NUTRITIONAL   RESTRICTION   OF   THE   GROWTH   RATE

OF

BROILER   BREEDER   REPLACEMENT   PULLETS

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INTRODUCTION

The primary objective in a feed restriction programme is the limitation
of mature body size so that utilisation of nutrients is improved and reproduc-
tivity enhanced. Feed restriction may be achieved by reducing feeding time,
limiting the amount of feed supplied to the animal, or feeding a diet so formu-
lated that feed intake will be controlled by the basic physiological mechanisms
known to regulate feed intake.

The effects of feed restrictions during the early growing period of man
and other species of animals have received considerable attention in recent
years. The evidence available to date is often conflicting and confusing, but
nevertheless certain characteristic effects of feed restriction seem to be
evident.

There is sufficient evidence to prove that restriction of the caloric inta
of dairy cattle during the growing period improves life-time production of fat
and/or milk (Hanson, 1956; Reid, 1971). The useful life of the dairy cow is
extended, and reproductive problems have been shown by the above workers to be
less of a problem for cattle reared on a restricted diet. Similarly, it has
been shown by Visscher, King & Lee (1952) that the reproductive life span of mi
can be greatly lengthened by reducing their caloric intake during growth.
McCay (1952) demonstrated that longevity of dogs and rats could be increased by
restricting the diet fed to these animals during growth. In fact, Reid (1959)
has noted that a lower plane of nutrition and the resultant retardation of earl
growth is associated with a prolongation of the life-span of protozoa, water
flies, fruit flies, silkworms, rats, mice and cattle.

Poultry geneticists developing broiler strains have placed great emphasis
on economically desirable characteristics in broilers, such as very rapid growt
from an early age, large body size, and the ability to convert feed efficiently
into body tissue. This has resulted in the development of broiler breeder
pullets which are different to the type of bird used for egg production. Withi

some form of restriction during growth these birds become overweight and obese by the time they are sexually mature. Their mortality is higher than normal, and layers of adipose tissue enveloping the reproductive organs cause egg prod tion from such birds to be considerably lower than the potential egg-laying ability of the strain. Also, feed consumption during the laying stage is high than it should be, due to the greater maintenance requirement of heavy birds a are therefore found to be unprofitable producers of day-old broiler chickens.

Some programme of dietary control is therefore desirable, and indeed essential, during the rearing period in order to produce pullets at point-of-l at a minimum cost, in a physical condition that will allow them to commence production at the desired age and weight, and which attain the reproductive potential characteristic of that genotype. To attain this goal, numerous feed restriction methods have been employed. These include the limitation of the amount of feed supplied to the birds, the reduction of feeding time, or the feeding of diets which have a low energy content or are deficient in protein o unbalanced in amino acid content. High fibre diets have also been employed in order to limit nutrient intake by means of the bulk of the ration.

Methods employed to restrict the daily amount of feed which the birds receive, or which restrict the feeding time, demand a high degree of manageria skill, and often require special facilities unavailable to some breeders. The most desirable method of feed restriction would be to feed a diet on an _ad lib_ basis. The diet should not be expensive, should not contain an excessive quan tity of fibre which might cause the broiler mother to habitually overconsume, and should enhance egg production.

The experiment reported in this dissertation was planned in an attempt to meet these requirements. Diets were fed _ad lib._, and since reports of conven tional dietary treatments fed _ad lib._ were unfavourable, an unconventional ste down in protein content of the diet was incorporated into the feeding programm It was hoped that this system might be more efficient than other _ad lib._ feedin schedules in reducing body weight at point-of-lay.

Physiological mechanisms responsible for changes in body weight and carcas

composition, as well as changes in the physiological characteristics of broiler

breeder pullets on a feed restriction programme have been virtually unexplored.

Consequently, the feed, energy and protein intake of the birds in this experi-

ment were carefully measured, and the influence of nutrient intake on weight ga

and body composition was determined. It was hoped that physiological control

mechanisms governing feed intake with respect to broiler breeder pullets might

be elucidated, and that these might be exploited in future experiments in attem

to control the energy intake by this type of bird.

Carcass composition studies in conjunction with a feed restriction program

are extremely useful in that this variate, or more specifically the percentage

body fat, is influenced to a large degree by nutritional changes. It is possib

that body weight might be sufficiently reduced in such a programme, whereas the

percentage of fat in the body might remain at a level too high for efficient eg

production. Consequently a check on the body fat percentage of birds on a broi

feed restriction programme is a useful measure of the efficacy of such a pro-

gramme. If a high correlation between a readily measurable character and per-

centage body fat could be found, the estimation of body fat could then become a

more feasible and, indeed, a routine operation. Blood analyses were carried ou

on birds whose carcass composition was also determined in order to ascertain th

correlation between body fat content and the protein and lipid concentrations c

blood serum, and the degree to which the latter are affected by dietary manipu-

lation.

Since the thyroid gland plays such an important role in the general metabo

of body tissues and in growth processes, it is likely to be involved, directly

indirectly, with the abnormal feed intake and carcass composition of broiler

breeder pullets. The high body fat content of broiler breeders, as reported in

the literature, suggests that hypothyroidism is involved in the deposition of

these large amounts of fat. Measurements of thyroid activity were made in the

present experiment to determine the degree of involvement of the thyroid gland

A more thorough understanding of thyroid activity under such conditions
will enable better use to be made of thyro-active and antithyroid substances.
The relationships between fat metabolism and growth rate, and between thyroid
activity and fat content may also assume practical importance in broiler pullet
rearing if these relationships could be identified.

CHAPTER I

The influence of dietary energy and protein
on feed consumption and growth
of broiler breeder pullets

1.1 Review

Most published work on restricted feeding during the rearing period

has been concerned with laying type pullets. Numerous reports have, however,

been published during the past fifteen years relative to different methods of

restricted feeding of broiler breeder replacement pullets during the growing

period. It is generally conceded that some form of dietary control is necessar

with broiler breeders, as the pullet, if allowed to eat to repletion during the

rearing period will attain an undesirable weight and carcass composition at

point-of-lay. Low egg production, poor fertility and high mortality is the res

of an overweight, obese flock at this age.

Research studies in the field of physiology have established the fact that

certain specific physiological mechanisms in animals regulate and maintain ener

balance. Such regulation and maintenance is brought about through adjustments

made in food intake, stored energy, work and heat production. Food intake is

normally the key factor in this regulation process, since it compensates for ar

changes in the other three variables. Mechanisms that control and regulate foc

intake are, therefore, of prime importance in the overall maintenance of energy

balance (Anand, 1961).

During the past 20 years, the physiological regulators of food intake have

been studied more and more as functions of the central nervous system (Grossmar

1963). From the research data acquired in these studies, five food intake regu-

latory mechanisms have been proposed as signals for the central nervous system.

These are:

(1)   a distension of the digestive tract as proposed by Janowitz &
        Grossman (1949, 1951), and Grossman (1955);

(2)   the "lipostatic" hypothesis of Kennedy (1952);

(3)   the "thermostatic" hypothesis of Strominger & Brobeck (1953);

(4)   the "glucostatic" hypothesis of Mayer (1953, 1955);

(5)   the concentration of serum amino acids (Mellinkoff, Frankland,
        Boyle & Greipel, 1956).

No single hypothesis can satisfactorily explain the mechanism controlling feed

intake.   According to Anand (1961) a multiple factor theory of regulation

appears to be most acceptable.

    Isaacs, Crawford, Wheeler, Quisenberry, Reid & Couch (1957) and Isaacs,

Reid, Davies, Quisenberry & Couch (1960) were amongst the first workers to

report on the feeding of self-restricting high fibre diets to growing broiler

strain pullets.   They noted an overconsumption of energy and a tendency by the

birds to consume a specific amount of protein.   These results refuted the feed

intake regulatory mechanisms thus far proposed.   Consequently a number of

workers deemed it necessary to study the energy requirements of broiler-strain

pullets and methods for restricting their caloric intake.

    Most of the five feed intake regulatory mechanisms have been used in

attempts to restrict the body weight of broiler breeders at point-of-lay.   The

most successful methods employed thus far have involved limiting the amounts of

feed offered or restricting the feeding time.   These methods are difficult to

implement, and demand a high degree of stockmanship and managerial skill

(Shane, 1970).   Consequently, attempts have been made to discover a feed intake

regulatory mechanism that could be used in an ad lib. feeding programme but

which would still succeed in limiting the weight of broiler breeder pullets at

point-of-lay.

    The present trial involved the ad lib. feeding of different protein and

energy levels in the diet.   The presentation of literature will therefore be

limited to that pertaining to restriction of body weight based on diets low in

Howes & Cottier (1965) attempted to control body weight and sexual maturit
of broiler pullets by feeding a ten per cent protein diet from eight to 26 week
of age. They found no delay in maturity because these birds compensated for th
protein deficiency by increased total food consumption. Body weight was not
restricted by this ration compared to the control weights. This ability to
compensate for protein deficiencies in the diet is contrary to the findings of
workers such as Waldroup, Damron & Harms (1966) and Scott, Nesheim & Young (196
with Leghorn pullets; their feed consumption tends to be depressed by low prot
diets. According to Singsen, Matterson, Tlustohowicz & Potter (1959), this
tendency amongst Leghorn-type pullets to eat only until their energy require-
ments are satisfied, and not to overconsume energy to satisfy their protein
requirements, is seldom found amongst broiler-type birds.

Britzman, Gray & Strandine (1965) published results of an experiment with
broiler pullets fed a high energy (2336 kcal productive energy (PE) per kg)
containing eleven per cent protein and a low energy diet (1170 kcal PE per kg)
with twelve per cent protein to two groups of birds. Feed consumption on the
two diets was 8,77 and 13,68 kg per bird and final live weights 2,88 and 2,79 k
per bird respectively.

Waldroup et al. (1966) evaluated a simplified low protein grower diet for
meat-type pullets, and found the control group (fed 16 per cent protein and
2167 kcal PE per kg) to be significantly heavier than the group fed a 12 per ce
1320 kcal PE per kg high fibre feed. This group was in turn heavier than the
group fed a low protein diet (ten per cent protein, 2264 kcal PE per kg). Ther
was also a significant difference in the amount of feed consumed by the three
groups of birds. Consumption was highest by the birds on the high fibre diet,
and lowest by the birds on the low protein diet.

Summers, Pepper, Slinger & McConachie (1967) fed broiler pullets a control
diet containing 13,4 per cent protein and 1118 kcal metabolizable energy (ME)
per pound (lb); a diet containing feather meal (13,7 per cent protein, 1372 kc
ME per lb), and a diet containing 80 per cent wheat bran (15 per cent protein,

750 kcal ME per lb). Although the feed consumption of the birds fed wheat bran
was 50 per cent greater than that of the controls, these birds were significant
lighter than both other groups. Imbalances of amino acids were thought to play
an important role in the reduction of body weight of the birds fed the wheat
bran and the feather meal rations. The workers concluded that the reduction in
live weight at point-of-lay, and not delay of age at sexual maturity, was the
important objective in restricting pullets.

Fuller (1966) demonstrated that energy restriction during the growing period
delayed maturity in pullets of heavy breeds to a greater extent than did protein
restriction. The lowest level of protein fed in his trial was twelve per cent.
The restricted group had a far lower carcass fat content at maturity and laid
more and significantly larger eggs than did the controls which were not delayed
in maturity.

Fuller, Potter & Kirkland (1969) conducted a further experiment in an
attempt to ascertain which of the factors, leanness or age at sexual maturity,
may have been responsible for the beneficial effects on subsequent productivity
Energy restriction and declining daylength were simultaneously employed in an
attempt to delay maturity with and without an accompanying decrease in carcass
fat. The birds on a restricted energy diet exhibited reduced carcass fat and
an increased age at sexual maturity. Decreasing daylength also delayed maturi
but within each feeding treatment pullets delayed in this manner were fatter a
maturity than those receiving increasing daylength. The advantages of this
method of delay were considerably less for pullets housed in the summer than f
those housed in the winter, which was interpreted as a relationship between
obesity and the effects of temperature. The workers concluded that the advan-
tages of decreasing daylength are additive to those of restricted energy intak

Summers, Pepper & Moran (1969) realised that a feed restriction programme
would be more effective, and would subject the birds to less stress, if the
programme was started at day-old rather than at eight weeks, as was practised

by Howes & Cottier (1964), Singsen et al. (1965) and Waldroup et al. (1966).
Cannibalism and feed medication, two problems involved in conventional restrict
feeding programmes would be reduced or eliminated. The birds kept on a 14 per
cent protein diet from day-old in the experiment of Summers et al. (1969) were
246 g lighter, and consumed an average of 10,2 g of feed a day less by 24 weeks
of age than did the birds kept on a 20 per cent protein diet to eight weeks of
age.

As there are numerous advantages to a feed restriction programme, it was
decided to test an unconventional system in this trial. The system was devised
in an attempt to overcome the unfavourable effects of restricting the body weig
of broiler breeder pullets by dietary manipulation. The programme used consis
of a reduction in the protein level of the diet when the broilers were twelve
weeks of age. It was expected that the intake of nutrients from a diluted fee
would be reduced initially because the birds would be unable to consume a suff.
cient volume of feed to compensate for its reduced nutrient concentration. Th
difference in nutrient intake would result in a difference in body weight gain
of the treatment groups so that within a short time the weights of the birds
should be different. The difference in body weights should then result in fur
differences in feed intake resulting in an even greater disparity in the weigh
gains.

Body weights, feed intake and the effect on age at sexual maturity were
measured to ascertain the extent to which this programme might be of benefit i
restricting the body weight of broiler breeder pullets at point-of-lay. The e
production of a small number of birds from each treatment was recorded in orde
to observe the effects of these dietary manipulations on the initial fourteen
weeks of lay.

1.2 Experimental procedure

The study was conducted at Ukulinga, the Research Farm associated wit the Faculty of Agriculture, University of Natal in Pietermaritzburg.

A total of 360 White Rock females, five weeks of age were used in the experiment. These birds were weighed, wingbanded and placed in 36 pens such th the total weight of the ten birds in each pen was the same for all pens. The pens were in the form of sunporches with a floor area of one square metre and each pen had one metre each of feeding and watering space. Feed and water were supplied ad libitum. Hessian blinds were used in the initial weeks to reduce exposure of the birds to cold winds which were experienced at that time.

The diets employed in the first seven weeks of the experiment were at thre protein levels and two energy levels. After twelve weeks of age the treatments were subdivided as shown in Table 1.1.

TABLE 1.1 Protein and energy levels used in the experiment, indicating the subdivision of protein treatments at twelve weeks of age

Growing treatment	Period 5 to 12 weeks		Period 13 to 22 weeks	
	Protein (percent)	Energy (kcal/kg)	Protein (percent)	Energy (kcal/kg)
AA	16,12	2260	16,12	2260
AB	16,12	2260	13,95	2260
AC	16,12	2260	11,78	2260
BB	13,95	2260	13,95	2260
BC	13,95	2260	11,78	2260
CC	11,78	2260	11,78	2260
DD	16,12	2978	16,12	2978
DE	16,12	2978	13,95	2978
DF	16,12	2978	11,78	2978
EE	13,95	2978	13,95	2978
EF	13,95	2978	11,78	2978
FF	11,78	2978	11,78	2978

Treatments were randomly allocated to the pens, there being three repli-
cations of each treatment.

The composition of the diets fed in the experiment are presented in
Table 1.2, and the composition of the vitamin and mineral premixes in Table 1.2

Protein, energy and amino acid composition of the diets was formulated
according to a generalized matrix as used by the Poultry Science section at the
University of Natal. The amino acid composition of the diets is presented in
Table 1.3.

TABLE 1.2 Composition of the diets fed in the experiment. (Percent-
ages, except that of energy is given in kcal/kg).

Ingredient	Diets					
	A	B	C	D	E	F
Yellow Mealie Meal	39,41	41,65	43,89	32,09	34,35	36,59
Germ Meal	14,72	15,85	16,98	11,70	12,84	13,97
Bran	14,72	15,85	16,98	11,70	12,84	13,97
Lucerne Meal	4,91	5,28	5,66	3,90	4,28	4,66
Rice Hulls	6,18	6,55	7,13	4,91	5,38	5,86
Sunflower oilcake	8,57	5,71	2,86	10,24	7,37	4,50
Fishmeal	7,50	5,00	2,50	8,96	6,45	3,94
Monocalcium phosphate	0,08	0,46	0,84	0,08	0,42	0,80
Limestone	2,62	2,80	2,98	2,46	2,65	3,16
Vitamin Premix	0,12	0,12	0,12	0,12	0,12	0,12
Mineral Premix	1,40	1,40	1,40	1,40	1,40	1,40
Oil	-	-	-	12,50	12,50	12,50
Protein	16,12	13,95	11,78	16,12	13,95	11,78
Fibre	9,466	9,466	9,467	8,232	8,232	8,232
Energy	2260	2260	2260	2978	2978	2978
Calcium	1,530	1,530	1,530	1,530	1,530	1,530
Phosphorus	0,701	0,700	0,700	0,701	0,700	0,700
Density (m^3/metric ton)	1,686	1,716	1,742	1,575	1,575	1,607

TABLE 1.2.1 The composition of the vitamin and mineral premix
formulations used in the diets

Vitamin Premix	mg/kg	Mineral Premix	g/100 kg
Thiamine	25	KH_2PO_4	1050
Riboflavin	16	NaCl	800
Ca-pantothenate	20	Fe	50
Pyridoxine	6	$MgSO_4$	250
Biotin	0,60	$MnSO_4.H_2O$	20
Folic acid	4	KI	1
Menadione	5	$CuSO_4$	1,28
Vitamin B_{12}	0,02	$ZnCO_3$	20
Nicotinic acid	150	$Na_2MoO_4.H_2O$	1
Ascorbic acid	250		
Vitamin A (6600 I.U./g)	150		
Vitamin D (80 000 I.U./g)	7,20		
Alpha-tocopherol succinate	4,20		
Maize starch	67		

TABLE 1.3 Estimated amino acid composition of the growing diets
(percent of diet)

	N.R.C. Requirement[1]		Diets					
	6-12 weeks	13-20 weeks	A	B	C	D	E	F
Arginine	0,89	0,70	0,98	0,80	0,63	1,03	0,85	0,67
Lysine	0,80	0,62	0,73	0,60	0,50	0,73	0,63	0,50
Histidine	0,36	0,28	0,32	0,28	0,24	0,32	0,28	0,24
Methionine	0,36	0,28	0,35	0,29	0,23	0,36	0,30	0,24
Cysteine	0,27	0,21	0,41	0,35	0,30	0,41	0,35	0,30
Tryptophan	0,18	0,14	0,16	0,14	0,12	0,16	0,14	0,14
Phenylalanine	0,62	0,49	0,57	0,50	0,43	0,57	0,50	0,43
Tyrosine	0,53	0,42	0,41	0,35	0,29	0,42	0,36	0,30
Leucine	1,25	0,97	1,16	1,03	0,90	1,04	1,01	0,87
Isoleucine	0,67	0,52	0,51	0,51	0,42	0,62	0,53	0,43
Threonine	0,62	0,49	0,75	0,66	0,57	0,74	0,65	0,56
Valine	0,77	0,60	0,80	0,68	0,57	0,81	0,69	0,57

(1) Scott et al., 1969.

1.2.1 Body weight and feed intake from five to 22 weeks of age

The birds were five weeks of age in July, 1970, at the initiatic of the experiment. As no artificial lighting was available in the pens used, t birds were subjected to the normal seasonal increase in daylight length during the course of the experiment.

Feed consumption was measured each time the birds were weighed. The birds were weighed individually after a fasting period of 14 hours, and weighings too place at two- or three-weekly intervals throughout the growing period. At 12, 17 and 22 weeks of age two birds from each pen were removed at random and sacrificed. Tests conducted on these birds are reported in subsequent chapters The removal of these birds had the effect of increasing the floor space for the remaining birds in the pens. The main experiment was terminated when the birds reached the age of 22 weeks.

A 6 x 2 factorial analysis was performed on data at seventeen and twenty-two weeks of age to test for significant differences between treatments. The normal equations at twelve weeks were solved directly by putting linear combinations of the effects, as dictated by the numbers in the subclasses, equal to zero. This was necessary as the analysis of variance at twelve weeks took the form of a 3 x 2 factorial with different but proportional numbers of observatic in the subclasses. In this case the following applied:

	Protein 1	Protein 2	Protein 3
Energy 1	3	2	1
Energy 2	3	2	1

If α is the energy effect, $\alpha_1 + \alpha_2 = 0$ (assumed) and if Υ is the protein effect, $3\Upsilon_1 + 2\Upsilon_2 + \Upsilon_3 = 0$ (assumed). This makes the effects orthogonal, and the analysis of variance was then obtained from a 6 x 2 factorial design with

two modifications:

The S.S. due to protein

$$= \frac{Y_{i1}^2}{18} + \frac{Y_{i2}^2}{12} + \frac{Y_{i3}^2}{6} - \frac{\Sigma Y^2}{36}$$

and S.S. due to P x E

$$= \frac{\Sigma Y_{ij}^2}{n_{ij}} - C.F. - S.S. (P) - S.S. (E)$$

1.2.2 Age at sexual maturity; egg production; egg weight and body weight at 36 weeks of age

At 22 weeks of age, after two birds had been removed from each pen, the remaining birds were grouped together according to their treatments prior to this age. Twelve breeding pens were used to house these birds and a standard laying mash was fed _ad lib_.

The age at sexual maturity was recorded for each hen after the birds had been housed, although some had actually reached sexual maturity before 22 weeks of age. In these cases, only the pen as such was recorded as containing a laye

Egg weight was measured for the first two months of lay, and egg productio was recorded to 36 weeks of age. At this stage the experiment was terminated; the birds were weighed and two birds from each treatment were sacrificed for purposes of carcass analysis. These results are presented in CHAPTER II.

1.3   Results

1.3.1   Body weights

Mean body weights of the broiler pullets on each of the various treatments at 12, 17 and 22 weeks of age are presented in Table 1.4. The standard error of a single treatment mean and the coefficient of variation are given for each age group. As the treatments represent a classified set, the analysis of variance table is not presented. Significant differences ($p < 0.05$ between treatment means are indicated within each age group.

Significant differences in mean body weights were evident at 12 weeks of age. Within each energy level, protein treatments did not differ significantly from each other, although as the protein level decreased, body weights also decreased in most instances. Within protein levels, however, birds receiving high energy diets were significantly heavier than birds on low energy diets at two of the protein levels under study.

Very few significant differences in body weight were evident between treatments at 22 weeks of age. No significant differences existed between protein treatments within either of the two energy levels under study. In only one case was there a significant difference in body weights between energy levels within protein treatments, this difference being between Treatments AC and DF.

TABLE 1.4 Effect of different dietary treatments on mean body weight[1] (kg) at 12,17 and 22 weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary Protein Level (Percent)						S.E. C.V.
		16,12			13,95		11,78	
12	2260	2,019^a			2,061^{ad}		1,911^a	(2) 0,1013
	2978	2,252^b			2,236^{bc}		2,088^{acd}	(3) 4,78
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	3,000^{ab}	2,806^{cd}	2,722^c	2,906^{ad}	2,897^{acd}	2,818^{cd}	(2) 0,1097
	2978	3,110^{ab}	3,012^{ab}	3,140^a	3,019^{ab}	3,039^{ab}	2,956^{bd}	(3) 3,71
22	2260	3,888^{ab}	3,598^a	3,587^a	3,844^{ab}	3,689^{ac}	3,731^{ab}	(2) 0,252
	2978	4,059^{bc}	3,977^{abc}	4,106^b	3,802^{abc}	3,906^{abc}	3,922^{abc}	(3) 6,56

(1) Average initial starting weight at five weeks was 574 g.

(2) Standard error of a single treatment mean.

(3) Coefficient of variation (percent).

a, b, c, d Values not significantly different from others within the same age group ($P < 0,05$).

1.3.2 Feed consumption

Mean values for feed consumption, energy intake and protein inta
from five weeks of age are presented in Tables 1.5, 1.6 and 1.7. Significant
differences between treatments within each age group, together with standard
errors of treatment means and coefficients of variation for each age group are
indicated.

Feed consumption at 12 weeks differed significantly between energy levels
at each protein level studied, consumption being lower in the case of birds on
the high energy diets. Significant differences also existed between energy lev
within protein treatments, being higher amongst the birds on low protein diets.

At 17 weeks a number of significant differences in feed consumption betwee
treatments existed. In all cases but one, the feed consumption of birds on hig
energy diets was significantly lower than that of birds on low energy diets.
Significant differences between protein treatments within energy treatments wer
also evident.

In only two cases at 22 weeks did feed consumption not differ significantl
between energy levels and within protein levels. These exceptions were between
treatments AC and DF and between CC and FF. Few differences existed between th
feed consumption results at each energy level. There was a significant inter-
action between protein and energy at 22 weeks, with birds on low energy diets
consuming less feed as the protein level decreased, while the birds on the high
energy diets consumed more as the protein level was decreased. The table of
Analysis of Variance for feed consumption at 22 weeks is presented in the
APPENDIX I.

Energy consumption followed a very similar pattern to feed consumption at
each of the three ages studied. At 22 weeks there was no significant differenc
between the energy intake of birds on the low energy diets, and only the energy
consumption of birds on Treatment FF proved to be significantly greater than
that of birds on any of the high energy treatments.

Protein consumption proved to be significantly affected by the dietary treatments. By 12 weeks of age, birds on the low protein low energy treatment had consumed significantly less protein than the remaining birds on the low energy diets. However, no significant differences in protein intake were evid within the high energy treatments.

By 17 weeks, after the protein treatments had been subdivided, birds on t low energy treatments again demonstrated significant differences in the amount of protein consumed. Birds on high energy diets once again exhibited no signi ficant differences between treatments, except that birds on Treatment FF had consumed significantly less protein by 17 weeks of age than had the birds on Treatment DD.

Significant differences in the amount of protein consumed existed within both high and low energy levels by 22 weeks of age. As a result of a decrease in protein levels at 12 weeks of age, birds on both the low and the high energ treatments, whose protein content was reduced to 11,78 per cent, consumed sign ficantly less protein than did the birds remaining on the higher protein diets The protein consumption of these birds switched to low protein diets did not differ significantly from the amount of protein consumed by the birds remainin on an 11,78 per cent protein diet from five weeks of age.

1.3.3 Correlations between body weight, feed consumption, energy and protein intake

Total and partial correlation coefficients between body weight, feed consumption, energy intake and protein intake are presented in Table 1.8. The results are given according to the three age groups under study and significant differences at $P < 0,05$ and $P < 0,01$ are indicated.

Both total and partial correlation coefficients between energy intake and body weight were significant throughout the growing period. Similarly, feed intake seemed to be governed by protein intake as indicated by the significant total and partial correlation coefficients between these two variates.

Correlation coefficients between feed intake and body weight are negative, with the partial correlation coefficients being significant. This picture is almost identical to the correlations between protein intake and energy intake, with partial correlation coefficients being negative and significant.

TABLE 1.5 Effects of different diets on mean feed consumption (kg) from five weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary Protein level (per cent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	5,869[a]		6,588[b]		6,011[a]		(2) 0,3909
	2978	5,162[c]		6,114[a]		6,879[b]		(3) 6,57
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	11,258[cf]	11,551[ae]	10,943[cf]	12,416[a]	11,969[ac]	11,393[ac]	(2) 0,684
	2978	9,419[bd]	9,236[b]	10,407[d]	10,323[bd]	10,784[ef]	11,001[cef]	(3) 6,28
22	2260	18,136[abe]	18,040[abe]	17,068[aef]	18,810[b]	18,779[b]	18,140[abe]	(2) 0,973
	2978	14,637[c]	14,978[c]	15,512[cdf]	15,591[cdf]	16,133[cdf]	17,050[de]	(3) 5,75

a ... f; (2), (3): See footnotes to Table 1.4.

TABLE 1.6 Effects of different dietary treatments on mean energy consumption ($\frac{kcal}{1000}$) from five weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	$13{,}264^{a}$		$14{,}887^{bc}$		$13{,}584^{ab}$		(2) 0,1139
	2978	$15{,}372^{c}$		$18{,}208^{d}$		$20{,}486^{e}$		(3) 7,34
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	25,442 abf	26,102 abf	24,729 a	28,058 bgh	27,048 abf	25,747 abf	(2) 0,1977
	2978	28,050 cdfg	27,506 adfg	30,992 ceh	30,744 cdh	32,116 e	32,762 e	(3) 6,99
22	2260	40,985 ad	40,768 ad	38,570 a	42,506 ade	42,437 ade	40,992 ad	(2) 0,2795
	2978	43,592 bd	44,605 bd	46,196 be	46,432 bce	48,046 bc	50,776 c	(3) 6,38

a ... h; (2), (3): See footnote to Table 1.4.

TABLE 1.7 Effects of different diets on mean protein consumption per bird (g) from five weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (per cent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	946,20[a]		922,99[a]		708,50[c]		(2) 63,63
	2978	795,86[b]		830,52[b]		810,33[bc]		(3) 7,45
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	1814,6[a]	1739,7[a]	1543,7[be]	1732,3[a]	1550,7[bf]	1342,3[d]	(2) 100,99
	2978	1519,0[cef]	1363,0[cde]	1379,3[cdef]	1440,3[cdef]	1354,3[cd]	1295,3[d]	(3) 6,70
22	2260	2923,0[a]	2644,7[b]	2265,0[c]	2624,0[b]	2353,0[c]	2137,3[cf]	(2) 134,04
	2978	2360,0[cd]	2163,7[cdef]	1980,0[ef]	2175,0[cde]	1944,0[f]	2007,3[ef]	(3) 5,82

a ... f; (2), (3): See footnotes to Table 1.4.

TABLE 1.8 Total and partial correlation coefficients between body weight, energy, feed and protein intake at three ages during the growing period (partial correlation coefficients given in brackets)

Age	Variate	Body Weight	Feed Intake	Energy Intake
12 weeks	Feed intake	-0,1513 (-0,6615)**		
	Energy intake	0,5100** (0,6883)**	0,5026** (0,7979)**	
	Protein intake	-0,0149 (0,4304)*	0,4193* (0,5443)**	-0,0847 (-0,3367)
17 weeks	Feed intake	-0,2330 (-0,5310)**		
	Energy intake	0,5779** (0,6956)**	0,0977 (0,6377)**	
	Protein intake	-0,0516 (0,4527)**	0,5506** (0,5995)**	-0,2659 (-0,5036)**
22 weeks	Feed intake	-0,1560 (-0,3705)*		
	Energy intake	0,5519** (0,6154)**	-0,0800 (0,5058)**	
	Protein intake	-0,0252 (0,3834)*	0,5953** (0,6146)**	-0,3160 (-0,4651)**

* indicates significance at $P < 0,05$.

** indicates significance at $P < 0,01$.

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	Protein intake	-0,0516 (0,4527)**	0,5506** (0,5995)**	-0,2659 (-0,5036)**
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	Protein intake	-0,0252 (0,3834)*	0,5953** (0,6146)**	-0,3160 (-0,4651)**

* indicates significance at $P < 0,05$.

** indicates significance at $P < 0,01$.

1.3.4   Age at sexual maturity;   egg production;   egg weight and body
        weight at 36 weeks of age

        The mean age at sexual maturity (ten per cent production), egg

production, egg weight and body weight of the birds remaining after 22 weeks o

age are presented in Table 1.9.   The birds were 36 weeks of age at the termi-

nation of the experiment.

        Statistical evaluation could not be performed on these results as there

were no replications of treatments in the laying period.   The results are there

fore presented simply as observations of the effects of the various treatments

on subsequent production.

        With the exception of Treatment CC, the birds receiving the same ration

throughout the growing period were heavier at 36 weeks than the birds receivin

a step-down protein ration at 12 weeks of age.   In most cases the magnitude of

body weight restriction at 22 weeks appeared to persist to 36 weeks of age.

Birds on Treatment AC, for example, were lighter at 22 and 36 weeks of age than

those on Treatments AB or AA.   Similarly the birds on Treatment BC were lighte

at these ages than those on Treatment BB.

        No trend could be easily identified with regard to the effect of growing

treatments on three-month egg production or egg weight.   Age at ten per cent

production, however, appeared to be delayed by the step-down protein treatment

at the low energy level, but the opposite appeared to be the case with the high

energy treatments.

TABLE 1.9   Mean age at sexual maturity (ten per cent production), three
            month egg production per hen, egg weight, and body weight at
            36 weeks of age

Treatment	Body weight at 36 weeks (kg)	Age at ten percent prod. (days)	Mean three month total egg prod./hen	Egg weight (g)
AA	4,127	150	40	53
AB	3,826	156	36	55
AC	3,798	164	48	51
BB	4,229	142	55	54
BC	3,736	147	40	53
CC	3,853	158	48	54
DD	4,044	160	51	51
DE	3,560	162	27	54
DF	3,806	158	34	49
EE	4,027	163	47	51
EF	3,932	157	51	52
FF	4,056	159	46	53

1.4 Discussion

1.4.1 Body weights

Body weights of birds at 22 weeks of age in this experiment wer
in excess of the weights recorded by Fuller et al. (1969) at 20 weeks;
Isaacs et al. (1957) and Isaacs et al. (1960) at 21 weeks; and Waldroup et al.
(1966) and Summers et al. (1969) at 24 weeks. Considering the rapid strides
made in improving body weights of broilers in the past few years it is conceiv
that the strain used in this experiment was faster-growing, and hence more dif
cult to restrict, than strains of White Rocks used in feed restriction trials
three or more years ago. This possibility makes the efficient restriction of
body weight at point-of-lay even more difficult than it proved to be a number
years ago and should be borne in mind when comparing results of restriction
treatments carried out from year to year.

Significant differences in the body weights of birds on the different
treatments were observed throughout the trial period. In almost all cases the
birds on low energy diets were lighter than those fed diets high in energy. H
the amount of feed consumed by these broilers been based on a specific require
for energy (as suggested by Hill & Dansky, 1954; Hill, 1962; Gleaves, Tonkin
Dunkelgold, Thayer, Sirney & Morrison, 1963; Wells, 1963; Gill, Payne & Lewi
1965) it would be expected that energy consumption would not differ significan
between energy or protein levels, with the consequence that body weights shoul
not differ significantly. This in fact was not the case in the present experi
as there proved to be a significantly higher consumption of energy by the bird
fed the high energy diets. The birds tended therefore to overconsume energy,
and were not restricted by the amount consumed. This fact has been noted in
broiler breeders by Singsen et al. (1959) who have proved that broilers tend t
overconsume energy above their requirements.

Table 1.10 was drawn up to show the protein and energy consumed by the bi on both the high and the low energy diets at three different stages during the growing period. These figures are compared in the table with the estimated requirements for protein and energy according to Robinette (1964). It is obvi that energy and protein consumption were far in excess of the levels required adequate feed restriction. The comparison demonstrates further that the feed consumption by the birds on high energy diets was in greater excess of the requirements than the consumption of those birds on low energy diets. This ov consumption could therefore be held responsible for the excessively large body weights obtained in the present experiment.

Birds on the low energy treatment that were switched from a high to a low protein level at 12 weeks (Treatment AC), although not significantly lighter than any other low energy treatment at 22 weeks, nevertheless proved to be significantly lighter ($P < 0,05$) than the birds on two of the high energy trea ments. Similarly the birds on Treatment BC were significantly lighter than th heaviest group of birds on the high energy treatments. Thus, lowering the protein level in the diet at 12 weeks, if the ration is of a low energy type, appears to be of some merit in a broiler breeder rearing programme, and the incorporation of such a step-down protein treatment in this trial was successf in limiting weight gain. It must be emphasised, however, that the step-down protein treatment must be used in conjunction with a low energy ration, since the step-down high energy treatments were not successful in regulating weight gains.

This point will be discussed in more detail later.

TABLE 1.10/

TABLE 1.10 Comparison of the mean body weights and the energy and protein intakes per bird, for three periods at two energy levels, with the recommendations of Robinette (1964)

Period (weeks)	Desired[1] body weight (kg)	Energy level (kcal/kg)	Body weight attained (kg)	Energy intake ($\frac{kcal}{100}$)		Protein intake (g)	
				Requirement (1)	Consumed	Requirement (1)	Consumed
5 - 12	1,590	2260	2,015	102,20	138,58	630	897
		2978	2,219		171,69		809
5 - 17	2,090	2260	2,858	189,35	261,87	1116	1621
		2978	3,046		303,62		1392
5 - 22	2,500	2260	3,722	287,35	410,43	1660	2491
		2978	3,962		466,08		2112

(1) Data according to Robinette (1964) - modified.

1.4.2 Feed consumption

As no data on feed consumption between five and 22 weeks of age was available in the literature, no direct comparison with the feed consumed in this trial could be made. Most workers in this field (Isaacs et al., 1957; Isaacs et al., 1960; Waldroup et al., 1966; Summers et al., 1969) have recor feed consumption from eight to 24 weeks of age. Nevertheless, in most cases t reported feed intakes are lower than in the present study. The larger body weights recorded in this trial are an indication of this difference.

There was a significant difference between treatments in the amount of fe consumed. Birds on the high energy and high protein treatments consumed signi ficantly less feed than the birds on low energy diets. This is in agreement w the work of Hill & Dansky (1954), Berg & Bearse (1956), Hill (1962) and others

who found under ad lib. conditions that the energy content of a diet is pre-

dominant in governing feed intake. Energy is not the only governing factor,

however, as other factors such as protein content and the weight and density of

feed have also been shown to exert a significant influence on daily feed intake

(Gleaves, 1966). This point is illustrated by the feed consumption of the

birds to 12 weeks, and also from 13 to 17 weeks of age. Birds on high energy

treatments to 12 weeks exhibited to a marked degree a need to satisfy their

protein requirements, without regard to energy consumption. It can be seen fro

Tables 1.5, 1.6 and 1.7 that feed consumption in the case of birds on Treatment

F was higher than that of birds on Treatment E which was in turn higher than th

consumption on Treatment D. Energy consumption followed the same pattern, bein

significantly different at all three levels of protein fed. Protein consumptio

by the birds on high energy rations, however, did not differ significantly

between treatments indicating that the birds were successful in satisfying thei

protein requirements irrespective of the energy level of the diet. On the othe

hand, probably due to the bulk of the rations (Fisher & Weiss, 1956), those bir

receiving a low energy, low protein diet consumed significantly less protein to

12 weeks of age than did the birds on the two other low energy treatments. The

same picture was evident between 13 and 17 weeks, after the protein levels had

been reduced on a number of the treatments. The figures of feed consumption

between 13 and 17 weeks are given in Table 1.11. It is evident from this table

that birds switched over to a lower protein level at 12 weeks either increased

(in the case of the high energy diets) or decreased (in the case of the low

energy diets) their feed intake compared to the birds remaining on the high

protein treatments.

TABLE 1.11 Effect on subsequent feed intake, energy intake and
           protein intake of lowering the level of protein in the
           diet at 12 weeks of age

Treatment	Period 13 - 17 weeks			Period 18 - 22 weeks		
	Feed intake (kg)	Energy intake (kcal)	Protein intake (g)	Feed intake (kg)	Energy intake (kcal)	Protein intake (g)
AA	5,433	12 278	875,8	6,870	15 526	1107,4
AB	5,635	12 735	786,1	6,490	14 667	905,4
AC	5,076	11 472	597,9	6,125	13 843	721,5
BB	5,745	12 984	801,4	6,394	14 450	891,9
BC	5,462	12 344	643,4	6,813	15 397	802,6
CC	5,382	12 163	634,0	6,746	15 246	794,7
DD	4,195	12 493	676,2	5,219	15 542	841,7
DE	4,270	12 716	595,7	5,742	17 099	801,0
DF	5,277	15 715	621,6	5,105	15 203	601,4
EE	4,063	12 099	566,8	5,268	15 688	734,9
EF	4,817	14 345	567,4	5,349	15 929	630,1
FF	4,122	12 275	485,6	6,049	18 014	712,6

Conflicting reports appear in the literature pertaining to this subject.
Donaldson, Combs, Romoser & Supplee (1955) maintain that the widening of a
calorie/protein ratio by decreasing the level of protein in isocaloric rations
results in an increased feed intake. Similarly Combs, Bossard, Childs &
Blamberg (1964), in studies with growing chickens, fed rations containing diffe-
rent amounts of identical protein mixtures and found that voluntary energy con-
sumption increased in relation to energy needs as the protein level was reduced
Sibbald, Slinger & Ashton (1960) fed diets diluted with cellulose or kaolin and
found that the birds attempted to compensate for the reduced nutrient concen-
tration by increased feed intake. They were not successful in this, however,

resulting in lower body weights. These observations are in agreement with the

feed consumption figures obtained in this experiment where the protein levels

the high energy diets were reduced.

Waldroup & Harms (1962) found that laying type pullets did not overconsume

energy to meet their protein requirements. Also, Waldroup et al. (1966) noted

a tendency for feed consumption to be positively related to the protein content

of the diet in broiler breeder pullets, which agrees with the feed consumption

results of the birds on low energy diets in this experiment.

Anand (1961) has stated that on the basis of existing evidence it would

seem unwise to designate a single specific factor as being solely responsible

for feed intake regulation. The present experiment appears to confirm this

statement, as energy intake, protein intake and distension of the digestive

tract have all played a part in the regulation of food intake.

1.4.3 Correlations between feed intake, energy intake, protein intake and body weight

The highly significant positive total and partial correlation

coefficients between energy intake and body weight, and the negative total

(and significant partial) correlation coefficients between feed intake and body

weight are in agreement with the work of Hill & Dansky (1954), who noted such

a relationship in young chickens. They found that in spite of an increased

feed consumption as the dietary energy level was reduced, the total energy inta

was decreased progressively resulting in lower body weights, as was found in

this trial. Working with broiler breeder pullets, Britzman et al. (1965) and

Isaacs et al. (1966) reported very similar correlations. Waldroup et al. (1966

however, found heavier body weights with a low energy than with a high energy

diet. The low energy diet which these workers tested contained a higher protei

level than did the high energy diet. In consuming more feed due to the low

energy nature of the diet these birds will have consumed more protein than their high energy counterparts, and consequently would have been expected to be heavier.

The significant positive partial correlation coefficients between protein intake and body weight, with the effects of feed and energy intake eliminated, are in agreement with the results of Isaacs et al. (1960) and Waldroup et al. (1966). This in effect means that body weight of birds can be restricted by limiting their protein intake, proving that protein intake as well as energy intake should be controlled in order to attain a particular body weight at point-of-lay. In White Leghorns and similar breeds, protein intake can be relatively easily regulated by dietary manipulation. In the case of broiler breeders, however, it appears that this control can only be achieved by means of a system that supplies only a certain amount of protein and energy to the birds each day.

The correlation between feed intake and protein intake confirms the earlier observation and discussion concerning the fact that these birds exhibited a tendency to consume feed according to the level of protein in the diet, as in the case of the birds on low energy diets. This tendency must have predominated over the treatments for the correlation coefficient to be significant, in spite of the higher feed consumption, associated with low protein diets which are high in energy.

The significant total and partial correlation coefficients between energy intake and feed intake to 12 weeks of age seems to indicate that to this age the broilers consumed feed according to their energy requirements. After 12 weeks however, the total correlation coefficients decrease considerably, and may be regarded as zero. However, the partial correlation coefficients, eliminating protein intake and body weight effects, are significant. Thus the effects of eating to satisfy energy requirements (or according to the level of energy in the diet) have been masked by the high correlation between protein intake and

feed intake, which has presumably taken priority in governing the feed intake mechanisms.

1.4.4 Age at sexual maturity; egg production; egg weight and body weigh at 36 weeks of age

It must be emphasised that due to lack of numbers of birds avail able after 22 weeks of age no replication of treatments was possible. Consequently statistical analyses on these egg production results are not possible.

The fact that the mean weight of birds on some of the treatments appeared to decline by 36 weeks is due to the removal of six birds from each group at 22 weeks, and also to the fact that a number of birds died during the first three months of lay. Body weights between treatments at 36 weeks exhibited a similar pattern to that seen at 22 weeks of age. This indicates that a feed restriction programme such as the programme under study has the effect of inter fering with body weight gains throughout the period of growth, thereby affectin the weight at somatic maturity. This is in agreement with numerous reports tha body weight at sexual maturity is normally maintained throughout the laying period (Berg & Bearse, 1961; Bullock, Morris & Fox, 1963; MacIntyre & Gardine 1965; Harms et al., 1968; Fuller et al., 1969.)

Published work on the effect of controlling the feed intake of broiler breeders during lay is very limited. Singsen et al. (1959) maintain that some form of control is essential. They proved that ad lib. feeding resulted in higher mortality and obesity, with a consequent lower hen-housed average egg production. Rearing birds on an ad lib. feeding basis has an advantage in that once the birds have been placed on a laying diet, controlled feeding is not as important as it would have been had a limited-time or skip-a-day feeding schedu been used in the rearing period. Birds that have been reared in this latter

manner will tend to overconsume immediately they are placed on an ad lib.

feeding schedule. The gross overconsumption leads to a rapid gain in weight in

the ensuing weeks, in turn nullifying the advantages gained by the initial body

weight restriction. The present results are encouraging in that the effects of

the restriction programme on body weight depression persisted to 36 weeks of

age, thus obviating the need to apply any form of controlled feeding during the

laying period.

Many reports on the effects of restricted feeding during the rearing period

agree that feed restriction causes a delay in sexual maturity (Isaacs et al.,

1960; Fuller, 1966; Fuller et al., 1969). Fuller (1966) found that energy

restriction during the growing period delayed maturity to a much greater degree

than did protein restriction. The lowest level of protein which he fed was

12 percent, and he recognized that lower levels of protein may have resulted in

delayed maturity as found by Waldroup & Harms (1962). The results of the

present experiment suggest that low protein diets may delay sexual maturity to

a certain extent, if fed in conjunction with low energy diets. It must be

remembered however that these birds were reared on an increasing daylength which

has the effect (King, 1961) of accelerating sexual maturity. For this reason

the average age at sexual maturity was lower than would normally be the case

with these birds had they been reared on a decreasing lighting pattern. Fuller

et al. (1969) stated that energy restriction reduced carcass fat and increased

age at sexual maturity more than did a decreasing lighting programme. They

found the advantages of decreasing daylength to be additive to those of restric-

ing energy. It would appear essential to rear broiler breeder pullets on a decreas-

ing lighting programme, but it is suggested that the hours of lighting be kept

to a minimum so as to reduce feeding time.

Reports on the effects of restricted feeding on egg production and egg

weight are somewhat conflicting. However no reports are available to show that

a restricted feeding programme in the rearing period adversely affected egg

production. Most reports (Singsen et al., 1959; Isaacs et al., 1960;

Fuller, 1966; Fuller et al., 1969) indicate that a feed restriction programme improves egg production and increases egg weights. These two variables in the present study showed no trend, and appeared to behave in an entirely random manner. Very few birds were used in this section of the trial so no reliance can be placed on these results.

1.5 Conclusions

1. Although there were significant differences in body weights between treatments, body weight was not restricted sufficiently by any of the ad lib. feeding treatments. The desired weight was 1098 g lower than the lowest body weight recorded in the trial at 22 weeks. It appears therefore that no conventional ad lib. feeding system will reduce the body weight of broiler breeder pullets to the recommended level at point-of-lay.

2. In almost all cases, the high energy diets were responsible for heavier birds throughout the experimental period compared to the weight of birds on low energy diets. This difference is ascribed to the fact that feed consumption was regulated to a very limited extent by normal physiological mechanisms, resulting in an overconsumption of energy. This is in accordance with research reports mentioned in this study, and with the practical experience of poultrymen dealing with this type of bird.

3. From the feed consumption results at 12 weeks it appears that broilers fed a high energy diet tend to consume feed according to a need for a specific amount of protein. The consequence of this is that feed and energy consumption are excessive if birds receive a low protein diet. Conversely, intake is somewhat restricted by the bulk of the ration on a low protein low energy diet.

4. A decrease in protein level in conjunction with a high energy diet stimulates feed intake, with a consequent increase in body weight compared wit the birds fed the high energy, high protein diet.

5. Various theories are postulated in an attempt to explain the observed protein level-feed intake interaction. Some combination of these theories might be regarded as the most reasonable explanation for the results obtained.

6. Body weight at 36 weeks appeared to be influenced by dietary treatmen during the rearing period. This is an advantage of a restriction programme as birds do not overconsume feed when placed on a laying ration. The latter occu with other feed restriction methods such as the skip-a-day system.

7. Age at sexual maturity appeared to be delayed by a step-down protein treatment at low energy levels, but egg production and egg weight were apparen unaffected by the rearing treatments.

8. A single decrease in protein level of the low energy diet had the eff of decreasing feed intake and consequently body weight. Future possibilities restriction programmes might be the use of two or even more decreases in the protein level during the growing period, in conjunction with a low energy, high fibre diet.

CHAPTER II

The influence of restricted feeding on the
body composition of broiler breeder pullets

2.1   Review

Apart from the fact that body composition and the distribution of chemically or physically defined components in the carcass serve as indices of the nutritive and economic value of meat, the body composition of a bird prior to onset of lay has a bearing on the laying capabilities of that bird. Scott et al. (1969) make it clear that excess fat which is laid down in adipose tiss surrounding the reproductive organs will interfere with optimal egg production as well as predisposing the birds to greater stress with consequent high morta during hot weather. Evidence is accruing to show (Reid, 1971) that the body c position of poultry is pliant to nutritional treatments, i.e. body composition yields to dietary manipulation independently of the changes in body mass.

Very little work on the body composition of broiler breeder pullets on a feed restriction programme has been reported. Fuller et al. (1969), in an att to separate the effects of obesity from those of age at sexual maturity on sub sequent reproductive performance of White Rock pullets, subjected these birds a restricted energy intake, decreasing daylength, or both, during the growing period. Controls were full-fed and experienced an increasing daylength. A restriction of energy intake by one-third decreased the carcass fat from 25 pe cent on the control diet to 19,9 per cent and 23,8 per cent respectively with increasing and decreasing daylength. Full-fed pullets experiencing the decrea ing daylength pattern had carcasses containing 30,1 per cent fat. Because the egg production results paralleled these four restriction treatments, Fuller et al. (1969) concluded that the advantages of a decreasing daylength are addi tive to those of restricted energy.

Edwards, Ashour & Nugara (1971) conducted three experiments to determine the influence of various dietary fats on the carcass composition of broilers and on the fatty acid composition of adipose tissue. In one experiment they reared broilers to 20 weeks of age, the sexes being kept separate. They found that males and females differed considerably in carcass composition, particula with regard to moisture and fat content. The increase in fat content of the females occurred much earlier than in the males. However, for a given weight bird the protein and ash contents were approximately the same for both sexes. At 20 weeks the fat content of the female carcasses averaged 14,6 percent.

Numerous studies have been conducted on the effects of dietary protein an energy on the carcass composition of birds at an early stage of development. Fraps (1943) reported the chemical composition of growing chickens, used in studies on the utilization of the energy of feeds by these chickens. He found that the substitution of cottonseed oil for part of the corn meal in a standar ration produced chickens with a much higher than normal fat content. Substitution of corn meal with feeds of a lower productive energy content than that of corn meal produced leaner chickens. Hill & Dansky (1954) studied the effec of dietary energy level on growth and feed consumption by chickens. They show that when the energy content of the diet was lowered by adding oat hulls (from 0 to 40 per cent in the diet) the carcass fat content decreased from 26,8 to 16,1 per cent on a dry weight basis. Other workers (Newell, Fry & Thayer, 195 Rand, Kummerow & Scott, 1957; Spring & Wilkinson, 1957; Summers et al., 1965 Thomas, 1966; and Dean, 1967) have noted that specific changes in dietary fat protein or energy level produce changes in total body composition of chickens and ducks. These compositional changes are mainly in the ratio of moisture to fat, with protein level remaining relatively constant.

Donaldson, Combs & Romoser (1956) reported that as the ratio of energy to protein in the ration was widened, the energy intake and carcass fat depositio were increased and the water content of the carcass was decreased. Thus a hig

significant positive correlation between calorie/protein ratio of the ration
and tissue fat was evident, while highly significant negative correlations were
obtained between the calorie/protein ratio and water or protein content of the
carcass. The total fat and water content of the carcass remained constant unti
a calorie (productive energy)/protein ratio of 50 was exceeded. The fat deposi
ed in the carcass was in excess of the water displaced when this ratio in the
diets was widened to above 50. Widely different methods of dietary manipulatio
may be utilized to give different calorie/protein ratios, yet this ratio will
still have a high positive correlation with tissue fat in the birds fed these
rations (Edwards et al., 1971).

As reproductive fitness appears to be related to body composition (Fuller
et al., 1969) it would seem necessary to check the body composition of birds or
a feed restriction programme. Accordingly, the effect of the dietary energy
and protein intake on body composition was checked throughout the growing perio
during the experiment reported in CHAPTER I. It was hoped that this informati
might be of value in indicating the most suitable restriction treatment with
regard to the limitation of both body weight and carcass fat percentage.

2.2 Experimental procedure

Birds used for the carcass analysis studies were a representative
sample of the White Rock females used in the feed restriction trial reported in
CHAPTER I.

Carcass analyses for water, fat and protein were determined on a sample of
six pullets from each treatment at 12, 17 and 22 weeks of age. Before the bird
were killed a blood sample (20 ml) was taken from each. A heart puncture
technique was used for this purpose. After the birds had been killed (dislo-
cation of the neck between the atlas vertebra and the occipital bone), the
thyroid glands of each bird were removed for further study. Results of the

blood and thyroid analyses are presented and discussed in subsequent chapters.

The carcasses were placed individually in trays in a moisture-extracting oven, and it was found that constant-weight was attained after 14 days at a temperature of 95°C. The birds were removed after this period, and the percen̄ age water in each carcass was calculated from the wet and moisture-free weight̄

The water-free carcass was passed several times through an electric meat-grinder in order to ensure thorough mixing and grinding. Total lipid was deter̄ mined on a sample of the ground carcass using the method of DuPreez, Wessels & Stokoe (1971) which entails extraction of fat using chloroform and methanol (2:1) followed by diethyl ether (APPENDIX III). Nitrogen determinations were conducted in triplicate on the fat-free samples using a semi-micro Kjeldahl technique (A.O.A.C., 1966) (APPENDIX II).

All results were converted to percentages on a wet basis. A factorial analysis identical to that described in CHAPTER I was performed on the data. Total and partial correlation coefficients were calculated between the carcass components and feed, energy and protein intake. The correlations between carc̄ components and nutrient intake were determined in an attempt to ascertain the extent to which the carcass components of growing White Rock pullets could be manipulated by dietary means.

The birds killed at 36 weeks of age for carcass composition studies, reported in CHAPTER I, were placed in a drying oven for 14 days at 95°C. Carcass water was calculated by difference, and carcass fat was obtained using the regression equation of water on fat obtained from the carcass analyses at 12, 17 and 22 weeks of age.

2.3   Results

2.3.1   Body composition to 22 weeks of age

Mean body composition of birds on each of the treatments descri

in CHAPTER I are presented in Tables 2.1, 2.2 and 2.3. The tables indicate

respectively, body water, body fat on a wet basis, and body protein on a wet

basis. Standard errors and coefficients of variation are given for each varia

at the three age groups under study, and significant differences between treat-

ments are indicated.

At 12 weeks of age neither the low energy diets nor the high energy diets

produced significant differences in body water over the three protein levels.

Significant differences existed, however, between energy levels at each of the

three dietary protein levels, being lower in birds on the high energy diets.

A number of significant differences in body water between treatments were

evident at 17 weeks. These differences occurred in birds on the high energy

diets, and also in birds within protein treatments between the high and low

energy diets. No significant differences in body water occurred between any o

the birds on the low energy diets. This situation was essentially the same as

that at 22 weeks, where significant differences occurred in a few instances bu

only between protein treatments within the high energy diets, and between energ

levels within each protein treatment.

A highly significant negative correlation was obtained between body water

and body fat. Consequently the results of the body fat content of birds on th

different treatments followed an almost equal but opposite pattern to the resu

of the body water content of these birds. In the case of body fat content,

however, Treatment F differed significantly from Treatments D and E at 12 weeks

but at 17 and 22 weeks there were fewer significant differences within the ener

levels. Other significant differences proved to be similar to those between t

body water contents of birds on the various treatments.

Tests of significance indicated that, throughout the growing period, body

protein remained within very narrow limits for most of the energy and protein

treatments under consideration.

2.3.2 Correlations between body constituents and nutrient intake

Total and partial correlation coefficients between body water,

body fat and body protein, and between these components and feed, energy and

protein intake are presented in Table 2.4. The values are grouped according t

the three age groups under study. Significant differences between variates ar

indicated at $P < 0,01$ and $P < 0,05$.

At 12 weeks of age, a highly significant negative correlation ($P < 0,01$)

was found to exist between body water and body fat on a wet basis. Consequent

the values for each of these variates were found to be almost equal but opposi

when between-treatment means were analysed. At the same age, energy intake,

protein intake and body weight were correlated with body water. Body fat was

correlated positively with energy intake and negatively with protein intake.

At 17 weeks of age, both body fat and body protein were significantly

negatively correlated with body water. Protein and feed intake were signifi-

cantly positively correlated with body water, and negatively with body fat.

The significant negative correlation between body water and body fat was

evident at 22 weeks of age, as was the negative correlation between feed intak

and body fat. Body fat was found to be positively correlated with both energy

intake and body weight, this correlation proving to be significant. The

negative correlation between body water and body weight which was significant

at 12 weeks, was again significant at 22 weeks of age.

TABLE 2.1 The water content of the carcasses of birds randomly selected from each treatment at three ages during the growing period (expressed as a percentage of the live body weight)

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12	13.95	11,78				
12	2260	65,29[a]	64,84[a]	62,48 ac				(2) 2,208
	2978	61,41[bc]	60,64[bc]	58,57 b				(3) 3,52
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	58,34 a	55,18 ab	54,52 ab	57,23 ab	55,77 abe	57,08 ab	(2) 3,322
	2978	48,48 c	52,62 bcd	53,04 abd	53,56 abd	50,14 cd	50,69 cde	(3) 6,16
22	2260	51,84 ae	50,68 ab	53,12 ae	52,37 ac	49,99 ad	46,56 ad	(2) 5,468
	2978	41,44 d	48,66 ad	42,60 bd	44,82 bcde	44,71 bcde	52,91 a	(3) 11,31

a ... e; (2), (3): See footnotes to Table 1.4.

TABLE 2.2 The body fat content of the carcasses of birds randomly selected from each treatment at three ages during the growing period (expressed as a percentage of live body weight)

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12			13,95		11,78	
12	2260	14,25[a]			15,26[a]		ab 16,56	(2) 1,977
	2978	17,92[b]			18,27[b]		c 21,33	(3) 11,77
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	a 19,84	ac 22,79	ac 23,44	ac 21,45	ac 22,12	ac 21,77	(2) 2,806
	2978	b 27,85	bc 25,53	bc 24,78	abc 23,88	b 27,91	bc 25,96	(3) 11,72
22	2260	ab 24,73	abcde 28,09	a 23,47	abc 26,15	abcd 27,67	abcdef 29,93	(2) 4,617
	2978	f 36,26	bcef 32,31	ef 35,81	cef 33,17	def 34,37	abcd 27,04	(3) 15,43

a ... f; (2), (3): See footnotes to Table 1.4.

TABLE 2.3 The protein content of the carcasses of birds randomly selected from each treatment at three ages during the growing period (expressed as a percentage of live body weight)

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	18,13[a]		18,05[a]		18,24[a]		(2) 1,417
	2978	17,50[a]		17,71[a]		17,18[a]		(3) 7,95
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	18,21[a]	18,20[a]	17,98[a]	17,61[a]	17,91[a]	17,81[a]	(2) 1,714
	2978	17,92[a]	17,24[a]	17,96[a]	18,34[a]	17,17[a]	18,69[a]	(3) 9,56
22	2260	17,88[ab]	18,36[ab]	17,85[ab]	17,91[ab]	17,87[ab]	16,64[a]	(2) 1,422
	2978	18,37[ab]	17,86[ab]	17,38[ab]	19,15[b]	16,26[a]	19,08[b]	(3) 8,06

a, b; (2), (3): See footnotes to Table 1.4.

TABLE 2.4 Total and partial correlation coefficients between carcass con-
stituents and nutrient intake, and between body water, body fat
and body protein at three ages during the growing period

Age (weeks)	Variate	Body fat	Body protein	Energy intake	Protein intake	Feed intake	Body weight
12	Body water	-0,8720**	0,1608	-0,5594**	0,4381**	0,0542	0,3966*
		(-0,7518)**	(-0,1181)	(-0,1220)	(0,1470)	(0,0463)	(-0,1171)
	Body fat		-0,2240	0,5543**	-0,4358*	-0,0368	0,3297
			(-0,1261)	(0,2213)	(-0,0403)	(-0,1285)	(-0,1550)
	Body protein			-0,1827	0,1695	0,0566	-0,1137
				(-0,1384)	(0,0438)	(0,1056)	(0,0371)
17	Body water	-0,9268**	-0,3601*	-0,3412*	0,4441**	0,4664**	-0,2501
		(-0,9268)**	(-0,7200)**	(-0,1532)	(0,0136)	(0,1770)	(0,0069)
	Body fat		0,1061	0,3395	-0,4614**	-0,4798**	0,2559
			(-0,6284)**	(-0,0154)	(-0,0118)	(0,0146)	(-0,0298)
	Body protein			-0,0059	0,0238	0,0258	-0,0652
				(-0,1084)	(0,0487)	(0,1481)	(-0,0429)
22	Body water	-0,9427**	0,0022	-0,3070	0,2254	0,2960	-0,3754*
		(-0,9512)**	(-0,2882)	(0,4465)**	(0,1184)	(-0,4260)**	(-0,3050)
	Body fat		-0,0493	0,4149*	-0,3381	-0,4232*	0,3821*
			(-0,3062)	(0,4979)**	(0,1116)	(-0,4969)**	(-0,2490)
	Body protein			0,0393	0,0705	-0,1029	-0,0582
				(0,3092)	(0,2604)	(-0,3511)*	(-0,2436)

* indicates significance at P < 0,05.
** indicates significance at P < 0,01.

2.3.3 Body composition at 36 weeks of age

Means for body water, body fat and body weight of two birds fro

each treatment at 36 weeks of age are presented in Table 2.5. Corresponding

means for birds at 22 weeks of age are included for comparative purposes. As

only few birds were available for these determinations, which were not origin-

ally intended, no statistical analyses were carried out on these results. The

values are given purely as observations of the effects of feed restriction

during the growing period on body weight and carcass composition at 36 weeks.

TABLE 2.5 Comparison of mean body water, body fat and body weight
at 22 and 36 weeks of age of birds on different diet-
ary treatments to 22 weeks

Dietary treatment	Body Water (%)		Body Fat (%)		Body Weight (kg)	
	22 Weeks	36 Weeks	22 Weeks	36 Weeks	22 Weeks	36 Weeks
AA	51,84	48,30	24,73	28,95	3,888	4,127
AB	50,68	47,12	28,09	28,65	3,598	3,826
AC	53,12	51,07	23,47	20,85	3,587	3,798
BB	52,37	51,47	26,15	30,10	3,844	4,229
BC	49,99	52,72	27,67	24,35	3,689	3,736
CC	46,56	50,42	29,93	26,75	3,731	3,853
DD	41,44	53,71	36,26	23,05	4,059	4,044
DE	48,66	52,16	32,31	24,95	3,977	3,560
DF	42,60	48,79	35,81	28,50	4,106	3,806
EE	44,82	54,26	33,17	22,70	3,802	4,027
EF	44,71	50,00	34,37	27,20	3,906	3,932
FF	52,91	53,52	27,04	23,50	3,922	4,056

2.3.4 Regression of body fat on body water

The highly significant negative correlation coefficient between body water and body fat prompted the calculation of a regression equation of body fat on body water. The analysis of variance for testing the significance of the simple linear regression of carcass fat on body water is given in Table 2.6.

TABLE 2.6 Analysis of variance for testing the significance of the simple linear regression of carcass fat on body water (data at 12, 17 and 22 weeks being grouped together)

ANALYSIS OF VARIANCE

Source	D.F.	Mean Squares	F
Due to linear regression	1	10 770,42	2953,388[**]
Deviations from regression	214	3,6468	
TOTAL	215		

The regression equation expressing body fat in terms of body water is as follows:-

Y = 79,7 - 1,05X

where Y is the percentage of body fat on a wet basis;

X is the percentage of body water in the carcass.

2.4 Discussion

2.4.1 Body water, body fat and body protein

The fact that the body composition of the birds in this trial proved to be pliant to nutritional changes agrees very favourably with most of the published work on body composition studies with poultry. Amongst others, Hill & Dansky, (1951 and 1954); Donaldson et al., (1956); Summers et al., (19 Marion & Woodroof (1966); Du Preez, Du Plessis & Erasmus (1967) and Edwards et al. (1971) have all noted the effect of dietary energy and protein levels or body composition of broilers. These effects, as proved in this trial, demonstrate that the percentage of body fat increases as the energy level of the die increases, or as the protein level of the diet decreases. This is particularly noticeable at 12 weeks, and to a lesser extent at 17 and 22 weeks of age (see Table 2.2). Except for a few treatment means, the trend of increasing body fat or decreasing body water, follows the expected values for all the treatments.

The actual values obtained for the percentage of body fat on a wet basis are slightly higher at 22 weeks than the values obtained by Fuller et al., (19 However, the body weights were considerably higher than those recorded by Fulle It is conceivable that the major portion of the additional body weight of the birds in the present trial would be body fat.

The protein content of the carcasses remained surprisingly constant betwee treatments and at different ages. This agrees with the results of Fuller et al (1969) and Edwards et al. (1971). Bailey & Zobrisky (1968) state that the animal body is able to maintain to a high degree its essential protein composit regardless of differences in age, weight or nutrient intake. The present resul certainly support this statement.

Reid (1959) noted that a lower plane of nutrition and the resultant retardation of early growth are associated with the prolongation of the life span in

wide variety of animal and insect species. These observations, together with

the remarks of Scott et al. (1969) that overfat birds do not reproduce efficie

indicate that birds on the high protein, low energy diet in this experiment wo

be expected to show greatest reproductive fitness in the laying stage. The bi

on this treatment (AA) exhibited a significantly lower body fat content than m

of the birds on the high energy diets. The body fat content of these birds wa

also lower than that of all other birds fed low energy diets except for one di

(AC), although these differences were not significant. To keep the body fat

content of broiler breeder pullets as low as is possible with an ad lib. diet,

it would therefore appear that a high protein, low energy diet, i.e. a diet wi

a low calorie to protein ratio, would be required to be fed.

It is difficult to explain the fact that at 22 weeks the birds on Treatme

AC showed a body fat content lower than that of the birds on the high protein

low energy diet. This was not to be expected on the evidence that a lowering

the protein level in the diet results in an increased fat content (Petersen,

Grau & Peak, 1954). However this difference did not prove to be statistically

significant.

2.4.2 Correlations between carcass constituents and energy, protein and feed intakes and body weight

The highly significant negative correlation between body water

and body fat (percentages, on a wet basis) has been shown to exist in many

animals and birds (Brody, 1945; Combs & Robel, 1962; Summers et al., 1965;

Du Preez et al., 1969). A correlation of -0,97 in this trial was obtained by

grouping together data from all birds analysed, without regard to age or dieta

treatment imposed. It would appear therefore that a very accurate estimation o

carcass fat content can be made of carcasses whose water content is known. As

the water content of a bird is relatively easy to measure, this relationship

would appear to be very useful in carcass composition studies. However, in
young rats and chickens where a significant positive correlation between body
water and body nitrogen has been demonstrated (Bender & Miller, 1953;
De Muelenaere, Quicke & Wessels, 1960; and Combs & Robel, 1962) the negative
relationship between body fat and body water might not be as marked as indicate
in the present study. It is expected that as fat deposition begins to take
place in increasing amounts, with a corresponding "flattening" of the body
protein curve, the positive relationship between body water and body nitrogen
will diminish and then approximate zero. The low correlation between body wate
and body protein in the present experiment confirms this fact.

It would therefore appear that the method of Bender & Miller (1953) and
De Muelenaere et al. (1960) of estimating carcass nitrogen from body water is
suitable only for young rats and chickens. Similarly, estimating percentage f
from percentage water is valid only within limits and these limits must be
established separately for ages and species.

A number of the correlations in Table 2.4 are difficult to interpret. Th
significant negative total correlation found to exist between body water and
body protein at 17 weeks of age was totally unexpected. It is possible that tl
change of diet at 12 weeks had the effect of temporarily upsetting the normal
balance between the various body components as shown by the total correlation
coefficient between feed intake and body water, which was significant only at
17 weeks, and by the positive total correlation coefficient (non-significant)
between body protein and body fat at the same age.

The percentage fat in the carcasses tended to be positively related to bo
body weight and energy intake, but negatively related to both protein intake a
feed intake at all three ages studied. In support of the positive body weight
to body fat correlations, Hill & Dansky (1951 and 1954), Summers et al. (1965)
Du Preez et al. (1966), Thomas (1966) and Wethli (1968) found that by increasi
the level of dietary energy they obtained increased carcass fat, and this was

accompanied by heavier body weights. This would also account for the positive

relationship between carcass fat and energy intake, due to the fact that these

broilers tended to overconsume energy above their energy requirements. Accord

to Dickerson (1947) and Reid et al. (1968), a strong negative relationship

between feed conversion and body fat is indicative of low activity and large

appetite, which agrees well with the state of the birds in this trial.

The negative correlation between feed intake and carcass fat is an indi-

cation of the widely different energy levels used in this experiment. Birds o

the high energy treatments consumed less feed than those on the low energy

treatments, but nevertheless consumed more energy. Consequently these birds

exhibited a higher body fat content than the birds on the low energy diets.

Similarly, birds on the low protein treatments consumed less protein but more

energy than the birds receiving high protein diets, consequently showing a

higher body fat content. This trend was noticeable especially at 12 weeks, bu

persisted to a lesser degree throughout the growing period.

It is therefore reasonable to assume that the fat content of a carcass is

positively related to the amount of energy consumed by the bird, and any metho

whereby this energy intake can successfully be reduced, will result in a leane

carcass. The birds with a leaner body composition will in turn exhibit more

efficient reproductivity (Fuller et al., 1969).

A leaner carcass is, however, not necessarily related to a lower body

weight. Cohn (1963) and Reid et al. (1968) observed in rats that the frequenc

of feeding had a marked effect on body composition, but no effect on body weig

gain. Rats ingesting their daily allowance in two meals utilized a far higher

proportion of their dietary energy for fattening than did those allowed to

nibble continuously. These workers reported that the rats fed ad lib. had a

carcass fat content 6,4 per cent lower than the others. There are two questio

that arise from these observations. Firstly, is body weight the most importan

variate that should be limited in a broiler feed restriction programme, or

should carcass fat content be the criterion governing the method of restriction

The other question concerns the effect on body composition of a skip-a-day, or

limited-time feeding schedule as used by many large broiler breeding enterprise

With feeding time being limited it might be expected that the restricted birds

will tend to deposit more body fat as a consequence of "gorging" themselves on

every occasion that feed is presented.

The present data illustrate the point that a reappraisal of the variate to

be limited at point-of-lay is necessary. Birds on Treatments AA and EF at

22 weeks exhibited very similar mean body weights. Carcass fat content differe

however by 9,64 per cent (P nearly < 0,01). It would appear obvious from the

observations of the effects of obesity on reproductive fitness quoted previousl

that birds from Treatment AA would be more efficient egg producers than birds

from Treatment EF, even though their body weights were so similar at 22 weeks.

A reappraisal of the methods and the aims of feed restriction programmes as

applied to broiler breeder pullets is clearly required.

2.4.3 Carcass composition at 36 weeks of age

It appears that the body fat content of broiler hens is less

subject to variation near somatic maturity than at point-of-lay. This is demon

strated by the markedly lower carcass fat content of the birds at 36 weeks that

were grown on high energy diets. Little variation between the respective carca

compositions of birds on the low energy rearing diets was evident at 22 and 36

weeks of age. In fact, all the birds analysed at 36 weeks had a carcass fat

content approximating 25,8 per cent - the mean of all values obtained, i.e. the

range of carcass fat values had decreased considerably by 36 weeks of age.

An interesting observation concerning body fat at 36 weeks is the close

similarity between the trend in mean body fat percentages at this age and the

trend in body weight at 22 weeks. This suggests that dietary treatments durin₁
the rearing stage have a direct effect on body composition even to 36 weeks of
age.

2.5 Conclusions

1. Body fat content was related to the energy intake of the diet, being
lower in birds on low energy diets where the intake of energy was relatively
limited.

2. Similarly, body fat content was related to the protein content of the
diet, being lower for the high protein diets. This is due to the lower energy
intake of birds receiving the higher protein diets.

3. There was very little variation in the protein content of the carcasse₁
analysed, proving that the animal body maintains to a high degree the essentia₁
protein composition, regardless of differences in age, weight or nutrient intal₁

4. A highly significant negative correlation ($r = -0,97$) was obtained
between percentage carcass fat on a wet basis and percentage body water. A
regression of body fat in terms of body water ($Y = 79,7 - 1,05X$) was calculate₁
by grouping together data from the three ages under study. This equation may ₁
useful in estimating the percentage carcass fat from the easily-measured body
water content. Further study is required to ascertain the limits within which
this relationship remains valid.

5. Further research is needed to ascertain to what extent body weight an₁
percentage carcass fat should be limited in order to improve reproductivity.
the present study, birds on the low energy rations had both a lower body weigh₁
and body fat content than the birds on the high energy rations, and would

consequently be more favoured. However, there were cases where body weights were very similar between treatments, but body fat content differed significan In the latter cases a leaner carcass would presumably be favoured.

CHAPTER III

The effects of dietary protein and energy levels
in a feed restriction programme
on thyroid activity

3.1 Review

The thyroid gland and its secretions are necessary for normal growth

and development, although there is much conflicting evidence as to the influenc

of the thyroid gland on growth rate. Biellier & Turner (1957) considered that

there was a close relationship between early growth rate and thyroid secretion

rate, and Kamar (1961) assumed that the thyroid gland was associated with growt

because of its effect on metabolic rate and processes of cell division. The

gland has an important influence on the rate of absorption of foodstuffs from

the digestive tract, and on gastric motility of animals (Samuels, 1947). It

also plays a role in the lipid metabolism of the body (Gorbman & Bern, 1962;

Zarrow, Yochim & McCarthy, 1964). Wethli (1968) found the thyroid weight of

broilers to be positively related to the carcass fat content of the body.

Thyroid activity has been arbitrarily defined by Solomon & Dowling (1960)

as the rate at which the gland delivers biologically active hormones to the cir

culation. This activity can be measured in several different ways, some of wh:

are inaccurate whilst others do not give the thyroid activity of individual

animals (Mellen & Hardy, 1957). Kamar (1961) has emphasized the difficulty of

obtaining a reliable criterion of thyroid activity in chickens. In broiler

breeder feed restriction experiments, methods such as measuring the protein-

bound iodine in the blood, administration of thyroid active or goitrogenic sub-

stances, or radioactive iodine techniques (described by Mellen & Hardy, 1957)

to indicate thyroid activity are unsuitable.

Kamar (1961) recommends the use of histological examinations as a suitabl

and practical method of measuring thyroid activity. These t---b-:----

the rate of elaboration and secretion of the active principal of the "physio-
logically normal" thyroid gland can be reflected histologically. These changes
are actually an index of thyrotropic hormone secretion by the anterior pituitar
gland, but they nevertheless serve as an excellent indicator of thyroid activit
since the function of the thyroid is mediated directly by the pituitary
(Zarrow et al., 1964).

The thyroid has a greater capacity than any other endocrine gland to store
its secretions, and this is reflected in its histological structure. The gland
is composed of numerous follicles, each follicle being lined by a secretory
epithelium, composed of a single layer of cuboidal or low columnar cells. The
closed cavities of the follicles contain a homogeneous, gelatinous fluid, known
as the colloid, which is a storage product of the secretory epithelium (Turner,
1967).

It is generally accepted that the colloid content of the thyroid gland is
inversely related to its activity. Uhlenhuth, Schenthal, Thompson, Mech &
Algire (1945) considered the release and storage of colloid from the thyroid
follicles to be one of the most significant component functions of the secretor
activity of the gland, and consequently strongly recommended the use of colloid
content as a measure of thyroid activity. Lamberg (1953) noted a favourable
correlation between colloid per cent and basal metabolic rate. However, a wide
discrepancy of opinion exists in the literature with regard to thyroid size as
a measure of activity.

Huston, Edwards & Williams (1962) state that total thyroid output is not
necessarily determined by gland size, since at high temperatures the thyroids
are smaller relative to body weight but are more active per unit of body tissue
than in similar fowls held at lower temperatures. Winchester (1940) noted that
thyroid size and metabolic rate are closely related in hens. This was contra-
dicted by Mellen, Hill & Dukes (1954) with growing chickens. Mason, Bogart &
Kreuger (1956) and Mason, Nicholson, Bogart & Kreuger (1960) found that mice wi

large thyroid glands tended to have low metabolic rates, and _vice_ _versa_.
Mason _et_ _al._ (1956) were of the opinion that heavy thyroids may be associated
with impaired iodine metabolism which would increase gland size without a
proportional increase in functional capacity. Mueller & Amezcua (1959) obtaine
low correlations between thyroid secretion, determined with radioactive iodine,
and thyroid weight. Schultze & Turner (1945), Lever (1948), Tala (1952) and
Marshall (1961) all agree that thyroid weight is a very unreliable measure of
thyroid activity, since, in addition to colloid accumulation, increases in the
amount of stroma and vascularity of the gland may seriously distort the activit
values based on thyroid weight. Wethli (1968) contends that under conditions
of relative inactivity it is logical to conclude that the amount of colloid
will tend to influence the size of the gland, and a positive correlation betwee
thyroid weight and colloid per cent is to be expected. This also confirms the
positive correlation obtained by van Tienhoven, Williamson, Tomlinson &
MacInnes (1966) between colloid per cent and thyroid weight.

Wethli (1968) concluded that under "normal" conditions in growing chickens
when the thyroid gland is in a relatively "resting" state, thyroid weight is a
doubtful measure of the activity of the gland, tending, in fact, to be inverse:
related to the activity. However, thyroid weight may be useful as an indicato:
of activity under more extreme "abnormal" conditions, where greater differences
in activity, caused by more marked variations in the actual cellular contents
of the glands are considered.

Many workers, including Hoffman & Shaffner (1950), El-Ibiary & Shaffner
(1951), Hoffmann, Shaffner & Comstock (1953), Shaklee & Knox (1956) and Burger,
Lorenz & Clegg (1962) have found that thyroid weight, although not always an
accurate criterion, does provide some useful information regarding the activit)
of the gland in poultry.

Expressing thyroid weight as a percentage of body weight was considered by
Breneman (1954) to be advantageous when comparing experimental animals of the

same age. Oakberg (1951) and Hoffmann et al. (1953) strongly disagreed with this procedure, pointing out that ratios tend to underestimate differences in gland weight, and that gland weight usually becomes relatively smaller as body increases, so that heavier birds may be penalized. They recommend the use of regression and covariance to remove the effect of body weight on gland size. Burger et al. (1962), Snedecor & Mellen (1965) and Farrington & Mellen (1967) disagree with these workers. They maintain that adjustment of organ weight is only justifiable when a direct dependence of the organ weight on body weight exists. They include the thyroid gland as one of the organs for which correcti for body weight is of doubtful value, as the growth of the gland is affected specifically by hormonal influences which have a less-direct effect on general body growth.

From the above it was decided in this experiment to measure absolute and relative thyroid weight, as well as colloid percentage, and to use these three measurements as an indication of thyroid activity. It was hoped that correlat: between the various measurements might throw more light on the relationships between these variates.

The effect of thyroid activity on physiological processes of the body suck as growth, feed consumption and body fat deposition have not been well document for the chicken. Although Grossie & Turner (1965) and Hahn, Ishibashi & Turne: (1965) were of the opinion that thyroxin was only one of a number of hormones which influenced feed consumption, and Samuels (1947) thought that the body ha a certain power of adjustment with regard to the utilization of foodstuffs which is not under endocrine control, Wethli (1968) obtained consistent positive correlations between feed consumption and thyroid weight as well as between fee consumption and colloid per cent.

It is generally accepted that hypothyroidism is associated with fattening (Brody, 1945), whereas hyperthyroidism leads to the depletion of body fat depo: (Gorbman & Bern, 1962). Van Tienhoven & Cole (1962) and Cole (1966) have show

that hereditary obesity in chickens is partly due to hypothyroidism. Nalbando

& Card (1943) also observed that hypophysectomy of growing chickens caused a

reduced basal metabolic rate, decreased thyroid activity, and deposition of

large amounts of fat. Gribble & Peters (1951) give a reason for the above,

stating that thyroxine promotes fat catabolism by increasing the activity of

coenzyme A, and has the effect of increasing water retention.

Further evidence of a negative relationship between thyroid activity and

body fat content is provided by the data of Silberberg & Silberberg (1950) and

Fisher & Griminger (1963). Dickerson (1947) maintained that variations in

pituitary function, particularly the hypothalamus, may be a primary factor

causing feed intake to exceed feed requirements, with consequent obesity.

Secondary depression of thyroid activity may reduce metabolic needs without

affecting appetite and so produce the same effect. Silverstein, Sokoloff,

Mickelsen & Jay (1960) showed that the feeding of obesity-producing diets to

mice resulted in increased thyroid weights.

It is important to distinguish between fat deposition in the growing perio

and the fattening of the mature animal. It is likely that the supposition that

feed requirements are greater for the deposition of fatty tissue, because of it

higher energy content, than for non-fatty tissue will hold during the fattening

period (Wethli, 1968). On the other hand, the ability of some animals to fatte

more rapidly than others during the growing stage seems to be associated with

lower feed requirement per unit gain in weight. Heavy birds with a higher fat

content are possibly at a more advanced physiological age than smaller birds,

and their higher fat content may simply be due to this fact. Wethli surmises

that since these larger birds also grew faster, they consequently were more

efficient converters of feed during the period preceding the measurement of fa

content.

Differences in thyroid activity due to the treatments imposed during the

experimental period were determined by means of the same factorial analysis

described in CHAPTER I, so as to ascertain the effects of thyroid activity on weight gain, feed consumption and carcass fat content. Correlations on all data pertaining to thyroid activity were computed in order to study the relatio ships between the three measures of thyroid activity used in the trial, and weight gain, feed, energy and protein intake, as well as carcass fat. These results deal specifically with broiler breeder pullets between 12 and 22 weeks of age and on a feed restriction programme.

3.2 Experimental procedure

Six birds from each treatment in the broiler feed restriction experi- ment reported in CHAPTER I were removed at 12, 17 and 22 weeks. After the neck had been dislocated the thyroid glands were removed from each bird, the dis- section following the suggestions of Wethli (1968).

A ten per cent formalin solution was used as a fixative, the glands from each chicken being stored separately. Most of the surrounding tissue was remov before fixation, the remainder being easily removed just prior to the drying an weighing of each thyroid. Weighing was carried out to the nearest 0,1 mg. After fixation and weighing the thyroids were washed in water, and microscopic sections prepared by conventional paraffin wax techniques. Staining was accomplished with the use of Ehrlich's Acid Haematoxylin and Eosin.

A Visopan projection microscope was used for the measurements of colloid per cent. The projection head had a magnification of 10 X, and a finely- graduated, movable measuring rule was attached. A total magnification of 500 X was employed. The method of Uotila & Kannas (1952) was followed, two obliquel crossed lines, each 13 cm long, drawn on a piece of thin plastic sheeting being used as the criterion for measuring colloid percentage. Each field was selecte at random and measurements were made in all regions of the gland. The content

of the follicular lumen was regarded as colloid, and with the aid of a ruler

the amount of space along the line occupied by follicular lumen was measured.

This was repeated for three sections from each left and right thyroid gland,

making a total of twelve measurements per chicken. The mean of these measurem

was expressed as a percentage and regarded as a criterion of the activity of t

entire gland.

A factorial analysis identical to that described in CHAPTER I was perform

on the data. Total and partial correlation coefficients were calculated betwe

the three measures of thyroid activity and body weight, nutrient intake and

carcass fat.

3.3 Results

3.3.1 Absolute and relative thyroid weights

Mean absolute and relative thyroid weights of the broiler pulle

on each of the treatments at 12, 17 and 22 weeks of age are presented in Table

3.1 and 3.2 respectively. The standard error of a single treatment mean and t

coefficient of variation are given for each age group. Significant difference

$(P < 0,05)$ between treatment means are indicated within each age group.

At 12 weeks the mean absolute thyroid weights were significantly affected

by the three protein treatments, the lower protein levels having a depressing

effect on thyroid weight. The two energy levels had no significant effect on

thyroid weight. At 17 and 22 weeks the protein treatments appeared to be

responsible for differences in the absolute thyroid weights. Energy levels of

the diets fed had little effect on these weights at 17 weeks, and no significa

effect at 22 weeks.

Relative thyroid weight appeared to be affected by both the protein and energy levels of the diet at 12 weeks, with the high energy diets having a depressing effect on this variate. In most instances at 17 and 22 weeks, high energy diets had a depressing effect on relative thyroid weight, but this difference was significant only in a few instances.

TABLE 3.1 Effect of different dietary treatments on absolute thyroid weight (mg) at 12, 17 and 22 weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12			13,95		11,78	
12	2260	$270,66^a$			$216,16^{ab}$		$187,00^b$	(2) 58,306
	2978	$231,44^{ab}$			$225,16^{ab}$		$161,33^b$	(3) 25,56
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	$386,33^{abd}$	$385,00^{abd}$	$451,00^{ace}$	$345,67^{abd}$	$305,00^{bd}$	$415,67^{bc}$	(2) 69,504
	2978	$552,33^e$	$355,67^{dc}$	$331,67^{dc}$	$326,00^{dc}$	$383,67^{dc}$	$268,00^d$	(3) 18,50
22	2260	$493,33^{ab}$	$544,67^a$	$510,67^{ab}$	$458,67^{abc}$	$427,33^{abc}$	$372,67^{bc}$	(2) 79,142
	2978	$484,67^{ab}$	$523,33^a$	$506,67^{ab}$	$455,33^{abc}$	$505,67^{ab}$	$341,67^c$	(3) 16,88

a ... e; (2), (3): See footnotes to Table 1.4.

TABLE 3.2 Effect of different dietary treatments on relative
thyroid weight (mg/100 g body weight) at 12, 17 and
22 weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	12,802^a		10,833^{ab}		9,720^{ab}		(2) 2,774
	2978	10,302^{ab}		10,398^{ab}		7,857^b		(3) 25,70
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	16,183^{ab}	12,817^{ac}	15,287^{abc}	11,770^{ac}	12,497^{ac}	14,670^{abc}	(2) 3,450
	2978	20,897^b	10,987^c	10,360^c	10,803^c	12,660^a	9,763^c	(3) 26,08
22	2260	12,713^{ab}	14,877^a	14,213^a	13,040^{ab}	10,730^{bc}	10,373^{bc}	(2) 1,959
	2978	11,947^{abc}	12,863^{ab}	11,713^{abc}	12,097^{abc}	12,330^{ab}	8,707^c	(3) 16,15

a ... c; (2), (3): See footnotes to Table 1.4.

3.3.2 Colloid percentage

Mean values for colloid percentage in the thyroid glands of the
broiler pullets on each of the dietary treatments at 12, 17 and 22 weeks are
presented in Table 3.3. The standard error of a single treatment mean and the
coefficient of variation for each age group are also presented. Significant
differences between treatment means (P < 0,05) are indicated in the Table.

Very little variation was noticeable at 17 weeks of age, and no significar
differences were observed at 12 or at 22 weeks of age. In most cases, where
differences occurred, the birds on low energy diets had a slightly greater
percentage of colloid in their thyroid glands than birds fed high energy diets.

TABLE 3.3 Effect of different dietary treatments on mean colloid per cent at 12, 17 and 22 weeks of age

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (percent)						S.E. C.V.
		16,12		13,95		11,78		
12	2260	69,55[a]		74,16[a]		66,00[a]		(2) 9,645
	2978	76,90[a]		72,65[a]		73,33[a]		(3) 13,26
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	84,33[ab]	84,33[ab]	86,67[a]	84,00[ab]	82,00[ab]	85,67[ad]	(2) 3,113
	2978	85,33[ac]	79,67[b]	79,33[b]	80,67[bcd]	80,67[bcd]	80,33[bc]	(3) 3,76
22	2260	85,33[a]	88,67[a]	85,00[a]	87,33[a]	88,00[a]	88,00[a]	(2) 3,228
	2978	88,33[a]	87,67[a]	86,67[a]	90,67[a]	86,00[a]	88,00[a]	(3) 3,69

a ... d; (2), (3): See footnotes to Table 1.4.

3.3.3 Correlations between thyroid activity and nutrient intake, body weight and body fat

Total and partial correlation coefficients between absolute and relative thyroid weights, colloid percentage, body weight, body fat and feed, energy and protein intake at 12, 17 and 22 weeks are presented in Table 3.4. Values found to differ significantly are indicated.

Both total and partial correlation coefficients between absolute and relative thyroid weight proved to be highly significant, as expected. Colloid percentage proved to be highly significantly correlated with absolute thyroid weight at 17 weeks, and with relative thyroid weight at both 12 and 17 weeks.

Significant partial correlation coefficients between both absolute and relative thyroid weight and body weight were noted at 22 weeks.

At 12 weeks of age colloid percentage was the only measure of thyroid activity found to be correlated (partial correlation) to body weight, and at 17 weeks of age a significant total correlation between colloid percentage and body weight existed.

Very few significant correlations between feed, energy or protein intake and thyroid activity measurements were found to exist, the majority being present only at 17 weeks of age.

Body fat was both positively and negatively correlated with thyroid activ measurements, there being only one coefficient that proved to be significant. This was between colloid per cent and body fat at 17 weeks.

TABLE 3.4/

TABLE 3.4 Total and partial correlation coefficients between absolute and rel-
tive thyroid weight, colloid percentage, body fat, energy, protei
and feed intake, and body weight at 12, 17 and 22 weeks of age,
(partial correlations being given in brackets)

Age (weeks)	Variate	Relative thyroid weight	Colloid percent	Body fat	Energy intake	Protein intake	Feed intake	Body weigh
12	Absolute thyroid weight	0,943** (0,942)**	0,338 (-0,233)	-0,132 (-0,057)	-0,287 (0,226)	0,307 (0,157)	-0,250 (-0,421)*	0,00! (0,15
	Relative thyroid weight		0,384* (0,372)*	-0,164 (0,048)	-0,323 (-0,237)	0,337 (-0,010)	-0,124 (0,348)*	-0,13 (-0,21
	Colloid percent			0,383* (0,385)*	0,094 (-0,166)	-0,058 (-0,047)	-0,121 (0,082)	0,29 (0,38
17	Absolute thyroid weight	0,800** (0,740)**	0,420* (0,108)	0,083 (-0,035)	-0,318 (0,240)	0,132 (0,189)	-0,251 (-0,342)*	-0,05 (-0,30
	Relative thyroid weight		0,442** (0,207)	0,043 (0,183)	-0,357* (-0,390)*	0,212 (-0,088)	-0,160 (0,239)	0,06 (0,44
	Colloid percent			-0,286 (-0,103)	-0,475** (-0,070)	0,348* (0,113)	0,183 (0,068)	-0,38 (-0,23
22	Absolute thyroid weight	0,893** (0,931)**	0,110 (0,211)	0,041 (-0,074)	-0,111 (0,033)	0,211 (-0,086)	-0,126 (-0,211)	0,11 (0,49
	Relative thyroid weight		-0,001 (-0,187)	-0,093 (0,090)	-0,291 (-0,061)	0,338 (0,206)	0,040 (0,130)	-0,16 (-0,49
	Colloid percent			0,017 (-0,057)	0,060 (0,024)	-0,070 (0,006)	-0,116 (-0,042)	0,08 (-0,04

* indicates significance at P < 0,05.

** indicates significance at P < 0,01.

3.4 Discussion

3.4.1 Absolute and relative thyroid weights

Since no reports could be found in the literature concerning thyroid weights of broiler pullets to the age of 22 weeks, and because thyroid activity is influenced by so many factors, it was difficult to compare values obtained in this study with those obtained by other research workers. Wethli (1968) obtained thyroid weights of 62,22 to 79,70 mg in White Rock pullets at 12 weeks of age. These weights are much lower than those obtained in the present study at 12 weeks of age. The body weights of the birds in the present experiment were over 600 g (or 30 per cent) heavier than the pullets in Wethli experiment, and this may in part be responsible for the wide discrepancy in results. The thyroid weights are also larger than those obtained by van Tienh et al. (1966), and by Nestor & Jaap (1963). However, Shaklee & Knox (1956) published a significant linear regression coefficient of thyroid weight on bod weight of 9,85 mg per 100 g body weight, using New Hampshires. This figure agrees very well with the relative thyroid weights obtained in this trial, and consequently also with absolute thyroid weights.

It is well known that the size of the thyroid gland is influenced by seve variables such as age, sex, climatic conditions, diet, activity and species (Sturkie, 1965). With age and sex differences eliminated, there remain many variables which could have caused the differences in thyroid weight between th results and those of Wethli (1968), not least of these being the different str of White Rock pullets used in the two experiments.

In proportion to total body weight, the thyroid in the domestic fowl does not vary markedly with increasing age, whereas absolute thyroid weight is foun to increase with age (Sturkie, 1965). The results of this experiment agree we with these findings, as relative thyroid weight remained reasonably constant

throughout the period, whereas absolute thyroid weight was found to increase with age.

Coefficients of variation in absolute and relative thyroid weights at the three ages under study were slightly higher than those of Wethli (1968), but agree very favourably with the values recorded by Hoffmann & Shaffner (1950) with regard to absolute thyroid weight.

3.4.2 Colloid percentage

The interpretation of the histological results is based on the assumption that those processes concerned with the elaboration of thyroid horm proceed at a faster rate in glands with a relatively high columnar follicular epithelium and a small amount of stored colloid, while a lower, more flattened epithelium and larger quantities of colloid are characteristic of less-activel secreting glands (Uotila & Kannas, 1952; Chai, 1958; Jacobs, 1958; Solomon Dowling, 1960; Turner, 1960; Silverstein & Bates, 1961; Martin, 1964 and Wethli, 1968).

The percentage of colloid material in the thyroid glands of birds in this experiment compares very favourably (12 weeks of age) with the corresponding values recorded by Wethli (1968), but is somewhat lower than the comparable results of 85,8 per cent recorded by van Tienhoven et al. (1966). The values obtained indicate that a very large proportion of the thyroid glands of the bi in this experiment consisted of colloid. This would indicate relative hypo-thyroidism, and a tendency for the birds to be somewhat obese. This was actua the case, as discussed in CHAPTER II.

There was very little variation in the colloid percentage in this trial, at each age studied, indicating that the dietary treatments imposed on these birds did not cause thyroid activity (as measured by colloid percentage) to va

between treatments. These results contradict the suggestion by Wethli (1968)
that differences in colloid percentage might be due to dietary factors. Howeve
the birds Wethli worked with were younger than the birds used in the present
study, and at an early age broilers are very efficient converters of feed into
body weight. In such a situation a correlation between dietary treatments and
thyroid activity might be expected.

3.4.3 Correlations

The positive correlations between colloid percentage and absolut
thyroid weight (significant at 17 weeks) agrees well with the work of Wethli
(1968) in cases where the thyroid gland is in a relatively "resting" state. Th
very large percentage of colloid in the thyroid glands of the pullets in this
trial at all ages studied, indicates that these glands were not actively secret
ing thyroid hormone, and would thus be regarded by Wethli as being in a "restir
state. He points out that in this state thyroid weight would not be a reliable
measure of thyroid activity, weights tending in fact to be inversely related to
the activity of the gland. In such a case thyroid activity would be positively
correlated with thyroid weight, as was the case in the present trial.

In only one instance during the trial was absolute thyroid weight and body
weight significantly correlated, this being at 22 weeks, where there was a
significant partial correlation between these two variates. Relative thyroid
weight was negatively correlated with body weight at 12 and at 22 weeks, but th
correlation at 17 weeks was positive, the partial correlation coefficient at th
age being significant. These results contradict the highly significant corre-
lations between these variates obtained with chickens by El-Ibiary & Shaffner
(1951), Shaklee & Knox (1956), Draper & Firth (1957), Burger et al. (1962),
Nestor & Jaap (1963) and Wethli (1968). However, it must be remembered that th
birds in this trial were older than most of the birds studied in other investi-
gations, and that these birds at this age are vastly different in their the

overconsumed feed and energy, and hence are overweight and obese compared to normally-developing birds.

Thyroid weight appears to be related to the level of protein in the diet, but is not correlated with protein intake. Energy level of the diet seems to have little or no effect on thyroid weight, especially at 17 and 22 weeks of ag At 12 weeks of age the relationship between thyroid weight and protein content of the diet was most pronounced (Table 3.1). The reason for this is not known, and speculation is made difficult when the correlations between thyroid weight and either feed, energy or protein intake are also considered. The absence of a correlation between any of these variates could be interpreted in two ways. Either some factor other than nutrient intake (but related to the protein conte of the diet) is involved in the determination of thyroid weight, or alternative thyroid weight has little influence on feed intake. The design of this experi- ment was such that it is not possible to differentiate between these two alter- natives.

Colloid percentage and body weight were positively correlated at 12 weeks, negatively correlated at 17 weeks, and there was no correlation at 22 weeks of age. It is difficult to explain the change from a positive to a negative correlation at 17 weeks of age. Colloid percentage was significantly negativel correlated with energy intake and significantly positively correlated with prot intake at 17 weeks, these results contradicting the results at 12 and at 22 wee of age. A possible explanation for this contradiction is that the treatments were split up after 12 weeks, and in the period 12 to 17 weeks the birds that were switched to low protein rations would have undergone a period of adjustmer to their new rations. Energy consumption in this period was higher amongst the birds on high energy diets that were switched to lower protein diets, but the opposite was true of the birds on low energy diets that were switched to low protein rations (as discussed in CHAPTER I). This upheaval in adjusting to the new diets could have been reflected in the thyroid activity measurements at

17 weeks. By 22 weeks feed consumption had reverted to normal, and thyroid activity was once again negatively related to body weight and energy intake.

In this experiment, because the mean body weights of all treatments were essentially the same at the start of the experiment, weight gain or rate of growth may be considered to be equivalent to body weight. Hence, the correlat[ion] of thyroid activity and body weight refer equally to weight gain. Many worker[s] have indicated that rapidly growing chickens exhibit a higher thyroid gland activity than those which grow more slowly (Kamar & Mostageer, 1960; Kamar, 1961; Tanabe, 1965; and Farrington & Mellen, 1967). Biellier & Turner, (195[1]) state that the high correlation between thyroid secretion rate and growth rate of young chickens, turkeys (Smyth & Fox, 1951) and ducks (Biellier & Turner, 1951; Hoffmann, 1950) was added evidence for a direct relationship between thyroid activity and growth.

Wethli (1968) could not demonstrate this positive correlation between thyroid activity and growth. He attributed this to complex environmental fact[ors] or to the fact that the avian thyroid gland plays only a permissive role in th[e] growth process, facilitating rather than stimulating growth. One other point which might be used to interpret the present results is the fact that the thyr[oid] gland seems to be more involved with maturation than with growth (Khamsi & Eayrs, 1966). According to Brody (1945) and Hoffmann (1950) thyroxin may init[ially] accelerate growth of young animals, but, because of its maturation effect will eventually result in premature cessation of growth. It is unlikely that this effect was pronounced in the present study, considering the relative inactivit[y] of the glands and the large size of the birds.

Finally, Westerfield, Richert & Ruegamer (1964) state that the effect of thyroid hormones on metabolism and growth may be less pronounced in birds than in mammals.

The significant positive correlation between body fat and colloid percent[age] agrees with the results of Silberberg et al. (1950), van Tienhoven & Cole (196[ ])

Fisher & Grimínger (1963) and Wethli (1968), indicating that thyroid activity

is negatively associated with fat deposition. Little or no correlation betweer

thyroid activity and body fat at either 17 or 22 weeks of age, however, indica

once again that these birds should be regarded as "abnormal" with regard to

thyroid activity, as proposed by Wethli (1968). Differences in thyroid activi

among chickens that are obese and overweight are obviously much lower, and hen

not as easily recognisable as differences caused by relatively active thyroids.

3.4.4   General

A field of study receiving much attention lately, due to the

economic advantages of such a scheme, is the use of sex-linked dwarfism in a

broiler mother line used for the production of commercial broilers. The genet

and morphological characteristics of this condition have been described by

Hutt (1959). Dwarfism in the fowl, by analogy with dwarfism in the mouse

(Carsner & Rennels, 1960; Bartke, 1964; Lewis, Cheever & van der Laan, 1965),

might be due to hormonal deficiencies. Van Tienhoven et al. (1966) found the

colloid percentage of the thyroid glands to be much lower in dwarf chickens th

in others, and noted that their thyrotropic hormone secretion was lower. Alth

they were uncertain as to the physiological reason for this dwarfism, they

suspected that growth hormone accumulated in dwarf chickens instead of being us

normally. Further studies in this field might elucidate the basis of dwarfism

in birds. In turn, dwarfism might be of use to geneticists who have the probl

of breeding a bird that grows very rapidly to nine weeks of age, then slows do

this growth rate to such an extent as to reach a desirable weight at point-of-

lay.

The use of mild doses of goitrogenic agents, such as thiouracil, in the

feeding of broiler breeder pullets should be investigated. Andrews & Schnetzl

(1946) and Shaffner (1951) reported that thiouracil in the diet slightly impro

efficiency of feed utilization in poultry, with a depressing effect on both
feed consumption and growth. This effect is probably due to a reduced energy
expenditure, and a relatively lower maintenance requirement brought about by
thiouracil administration (Swift & French, 1954). Dosages are, however, very
important. Different strains and even individuals within a strain may react
differently to a given level of these substances (Glazener, Shaffner & Jull,
1949). Post (1963) discusses the problems involved in finding the optimum lev
of thyroid activity for each animal. The effects of thiouracil treatment to
limit body weight at point-of-lay, on subsequent egg production also requires
attention before such a scheme can be generally accepted.

3.5 Conclusions

1. Thyroid activity was measured by absolute and relative thyroid weight
and colloid percentage, these being the most suitable measures of activity for
the type of experiment reported here. These measures were not evaluated in
terms of more accurate measures of thyroid secretion rate such as the goitre-
prevention, or the radio-iodine assay methods, which are unsuitable for measur
ing the thyroid activity of individual birds.

2. Absolute and relative thyroid weights appeared to be influenced by
the protein level of the diet to a greater extent than by the energy level.
These thyroid measurements were nearly significantly correlated with both prot
and energy intake ($P < 0,05$).

3. The colloid content of the thyroid glands varied very little between
treatments at any of the ages studied, the glands in all cases exhibiting very
high colloid percentages. Glands of this nature are characteristic of "restin
glands with colloid being stored to a greater extent than being secreted.

4. Body fat and colloid percentage were significantly correlated at 12 weeks of age. This was to be expected considering the fact that the glands exhibited relative hypothyroidism with a consequent high body fat content. By 17 weeks, however, the values for colloid percentage had become so similar between treatments that colloid percentage was no longer correlated with body fat content at that age. This situation persisted to 22 weeks of age.

5. Measures of thyroid activity appear to indicate greater differences in the thyroids of physiologically very active birds. Under such conditions diet, environmental conditions and age might have a greater effect on thyroid activity than in the case of a "resting" gland.

6. Literature pertaining to this subject is reviewed in order to confirm these findings, and to speculate on the value of feeding goitrogenic agents to broiler breeder pullets.

CHAPTER IV

Serum protein and lipid content of
broiler breeder pullets
subjected to feed restriction

4.1 Review

4.1.1 Total serum proteins

There has been considerable interest in the possible use of

serum protein levels or the albumin-to-globulin ratio as a sensitive biochemica

index for appraising protein nutritional status. Feeding low protein diets has

been reported to be associated with decreased total serum protein and serum

albumin levels in rats by Allison (1955), in man by Scrimshaw & Behar (1961),

and in chickens by Leveille, Feigenbaum & Fisher (1960) and by Leveille &

Sauberlich (1961). Schendler, Hansen & Brock (1962) used serum albumin levels

to predict the degree of protein depletion in patients suffering from kwashiork

According to Arroyave (1962), plasma albumin levels are usually lower in

populations with a low socioeconomic standard of living and with a diet low in

protein of good quality. Albanese (1959), however, has cited cases where low

protein intakes have not been associated with low plasma protein levels; in

fact, above-normal levels have been observed in relatively undernourished indiv

duals. Graham, Cordano & Baertl (1966) found that inadequate caloric intakes o

infants, although adversely affecting weight gain, favour the synthesis of seru

albumin. Conversely, when the protein in the diet is apparently adequate, high

caloric intakes which favour rapid gain and nitrogen retention can decrease the

serum albumin level.

Thomas & Combs (1967) conducted experiments with ten-day-old chickens to determine the effect of varying the protein and energy intake on body composit: and serum protein levels, and the relationship between body composition and sei protein levels. After a 14-day feeding period, they found that when dietary protein levels were reduced without changing the energy level, both total serun protein and albumin levels were reduced. When the daily energy allowance was reduced without changing the protein intake, however, there was a rise in both total serum protein and albumin levels. They noted a very high correlation between either total serum protein or albumin levels and body composition data, and suggested that serum protein or albumin levels may serve as an index of boc composition at a given age, rather than as an index of an adequacy of dietary protein. This hypothesis would explain why the serum protein levels rise with caloric-deficient diets, since under caloric restriction the percentage body fa decreases.

The data on total serum proteins in this trial were obtained in an attempt to clarify the above discrepancies in the literature, and to establish whether a relationship existed between serum protein and either body composition or nutritional status in broiler breeder pullets subjected to feed restriction.

4.1.2 Total serum lipids

In past years a considerable amount of literature has been pub-lished relating to the effects of changes in composition of the diet, particu-larly in the lipids, on plasma lipids and atherosclerosis (Bieri, Pollard & Briggs, 1957; Weiss & Fisher, 1957; Stamler, Pick & Katz, 1959; Feigenbaum, Fisher, Leveille, Weiss & Griminger, 1961, and others). However, very little work relating to effects of age, sex, nutrition or environment on total serum lipids has been forthcoming. Sturkie (1965) reviewed some of the literature pertaining to serum lipids, but reported no values for the total serum lipids of growing female chickens.

Heald & Badman (1963) showed that a vast mobilisation of free fatty acid
and total lipids in the blood occurred about two weeks before laying commenced
coinciding with an increase in plasma protein levels and with the growth of
ovarian follicles. This they attributed to the effects of oestrogenic secreti
A sharp drop in these variates occurs immediately after laying commences,
coinciding with oviposition.

It has been demonstrated in starving mammals (Masoro, 1962; Kronfield,
1965) and in the starving immature bird (Heald & Rookledge, 1964) that the lev
of plasma-free fatty acids are markedly elevated. According to Heald (1966)
these increased levels may be the result of increased glucagon secretion, as
glucagon, at least in the domestic fowl, can be considered to play a significa
role in regulating the levels of plasma-free fatty acids.

The effects of tallow and of cocoa butter on growth and on serum lipids i
chickens was compared by Yacowitz, Fleischmann & Bierenbaum (1964). They
regarded the differences obtained in the total serum lipids as being due to po
absorption of cocoa butter and not to differences in feed consumption resultin
in lower body weights and lower serum lipid levels in the chickens fed cocoa
butter.

It seemed reasonable to test the effects of different levels of dietary
protein and energy on the total serum lipids of White Rock pullets being used
the feed restriction experiment reported in CHAPTER I. In addition, the corre
lation between total serum lipids and body fat was calculated in order to
ascertain if such a relationship did exist. A significant correlation between
these two variates would be of considerable benefit in broiler breeding enter-
prises, and in breeding projects, the aim of which is to breed a bird (ducks,
for example) with a low body fat content. The percentage of carcass fat in th
body could then be ascertained without resorting to slaughter techniques.

4.2 Experimental procedure

Blood samples to be used in the determinations of total serum protein
and lipids were obtained from birds which had been removed from the experiment
at 12, 17 and 22 weeks of age for studies of carcass composition and thyroid
activity. A total of 20 ml of blood was withdrawn from each bird by means of
a heart puncture, prior to the birds being killed. The blood was deposited int
heparinized centrifuge tubes and the blood cells and plasma separated by centri
fugation. The plasma was stored in sealed 6 ml plastic tubes in a refrigerator
at -5^{0}C. A fibrin clot had formed in each tube by the time blood analyses were
carried out, and serum was therefore used in the determinations.

Total serum protein was determined using the methods of Mokrasch & McGilve
(1956) and Leggett Bailey (1962) (APPENDIX IV), readings being taken on a Bausc
& Lomb Spectronic 20 photometer. Total serum lipids were determined using a
modified technique developed by Baryshkov et al. (1966) (APPENDIX V).

It was found that the values of serum protein and serum lipid in the blood
samples at 22 weeks of age fluctuated excessively within treatments, presumably
due to the mobilisation of serum proteins and lipids for egg production (Heald
& Badman, 1963). Consequently these values are not presented. Also, the metho
of Leggett Bailey (1962) was found to be unsuitable for a number of blood sampl
at 22 weeks, possibly due to the high serum lipid content in the blood at this
age.

Factorial analyses similar to those reported in CHAPTER I were carried out
on the data at 12 and 17 weeks of age. Correlations between the total serum
protein or serum lipid values and body fat were calculated, as well as between
total serum protein and body protein.

4.3 Results

4.3.1 Total serum proteins and lipids

Mean total serum protein values for the birds removed from the experiment at 12 and 17 weeks of age, and regarded as being representative samples of the groups on the various dietary treatments, are presented in Table 4.1. Similarly, mean total serum lipids for these birds at 12 and 17 weeks are presented in Table 4.2.

A widening of the calorie to protein ratio at 12 weeks had the effect of reducing the total serum protein level. This was not as noticeable at 17 weeks although in all cases where the protein level at 12 weeks had been reduced to the lowest protein level, a reduction in the total serum protein level was evident. Birds that remained on the same diet from five to 17 weeks appeared to have very similar total serum protein levels. In only two cases were there significant differences between treatment means.

Total serum lipid levels were reduced in a similar manner to the reduction in serum protein levels with a widening of the calorie to protein ratio at 12 weeks. At 17 weeks, however, no significant trend could be seen in the mean serum lipid values.

TABLE 4.1 Mean total serum proteins at 12 and 17 weeks of age (g/100 ml)

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (per cent)						S.E. C.V.
		16,12			13,95		11,78	
12	2260	4,86†			3,84		3,80	(2) 0,2966
	2978	3,84			3,90		3,68†	(3) 14,47
		16,12	13,95	11,78	13,95	11,78	11,78	
17	2260	4,23	4,54	3,83	4,00	3,83	4,09	(2) 0,2767
	2978	4,37	4,21	3,94	3,88	3,65†	4,64†	(3) 13,48

† Within age groups, values with this superscript differ significantly from each other (P < 0,05).

(2), (3): See footnotes to Table 1.4.

TABLE 4.2 Mean total serum lipids at 12 and 17 weeks of age (mg/100 ml)

Age (weeks)	Dietary energy level (kcal/kg)	Dietary protein level (per cent) 16,12	13,95	11,78	S.E. C.V.
12	2260	598,4^a	451,8^{bc}	296,2^e	(2) 0,4967
	2978	468,4^{ab}	421,8^{cd}	395,2^d	(3) 29,42

Age (weeks)	Dietary energy level (kcal/kg)	16,12	13,95	11,78	13,95	11,78	11,78	S.E. C.V.
17	2260	287,4^{ad}	212,6^a	312,2^a	276,2^{ad}	307,4^{ad}	494,0^{cd}	(2) 0,5374
	2978	683,6^b	419,0^{cd}	579,8^b	398,2^{cd}	304,0^a	393,6^{acd}	(3) 27,63

a ... e; (2), (3): See footnotes to Table 1.4.

4.3.2 Correlations

Correlations between total serum protein values obtained at 12 and 17 weeks and the corresponding body fat and body protein values, as wel as between total serum lipids and body fat at 12 and 17 weeks of age are presented in Table 4.3.

The results indicate a significant negative relationship between total serum protein and body fat at 12 weeks of age, this being the only correlation found to be significant. The slight positive correlation between total serum lipid and body fat at 17 weeks of age is almost significant at $P < 0,05$. At 12 weeks, however, there is no relationship between these two variates.

TABLE 4.3 Correlation coefficients between total serum protein and both body protein and body fat, and between total serum lipids and body fat at 12 and 17 weeks of age

Variate	Total serum protein		Total serum lipids	
	12 weeks	17 weeks	12 weeks	17 weeks
Body protein	0,064	0,187		
Body fat	-0,426**	0,072	0,041	0,275

** indicates significance at $P < 0,01$.

4.4 Discussion

4.4.1 Total serum proteins

The values obtained for total serum protein levels in birds at 12 and 17 weeks of age agree well with the values recorded by Perk, Perek,

Loebl & Allalouf (1960) and Brandt, Clegg & Andrews (1951) for White Leghorns
at 12 weeks and New Hampshires at 16 weeks respectively. The values obtained
by Thomas & Combs (1967) are lower than those obtained in the present trial,
but the birds in that experiment were only 25 days old, so a lower serum prote.
level would be expected.

Serum protein levels decreased as the calorie to protein ratio widened.
This confirms the suggestion by Thomas & Combs (1967) that serum protein levels
are an indication of body composition at a given age, due to the fact that at
wider calorie to protein ratios the birds had become fatter. On the evidence
presented by these workers, it would be expected that total serum protein shoul
be correlated with body fat and with body protein. The correlation with body
fat was highly significant and negative at 12 weeks. The fact that the percent
age of protein in the carcass becomes fairly constant by 12 weeks would account
for the fact that total serum protein is no longer correlated with body protein
from 12 weeks of age, which is the case at 25 days of age according to Thomas
& Combs (1967).

The mobilisation of body protein, which is reflected by changes in blood
components just prior to the onset of lay (Vanstone, Maw & Common, 1955), due
to the presence of phosphoprotein (Bell & McIndoe, 1962; Heald & Badman, 1963)
would account for the variation in the mean serum protein values obtained at
17 weeks. This would also be responsible for nullifying the correlation betwee
total serum protein and body fat at 17 weeks of age. A number of birds began
laying just after 18 weeks of age, and the levels of total serum protein would
be expected to rise approximately two weeks before the onset of laying.

From the above it might therefore be concluded that total serum proteins
are affected by the diet inasmuch as high calorie to protein ratios tend to be
related to overfatness. This is reflected in the blood by a decrease in the
total serum protein level. Consequently total serum proteins are an index of
body composition at an early age, but only of body fat when the bird ages and
the percentage protein in the carcass becomes constant.

4.4.2 Total serum lipids

No directly comparable results are reported in the literature
with which to compare the values obtained for total serum lipids in the broile
breeder pullets at 12 and 17 weeks of age. The majority of work on serum lipi
in birds has been concerned with the cholesterol levels in blood, and the effe
of oestrogen on the mobilisation of fats just prior to the commencement of lay
ing. Non-laying hens were found to have a serum lipid level of 550 mg/100 ml
(Greenberg, Larson, Pearson & Burmester, 1936). Yacowitz et al. (1964) publis
values of 586 and 388 mg/100 ml for the total serum lipids of young chickens
fed tallow and cocoa butter respectively. These results are of the same
magnitude as those obtained in this trial.

From the results at 12 weeks of age (Table 4.2) total serum lipids and
body fat might be expected to show a high positive correlation, since each sho
a very similar trend between dietary treatments. However, due to a high coeff
cient of variation in the serum lipid results, the correlation coefficient
between serum lipids and body fat was actually low and non-significant. This
variation could be due to the method employed to determine total serum lipids.
As only 0,05 ml of serum were used per determination, a small error at this
level would actually be of large magnitude when converted to a figure expresse
as mg/100 ml. The second factor which might have caused this variation is the
fact that the analyses were carried out 12 months after the blood had been
collected. Difficulties were experienced with the refrigerator used to store
these blood samples, and it is possible that some degradation and hydrolysis
might have taken place during storage. Serum proteins were determined approxi
mately six months prior to the lipid determinations, and were not, therefore,
subjected to identical storage difficulties.

The fact that total serum lipids and body fat were not related in birds
at 12 weeks of age is disappointing, as this correlation could have been usefu
in numerous ways. Presumably total serum lipids do not indicate nutritional
status of the bird to the same extent as total serum proteins.

4.5 Conclusions

1. Total serum protein in birds is significantly negatively correlated with body fat at 12 weeks of age. This variate can thus be used as an index o body composition at a given age as suggested by Thomas & Combs (1967). The relationship disappeared at 17 weeks in this trial, due probably to the mobili sation of serum proteins prior to laying.

2. No correlations exist between total serum protein and body protein, or between total serum lipid and body fat at either 12 or 17 weeks of age. Reasons for these results are suggested.

3. Both total serum protein and total serum lipid can be used to predict the nutritional status of birds at 12 weeks of age. It appears that total serum lipid may be subject to large variations due to factors other than the ration fed. Reasons are given which might explain this variation.

SUMMARY

The aim of the experiment which has been described was to ascertain the feasibility of using a diet fed ad lib., to control the body weight of White Rock pullets at point-of-lay at a level below that attained when allowed to ea to repletion. There are numerous advantages to the ad lib. feeding of broiler pullets, although it is generally thought that this method is not as efficient in regulating body weights as are limited-time or skip-a-day feeding systems. Consequently step-down protein treatments at 12 weeks of age were incorporated into the trial in an attempt to introduce an unconventional factor into an ad lib. feeding schedule which might make such a system more efficient.

Three protein levels at two widely different energy levels were fed to 360 White Rock pullets beginning at five weeks of age. At twelve weeks the si treatments were split into twelve, by decreasing the levels of protein in the original diets. The high protein treatments were subdivided into high, medium and low, and the medium protein treatments into medium and low protein levels. The low protein treatment was maintained throughout the test period.

Although the birds receiving low energy diets were significantly lighter in weight than birds fed high energy diets, body weight was not restricted sufficiently by any of the treatments to warrant the use of this type of feedi in order to restrict the growth rate of broiler breeder pullets. The main reason for this is the fact that these broilers tended to overconsume energy and weight gains were consequently not restricted by the energy or protein content of the feed.

Birds on high energy diets tended to consume feed according to a need for protein in cases where protein levels were decreased at 12 weeks of age. On low energy diets the position was reversed, and these birds consumed less feed than the birds remaining on the high protein diets. Consequently the body weight of these birds was lower than that of the controls. A possibility exis

that a feed restriction programme incorporating a number of decreases in dieta
protein level throughout the growing period might inhibit the feed intake
regulatory mechanisms to such an extent that the body weight of broilers at
point-of-lay might be successfully controlled.

The different dietary energy and protein levels tested in the experiment
brought about significant changes in body composition, mainly with regard to
body fat and body water. Body protein remained fairly constant throughout the
period indicating that this body component is relatively unaffected by age
(from 12 weeks in this case), nutritional status or weight. Correlations amon
the measures of body composition and nutrient intake confirmed that excessive
energy intake was responsible for the high body fat content of the birds. Thi
overconsumption of energy was accentuated by the feeding of high energy or low
protein diets. The fact that some birds were relatively small and still over-
fat at point-of-lay, should lead to a reappraisal of the desired weight and bo
composition of broiler breeders at point-of-lay. Body fat and body water
content were found to be highly significantly negatively correlated, and a
regression of body fat on body water is presented. This may be of value in
future studies of the carcass composition of broilers.

In a feed restriction programme both energy intake and protein intake mus
be controlled. A decrease in energy intake was shown to reduce both body weig
and body fat at 22 weeks of age, and consequently energy intake should be
restricted if these two variates are to be controlled. However, the fact that
protein intake appeared positively correlated with body weight in this trial,
and that low protein levels are conducive to body fat deposition, indicates th
there is likely to be an optimum level of protein in the diet which would favo
both a slower growth rate and a leaner carcass. More research is needed to
ascertain the optimum level of daily protein intake of broiler breeder pullets

The thyroid gland plays a significant role in growth and development and
may be expected to provide an index of a bird's performance. In the case of

large, inactive birds, the thyroid gland is composed almost entirely of colloi

and is consequently itself enlarged and inactive. Few significant differences

between treatments with regard to thyroid activity were evident in the present

trial due to the relative inactivity of the thyroid glands of these broiler

breeder pullets. Smaller birds such as those of the Leghorn breed would be

expected to exhibit somewhat greater thyroid activity than broiler-type pullet

and on available evidence this activity would tend to be affected to a greater

extent by dietary factors than was the case in the present trial. The most

significant finding was the dependence of thyroid size on the protein content

of the diet. This indicates that the size of the thyroid gland may be a measu

of nutritional status of an animal at a particular age.

Blood analyses which were carried out at 12 and 17 weeks of age indicate

that body composition of birds prior to onset of lay can be judged by the leve

of total serum protein and serum lipid. Total serum protein was significantly

correlated with body fat content at 12 weeks, but total serum lipid and body

fat were not significantly correlated at either 12 or 17 weeks of age.

In order to be successful in limiting body weight of broilers at point-of-

lay the essential factor to be considered appears to be the limitation of energ

intake. This cannot apparently be achieved by feeding a diet ad lib., as feed

consumption of broilers is not regulated by the known physiological mechanisms

that normally maintain energy balance. Further research is needed in order

to devise diets which will provide the birds with the needed amounts of nutrier

but which will not lead to overconsumption even when provided ad libitum. It

possible that diets low in lysine may meet these requirements. More research

also needed in the field of goitrogenic feed additives which may aid in achiev-

ing the objective of regulated growth in broiler breeder pullets.

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APPENDIX I

Analysis of variance of feed consumption
from five to 22 weeks of age

Analysis of Variance

Source	DF	Mean Squares	F
Protein	5	9,076	10,34[**]
Energy	1	56,791	64,68[**]
P × E	5	4,338	4,94[**]
Error	24	0,878	
Total	35		

[**] indicates significance at $P < 0,01$.

APPENDIX   II

Modified semi-micro Kjeldahl technique
nitrogen determinations

Reagents:

1. Concentrated sulphuric acid (A.R.Q.)

2. Copper catalyst tablets.

3. Mercury catalyst tablets.

4. Saturated boric acid solution:-

> Weigh 500 g boric acid (A.R.Q.) and dissolve in distilled
> water by heating slightly. Make up to 10 litres.

5. Indicator:-

> 8,4 mg methyl red and 42 mg bromocresol green are dissolved
> in 150 ml ethyl alcohol.

6. Pumice stone.

7. Zinc powder.

8. Sodium hydroxide and sodium thiosulphate solution:-

> Dissolve 5 kg 99 per cent NaOH flakes in 10 litres distilled
> water. Add 500 g $Na_2S_2O_3.5H_2O$.

9. Standardised HCl solution:-

> Make up 0,5N HCl solution with Titrisol capsule. Dilute to
> 0,05N with distilled water.

Method:

1. Weigh out a sample of the ground, fat-free, moisture-free carcass onto cigarette paper. The weight should be approximately 40 mg.

2. The cigarette paper is folded and placed into a 100 ml Kjeldahl flask. To this is added one copper and one mercury catalyst tablet.

3. Approximately 10 ml H_2SO_4 is added.

4. Digestion takes place on stoves inside a fume cupboard, until a clear fluid remains. This usually takes from two to three hours.

5. The digest is cooled, and then shaken together with a small quantity of distilled water.

6. The contents of the Kjeldahl flask are then transferred to a 100 ml volumetric flask, washing the Kjeldahl flask thoroughly in order to transfer all traces of digest to the volumetric flask.

7. The solution is made up to volume with distilled water, and shaken thoroughly.

8. To each of three Kjeldahl flasks add 10 ml of this solution.

9. One portion of pummice stone and a number of glass beads are added to each flask.

10. 100 ml conical flasks containing ca. 10 ml of boric acid solution together with one drop of indicator are placed under the distillation apparatus.

11. A pinch of zinc powder is then added to each Kjeldahl flask.

12. Pour ca. 20 ml of $NaOH-Na_2S_2O_3$ solution into Kjeldahl flask, and connect immediately to distillation apparatus.

13. Distillation continues until the original volume has been approximately doubled. The colour will have changed to blue.

14. The conical flasks are removed from the apparatus, and replaced with containers of distilled water, after which the stoves are switched off. Water will be drawn into the apparatus, and in this way the system will be cleaned.

15. Boric acid solution is titrated with standardised HCl.

16. Percentage protein is calculated from the following formula:-

$$\text{Per cent Protein} = \left[\frac{\text{amount of HCl} \times 0,3501}{\text{sample weight} \times 0,10} \right] \times 6,25 \ .$$

(Method based on A.O.A.C., 1966)

APPENDIX III

Fat extraction for the determination
of carcass fat

1. Work with well-mixed, ground representative samples, which have been dried
    for at least eight hours. Determinations are made in duplicate.

2. Weigh out approximately 10 g of the sample into a plastic bottle.

3. Cover sample in bottle with solvent consisting of two parts chloroform
    and one part methanol.

4. Bottles are shaken for four hours on a mechanical shaker. The contents
    are then poured into a weighed, marked filter paper in a funnel on a
    250 ml conical flask.

5. Solids are transferred back into bottle after most of the solvent has
    filtered off.

6. The plastic bottle is filled once more with solvent and shaken for a
    further four hours.

7. The contents are then emptied once again onto the filter paper, the bottle
    being rinsed with solvent to remove all traces of sample.

8. Filter paper is folded and inserted into a soxlett extraction tube, fat
    being extracted with diethyl ether for four hours.

9. Remove paper and dry in oven for four hours. After cooling has taken place
    in a desiccator the filter paper plus fat-free material is weighed. Fat
    content is calculated by difference.

(Method of Du Preez, Wessels & Stokoe, 1969)

APPENDIX   IV

Technique for the estimation of
total serum proteins

1. Biuret Reagent, (Mokrasch & McGilvery, 1956).

The reagent contains 230 ml of 18 to 20 M NaOH, 2,5 g of $CuSO_4.5H_2O$

and 50 ml of 28 per cent ammonia per litre.

2. Method, (Leggett Bailey, 1962).

To carry out the test, 4,0 ml of the reagent is added to 1,0 ml of

blood serum, and the mixture is allowed to stand for 30 min at room

temperature. The extinction is read at 540 to 650 mμ.

3. A standard curve is produced using bovine serum albumen, and this

regression is used to calculate total serum protein in g/100 ml.

APPENDIX   V

Technique for the estimation of
total serum lipids

The modified technique of Barȳshkov et al. (1966) involves the use of
sulphuric acid, phosphoric acid and vanillin.

0,1 ml of serum is mixed with 4 ml c. H_2SO_4 and the solution is kept on
a steam bath for 20 minutes. After cooling, 16 ml of concentrated $H_3PO_4^-$: 0,6
per cent aqueous vanillin (4 : 1) is carefully mixed with the solution. After
one hour the extinction is measured at 415 mu.

A calibration graph is prepared by using glycerol trioleate dissolved
in diethyl ether. (In the present experiment, 1 ml of trioleate was dissolved
in 12 500 ml of diethyl ether, and quantities from 1 ml to 10 ml were used
for the calibration graph. Diethyl ether was evaporated off with the use of
liquid nitrogen before digestion with H_2SO_4 took place.)