5-12-2012

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Leonel Mejia

EVALUATION OF VARYING DIGESTIBLE LYSINE LEVELS ON THE REPRODUCTIVE PARAMETERS OF COBB 500 BROILER BREEDERS AND THE PERFORMANCE OF THEIR PROGENY

By

Leonel Mejia

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Agricultural Science
in the Department of Poultry Science

Mississippi State, Mississippi

May 2012

EVALUATION OF VARYING DIGESTIBLE LYSINE LEVELS ON THE

REPRODUCTIVE PARAMETERS OF COBB 500 BROILER

BREEDERS AND THE PERFORMANCE

OF THEIR PROGENY

By

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Title of Study: EVALUATION OF VARYING DIGESTIBLE LYSINE LEVELS ON THE REPRODUCTIVE PARAMETERS OF COBB 500 BROILER BREEDERS AND THE PERFORMANCE OF THEIR PROGENY

Pages in Study: 100

Candidate for Degree of Doctor of Philosophy

The effect of decreasing digestible lysine (dLys) intake by broiler breeder (BB) hens from 35 to 45 wk of age on their reproductive performance and performance of their progeny was evaluated. Two types of diets were fed: a diet from commercially available ingredients consisting of dLys intakes of 1,200 (IDL) and 1,010 mg/hen/day (ID) and a semi-purified diet with dLys intakes of 1,010 (SPL) and 600 mg/hen/day (SP). Hens fed the SPL and SP diets had lower hen-day egg production compared to BB hens fed the IDL and ID diets. Fertility and hatchability of eggs set were lowest *(P <0.05)* for hens fed the SPL diets. Chick weight at hatch was lower (*P<0.05*) for those that came from the SP and SPL-fed hens, but 42 and 56 day body weights (BW) were similar for all treatments. Marginal improvements (*P<0.10*) in FCR were seen at 42 and 56 days for chicks from ID-fed hens compared to IDL hens. A decrease in daily intake of dLys appeared to improve BB reproductive performance when hens were fed a semi-purified diet and the same response was not observed in hens fed a diet from commercially available

ingredients. Furthermore, the progeny study revealed marginal improvements in some live performance parameters.

In a second study, a diet based from corn-soybean meal and formulated to a dLys intake of 1,000 mg/hen/day (CS) and three diets composed primarily of corn, soybean meal, and DDGS with 1,000 (DDGS-1,000), 800 (DDGS-800), and 600 (DDGS-600) mg of dLys/hen/day were fed to evaluate the reproductive performance of BB hens from 24 to 42 wk of age. Feeding diets composed of commercially available ingredients with dLys intake levels below 1,000 mg/hen/day did not impact BB reproductive performance. Reduced BW, carcass and breast weight, and higher ($P<0.05$) back half weight at 42 days of age was observed from broilers that came from 26 wk old BB hens fed the DDGS-600 diet. Reducing dLys intake in later BB hen ages did not impact progeny performance or carcass characteristics. This suggests that Lys may be in dietary surplus concentrations for commercial breeders under current practical conditions.

DEDICATION

This dissertation is dedicated to God, family, and friends, who were always supportive of what I did for no other reason than their love for me. Also, to my dearest nephews, whom I have not been able to spend enough time with: Luis Fernando Zuñiga, Santiago, Ian, and Mia Zapata, Aiden Reynaud, and Daniel and Andrea Mejia, with all my love.

ACKNOWLEDGEMENTS

First, I would like to thank my PhD committee members. Thanks to Dr. Alex Corzo, my PhD advisor, for giving me the opportunity to continue my graduate studies, for his knowledge, advice, and guidance during my program at Mississippi State University. I would like to express immense appreciation to Dr. Michael Kidd for his advice, motivation, and above everything else, for being a friend and mentor all this time. Thanks to Dr. Chris McDaniel and Dr. David Peebles for providing me with guidance, advice, and for always having an open door policy to answer questions throughout my graduate program.

This dissertation was made possible with the help of many people. Holly Parker, Sharon Womack, Brad Dewberry, and the Mississippi State poultry farm crew were essential to the work and data collection presented in this document and all the other separate studies that were done by the poultry nutrition lab. I would like to thank my fellow graduate students: Robert Loar, II, Michael Dooley, Keyla Lopez, Derrick Everett, Melissa Haines, C. Obi, and Adebayo Sokale; thank you for your patience, collaboration, and friendship. A special thanks to North Carolina State University and University of Illinois professors and friends, who I consider family, for always supporting me throughout my graduate studies. To my Zamorano professors, who have inspired and motivated me since I was an undergraduate student. Additional thanks to Dr. Paul Tillman for his professional guidance and friendship during my PhD program. Also,

thanks to Freddy Madrigal, Veronica Ferrera, and Cargill-Honduras for their financial support and motivation the past three years of my program.

Lastly, I would like to thank Diego Bohorquez, Mireille Arguelles, Guillermo Gaona, Victor Naranjo, and Cesar Coto for their countless words of support during scientific meetings and my graduate degrees. Also, special appreciation to my life-long friends Sonia and Ray-Scott Miller, Lucia Orantes, Alba Collart, Adriana Gaitan, Diego Zuniga, Wilmer Pacheco, Arnulfo Pineda and Miguel Castillo for their unconditional friendship.

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CHAPTER I

INTRODUCTION

In today's economy, the poultry industry has an ongoing effort to maximize profitability, with significant emphasis given to minimizing production costs such as feed ingredient prices which have pushed the costs of meat and eggs to record levels (Donohue and Cunningham, 2009). One of these opportunities has been through the selection practices employed by the broiler breeder (BB) industry focused on egg production, sexual maturity, fertility, egg size, heat resistance and viability (Hardiman, 1999), and has resulted in improved growth rates, reduced feed conversion, decreased age to slaughter, and increased meat yields in the growing broiler (Havenstein *et al*., 2003).

BB nutrition, most of the times, is evaluated in terms of egg production and hatchability, without taking into consideration fertility or live performance of the growing chick (Leeson, 2005). Vertical integration of the poultry industry has focused on live performance parameters of the growing broiler due to its economic importance in the industry. However, it is very complex to separate maternal nutrition from the embryo. An integrated approach to feed formulation, BB, and progeny performance is needed to maximize the potential of the modern BB and its offspring.

Current nutritional approaches in BB feed formulation include a balance of amino acids to energy in diets that are focused not only in maintaining egg production but also maximizing chick production (de Beer, 2011). According to de Beer (2011), when formulating to minimum crude protein (CP) levels, lysine (Lys) levels can often be found

to be over 40% of the requirement. Excess dietary Lys can drive muscle deposition (de Beer, 2011) and may lead to increased body weight by BB which can have a negative effect on reproductive performance (Lopez and Leeson, 1995). Coon *et al*. (2006) observed that feeding high levels of digestible Lys and isoleucine (Ile) caused a decrease in fertility in a study that employed semi-purified diets. Therefore, the following chapters will explore the effect that reduced dietary Lys intake has on the reproductive parameters of Cobb 500 BB hens and the live performance and carcass characteristics of their progeny.

CHAPTER II

LITERATURE REVIEW

Dietary amino acid requirements for broiler breeders

The amino acid requirement of poultry includes two components, a requirement for maintenance and for tissue accretion. In the case of the broiler breeder (BB), a third component exists, egg production (Coon *et al*., 2006). There are very few reports available regarding the requirements of protein and energy throughout the different phases of the laying period (pre-, peak, and post-peak egg production). Bowmaker and Gous (1991) stated that the amino acid composition of the diet is critical for BB performance, which may suggest that crude protein requirements could be reduced, as long as amino acid requirements of the BB are met (Lopez and Leeson, 1994a). However, it is important to mention that past research was not able to elucidate the independent effects of protein and amino acids on BB performance (Lopez and Leeson, 1994a). Furthermore, variability among BB strains, feed intake levels, production stages, and environmental conditions, among many others, make the determination of amino acid requirements for BB a complex problem.

Limited data is available to address the specific requirements for metabolizable energy (ME) and protein of BB with different body weights and phases of the laying period. Past research has focused on the protein needs of BB during the critical period of onset of egg production (Pearson and Herron, 1980; Bornstein and Lev, 1982; McDaniel, 1983; Brake *et al*., 1985; and Bowmaker and Gous, 1989) or during the entire laying

period (McDaniel *et al*., 1981 and Wilson and Harms, 1986) with varying daily protein requirements found. Also, the proposed ME requirements in the literature for the adult BB range between 220 to 450 kcal/hen per day (Waldroup *et al*., 1976; Bornstein *et al*., 1979; Pearson and Herron, 1981; Spratt and Leeson, 1987; NRC, 1994; Attia *et al*., 1995; and Leeson, 2003). Pearson and Herron (1981) reported that egg production was maximized with consumption levels of 413 kcal of ME and 19.5 g of protein/hen per day. Furthermore, Spratt and Leeson (1987) reported lower ME (385 kcal) and protein (19 g/hen per day) consumption levels to maximize egg production. It is important to mention that Pearson and Herron (1981), used BB in floor pens while Spratt and Leeson (1987) used BB in cages, which is possibly one of the reasons that daily ME and protein requirements were different. Recent information from primary breeding companies are recommending 390 kcal of ME/hen and 21.8 g of protein/hen per day for Cobb BB in cages between 23 and 40 wk of age (Cobb, 2008) and a consumption of 339 to 454 kcal of ME and 18.8 to 25.1 g of protein/hen/per day in Ross BB of the same age (Aviagen, 2007).

There is limited information on the specific amino acid requirements of BB (Lopez and Leeson, 1994a). In broilers, Lys and methionine (Met) have a significant effect on carcass composition (Leclercq, 1998), especially on breast meat development (Tesseraud *et al*., 1996). The previous stated amino acids are of great importance in BB feeds because of their influence in growth and development of the bird and their impact on egg production, weight and egg mass (Harms and Ivey, 1992).

Waldroup *et al*. (1976) performed a study using BB hens 24 wk of age to determine the optimum daily protein consumption level to maximize egg production performance. Results concluded that an optimum daily intake between 20 and 22 g of

protein/hen per day were recommended for increased egg production. These levels of daily protein intake contained 866 and 1,003 mg of Lys/hen per day and 340 and 360 mg of Met/hen per day, respectively. Pearson and Herron (1981) recommended daily Lys intake levels of 970 mg/hen per day and 19.5 g of protein/hen per day. In contrast, results obtained from Wilson and Harms (1984) determined that BB had a better egg production and egg weight performance with a daily Lys intake of 808 mg/hen per day and 18.6 g of protein/hen per day. The daily Lys and protein intake levels recommended by Wilson and Harms (1984) are lower than those recommended by Pearson and Herron (1981).

A study performed by Soares *et al*. (1988) evaluated feeding four different levels of total Lys (790, 915, 1,040, and 1,165 mg/hen per day) to BB between 45 and 60 wk of age. The authors determined that the optimum Lys level to obtain higher egg production was 1,022 mg/hen per day. The consumption of Lys had no effect on final BB body weight, fertility, hatchability or initial chick body weight. In a later study by Bowmaker and Gous (1991), the authors evaluated the effect of feeding varying levels of Lys and Met on BB performance. The results indicated that performance was affected when these amino acids were fed in their lower levels, and that egg production rather than egg weight was most affected. Furthermore, the results revealed that feeding levels between 918 to 1,272 mg of Lys/hen per day and 335 to 524 mg of Met/hen per day yielded greater egg production.

Harms and Ivey (1992) used 40 wk old Arbor Acres BB to determine the Lys requirement in a post-peak egg production phase. The seven experimental diets contained different total Lys levels, between 626 and 938 mg/hen per day for a period of eight weeks. The results determined that the optimum daily intake of total Lys for egg production, egg weight, and egg mass were 824, 806, and 819 mg/hen per day,

respectively, when they were provided with at least 18.55 g of protein/hen per day. In addition, the authors in the same study were able to conclude that BB had acceptable performance with daily intakes of 170, 921, 605, 778, and 625 mg/hen per day of tryptophan (Trp), arginine (Arg), threonine (Thr), valine (Val), and Ile, respectively, with a daily intake of 16.7 g of protein/hen per day. Most of the past literature has concentrated on evaluating the impact of dietary Lys and Met on BB performance. Research is sparse on the requirements of other essential amino acids like Trp, Arg, Thr, Val, and Ile which may have an effect in BB performance parameters.

In another study, Harms and Russell (1995a) used 32-wk old Arbor Acres BB to determine their Lys and protein requirements. The diets consisted of varying total Lys levels between 627 and 900 mg/hen per day and crude protein (CP) levels between 8.9 and 11.5%, respectively. Results obtained from the study concluded that a daily total Lys intake of 845 mg/hen per day was required for obtaining optimum egg production, egg weight and egg mass. In two other additional studies, the same authors evaluated the requirement of total Met in 32-wk old Arbor Acres BB for a period of eight wk (Harms and Russell, 1995b). They concluded that the total Met daily requirement was of 335 and 323 mg/hen per day, respectively, for each individual study. These recommended levels of Lys and Met were dependent on the levels of egg production and egg weight, because as these reproductive characteristics increase, the daily requirements of both amino acids also increase.

A study by Fisher (1998), used Ross 308 BB to determine the amino acid requirements during pre- (29 wk of age), peak (31 wk of age), and post-peak (64 wk of age) egg production phases. The requirements for each individual amino acid, a calculation of the percentage of the total requirement for maintenance, egg production,

body growth and flock variability, and a calculated amino acid requirement for BB relative to Lys was determined for each individual production phase. Fisher (1998) stated that the main problem in making comparisons among studies relied on the maintenance requirement, especially for Lys. The question of Lys requirement for maintenance is clearly a major issue in establishing a requirement for all other parameters and needs further experimentation.

Recently, Coon *et al*. (2006) conducted several experiments using Cobb 700 BB to determine the digestible amino acid requirements at peak egg production. These requirements were determined using semipurified diets in which a specific amino acid was added at increasing levels while all other amino acids were kept constant. Results obtained for digestible Arg, Ile, total sulfur amino acids (TSAA), Thr, and Val were higher than those determined by Fisher (1998) in a previous study. The requirement obtained for Lys was similar between both studies. It is possible that the use of a modern high-yield BB strain by Coon *et al*. (2006) resulted in a higher digestible amino acid requirement than the strain used in the previous study by Fisher (1998).

It becomes apparent that most of the current literature differs in the BB requirements from the previous published studies. This may be due to the current genetic selection programs focused on egg production, sexual maturity, fertility, egg size, heat resistance and viability (Hardiman, 1999) which have resulted in improved growth rates, reduced feed conversion, decreased age to slaughter, and increased meat yields in the growing broiler (Havenstein *et al*., 2003). Additionally, the selection of animals within and between lines (crossbreeding) has resulted in the development of diverse commercial crosses that are designed to improve traits of economic importance to the meat and poultry industry (Mehaffey *et al*., 2006), which may not have been commonly done in

previous years. Therefore, research is needed to fully understand modern high-yielding BB requirements in each egg production phase in order to obtain their full genetic potential which may be beneficial to the poultry industry.

Dietary amino acid requirements for broiler breeder fertility

Very little data exists that suggest that there is an amino acid requirement for fertility. Larbier (1973) determined that free amino acids had a specific role in the development of the embryo, but he could not establish how that result could have an influence in practical decisions. The results he obtained demonstrated that no evidence existed on a possible requirement other than a requirement for egg production.

Recent literature is not consistent with the results obtained by Larbier (1973). Lopez and Leeson (1994b) concluded that the protein level fed in BB diets had an effect on fertility. They attributed a beneficial effect in low protein diets on the body weight of the BB hen and therefore, a higher rate of sexual activity. Pearson and Herron (1981) observed a decrease in fertility associated with a high energy intake by BB. Furthermore, Wilson *et al*. (1983) observed that when high body weights in BB before 30 wk of age are obtained, fertility is affected throughout the entire production period. The previous studies may imply that body weight control, rather than energy or protein intake, is the major factor affecting fertility.

In a study by Coon *et al*. (2006), he observed that some amino acids had an effect on fertility. The requirements were determined by feeding semipurified diets in which a specific amino acid was added at increasing levels while all other amino acids were kept constant. The BB were then artificially inseminated with 5×10^7 sperm weekly, eggs were collected and fertility was determined. A reduction in fertility was observed when

increasing levels of Lys and Ile were fed to BB hens. It is important to mention that this response was observed when feeding a semipurified diet, which is not commonly used in the industry. Previous studies, using broiler chicks that were fed low CP-semipurified diets have shown that growth performance is inferior to broilers fed a standard high-CP diet (Fancher and Jensen, 1989; Bregendahl *et al*. 2002). A possible explanation by Coon *et al*. (2006) was that the reduction in fertility may be caused by a change in the micro-environment of the sperm storage tubules in the BB hen. This change may be caused by the use of a low-CP semipurified diet.

Because research is sparse on evaluating if an amino acid requirement for fertility of high-yielding BB exists, current research should focus on feeding diets composed only of commercially available ingredients. Such research could determine if increasing intake of dietary amino acids has an effect on the reproductive parameters of the BB, and if correlation exists with progeny performance.

Nutritional optimization of maternal feed to optimize progeny performance

The profitability of the poultry industry depends largely on hatching a viable broiler chick and will be related to improved performance in livability, growth rate to slaughter, feed efficiency, and carcass value (Kidd, 2003; Hocking, 2007). Moreover, the physiological status of the chick at hatching is greatly influenced by the nutrition of the BB hen (Kenny and Kemp, 2005). Therefore, nutritional decisions for BB impact the overall economic performance of a poultry complex.

Nutritional modifications in varying levels of ME, protein, minerals, vitamins, fat and alternative protein sources in BB feeds have been previously evaluated on progeny live performance. The previous nutritional modifications have been shown to alter body

weight, carcass traits, immune status, bone development and disease resistance in the newly hatched chick and throughout their whole production cycle.

Protein modifications in broiler breeder feed and its effects on broiler progeny

Previous research has addressed the impact of modifying CP in BB feed on reproductive parameters (Waldroup *et al*., 1976; Pearson and Herron, 1980; Pearson and Herron, 1981; Bornstein and Lev, 1982; McDaniel, 1983; Wilson and Harms, 1984; Brake *et al*., 1985; Proudfoot *et al*., 1985; Spratt and Leeson, 1987; Soares *et al*., 1988; Bowmaker and Gous, 1989; Harms and Ivey, 1992; Lopez and Leeson, 1994; Harms and Russell, 1995a; Harms and Russell, 1995b; Lopez and Leeson, 1995 and Fisher, 1998). In contrast, there are very few comprehensive studies on the effect of modifying dietary protein and amino acids in BB diets on progeny live performance (Wilson and Harms, 1984; Proudfoot *et al*., 1985; Lopez and Leeson, 1994; Lopez and Leeson, 1995a; Lopez and Leeson, 1995b; Halle, 1999; and Brake *et al*., 2008). These studies have yielded inconclusive results for body weight at various stages, feed efficiency, and carcass traits. In the previous studies mentioned above, it is believed that authors have confounded dietary CP intake with the intake of limiting amino acids (Lopez and Leeson, 1994b) which could possibly explain the inconclusive results obtained in the literature.

Wilson and Harms (1984) were some of the first authors to evaluate if protein manipulation in BB diets has an effect on progeny performance. Varying dietary levels of protein, TSAA, calcium, and phosphorus to furnish 96.3, 92.5, 89.4, and 86.6% in Experiment 1 and 92.5, 89.4, 86.6, 83.4, and 80.9% in Experiment 2 of suggested recommendations were fed to Cobb BB. Moreover, the levels of Lys, arginine, and tryptophan also changed as a result of the reductions in corn and soybean meal. No

significant effect was observed for any of the reproductive parameters evaluated. Furthermore, body weight of broilers hatched from 39- and 56- wk old BB was unaffected at 49 d of age by the BB dietary treatment. Proudfoot *et al*. (1985) fed BB diets containing 15 or 17% CP and evaluated progeny live performance. No significant effect was observed for body weight or feed intake at 41 d of age. Feed conversion was significantly improved in broilers that were hatched from BB that were fed a 15% CP diet at 41 wk of age.

Three studies were performed by Lopez and Leeson (1994a, 1995a, 1995b) to further evaluate the effects of feeding varying levels of dietary protein on subsequent progeny live performance. Lopez and Leeson (1994a) fed four different levels (9, 11, 13, or 15%) of CP and isoenergetic diets for a four wk period to 58-wk old Arbor Acres BB hens. Egg production, fertility, and hatchability were unaffected by the dietary treatments. Egg weight was observed to be lower for hens fed either a 9 or 11% CP diet, which resulted in lower chick weight at hatch. After a 49 d growout period, no differences were observed for body weight, carcass weight, and breast meat yield. In a second study, Lopez and Leeson (1995a) fed 18-wk old BB pullets diets varying in CP (10, 12, 14, and 16%) and observed lower egg weight and chick weight at hatch from the hens fed the lower CP levels (10 and 12%). A subsequent study by Lopez and Leeson (1995b) evaluated feeding the previous CP levels (Lopez and Leeson, 1995a) to Hubbard BB hens on progeny live performance and carcass characteristics. Eggs and chick weight at hatch was lower for BB hens fed either a 10 or 12% CP diet, but feed conversion was significantly improved at 21 d for these chicks. No significant effects were observed for body weight, mortality, carcass weight or breast meat weight at 48 d of age. These studies support the conclusion that dietary protein levels may be reduced in BB diets without

affecting reproductive performance, progeny live performance and carcass characteristics.

Energy modifications in broiler breeder feed and its effect on broiler progeny

Most of the literature regarding maternal energy concentrations in BB feeds has been evaluated in conjunction with feeding varying levels of dietary protein (Aitken *et al*., 1969; Pearson and Herron, 1981; Proudfoot and Hulan, 1986; Proudfoot and Hulan, 1987; Spratt and Leeson, 1987; Brake *et al*., 2003; Romero-Sanchez *et al*., 2008). Other experiments have evaluated the effect that type and level of added fat in the diet on egg composition and subsequent progeny live performance and carcass characteristics (Proudfoot *et al*., 1982; Jiang *et al*., 1990; Attia *et al*., 1993; Cherian and Sim, 1993; Attia *et al*., 1995; Latour *et al*., 1996; Halle, 1999; Peebles *et al*., 1998; Peebles *et al*., 1999; Peebles *et al*., 2002a; Peebles *et al*., 2002b; Enting *et al*., 2007a; Enting *et al*., 2007b). Some of the previous research mentioned above has yielded improvements in live performance and carcass characteristics in commercial-type growout studies performed on the progeny.

Aitken *et al*. (1969) fed a low (14.6% CP, 2,490 kcal/kg ME) and high (17.5% CP, 2,880 kcal/kg ME) density diet to broiler breeder hens. Egg weight was higher for the hens fed the high density diet. Additionally, progeny from the hens fed the high density dietary treatment had higher body weights at 42 and 63 d of age. Pearson and Herron (1981) fed 21 wk-old BB hens three different levels of ME (1.88, 1.73, and 1.52 MJ of apparent ME/hen per day) with two varying intake levels of dietary protein (19.4 or 27.2 g CP/hen per day) for a 45 wk period and evaluated live performance of their progeny. No differences were observed for growth rate, feed conversion ratio and mortality from

chicks hatched on wk 36, 51, and 52. An additional study by Proudfoot and Hulan (1986) evaluated feeding BB pullets (15 wk of age) two diets varying in energy and protein (13.1% CP and 12.2 MJ/kg of diet or 16.0% CP and 11.3 MJ/kg of diet) for a 5 wk period. Then during their laying period (20 to 60 wk of age) they fed diets that contained different levels of protein (15 or 17% CP) and energy (11.1 and 11.5 MJ/kg of diet). They found no overall effect on progeny performance. The authors also determined that replacing soybean meal with canola meal in BB hen diets had no beneficial effects on the progeny (Proudfoot and Hulan, 1987). Spratt and Leeson (1987) fed two levels of CP (19 or 25 g/hen per day) and three levels of ME (325, 385, and 450 kcal/hen per d) to BB hens. Results indicated a higher body weight in male chicks from the BB hens fed 450 kcal/kg of ME at 1 and 20 d of age. Additionally, these same chicks were observed to have a higher carcass protein and decreased carcass fat at 41 d of age, when carcass characteristics were evaluated. In a more recent study, Brake *et al*. (2003) evaluated different CP and energy levels in BB diets to determine the effects on four growout-progeny studies performed at different BB ages. Increasing the CP and energy levels resulted in higher male chick body weights at 21 d of age in three of the four hatches. In contrast, female chicks were only observed to have a higher body weight in one of the four hatches. Furthermore, Romero-Sanchez *et al*. (2008) evaluated two different cumulative feeding programs (low and high density in CP and ME, respectively) in male BB and its effects on reproductive and progeny performance parameters. Twenty-nine wk of age BB males fed the high density diet produced male broilers that exhibited lower body weight at 42 d. This response could have been an effect of the BB with greatest genetic potential not mating due to their high BW and only the small BB males being able to mate. Also, in the same study dietary treatments were observed to have no impact

on broilers that were hatched from eggs collected at 32 wk of age. In contrast, female and male broilers that hatched from eggs collected at 48 wk of age were lower when BB males were submitted to a low density feeding program throughout the production period. The previous response could be explained that when BB males were fed a low density diet the large males would be the first to go out of semen production and would contribute little to the production of offspring from that male treatment group allowing only the smaller males to sire offspring (Bramwell *et al*., 1996). Bramwell *et al.* (1996) fed full- and half-brother sires, which were assessed as genetically equivalent, diets varying in ME levels and observed no effect in offspring BW at 0, 3, or 6 wk of age.

The type and level of fat added in the maternal diet has been shown to have an effect in fatty acid composition of the yolk, and thus causing an impact in progeny performance. Jiang *et al*. (1990) fed laying hens a diet containing either 0 or 1% cholesterol and observed that hen cholesterol levels impact the metabolism of the developing embryo and the post-hatch chick. In two studies by Attia *et al*. (1993, 1995), BB males were fed high-energy diets and progeny body weight was improved at 42 d of age. It is possible that this response was influenced by inadvertently assigning BB males with increased genetic potential to the higher energy diets and yielding higher offspring BW (Bramwell *et al*., 1996) or the presence of supernumerary sperms in eggs laid by hens inseminated from the same BB males (Attia *et al*., 1995). Cherian and Sim (1993) fed maternal diets varying in oleic, linolenic, and linoleic acid composition and found that the fatty acid composition of the hatched progeny was significantly altered by egg yolk lipids. In a later study by Latour *et al*. (1996), BB hens fed diets varying in corn and poultry oil levels yielded no effects on serum concentrations of lipids and glucose, or relative yolk sac weight at d 18 of incubation from BB hens at 26, 36, and 46 wk of age.

In a similar study by Halle (1999), different types of fats, either palm butter or safflower oil in BB hen diets produced no effects on fertility, hatchability or progeny performance of 35 d old broilers. However, this study did not evaluate if the type of fat had an impact on the egg contents or the developing embryo.

Various studies were performed by Peebles *et al*. (1998, 1999, and 2002) evaluating different types of dietary fat in BB diets and their impact on embryo development and further progeny performance. Feeding Arbor Acres BB hens (22 wk of age) isocaloric diets that contained three types of fat (corn oil, poultry fat and lard) and two levels (1.5 or 3.0%) during a 40 wk period had no influence on feed consumption, relative body weight gain, and feed conversion of broiler progeny in a 21 d-old growout from eggs set from 52 and 62 wk-old BB hens (Peebles *et al*., 1998). Forty-three day-old growout studies performed on the progeny determined that corn oil in BB hen diets significantly increased live body weight and chilled carcass yield (Peebles *et al*., 1999). Additionally, broilers from BB hens that had corn oil as a dietary fat source in the diet were observed to have increased relative front-half yields when compared to broilers from BB hens that had poultry fat as a dietary fat source in the diet. They also determined that BB age influenced broiler slaughter yield and that fat added at a 1.5% was effective. In a follow-up study, Peebles *et al*. (2002a) fed 22 wk-old Arbor Acres BB hens' diets that provided high (467 calories/d), moderate (449 calories/d) and low (430 calories/d) levels of ME, which were achieved by either adding poultry fat or corn oil at 1.5 or 3.0%. Eggs were collected at 29 wk of age and no differences were observed among treatments for hatchability or early, late, and pipped embryonic mortalities. In addition to the embryonic parameters evaluated, dietary treatments had no effect on 42 d old broiler performance parameters. This study concluded that adding 1.5 or 3.0% corn oil or poultry

fat had no effect on embryogenesis and progeny performance, indicating that either type of fat source could serve as an alternative in BB diets. Two growout studies from eggs collected at wk 29 and 36 were performed (Peebles *et al*., 2002b) with BB hens in the previous study (Peebles *et al*., 2002a) and progeny performances in conjunction with liver characteristics were evaluated. Breeder age influenced d 43 progeny live body weight, total carcass and front half-yields and liver moisture contents. Also, wet and dry liver weights were higher for female broilers compared to those male broilers from 29 wk-old BB hens. Contrast analyses determined that live body weight was higher for broilers that came from BB hens that were fed a low energy diet when compared to a moderate diet in which BB hens had a dietary fat inclusion of 3.0%. Wet and dry liver weights were lower in broilers from BB hens that were fed a 3.0% poultry fat diet. It becomes apparent that ME levels and fat levels in BB diets have an impact on progeny live performance and liver characteristics.

Recent studies by Enting *et al*. (2007a, 2007b) have evaluated the effects that low or high density diets have on BB performance during the laying period as well as on the embryonic development and the immune status of offspring. Enting *et al*., (2007a) fed diets varying in ME levels from 4 to 60 wk of age to Cobb 500 BB hens and observed that low-density diets increased 1-d and 38 d-old chick body weight. In contrast, IgM titers were shown to decrease in broilers from eggs collected at 29 wk of age when fed low density diets, but the same IgM titers were observed to increase in broilers from eggs collected at 60 wk of age. Furthermore, decreasing density in Cobb 500 BB hen diets during the laying period (25 to 60 wk of age) improved the rate of lay with higher egg weights but lower hatchability (Enting *et al*., 2007b). Feeding lower density diets to 29 wk old BB hens resulted in better development of the perivitelline layer and heart and

embryo weight. Feeding low density diets may improve progeny performance and immune status of the broiler chick and aid embryonic development but is dependent on the BB hen age.

Research regarding energy levels and fat sources in maternal diets has been shown to impact offspring performance. However, research is sparse on feeding varying density diets to current high-yielding BB strains to examine their impact on reproductive and progeny parameters. With the current motion to eradicate anti-microbial drugs from feeds, fat sources may be a potential alternative to enhance immune characteristics in the developing embryo with a further effect on growth performance parameters. Also, research is needed to evaluate if a possible carryover effect may exist in feeding essential fatty acids (omega-3 and omega-6) to BB hens to improve progeny fat quality that may eventually be consumed.

Modifications in vitamin and mineral concentrations in broiler breeder diets and its effects on broiler progeny

Several comprehensive reviews have addressed the impact that maternal vitamin and mineral nutrition have on egg contents, the developing embryo, the immune status of the chick and progeny live performance (Kidd, 2003; Hocking, 2007; Calini and Sirri, 2007). It is understood that the composition of the hen's egg must contain all the available nutrients for embryo development that have the potential to prepare the chick for emergence and hatching and that produce a healthy and viable chick which has a developed gastrointestinal tract to utilize its first feed (Moran, 1997). The vitamin and mineral content of the egg are dependent on the maternal diet and cases of deficiencies, excesses or imbalances may affect fertility, hatchability, nutrient reserves for the

developing embryo and chick viability and growth (Whitehead *et al*., 1985; Wilson, 1997).

Past research was concerned in evaluating mineral concentrations and requirements for BB hen reproductive performance. Alterations in the concentration of macro-minerals, such as calcium, phosphorus, sodium, potassium, and chloride, found in current industry diets are not likely to have an impact on progeny live performance (Kidd, 2003). Therefore, more recent research has been focused on the carryover effect that micro-minerals from the BB hen may have on progeny performance and the immune status of the chick (Kidd, 2003). Calcium and calcium-interactions with other minerals have been shown to impact chick hatching weight (Buckner *et al*., 1925; Berg *et al*., 1962; Heth *et al*., 1966). Phosphorus nutrition in BB hens has also been evaluated in progeny performance parameters. Previous research has indicated that increasing phosphorus levels in maternal diets increases egg weight (Singsen *et al*., 1962; Taylor, 1965) but has no effect in weight at hatch, two or seven wk-old broilers (Triyuwanta *et al*., 1992). Even though no effects were observed in live performance parameters, Triyuwanta *et al*. (1992) observed an improvement in tibia maximal force, elastic force and ash content in day old chicks. Furthermore, Shim *et al*. (2008) fed a P-deficient diet to BB hens and determined that chicks from young BB (27 wk of age) had improved growth and that longer egg storage (10 d) from eggs obtained from older BB (61 wk of age) resulted in higher P rickets scores and incidence. A relationship between BB age and egg storage time may only be applied when BB hens are fed P-deficient diets, which may be a typical result of high phosphorus prices or low phosphorus consumption by BB hens in a commercial environment (Shim *et al*., 2008).

Zinc has been known to impact early progeny livability and improve the immune status of the chick (Kidd, 2003). Early research on supplementing dietary zinc in BB diets was observed to have no effect on progeny 21 d-old weights or zinc, copper, and iron metabolism (Stahl *et al*., 1986; Stahl *et al*., 1990), but in the case of these studies no immune response was measured. In contrast, Flinchum *et al*. (1989) fed 80 mg/kg of zinc-methionine complex, which is an inorganic form of zinc, to leghorns hens and observed that chicks from those hens had an improved survival rate against an *Escherichia coli* challenge. In addition, Kidd *et al*. (1992) supplemented BB hen diets with 40 mg/kg zinc oxide or zinc methionine and observed an improvement in cellular immunity of chicks that came from BB hens that were supplemented with zinc methionine in the diet. In another study, BB hens were supplemented with 40 mg/kg zinc oxide or zinc methionine in a corn-soybean meal or sorghum-soybean meal diet, and regardless of the diet type, chicks from hens supplemented with zinc methionine were observed to have improved cellular immunity whereas chicks from hens supplemented with zinc oxide were shown to have improved humoral immunity (Kidd *et al*., 1993). Furthermore, Kidd *et al*. (2000) supplemented Hybrid turkey hens with either zinc sulfate or zinc methionine and concluded that chicks from the hens that were supplemented with an organic source of zinc had a heavier bursa of Fabricius, higher blood leukocyte concentrations at 7 d of age, and greater cutaneous basophil hypersensitivity (CBH) response to phytohemagglutinin-P. These results can be interpreted as an improvement in immune organ development in the progeny from hens fed an organic source of zinc. In a more recent study, Virden *et al*. (2003) fed Cobb 500 BB diets supplemented with zinc sulfate and manganese sulfate, zinc amino acid complex and manganese sulfate, or zinc and manganese from an amino acid complex during the laying period and evaluated the

carryover effect on progeny performance. Growth performance and carcass characteristics were not improved by mineral supplementation, but progeny from BB fed diets supplemented with zinc and manganese amino acid complexes (organic sources) were observed to have improved livability from 1 to 18 d of age (Virden *et al*., 2003). Using the same BB hens and dietary treatments, Virden *et al*. (2004) observed that progeny from BB hens fed a supplemental organic zinc and manganese diet had higher bursa weight, increased CBH to phytohemagglutinin-P, and increased absolute left ventricle plus septum weight and total ventricular weight. Therefore, it can be determined that these chicks had an improved cardiac functional capacity and immunity. From the previous results, it becomes apparent that organic sources of zinc and manganese may have a better carryover effect than inorganic sources in the immune status of the progeny, which may lead to improved immunity and disease resistance of the chicks.

Other minerals such as selenium (Se), fluoride, magnesium (Mg), and iodine have been studied mainly for their effect on egg composition and production parameters (Latshaw and Osman, 1975; Utterback *et al*., 2005; Reis *et al*., 2009; Bennett and Cheng, 2010; Wang *et al*., 2010; Merkley, 1981; Machalinski, 1996; Sell *et al*., 1967; Atteh and Leeson, 1983; Christensen and Edens, 1985; Hess and Britton, 1997; Lee *et al*., 1936; Johnson, 1936; Rogler *et al*., 1961; Perdomo *et al*., 1966; Arrington *et al*., 1967; Christensen *et al*., 1991). Sparse research has been focused on the impact these minerals may have on progeny performance. Progeny studies performed by feeding a deficient or fortified selenium level using Single Comb White Leghorn hens concluded that chicks from hens fed a deficient selenium diet had inferior growth (Cantor and Scott, 1974). Paton *et al*. (2002) evaluated the effect of feeding a dietary source (inorganic or organic) and level of Se on the Se uptake by the chick. Feeding increasing levels of Se as Se yeast

(organic) resulted in greater rates of Se deposition in yolks and whites and in embryos at various stages of incubation compared with feeding Se selenite (Paton *et al*., 2002). Wang *et al*. (2009) fed varying levels of Se yeast with one of three Met levels to 52 wk-old Lang Shan hens and concluded that the meat from 21 d old chicks from hens supplemented with Met and Se yeast had improved color, water-holding capacity, and oxidative stability. Merkley and Sexton (1982) and Van Toledo and Combs (1984) supplemented hen diets with 100 mg/kg or levels between 0 to 1,200 mg/kg in increments of 300 mg/kg of fluoride, respectively and observed no improvements in progeny growth or feed conversion. Sell *et al*. (1967) fed Single Comb White Leghorn hens Mg deficient diets and observed high embryonic mortality at D 19 and 20 of incubation and chicks that were able to hatch died within 2 days. Perdomo *et al*. (1966) and Arrington *et al*. (1967) fed high levels of iodine to hens and observed increased embryonic deaths and delayed hatching time, concluding that excess iodine has a detrimental effect on hatchability of chicks from hens fed high levels of iodine.

Several studies have been performed to evaluate the carryover effect of vitamin supplementation on progeny live performance, immune responses, skeletal development, and carcass characteristics. Hill *et al*. (1961) fed varying levels of vitamin A to laying hens and evaluated progeny growth and livability. Progeny from hens fed 2,268 IU vitamin A/kg of diet had accelerated growth over progeny fed the lower levels. In contrast, progeny from hens fed 363 IU vitamin A/kg of diet had depressed growth. In a similar study, progeny from hens fed a high level of vitamin A (210,000 IU/kg of diet) were observed to have a prolonged hatch time when compared to chicks from hens fed a lower vitamin A level (10,000 IU vitamin A/kg of diet) (March *et al*., 1972). Furthermore Surai *et al*. (1998) fed Rhode Island Red hens varying levels of vitamin A, ranging from

0 to 120 µg/g retinol equivalents, and observed that the concentration of vitamin A in the yolk of the hens' eggs was increased with dietary supplementation and that vitamin E and carotenoids were significantly reduced with higher levels of vitamin A being fed. Past research has determined that high levels of vitamin A interfere with vitamin E absorption by the chick (Combs and Scott, 1974; Abawi *et al*., 1985; Abawi and Sullivan, 1989) and could possibly explain the decreased liver vitamin E, carotenoids, and ascorbic acid levels found in the progeny from hens fed higher levels of vitamin A (Surai *et al*., 1998). Research regarding vitamin E has been focused on evaluating its effect on hen reproductive performance (Olson *et al*., 1962; Tengerdy and Nockels, 1973; Siegel *et al*., 2001), and its influence on the immune status of the chick (Haq *et al*., 1996; Gore and Qureshi, 1997; Leshchinsky and Klasing, 2001; Siegel *et al*., 2006). Jackson *et al*. (1978) supplemented varying levels of vitamin E to laying hens and observed that progeny chicks from hens fed supplemental vitamin E had improved agglutination titers, which could be interpreted as improved humoral immunity in the progeny. Studies performed by Surai *et al*. (1997, 1999, 2000) have evaluated the carryover effect of vitamin E to the progeny and indicated that feeding vitamin E to BB hens increases α-tocopherol levels in eggs and the subsequent hatching chick. Also, Surai *et al*. (2000) concluded that the antioxidant status of the chick could be improved by feeding BB hens' higher levels of vitamin E and Se. In addition, Hossain *et al*. (1998) fed graded levels of supplemental vitamin E to Ross BB hens and observed that vitamin E content in eggs increased by increasing levels of vitamin E. Also, antibody titers to kill Newcastle vaccine were significantly higher with increasing levels of vitamin E. Furthermore, Siegel *et al*. (2001) was able to reduce chick mortality from chicks of young breeders by feeding high levels of vitamin E, which enhanced the humoral immunity.

Carryover of vitamin D from the hen to the progeny may be a factor to consider when improving the skeletal development for the young chick or poult (Coto *et al*., 2010a). Previous studies determined that 90% of the vitamin deposited in the egg occurs as vitamin D_3, with only 5% occurring as 25-OH (Fraser and Emtage, 1976). However, in the chick as much as 80% of the activity of the circulating vitamin D is attributed to 25-OH (Ovesen *et al*., 2003). Griminger (1966) and Ameenuddin *et al*. (1986) fed high levels of vitamin D (cholecalciferol) to BB hens and demonstrated increased mineralization of the tibia and heightened tibia ash. Atencio *et al*. (2005a, 2005b, 2005c) performed several studies to evaluate the carryover effect of different sources and levels of vitamin D_3 on progeny live performance and general health parameters. Feeding high levels of vitamin D_3 (2,000 or 4,000 IU of vitamin D_3/kg of diet) to Ross BB hens resulted in progeny chicks obtaining higher body weight gains and tibia ash and reduced incidence of Ca rickets (Atencio *et al*., 2005a). A second study by Atencio *et al*. (2005b) concluded that when comparing vitamin D sources, 25-hydroxycholecalciferol had greater potency than D_3 only at low levels of supplementation. In a later study, BB hens were fed various levels of vitamin D_3 (0 to 4,000 IU/kg of diet) and their progeny were fed two levels of Ca (0.63 or 0.90%) and six levels of 25-OHD_3 (0 to 40µg/kg of diet), in two separate studies, to evaluate the performance and bone abnormalities of the progeny (Atencio *et al*., 2005c). Their results indicated that chicks from hens fed the highest levels of vitamin D_3 and fed high levels of Ca or 25-OHD_3 had highest body weight gain and tibia ash, and lower incidence of TD and Ca rickets. Furthermore, Driver *et al*. (2006) fed low (250 IU of vitamin D_3/kg of diet) or high (2,000 IU of vitamin D_3/kg of diet) levels of vitamin D_3 to Ross BB hens and observed that chicks from BB hens that were fed a low vitamin D_3 level had lower body weight gain and feed intake and higher

TD scores and severe TD incidence, thus indicating a lower carryover effect to the progeny. In more recent research, Coto *et al*. (2010a, 2010b) concluded that feeding increasing levels of 25-OHD_3 to Cobb 500 BB hens increased 25-OH levels in plasma and egg yolk and improved egg shell thickness, hen-day egg production, and egg mass; and at the same time improved feed conversion and body weight in 21 d-old chicks and tibia ash in 1 d-old chicks. However, it is important to mention that most of these improvements were more noticeable at low levels of vitamin D supplementation with no difference at higher levels in the hens' diet.

Vitamin K, biotin, riboflavin, vitamin B_{12}, pantothenic acid, thiamin, niacin, folic acid, and pyridoxine have been less studied recently and most of the work has been done using Single Comb White Leghorn hens, which is very different than the BB hen. However, some work has indicated that when these vitamins are deficient in a BB diet, egg production, egg contents, and in some cases the progeny are affected. Lavelle *et al*. (1994) fed vitamin K deficient diets to laying hens and observed that blood clotting time increased in the progeny of these hens. When deficient biotin diets were fed to hens, leg abnormalities (Cravens *et al*., 1944) and reduced plasma biotin at hatch, growth, and viability (Whitehead *et al*., 1985) in the progeny were shown, but Leeson *et al*. (1979) observed no effect in male and female growth at 0 and 21 d of age. In contrast, when Brewer and Edwards (1972) fed increasing levels of biotin, a higher body weight was observed in progeny of hens that were supplemented with the higher levels. Progeny from hens fed riboflavin deficient diets were shown to have leg abnormalities such as curled toe paralysis (Whitehead *et al*., 1993), but in a previous study by Leeson *et al*. (1979) the authors, did not see growth performance to be affected. Feeding high levels of vitamin B_{12} (10 μg/kg of diet) and pantothenic acid (6-9.9 mg/kg of diet) to hens were observed to

have a positive impact on the progeny, such as enhanced body weight gain at 21 d (Patel and McGinnis, 1977) and improved growth rate and livability (Beer *et al*., 1963; Balloun and Phillips, 1957), respectively. In the case of the above mentioned vitamins and the water soluble vitamins, research is sparse mainly because these vitamins are usually included in the vitamin premixes in higher than required levels so that deficiencies do not occur in the field (Kidd, 2003).

Maternal nutrition impacts progeny performance, livability, growth and immune parameters in the growing chick. It is imperative that current research regarding BB hen nutrition is evaluated not only in terms of reproductive parameters but also the impact it may have on the progeny. Future research should concentrate on enhancing the immune status, disease resistance, and skeletal development without compromising growth performance and carcass characteristics of the progeny from modern BB high-yielding strains.

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CHAPTER III

DIETARY INFLUENCE OF DIGESTIBLE LYSINE CONCENTRATION ON COBB 500 BROILER BREEDER REPRODUCTIVE PERFORMANCE

Abstract

A study was conducted to examine the reproductive parameters of Cobb 500 broiler breeder hens fed two different types of diets varying in digestible Lys (dLys) concentration. A total of 240 Cobb 500 broiler breeder pullets were placed in individual cages and given experimental diets from 35 to 45 wks of age. Treatments 1 and 2 were diets formulated using only commercially available feed ingredients and consisted of dLys intakes of 1,200 (IDL) and 1,010 mg/hen per d (ID). Treatments 3 and 4 consisted of semi-purified diets with the inclusion of L-glutamic acid to maintain isonitrogenous conditions with dLys intakes of 1,010 (SPL) and 600 mg/hen per d (SP). Hens fed the SPL and SP diets had lower hen-day egg production than hens fed the ID diet with hens receiving the IDL diet yielding intermediate values. Hens fed the SP diet had the lowest ($P < 0.05$) egg weight but no differences were observed among dietary treatments for egg specific gravity. Fertility and hatchability of eggs set were lowest $(P < 0.05)$ for hens fed the SPL dietary treatment. No differences were observed for early and middle embryonic mortality, contaminated or pipped eggs. Late embryonic mortality was observed to be higher $(P < 0.05)$ in hens fed the SP diet. A decrease in the daily intake of dLys appeared to improve broiler breeder reproductive performance when hens were fed a semi-purified

diet. In contrast, the same effect was not observed when hens were fed a standard industry-type diet that contained less lysine.

Introduction

Nutritional studies evaluating reproductive performance of broiler breeders (BB) have been focused primarily on delaying sexual maturity (Romero et al., 2009), varying photoperiod length during the laying period (Lewis et al., 2010), feed restriction during the grower phase (Waldroup et al., 1976), improving feeding regimens (de Beer et al., 2007; Ekmay et al., 2010), and the influence of protein and energy intakes (Walsh and Brake, 1999; Brake et at., 1985; Romero et al., 2009; Enting et al., 2007) on the reproductive performance of BB hens. However, most of the current research efforts are being made to address the concerns of the broiler breeder paradox, which states the impossibility of reconciling production requirements without relying on severe feed restriction programs (Decuypere et al., 2006).

The requirement of any amino acid by BB includes three components, requirements for maintenance, tissue accretion and egg production (Coon et al., 2006). According to de Beer (2011), commercial BB feeds are often formulated to minimum CP levels, resulting in amino acid levels significantly higher than the requirement. Previously, Bowmaker and Gous (1991) stated that the amino acid composition of the diet is critical for BB performance, which may suggest that protein requirements could be reduced, as long as amino acid requirements of the BB are met (Lopez and Leeson, 1994a). Excess amino acids may lead to an increase in muscle deposition by the BB, which is not a desirable trait for these birds (de Beer, 2011). Moran (2007) concluded that marginal dietary deficiencies, and possibly in amino acids, may cause marginal

deficiencies in the offspring. On the other hand, Wilson (1997) observed that high levels of certain nutrients, which may include amino acids, could be deposited by the hen and may cause problems related to toxicity in the progeny. Furthermore, Coon et al. (2006) when feeding semipurified diets, observed a decrease in fertility when feeding high levels of dLys and isoleucine. To the author's knowledge, studies evaluating the effect of dLys intake levels on broiler breeder reproductive performance have only been done using semipurified diets (Coon et al., 2006). Therefore, the objective of the current study was to evaluate two types of diets, a semipurified and industry type diet, with various dLys intake levels, on the reproductive parameters of Cobb 500 broiler breeder hens.

Materials and methods

Dietary treatments

A pre-breeder diet (CP 16%, 1.50% Ca, and 0.63% dLys) was fed from 21 to 24 wk of age, while experimental breeder-phase diets (CP 16%, 3.0% Ca) were fed from 35 to 45 wk of age, respectively (Table 3.1). The experimental treatments consisted of two types of diets; a diet composed only of commercially available feed ingredients and a semi-purified diet, each providing two different dLys concentrations and fed from 35 to 45 wk of age (Table 3.1). A common diet composed only from commercially available ingredients was used to obtain treatment 1 and 2 diets. Treatment 1 and 2 diets resulted from one of two aliquots of the common industry diet that was reblended with either L-Lys HCl or an inert filler (sand). Therefore, hens fed treatment 1 and 2 diets (only commercially available ingredients) were fed to calculated daily dLys intake levels of 1,200 [IDL] and 1,010 mg/hen per d [ID], respectively, accomplished by replacing the portion of the IDL diet composed of L-Lys-HCl with an inert filler (sand). A common

semi-purified diet was used to obtain treatment 3 and 4 diets. Treatment 3 and 4 diets resulted from one of two aliquots of the common semi-purified diet that was reblended with either L-Lys HCl or sand as an inert filler. Therefore, hens fed treatment 3 and 4 diets were fed to calculated dLys daily intake levels of 1,010 mg/hen per d [SPL], simulating the dLys intake level of the ID diet, and another diet devoid of the L-Lys-HCl which was replaced by an inert filler (sand) and calculated to provide 600 mg/hen per d [SP]. For the semipurified diets, in an attempt to maintain isonitrogenous conditions similar to those of the commercial-type diets while allowing for a further reduction of dLys, the semi-purified diets were supplemented with L-glutamic acid. All other nutrients, except for dLys, were satisfied for all the experimental treatments per the primary breeder company's recommendations (Cobb, 2008). Feed intake was restricted to 390 kcal/d; which resulted in a daily intake of 136 g/hen (C. Wiernusz, Cobb-Vantress Nutrition Director; Personal communication). All hens were fed their respective treatment diets everyday and at the same time, following the recommendation from the primary breeding company.

Bird husbandry

A total of 260 Cobb 500 broiler breeder pullets and 40 cockerels, 20 wk of age, were obtained from a commercial blackout rearing house under an 8L:16D lighting program. The pullets had been housed in a curtain sided house and placed in individual cages (60 × 50 × 40 cm). The photoperiod was then extended with artificial light to 14 and 15 h at 22 and 24 wk of age and to 15.5 and 16 h at 5 and 50% egg production, respectively. At 35 wk of age, hens that had not reached the required BW were discarded and six replicate groups of 10 broiler breeder hens each (10 adjacent cages containing 1

hen/cage, 60 × 50 × 40 cm) were allotted to the four dietary treatments (total= 240 hens) in a completely randomized design so that mean BW was similar for each treatment. The roosters for artificial insemination were housed in a separate solid-side wall house in individual cages (64 × 48 × 48 cm), where the photoperiod was extended with artificial light to 16 h and they received an industry standard broiler breeder diet (CP 16%, AME 2,860 kcal/kg, 3.0% Ca, 0.45 available P, and 0.65% dLys).

Egg production and performance

Egg production performance was measured for a 10 wk period after initiating the feeding of the experimental diets. Egg production and hen mortality were recorded daily throughout the 10 wk experimental period. Egg weight and egg specific gravity, using Archimedes' principle as described by Peebles and McDaniel (2004), was measured daily for all eggs produced during wk 39, 43, and 45.

Semen collection, dilution and artificial insemination

On wk 36, 37, 38, 40, 41, 42, 44, and 45 semen was collected and used for artificial insemination to provide fertilized eggs for sperm-egg penetration, fertility, and hatchability analyses. Semen was collected using the abdominal massage method of Burrows and Quinn (1937). Immediately after collection, semen was diluted 1:1 with Beltsville Poultry Semen Extender (Continental Plastic Corp., Delavan, WI). Within 30 min of collection, 50 μL of diluted semen was inseminated into each hen.

Sperm-egg penetration assay

Eggs collected on wk 37, 41, and 45 were used to perform the sperm egg penetration assay. Eggs were collected daily for each of the 8 days post-insemination of each week. This assay provided the quantitative determination of the rate of sperm

penetration of the perivitelline layer in oviposited eggs *in vivo*. This assay was performed with freshly laid eggs and can be used as a predictive measure of fertility. The methodology used for this assay is detailed in the study by Bramwell *et al*. (1995).

Incubation

Eggs collected from wk 36, 38, 40, 42 and 44 were stored at 18.3 °C over the 8 days post-insemination and then set in a Natureform (Model No. 2340, Jacksonville, FL)incubator at 37.5 °C and 55% RH for 516 h. Eggs were randomly distributed in the incubator according to day post-insemination and replicate group. Eggs were candled on d 11 of incubation and transferred for hatching on d 18 of incubation. During candling, eggs were characterized as being infertile, cracked, contaminated, or containing early dead embryos (less than 7 d), or middle dead embryos (7 to 14 d). Eggs were transferred to hatching baskets at d 18 of incubation, where they were randomly distributed according to the day post-insemination and replicate group. After hatching (d 21.5 of incubation), the number of live chicks, initial chick BW, middle and late dead embryos (15 to 21 d) and dead pips was determined.

Statistical analysis

Data were analyzed as a split-split plot design using the GLM procedures of SAS® (2010). Whole plots were replicated by the randomized groups of 10 hens each. Weeks of the study were considered split plots, whereas for fertility and hatchability data, days post-insemination were considered split-split plots. Fisher's-protected LSD test was used to determine significant differences ($P < 0.05$) among treatment means.

Results

Validating the experimental treatments imposed on the hens, the amino acid analyses of the experimental diets were in agreement with formulated values for the IDL and ID diets. The IDL and ID diets were formulated to total Lys concentration values of 0.98 and 0.82%, and resulted in analyzed total Lys values of 0.99 and 0.87%, respectively. Additionally, the SPL and SP diets were formulated to 0.82 and 0.49% total Lys values, and resulted in analyzed total values of 0.70% and 0.50%, respectively. The intention was to formulate the ID and SPL diets to have the same dLys content and similar total dLys values, but the total amino acid analyses reported lower than expected values for the SPL diet. Considering that the SP diet has a close calculated vs. analyzed value (0.49 vs 0.50%), it is possible that a possible sampling error and/or along with laboratory experimental variability might led to the lower value reported by the SPL, considering it was the only diet not in agreement with the formulated value, Additionally, it should also be reiterated that the SP and SPL diets came from a common mixing batch, and then each aliquot reblended with either the L-Lys-HCl or the inert filler, therefore the authors feel confident that the experimental dietary treatments imposed are valid despite the low total Lys concentration observed by the SPL diet.

Throughout the study, no significant 2- or 3-way interactions of dietary treatments with weeks or days postinsemination were observed, therefore only the main effect of diet is addressed. Hens fed the ID diet had higher ($P < 0.05$) hen-day egg production than hens fed the SPL and SP diets (Table 3.2). Hens fed the SP diet, which had the lowest dLys intake level of all the treatments (600 mg/hen per d), had similar ($P > 0.05$) hen-day egg production with the hens fed the SPL diet (Table 3.2). Therefore, decreasing the dLys

intake in the commercial-type or semi-purified diets had no detrimental effect on hen-day egg production (Table 3.2).

Hens fed the SP diet produced smaller ($P < 0.05$) eggs when compared to hens fed the other treatments (Table 3.2). However, decreasing the dLys intake in the commercial-type diets had no influence on egg weight. Also, for egg specific gravity, no differences were detected among treatments for the 35 to 45 wk period (Table 3.2). Additionally, no differences were observed for initial chick BW between hens fed the IDL and ID diets (Table 3.2), but those hens fed the SP diet produced chicks with lower ($P < 0.05$) initial BW when compared to chicks from the hens that were fed the SPL dietary treatment or both industry type diets (Table 2). Thus, decreasing the dLys intake when feeding semi-purified diets negatively affected initial chick BW as expected since the diets were deficient in Lys according to NRC (1994) recommendations.

Overall fertility is shown in Table 3.3. When decreasing the dLys intake levels in semi-purified diets, the hens improved in fertility ($P < 0.05$) (Table 3.3). Reducing the dLys intake in hens fed the industry-type diets resulted in no differences in the percentage of late dead, while those fed the SP diet had a lower ($P < 0.05$) percentage of late dead than the hens fed the SPL diet (Table 3.3). Thus, increasing the daily intake of dLys in semi-purified diets led to an increase ($P < 0.05$) in the percentage of late dead. Furthermore, the lower fertility and the higher incidence of late dead in the SPL dietary treatment caused a lower ($P < 0.05$) hatchability of eggs set by the hens fed the same diet when compared to the hens fed the SP diet (Table 3.3). No differences were observed for early and middle dead, contaminated, cracked, pipped eggs or hatch of fertile (Table 3.3). Results from the sperm egg penetration assay yielded no significant difference among treatments (Table 3.3).

Discussion

Past research has elucidated protein responses in poultry diets, but current literature has few reports on the effects of amino acids on BB performance. Current commercial BB feeds are often being formulated to minimum CP levels, which often lead to dietary excess of amino acids (de Beer, 2011). An excess of protein and amino acids has been shown to have negative effects on BB performance (Lopez and Leeson, 1995). Coon et al. (2006), using semi-purified diets, reported a reduction in fertility with increasing Lys and Ile concentrations. Conversely, Bowmaker and Gous (1991) observed a poor performance in broiler breeders when feeding low levels of Lys and Met.

Bowmaker and Gous (1991) reported that small increments in intake of total Lys that were close to the optimum resulted in equal responses in rate of lay. In parallel with the present study, it is possible that the optimum daily need of dLys in commercial-type diets for BB is close to 1,010 mg/hen per d during the 35 to 45 wk period, and that an increment of 190 mg/hen per d did not result in a significant improvement in hen-day egg production during the present study. Further, it appears that when feeding a semi-purified diet to BB from 35 to 45 wk of age, optimum daily need of dLys does not exceed 630 mg/hen/d and that increasing the dLys intake results in no improvement in hen-day egg production. Although the hens that were fed the ID and SPL dietary treatments in the current study likely had the same dLys intake, it remains unclear why the hens that were fed the SPL diet had a lower hen-day egg production. It is possible that the high level of the glutamic acid in the semi-purified diet had a negative impact on the performance of the bird and may not be an effective replacement of intact protein sources. Experiments performed in broiler chicks that were fed low CP –semi-purified diets have shown that growth performance is inferior to those of broilers fed a standard high-CP diet (Fancher

and Jensen, 1989; Ferguson *et al*., 1998; Bregendahl *et al*., 2002). The reasons of inferior performance remain elusive, but the inclusion of crystalline amino acids could be affecting the dietary acid-base balance in the bird (Fancher and Jensen, 1989; Kerr, 1993).

Previous research has determined that a reduced protein intake by older breeders results in reduced egg weights (Lopez and Leeson, 1994a). Also, Prochaska *et al*. (1996) observed a reduction in egg weight when feeding low levels of Lys in 42 wk-old Hy-Line W-36 hens. This reduction in egg weight may be explained by a reduction in albumen weight, solids and protein when hens are fed low levels of Lys (Prochaska *et al*., 1996) and could partially explain the results observed in our study. Spratt and Leeson (1987) observed an increase of 1.2 g in egg weight when feeding a 16.7 % CP diet, when compared to eggs laid by hens that were fed a 12.7 % CP diet. In the current study, a dLys intake of 630 mg/hen per d from a semi-purified diet led to the lowest ($P < 0.05$) egg weight of all treatments. A decrease in the dLys intake from 1,200 to 1,010 mg/hen per d in commercial-type diets did not affect egg weight. It appears that a dLys intake level above of 1,010 mg/hen per d may not be needed in commercial-type diets for optimum for egg weight.

Candling fertility can be affected by a wide range of factors. Male performance (Romero-Sanchez *et al*., 2008), shell quality (McDaniel *et al*., 1979), and heavy body weight (Bilgili and Renden, 1985), are among some of the factors that affect candling fertility in BB. The results of this experiment indicate that when increasing the dLys intake of BB, candling and egg breakout fertility is reduced only when feeding a semi-purified diet. A similar reduction in candling fertility was observed by Coon *et al*. (2006) when feeding a semi-purified diet with high levels of dLys. However, the sperm egg

penetration assay allows us to measure fertilization directly by obtaining the number of holes in the perivitelline layer produced by sperm at the moment of fertilization (Bramwell *et al*., 1995). No differences were observed among treatments for the number of sperm holes penetrating the perivitelline layer of the eggs indicating that fertilization was actually similar across all treatments. This difference between candling fertility and sperm-egg penetration could be the result of eggs being classified as infertile by egg breakout actually undergoing very early embryonic mortality that cannot be detected by the un-aided eye. The decrease in hatchability of eggs set from the hens that were fed the SPL dietary treatment was primarily due to this apparent decrease in candling fertility which, as previously mentioned, may have in fact been very early embryonic mortality. Additionally, the increase in percentage of late dead embryos for the SPL treatment also contributed to the lower hatchability of eggs set. The dLys intake of 1,010 mg/hen per d in the semi-purified diet was possibly higher than the optimum level and may have caused a higher percentage of late dead embryo and perhaps even a higher percentage of very early deads that were incorrectly classified as infertile eggs by candling and egg breakout. Pearson and Herron (1982) observed a decrease in hatchability when feeding breeders a low energy-high protein diet as a result of increased embryonic mortality in middle dead and unhatched pips. However, it is important to mention that most of the past research on the effects of CP on hatchability and embryo development is confounded by lack of information about amino acid content or other nutrients in the diet (Wilson, 1997).

Chicks that hatched from the hens fed the IDL and ID dietary treatments from 35 to 45 wk of age had similar BW. Lopez and Leeson (1994a) fed broiler breeder hens increasing CP levels (9 to 15%) and after 528 h of incubation observed a reduction in

chick BW from the hens that were fed 9 and 11% CP levels. Perhaps a reduction of 190 mg of dLys/hen per d in commercial-type diets was not sufficient to cause a deficiency in the chicks which may suggest that the dLys needs of BB may be overestimated. However, the same results of Lopez and Leeson (1994a) are similar to the effect that was observed on the chick BW from hens that were fed the SPL and SP diets. A dLys intake of 600 mg/hen per d resulted in lower egg weight and chick BW at hatch. Our results are in agreement with previous research, which has observed a positive correlation between egg weight and chick weight (Wiley, 1950; Goodwin, 1961; McNaughton *et al*., 1978).

Overall, decreasing the dLys intake levels from 1,010 to 600 mg/hen per d from 35 to 45 wk of age improves broiler breeder reproductive performance only when feeding a semi-purified diet. The mechanism of how decreased Lys intake positively affects reproductive performance only when feeding a semi-purified diet still remains unclear. However, under practical conditions, it appears that commercial type diets may not need to be formulated to levels above 1,010 mg/hen per d of dLys. Furthermore, research regarding feeding lower intake levels of dLys to BB hens using only commercial available ingredients needs to be assessed. Additionally, various Lys intake levels of BB have yet to be correlated to progeny performance.

Table 3.1 Composition of experimental diets (as-is basis).

Ingredient	Industry	Semi-purified
Corn	67.97	60.81
Soybean meal	20.70	8.70
Wheat middlings	6.00	12.82
Poultry oil	1.00	1.00
Calcium carbonate	6.70	6.71
Dicalcium phosphate	1.81	1.84
Sodium bicarbonate	0.27	-
L-Lysine-HCl	0.18	0.38
DL-Methionine	0.09	0.23
L-Threonine	-	0.14
L-Tryptophan	-	0.06
L-Isoleucine	-	0.10
L-Valine	-	0.05
L-Arginine	-	0.17
L-Glutamic acid	-	6.59
Vitamin premix[1]	0.06	0.06
Mineral premix[2]	0.07	0.07
Calculated composition		
CP, %	16.0	16.0
AME, kcal/kg	2,860	2,860
Calcium, %	3.00	3.00
Available phosphorus, %	0.45	0.45
Sodium, %	0.19	0.19
Lysine, % digestible	0.88	0.74
Methionine, % digestible	0.38	0.41
TSAA, % digestible	0.57	0.57
Threonine, % digestible	0.54	0.49
Isoleucine, % digestible	0.61	0.50
Valine, % digestible	0.68	0.53
Tryptophan, % digestible	0.17	0.17
Arginine, % digestible	0.97	0.80

[1]The vitamin premix contained per kilogram of diet: vitamin A from retinyl acetate, 60,000 IU; vitamin D_3, 2,167 IU; vitamin E from DL-α-tocopheryl acetate, 33 IU; vitamin B_{12}, 0.01 mg; vitamin B_6 from pyridoxine mononitrate, 3 mg; niacin, 27 mg; d-pantotenic acid, 10 mg; menadione, 2 mg; folic acid, 0.87 mg; thiamine from thiamine mononitrate, 3 mg; ethoxyquin, 67 mg; d-biotin, 0.3 mg; and riboflavin, 7 mg.
[2]The mineral premix contained: zinc as zinc sulfate, 11.02%; manganese as manganese sulfate, 8.82%; iron as ferrous sulfate monohydrate, 5.29%; copper as copper sulfate, 1.03%; iodine as ethyllenediamine dihydroiodide, 2,600 ppm; selenium as sodium selenite, 400 ppm.

Table 3.2 Egg production parameters and chick BW of Cobb 500 broiler breeder hens fed two types of diets with various digestible lysine intakes from 35 to 45 wk of age.

Treatment[1]	Hen-day egg production	Egg weight	Egg specific gravity	Chick initial BW
	--------%--------	-------g/egg-----		-----g/chick-----
IDL	72.01[ab]	64.8[a]	1.077	43.92[a]
ID	73.31[a]	65.0[a]	1.077	44.33[a]
SPL	67.34[c]	64.0[a]	1.078	43.70[a]
SP	68.49[bc]	62.7[b]	1.077	42.61[b]
Pooled SEM	1.473	0.383	0.0007	0.321
P-value	0.029	0.00001	0.472	0.006

[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$)
[1]Industry-type diets: IDL= 1,200 mg of dLys/hen per d; and ID= 1,010 mg dLys/hen per d. Semi-purified type diets: SPL= 1,010 mg dLys/hen per d; SP= 600 mg dLys/hen per d.

Table 3.3 Reproductive performance of Cobb 500 broiler breeder hens fed two types of diets with various digestible lysine intakes from 35 to 45 wk of age.

Treatment[1]	Fertility	Early dead	Middle dead	Late dead	Pip	Hatch of set	Hatch of fertile	Sperm Holes[2]
	----------%----------							
IDL	88.92[a]	2.32	0.73	0.83[b]	1.04	83.92[a]	94.51	18.90
ID	86.88[a]	3.13	0.93	1.05[b]	0.52	81.38[ab]	93.56	20.37
SPL	79.63[b]	2.70	1.03	1.81[a]	1.25	72.94[c]	91.28	16.22
SP	84.93[a]	3.51	0.98	0.91[b]	0.66	78.73[b]	92.73	19.76
Pooled SEM	1.528	0.588	0.202	0.235	0.275	1.400	0.0001	1.802
P-value	0.003	0.638	0.629	0.019	0.239	0.0002	0.134	0.154

[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$)
[1]Industry-type diets: IDL= 1,200 mg of dLys/hen per d; and ID= 1,010 mg dLys/hen per d. Semi-purified-type diets: SPL= 1,010 mg dLys/hen per d; SP= 600 mg dLys/hen per d.
[2]Holes in the germinal disc perivitelline layer (1.35mm^2 area)

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CHAPTER IV

DIGESTIBLE LYSINE INTAKE LEVEL EFFECTS ON COBB 500 BROILER BREEDER HEN REPRDUCTIVE PERFORMANCE

Abstract

A study was conducted to examine the reproductive parameters of Cobb 500 broiler breeder hens fed different daily consumption levels of dig Lys (mg/hen/d). A total of 240 Cobb 500 broiler breeder pullets and 40 cockerels, 20 wk of age, were obtained from a commercial blackout rearing house and placed in individual cages. A common breeder diet (16% CP, 3.0% Ca, 0.65% dig Lys) was fed from 20 to 24 wk of age. Experimental diets were fed from 24 to 42 wk of age. Treatment 1 diet was corn-soybean based and formulated to a dig Lys intake of 1,000 mg/hen/d (CS). The remaining three treatments consisted of a diet composed primarily of corn and DDGS, and had three different levels of dig Lys intake: 1,000 (DDGS-1,000), 800 (DDGS-800), and 600 (DDGS-600) mg/hen/d. The study was a completely randomized design, and each treatment was replicated 6 times. Each replicate consisted of a group of 10 hens. Hens were artificially inseminated on wk 25, 30, 35, and 41 with 50 µL of undiluted semen obtained from the Cobb 500 roosters. Hen-day egg production and egg weight were observed to be similar among all treatments. Fertility parameters were unaffected by the dig Lys intake levels fed. Additionally, hatchability was unaffected, likely because early dead, middle dead, late dead, contaminated or pipped eggs were similar for all treatments. Feeding dig Lys below 1,000 mg/hen/d did not impact broiler breeder reproductive

performance, thus suggesting that Lys may be in dietary surplus concentrations for commercial breeders under current practical conditions.

Introduction

Current nutritional approaches in broiler breeder (BB) formulation include a balance of amino acids to energy, in diets that focus on maintaining the potential for egg production and maximizing chick production (de Beer, 2011). Efficient reproduction, management, livability, and health status of the female BB must be maintained while retaining the genetic potential for rapid growth and breast development to be passed on to their progeny (Richards *et al*., 2010; de Beer, 2011). Previous research has shown that growth and reproductive efficiency are negatively related traits (McDaniel *et al*., 1981; Wilson and Harms, 1986; Lopez and Leeson, 1994a; Robinson *et al*., 1995; Wilson *et al*., 1995; Hudson *et al*., 2001). Therefore, one of the goals of a nutritionist should be to minimize the effect that nutrition and management have on the reproductive parameters without affecting the production traits of the BB hen.

Previous nutritional studies on BB have been focused on evaluating dietary protein levels (Lopez and Leeson, 1994a; Walsh and Brake, 1999; Romero *et al*., 2009) and feed programs (Wilson *et al*., 1995; Goerzen *et al*., 1996; Enting *et al*., 2007; Ekmay *et al*., 2010) during various production stages. There is sparse research regarding the specific amino acids needs of BB, and often, dietary CP intake has been confounded with intake of limiting amino acids (Lopez and Leeson, 1994b). According to de Beer (2011), when formulating to minimum CP levels, Lys levels can often be found to over 40% of the requirement. Excess dietary Lys can drive muscle deposition (de Beer, 2011), and

may lead to increase body weight by BB which can have a negative effect on reproductive performance (Lopez and Leeson, 1995). Coon *et al*. (2006) observed that feeding high levels of digestible Lys [dLys] and Ile caused a decrease in fertility, in a study that employed semi-purified diets. Furthermore, recent research from our lab suggests that decreasing dLys intake by BB may corroborate the improvement in fertility when feeding semi-purified diets (Mejia *et al*., 2012). Therefore, the objective of the current study was to evaluate the effect that reduced dietary Lys intake has on the reproductive parameters of Cobb 500 BB hens when fed diets composed only of commercially available ingredients.

Materials and Methods

Dietary treatments

A common breeder diet (16% CP, 1.50% Ca, and 0.63% dLys) was fed from 21 to 24 wk of age, while experimental breeder-phase diets were fed from 24 to 42 wk of age (Table 4.1). A common industry-type diet composed of corn and soybean meal with the inclusion on L-Lys HCl was fed to meet a daily dLys intake level of 1,000 mg/hen/d [CS] and served as a control only for the DDGS-1,000 diet. Treatments 2, 3, and 4 resulted from one of three aliquots of a common diet composed primarily of corn, corn distiller's grains with solubles (DDGS), and soybean meal. Each of these aliquots was subsequently reblended with either L-Lys HCl and/or sand to obtain progressive calculated daily dLys intake levels of 1,000 [DDGS-1,000], 800 [DDGS-800], or 600 mg/hen/d [DDGS-600]. All other nutrients, except for dLys, were satisfied for all the experimental treatments per the primary breeder company's recommendations (Cobb, 2008). Feed intake was

restricted to 390 kcal/d, which resulted in a daily intake of 136 g/hen (C. Wiernusz, 2010). All hens were fed their respective dietary treatments on a daily basis and at the same time of the day, following the recommendation from the primary breeding company.

Bird husbandry

A total of 260 Cobb 500 broiler breeder pullets and 40 cockerels, 20 wk of age, were obtained from a commercial blackout rearing house under a 8L:16D lighting program. The pullets were placed in a curtain-sided house and allocated in individual cages (60 × 50 × 40 cm). The photoperiod was then extended with artificial light to 14 and 15 h at 22 and 24 wk of age, and then to 15.5 and 16 h at 5 and 50% egg production, respectively. At 24 wk of age, hens that had not reached the required BW were discarded. Six replicate groups of 10 broiler breeder hens each (10 adjacent cages containing 1 hen/cage, 60 × 50 × 40 cm) were allotted to the four dietary treatments (total= 240 hens) in a completely randomized design, so that mean BW was similar for each treatment. The roosters for artificial insemination were housed in a separate solid-sided house in individual cages (64 × 48 × 48 cm), where the photoperiod was extended with artificial light to 16 h, and they received an industry standard broiler breeder diet (CP 16%, AME 2,860 kcal/kg, 3.00% Ca, 0.45% available P, and 0.65% dLys). All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Egg production and performance

Egg production performance was measured for a 19 wk period and corresponded to periods prior to-, during-, and post-peak of production. Egg production and hen

mortality were recorded daily throughout the 19 wk experimental period. Egg weight was measured daily for all eggs laid during wk 26, 31, 36, and 42.

Semen collection and artificial insemination

At 25, 30, 35, and 41 wk of age, semen was collected and used for artificial insemination to provide fertilized eggs for fertility and hatchability analyses. Semen was collected using the abdominal massage method of Burrows and Quinn (1937). Immediately after collection, 50 µL of undiluted semen was obtained from the Cobb 500 roosters and inseminated into each hen.

Incubation

Eggs collected from wk 26, 31, 36, and 42 were stored at 18.3 °C over the 8 d post-insemination period, and then set in a Natureform (Model No. 2340, Jacksonville, FL) incubator at 37.5 °C and 55% RH for 516 h. Eggs were randomly distributed in the incubator according to day post-insemination and replicate group. Eggs were candled on d 14 of incubation and transferred for hatching on d 18 of incubation. During candling, eggs were characterized as being infertile, cracked, contaminated, or containing early dead embryos (less than 7 d), or middle dead embryos (7 to 14 d). Eggs were transferred to hatching baskets at d 18 of incubation, where they were randomly distributed according to the day post-insemination and replicate group. After hatching (d 21.5 of incubation), the number of live chicks, initial chick BW, middle and late dead embryos (15 to 21 d) and dead pips was determined.

Statistical analysis

Data were analyzed as a split-split plot design using the GLM procedures of SAS® (2010). Whole plots were replicated by the randomized groups of 10 hens each.

Weeks of the study were considered split plots, whereas for fertility and hatchability data, days post-insemination were considered split-split plots. Fisher's-protected LSD test was used to determine significant differences ($P < 0.05$) among treatment means.

Results and Discussion

Validating the experimental treatments imposed on the hens, the amino acid analyses of the experimental diets were in agreement with formulated values. The CS and DDGS-1,000 were formulated to total Lys concentration values of 0.83 and 0.87%, and resulted in analyzed total Lys values of 0.89 and 0.87%, respectively. Additionally, the DDGS-800 and DDGS-600 were expected to contain 0.73 and 0.58% total Lys values, and resulted in analyzed total Lys values of 0.72 and 0.63%, respectively. Throughout the study, no significant 2-or 3-way interactions of dietary treatments with wk or d postinsemination were observed, therefore only the main effect of diet is addressed in this manuscript.

Hen-day egg production, egg weight and initial chick weight were similar ($P > 0.05$) for the hens fed the CS and all the DDGS dietary treatments (Table 4.2). Hens fed the DDGS-1,000 diet, which contained the same dLys level of 1,000 mg/hen/d as those fed the CS diet, yielded similar egg production, egg weights and initial chick weight despite having the inclusion of an alternative feed ingredient such as DDGS. Past research has found it difficult to dissociate the effects of protein from those limiting amino acids on the performance of BB hens (Harms, 1992). Spratt and Leeson (1987) observed no differences in hen-day egg production when feeding low (19 g/hen/d) or high (25 g/hen/d) protein intakes to broiler breeders from 25 to 40 wk of age. Previous studies regarding the effect of Lys per se have reported that small increments in total Lys

close to an optimum resulted in equal responses in rate of lay and egg weight (Bowmaker and Gous, 1991). Furthermore, many workers have reported that egg weight is positively correlated with chick weight (Wiley, 1950; Goodwin, 1961; Morris *et al*., 1968, Washburn *et al*., 1974; and McNaughton *et al*., 1978) and results from the current study determined that decreasing dLys intake on BB hens had no effect on egg weight or chick initial weight (Table 4.2).

Reproductive parameters in BB hens were unaffected by the dLys intake levels fed to the hens (Table 4.3). Fertility has been shown to improve when low levels of dLys are fed in a semipurified diet (Coon *et al*., 2006), although Mejia *et al*. (2012) did not observe the same effect when decreasing dLys intake levels were fed in an industry-type diet to Cobb 500 BB hens from 35 to 45 wk of age. Hatchability of eggs set and hatch of fertile eggs were similar between treatments, possibly because early, middle, and late dead, contaminated or pipped eggs yielded similar results among all treatments (Table 4.3). Previous research revealed that increasing the daily intake of dLys in semi-purified diets leads to a lower fertility and a higher incidence in the percentage of late dead, causing a lower hatchability of eggs set, but the same response was not observed when feeding BB hens an industry-type diet (Mejia *et al*., 2012). Past research by Pearson and Herron (1982) observed that feeding breeders a low energy-high protein diet decreased hatchability due to increased embryonic mortality in middle dead and unhatched pips. However, it is important to mention that most of the research on the effects of CP on hatchability and embryo development may have been confounded by lack of information about amino acid content or other nutrients in the diet (Wilson, 1997).

On a different note, the utilization of DDGS in broiler diets has become a plentiful and relatively inexpensive feed ingredient in many areas. Nutritional variability

among DDGS sources (Cromwell *et al*., 1993; Batal and Dale, 2006; Fastinger *et al*., 2006), inclusion limits for broilers and laying hens at various production stages (Lumpkins *et al*., 2004; Loar *et al*., 2010a; Loar *et al*., 2010b; Lumpkins *et al*., 2005; Mejia *et al*., 2011), product storage (Rosentrater, 2006) and feed throughput and pellet quality (Behnke, 2007; Loar *et al*., 2010a) among others have become a major concern when formulating diets with DDGS. To our knowledge, no study had reported the impact that DDGS inclusion could have on BB fertility and production. Results from our current study indicate that inclusion levels of 25% of DDGS in BB diets had no effect in performance, thus suggesting that an older bird with a more mature gastrointestinal tract may have a better tolerance to high DDGS inclusion rates when compared to a young chick or growing broiler.

Overall, decreasing the dLys intake in commercial-type diets from 24 to 42 wk of age did not have any impact in broiler breeder performance. It appears that under practical conditions, Lys intake is possibly being fed in surplus to BB hens.

Table 4.1 Composition of experimental diets (as-is basis)

Ingredient	Control	Lys titration diet
Corn	68.92	57.18
Soybean meal	21.70	8.06
Distillers dried grains with solubles	-	25.00
Poultry oil	0.13	0.10
Calcium carbonate	6.63	6.97
Dicalcium phosphate	1.85	1.44
Sodium chloride	0.33	0.13
Sodium bicarbonate	0.11	0.09
L-Lysine-HCl	0.02	0.37
DL-Methionine	0.17	0.19
L-Threonine	-	0.08
L-Tryptophan	-	0.04
L-Isoleucine	-	0.11
L-Valine	-	0.02
L-Arginine	-	0.10
Vitamin premix[1]	0.06	0.06
Mineral premix[2]	0.07	0.07
Calculated composition		
CP, %	15.2	14.5
AME, kcal/kg	2,860	2,860
Calcium, %	3.00	3.00
Available phosphorus, %	0.45	0.45
Sodium, %	0.19	0.19
Lysine, % digestible	0.74	0.74
TSAA, % digestible	0.63	0.63
Threonine, % digestible	0.54	0.52
Arginine, % digestible	0.99	0.77

[1]The vitamin premix contained per kilogram of diet: vitamin A from retinyl acetate, 60,000 IU; vitamin D_3, 2,167 IU; vitamin E from DL-α-tocopheryl acetate, 33 IU; vitamin B_{12}, 0.01 mg; vitamin B_6 from pyridoxine mononitrate, 3 mg; niacin, 27 mg; d-pantotenic acid, 10 mg; menadione, 2 mg; folic acid, 0.87 mg; thiamine from thiamine mononitrate, 3 mg; ethoxyquin, 67 mg; d-biotin, 0.3 mg; and riboflavin, 7 mg.
[2]The mineral premix contained: zinc as zinc sulfate, 11.02%; manganese as manganese sulfate, 8.82%; iron as ferrous sulfate monohydrate, 5.29%; copper as copper sulfate, 1.03%; iodine as ethyllenediamine dihydroiodide, 2,600 ppm; selenium as sodium selenite, 400 ppm.

Table 4.2 Egg production parameters and chick BW of Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels from 24 to 42 wk of age

Treatment[1]	Hen-day egg production	Egg weight	Chick initial BW
	--------%--------	-------g/egg-----	-----g/chick-----
CS	66.26	59.63	41.40
DDGS-1,000	66.60	59.10	41.33
DDGS-800	60.73	59.76	42.51
DDGS-600	62.70	58.69	40.87
Pooled SEM	2.090	0.389	0.560
P-value	0.17	0.25	0.18

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d. DDGS-type diets: DDGS-1,000= 1,000 mg dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 4.3 Reproductive performance of Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels from 24 to 42 wk of age.

Treatment[1]	Fertility	Early dead	Middle dead	Late dead	Pip	Hatch of set	Hatch of fertile
	----------%----------						
CS	87.94	4.87	0.31	1.94	0.90	79.73	90.81
DDGS-1,000	90.03	3.99	0.38	2.39	1.43	81.76	91.00
DDGS-800	87.00	5.35	0.52	1.44	0.55	78.92	91.05
DDGS-600	85.51	2.73	0.43	2.17	0.76	79.30	92.66
Pooled SEM	2.243	0.835	0.227	0.412	0.363	2.185	0.012
P-value	0.65	0.17	0.88	0.62	0.54	0.87	0.63

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d. DDGS-type diets: DDGS-1,000= 1,000 mg dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; and DDGS-600= 600 mg dLys/hen/d.

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CHAPTER V

EVALUATION OF CARRYOVER EFFECTS OF DIETARY LYSINE INTAKE BY COBB 500 BROILER BREEDER HENS ON PROGENY LIVE PERFORMANCE

Abstract

Two experiments were conducted to examine the progeny performance of broiler breeder hens that were fed diets differing in digestible lysine (dLys). A total of 240 Cobb 500 broiler breeder pullets and 40 cockerels, 20 wk of age, were used for each experiment. In Experiment 1, treatment diets were fed from 35 to 45 wk of age. Treatment 1 and 2 diets were formulated with commonly used feed ingredients and had dLys daily intakes of 1,200 (IDL) and 1,010 mg/hen/d (ID), respectively. Treatments 3 and 4 were composed of semi-purified diets formulated to contain dLys intakes of 1,010 (SPL) and 600 mg/hen/d (SP), respectively. Chicks corresponding to eggs collected from wk 42 were grown to 56 d of age. Chick weight at hatch was lower ($P<0.05$) for those that came from the SP and SPL-fed hens, but 42 and 56 d BW was similar for all treatments. Marginal improvements ($P<0.10$) in FCR were seen at 42 and 56 d for chicks from ID-fed hens compared to IDL hens. For Experiment 2, diets were fed to hens from 24 to 42 wk of age. Treatment 1 was a corn-soybean meal-based diet formulated to have a dLys intake of 1,000 mg/hen/d (CS). Treatments 2, 3, and 4 had the inclusion of DDGS with dLys intake levels of 1,000 (DDGS-1,000), 800 (DDGS-800), and 600 (DDGS-600) mg/hen/d, respectively. Progeny performance was evaluated from eggs collected at wk 26, 31, and 36. Chick hatch weight was similar for all 3 hatches. Chicks from hens at 26

wk and fed DDGS-600 diets resulted in lower ($P<0.05$) BW, carcass and breast weight, and higher ($P<0.05$) back half weight, at 42 d of age. No effects were observed for any parameter at 56 d. Growout studies performed on eggs laid during wk 31 and 36 revealed that dLys intake levels fed had no effect on live performance or carcass characteristics of the progeny. In conclusion, the results from Experiments 1 and 2 may not be comparable. Therefore, the potential impact of dietary Lys in the breeder hen diet on progeny performance needs to be further evaluated.

Introduction

Traditionally, broiler breeder (BB) research has evaluated ways to maximize the production and hatchability of eggs. Recent attempts have been to establish the relationship between BB hen nutrition and its impact on progeny performance (Kidd, 2003; Hocking, 2007; Calini and Siri, 2007). Early chick growth, livability, market age, feed efficiency, uniformity and breast meat yield among many others may be considered as chick quality endpoints to be evaluated in the progeny. Nutrient manipulations in CP (Wilson and Harms, 1984; Proudfoot *et al*., 1985; Lopez and Leeson, 1994; Lopez and Leeson, 1995), energy (Aitken *et al*., 1969; Pearson and Herron, 1981; Proudfoot and Hulan, 1987; Spratt and Leeson, 1987; Romero-Sanchez *et al*., 2008), type of fat (Proudfoot *et al*., 1982; Cherian and Sim, 1993; Attia *et al*., 1995; Latour *et al*., 1996; Peebles *et al*., 1998; Peebles *et al*., 1999; Peebles *et al*., 2002; Enting *et al*., 2007), mineral (Heth *et al*., 1966; Taylor, 1965; Kidd *et al*., 1992; Triyuwanta *et al*., 1992; Virden *et al*., 2003; Shim *et al*., 2008;) and vitamin (Balloun and Phillips, 1957; March *et al*., 1972; Ameenuddin *et al*.,1986; Surai *et al*., 1998; Leshchinsky and Klasing, 2001;

Atencio *et al*., 2005; Siegel *et al*., 2006; Coto *et al*., 2010) nutrition in BB have been shown to affect progeny live performance.

According to de Beer (2011), commercial BB feeds are often formulated to minimum CP levels, resulting in amino acid levels significantly higher than the requirement and may lead to an increase in muscle deposition by this type of bird, which is not a desirable trait. Additionally, Coon *et al*. (2006) observed a decrease in fertility when feeding high levels of dLys and isoleucine, but this was done by feeding a semipurified diet and the potential effect that this may have on chick growth was not evaluated. Past research has shown that growth performance in broilers and BB reproductive performance is inferior when feeding low CP-semipurfied diets as compared to a standard CP-diet (Fancher and Jensen, 1989; Ferguson *et al*., 1998; Bregendahl *et al*., 2002; Mejia *et al*., 2012). The reasons for inferior performance remain unclear, but the inclusion of crystalline amino acids and less soybean meal may cause a lack of sufficient nitrogen pool to provide synthesis of nonessential amino acids or an imbalance among certain amino acids and even an alteration in the dietary acid-base balance of the bird (Fancher and Jensen, 1989; Kerr, 1993; Si *et al*., 2004). Furthermore, to the authors' knowledge past research has elucidated the impact of dLys on BB reproductive performance only when feeding a semipurified diet (Coon *et al*., 2006; Mejia *et al*., 2012a). However, a study from our lab evaluated feeding diets, composed from commercially available ingredients that had the inclusion of corn distillers dried grain with solubles (DDGS), with varying levels of dLys on BB hens and observed that decreasing the dLys intake when feeding this type of diets to Cobb 500 BB hens had no detrimental effect on reproductive performance and chick weight at hatch (Mejia *et al*., 2012b). DDGS has become a readily available and inexpensive feed ingredient that has

been used in broiler and laying hen rations (Lumpkins *et al*., 2005; Loar *et al*., 2010; Mejia *et al*., 2011; Masa'deh *et al*., 2011; Loar *et al*., 2012). A previous study revealed that the inclusion of up to 25% DDGS in a BB diet had no detrimental effect on the reproductive parameters of this type of bird (Mejia *et al*., 2012b). In general, very little information is available in the literature on the effects of decreasing amino acid intake by BB hens and whether or not this practice may affect subsequent progeny live performance and carcass traits. Therefore, the objective of the current studies were to evaluate two types of diets, a semipurified and/or industry type diet, with various dLys intake levels during different production phases of the BB hen on subsequent progeny live performance and carcass characteristics.

Materials and Methods

Dietary treatments

A total of 240 Cobb 500 broiler breeder pullets and 40 cockerels, 20 wk of age, were used for each experiment. In Experiment 1, treatment diets were fed from 35 to 45 wk of age. Treatment 1 and 2 diets were formulated with commonly used feed ingredients and had dLys intakes of 1,200 (IDL) and 1,010 mg/hen/d (ID), respectively. Treatments 3 and 4 were composed of semi-purified diets formulated to contain dLys intakes of 1,010 (SPL) and 600 mg/hen/d (SP), respectively. The methodology used in this experiment for feed mixing, BB husbandry and feed consumption, egg production and performance, semen collection, dilution and artificial insemination, and incubation was published previously by Mejia *et al*. (2012a).

For Experiment 2, diets were fed to hens from 24 to 42 wk of age. Treatment 1 was a corn-soybean meal-based diet formulated to have a dLys intake of 1,000 mg/hen/d

(CS). Treatments 2, 3, and 4 had the inclusion of DDGS with dLys intake levels of 1,000 (DDGS-1,000), 800 (DDGS-800), and 600 (DDGS-600) mg/hen/d, respectively. The methodology used in this experiment for feed mixing, BB husbandry and feed consumption, egg production and performance, semen collection, artificial insemination, and incubation was published previously by Mejia *et al*. (2012b).

Bird husbandry-Progeny studies

A progeny growout study for Experiment 1 was conducted with eggs collected at 42 wk of age and chicks were grown to 56 d of age. For Experiment 2 growout studies, eggs were collected at 26, 31, and 36 wk of age and chicks were grown to 56 d of age. For each individual growout study, day-old chicks were randomly distributed in each of 56 floor pens (12 birds/pen; 672 birds total; 0.09m^2/bird) of a solid-wall facility, and each pen was equipped with a hanging feeder, a nipple drinker line (3 nipple drinkers/pen), and built up litter. Birds consumed feed and water on an ad libitum basis. The lighting program was 23L:1D, and ventilation was accomplished by negative air pressure. Mortality was recorded daily, and all bird procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Broilers were fed a common starter diet from 1 to 14 d of age, grower diet from 14 to 28 d of age, finisher from 28 to 42 d of age, and withdrawal diet from 42 to 56 d of age (Table 5.1). All diets were least-cost formulated, and were mainly composed of commercially available ingredients, corn and soybean-meal with the inclusion of corn distillers dried grain with solubles and Pro-Plus, an animal by product blend. Diets were formulated using calculated digestible amino acid values from published digestibility coefficients (Ajinomoto, 2004) by using the analyzed total amino acid content of the

ingredients. Essential digestible amino acids were maintained in all diets by setting minimum formulation ratios relative to digestible Lys as follows: TSAA, 75; Thr, 66; Val, 76; Ile, 68; Trp, 16; and Arg, 105 and following previously published recommendations (Lemme *et al*., 2004). All other essential nutrients were formulated to meet or exceed NRC (1994) nutrient recommendations. For each individual growout progeny study, the dietary dLys treatment of the BB represented the progeny treatments.

Evaluation criteria

In Experiment 1, birds in each pen were weighed collectively at the beginning and end of the finisher and withdrawal feed phase. In Experiment 2 growout progeny studies, birds in each pen were weighed collectively at the beginning and end of each individual feed phase. Feed consumption and mortality were monitored throughout the studies and feed conversion was corrected for the weight of mortality and represents the following: (g of feed consumed by all birds in a pen) / (g of BW per pen + weight of dead birds).

In Experiment 1, at 56 d of age, 6 broilers (3 males and 3 females) from each pen were randomly selected for processing. In Experiment 2, broilers from eggs collected at 26 wk of age were processed at 42 d of age (4 broilers; 2 males and 2 females) and at 56 d of age (6 broilers; 3 males and 3 females). For all the other growout studies performed in Experiment 2, 6 broilers (3 males and 3 females) were randomly selected for processing only at 56 d of age. During processing of both Experiments, broilers were hung by their feet in steel shackles and were electrically stunned by placing their heads in a saturated saline bath (11.5 volts, <0.5mA AC to DC current for 5 s). The shackle line speed was constant and set so that approximately 22 broilers were stunned per min. Unilateral neck cutting was mechanically performed immediately after stunning, and

bleeding lasted for 140 s. Upon completion of exsanguination, the broilers were scalded at 53.3°C for 191 s and picked for 35 s using a rotary drum picker. Carcasses were then manually removed of heads and paws, and transferred to a separate room where they were mechanically eviscerated. Carcasses were removed from the line, their abdominal fat removed and hot carcass and abdominal fat pad weight was recorded for each individual bird. Broiler carcasses were submerged in ice water in metal containers (173 cm in length, 85 cm in width, and 68.5 cm in depth). At 4 h postmortem, breast (boneless and skinless) muscles, wings, and back halves (drumsticks and thighs) were manually deboned and their weight was recorded.

Statistical analysis

A completely randomized block design was used to test the effects of BB treatment on the progeny. Each treatment was replicated 14 times, where the pen represented the experimental unit for analyses in the study. Since broilers were reared as straight run, the number of males and females in each floor pen (experimental unit) was used to calculate a male-to-female ratio in each and was used as a covariate in each individual growout study. Block corresponded to location in the experimental broiler house. When significant differences existed in Experiment 1 ($P < 0.10$) and Experiment 2 ($P < 0.05$) among treatments, the Fisher's protected least significant difference test was used to separate treatment means (Steel and Torrie, 1980).

Results and Discussion

Experiment 1

A reduction in dLys intake by BB hens, when feeding industry-type diets, had no detrimental effect (*P > 0.10*) on progeny live BW at 42 or 56 d of age (Table 5.2).

However, broilers from BB receiving the ID diet had a lower ($P < 0.10$) feed consumption only at 42 d of age, which lead to an improved feed conversion during the same time period (Table 5.2). Furthermore, the same broilers displayed improved feed conversion at 56 d of age (*P < 0.10*) when compared to broilers from BB receiving the IDL treatment (Table 5.2). It is believed that current BB feeds are being formulated to minimum CP levels, which may lead to dietary excess of amino acids (de Beer, 2011). Results from our study, may indicate that a reduction of 190 mg dLys/hen per day did not affect live BW of the progeny and marginally improved feed conversion, which may indicate that a daily dLys intake of 1,200 mg/hen per day is probably in excess for the BB. Lopez and Leeson (1994a) fed low CP daily intake levels to BB hens and observed a lower chick weight at hatching, but after a 49 d growout period, the difference was no longer apparent, which is a similar growout period to the current study. In another study, Wilson and Harms (1984) decreased the daily intake of Lys and other essential amino acids in BB feeds and observed no detrimental effect in the BW of the progeny at 49 d of age. In addition, the marginal improvement observed only in feed conversion and not in live BW from broilers that came from BB fed the ID diet (1,010 mg/hen per day) was similar to the feed conversion improvement observed by other studies (Proudfoot *et al*., 1985; Lopez and Leeson, 1995) in progeny chicks that came from BB hens fed a low CP diet.

Also, in the current study, when BB hens were fed a semipurified diet, a reduction in dLys intake caused a lower (*P < 0.10*) chick BW at hatch, but all other live performance parameters remained unaffected (Table 5.2). The lower chick weight at hatch from chicks that came from BB hens fed the SP diet may have been caused by the low egg weights observed in the study (Mejia *et al*., 2012a). Previous research has

observed a positive correlation between egg weight and chick weight (Wiley, 1950; Goodwin, 1961; McNaughton *et al*., 1978), which is in agreement with our results.

Furthermore, no differences in carcass characteristics were observed among dietary treatments (Table 5.3). To the authors' knowledge, the effect of dLys intake on progeny carcass yields has not been previously reported. However, Spratt and Leeson (1987) observed in male offspring from BB hens fed a high protein level (25 g/hen per day) diet contained higher carcass protein than offspring from BB hens fed a low protein diet (19 g/hen per day). It is known that protein yield and carcass quality are related to BB nutrition (Spratt and Leeson, 1987), but it is possible that the reduction of only dLys and not CP in both type of diets did not affect any processing yield parameter in our study.

Experiment 2

Three growout progeny studies were performed from eggs collected at 26, 31, and 36 wk of age, corresponding to the pre-, peak, and post-peak egg production phase of the BB hen. Broilers from eggs collected at 26 wk of age were observed to have no significant differences among treatments for BW at hatch and BW, feed consumption, feed conversion, and mortality at 14 or 28 d of age (Table 5.4). Lopez and Leeson (1994a; 1994b) fed varying CP levels to BB hens and observed that a reduction of up to 6% CP did not impact live performance parameters from broilers at7, 21 or 35 d of age, which are similar growout phases evaluated in our study. Additionally, a previous study by Wilson and Harms (1984) observed no significant effect on progeny BW at 49 d of age from BB hens fed diets with varying levels of Lys and other amino acids. At 42 d of age, broilers from BB fed the DDGS-600 diet had the lowest ($P < 0.05$) BW of all

treatments (Table 5.5). This yielded lower ($P < 0.05$) carcass and breast meat weights, but a higher ($P < 0.05$) back half weight in the processing parameters of these broilers at 42 d of age (Table 5.6). Past research has shown that feeding increasing levels of Lys in broilers improves BW gain and carcass yield (Kidd *et al*., 1998; Kerr *et al*., 1999). Furthermore, feeding diets that have a high digestible Arg:Lys ratio to 42 d-old broilers improves back-half (drums and thighs) yield (Costa *et al*., 2001). Although BW at hatch, 14, 28, and 56 d of age remained unaffected by the BB dietary treatment, it remains unclear why the progeny from BB hens fed the DDGS-600 diet had a lower BW at 42 d of age. It is possible that in our study the BB hens fed the DDGS-600 diet were fed a low Lys level in the diet which yielded a diet with an increased digestible Arg:Lys ratio, and may have influence the progeny bird to allocate more nutrients for back half (drums and thighs) development at this young age rather than breast meat. No significant differences were observed for carcass traits among treatments at 56 d of age (Table 5.7). Lopez and Leeson (1994a) observed that a reduced CP intake in BB did not adversely affect carcass characteristics of offspring grown to a 49 d period, which is similar to that obtained from progeny of the current study.

Progeny studies from eggs collected at 31 and 36 wk of age were unaffected by BB hen dietary treatment and no differences were observed for all live performance parameters (Table 5.8 and Table 5.9; Table 5.11 and Table 5.12). Furthermore, decreasing the dLys intake levels in BB had no detrimental effect ($P > 0.05$) on carcass characteristics of the progeny at 56 d of age (Table 5.10; Table 5.13). The explanation for these results should be similar to that of Experiment 1. Previous research has indicated that feeding low CP levels, a parameter more sensitive than Lys, to BB hens has no adverse effect on live performance and carcass characteristics of the progeny (Wilson and

Harms, 1984; Lopez and Leeson 1994a; Lopez and Leeson, 1995) which was observed in the results of the progeny studies from eggs collected at 31 and 36 wk of age. In a more recent study, Ekmay *et al*. (2011) determined the partitioning of Lys stable isotopes in BB hens. They concluded that during early lay and peak production periods, between 60 to 76% of Lys found in egg albumen relied on skeletal muscle reserves than on dietary protein, suggesting that skeletal muscle may act as an intermediary organ that partitions Lys. The response observed by Ekmay *et al*. (2011) could possibly explain why decreasing dietary Lys intake levels to BB hens in our study had little or no impact on progeny performance or carcass characteristics.

Overall, the results from Experiments 1 and 2 were not complementary of each other. The limitations of feeding semi-purified diets to BB hens, the different dLys intake levels used for both experiments, the changes in amino acid ratios relative to Lys observed in the different diets, the inclusion of DDGS, and the different progeny growout ages used may have been the possible causes of the variant responses observed between Experiments 1 and 2. Therefore, the potential impact of dietary Lys in the breeder hen diet on progeny performance needs to be further evaluated. Also, studies should evaluate if amino acid intake levels may have a potential impact in later BB production phases and their corresponding offspring, which was not evaluated in these studies.

Table 5.1 Composition of experimental diets (as-is basis)

Ingredient	Starter	Grower	Finisher	Withdrawal
Corn	59.7	62.9	66.5	69.8
Soybean meal	31.8	28.4	24.5	20.9
Distillers dried grains with solubles	2.0	2.0	2.0	2.0
Animal byproduct blend[1]	2.0	2.0	2.0	2.0
Poultry oil	1.37	1.71	2.07	2.45
Calcium carbonate	1.03	0.99	0.95	0.94
Dicalcium phosphate	0.81	0.72	0.86	0.83
Sodium chloride	0.35	0.37	0.32	0.33
L-Lysine-HCl	0.22	0.20	0.20	0.18
DL-Methionine	0.30	0.27	0.24	0.20
L-Threonine	0.07	0.07	0.06	0.05
Vitamin/mineral premix[2]	0.25	0.25	0.25	0.25
Coccidiostat[3]	0.05	0.05	0.05	0.05
Choline chloride	0.02	----	----	----
Phytase enzyme[4]	0.02	0.02	0.02	0.02
Calculated composition				
CP, %	21.4	20.0	18.3	16.8
AME, kcal/kg	3,025	3,075	3,125	3,175
Calcium, %	0.88	0.84	0.80	0.78
Available phosphorus, %	0.44	0.42	0.40	0.39
Sodium, %	0.22	0.22	0.20	0.20
Lysine, % digestible	1.20	1.10	1.00	0.90
TSAA, % digestible	0.90	0.84	0.77	0.70
Threonine, % digestible	0.79	0.74	0.68	0.62

[1] ProPlus is an animal by-product blend, with a CP content of 60% (H. J. Baker & Bros., Inc.; Little Rock, AR).

[2] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.1 mg.

[3] Dietary inclusion of coccidiostat (Bio-Cox 60, Alpharma LLC, Bridgewater, NJ) provides 60 g of salinomycin sodium per 907.2 kg of feed.

[4] DSM Nutritional Products, Inc. Parsippany, NJ.

Table 5.2 Experiment 1 live performance of progeny (d 0 to 56) from Cobb 500 broiler breeder hens fed 2 types of diets with various digestible lysine intake levels.

		42 d of age				56 d of age			
Treatment[1]	BW 0d	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	--g/bird--	----------g/bird----------			----%---	----------g/bird---------			-----%----
IDL	52.76[ab]	2,582.7	4,417.9[a]	1.70[a]	0.00	3,033.1	6,254.6	2.06[a]	1.19[ab]
ID	53.41[a]	2,560.7	4,275.1[b]	1.65[b]	0.00	2,993.6	6,094.9	2.00[b]	0.00[b]
SPL	52.61[b]	2,589.5	4,353.2[ab]	1.61[a]	1.79	2,978.9	6,117.2	2.06[a]	4.76[a]
SP	51.16[c]	2,595.2	4,354.4[ab]	1.68[ab]	1.19	3,050.7	6,191.9	2.02[ab]	4.17[ab]
Pooled SEM	0.003	27.34	69.21	0.012	1.49	47.027	73.194	0.015	1.522
P-value	0.0001	0.405	0.086	0.081	0.471	0.544	0.246	0.076	0.087

[a,b] Means within a column with no common superscript differ significantly ($P < 0.10$).

[1]Industry-type diets: IDL= 1,200 mg of dLys/hen per d; and ID= 1,010 mg dLys/hen per d. Semi-purified-type diets: SPL= 1,010 mg dLys/hen per d; ID= 600 mg dLys/hen per d.

Table 5.3 Experiment 1 carcass characteristics of progeny (d 0 to 56) from Cobb 500 broiler breeder hens fed 2 types of diets with various digestible lysine intake levels.

Treatment[1]	Fat, g	Fat,[2] %	Breast, g	Breast,[2] %	Back Half, g	Back Half,[2] %	Wings, g	Wings,[2] %
IDL	61	1.97	689	22.03	920	29.42	254	8.21
ID	59	1.88	690	21.82	910	29.26	254	8.12
SPL	60	1.96	672	22.15	914	29.47	252	8.22
SP	59	1.92	682	22.07	900	29.31	252	8.18
Pooled SEM	2.0	0.067	15.0	0.202	11.0	0.132	3.0	0.050
P-value	0.938	0.856	0.839	0.495	0.548	0.872	0.878	0.558

[1]Industry-type diets: IDL= 1,200 mg of dLys/hen per d; and ID= 1,010 mg dLys/hen per d. Semi-purified-type diets: SPL= 1,010 mg dLys/hen per d; ID= 600 mg dLys/hen per d.
[2]Percentage of live weight

Table 5.4 Experiment 2 live performance of progeny (d 0 to 28) from eggs collected at 26 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

		14 d of age				28 d of age			
Treatment[1]	Chick initial BW	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	------g/bird------	----------g/bird----------			-------%-----	----------g/bird----------			-----%----
CS	36.7	332.8	416.1	1.25	1.28	1,367.7	1,961.7	1.43	2.56
DDGS-1,000	36.3	330.7	410.5	1.24	1.79	1,377.6	1,976.7	1.43	1.79
DDGS-800	35.8	333.8	423.5	1.24	1.92	1,404.4	2,009.1	1.42	3.21
DDGS-600	35.4	332.6	415.7	1.25	1.92	1,371.2	1,978.1	1.43	1.92
Pooled SEM	0.32	3.58	4.98	0.008	0.950	11.32	16.05	0.006	1.014
P-value	0.390	0.935	0.335	0.621	0.965	0.094	0.203	0.653	0.765

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 5.5 Experiment 2 live performance of progeny (d 0 to 56) from eggs collected at 26 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

	42 d of age				56 d of age			
Treatment[1]	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	----------g/bird----------			-------%-----	----------g/bird----------			------%-----
CS	2,592.3[ab]	4,173.1	1.60	3.21	3,817.4	6,670.5	1.77	5.13
DDGS-1,000	2,637.0[a]	4,199.9	1.60	2.38	3,801.8	6,729.5	1.76	2.98
DDGS-800	2,637.0[a]	4,248.8	1.60	3.85	3,801.4	6,642.0	1.73	3.85
DDGS-600	2,579.5[b]	4,159.0	1.61	1.92	3,798.6	6,621.9	1.72	3.33
Pooled SEM	16.68	26.83	0.006	1.219	32.21	0.015	0.019	1.357
P-value	0.033	0.186	0.434	0.653	0.896	0.527	0.302	0.697

[a,b] Means within a column with no common superscript differ significantly ($P < 0.05$).
[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 5.6 Experiment 2 carcass characteristics of progeny (d 0 to 42) from eggs collected at 26 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

Treatment[1]	Carcass, g	Dress,[2] %	Fat, g	Fat,[2] %	Breast, g	Breast,[2] %	Back Half, g	Back Half,[2] %	Wings, g	Wings,[2] %
CS	1,845.0[a]	68.5	27	1.04	604[a]	22.28	732	27.37[b]	204	7.71
DDGS-1,000	1,803.0[ab]	68.5	27	1.02	590[ab]	22.26	727	27.63[b]	206	7.83
DDGS-800	1,838.0[a]	68.9	26	0.97	598[a]	22.42	741	27.76[ab]	208	7.76
DDGS-600	1,760.0[b]	68.2	26	0.99	566[b]	21.96	732	28.13[a]	204	7.80
Pooled SEM	18.0	0.23	1.0	0.050	9.0	0.214	8.0	0.151	2.0	0.057
P-value	0.008	0.213	0.789	0.752	0.032	0.509	0.657	0.011	0.480	0.527

[a,b] Means within a column with no common superscript differ significantly ($P < 0.05$).
[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.
[2]Percentage of live weight

Table 5.7 Experiment 2 carcass characteristics of progeny (d 0 to 56) from eggs collected at 26 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

Treatment[1]	Carcass, g	Dress,[2] %	Fat, g	Fat,[2] %	Breast, g	Breast,[2] %	Back Half, g	Back Half,[2] %	Wings, g	Wings,[2] %
CS	2,768.0	71.2	54	1.42	966	25.11	1,070	27.96	296	7.62
DDGS-1,000	2,742.0	71.3	51	1.27	949	24.87	1,090	28.26	292	7.61
DDGS-800	2,738.0	71.3	47	1.22	962	24.99	1,080	28.90	290	7.64
DDGS-600	2,723.0	71.3	49	1.31	968	25.13	1,080	27.89	289	7.50
Pooled SEM	30.0	0.20	2.0	0.182	15.0	0.224	14.0	0.163	4.0	0.050
P-value	0.789	0.909	0.197	0.101	0.793	0.849	0.831	0.155	0.555	0.235

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.
[2]Percentage of live weight

Table 5.8 Experiment 2 live performance of progeny (d 0 to 28) from eggs collected at 31 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

		14 d of age				28 d of age			
Treatment[1]	Chick initial BW	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	---g/bird---	----------g/bird----------			------%-----	----------g/bird----------			------%-----
CS	39.3	371.3	425.1	1.14	1.67	1,443.1	2,068.1	1.43	2.22
DDGS-1,000	39.3	377.5	429.7	1.14	1.11	1,466.8	2,092.8	1.43	5.00
DDGS-800	39.3	359.3	403.9	1.12	5.56	1,431.5	2,033.3	1.42	6.11
DDGS-600	38.5	368.4	420.9	1.14	3.89	1,447.0	2,070.9	1.42	4.44
Pooled SEM	0.31	8.17	11.28	0.009	1.504	16.03	26.16	0.006	1.948
P-value	0.192	0.482	0.409	0.219	0.146	0.495	0.455	0.862	0.553

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 5.9 Experiment 2 live performance of progeny (d 0 to 56) from eggs collected at 31 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

	42 d of age				56 d of age			
Treatment[1]	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	----------g/bird----------			-------%-----	----------g/bird----------			------%------
CS	2,554.0	4,295.4	1.68	3.33	3,640.5	6,939.2	1.91	7.78
DDGS-1,000	2,574.8	4,318.1	1.68	5.00	3,676.9	7,001.2	1.92	8.33
DDGS-800	2,553.6	4,268.9	1.67	7.22	3,621.0	6,874.4	1.90	10.00
DDGS-600	2,536.7	4,263.2	1.67	4.44	3,632.8	6,923.9	1.92	7.22
Pooled SEM	24.70	30.25	0.010	2.035	22.71	43.96	0.011	2.366
P-value	0.883	0.683	0.733	0.518	0.765	0.425	0.312	0.829

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d

Table 5.10 Experiment 2 carcass characteristics of progeny (d 0 to 56) from eggs collected at 31 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

Treatment[1]	Carcass, g	Dress,[2] %	Fat, g	Fat,[2] %	Breast, g	Breast,[2] %	Back Half, g	Back Half,[2] %	Wings, g	Wings,[2] %
CS	2,626.0	70.1	61	1.67	881	23.66	1,060	28.49	286	7.67
DDGS-1,000	2,599.0	70.6	62	1.73	868	23.69	1,055	28.43	281	7.59
DDGS-800	2,608.0	70.5	63	1.69	866	23.33	1,065	28.73	283	7.66
DDGS-600	2,589.0	70.7	60	1.69	881	23.95	1,053	28.69	283	7.70
Pooled SEM	20.0	0.19	2.0	0.050	10.0	0.193	9.0	0.167	2.0	0.052
P-value	0.600	0.923	0.667	0.856	0.592	0.168	0.827	0.500	0.423	0.478

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d

[2]Percentage of live weight

Table 5.11 Experiment 2 live performance of progeny (d 0 to 28) from eggs collected at 36 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

		14 d of age				28 d of age			
Treatment[1]	Chick initial BW	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	--g/bird--	----------g/bird----------			------%-----	----------g/bird----------			-----%-----
CS	39.9	412.3	473.9	1.14	1.19	1,463.8	2,084.6[b]	1.42	1.78
DDGS-1,000	40.5	428.3	495.9	1.15	0.59	1,484.3	2,142.7[a]	1.43	0.59
DDGS-800	40.6	428.8	487.9	1.14	0.00	1,479.1	2,125.6[ab]	1.43	1.19
DDGS-600	38.7	426.0	490.8	1.15	0.00	1,515.7	2,155.0[a]	1.42	0.59
Pooled SEM	0.13	4.74	6.34	0.009	0.490	11.48	16.51	0.007	0.741
P-value	0.212	0.056	0.124	0.908	0.233	0.122	0.047	0.736	0.730

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 5.12 Experiment 2 live performance of progeny (d 0 to 56) from eggs collected at 36 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

	42 d of age				56 d of age			
Treatment	BW	Feed intake	FCR	Mortality	BW	Feed intake	FCR	Mortality
	----------g/bird----------			-------%-----	----------g/bird----------			-------%-----
CS	2,636.5	4,341.3	1.66	1.79	3,748.5	7,186.7	1.91	3.57
DDGS-1,000	2,635.5	4,396.4	1.67	0.59	3,731.2	7,140.3	1.92	1.19
DDGS-800	2,618.2	4,408.9	1.67	2.38	3,648.7	7,168.1	1.94	2.98
DDGS-600	2,672.4	4,434.0	1.66	1.19	3,760.6	7,186.7	1.91	1.19
Pooled SEM	20.14	36.45	0.008	1.058	31.90	67.95	0.011	1.317
P-value	0.945	0.237	0.852	0.812	0.775	0.591	0.483	0.602

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.

Table 5.13 Experiment 2 carcass characteristics of progeny (d 0 to 56) from eggs collected at 36 wk of age from Cobb 500 broiler breeder hens fed diets with various digestible lysine intake levels.

Treatment[1]	Carcass, g	Dress,[2] %	Fat, g	Fat,[2] %	Breast, g	Breast,[2] %	Back Half, g	Back Half,[2] %	Wings, g	Wings,[2] %
CS	2,740.0	72.0	54	1.463	909	23.96	1,090	28.54	287	7.55
DDGS-1,000	2,713.0	71.8	53	1.425	910	23.99	1,078	28.60	291	7.64
DDGS-800	2,705.0	72.3	57	1.542	892	23.85	1,073	28.63	288	7.64
DDGS-600	2,691.0	72.1	55	1.509	892	23.96	1,070	28.55	283	7.61
Pooled SEM	25.0	0.17	2.0	0.056	12.0	0.185	11.0	0.155	2.0	0.055
P-value	0.588	0.410	0.508	0.483	0.598	0.961	0.610	0.977	0.077	0.652

[1]Corn-Soybean meal diet: CS= 1,000 mg of dLys/hen/d; DDGS-based diets: DDGS-1,000= 1,000 mg of dLys/hen/d; DDGS-800= 800 mg dLys/hen/d; DDGS-600= 600 mg dLys/hen/d.
[2]Percentage of live weight

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CHAPTER VI

CONCLUDING STATEMENTS

Conclusions

Decreasing the dLys intake levels from 1,010 to 600 mg/hen per d from 35 to 45 wk of age improves broiler breeder reproductive performance only when feeding a semi-purified diet. The mechanism of how Lys intake negatively affects reproductive performance only when feeding a semi-purified diet still remains unclear. However, under practical conditions, it appears that commercial type diets may not need to be formulated to levels above 1,010 mg/hen per d of dLys. Furthermore, research regarding feeding lower intake levels of dLys to BB hens using only commercially available ingredients needs to be assessed.

In order to further elucidate the impact of dLys intake levels on BB hens, a study evaluated feeding decreasing levels of dLys in commercial-type diets from 24 to 42 wk of age. Results indicated that feeding low levels of dLys (600 mg/hen per day) to BB hens did not have any impact on broiler breeder performance. It appears that under practical conditions, Lys intake is possibly being fed in surplus to BB hens.

Nutrient modifications in BB diets have been shown to impact live performance, carcass characteristics, and the immune status of the chick. Therefore, two experiments evaluated the impact that reducing dLys intake in BB hens may have on progeny live performance parameters. Evaluating the progeny live performance and carcass

characteristics results from Experiments 1 and 2 were not complementary of each other. Therefore, the potential impact of dietary Lys in the breeder hen diet on progeny performance needs to be further evaluated. Also, studies should evaluate if amino acid intake levels may have a potential impact in later BB production phases and their corresponding offspring, which was not evaluated in these studies.