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Evaluation of Feeding Different Digestible Lysine Intake Levels on Semen Characteristics and Body Weight of Broiler Breeders During Pre-Peak and Peak Production

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Abstract: Because research revealing the impact of Lys on reproduction in Broiler Breeders (BB) is sparse, this study was conducted to evaluate the impact of digestible Lys (dLys) on BB semen characteristics and BW. Eighty males were caged individually from 20 to 39 wk of age. Treatment 1 and 2 diets had the same level of dLys (1,000 mg/rooster/day) in a corn-soybean meal based diet (Soy 1000) and distillers dried grains with solubles (DDGS; DDGS 1000) diet, respectively. Treatment 3, 4 and 5 diets had the inclusion of DDGS in order to titrate dLys intake levels of 850 (DDGS850), 700 (DDGS700) and 550 (DDGS550) mg/rooster/day, respectively. Body weight and semen samples were determined every 2 wk from 26 to 38 wk of age. Immediately after semen collection, samples were analyzed for semen volume, sperm viability, sperm concentration and the Sperm Quality Index (SQI). BW of roosters fed Soy 1,000 was higher than the other treatments from wk 26 through wk 38. This excess weight could be due to over estimating the energy content of DDGS resulting in diets that were not isocaloric. At 28 wk and continuing through wk 38, the percentage of dead sperm was highest in roosters fed Soy 1000. Also, at wk 38 plasma testosterone concentrations were higher for roosters fed Soy 1000. In conclusion, varying levels of dLys (1,000-550 mg/rooster/day) in a DDGS based diet does not appear to cause adverse effects on BB male semen quality during pre-peak and peak production.

Key words: lysine, DDGS, semen quality, body weight

INTRODUCTION

Nutrition is important in the pre-puberty, puberty and sexual maturity stages of Broiler Breeder (BB) male production. For example, malnutrition or over eating could lead to excess body weight loss or gain in any of these three stages of sexual maturity possibly affecting sperm production. Therefore, poor dietary management can drastically impact a rooster's fertility (Leeson and Summers, 2000). Poultry integrators practice feed restriction in BB to avoid excess weight gain (Pym and Dillon, 1974). Feed restriction is the reduction in the amount of feed a bird is allowed to eat at a given time; therefore their diet must have an adequate amount of nutrients in a small volume of feed. For example, rooster diets should meet the requirement for the 3 essential and most limiting Amino Acids (AA) which are Met, Lys and Thr in order that the rooster may receive enough protein which is essential for sperm production (Brown and McCartney, 1986).

Several trials have shown that throughout the life of the chicken, Lys is essential (Kidd *et al.*, 2004; Dozier *et al.*, 2010). The effect of Lys inclusion in the broiler diet has been studied and shown to improve production such as growth rate, immune response and breast meat yield (Corzo *et al.*, 2006; Pahm *et al.*, 2009; Dozier *et al.*, 2010). However in BB, excess dietary AA may lead to

increased muscle deposition (De Beer, 2011) resulting in obesity and poor mating ability. Also, high levels of Lys present in some ingredients such as a typical corn-soybean meal based diet may cause difficulty when formulating diets for lower levels of digestible Lys (dLys). However, a diet with the inclusion of Distiller Dried Grains with Solubles (DDGS) may allow for diet formulation with lower levels of dLys because there is a low concentration of dLys in DDGS. For example, Mejia *et al.* (2012b) used varying levels of dLys at 1,000, 800 and 600 mg/hen/day in a DDGS based diet and reported that fertility parameters of BB hens were not affected.

When using Cobb 500 BB males during post peak production, between 45 to 49 wk of age, Obi *et al.* (2012) reported that semen volume, sperm concentration and the Sperm Quality Index (SQI) were similar when roosters were fed dLys varying from 1,000 to 550 mg/rooster/day in a DDGS based diet. However, roosters fed 550 mg/rooster/day dLys in the DDGS based diet showed a higher percentage of dead sperm when compared to the other diets (Obi *et al.*, 2012). Because dLys impacted the percentage of dead sperm in post peak BB males, it was suggested that semen characteristics should be examined during pre-peak and peak production of BB males. This would determine if this dietary effect also exists earlier in sperm production.

Therefore, this study was conducted during the pre-peak and peak period of BB reproduction to evaluate the effect of dLys on BB male semen quality and BW.

MATERIALS AND METHODS

Bird husbandry and dietary treatment: For this trial, 80 Cobb MX BB males (20 wk of age) were obtained from a local poultry integrator. The feed restriction program utilized for this trial was recommended by the primary BB company (136 g/rooster/day). All birds were individually weighed at 2 wk intervals throughout the trial beginning at 20 wk of age. Feed was weighed and added on a daily basis to individual feed troughs to ensure that feed restriction was strictly followed. The experiment lasted for 19 wk (20 to 39 wk of age).

Males were randomly assigned to individual cages (cage size: 60*50*40 cm) to facilitate semen collection and placed on 5 different dietary treatments immediately after being caged (16 rooster/dietary treatment). Treatment 1 was a typical corn-soybean meal based diet with 1,000 mg dLys/rooster/day (CP 15.2%, AME 2, 860 kcal/kg, Ca 3.0 and dLys 0.74; Table 1). Treatment 2 was prepared using the same level of dLys as treatment 1 but with the inclusion of 25% DDGS (CP 14.5% and dLys 0.77). Treatments 3, 4 and 5 were prepared using the same amount of DDGS as in treatment 2 but with inclusions of 850, 700 and 550 mg of dLys/rooster/day (Obi *et al.*, 2012) (Table 1). All birds were treated in accordance with the Guide for Care and Use of

Table 1: Composition of treatment diets (% , as-is basis) fed during pre-peak and peak semen production

Ingredients	Corn-soybean meal diet ¹	DDGS diet ²
Corn	68.91	57.33
Distillers dried grains w/solubles	-	25
Poultry oil	0.13	0.05
Soybean meal-48% CP	21.70	7.92
Calcium carbonate	6.63	6.97
Dicalcium phosphate	1.85	1.44
Salt, plain (NaCl)	0.33	0.08
Sodium bicarbonate	0.11	0.11
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/mineral	0.06	0.06
Mineral premix	0.08	0.08
L-Lysine	0.02	0.42 ³
Calculated composition		
Dig-Lysine	0.74	0.77
Crude protein	15.22	14.50
Calcium	3.0	3.0
AME (kcal/kg)	2,860	2,860

¹Diet for 1,000 mg of digestible lysine (control diet).

²Distiller dried grain with soluble with 1,000mg of digestible lysine (diet 2).

³Diet 2 had inclusion of L-Lys. at 0.42% without sand; diet 3 had inclusion of L-Lys. at 0.28% and 0.14% sand; diet 4 had inclusion of L-Lys. at 0.14% and 0.28% sand; diet 5 without L-Lys and 0.42% sand.

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Semen analysis: When birds were 26 wk of age, semen samples were collected from individual males by abdominal massage (Burrows and Quinn, 1937) using a single semen collector (Cecil and Bakst, 1985). Semen samples were immediately analyzed for the following semen characteristics: Semen volume, sperm concentration, percentage of dead sperm and the Sperm Quality Index (SQI). Semen volume was measured using a graduated vial (Christensen, 1995) and sperm concentration was evaluated using a Micro-Reader 1 (IMV International, Maple Grove, MN; King and Donoghue, 2000). For each male, 2 readings were used to determine sperm concentration.

The fluorometric method of Bilgili and Renden (1984) was used to evaluate the percentage of dead spermatozoa in the semen sample. To determine overall semen quality, neat semen was diluted 10-fold and evaluated using a Sperm Quality Analyzer® (Medical Electronic Systems Ltd., Migdal Haemek, Israel) which determines the SQI (McDaniel *et al.*, 1998), a measure of sperm motility. For both the percentage of dead sperm and the SQI, 2 readings were obtained for each determination.

Hormone analysis: To determine if dLys had any effect on reproductive hormones, plasma from each male was analyzed for testosterone and estradiol. At the end of the experiment (39 wk of age), blood samples were collected from the wing vein of each male and placed into a lithium heparin tube and centrifuged immediately to separate blood components (Straková *et al.*, 2002). The plasma was collected into eppendorf tubes and frozen at -20°C. The blood samples were later thawed and analyzed using chicken testosterone and estradiol ELISA kits (Cusabio Biotech®). After blood collection, all birds were euthanized by cervical dislocation. Testes, liver and the breast muscle of each male was collected and weighed.

Statistical analysis: Data in this experiment were evaluated using a randomized complete block design with a split plot over bird age. Individually caged birds were the experimental units and area of the house was the blocking factor. All data were analyzed using the Proc GLM option of SAS (2010). Treatment means were separated using Fisher's protected LSD at P<0.05.

RESULTS AND DISCUSSION

In managing BB, several factors have been shown to affect reproductive performance and semen quality, including nutrition (Wilson *et al.*, 1979). Nutrition is very important during the puberty, peak and post-peak stages of BB males (Leeson and Summers, 2000). An

excessive or reduced feed intake at these stages will affect semen quality (Wilson *et al.*, 1979). For example, Hocking and Bernard (1997) reported decreased sperm concentration in caged BB males that were fed 16% Crude Protein (CP) as compared to 12% CP. Buckner and Savage (1986) reported that no differences were observed in semen volume with 9% CP inclusion in the diet when compared with 7% CP.

AA are the building blocks of protein and can be effectively added into the diet to supplement for protein requirements (Lopez and Leeson, 1995). In broiler production, AA, especially Lys, have been reported to affect BW (Dozier *et al.*, 2009), optimum growth and yield of marketable meat products (Corzo *et al.*, 2006). In BB hens, Novak *et al.* (2006) reported that egg production could be maintained if CP intake is reduced from 18.9 to 17.0 g/hen/day with supplemental Met, Lys, Thr and Trp. Mejia *et al.* (2012a) reported that the inclusion of dLys at 600 mg/hen/day in a semi-purified diet improved egg production as compared to hens fed 1,010 mg dLys/hen/day. Furthermore, Mejia *et al.* (2012b) reported that egg production and fertility were not affected when BB hens were fed dLys intake levels of 1,000, 800 or 600 mg/hen/day in a DDGS based diet. Although, not much research has been conducted studying the effect of dietary Lys or DDGS on the fertility in BB males, Obi *et al.* (2012) reported that semen volume, sperm concentration and the SQI was not affected when roosters were fed dLys intakes ranging from 1,000 to 550 mg/rooster/day in a DDGS based diet.

In the current study, roosters that were fed a corn-soybean meal based diet with 1,000 mg dLys had a higher BW in comparison to the roosters fed the DDGS based diet with the same level of dLys (1,000 mg/rooster/day; Fig. 1). Roosters fed the DDGS1000 BW leveled off at 32 wk of age and then started a minimal decline until the end of the experiment. However, at 38 wk of age (Fig. 2), this dietary treatment yielded BW which was close to the primary breeder recommended BW for Cobb MX males. In addition, roosters fed the corn-soybean meal based diet with 1,000 mg of dLys had a greater BW than the other roosters during every week of the study beginning at 26 wk of age. Attia *et al.* (1995) reported an increase in BW of caged males with an increase in energy intake. Also, caged males are not as active as when they are housed with hens and continuously mating hens, therefore burning energy. In this current study, increased BW was possibly a result of an overestimation of metabolizable energy in the DDGS that was used in formulating the diet which yielded diets that were not isocaloric. The effect of BW was also evident for the percentage of breast weight (Fig. 3). The breast meat weight was greater for the roosters fed the corn-soybean meal based diet (1,000 mg dLys/rooster/day) when compared to males from the DDGS 1,000 and DDGS 550 dietary treatments.

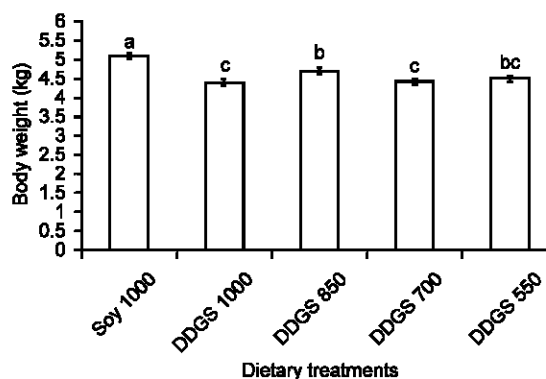


Fig. 1: Mean body weight during pre-peak and peak semen production. Each bar represents the average body weight for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles based diet with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine.

^{a-c}Means not sharing a common superscript differ (P<0.05; SEM = 0.085)

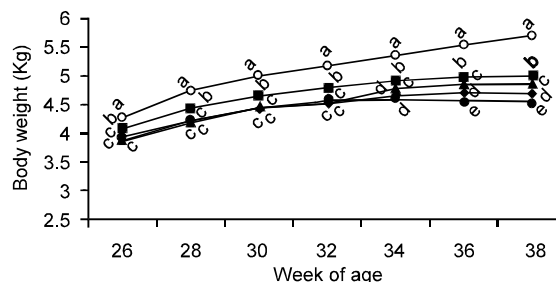


Fig. 2: Treatment by week interaction for body weight during pre-peak and peak semen production. Individual males (26-38 wk of age n = 16 roosters per treatment). Dietary treatments were Soy 1000 (○)= Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles with an inclusion of 1000 (●), 850 (■), 700 (◆) and 550 (▲) mg of digestible lysine.

^{a-e}Means not sharing a common superscript differ at the same age (P<0.05)

Roosters fed DDGS 1,000 mg dLys/rooster/day exhibited a higher percentage of liver weight relative to BW (Fig. 4) in comparison to the percentage liver weight of the roosters fed Soy 1000. However, the percentage liver weight relative to BW across treatments (Fig. 4) was similar for roosters fed Soy 1000 and DDGS 850, 700 and 550 mg dLys/rooster/day as opposed to those fed DDGS 1000. Although, the percentage of liver weight was greater for the DDGS 1000 dietary treatment, the

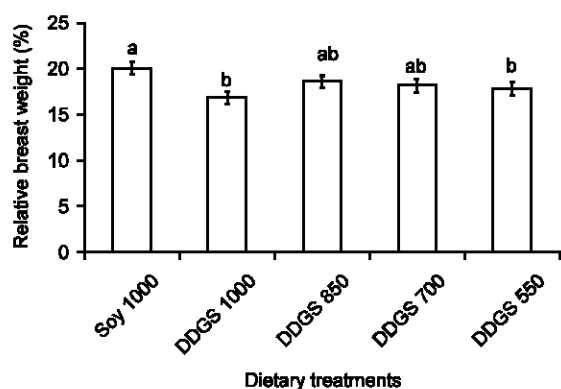


Fig. 3: Mean breast weight relative to body weight at 39 wk of age for each dietary treatment. Each bar represents the average breast weight relative to body weight for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine.
^{a-b}Means not sharing a common superscript differ (P<0.05; SEM = 0.70)

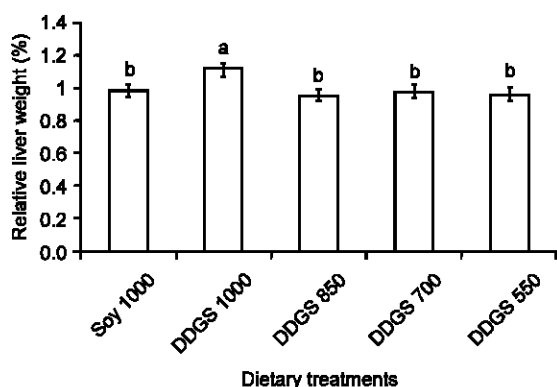


Fig. 4: Mean percentage liver weight relative to body weight at 39 wk of age for each dietary treatment. Each bar represents the average percentage liver weight relative to body weight for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine.
^{a-b}Means not sharing a common superscript differ (P<0.05; SEM = 0.04)

liver appeared normal in color and texture. Carew *et al.* (2005) reported that in growing chicks true liver weight significantly declined in Lys deficient chicks as

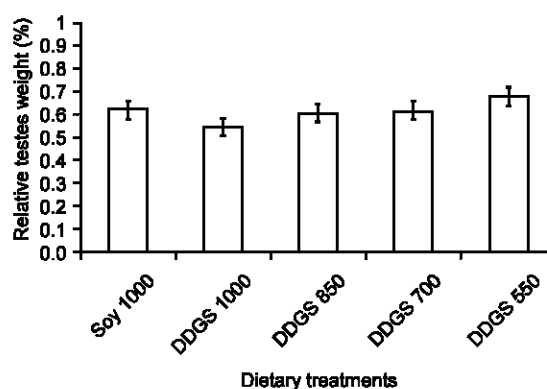


Fig. 5: Mean testes weight relative to body weight at 39 wk of age for each dietary treatment. Each bar represents the average testes weight relative to body weight for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine (P = 0.26; SEM = 0.04)

compared to chicks fed adequate Lys intake. However, when the liver weight was expressed as a percentage of BW, the differences in true liver weight disappeared. They suggested that the difference was not due to Lys deficiency but due to smaller body size in the Lys deficient chicks. As mentioned earlier in the current study, males fed the DDGS 1000 BW leveled off and started to decline beginning at 32 wk of age until the end of the trial. Because of this decline in BW, yet possibly not in liver weight, the percentage of liver weight in relation to the BW was greater for the males fed the DDGS 1000 diet.

Percentage of testes weight (Fig. 5) was similar for roosters fed a corn-soybean meal based diet with 1,000 mg dLys and those fed DDGS based diets with varying levels of dLys. Zhang *et al.* (1999) reported that testes weight was greater in roosters fed 16% CP than those fed 12% at 12 wk of age. However, Wilson *et al.* (1987) observed no differences in testes weight for roosters fed 12% or 16% CP at 53 wk of age. It appears that dLys or CP may not affect testes weight after sexual maturity because at this stage rapid growth of the testes has ceased in BB males. Wilson *et al.* (1988) reported a positive correlation between testes weight and spermatozoa number in BB males. This relationship is consistent with the present study because both testes weight and sperm concentration were not affected by dietary treatment.

During sexual maturity, testosterone concentration increases while estradiol concentration steadily declines in BB males (Weil *et al.*, 1999). Sharpe *et al.*

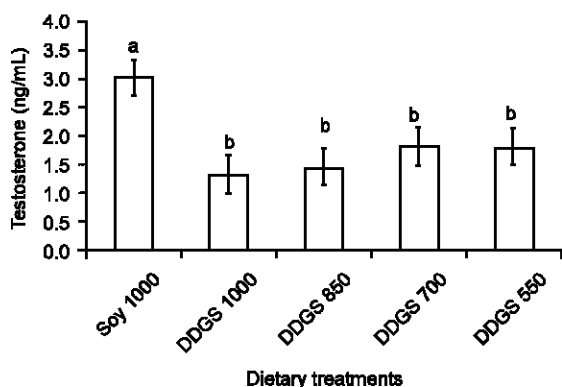


Fig. 6: Plasma testosterone concentrations at 39 wk of age for each dietary treatment. Each bar represents the average testosterone for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine. ^{a-b}Means not sharing a common superscript differ (P<0.05; SEM = 0.32)

(1988) reported that testosterone is necessary for spermatogenesis. However, testosterone concentration has been shown to decline after 30 wk of age in caged BB males (Sexton *et al.*, 1989b). In the present study, plasma testosterone concentration for the roosters fed the Soy 1000 diet were higher when compared to roosters fed the other dietary treatments (Fig. 6). The roosters fed Soy 1000 mg dLys which had the highest BW, also had the highest plasma testosterone concentration. Sexton *et al.* (1989a) reported an increase in testosterone concentration with an increase in BW of caged males. It could therefore be inferred that the elevated plasma testosterone concentration is a result of increased BW. Plasma estradiol concentrations (Fig. 7) were similar for roosters fed a corn-soybean meal based diet with 1,000 mg dLys and those fed DDGS based diets with dLys levels ranging from 1,000 to 550 mg/rooster/day. As BB males begin to pass peak production, the need for estradiol becomes minimal. Weil *et al.* (1999) reported that as fertility in BB males eventually declines with age, estradiol concentration remains constant while the testosterone concentration declines.

Semen characteristics such as semen volume (Fig. 8), sperm concentration (Fig. 9) and the SQI (Fig. 10) of roosters fed the corn-soybean meal based diet with 1,000 mg dLys/rooster/day were similar to the roosters fed the corn-DDGS based diet with the same level of dLys. Also, the inclusion of dLys varying from 850 to 550 mg/rooster/day in a DDGS based diet yielded similar

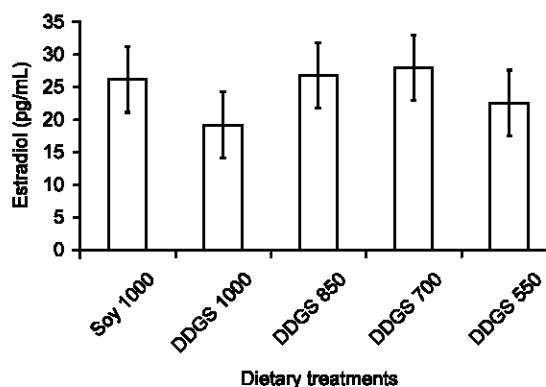


Fig. 7: Plasma estradiol concentrations at 39 wk of age for each dietary treatment. Each bar represents the average plasma estradiol for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine (P = 0.68; SEM = 5.01)

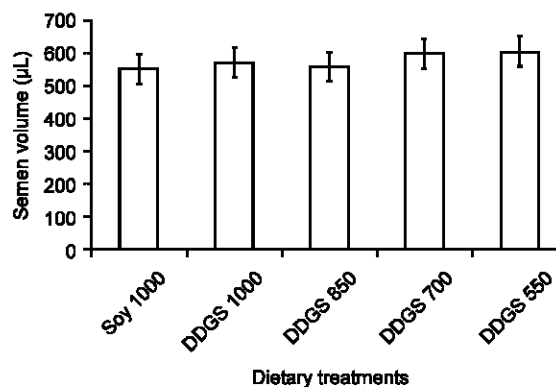


Fig. 8: Mean semen volume during pre-peak and peak semen production. Each bar represents the average semen volume for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine. Semen was collected once every week from 26, 28, 30, 32, 34, 36 and 38 weeks of age (P = 0.87; SEM = 45.02)

results for semen volume, sperm concentration and the SQI. These results are consistent with a previous trial by Obi *et al.* (2012) where semen volume, sperm concentration and the SQI were similar when roosters between 41 to 49 wk of age were fed the same amounts of dLys in a DDGS based diet. In addition, Wilson *et al.*

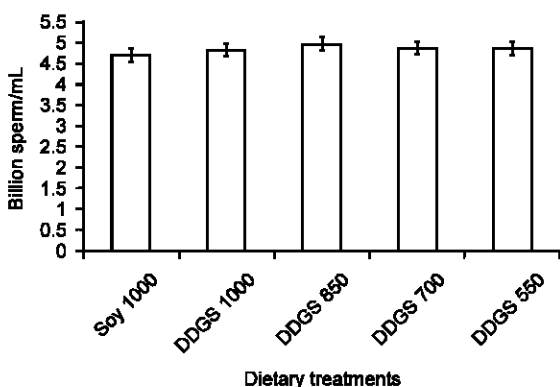


Fig. 9: Mean sperm concentration during pre-peak and peak semen production. Each bar represents the average sperm concentration for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine. Semen was collected once every week from 26, 28, 30, 32, 34, 36 and 38 weeks of age (P = 0.77; SEM = 0.16)

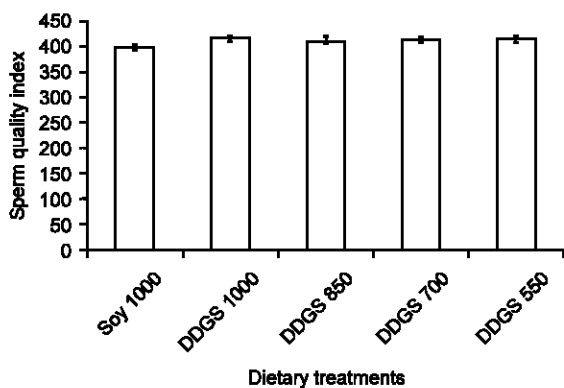


Fig. 10: Mean sperm quality index during pre-peak and peak semen production. Each bar represents the average sperm quality index for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine. Semen was collected once every week from 26, 28, 30, 32, 34, 36 and 38 weeks of age (P = 0.39; SEM = 5.93)

(1988) reported a positive correlation between testes weight and spermatozoa number in BB males and this report is consistent with the present study because both

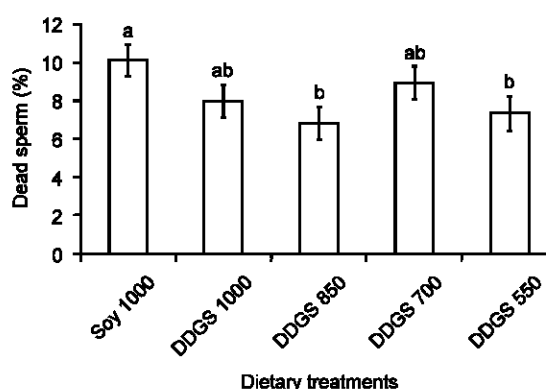


Fig. 11: Mean percentage of dead sperm during pre-peak and peak semen production. Each bar represents the average percentage of dead sperm for each dietary treatment (n = 16 roosters/treatment). Dietary treatments were Soy 1000 = Corn-soybean meal based diet with 1000 mg inclusion of digestible lysine (control diet). Other diets included 25% Distiller Dried Grains with Solubles (DDGS) with an inclusion of 1000, 850, 700 and 550 mg of digestible lysine. Semen was collected once every two week from 26, 28, 30, 32, 34, 36 and 38 weeks of age.

^{a-b}Means not sharing a common superscript differ (P<0.05; SEM = 0.86)

testes weight and sperm concentration was not affected by dietary treatment.

In this present study, roosters fed the Soy 1000 diet had the greatest percentage of dead sperm (Fig. 11). In contrast, Obi *et al.* (2012) reported a higher percentage of dead sperm in roosters fed 550 mg dLys/rooster/day as opposed to those fed a soy-bean meal based diet with 1,000 mg dLys or 1,000, 850 and 700 mg dLys in a DDGS based diet. Previous studies have shown that excess fleshing in roosters is detrimental to mating behaviors, libido (Wilson *et al.*, 1979; Leeson and Summers, 2000) and fertility (Hocking and Duff, 1989). Similarly, in turkeys, Alkan *et al.* (2002) reported a positive correlation between BW and abnormal spermatozoa, whereas a negative correlation of BW with sperm concentration and motility was found. The negative effect of high BW on fertility in caged males has also been demonstrated (McDaniel *et al.*, 1981; Yu *et al.*, 1992; Goerzen *et al.*, 1996). In the present study, it appears that roosters with the highest BW also had the greatest percentage of dead sperm (data not shown). Therefore, the increase in the percentage of dead sperm observed in the roosters fed the Soy 1000 diet could most logically be explained by the influence of the increased BW. In addition, Hocking and Bernard (2000) suggested that BW control is an important factor in maintaining high fertility in BB flocks.

Conclusion: Varying levels of dLys (1,000-550 mg/rooster/day) in a DDGS based diet does not appear to cause adverse effects on BB male semen quality during pre-peak and peak production. However, excess BW gain could increase the percentage of dead sperm and should be considered during BB male management for optimum fertilization or reproduction.

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