				NS	NS 2.5												
1/2	NS 2.5 S 3.5								S (P)					NS 4.5			

Hen number																	
2/2														LP			
3/2				S													
4/2									S								
5/2												S	SN 1.5				
6/2														SS 1			
7/2														S			
8/2	L (P)																
10/2									S								
11/2									SS 2.5						S		
13/2									S					NS 8			
14/2	NP 9								S		NS 9			S			
15/2			NS 7 P 0.5							NP 2.5	S			NS 8.5 P 0.5			
18/2									S					S			
19/2																	S (P)
20/2			S (P)														
22/2	S																S
24/2														S			
25/2														S (P)			

Appendix 3.40: The production of soft-shelled eggs by individual Hy-Line Brown hens in room 1 (12-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; E= earlier than expected; L= later than expected. Time interval shown to nearest half hour.

	Hen number							
Date	25	27	29	31	35	37	40	43
12/1								NS 9.5 P 0.5
13/1		F (P)						
18/1								SS
20/1								NS 8.5
21/1		NS 8.5						SS 7
22/1		S (P)						S
23/1								S
24/1					NS 9.5			NS 7.5
25/1					L (P)	L (P)		S (L)
26/1								NS 8.5
27/1							S (P)	S (P)
28/1		S				S		
29/1						S		
30/1						S		
1/2	S	S				NP 8		
2/2								LS 1.5
3/2		SN 0.5						L (P)
5/2		NS						
6/2			S					
7/2	S	NS						
10/2								S
12/2								S
13/2		NS						N 8 SS
16/2		S				SN 2		
17/2								S
19/2								S
20/2								S
24/2	S			SS				
26/2					NS			

Appendix 3.41: The production of soft-shelled eggs by individual Hy-Line Silver and Brown hens in room 2 (15-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour.

	Hen number										
Date	1	3	9	14	19	24		28	29	37	47
25/1				NP 7.5							
27/1			S (E,P)		F	NS 2.5					
28/1				S							
30/1				SS							
1/2										F (P)	
4/2								SS			
5/2		S							SS		
6/2		S									
7/2	S	S									
8/2			NS								
13/2											S
18/2						S					
21/2					S						
22/2					S (P)						
24/2									SS (E)		
26/2										S	

Appendix 3.42: The production of soft-shelled eggs by individual Hy-Line Silver and Brown hens in room 3 (18-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour.

	Hen number										
Date	15	20									
29/1	S										
7/2	S										
10/2	S										
12/2	S										
18/2		NP 6.5									

Appendix 3.43: The production of soft-shelled eggs by individual Hy-Line Silver hens in room 4 (12-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour.

	Hen number															
Date	1	2	3	4	5	7	9	10	11	12	13	14	15	17	18	24
4/1											F (P)					
5/1						N S										
6/1						S (E)										
7/1								S (P)		S (E)						
8/1										S (P)		N P 5. 5				
9/1											S			E (P)		
10/1			S						S (P)	S					F (P)	
11/1										S						
13/1													S (P)			
14/1			S N P 1. 5 7. 5	S (P)						S						
15/1										S N 2. 5	S					
17/1										S N 2						
18/1					N S 1. 5											
19/1							S							N P 2. 5		
20/1			S		SS 0. 5											

Hen number																
21/1												SS				
22/1															SS	
23/1	S														S	
24/1								SS								
25/1		NS0.5			SS7											
26/1			NP9									NS		NP8	S	
27/1		S		S(P)								NSSP8.50.5				
28/1		SP														
29/1				S(P)												
30/1												SN2				FN
31/1					SN1											
1/2												S				
2/2														NS1		
4/2		NP9										NS1				
6/2		S														
7/2					S											
9/2		NS8														
19/2														S	S	
20/2															S	

Appendix 3.44: The production of soft-shelled eggs by individual Hy-Line Brown hens in room 4 (12-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg at onset of lay; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour

	Hen number													
Date	25	26	27	28	30	33	34	35	37	38	40	42	46	48
5/1										F (P)				
7/1										S (P)	S (P)			
10/1						NF 5.5				NP				
13/1														S (P)
16/1			S							S (P)			S (P)	
18/1								NS 6		PN 1.5				
19/1								S (E)						
20/1	SN 4	S			NS 6		SS (E)			NS 9.5				
21/1					S (P)		S (P)							
23/1		S	SS P 7 0.5	S										
25/1			S											
26/1										S				
28/1				S (P)						NS 9.5				
29/1										N 9 SP				
30/1			SS											
31/1					S									
1/2										NS		SN 4.5		
6/2								S		S (P)				
7/2													S (P)	
8/2			NP 2.5							NP				
10/2		NP							S					

Hen number														
		4												
12/2			NS 1.5											
13/2										NS				
17/2														S
19/2										S				

Appendix 3.45: The production of soft-shelled eggs by individual Hy-Line Silver and Brown hens in room 5 (15-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour

	Hen number																
Date	6	7	9	11	12	21	22	24	28	29	30	31	32	38	39	41	45
17/1																S (E) (P)	
22/1								NF									
26/1						SS (E)											
27/1	S (E)						S										
30/1												SS					
31/1				S								S					
1/2								NP 9.5			SN 4						
2/2						S											
3/2						NS 8.5						S			S (P)		
4/2						S											S
6/2								S									
7/2																	S
8/2		S													S		
9/2		S															
10/2		S															
12/2				SN 0.5			S					NS 7		S (P)			
14/2													S				
16/2											SP						
19/2	NS 5			S (P)	S								S				
20/2	S (E)									NP 7							
25/2							NS 7.5		NP 1								
26/2			S														

Appendix 3.46: The production of soft-shelled eggs by individual Hy-Line Silver and Brown hens in room 6 (18-week photostimulation). Key: N= normal shelled egg; S= soft shell within a normal sequence at expected time; P= associated with a pause in the sequence; F= first egg; T= terminal egg of a sequence; (E)= earlier than expected; (L)= later than expected. Time interval shown to nearest half hour

Hen number											
Date	4	14	17	23		29	30	32	43		
9/2			S (E, P)								
10/2	NS 9										
13/2		S (E)									
17/2		S (E)						T			
18/2		S (E)									
20/2									S (E)		
23/2						SS (P) 2.5	NS 0.5				
24/2				S							

Appendix 3.47: Analysis of variance for mean egg weight during the first four weeks of lay for each treatment, using a split-plot design and testing for main effects.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.033	0.033	0.01	
Block.lights stratum					
Lights	2	134.236	67.118	16.84	0.056
residual	2	7.970	3.985	3.30	
Block.lights.strain stratum					
Strain	1	57.860	57.860	47.92	< 0.001
residual	5	6.037	1.207		
Total	11	206.136			

Tables of means

Grand mean	45.49		
lights	12 wks	15 wks	18 wks
	41.40	45.47	49.59
strain	HS	HB	
	43.29	47.68	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d.	1.412	0.634

Appendix 3.48: Analysis of variance for mean egg weight (excluding double-yolked eggs) during the first four weeks of lay for each treatment, using a split-plot design and testing for main effects.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.0002	0.0002	0.00	
Block.lights stratum					
Lights	2	159.2995	79.6497	22.66	0.042
residual	2	7.0285	3.5142	4.08	
Block.lights.strain stratum					
Strain	1	60.1664	60.1664	69.81	<0.001
residual	5	4.3095	0.8619		
Total	11	230.8041			

Tables of means

Grand mean	44.89		
lights	12 wks	15 wks	18 wks
	40.35	45.06	49.27
strain	HS	HB	
	42.65	47.13	

Standard errors of differences of means

Table	lights	strain
rep.	4	6
d.f.	2	5
s.e.d.	1.326	0.536

Appendix 3.49: Linear regression of mean egg weight (excluding double yolks) for the first four weeks of lay for the Hy-Line Silver strain on age at photostimulation.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	35.11220	35.11220	483.64	0.029
Residual	1	0.07260	0.07260		
Total	2	35.18480	17.59240		

Percentage variance accounted for: 99.6
Standard error of observations is estimated to be 0.269

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	21.710	0.965	22.49	0.028
Age at photostim.	1.3967	0.0635	21.99	0.029

Appendix 3.50: Linear regression of mean egg weight (excluding double yolks) for the first four weeks of lay for the Hy-Line Brown strain on age at photostimulation.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	46.5613	46.5613	52.35	0.087
Residual	1	0.8894	0.8894		
Total	2	47.4506	23.7253		

Percentage variance accounted for: 96.3
Standard error of observations is estimated to be 0.943

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	23.02	3.38	6.82	0.093
Age at photostim.	1.608	0.222	7.24	0.087

Appendix 3.51: Analysis of variance for mean yolk weight at 20 weeks of age, using a split-plot design and testing for main effects.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.08168	0.08168	0.40	
Block.lights stratum					
Lights	2	0.36102	0.18051	0.89	0.529
residual	2	0.40625	0.20312	3.13	
Block.lights.strain stratum					
Strain	1	0.19508	0.19508	3.01	0.143
residual	5	0.32407	0.06481		
Total	11	1.36809			

Table of means

Grand mean	9.646		
lights	12 wks	15 wks	18 wks
	9.870	9.620	9.448
Strain	HS	HB	
	9.773	9.518	

Standard errors of differences of means

Table	Lights	Strain
rep.	4	6
d.f.	2	5
s.e.d.	0.3187	0.1470

Appendix 3.52: Analysis of variance for mean yolk weight at 21 weeks of age, using a split-plot design and testing for main effects.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	0.00853	0.00853	0.71	
Block.lights stratum					
Lights	2	0.02705	0.01353	1.12	0.471
residual	2	0.02412	0.01206	0.26	
Block.lights.strain stratum					
Strain	1	0.11603	0.11603	2.51	0.174
residual	5	0.23097	0.04619		
Total	11	0.40670			

Tables of means

Grand mean	10.375		
lights	12 wks	15 wks	18 wks
	10.428	10.385	10.312
Strain	HS	HB	
	10.473	10.277	

Standard errors of differences of means

Table	Lights	Strain
rep.	4	6
d.f.	2	5
s.e.d.	0.0776	0.1241

Appendix 3.53: Fitted Gompertz equation for the regression of yolk weight on age, for Hy-Line Silver birds.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	3	91.1662	30.38873	581.30	<0.001
Residual	4	0.2091	0.05228		
Total	7	91.3753	13.05361		

Percentage variance accounted for: 99.6
Standard error of observations is estimated to be 0.229

Estimates of parameters

	Estimate
B	0.01771
M	-370.1
C	51123
A	-51107

Appendix 3.54: Fitted Gompertz equation for the regression of yolk weight on age, for Hy-Line Brown birds.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	3	45.7868	15.26227	307.03	<0.001
Residual	3	0.1491	0.04971		
Total	6	45.9359	7.65599		

Percentage variance accounted for: 99.4
Standard error of observations is estimated to be 0.223

Estimates of parameters

	Estimate
B	0.01972
M	-15.36
C	116.0
A	-101.1

Appendix 3.55: Linear regression of mean prime sequence length on age at photostimulation for the Hy-Line Silver hens.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	0.106	0.106	0.02	0.900
Residual	1	4.234	4.234		
Total	2	4.339	2.170		

Residual variance exceeds variance of response variate
Standard error of observations is estimated to be 2.06

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	87.54	7.37	11.88	0.053
Age at photostim.	-0.077	0.485	-0.16	0.900

Appendix 3.56: Linear regression of mean prime sequence length on age at photostimulation for the Hy-Line Brown hens.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	94.394	94.394	10.29	0.192
Residual	1	9.176	9.176		
Total	2	103.570	51.785		

Percentage variance accounted for: 82.3
Standard error of observations is estimated to be 3.03

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	41.0	10.9	3.78	0.165
Age at photostim.	2.290	0.714	3.21	0.192

Appendix 3.57: Linear regression of mean sequence length on age at photostimulation for the Hy-Line Silver hens.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	15.457	15.457	1.86	0.403
Residual	1	8.307	8.307		
Total	2	23.764	11.882		

Percentage variance accounted for: 30.1
Standard error of observations is estimated to be 2.88

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	11.0	10.3	1.06	0.481
Age at photostim.	0.927	0.679	1.36	0.403

Appendix 3.58: Linear regression of mean sequence length on age at photostimulation for the Hy-Line Brown hens.

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	9.9013	9.9013	26.05	0.123
Residual	1	0.3800	0.3800		
Total	2	10.2813	5.1406		

Percentage variance accounted for: 92.6
Standard error of observations is estimated to be 0.616

Estimates of parameters

	estimate	s.e.	t(1)	t pr.
Constant	9.56	2.21	4.33	0.145
Age at photostim.	0.742	0.145	5.10	0.123

Appendix 4.1: Fitted logistic equation for the regression of yolk weight on age, for Amber-Link hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	3	96.2479	32.0826	275.94	0.001
Residual	8	0.9301	0.1163		
Total	11	97.1780	8.8344		

Percentage variance accounted for: 98.7
Standard error of observations is estimated to be 0.341

Estimates of parameters

	Estimate
B	0.01268
M	-116.4
C	243.2
A	-224.7

Appendix 4.2: Parameter Estimates for the Linear Regression of Albumen Weight (g) on Yolk Weight (g) at Specific Ages During the Laying Cycle, for Amber-Link hens. (AW = a + b.YW)
(* denotes non-significance)

Age (weeks)	Range in yolk wt.	a	b	r^2 (%)	s (g)	Signif. Level
20	6.76- 10.55	15.978 (±3.216)	1.3839 (±0.3559)	14.5	2.551	P<0.001
22	7.36- 13.07	24.976 (±3.094)	0.6350 (±0.2793)	5.4	2.524	P<0.05
24	9.91- 14.33	26.418 (±4.873)	0.6606 (±0.3924)	3.0	2.959	n/s
26	11.14- 15.43	24.232 (±5.546)	0.9021 (±0.4111)	5.2	3.611	P<0.05
28	11.92- 15.90	26.636 (±5.138)	0.7097 (±0.3668)	4.1	3.228	n/s
30	11.93- 17.07	33.068 (±4.422)	0.2675 (±0.3016)	0.9	3.144	n/s
32	12.32- 18.44	21.751 (±4.649)	1.0411 (±0.3012)	11.7	3.013	p<0.01
36	13.04- 19.81	33.304 (±3.732)	0.3403 (±0.2334)	2.1	2.869	n/s
40	13.19- 19.08	19.757 (±4.743)	1.2027 (±0.2881)	16.7	2.982	p<0.001
50	15.17- 20.85	18.387 (±3.606)	1.1476 (±0.2017)	25.2	2.677	p<0.001
60	15.17- 22.43	35.37 (±4.83)	0.250* (±0.259)	0	3.410	n/s
70	14.13- 22.30	32.507 (±4.970)	0.4667* (±0.2686)	3.2	3.792	n/s

Appendix 4.3: Fitted exponential equation for the regression of albumen weight on yolk weight, for the collective data from 20 to 70 weeks for Amber-Link hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	13291.	6645.434	669.72	<0.001
Residual	1103	10945.	9.923		
Total	1105	24236.	21.933		

Percentage variance accounted for: 54.8
Standard error of observations is estimated to be 3.15

Estimates of parameters

	Estimate	s.e.
R	0.8821	0.0137
B	-50.97	4.29
A	45.37	1.16

Appendix 4.4: Linear regression of *ln* albumen weight on *ln* yolk weight for the collective data from 20 to 70 weeks for Amber-Link hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	10.944	10.9441	1464.90	<0.001
Residual	1104	8.248	0.007471		
Total	1105	19.192	0.017368		

Percentage variance accounted for: 57.0
Standard error of observations is estimated to be 0.0864

Estimates of parameters

	Estimate	s.e.	t(1104)	t pr.
Constant	2.3970	0.0314	76.26	<0.001
ln Yolk weight	0.4491	0.0117	38.27	<0.001

Appendix 4.5: Parameter Estimates for the Linear Regression of Shell Weight (g) on Egg Contents Weight (g) at Specific Ages During the Laying Cycle for Amber-Link hens (SW = a + b.ECW)
(* denotes non-significance)

Age (weeks)	Range in ECW	a	b	r^2 (%)	s (g)	Signif. Level
20	30.1-46.2	0.4123* (±0.5881)	0.09728 (±0.01565)	30.3	0.4620	P<0.001
22	35.1-50.4	1.0888* (±0.5523)	0.08185 (±0.01281)	31.2	0.3603	P<0.001
24	39.1-54.4	1.5704 (±0.5428)	0.07000 (±0.01152)	28.6	0.3575	P<0.001
26	43.2-62.3	1.9950 (±0.5205)	0.06117 (±0.01041)	28.2	0.3933	P<0.001
28	43.0-60.2	2.2026 (±0.6685)	0.05686 (±0.01320)	17.6	0.4442	P<0.001
30	45.0-60.6	2.6350 (±0.5620)	0.04701 (±0.01087)	17.5	0.3513	P<0.001
32	46.1-61.9	3.2140 (±0.6506)	0.03837 (±0.01220)	9.9	0.4287	P<0.01
36	45.4-66.9	1.9866 (±0.6825)	0.06486 (±0.01246)	21.3	0.4122	P<0.001
40	46.6-69.1	1.9919 (±0.5725)	0.06043 (±0.01021)	28.7	0.3672	P<0.001
50	47.2-72.0	1.9631 (±0.6227)	0.06433 (±0.01096)	26.4	0.4246	P<0.001
60	48.9-68.5	1.924 (±0.923)	0.0630 (±0.0157)	15.1	0.555	P<0.001
70	49.6-70.5	2.8337 (±0.8334)	0.04587 (±0.01396)	10.6	0.5817	P<0.01

Appendix 4.6: Fitted exponential equation for the regression of shell weight on egg contents weight, for the collective data from 20 to 70 weeks for Amber-Link hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	269.5	134.7321	703.12	<0.001
Residual	1103	211.4	0.1916		
Total	1105	480.8	0.4351		

Percentage variance accounted for: 56.0
Standard error of observations is estimated to be 0.438

Estimates of parameters

	Estimate	s.e.
R	0.97326	0.00551
B	-9.599	0.667
A	7.564	0.536

Appendix 4.7: Linear regression of *ln* shell weight on *ln* egg contents weight, for the collective data from 20 to 70 weeks for Amber-Link hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	11.386	11.386023	1443.18	<0.001
Residual	1104	8.710	0.007890		
Total	1105	20.096	0.018186		

Percentage variance accounted for: 56.6
Standard error of observations is estimated to be 0.0888

Estimates of parameters

	Estimate	s.e.	t(1104)	t pr.
Constant	-1.0825	0.0714	-15.16	<0.001
ln egg contents weight	0.6896	0.0182	37.99	<0.001

Appendix 4.8: Linear regression of *ln* albumen weight on *ln* yolk weight, for the collective data from 15 to 21 weeks, 37 and 64 weeks for Hy-Line Silver hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	8.955	8.954583	1045.54	<0.001
Residual	419	3.589	0.008565		
Total	420	12.543	0.029865		

Percentage variance accounted for: 71.3
Standard error of observations is estimated to be 0.0925

Estimates of parameters

	Estimate	s.e.	t(419)	t pr.
Constant	2.2485	0.0348	64.65	<0.001
ln yolk weight	0.5044	0.0156	32.33	<0.001

Appendix 4.9: Linear regression of *ln* shell weight on *ln* egg contents weight, for the collective data from 15 to 21 weeks, 37 and 64 weeks for Hy-Line Silver hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	13.355	13.35464	1065.6	<0.001
Residual	419	5.251	0.01253		
Total	420	18.606	0.04430		

Percentage variance accounted for: 71.7
Standard error of observations is estimated to be 0.112

Estimates of parameters

	Estimate	s.e.	t(419)	t pr.
Constant	-1.979	0.103	-19.30	<0.001
ln egg contents weight	0.9180	0.0281	32.64	<0.001

Appendix 4.10: Linear regression of *ln* albumen weight on *ln* yolk weight, for the collective data from 15 to 21 weeks and 37 weeks for Hy-Line Brown hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	5.347	5.34709	523.23	<0.001
Residual	326	3.331	0.01022		
Total	327	8.679	0.02654		

Percentage variance accounted for: 61.5
Standard error of observations is estimated to be 0.101

Estimates of parameters

	Estimate	s.e.	t(326)	t pr.
Constant	2.3879	0.0480	49.80	<0.001
ln yolk weight	0.5020	0.0219	22.87	<0.001

Appendix 4.11: Linear regression of *ln* shell weight on *ln* egg contents weight, for the collective data from 15 to 21 weeks and 37 weeks for Hy-Line Brown hens

Summary of analysis

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	8.587	8.58710	764.13	<0.001
Residual	326	3.664	0.01124		
Total	327	12.251	0.03746		

Percentage variance accounted for: 70.0
Standard error of observations is estimated to be 0.106

Estimates of parameters

	Estimate	s.e.	t(326)	t pr.
Constant	-2.016	0.125	-16.08	<0.001
ln egg contents weight	0.9310	0.0337	27.64	<0.001

Appendix 5.1: The Visual Basic code for the layer model

```
'Declarations

'Programmer: Shelley Anne Johnston, year 2004. sajohnston@nitrosoft.co.za
'Developed as part of a PhD thesis at the University of KwaZulu-Natal, South Africa

'This program calculates the ovulation time, time of lay, rate of lay, mean sequence
'length, egg weight and egg component weights for a theoretical flock of 100 hens for a
'period up to 500 days.

'All variables must be declared:

Option Explicit

'Arrays start at element 1:

Option Base 1

---------------------------------------------------------------------------

'DECLARES MODULE LEVEL VARIABLES:

    Dim HENAGE As Range
    Dim Numberofdays As Range
    Dim minimumOpenPeriod As Range
    Dim STARTTIME As Date
    Dim STARTTDAY As Single
    Dim DayNumber As Range
    Dim TIMEOFDAY As Range
    Dim TDAY As Single
    Dim FLOCKBREED As Range
    Public BREED As Integer

'Variables for internal cycle length

    Dim MeanD As Single
    Dim sdD As Single
    Dim MeanB As Single
    Dim sdB As Single
    Dim MeanC As Single
    Dim sdC As Single
    Dim MeanA As Single
    Dim sdA As Single
    Dim RandomD As Range
    Dim RandomB As Range
    Dim RandomC As Range
    Dim RandomA As Range
    Dim ICLD As Range
    Dim ICLB As Range
```

```
Dim ICLC As Range
Dim ICLA As Range
Dim MeanLag As Single
Dim sdLag As Single
Dim LAG As Range
Dim RandomLag As Range
Dim ICL(100) As Variant

'Variables for ovulation time

Dim MeanStartFM As Single
Dim sdStartFM As Single
Dim StartFM As Range
Dim RandomStartFM As Range
Dim MeanOpenPeriod As Single
Dim sdOpenPeriod As Single
Dim OpenPeriod As Range
Dim RandomOpenPeriod As Range
Dim DAYSOVULATION(100) As Variant
Dim OVRATE(100) As Variant
Dim PL1(100) As Variant
Dim PL2(100) As Variant
Dim PAS1(100) As Variant
Dim PS1(100) As Variant
Dim STARTPS1(100) As Variant
Dim HOURPS1(100) As Variant
Dim MINUTEPS1(100) As Variant
Dim SECONDPS1(100) As Variant
Dim FLOCKTIMEPS1 As Range
Dim TIMEPS1(100) As Variant
Dim Pb1(100) As Variant
Dim Pb2(100) As Variant
Dim Pb3(100) As Variant
Dim PS2(100) As Variant
Dim Pa2(100) As Variant
Dim TIMERC(100) As Variant
Dim REGCONC(100) As Variant
Dim TIMEFM(100) As Variant
Dim FOLLMAT(100) As Variant
Dim FMRC(100) As Variant
Dim OVULATION(100) As Variant
Dim OVTIME(100) As Variant
Dim STORETIMES(100) As Variant
Dim FLOCKSTORETIMES As Variant
Dim ADDTIMEFM(100) As Variant
Dim YESTERDAYOVTIME(100) As Variant
Dim FLOCKYESTERDAYOVTIME As Variant

'Variables for oviposition time and sequence length
```

```
Dim MeanINTERVAL As Single
Dim sdINTERVAL As Single
Dim Interval As Range
Dim RandomInterval As Range
Dim SEQUENCE(100) As Variant
Dim YESTERDAYSEQUENCE(100) As Variant
Dim OVIPOSITION(100) As Variant
Dim EGGSEQUENCE(100) As Variant
Dim OVIPSEQUENCE(100) As Variant
Dim LASTINTERVAL(100) As Variant
Dim STOREOVIPTIMES(100) As Variant
Dim TOTALEGGS(100) As Variant
Dim COUNTTFE As Range
Dim TIMEFIRSTEGG(100) As Variant
Dim STORETFE(100) As Variant
Dim FLOCKOVIPOSITION As Variant
Dim FLOCKSTOREOVIPTIMES As Variant
Dim FLOCKOVIPSEQUENCE As Variant
Dim FLOCKSTORETFE As Variant
Dim FLOCKTOTALEGGS As Variant
Dim FLOCKTIMEFIRSTEGG As Variant
Dim FLOCKLASTINTERVAL As Variant
Dim FINALINTERVAL As Range
Dim FLOCKEGGSEQUENCE As Variant
Dim FLOCKSEQUENCE As Variant
Dim CONVERTSEQUENCE(100) As Variant
Dim SequenceCounter As Integer
Dim FlockCurrent As Variant
Dim FlockCurrentSL As Range
Dim FlockPreviousSL As Range
Dim DAYMeanSL As Integer
Dim RemoveIO(100) As Variant
Dim FLOCKRemoveIO As Variant

'Variables for internal ovulations, soft shells and double yolks

Dim HenInternalOvulations As Range
Dim RandomInternalOvulations As Range
Dim InternalOvulations(100) As Variant
Dim NumberIO(100) As Variant
Dim PercentIO As Single
Dim ProportionIO As Single
Dim HenSoftShells As Range
Dim RandomSoftShells As Range
Dim SoftShells(100) As Variant
Dim NumberSS(100) As Variant
Dim PercentSS As Single
Dim ProportionSS As Single
Dim HenDoubleYolks As Range
```

```
Dim RandomDoubleYolks As Range
Dim DoubleYolks(100) As Variant
Dim NumberDY(100) As Variant
Dim PercentDY As Single
Dim ProportionDY As Single

'Variables for lighting programme

Dim RandomAFE As Range
Dim AFE As Range
Dim MinAFE As Range
Dim MeanAFE As Single
Dim FlockMeanAFE As Range
Dim sdAFE As Single
Dim AgePS As Variant
Dim AgePSdays As Integer
Dim Lightsoff As Range
Dim Constantphpd As Range
Dim Finalphpd As Range
Dim AFEConstantphpd As Single
Dim CHANGEphpd As Single
Dim MEANphpd As Single
Dim LIGHTSb As Single
Dim LIGHTSp As Range
Dim LIGHTSm As Range
Dim LIGHTSMeanm As Single
Dim LIGHTSsdm As Single
Dim AFEConstant10phpd As Single
Dim AFEAnyConstant As Range
Dim AnyConstant As Range

'Variables for egg component weights

Dim MeanYW As Single
Dim sdYW As Single
Dim RandomYW As Range
Dim YW As Variant
Dim AWHSMeanA As Single
Dim AWHSMeanB As Single
Dim SWHSMeanA As Single
Dim SWHSMeanB As Single
Dim AWHBMeanA As Single
Dim AWHBMeanB As Single
Dim SWHBMeanA As Single
Dim SWHBMeanB As Single
Dim AWALMeanA As Single
Dim AWALMeanB As Single
Dim SWALMeanA As Single
Dim SWALMeanB As Single
```

```
Dim MeanAforAW As Single
Dim MeanBforAW As Single
Dim MeanAforSW As Single
Dim MeanBforSW As Single
Dim sdAforAW As Single
Dim sdBforAW As Single
Dim sdAforSW As Single
Dim sdBforSW As Single
Dim AforAW As Range
Dim BforAW As Range
Dim AforSW As Range
Dim BforSW As Range
Dim RandomAforAW As Range
Dim RandomBforAW As Range
Dim RandomAforSW As Range
Dim RandomBforSW As Range
Dim YolkWeight(100) As Variant
Dim FLOCKYOLKWT As Variant
Dim AlbumenWeight(100) As Variant
Dim FLOCKALBUMENWT As Variant
Dim ShellWeight(100) As Variant
Dim FLOCKSHELLWT As Variant
Dim EggWeight(100) As Variant
Dim FLOCKEGGWT As Variant
```

'DECLARES MODULE-LEVEL CONSTANTS:

```
Private Const EXCL As Integer = 24
Private Const TINT1 As Date = "0:01:00"
Private Const TINT2 As Single = 0.016667
Private Const ENDTIME As Date = "18:00:00"
Private Const ENDTDAY As Integer = 18

'Constants for ovulation rate

Private Const PB As Integer = 8
Private Const PM As Single = 0.667
Private Const Pa1 As Single = 2.175
Private Const PAL1 As Single = 0.132475
Private Const PCL1 As Single = 0.020456
Private Const PAL2 As Single = 0.223662
Private Const PCL2 As Single = 0.071596
Private Const PCS1 As Single = -3
Private Const PAb1 As Single = 0.723662
Private Const PCb1 As Single = 0.071596
Private Const PAb2 As Single = 4.448
Private Const PCb2 As Single = 16.038
Private Const PBb2 As Single = 7.363
```

```
    Private Const PMb2 As Single = 0.734
    Private Const PAb3 As Single = 0.134213
    Private Const PCb3 As Single = 0.233199
    Private Const PAS2 As Single = 17.08095
    Private Const PCS2 As Single = -3.59621

'Constant for lighting programme

    Private Const LIGHTSk As Integer = 1
```

```
Sub StartProgram()

'Loads the first UserForm (that in turn loads the next UserForm) that explains the model
'to the user and requests input

    UserForm1.Show
End Sub
```

```
'STEP 1 OF THE PROGRAM

Sub INITIALISEMODEL()

'Initialises parameters and times and runs the model for the first minute of the first day,
'up to the point where ovulation times, if any, are stored

    ResetData
    QuitViewCharts
    SelectBreed
    LightingProgram
    CreatePopulation
    FILLARRAYDaysOvulation
    FILLARRAYICL
    FILLARRAYOvRate
    FILLARRAYParameterL1
    FILLARRAYParameterL2
    FILLARRAYParameterAS1
    FILLARRAYParameterS1
    ConvertToTime
    FILLARRAYParameterb1
    FILLARRAYParameterb2
    FILLARRAYParameterb3
    FILLARRAYParameterS2
    FILLARRAYParametera2
    SetStartTimes
    FILLARRAYTimeRC
    FILLARRAYRegConc
    FILLARRAYTimeFM
```

```
    FILLARRAYFollMat
    FILLARRAYIntersection
    FILLARRAYOvulation
    FILLARRAYOvTime
    FILLARRAYStoreTimes
    FILLARRAYResetTimeFM
End Sub
```

```
Sub ResetData()

'Erases previous flock data from the eleven different worksheets (model, ov.times,
'timefirstegg, ovip.times, seq.length, mean sl, yolk wt, albumen wt, shell wt, egg wt,
'seq.analyzer):

    Worksheets("model").Select
    Range("y5:ah104").Select
    Selection.ClearContents
    Range("f4:f5").Select
    Selection.ClearContents
    Range("as5:au104").Select
    Selection.ClearContents
    Range("aw5:aw104").Select
    Selection.ClearContents
    Range("bc5:bc104").Select
    Selection.ClearContents
    Range("bj5:bj104").Select
    Selection.ClearContents
    Range("bq5:bt104").Select
    Selection.ClearContents
    Range("bj5:bl104").Select
    Selection.ClearContents
    Worksheets("ov.times").Select
    Range("b5:cw504").Select
    Selection.ClearContents
    Range("a1").Select
    Worksheets("timefirstegg").Select
    Range("b5:b104").Select
    Selection.ClearContents
    Range("a1").Select
    Worksheets("ovip.times").Select
    Range("b5:cw505").Select
    Selection.ClearContents
    Range("a1").Select
    Worksheets("seq.length").Select
    Range("b5:cw505").Select
    Selection.ClearContents
    Range("a1").Select
    Worksheets("mean sl").Select
```

```
Range("b5:cw505").Select
Selection.ClearContents
Range("b516:cw1016").Select
Selection.ClearContents
Range("a1").Select
Worksheets("yolk wt").Select
Range("b5:cw505").Select
Selection.ClearContents
Range("a1").Select
Worksheets("albumen wt").Select
Range("b5:cw505").Select
Selection.ClearContents
Range("a1").Select
Worksheets("shell wt").Select
Range("b5:cw505").Select
Selection.ClearContents
Range("a1").Select
Worksheets("egg wt").Select
Range("b5:cw505").Select
Selection.ClearContents
Range("a1").Select
Worksheets("seq.analyzer").Select
Range("c4:cx504").Select
Selection.ClearContents
Range("a1").Select
Worksheets("model").Select
Range("a1").Select

'Empties the following arrays:

    ClearSequence
    ClearStoreTimes
    ClearStoreOvipTimes
    ClearTimeFirstEgg
    ClearTotalEggs
    ClearStoreTFE
    ClearYesterdayOvTime
    ClearYesterdaySequence
    ClearEggSequence
    ClearOvipSequence
End Sub
```

```
Sub ClearSequence()

'Empties the array SEQUENCE

    Dim I As Integer
    For I = LBound(SEQUENCE) To UBound(SEQUENCE)
```

```
    SEQUENCE(I) = Empty
  Next
End Sub
```

```
Sub ClearStoreTimes()

'Empties the array STORETIMES

  Dim I As Integer
  For I = LBound(STORETIMES) To UBound(STORETIMES)
    STORETIMES(I) = Empty
  Next
End Sub
```

```
Sub ClearStoreOvipTimes()

'Empties the array STOREOVIPTIMES

  Dim I As Integer
  For I = LBound(STOREOVIPTIMES) To UBound(STOREOVIPTIMES)
    STOREOVIPTIMES(I) = Empty
  Next
End Sub
```

```
Sub ClearTimeFirstEgg()

'Empties the array TIMEFIRSTEGG

  Dim I As Integer
  For I = LBound(TIMEFIRSTEGG) To UBound(TIMEFIRSTEGG)
    TIMEFIRSTEGG(I) = Empty
  Next
End Sub
```

```
Sub ClearTotalEggs()

'Empties the array TOTALEGGS

  Dim I As Integer
  For I = LBound(TOTALEGGS) To UBound(TOTALEGGS)
    TOTALEGGS(I) = Empty
  Next
End Sub
```

```
Sub ClearStoreTFE()

'Empties the array STORETFE

   Dim I As Integer
   For I = LBound(STORETFE) To UBound(STORETFE)
      STORETFE(I) = Empty
   Next
End Sub
```

```
Sub ClearYesterdayOvTime()

'Empties the array YESTERDAYOVTIME

   Dim I As Integer
   For I = LBound(YESTERDAYOVTIME) To UBound(YESTERDAYOVTIME)
      YESTERDAYOVTIME(I) = Empty
   Next
End Sub
```

```
Sub ClearYesterdaySequence()

'Empties the array YESTERDAYSEQUENCE

   Dim I As Integer
   For I = LBound(YESTERDAYSEQUENCE) To UBound(YESTERDAYSEQUENCE)
      YESTERDAYSEQUENCE(I) = Empty
   Next
End Sub
```

```
Sub ClearEggSequence()

'Empties the array EGGSEQUENCE

   Dim I As Integer
   For I = LBound(EGGSEQUENCE) To UBound(EGGSEQUENCE)
      EGGSEQUENCE(I) = Empty
   Next
End Sub
```

```
Sub ClearOvipSequence()

'Empties the array OVIPSEQUENCE
```

```
    Dim I As Integer
    For I = LBound(OVIPSEQUENCE) To UBound(OVIPSEQUENCE)
        OVIPSEQUENCE(I) = Empty
    Next
End Sub
```

```
Sub SelectBreed()

'Assigns values to the variables for determining the proportions of internal ovulations,
'soft shells and double yolks, according to the breed selected by the user

    Set FLOCKBREED = ActiveSheet.Range("f5")
    If BREED = 1 Then
        Let FLOCKBREED = "Hy-Line Silver"
        Let ProportionIO = 0.48
        Let ProportionSS = 0.38
        Let ProportionDY = 0.36
        ElseIf BREED = 2 Then
            Let FLOCKBREED = "Hy-Line Brown"
            Let ProportionIO = 0.28
            Let ProportionSS = 0.26
            Let ProportionDY = 0.36
        ElseIf BREED = 3 Then
            Let FLOCKBREED = "Amber-Link"
            Let ProportionIO = 0.39
            Let ProportionSS = 0.32
            Let ProportionDY = 0.36
        ElseIf BREED = 4 Then
            Let FLOCKBREED = "Other"
            Let ProportionIO = 0.48
            Let ProportionSS = 0.38
            Let ProportionDY = 0.36
        ElseIf BREED = Empty Then
            Let FLOCKBREED = "Hy-Line Silver"
            Let ProportionIO = 0.48
            Let ProportionSS = 0.38
            Let ProportionDY = 0.36
            Let BREED = 1
    End If
End Sub
```

```
Sub LightingProgram()

'Takes the user-defined variables for sunset, breed, initial and final photoperiods and
'the age at photostimulation, and predicts mean age at first egg
```

```
  Set Lightsoff = Worksheets("model").Range("az2")
  Set Constantphpd = Worksheets("lightprogram").Range("c8")
  Set Finalphpd = Worksheets("lightprogram").Range("c9")
  Set AgePS = Worksheets("lightprogram").Range("c7")
  Set Numberofdays = Worksheets("model").Range("j9")
  Set AFEAnyConstant = Worksheets("lightprogram").Range("c4")
  Set AnyConstant = Worksheets("lightprogram").Range("c5")

'Converts the age at photostimulation from weeks to days and assigns ranges to the
'variables

  Let AgePSdays = AgePS * 7
  Worksheets("lightprogram").Range("c10").Value = AgePSdays
  Worksheets("model").Range("f4").Value = AgePS
  CALCAFE
End Sub
```

```
Sub CALCAFE()

'Predicts mean age at first egg using the model of Lewis, Morris and Perry. Stores the
'output in the worksheet "lightprogram"

  AFEConstant10phpd = CalcAFEConstantphpd(AFEAnyConstant, AnyConstant)
  Worksheets("lightprogram").Range("c6").Value = AFEConstant10phpd
  Let LIGHTSMeanm = AFEConstant10phpd - 13
  Let LIGHTSsdm = -8.76 + 0.124 * LIGHTSMeanm
  Worksheets("lightprogram").Range("c13").Value = LIGHTSMeanm
  Worksheets("lightprogram").Range("c14").Value = LIGHTSsdm
  MEANphpd = 0.5 * (Constantphpd + Finalphpd)
  CHANGEphpd = Abs(Finalphpd - Constantphpd)
  LIGHTSb = LIGHTSk * (-1.763 + 0.1425 * CHANGEphpd - 0.0107 * CHANGEphpd *
CHANGEphpd  + 0.3574 * MEANphpd - 0.01687 * MEANphpd * MEANphpd)
  Set LIGHTSp = Worksheets("lightprogram").Range("c11")
  Set LIGHTSm = Worksheets("lightprogram").Range("c12")
  MeanAFE = CalcMeanAFE(LIGHTSp, LIGHTSm, AFEConstant10phpd, LIGHTSb,
AgePSdays)
  sdAFE = -8.76 + 0.124 * MeanAFE
  Worksheets("lightprogram").Range("c8").Value = Constantphpd
  Worksheets("lightprogram").Range("c9").Value = Finalphpd
  Worksheets("lightprogram").Range("c16").Value = MeanAFE
  Worksheets("lightprogram").Range("c17").Value = sdAFE
End Sub
```

```
Function CalcAFEConstantphpd(AFEAnyConstant, AnyConstant)

'Calculates the mean age at first egg for the breed as if the pullets had been reared on
'constant 10-hour daylengths.
```

```
  Dim AFEConstant As Single
  If AnyConstant <= 10 Then
    AFEConstant = AFEAnyConstant - 1.731 * (10 - AnyConstant)
    Else: AFEConstant = AFEAnyConstant - 0.301 * (AnyConstant - 10)
  End If
  CalcAFEConstantphpd = AFEConstant
End Function
```

```
Function CalcMeanAFE(LIGHTSp, LIGHTSm, AFEConstant10phpd, LIGHTSb,
AgePSdays)

'Calculates the mean age at first egg according to the user-defined age at photostimulation

  Dim MeanFlockAFE As Single
  If Finalphpd >= Constantphpd Then
    MeanFlockAFE = (1 - LIGHTSp) * AFEConstant10phpd + LIGHTSp * (1 -
LIGHTSm) * (AFEConstant10phpd - LIGHTSb * (AFEConstant10phpd - AgePSdays)) +
LIGHTSp * LIGHTSm * AFEConstant10phpd
    Else: MeanFlockAFE = (1 - LIGHTSm) * (AFEConstant10phpd + LIGHTSb *
AgePSdays) + LIGHTSm * AFEConstant10phpd
  End If
  CalcMeanAFE = MeanFlockAFE
End Function
```

```
Sub CreatePopulation()

'Assigns Excel worksheet ranges and values to Object variables, uses random number
'generation to create distributions of variables

  Set HENAGE = ActiveSheet.Range("j11")
  Set minimumOpenPeriod = ActiveSheet.Range("ag106")
  Set MinAFE = ActiveSheet.Range("y106")
  Set FlockMeanAFE = ActiveSheet.Range("c3")
  Set COUNTTFE = Worksheets("timefirstegg").Range("b106")
  Let MeanLag = 8.5
  Let sdLag = MeanLag * 0.02
  Let MeanStartFM = Lightsoff + 2.5
  Let sdStartFM = MeanStartFM * 0.05
  Let MeanINTERVAL = 0.020833
  Let sdINTERVAL = 0.00625
  Let MeanOpenPeriod = 7
  Let sdOpenPeriod = 0.35
  Let SequenceCounter = 0

'Assigns ranges and values to means and standard deviations for the parameters A and B
'for the allometric functions used to calculate egg component weights(AW = albumen
weight; SW = shell weight), for the breeds (HS = Hy-Line Silver; HB = Hy-Line Brown;
'AL = Amber-Link)
```

```
Set RandomYW = ActiveSheet.Range("bc5:bc104")
Set RandomAforAW = ActiveSheet.Range("bq5:bq104")
Set RandomBforAW = ActiveSheet.Range("br5:br104")
Set RandomAforSW = ActiveSheet.Range("bs5:bs104")
Set RandomBforSW = ActiveSheet.Range("bt5:bt104")
Set AforAW = ActiveSheet.Range("bu5:bu104")
Set BforAW = ActiveSheet.Range("bv5:bv104")
Set AforSW = ActiveSheet.Range("bw5:bw104")
Set BforSW = ActiveSheet.Range("bx5:bx104")
Let AWHSMeanA = 9.473515
Let AWHSMeanB = 0.5044
Let SWHSMeanA = 0.138207
Let SWHSMeanB = 0.918
Let AWHBMeanA = 10.8906
Let AWHBMeanB = 0.502
Let SWHBMeanA = 0.133187
Let SWHBMeanB = 0.931
Let AWALMeanA = 10.99015
Let AWALMeanB = 0.4491
Let SWALMeanA = 0.33875
Let SWALMeanB = 0.6896

'The breed determines the mean A and B values (for predicting albumen and shell weights)
'used in the random number generation

If BREED = 1 Or BREED = 4 Then
   Let MeanAforAW = AWHSMeanA
   ElseIf BREED = 2 Then
      Let MeanAforAW = AWHBMeanA
   ElseIf BREED = 3 Then
      Let MeanAforAW = AWALMeanA
End If
If BREED = 1 Or BREED = 4 Then
   Let MeanBforAW = AWHSMeanB
   ElseIf BREED = 2 Then
      Let MeanBforAW = AWHBMeanB
   ElseIf BREED = 3 Then
      Let MeanBforAW = AWALMeanB
End If
If BREED = 1 Or BREED = 4 Then
   Let MeanAforSW = SWHSMeanA
   ElseIf BREED = 2 Then
      Let MeanAforSW = SWHBMeanA
   ElseIf BREED = 3 Then
      Let MeanAforSW = SWALMeanA
End If
If BREED = 1 Or BREED = 4 Then
   Let MeanBforSW = SWHSMeanB
```

```
    ElseIf BREED = 2 Then
      Let MeanBforSW = SWHBMeanB
    ElseIf BREED = 3 Then
      Let MeanBforSW = SWALMeanB
  End If

  Let sdAforAW = MeanAforAW * 0.05
  Let sdBforAW = MeanBforAW * 0.05
  Let sdAforSW = MeanAforSW * 0.02
  Let sdBforSW = MeanBforSW * 0.02

'Ranges for random number generation

  Set RandomAFE = ActiveSheet.Range("y5:y104")
  Set RandomLag = ActiveSheet.Range("z5:z104")
  Set RandomStartFM = ActiveSheet.Range("aa5:aa104")
  Set RandomD = ActiveSheet.Range("ab5:ab104")
  Set RandomB = ActiveSheet.Range("ac5:ac104")
  Set RandomC = ActiveSheet.Range("ad5:ad104")
  Set RandomA = ActiveSheet.Range("ae5:ae104")
  Set RandomInterval = ActiveSheet.Range("af5:af104")
  Set RandomOpenPeriod = ActiveSheet.Range("ag5:ag104")
  Set RandomInternalOvulations = ActiveSheet.Range("bj5:bj104")
  Set RandomSoftShells = ActiveSheet.Range("bk5:bk104")
  Set RandomDoubleYolks = ActiveSheet.Range("bl5:bl104")

'Excel arrays from random number generation

  Set AFE = ActiveSheet.Range("ai5:ai104")
  Set LAG = ActiveSheet.Range("aj5:aj104")
  Set StartFM = ActiveSheet.Range("ak5:ak104")
  Set ICLD = ActiveSheet.Range("al5:al104")
  Set ICLB = ActiveSheet.Range("am5:am104")
  Set ICLC = ActiveSheet.Range("an5:an104")
  Set ICLA = ActiveSheet.Range("ao5:ao104")
  Set Interval = ActiveSheet.Range("ap5:ap104")
  Set OpenPeriod = ActiveSheet.Range("aq5:aq104")
  Set FINALINTERVAL = ActiveSheet.Range("ar5:ar104")
  Set HenInternalOvulations = ActiveSheet.Range("bm5:bm104")
  Set HenSoftShells = ActiveSheet.Range("bn5:bn104")
  Set HenDoubleYolks = ActiveSheet.Range("bo5:bo104")
  Set YW = ActiveSheet.Range("bd5:bd104")

'Selects parameters used in quadratic-by-linear functions to calculate ICL depending on the
'breed (1 = Hy-Line Silver; 2 = Hy-Line Brown; 3 = Amber-Link; 4 = Other)

  If BREED = 1 Or BREED = 3 Or BREED = 4 Then
    Let MeanD = 0.02279
    Let MeanB = 2.0469
```

```
    Let MeanC = 0.011
    Let MeanA = 22.4416
    Let sdD = MeanD * 0.01
    Let sdB = MeanB * 0.01
    Let sdC = MeanC * 0.01
    Let sdA = MeanA * 0.01
  ElseIf BREED = 2 Then
    Let MeanD = 0.02279
    Let MeanB = 2.0469
    Let MeanC = 0.011418
    Let MeanA = 22.5416
    Let sdD = MeanD * 0.01
    Let sdB = MeanB * 0.01
    Let sdC = MeanC * 0.01
    Let sdA = MeanA * 0.01
  End If

'Normally distributed random number generation with predicted mean AFE and standard
'deviation

   Application.Run "ATPVBAEN.XLA!Random", RandomAFE, 1, 100, 2, , MeanAFE,
sdAFE

'Normally distributed random number generation using mean Lag and standard deviation

   Application.Run "ATPVBAEN.XLA!Random", RandomLag, 1, 100, 2, , MeanLag,
sdLag

'Normally distributed random number generation for StartFM (time the first follicle begins
'its maturation process at sexual maturity)

   Application.Run "ATPVBAEN.XLA!Random", RandomStartFM, 1, 100, 2, ,
MeanStartFM, sdStartFM

'Normally distributed random number generation for D (used to calculate ICL)
   Application.Run "ATPVBAEN.XLA!Random", RandomD, 1, 100, 2, , MeanD, sdD

'Normally distributed random number generation for B (used to calculate ICL)
   Application.Run "ATPVBAEN.XLA!Random", RandomB, 1, 100, 2, , MeanB, sdB

'Normally distributed random number generation for C (used to calculate ICL)
   Application.Run "ATPVBAEN.XLA!Random", RandomC, 1, 100, 2, , MeanC, sdC

'Normally distributed random number generation for A (used to calculate ICL)
   Application.Run "ATPVBAEN.XLA!Random", RandomA, 1, 100, 2, , MeanA, sdA

'Normally distributed random number generation for Interval (interval between oviposition
'and subsequent ovulation)
```

```
   Application.Run "ATPVBAEN.XLA!Random", RandomInterval, 1, 100, 2, ,
MeanINTERVAL, sdINTERVAL

'Normally distributed random number generation for OpenPeriod (interval between sunset
'and start of open period for ovulation)
   Application.Run "ATPVBAEN.XLA!Random", RandomOpenPeriod, 1, 100, 2, ,
MeanOpenPeriod, sdOpenPeriod

'Normally distributed random number generation for AforAW (the allometric function
'parameter A used to calculate albumen weight)
   Application.Run "ATPVBAEN.XLA!Random", RandomAforAW, 1, 100, 2, ,
MeanAforAW, sdAforAW

'Normally distributed random number generation for BforAW (the allometric function
'parameter B used to calculate albumen weight)
   Application.Run "ATPVBAEN.XLA!Random", RandomBforAW, 1, 100, 2, ,
MeanBforAW, sdBforAW

'Normally distributed random number generation for AforSW (the allometric function
'parameter A used to calculate shell weight)
   Application.Run "ATPVBAEN.XLA!Random", RandomAforSW, 1, 100, 2, ,
MeanAforSW, sdAforSW

'Normally distributed random number generation for BforSW (the allometric function
'parameter B used to calculate shell weight)
   Application.Run "ATPVBAEN.XLA!Random", RandomBforSW, 1, 100, 2, ,
MeanBforSW, sdBforSW

'Uniformly distributed random number generation to identify the hens prone to Internal
'Ovulations
   Application.Run "ATPVBAEN.XLA!Random", RandomInternalOvulations, 1, 100, 1, ,
0, 1

'Uniformly distributed random number generation to identify the hens prone to laying eggs
'with soft shells
   Application.Run "ATPVBAEN.XLA!Random", RandomSoftShells, 1, 100, 1, , 0, 1

'Uniformly distributed random number generation to identify the hens prone to laying
'double-yolked eggs
   Application.Run "ATPVBAEN.XLA!Random", RandomDoubleYolks, 1, 100, 1, , 0, 1

'Sets the initial hen age so that the model checks for ovulations the day before the
'first hen is due to lay an egg

   Let HENAGE = MinAFE - 1

'Formats areas in Excel worksheet 'model' where the output of the random number
generation 'is stored: font, number format and background colour:
```

```
 Range("y5:ah104").Select
 Selection.NumberFormat = "0.000"
 With Selection.Interior
    .ColorIndex = 19
    .PatternColorIndex = xlAutomatic
 End With
 With Selection.Font
    .Name = "Comic Sans MS"
    .FontStyle = "Regular"
    .Size = 9
 End With
 Range("af5:af104").Select
 Selection.NumberFormat = "mm:ss"
 Range("ah5:ah104").Select
 Selection.NumberFormat = "hh:mm:ss"
 Range("bq5:bt104").Select
 Selection.NumberFormat = "0.000"
 With Selection.Font
    .Name = "Comic Sans MS"
    .FontStyle = "Regular"
    .Size = 9
 End With
 With Selection.Interior
    .ColorIndex = 19
    .PatternColorIndex = xlAutomatic
 End With
 Range("bj5:bl104").Select
 Selection.NumberFormat = "0.000"
 With Selection.Interior
    .ColorIndex = 19
    .PatternColorIndex = xlAutomatic
 End With
 With Selection.Font
    .Name = "Comic Sans MS"
    .FontStyle = "Regular"
    .Size = 9
 End With
 Range("a1").Select
End Sub
```

'OVULATION TIME

Sub FILLARRAYDaysOvulation()

'Fills the array DAYSOVULATION using the function CalcDaysOvulation to determine when each hen starts ovulating. Cumulates to give the time from first ovulation

```
    Dim DAYS As Variant
    DAYS = CalcDaysOvulation(AFE, HENAGE)
```

```
End Sub

---

Function CalcDaysOvulation(AFE, HENAGE)

'Compares each hen's predicted age at first egg to the flock age; assigns a value of 1
'(i.e. an ovulation is due to occur) the day before the hen is due to lay

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(DAYSOVULATION) To UBound(DAYSOVULATION)
    If Round(AFE(J), 0) - 1 > Round(HENAGE, 0) Then
      DAYSOVULATION(I) = 0
      ElseIf Round(AFE(J), 0) - 1 = Round(HENAGE, 0) Then
        DAYSOVULATION(I) = 1
        ElseIf DAYSOVULATION(I) < Round(HENAGE, 0) Then
          DAYSOVULATION(I) = DAYSOVULATION(I) + 1
    End If
  Let J = J + 1
  Next
  CalcDaysOvulation = DAYSOVULATION
End Function

---

Sub FILLARRAYICL()

'Fills the array ICL using the function CalcICL to determine each hen's internal cycle
'length

  Dim FLOCKICL As Variant
  FLOCKICL = CalcICL(ICLA, ICLB, ICLC, ICLD, DAYSOVULATION)

End Sub

---

Function CalcICL(ICLA, ICLB, ICLC, ICLD, DAYSOVULATION)

'Calculates the internal cycle length using a quadratic-by-linear equation and based on
'the time from first ovulation

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(ICL) To UBound(ICL)
    ICL(I) = ICLA(J) + ICLB(J) / (1 + ICLD(J) * DAYSOVULATION(J)) + ICLC(J) *
DAYSOVULATION(J)
  Let J = J + 1
  Next
```

```
   CalcICL = ICL
End Function
```

```
Sub FILLARRAYOvRate()

'Fills the array OvRate using the function CalcOvRate to determine the ovulation rate

   Dim FLOCKOVRATE As Variant
   FLOCKOVRATE = CalcOvRate(EXCL, ICL, LAG)

End Sub
```

```
Function CalcOvRate(EXCL, ICL, LAG)

'Calculates the ovulation rate using the equation of Emmans and Fisher

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(OVRATE) To UBound(OVRATE)
      If EXCL >= ICL(J) Then
         OVRATE(I) = 24 / EXCL
         Else: OVRATE(I) = LAG(J) / ((ICL(J) - EXCL) * (1 + LAG(J) / (ICL(J) - EXCL)))
      End If
   Let J = J + 1
   Next
   CalcOvRate = OVRATE
End Function
```

```
Sub FILLARRAYParameterL1()

'Fills the array ParameterL1 using the function CalcParameterL1 and based on the
'ovulation rate

   Dim FLOCKPL1 As Variant
   FLOCKPL1 = CalcParameterL1(PAL1, PCL1, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterL1(PAL1, PCL1, PB, PM, OVRATE)

'Calculates the value of L1 using a Gompertz function and the defined constants

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(PL1) To UBound(PL1)
```

```
    PL1(I) = PAL1 + PCL1 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterL1 = PL1
End Function
```

```
Sub FILLARRAYParameterL2()

'Fills the array ParameterL2 using the function CalcParameterL2 and based on the
'ovulation rate

  Dim FLOCKPL2 As Variant
  FLOCKPL2 = CalcParameterL2(PAL2, PCL2, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterL2(PAL2, PCL2, PB, PM, OVRATE)

'Calculates the value of L2 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(PL2) To UBound(PL2)
    PL2(I) = PAL2 + PCL2 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterL2 = PL2
End Function
```

```
Sub FILLARRAYParameterAS1()

'Fills the array ParameterAS1 (used to calculate S1) using the function CalcPAS1, so that
'ovulaton time is linked to Sunset

  Dim FLOCKPAS1 As Variant
  FLOCKPAS1 = CalcPAS1(Lightsoff, OpenPeriod, EXCL)
End Sub
```

```
Function CalcPAS1(Lightsoff, OpenPeriod, EXCL)

'Calculates the value of PAS1 based on the time the lights are turned off and the
'interval between lights off and the start of the open period for ovulation

  Dim I As Integer
  Dim J As Integer
  Let J = 1
```

```
  For I = LBound(PAS1) To UBound(PAS1)
    PAS1(I) = Lightsoff + OpenPeriod(J) - EXCL + 3
  Let J = J + 1
  Next
  CalcPAS1 = PAS1
End Function
```

```
Sub FILLARRAYParameterS1()

'Fills the array ParameterS1 using the function CalcParameterS1 and based on the
'ovulation rate

  Dim FLOCKPS1 As Variant
  FLOCKPS1 = CalcParameterS1(PAS1, PCS1, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterS1(PAS1, PCS1, PB, PM, OVRATE)

'Calculates the value of S1 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(PS1) To UBound(PS1)
    PS1(I) = PAS1(J) + PCS1 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterS1 = PS1
End Function
```

```
Sub ConvertToTime()

'Uses the Excel spreadsheet to convert the array PS1 from a decimal to a time variable.
'Stores the hour portion in an Excel range and formats the range

  Dim FLOCKHOURPS1 As Variant
  FLOCKHOURPS1 = CalcHourPS1(PS1)
  Worksheets("model").Select
  Worksheets("model").Range("a113:cv113").Value = FLOCKHOURPS1
  Worksheets("model").Range("a113:cv113").Select
  Selection.Copy
  Range("as5").Select
  Selection.PasteSpecial Paste:=xlAll, Transpose:=True
  Range("as5:as104").Select
  Selection.NumberFormat = "0"
```

```
'Stores the minute portion in an Excel range and formats the range

    Dim FLOCKMINUTEPS1 As Variant
    FLOCKMINUTEPS1 = CalcMinutePS1(HOURPS1, PS1)
    Worksheets("model").Range("a113:cv113").Value = FLOCKMINUTEPS1
    Worksheets("model").Range("a113:cv113").Select
    Selection.Copy
    Range("at5").Select
    Selection.PasteSpecial Paste:=xlAll, Transpose:=True
    Range("at5:at104").Select
    Selection.NumberFormat = "0.00"

'Stores the second portion in an Excel range and formats the range

    Dim FLOCKSECONDPS1 As Variant
    FLOCKSECONDPS1 = CalcSecondPS1(MINUTEPS1)
    Worksheets("model").Range("a113:cv113").Value = FLOCKSECONDPS1
    Worksheets("model").Range("a113:cv113").Select
    Selection.Copy
    Range("au5").Select
    Selection.PasteSpecial Paste:=xlAll, Transpose:=True
    Range("au5:au104").Select
    Selection.NumberFormat = "0.00"

'Combines the three time portions into a time variable in Excel, passes the Excel
'array back to Visual Basic

    Set FLOCKTIMEPS1 = Worksheets("model").Range("av5:av104")
    Dim FLOCKTIME2PS1 As Variant
    FLOCKTIME2PS1 = CalcFlockTime(FLOCKTIMEPS1)
    Worksheets("model").Range("a113:cv113").Value = FLOCKTIME2PS1
    Worksheets("model").Range("a113:cv113").Select
    Selection.Copy
    Range("aw5").Select
    Selection.PasteSpecial Paste:=xlAll, Transpose:=True
    Range("aw5:aw104").Select
    Selection.NumberFormat = "h:m:s"
    Range("as5:aw104").Select
    With Selection.Interior
        .ColorIndex = 20
        .PatternColorIndex = xlAutomatic
    End With
    Range("a1").Select
End Sub
```

```
Function CalcHourPS1(PS1)

'Stores the integer portion of PS1, representing the hour
```

```
  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(HOURPS1) To UBound(HOURPS1)
    HOURPS1(I) = Int(PS1(J))
  Let J = J + 1
  Next
  CalcHourPS1 = HOURPS1
End Function
```

```
Function CalcMinutePS1(HOURPS1, PS1)

'Subtracts the hour portion from PS1 and converts it to minutes

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(MINUTEPS1) To UBound(MINUTEPS1)
    MINUTEPS1(I) = (PS1(J) - HOURPS1(J)) * 60
  Let J = J + 1
  Next
  CalcMinutePS1 = MINUTEPS1
End Function
```

```
Function CalcSecondPS1(MINUTEPS1)

'Subtracts the minutes portion and converts it to seconds

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(SECONDPS1) To UBound(SECONDPS1)
    SECONDPS1(I) = (MINUTEPS1(J) - Int(MINUTEPS1(J))) * 60
  Let J = J + 1
  Next
  CalcSecondPS1 = SECONDPS1
End Function
```

```
Function CalcFlockTime(FLOCKTIMEPS1)

'Stores the Excel array FLOCKTIMEPS1 in the Visual Basic array TIMEPS1

  Dim I As Integer
  Dim J As Integer
  Let J = 1
```

```
  For I = LBound(TIMEPS1) To UBound(TIMEPS1)
    TIMEPS1(I) = FLOCKTIMEPS1(J)
  Let J = J + 1
  Next
  CalcFlockTime = TIMEPS1
End Function
```

```
Sub FILLARRAYParameterb1()

'Fills the array Parameterb1 using the function CalcParameterb1 and based on the
'ovulation rate

  Dim FLOCKPb1 As Variant
  FLOCKPb1 = CalcParameterb1(PAb1, PCb1, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterb1(PAb1, PCb1, PB, PM, OVRATE)

'Calculates the value of b1 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(Pb1) To UBound(Pb1)
    Pb1(I) = PAb1 + PCb1 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterb1 = Pb1
End Function
```

```
Sub FILLARRAYParameterb2()

'Fills the array Parameterb2 using the function CalcParameterb2 and based on the
'ovulation rate

  Dim FLOCKPb2 As Variant
  FLOCKPb2 = CalcParameterb2(PAb2, PCb2, PBb2, PMb2, OVRATE)
End Sub
```

```
Function CalcParameterb2(PAb2, PCb2, PBb2, PMb2, OVRATE)

'Calculates the value of b2 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
```

```
  Let J = 1
  For I = LBound(Pb2) To UBound(Pb2)
    Pb2(I) = PAb2 + PCb2 * Exp(-Exp(-PBb2 * (OVRATE(J) - PMb2)))
  Let J = J + 1
  Next
  CalcParameterb2 = Pb2
End Function
```

```
Sub FILLARRAYParameterb3()

'Fills the array Parameterb3 using the function CalcParameterb3 and based on the
'ovulation rate

  Dim FLOCKPb3 As Variant
  FLOCKPb3 = CalcParameterb3(PAb3, PCb3, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterb3(PAb3, PCb3, PB, PM, OVRATE)

'Calculates the value of b3 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(Pb3) To UBound(Pb3)
    Pb3(I) = PAb3 + PCb3 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterb3 = Pb3
End Function
```

```
Sub FILLARRAYParameterS2()

'Fills the array ParameterS2 using the function CalcParameterS2 and based on the
'ovulation rate

  Dim FLOCKPS2 As Variant
  FLOCKPS2 = CalcParameterS2(PAS2, PCS2, PB, PM, OVRATE)
End Sub
```

```
Function CalcParameterS2(PAS2, PCS2, PB, PM, OVRATE)

'Calculates the value of S2 using a Gompertz function and the defined constants

  Dim I As Integer
  Dim J As Integer
```

```
  Let J = 1
  For I = LBound(PS2) To UBound(PS2)
    PS2(I) = PAS2 + PCS2 * Exp(-Exp(-PB * (OVRATE(J) - PM)))
  Let J = J + 1
  Next
  CalcParameterS2 = PS2
End Function
```

```
Sub FILLARRAYParametera2()

'Fills the array Parametera2 using the function CalcParametera2 and based on the
'ovulation rate

  Dim FLOCKPa2 As Variant
  FLOCKPa2 = CalcParametera2(Pa1, PL1, PL2, EXCL)
End Sub
```

```
Function CalcParametera2(Pa1, PL1, PL2, EXCL)

'Calculates the value of a2 using constants and the other parameters in the regulator
'concentration function

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(Pa2) To UBound(Pa2)
    Pa2(I) = (Pa1 - (Pa1 * Exp(-PL1(J) * EXCL))) / (1 - Exp(-PL2(J) * EXCL))
  Let J = J + 1
  Next
  CalcParametera2 = Pa2
End Function
```

```
Sub SetStartTimes()

'Assigns ranges and values to Object variables; initialises Day Number and starting
'times

  Set DayNumber = Worksheets("model").Range("j10")
  Let DayNumber = 1
  Let STARTTDAY = Lightsoff + minimumOpenPeriod - EXCL

'(The time of day in decimal format, needed for the regulator concentration)

  Let TDAY = STARTTDAY
```

```
'Converts STARTTDAY from a decimal to a time variable STARTTIME.  The hour and
minute given by STARTTDAY are calculated by the functions CalcFlockStartTimeHour
and CalcFlockStartTimeMinute, stored in Excel and converted to a time format

   Dim FLOCKSTARTTIMEHOUR As Variant
   FLOCKSTARTTIMEHOUR = CalcFlockStartTimeHour(STARTTDAY)
   Worksheets("model").Range("ax5").Value = FLOCKSTARTTIMEHOUR
   Dim FLOCKSTARTTIMEMINUTE As Variant
   FLOCKSTARTTIMEMINUTE = CalcFlockStartTimeMinute(STARTTDAY)
   Worksheets("model").Range("ay5").Value = FLOCKSTARTTIMEMINUTE

'This time is stored in a variable STARTTIME and used to initialise TIMEOFDAY

   Let STARTTIME = Worksheets("model").Range("az5").Value
   Set TIMEOFDAY = Worksheets("model").Range("j5")
   Let TIMEOFDAY = STARTTIME
End Sub
```

```
Function CalcFlockStartTimeHour(STARTTDAY)

'Calculates the hour of the starting time, using the integer portion of STARTTDAY

   Dim StartTimeHour As Integer
   StartTimeHour = Int(STARTTDAY)
   CalcFlockStartTimeHour = StartTimeHour
End Function
```

```
Function CalcFlockStartTimeMinute(STARTTDAY)

'Calculates the minute of the starting time, using the fraction portion of STARTTDAY

   Dim StartTimeMinute As Single
   StartTimeMinute = (STARTTDAY - Int(STARTTDAY)) * 60
   CalcFlockStartTimeMinute = StartTimeMinute
End Function
```

```
Sub FILLARRAYTimeRC()

'Fills the array TimeRC (the time for the regulator concentration function) using the
'function CalcTimeRC

   Dim FLOCKTIMERC As Variant
   FLOCKTIMERC = CalcTimeRC(TIMEOFDAY, TIMEPS1, TDAY, EXCL)

End Sub
```

```
Function CalcTimeRC(TIMEOFDAY, TIMEPS1, TDAY, EXCL)

'Calculates the time used to determine the regulator concentration based on the time of
'day and S1.  The two times are equal between TIMEPS1 and midnight. From midnight to
'TIMEPS1 the TIMERC is greater than 24.

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(TIMERC) To UBound(TIMERC)
      If TIMEOFDAY >= TIMEPS1(J) Then
        TIMERC(I) = TDAY
         Else
         TIMERC(I) = EXCL + TDAY
      End If
   Let J = J + 1
   Next
   CalcTimeRC = TIMERC
End Function
```

```
Sub FILLARRAYRegConc()

'Fills the array RegConc (the concentration of the regulator substance) using the
'two-compartmental model contained in CalcRegConc

   Dim FLOCKREGCONC As Variant
   FLOCKREGCONC = CalcRegConc(Pa1, PL1, TIMERC, PS1, Pa2, PL2)

End Sub
```

```
Function CalcRegConc(Pa1, PL1, TIMERC, PS1, Pa2, PL2)

'Calculates the concentration of the regulator substance based on the time and the
'ovulatory cycle parameters L1, L2, S1, a1 and a2

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(REGCONC) To UBound(REGCONC)
      REGCONC(I) = 1 - ((Pa1 * Exp(-PL1(J) * (TIMERC(J) - PS1(J)))) - (Pa2(J) * Exp(-
PL2(J) * (TIMERC(J) - PS1(J)))))
   Let J = J + 1
   Next
   CalcRegConc = REGCONC
End Function
```

```
Sub FILLARRAYTimeFM()

'Fills the array TimeFM (the initial time for follicle maturation) using the function
'CalcTimeFM

   Dim FLOCKTIMEFM As Variant
   FLOCKTIMEFM = CalcTimeFM(AFE, HENAGE, EXCL, StartFM, TDAY)

End Sub
```

```
Function CalcTimeFM(AFE, HENAGE, EXCL, StartFM, TDAY)

'Calculates the time used to determine initial follicle growth, based on the time of
'day and the time the first follicle at sexual maturity commences maturation

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(TIMEFM) To UBound(TIMEFM)
      If Round(AFE(J), 0) - 1 = Round(HENAGE, 0) Then
         TIMEFM(I) = EXCL - StartFM(J) + TDAY
      Else: TIMEFM(I) = 0
      End If
   Let J = J + 1
   Next
   CalcTimeFM = TIMEFM
End Function
```

```
Sub FILLARRAYFollMat()

'Fills the array FollMat (the stage of follicle maturation) using the Gompertz function
'contained in CalcFollMat

   Dim FLOCKFOLLMAT As Variant
   FLOCKFOLLMAT = CalcFollMat(DAYSOVULATION, Pb1, Pb2, Pb3, TIMEFM,
PS2)
End Sub
```

```
Function CalcFollMat(DAYSOVULATION, Pb1, Pb2, Pb3, TIMEFM, PS2)

'Calculates the stage of follicle maturation based on the time and the ovulatory cycle
'parameters b1, b2, b3 and S2

   Dim I As Integer
```

```
  Dim J As Integer
  Let J = 1
  For I = LBound(FOLLMAT) To UBound(FOLLMAT)
    If DAYSOVULATION(J) = 1 Then
      FOLLMAT(I) = Pb1(J) * Exp(-Pb2(J) * Exp(-Pb3(J) * (TIMEFM(J) - 0)))
      Else: FOLLMAT(I) = Pb1(J) * Exp(-Pb2(J) * Exp(-Pb3(J) * (TIMEFM(J) -
PS2(J))))
    End If
  Let J = J + 1
  Next
  CalcFollMat = FOLLMAT
End Function
```

```
Sub FILLARRAYIntersection()

'Checks to see whether the two functions RegConc and FollMat have intersected, using the
'function CalcIntersection

  Dim FLOCKFMRC As Variant
  FLOCKFMRC = CalcIntersection(FOLLMAT, REGCONC)

End Sub
```

```
Function CalcIntersection(FOLLMAT, REGCONC)

'Calculates the difference between the two functions

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(FMRC) To UBound(FMRC)
    FMRC(I) = FOLLMAT(J) - REGCONC(J)
  Let J = J + 1
  Next
  CalcIntersection = FMRC
End Function
```

```
Sub FILLARRAYOvulation()

'Fills the array Ovulation with 0 or 1, using the function CalcOvulation and the
'difference between the functions

  Dim FLOCKOVULATION As Variant
  FLOCKOVULATION = CalcOvulation(FMRC)

End Sub
```

```
Function CalcOvulation(FMRC)

'Assigns the value 0 if the two function have not intersected (no ovulation) or 1 if an
'intersection has occurred (ovulation)

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(OVULATION) To UBound(OVULATION)
      If FMRC(J) >= 0 Then
         OVULATION(I) = 1
         Else: OVULATION(I) = 0
      End If
   Let J = J + 1
   Next
   CalcOvulation = OVULATION
End Function
```

```
Sub FILLARRAYOvTime()

'Fills the array OvTime using the function CalcOvTime

   Dim FLOCKOVTIME As Variant
   FLOCKOVTIME = CalcOvTime(OVULATION, TIMEOFDAY)
   Range("a1").Select
End Sub
```

```
Function CalcOvTime(OVULATION, TIMEOFDAY)

'Stores the ovulation time (equal to the time of day) if an ovulation has taken place

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(OVTIME) To UBound(OVTIME)
      If OVULATION(J) = 1 Then
         OVTIME(I) = TIMEOFDAY
         Else: OVTIME(I) = 0
      End If
   Let J = J + 1
   Next
   CalcOvTime = OVTIME
End Function
```

```
Sub FILLARRAYStoreTimes()

'Fills the array StoreTimes with the ovulation times predicted during the day

   FLOCKSTORETIMES = CalcStoreTimes(OVTIME)
End Sub
```

```
Function CalcStoreTimes(OVTIME)

'Stores the predicted ovulation times if an ovulation had occurred during that minute

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(STORETIMES) To UBound(STORETIMES)
      If OVTIME(J) <> 0 Then
         STORETIMES(I) = OVTIME(J)
         Else: STORETIMES(I) = STORETIMES(I)
      End If
   Let J = J + 1
   Next
   CalcStoreTimes = STORETIMES
End Function
```

```
'STEP 2 OF THE PROGRAM

Sub AddTime()

'Adds one minute intervals to TimeRC and TimeFM, recalculates the regulator
concentration and follicle maturation, checks for intersections and stores ovulation times.
Performs iterations for the specified number of days.

   Worksheets("model").Select
   Do Until DayNumber = Numberofdays + 1
      Do Until TIMEOFDAY >= ENDTIME
         Let TIMEOFDAY = TIMEOFDAY + TINT1
         Let TDAY = TDAY + TINT2
         FILLARRAYTimeRC
         FILLARRAYRegConc
         FILLARRAYAddTimeFM
         FILLARRAYFollMat
         FILLARRAYIntersection
         FILLARRAYOvulation
         FILLARRAYOvTime
         FILLARRAYStoreTimes
         FILLARRAYResetTimeFM
      Loop
```

```
'Runs through a list of subroutines at the end of each day

    EndDay
  Loop

'Stores the oviposition times and sequence data for the final day

  FILLARRAYSequence
  FILLARRAYOviposition
  FILLARRAYStoreOvipTimes
  FILLARRAYEggSequence
  FILLARRAYRemoveIO
  FILLARRAYOvipSequence
  FILLARRAYTotalEggs
  FILLARRAYYolkWeight
  FILLARRAYAlbumenWeight
  FILLARRAYShellWeight
  FILLARRAYEggWeight
  FINDROWOviposition
  FINDROWEggSequence
  FINDROWRemoveIO
  FINDROWMeanSL
  FINDROWYolkWeight
  FINDROWAlbumenWeight
  FINDROWShellWeight
  FINDROWEggWeight
  ConvertSequences
  SortSequenceData
  SequenceAnalyzer
  Worksheets("model").Select
End Sub
```

```
Sub FILLARRAYAddTimeFM()

'Fills the array AddTimeFM using the function CalcAddTimeFM

  Dim FlockAddTimeFM As Variant
  FlockAddTimeFM = CalcAddTimeFM(TINT2, DAYSOVULATION)

End Sub
```

```
Function CalcAddTimeFM(TINT2, DAYSOVULATION)

'Adds one-minute intervals to the time for follicle maturation once the hen has started
'ovulating (i.e. reached sexual maturity)
```

```
  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(TIMEFM) To UBound(TIMEFM)
    If DAYSOVULATION(J) > 0 Then
      TIMEFM(I) = TIMEFM(I) + TINT2
      Else: TIMEFM(I) = TIMEFM(I)
    End If
  Let J = J + 1
  Next
  CalcAddTimeFM = TIMEFM
End Function
```

```
Sub FILLARRAYResetTimeFM()

'Fills the array ResetTimeFM using the function CalcResetTimeFM

  Dim ResetTimeFM As Variant
  ResetTimeFM = CalcResetTimeFM(OVULATION)
  Range("a1").Select
End Sub
```

```
Function CalcResetTimeFM(OVULATION)

'Resets the TimeFM to zero (for the next follicle's maturation process) if an ovulation
'occurs during the day

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(TIMEFM) To UBound(TIMEFM)
    If OVULATION(J) = 1 Then
      TIMEFM(I) = 0
      Else: TIMEFM(I) = TIMEFM(I)
    End If
  Let J = J + 1
  Next
  CalcResetTimeFM = TIMEFM
End Function
```

```
'STEP 3 OF THE PROGRAM (Called by STEP 2: AddTime)

'Ends the day by storing oviposition times and sequence data.  Stores yolk weight and
'predicts egg weight
```

```
Sub EndDay()
   FILLARRAYSequence
   FILLARRAYLastInterval
   FILLARRAYInternalOvulations
   FILLARRAYNumberIO
   FILLARRAYOviposition
   FILLARRAYStoreOvipTimes
   RecalcRandomInterval
   FILLARRAYSoftShells
   FILLARRAYNumberSS
   FILLARRAYDoubleYolks
   FILLARRAYNumberDY
   FILLARRAYEggSequence
   FILLARRAYRemoveIO
   FILLARRAYOvipSequence
   FILLARRAYTotalEggs
   FILLARRAYTimeFirstEgg
   FillArrayYW
   FILLARRAYYolkWeight
   FILLARRAYAlbumenWeight
   FILLARRAYShellWeight
   FILLARRAYEggWeight

'Transfers data to Excel worksheets, finding the row corresponding to the day number

   FINDROWOvulation
   FINDROWOviposition
   FINDROWEggSequence
   FINDROWRemoveIO
   FINDROWMeanSL
   FINDROWYolkWeight
   FINDROWAlbumenWeight
   FINDROWShellWeight
   FINDROWEggWeight

'Progresses time, day and hen age

   Let DayNumber = DayNumber + 1
   Let HENAGE = HENAGE + 1
   Let TDAY = STARTTDAY
   Let TIMEOFDAY = STARTTIME

'Clears some arrays. Stores some data as yesterday's data

   FILLARRAYYesterdayOvtime
   FILLARRAYYesterdaySequence
   ClearStoreOvipTimes
   FILLARRAYDaysOvulation
```

```
'Sets up ovulation parameters at the start of the new day

   FILLARRAYICL
   FILLARRAYOvRate
   ClearStoreTimes
   FILLARRAYParameterL1
   FILLARRAYParameterL2
   FILLARRAYParameterS1
   ConvertToTime
   FILLARRAYParameterb1
   FILLARRAYParameterb2
   FILLARRAYParameterb3
   FILLARRAYParameterS2
   FILLARRAYParametera2
   FILLARRAYProgressTimeFM

End Sub
```

```
Sub FILLARRAYSequence()

'Fills the array Sequence with the position of the ovulation in the sequence, using
'consecutive numbers from 1 onwards

   FLOCKSEQUENCE = CalcSequence(STORETIMES)
End Sub
```

```
Function CalcSequence(STORETIMES)

'Progresses the position of the ovulation in the sequence if an ovulation time has been
'stored. If no ovulation occurred, the element is set to Empty to signal a pause day

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(SEQUENCE) To UBound(SEQUENCE)
      If STORETIMES(J) <> 0 Then
         SEQUENCE(I) = SEQUENCE(I) + 1
         Else: SEQUENCE(I) = Empty
         End If
   Let J = J + 1
   Next
   CalcSequence = SEQUENCE
End Function
```

```
Sub FILLARRAYLastInterval()
```

```
'Fills the array LastInterval (the interval between the last two ovipositions) using
'CalcLastInterval. Stores the array as FinalInterval in Excel and converts it to time
'format

  FLOCKLASTINTERVAL = CalcLastInterval(OVRATE)
  Worksheets("model").Range("a113:cv113").Value = FLOCKLASTINTERVAL
  Worksheets("model").Range("a113:cv113").Select
  Selection.Copy
  Range("ah5").Select
  Selection.PasteSpecial Paste:=xlAll, Transpose:=True
  Selection.NumberFormat = "hh:mm:ss"
  With Selection.Interior
    .ColorIndex = 19
    .PatternColorIndex = xlAutomatic
  End With
End Sub
```

```
Function CalcLastInterval(OVRATE)

'Calculates the last oviposition interval using a linear-by-linear function and based
'on the ovulation rate. Shorter ovulation sequences will have a longer interval between
'the last two ovipositions

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(LASTINTERVAL) To UBound(LASTINTERVAL)
    LASTINTERVAL(I) = (109513.0732 - 0.037 / (1 - 1.965 * OVRATE(J))) - _
    Int((109513.0732 - 0.037 / (1 - 1.965 * OVRATE(J))))
  Let J = J + 1
  Next
  CalcLastInterval = LASTINTERVAL
End Function
```

```
Sub FILLARRAYInternalOvulations()

'Fills the array InternalOvulations using the function CalcInternalOvulations

  Dim FlockInternalOvulations As Variant
  FlockInternalOvulations = CalcInternalOvulations(HenInternalOvulations,
ProportionIO)

End Sub
```

```
Function CalcInternalOvulations(HenInternalOvulations, ProportionIO)
```

```
'A random number is generated for the proportion of the flock expected to ovulate
'internally

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(InternalOvulations) To UBound(InternalOvulations)
      If HenInternalOvulations(J) <= ProportionIO Then
         InternalOvulations(I) = Rnd()
         Else: InternalOvulations(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcInternalOvulations = InternalOvulations
End Function
```

```
Sub FILLARRAYNumberIO()

'Fills the array NumberIO using the function CalcNumberIO. The % internal ovulations is
'predicted from hen age using a quadratic-by-linear function

   If BREED = 1 Or BREED = 3 Or BREED = 4 Then
      PercentIO = -58.08 + 67.17 / (1 + 0.001585 * HENAGE) + 0.04426 * HENAGE
      ElseIf BREED = 2 Then
         PercentIO = -29.78 + 38.32 / (1 + 0.002352 * HENAGE) + 0.02659 * HENAGE
   End If
   Dim FlockNumberIO As Variant
   FlockNumberIO = CalcNumberIO(InternalOvulations, ProportionIO, PercentIO)
End Sub
```

```
Function CalcNumberIO(InternalOvulations, ProportionIO, PercentIO)

'If the hen is expected to ovulate internally and the random number associated with the
'hen for the day is less than PercentIO, the element is given a value of 1.

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(NumberIO) To UBound(NumberIO)
      If InternalOvulations(J) <= (PercentIO / (ProportionIO * 100)) And
InternalOvulations(J) <> Empty Then
         NumberIO(I) = 1
         Else: NumberIO(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcNumberIO = NumberIO
```

```
End Function
```

```
Sub FILLARRAYOviposition()

'Fills the array Oviposition (oviposition times) using the function CalcOviposition

   FLOCKOVIPOSITION = CalcOviposition(SEQUENCE, STORETIMES, Interval,
YESTERDAYOVTIME,  FINALINTERVAL, InternalOvulations, NumberIO)
End Sub
```

```
Function CalcOviposition(SEQUENCE, STORETIMES, Interval,
YESTERDAYOVTIME, FINALINTERVAL, InternalOvulations, NumberIO)

'Calculates oviposition time relative to the associated ovulation time (mid-sequence) or
'using the array FINALINTERVAL (terminal oviposition). If an internal ovulation has
'occurred, no oviposition time is stored

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(OVIPOSITION) To UBound(OVIPOSITION)
      If YESTERDAYOVTIME(J) = 0 Then
         OVIPOSITION(I) = 0
         ElseIf SEQUENCE(J) > 1 And NumberIO(J) <> 1 Then
            OVIPOSITION(I) = STORETIMES(J) - Interval(J)
         ElseIf SEQUENCE(J) > 1 And NumberIO(J) = 1 Then
            OVIPOSITION(I) = 0
         ElseIf SEQUENCE(J) = 1 Then
            OVIPOSITION(I) = 0
         ElseIf SEQUENCE(J) = 0 And NumberIO(J) <> 1 Then
            OVIPOSITION(I) = YESTERDAYOVTIME(J) + FINALINTERVAL(J)
         ElseIf SEQUENCE(J) = 0 And NumberIO(J) = 1 Then
            OVIPOSITION(I) = 0
      End If
   Let J = J + 1
   Next
   CalcOviposition = OVIPOSITION
End Function
```

```
Sub FILLARRAYStoreOvipTimes()

'Fills the array STOREOVIPTIMES using the function CalcStoreOvipTimes

   FLOCKSTOREOVIPTIMES = CalcStoreOvipTimes(OVIPOSITION)
End Sub
```

```
Function CalcStoreOvipTimes(OVIPOSITION)

'Stores the oviposition times in STOREOVIPTIMES if an egg has been laid that day

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(STOREOVIPTIMES) To UBound(STOREOVIPTIMES)
      If OVIPOSITION(J) <> 0 Then
         STOREOVIPTIMES(I) = OVIPOSITION(J)
         Else: STOREOVIPTIMES(I) = STOREOVIPTIMES(I)
      End If
   Let J = J + 1
   Next
   CalcStoreOvipTimes = STOREOVIPTIMES
End Function
```

```
Sub RecalcRandomInterval()

'Uses the random number generation to produce a new array of the variable Interval
'(between oviposition and the associated ovulation) for the next day. First clears
'the range

   Worksheets("model").Select
   Range("af5:af104").Select
   Selection.ClearContents
   Application.Run "ATPVBAEN.XLA!Random", RandomInterval, 1, 100, 2, ,
MeanINTERVAL,  sdINTERVAL

'Formats the range (background colour and time format in minutes and seconds)

   Range("af5:af104").Select
   Selection.NumberFormat = "mm:ss"
   With Selection.Interior
      .ColorIndex = 19
      .PatternColorIndex = xlAutomatic
   End With
   With Selection.Font
      .Name = "Comic Sans MS"
      .FontStyle = "Regular"
      .Size = 9
   End With
End Sub
```

```
Sub FILLARRAYSoftShells()

'Fills the array SoftShells using the function CalcSoftShells
```

```
   Dim FlockSoftShells As Variant
   FlockSoftShells = CalcSoftShells(HenSoftShells, ProportionSS)
End Sub
```

```
Function CalcSoftShells(HenSoftShells, ProportionSS)

'A random number is generated for the proportion of the flock expected to produce
'soft-shelled eggs

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(SoftShells) To UBound(SoftShells)
      If HenSoftShells(J) <= ProportionSS Then
         SoftShells(I) = Rnd()
         Else: SoftShells(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcSoftShells = SoftShells
End Function
```

```
Sub FILLARRAYNumberSS()

'Fills the array NumberSS using the function CalcNumberSS. The % soft shells is
predicted from hen age using a line plus exponential function

   If BREED = 1 Or BREED = 3 Or BREED = 4 Then
      PercentSS = -0.8006 + 1202.6 * (0.952622 ^ HENAGE) + 0.0036895 * HENAGE
      Else: PercentSS = -0.8134 + 714.6 * (0.953762 ^ HENAGE) + 0.0037948 *
HENAGE
   End If
   Dim FlockNumberSS As Variant
   FlockNumberSS = CalcNumberSS(SoftShells, PercentSS, ProportionSS)
End Sub
```

```
Function CalcNumberSS(SoftShells, PercentSS, ProportionSS)

'If the hen is expected to lay a soft-shelled egg and the random number associated with
'the hen for the day is less than PercentSS, the element is given a value of 1.

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(NumberSS) To UBound(NumberSS)
```

```
    If SoftShells(J) <= (PercentSS / (ProportionSS * 100)) And SoftShells(J) <>
Empty Then
        NumberSS(I) = 1
        Else: NumberSS(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcNumberSS = NumberSS
End Function
```

```
Sub FILLARRAYDoubleYolks()

'Fills the array DoubleYolks using the function CalcDoubleYolks

   Dim FlockDoubleYolks As Variant
   FlockDoubleYolks = CalcDoubleYolks(HenDoubleYolks, ProportionDY)

End Sub
```

```
Function CalcDoubleYolks(HenDoubleYolks, ProportionDY)

'A random number is generated for the proportion of the flock expected to produce
'double-yolked eggs

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(DoubleYolks) To UBound(DoubleYolks)
      If HenDoubleYolks(J) <= ProportionDY Then
         DoubleYolks(I) = Rnd()
         Else: DoubleYolks(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcDoubleYolks = DoubleYolks
End Function
```

```
Sub FILLARRAYNumberDY()

'Fills the array NumberDY using the function CalcNumberDY. The % double yolks is
predicted from hen age using an exponential function

   PercentDY = -0.000475 + 8786.66 * (0.9403199 ^ HENAGE)
   Dim FlockNumberDY As Variant
   FlockNumberDY = CalcNumberDY(DoubleYolks, PercentDY, ProportionDY)
```

```
End Sub
```

```
Function CalcNumberDY(DoubleYolks, PercentDY, ProportionDY)

'If the hen is expected to lay a double-yolked egg and the random number associated with
'the hen for the day is less than PercentDY, the element is given a value of 1

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(NumberDY) To UBound(NumberDY)
      If DoubleYolks(J) <= (PercentDY / (ProportionDY * 100)) And DoubleYolks(J) <>
Empty Then
         NumberDY(I) = 1
         Else: NumberDY(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcNumberDY = NumberDY
End Function
```

```
Sub FILLARRAYEggSequence()

'Fills the array EGGSEQUENCE using the function CalcEggSequence. This data is used in
'the Sequence Analyzer program

   FLOCKEGGSEQUENCE = CalcEggSequence(STOREOVIPTIMES,
YESTERDAYOVTIME, NumberSS,  NumberIO, NumberDY)

End Sub
```

```
Function CalcEggSequence(STOREOVIPTIMES, YESTERDAYOVTIME, NumberSS,
NumberIO, NumberDY)

'If a normal egg has been laid that day, the letter 'A' is stored. If an internal ovulation
'has occurred, the letter 'C' is stored. If a soft shell has been laid, the letter 'B'
'is stored. If a double yolk has been laid, the letter 'D' is stored

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(EGGSEQUENCE) To UBound(EGGSEQUENCE)
      If STOREOVIPTIMES(J) <> 0 And NumberSS(J) <> 1 And NumberDY(J) <> 1
Then
         EGGSEQUENCE(I) = "A"
         ElseIf STOREOVIPTIMES(J) <> 0 And NumberSS(J) = 1 Then
            EGGSEQUENCE(I) = "B"
```

```
        ElseIf STOREOVIPTIMES(J) <> 0 And NumberSS(J) <> 1 And
NumberDY(J) = 1 Then
            EGGSEQUENCE(I) = "D"
        ElseIf YESTERDAYOVTIME(J) <> 0 And STOREOVIPTIMES(J) = 0 And
NumberIO(J) = 1 Then
            EGGSEQUENCE(I) = "C"
        Else: EGGSEQUENCE(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcEggSequence = EGGSEQUENCE

End Function
```

```
Sub FILLARRAYRemoveIO()

'Fills the array RemoveIO, using the function CalcRemoveIO

   FLOCKRemoveIO = CalcRemoveIO(EGGSEQUENCE)

End Sub
```

```
Function CalcRemoveIO(EGGSEQUENCE)

'Retains normal, soft-shelled and double-yolked eggs in the sequence profile but removes
'internal ovulations, so that they create pauses in egg sequences

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(RemoveIO) To UBound(RemoveIO)
      If EGGSEQUENCE(J) = "C" Then
         RemoveIO(I) = Empty
         Else: RemoveIO(I) = EGGSEQUENCE(J)
      End If
   Let J = J + 1
   Next
   CalcRemoveIO = RemoveIO
End Function
```

```
Sub FILLARRAYOvipSequence()

'Fills the array OVIPSEQUENCE with the position of the egg in the sequence, using
'consecutive numbers from 1 onwards
```

```
   FLOCKOVIPSEQUENCE = CalcOvipSequence(STOREOVIPTIMES)
End Sub
```

```
Function CalcOvipSequence(STOREOVIPTIMES)

'Progresses the position of the egg in the sequence if an oviposition time has been
'stored.

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(OVIPSEQUENCE) To UBound(OVIPSEQUENCE)
      If STOREOVIPTIMES(J) <> 0 Then
         OVIPSEQUENCE(I) = OVIPSEQUENCE(I) + 1
         Else: OVIPSEQUENCE(I) = Empty
      End If
   Let J = J + 1
   Next
   CalcOvipSequence = OVIPSEQUENCE
End Function
```

```
Sub FILLARRAYTotalEggs()

'Fills the array TOTALEGGS using the function CalcTotalEggs.  Cumulates the number of
eggs laid by each hen

   FLOCKTOTALEGGS = CalcTotalEggs(OVIPOSITION)
End Sub
```

```
Function CalcTotalEggs(OVIPOSITION)

'If an oviposition has occurred that day, one egg is added to the total

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(TOTALEGGS) To UBound(TOTALEGGS)
      If OVIPOSITION(J) <> 0 Then
         TOTALEGGS(I) = TOTALEGGS(I) + 1
         Else: TOTALEGGS(I) = TOTALEGGS(I)
      End If
   Let J = J + 1
   Next
   CalcTotalEggs = TOTALEGGS
End Function
```

```
Sub FILLARRAYTimeFirstEgg()

'Fills the array TIMEFIRSTEGG using the function CalcTimeFirstEgg. Continues until all
'100 hens have a recorded time for first egg. The range object COUNTTFE keeps a sum of
'the number of first eggs. Stores the times in STORETFE

   If COUNTTFE < 100 Then
      FLOCKTIMEFIRSTEGG = CalcTimeFirstEgg(TOTALEGGS, STOREOVIPTIMES)
      FILLARRAYStoreTFE
      Else: FLOCKTIMEFIRSTEGG = 0
   End If
End Sub
```

```
Function CalcTimeFirstEgg(TOTALEGGS, STOREOVIPTIMES)

'If the egg is the first one laid by a hen, the oviposition time is stored in TIMEFIRSTEGG

      Dim I As Integer
      Dim J As Integer
      Let J = 1
      For I = LBound(TIMEFIRSTEGG) To UBound(TIMEFIRSTEGG)
         If TOTALEGGS(J) = 1 Then
            TIMEFIRSTEGG(I) = STOREOVIPTIMES(J)
            Else: TIMEFIRSTEGG(I) = TIMEFIRSTEGG(I)
         End If
      Let J = J + 1
      Next
      CalcTimeFirstEgg = TIMEFIRSTEGG
End Function
```

```
Sub FILLARRAYStoreTFE()

'Fills the array STORETFE using the function CalcStoreTFE. Transfers the times to the
'Excel worksheet "timefirstegg" for a frequency analysis. Called by sub procedure
'FILLARRAYTimeFirstEgg

   FLOCKSTORETFE = CalcStoreTFE(TIMEFIRSTEGG)
   Worksheets("timefirstegg").Select
   Range("a113:cv113").Value = FLOCKSTORETFE
   Range("a113:cv113").Select
   Selection.Copy
   Range("b5").Select
   Selection.PasteSpecial Paste:=xlAll, Transpose:=True

'Formats the data to a time and sets the background colour
```

```
  Range("b5:b104").Select
  Selection.NumberFormat = "h:mm:s"
  With Selection.Interior
    .ColorIndex = 35
    .Pattern = xlSolid
    .PatternColorIndex = xlAutomatic
  End With
  Range("a1").Select
  Worksheets("model").Select
  Range("a1").Select
End Sub
```

```
Function CalcStoreTFE(TIMEFIRSTEGG)

'Stores the time of first egg daily for each hen in the array STORETFE. If the egg was not
'a first egg, leaves the stored time unaltered

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(STORETFE) To UBound(STORETFE)
    If TIMEFIRSTEGG(I) <> 0 Then
      STORETFE(I) = TIMEFIRSTEGG(J)
      Else: STORETFE(I) = STORETFE(I)
    End If
    Let J = J + 1
  Next
  CalcStoreTFE = STORETFE
End Function
```

```
Sub FillArrayYW()

'Calculates the mean yolk weight, using Gompertz or logistic equations based on the hen
'age and the breed. (Hy-Line Silver = 1; Hy-Line Brown = 2; Amber-Link = 3; Other = 4)

  If BREED = 1 Or BREED = 4 Then
    MeanYW = -51107 + 51123 * Exp(-Exp(-0.01771 * (HENAGE + 370.1)))
    ElseIf BREED = 2 Then
      MeanYW = -101.1 + 116 * Exp(-Exp(-0.01972 * (HENAGE + 15.36)))
    ElseIf BREED = 3 Then
      MeanYW = -224.7 + 243.2 / (1 + Exp(-0.01268 * (HENAGE + 116.4)))
  End If
  Let sdYW = MeanYW * 0.05

'Clears the range for the new random number generation

  Worksheets("model").Select
```

```
   Range("bc5:bc104").Select
   Selection.ClearContents

'Normally distributed random number generation for Yolk weight

   Application.Run "ATPVBAEN.XLA!Random", RandomYW, 1, 100, 2, , MeanYW,
sdYW

'Formats the area

   Range("bc5:bc104").Select
   Selection.NumberFormat = "0.00"
   With Selection.Interior
      .ColorIndex = 19
      .PatternColorIndex = xlAutomatic
   End With
   With Selection.Font
      .Name = "Comic Sans MS"
      .FontStyle = "Regular"
      .Size = 9
   End With
End Sub
```

```
Sub FILLARRAYYolkWeight()

'Fills the array YolkWeight using the function CalcYolkWeight

   FLOCKYOLKWT = CalcYolkWeight(STOREOVIPTIMES, EGGSEQUENCE, YW)
End Sub
```

```
Function CalcYolkWeight(STOREOVIPTIMES, EGGSEQUENCE, YW)

'If an egg has been laid and the time stored, the yolk weight is calculated from Gompertz
'or logistic functions (depending on the breed) and hen age

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(YolkWeight) To UBound(YolkWeight)
      If STOREOVIPTIMES(J) <> Empty And EGGSEQUENCE(J) <> "B" And
EGGSEQUENCE(J) <> "D" Then
         YolkWeight(I) = YW(J)
      ElseIf STOREOVIPTIMES(J) <> Empty And EGGSEQUENCE(J) <> "B" And
EGGSEQUENCE(J) = "D" Then
         YolkWeight(I) = YW(J) + YW(J)
         Else: YolkWeight(I) = Empty
```

```
    End If
  Let J = J + 1
  Next
  CalcYolkWeight = YolkWeight
End Function
```

```
Sub FILLARRAYAlbumenWeight()

'Fills the array AlbumenWeight using the function CalcAlbumenWeight

  FLOCKALBUMENWT = CalcAlbumenWeight(YolkWeight, AforAW, BforAW)
End Sub
```

```
Function CalcAlbumenWeight(YolkWeight, AforAW, BforAW)

'Predicts the albumen weight from the yolk weight, using allometric function parameters
'for the breed

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(AlbumenWeight) To UBound(AlbumenWeight)
    If YolkWeight(J) <> Empty Then
      AlbumenWeight(I) = AforAW(J) * YolkWeight(J) ^ BforAW(J)
      Else: AlbumenWeight(I) = Empty
    End If
  Let J = J + 1
  Next
  CalcAlbumenWeight = AlbumenWeight
End Function
```

```
Sub FILLARRAYShellWeight()

'Fills the array ShellWeight using the function CalcShellWeight

  FLOCKSHELLWT = CalcShellWeight(YolkWeight, AlbumenWeight, AforSW,
BforSW)
End Sub
```

```
Function CalcShellWeight(YolkWeight, AlbumenWeight, AforSW, BforSW)

'Predicts the shell weight from egg contents weight, using allometric function parameters
'for the breed

  Dim I As Integer
```

```
  Dim J As Integer
  Let J = 1
  For I = LBound(ShellWeight) To UBound(ShellWeight)
    If YolkWeight(J) <> Empty Then
      ShellWeight(I) = AforSW(J) * (YolkWeight(J) + AlbumenWeight(J)) ^ BforSW(J)
      Else: ShellWeight(I) = Empty
    End If
  Let J = J + 1
  Next
  CalcShellWeight = ShellWeight
End Function
```

```
Sub FILLARRAYEggWeight()

'Fills the array EggWeight using the function CalcEggWeight

  FLOCKEGGWT = CalcEggWeight(YolkWeight, AlbumenWeight, ShellWeight)
End Sub
```

```
Function CalcEggWeight(YolkWeight, AlbumenWeight, ShellWeight)

'Predicts the egg weight by adding the weights of the three components

  Dim I As Integer
  Dim J As Integer
  Let J = 1
  For I = LBound(EggWeight) To UBound(EggWeight)
    If YolkWeight(J) <> Empty Then
      EggWeight(I) = YolkWeight(J) + AlbumenWeight(J) + ShellWeight(J)
      Else: EggWeight(I) = Empty
    End If
  Let J = J + 1
  Next
  CalcEggWeight = EggWeight
End Function
```

```
Sub FINDROWOvulation()

'Transfers the array containing the ovulation times to Excel.  Finds the correct row
'in the worksheet "ov.times" alongside the day number

  Worksheets("ov.times").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5: a504")
  Dim I As Integer
    For I = 5 To 504
      If Cells(I, 1).Value = DayNumber Then
```

```
            Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKSTORETIMES
        End If
    Next
End Sub
```

```
Sub FINDROWOviposition()

'Transfers the array containing the oviposition times to Excel.  Finds the correct row
'in the worksheet "ovip.times" alongside the day number

    Worksheets("ovip.times").Select
    Dim RangeToSearch As Range
    Set RangeToSearch = ActiveSheet.Range("a5: a505")
    Dim I As Integer
        For I = 5 To 505
            If Cells(I, 1).Value = DayNumber Then
                Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKSTOREOVIPTIMES
            End If
        Next
End Sub
```

```
Sub FINDROWEggSequence()

'Transfers the array containing the sequence data "A, B, C or D" to Excel.  Finds the
'correct row in the worksheet "seq.length" alongside the day number

    Worksheets("seq.length").Select
    Dim RangeToSearch As Range
    Set RangeToSearch = ActiveSheet.Range("a5:a505")
    Dim I As Integer
        For I = 5 To 505
            If Cells(I, 1).Value = DayNumber Then
                Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKEGGSEQUENCE
            End If
        Next
End Sub
```

```
Sub FINDROWRemoveIO()

'Transfers the array containing the sequence data "A, B, or D" to Excel.  Finds the
'correct row in the worksheet "seq.analyzer" alongside the day number

    Worksheets("seq.analyzer").Select
    Dim RangeToSearch As Range
    Set RangeToSearch = ActiveSheet.Range("b4:b504")
    Dim I As Integer
```

```
    For I = 4 To 504
      If Cells(I, 2).Value = HENAGE Then
        Range(Cells(I, 3), Cells(I, 102)).Value = FLOCKRemoveIO
      End If
    Next
End Sub
```

```
Sub FINDROWMeanSL()

'Transfers the array containing the position of the oviposition in the sequence to Excel.
'Finds the correct row in the worksheet "mean sl" alongside the day number
  Worksheets("mean sl").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a505")
  Dim I As Integer
  For I = 5 To 505
    If Cells(I, 1).Value = DayNumber Then
      Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKOVIPSEQUENCE
    End If
  Next
End Sub
```

```
Sub FINDROWYolkWeight()

'Transfers the array containing the yolk weights to Excel. Finds the correct row in the
'worksheet "yolk wt" alongside the day number

  Worksheets("yolk wt").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a505")
  Dim I As Integer
  For I = 5 To 505
    If Cells(I, 1).Value = DayNumber Then
      Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKYOLKWT
    End If
  Next
End Sub
```

```
Sub FINDROWAlbumenWeight()

'Transfers the array containing the albumen weights to Excel. Finds the correct row in the
'worksheet "albumen wt" alongside the day number

  Worksheets("albumen wt").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a505")
```

```
  Dim I As Integer
  For I = 5 To 505
    If Cells(I, 1).Value = DayNumber Then
      Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKALBUMENWT
    End If
  Next
End Sub
```

```
Sub FINDROWShellWeight()

'Transfers the array containing the shell weights to Excel. Finds the correct row in the
'worksheet "shell wt" alongside the day number

  Worksheets("shell wt").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a505")
  Dim I As Integer
  For I = 5 To 505
    If Cells(I, 1).Value = DayNumber Then
      Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKSHELLWT
    End If
  Next
End Sub
```

```
Sub FINDROWEggWeight()

'Transfers the array containing the egg weights to Excel. Finds the correct row in the
'worksheet "egg wt" alongside the day number

  Worksheets("egg wt").Select
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a505")
  Dim I As Integer
  For I = 5 To 505
    If Cells(I, 1).Value = DayNumber Then
      Range(Cells(I, 2), Cells(I, 101)).Value = FLOCKEGGWT
    End If
  Next
  Worksheets("model").Select
End Sub
```

```
Sub FILLARRAYYesterdayOvtime()

'Fills the array YESTERDAYOVTIME using the function CalcYesterdayOvTime at the
end of the day
```

```
   FLOCKYESTERDAYOVTIME = CalcYesterdayOvtime(STORETIMES)
End Sub
```

```
Function CalcYesterdayOvtime(STORETIMES)

'Stores the ovulation times for the day in YESTERDAYOVTIME after the day has
progressed

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(YESTERDAYOVTIME) To UBound(YESTERDAYOVTIME)
      YESTERDAYOVTIME(I) = STORETIMES(J)
   Let J = J + 1
   Next
   CalcYesterdayOvtime = YESTERDAYOVTIME
End Function
```

```
Sub FILLARRAYYesterdaySequence()

'Fills the array YesterdaySequence with the position of the ovulation in the sequence,
'before the next day's calculations begin

   Dim FLOCKYESTERDAYSEQUENCE As Variant
   FLOCKYESTERDAYSEQUENCE = CalcYesterdaySequence(SEQUENCE)
End Sub
```

```
Function CalcYesterdaySequence(SEQUENCE)

'Stores the array SEQUENCE in the array YESTERDAYSEQUENCE after the day has
progressed

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(YESTERDAYSEQUENCE) To UBound(YESTERDAYSEQUENCE)
      YESTERDAYSEQUENCE(I) = SEQUENCE(J)
   Let J = J + 1
   Next
   CalcYesterdaySequence = YESTERDAYSEQUENCE
End Function
```

```
Sub FILLARRAYProgressTimeFM()
```

```
'Fills the array TIMEFM using the function CalcProgressTimeFM.  At the end of the
day the time is put forward to STARTTIME on the following day.  Follicle maturation
needs to be progressed.

   Dim FLOCKPROGRESSTIMEFM As Variant
   FLOCKPROGRESSTIMEFM = CalcProgressTimeFM(AFE, HENAGE, EXCL,
StartFM, TDAY,  DAYSOVULATION, ENDTDAY, STARTTDAY)
End Sub
```

```
Function CalcProgressTimeFM(AFE, HENAGE, EXCL, StartFM, TDAY,
DAYSOVULATION, ENDTDAY, STARTTDAY)

'Recalculates the time the follicle has been maturing

   Dim I As Integer
   Dim J As Integer
   Let J = 1
   For I = LBound(TIMEFM) To UBound(TIMEFM)
      If Round(AFE(J), 0) - 1 = Round(HENAGE, 0) Then
         TIMEFM(I) = EXCL - StartFM(J) + TDAY
         ElseIf DAYSOVULATION(J) > 0 Then
            TIMEFM(I) = TIMEFM(I) + (EXCL - ENDTDAY) + STARTTDAY
         Else: TIMEFM(I) = TIMEFM(I)
      End If
   Let J = J + 1
   Next
   CalcProgressTimeFM = TIMEFM
End Function
```

```
Sub ConvertSequences()

'Converts the sequence data (position in sequence) in worksheet "mean sl" at the end of
'the model run. Starts at the last day of model output.  Converts eg. a 3-egg sequence
'from 1-2-3 to 3-3-3, the format needed to calculate mean sequence length daily for the
'flock.

      Worksheets("mean sl").Select
      Let DAYMeanSL = DayNumber
      Dim SequenceCurrent As Range
      Dim SequencePrevious As Range
      Set SequenceCurrent = ActiveSheet.Range("b507:cw507")
      Set SequencePrevious = ActiveSheet.Range("b506:cw506")
      Do Until DAYMeanSL = 2

'Calls sub procedures and function

      FINDROWConvertNameSL
```

```
    FlockCurrent = CalcConvertSequence(SequenceCurrent, SequencePrevious)
    Worksheets("mean sl").Range("b509:cw509").Value = FlockCurrent
    FINDROWConvertMeanSL
    Let DAYMeanSL = DAYMeanSL - 1
    Loop
    Let SequenceCounter = 1
End Sub
```

```
Sub FINDROWConvertNameSL()

'Finds the last two days of sequence data; stores data in named ranges "FlockCurrentSL"
'and FlockPreviousSL"
  Dim RangeToSearch As Range
  Set RangeToSearch = ActiveSheet.Range("a5:a504")
  Dim I As Integer
    For I = 5 To 504
      If Cells(I, 1).Value = DAYMeanSL Then
        Range(Cells(I, 2), Cells(I, 101)).Name = "FlockCurrentSL"
      End If
    Next
    For I = 5 To 504
      If Cells(I, 1).Value = DAYMeanSL - 1 Then
        Range(Cells(I, 2), Cells(I, 101)).Name = "FlockPreviousSL"
      End If
    Next
End Sub
```

```
Function CalcConvertSequence(SequenceCurrent, SequencePrevious)

'Converts the numbers representing the position of each ovulation in the sequence to
'numbers representing the total number in the sequence, leaving pauses empty

  Dim I As Integer
  Dim J As Integer
  Let J = 1
    For I = LBound(CONVERTSEQUENCE) To UBound(CONVERTSEQUENCE)
      If SequencePrevious(J) = 0 Then
        CONVERTSEQUENCE(I) = Empty
        ElseIf SequencePrevious(J) < SequenceCurrent(J) Then
          CONVERTSEQUENCE(I) = SequenceCurrent(J)
        ElseIf SequenceCurrent(J) = 0 Then
          CONVERTSEQUENCE(I) = SequencePrevious(J)
      End If
    Let J = J + 1
    Next
  CalcConvertSequence = CONVERTSEQUENCE
End Function
```

```
Sub FINDROWConvertMeanSL()

'Transfers the converted data to the correct place in the worksheet "mean sl"

   Dim RangeToSearch As Range
   Set RangeToSearch = ActiveSheet.Range("a5:a505")
   Dim I As Integer
      For I = 5 To 505
         If Cells(I, 1).Value = DAYMeanSL - 1 Then
            Range(Cells(I, 2), Cells(I, 101)).Value = FlockCurrent
         End If
      Next
End Sub
```

```
Sub SortSequenceData()

'Sorts the sequence data in descending order according to mean sequence length, so that
'the flock may be split into thirds (top, middle, bottom) and mean sequence lengths for
'each third can be plotted.

   Worksheets("mean sl").Select
   Range("b5:cw505").Select
   Selection.Copy
   Range("b516").Select
   ActiveSheet.Paste
   Range("b516:cw1017").Select
   Selection.Sort Key1:=Range("b1017"), Order1:=xlDescending, Header:= _
      xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:=xlLeftToRight,
DataOption1:=xlSortNormal
   Range("a1").Select
   Worksheets("model").Select

End Sub
```

```
Sub SequenceAnalyzer()

'Stores the breed in the worksheet designed for importing into the Sequence Analyzer
program

   If BREED = 1 Then
      Let Worksheets("seq.analyzer").Range("c2").Value = "HS"
      ElseIf BREED = 2 Then
         Let Worksheets("seq.analyzer").Range("c2").Value = "HB"
      ElseIf BREED = 3 Then
         Let Worksheets("seq.analyzer").Range("c2").Value = "AL"
```

```
    ElseIf BREED = 4 Then
      Let Worksheets("seq.analyzer").Range("c2").Value = "Other"
    Else: Let Worksheets("seq.analyzer").Range("c2").Value = "HS"
  End If
  Range("a1").Select
End Sub
```

```
Sub ViewChartOvulationRate()

'View chart of flock ovulation rate

  Worksheets("ov.times").Select
  Range("dj1").Select
End Sub
```

```
Sub ViewChartRateofLay()

'View chart of flock rate of lay

  Worksheets("ovip.times").Select
  Range("dn32").Select
End Sub
```

```
Sub ViewChartOvipositionTimes()

'View chart of distribution of oviposition times

  Worksheets("ovip.times").Select
  Range("dn1").Select
End Sub
```

```
Sub ViewChartTimeFirstEgg()

'View chart of distribution of times of first eggs

  Worksheets("timefirstegg").Select
  Range("n1").Select
End Sub
```

```
Sub ViewChartMeanSL()

'View chart of mean sequence lengths over time

  Worksheets("mean sl").Select
```

```
   Range("di1").Select
End Sub
```

```
Sub ViewChartSLThirds()

'View chart of mean sequence lengths with the flock divided into thirds

   Worksheets("mean sl").Select
   Range("di31").Select
End Sub
```

```
Sub ViewChartMeanYW()
'View chart of mean yolk weights over time

   Worksheets("yolk wt").Select
   Range("di1").Select
End Sub
```

```
Sub ViewChartYolkPercent()

'View chart of yolk weight as a % of total egg weight

   Worksheets("yolk wt").Select
   Range("di32").Select
End Sub
```

```
Sub ViewChartMeanAW()

'View chart of mean albumen weights over time

   Worksheets("albumen wt").Select
   Range("di1").Select
End Sub
```

```
Sub ViewChartAlbumenPercent()

'View chart of albumen weight as a % of total egg weight

   Worksheets("albumen wt").Select
   Range("di31").Select
End Sub
```

```
Sub ViewChartMeanSW()

'View chart of mean shell weights over time

   Worksheets("shell wt").Select
   Range("di1").Select
End Sub
```

```
Sub ViewChartShellPercent()

'View chart of shell weight as a % of total egg weight

   Worksheets("shell wt").Select
   Range("di32").Select
End Sub
```

```
Sub ViewChartMeanEW()

'View chart of mean egg weight over time

   Worksheets("egg wt").Select
   Range("dl1").Select
End Sub
```

```
Sub ViewChartMeanEO()

'View chart of mean egg output over time

   Worksheets("egg wt").Select
   Range("dl31").Select
End Sub
```

```
Sub ViewChartMeanAFE()

'View chart of distribution of mean ages at first egg

   Worksheets("lightprogram").Select
   Range("p1").Select
End Sub
```

```
Sub ViewChartYWvEW()

'View chart of yolk weight v. egg weight at a given age
```

```
   Worksheets("egg wt").Select
   Range("dl51").Select
End Sub
```

```
Sub ViewChartAWvEW()

'View chart of albumen weight v. egg weight at a given age

   Worksheets("egg wt").Select
   Range("dl72").Select
End Sub
```

```
Sub ViewChartSWvEW()

'View chart of shell weight v. egg weight at a given age

   Worksheets("egg wt").Select
   Range("dl93").Select
End Sub
```

```
Sub ViewChartPercentYvEW()

'View chart of % yolk v. egg weight at a given age

   Worksheets("egg wt").Select
   Range("dl113").Select
End Sub
```

```
Sub ViewChartPercentAvEW()

'View chart of % albumen v. egg weight at a given age

   Worksheets("egg wt").Select
   Range("dl133").Select
End Sub
```

```
Sub ViewChartPercentSvEW()

'View chart of % shell v. egg weight at a given age

   Worksheets("egg wt").Select
   Range("dl153").Select
End Sub
```

```
Sub ViewChartInternalOvulations()

'View chart of distribution of internal ovulations

   Worksheets("seq.length").Select
   Range("dx1").Select
End Sub
```

```
Sub ViewChartPercentIO()

'View chart of % internal ovulations over time

   Worksheets("seq.length").Select
   Range("dx71").Select
End Sub
```

```
Sub ViewChartDoubleYolks()

'View chart of distribution of double yolks

   Worksheets("seq.length").Select
   Range("ea31").Select
End Sub
```

```
Sub ViewChartPercentDY()

'View chart of % double yolks over time

   Worksheets("seq.length").Select
   Range("ea92").Select
End Sub
```

```
Sub ViewChartSoftShells()

'View chart of distribution of soft shells

   Worksheets("seq.length").Select
   Range("ea51").Select
End Sub
```

```
Sub ViewChartPercentSS()

'View chart of % soft shells over time
```

```
   Worksheets("seq.length").Select
   Range("ea112").Select
End Sub
```

```
Sub QuitViewCharts()

'Quit view chart menu. Return to cell a1 on each worksheet

   Worksheets("ov.times").Select
   Range("a1").Select
   Worksheets("ovip.times").Select
   Range("a1").Select
   Worksheets("timefirstegg").Select
   Range("a1").Select
   Worksheets("seq.length").Select
   Range("a1").Select
   Worksheets("mean sl").Select
   Range("a1").Select
   Worksheets("yolk wt").Select
   Range("a1").Select
   Worksheets("albumen wt").Select
   Range("a1").Select
   Worksheets("shell wt").Select
   Range("a1").Select
   Worksheets("egg wt").Select
   Range("a1").Select
   Worksheets("lightprogram").Select
   Range("a1").Select
   Worksheets("model").Select
   Range("a1").Select

End Sub
```

Appendix 5.2: The values for the parameters A, B, D and C substituted in Equation 5.5 to predict internal cycle length for three hens (shown in Figure 5.3)

	A	B	D	C
Hen 3	19.975	5.034	0.02486	0.02618
Hen 4	16.590	7.350	0.00907	0.02000
Hen 5	18.942	9.076	0.03138	0.03396

Appendix 5.3: The means of the parameters used in Equation 5.5 to predict ICL for Hy-Line Silver and Hy-Line Brown hens

Variable	Hy-Line Silver	Hy-Line Brown
D	0.02279	0.02279
B	2.0469	2.0469
C	0.011	0.011418
A	22.4416	22.5416

Appendix 5.4: Parameter estimates for the linear-by-linear equation fitted to the final oviposition interval data.

Response variate: interval
Explanatory: x (ovulation rate)
Fitted curve: A + B / (1 + D. x)

Summary of analysis

	d.f.	s.s.	m.s.	v.r	F pr.
Regression	2	0.008138	0.004069	7.27	< 0.008
Residual	13	0.007273	0.0005595		
Total	15	0.015411	0.0010274		

% variance accounted for: 45.5
S.E. of observations estimated to be 0.0237

Estimate of parameters

	estimate	s.e.
D	-1.965	±0.986
B	-0.037	±0.103
A	109513.07	±0.0845

INSTRUCTIONS ON USING THE LAYER MODEL

1. The model was developed in Excel, Microsoft Office 2003. Although programmed in Visual Basic, Excel is needed to display worksheets and charts.
2. A 17-inch monitor is preferable for viewing the charts.
3. A Pentium 4 processor will minimise the runtime.
4. It takes about one minute to calculate flock egg production for 24 days.
5. Two files are included on the cd; 'Layer Model' and 'Layer Model test version'. The files are identical, but it is suggested that you run the test version and keep the other file complete with 500 days of egg production records, for viewing.
6. When opening the file in Excel, a Security Warning appears. The macros need to be enabled.
7. In order for the random number generator to work, the Add-Ins must be installed on your computer. In Excel, click on Tools, Add-Ins and tick the Analysis ToolPak.
8. Run the model by clicking on the 'run model' button found in cell J1 in the worksheet 'model'.
9. To terminate the model prematurely, press Esc on the keyboard. Select End from the pop-up menu. If you select Debug by mistake, select Run and Reset from the menu bar, and click on the Excel icon to return to Excel from Visual Basic.
10. Once the model has finished running, you can look at different worksheets. The charts are to the right of the flock summaries, starting in row 1. Use the View Charts option from the form or the View Charts button in cell J3 in the worksheet 'model' for easy access to all charts.
11. In older versions of Excel, the model may produce an error message at the end – 'Compile error; variable not defined'. This means that you will not be able to see the mean sequence lengths for the flock divided into thirds, but the rest of the results are available. Click OK, then select Run and Reset from the menu bar and click on the Excel icon to return to Excel. (You may be able to correct this by deleting the highlighted portion of the code plus a bit extra:

    DataOption1:=xlSortNormal

    as well as the underscore and comma (, _) at the end of the previous line).