THE RESPONSE OF BROILER BREEDER HENS TO DIETARY LYSINE: HATCHABILITY, EMBRYO GROWTH AND SUBSEQUENT OFFSPRING PERFORMANCE

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Submitted in fulfilment
of the requirements for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

in the Discipline of Animal and Poultry Science
School of Agricultural Sciences and Agribusiness
Faculty of Science and Agriculture
University of KwaZulu-Natal
Pietermaritzburg
2011

ABSTRACT

This study was conducted to determine the effects of breeder hen dietary lysine on offspring performance. In trial 1, Cobb 500 broiler breeder hens were fed 1200, 1070, 930 and 800 mg lysine/bird/d from 26 to 60 weeks of age or provided a gradual decrease from 1200 to 800 mg lysine/bird/d or a gradual increase from 800 to 1200 mg lysine/bird/d. Feed allocation was constant for all treatments at 160 g/bird/d. From each of these treatments 84 eggs were collected at 38, 48 and 60 weeks of age and incubated. The different lysine treatments did not have a significant effect on the percentage hatch of the eggs at any of the recorded ages. However, hatchability, unexpectedly, decreased linearly with increasing dietary lysine, although the R^2 for this was low. In trial 2, the effect of the maternal dietary lysine intake on the egg weight, yolk and albumen weights, embryo heat production and embryo growth rates of three genotypes (broiler, broiler x layer, and layer) and growth rates were assessed. The hens were fed either 920 mg lysine/bird/d (medium) or 816 mg lysine/bird/d (low). From each group 60 eggs were collected, weighed and then incubated. During the incubation from d 2 to d 18, 3 eggs were removed from each group every 2 d for eggshell temperature measurement and embryo weight measurement. There was a significant effect of lysine level on the embryo heat production of the broiler genotype with the birds from the high lysine treatment producing more heat and attaining a higher d 18 embryo weight. In trial 3, once the chicks had hatched, chick weight was recorded at 1, 7, 14, 21, 28 and 35 d. The chicks received a commercial starter and grower feed *ab libitum*. The broiler progeny from the birds on the high lysine treatment remained significantly heavier until d 14. The results indicate that an increased maternal lysine level improves the performance of a faster growing genotype up to 14 d of age.

PREFACE

The experimental work described in this dissertation was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from January 2009 to December 2009, under the supervision of Dr. Nicola C. Tyler and Dr. Mariana Ciacciariello.

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

DECLARATION - PLAGIARISM

I, Shaun Bernard Ruck declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed: ……

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following people and institutions for their invaluable contribution to this dissertation:

Dr. Nicola Tyler for her understanding, supportive nature and constant willingness to go the extra mile. Thank you for helping me keep it together.

Dr. Mariana Ciacciariello, my own “Jedi Master”, for her wonderful insights, invaluable input and direction and most importantly a firm guiding hand when required.

The Protein Research Foundation for a generous bursary which allowed me to pursue my studies in this field.

The National Research Foundation for funding.

My dearest mother, Jennifer Scarr, for giving me the support and encouragement to reach for the stars and to not give up until I reach them.

Dr. Oscar Blanco, for invaluable advice and guidance.

My fellow Masters students: Jessica Mattchett, Jamila Patel, Thami Khetani, Masefo Mokoma and Cheralyn Martins. It has been a pleasure to work alongside you.

The laboratory staff in the Animal and Poultry Department for their technical assistance and helpful insights, and Jolene Thomas for administrative assistance.

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1. GENERAL INTRODUCTION

It has been shown in the available literature that egg composition can be influenced by the nutrient composition of the maternal diet. Supplementation of the diet of a hen with vitamin A has an effect on the vitamin A, vitamin E, carotenoids and ascorbic acid in the embryonic liver and can influence the antioxidant status of the chick (Surai *et al*., 1998). It has also been shown that maize-based, as opposed to wheat-based, maternal diets improved the antioxidant status of the chick during embryonic development (Surai & Sparks, 2001). However, there is currently little research on the influence that the maternal diet has on the protein status of the chick. Most research that has been conducted on the influence of maternal dietary protein intake has indicated that it has no significant effect on the chick performance from hatch, although most of this research focused on the crude protein (CP) level in the diet rather than the amino acid (AA) concentrations and ratios (Lopez & Leeson, 1994b; Lopez & Leeson, 1995b). Al-Murrani (1978) determined that the protein levels in the egg were the main limiting factor to the development of the chick. It was further found by Al-Murrani (1982) that supplementing the egg with AA *in ovo* improved the development of the embryo. A developing embryo undergoes several metabolic processes such as tissue formation, which involve protein. These processes utilise energy and produce heat, thereby measuring the heat produced the metabolic activity of the embryo can be inferred. Thus a faster growing and developing embryo will produce more heat than a slower growing one (Boerjan, 2006). This can be corroborated by measuring the actual growth of the embryo. Thus an investigation of embryo growth and heat production could also be a useful indicator of the influence of maternal diet on egg composition and embryo performance. This would have important implications for the broiler industry as the maternal input contributes less than 7% of the feed consumed by the broiler over the production cycle (Calini & Sirri, 2007).

As with the embryo development, most reports on the impact of maternal CP intake on chick performance have shown no significant improvement with an increase in maternal CP intake (Pearson & Herron, 1981; Spratt & Leeson, 1987; Lopez & Leeson, 1994b), although this could also be due to a lower sensitivity to increased protein in older

genotypes. As the main focus has been on CP rather than any specific AA there is a possibility that an improvement in not only the day-old chick but also the growing broiler may be possible by focusing on specific AA. This would have important ramifications for broiler production. An investigation of how dietary protein in the maternal diet influences the hatch, embryo development and post-hatch growth would provide useful information for improving chick performance.

The work presented in this dissertation is a study of the influence of maternal dietary lysine on the hatchability, embryo growth and subsequent offspring performance of broiler chickens.

2. LITERATURE REVIEW

2.1 INTRODUCTION

In the past, chicken breeds were dual purpose, with the same type producing both eggs and meat with a disadvantage of limiting the maximum potential output of these birds for either meat or eggs. However, modern selection methods have produced birds that are aimed at maximising a particular facet of production in the chicken. This has resulted in two main types of chickens being developed: the commercial layer and the broiler (NRC, 1994). As the name suggests, the layer hen has been genetically selected to maximise egg output. This, through reducing the body size and maintenance requirements, has allowed for more of the ingested nutrients to move into egg production. The broiler on the other hand has been selected primarily for meat production and so has the potential for a greater growth rate and larger final bodyweight than the layer. This, in turn, has reduced the broiler's ability to produce eggs. One of the main reasons that broiler egg production is worse than layers is due to the potential to become over weight after peak production (Lopez & Leeson, 1995a).

Protein is an essential building block of all life. It is formed from a combination of amino acids (AA) and allows for the formation of complex organic structures such as muscle fibres, feathers, the bone matrix and soft tissue organs (NRC, 1994). There are 22 AA that are used in varying combinations to form the proteins found in animals such as birds (NRC, 1994; Leeson & Summers, 2001). Of these 22 common AA that form the macronutrient of protein, 10 are essential for growth and production in birds (Murphy, 1994). Protein is synthesised from the DNA template of a cell which uses RNA to create the code for the formation of each protein molecule from available AA. As the synthesis of protein molecules requires the presence of adequate and balanced amounts of the required AA, those that cannot be synthesised within the body itself must be provided from ingested protein. Amino acids that are not needed in the body for protein synthesis are degraded for either glucose synthesis, fat synthesis or degraded directly to carbon dioxide and water (Leeson & Summers, 2001).

With the high popularity of chicken products production systems are continually revised and updated to achieve optimal outputs. Most improvements in broiler production focus on the broiler from hatch to marketing (Morris & Njuru, 1990; Mack *et al*., 1999). However, more attention is being given to the impact that the broiler breeder has on the performance of its offspring, and in particular the maternal nutritional effects (Lopez & Leeson, 1995b; Decuypere *et al*., 2001; Calini & Sirri, 2007). Most of the available literature focuses mainly on the maternal dietary energy, vitamins, fatty acids and antioxidants with protein and particularly AA being less prevalent. As such the aim of this chapter is to review the relevant literature on maternal requirements for dietary protein and AA and the effect this has on offspring performance.

2.2 DIETARY REQUIREMENTS FOR PROTEIN

The protein requirements of livestock vary depending on several factors such as previous diet, environment and genetics (Mack *et al.*, 1999). The dietary requirements for protein of chickens are, more accurately, the requirements of the birds for the AA contained within the protein (NRC, 1994). Although plants can synthesise all 22 required AA, animals, including chickens, can only synthesise 12 (the non-essential AA). The remaining AA that are required by the animals must be acquired through the diet and are known as nutritionally essential AA (NRC, 1994; Leeson & Summers, 2001). Lysine and threonine are both essential AA, while leucine, isoleucine and valine have intermediate precursors that allow limited synthesis (Leeson & Summers, 2001). During protein synthesis AA are bonded together to form long chains in a specific sequence. Then the chains may interlock to form specific proteins. However, as an intermediate precursor, AA are limited in availability and, due to the dynamic state of body proteins, with synthesis and degradation occurring on a continual basis, sufficient intake of AA is required (NRC, 1994).

2.2.1 Protein Requirements for Growth

While energy is required for chemical processes in the body to occur there can be no growth and development without protein. The protein content of an animal can provide information as to the requirements of that animal for growth and maintenance (Leeson

& Summers, 2001). There is a close agreement between the AA found in the carcass of a chicken and the AA required for the growth of a chicken as determined by nutritional studies (Leeson & Summers, 2001). This is supported by Pearson & Herron (1982) who reported that broiler breeders fed higher protein diets (19.5, 23, 27 g CP/bird/d) from 21 to 60 weeks had a higher carcass protein content and lower fat content than birds fed on a low protein diet (16.5 g CP/bird/d). This was attributed to the low protein allowance being insufficient to support the same body protein gain as at higher protein levels. This resulted in the birds storing the surplus energy from the allowance as fat. It was also found that bodyweight gain increased linearly with increasing energy intake.

Most of the growth in broiler breeders is due to the deposition of protein (NRC, 1994). Thus, broiler breeders require sufficient balanced AA in the feed to meet their growth demands. These fast growing birds also have higher daily energy requirements compared to slower growing birds and so will eat more feed of an equal energy level under *ad libitum* conditions (Morris & Njuru, 1990). Because of this high intake of feed, it may be possible to feed a lower protein concentration in relation to the energy content.

2.2.2 Protein Requirements for Growth in Broilers

Although broiler breeders and, by extension, broilers are derived from similar ancestral stock to commercial layers, intensive breeding programs have resulted in genetic differences, particularly in terms of nutritional requirements and production. It is widely acknowledged that broilers have different growth requirements and parameters from layers (Morris & Njuru, 1990; Bowmaker & Gous, 1991; NRC, 1994; McLoughlin, 1998; Leeson & Summers, 2001). The four most commonly accepted dietary factors affecting growth and production are energy, protein, minerals, and vitamins (NRC, 1994). Of these, energy has been exhaustively studied and the vitamin requirements are generally well researched, but due to limitations in analysis and availability in synthetic form, only certain essential AA have been thoroughly researched.

To maximise efficiency, broilers need to attain a given production weight with the minimal input of resources. Food quality and quantity, ambient temperature, availability

of water, and other factors affect the ability of broiler chicks to attain their potential growth rate. One of the most important factors is feed composition, as it is the input of these nutrients that translates directly to the development of the chicken. The majority of weight increase in growing broilers is a result of the increase in the weight of tissue protein (NRC, 1994; Mabusela, 2004). Broilers have a greater potential, up to a point, for protein deposition than commercial layers (Morris & Njuru, 1990). As such, the faster growth of broilers is due to a greater rate and amount of protein deposition.

In an experiment to determine the protein requirements of broilers and layers, Morris & Njuru (1990) found that broiler chicks consumed about twice the quantity of feed compared to the layer chicks. It was also found that, while the maximum effective dietary CP content for layers was 188 g CP/kg feed, broilers showed significant growth responses up to the maximum protein treatment of 251 g/kg feed. For broilers the best feed conversion efficiency was at 230 g CP/kg feed. Gous & Morris (1985) found that a decline in the concentration of dietary lysine results in an increase in the carcass fat content, this was caused by the birds consuming more feed to meet the lysine requirement which resulted in the over consumption of energy that was then deposited as fat. This is supported by the results from Morris & Njuru (1990). Gous & Morris (1985) also found that the increase in fat content was related to the voluntary intake of feed by the birds on various diets. Feed intake and dietary amino acid content are not independent, so when the lysine content in the diets of the experimental birds decreased the feed intake increased to meet the lysine requirements. This resulted in the excess energy that was consumed being stored as fat. In both trials lysine was the first limiting AA with the other essential AA provided in excess of requirement.

2.2.3 Protein Requirements for Growth, Development and the effect of pre-lay protein intake on egg production in Broiler Breeders

The first stage for nutritional intake by the broiler breeder is in the initial growth phase after hatching. At hatching the chicken ceases to be solely dependent on the nutrient reserves from the egg and is able to supplement these reserves with direct nutrient intake. During this phase the nutrition needs to be tailored to the requirements of the bird for growth and development. Once the early growth phase is over, but before the

birds become reproductively active, they enter the pre-breeder phase. Nutrition in this phase is particularly important as it affects the reserves of the bird before coming into lay. Both Brake *et al.* (1985) and de Beer & Coon (2006) found that increasing the protein content fed to pre-breeder broiler breeders improved the performance of hens coming into lay.

In the growing and developing bird, growth rate, feathering and skeletal development are the major factors that utilise the majority of the nutrient intake (Leeson & Summers, 2001). A low protein diet invariably depresses the growth rate of pullets which reduces mature body weight and adult performance (NRC, 1994). As broiler breeder pullets will eat to meet the maximum growth potential (i.e. that of a broiler) they need to be fed on a restricted diet to prevent obesity and a resultant reduced reproductive capacity. Hence, both the protein and energy in the diet need to be balanced to the requirements of the birds within the controlled diet. Cave (1984) found that feeding pre-lay broiler breeder pullets a higher level of CP (181 *vs* 151 g CP/kg feed) resulted in a significant increase in the number of eggs produced over the period of 35 to 56 weeks of age, while the age at 50% production, egg weight and hatchability were not affected. They concluded that as the high protein pre-breeder diet resulted in a higher total egg output, the feed consumed per egg was lower when all birds were fed on the basis of equal weight of feed per bird.

De Beer & Coon (2006) found that hens fed a high protein pre-breeder diet had a higher total carcass protein content than those fed a low protein pre-breeder diet. The findings of Brake *et al.* (1985) are in agreement with this, where it was found that birds fed an additional 140 g CP/bird pre-breeder diet produced an additional 5.7 eggs between 24 and 64 weeks. De Beer & Coon (2006) also found that hens fed a high protein pre-breeder diet produced larger eggs than those fed a low protein pre-breeder diet. They found that low dietary protein levels, 520.3 g cumulative CP intake, between 16 and 21 weeks of age resulted in reduced egg production (165.8±3.8 compared to 170.9±4.6 for 630.2 g cumulative CP intake), while excess protein during this period had little effect on egg production. Brake *et al.* (1985) found that broiler breeders fed a high energy (1.97 MJ ME/bird/d) and low protein (22.4 g CP/bird/d) or low energy (1.90 MJ ME /bird/d) and high protein (28.8 g CP/bird/d) diets had a lower percentage of fertile eggs

when compared to birds fed diets that were either high or low in both protein and energy during the prebreeder period from 18 to 23 weeks, suggesting the necessity for a more balanced feed. However, high energy and low protein as well as low energy and high protein diets resulted in the production of larger eggs during weeks 28 to 44 due to a delay in age at sexual maturity. Birds on the high energy and high protein ration had an earlier age at sexual maturity with, as expected, the production of the smallest eggs. However, the relatively small difference between the energy allowances may also have affected the results and increasing the differences may produce a more marked result. De Beer & Coon (2006) found that better egg output was recorded from hens on a higher CP prebreeder feed, although they were the same size as those from lower CP prebreeder feeds This agrees with the findings of Brake *et al.* (1985) where birds fed low energy and protein diets had significantly poorer egg production than those on low energy and high protein diets.

Pearson & Herron (1982) found that the number of eggs produced by broiler breeders was significantly affected by the apparent metabolisable energy (AME) content of the feed. However, the effect of the dietary protein content was dependent on the available energy. Birds given lower energy diets matured later and at a lower bodyweight than those on higher energy diets. It was also shown that the energy intake had a significant effect on egg weight with increasing energy in the diet resulting in a linear increase in egg weight, until a plateau was reached with no increase in egg size, but rather an increase in body weight, in birds fed energy levels above 1.61 MJ/bird/d. Protein intake during the laying period had no significant effect on body weight and bodyweight gain, but an increase in energy allowance resulted in a linear increase in bodyweight gain (Pearson & Herron, 1982). In this trial, although low energy intake delayed sexual maturity, it was found that the birds did not have to reach a particular bodyweight before they commenced laying. However, a reduction in available energy did delay the onset of lay and also resulted in a lower bodyweight at the commencement of lay due to retarded growth.

2.3 PROTEIN REQUIREMENTS FOR EGG FORMATION

Calculations of the nutrient requirements of broiler breeders are generally based on results from experiments using laying hens (Bowmaker & Gous, 1991; NRC, 1994). As broiler breeders have been genetically selected for meat rather than egg production, unlike commercial layers, egg output and the uniformity of egg production is expected to be lower than in layer flocks (Bowmaker & Gous, 1991). A major shortcoming, generally found in broiler breeder nutrition trials, is that birds are housed in groups which share the same food source (Bowmaker & Gous, 1991). As broiler breeders have been selected for growth potential rather than egg production, invariably there is a very high desired feed intake. This often results in differences in the feed intake between birds, on a restricted feed intake, with the stronger more dominant birds eating more than the weaker birds (Bowmaker & Gous, 1991; Lopez & Leeson, 1995a) which may distort the results as there will be variation in the size between the birds (Bowmaker & Gous, 1991). This in turn can impact the applicability of the results especially if there is limited available information, as is the case with broiler breeders. The rapid rate of progress and development of broiler breeder genotypes has also potentially outpaced the applicable research, thus, indicating a need for more extensive determination of broiler breeder-specific nutrient requirements for egg production.

The avian egg contains about 12% protein (Leeson & Summers, 2001), 11% lipids and carbohydrates, as well as 66% water (Romanoff & Romanoff, 1949). The remainder consists of inorganic matter (Romanoff & Romanoff, 1949; Murphy, 1994). The total protein of the egg is divided into the albumen (55%), the yolk (42%), and the shell, (3%) (Leeson & Summers, 2001). Protein is the main constituent of the albumen after water which comprises about 88% (Romanoff & Romanoff, 1949). In an experiment in which the amino acid composition of Zebra Finches (*Taeniopygia guttata)* and Pigeons (*Columbia livia)* was analysed (Murphy, 1994), it was found that the protein concentration was higher in the yolk (13%) than the albumen (9%). However, the albumen had a greater mass than the yolk and was found to contain about 55-60% of the total egg protein which is in agreement with Leeson & Summers (2001). It was also found that leucine, lysine, and valine were the most abundant AA in the eggs, with tryptophan, histidine, methionine, and tyrosine being the least abundant. In addition, if

any of the macronutrients are limited in the diet then egg production may be reduced (Murphy, 1994).

Bolton *et al.* (1992) found that mated pairs of Black-backed gulls that were fed a protein supplement of fish (*Trachurus trachurus* 200 g/pair/day) produced larger eggs at the end of the productive cycle than those that received an extra energy supplement. Larger clutches were also observed, while an energy supplement of a similar calorific value had no effect. The difference in egg size was attributed primarily to the yolk protein content as the mass of yolk fat and the albumen mass were the same across both treatments and the control. However, it was also noted that this occurred in a breeding year with limited natural feed sources, suggesting that the improvements in egg production may have also been caused by an improvement in the body condition of the gulls, and their ability to produce eggs, rather than any direct effect on egg production. In a second treatment, during a year with no apparent feed shortages, gulls fed supplementary egg protein produced eggs that were about 10% larger than those that did not receive additional protein or received fish supplements as before. Gulls that received additional egg-based protein not only produced eggs that were larger but also chicks that were skeletally larger and heavier than those that did not receive additional protein. It was concluded that the provision of supplementary nutrients required for egg production allows female gulls to produce eggs that are larger than they would normally be able to produce. The authors also concluded that provision of specific AA to the female gulls was important for egg production and that these may have been limited in the natural diet. This was in part due to eggs, from birds that received egg protein supplement, having higher levels of methionine and tryptophan compared to the fish samples. So it is not specifically the provisioning with extra protein that is beneficial, but rather the addition of specific AA and the quality of the feed source. An additional, and potentially useful, conclusion from this study was that egg production (clutch length and egg size) in these birds was limited by an AA rather than an energy deficit.

With protein being the second largest constituent of the chicken egg (Romanoff & Romanoff, 1949; Lunven *et al.*, 1973; Murphy, 1994), the protein requirement of the hen is at its highest during the laying period (Robbins, 1981; Bolton *et al.*, 1992; Fisher, 1998; Leeson & Summers, 2001). Egg production comprises 72% of the total protein

requirements from onset of lay to 42 weeks and 79% from 42 weeks to 72 weeks (Leeson & Summers, 2001). Robbins (1981) found that, for wild birds, the increase in the protein requirement for egg production above the basal level, by 86% to 232%, during lay was much higher than that of the energy requirement, which increased by 34% to 135 %. This indicates the important role of dietary protein in egg production.

Protein, in general, and AA such as; lysine, tyrosine, phenylalanine (Murphy, 1994), and methionine (Leeson & Summers, 2001), have a large influence on egg size. McLoughlin (1998) found that increasing the tryptophan concentration in the diets of both broiler breeders and laying hens increased the rate of lay, although it had a greater effect on the laying hens. It was also found that while the tryptophan content in the diet affected the weight of the eggs produced by the laying hens, it had no significant effect on the weight of the eggs produced by the broiler breeder hens. Beckerton & Middleton (1982) found that increasing the dietary protein levels for Ruffed grouse resulted in linear increases for chick survival, chick weight at hatching, hatching success, clutch size, duration of lay, rate of lay, weight of the first egg, and average egg weight. By providing Blue tits with extra protein supplements that contained the AA required for egg production a greater number of eggs were produced (Ramsay & Houston, 1998).

An increase in the dietary protein of broiler breeders from 14% to 16 and 18% CP resulted in an increase in egg size, primarily as a result of increased albumen weight, with mean yolk weight being unaffected (Joseph *et al*., 2000). While this is in disagreement with the findings of Bolton *et al.* (1992) who found the increase in egg weight was due to the increase in yolk weight, both agree that the increase in the egg size is linked to an increase in the overall protein content of the egg. Penz & Jensen (1991) found that, while an increase of CP from 13% to 16% improved the body weight and egg weight of layers, although increasing only essential AA did not improve either egg or body weight. It was also found that the smaller eggs from the 13% CP diet had a lower proportion of albumen compared to the 16% CP diet. This may indicate a potential trend that is in accordance with the findings of Joseph *et al*. (2000). Lopez & Leeson (1995a) also found that the smaller eggs produced by broiler breeders on a low (10%) protein diet had a lower relative albumen content compared to those on a higher (16%) protein diet. Broiler breeder hens with low (14%) CP diets had a reduced rate of

egg production compared to those on higher (18%) CP diets up to 29 weeks (Joseph *et al.,* 2000). Between 25 and 29 weeks of age broiler breeder hens on high (18%) dietary CP levels produced more eggs than those on a standard (16%) or below-standard (14%) levels of protein. High protein levels also resulted in a greater proportion of settable (over 52 g in weight) eggs.

Lopez & Leeson (1995a) found that broiler breeders fed 16% protein attained a higher (89%) and more persistent peak of production and produced larger eggs than those fed a low protein (10%) diet (85%). Birds fed 16% protein also produced eggs with fewer deformities at 60 weeks. In a trial by Lopez and Leeson (1994a), 45 week-old broiler breeder hens were fed one of four diets containing; 14, 16, 18 or 20% CP, for a period of 10 weeks. The lysine and methionine levels in the feed were kept constant for all of the feeds at 0.90% and 0.38% respectively. Total egg production was significantly lower in the 20% CP diet compared to the lower protein diets. The average egg weight of all the diets was similar, and it was concluded that maternal protein intake above 21 g/bird/d had no effect on egg weight, hatchability, chick weight, or subsequent offspring performance. The lack of response was thought to be due to the limiting AA remaining constant across all treatments. It was also found that the changes in dietary protein had no effect on egg components and egg composition. It was concluded that changing the dietary protein levels of the hens would more likely result in a change in the rate of egg production, rather than the egg composition.

Due to the controlled nature of broiler breeder feeding, all nutrients and dietary elements need to be provided within the portion specified by the energy supply (Fisher, 1998). Heck *et al*. (2004) found that feeding an industry standard broiler breeder genotype *ad libitum* resulted in low egg production with a low proportion of settable eggs as well as high mortality. This was primarily due to the overweight nature of the birds. Morris & Gous (1988) found that decreasing the protein in the feed had a greater impact on the numbers of eggs produced, resulting in lower egg production, and not the weight of the eggs. The decrease in egg weight seldom fell below 90% of the maximum value. Taking into account the work of Bolton *et al.* (1992) on Black-backed gulls it may be possible that increasing the dietary protein results in an increase in egg size and number, while a decrease in dietary protein will reduce the egg numbers rather than the

egg weight. Bolton *et al*. (1992) also found that an increased egg size results in an increased chick size and so a greater chance of survival, as did Wilson (1991) and Vieira & Moran (1998). Thus, a reduction in egg number rather than egg size, when protein is limited, will prevent smaller, less viable chicks. The benefit is that under reduced protein levels, although there will be a lower number of chicks produced they will be more viable, so that the energy investment in producing a chick is not wasted. This is of potential interest for production systems where the production of the broiler for market, rather than just egg production, is the objective. However, it is also possible that once passed a certain threshold in dietary concentration, egg size is reduced in favour of clutch size in order to maintain at least some chance of reproductive success.

2.4 NUTRITION OF THE EMBRYO

The AA from the yolk sac are transported via the blood vessels to the embryo which uses them for growth and development (Freeman & Vince, 1974). These include intact proteins required for normal development and AA for the production of protein or new AA (Freeman & Vince, 1974). Amino acids and vitamins are required during the early incubation period primarily to promote growth, however, at this stage glucose is more important (Romanoff, 1960).

Lopez & Leeson (1995b) found that, while broiler chicks from 30-week-old hens fed higher protein levels were significantly heavier at hatch, this effect diminished with age until there was no difference in weight by 7 d for female chicks and 21 d for male chicks. When Finkler *et al.* (1998) removed either 20% of the albumen or 20% of the yolk from pre-incubation chicken eggs, the metabolism of chicken embryos was not significantly affected by the removal of either of the components. However, the mass of the 20 d embryos was significantly reduced in both cases. The removal of 20% of the albumen also significantly reduced the mass of the wet, yolk-free, embryo at 20 d, compared to the control. This was not found for the treatment of removal of 20% of the yolk. The removal of either 20% of the albumen or the yolk had no effect on the dry yolk-free embryo mass. The removal of 20% of the yolk, but not the albumen, significantly affected the mass of the dry yolk-sac (Finkler *et al.* 1998). However, both

treatments affected the mass of the wet yolk-sac. The removal of the albumen was the only treatment in this experiment which significantly affected the embryo tibiotarsus length. It was concluded that the water content of the egg, in particular the albumen, was the major determining factor of near-term embryo and hatching size in chickens. It was further concluded that the degree to which yolk is assimilated into the tissue of the developing embryo was not influenced by changing the yolk content of the egg. Rather this change resulted in a difference in the amount of residual yolk remaining in the yolk sac at the end of incubation. While the water content of the albumen may indeed play an important role in determining the mass of the hatching chick the importance of the protein content of the albumen, contributing 67% of the protein content of the egg cannot be ignored (Romanoff & Romanoff, 1949).

Pal *et al*. (2002), using equalised egg weights for broiler and layer hens, found that the wet- and dry-weight of embryos was significantly affected by genotype, with the broiler embryos being heavier from 12 to 20 d of incubation. The embryonic growth rate was also affected by environmental factors such as temperature and relative humidity for the different genotypes. The average nitrogen concentration was found to be significantly higher in the broiler compared to the layer embryos, although this parameter was not affected by the stage of development. It was concluded that this was due to superior tissue development in the broiler embryos. The total ash content of the embryos was not significantly affected by either the strain or the stage of development. However, the water content was significantly affected by the stage of development. Everaert *et al.* (2008), using eggs from 48-week-old Cobb-500 and Isa Brown flocks, found that from d 4 of embryonic development until hatch, the absolute yolk-free embryos of broilers were significantly heavier than their layer counterparts. At hatch both the broiler and layer yolk-free chicks weighed 62% of their initial egg weight. There was however, a significant difference between them when the yolk was included. The broiler chicks weighed 75% of their initial egg weight and the layer chicks weighed 72% of their initial egg weight. After 14 d of embryonic development until hatch the absolute yolk weight of the broiler embryos was significantly higher than the layer embryos. While the relative yolk weight decreased during development in both strains it was still significantly higher in the broiler strain from 14 d of embryonic development until hatch. Although the duration from setting to hatching was similar in both strains, the

duration from setting in the incubator to external pipping was significantly shorter in eggs from the broiler strain, while the duration from external pipping to hatching was significantly shorter in eggs from the layer strain. The period from internal to external pipping was also similar for both strains, but commenced significantly earlier for the broiler strain compared to the layer strain. This resulted in a longer period between internal pipping and emergence in the broiler embryos compared to the layer embryos. The authors theorised that this was due to the broiler embryos utilising a higher proportion of the available energy in the egg during incubation. This resulted in them having lower energy reserves to utilise for the hatching process and so they took longer. However, Janke *et al.*(2004) found that broiler embryos of the Ross 308 and 508 strains hatched a day earlier than those of the White Leghorn strain. This earlier hatch, in comparison with the findings of Everaert *et al*. (2008), may have been due to differences between hens for egg provisioning. However, they also acknowledged that insufficient eggs were used to determine the extent of this occurrence. According to Pal *et al*. (2002), broiler embryos have a faster rate of growth from 12 d to 20 d of incubation, achieving a higher weight from equalised egg weights. As they found no effect of genotype or stage of development on total ash content, but did find an effect of genotype on average embryo nitrogen concentration, it may be concluded that the differences observed were due to protein assimilation and deposition in the embryos. Therefore, potentially the broiler embryos have a greater capacity for the utilisation of protein. However, Everaert *et al*. (2008) found that although yolk-free broiler embryos were heavier than layer embryos at hatch, they both maintained a common ratio to the initial egg weight. It was only with the inclusion of the yolk sac that the broiler embryos achieved a higher ratio to initial egg weight compared to the layer embryos. The broiler embryos also maintained a higher yolk sac weight than the layer embryos from d 14 of incubation onwards. As Pal *et al*. (2002) did not consider yolk free embryo weight it is possible that their findings were influenced by a larger yolk sac in the broiler embryos. The increased chick weight, regardless of whether it is due to a larger yolk sac or greater embryo growth, nonetheless gives broiler embryos a greater potential for growth.

2.4.1. Importance of Protein in Embryo Metabolism

The selection for rapid growth in broilers is changing the embryo metabolism, and the availability of nutrients and oxygen to the embryo can determine the rate of embryonic growth via the rate of bio-synthesis of tissue (Boerjan, 2006). There is also a strong physiological relationship between the amount of metabolic heat produced and the rate of bio-synthesis of tissues. Boerjan (2006) found that the increased metabolic heat production in modern broilers compared to traditional meat-producing birds is due to a higher growth rate. As such, it is very likely that modern broiler embryos have an increased requirement for protein in order to meet the increased growth potential. Al-Murrani (1978) found that at the start of incubation (0 d) the protein content of larger eggs (over 59 g) was significantly higher than that of smaller eggs (under 59 g), indicating a potentially higher chick weight at hatch. At 7 and 14 d of incubation the embryos within the eggs of different sizes contained similar amounts of protein with a significant difference arising only after 14 d, with the embryos from the larger eggs containing more protein. This reflected the larger size of the embryo of the larger eggs from 14 d of incubation. Both the eggs and the embryos from the larger eggs contained more fat. It was concluded that after 14 d of incubation the embryo growth is greatly affect by the protein content of the egg.

In a direct comparison between broilers and layers, using eggs of similar weight, it was found that there was no difference in the protein content of the eggs before incubation (Ohta *et al*., 2004). Throughout incubation there was no difference in total egg weight between the broilers and the layers. However, the weights of the broiler embryos were significantly greater than those of the layers on d 14 and 19 of incubation, indicating more effective utilisation of available protein. It was concluded that the accumulation of protein, and growth in the broiler embryos, was faster than that in the layers, as well as the increased yolk consumption, suggesting a difference in the embryonic growth between broilers and layers.

Ohta *et al.* (1999) analysed the CP, crude fat (CF), and moisture contents of commercial Cobb broiler embryos and eggs. It was found that the combined weight of embryo, egg and shell had decreased to 88% of pre-incubation weight by d 19 of incubation. The

moisture content of the embryos and eggs was consistently higher and the CF lower, during incubation, than the CP from the start of incubation until d 19. During the period of incubation the CP, CF, and moisture of the egg all decreased while the same components in the embryo increased. As incubation time increased, total CP did not change, total CF decreased exponentially to 69% of the initial amount and moisture decreased to 90% of the initial amount recorded. On d 19 of incubation the ratios of transferred CP, CF, and moisture to the embryo were 58, 29, and 64%, respectively, of the initial egg contents. The total AA contents present in the egg decreased during incubation, most likely to form proteins. Al-Murrani (1982) found that chicks hatched from eggs that received supplement injections of protein at d 0 of incubation were consistently and significantly heavier than those from un-supplemented eggs. At 56 d of age the mean weight of the supplemented chicks was 116 g higher than the un-supplemented chicks. It was also found that at 20 d of incubation the embryo protein content of the supplemented eggs was significantly higher which was reflected by higher embryo weights. These results suggest that the provision of additional protein and particularly AA may improve the development and growth of broiler embryos.

Ohta *et al.* (2001) evaluated the effects of *in ovo* administration of AA on broiler embryos and chicks. Amino acids were injected into the yolk of the embryo on d 7 of incubation. It was found that the *in ovo* AA injection resulted in a significantly higher chick body weight at hatch, relative to initial egg weight compared to non-injected chicks. This implies that a lack of nutrient provision in the egg, rather than the size of the egg, may be a constraint on embryo growth. Ohta *et al*. (2004), also using AA *in ovo* injections, determined that there was a significantly higher body weight relative to the initial egg weight in the AA-injected treatment. The injection of the AA resulted in an increase in the plasma concentration of lysine over that of the control group. Supplementation also significantly decreased the AA plasma levels, except glutamic acid and lysine, at hatch (Ohta *et al.*, 2001). However, *in ovo* injection of water did not affect the AA profile, but the AA ratio to lysine was reduced by the *in ovo* injection of AA at d 7. The 14 and 19 d yolk, albumen, and embryo weights were not significantly different between the supplemented and un-supplemented treatments. At d 19 the AA contents of the embryo, yolk, and albumen, were significantly increased in the supplemented eggs. This was concluded to be due to AA injection at d 7 stimulating AA

utilisation. However, the injection had no significant effect on the chick weights. At d 19, the supplemented and un-supplemented eggs had similar AA content for all AA, except lysine which was significantly increased by supplementation. It was concluded that the utilisation of protein, rather than individual AA utilisation, by the embryo may be improved when an AA solution, identical to the makeup of the egg, is injected at d 7 of incubation. Hence, the artificial manipulation of the egg composition has the potential to improve the chick performance, and therefore the possibility exists that if egg composition is changed through the maternal diet, it could influence the hatching chick weight.

The yolk sac of the chick needs to supply the protein that the chick requires until it is able to ingest feed. According to Rol'nik (1970) the yolk sac at hatching has a higher protein concentration than at oviposition. Thus, the non-protein nutrients are used up at a different rate than protein. This is most likely due to the presence of complex proteins that are only required after hatching, but before the chick is able to synthesise or ingest them. As the protein content of the egg is a very important factor determining the chick performance, it needs to be of the required composition. This includes simple AA as well as complex protein molecules. In order to achieve this, the hen needs to be fed to meet the protein requirements for egg production.

2.5 IMPLICATIONS

The egg, and the embryo it contains, is of utmost importance in the production of as many viable chicks as possible. One of the most valuable ways of assisting the hen is by ensuring that the feed provides as many of the required nutrients in the best possible ratios. As broiler breeders are mostly fed on a restricted basis, often on the basis of energy requirements, it is important to ensure that the feed received meets the requirements for maintenance and egg production and to meet the demand of the changing requirements of improved genetics. When a bird lays an egg it is provided with nutrients for embryonic growth and development. As energy, protein, and vitamins are required for the embryo to develop normally, these nutrients need to be provided in sufficient quantities when the egg is formed. However, as modern broiler breeder genotypes have been selected for rapid growth it is possible that the embryo also has a

high potential for growth. The embryo, in turn, may have different requirements from those that the hen is genetically programmed to provide. As most genotype improvement has focused on the chick after hatch, it is likely that the reproductive system of the hen has not developed to match the improved embryo. So it is possible that the reproductive tract of the broiler breeder hen is only producing a larger version of the egg that was produced by the original genetic stock, thus resulting in an under-provisioned egg for the modern broiler chick. This means that there is a strong possibility that the hen is unable to provide the necessary provisioning in the egg for the embryo to grow at its genetic potential. This is also supported by improved embryo growth after the *in ovo* supplementation of nutrients (Ohta *et al*. 2001; Ohta *et al*. 2004). The net result of this is that the egg production and provisioning may not only be influenced by the maternal diet but also by the reproductive system. This indicates a far reaching situation that could degrade further as the production capacity of broilers is increased. As illustrated in this review, broilers and broiler breeders require more protein for growth and production than layers, and therefore the nutrient requirements of layers should not be used in formulating feed for broiler breeders. Lopez & Leeson (1995a) found that an increase in the protein of broiler breeder diets increased the hatchability of the eggs. So there is a possibility that the eggs of broiler breeders are not sufficiently provisioned with protein which may result in decreased hatchability with economic implications.

The information presented in this review encompasses the majority of available research in this specific area, and there appears to be a lack of research on the transfer of maternal protein and AA to the embryo. Thus, there is a need to establish what the maternal requirement of lysine is that results in production of the most desirable (largest and healthiest) chicks.

3. THE EFFECT OF MATERNAL DIETARY LYSINE INTAKE ON THE HATCHABILITY OF BROILER BREEDER EGGS

3.1 INTRODUCTION

The primary focus of broiler breeder production is maximum production of viable day old broiler chicks. Many studies have focused on various factors that affect the chick at hatch. Factors such as egg storage (Decuypere *et al*., 2001; Tona *et al.*, 2003), egg weight (Morris *et al.,* 1968; Al-Murrani, 1978; French, 1997), maternal age (Lopez & Leeson, 1994a; Suarez *et al*., 1997; Heier & Jarp, 2001), and incubation conditions (French, 1997; Decuypere *et al.*, 2001; Boerjan, 2006) have all been examined.

Pearson & Herron (1981) fed broiler breeder hens diets of varying energy and protein levels and found an interaction between protein and energy and its effect on hatchability from 21 to 36 weeks of age. At a protein allowance of 27 g/bird/d the hatchability was observed to decrease with a decreasing dietary energy. A similar, although non-significant, trend was also observed from 37 to 50 weeks of age. It was also observed that a significant increase in embryo deaths in the second week of incubation was associated with a ratio of 17.8 g CP : 1 MJ AME. There was no significant impact of maternal CP or energy intake observed on the performance of the progeny. Lopez & Leeson (1995a) found that a high maternal dietary CP intake (16%) resulted in a significantly reduced fertility compared to a lower (14%) CP intake. The fertility improved further with a 12% CP intake although this improvement was not significant. No significant effect of dietary protein on hatchability was found, unlike that found by Pearson & Herron (1981), although this could be due to the utilisation of a fixed level of dietary energy. Lopez & Leeson (1995a) also observed an increase in egg size with increasing dietary protein, similar to that observed by Pearson & Herron (1981). Unfortunately most of the studies on the impact of maternal dietary protein for broiler breeders conducted more than 15 years ago. Improved genotypes have a higher potential for growth. Due to this, the research on the older genotypes may not sufficiently provide

for the genetic potential of the new genotypes. In addition to this the research focused on CP rather than AA. As such, more research on improved genotypes, using modern feed evaluation structures, is required.

This chapter focuses on the effect that protein and, in particular, lysine has on the hatchability of broiler chicks, when included at different levels in the maternal diet at three different maternal ages.

3.2 MATERIALS AND METHODS

Female (n=900) and male (n=90) Cobb 500 broiler breeders were divided into 18 groups, each composed of 50 Cobb 500 broiler breeder females and 5 Cobb 500 broiler breeder males, which were placed in floor pens in a naturally ventilated house at 21 weeks of age. For the first week after arrival the hens received 110 g/bird/d of a commercial broiler breeder ration. This was increased by 10 g each week until the birds were receiving 160 g feed/bird/d at 26 weeks of age. At this time each pen was randomly assigned one of six dietary treatments differing in lysine concentration. This resulted in each treatment having three replications with 150 females and 15 males. Each of the treatments was formulated using two basal feeds, A and B (Table 3.1, 3.2). These were blended to form each of the six treatment feeds (Table 3.3, 3.4). The first four consisted of constant lysine concentrations providing 1200, 1070, 930 and 800 mg lysine/bird/d. The last two treatments consisted of dietary lysine levels that were changed every two weeks by thoroughly blending different ratios of the basal feeds as the birds progressed from 26 to 60 weeks of age. Feed allocation remained constant for all treatments at 160 g/bird/d. The lysine concentration in Treatment 5 decreased from 1200 to 800 mg/bird/d. Treatment 6 provided an increasing concentration from 800 to 1200 mg/bird/d. All of the treatments were formulated to provide the birds with 1,9 MJ metabolisable energy (ME) per d at an intake of 160 g feed/bird/day. The trial was terminated when the birds were 60 weeks of age. As this experiment focused on the maternal dietary effects, the male birds received a 120 g/bird/d standard commercial broiler breeder diet (Table 3.5) throughout the experiment. The males were fed in separate feeders that were raised above the reach of the females. The females received feed in feed troughs with male exclusion grids and prevented the males from accessing

the female feed. The chickens were provided with 12 h light/d with artificial lights used in the early morning and late afternoon as required.

Table 3.1 *Basal feed raw material ingredient composition (% inclusion)*

Ingredients	Basal A	Basal B
Maize	57.062	67.465
Wheat bran	9.176	9.120
Soybean full fat	24.826	14.311
L-lysine HCl	0.067	
DL methionine	0.109	0.025
L-threonine		0.545
Vitamin andmineral premix	0.250	0.250
Limestone	6.852	6.917
Salt	0.168	0.276
Monocalcium phosphate	0.488	0.502
Sodium bicarbonate	0.486	0.194
Potassium carbonate	0.515	0.394

Table 3.2 *Nutrient composition of the basal feeds (Calculated)*

	Basal A		Basal B	
Nutrients	Total	Digestible	Total	Digestible.
AMEn (MJ/kg)	11.9		11.9	
Lysine (%)	0.847	0.750	0.568	0.500
Methionine (%)	0.357	0.328	0.240	0.219
Methionine + Cystine (%)	0.660	0.563	0.510	0.432
Threonine (%)	0.590	0.499	1.000	0.932
Tryptophan (%)	0.174	0.145	0.129	0.107
Arginine (%)	1.010	0.910	0.762	0.686
Isoleucine (%)	0.660	0.572	0.501	0.434
Leucine (%)	1.450	1.310	1.260	1.160
Histidine (%)	0.441	0.392	0.362	0.324
Phenylalanine + Tyrosine (%)	1.260	1.100	1.00	0.882
Valine (%)	0.778	0.672	0.631	0.547

Table 3.3 *Basal feed blends for treatments 1 to 4*

Treatment 1	Treatment 2		Treatment 3		Treatment 4
Basal A(%)	Basal A(%)	Basal B(%)	Basal A(%)	Basal B(%)	Basal B(%)
100	67	33	33	67	100

Table 3.4 *Basal feed blends for treatments 5 and 6 from 26 to 60 weeks*

Age (weeks)	Treatment 5		Treatment 6	
	Basal A(%)	Basal B(%)	Basal A(%)	Basal B(%)
26	100	0	0	100
28	94	6	6	94
30	87	13	13	87
32	81	19	19	81
34	75	25	25	75
36	69	31	31	69
38	63	37	37	63
40	56	44	44	56
42	50	50	50	50
44	44	56	56	44
46	37	63	63	37
48	31	69	69	31
50	25	75	75	25
52	19	81	81	19
54	13	87	87	13
56	6	94	94	6
58	0	100	100	0
60	0	100	100	0

Table 3.5 *Commercial broiler breeder diet composition*

Nutrients	Protein	Moisture	Fat	Fibre	Calcium	Phosphorus	Total Lysine
% inclusion	14	12	15	7	2.8 – 3.0	0.5	0.6

Twenty eight eggs from each pen (a total of 84 eggs per treatment) were collected at 38, 48 and 60 weeks of age. They were labelled and incubated within 4 h of collection. The eggs were randomised, set and incubated for 18 d after which they were placed, according to their original pen, into compartmentalised hatching trays, for a further 3 d. Two identical single-stage incubators (capacity 768 eggs each) were used with the eggs split evenly between them. This was done as the incubators were originally designed to function as multi-stage and so had limited hatching tray capacity. An automatic turning system rotated the trays every 90 minutes. The incubators were humidified using standing water trays and adjustable vents and heated via a heating fan with an electrical element. The temperature was controlled with an electronic temperature sensor and onboard monitoring and control system. The incubators were maintained at 37 ^{o}C and between 50 and 60% Relative Humidity (RH). During the first incubation at 38 weeks the automatic turning system malfunctioned and the trays had to be turned using the manual override. This resulted in insufficient rotations of the incubator trays as they were only rotated every 4 hours. During the third incubation at 60 weeks low atmospheric RH resulted in a low incubator RH. On d 21 of incubation the percentage hatch was recorded for each treatment.

The overall percentage hatch and that for each age were subjected to ANOVA. The first four, constant lysine level, treatments were also subjected to a simple linear regression with age as a group, to determine the response of percentage hatch to lysine level. All significant values are at the 0.05 level unless stated otherwise. Statistical analyses were performed using Genstat (2010).

Ethical approval from the University of KwaZulu Natal ethics committee was obtained (reference 051/09/Animal) prior to the start of the experiment, and the code of conduct was adhered to throughout.

3.3 RESULTS AND DISCUSSION

The average percentage hatch of eggs from the 48-week-old hens was unexpectedly higher than that of the 38- and 60-week-old hens (Table 3.6). There was no significant difference in the average percentage hatch between the eggs from the 38- and 60-week-old birds. Only the age of the hens had a significant effect on hatchability. Hens with a dietary lysine intake of 1070 mg/bird/d at 38 weeks of age produced eggs that had a significantly lower hatch percentage than from hens fed 1200 and 930 mg lysine/bird/d (Table 3.6). However, this is most likely due to the high variation experienced between treatments rather than due to any specific treatment effect. There was a large distribution of response within treatments, indicating that within-treatment variation was probably too large to observe differences between treatments. The malfunction of the incubator at 38 weeks and the reduced RH at 60 weeks may have negatively affected the results and induced additional variation. The percentage hatch, at all ages, was also lower than expected in comparison to other reported results (Pearson & Herron, 1981; Lopez & Leeson, 1995b). Increasing or decreasing the dietary lysine levels produced no significant improvements in the percentage hatch at any of the ages examined (Table 3.6). As only the percentage hatch and not the percentage hatch of fertile was recorded it is also possible that there may have been an effect of lysine on fertility, which would in turn have impacted the percentage hatch.

Hatchability decreased linearly with increasing dietary lysine, although the R^2 for this was low (R^2 = 13.4; $p<0.09$). A similar result was found by Pearson & Herron (1982) who found that a low hatchability was associated with a high dietary protein (27 g/bird/d), particularly when the energy intake decreased. During the early part of lay (22-36 weeks) a decrease in hatchability occurred when the protein to energy ratio was more than 15 g CP : 1 MJ AME in the diet (Pearson & Herron, 1981). However, the low R^2 observed in this trial may indicate that the range of lysine used may have been insufficient to induce a notable reaction. This is consistent with the findings of several other researchers (Lopez & Leeson, 1994a; Lopez & Leeson, 1995a) who found no significant effect of lower protein levels on hatchability.

Table 3.6 *Percentage hatch of eggs from hens at 38, 48, and 60 weeks of age for each treatment, including standard error of the means (S.E.M).*

	Lysine intake (mg/bird/d)	Percentage Hatch	S.E.M	p-value
Main effects				
Lysine intake	1200	67.9	5.29	0.438
(mg/bird/d)	1070	63.1	6.16	
	930	74.2	4.60	
	800	73.4	4.88	
	1200 to 800	67.5	4.89	
	800 to 1200	75.8	3.61	
Hen Age (weeks)	38	63.5^{b}	2.82	0.002
	48	79.6^{a}	3.28	
	60	67.9^{b}	3.39	
Hen Age (weeks)				
38	1200	71.4^{efg}	2.06	0.733
	1070	47.6^{h}	6.63	
	930	71.4^{efg}	2.06	
	800	61.9^{fgh}	8.33	
	1059	59.5^{gh}	5.19	
	941	69.0^{efgh}	6.63	
48	1200	76.2^{efg}	6.30	
	1070	72.6^{efg}	13.7	
	930	83.3^{ef}	8.33	
	800	83.3^{ef}	9.74	
	941	75.0^{efg}	8.25	
	1059	86.9^{e}	2.38	
60	1200	56.0^{gh}	13.4	
	1070	69.0^{efgh}	6.30	
	930	67.9^{efgh}	10.7	
	800	75.0^{efg}	2.06	
	800	67.9^{efgh}	11.4	
	1200	71.4^{efg}	3.57	

Values in a column without a common superscript (a,b) and (e,f,g,h) differ significantly ($p<0.05$)

Spratt & Leeson (1987), using maternal dietary protein levels of 19 and 25 g CP/bird/d, found no significant effect of dietary protein on hatchability of eggs. Ohta *et al.* (2001), using eggs from a 52 week old flock, found no significant effect of *in ovo* administration of AA at 7 d on percentage hatchability.

As the hatchability of the eggs may not provide a clear indication of the effect of maternal dietary protein on the offspring, several other parameters need to be considered. Factors such as temperature of the incubator (French, 1997) and its effect on the egg (Hulet *et al.*, 2007), as well as the heat production of the developing embryo itself (Tona *et al.*, 2004; Sato *et al.*, 2006; Everaert *et al.*, 2008) impact the development of the embryo. In addition to this, the genetic potential of the embryo can also have a significant impact on the embryo development and hatching (Morris & Njuru, 1990; Mumramatsu *et al.*, 1990; Pal *et al.*, 2002; Heck *et al.*, 2004; Janke *et al.*, 2004; Sato *et al*., 2006; Everaert *et al.*, 2008). From d 4 of incubation until hatching absolute yolk-free embryos were found to be significantly heavier in broiler than in layer embryos (Everaert *et al*., 2008). Similarly, Ohta *et al.* (2004) found that the broiler embryos were only significantly heavier from d 14 onwards. Janke *et al.* (2004) found that the body temperature of layer embryos was significantly lower than broilers during two periods within incubation. The average difference in between the two sets of embryos was 0.5 ^{o}C. Lourens *et al.* (2006) found that larger eggs had a higher heat production. The relative O_2 consumption in broiler embryos was significantly lower than that of layers during incubation (Sato *et al*., 2006).

3.3 CONCLUSIONS

Although, as selection for improved growth rate may be limited by the egg composition, increasing lysine concentration in the maternal diet was expected to improve embryo growth and viability (Al-Murrani, 1978; Bolton *et al.*, 1992; Lopez & Leeson, 1995a; Finkler, 1998), this was not found to be the case. This could be due to the large within-treatment variation observed as well as other factors contributing to hatchability, especially considering the hatch percentages were lower than expected across all treatments. The high variation, as well as the reduced hatchability, was most likely

caused by either the incubator complications or possible fertility variations. It would also have been useful to have analysed results of the feeds to verify the treatments.

4. THE EFFECT OF MATERNAL DIETARY LYSINE ON EMBRYONIC GROWTH AND EMBRYONIC TEMPERATURE OF THREE CHICKEN GENOTYPES

4.1 INTRODUCTION

Many modern broiler studies focus on the chick after it has hatched until it is marketed. There are conflicting reports on whether the maternal diet can influence the performance of the chick. It is thought by some that the diet of the hen can influence the performance of the chick (Al-Murrani, 1978; Spratt & Leeson, 1987; Lopez & Leeson, 1994b; Lopez & Leeson, 1995b; Calini & Sirri, 2007) and that a larger chick at hatch results in a larger bird at marketing (Morris, 1968; Al-Murrani, 1978; Sklan *et al.*, 2003; Hulet *et al*., 2007). In addition, most of the available research has been carried out on older genotypes. With the continuous rapid improvement in new genotypes this information may not be as applicable as it once was. Most research on the influence of protein in the maternal diet has indicated that it has no significant effect on the chick performance from hatch. Most of this research focused on the CP level in the diet rather than the AA concentrations and ratios. Al-Murrani (1978) determined that the protein levels in the egg were the main limiting factor to the development of the chick. It was further found by Al-Murrani (1982) that supplementing the egg with AA *in ovo* improved the development of the embryo. A similar result was found by Ohta *et al.* (1999) in which the AA had been balanced to those found in the egg and embryo. As *in ovo* administration of AA, balanced to the requirements of the embryo, has been shown to improve the embryo growth (Ohta *et al.*, 1999), and since additional CP in the maternal diet has failed to produce an improvement in embryo weight, there is a paradox that needs to be resolved. Is the hen capable of improving the AA provisioning of the egg or can this only be achieved by artificial means?

To provide a preliminary indication of this, an experiment was conducted to evaluate two different maternal lysine levels on 3 genotypes of different growth rate, on the egg constituents, embryo growth, embryo temperature, and hatching percentage.

4.2 MATERIALS AND METHODS

Naturally-mated Cobb 500 hens and roosters produced eggs of the fast-growing genotype (F), Hy-Line Brown hens and roosters produced eggs of the slow-growing genotype (S) and a cross of Hy-Line Brown roosters and Cobb 500 hens produced eggs of the medium-growth genotype (M). The birds were placed on wood shavings on a solid concrete floor in 3m x 3m floor pens in an open-sided naturally ventilated house. Two replicates of ten females and one male were used for each genotype and feed combination, resulting in a total of 12 pens. The male and female birds for both the Cobb 500 and the Hy-Line brown were fed in separate feeders. The females were fed in metal troughs with male exclusion grids that prevented the male access to the female feed by providing limited size opening to the trough. The males were fed in separate raised feeders with no access barriers. It was assumed that the males would consume the available feed before the females had finished and thereby prevent the females from access to the male feed. All of the pens received water from suspended bell drinkers.

A high- and a low-protein feed were formulated (Table 4.1). The high- and the low-protein feeds were mixed in a 1:1 ratio to produce Treatment 1, a diet containing a medium protein level (MP), which provided 920 mg lysine/bird/d. The low-protein feed (LP) provided Treatment 2 which provided 816 mg lysine/bird/d (Table 4.2) Each treatment was replicated within each genotype group. The feeds were analysed at the University of KwaZulu-Natal Animal and Poultry Science Department. The samples were freeze-dried for 72 hours for moisture determination (AOAC, 1990). Crude protein was determined with a LECO FP2000 Nitrogen Analyser, based on the Dumas combustion method (AOAC, 1990). AME was measured using the method of Fisher (1982) in which 50g of the diet is given by tube to cockerels (Sibbald, 1976), following a 48 h fasting period, and excreta are collected over the following 48 h. The AME value was corrected to zero N retention and to reflect an intake of 80 g/d (AMEn80). The results of the analysis showed the expected levels of protein (Table 4.3). Unfortunately, due to constraints, the AA profiles of the feeds were not analysed. However, the AA levels were assumed to be as expected. The feed allocation for all genotypes was 160 g/bird/d. Both the Cobb 500 hens and the Hy-Line hens were placed on the trial feed 2 weeks before the start of egg collection. As the aim of the trial was to determine the

maternal dietary effect on the progeny only the hens received the treatment diets. All male birds were provided with a commercial broiler breeder feed at 120 g/bird/d (16% CP). Prior to this experiment all birds had received industry standard feed rations (16% CP) at recommended feed intake levels.

Table 4.1 *Basal feed raw material ingredient composition*

Ingredients (% inclusion)	HP	LP
maize	56.0	63.5
wheat bran	11.3	12.0
soybean full fat	18.7	12.3
sunflower oil cake	6.4	4.3
DL methionine	0.05	0.01
vit+min premix	0.15	0.15
limestone	6.5	6.5
salt	0.21	0.20
monocalcium phosphate	0.42	0.42
sodium bicarbonate	0.35	0.37
potassium hydroxide	-	0.14

Table 4.2

Calculated amino acid for two trial feeds at 920 mg lysine/bird/d (MP) and 816 mg lysine/bird/d (LP) with an expected feed intake of 160 g/bird/d

Amino acid	Calculated g/kg	
	HP	LP
Lysine	6.40	5.10
Methionine	2.90	2.30
Methionine & Cystine	4.90	3.90
tryptophan	1.50	1.20
Threonine	4.10	3.30
Isoleucine	4.60	3.70
Arginine	5.80	4.60
Leucine	7.40	5.90
Histidine	2.20	1.80
Phenylalanine & tyrosine	7.90	6.30
Valine	5.40	4.30
AMEn	11.5	11.5

Table 4.3 *Results of laboratory analysis for two trial feeds at 920 mg lysine/bird/d (MP) and 816 mg lysine/bird/d (LP) with an expected feed intake of 160 g/bird/d*

Feed	Protein (%)	Moisture (%)	AMEn (MJ/kg)
HP	16.6	11.3	12.0
LP	12.4	11.8	11.7

The females were placed in the pens 3 d before the males were introduced. This was to allow them to become accustomed to the pens as the layers were sourced from a battery cage system.

The number of eggs produced, as well as the total egg weight per pen was recorded daily from the day the roosters were introduced for 18 d. Eggs were collected for incubation from the d 7 for to d 17, marked by treatment, weighed, and stored in a cool room at 18 ^{o}C for the duration of the collection period. Although the storage time for the collected eggs was up 10 d, which is expected to impact hatchability, this long

collection period was used to ensure sufficient eggs were collected to achieve viable results. Once the collection was complete 60 eggs per trial group were placed in an incubator at 37^{o}C and 60% RH with 90 minute rotation of the eggs for 18 d, and transferred to hatching baskets with dividers, to keep each treatment separate. The Cobb 500 hens were all 47 weeks old, and the Hy-Line Brown hens were 32 weeks old at the start of collection. This difference in age was due to availability of birds for the trial.

During incubation, 3 eggs from each trial group were removed from the incubator every 2 d. The egg shell temperature was immediately measured at three random locations around each egg using a Braun thermoscan 1RT 4520 digital thermometer and then the average was recorded. As the thermal conductance of the egg shell is high, in comparison with that of the surrounding air (Sotherland *et al*., 1987) a measurement of the egg shell temperature provides a reasonable estimate of the embryo temperature (French, 1997). This method allows the chick to grow and develop normally throughout incubation, as opposed to more invasive methods were a probe is implanted in the egg and may hinder growth. After the temperature was measured the eggs were weighed and developing embryos were sacrificed by breaking the egg into a petri dish so that the embryo could be removed and weighed on a digital scale. On d 2, as the developing embryo was too small to get a meaningful weight measurement with the available equipment the albumen and yolk weights were recorded instead. On d 21 the overall percentage hatch and percentage hatch of fertile, measured using a break-out analysis, were recorded for each treatment.

The embryo temperature, embryo weight, albumen weight, yolk weight, egg weight, percentage hatch and percentage hatch of fertile were subjected to an ANOVA using the combined data for all three genotypes separated for each lysine level. The above parameters were also separated by genotype and analysed against the two lysine levels using ANOVA. The change of embryo weight for each genotype was analysed individually using an exponential standard curve regression grouped by lysine level. Each genotype and dietary treatment combination were then analysed individually. Although it resulted in negative asymptotes the origins of the equation were left unconstrained. As the embryo experiences exponential growth without reaching a plateau before hatching this was deemed to be the cause of the negative shape of the

exponential curve. As such the negative asymptote is a mathematical rather than a biological cause. As the equation should only be used to predict the embryo weight over 0 g the negative asymptote was not seen to be an issue. All significant values are at the 0.05 level unless stated otherwise. Statistical analyses were performed using Genstat (2010).

Ethical approval from the University of KwaZulu Natal ethics committee was obtained prior to the start of the experiment (reference 051/09/Animal), and the code of conduct was adhered to throughout.

4.3 RESULTS AND DISCUSSION

The embryo temperature of eggs from hens fed MP was significantly affected by genotype, with the eggs from M recording the highest average temperature and those from S the average lowest (Table 4.4). This was also observed for most days of incubation. For hens fed LP the F embryo temperature was significantly less than S, with the M genotype being non-significant from either. For the F genotype the dietary treatment had a significant effect on the embryo temperature with the MP diet resulting in eggs with a higher temperature. The M genotype followed the same trend with the higher lysine level resulting in significantly higher embryo temperature than the lower lysine level. However, for the S genotype the LP resulted in a significantly higher embryo temperature than the MP.

Table 4.4 *Embryo temperature during incubation (°C) for fast (F), medium (M) and slow (S) growing genotypes from hens fed 920 mg lysine/bird/d (MP) and 816 mg lysine/bird/d (LP) over 18 days of incubation*

	Embryo Temperature (°C)						
Main Effects							
Day of Incubation	MP	LP	p-value	F	M	S	p-value
2	36.3	36.5	0.300	36.2	36.4	36.5	0.420
4	36.9	37.1	0.080	36.8^{b}	37.2^{a}	37.0^{ab}	0.050
6	36.6	36.2	0.118	36.7^{a}	36.6^{a}	36.0	0.011
8	36.6	36.6	0.735	36.7	36.6	36.5	0.336
10	36.7	36.5	0.319	36.4	36.8	36.5	0.206
12	36.7	36.5	0.319	36.4	36.8	36.5	0.206
14	36.5	36.6	0.494	36.6	36.8	36.4	0.168
16	36.9	37.2	0.230	37.1^{a}	37.4^{a}	36.5^{b}	0.013
18	36.9	36.6	0.148	36.8^{a}	37.1^{a}	36.4^{b}	0.005
Average Embryo Temperature							
	Lysine						
Genotype	LP	MP	p-value				
F	36.5^{g}	36.8^{ef}	0.001				
M	36.6^{fg}	37.1^{e}					
S	36.8^{ef}	36.1^{h}					
	F		M		S		
Day of Incubation	MP	LP	MP	LP	MP	LP	p-value
2	36.2	36.1	36.4	36.5	36.2	36.8	0.378
4	36.9^{abc}	36.7^{ab}	37.1^{bcd}	37.3^{d}	36.7^{a}	37.3^{cd}	0.002
6	36.6^{bcd}	36.8^{cd}	37.1^{d}	36.2^{abc}	36.1^{ab}	35.8^{a}	0.003
8	36.8^{b}	36.7^{ab}	36.6^{ab}	36.5^{ab}	36.3^{a}	36.7^{ab}	0.317
10	36.8^{bc}	36.0^{a}	37.2^{c}	36.5^{ab}	36.1^{a}	36.9^{bc}	0.001
12	36.8^{bc}	36.0^{a}	37.2^{c}	36.5^{ab}	36.1^{a}	36.9^{bc}	0.001
14	36.5^{bc}	36.6^{bc}	37.2^{d}	36.4^{b}	35.8^{a}	36.9^{cd}	0.001
16	37.5^{c}	36.8^{b}	37.4^{c}	37.4^{c}	35.7^{a}	37.4^{c}	0.001
18	36.8^{b}	36.9^{b}	37.6^{c}	36.5^{ab}	36.3^{a}	36.5^{ab}	0.001

Values in a row without a common superscript (a,b,c,d) differ significantly ($p<0.05$)

Values for average embryo temperature without a common superscript (e,f,g,h) differ significantly ($p<0.05$)

Janke *et al.* (2004) found that from d 12 to 15 and 18 to 20 of incubation, the temperature of White Leghorn layer embryos was significantly lower than Ross 308 and 508 broiler embryos (Table 4.5). Tona *et al.* (2004) found that embryos from a standard heavy broiler line had a significantly higher heat production at 18 d of incubation compared to slower growing broiler lines. The availability of nutrients and oxygen determines the rate of tissue bio-synthesis of the embryo and so the rate of growth. Thus, a strong physiological relationship exists between the rate of metabolic heat production and bio-synthesis (Boerjan, 2006), and suggests that parents fed the higher lysine level in this experiment produced embryos that had a higher capacity for growth with both the F and M genotypes. Boerjan (2006), determined that the d 18 metabolic heat production was 26% higher for Ross 308 broilers compared to White Leghorn layers. From this, it was determined that a higher metabolic heat production by modern broiler breeds, compared to a slower growing breed, was the result of a higher growth potential. However, French (1997) determined that the thermal conductance of the egg, accounting for incubator air speed, scales with egg mass to the power of 0.53. Thus, as egg mass increases, thermal conductance does not increase proportionally, so larger eggs should have greater difficulty in losing metabolic heat produced by the embryo. However, this was only expected to have a significant impact for large eggs towards the end of incubation. The findings of Lourens *et al.* (2006), with eggs from Hybro G grandparent stock, that large eggs (70-72 g) had a higher heat production compared with small eggs (54-56 g) from d 15 to d 18 of incubation are in agreement with this. It is, therefore, possible that the egg shell temperature may be influenced by factors other than the embryonic metabolic heat production, such as egg size, and may explain the differences seen between genotypes.

Table 4.5 *Embryo temperatures during incubation for Ross 308, Ross 508, and White Leghorn modified from Janke et al. (2004)*

Day of Incubation	Embryo Temperature (°C)		
	Ross 308	Ross 508	White Leghorn
10	37.6	37.6	
12	38.2[a]	38.2[a]	37.9[b]
14	38.9[a]	39.0[a]	38.3[b]
16	39.4	39.6	39.0
18	39.7[a]	39.6[a]	39.3[b]
20	40.5[a]	40.5[a]	39.0[b]

White Leghorn data adapted from Janke *et al.* (2004), values in a row without a common superscript (a,b) reported to differ significantly ($p<0.05$)

The S genotype eggs had a higher heat production from parents fed LP *vs* MP. This indicates that the MP treatment may have induced some maternal response that affected the embryo. However, the exact nature of the factors that have induced this result, as well as the possible processes involved, are not clear. It is possible that the higher lysine in the maternal diet may have resulted in an oversupply of lysine in the egg. This, in turn, may have impacted the metabolic heat production. This may have been caused by unbalancing of the AA ratios in the egg or by the excess lysine producing some "toxic" effect that negatively impacted the metabolic process. Further research is necessary in order to clarify these findings and determine possible repercussions.

For eggs from hens on LP from d 8 of incubation onwards, the weight of S was significantly lower than F (Table 4.6). Different genotypes resulted in different rates of embryonic growth (Figure 4.1).

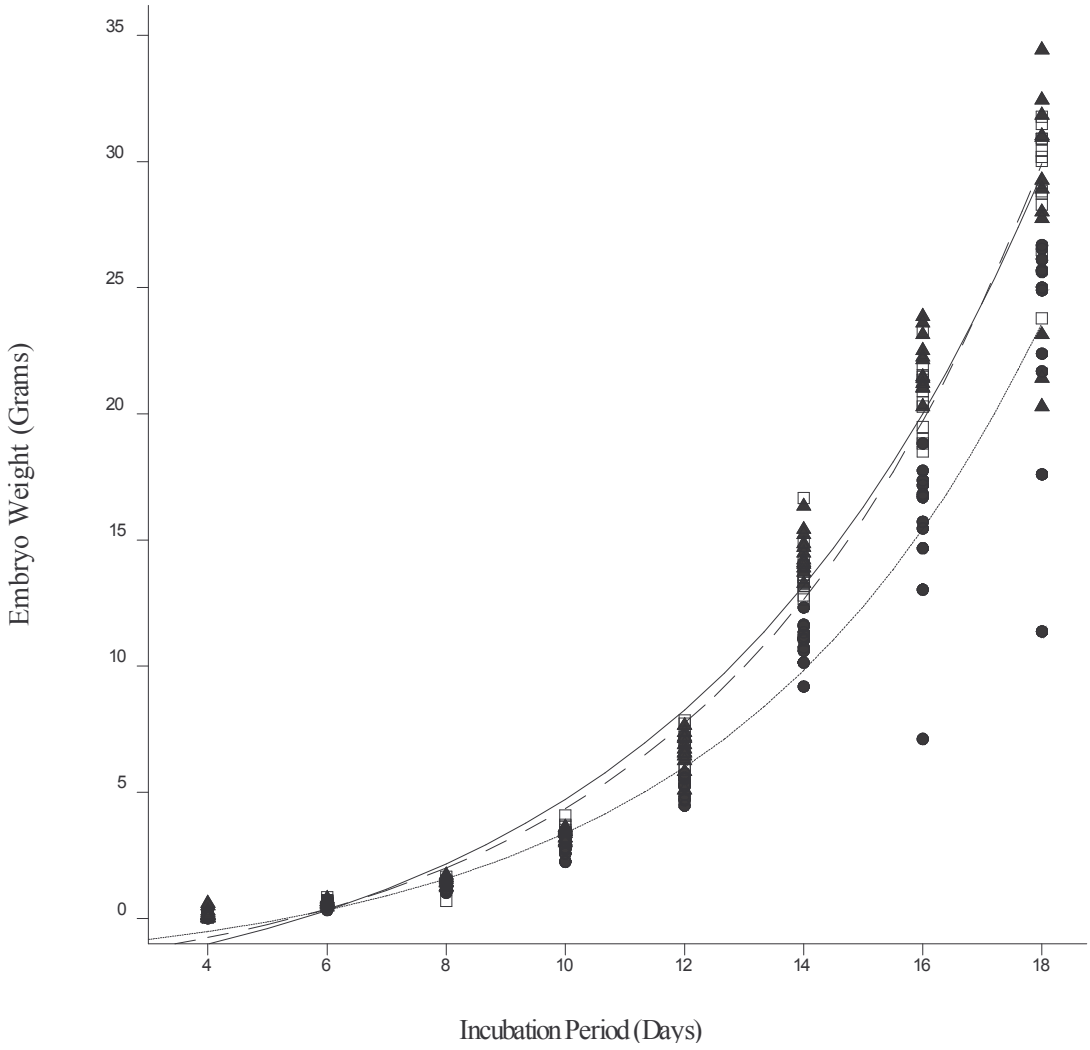

Figure 4.1 *Embryo weight from d 4 to d 18 of incubation, grouped by genotype without constrained origin, for fast (▲)(——), medium (□)(– –), and slow (●)(·····) growing genotypes*

The slow genotype had the lowest growth rate

$y = -2.360 + 0.870*1.21^{x}$ ($p<0.05$, $R^2=0.96$, asymptote SE =0.74 rate SE = 0.25, shape SE = 0.02)

followed by the M genotype.

$y = -3.30 + 1.22*1.20^{x}$ ($p<0.05$, $R^2=0.96$, asymptote SE =0.77 rate SE = 0.27, shape SE = 0.02)

The F growth rate and shape of the curve was significantly different for birds on MP and LP (Figure 4.2)

Lysine 920 mg/bird/d

$y = -3.37 + 1.23*1.21^{x}$ ($p<0.05$, $R^2=0.96$, asymptote SE =1.10 rate SE = 0.38, shape SE = 0.02)

Lysine 816 mg/bird/d

$y = -5.84 + 2.63*1.15^{x}$ ($p<0.05$, $R^2=0.96$, asymptote SE =1.75 rate SE = 0.91, shape SE = 0.02)

Where y = average weight (grams) and x = duration of incubation from d 0 (d).

Table 4.6 *Average embryo weights during incubation for fast (F), medium (M) and slow (S) growing genotypes from hens fed 920 mg lysine/bird/d (MP) and 816 mg lysine/bird/d (LP) over 18 days of incubation including main effects*

	Embryo weight (g)						
Day of incubation	MP	LP	p-value	F	M	S	p-value
4	0.146	0.091	0.191	0.189^{a}	0.113^{ab}	0.053^{b}	0.021
6	0.602	0.593	0.806	0.633^{a}	0.633^{a}	0.527^{b}	0.013
8	1.340	1.330	0.951	1.470^{a}	1.360^{a}	1.180^{b}	0.002
10	3.070	3.270	0.147	3.370^{a}	3.450^{a}	2.710^{b}	0.001
12	6.160	6.240	0.812	6.640^{a}	6.720^{a}	5.230^{b}	0.001
14	12.900	13.300	0.579	14.500^{a}	13.800^{a}	11.000^{b}	0.001
16	19.300	19.500	0.884	22.000^{a}	20.600^{a}	15.6200^{b}	0.001
18	27.900	26.000	0.215	28.300^{a}	29.300^{a}	23.2800^{b}	0.001

Day of incubation		F	M	S	p-value
4	MP	0.280^{a}	0.098^{b}	0.058^{b}	0.030
6		0.633^{a}	0.605^{a}	0.568^{a}	0.472
8		1.510^{a}	1.340^{ab}	1.170^{b}	0.070
10		3.340^{a}	3.310^{a}	2.580^{b}	0.001
12		6.740^{a}	6.730^{a}	5.010^{b}	0.001
14		14.800^{a}	13.400^{b}	10.700^{c}	0.001
16		22.000^{a}	21.300^{a}	14.700^{b}	0.001
18		31.100^{a}	28.900^{a}	23.800^{b}	0.002
4	LP	0.098^{ab}	0.128^{a}	0.047^{b}	0.048
6		0.633^{a}	0.662^{a}	0.485^{b}	0.017
8		1.430^{a}	1.380^{a}	1.200^{b}	0.008
10		3.400^{a}	3.590^{a}	2.840^{b}	0.001
12		6.540^{a}	6.710^{a}	5.450^{b}	0.036
14		14.300^{a}	14.200^{a}	11.400^{b}	0.001
16		22.000^{a}	19.900^{b}	16.500^{c}	0.001
18		25.500^{ab}	29.700^{a}	22.700^{b}	0.045

Values in a row without a common superscript differ significantly ($p<0.05$)

Everaert *et al.* (2008) observed that from d 4 of incubation until hatching, the absolute yolk-free broiler embryos were significantly heavier than the layer embryos. This is in agreement with Pal *et al.* (2002) who found that both wet and dry weights of broiler embryos were found to be greater than the equivalent layer values from 12 to 20 d of incubation, and Sato *et al.* (2006) found the same pattern for whole embryo weight from 12 to 18 d of incubation.

There was a clear distinction between the embryo weights of the F and M genotypes compared to the S genotype (Table 4.6). From d 6 of incubation the weight of the S genotype was significantly lower than the other two (Table 4.6). Unpublished data from trials conducted concurrently suggests that the AA profile of the egg can be influenced by the diet of the hen. So, a reduction in the lysine level may have resulted in a sub-optimal amino acid balance in the egg. Al-Murrani (1978) compared the embryo development in large (59 to 69 g) and small (46 to 56 g) eggs as well as the egg protein, fat and moisture contents. It was found that of the three, protein was the only component of the egg not in excess of requirements for incubation and, in comparison with the growth, that protein, rather than egg size, limited the embryo growth. This is agreement with the current findings. However, Ohta *et al.* (2001) discovered that broiler embryos that received an AA injection on d 7 of incubation exhibited no significant difference in embryo weight on d 14 or 19 of incubation, although the AA present in the chicks was increased by the *in ovo* injection on d 19 of incubation. Ohta *et al.* (2004) found that although *in ovo* AA injections on d 7 of incubation did not significantly improve chick weight at hatch it did significantly improve the chick weight relative to the initial egg weight compared to the control. So it may not only be the amount of protein that the egg is provisioned with, but also the ratios of the AAs, as determined by what the hen has available, that affect the embryo development.

As the majority of the growth of the embryo happens towards the end of the incubation period (Romanoff, 1960), differences between treatments should be discernible at this stage. As expected, the weight of the F-genotype was significantly affected by the lysine concentration in the maternal diet at d 18 of incubation with a higher embryo weight resulting from eggs incubated from parents fed MP (Figure 4.2). This is probably linked

to the higher embryo heat production observed from eggs incubated from parents fed MP for the F and M genotypes. Ohta *et al.* (2004) found that on d 14 and 19 of incubation the broiler embryo weight was significantly greater than the layer embryo weight. It was also found that, in addition to the faster consumption of yolk in broiler embryos, embryonic growth and accumulation of protein was higher in broiler embryos compared to layer embryos which infers a difference in the embryonic growth between broilers and layers.

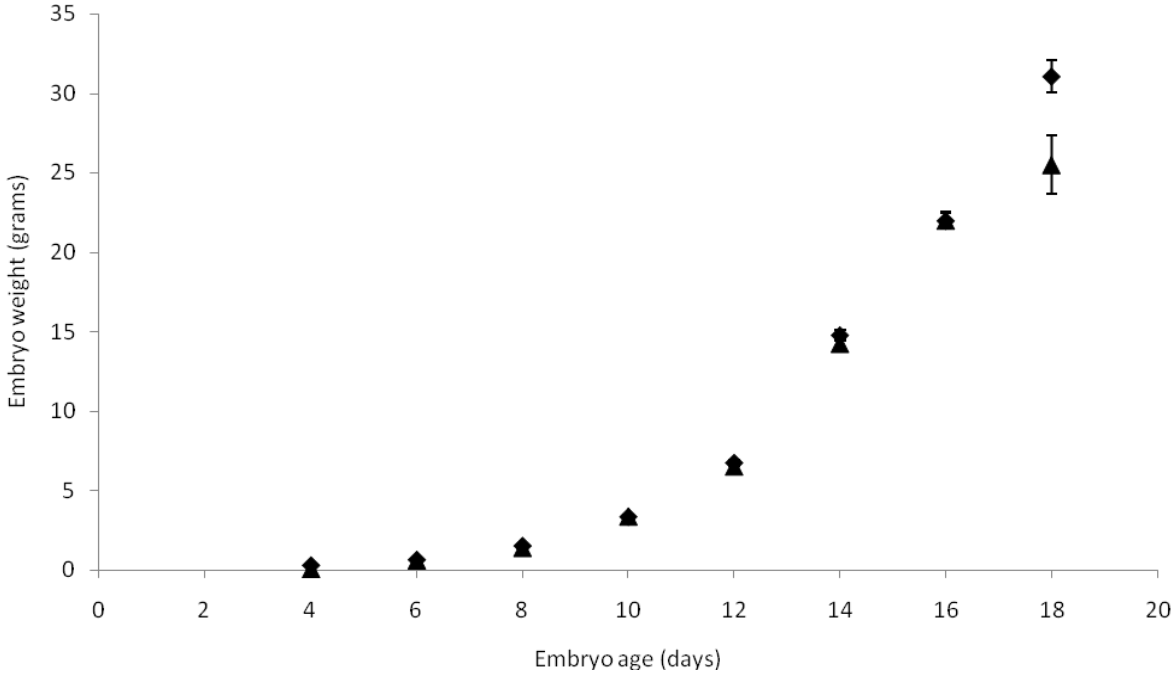

Figure 4.2 *Fast growing genotype embryo weight from d 4 to d 18 of incubation of eggs from parents fed 920 mg lysine/bird/d (◆) or 816 mg lysine/bird/d,(▲) ± standard error of the mean*

The egg weight was significantly affected by the maternal lysine treatments for both the F and S genotypes but not for M (Table 4.7). Pearson & Herron (1981) observed a significant increase in egg weight from broiler breeders that received a high (27.0 g/bird/d) level of protein compared to a low (19.4 g/bird/d) level, as did Spratt & Leeson (1987) for 25 g protein/bird/d and 19 g protein/bird/d. Hens of the F genotype on MP produced eggs with a significantly higher weight compared to those fed LP and hens of the S genotype on LP produced eggs with a significantly higher weight compared to those fed MP. As with the embryo heat production, the F genotype hens

appear to have benefited more from the MP by producing larger eggs than those on the LP. This may indicate that the lower lysine level of 816 mg lysine/bird/d could have had a negative impact in some physiological processes within the hen. This would most likely involve the egg production process and the lower dietary lysine may have resulted in reduced lysine deposition in the egg or a change in the amino acid ratios in the egg. Which, in turn, may have resulted in a retardation of the growth and development of the broiler embryo. The S genotype hens also followed the same trend as that found in the embryo heat production. As with the embryo heat production there is need for further research in order to determine the exact nature of the effect of these lysine levels on both the F and S genotype hens.

Table 4.7 *Average egg, albumen and yolk weight and albumen to yolk ratio for fast (F), medium (M), and slow (S) growing genotypes from hens fed 920 mg lysine/bird/d (MP) or 816 mg lysine/bird/d (LP)*

	Genotype	MP	LP	p-value
Average egg weight (g)	F	64.80[a]	62.50[b]	0.006
	M	65.00	64.80	0.879
	S	53.50[b]	56.20[a]	0.004
Albumen Weight (g)	F	35.30[a]	31.60[b]	0.028
	M	32.50	30.40	0.530
	S	26.70	29.60	0.078
Yolk Weight (g)	F	23.70	24.20	0.725
	M	23.50	28.60	0.133
	S	18.50	16.50	0.168
Albumen : Yolk (g/g)	F	1.53	1.31	0.168
	M	1.39	1.18	0.372
	S	1.49	1.80	0.077

Values in a row without a common superscript differ significantly ($p<0.05$)

The albumen weight was significantly affected by the maternal lysine treatments for the F-genotype but not for M and S-genotypes. It followed the same trend as the egg weight (Table 4.7). However, there was no significant difference in the yolk weights within each genotype for the two treatments. Thus, the differences in the albumen weight could

have contributed to the differences in the egg weight in all three genotypes. Although the albumen weight was only significantly higher for the F genotype, it did follow the same trend as the egg weight for all three genotypes. The difference in albumen weights between the two treatments for the F genotype may account for the difference in embryo weight observed on d 18 of incubation, and may indicate a potential benefit for the embryo. As with the first experiment, the percentage hatch and percentage hatch of fertile were not affected by genotype or treatment. So while the growth and size parameters of the embryo provide an insight into its potential growth and the effect of the treatments, measuring the hatch does not appear to provide any useful congruent parameters.

4.3 CONCLUSIONS

The effect of the two maternal lysine levels appears to have been more pronounced on the F and S genotypes. A higher performance was observed for the F genotype that received the higher lysine treatment and for the S genotype that received the lower lysine treatment. So while increasing the lysine may have been beneficial for the F genotype it appears that this was not the case for the S genotype. As the reasons for this are not clearly understood there is a need for further research investigation the mechanisms involved in the utilisation of maternal protein and egg formation to understand how the nutrition of the hen affects the performance of the progeny.

5. THE EFFECT OF MATERNAL DIETARY LYSINE ON CHICK GROWTH OF THREE CHICKEN GENOTYPES

5.1 INTRODUCTION

As with the embryo development, most reports on the impact of maternal CP on the chick performance have shown no significant improvement with an increase in maternal CP (Pearson & Herron, 1981; Spratt & Leeson, 1987; Lopez & Leeson, 1994b). However, larger eggs produce larger chicks which have an advantage in early weight gain (Vieira & Moran, 1998). Larger chicks at hatch can also lead to larger chicks at marketing with each 1 g increase at hatch leading to an 8 to 13 g increase at marketing (Wilson, 1991). As growth does not start at hatch but rather with embryonic development, it may be short-sighted to not consider growth from the start of embryonic development. As such, any maternal impact may carry through to the growing chick. The consideration of the impact of maternal dietary protein has, so far, as with the embryo, been limited to CP rather than AA. If there is an impact of maternal AA intake on the growth and development of the embryo then it is possible that it may also be true for the chick. This is of particular interest considering that, of the total feed consumed by the chick during growth, the maternal contribution is less than 7% (Calini & Siri, 2007). If an improvement in the performance of the chick can indeed be achieved via the maternal nutrition then this will result in an additive effect that may improve chick performance in conjunction with the chick nutrition.

At 6 weeks of age the body weights of broilers are five times greater than those of layers (Zhao *et al.*, 2004), and there is a possibility that the chicks of different genotypes may respond differently to changes in maternal nutrition.

Therefore, the objective of this experiment was to determine if the maternal dietary lysine intake has any influence on the chick performance of different genotypes by measuring the growth of chicks after hatch, from different maternal genotypes fed different levels of lysine.

5.2 MATERIALS AND METHODS

For this experiment the chicks that hatched from the previous experiment were used (see section 4.2). For the fast-growing genotype (F) naturally-mated Cobb 500 hens and roosters were used, Hy-Line Brown hens and roosters produced the slow-growing genotype (S) and a cross of Hy-Line Brown roosters and Cobb 500 hens produced the medium-growth genotype (M). Two groups of parent flocks were used for each genotype. Each group of parent flocks of each genotype received either; treatment 1, a diet containing a medium protein level (MP), which provided 920 mg lysine/bird/d or treatment 2, a low-protein feed (LP) which provided 816 mg lysine/bird/d. As chicks from the previous trial were used there was some difference in numbers due to variations in hatchability and fertility. The numbers of chicks for each group were: F genotype MP (n=30), LP (n=14); M genotype MP (n=50), LP (n=37); S genotype MP (n=40), LP (n=48). The chicks were separated into groups of 5 based on genotype and maternal lysine treatment. Each group was treated as a individual unit with the average weight being used for measurements. Each group was placed in a separate 0.5 m x 0.5 m wire cage with free access to feed from troughs and water from nipple drinkers. These cages were raised 1 m off the floor and arranged in rows two cages wide and eight cages deep. The entire cage system was kept in an enclosed brooder room with a sealed concrete floor and wood shavings 5 cm deep under each row. The air temperature in the room was maintained using a gas heater and PVC ventilation tubing. The initial temperature in the room when the chicks were placed was 30^{o}C, which was decreased linearly to 21^{o}C at d 21.

At 21 d the chicks were wing-banded, to allow dietary treatment and group identification. They were then moved out of the brooder room and into a floor pen system on a wood shaving floor with each genotype group in its own pen. Each original group of 5 was maintained for weight measurement. The pens were in an open-sided house with natural ventilation. As the impact of the maternal nutrition was the main focus for this trial the same commercial feed was fed to all the chicks. The chicks were fed a commercial broiler starter for 21 d and broiler grower (Table 5.1) for 14 d *ad libitum* from feed troughs placed in the pens. All of the pens received water from suspended bell drinkers.

Table 5.1 *Chemical composition of commercial broiler starter and grower feeds*

Feed	Protein (%)	Moisture (%)	Fat (%)	Fibre (%)	Calcium (%)	Phosphorus (%)	Total lysine (%)
Broiler Starter	20	12	2.5	5	0.8-1.2	0.6	1.2
Broiler Grower	18	12	2.5	5	0.7-1.2	5.5	1.0

On d 1 and every seven days the chick weight was recorded. This continued to d 35 when the last measurement was taken. The average weight gain was determined by subtracting the average chick weight from the average chick weight of the previous measurement, i.e. average chick weight on d 14 minus average chick weight on d 7. The cumulative weight gain was determined by addition of the average weight gain for the given period and the average weight gain from the previous measurement, ie: for d 14, average chick weight gain to d 7 plus average chick weight gain to d 14.

The average chick weight, average chick weight gain and cumulative weight gain were subjected to ANOVA to determine main effects and interactions. The above parameters were also separated by genotype and analysed against the two lysine levels using ANOVA. The effects of age on chick weight and cumulative weight gain for both maternal lysine treatments were analysed using an exponential standard curve to determine response of chick weight and cumulative weight gain to lysine level, with genotype as a group. Each genotype and treatment combination were then analysed individually determine the equations of the curves. For the equations the origin was left unconstrained. Although this resulted in a negative asymptote it was deemed to be a more meaningful equation as it accounted for the chicks hatching at a weight above 0 g. The negative nature of the asymptote was attributed to the nature of the growth curve and the period that was analysed, ie. during the accelerating growth phase. As the measured period was only during the exponential growth phase, rather than considering the entire growth pattern the equation can only be used to predict values from this

phase. All significant values are at the 0.05 level unless stated otherwise. Statistical analyses were performed using Genstat (2010).

Ethical approval from the University of KwaZulu Natal ethics committee was obtained prior to the start of the experiment, and the code of conduct was adhered to throughout.

5.3 RESULTS AND DISCUSSION

For both maternal lysine treatments the F and M genotypes produced significantly heavier chicks than those of the S genotype. In addition to the expected differences in chick weight between the three genotypes there was also a difference between the lysine treatments for chicks from both the F and S genotypes (Table 5.2).

Table 5.2 *Average chick weight at hatch for fast (F), medium (M), and slow (S) growing genotypes from hens fed 920 mg lysine/bird/d (MP) or 826 mg lysine/bird/d (LP)*

Genotype	Average Chick Weight (g)	
	MP	LP
F	50.1^{a1}	47.1^{b1}
M	48.9^{a1}	49.0^{a1}
S	39.6^{b2}	42.8^{a2}

[a, b] Values in a row without a common superscript differ significantly ($p<0.05$)

[1, 2] Values in a column without a common superscript differ significantly ($p<0.05$)

As seen in Chapter 3, the F genotype responded more favourably to the higher lysine level, and the S genotype to the lower lysine level. This indicates that there may be some influence of the lysine on the performance of the chick up to hatch. Several studies have reported that higher body weight at hatch results is a greater body weight at marketing (Shanawany, 1984; Sinclair *et al*., 1990; Vieira & Moran, 1998; Sklan *et al*., 2003), so it is reasonable to assume that these chick size differences may be carried through into post-hatch growth.

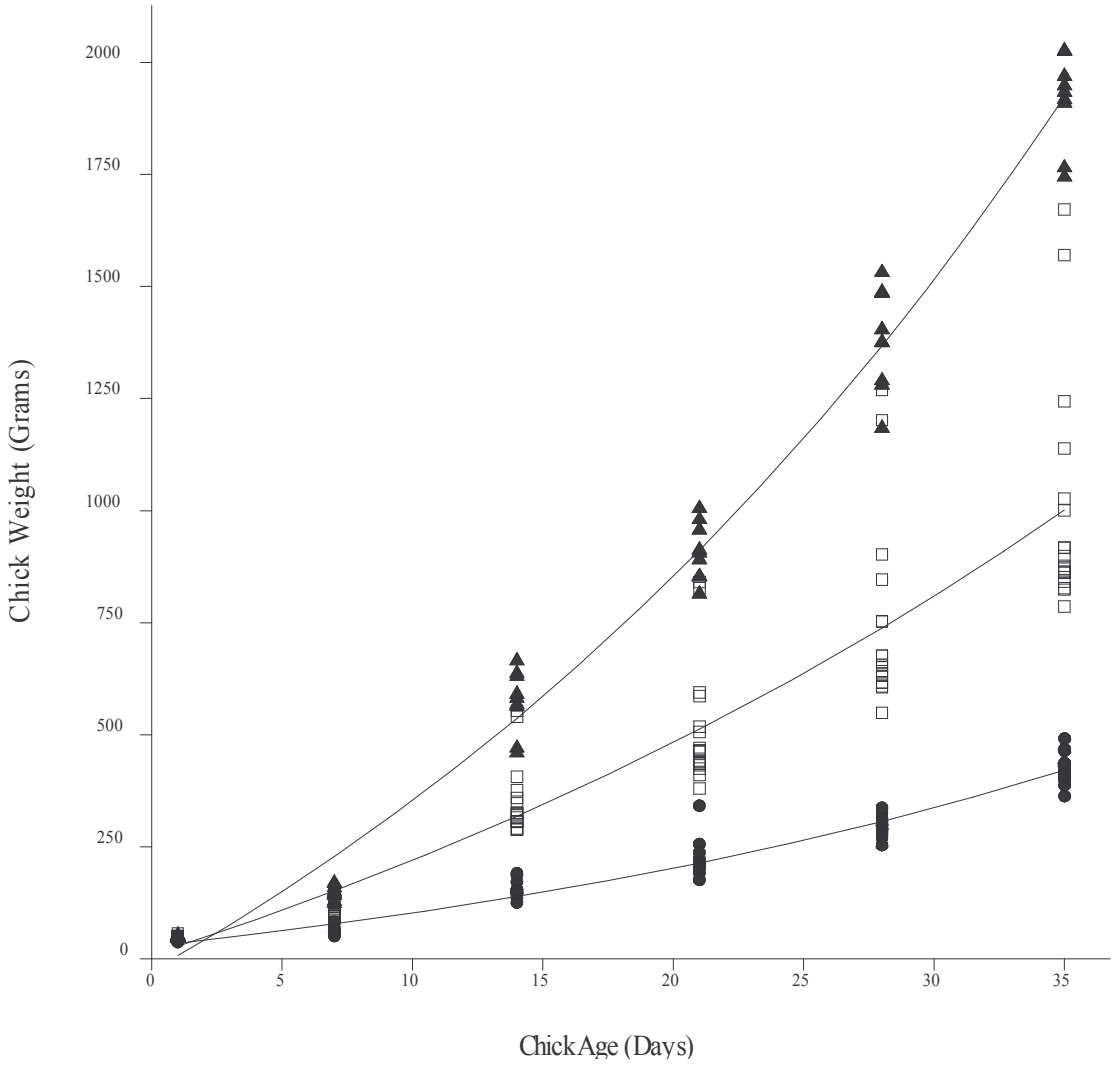

Figure 5.1 *Chick weight from hatch to d 35 grouped by genotype without constrained origin, for fast (▲), medium (□), and slow (●) growing genotypes*

The chick weight for all three genotypes increased in a positive exponential manner with the F genotype growing the fastest and the S genotype the slowest (Figure 5.1). The combined exponential regression of all three genotypes revealed a similar type of response with all three following the same exponential shape, despite significant differences in the rate:

F genotype

$y = -1203 + 1176*1.03^x$ ($p<0.05$, $R^2=0.95$, asymptote SE =319, rate SE = 296, shape SE = 0.01)

M genotype

$y = -859 + 868*1.02^x$ ($p<0.05$, $R^2=0.95$, asymptote SE =366, rate SE = 350, shape SE = 0.01)

S genotype

$y = -184 + 212*1.03^x$ ($p<0.05$, $R^2=0.95$, asymptote SE =203, rate SE = 186, shape SE = 0.02)

Where y = body weight (grams) and x = age from hatch (d).

Tullett & Burton (1983) found that although broiler chicks were not significantly different to broiler cross layer chicks at hatch, they were significantly heavier from d 7 to 56. Layer chicks were found to be significantly lighter at hatch and from d 7 to 56, which is in agreement with this study.

There was no significant difference between the weight of chicks from parents fed MP or LP within or between genotypes when taken over the 35 d period (Figure 5.2). Spratt & Leeson (1987) utilised two CP levels of 19 and 25 g/bird/d on 29, 32, 36, and 40 week old broiler breeder hens and found that it had no significant effect on the growth of the offspring from 1 to 41 d. Lopez & Leeson (1994a) found that a maternal protein intake above 21 g/bird/d had no significant effect on chick weight or subsequent offspring performance. Lopez & Leeson (1994b) reported that feeding hens a dietary CP range of 13 g/bird/d to 22 g/bird/d at a constant lysine level of 0.90% had no significant effect on the offspring growth to 49 d of age. Lopez & Leeson (1995b) evaluated the offspring performance from hens at 30 and 52 weeks of age, using a range of hen dietary CP intake from 16 g/bird/d to 26 g/bird/d with a constant lysine inclusion of 0.82%. They found that maternal dietary CP intake level had a significant effect on the chick weight with an increase in CP resulting in an increase in the chick weight. It was also found that chicks from the 52 week old flock that received 19 g/bird/d CP were consistently heavier through to 48 d of age. The chicks from the same flock with the 16 g/bird/d maternal CP level were consistently the lightest and significantly lighter than those from the 19 g/bird/d maternal CP group. However, all of this research focused primarily on the CP level in the maternal diet rather than the component AA. As such, the effect found by Lopez & Leeson (1995b) may have had more to do with either the levels of particular AA or the AA balance in the diet. However, Pearson & Herron

(1981) used four maternal lysine levels of 970, 1110, 1260, 1450 mg/bird/d and evaluated the offspring performance to d 56 and no significant difference in the growth rates of the chicks was observed.

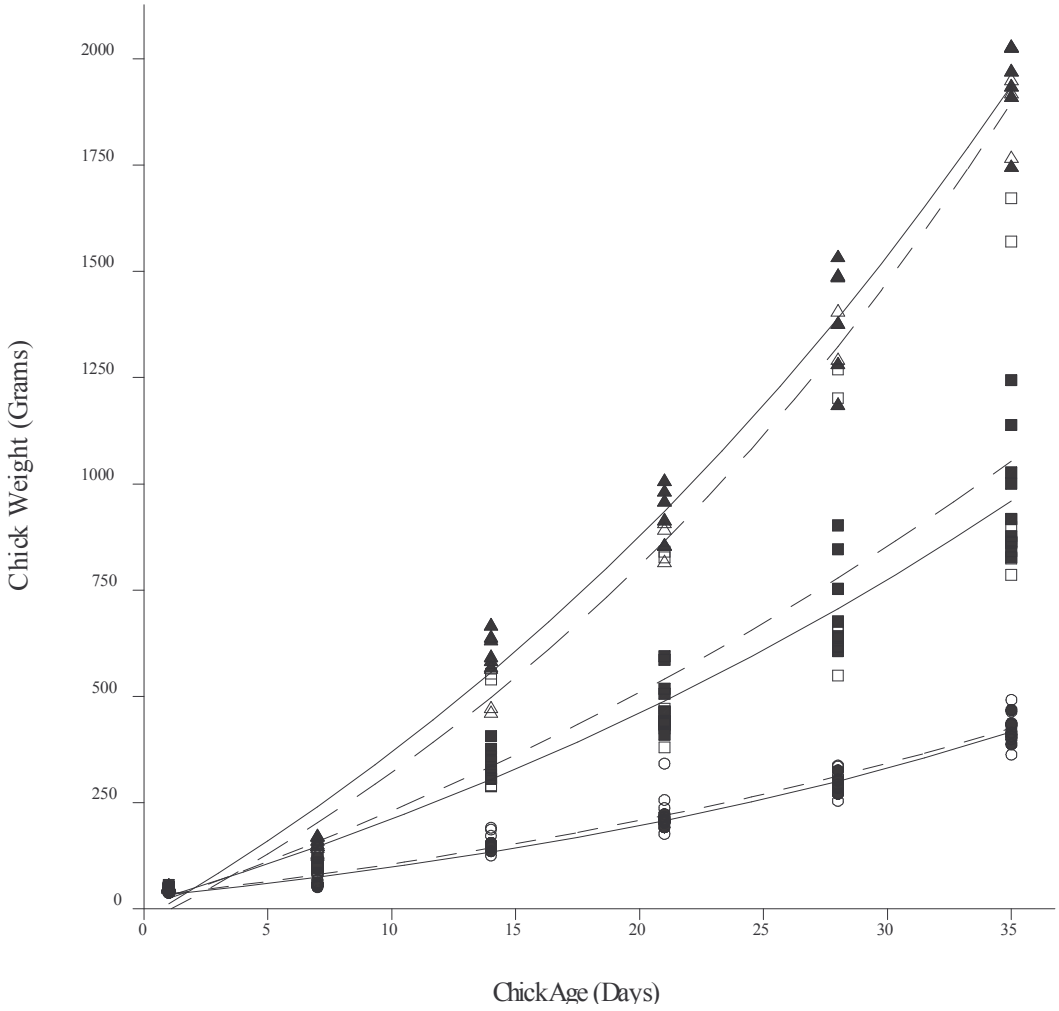

Figure 5.2 *Chick weight from hatch to d 35, for fast growing genotypes with maternal lysine of 920 mg lysine/bird/d (▲)(——) and 816 mg lysine/bird/d (△)(– – –), medium growing genotypes with maternal lysine of 920 mg lysine/bird/d (■)(——), and 816 mg lysine/bird/d (□)(– – –), and fast growing genotypes with maternal lysine of 920 mg lysine/bird/d (●)(——), and 816 mg lysine/bird/d (○)(– – –)*

From d 1 to d 14 of the growth period, within the F genotype, there was a significant difference between the two treatments with heavier chicks from MP:

MP

$y = -693 + 702*1.02^x$ (R^2=0.51, asymptote SE =726, rate SE = 681, shape SE = 0.02)
LP
$y = -598 + 608*1.03^x$ (R^2=0.51, asymptote SE =785, rate SE = 736, shape SE = 0.02)
Where y = body weight (grams) and x = age from hatch (d).

This follows a similar pattern to that observed between the maternal lysine levels during the embryo development, yet at 21 d this trend has been lost. Two possible causes for this are that either compensatory growth from LP has allowed the chicks to catch up with those from MP, or the formulation of the commercial diet was not sufficient for the chicks from MP to capitalise on their advantage. Vieira & Moran (1998) found that chicks that hatched from heavy eggs (65.3 g), regardless of strain, had a higher early weight gain compared to light eggs (57.1 g). However, this initial advantage diminished as the chicks grew larger. So there may be other factors that are influencing the growth of the chicks. Sklan (2001) found that the growth process of the chick was regulated, at least in part, by muscle growth rather than by the development of the gastro-intestinal tract.

5.4 CONCLUSIONS

As the chicks develop they are able to process the required AA more effectively and thus can achieve the required compensatory growth diminishing the advantage of the larger chicks. This may explain the growth patterns found in this experiment. However, it is also possible that there may be other factors that are affecting the chick. As most research focuses on the chick after hatch and does not account for the maternal and embryonic influence, there is a need to further explore this area to better understand the mechanisms involved.

6. GENERAL CONCLUSIONS

The work presented in this dissertation has shown that the maternal lysine intake may affect the developing embryo to some degree. The percentage hatch was not affected by the wide range of lysine treatments used. This indicates that either lysine has no impact on hatchability or the range was not wide enough to elicit a response. Additionally the high variation observed may have been caused by the complications with the incubator which may have masked a possible lysine response. As a depressed hatching percentage is indicative of severe problems with the developing embryo, it may be assumed that broiler breeders can tolerate a wide range of protein without impacting the embryo to the degree that it cannot hatch.

It was observed that broiler breeder hens, at 47 weeks, fed a low lysine level produced offspring that did not grow as well as those from a higher lysine level. The higher lysine level also produced heavier eggs which in turn allowed for larger broiler chicks at hatch. As the advantage that the chicks experienced from the higher maternal lysine continued through to d 14 posthatch there is definitely some benefit conferred by the maternal lysine. This was also demonstrated by the commercial layer offspring where the higher maternal lysine resulted in a reduction in the growth rate of the embryos. As the trend for the broiler embryos from the higher lysine treatment was to grow faster until d 14 posthatch, there is a definite need to determine if this advantage can be carried further by improving the diet of the chicks. If this advantage can be carried through to marketing age it may provide a valuable tool to improve the broiler performance by maximising their genetic growth potential.

The comparison of growth rates of the broiler, layer and broiler cross layer genotypes provides some useful insight into the development of the broiler as a fast growing bird. The growth rate of the cross genotype was close to half-way between that of the broiler and layer genotypes. While this was expected it was none the less of interest for comparing the other two genotypes. The growth rate of the broiler was higher than that of the layer genotype, not only after hatch, as expected, but also before hatch. This indicates that the genetic improvements in broiler growth performance have also

affected the embryo. The cross genotype, while being between the broiler and layer genotypes during growth after hatch, was more similar to that of the broiler during incubation. While this is likely to be an unintended effect of utilising broiler hens for the cross, it does provide a valuable insight into the matter of egg size and egg provisioning. As broiler breeders lay larger eggs the cross genotype would have had not only more space, but also more available nutrients. As can be seen, from hatch, the broiler genotype has a vastly superior growth rate. However, the broiler and cross embryos shared similar embryonic growth rates. This means that some factor was allowing the cross genotype embryo to grow at a similar rate to that of the broiler. As the layer genotype not only had a slower embryo and chick growth rate but also a smaller egg size, it is possible that the larger egg allowed more space for the cross genotype embryo to grow at a higher rate. However, it is also possible that the larger amount of available nutrients allowed the cross genotype embryo to grow to its maximum genetic potential. In which case, this indicates that the broiler embryo, which should have had a higher embryo growth rate as it has a higher chick growth rate, was limited in its embryonic growth. So, a larger egg may have allowed the broiler embryos to grow to their maximum potential, but this would most likely would have been due to more available nutrition, rather than because there was more available space.

The most intriguing development from this research is the different growth curves experienced by the broiler embryos from the two maternal lysine levels. This demonstrates that while the common consensus is that broiler breeders should be fed according to egg output, this does not allow the embryo to reach its genetic potential for growth while in the shell. As it has already been noted that the shell itself does not exert a limiting influence on the growth of the embryo, feeding the hen to ensure optimal embryo growth is a logical conclusion. However, as there are no previous records of this phenomenon in the available literature, there is a clear need for it to be further studied. It is possible that the maternal lysine may have a direct effect on the egg production and so influencing the embryo growth. However, this is not the only possible cause. Another cause may be better understood by taking a more comprehensive perspective. The higher maternal lysine intake may affect another process rather than having a direct effect on the egg production. This process may then affect an additional process within the hen, and so on, resulting a in a cascade effect whereby multiple

physiological aspects within the hen are changed in some, probably minor, way. The net result is that the egg produced may not have a higher protein or lysine content, but rather be improved in some other manner such as the ratios or provisioning of nutrients or other components within the egg. This "cascade" may also affect the developmental cues of the developing embryo. However, more research into the egg formation and embryo developmental processes is required before this can be determined. If this can be built upon it will provide valuable insights into reducing the production period from egg to market for broilers which is of value to the commercial industry.

During this study other areas that could benefit from further research became apparent, such as

- Were the results observed caused by a direct effect of lysine on the maternal provisioning of AA in the egg or did the changes in the maternal diet have an indirect effect on the embryo such as altering other components in the egg?
- Does changing the lysine intake affect the physiological process of the hen and what effect may this have on reproduction?
- Can the duration of the spread of hatch from broiler breeder eggs be reduced by the maternal diet?
- What influence do the improved genetic strains of broiler breeders have on the length of the incubation period, and can it be shortened through either maternal diet or selective breeding programs?
- How does changing the maternal AA intake influence the AA content of the egg and that of the developing embryo?
- How does changing the maternal AA intake influence the yolk and albumen components of the egg?
- Can the maternal diet be used to influence the size of egg produced by broiler breeders, and what influence does egg size have on the developing embryo in improved strains?
- How does the maternal diet influence the egg production in the modern broiler breeder?

- Can decreasing the protein or AA in the maternal diet increase the chick performance by reducing the number of eggs produced and so improving the provisioning of AA and protein in each egg?
- Are the reproductive tracts of modern broiler breeder hens capable of optimum egg formation in terms of required nutrition for broiler embryos?
- Does the chicken embryo have any growth control or regulatory system and how do they influence the embryo growth and development?
- Can the chicken embryo limit its rate of growth and nutrient utilisation based on the amount of nutrition available in the egg?

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