FEEDING SUPPLEMENTATION AND FEEDING PROGRAM IMPACTS ON BROILER BREEDER REPRODUCTION

by

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(Under the Direction of Jeanna L. Wilson)

ABSTRACT

Genetic selection for increased growth rate in our broilers makes feed restriction programs for broiler breeders essential for managing body weight, flock uniformity, and reproductive performance. Ensuring that breeders achieve and maintain their proper body weight is conducive to good health and reproductive efficiency. However, due to the high level of feed restriction welfare issues such as frustration and hunger are prominent. Qualitative feed restriction can be used to mitigate these behaviors as well as limit growth rate. Whole oats, wheat middling's, and large particles of corn were used to make a high fiber and bulky diet so that it could be fed on an every-day (ED) or skip-a-day (SAD) basis. The use of this diet on an ED basis shows an improvement in performance with increased body weight, improved uniformity, improved egg weight, improved eggshell quality, and earlier onset of lay. These results suggest that ED feeding can improve broiler breeder reproductive parameters.

Roosters play an essential role in flock fertility and their nutritional needs must be optimized for longevity of flock fertility. In the US, roosters are commonly fed a hen diet that is over formulated for their nutritional needs. Utilization of a diet tailored to a

rooster's nutritional needs with lower levels of calcium and protein improves early in life semen production as well as reduces microanatomy lesions in the kidneys. Supplementing these tailored diets with omega-3 fatty acids or organic selenium can further improve male reproduction parameters by increasing flock fertility and semen quality. The results suggest that the utilization of diets tailored to a rooster's nutritional needs then supplemented with an omega-3 fatty acid or organic selenium product can improve male reproduction parameters and flock fertility.

INDEX WORDS: Broiler breeders, Whole oats, Every-day feeding, Fertility, Semen quality, Rooster diet

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DEDICATION

I dedicate this work to my husband and the Gosnell family: Thank you all for your unconditional love and support in every step of the way. To my husband, Jedidiah Clark who always encouraged me to do my very best and was always willing to help me however he could. To Fran Gosnell, who has been a role model for perseverance and unconditional love. To John Gosnell, who has been a role model for hard work and dedication. To the rest of the Gosnell family, who always supported me and knew how to make me smile. I love each of you very much.

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CHAPTER 1

INTRODUCTION

Due to the success of genetic selection for improved growth rate and feed conversion in broilers the need for feed restriction in broiler breeders is essential. If broiler breeders are fed *ad libitum,* they will over consume their nutrient requirements for growth and maintenance. This leads to overweight birds that can drastically impact laying performance, fertility, lameness, and death (de Jong and Guémené, 2011; van Krimpen and de Jong, 2014). Ensuring proper health and wellness of broiler breeders through feed restriction programs assists in maintaining optimal levels of reproductive performance as well as bird livability. Broiler breeders are responsible for providing fertile eggs to hatcheries to produce broilers for meat consumption. On average, 29,000 parent stock broiler breeder hens are responsible for 4 million broilers that will enter the food supply (Cobb-Vantress, 2022). This makes maximizing broiler breeder performance through management and nutrition essential for our food supply in the US.

Management of body weight can be difficult due to the increase in voluntary feed intake in broiler breeders due to genetic selection (Richards et al., 2010). Researchers have examined many different dietary manipulations to reduce hunger, optimize reproduction, and improve welfare in broiler breeders (Zuidhof et al., 1995; Savory and Lariviére, 2000; Sanilands et al., 2005; Aranibar et al., 2020). Due to the negative correlation between growth rate and

reproductive competence (Siegel and Dunnington, 1985), understanding how to appropriately use management tools such as feeding programs and dietary manipulations to limit growth rate and optimize reproductive performance is crucial for broiler breeder management.

Primary breeders have guides that assist in determining nutrient requirements, feed allocation, and target body weights to maximize reproduction and bird health. However, due to genetic improvements, the level of feed restriction is very high. Commercially applied feeding programs restrict feed intake of broiler breeders during rearing from 25-55% of the intake of *ad libitum* fed broiler breeder at the same age (Savory et al., 1996; Renema et al., 2007). This level of feed restriction has lessened reproductive dysfunctions due to overweight birds such as reduced egg production in the females (Robinson et al., 1991; van der Klein et al., 2018) and incomplete copulations for males and females (Hocking and Duff, 1989). These findings suggest that controlling body weight will improve flock fertility as well as the total number of fertile eggs. Despite these reproductive improvements, it has been well documented that this level of feed restriction causes feeding frustration, chronic hunger, and polydipsia (Savory and Maros, 1993; Hocking et al., 2001; de Jong et al., 2002; Sandilands et al., 2005; D'Eath et al., 2009; Dixon et al., 2014).

The traditional use of skip-a-day (SAD) feeding to limit body weight in broiler breeder pullets has proven to be effective (Bartov et al., 1988; de Beer and Coon, 2007). SAD feeding is combining feed allotment of 2 d and feeding them one day and fasting the next. Since 2 d worth of feed is presented to the birds every other day, it increases the total volume of feed on each feed day. This allows for less feed aggressive birds to receive adequate amounts of feed when the more feed aggressive slow their feed intake or leave the feeder for water (Hadinia et al., 2018). However, SAD feeding also causes birds to be less efficient with feed due to inconsistency in

nutrient supplies (de Beer et al., 2007; Zuidhof et al., 2015) as well as compromising welfare (Tolkamp et al., 2005).

New management strategies are being investigated and implemented to achieve desired reproduction as well as welfare. Some of those strategies are qualitative feed restriction, alternative feed restriction, and precision feeding. Qualitative feed restriction is currently one of the main strategies aiming to increase feed volume by lowering the density of the diet. This increase in feed volume has great potential for every-day (ED) feeding. By diluting the diet using high fiber sources and increasing the volume there will be increased feeding times and increased gut fill therefore decreasing feeding frustration. Most of the fiber sources that have been researched are oat hulls, ground unmolested sugar beet pulp, sunflower meal, and soybean hulls (Hocking et al., 2004; de Jong et al., 2005; Morrisey et al., 2014; Aranibar et al., 2020). While in most of these studies birds' behavior and performance was positively impacted, the level of dilution and ingredient source that leads to peak performance has yet to be identified. More research should be conducted to evaluate different feed ingredients and performance parameters.

Changes in feeding strategies and management practices due to physiological requirements of roosters and hens has a direct impact on rooster reproduction. Rooster reproduction can be impacted due to management, environmental conditions, and nutrition. The testicles of male poultry are located within the body, unlike mammals. This makes them very susceptible to damage during sperm production and causes a reduction in fertility due to temperature fluctuation of the environment (Daghir and Jones, 2008). While environment plays a role in rooster reproductive success, the most influential is nutrition. For instance, a dietary reduction in crude protein can cause negative effects on rooster fertility and sperm quality (Buckner et al., 1986; Tyler and Bekker, 2012). In contrast, when males are fed higher levels of

crude protein, like what is in a hen diet, it can reduce sperm production and viability (McDaniel, 1985).

It is a common management program in the US to feed males a diet that is tailored to a hen's nutritional needs (*i.e.*, high protein and calcium). Some of the main reasons for this is simply logistics and convenience. If males receive the same diet as the hens, it diminishes the need for an extra male feed bin on the farm, another diet to mix at the feed mill, and a reduction of risk for feeding the incorrect feed to the males or the hens. Males that are fed a hen diet have been shown to have a comparatively earlier decline in fertility rate, requiring the utilization of a higher number of males and increased culling practices (Hocking, 1989). Excessive uptake of minerals by males through feeding a hen diet can also cause detrimental issues to a broiler breeder flock. Waldroup (1996) showed that excessive intake of minerals such as inorganic zinc, manganese, and calcium caused a decrease in male performance.

Broiler breeder male management requires attention to body weight, testes development, and sperm production. The implementation of a nutritional management plan for broiler breeder males would make increased production goals and prolonged reproductive life a more achievable goal (Goncalves et al., 2015). Assisting in this goal of improved reproductive performance is the use of a male feed supplemented with products that have the potential to improve male reproductive qualities. The use of organic trace minerals for improving reproduction in broiler breeder males is of great importance due to the essential physiological and biochemical processes they are involved in (Leeson et al., 2005) and their better absorption and utilization rate in comparison to inorganic trace minerals (Spears, 1996). Another avenue of diet supplementation is the use of omega-3 fatty acids that are important for membrane flexibility, testosterone secretion, and fertilization capacity of eggs (Gulliver et al., 2012).

Selenium (Se) is a trace mineral that is important to produce glutathione peroxidase (GPX). GPX is an antioxidant enzyme that assists in the protecting cells from oxidative damage (Moslemi and Tavanbakhsh, 2011). Sperm cells are subject to such oxidation which can limit fertilizing capacity and semen quality. Se plays a key role in ensuring there is adequate GPX production so that it can act as a free radical scavenger and limit oxidation of sperm cells (Bains and Shaw, 1997). Also, without Se there is an impairment in testis development and an increase in the production of abnormal sperm cells (Xu et al., 2015; Kothari and Chaudhari, 2016). Organic Se, such as selenomethionine (SeMet), are not toxic like the inorganic form sodium selenite and creates a stronger antioxidant reaction (Stewart et al., 1999; Sun et al., 1997).

Zinc (Zn) is a trace mineral that is important for the regulation of sex and growth hormones and the formation of various enzymes (Naz et al., 2016; Jafari et al., 2020). Most Zn is supplemented in poultry feeds in inorganic forms such as Zn Oxide (ZnO) or Zn Sulphate (ZnS) (Leeson and Summers, 1997). Due to the bioavailability of organic forms of Zn, several reproductive benefits can be seen through its supplementation in poultry diets such as increased fertility, sperm motility, and decreased apoptosis of cells (Naz et al., 2016; Abd El-Hack et al., 2017; Zhandi et al., 2020).

Docosahexaenoic acid (DHA) is and omega-3 fatty acid that has been shown to improve growth, fertility, and immunity in poultry (Lee et al., 2019). The inclusion of omega-3 fatty acids in poultry diets can be deposited into soft tissues, seminal fluid, egg yolks, and meat to increase their fatty acid profiles (Van Elswyk, 1997; Hudson and Wilson, 2003). It has been well documented that the inclusion of products that are high in omega-3 fatty acids improve fertility in broiler breeders (Kelso et al., 1997; Blesbois et al., 1997; Surai et al., 1998), however, due to

product stability of the oils that are high in omega-3's they are difficult to use on a commercial scale. The main objectives of these studies were:

1. To evaluate feed supplements and feeding programs on broiler breeder growth and reproductive performance.
2. To evaluate the impact of an every-day feeding program compared to a skip-a-day feed program on body weight uniformity, intestinal development, and reproductive performance.
3. To evaluate rooster reproductive performance when fed a diet tailored to their nutritional needs compared to a standard US hen diet which they are typically fed.
4. To evaluate the use of organic trace minerals and shelf stable omega-3 fatty acids on rooster semen quality and reproductive performance.

REFERENCES

Abd El-Hack, M. E., M. Alagawany, M. Arif, M. T. Chaudhry, M. Emam, and A. Patra. 2017. Organic or inorganic zinc in poultry nutrition: a review. World's Poult Sci J. 73:904–915.

Aranibar, C. D., C. Chen, A. J. Davis, W. I. Daley, C. Dunkley, W. K. Kim, C. Usher, A. B. Webster, and J. L. Wilson. 2020. Impact of an alternative feeding program on broiler breeder pullet behavior, performance, and plasma corticosterone. Poult. Sci. 99:829-838.

Bains, J. S., and C. A. Shaw. 1997. Neurodegenerative disorders in human: the role of glutathione in oxidative stress-mediated neuronal death. Brain Research Reviews. 25:335-358.

Bartov, I., S. Bornstein, Y. Lev, M. Pines, and J. Rosenberg. 1988. Feed restriction in broiler breeder pullets: skip-a-day versus skip-two-days. Poult. Sci. 67:809–813.

Blesbois, E., M. Lessire, I. Grasseau, J. M. Hallouis, and D. Hermier. 1997. Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. Biol Reprod. 56:1216–1220.

Bruggeman, V., O. Onagbesan, E. D'Hondt, E. Buys, M. Safri, D. Vanmontfort, L. Berghman, F. Vandesande, and E. Decuypere. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poult. Sci. 78:1424–1434.

Buckner R. E., J. A. Renden, and T. F. Savage. 1986. The effect of feeding programs on reproductive traits and selected blood chemistries of caged broiler breeder males. Poult. Sci. 65:85-91.

Cobb-Vantress. 2022. Breeder Production Pyramid. Cobb-Vantress, Siloam Springs, AR.

D'Eath, R.B., B. J. Tolkamp, I. Kyriazakis, and A. B. Lawrence. 2009. 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. Anim. Behav. 77:275–288.

Daghir, N. J., and R. Jones. 2008. Breeder and hatchery management in hot climates. Chapter 11. DAGHIR, N.J. (Ed.) Poultry Production in Hot Climate, 2nd Ed., 294-329 (CAB International, UK).

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

de Jong I. C., M. Fillerup, and H. J. Biokhuis. 2005. Effect of scattered feeding and feeding twice a day during rearing on indicators of hunger and frustration in broiler breeders. Appl. Anim. Behav. Sci. 92:61-76.

de Jong I.C., S. van Voorst, D. A. Ehlhardt, H. J. Blokhuis. 2002. Effects of restricted feeding on physiological stress parameters in growing broiler breeders. Br. Poult. Sci. 43:157–168.

de Jong, I. C., and D. Guémené. 2011. Major welfare issues in broiler breeders. World's Poult. Sci. J. 67:73-82.

Dixon, L. M., S. Brocklehurst, V. Sandilands, M. Bateson, B. J. Tolkamp, and R. B. D'Eath. 2014. Measuring motivation for appetite behavior: food restricted broiler breeder

chickens cross a water barrier to forage in an area of wood shavings without food. PLoS 9: e102322.

Goncalves, F., V. Santos, G. Farina, C. Oliveria, M. Anciuti, and F. Rutz. 2015. Nutritional aspects and reproductive management of roosters in Brazil. World's Poult. Sci. J. 71:515-522.

Gulliver, C. E., M. A. Friend, B. J. King, and E. H. Clayton. 2012. The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim. Repro. Sci. 131:9-22.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Energy partitioning by broiler breeder pullets in a skip-a-day and precision feed systems. Poult. Sci. 97:4279-4289.

Hocking P. M., and S. R. I. Duff. 1989. Musculo-skeletal lesions in adult male broiler breeder fowls and their relationships with body weight and fertility at 60 weeks of age. Br. Poult. Sci. 4:777-784.

Hocking, P. M. 1989. Effect of dietary crude protein concentration on semen yield and quality in male broiler breeder fowls. Br. Poult. Sci. 30:935-945.

Hocking, P. M., M. H. Maxwell, G. W. Robertson, and M. A. Mitchell. 2001. Welfare assessment of modified rearing programs for broiler breeders. Br. Poult. Sci. 42:424-432.

Hocking, P. M., V. Zaczek, E. K. M. Jones, and M. G MacLeod. 2004. Different concentrations and sources of dietary fibre may improve the welfare of female broiler breeders. Br. Poult. Sci. 45:9-19.

Hudson, B. P., and J. L. Wilson. 2003. Effects of dietary Menhaden oil on fertility and sperm quality of broiler breeder males. J. Appl. Poult. Res. 12(3):341-347.

Jafari, M., M. Irani, and V. Rezaeipour. 2020. Effect of different dietary zinc sources in the semen quality, testicular histology and sex hormone concentration in broiler breeder roosters. Italian J. Anim. Sci. 20:489-496.

Kelso, K., S. Cerolini, B. Speake, L. Cavalchini, and R. Noble. 1997. Effects of dietary supplementation with α-linolenic acid on the phospholipid fatty acid composition and quality of spermatozoa in cockerel from 24 to 72 weeks of age. J. Reprod. Fertil. 110:53–59.

Kothari, R. P., and A. R. Chaudhari. 2016. Zinc levels in seminal fluid in infertile males and its relation with serum free testosterone. J. Clinic. Diag. Res. DOI: 10:CC05-CC10.

Lee, S. A., N. Whenham, M. R. Bedford. 2019. Review on docosahexaenoic acid in poultry and swine nutrition: Consequences of enriched animal products on performance and health characteristics. Anim. Nutr. 5:11-21.

Leeson S., and J. D. Summers. 1997. Commercial poultry nutrition. 2nd ed. Guelph (Canada): University Books.

Leeson, S., J. Taylor-Pickard, and L. Tucker. 2005. Trace mineral requirements of poultry-validity of the NRC recommendations. Re-defining mineral nutrition. Nottingham: University Press: 107-117.

McDaniel, G. R. 1985. Separate feeders for male and female breeders. Zootech. Int. 11:54-58.

Morrisey K. L., T. Widowshi, S. Leeson, V. Sandilands, A. Arnone, and S. Torrey. 2014. The effect of dietary alterations during rearing on growth, productivity, and behavior in broiler breeder females. Poult. Sci. 93:285-295.

Moslemi, M. K., and S. Tavanbakhsh. 2011. Selenium-vitamin E supplementation in infertile men: effects in semen parameters and pregnancy rate. Int. J. Gen. Med. 4:99-104.

Naz, S., M. Idris, M. A. Khalique, I. A. Zia-Ur-Rahman Alhidary. M. M. Abdelraham, and S. Ahmad. 2016. The activity and use of zinc in poultry diets. World's Poult. Sci. J. 1:159-167.

Renema, R. A., M. E. Rustard, and F. E. Robinson. 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. Worlds Poult. Sci. J. 63:457–472.

Richards M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMutry. 2010. Feed intake regulation for the female broiler breeder: in theory and in practice. J. Appl. Poult. Res. 19:182-193.

Robinson, F. E., N. A. Robinson, and T. A. Scott. 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler breeder hens. Can. J. Anim. Sci. 71:549-556.

Sandilands, V., B. J. Tolkamp, and I. Kyriazakis. 2005. Behavior of food restricted broilers during rearing and lay- effects of an alternative feed method. Physiol. Behav. 85:115-123.

Savory, C. J., and J-M. Lariviere. 2000. Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. Appl. Anim. Behav. Sci. 69:135–147.

Savory, C. J., and K. Maros. 1993. Influence of degree of food restriction, age and time of day on behavior of broiler breeder chickens. Behav. Process. 29:179-189.

Savory, C. J., P. M. Hocking, J. S. Mann, and M. H. Maxwell. 1996. Is broiler breeder welfare improved by using qualitative rather than quantitative food restriction to limit growth rate? Animal Welfare 5:105-127.

Siegel, P. B., and E. A. Dunnington, 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in: Poultry Genetics and Breeding. W. G. Hill, J. M. Manson, and D. Hewitt, ed. British Poultry Science, Longman Group, Harrow, Essex, England.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Tech. 58:151-163.

Stewart, M. S., J. E. Spallholz, K. H. Neldner, K. H. Pence. 1999. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Rad. Bio. Med. 26:42-48.

Sun, E., H. Xu, D. Wen, P. Zuo, J. Zhou, J. Wang. 1997. Inhibition of lipid peroxidation. Biological Trace Element Research. 59:87-92.

Surai, P. F., E. Blesbois, I. Grasseau, T. Chalah, J. P. Brillard, and G. J. Wishart. 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 120:527–533.

Tolkamp, B. J., V. Sandiland, and I. Kyriazakis. 2005. Effects of qualitative feed restriction during rearing on the performance of broiler breeders during rearing and lay. Poult. Sci. 84:1286–1293.

Tyler, N. C., and H. A. Bekker. 2012. The effect of dietary crude protein on the fertility of male broiler breeders. S. Afr. J. Anim. Sci. 42:304-309.

van der Klein S.A.S., G. Y. Bédécarrats, and M. J. Zuidhof. 2018. The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight. Poult. Sci. 97:3286–3294.

Van Elswyk, M.E. 1997. Comparison of n-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: A review. Br. J. Nutr. 78 (1): 61-69.

van Krimpen, M. M., and I. C. de Jong. 2014. Impact of nutrition on welfare aspects of broiler breeder flocks. World's Poult. Sci. J. 70:139-150.

Waldroup, P. 1996. Bioassays remain necessary to estimate phosphorus, calcium bioavailability. Feedstuffs 68: 13-20.

Xu, X., X. Chen, H. Hu, A. B. Dailey, and B. D. Taylor. 2015. Current opinion on the role of testosterone in the development of prostate cancer: a dynamic model. BMC cancer 15:806.

Zhandi, M., A. Talebnia-Chalanbar, A. Towhidi, M. Sharafi, A. R. Yousefi, and S. M. H. Hussaini. 2020. The effect of zinc oxide on rooster semen cryopreservation. Br. Poult. Sci. 61:188–194.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

Zuidhof, M. J., F. E. Robinson, J. J. R. Feddes, R. T. Hardin, J. L. Wilson, R. I. Mckay, and M. Newcombe. 1995. The effects of nutrient dilution on the well-being and performance of female broiler breeders. Poult. Sci. 74:441-456.

CHAPTER 2

LITERATURE REVIEW

For more than five decades the poultry industry has been one of the most successful sectors of production agriculture. This great success would not be possible without the improvement in genetic selection and feed manufacturing for production poultry. While these improvements in genetics and feed manufacturing have allowed for a cheap and sustainable protein source for consumers, the issues of animal welfare have become of great concern. Due to the increased growth rate and yield in broilers brought on by genetic selection, this can lead to excessive weight gain and health issues such as lameness, poor reproductive performance, and ruptured tendons in broiler breeders (Hocking and Duff, 1989 and de Jong and Guémené, 2011).

Feed restriction of broiler breeders is necessary to manage body weight (BW) to maintain good health and reproductive competency. However, the feed allocation is severely restricted when compared to what they would typically consume *ad libitum.* Feed restriction can be approximately 33-50% of their *ad libitum* consumption during rearing (Savory and Kostal, 1996; de Jong et al., 2002). This can lead to behavioral changes in the bird that indicate stress, frustration, boredom, and hunger (de Jong and Jones, 2006; Arrazola et al., 2019; Aranibar et al., 2020).

Excess Body Weight and Feed Restriction of Males

Excessive weight gain and breast muscle deposition in broiler breeder males has impactful influences on flock fertility. Broiler breeders rely on natural mating and complete copulations in order to obtain fertile eggs. A single broiler breeder male can be responsible for mating a dozen hens. Therefore, his impact on fertility can be critical. When broiler breeder males become overweight and over fleshed it can become physically impossible for them to complete a copulation with a female (Hocking and Duff, 1989). Underweight broiler breeder males can also dramatically influence flock fertility. These underweight males typically do not achieve proper BW which impacts testicular development and spermatogenesis (McDaniel et al., 1981). BW can also influence mating behaviors that will directly influence flock fertility as well. There is a negative relationship between BW and mating activity (Burke and Mauldin, 1985). This decrease in mating activity directly affects the flock's fertility. Heavy males have an increase in semen volume and higher spermatozoa levels early in production (Sexton et al., 1989), but in contrast, excessive weight can lead to a severe decline in fertility later in life as well as a reduction in testes size (Robinson and Wilson, 1996). Severe feed restriction of males can decrease semen volume, semen concentration, and fertility (Parker and McSpadden, 1943) as well as decreased testes size (McCartney and Brown, 1980). Feed restriction of male broiler breeders to achieve fixed body weight targets may be counterproductive because the nutrient requirements for both growth and reproductive function of modern genotypes may not be met under such circumstances (Romero-Sanchez et al., 2007).

Controlling the BW and male's overall body conformation can assist in more completed copulations shown through cloacal contact (Bilcik et al., 2005). These researchers (Bilcik et al., 2005; Fragoso et al., 2013) reported that heavier males increased mating frequency yet were not

able to complete the matings. It can be concluded that while an increase in BW can assist in spermatozoa concentration and semen volume earlier in life (Sexton et al., 1989), the excess BW can influence their conformation in such a way that it prevents successful copulation. This excessive weight can cause welfare issues such as lameness and ruptured tendons and ligaments (Hocking and Duff, 1989). These physical impairments can also create a scenario where males simply become unwilling or unable to mate. Heavier males also have an increased frequency of forced matings (Bilcik and Estevez, 2005) which can lead to injury of the hens that will encourage the hen to become unreceptive and decrease flock fertility overtime (Jones et al., 2001).

Feed restriction of broiler breeder males is a successful and common strategy to manage BW to assist in optimizing reproductive performance (Brown and McCartney, 1983; Buckner et al., 1986; Vaughters et al., 1987). While feed restriction of these males can decrease overall size when compared to full fed males (Robinson and Wilson, 1996) it can be done in such a way that there are no negative impacts on fertility and hatchability (Buckner et al., 1986). When feed restriction is used for males, it can reduce the frame size and BW (Vaughters et al., 1987) but improve the maturation of the reproductive tract (Scogin et al., 1980). This suppression is beneficial to increase the maturity of the reproductive tract prior to photo stimulation due to the slower growth rate (Scogin et al., 1980). These authors suggest feed restriction actually improves the development of the reproductive tract.

In comparison to females, the research on feed restricting broiler breeder males is limited (Borges et al., 2006). However, while these males only comprise approximately 10% of our flocks, they represent 50% of the offspring genetics (Silveira et al., 2014). This means understanding the importance of their feeding programs and how the programs impact males are

critical to the poultry production process. The main overarching problem for these broiler breeders is that fact that fertility declines dramatically during late reproduction (Creel et al., 1990; Walsh and Brake, 1997). This problem has been linked to a reduction in mating frequency and efficiency due to excessive weight gain (Duncan et al., 1990; Hocking 1990; Hocking and Bernard, 2000). The use of a feed restriction program is a viable method to manage BW of broiler breeder males while positively impacting reproduction, fertility, and animal welfare.

Excess Body Weight and Restriction of Females

A paradox in the selection for broiler breeder females is the increase pressure for fast growth rate and a high rate of egg production (Renema and Robinson 2004). Selecting for increased growth rate, leads to overweight females that have reduced egg production, fertility, ovulation frequency, as well as egg quality. Wilson and Harms (1986) showed that overweight females that had been fed *ad libitum* peaked early in egg production then rapidly declined when compared to females whose body weight had been managed via feed restriction. This also agrees with the findings of Robinson and Wilson (1996) where heavier females had decreased egg production. Robinson and Wilson (1996) attributed these findings to the body's lack of ability to regulate the follicle maturation leading to multiple-yolked eggs and sporadic laying problems.

Many factors are known to influence flock fertility and one of those would be female BW. McDaniel et al. (1981) showed a negative correlation between BW and fertility, hatchability, and egg production. It has also been shown that the duration of fertility is decreased when females are overweight (Goerzen et al., 1996). The decline in the flock's duration of fertility can be attributed to ovarian regression and hormonal regulation (Renema et al., 1999). This also leads to the formation of excess large yellow follicles which can result in an increase in unsettable eggs (Hocking et al., 1987). Renema et al. (1999) also showed that excessive body

weight can cause early maturation prior to photostimulation. This could have negative impacts on the lay cycle of broiler breeder hens.

Increased BW in females has been shown to have a positive correlation with egg size yet a negative correlation with shell quality (McDaniel et al., 1981). While an increase in egg size results in a larger chick, a decrease in the shell quality can impact chick quality and growth (Wilson, 1991). Maternal nutritional factors can impact egg quality, embryonic development, and chick quality. Dietary protein levels and energy can increase egg and chick weights (Enting et al., 2007a). Females that receive excess nutrients are overweight and have higher rates of embryonic mortality and loss of eggshell calcium (Robinson et al., 1993). This impact on shell quality would directly impact the embryonic mortality due to the difficulty in regulating temperature and moisture loss in the incubator. In a recent study, Bowling et al. (2018) correlated increased maternal feed restriction to greater corticosterone levels in chicks as well as lower 6 wk body weight of male offspring. This research indicates that while feed restriction is critical it can have a lasting negative impact on offspring. Humphreys (2020) suggests that females 21% above recommended BW guidelines will produce increased broiler weights by 3.9 to 4.1%. While this research shows increased profits from broiler production due to increased growth, it also shows an increase in feed costs for the hens. Other research suggests that birds carrying excess weight suffer from physical issues such as lameness and ruptured tendons as well as poor reproductive efficiency (Hocking and Duff, 1989 and de Jong and Guémené, 2011).

Females must be feed restricted to control BW for production efficiency as well as welfare competency. Feed restriction must be started during the rearing period for the females and continued until flock termination. The allocation of the feed is restricted to approximately one-third of the desired intake of *ad libitum* fed birds during rearing (de Beer and Coon, 2007).

The most critical times for limiting body weight during rearing is between 7-15 weeks of age (Bruggeman et al., 1999). This time of restriction proved to be the most impactful for future reproduction. However, Bruggeman et al. (1999) also noted that it is equally critical to production in how these females are feed restricted coming into lay. This suggests that feed restriction of females is critical during their entire life. The increase seen in egg production, hatchability, egg quality, and reduced mortality (McDaniel et al., 1981; Leeson and Summers, 1985; Hocking et al., 1993) reiterates the point that feed restriction is essential to the performance and sustainability of commercial broiler breeder flocks.

Feed Restriction Programs

Feed restriction controlling the weight of broiler breeders has been successfully adopted within the industry for many decades now. Some early and satisfactory feed restriction programs for meat-type chicken production was simply to use low protein diets to delay sexual maturity (Waldroup et al., 1966; Harms et al., 1968). The most used method of feed restriction in the US is the skip-a-day method which was suggested by Luckham et al. (1963). While feed restriction programs may be similar between poultry companies the level of restriction differs greatly among companies. Most feeding programs will be based upon the recommendations from the breeding companies. Skip-a-day feeding, or a variation of that method is the most common. The top four common feeding programs are:

1. Skip-a-day (SAD) – birds are fed every other day receiving no feed on the off day.
2. 5/2 – birds receive 5 feed days per week and 2 days per week with no feed (not consecutive).
3. 4/3 – birds receive 4 feed days per week and 3 days per week with no feed (not consecutive).

4. Every Day (ED) – birds receive feed every day.

Each of these feed restriction programs have advantages as well as disadvantages depending on variables such a feed, feeder type, and feed time. The SAD method is advantageous when using a nutrient dense diet. This method will allow for longer feeding periods due to the birds receiving double the amount feed at one time. This also helps to eliminate feed distribution issues by increasing the volume of feed delivered on each feed day. This will assist in increasing flock uniformity by allowing the smaller, less aggressive birds access feed. However, the SAD and 4/3 programs must be monitored for over consumption and pendulous crops (Cobb Management Guide, 2020).

The 5/2 and 4/3 feeding programs are a variation of the SAD method. The 5/2 method will not have the same level of feed volume on each feed day as the 4/3 or SAD feeding method, but it decreases the number of days per week that the birds go without feed. Research suggests that SAD, 5/2, and 4/3 feeding of broiler breeder pullets has no significant impact on BW, uniformity, and egg production when compared to each other (de Beer and Coon, 2007).

ED feeding programs are appealing from an animal welfare perspective when compared to SAD, 5/2, or 4/3 feeding where birds are subject to days of fasting. Birds on an ED feeding program are still as quantitatively restricted as those with SAD fed allocation, but they are offered feed every day. However, one downfall of feeding pullets daily is that the severity of feed restriction required to optimize performance when coupled with a nutrient dense diet makes the daily volume of feed very small. This can lead to shortened feeding time, feed distribution issues, BW, and uniformity problems (Bartov et al., 1988).

ED fed birds are consistently heavier than SAD (Leeson and Summers, 1985; Kantanbaf et al., 1989). This can be attributed to the bird's ability to utilize and mobilize nutrients on the

off-feed day (Richards et al., 2010). This difference in BW will impact the onset of sexual maturity with those birds being heavier (ED) achieving sexual maturity earlier (Wilson et al., 1989). de Beer and Coon (2007) also found that ED feeding led to higher levels of egg production, egg production peaks, and egg weights.

Consequences of Feed Restriction

Feed restriction of any kind will always be criticized due to behavioral changes such as frustration, boredom, hunger, pecking, drinking, and pacing (de Jong and Jones, 2006; Arrazola et al., 2019; Aranibar et al., 2020). Birds that experience a certain degree of hunger have increased foraging activity (van Emous et al., 2015) as well as pecking behavior believed to be associated with a lack of satiety and a need to find food (Hocking, 2006; Savory et al., 1993). Feeding programs directed to improving animal welfare are encouraging of natural behavior (ex. preening) as well as a comfortable state of the animals. Feed restricted birds exhibit fewer comfort behaviors (de Jong et al., 2003; Putterflam et al., 2006). These behaviors are exacerbated in birds that have a fasting day (de Jong et al., 2005). This indicates that while feed restriction has a positive influence on production, the hunger that is created in the bird is stressful.

While there is not much research on the impact of blooms of *Salmonella* and *Campylobacter* occurring in pullets on the off-feed day it is speculated that there may be increases in their prevalence and colonization within fasting breeder pullets. In broilers it is well documented that feed withdrawal and cooping increase *Salmonella* levels in broilers (Ramirez et al., 1997; Corrier et al., 1999; Buhr et al., 2017). Intestinal permeability is associated with increased fasting times as well as making birds more susceptible to disease (Vicuna et al., 2015; Galarza-Seeber et al., 2016; Gilani et al., 2018). Wilson et al. (2018) found that broiler breeder pullets that were fasted (SAD feeding) had significantly higher recovery rates of *Salmonella* and

Campylobacter when compared to broiler breeders fed ED. These findings suggest that increased fasting times associated with SAD feeding could impact intestinal development, health, and disease prevalence. Findings by Montiel (2016) showed that broiler breeder pullets receiving broadcast feedings ED exhibited lower *Salmonella* colonization rates as well as they cleared *E. coli* faster than broiler breeder pullets fed SAD. Walstra et al. (2010) also found that stress in early stages of life (rearing) associated with SAD feeding can negatively impact immune responses to infectious bronchitis virus. These findings suggest that feeding programs with off-feed days such as SAD feeding have a negative impact in the immune response in broiler breeder pullets. However, in broilers and broiler breeders it has also been shown that birds that had more fasting had greater numbers of *Lactobacillus* spp colonies in the crop (Dalal, 2016; Fondevila et al., 2020) which assists in lowering the pH of the crop therefore increasing nutrient absorption (Boling et al., 2001). Perhaps some amount of fasting is physiologically important for the bird's health. The colonization of *Lactobacillus* strains and increase in the pH of the crop associated with fasting has also been shown to inhibit bacteria such as *Escherichia coli* (Jin et al., 1996).

Based on previous research, it is clear that feed restriction has positive effects on broiler breeder performance; however, it should be noted that feed restriction drastically alters their behavior as well as physiology. With consistent increases in genetic selection for growth and feed efficiency in broilers; it will increase the need for more aggressive feed restriction in broiler breeders causing a strong demand to better evaluate animal welfare and feeding programs. New feeding methods and additives for current feeding methods need to be continuously evaluated for validity for growth, reproduction, and welfare and these changes must be implemented.

New Strategies for Broiler Breeder Management

Due to the increased pressure for better animal welfare while also supplying the demand for a cheap and sustainable protein source, a management plan that can be aligned with health, welfare, and reproductive performance is essential.

Qualitative Feed Restriction

One method would be the switch from quantitative restriction of feed to qualitative. The use of low-density diets (qualitative restriction) in comparison to high-density diets (quantitative restriction) could be used as a possible method for reduced stress and hunger via increased feed intake time and filled gastrointestinal tract. However, it should be noted that many of the fibrous materials that are used to dilute the nutrients of the diets can lead to wet manure, which can in turn cause footpad lesions and hock burns (Ekstrand et al., 1997;1998). Some feeding products that have proven to increase feeding times while diluting the diet are oat hulls and an appetite suppressant (calcium propionate) (Tolkamp et al., 2005). In this study they observed that the birds on the qualitative restricted diet spent more time eating than those on the quantitative restricted diet. They also noted significant behavioral changes in non-feeding oral activities. This agrees with other research where an increase in time spent eating and comfort behaviors were seen and a decrease in pecking (de Jong et al., 2005 and Hocking et al., 2004; Hocking 2006). Other researchers indicate that calcium propionate can decrease palatability of feed (Arrazola and Torrey, 2019). While this decreases their feed intake it also causes an avoidance of feed therefore potentially decreasing the feeling of satiety in the birds.

The overall goal of a qualitative restricted feeding program for broiler breeders is to manipulate the nutrient content of the diet to diminish abnormal behaviors as well as hunger. When birds are hungry, they can be unnecessarily stressed, having a negative impact on

reproduction, development, and performance. Fault bars in the feathers can be seen in birds that are experiencing high levels of stress due to hunger from feed restriction as well as insufficient dietary protein (Jovani and Diaz-Real, 2012). This suggests that plumage condition from stress is negatively impacted which could lead to skin injuries that potentially reduce mating activity in broiler breeders or invite infection or injury. The utilization of low-density diets using higher fiber feedstuffs have been shown to decrease stress as measured by plasma corticosterone concentrations (Hocking, 2003; de Jong et al., 2005; Sandilands et al., 2006). However, this decrease in corticosterone is not consistently replicated week to week or between studies (Zuidhof et al., 1995; Savory et al., 1996; de Jong et al., 2005). Perhaps this is an indication that the evaluation of stress in broiler breeders needs to be reassessed and standardized or is confounded with restriction or level of restriction.

A commonly used fiber feedstuff is oat hulls. Zuidhof et al. (1995) found that hens fed 15% ground oat hulls had improved nutrient digestibility, egg production as well as flock uniformity when compared to a standard nutrient diet. In this same study these positive results were not seen when oat hulls were increased to 30 %. Enting et al. (2007b) suggests that where broiler breeders were fed some mixtures of fiber sources increasing from at 12 to 23 %, nutrient digestibility decreased. However, Enting et al. (2007a) found that using certain fiber types increased fertility and improved egg production. This agrees with Mohiti-Asli et al. (2012) that increased fiber has a positive impact on production parameters as well as managing body weight.

The use of alternative qualitative restriction diets needs to be further evaluated to determine their total impact on broiler breeder welfare and reproduction. Based on previously mentioned research the trend for restriction method appears to be moving towards increasing normal behaviors which would be an indication of improved animal welfare. However, some

suggest that the negative behaviors such as pecking have simply been redirected from each other to feed since feed clean up time is increased (Mason et al., 2007). Another limiting factor to the use of these low-density diets is the issue of accurately achieving target body weights (Savory et al., 1996; Savory and Lariviere, 2000; Tolkamp et al., 2005). Aranibar et al. (2020) attempted an alternative feeding program using a standard diet on a normal SAD feeding method, but then added a low-density ingredient on top of the diet during feeding or giving these breeder pullets the low-density feed product on the off-feed day to reduce hunger and feeding frustration. The addition of the low-density feed product on the off-feed day showed improved body weight and egg production. Future research using bulky, large particle sizes, and high fiber feedstuffs should be evaluated to test their usefulness on body weight gains, reproduction, and welfare in qualitative feed restriction of broiler breeders during rearing.

Improvements in Genetic Selection

Because of the drastic increase demand for poultry as an inexpensive source of protein, there has been pressure to genetically improve meat yield. However due to this pressure to increase meat yield in broilers, reproductive performance has suffered with males' ability to fertilize eggs declining 0.5 % per generation (Reddy and Sajadi, 1990). It has been proposed to potentially use dwarf hens due to their reproductive fitness when fed *ad libitum* or simply accept lower broiler productivity (Decuypere, et al., 2010). The benefits of dwarf broiler breeders come from their low levels of feed consumption and greater reproductive performance, however, the progeny of the dwarf hen is not as heavy as those from standard meat lines (Merat, 1990).

Due to smaller chick size from a dwarf hen, it would take the broiler longer to reach market weight which would lead to less broilers produced overtime or an increased need for additional broiler grow out facilities. This coupled with the hens reduced feed efficiency would

come at great economic expense. More feed will be required to grow a hen to proper weight prior to photostimulation than on a standard line of hens. While using dwarf lines or changing genetics can reduce welfare impacts such as hunger and lameness, it will require many changes in management practices as well as it would take many years for genetic changes to impact our daily food supply from the pedigree level.

Rooster Nutrition

Nutrition is critical for growth, maintenance, and reproduction. A variety of factors such as age, stress, and genetic background can influence semen characteristics and reduce fertility (Rosenstrauch et al., 1994; Chen et al., 2016; Sun et al., 2019). Fat types and minerals can have positive and negative influence on semen quality, body confirmation, and fertility (Fouad et al., 2020).

Fat types

Fatty acids are present in high quantities in rooster spermatozoa and seminal plasma, and they are typically in the form of n-6 polyunsaturated fatty acids, specifically docosatetraenoic acid (C22:4 n6) (Kelso et al., 1996; Cerolini et al., 1997a; Surai et al., 1998). Due to the high levels of polyunsaturated fatty acids in rooster semen, it makes spermatozoa very susceptible to lipid oxidation and deterioration which can limit the fertilizing capacity (McLeod, 1943; Wales et al.,1959; Surai et al., 1998; Ferrini et al., 2010; Asl et al., 2018). Corn oil, many types of fish oils, and linseed oil have all been used to try and alter the spermatozoa characteristics and increase flock fertility.

Lipids are important for proper sperm function and proper fertilization. They assist in the fusing properties of membranes as well as flagella flexibility (Cerolini et al., 1997b). Hudson and Wilson (2003) found that adding Menhaden oil to rooster diets, which is rich in n-3 fatty acids,

increased the number of fertile eggs. Surai and Sparks (2000) found similar results of increased fertility when using tuna oil which also contains n-3 polyunsaturated fatty acids. These findings of increased fertility when fed diets enriched with n-3 polyunsaturated fatty acids are attributed to increases in semen volume, semen concentration, and increased motility (Surai et al., 2000; Cerolini et al., 2005) and are linked to energy production enhanced by n-3 fatty acids promoting mitochondria activity (Kamali Sangani et al., 2017; Asl et al., 2018). However, high prices for these fish oils as well as organoleptic and heat sensitivity or stability issues has encouraged a demand for alternative products to increase semen quality and fertility (Tan et al., 2018; Fremopong et al., 2019). Linseed oil is another n-3 polyunsaturated fatty acid used in dietary supplementation that does not go rancid as quickly as fish oils. Linseed oil in rooster diets has been shown to increase fertility, sperm motility, and fatty acid profiles in roosters (Kelso et al., 1997; Ferrini et al., 2010; Zanussi et al., 2019).

Polyunsaturated fatty acids act as defense system to reduce oxidative stress (Hill et al., 2011) by protecting mitochondria from reactive oxygen species (ROS) (Andreyev et al., 2015). Chicken semen can have thiobarbituric acid reactive substances (TBARS) produced by lipid peroxidation and correlated with motility impairment (Fujihara and Howarth, 1978). Wishart (1984) also found that with the formation of high levels of malondialdehyde (MDA) in chicken semen, fertilizing ability was lost. This also agrees with Cecil and Bakst (1993). Froman and Thurston (1981) suggested that lipid peroxidation plays a significant role in semen quality and fertility in turkeys. Increased lipid peroxidation was associated with decreases in polyunsaturated fatty acids. This process of cell damage due to a lack of defense barrier via polyunsaturated fatty acids should be considered important in gaining a better understanding of the dilemma in broiler breeder male infertility.

Selenium

Selenium is a trace mineral that is found naturally in feedstuffs. The levels found in many feedstuffs is insufficient and it must be added to poultry diets. The forms of selenium found in grains is primarily in the organic form of selenomethionine (SeMet) and can be metabolized the same way as methionine (Wolffram, 1999). The inorganic forms of selenium are sodium selenite or selenate which are passively absorbed in the intestines and are excreted via urates because they are not easily retained in the tissues (Apsite et al., 1994; Wolfram, 1999). SeMet can be stored in the body as proteins making it more bioavailable (Henry and Ammerman, 1995). Feeding SeMet allows for increases selenium reserves for the body to pull from when it recognizes oxidative stress. Selenium is essential to produce the enzyme glutathione peroxidase which protects against oxidative damage (Surai and Fisinin, 2014). Glutathione peroxidase is found in the sperm head and tail midpiece mitochondria which assists in spermatozoa maturation (Godeas et al., 1997; Ursini et al., 1999).

Roosters that were fed a diet that was deficient in selenium were found to have a decrease in glutathione peroxidase production and an increase in lipid peroxidation in testes (Shi et al., 2014). Surai et al. (1998) found that adequate amounts of selenium in poultry diets increased glutathione peroxidase activity and decreased lipid peroxidation in testes and semen. Proper levels of selenium have also been shown to decrease the percentage of abnormal sperm, increase sperm motility and increase Sertoli cells count (Song et al., 2015; Biswas et al., 2017; Wang et al., 2017). This suggests that selenium plays a critical role in the fertilization capacity of rooster semen as well as flock fertility.

Zinc

The dietary zinc requirements for chickens have been set at 40mg/kg of feed by the National Research Council (NRC, 1994). Like many other trace minerals, zinc can be added in its inorganic form or organic forms such as zinc-methionine or zinc-propionate. The organic forms of trace minerals such as zinc are increasing in use due to their bioavailability (Kidd et al., 1996; Sahin et al., 2005). Zinc is a dietary trace mineral that is supplemented in poultry diets for many reasons such as cell proliferation, feather development, endocrine secretion, antioxidation, and hatchability of fertile eggs (Naz et al., 2016). This suggests that zinc plays a large role in the fertilization capacity of broiler breeder males largely due to the antioxidation capacity against ROS that prevents membrane damage (Rahman et al., 2014). Protecting the membranes surrounding the spermatozoa as well as in the hen's sperm storage tubules can assist in the longevity of flock fertility. The crucial part of zinc's defense system is its role in the antioxidant enzyme superoxide dismutase which assists in protection from ROS (Niles et al., 2008).

Dietary zinc also plays a critical role in the regulation of endocrine hormones. However, it should be noted that zinc, just like other trace minerals should be fed at recommended levels to prevent toxicity. If zinc is fed at too high a level it can increase serum corticosterone levels as well as cortisol levels that can cause apoptosis of cells and regression of the reproductive tract (Sundaresan et al., 2008; Khan et al., 2013). While the research is limited on the specific impacts that dietary zinc has on broiler breeder males, it has been shown to be involved in important spermatozoa functions via copper-zinc superoxidase (Khan, 2011) as well as prevent oxidative damage to spermatozoa (Chia et al., 2000). Excess dietary zinc has been shown to have negative effects on rooster reproductive qualities by decreased testes and seminiferous tubule size (Rahman et al., 2014). Zinc is needed for testosterone metabolism, testicular growth, sperm

production, motility, reducing excess estrogen in male reproductive tissues and progesterone synthesis (Brown and Pentland, 2007). In the study performed by Manmood and Al-Daraji in 2011, there was significant differences seen in the sperm penetration of roosters supplemented with 75mg Zn/kg of the diet. However, no significant differences were found in both percentage of embryonic mortality and hatchability of fertile eggs. These results agreed with previous research (Durmus et al., 2004). Trace mineral deficiencies can cause impaired growth and abnormal developments in major organ systems. Therefore, supplementation of zinc is important and should be monitored closely via the use of added trace minerals as well as what is occurring naturally in feedstuffs.

Conclusion

Genetic selections for increased growth rate and efficiency in broilers has a tremendous impact on broiler breeder body weight, body conformation, and reproduction. The high levels of feed consumed by broiler breeders due to the impact of genetic selection leads to overweight males and females resulting in a decrease in reproductive efficiency and economic loss for the poultry industry. Feed restriction is currently the best practice that prevents both males and females from becoming overweight. However, research shows that while feed restriction assists in managing body weight and reproductive efficiency it can also cause negative effects on reproduction when mismanaged. Several alternatives have been proposed and researched to assist in the broiler breeder paradox, more research needs to be conducted to gain a better understanding of nutritional needs and welfare improvements.

The use of organic trace minerals and fats in the diets of broiler breeder males have been vaguely explored regarding proper inclusion levels as well as the most valuable time in life to include them in the diet. Wang et al. (2017) suggests that including Se at 0.5 ppm in pigeon diets

will decrease dead sperm, increase fertility, as well as increase GPX expression. This inclusion rate is 0.2 ppm above the legal limit in the US. There have been some improvements shown in using these products in production parameters such as fertility, semen quality, and semen volume. However, the full understanding of their total potential during rearing and production phase as well as the birds' specific nutritional requirements needs further research.

As studies have shown in the past, many different factors contribute to the reproductive potential and reproductive longevity of broiler breeder males and females. This can be seen in the relationship between semen quality parameters and management practices such as feed restriction as well as in different feed additives. With the genetics of broiler breeders constantly improving for breast meat deposition, feed conversion, and fast growth a continued decline in reproductive function and fertility can be expected. Each of those three genetic changes will pose their own unique problem for broiler breeders.

Despite the increase in research for alternative feeding programs for broiler breeders during rearing as well as the use of feed additives to increase broiler breeder male fertilizing capacity, the answer to the broiler breeder mystery of increased fertility and production remains unsolved. However, with emerging feeding programs, better nutritional understanding, as well as consumer demand will lead to feeding programs that improve broiler breeder growth, reproduction, and physiology without having a negative economic and welfare impact. The area of alternative feeding program research will continue to be influential for the poultry industry.

REFERENCES

Andreyev, A. Y., H. S. Tsui, G. L. Milne, V. V. Shmanai, A. V. Bekish, M. A. Fomich, M. N. Pham, Y. Nong, A. N. Murphy, C. F. Clarke, M. S. Shchepinov. 2015. Isotope-reinforced polyunsaturated fatty acids protect mitochondria from oxidative stress. Free Radic. Biol. Med. 82:63-72.

Aranibar, C. D., C. Chen, A. J. Davis, W. I. Daley, C. Dunkley, W. K. Kim, C. Usher, A. B. Webster, and J. L. Wilson. 2020. Impact of an alternative feeding program on broiler breeder pullet behavior, performance, and plasma corticosterone. Poult. Sci. 99:829-838.

Arrazola, A., and S. Torrey. 2019. Conditioned place avoidance using encapsulated calcium propionate as an appetite suppressant for broiler breeders. PLoS ONE 14: e0206271.

Asl, R. S., F. Shariatmadari, M. Sharafi, M.A.K. Torshizi, and A. Shahverdi. 2018. Dietary fish oil supplemented with vitamin E improves quality indicators of rooster cold-stored semen through reducing lipid peroxidation. Cryobiology 84:15-19.

Aspite, M., B. Pitrans, and A. Atlavin. 1994. Absorption of ^{75}Se-selenate and ^{75}Se-Selenite in chicks. In: Mengen-und-Suprenelemente, Edited by Anke, M. and Meissner, D, Arbeitstagung, Jena, Page 188.

Bartov, I., S. Bornstein, Y. Lev, M. Pines, and J. Rosenberg. 1988. Feed restriction in broiler breeder pullets: Skip-a-day versus skip-two-days. Poult. Sci. 67:809–813.

Bilcik, B., and I. Estevez. 2005. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. Appl. Anim. Behav. Sci. 92:307-323.

Bilcik, B., I. Estevez, and E. Russek-Cohen. 2005. Reproductive success of broiler breeders in natural mating systems: the effect of male-male competition, sperm quality, and morphological characteristics. Poult. Sci. 84:1453-1462.

Biswas, A., J. Mohan, A. B. Mandal, and N. Lal. 2017. Semen characteristics and biochemical composition of cloacal foam of male Japanese quails **(***Coturnix coturnix Japonica***)** fed diet incorporated with selenium. J. Anim. Physiol. Anim. Nutr. 101:299-235.

Boling, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci. 80:783-788.

Borges, C. A. Q., H. S. Rostagno, J. H. Vilar de Silva, L. F. T. Albino, C. A. A. Torres, J. J. Filho, and M. L. G. Riberio. 2006. Energy requirements and carcass composition of male broiler breeders as a function of energy intake during the reproduction period. R. Bras. Zootec. 35(5):1978-1984.

Bowling, M., R. Forder, R. J. Hughes, S. Weaver, and P. I. Hynd. 2018. Effects of restricted feed intake on broiler breeder hens on their stress levels and the growth and immunology of their offspring. Transl. Anim. Sci. 2:263-271.

Brown, H. B., and M. G. McCartney. 1983. Effects of dietary restriction on reproductive performance of broiler breeder males. Poult. Sci. 62:1885-1888.

Brown, L., and S. Pentland, 2007. Health infertility organization: Male infertility-improving sperm quality Acubalance wellness Centre Ltd. Onwest 8th Ave. In Vancouver BC. Canada.

Bruggeman, V., O. Onagbesan, E. D'Hondt, E. Buys, M. Safri, D. Vanmontfort, L. Berghman, F. Vandesande, and E. Decuypere. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poult. Sci. 78:1424–1434.

Buckner, R. E., J. A. Renden, and T. F. Savage. 1986. The effect of feeding programs on reproductive traits and selected blood chemistries of caged broiler breeder males. Poult. Sci. 65:85–91.

Buhr, R. J., D. V. Bourassa, A. Hinton Jr., B. D. Fairchild, and C. W. Ritz. 2017. Impact of litter Salmonella status during feed withdrawal on Salmonella recovery from the broiler crop and ceca. Poult. Sci. 96:4361-4369.

Burke, W. H., and J. M. Mauldin. 1985. Reproductive characteristics of broiler breeder males from flocks with low fertility. Poult. Sci. 64(Suppl.1):73.

Cecil, H. C., and M. R. Bakst. 1993. In vitro lipid peroxidation of turkey spermatozoa. Poult. Sci. 72:1370-1378.

Cerolini, S., K. A. Kelso, R. C. Noble, B. K. Speake, F. Pizzi, and L. G. Cavalchini. 1997b. Relationship between spermatozoan lipid composition and fertility during aging of chickens. Biol. Reprod. 57:976–980.

Cerolini, S., P. F. Surai, B. K. Speake, and N. H. C. Sparks. 2005. Dietary fish and evening primrose oil with vitamin E effects on semen variables in cockerels. Br. Poult. Sci. 46:214-222.

Cerolini, S., P. Surai, A. Maldjian, T. Gliozzi, and R. Noble. 1997a. Lipid composition of semen in different fowl breeders. Poult. Avian Bio. Rev. 8:141-148.

Chen, Y. C., H. C. Liu, L. Y. Wei, J. F. Huang, C. C. Lin, E. Blesbois, M. C. Chen. 2016. Sperm quality parameters and reproductive efficiency in Muscovy duck **(***Cairina moschata*). J. Poult. Sci. 53:223-232.

Chia, I., C. Ong, L. Chua, M. Ho, and S. Tay. 2000. Comparison of various sperm parameters and plasma zinc concentrations on blood and seminal of fertile and infertile men. J. of Andro. 21:53-57.

Cobb-Vantress. 2020. Breeder Management Guide. Cobb-Vantress, Siloam Springs, AR.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Presence of Salmonella in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poult. Sci. 78:45-49.

Creel, L. H., D. Maurice, W. C. Bridges, and L. W. Grimes. 1990. A model to describe and predict post-peak changes in broiler hatchability. J. Appl. Poult. Res. 7:85-89.

Dalal, S. 2016. Effect of photoperiod and dietary strategies on crop microbial ecology and health of broiler chickens. PhD. Dissertation. University of Saskatchewan, Saskatoon, Canada.

de Beer M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927-1939.

de Jong I. C., A. Van Voorst, and H. J. Blokhuis. 2003. Parameters for quantification of hunger in broiler breeders. Phys. and Behav. 78:773-783.

de Jong I. C., and B. Jones. 2006. Feed restriction and welfare in domestic birds. Feeding in Domestic Vertebrates 1:120-135.

de Jong I. C., M. Fillerup, and H. J. Biokhuis. 2005. Effect of scattered feeding and feeding twice a day during rearing on indicators of hunger and frustration in broiler breeders. Appl. Anim. Behav. Sci. 92:61-76.

de Jong I. C., S. Van Voorst, D. A. Ehlhardt, and H. J. Blokhuis. 2002. Effects of timing and durations of feed restriction during rearing on reproductive characteristic in broiler breeder females. Poult. Sci. 78:1424-1434.

de Jong, I. C., and D. Guémené. 2011. Major welfare issues in broiler breeders. World's Poult. Sci. J. 67:73-82.

Decuypere, E., V. Bruggeman, N. Everaert, Y. Li, R. Boonen, J. De Tavernier, S. Janssens, and N. Buys. 2010. The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. Br. Poult. Sci. 51(5):569-579.

Dumus, I., C. Atasoglu, C. Mizrak, S. Ertas, and M. Kaya. 2004. Effect of increasing zinc concentration in the diets of brown parent stock layers on various production and hatchability traits. Arch-Tierz, Dummerstorf, 5:483-489.

Duncan, I. J. H., P. M. Hocking, and E. Seivewright. 1990. Sexual behavior and fertility in broiler breeder domestic fowl. App. Anim. Behav. Sci. 26:1-12.

Ekstrand, C., B. Algers, and J. Svedberg. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. Prev. Vet. Med. 31:167-174.

Ekstrand, C., B. Algers, and J. Svedberg. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. Prev. Vet. Med. 31:167-174.

Ekstrand, C., T. E. Carpenter, I. Anderson, and B. Algers. 1998. Prevalence and control of foot-pad dermatitis in broilers in Sweden. Br. Poult. Sci. 39:318-324.

Enting, H., T. A. M. Kruip, M. W. A Verstegen, and P. J. van der Aar. 2007b. The effect of low-density diets on broiler breeder performance during the laying period and on embryonic development of their offspring. Poult. Sci. 86:850-856.

Enting, H., W. J. A. Boersma, J. B. W. J. Cornelissen, S. C. L. van Widen, M. W. A. Verstegen, and P. J. van der Aar. 2007a. The effect of low-density diets broiler breeder diets on performance and immune status of their offspring. Poult. Sci. 86:282-290.

Ferrini, G., E. G. Manzanilla, D. Menoyo, E. Esteve-Garcia, M.D. Baucells, and A.C. Barroeta. 2010. Effects of dietary n-3 fatty acids in fat metabolism and thyroid hormone levels when compared to dietary saturated fatty acids in chickens. Livest. Sci. 131:287-291.

Fondevila, G., J. L. Archs, L. Cámara, A. F. de Juan, and G. G. Mates. 2020. The length of the feed restriction period affects eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract of young broilers. Poult. Sci. 99:1010-1018.

Fouad, A. M., H. K. El-Senousey, D. Ruan, W. Xia, W. Chen, S. Wang, and C. Zheng. 2020. Nutritional modulation of fertility in male poultry. Poult. Sci. 99(11):5637-5646.

Fragoso, S. J., D. M. Pizarro, J. C. M. Abad, P. I. Casanovas, A. Rodriguez-Bertos, and K. Barger. 2013. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). Reprod. Dom. Anim. 48:345-352.

Frempong, N. S., T. N. Nortey, C. Paulk, and C. R. Stark. 2019. Evaluating the effect of replacing fish meal in broiler diets with either soybean meal or poultry by-product meal on broiler performance and total feed cost per kilogram of gain. J. Appl. Poult. Res. 28:912-918.

Froman, D. P., and R. J. Thurston. 1981. Chicken and turkey spermatozoal superoxide dismutase: A comparative study. Bio. Repro. 24:193-200.

Fujihara, N., and B. Howarth. 1978. Lipid peroxidation in fowl spermatozoa. Poult Sci. 57:1766-1768.

Galarza-Seeber, R., J. D. Latorre, L. R. Bielke, V. A. Kuttappan, A. D. Wolfenden, and X. Hernandez-Velasco. 2016. Leaky gut and mycotoxins: aflatoxin B1 does not increase gut permeability in broiler chickens. Front. Vet. Sci. 3:10.

Gilani, S., G. S. Howarth, G. Nattrass, S. M. Kitessa, R. Barekatain, R. Forder, C. D. Tran, and R. J. Hughes. 2018. Gene expression and morphological changes in the intestinal mucosa associated with increased permeability induced by short-term fasting in chickens. J. Anim. Physiol. Anim. Nutr. 102(2):653-661.

Godeas, C., F. Tramer, M. Soranzo, G. Sandri, and E. Panfili. 1997. Distribution and possible novel role of phospholipid hydroperoxide glutathione peroxidase in rat epididymal spermatozoa. Bio. of Repro. 57:1502-1508.

Goerzen, P. R., W. L. Julsrud, and F. E. Robinson. 1996. Duration of fertility in *ad libitum* and feed-restricted caged broiler breeders. Poult. Sci. 75:962-965.

Harms, R. H., B. L. Damron, and H. R. Wilson. 1968. Performance of broiler breeder pullets as influenced by composition of grower and layer diets. Br. Poult. Sci. 9:359-366.

Henry, P. R., and C. B. Ammerman. 1995. Selenium and bioavailability. In: Bioavailability of nutrients for animals: Amino acids, minerals, and vitamins. Edited by: Ammerman. 235-256.

Hill, S., K. Hirano, V. V. Shmanai, B. N. Marbois, D. Vidovic, A. V. Bekish, B. Kay, V. Tse, J. Fine, C. F. Clarke, and M. S. Shchepinov. 2011. Isotope-reinforced polyunsaturated fatty acids protect yeast cells from oxidative stress. Free Radic. Biol. Med. 50:130-138.

Hocking P. M. 2003. Welfare of broiler breeder and layer females subjected to food and water control during rearing: quantifying the degree of restriction. Brit. Poult. Sci. 34:53-64.

Hocking P. M., A. B. Gilbert, M. Walker, and D. Waddington. 1987. Ovarian follicular structure of White Leghorns fed ad-libitum and dwarf and normal broiler breeders fed *ad-libitum* or restricted until point of lay. Brit. Poult. Sci. 28:493-506.

Hocking P. M., and S. R. I. Duff. 1989, Musculo-skeletal lesions in adult male broiler breeder fowls and their relationships with body weight and fertility at 60 weeks of age. Br. Poult. Sci. 4:777-784.

Hocking P. M., M. H. Maxwell, and M. A. Mitchell. 1993. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. Brit. Poult. Sci. 34:443-458.

Hocking, P. M. 1990. The relationships between dietary crude protein, body weight, and fertility in naturally mated broiler breeder males. Brit. Poult. Sci. 31:743-757.

Hocking, P. M. 2006. High-fibre pelleted rations decrease water intake but do not improve physiological indexes of welfare in food-restricted female breeders. Brit. Poult. Sci. 47:19-23.

Hocking, P. M., and R. Bernard. 2000. Effect of the age of male and female broiler breeders on sexual behavior, fertility, and hatchability of eggs. Br. Poult. Sci. 41:370-376.

Hocking, P. M., V. Zaczek, E. K. M. Jones, and M. G MacLeod. 2004. Different concentrations and sources of dietary fibre may improve the welfare of female broiler breeders. Br. Poult. Sci. 45:9-19.

Hudson, B. P., and J. L. Wilson. 2003. Effects of dietary Menhaden oil on fertility and sperm quality of broiler breeder males. J. Appl. Poult. Res. 12(3):341-347.

Humphreys, K. D. 2020. Implications of altering maternal feed availability and feeding system on offspring performance. MSc. Thesis. University of Alberta, Edmonton, AB, Canada.

Jin, L. Z., Y. W. Ho, N. Abdullah, M.A. Ali, and S. Jalaludin. 1996. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. Lett. Appl. Microbiol. 23:61-71.

Jones, E. K. M., N. B. Prescott, P. Cook, R. P. White, and C. M. Wathes. 2001. Ultraviolet light and mating behavior in domestic broiler breeders. Br. Poult. Sci. 42:23-32.

Jovani, R., and J. Diaz-Real. 2012. Fault bars timing and duration: the power of studying feather fault bars and growth bands together. J. Avian Biol. 43:97-101.

Kamali Sangani, A., A. A. Masoudi, and R. Vaez Torshizi. 2017. Association of mitochondrial function and sperm progressivity in slow-and fast-growing roosters. Poult. Sci. 96:211-219.

Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel. 1989. Restricted feeding in early and late feathering chickens. Growth and physiological responses. Poult. Sci. 68:344–351.

Kelso, K. A., S. Cerolini, B. K. Speake, L. G. Cavalvhini, and R. C. Noble. 1997. Effects of dietary selenium supplementation on seminiferous tubules and SelW, GPx4, LHCGR, and ACE expression in chicken testis. Biol. Trace. Elem. Res. 173:202-209.

Kelso, K. A., S. Cerolini, R. C. Noble, N. H. Sparks, and B. K. Speake. 1996. Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. J. Reprod. Fertil. 106:201-206.

Khan, R. U. 2011. Antioxidants and poultry semen quality. World's Poult. Sci. 67(2):297-308.

Khan, R. U., Z. Rahman, I. Javed, and F. Muhammad. 2013. Supplementation of vitamins, probiotics and proteins on oxidative stress, enzymes and hormones in post-molt male broiler breeders. Arch. Anim. Breed. 56:607-616.

Kidd, M. T., P. R. Ferket, and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunity. World's Poult. Sci. J. 52(3):309-324.

Leeson, S., and J. D. Summers. 1985. Effect of cage versus floor rearing and skip-a-day versus everyday feed restriction on performance of dwarf broiler breeders and their offspring. Poult. Sci. 64:1742-1749.

Luckham, D. G., S. J. Slinger, I. R. Slinger, I. R. Sibbald, and G. C. Ashton. 1963. Methods of restricting feed or energy intake of growing Leghorn pullets and their effect on subsequent reproductive performance. Poult. Sci. 42:1285. (Abstr).

Manmood, H. M., and J. Al-Daraji. 2011. Effect of dietary supplementation with different level of zinc on sperm egg penetration and fertility traits of broiler breeder chicken. Pak. J. Nutr., 11:1083.

Mason G., R. Clubb, N. Lathman, and S. Vickery. 2007. Why and how should we use environmental enrichment to tackle stereotypic behavior? Appl. Anim. Behav. Sci. 102:163-188.

McCartney, M. C., and H. B. Brown. 1980. Effects of feed restriction on reproductive performance of broiler-breeder males. Poult. Sci. 59:2583-2585.

McDaniel, G. R., J. Brake, and R. D. Bushong. 1981. Factors affecting broiler breeder performance. Relationship of daily feed intake level to reproductive performance of pullets. Poult. Sci. 60:307-312.

McLeod, J. 1943. The role of oxygen in the metabolism and motility of human spermatozoa. American J. of Physiol. 138:512-518.

Merat, P. 1990. Pleiotropic and associated effects of major genes. Pages 429–467 in R. D. Crawford, ed. Poultry Breeding and Genetics. New York.

Mohiti-Asli, M., M. Shivazad, M. Zaghari, M. Rezaian, S. Aminzadeh, and G. G. Mateos. 2012. Effects of feeding regimen, fiber inclusion, and crude protein content of the diet on

performance and egg quality and hatchability of eggs of broiler breeder hens. Poult. Sci. 91:3097-3106.

Montiel, E. R. 2016. Influence of feeding programs on innate and adaptive immunity in broiler breeders. PhD. Dissertation. University of Georgia, Athens, Ga.

National Research Council. 1994. Nutrient requirements for poultry. 9th edition p. 14.

Naz, S., M. Idris, M. A. Khalique, Zia-Ur-Rahman, I. A. Alhidry, M. M. Abdelrahman, R. U. Khan, N. Chand, U. Farooq, and S. Ahmad. 2016. The activity and use of zinc in poultry diets. World's Poult. Sci. J. 72:159-167.

Niles, B. J., M. S. Clegg, L. A. Hanna, S. S. Chou, T. Y. Momma, H. Hong, and C. L. Keen. 2008. Zinc deficiency-induced iron accumulation, a consequence of alterations in iron regulatory protein-binding activity, iron transporters and iron storage proteins. J. Biol. Chem. 283:5168-5177.

Parker, J. E., and B. J. McSpadden. 1943. Influence of feed restriction on fertility in male domestic fowls. Poult. Sci. 22:170-177.

Putterflam, J., F. Merlet, J. M. Faure, P. M. Hocking, and M. Picard. 2006. Effects of genotype and feed restriction on the time-budgets of broiler breeders at different ages. Appl. Anim. Behav. Sci. 98:100-113.

Rahman, H., M. S. Qureshi, and R. U. Khan. 2014. Influence of dietary zinc in semen traits and seminal plasma antioxidant enzymes and trace minerals of beetle bucks. Repro. In Dom. Anim. 49:1004-1007.

Ramero-Sanchez, H., P. W. Plumstead, and J. Bracket. 2007. Effect of feeding programs and dietary crude protein during rearing on body weight and fertility of broiler breeder males. Poult. Sci. 86:168-174.

Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, M. E. Hume, D. E. Corrier, J. R. DeLoach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of Salmonella in the crops and ceca of market age broiler chickens. Poult. Sci. 76:654-656.

Reddy, R. P. K., and A. Sajadi. 1990. Selection for growth and semen traits in the poultry industry: What can we expect in the future? Cont. of Fert. In Dom. Birds. 47-59.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I McKay. 1999. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629-639.

Renema, R.A., and F. E. Robinson. 2004. Defining normal: comparison of feed restriction and full feeding of female broiler breeders. World's Poult. Sci. 60:511-525.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMutry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. of Appl. Poult. Res. 19:182-193.

Robinson, F. E., and J. L. Wilson. 1996. Reproductive failure in overweight male and female broiler breeders. Anim. Feed Sci. and Tech. 58:143-150.

Robinson, F. E., J. L. Wilson, M. W. Yu, G. M. Fasenko, and R. T. Hardin. 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. Poult. Sci. 72:912-922.

Rosenstrauch, A., A. A. Degen, M. Friedländer. 1994. Spermatozoa retention by Sertoli cells during the decline in fertility in aging roosters. Biol. Reprod. 50:129-136.

Sahin, K., M. O. Smith, M. Onderci, N. Sahin, M. F. Gursu, and O. Kucuk. 2005. Supplementation of zinc from organic and inorganic source improves performance and antioxidant status of heat-distressed quail. Poult. Sci. 84(6):822-887.

Sandilands, V., B. J. Tolkamp, C. J. Savory, and I. Kyriazakis. 2006. Behaviour and welfare of broiler breeders fed qualitatively restricted diets during rearing: are there viable alternatives to quantitative restriction? Appl. Anim. Behav. Sci. 96:53-67.

Savory, C. J., and J-M. Lariviere. 2000. Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. Appl. Anim. Behav. Sci. 69:135–147

Savory, C. J., and L. Kostal. 1996. Temporal patterning of oral stereotypies in restricted-fed fowls: 1. Investigations with a single daily meal. Int. J. Comp. Psychol. 9:117–139.

Savory, C. J., K. Maros, and S. M. Rutter. 1993. Assessment of hunger in growing broiler breeders in relation to a commercial restricted feeding program. Animal Welfare 2:131-152.

Savory, C. J., P. M. Hocking, J. S. Mann, and M. H. Maxwell. 1996. Is broiler breeder welfare improved by using qualitative rather than quantitative food restriction to limit growth rate? Animal Welfare 5:105-127.

Scogin, V., G. C. Harris Jr., J. Benson, and T. L. Barton. 1980. Effects of feeding program during the growing period on body weight, semen production, and certain blood serum components of broiler breeder cockerels. Poult. Sci. (Suppl.1):1571.

Sexton, K. J., J. A. Renden, D. N. Marple, and R. J. Kemppainen. 1989. Effects of *ad libitum* and restricted feeding on semen quantity and quality, body composition, and blood chemistry of caged broiler breeder male. Poult. Sci. 68:569-576.

Shi, L., H. Zhao, Y. Ren, X. Yao, R. Song, an W. Yue. 2014. Effects of different levels of dietary selenium on the proliferation of spermatogonial stem cells and antioxidant status in testis of roosters. Anim. Reprod. Sci., 149:266-272.

Silveira, M. M., A. G. de Freitas, C. A. Moraes, F. S. Gomes, F. H. Litz, J. M. S. Martins, N. S. Fagundes, and E. A. Fernandes. 2014. Feeding management strategy for broiler breeder males and its effects on body weight, hatchability, and fertility. Braz. J. Poult. Sci. 16(4):397-402.

Song, R., X. Yao, L. Shi, Y. Ren, and H. Zhao. 2015. Effects of dietary selenium on apoptosis of germ cells in the testis during spermatogenesis in roosters. Theriogenology. 84:583-588.

Sun, Y., F. Xue, Y. Li, L. Fu, H. Bai, H. Ma, S. Xu, J. Chen. 2019. Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. Poult. Sci. 98:4182-4189.

Sundaresan, N. R., D. Anish, K. V. H. Sastry, V. K. Saxena, K. Nagrajan, J. Subramani, M. D. M. Leo, N. Shit, J. Mohan, M. Saxena, and K. A. Ahmed. 2008. High doses of dietary zinc induce cytokines, chemokines, and apoptosis in reproductive tissues during regression. Cell Tissue Research 332:543-554.

Surai, P. F., and N. H. C. Sparks. 2000. Tissue-specific fatty acid and α-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci. 79:1132-1142.

Surai, P. F., and V. I. Fisinin. 2014. Selenium in poultry breeder nutrition: an update. Anim. Feed Sci. Technol. 191:1-15.

Surai, P. F., R. C. Noble, N. H. C. Sparks, and B. K. Speake. 2000. Effect of long term supplementation with arachidonic or docosahexaenoic acid on sperm production in broiler chicken. J. Reprod. Fertil. 120:257-264.

Surai, P., I. Kostjuk, G. Wishart, A. Macopherson, B. Speake, R. Noble, I. Ionov, and E. Kutz. 1998. Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes, and liver. Biol. Trace Elem. Res. 64:119-132.

Tan, L., D. Rong, Y. Yang, and B. Zhang. 2018. The effect of oxidized fish oils on growth performance, oxidative status, and intestinal barrier function in broiler chickens. J. Appl. Poult. Res. 28:31-41.

Tolkamp, B. J., V. Sandilands, and I. Kyriazakis. 2005. Effects of qualitative feed restriction during rearing on the performance of broiler breeders during rearing and lay. Poult. Sci. 84:1286-1293.

Ursini, F., S. Heim, M. Kiess, M. Maiorino, A. Roveri, J. Wissing, and L. Flohe. 1999. Dual function of selenoprotein PHGPx during sperm maturation. Science 285:1393-1396.

van Emous R. A., R. P. Kwakkel, M. M. van Krimpem, and W. H. Kendriks. 2015. Effects of dietary protein levels during rearing and different dietary energy levels on behavior and feather cover in broiler breeder females. Poult. Sci. 168:45-55.

van Emous, R. A., R. P. Kwakkel, M. M. van Krimpen, and W. H. Hendriks. 2014. Effects of growth pattern and dietary protein level during rearing on feed intake, eating time, eating rate, behavior, plasma corticosterone concentration, and feather cover in broiler breeder females during rearing and laying period. Appl. Anim. Behav. Sci. 150:44-54.

Vaughters, P. D., G. M. Pesti, and B. Howarth, Jr. 1987. Effects of feed composition and feeding schedule on growth and development of broiler breeder males. Poult. Sci. 66:134–146.

Vicuña, E. A., V. A. Kuttappan, G. Tellez, X. Hernandez-Velasco, R. Seeber-Galarza, J. D. Latorre, O. B. Faulkner, A. D. Wolfenden, B. M. Hargis, and L. R. Bielke. 2015. Does

titration of FITC-D for optimal measurement of enteric inflammation in broiler chicks. Poult. Sci. 94(6):1353-1359.

Waldroup, P. W., B. L. Damron, and R. H. Harms. 1966. The effect of low protein and high fiber grower diets on performance of broiler pullets. Poult. Sci. 45:393-401.

Wales, R. G., I. G. White, and D. R. Lammond. 1959. The spermicidal activity of hydrogen peroxide *in vitro* and *in vivo*. J. of Endo. 18:236-244.

Walsh, T. J., and J. Brake. 1997. The effect of nutrient intake during rearing of broiler breeder females on subsequent fertility. Poult Sci. 76:297-305.

Walstra, I., J. ten Napel, B. Kemp, H. Schipper, and H. van der Brand. 2010. Early life experience affect the adaptive capacity of rearing hens during infectious challenges. Animal, 4:1688 – 1696.

Wang, Y., H. M. Yang, W. Cao, and Y. B. Li. 2017**.** Effect of selenium supplementation on pigeon reproductive performance, selenium concentration and antioxidant status. Poult. Sci. 96:3407-3413.

Wilson, H. R. 1991. Interrelationships of egg size, chick size, post hatching growth and hatchability. J. World's Poult. Sci. 47:5-20.

Wilson, H. R., and R. H. Harms. 1986. Performance of broiler breeders as affected by body weight during the breeder season. Poult Sci. 65(6)1052-1057.

Wilson, H. R., D. R. Ingram, F. B. Mather, and R. H. Harms. 1989. Effect of daily restriction and age at initiation of a skip-a-day program for young broiler breeders. Poult. Sci. 68:1442–1446.

Wilson, K. M., D. V. Bourassa, B. L. McLendon, J. L. Wilson, and R. J. Buhr. 2018. Impact of skip-a-day and every-day feeding programs of broiler breeder pullets on the recovery of Salmonella and Campylobacter following challenge. Poult. Sci. 97(8):2775-2784.

Wishart, G. J. 1984. Effects of lipid peroxide formation in fowl semen on sperm motility, ATP content, and fertilizing ability. J. Repro. and Fert. 71:113-118.

Wolffram, S. 1999. Absorption and metabolism of selenium: difference between inorganic and organic sources. In: Biotech. in the Feed Industry. Lyons, T.P. and K.A. Jacques, ed. Nottingham University Press, Nottingham NG11 0AX, UK. Proc. 15th Annual Symp. 15, 547-566.

Zanussi, H. P., F. Shariatmadari, M. Sharafi, and H. Ahmadi. 2019. Dietary supplementation with flaxseed oil as source of omega-3 fatty acids improves seminal quality and reproductive performance in aged broiler breeder roosters. Theriogenology. 130:41-48.

Zuidhof, M. J., F. E. Robinson, J. J. R. Feddes, R. T. Hardin, J. L. Wilson, R. I. McKay, and M. Newcombe. 1995. The effects of nutrient dilution on the well-being and performance of female broiler breeders. Poult. Sci. 74:441-456.

CHAPTER 3

IMPACT OF EVERY-DAY VERSUS SKIP-A-DAY FEEDING OF BROILER BREEDER PULETS DURING REARING ON BODY WEIGHT UNIFORMITY AND REPRODUCTIVE PERFOMANCE

[1]Sweeney, K. M., C. D. Aranibar, W. K. Kim, S. M. Williams, L. P. Avila, J. D. Starkey, C. W. Starkey, and J. L. Wilson. Accepted on April 25, 2022, to *Poultry Science*.

ABSTRACT

Genetic selection for increased growth rate in broilers makes feed restriction programs such as skip-a-day (SAD) feeding, for broiler breeders essential to managing body weight, flock uniformity, and reproductive performance. The objective of this experiment was to compare intestinal development, weight gain of breeder pullets, and reproductive performance (22-45 wk) when fed a high fiber diet (3.8% crude fiber) on either an every-day (ED) or SAD basis during rearing. The same developer ration and feed amounts were fed to both treatments. Day-old Ross 708 pullet chicks (n=912) were randomly distributed into 4 floor pens (n=228/pen, 2 pens/treatment). At 20 wk of age all birds were weighed, and the coefficient of variation (CV) and average body weight was calculated for each treatment. Birds were then distributed into 10 lay pens (n=35 birds/pen, 5 pens/treatment) at 21.5 wk of age. Light was increased from 8 h to 15.25 h at move to the lay facility, and all birds were daily fed for the remainder of the study. Data were analyzed by SAS SLICE using a significance level of $P \leq 0.05$. During lay, 25% of the birds from each treatment were weighed weekly to adjust feed and monitor body weight. At 21 wk the ED fed pullets were more uniform (P=0.0007) than the SAD fed pullets. Eggs were collected daily and set for hatch every 4 wk from 28 to 42 wk of age. No significant difference in the hatch data were observed. The ED fed birds achieved first egg at 166 d of age while the SAD fed birds achieved first egg at 173 d of age. Specific gravity was measured every 2 wk from 30-40 wk, with ED reared birds having better overall eggshell quality (P=0.02) and greater overall egg weight (P<0.0001) than those fed SAD. Feeding a high fiber diet on an ED basis during rearing, improved body weight uniformity in rearing, encouraged early lay, improved eggshell quality and increased egg weight.

Key Words: ED feeding, shell quality, body weight uniformity, broiler breeder pullet, intestinal development

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INTRODUCTION

Broiler growth rate has increased through genetic selection leading to a critical need for feed restriction programs for broiler breeders to control body weight (BW) and reproductive efficiency (Harms and Ivey, 1992; Robinson et al., 1993; Lopez and Lesson, 1995; de Beer and Coon, 2006). The skip-a-day (SAD) feeding program became the method of choice for feed restricting broiler breeders due to its ease of application, good results, and ample feed distribution (Hudson et al., 1999). When using a SAD feeding method, feed cleanup time is increased which allows for more equal opportunities for feed intake thus a more uniform flock. The body weight uniformity of the flock is critical for broiler breeder management because it impacts feed allocation, egg size uniformity, and incubation conditions.

Feed restriction is critical for managing broiler breeders to mitigate excessive BW gain as well as over fleshing. Without the feed restriction programs, broiler breeders will develop severe health and reproductive dysfunction (ruptured tendons, lameness, decreased livability, poor fertility, decreased laying performance) once in the lay period (Hocking and Duff, 1989; de Beer and Coon, 2006; de Jong and Guémené, 2011). While feed restriction assists to manage BW and production goals it cannot be ignored that there are negative impacts of feed restricting broiler breeders. Female broiler breeders are feed restricted to approximately 25-55% of their *ad libitum* feed intake during rearing (Savory et al., 1996) which can lead to chronic hunger and feeding anxiety as well as general welfare issues (Aranibar et al., 2020). Every-day (ED) feeding pullets would help to alleviate some welfare issues. However, due to the use of high-density feeds commonly fed in the US poultry industry and the current widespread use of older chain and pan feeders, ED feeding is impractical and can lead to feed distribution issues, BW, and flock uniformity issues (Bartov et. al, 1988). In contrast, SAD feeding physiologically disrupts the

bird's natural tendency to forage daily. Richards et al. (2010) found birds on a SAD program were less feed-efficient due to their inability to store and mobilize nutrients when receiving such a large quantity one day and fasting the next.

One method to increase the success of an ED feeding program is to implement a low-density and high fiber diet. Feeding is a huge production cost and has evolved as such for rearing birds even though our understanding of physiology and metabolism of feedstuffs has advanced tremendously (Kiarie and Mills, 2019). Birds have a high sense of hunger which can be alleviated by low-density high-volume feeds. While the highly concentrated diets are easier to mill, store and transport they offer little assistance to improve feed distribution and a sense of satiety in the bird. Bulky high fiber diets however can assist in feed distribution issues as well as aiding in a sense of satiety in the bird (Savory et al., 1996). Understanding how these birds utilized these high fiber feed stuffs can assist in these production costs as well as potentially improve the bird's production goals. It is widely known that increasing dietary fiber in poultry diets impact the small intestine by increasing epithelial cell turnover rate, villi height, and intestinal viscosity (Chiou et al., 1996; Sklan et al., 2003; Tejada and Kim, 2020). The diet used for this experiment contained high fiber cereal grain feedstuffs (wheat middling's and whole oats) that are known to decrease the digestion of protein and fats due to the non-starch polysaccharides (NSPs) surrounding the starch particles in the grain (Kumar et. al, 2012). These products allow an increase in overall feed volume on each feed day while maintaining proper nutrition. To assist in increasing the volume and diluting the diet, whole oats were also utilized. Due to the hull of the oat, they are a fibrous feedstuff with approximately 75% as much energy as corn and are approximately 12% fiber (Commercial Production. Chicken, Meat and Egg, p

218). Research has shown that feeding fibrous products such as oats stimulates digestive tract development in pullets (Ernst et al., 1994).

The current study investigated if feeding broiler breeder pullets, a low-density high fiber diet on an ED feeding program can improve broiler breeder pullet intestinal development without causing a negative influence on overall performance. The main goal of this study was to determine if feeding ED or SAD impacted BW uniformity, performance or intestinal development. So, both feeding methods received equal amounts of the same diet. The only difference being the delivery of feed either ED or SAD. Feeding large particle sizes of corn and whole oats as well as fibrous feedstuffs has the potential to eliminate feed distribution issues on an ED feeding program as well as eliminate the need for a fasting day. We hypothesize that feeding broiler breeder pullets a low-density high fiber diet with large particle sizes on an ED basis will stimulate intestinal development in rearing and not negatively impact a flock's reproductive performance during the lay period.

MATERIALS AND METHODS

A total of 912 one-day-old (Ross 708) pullets were raised in 4 floor pens with new pine shavings in an environmentally controlled solid side wall poultry house. At 4 wk of age, 2 replicate pens (7.3 x 4.6m^2, 228 pullets, 147.3 cm^2/bird) were allocated to each feeding program (456 birds/treatment). The two pens were housed in the same room separated by a chain-link partition and fed on the same feeding program. All birds were fed a standard starter ration (2,910 kcal/kg, 18% CP) for the first 3 wk of age, followed by a grower diet (2,820 kcal/kg, 15.4% CP, 1.07% Ca) to 22 wk of age (Table 1). A pre-breeder diet (2,820 kcal/kg, 14.7% CP, 1.5% Ca) was used from 22 to 25 wk (Table 1) until the hens reached 5% egg production. For the lay

portion of the study, they were fed a breeder diet (2,735kcal/kg, 14.8% CP, 3.4% Ca) from 25 to 45 wk (Table 1). The feeding programs (ED or SAD) were started at 4 wk and continued through 22 wk of age with 2 pens of pullets within a room receiving a limited amount of feed daily (everyday, ED) and the other 2 pens receiving twice their daily amount on 1-day and no feed on the following day (skip-a-day, SAD). Both groups received the same feed and same feed amount over a 2-d period as well as for the duration of the trial. During rearing, the pullets ate from a chain feeder (14.3 m, 6.3 cm/bird) and water was provided *ad libitum* by nipple drinker line (6.1 m with 40 nipples). All birds were wing banded at 4 wk of age to track growth rate. The photoperiod to 22 wk of age was 23 h of light:1 h of darkness (23L:1D) for the first 3 d, followed by an 8L:16D pattern until 22 wk of age. The photoperiod was increased to 15.25L:8.75D at 22 wk of age and remained constant until the end of the study at 45 wk of age.

At 21.5 wk pullets were distributed to 10 lay pens (n=35 birds/pen, 5 pens/treatment) based on the 20 wk BW and CV so that all pens within a treatment were similar to the rearing CV for that treatment. Lay pens were 2/3 raised slats and 1/3 litter, and each pen was equipped with a nest section of roll out nest containing 6 nest holes. Once moved to the lay facility all birds began feeding on a daily basis per industry standard. Through the production period, feed was adjusted based on weekly body weights and egg production according to the Aviagen Breeder Management Guide Recommendations (Aviagen, 2016). All birds were fed equal amounts of feed for the life of the flock.

Eggs were collected 4 to 5 times per day by pen and percentage total egg production calculated by week on a hen day basis (number of eggs laid per wk was divided by 7-d and by the number of birds per pen). Every 4 wk through 45 wk of age, eggs were collected and stored for no more than 7 d. From each treatment pen, 90 eggs were sorted and placed in a Natureform

incubator (Natureform INC, Jacksonville, FL) at 37.5 ° C and 53% relative humidity. Eggs were candled at 10 d of incubation to determine (%) fertility, early dead and cracked eggs. At hatch, all first quality chicks were counted to determine percentage hatchability. Unhatched eggs were opened, and middle and late dead embryos counted along with pipped, contaminated, abnormal shelled eggs and cull chicks and percentage of incubated eggs were calculated. All procedures were approved by the University of Georgia Animal Care and Use Committee.

TMEn Determination (in Leghorn roosters)

The nutrient availability in whole oats were determined by standard TMEn (Nitrogen-corrected true metabolizable energy) methods described by Sibbald (1976) and modified by Dale and Fuller (1984). Eight Single Comb White Leghorn roosters were fasted for 30 h to empty the digestive tract. Roosters were in an individual cage measuring 30 cm wide, 45 cm deep and 50 cm high. Each cage was equipped with a nipple drinker for *ad libitum* access of water and a stainless-steel excreta collection pan. Roosters were precision fed 35 g of whole oats and excreta were collected for 48 h post feeding.

Excreta were collected from each individual pan, dried, and weighed. Crude protein and moisture of the feces and whole oats were determined (AOAC, 2006, by the University of Georgia Agricultural and Environmental Laboratories, Athens, GA), with gross energy of feed and feces determined with a bomb calorimeter (University of Georgia Agricultural and Environmental Laboratories, Athens, GA).

Digestible Amino Acid Determination

The determination of the digestible amino acid coefficients of the whole oats followed the same procedures utilized for the TMEn determination except that cecectomized Single Comb White Leghorn roosters were utilized. The amino acid content of the whole oats and feces were

determined (AOAC, 2006, and University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MD) for calculation of the digestible amino acid coefficients for the whole oats.

Growth and Productivity

A sample of individual BW (25% of the birds from each pen) were taken prior to feeding (*ad libitum* water) during rearing weekly and all birds were weighed at 4, 8, 12, 16, and 20 wk. Once in lay, 25% of the birds from each treatment were weighed individually and approximately 7 h after feeding (*ad libitum* water) weekly through 45 wk of age. Coefficient of variation (CV) for BW was calculated on a per pen basis during rearing as a measure of flock uniformity. In lay, BW for each treatment was calculated per pen (n=35 birds/pen, 350 birds/treatment and total of 700 birds). Egg production was determined on a daily per pen basis from 23 to 45 wk of age.

Digestive Tract Sample Collection

To evaluate the impact of ED feeding on digestive tract development and intestinal morphology five pullets from each pen were randomly selected at 8, 12, 16, and 20 wk of age for necropsy and morphometric analyses as described by (Teng et al., 2017). The entire digestive tract was removed, and individual parts were weighed, and their percentage of body weight was calculated. Samples of the duodenum, jejunum and ileum were taken and fixed with 10% buffered formalin. The duodenum sample was taken at the distal end and sliced approximately into 2 cm sections that included part of the pancreas. A section of the jejunum was taken by measuring using the Meckel's Diverticulum and placing it even with the distal end of the duodenum and cutting at the apex of the loop created. The ileum section taken was created by taking Meckel's Diverticulum and placing it even with the proximal end of the ceca and slicing a 2 cm at the apex of the loop created. Routine tissue processing, embedding, sectioning, and

staining of slides was performed for measuring villi height and crypt depth using Lecia Application Suite V 4.8 software program. The ratio of villi height and crypt depth was calculated.

Specific Gravity and Egg Weight

Specific gravity was measured using a flotation method as described by Harms et al. (1990). A days' worth of settable eggs from each pen (n=5 pens/treatment) were collected every two weeks from 30 to 40 wk of age. Eggs were allowed to equilibrate to the temperature of the room that the saline solutions were stored in (21° C) for a minimum of twelve hours. Saline densities were as follows: 1.060, 1.065, 1.070, 1.075, 1.080, 1.085, 1.090, and 1.095 g per cm^3. A weighted mean of eggshell quality was determined by pen.

A days' worth of settable eggs from each pen (n=5 pens/treatment) were collected every two weeks from 29 wk to 41 wk of age. Individual eggs were weighed using a Mettler Toledo bench scale (Mettler Toledo, Columbus, OH). An average egg weight was calculated per pen.

Statistical Analysis

Body weight, gastrointestinal measurements, egg production, hatch data, and specific gravity were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Slice analysis specifies effects to test for differences between interactions LS-mean, to produce tests of simple effects (Winer, 1971). This method evaluates the effect of the treatment and minimizing the impact of time (week). Differences were deemed to be significant when the P-value was less than or equal to 0.05.

RESULTS

Body Weight and Uniformity

Body weight and body weight uniformity were not significantly different at 4 wk (ED 326.9 g, 20.5 CV and SAD 354.8 g, 22.5 CV) when the feeding treatment was implemented (Table 2). However, by 20 wk of age BW was significantly different ($P \leq 0.05$) between the feeding programs with the ED pullets being heavier than the SAD pullets (1948.1 g, 1895.6 g, respectively). Body weight was only significantly different at wk 23 and 34 during the laying period ($P<0.05$) (data not shown). The ED feeding program significantly improved body weight uniformity at 12, 16 and 20 wk of age (Table 3). During the laying period, body weight uniformity did not differ between the hen groups fed either ED or SAD as pullets (data not shown).

Feed Intake and TMEn

Feed intake was the same throughout the study for both treatments and was adjusted weekly to meet recommended breeder target BW during rearing (data not shown). For the lay phase of the experiment (22-45 wk) feed intake also did not differ (data not shown).

The TMEn value of whole oats was approximately 9.8% CP (Table 4) and 11.5% fiber. Based on these findings we included whole oats at a rate of 8% in the grower diet (Table 1).

Egg production and Eggshell Quality

Hens fed on the ED program achieved first egg one week prior (166 d) to the hens fed on the traditional SAD feeding program (173 d) during rearing. Overall egg production was not significantly different between treatments. However, there were 2 wk that had significant differences in egg production at wk 26 ($P=0.04$) and 39 ($P=0.01$) (Figure 1). At wk 26, the hens that were fed ED during rearing had 6% better egg production than those fed SAD during

rearing. At wk 39, those hens fed SAD during rearing had 7% better egg production than those fed ED during rearing. The weekly egg weights were not significantly different, but overall egg weights were significantly different from 29-41 wk of age (60.4 g and 59.1 g for the ED and SAD, respectively) (Figure 2).

Overall specific gravity was significantly different with hens fed on an ED basis having improved shell quality when compared to those fed SAD as pullets (Figure 3, $P=0.02$). When compared by week the specific gravity comparisons were not different.

Intestinal Morphology and Organ Weights

In the duodenum there was no significant differences in villi height or crypt depth between the SAD and ED fed pullets (Table 5). In the jejunum, there were significant differences in the crypt depth at 8 and 20 wk of age ($P\leq0.05$) with the ED-fed pullets having greater crypt depths than the SAD pullets. Also, in the jejunum there was a significant difference ($P=0.05$) in the villi height at 16 wk (1580.0 µm and 1443.4 µm for the ED and SAD, respectively). In the ileum, villi height was significantly greater at 16 and 20 wk of age ($P<0.02$) in the SAD pullets. Jejunum villi height to crypt depth ratio was significantly different ($P<0.04$) at 8, 16, and 20 wk of age with those pullets fed SAD having a greater ratio than those fed ED (Table 6).

Crop (8 and 12 wk), liver (8, 12, and 20 wk), and ileum (8, 16, and 20 wk) weights as a percentage of BW were significantly different ($P<0.05$) between the treatments (Table 7). The pullets fed on the SAD program had greater liver, crop, and ileum weights as a percentage of BW than the ED fed pullets.

DISCUSSION

The results from this current research indicated that there are significant benefits to feeding broiler breeder pullets a low-density high fiber diet containing large grain particles or

whole grains on an ED feeding program. Providing feed each day (ED) improved flock uniformity in rearing as well as allowed the ED fed pullets to achieve target body weight before the SAD pullets at the time of photostimulation. The pullets reared on the ED feeding program had greater overall egg weights and eggshell quality during the lay phase of this experiment. The reasons for this are not well understood. Possibly, the increase in villi height in the jejunum and ileum at 16 and 20 wk in the small intestine of the pullets could explain these results. Perhaps the ED pullets were able to better absorb and utilize nutrients at critical times during development of the intestine leading to more efficient weight gain, medullary bone development and reproductive tract development than the SAD fed pullets. Future research should be conducted to better understand the impact of feeding methods on intestinal development and nutrient utilization. The SAD fed birds had a larger overall digestive tract relative to body weight, and perhaps the larger digestive tract developed to accommodate the large bolus of feed they received on the feed day.

It should be noted that further research should also be conducted on feeder types and their ability to aid in the delivery of feed on an ED basis. This study was based on the impacts of delivery method on pullet development and reproductive performance. Feeding equipment type was not used as an evaluation parameter for this study. However, this research can aid in the implementation of ED feeding due to the current use of older types of chain feeders still in use in the US, that can only deliver feed at approximately 18 to 27 m/min in houses that range from 91 to 153 m long (Wilson, 2003). The attraction to these chain feeders is their longevity and ease of use. Pan feeders offer more rapid delivery through the house but can be more mechanically complex and expensive. More modern chain feeders have variable speed control settings that can

distribute feed to a flock at 36 m/min (van de Sluis, 2011). The ability to feed flocks on an ED basis must take into consideration the feed formulation as well as feeding equipment.

Body Weight, Uniformity, and Intestinal Morphology

Extensive research with whole oats indicates they offer poultry approximately 2,756 kcal/kg (Ahiew et al, 2018) and non-starch polysaccharides comprise 300 g/kg of whole oats due to the hull (Knudensen and Bach, 1997). This low protein and bulky feedstuff increased the feed volume and improved feed distribution providing more opportunity for birds to eat than a standard concentrated diet. In our study both treatment groups received the same bulky diet increasing the opportunity for the birds to feed. In addition to the whole oats, we used wheat middling's to increase the fiber content of this diet. High fiber diets have proven to modulate intestinal morphology of poultry (Han et al., 2017; Tejeda and Kim, 2021). The increased villus height seen in the pullets on the ED treatment increased the overall surface area of the small intestine which could explain the greater nutrient absorption and utilization. Enzyme activity is greatest in the jejunum and the enzymes are located in the epithelial cells of the villi which are important for the last steps of digestion (Denbow, 2015). Also, while not significant there were consistently smaller crypts in the ileum of the ED fed pullets. A decrease in crypt depth is correlated to an improvement in digestion (Seyyedin and Nazem, 2017).

ED feeding also decreased the time pullets were without feed. Increasing fasting time has shown to increase gut permeability (Gilani et al., 2017). This means that the intestinal walls become more easily permeable to things such as bacteria and toxins which can cause inflammatory reactions in digestive organs such as the liver (Bischoff et al., 2014). Fasting also increases intestinal sloughing and decrease villi height in as little as 24 h (Yamauchi et al., 1997). These factors together can negatively impact nutrient utilization. This is perhaps why the

ED fed pullet gains more weight on the same amount of feed as the SAD fed pullets (BW and uniformity). However, the increase in the villus height to crypt depth ratio in the SAD fed pullets should be noted. An increase in this ratio is an important reflection of the improvement of absorption and digestion of nutrients (Hou et al., 2010, Hou et al., 2012, and Yao et al., 2012). Perhaps the SAD fed pullets directed their nutrient allocations to building a larger digestive tract instead of bone and muscle to process the large bolus of feed on the feed day. Nutrients could also have been allocated to the digestive tract for repairs from intestinal sloughing on the off-feed days as well. This can be indirectly seen in the relative percentage of body weight of the digestive tract of the SAD pullets when compared to the ED fed pullets. High dietary fibers have been shown to have a harsh effect on the intestinal walls which leads to nutrient loss (Leterme et al., 1998). Perhaps such a large bolus of high fiber feed on the SAD fed pullets impacted breakage and cell loss in the intestines as well, leading to the SAD pullets being under weight and slower to sexually mature.

Productivity and Eggshell Quality

Several factors impact the successful stimulation of broiler breeder pullets into lay and production of high-quality eggs. Achieving proper BW prior to the time of photostimulation is important to optimum reproductive performance. Researchers report that pullets fed on an ED basis started to lay at a younger age and had increased egg production (de Beer and Coon, 2007; Katanbaf et al., 1989; Wilson et al., 1989). This study agrees with these reports that pullets fed ED during rearing achieved first egg (sexual maturity) one week prior to the pullets fed SAD during rearing.

The development of the reproductive tract during rearing and at time of photo-stimulation is critical for broiler breeders. However, equally as critical can be the development of bones and

more specifically their medullary bones that act as a reservoir for calcium for birds during lay (Prondvia and Stein, 2014). During the rearing phase the pullet is developing muscle, bones, feathers, and their reproductive tract. Nutrition and the utilization of nutrients are critical for all aspects of development. Bone formation requires nutrients such as calcium, protein, magnesium, phosphorus, vitamin D, potassium, and fluoride (Palacious, 2006). In this study ED fed pullets had longer villi, greater villi height to crypt depth ratios, and higher and more uniform body weights, suggesting that better intestinal morphology of the ED fed pullets allowed for improved nutrient utilization as well as bone and reproductive tract development. We see the impacts of this nutrient utilization in improved shell quality measured by specific gravity of the ED verses the SAD fed pullets. If the pullets fed ED were able to deposit and build more dense medullary bone this is perhaps an explanation for the better overall eggshell quality when compared to the SAD fed pullets. Bone density and mineral content of the bones of ED and SAD pullets and hens should be evaluated in future studies to confirm this observation.

CONCLUSION

Feed restriction of broiler breeders is necessary due to genetic selection for increased growth of broilers; however, our use of high-density diets has made feed restriction of broiler breeders challenging. These feed restriction programs are critical for BW management, flock uniformity, and reproductive performance. The traditional SAD feed restriction program using these high-density feeds can lead to welfare issues and underperformance. In this study, we showed an improvement in achieving first egg, intestinal development and eggshell quality in hens fed a low-density high fiber diet ED as a pullet. In addition, ED fed pullets had increased BW at time of stimulation, improved flock uniformity, overall egg weight, overall specific gravity, and encouraged longer and more robust villi compared to the SAD fed pullets. We

conclude that the difference in these parameters mentioned are attributed to the method of delivery (SAD or ED) and the physiological impact on the bird utilizing a low-density high fiber diet.

REFERENCES

Ahiwe, E. U., A. A. Omede, M. B. Abdallh, and P. A. Iji. 2018. Managing dietary intake by broiler chickens to reduce production costs and improve product quality. Animal Husbandry and Nutrition. Chapter 6.

Aranibar, C. D., C. Chen, A.J. Davis, W.I. Daley, C. Dunkley, W.K. Kim, C. Usher, A.B. Webster, and J.L. Wilson. 2020. Impact of an alternate feeding program on broiler breeder pullet behavior, performance, and plasma corticosterone. Poult. Sci. 99:829-838.

Aviagen. 2016. Ross 708 Aviagen Parent Stock Nutrient Specifications. Aviagen Inc., Huntsville, AL.

Aviagen. 2016. Ross 708 Aviagen Parent Stock Performance Objectives. Aviagen Inc., Huntsville, AL.

Bartov, I., S. Bornstein, Y. Lev, M. Pines, and J. Rosenberg. 1988. Feed restriction in broiler breeder pullets: Skip-a-day versus skip-two-days. Poult. Sci. 67:809–813.

Bischoff S. C., G. Barbara, W. Burrman, T. Ockhuizen, J. D. Schulzke, M. Serino, H. Tilg, A. Watson, and J. M. Wells. 2014. Intestinal permeability – A new target for disease prevention and therapy. BMC Gastroenterol. 14:189.

Chiou, P. W., T. W. Lu, J. C. Hsu, and B. Yu. 1996. Effect of different sources of fiber on the intestinal morphology of domestic geese. Asian-Austral. J. Anim. Sci. 9:539-550.

Dale, N., and L. H. Fuller. 1984. Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations. Poult. Sci. 63:1008-1012.

de Beer, M., and C. N. Coon. 2006. The effects of increased protein intake during the starter and prebreeder periods on reproductive performance of ultra-high yield broiler breeder hens. Int. J. Poult. Sci. 5:812-821.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927-1939.

de Jong, I. C., and D. Guémené. 2011. Major welfare issues in broiler breeders. World's Poult. Sci. 67:73-82.

Denbow, D. M. 2015. Sturkie's Avian Physiology. Gastrointestinal anatomy and physiology. Chapter 14 page 354.

Ernst, R. A., P. Vohra, F.H. Kratzer, and O. Ibanga. 1994. A comparison of feeding corn, oats, and barley on the growth of white leghorn chickens, gastrointestinal weights of males, and sexual maturity of females. J. Appl. Poult. Res. 3:253-260.

Gilani, S., G. S. Howarth, C. D. Tran, R. Barekatain, S. M. Kitessa, R. E. A. Forder, and R. J. Hughes. 2017. Reduced fasting periods increase intestinal permeability in chickens. J. Anim. Physiol. Anim. Nutr. 102(1):486-492.

Han, H. Y., K. Y. Zhang, X. M. Ding, S. P. Bai, Y. H. Luo, J. P. Wang, and Q. F. Zeng. 2017. Effect of dietary fiber levels on performance, gizzard development, intestinal morphology, and nutrient utilization in meat ducks from 1 to 21 days of age. Poult. Sci. 96 (12):4333-4341.

Harms, R. H., A. F. Rossii, D. R. Sloan, R. D. Miles, and R. B. Christmas. 1990. A method estimating shell weight correcting specific gravity for egg weight in eggshell quality studies. Poult. Sci. 69: 48-52.

Harms, R. H., and F. J. Ivey. 1992. An evaluation of the protein and lysine requirement for broiler breeder hens. J. Appl. Poult. Res. 1:308-314.

Hocking P. M., and S. R. I. Duff. 1989. Musculo-Skeletal lesions in adult male broiler breeder fowls and their relationships with body weight and fertility at 60 weeks of age. Brit. Poult. Sci. 4:777-784.

Hou, Y., L. Wang, B. Ding, Y. Liu, H. Zhu, J. Liu, Y. Li, X. Wu, Y. Yin, and G. Wu. 2010. Dietary alpha-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. Amino Acids 39(2):555-564.

Hou, Y., L. Wang, W. Zhang, Z. Yang, B. Ding, H. Zhu, Y. Liu, Y. Qiu, Y. Yin, and G. Wu. 2012. Protective effects of N-acetylcysteine on intestinal function of piglets challenged with lipopolysaccharide. Amino Acids 43(3) 1233-1242.

Hudson, H. A., J. L. Wilson, G. N. Rowland, R. J. Buhr, and W. M. Britton. 1999. Feed restriction affects bone properties of the broiler breeder pullet femur. J. Appl. Poult. Res. 8:400-407.

Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel. 1989. Restricted feeding in early and late feathering chickens. 2. Reproductive responses. Poult. Sci. 68:352–358.

Kiarie, E. G., and A. Mills. 2019. Role of feed processing on gut health and function in pigs and poultry: conundrum of optimal particle size and hydrothermal regimens. Front. Vet. Sci. 6: 19.

Knudsen, K., E. Bach, and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. Digestibility and bulking properties of polysaccharides and other major constituents Br. J. Nutr. 65: 217-232.

Knudsen, K., and E. Bach. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. and Tech. 67 (4): 319-338.

Kumar, V., A. K. Sinha, H. P. S. Makkar, G. de Boeck and K. Becker. 2012. Dietary role of non-starch polysaccharides in human nutrition: A review. Crit. Rev. Food. Sci. and Nutr. 52(10):899-935.

Leterme, P., E. Froidmont, F. Rossi, and A. Thewis. 1998. The high water-holding capacity of pea inner fibers affects the ileal flow of endogenous amino acids in pigs. J. Agric. Food Chem. 46(5) 1927-1934.

Lopez, G., and S. Leeson. 1995. Response of broiler breeders to low-protein diets. 1. Adult breeder performance. Poult. Sci. 74:685-695.

Palacios, C. 2006. The role of nutrients in bone health, from A to Z. Crit. Rev. Food. Sci. Nutr. 46(8):621-628.

Prondvai, E., and K. Stein. 2014. Medullary bone-like tissue in the mandibular symphyses of a pterosaur suggests non-reproductive significance. Sci. Rep. 4: 6253.

Richards M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMutry. 2010. Feed intake regulation for the female broiler breeder: in theory and in practice. J. Appl. Poult. Res. 19:182-193.

Robinson F. E., J. L. Wilson, M. W. Yu, G. M. Fasenko, and R. T. Hardin. 1993. The relationship between body weight and reproductive efficiency in meat-type chickens. Poult. Sci. 72:912-922.

Savory C. J., P. M. Hocking, J. S. Mann, and M. H. Maxwell. 1996. Is broiler breeder welfare improved by using qualitative rather than quantitative food restriction to limit growth rate? Anim. Welfare 5:105-127.

Seyyedin, S., and M. Nazem. 2017. Histomorphometric study of the effect of methionine on small intestine parameters in rat: and applied histologic study. Folia. Morphologica. 76(4) 620-629.

Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feeding stuffs. Poult. Sci. 55:303-308.

Sklan, A. S., and I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br. Poult. Sci., 4:735-740.

Tejeda, O. J., and W. K. Kim. 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens, Poult. Sci. 99(12):6828-6836.

Tejeda, O. J., and W. K. Kim. 2021. Effects of fiber type, particle size, and inclusion level on the growth performance, digestive organ growth, intestinal morphology, intestinal viscosity, and gene expression of broilers. Poult. Sci. 100 (10):101397.

Teng, P. Y., C. L. Chang, C. M. Huang, S. C. Chang, and T. T. Lee. 2017. Effects of solid-state fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on growth performance and intestinal microbiota in broiler chickens. Italian J. of Anim. Sci. 16:552-562.

van de Sluis, W. 2011. Revival of the chain feeder. Poultry World.

Wilson, J. L. 2003. Feeding breeders, theory, and practical application. University of Georgia Cooperative Extension Service. Poultry Tip.

Wilson, H. R., D. R. Ingram, F. B. Mather, and R. H. Harms. 1989. Effect of daily restriction and age at initiation of a skip-a-day program for young broiler breeders. Poult. Sci. 68:1442–1446.

Winer, B. J. 1971. Statistical Principles in Experimental Design. Second Edition, New York: McGraw-Hill Book Co.

Yamauchi, K., H. Kamisoyama, and Y. Isshiki. 1997. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. Br. Poult. Sci. 38: 225-228.

Yao, K., Y. Yin, X. Li, P. Xi, J. Wang, J. Lei, Y. Hou, and G. Wu. 2012. Alpha-ketoglutarate inhibits glutamine degradation and enhances protein synthesis in intestinal porcine epithelial cells. Amino Acids 42(6): 2491-2500.

Table 1. Composition of diets.

Ingredients, %	Grower	Pre-Breeder	Layer
Corn, coarse ground	57.21	59.20	60.39
Wheat middling's	16.03	16.39	7.00
Soybean meal	14.15	14.02	17.00
Oats, whole	8.00	4.00	4.00
Defluorinated Phosphorus	1.68	1.67	-
Dicalcium Phosphate	-	-	1.70
Limestone (fine)	1.20	2.49	3.80
Oyster shell (coarse)	-	0.50	3.80
Soybean oil	0.50	0.50	1.30
Salt	0.30	0.30	0.30
Choline chloride 60%	-	-	0.10
Trace mineral premix[1,2]	0.08[1]	0.08[1]	0.10[2]
Vitamin Premix[3,4]	0.80[3]	0.80[3]	0.03[4]
D3 premixed hand-add[5]	-	-	0.25[5]
DL-methionine	0.05	0.05	0.19
L-threonine	-	-	0.04
Total	100.0	100.0	100.0
Calculated Analysis			
Crude Protein, %	15.4	14.7	14.8
Ca, %	1.07	1.50	3.40
P, %	0.72	0.80	0.60
Available P, %	0.44	0.40	0.30
Metabolizable energy, kcal per kg of diet	2,820.0	2,820.0	2,735.0
Digestible lysine, %	0.72	0.70	0.60
Digestible methionine, %	0.29	0.43	0.40

[1]Supplied per kg of diet: Mn, 107.20 mg as Mn sulfate; Fe, 21.25 mg as ferrous sulfate; Cu, 3.20 mg as basic copper chloride; I, 0.80 mg as calcium iodate; Se, 0.32 mg as sodium selenite.
[2]Supplied per kg of diet: Mn, 109 mg as Mn sulfate; Zn, 90 mg as Zn sulfate; Fe, 27 mg as ferrous sulfate; Cu 7 mg as basic Cu chloride; I, 1.3 mg as ethylenediamine hydroiodide; Se, 0.3 mg as sodium selenite.
[3]Supplied per kg of diet: vitamin A, 17600 IU; vitamin D3, 3527 IU; vitamin E, 35.3mg; vitamin B12, 0.04 mg; thiamine, 7.0 mg; riboflavin, 14.1 mg; menadione, 3.5 mg; vitamin B6, 7.0 mg; niacin, 141.0 mg; panthothenic acid, 35.2 mg; folic acid, 1.8 mg; biotin, 0.4 mg.
[4]Supplied per kg of diet: vitamin A, 11,000 IU; vitamin D3, 2,240 IU; vitamin E, 63 mg; vitamin K, 4.2 mg; thiamine, 3 mg; riboflavin, 12 mg; pyridoxin, 4.2 mg; cobalamin, 0.03 mg; niacin, 55 mg; pantothenic acid, 18.3 mg; folic acid, 2.8 mg; biotin, 0.46 mg.
[5]Supplied per kg of diet: 2,760 IU of D3 in addition to 2,240 IU already provided.

Table 2. The mean body weight of pullets (g) at 4, 8, 12, 16 and 20 wk of age as affected by the skip-a-day and every-day feeding program (SAD and ED).

Weeks	SAD[c]	ED[d]
4	354.8	326.9
8	689.7	645.6
12	1057.8	1019.6
16	1474.6	1461.0
20	1895.6[a]	1948.1[b]

[a- b] Treatments significant within period (P<0.05).
[c]SAD = Skip-a-day feeding program.
[d]ED = Every-day feeding program.

Table 3. The coefficient of variation of body weight (%) at 4, 8, 12, 16, and 20 wk of age during the rearing period as affected by the different feeding programs skip-a-day and every-day feeding program (SAD and ED).

Weeks	SAD[c]	ED[d]
4	22.5[a] (n=429)	20.5[a] (n=431)
8	18.8[a] (n=407)	16.1[a] (n=418)
12	17.9[b] (n=406)	13.1[a] (n=415)
16	16.8[b] (n=393)	11.9[a] (n=404)
20	15.8[b] (n=350)	11.2[a] (n=379)

[a- b] Treatments significant within period ($P<0.05$).
[c]SAD = Skip-a-day feeding program.
[d]ED = Every-day feeding program.

Table 4. Crude protein, amino acid content, and digestibility coefficient of whole oats.[1]

Amino Acid	Feed Content (%)	Digestibility Coefficient (%)
Crude Protein	9.82	
Alanine	0.450	79.7
Arginine	0.580	91.2
Aspartic Acid	0.760	84.0
Cysteine	0.310	80.1
Glycine	0.480	4.4
Glutamic Acid	1.820	91.5
Histidine	0.210	89.5
Isoleucine	0.370	83.1
Leucine	0.690	87.5
Methionine	0.160	81.4
Phenylalanine	0.480	88.3
Proline	0.490	87.6
Serine	0.400	83.6
Threonine	0.320	81.8
Tryptophan	0.090	89.5
Tyrosine	0.240	87.4
Valine	0.510	81.5

[1]Values are reported on an as fed basis. The dry matter content of whole oats was 88%.

Table 5. Histolgical analysis data as affected by the feeding programs skip-a-day and every-day feeding (SAD and ED) at 8, 12, 16, and 20 weeks. Each value represents the mean of the villus height (µm) and crypt (µm) depth of the pullet. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Treatment	WK	Doudenum Villi	Doudenum Crypt	Jejunum Villi	Jejunum Crypt	Ileum Villi	Ileum Crypt
SAD[c] (n=10)	8	1864.0	188.7	1389.0	114.1[a]	1080.5[a]	135.5
ED[d] (n=9)		1873.1	193.6	1394.6	173.9[b]	1139.3[a]	131.0
SAD[c] (n=10)	12	2037.7	132.6	1410.0	133.7[a]	1010.3[a]	115.6
ED[d] (n=10)		1937.0	132.4	1313.0	138.8[a]	963.4[a]	111.6
SAD[c] (n=10)	16	2041.4	147.9	1443.4[a]	120.1[a]	1111.1[a]	134.3
ED[d] (n=10)		2060.0	147.5	1580.0[b]	139.4[a]	1005.3[b]	119.7
SAD[c] (n=10)	20	2071.9	160.1	1560.6	115.0[a]	1125.2[a]	131.8
ED[d] (n=10)		2015.3	141.6	1570.0	156.9[b]	1001.9[b]	122.1

[a- b] Treatments significant within period (P<0.05).

[c]SAD = Skip-a-day feeding program.

[d]ED = Every-day feeding program.

Table 6. Histological data as affected by the feeding programs skip-a-day and every-day feeding (SAD and ED) at 8, 12, 16, and 20 weeks. Each value represents the mean ratio of the villus height to crypt depth expressed as a percentage. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Treatment	WK	Doudenum	Jejunum	Ileum
SAD[c] (n=10)	8	10.4	13.3[a]	8.9
ED[d] (n=9)		10.1	8.3[b]	9.1
SAD[c] (n=10)	12	16.1	11.2[a]	9.3
ED[d] (n=10)		15.1	10.5[a]	9.0
SAD[c] (n=10)	16	15.2	13.5[a]	8.7
ED[d] (n=10)		14.2	11.5[b]	8.9
SAD[c] (n=10)	20	13.7	14.1[a]	9.0
ED[d] (n=10)		15.3	11.0[b]	8.7

[a- b] Treatments significant within period (P<0.05).

[c]SAD = Skip-a-day feeding program.

[d]ED = Every-day feeding program.

Table 7. Necropsy data as affected by the feeding programs skip-a-day and every-day feeding (SAD and ED) at 8, 12, 16, and 20 weeks. Each value represents the mean of the organ weight expressed as a percentage of the body weight of the pullet. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Treatment	WK	Crop	Liver	Ileum
SAD[c] (n=10)	8	0.713[a]	1.893[a]	0.818[a]
ED[d] (n=9)		0.523[b]	1.660[b]	0.690[b]
SAD[c] (n=10)	12	0.635[a]	1.530[a]	0.594[a]
ED[d] (n=10)		0.520[b]	1.300[b]	0.521[a]
SAD[c] (n=10)	16	0.426[a]	1.136[a]	0.486[a]
ED[d] (n=10)		0.361[a]	1.125[a]	0.367[b]
SAD[c] (n=10)	20	0.456[a]	1.524[a]	0.461[a]
ED[d] (n=10)		0.398[a]	1.119[b]	0.337[b]

[a- b] Treatments significant within period (P<0.05).

[c]SAD = Skip-a-day feeding program.

[d]ED = Every-day feeding program.

Figure 1. Egg production curve as affected by the different feeding programs. Feeding programs were are as follows: SAD = skip-a-day feeding program **(—)**, ED = every-day feeding program (- -). Each value represents the mean percentage of total eggs produced within each feeding program. There were significant differences (P<0.05) between the feeding programs at 26 and 39 wk of age and no overall differences for the production period.

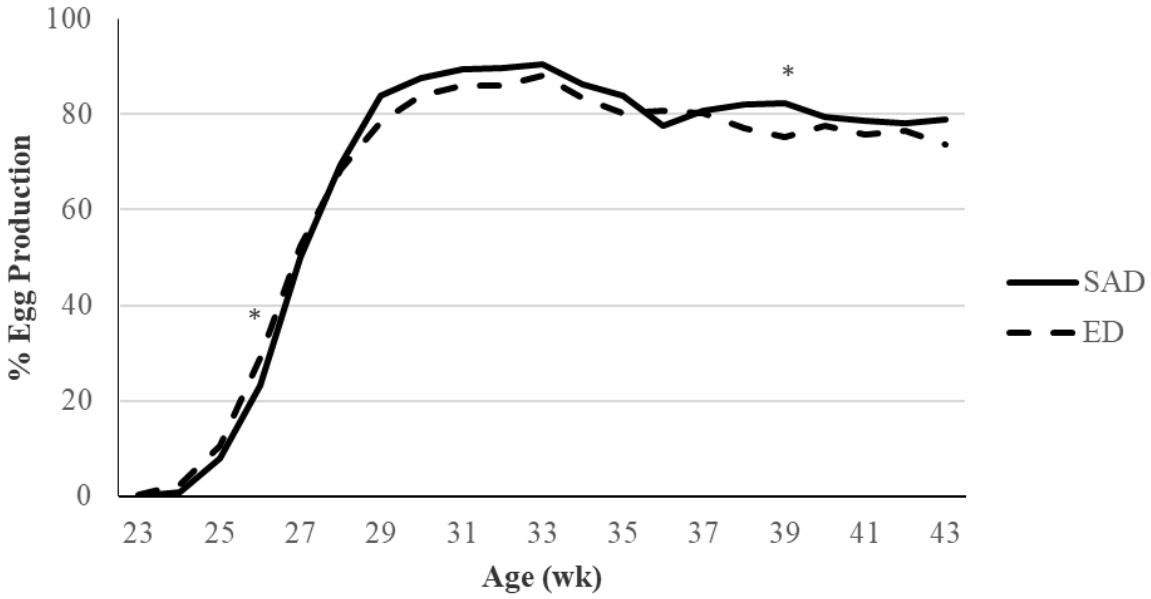


Figure 2. Mean egg weight affected by different feeding programs. Feeding programs are as follows: SAD = skip-a-day feeding program **(—)**, ED = every-day feeding program (- -). Each value represents the mean weight of total eggs produced on a single day within each feeding program. The means for the overall period are 60.4 g and 59.1 g for the ED and SAD feeding programs, respectively. There were significant differences (P<0.001) for the overall production period between the feeding programs.

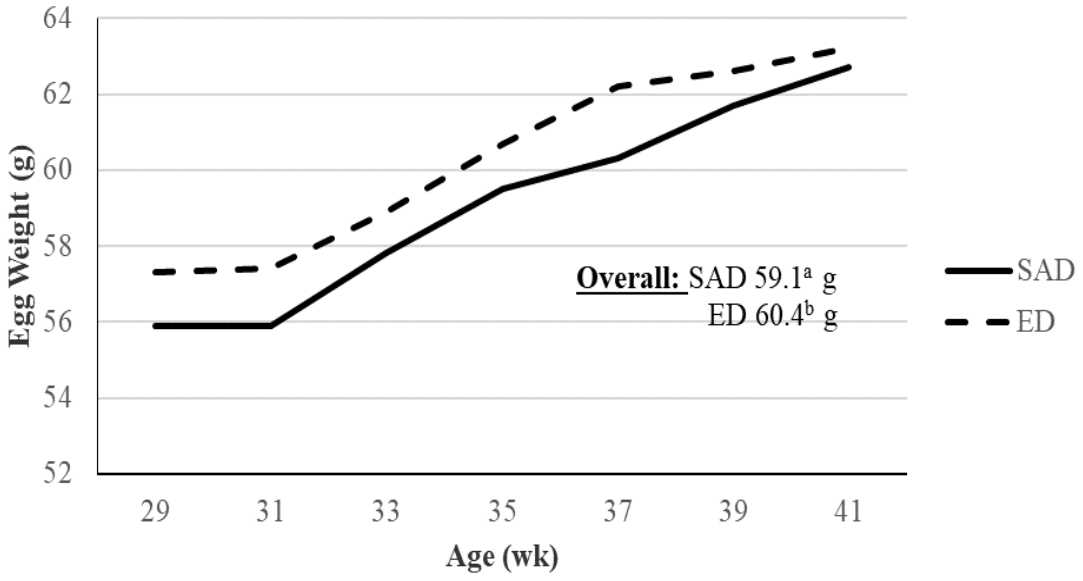
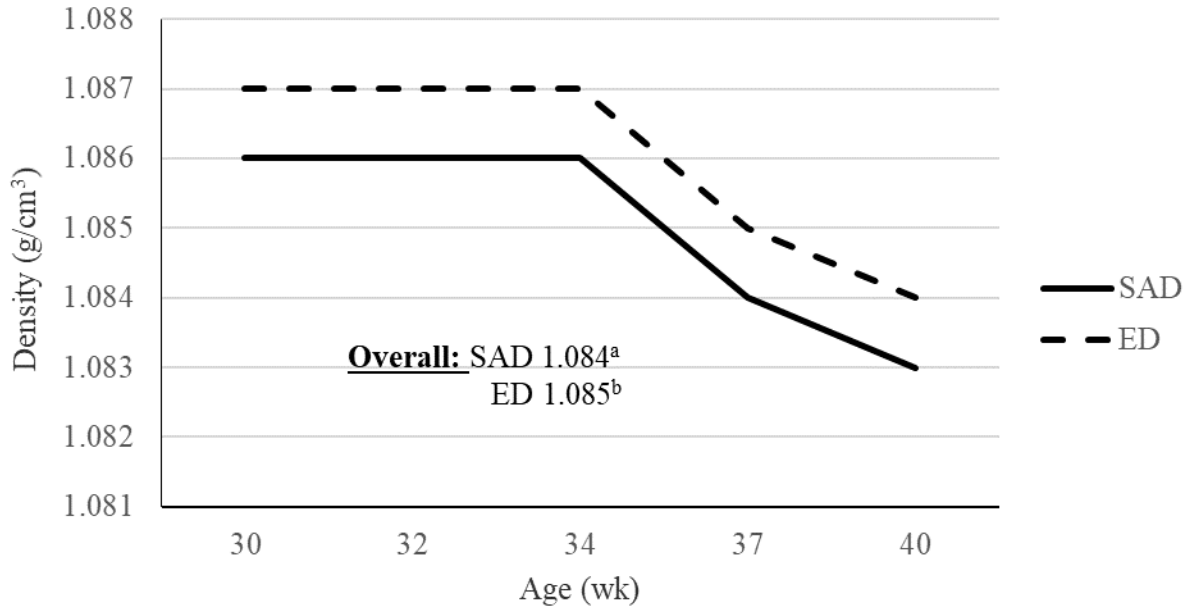


Figure 3. Mean eggshell density affected by different feeding programs during rearing. Feeding programs are as follows: SAD = skip-a-day feeding program **(—)**, ED = every-day feeding program (- -). Each value represents a weighted average of eggshell density of the total number eggs within each feeding program. Overall specific gravity was different between treatments (P=0.02).



CHAPTER 4

DIETARY INFLUENCE ON ROOSTER SEMEN QUALITY AND REPRODUCTIVE PERFORMANCE

[1]Sweeney, K. M., C. D. Aranibar, A. P. Benson, M. S. Franca, B. P. Hudson, B. Fancher, S. Garcia-Gomora, and J. L. Wilson. To be submitted to *Poultry Science*.

ABSTRACT

In the US, male broiler breeders are sometimes fed a female diet out of convenience, but roosters have specific dietary requirements not met by a hen formulated diet that has too high a level of calcium (Ca) or crude protein (CP) for roosters. In this study, a hen basal diet with 2750 kcal/kg, 14 % CP, and 3 % Ca and a male basal diet with 2750 kcal/kg, 12.4 % CP, and 0.70 % Ca were mixed. The hen basal was split into two diets providing either 110 ppm zinc from zinc sulfate (Hen-ZnS) exclusively or a 50:50 combination of organic zinc and zinc sulfate (Hen-OZn) in the mineral premix. The male basal diet was split into two treatments: one with 0.018 % DHA inclusion/ton and one without. A total of 63 Ross Yield Plus roosters were assigned to each dietary treatment (9 replicates, 7 roosters per replicate) starting at 25 wk of age. Approximately 25 to 30% of males in each treatment were weighed weekly. Percentage of roosters producing semen, semen volume, sperm concentration, and sperm mobility were measured every 4 wk from 33 to 65 wk. Individually caged Ross 708 hens were artificially inseminated with 0.05 mL pooled and diluted semen from a replicate group of 7 males. Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 days' post-insemination and incubated. An objective of this study focused on whether the Organic Zn or DHA supplemented diet would improve reproduction parameters, as well as the morphological structure of the kidneys and testes. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was $P \leq 0.05$. Neither treatment diet had a significant influence on body weight, semen volume, concentration, or mobility to 65 wk of age. Semen production was influenced by the STDm diet at 31 wk with 6.3 % more males in semen production on that diet. There were significant differences between treatments for testes and kidney histomorphology. Specific diet formulations

for roosters may be warranted to enhance reproduction, but the mix of Zn sulfate and organic Zn had limited impact on reproductive measurements in this trial.

Key Words: Rooster diet, DHA, Organic Zinc, Calcium, Semen Quality

INTRODUCTION

Adult broiler breeder males are often fed a hen diet that exceeds their nutritional requirements for protein, amino acids, and calcium instead of a lower-density male ration. Rooster fertility is a complicated research area, especially when birds are evaluated in a floor setting because of the impact that sexual behavior of both roosters and hens has on fertility of naturally mating birds. Improving flock fertility is the ultimate goal. In this study evaluating rooster semen volume, sperm mobility, and longevity of semen production across a population of roosters are important evaluations of whether dietary changes are influencing potential fertility. The majority of roosters in the US poultry industry are fed a hen diet that is specifically formulated for egg production, and results in too dense a nutrient consumption making it more difficult to control rooster body weight. This may lead to body weight uniformity problems, high mortality, the need to cull or remove roosters, more spiking and lower flock fertility. In addition to diet density, hen diets typically have nearly 2 % more calcium to support strong eggshells. Roosters do not need the higher calcium in the diet, and it has been speculated that high calcium consumption levels might harm semen production. Increased Ca levels in broiler breeder male diets have shown increased prevalence of epididymal stones which causes reproductive dysfunction (Janssen et al., 2000; Mahecha et al., 2002).

Investigating the impacts of nutritional factors on reproduction, semen quality and fertility on broiler breeder males is critical for better management practices. A broiler breeder male is responsible for over 1,000 fertile eggs in a production period (McGary et al., 2002) which can directly affect the number of broilers placed. Nutritional factors such as Ca, fats, trace minerals, amino acids, and probiotics can play a positive or negative role in rooster reproduction and fertility depending on how they are mixed and fed (Fouad et al., 2020).

Trace minerals are important for many physiological functions, but one of the most important for broiler breeder males and females is reproduction. Trace minerals facilitate enzyme development, hormonal regulation, gonadal development, and much more. Among these critical trace minerals for physiological functions is zinc (Zn) which must be added to the diet due to the low Zn values in feedstuffs. In years past, inorganic forms of Zn such as Zn sulfate or Zn oxide have been used. Zn is needed for good egg production, hatchability, proper feathering, and growth (Coon, 2001). Dietary Zn is also very important for cell division to produce viable spermatozoa. In addition, Zn is needed for testosterone metabolism, testicle development, sperm production, sperm motility, and reducing excess estrogen in male reproductive tissues (Amen and Al-Daraji, 2011). Zn also impacts gonadotropic hormones through pituitary influence (Hurley and Doane, 1989). In a recent study it was found that organically bound trace minerals such as Zn may be absorbed more effectively via the amino acid or peptide transport pathways which leads to greater utilization (Muszynski et al., 2018).

Docosahexaenoic acid (DHA) is a long chain omega-3 fatty acid that has proven to have health benefits for soft tissue development such as the brain in a developing human fetus as well as helping reduce inflammation (Horrocks and Yeo, 1999). Feeding a DHA supplement to layers enriches the egg with an omega-3 fatty acid profile, and when fed to broilers changes the fatty acid profile of the meat. DHA can be found in fish oil which has been proven to significantly benefit broiler breeder males. Cobb broiler breeder males fed a Menhaden oil supplemented diet had higher fertility as well as the omega-3 to omega-6 fatty acid ratio was greatly influenced (Hudson and Wilson, 2003). In addition to other findings, polyunsaturated fatty acids like DHA have been shown to increase semen volume, semen concentration, and increased sperm mobility (Surai et al., 2000; Cerolini et al., 2005). Due to organoleptic problems, cost, and product

stability, using an alternative to fish oil that is equally as effective is a thriving area of research. DHAgold™ (DSM) is a dried whole-cell algae product that contains high levels of docosahexaenoic acid (DHA). Using a powdered form of DHA with a long and stable shelf life could potentially make it easier for feed mills to use and store while also improving bird performance. The dietary objectives of this study were:

1. Evaluate the impact of a diet tailored to male's dietary needs on semen quality, flock fertility, and histomorphology of kidneys and testicles compared to males fed a hen diet.

2. Evaluate the semen quality, flock fertility, and histomorphology of kidneys and testicles of roosters fed a hen diet supplemented with organic zinc compared to males fed a standard hen diet.

3. Evaluate the impact of a DHA supplemented diet on semen quality, flock fertility, and histomorphology of kidneys and testicles compared to a standard male diet.

MATERIALS AND METHODS

A total of 350 Ross Yield Plus cockerels were raised in two floor pens (7.3 x 4.6 m^2, 175 cockerels) with new pine shavings in an environmentally controlled solid side walled poultry house. At 4 wk, they were allocated to a traditional skip-a-day feeding program. The two pens were housed in the same room separated by a chain-link partition and fed on the same feeding program. All birds were fed a common standard starter ration (2,910 kcal/kg, 18 % CP) for the first 4 wk of age, followed by a grower diet (2,820 kcal/kg, 15 % CP) to 25 wk of age (Table 1). During rearing, the cockerels ate from a chain feeder (14.3 m) and water was provided *ad libitum* by nipple drinker line (6.1 m with 40 nipples). All birds were wing banded at 4 wk of age to

track growth rate. The photoperiod to 22 wk of age was 23 h of light:1 h of darkness (23 L:1 D) for the first 3 d, followed by an 8 L:16 D pattern until 22 wk of age. The photoperiod was increased to 15.25 L:8.75 D at 22 wk of age and remained constant until the end of the study at 65 wk of age.

At 22 wk of age 252 cockerels were transferred into individual rooster cages (59.7×58.4× 35.5 cm; 1 rooster per cage). The facility was enclosed with forced air heaters and evaporative cooling to keep the environment comfortable for the birds. Water was supplied free choice by a nipple drinker. Each treatment was represented by 9 replicate groups of 7 roosters. The body weight (BW) and variation of body weight (coefficient of variation, CV) across all treatments were similar at the start of the dietary treatments. To prevent feed from moving within the feed trough, an extra rooster was positioned between the feed treatments that created a physical barrier to prevent roosters within feed treatment consuming the incorrect feed. All males were fed one of four diets. A common breeder hen basal and male basal diet was mixed based on Aviagen standards (Aviagen, 2016a). The hen basal was divided into 2 treatments: a hen diet with Zn sulfate (Hen-ZnS: 2,750 kcal/kg, 14.1 % CP, 3.0 % Ca, 110 ppm Zn Sulfate), and a hen diet with a 50:50 mix of Zn sulfate and organic Zn (Hen-OZn: 2,750 kcal/kg, 14.1 % CP, 3.0 % Ca, 55 ppm Zn Sulfate + 55 ppm Organic Zn) (Table 2). Hen-ZnS was used at the standard control diet since it is commonly fed in the industry. The male basal was also divided into two treatments: a standard male diet (STDm: 2,750 kcal/kg, 12.4 % CP, 0.70 % Ca) and a standard male diet with DHA (STD-DHA: 2,750 kcal/kg, 12.4 % CP, 0.70 % Ca, 0.045 % DHA) (Table 3). The roosters were fed the treatment or control diets from 25-65 wk. Males on the hen diet were switched to a breeder 2 diet at 40 wk of age that increased the Ca to 3.24 %. Birds were vaccinated using a standard industry vaccination program.

From 26 to 32 wk of age, roosters were allowed to habituate to the cages as well as to the handlers for semen collection via abdominal massage. During this time the rooster's vent feathers were trimmed to help ensure semen samples were as free from foreign material and feces as possible. Semen production, quality, and volume was evaluated on every 4 wk basis from 32 to 65 wk of age. Feeding the STDm diet to adult roosters versus those on a hen diet, allowed us to evaluate if the high crude protein and calcium had an impact on the number of roosters that matured and stayed in semen production. At wk 45, 55, and 65 semen was pooled from each replicate group and analyzed for lipid peroxidation by a Thiobarbituric Acid Reactive Substances (TBAR) test.

At 65 wk of age all males were euthanized, weighed and testes were removed and weighed. A section of the kidney was also removed during necropsy, and testes and kidney samples were placed in a 10 % formalin solution for histological analysis of normal or abnormal spermatozoa production. All procedures were approved by the University of Georgia Animal Care and Use Committee.

Body Weight and Uniformity

A sample body weight (BW) (25 % of the birds from each pen) was taken weekly and all birds were weighed at 4, 8, 12, 16, and 20 wk of age during rearing. Once photostimulated, 25 % of the birds from each treatment were weighed weekly through 65 wk of age. CV for BW was calculated on a per pen basis during rearing as a measure of flock uniformity. Post photostimulation, BW for each treatment was calculated on a treatment basis (n=7 birds per rep, 63 birds per treatment and total of 252 roosters).

Testosterone Determination from Blood Plasma

At 53 wk of age blood was collected from the brachial vein and placed in an ethylenediaminetetraacetic acid (ETDA) vacutainer (Becton Dickinson, Franklin Lakes, NJ). The blood samples were placed on ice immediately after collection, later centrifuged at 1000 x g 4° C for 10 min. Plasma was subsequently collected after centrifugation and stored in Eppendorf tubes at -80° C. A free testosterone AccuBind® ELISA test (Monobind Inc., Lake Forest, CA) was used as a quantitative measurement of free testosterone via an Enzyme Immunoassay (EIA). This was used as a more accurate hormone analysis than total testosterone levels because free hormone is a more accurate measure of biological activity since it is not bound to transport proteins.

Malondialdehyde analysis using Thiobarbituric Acid Test

Semen was collected free of contaminants using abdominal massage from one replicate of roosters at a time (7 roosters). Immediately after collecting semen, 200 µL of semen from each male per replicate was pipetted into a 16 x 100 mm test tube. Semen samples were brought up to an equivalent volume of 4 mL with ice cold phosphate buffered saline solution (PBS) with 1 mM of EDTA. Sperm was washed with PBS (w/EDTA) at 1500 x g 4° C for 20 min. A Lowery assay was performed on a portion of the lysate to equilibrate total protein of the samples and the remainder of the sample was stored at -80° C. Each replicate group was analyzed for lipid peroxidation by a TBARS analysis using a QuantiChrom TBARS Assay Kit (Bioassay Systems, Hayward, CA).

Fatty Acid Profile Determination

Semen was collected via abdominal massage and prepared for analysis as described by (Hudson and Wilson, 2003). The fatty acid profiles of the diet and semen samples were

determined by gas chromatography (Shimadzu, model 14 A, Tokyo, Japan) with a flame ionization detector, as described previously (Cromwell et al., 2011). Samples were transmethylated according to the methods of Park and Goins (1994) and 2 mg of tridecanoic acid (C13:0) was added as an internal standard before processing. Methyl esters were isolated in hexane, anhydrous sodium sulfate was added to remove any residual water, and samples were stored at 4° C until analyzed.

Fatty acid methyl esters were separated on a Phenomenex, ZBWax Plus wide-bore capillary column (60 m × 0.53 mm, 1.00 μm film thickness; Phenomonex, Torrance, CA) with nitrogen as the carrier gas. Initial column temperature was 160° C, temperature was held for 10 min and increased at a rate of 5° C/min until 220° C. Injector temperature was 250° C and detector temperature was 260° C. Peaks were identified by comparison of retention times of known standards (Nu-Chek Prep, Elysian, MN).

Fertility Determination

Individually caged Ross 708 hens that were of equivalent age to the roosters were artificially inseminated with 0.05 mL of pooled and diluted semen from a replicate group of 7 roosters at 46, 52, 56, 60, and 64 wk of age. At each evaluation age, hens were artificially inseminated once with semen from the same assigned group of males each time. If ejaculates were less than 50 μL they were discarded. Otherwise, semen from each male within a replicate of 7 males was pooled then diluted accordingly. Semen was diluted using Beltsville Chicken Extender II (Continental Plastic Corp, Delavan, WI) to a minimal dose of 7.5×10^7 sperm concentration. Semen from each replicate was used to inseminate a maximum of 8 hens. Eggs were collected for 14 days (2-14 d) post a single insemination and incubated. Eggs were

collected 3 to 4 times per day by individual cage. Eggs were sorted and incubated by replicate group. After each insemination, eggs were stored for no more than 4 d. From each day after insemination all eggs were set in a Natureform incubator (Natureform Inc., Jacksonville, FL) at 37.5° C and 53 % relative humidity. Fertility was determined by gross evaluation after opening the eggs that had been incubated for 5 to 7 d.

Semen Production and Sperm Mobility Index

At 25 wk of age, all roosters were trained to the semen collection process using the abdominal massage method (Burrows and Quinn, 1937). Semen was collected weekly through 65 wk of age. The percentage of males producing semen was calculated as a percentage of the live males at each collection period everting the phallus and the presence of semen.

A 6% (wt/vol) Accudenz® was prepared along with a 320 mmol/kg mobility buffer as described by Froman and McLean (1996). Froman and McLean's technique was used to determine semen mobility and concentration of each individual rooster using a spectrophotometer SP-830 (Barnstead|Turner, Dubuque, IA).

Histology

At 65 wk of age all roosters were weighed and euthanized for necropsy. A portion of the kidney was removed and placed in 10 % formalin for further evaluation. Testicles were removed whole and weighed to calculate their relative percentage of overall BW. Post weighing, a 2 cm midsection of the testis containing a portion of the epididymis was placed in 10 % formalin.

For both the kidney and testicles, routine tissue processing, embedding, sectioning, and staining of slides was performed. Kidneys were measured for the presence of lesions in renal tubular necrosis (scaring or inflammation of tubules), tubular nephrosis (basophilic homogenous material), tubular mineralization (dark blue mineralized material in tubules) and interstitial

inflammation (scaring or inflammation in interstitial tissue). Testicles were measured for atrophy of seminiferous tubular epithelium (attenuated epithelium or no spermatogenesis), sperm retention (tangled mass of sperm cells), cystic dilatation of tubules (sac-like membranous tissue), interstitial inflammation (scaring or inflammation in interstitial tissue) and intratubular inflammation (scaring or inflammation within the tubules). The epididymis was analyzed for atrophy (degeneration of epithelium), interstitial inflammation (scaring or inflammation in interstitial tissue), intratubular inflammation (scaring or inflammation within the tubules) and lithiasis (calculus/epididymal stones) in the epididymis. The scoring method (0-4) was based on the percentage of tissue affected by the lesion (Table 4).

Statistical Analysis

Body weight, semen quality measurements, TBAR, and fertility were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Slice analysis specifies the effect to test for differences between interactions LS-mean, to produce tests of simple effects (Winer, 1971). This method evaluates the effect of the treatment and minimizing the impact of time (week). Differences were deemed to be significant when the P-value was less than or equal to 0.05.

RESULTS

HEN ZINC SULFATE TO STANDARD MALE (HEN-ZNS V STDM)

Body Weight and Uniformity

During rearing the BW was similar to the target BW according to the Aviagen guide (2016b) and flock uniformity was similar between pens (12.1 CV). While males were on the different dietary treatments, BW of the roosters on the Hen-ZnS diet were consistently heavier than those on the STDm diet during the treatment period (25-65 wk) (Figure 1). CV was not

significantly different between the treatments during the production period (25-65 wk) (data not shown).

Testosterone Concentration

Testosterone is important for the development of testicles as well as secondary sex characteristics in birds. When testosterone levels decrease this can impact semen production as well as *libido*. Testosterone levels were measured at 53 wk when males are considered past their reproductive peak to determine if the STDm diet would increase the longevity of testosterone concentrations. No significant differences (P=0.256) were found in testosterone concentrations between the males fed the STDm diet (0.334 ng/mL) and those fed the Hen-ZnS diet (0.576 ng/mL).

Thiobarbituric Acid Test

Free radical cell damage is of great concern in broiler breeder male reproduction. Many types of stress can create the antioxidant and free radical imbalance that causes irreputable damage to the spermatozoa. A TBAR analysis was performed to detect malondialdehyde (MDA) levels between the STDm diet and the Hen-ZnS. This was to determine if increased calcium levels in a hen diet causes unwarranted stress on the males because their body process and excrete the excess calcium. While there were no significant differences at 45, 55, and 65 wk or overall, the roosters consuming the Hen-ZnS diet consistently had greater levels of MDA when compared to the STDm roosters (Figure 2).

Sperm Mobility Index and Males in Production

All semen quality parameters are important however the simple measurement of semen production status is equally important. If the roosters are going out of semen production early or coming into semen production later, it limits their effectiveness or fertile egg numbers due to

fewer mating males in the flock. Roosters on the STDm diet came into semen production earlier than those on the Hen-ZnS (STDm 96.8 % at 31wk; Hen-ZnS 90.5 % at 31 wk) (Figure 3). While not significant, roosters fed the STDm diet consistently had a higher percentage of males in semen production compared to those roosters on the Hen-ZnS diet.

Semen mobility is a critical measure of normal morphology of spermatozoa and forward motion or movement of the spermatozoa. If the mobility index is low, this would indicate deformed or abnormal spermatozoa and less movement. There were no significant differences between the Hen-ZnS male and the STDm males for sperm mobility or concentration (data not shown).

Fertility

The fertility analysis performed was to replicate a worst-case scenario. For instance, if a hen were only being mated once every 14 d or if male to female ratios were low in a house this measure of fertility would be similar to this real-world scenario. By increasing the overall spermatozoa quality through feed supplementation, the goal is to encourage more healthy spermatozoa to be stored in the hens' sperm storage tubules as well as make its way to the infundibulum for fertilization. There were no significant differences in fertility between the males on the STDm diet and the Hen-ZnS diet at any insemination time point (data not shown).

Histology

The kidney, epididymis, and testis of each rooster were submitted for histological analysis to determine if feeding males, a female diet would impact these tissue morphologies. The use of a high calcium diet such as the Hen-ZnS would cause damage to the kidneys and increase the prevalence of epididymal stones which is supported by Janssen et. al. (2000). For the kidneys, the males fed the Hen-ZnS had significantly higher presence of lesions ($P<0.05$) in the

categories of tubular nephrosis and interstitial inflammation (Table 5). However, in the testes we observed significantly higher levels ($P<0.05$) of lesions scores in males fed the STDm diet in the categories of sperm retention and cystic tubules (Table 6). In the epididymis, the only significant difference ($P<0.05$) observed was inflammation in the tubules with those males fed the STDm diet having an increased prevalence of lesions when compared to the Hen-ZnS males.

HEN ZINC SULFATE TO HEN ORGANIC ZINC (HEN-ZNS V HEN-OZN)

Body Weight and Uniformity

During rearing the BW was very similar to the target BW (Aviagen guide, 2016b). Flock uniformity was likewise similar between pens (12.1 CV). BW of the roosters were not significantly affected by the treatment diets (25-65 wk) (Figure 4).

Testosterone Concentration

Testosterone is essential for regulation of libido and production of sperm, and Zn aids in the balance of this hormone. Testosterone was measured at 53 wk of age. In this study, there were no significant differences in the testosterone concentrations between treatment groups (Figure 5).

Thiobarbituric Acid Test

Malondialdehyde occurs as a result of lipid peroxidation of fatty acids. Lipid peroxidation in rooster reproduction can degrade the spermatozoa within the sperm storage tubules (Surai et al., 1998). Zn is crucial in the diet of these birds since it is important for sperm oxidative metabolism as well as lipid and sperm membrane stability (Chia et al., 2000 and Fallah et. al, 2018). The TBAR test was used on semen to analyze oxidation at three different critical time points in this study. There were no significant differences at wk 45, 55 or 65 as well as there were no overall significant differences between the two dietary treatments (data not shown).

Sperm Mobility Index and Males in Production

Zinc is important for many physiological functions and for this study the focus was on zinc's potential impact on semen quality and semen production. Testing for semen mobility gives an accurate indication of spermatozoa's overall structural integrity. A significant difference (P=0.042) in sperm mobility was observed at 57 wk (0.416 absorbance for Hen-ZnS and 0.341 absorbance for Hen-OZn, respectively). Semen concentration is an indicator for healthy spermatogenesis. No significant differences were observed between the Hen-ZnS and Hen-OZn dietary treatments for semen concentration (data not shown). Males in production were not significantly impacted by these two dietary treatments (data not shown)

Fertility

Late in life fertility for broiler breeders is known to decline which leads to an increased need for spiking programs and potentially selling flocks early. There are many factors that contribute to flock fertility, but one of the most important factors for males is developing and maintaining healthy testicles is critical for flock fertility. The goal of using organic Zn was to encourage the maintenance of healthy testicles as well as reduce the sperms' oxidative metabolism (Fallah et al., 2018). Fertility was analyzed by day and week post-insemination as well as overall flock fertility. In week 1 post-insemination there were significant differences at wk 46 (P=0.032) and 56 (P=0.0004). At 46 wk Hen-OZn had higher fertility (80.0 %) than the Hen-ZnS (72.8 %) fed males. However, at 56 wk the Hen-ZnS had higher fertility (79.9 %) than the Hen-OZn (67.2 %) fed males. In week 2 post-insemination there were no significant differences among the fertility measurements between the two dietary treatments. There were significant differences observed in overall flock fertility between the treatments with the Hen-

ZnS fed males having better overall flock fertility than the Hen-OZn fed males (54.6 % Hen-ZnS and 51.9 % Hen-OZn, respectively).

Histology

As previously mentioned, zinc deficiency can lead to compromised testes morphology (Croxford et al., 2011). The use of organic zinc has been proven to improve zinc utilization as well as zinc retention in tissues (Muszynski et al., 2018). However, no significant differences were observed in the kidney, testes, and epididymis morphology when comparing the males fed the Hen-ZnS diet and those fed the Hen-OZn diet (data not shown).

STANDARD MALE DIET TO STANDARD MALE DIET WITH DHA (STDM V STD-DHA)

Body Weight and Uniformity

During rearing the BW was very similar to the target BW according to the Aviagen guide (2016b) and flock uniformity was similar between pens (12.1 CV). BW of the roosters were not significantly affected by the treatment diets (STDm v STD-DHA, 25-65 wk) (Figure 6).

Testosterone Concentration

A goal of using a diet tailored to the needs of a rooster is to assist in managing his body weight. Overweight males have more structural health issues and have difficulty in completing mating's due to excess BW. It has been shown that a male diet that is lower in protein improves male body weight management (Wilson et.al., 1987) and breast muscle deposition (Hocking, 1990). BW can negatively impact testosterone levels as well. In a study performed on humans, overweight males supplemented with a DHA product had increased testosterone levels. Previous research agrees that it is easier to control body weight with a male diet and with the added DHA product increase testosterone levels (Abbott et al., 2020). The STD-DHA supplemented roosters

(0.627 ng/mL) had significantly greater (P=0.008) testosterone concentrations when compared to males fed a STDm diet (0.334 ng/mL).

Thiobarbituric Acid Test

DHA has been proven to act as an antioxidant and significantly decrease the formation of malondialdehyde in the body (Véricel et al., 2003 and Li et al., 2021). As previously mentioned, oxidative stress that damages cells come from an imbalance of free radicals and antioxidant. The inclusion of DHA in these diets was to aid in reducing oxidative stress. There were no significant differences in malondialdehyde concentrations of semen between the STDm and STD-DHA males (data not shown).

Sperm Mobility Index and Males in Production

Improving semen quality was a major goal for using DHA in the feed with the thought that feeding DHA would act as an antioxidant and increase viable spermatozoa via analysis of semen concentration and mobility. Semen concentration was significantly different (P=0.03) at 62 wk with the males on the STD-DHA diet (3.53 bil/mL) having greater semen concentration than those on the STDm diet (3.05 bil/mL). Sperm mobility and males in semen production was similar between these two dietary treatments (data not shown).

Fertility

Penetration of the perivitelline membrane by the spermatozoa is vital for producing fertile eggs. Feeding a polyunsaturated acid (PUFA) has been proven to increase fertility by affecting the sperm membrane structure (Belsbois et al., 1997; Christensen et al., 1998). Fish oil has been a widely used product, but due to product instability and cost it is not common in breeder diets. Using a powdered DHA product assists in product handling issues as well as product sustainability. Wk 1 fertility post insemination was consistently numerically higher for the

roosters on the STD-DHA diet than those on the STDm diet (Figure 7). A significant difference ($P<0.05$) in fertility was seen at 62 wk with the males on the STDm diet having better fertility than those males on the STD-DHA. Overall fertility was higher for the males on the STD-DHA diet than those on the STDm diet (Figure 8).

Histology

There were significant differences ($P<0.05$) between those males fed the STDm diet having lower kidney lesion scores than those males fed the STD-DHA diet for the categories of tubular nephrosis and interstitial inflammation (Table 7). We also observed significant differences in the testis with those males fed the STD-DHA diet having significantly ($P<0.05$) lower lesion scores in all categories except interstitium inflammation and inflammation in tubules (Table 8). Also, those males fed the STD-DHA diet had significantly lower ($P<0.05$) lesion scores in the epididymis in atrophy of ducts and overall lesion scores (Table 9).

Fatty Acid

The fatty acid composition of the semen was influenced by the supplementation of DHA. Those males fed the STD-DHA diet had higher C22:6 n3 (DHA) content compared to those males fed the STDm diet (Table 10).

DISCUSSION

The results from this current research indicated that there were no negative impacts measured of feeding broiler breeder males a diet that is tailored to a hens' nutritional needs compared to those males fed a diet for their specific nutritional needs. Providing these broiler breeder males with the supplementation of either organic zinc at 110 ppm or DHA at 54 mg/day showed no significant improvement in weekly semen quality or fertility. However, based on the results of the overall fertility there is significant improvement in overall flock fertility when

feeding a male diet supplemented with DHA. Perhaps the level of DHA provided was not high enough or the DHA supplementation began too late to impact testicular development and spermatogenesis. According to Simpopulous et al. (1999) developing children need anywhere from 60 - 80 mg/day of DHA based on BW. We did not meet that level of supplementation of DHA, nor did we feed the product during critical times of reproductive development such as photostimulation (treatments began at 25 wk). Perhaps this potentially explains the limited differences observed in this experiment.

Feeding a hen formulated diet to broiler breeder males did impact the mean lesion scores for the kidneys influencing tubular nephrosis interstitial inflammation as well as total overall lesion scores by increasing their prevalence when compared to those males fed a male diet. We suspected based on previous literature that we would see the increased prevalence of lesion scores in the testes and epididymis of males fed the high calcium hen diet. However, the opposite was observed with males on the male diet having significantly increased inflammation and overall lesion scores.

Body weight

The recommendation for protein and calcium for a Ross 708 hen is 14-15 % CP and 3.0-3.5 % Ca depending on their age during the lay cycle while the Yield plus male only needs 11.5 % CP and 0.70 % Ca according to the Aviagen guidelines (2016a). Hens need protein for not only their growth and immunity, but also for egg production. Egg proteins are crucial for broiler breeder eggs because protein synthesis is essential during the early stages of embryogenesis (Alberts et al., 2002). While roosters need protein to maintain a healthy growth rate, their requirement for protein is presumably lower than a hen since the rooster does not have the daily task of making an egg. Increased dietary protein being fed to males, such as the protein level in a

hen ration, have been proven to significantly increase BW (Wilson et al., 1987) as well as significantly reduce the number of males in semen production (Wilson et al., 1971). Controlling male BW is critical for livability and successful copulation in meat strains. Due to the increased growth potential, more specifically for the deposition of breast muscle, high protein levels can increase muscle deposition making in physically difficult for roosters to mate. Having this increase in growth and difficulty in mating leads to the need for spiking programs which increases the risk of spreading disease. This also leads to multiple ages of birds in one flock which make it difficult to manage nutritional needs. In this study, male BW management is not a true representation of field flocks due to the lack of physical activity. However, seeing the consistencies in lower BW for the males fed the STDm diet versus those fed the Hen-STD we can see the potential for these diets to assist in managing BW.

Fertility

Fertility was one of the most critical measurements taken during this experiment. It has been well documented that as these broiler breeder males age flock fertility and semen quality decline significantly (Lake, 1989; Sexton et al., 1989; Rosenstrauch et al., 1994). Improving life of flock fertility would have significant monetary benefits for breeder farmers and poultry integrators. Males fed the Hen-ZnS diet had better overall flock fertility and fertility was significantly greater at 46 wk of age. However, males supplemented with organic zinc (Hen-OZn) had higher late in life fertility at 56 wk of age. Perhaps organic Zn supplementation improves late in life fertility by sustaining semen quality but has little impact on younger males when semen quality is at a natural peak in quality.

Those males fed the STD-DHA diet had better overall flock fertility when compared to the STDm fed males. This overall improvement in flock productivity indicates that feeding DHA

enhances reproduction in adult roosters. DHA is known to have anti-inflammatory characteristics, impact soft tissue development, as well as testosterone synthesis (Swanson et al., 2012 and Abbott et al., 2020). Given that weekly differences in fertility were not observed suggested that perhaps the males should have received the DHA supplementation in their feed prior to photostimulation in order to have maximum seminiferous cell proliferation during testicular development. Also, due to the anti-inflammatory characteristics of DHA and testosterone synthesis properties perhaps males needed a higher inclusion level in the diet, but especially later in life. The diet inclusion level coupled with the daily restriction of feed provided only 54 mg/day of DHA intake. Further research should be conducted to investigate the impact on early in life feeding of DHA and higher inclusion rates.

Histology and Testosterone

Based on the histological analysis we can infer that the higher calcium hen diet (Hen-ZnS) fed to broiler breeder males has a negative impact on tubular nephrosis, interstitial inflammation, as well as total lesion scores when compared to those males fed the lower calcium diet (STDm). Excess calcium is eliminated from the body in the form of uric acid which can negatively affect Ca channels in the kidneys (Tyler et al., 2021). Perhaps the significant increase in overall lesion scores in the kidney as well as the significant increase of the lesion scores in tubular nephrosis indicates Ca damage since the functional unit for eliminating waste and excess nutrients of the kidney is the nephron. While Ca is important for gamete function as well as sperm motility (Nguyen et at., 2016), other research has reported more negative impacts during the production period for roosters (25-65 wk) on sperm concentration, and testicular function when Ca is fed in excess (Hocking and Bernard, 1997; Tyler and Bekker, 2012).

While testosterone is important for many reproductive functions in the male, when measured late in life (53 wk) testosterone level was not different in roosters supplemented with these dietary treatments.

Semen Quality and TBAR

Sperm mobility is a critical component of fertility. Determining the mobility is an indication of the overall structure of the spermatozoa. If the mobility is lower that would indicate deformations that would drastically impact the spermatozoa's ability to successfully navigate the hen's reproductive tract to the site of fertilization in the infundibulum. While sperm mobility and concentration were not significantly different consistently week to week there was clearly evidence that semen quality was improved based on the fertility findings for those males on the STD-DHA diet. Perhaps this can be more strongly correlated with the overall decrease in lipid oxidation seen in the TBAR analysis of the semen which agrees with Blesbois et al. (1997) and Casanovas (1999).

CONCLUSION

With the ever-growing demand for an affordable and sustainable protein source it is critical that we look for new and innovative ways to manage our broiler breeders to ensure that we have ample fertility and hatch in order to supply broilers for the food market. Making a change to a specific male feed in the US is no small task. Most companies will need to install feed bins on the farms, purchase more feed trucks, increase milling capacity, and increase labor. However, if feeding males tailored to their nutritional needs could potentially impact flock fertility in a positive manner this would directly increase the number of broilers hatched without placing additional breeder flocks.

Feeding males a diet tailored to their needs (STDm) brought them into semen production significantly earlier than those fed a hen diet (Hen-ZnS). This could drastically impact a flock's fertility since this semen production difference was seen while hens would be in their peak of egg production. If males are not in semen production and mating hens, then infertile eggs are being sent to the hatchery, reducing broiler chick numbers.

Feeding the DHA product showed significant improvement in overall flock fertility and should be further investigated. Perhaps the inclusion level was too low, or the males needed to be on the supplement at an earlier age. Providing Zn is very important for semen and semen production but in this study, organic Zn showed no consistent positive or negative impact on the parameters tested. Perhaps the organic Zn supplementation was too high on top of the Zn that is already found in the feed ingredients of the diet. Future research should be conducted to evaluate the levels of organic Zn inclusion or use the organic Zn product in a male tailored diet.

REFERENCES

Abbott, K., T. L. Burrows, S. Acharya, R. N. Thota, and M. L. Garg. 2020. Dietary supplementation with docosahexaenoic acid rich fish oil increases circulating levels of testosterone in overweight and obese men. Prostaglandins Leukot Essent. Fatty Acids. 163:102204.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2002. Eggs. Molecular biology of the cell. 4th edition. New York. Garland Science.

Amen, H. M. M., and H. J. Al-Daraji. 2011. Effect of dietary supplementation with different level of zinc on sperm egg penetration and fertility traits of broiler breeder chicken. Pakistan J. Nut. 10(11):1083-1088.

Aviagen. 2016a. Ross 708 Aviagen Parent Stock Nutrient Specifications. Aviagen Inc., Huntsville, AL.

Aviagen. 2016b. Ross 708 Aviagen Parent Stock Performance Objectives. Aviagen Inc., Huntsville, AL.

Blesbois, E., M. Lessire, I. Grasseau, J. M. Hallouis, and D. Hermier. 1997. Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. Biol. Reprod. 56:1216–1220.

Burrows, W. H., and J. P. Quinn. 1937. The collection of spermatozoa from the domestic turkey. Poult. Sci. 16:19-24.

Casanovas, P. 1999. Methods of alleviating the age-related decline in fertility of broiler breeder males. M.S. Thesis, University of Georgia, Athens, GA.

Cerolini, S., P. F. Surai, B. K. Speake, and N. H. C. Sparks. 2005. Dietary fish and evening primrose oil with vitamin E effects on semen variables in cockerels. Br. Poult. Sci. 46:214-222.

Chia, I., C. Ong, L. Chua, M. Ho, and S. Tay. 2000. Comparison of various sperm parameters and plasma zinc concentrations on blood and seminal of fertile and infertile men. J. Andro. 21:53-57.

Christensen, V. L., J. L. Grimes, W. E. Donaldson, and B. D. Fairchild. 1998. Sperm cell integrity and storage ability of turkey semen as influences by dietary fat source. Poult. Sci. 68 (Abstr).

Coon, C.N. 2001. Chicken Meat and Egg Production. Springer Science and Business, LLC. 5th ed.

Cromwell, G. L., M. J. Azain, O. Adeola, S. K. Baidoo, S. D. Carter, T. D. Crenshaw, S. W. Kim, D. C. Mahan, P. S. Miller, and M. C. Shannon; North Central Coordinating Committee on Swine Nutrition. 2011. Corn distillers dried grains with solubles in diets for growing-finishing pigs: a cooperative study. J. Anim. Sci. 89:2801–2811.

Croxford, T. P., N. H. McCormick, and S. L. Kelleher. 2011. Moderate zinc deficiency reduces testicular Zip6 and Zip10 abundance and impairs spermatogenesis in mice. J. Nutr. 14:359–65.

Commerical Chicken Meat and Egg Production. 2001. 5th edition, edited by D.D. Bell and W.D. Weaver, Jr.

Fallah, A., A. Mohammad-Hasani, and A. H. Colagar. 2018. Essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization. J. Reprod. Infertil. 19:69-81.

Fouad, A. M., H. K. El-Senousey, D. Ruan, W. Xia, W. Chen., S. Wang, and C. Zheng. 2020. Nutritional modulation of fertility in male poultry. Poult. Sci. 99:5637-5646.

Froman, D. P., and D. J. McLean. 1996. Objective measurement of sperm mobility based upon sperm penetration of Accudenz ®. Poult. Sci. 75:776-784.

Hocking, P. M., and R. Bernard. 1997. Effects of dietary crude protein and food intake on the production of semen in two lines of broiler breeder males. Br. Poult. Sci. 38:199-202.

Hocking, P.M. 1990. The relationships between dietary crude protein, body weight, and fertility in naturally mated broiler breeder males. Br. Poult. Sci. 31:743- 757.

Horrocks, L. A., and Y. K. Yeo. 1999. Health benefits of docosahexaenoic acid (DHA). Pharmacological Res. 40:211-225.

Hudson, B. P., and J. L. Wilson. 2003. Effects of dietary menhaden oil on fertility and sperm quality of broiler breeder males. J. Appl. Poult. Res. 12(3):341-347.

Hurley, W. L., and R. M. Doane. 1989. Recent developments in the role of vitamins and minerals in reproduction. J. Dairy Sci. 72:784-804.

Janssen, S. J., J. D. Kirby, R. A. Hess, M. Rhoads, D. Bunick, K. L. Bailey, C. M. Parsons, H. Wang, and J. M. Barber. 2000. Identification of epididymal stones in diverse rooster populations. Poult. Sci. 79:568-574.

Lake, P.E. 1989. Recent progress in poultry production. World's Poult. Sci. J. 45:53-59.

Li, J., B. L. R. Pora, K. Dong, and J. Hasjim. 2021. Health benefits of docosahexaenoic acid and its bioavailability. A review. Food Sci. Nutr. 9:5229-5243.

Mahecha, G. A., C. A. Oliveira, K. Balzuweit, and R. A. Hess. 2002. Epididymal lithiasis in roosters and efferent ductule and testicular damage. Reprod. 124:821-834.

McGary, S., I. Estevez, M. R. Bakst, and D. L. Pollock. 2002. Phenotypic traits as reliable indicators of fertility in male broiler breeders. Poult. Sci. 81:102-111.

Muszyński, S., E. Tomasexwska, M. Kwiecień, P. Dobrowolski, and A. Tomczyk-Warunek. 2018. Subsequent somatic axis and bone tissue metabolism responses to low zinc-diet with or without phytase inclusion in broilers. PLoS ONE. 13: e0191964.

Nguyen, T. M. D., A. Duittoz, C. Praud, Y. Combarnous, and E. Blesbios. 2016. Calcium channels in chicken sperm regulate motility and acrosome reaction. FEBS J. 283:1902-1920.

Park, P., and R. E. Goins. 1994. In situ preparation of flame for analysis of fatty acid composition in food. J. Food Sci. 59:1262–1266.

Rosenstrauch, A., A. Allen Degen, and M. Friedländer. 1994. Spermatozoa retention by sertoli cells during the decline in fertility in aging roosters. Physiol. Reprod. 50:129-136.

Sexton, K.J., J.A. Renden, D.N. Marple, and R.J. Kempainen, 1989. Effects of dietary energy on semen production, fertility, plasma testosterone, and carcass composition of broiler breeder males in cages. Poult. Sci. 68:1688-1694.

Simopoulos, A. P., A. Leaf, and N. Salem Jr. 1999. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann. Nutr. Metab. 43:127-130.

Surai, P. F., E. Blesbois, I. Grasseau, T. Chalah, J. P. Brillard, and G. J. Wishart. 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 120:527-533.

Surai, P. F., R. C. Noble, N. H. C. Sparks, and B. K. Speake. 2000. Effect of long-term supplementation with arachidonic or docosahexaenoic acid on sperm production in broiler chickens. J. Reprod. Fertil. 120:257-264.

Swanson, D., R. Block, and S. A. Mousa. 2012. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. Adv. Nutr. 3:1-7.

Tyler, N. C., and H. A. Bekker. 2012. The effect of dietary crude protein on fertility of male broiler breeders. S. Afr. J. Anim. Sci. 42:304-309.

Tyler, N. C., M. Namntu, and M. Ciacciariello. 2021. Research note: The effect of crude protein and calcium intake on fertility of male broiler breeders. Poult Sci. 100:101284.

Véricel, E., A. Polette, S. Bacot, C. Calzada, and M. Lagarde. 2003. Pro- and antioxidant activities of docosahexaenoic acid on human blood platelets. J. Thromb. Haemost. 1:566-572.

Wilson, H. R., L. O. Rowland Jr., and R. H. Harms. 1971. Use of low protein grower diets to delay sexual maturity of broiler breeder males. Br. Poult. Sci. 12:157-163.

Wilson, J. L., G. R. McDaniel, and C. D. Sutton. 1987. Dietary protein levels for broiler breeder males. Poult. Sci. 66:237-242.

Winer, B. J. 1971. Statistical Principles in Experimental Design. Second Edition, New York: McGraw-Hill Book Co.

Table 1. Composition of developer diet.

Ingredients, %	Developer
Corn, coarse ground	57.21
Wheat middling's	16.03
Soybean mean	14.15
Oats, whole	8.00
Defluorinated Phosphorus	1.68
Limestone (fine)	1.20
Soybean oil	0.50
Salt	0.30
Trace mineral premix[1]	0.08
Vitamin Premix[2]	0.80
DL-methionine	0.05
Total	100.00
Calculated Analysis	
Crude Protein, %	15.4
Ca, %	1.07
P, %	0.72
Available P, %	0.44
Metabolizable energy, kcal per kg of diet	2,800.00
Digestible lysine, %	0.72
Digestible methionine, %	0.29

[1]Supplied per kg of diet: Mn, 107.20 mg as Mn sulfate; Fe, 21.25 mg as ferrous sulfate; Cu, 3.20 mg as basic copper chloride; I, 0.80 mg as calcium iodate; Se, 0.32 mg as sodium selenite.

[2]Supplied per kg of diet: vitamin A, 17600 IU; vitamin D3, 3527 IU; vitamin E, 35.3mg; vitamin B12, 0.04 mg; thiamine, 7.0 mg; riboflavin, 14.1 mg; menadione, 3.5 mg; vitamin B6, 7.0 mg; niacin, 141.0 mg; panthothenic acid, 35.2 mg; folic acid, 1.8 mg; biotin, 0.4 mg.

Table 2. Composition of broiler breeder hen diets fed to roosters.

	Treatment	
Ingredients**, %**	Hen-ZnS	Hen-OZn
Corn, coarse ground	62.78	62.78
Wheat middling's	8.50	8.50
Soybean meal	16.37	16.37
Soy hulls	-	-
Oats, whole	2.50	2.50
Dicalcium phosphate	1.29	1.29
Limestone (fine)	6.85	6.85
Soybean Oil	0.50	0.50
Salt	0.29	0.29
Sodium bicarbonate	0.29	0.29
Trace mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.11	0.11
DL-methionine	0.16	0.16
Choline cl 60%	0.10	0.10
Propionic acid	0.05	0.05
Santoquin 66.7%	0.02	0.02
L-Threonine	0.04	0.04
Zn sulfate 36%[3]	0.03	0.015
Availa-Zn 12%	-	0.046
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	14.05	14.05
Ca, %	3.00	3.00
P, %	0.60	0.60
Available P, %	0.35	0.35
Metabolizable energy, kcal per kg of diet	2,750.0	2,750.0
Digestible lysine, %	0.58	0.58
Digestible methionine, %	0.36	0.36

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Fe, 50 mg as ferrous sulfate; Cu, 10 mg as basic copper chloride; I, 2 mg as calcium iodate; Se, 0.3 mg as sodium selenite.

[2] Supplied per kg of diet: vitamin A, 13.503 IU; vitamin D3, 3,500 IU; vitamin E, 135 mg; vitamin B12, 0.05 mg; thiamine, 5 mg; riboflavin, 16 mg; menadione, 6 mg; vitamin B6, 7 mg; niacin, 65mg; panthothenic acid, 22 mg; folic acid, 3.7 mg; biotin, 0.40 mg.

[3] Supplied per kg of diet: Zn, 0.0004 as Zn sulfate.

Table 3. Composition of broiler breeder rooster diets.

	Treatment	
Ingredients**, %**	STDm	STD-DHA
Corn, coarse ground	61.61	61.61
Wheat middling's	20.00	20.00
Soybean meal	8.50	8.50
Soy hulls	3.62	3.62
Oats, whole	2.50	2.50
Dicalcium phosphate	1.20	1.20
Limestone (fine)	0.99	0.99
Soybean Oil	0.50	0.50
Salt	0.30	0.30
Sodium bicarbonate	0.28	0.28
Trace mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.11	0.11
DL-methionine	0.10	0.10
Choline cl 60%	0.06	0.06
Propionic acid	0.05	0.05
Santoquin 66.7%	0.02	0.02
L-Threonine	-	-
Zn sulfate 36%[3]	0.03	0.03
DHAgold™	-	0.25
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	12.45	12.45
Ca, %	0.70	0.70
P, %	0.60	0.60
Available P, %	0.35	0.35
Metabolizable energy, kcal per kg of diet	2,750.0	2,750.0
Digestible lysine, %	0.45	0.45
Digestible methionine, %	0.29	0.29

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Fe, 50 mg as ferrous sulfate; Cu, 10 mg as basic copper chloride; I, 2 mg as calcium iodate; Se, 0.3 mg as sodium selenite.
[2] Supplied per kg of diet: vitamin A, 13.503 IU; vitamin D3, 3,500 IU; vitamin E, 135 mg; vitamin B12, 0.05 mg; thiamine, 5 mg; riboflavin, 16 mg; menadione, 6 mg; vitamin B6, 7 mg; niacin, 65mg; panthothenic acid, 22 mg; folic acid, 3.7 mg; biotin, 0.40 mg.
[3] Supplied per kg of diet: Zn, 0.0004 as Zn sulfate.

Table 4. Lesion scoring template for histology analysis.

Lesion score	
0	Lesion not present
1	<25% of the tissue affected
2	25-50% of the tissue affected
3	50-75% of the tissue affected
4	>75% of the tissue affected

Table 5. Comparison of kidney lesions for Hen-ZnS v STDm at 65 wk. Each value represents the mean lesion score. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Mean ± SD lesion scores in kidneys

Group					
	Tubular Necrosis	**Tubular Nephrosis**	**Tubular Mineralization**	**Interstitial Inflammation**	**Total Lesion Score**
STDm[c] (n=61)	0.049 [a] ± 0.218	0.147[a] ± 0.357	0.147[a] ± 0.357	0.836[a] ± 0.373	1.180[a] ± 0.866
Hen-ZnS[d] (n=58)	0.051[a] ± 0.223	0.931[b] ± 0.413	0.206[a] ± 0.408	1.017[b] ± 0.295	2.206[a] ± 0.789

[a- b] Treatments significant within period (P<0.05).

[c]STDm= Standard male diet.

[d]Hen-ZnS = Standard hen diet.

Table 6. Comparison of testis lesions for Hen-ZnS v STDm at 65 wk. Each value represents the mean lesion score. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Mean ± SD lesion scores in testis						
Group	**Atrophy**	**Sperm retention**	**Cystic tubules**	**Inflammation (interstitium)**	**Inflammation (tubules)**	**Total lesion score**
STDm[c] (n=61)	1.114[a] ± 1.050	0.737[a] ± 0.544	0.885[a] ± 0.818	0.622[a] ± 0.521	0.114[a] ± 0.321	3.475[a] ± 2.203
Hen-ZnS[d] (n=58)	0.819[a] ± 1.132	0.459[b] ± 0.502	0.491[b] ± 0.648	0.590[a] ± 0.495	0.213[a] ± 0.412	2.573[b] ± 2.156

[a- b] Treatments significant within period (P<0.05).

[c]STDm= Standard male diet.

[d]Hen-ZnS = Standard hen diet.

Table 7. Comparison of kidney lesions for STDm v STD-DHA at 65 wk. Each value represents the mean lesion score. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

	Mean ± SD lesion scores in kidneys				
Group	**Tubular Necrosis**	**Tubular Nephrosis**	**Tubular Mineralization**	**Interstitial Inflammation**	**Total Lesion Score**
STDm[c] (n=61)	0.049 [a] ± 0.218	0.147[a] ± 0.357	0.147 [a] ± 0.357	0.836[a] ± 0.373	1.180 [a] ±0.866
STD-DHA[d] (n=58)	0.052[a] ± 0.230	0.432[b] ± 0.535	0.115[a] ± 0.477	0.947[b] ± 0.295	1.158[a] ± 0.602

[a- b] Treatments significant within period (P<0.05).

[c]STDm= Standard male diet.

[d]STD-DHA = Standard male diet with DHA supplementation.

Table 8. Comparison of testis lesions for STDm v STD-DHA at 65 wk. Each value represents the mean lesion score. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

	Mean ± SD lesion scores in testis					
Group	**Atrophy**	**Sperm retention**	**Cystic tubules**	**Inflammation (interstitium)**	**Inflammation (tubules)**	**Total lesion score**
STDm[c] (n=61)	1.114[a] ± 1.050	0.737[a] ± 0.544	0.885[a] ± 0.818	0.622 [a] ± 0.521	0.114[a] ± 0.321	3.475[a] ± 2.203
STD-DHA[d] (n=60)	0.521[b] ± 0.686	0.414[b] ± 0.504	0.398[b] ± 0.559	0.621[a] ± 0.495	0.191[a] ± 0.393	2.146[b] ± 2.031

[a- b] Treatments significant within period (P<0.05).

[c]STDm= Standard male diet.

[d]STD-DHA = Standard male diet with DHA supplementation.

Table 9. Comparison of epididymal lesions for STDm v STD-DHA at 65 wk. Each value represents the mean lesion score. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

Mean ± SD lesion scores in testis

Group	Atrophy	Inflammation (interstitium)	Inflammation in tubules	Lithiasis	Total lesion score
STDm[c] (n=61)	0.655[a] ± 0.834	1.081[a] ± 0.918	0.180[a] ± 0.428	0.524[a] ± 0.848	2.442[a] ± 2.254
STD-DHA[d] (n=60)	0.063[b] ± 0.305	1.132[a] ± 0.562	0.041[a] ± 0.152	0.223[a] ± 0.544	1.394[b] ± 0.911

[a- b] Treatments significant within period (P<0.05).

[c]STDm= Standard male diet.

[d]STD-DHA = Standard male diet with DHA supplementation.

Table 10. Fatty acid composition of broiler breeder rooster semen feeds containing either 0.25 % Docosahexaenoic acid (STD-DHA) or a Standard Male Diet (STDm).

Fatty acid	STD-DHA (%)	STDm (%)
Saturate		
C14:0	0.50	0.08
C16:0	15.20	14.50
C18:0	2.05	2.29
Monounsaturate		
C16:1 n7	0.22	0.22
C18:1 n9	20.65	21.80
PUFA[c] n3		
C22:6 n3	0.95	0
PUFA n6		
C18:2 n6	55.95	56.32

Figure 1. The weekly mean sample body weight (g) of roosters as affected by the dietary treatment. Dietary programs were as follows: Hen-ZnS **(—**), and STDm (- -). Values represent the mean body weight of 25-30 % of the roosters for these dietary treatments on a weekly basis. There were not significant differences (P<0.05) between the two dietary treatments during the production period.

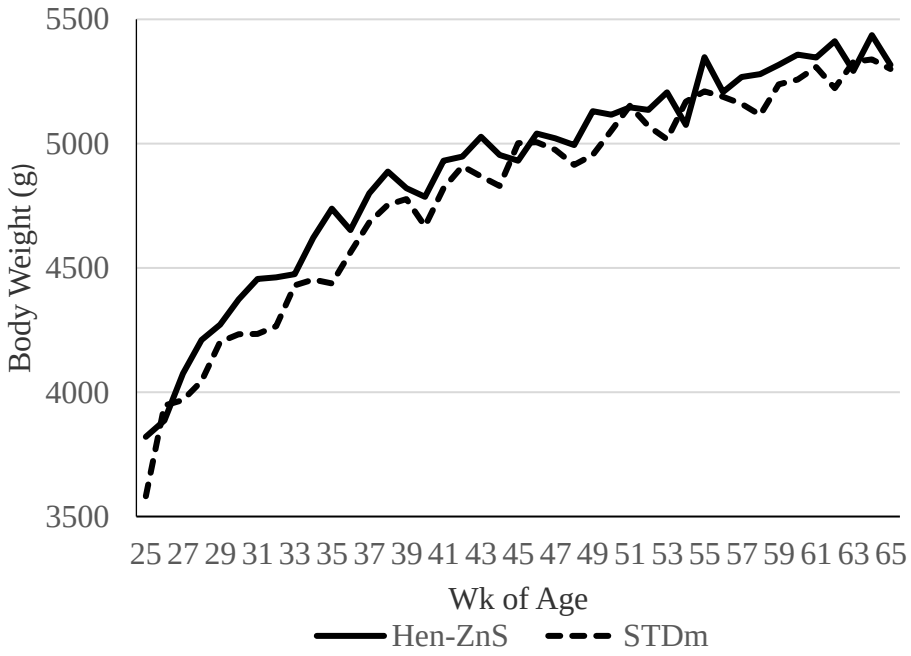


Figure 2. The mean malondialdehyde level of rooster semen (nM/mL) as affected by the dietary treatment. Dietary programs were as follows: Hen-ZnS (solid black column) and STDm (shaded column). There were no significant differences (P<0.05) between the two dietary treatments during the production period.

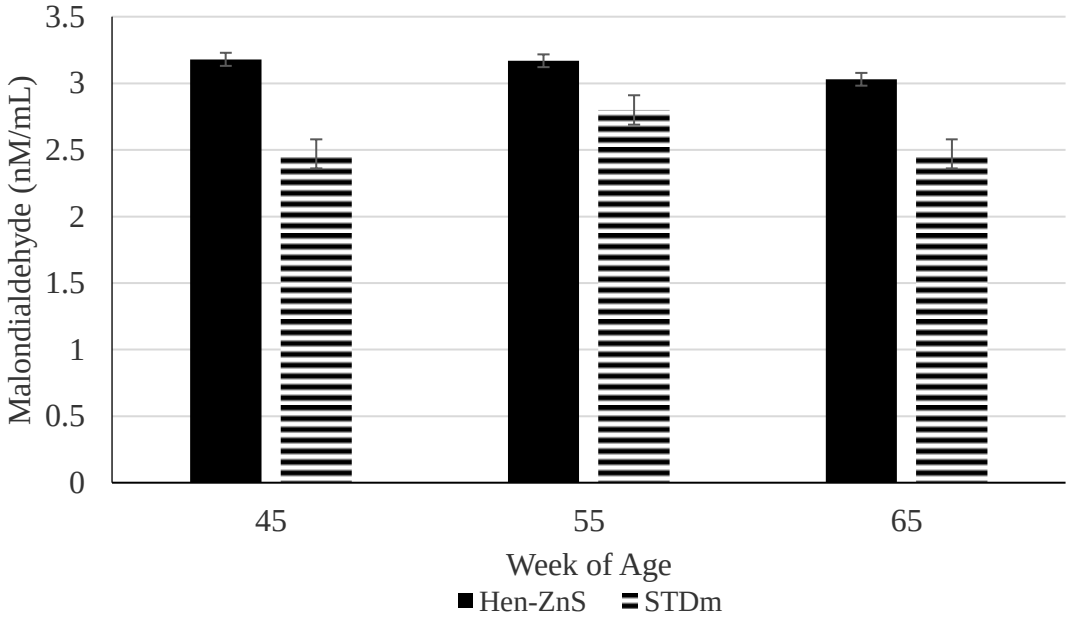


Figure 3. The mean of roosters in semen production (%) as affected by the dietary treatment. Dietary programs were as follows: Hen-ZnS **(—**) and STDm (- -). There were significant differences (P<0.05) between the two dietary treatments at 31 wk. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments.

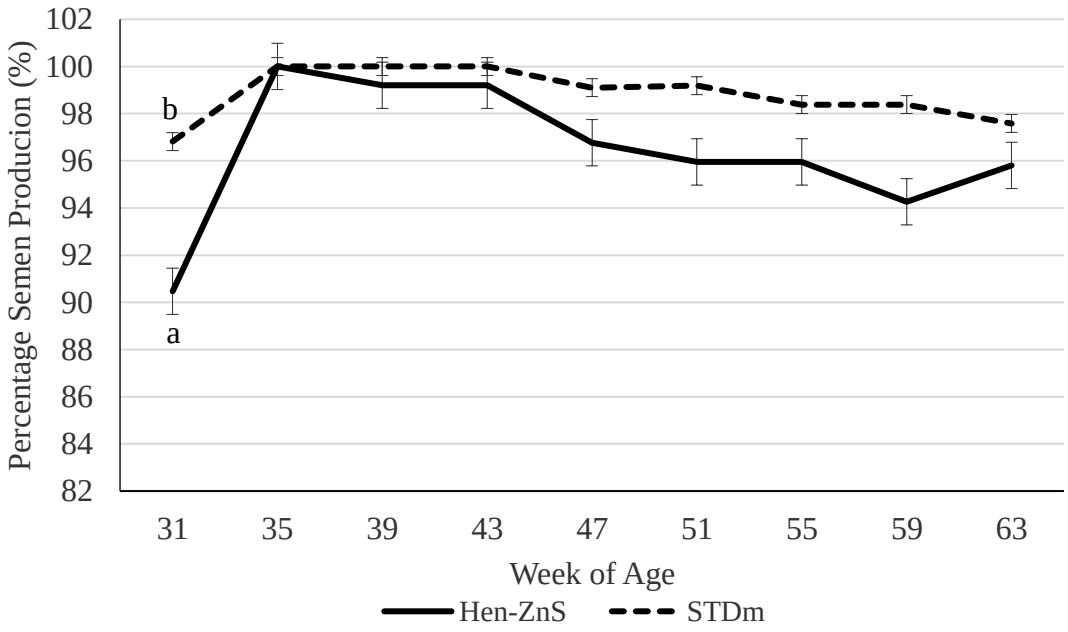


Figure 4. The mean body weight (g) as affected by the dietary treatment. Dietary programs were as follows: Hen-ZnS **(—**), and Hen-ZnO (- -). There were no significant differences (P<0.05) between the two dietary treatments during the production period.

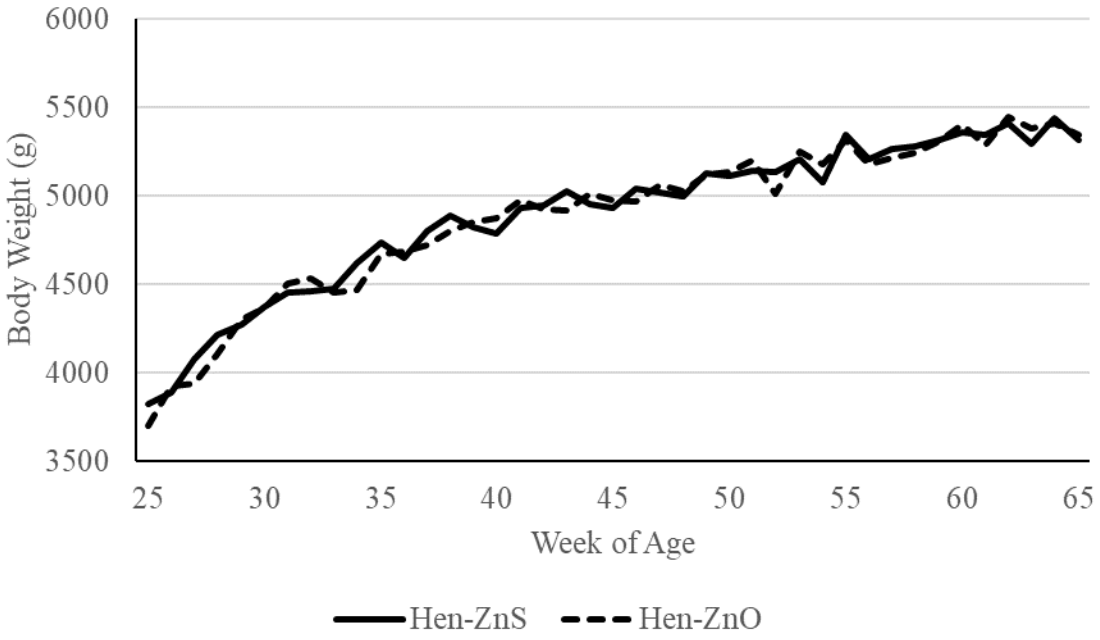


Figure 5. The mean testosterone concentration of blood plasma (ng/mL) as affected by the dietary treatment. Dietary programs were as follows: Hen-ZnS (solid black column) and Hen-OZn (shaded column). There were not significant differences (P<0.05) between the two dietary treatments during the production period.

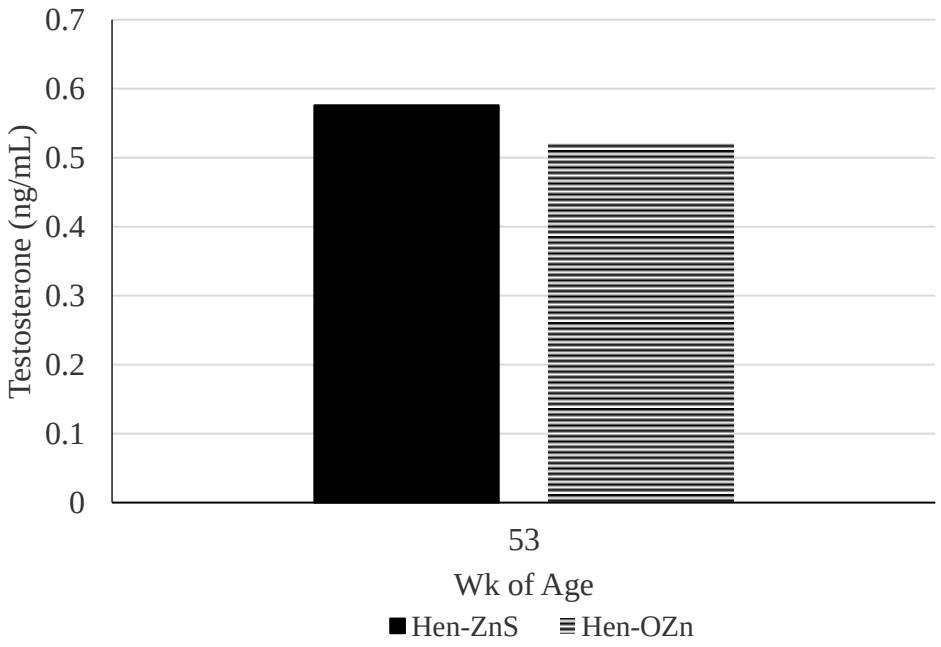


Figure 6. The mean body weight (g) as affected by the dietary treatment. Dietary programs were as follows: STDm **(—)**, and STD-DHA (- -). There were no significant differences (P<0.05) between the two dietary treatments during the production period.

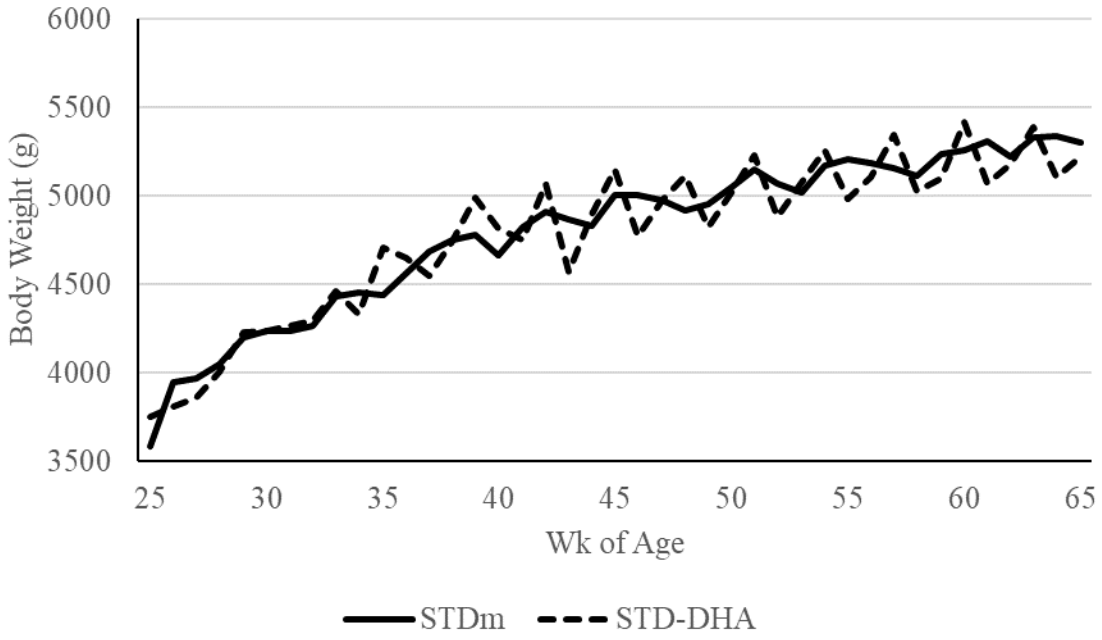


Figure 7. The mean fertility (%) week 1 (2d – 7d) post insemination as affected by the dietary treatment. Dietary programs were as follows: STDm **(—**), and STD-DHA (- -). There were no significant differences (P<0.05) between the two dietary treatments during the production period.

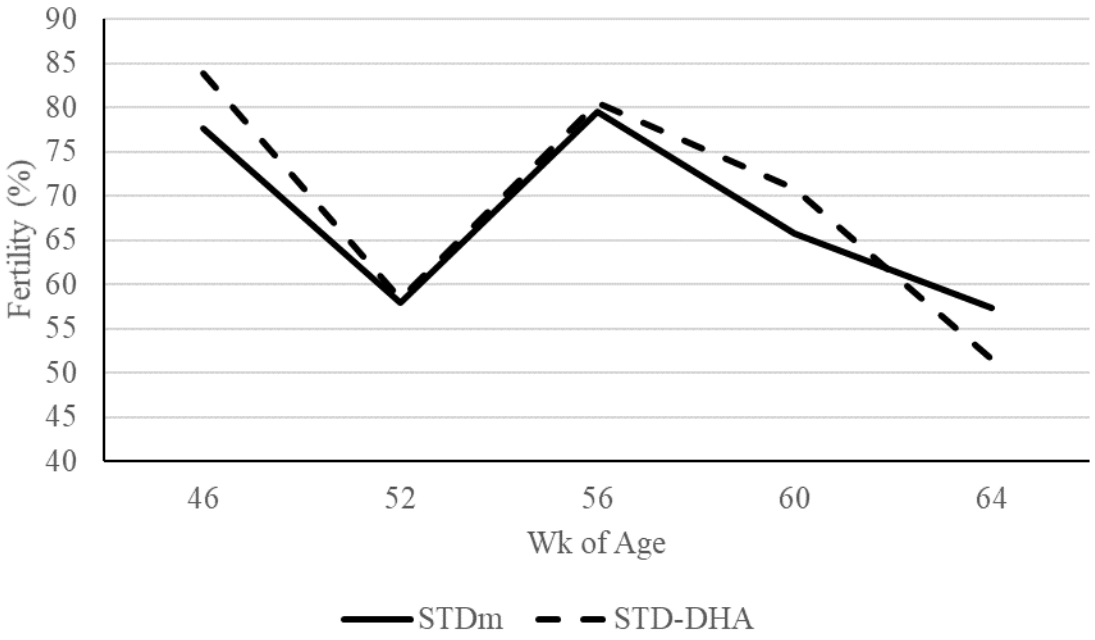
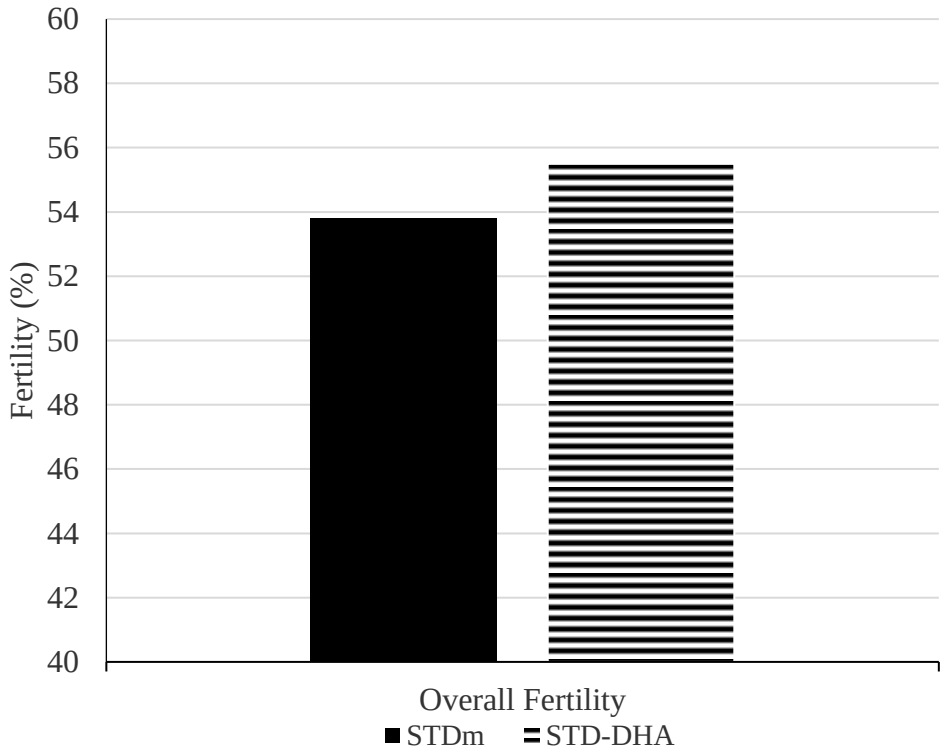


Figure 8. The mean fertility (%) overall (2d – 14d) post insemination as affected by the dietary treatment. Dietary programs were as follows: STDm (solid black column), and STD-DHA (shaded column). There were no significant differences (P<0.05) between the two dietary treatments during the production period.



CHAPTER 5

COMPARISON OF A STANDARD MALE DIET TO AN ORGANIC SELENIUM SUPPLMENTED MALE DIET ON ROOSTER SEMEN QUALITY AND REPRODUCTIVE PERFORMANCE

[1]Sweeney, K. M., L. P. Avila, B. P. Hudson, and J. L. Wilson. To be submitted to *Poultry Science*.

ABSTRACT

Roosters have specific dietary requirements not met by a hen formulation. Hen diets have too high a level of calcium and protein for roosters. Excess calcium can cause stress on the kidneys and excess protein can make managing male BW and breast fleshing difficult. In this study, a basal diet with 2720 kcal/kg energy, 12 % crude protein, 0.74 % calcium, and 0.15 ppm of sodium selenite was mixed specific to the rooster's needs according to primary breeder guidelines. The basal was split into two diets with the male control (MC) diet containing a total of 0.30 ppm of Se from sodium selenite and male organic (MO) diet having 0.15 ppm of Se from sodium selenite and 0.15 ppm of Se from organic selenium (Se). The trace mineral Se is important for testosterone metabolism and sperm motility. The objective of this study was focused on evaluating if the dietary inclusion of organic Se would improve rooster semen production and quality compared to a diet containing the inorganic form, sodium selenite. A total of 80 Ross Yield Plus roosters were assigned to each dietary treatment (8 replicates, 10 roosters per replicate). During the production phase, approximately 25 to 30 % of males in each treatment were weighed weekly, with groups rotating over time. Percentage of roosters producing semen, semen volume, sperm concentration, and sperm mobility were measured every 5 wk from 25 to 65 wks. Individually caged Ross 708 hens were artificially inseminated with 0.05 mL pooled and diluted semen from a replicate group of males. Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 days' post-insemination and incubated for a fertility analysis. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was $P \leq 0.05$. Neither of the diets had significant influence on BW, semen volume, or semen concentration. Sperm mobility was significantly impacted from 34-65 wk ($P<0.05$) with the

males fed the MO diet having greater sperm mobility. Organic Se positively impacts fertility and semen mobility in broiler breeder flocks.

Key words: Rooster diet, semen quality, fertility, selenium, organic selenium

INTRODUCTION

Many factors play a role in flock fertility but one of the most influential is nutrition. From fats to trace minerals, vitamins, amino acids, and probiotics they are all important factors for reproduction in avian species (Fouad et al., 2020). Trace minerals such as zinc (Zn), Selenium (Se), and Manganese (Mn) are all important for proper reproductive function and must be added to poultry feed due to their low values in feedstuffs (Smith and Akinbamijo, 2000). Se is needed for the production of selenoproteins which are essential for limiting oxidation in sperm cells (Leonhard, 2000). Selenoproteins are used in the production of antioxidant enzymes that limit the formation of reactive oxygen species (ROS) that cause lipid oxidation. If Se is deficient in a diet, sperm motility is reduced (Corah and Ives, 1992). Se has such a great influence on spermatozoa due to the structural composition of the mid piece of the sperm (Foresta et al., 2002). It is also needed for the Se-dependent enzyme Glutathione peroxidase (GPX) which is important for the antioxidant system in avian species (Surai et al., 2000).

Se is a trace mineral that has long been added to poultry diet in the inorganic salt form of sodium selenite. However, the use of organic forms such as selenomethionine and Se yeast have shown to be more bioavailable (Cantor et al., 1982). Inclusion of these bioavailable forms leads to an increase in Se content in eggs (Payne at al., 2005) as well as in developing embryos (Paton et al., 2002). Organic Se supplementation has also been shown to improve rooster sperm viability, morphology, and semen concentration (Chauychu-Noo et al., 2021). It has been proven to increase GPX activity and reduce lipid peroxidation in domestic fowl semen (Ebeid, 2009). In contrast, other research suggests that selenomethionine decreases normal sperm counts in broiler breeders (Edens and Sefton, 2002)

In the US, the legal inclusion rate of Se in a diet for broiler breeders is 0.30 ppm of Se selenite, Se yeast, or selenomethionine. A combination of inorganic and organic Se has proven to be more efficiently absorbed in equal amounts than a comparable amount of Se yeast (Han et al., 2017). This author also suggests that the cost of using solely organic Se may limit use in many feed mills. For this study Se from sodium selenite and Se from an organic Se product were mixed in equal parts to achieve 0.30 ppm of total added Se in the final diet. The objective of this study was to analyze flock fertility and semen quality of broiler breeder roosters fed a male formulated diet then supplemented with 0.15 ppm of Se from organic Se and 0.15 ppm of Se from sodium selenite (total = 0.30 ppm of Se) compared to the same male diet that included only 0.30 ppm of Se from sodium selenite.

METHODS

A total of 200 Ross Yield Plus cockerels were obtained for a commercial integrator at 8 wk and were divided equally among four floor pens (3.5 m x 3 m) with new pine shavings in an environmentally controlled solid side walled poultry house. They were fed a common developer diet on a traditional skip-a-day basis. The four pens were housed in the same room separated by a chain-link partition and cockerels were fed from a standard pan feeder (5 cm/opening with 14 openings) with water provided *ad libitum* by nipple drinker line (3 m with 20 nipples). All birds were wing banded at 10 wk of age to track growth rate and assign treatments. A group of 300 Ross 708 pullets of the same age were also reared in the same manner for artificial insemination during the production period. The pullets were reared using the male control diet which was a standard developer diet. At 5 % egg production the pullets were switched to a standard hen ration based on the Aviagen guidelines (Aviagen 2018). At 16 wk the four pens of cockerels were divided into two treatments with 8 replicates per treatment of 10 birds (n=80 birds/trt). The same

basal developer was used but split into two treatments, the male control (MC) (2820 kcal/kg, 14.9 % CP, 0.30 ppm of Se supplied by sodium selenite) and male organic (MO) (2820 kcal/kg, 14.9 % CP, 0.15 ppm of Se supplied by sodium selenite, 0.15 ppm supplied by organic Se) (Table 1). The photoperiod to 22 wk of age was 23 h of light:1 h of darkness (23 L:1 D) for the first 3 d, followed by an 8 L:16 D pattern until 22 wk of age. The photoperiod was increased to 15.25 L:8.75 D at 22 wk of age and remained constant until the end of the study at 65 wk of age.

At 22 wk 160 cockerels were transferred into individual rooster cages (59.7×58.4×35.5 cm; 1 rooster per cage). The facility was enclosed with forced air heaters and evaporative cooling to keep the environment comfortable for the birds. Water was supplied free choice by a nipple drinker. Each treatment was represented by 8 replicate groups of 10 roosters. The body weight (BW) and variation of BW across all treatments were similar at the start of the dietary treatments. To prevent feed from moving within the feed trough, an extra rooster was positioned between the feed treatments that created a physical barrier to prevent roosters within feed treatment consuming the incorrect feed.

At 22 wk of age the males were switched to a restricted amount of feed on an every-day basis. The treatments for the production period were as follows: MC (2720 kcal/kg, 12.1 % CP, 0.30 ppm of Se supplied by sodium selenite) and MO (2720 kcal/kg, 12.1 % CP, 0.15 ppm of Se supplied by sodium selenite, 0.15 ppm of Se supplied by organic Se) (Table 2).

Body Weight and Uniformity

A sample BW (25 % of the birds from each treatment) was taken weekly and all birds were weighed at 8 and 20 wk to divide them equally into their respective treatment. Once photostimulated, 25 % of the birds from each treatment were weighed weekly through 65 weeks of age. Coefficient of variation (CV) for BW were calculated on a per pen basis during rearing as

a measure of flock uniformity. Post photostimulation, BW and CV for each treatment was calculated on a treatment basis (n=10 birds per rep, 80 birds per treatment and total of 160 roosters).

Malondialdehyde analysis using Thiobarbituric Acid Test

Semen was collected free of contaminants using abdominal massage from one replicate of roosters at a time (10 roosters). Immediately after collecting semen, 200 µL of semen from each male per replicate was pipetted into a 16 x 100 mm test tube. Semen samples were brought up to an equivalent volume of 4 mL with ice cold phosphate buffered saline solution (PBS) with 1 mM of EDTA. Sperm was washed with PBS (w/EDTA) at 1500 x g 4° C for 20 min. A Lowery assay was performed on a portion of the lysate to equilibrate total protein of the samples and the remainder of the sample was stored at -80° C. Each replicate group was analyzed for lipid peroxidation by a TBARS analysis using a QuantiChrom TBARS Assay Kit (Bioassay Systems, Hayward, CA).

Semen Production, Semen Volume, and Sperm Mobility Index

At 25 wk of age, all roosters were trained to the semen collection process using the abdominal massage method (Burrows and Quinn, 1937). Semen was collected weekly through 65 wk of age. The percentage of males producing semen was calculated as a percentage of the live males at each collection period everting the phallus and the presence of semen.

Semen volume was measured once every 5 wk from 25 to 65 wk of age. Semen was collected from individual males and free of contaminants using the abdominal massage method. Volume was measured indirectly by weight (1 mL =1 g) (Brillard and de Reviers, 1981). Semen was collected into a pre-weighed and labeled 16 x 100 mm glass test tube. The exterior of each tube was wiped clean and weighed again for the collective weight of semen and tube. The initial

weight of the tube was subtracted from the tube weight with semen to determine semen volume (*initial tube wt- tube wt with semen = semen volume*).

A 6 % (wt/vol) Accudenz® was prepared along with a 320 mmol/kg mobility buffer as described by Froman and McLean (1996). Froman and McLean's technique was used to determine semen mobility and concentration of each individual rooster using a visible spectrophotometer Genesys 40 (Thermo Fisher Scientific, Madison, WI).

Fertility Determination

Individually caged Ross 708 hens that were of equivalent age to the roosters were placed on a standard hen diet (Table 3). The hens were artificially inseminated with 0.05 mL of pooled and diluted semen from a replicate group of 10 roosters at 31, 36, 41, 46, 52, 58 and 62 wk of age. Hens were placed into replicate groups of 12 with similar BW, CV, and egg production prior to the first insemination. If the egg production of a replicate group of hens varied in comparison to the overall egg production, hens were reassigned within their assigned treatment of males to equilibrate egg production. Hens were artificially inseminated once per evaluation period with semen from the same assigned group of males each evaluation period. If ejaculates were less than 50 µL they were discarded. Otherwise, semen from each male within a replicate of 10 males was pooled then diluted accordingly. Semen was diluted using Beltsville Chicken Extender II (Continental Plastic Corp, Delavan, WI) to a minimal dose of 7.5×10^7 sperm concentration. Semen from each replicate was used to inseminate a maximum of 12 hens. Eggs were collected for 14 d (d 2 - d 14) post a single insemination and incubated. Eggs were collected 3 to 4 times per day by individual cage. Eggs were sorted and incubated by replicate group. Eggs were stored for no more than 4 d. From each day after insemination all eggs were set in a Natureform incubator (Natureform Inc., Jacksonville, FL) at 37.5° C and 53 % relative

humidity. Fertility was determined by gross evaluation after opening the eggs being incubated for 5 to 7 d of incubation.

Histology

At 65 wk all males were weighed and then euthanized via CO_2 stunning and cervical dislocation. Testicles were removed and weighed, and their percentage of BW was calculated. After weighing, testicles from three males from each replicate were chosen for histological analysis. A 2 cm midsection containing a portion of the epididymis was placed in 10 % formalin. The testicles underwent routine tissue processing, embedding, sectioning and staining of slides was performed for measuring the diameter of the seminiferous tubules and epithelium thickness as described by Wilson et al. (2018) and de Melo et al. (2013). A Keyence BZ-X800 microscope (Keyence Corporation of America, Itasca, IL) was used to take pictures of the histology slide and ImageJ was used to take the measurements.

Glutathione

Semen was collected free of contaminants using abdominal massage from one replicate of roosters at a time (n=10). Immediately after collecting semen, 200 µL of semen from each male per replicate was pipetted into a 16 x 100 mm test tube. Semen was centrifugated $0.1x10^9$ spermatozoa at 1500 x g for 10 min at 4° C, then the supernatant was discarded. The remainder was transferred to a tube on ice and centrifuge at 250 × g for 10 min at 4° C and the supernatant was discarded again. The cell pellet was resuspended in ice-cold PBS (50µl) and transferred to a microcentrifuge tube on ice. The cells were centrifuged at 1500 x g for 10 min at 4° C, the supernatant was discarded, and the tube was placed on ice. The pellet was sonicated in 0.5 of PBS for 15 s and repeated at least 5 times. Samples were centrifuged again at 1,500 × g for 10 min at 4° C. Supernatant was collected and stored at -80 °C. Each replicate group was analyzed

directory for NADPH consumption in the enzyme coupled reactions using a Glutathione Peroxidase Assay Kit (Bioassay Systems, Hayward, CA).

Total Selenium of Semen and Testicles

Semen was collected free of contaminants using abdominal massage from one replicate of roosters at a time (n=10). Immediately after collecting semen, a minimum 500 µL of semen from each male per replicate was pipetted into a 15 mL screw top test tube. Samples were centrifuged at 1500 g for 15 min and supernatant was poured off. Samples were then frozen at 0° C then shipped to Michigan State University-Veterinary Diagnostic Laboratory (MSU) on icepacks overnight. The semen was weighed and digested overnight in a 95° C oven, using approximately 10x the mass of nitric acid. The digested samples were diluted with water to 25x the sample mass.

At 65 wk of age from each replicate treatment, testicles from three males per replicate treatment were packaged in a 0.9 % buffered saline solution and frozen at 0° C then shipped to Michigan State University (MSU) on icepacks overnight. At MSU Samples were stored in a freezer kept at ≤5˚ C until they were thawed for preparation. The testicles were homogenized using a Precellys Tissue Homogenizer (Bertin Technologies SAS, Montigny-le-Bretonneux, France). The homogenate was weighed and digested overnight in a 95° C oven, using approximately 10x the dry mass of nitric acid (BDH 67-70 % Nitric Acid, VWR Analytical, Radnor, PA). A separate portion was dried overnight in a 75° C oven to determine the dry matter fraction and calculate the dried sample mass. The digested samples were diluted with water to 100x the dried tissue mass.

Semen and testicles were analyzed by elemental analysis followed the method of (Wahlen et al., 2005), using an Agilent 7900 Inductively Coupled Plasma – Mass Spectrometer

(ICP/MS) (Agilent Technologies Inc., Santa Clara, CA). An aliquot of each diluted tissue digest and calibration standard was diluted 20-fold with a solution containing 0.5 % EDTA and Triton X-100, 1 % ammonium hydroxide, 2 % butanol, and 5 ppb of scandium and 7.5 ppb of germanium, rhodium, indium, and bismuth as internal standards. The ICP/MS was tuned to yield a minimum of 7500 cps sensitivity for 1 ppb yttrium (mass 89), less than 1.0 % oxide level as determined by the 156/140 mass ratio and less than 2.0 % double charged ions as determined by the 70/140 mass ratio. Elemental concentrations were calibrated using a linear curve of the analyte-internal standard response ratio. Standards were from Inorganic Ventures (Inorganic Ventures INC, Christiansburg, VA). A second source calibration check standard from High Purity Standards (High Purity Standards, Charleston, SC) were also used.

Statistical Analysis

Body weight, semen quality measurements, TBAR, and fertility were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Slice analysis specifies the effect to test for differences between interactions LS-mean, to produce tests of simple effects (Winer, 1971). This method evaluates the effect of the treatment and minimizing the impact of time (week). Differences were deemed to be significant when the P-value $\leq$ to 0.05.

RESULTS

Body weight, Uniformity, and Semen Volume

Body weight was not significantly impacted by the dietary treatments during rearing nor during the production phase of the project (data not shown). Uniformity was also unaffected by the dietary treatments (data not shown).

Semen volume is used as an indicator of testicle size. For this study there were no significant differences in semen volume for the duration of the production period as well as there were no differences in testicle size or testicle weight as a percentage of BW (data not shown) measured at 65 wk of age.

Semen Production and Sperm Mobility Index

For this study semen concentration was significantly different ($P<0.05$) at 56 wk of age with those males fed the MC diet having higher semen concentration than those males fed the MO diet (Figure 1). Sperm mobility was significantly different at 38, 48, and 62 wk of age with those males fed the MC diet have lower mobility scores than males fed the MD diet at 48 and 62 wk (Figure 2). For this study, semen production was not significantly different between the two dietary treatments (data not shown).

Fertility analysis

Fertility was analyzed 1 wk post the minimal single dose (2-7 d), 2 wk post the minimal single dose, and overall post the single minimal dose after each insemination. Fertility was significantly increased ($P<0.05$) during the 1 wk analyses at 36, 41, 46, 52, 58, and 62 wk with those males fed the MO diet having higher fertility than those fed the MC diet (Figure 3). A significant improvement ($P<0.05$) in fertility for the males on the MO diet was also seen during the wk 2 analyses at 31, 41, 46, 52, and 62 wk of age (Figure 4) as well as overall fertility at wk 31, 36, 41, 46, 52, 58, and 62 (Figure 5).

Testicle Analysis

Seminiferous tubule diameter and germinal epithelium were measured as an indication of normal spermatogenesis late in life (65 wk). There were no significant differences between the two dietary treatments in seminiferous tubule diameter or germinal epithelium (data not shown).

Glutathione Peroxidase

While GPX activity was not significantly different between those males fed the MO diet and those fed the MC diet, the males on the MO diet had higher levels of GPX activity at each sampling (Figure 6) as well as overall (510.72 U/L and 491.13 U/L, MO and MC, respectively).

Malondialdehyde

Increased levels of MDA are indicative of cellular damage caused by lipid peroxidation. The addition of Se has been shown to decrease the lipid peroxidation in rooster testicles (Surai et al., 1998). However, there were no significant differences in MDA in the semen samples between those males fed the MO diet and those males fed the MC (data not shown).

Total Selenium

Due to the antioxidant capacity of Se, total Se of semen at two time points (50 and 65 wk) and total Se of the testicles were analyzed at wk 65 when the flock was terminated. There were significant differences ($P<0.05$) in the semen (Figure 7) and testicles (1.834 ug/g and 2.200 ug/g, MC and MO, respectively) at 65 wk with those males on the MO diet having higher levels of total Se than those fed the MC diet.

DISCUSSION

Body Weight, Uniformity, Semen Volume, and Semen Production

One of the more critical reasons to utilize a feed that is tailored more to a rooster's specific dietary needs is to assist in managing BW and breast fleshing. One major difference in these diets when compared to diets that have recently been fed to roosters in the US is the CP levels. Sometimes, adult broiler breeder males are fed a hen ration that is approximately 14-16 % CP. It has been reported that feeding high levels of CP will lead to over-fleshing of broiler breeders (de Beer, 2009). In this study with the inclusion of organic Se in a low protein male

diet, neither BW nor BW uniformity were different between the dietary treatments. Wilson et al. (1987) showed that broiler breeder males fed a lower protein diet had similar semen production and semen volume as those fed a high protein diet. This study used a low protein male diet (MC) and a low protein male diet supplemented with organic Se (MO). There were no differences in semen volume or semen production between the dietary treatments.

Sperm Mobility Index and Fertility

Supplementation of organic Se to a male diet improved reproductive characteristics such as semen quality and testicular development. Fertility is dependent on the production of quality semen from males and their ability to transfer semen to hens during natural mating. When fertility decreases, young males may be added to the flock (spiking) to increase fertility, or if the fertile eggs are not needed the flock maybe sold at an early age. However, spiking increases the risk of disease by adding males from other farms and flock sources, and selling a flock early increases the time between flocks and will negatively impact grower income. The production of high-quality semen is also essential for proper flock fertility. One of the methods used in this study to measure semen quality was to determine semen concentration in billions of spermatozoa per mL as well as sperm mobility. Sperm mobility determines if structural development was normal allowing movement of the spermatozoa.

It has been proven that dietary supplementation of antioxidants can improve semen quality parameters such as motility, concentration, and viability (Danikowski et al., 2002; Lin et al., 2005). Selenoproteins have been identified to be directly and indirectly involved in the antioxidant system to limit oxidative damage (Lei, 2017). In the avian species, the use of Se in diets have proven to improve semen quality (Słowińska et al., 2011; Biswas et al., 2017). Since organic trace minerals are more bioavailable, the improvements seen in semen mobility in those

males fed the MO diet were expected. Perhaps this can also explain the differences seen in fertility with males consuming the MO diet having improved fertility when compared to those males fed the MC diet. Without proper semen mobility the movement of a spermatozoa to the site of fertilization in the infundibulum is limited. Arguably, semen mobility is more important than semen concentration when evaluating semen. Froman and McLean (1996) categorized males into minimal and maximal semen mobility groups to determine the correlation between semen mobility and fertility through an equivalent semen dose to hens via artificial insemination. A 12 % increase in fertility was measured by Froman and McLean (1996) with those males categorized as with minimal semen mobility having 84 % fertility and those with maximal semen mobility having 96 % fertility, respectively. Since mobility allows for the indirect evaluation of the structure of the spermatozoa, it is a good indication of the viability of the spermatozoa as well.

Total Selenium

Unlike the inorganic forms of Se, organic Se can be stored in the Se reserves in the body (Surai and Fisinin, 2014). Organic Se can be transferred from parent to progeny better to increase antioxidant defense (Fisinin et al., 2008) as well as have positive effects on meat quality in broilers (Surai, 2006). The significant increase in Se in semen at 65 wk of age agrees with other studies mentioned earlier. Perhaps this also played a role in increasing the fertility later in life. Se is important for sperm function (Hawkes et al., 2009) as well as antioxidant defense that integrates selenoproteins, particularly the selenoprotein GPX. This is known to help with the reduction of oxidative damages (Surai and Fisinin, 2014) as well as improve reproductive performance (Wang et al., 2017). Many factors that can be controlled such as environment and BW can impact fertility (McDaniel et al., 1995). However, one factor that dramatically impacts

fertility that cannot be controlled is age (Bramwell et al., 1996). Through simple feed additives, such as vitamin E, Se, and other antioxidants we have seen improvement in broiler breeder fertility as they age. The goal of these feeding supplements is simply to assist in maintaining profitable level of fertility as fertility naturally declines with age.

CONCLUSION

The use of Se is essential for many biological functions. However, the use of Se in its inorganic form such as sodium selenite can be a limiting factor when used in broiler breeder male diets. Sodium selenite can be easily reduced to its unavailable form when mixed improperly with ascorbic acid. The chemical reaction that occurs causes the loss of biological activity (Robinson et al., 1985; Gosetti et al., 2007). With the use of organic forms of Se it would eliminate this risk, increase the bioavailability of Se, as well as encouraging storage in the body for later use (Surai and Fisinin, 2014). With the findings of this study, it allows us to better understand the importance and impact the organic Se can have on broiler breeder flocks. It is important to highlight that organic Se was only fed to the males in this study and such dramatic positive increases were seen in fertility. Future research should be conducted to evaluate the optimal level of organic Se in broiler breeder male's diets, determine if fed at an earlier age could show further improvements, as well as to evaluate the impact of organic Se on broiler breeder flocks when fed to hens and roosters.

REFERENCES

Aviagen. 2018. Ross 708 Aviagen parent stock nutrient specifications. Aviagen Inc., Huntsville, AL.

Biswas, A., J. Mohan, A. B. Mandal, and N. Lal. 2017. Semen characteristics and biochemical composition of cloacal foam of male Japanese quails (*Coturnix coturnix Japonica*) fed diet incorporated selenium. J. Anim. Physiol. Anim. Nutr. 101:229-235.

Bramwell, R. K., C. D. McDaniel, J. L. Wilson, and B. Howarth. 1996. Age effect of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. Poult. Sci. 75:755-762.

Brillard, J. P., and M. de Reviers. 1981. Testis development and daily sperm output in guinea-fowl raised under constant daily photoperiods. Reprod. Nutr. Develop. 21(6b):1105-1112.

Burrows, W. H., and J. P. Quinn. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poult. Sci. 16:19-24.

Cantor, A. H., P. D. Moorhead, and M. A. Musser. 1982. Comparative effects of sodium selenite and selenomethionine upon nutritional muscular dystrophy, selenium-dependent glutathione peroxidase, and tissue selenium concentrations of turkey poults. Poult. Sci. 61:478-484.

Chauychu-Noo, N., P. Thananurak, W. Boonkum, T. Vongpralub, and V. Chankitisakul. 2021. Effect of organic selenium dietary supplementation on quality and fertility of cryopreserved chicken sperm. Cryobiology. 98:57-62.

Corah, L. R., and S. Ives. 1992. Trace minerals in cow herd nutrition programs. Agri-Practice. 13:5-7.

Danikowski, S., H. P. Sallmann, I. Halle, and G. Flachowsky. 2002. Influence of high levels of vitamin E on sperm parameters of cock. J. Anim. Physiol. Anim. Nutr. 86:376-382.

de Beer, M. 2009. Current approaches to feeding broiler breeders. Pages 104-114 in Proc. 17th Eur. Symp. Poult. Nutr., Edinburg, Scotland. World's Poult. Sci. Assoc., Attleborough, United Kingdom.

de Melo, F. C. S. A., T. P. de Sousa, K. L. C. Costa, S. L. P. da Matta, F. R. de Melo, and R. de Mattos Santa-Rita. 2013. Descriptive morphology and stereology of the tubular compartment in the wild rodent *Hylaeamys megacephalus* (Rodentia:Cricetidae) from central Brazil. Anim. Reprod. Sci. 138:110-117.

Ebeid, T. A. 2009. Organic selenium enhances the antioxidative status and quality of cockerel semen under high ambient temperature. Br. Poult. Sci. 50:641-647.

Edens, F. W., and A. E. Sefton. 2002. Selenomethionine supplementation to diets of broiler breeders improves performance. Poult. Sci. 81:91 (Abstr.)

Fisinin, V. I., T. T. Papazyan, and P. F. Surai. 2008. Selenium in poultry nutrition. In: Current advances in se research and applications. Surai, P. F. and Taylor-Pickard J eds. Wageningen Academic Publishers, Wageningen, The Netherlands. 1:221-261.

Foresta, C., L. Flohé, A. Garolla, A. Roveri, F. Ursini, and M. Maiorino. 2002. Male fertility is linked to the Selenoprotein Phospholipid Hydroperoxide Glutathione Peroxidase. Bio. Reprod. 67:967-971.

Fouad, A. M., H. K. El-Senousey, D. Ruan, W. Xia, W. Chen, S. Wang, and C. Zheng. 2020. Nutritional modulation of fertility in male poultry. Poult. Sci. 99(11):5637-5646.

Froman, D. P., and D. J. McLean. 1996. Objective measurement of sperm mobility based upon sperm penetration of Accudenz ®. Poult. Sci. 75:776-784.

Gosetti, F., P. Farascarolo, S. Polati, C. Medana, V. Gianotti, P. Palma, R. Aigotti, C. Baiocchi, and M. C. Gennaro. 2007. Speciation of selenium in diet supplements by HPLC-MS/MS methods. Food Chemistry. 105:1738-1747.

Han, X. J., P. Qin, W. X. Li, Q. G. Ma, C. Ji, J. Y. Zhang, and L. H. Zhao. 2017. Effect of sodium selenite and selenium yeast on performance, egg quality, antioxidant capacity, and selenium deposition of laying hens. Poult. Sci. 96(11):3973-3980.

Hawkes, W. C., Z. Alkan, and K. Wong. 2009. Selenium supplementation does not affect testicular selenium status or semen quality in North American men. J. Andro. 30:525-533.

Lei, X. 2017. Avian selenogenome: response to dietary Se and protection against oxidative insults. Poult. Sci. 96:220.

Leonhard, M. S. 2000. Why do trace elements have an influence on fertility? Tierarztl. Prax. Ausg. G, Grosstiere Nutztiere. 28:60-65.

Lin, Y. F., S. J. Chang, J. R. Yang, Y. P. Lee, and A. L. Hsu. 2005. Effects of supplemental vitamin E during the mature period on the reproduction performance of Taiwan native chicken cockerels. Br. Poult. Sci. 46:366-373.

McDaniel, C. D., R. K. Bramwell, J. L. Wilson, and H. Birkett Jr. 1995. Fertility of male and female broiler breeders following exposure to elevated ambient temperature. Poult. Sci. 6: 1029-1038.

Paton, N. D., A. H. Cantor, A. J. Prescatore, M. J. Ford, and C. A. Smith. 2002. The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos. Poult. Sci. 81:1548-1544.

Payne, R. L., T. K. Lavergne, and L. L. Southern. 2005. Effect of inorganic versus organic selenium on hen production and egg selenium concentration. Poult. Sci. 84:232-237.

Robinson, M. F., C. D. Thomson, and P. K. Huemmer. 1985. Effect of a megadose of ascorbic acid, a meal and orange juice on the absorption of selenium as sodium selenite. New Zealand Medical J. 98:627-629.

Słowińska, M., J. Jankowski, G. J. Dietrich, H. Karol, E. Liszewska, J. Glogowski, and K. Kozłowski. 2011. Effect of organic and inorganic forms of selenium in diets on turkey semen quality. Poult. Sci. 90:181-190.

Smith, O. B., and O. O. Akinbamijo. 2000. Micronutrients and reproduction in farm animals. Anim. Reprod. Sci. 60: 549-560.

Surai, P. F. 2006. Selenium in nutrition and health. Nottingham University Press, Nottingham, UK.

Surai, P. F., and V. I. Fisinin. 2014. Selenium in poultry breeder nutrition: an update. Anim. Feed Sci. Technol. 191:1-15.

Surai, P. F., R. C. Noble, N. H. C. Sparks, and B. K. Speake. 2000. Effect of long term supplementation with arachidonic or docosahexaenoic acid on sperm production in broiler chicken. J. Reprod. Fertil. 120:257-264.

Wahlen, R., L. Evans, J. Turner, and R. Hearn. 2005. The use of collision/reaction cell ICP-MS for the determination of elements in blood and serum samples. Spectroscopy. 20:84-89.

Wang, Y., H. M. Yang, W. Cao, and Y. B. Li. 2017. Effect of selenium supplementation on pigeon reproductive performance, selenium concentration and antioxidant status. Poult. Sci. 96:3407-3413.

Wilson, F. D., D. I. Johnson, D. L. Magee, and F. J. Hoerr. 2018. Testicular histomorphometries including Sertoli cell quantitation for evaluating hatchability and fertility in commercial broiler-breeder roosters. Poult. Sci. 97:1738-1747.

Wilson, J. L., G. R. McDaniel, and C. D. Sutton. 1987. Dietary protein levels for broiler breeder males. Poultry Sci. 66: 237-242.

Winer, B. J. 1971. Statistical Principles in Experimental Design. Second Edition, New York: McGraw-Hill Book Co.

Table 1. Composition of broiler breeder grower diets.

	Treatment	
Ingredients**, %**	MC	MO
Corn, coarse ground	58.40	58.39
Wheat middlings	2.00	2.00
Soybean meal	16.76	16.76
Soy hulls	4.00	4.00
Oats, whole	13.00	13.00
Mono-dicalcium phosphate	2.13	2.13
Limestone (fine)	1.48	1.48
Soybean Oil	0.56	0.56
Salt	0.39	0.39
Sodium bicarbonate	0.12	0.12
Trace mineral premix[1]	0.10	0.10
Sodium selenite blend[2]	0.01	-
Hydroxy- selenomethionine blend[3]	-	0.02
Vitamin premix[4]	0.67	0.67
L-Lysine HCL	0.04	0.04
DL-methionine	0.22	0.22
L-Threonine	0.12	0.12
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	14.99	14.99
Ca, %	1.00	1.00
P, %	0.81	0.81
Available P, %	0.44	0.44
Metabolizable energy, kcal per kg of diet	2,820.0	2,820.0
Digestible lysine, %	0.61	0.61
Digestible methionine, %	0.41	0.41

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Zn, 110 mg as Zn sulfate; Fe, 40 mg as ferrous sulfate; Cu, 16 mg as copper sulfate; I, 0.8 mg as calcium iodate and 0.5 as ethylenediamine dihydroiodide; Se, 0.15 mg as sodium selenite.

[2]Supplied per kg of diet: Se, 0.15 mg as sodium selenite = male control diet (MC).

[3] Supplied per kg of diet: Se, 0.15 mg as hydroxy-selenomethionine = male organic Se diet (MO).

[4] Supplied per kg of diet: vitamin A, 14,300 IU; vitamin D3, 2,866 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5.7 mg; riboflavin, 11.5 mg; menadione, 2.9 mg; vitamin B6, 5.7 mg; niacin, 115 mg; pantothenic acid, 29 mg; folic acid, 1.4 mg; biotin, 0.29 mg.

Table 2. Composition of broiler breeder rooster diets.

	Treatment	
Ingredients**, %**	MC	MO
Corn, coarse ground	62.68	62.67
Wheat middling's	15.00	15.00
Soybean meal	7.60	7.60
Soy hulls	8.00	8.00
Oats, whole	2.30	2.30
Mono-dicalcium phosphate	1.49	1.49
Limestone (fine)	1.05	1.05
Soybean Oil	0.35	0.35
Salt	0.31	0.31
Sodium bicarbonate	0.17	0.17
Trace mineral premix[1]	0.10	0.10
Sodium selenite blend[2]	0.01	-
Hydroxy- selenomethionine blend[3]	-	0.02
Vitamin premix[4]	0.67	0.67
L-Lysine HCL	0.04	0.04
DL-methionine	0.11	0.11
L-Threonine	0.04	0.04
Choline Cl. 60%	0.08	0.08
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	12.1	12.1
Ca, %	0.74	0.74
P, %	0.71	0.71
Available P, %	0.35	0.35
Metabolizable energy, kcal per kg of diet	2,720.0	2,720.0
Digestible lysine, %	0.44	0.44
Digestible methionine, %	0.26	0.26

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Zn, 110 mg as Zn sulfate; Fe, 40 mg as ferrous sulfate; Cu, 16 mg as copper sulfate; I, 0.8 mg as calcium iodate and 0.5 as ethylenediamine dihydroiodide; Se, 0.15 mg as sodium selenite.

[2]Supplied per kg of diet: Se, 0.15 mg as sodium selenite = male control diet (MC).

[3] Supplied per kg of diet: Se, 0.15 mg as hydroxy-selenomethionine = male organic Se diet (MO).

[4] Supplied per kg of diet: vitamin A, 14,300 IU; vitamin D3, 2,866 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5.7 mg; riboflavin, 11.5 mg; menadione, 2.9 mg; vitamin B6, 5.7 mg; niacin, 115 mg; pantothenic acid, 29 mg; folic acid, 1.4 mg; biotin, 0.29 mg.

Table 3. Composition of broiler breeder hen diets.

	Treatment
Ingredients**, %**	Hen
Corn, coarse ground	57.22
Wheat middlings	4.00
Soybean meal	17.50
Soy hulls	2.50
Oats, whole	6.75
Mono-dicalcium phosphate	1.63
Limestone (fine)	3.65
Oyster Shell	3.65
Soybean Oil	1.57
Salt	0.23
Sodium bicarbonate	0.20
Trace mineral premix[1]	0.09
Vitamin premix[2]	0.59
L-Lysine HCL	0.04
DL-methionine	0.21
L-Threonine	0.17
Total	100.00
Calculated Analysis	
Crude protein, %	14.43
Ca, %	3.1
P, %	0.68
Available P, %	0.34
Metabolizable energy, kcal per kg of diet	2,750.0
Digestible lysine, %	0.61
Digestible methionine, %	0.40

[1] Supplied per kg of diet: Mn, 100 mg as Mn sulfate; Zn, 100 mg as Zn sulfate; Fe, 20 mg as ferrous sulfate; Cu, 3 mg as basic copper chloride; I, 0.75 mg as calcium iodate; Se, 0.3 mg as sodium selenite.

[2] Supplied per kg of diet: vitamin A, 12,500 IU; vitamin D3, 2,505 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5 mg; riboflavin, 10 mg; menadione, 2.5 mg; vitamin B6, 5 mg; niacin, 100 mg; pantothenic acid, 25.1 mg; folic acid, 1.3 mg; biotin, 0.25 mg.

Figure 1. Semen concentration as affected by the different dietary treatments.: MC = male control diet (■), MO = male diet with organic Se (□). Each value represents the mean absorbance of neat semen within each dietary treatment. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 56 wk of age.

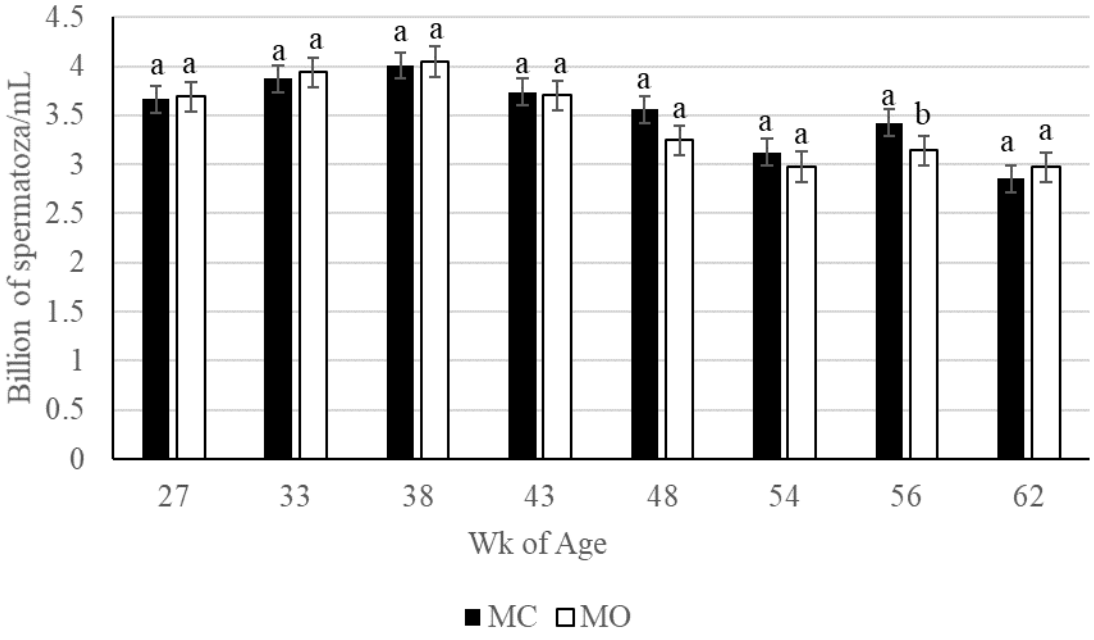


Figure 2. Semen mobility as affected by the different dietary treatments: MC = male control diet (■), MO = male diet with organic Se (□). Each value represents the mean absorbance of diluted semen within each dietary treatment. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 38, 48, and 62 wk of age.

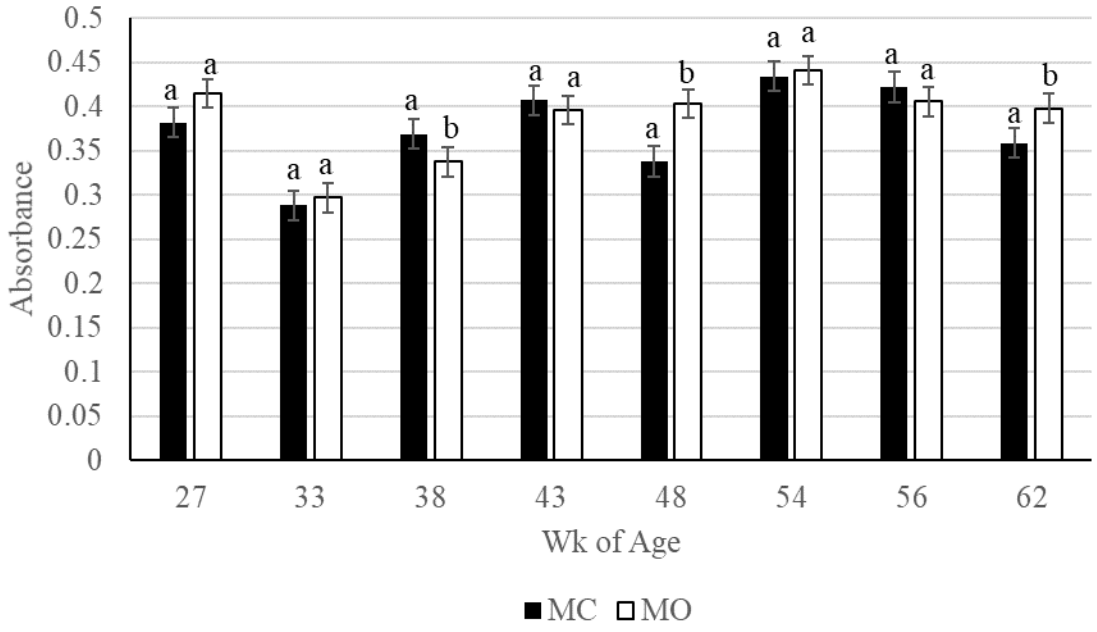


Figure 3. Fertility for wk 1 post insemination as affected by the different dietary: MC = male control diet **(—)**, MO = male diet with organic Se (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 36, 41, 52, 58, and 62 wk of age.

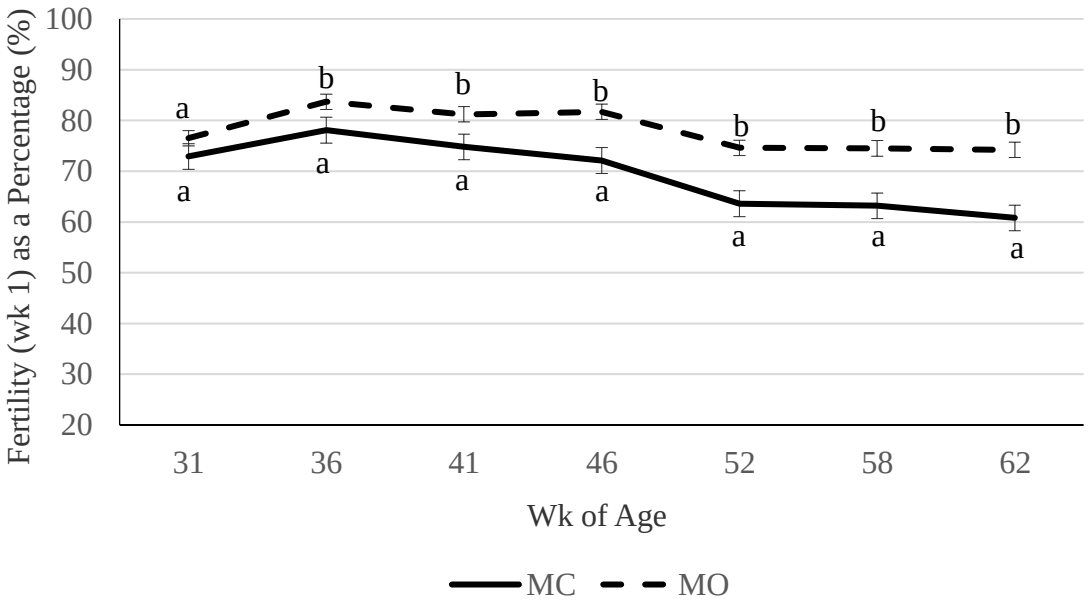


Figure 4. Fertility for wk 2 post insemination as affected by the different dietary treatments: MC = male control diet **(—)**, MO = male diet with organic Se (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 31, 41, 46, 52 and 62 wk of age.

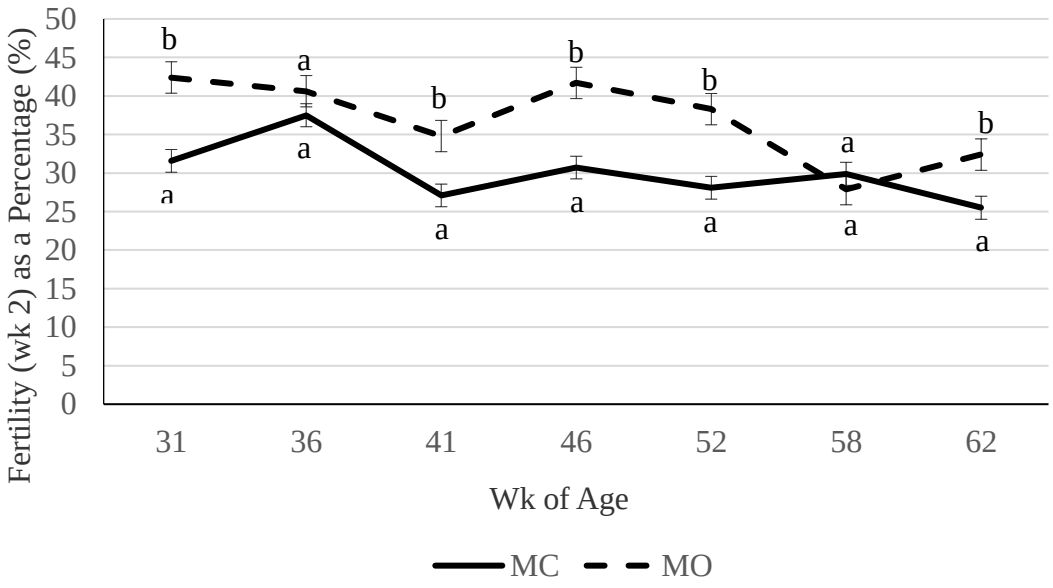


Figure 5. Overall fertility (wk 1 and wk 2) post insemination as affected by the different dietary treatments: MC = male control diet **(—)**, MO = male diet with organic Se (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 31, 36, 41, 52, and 62 wk of age.

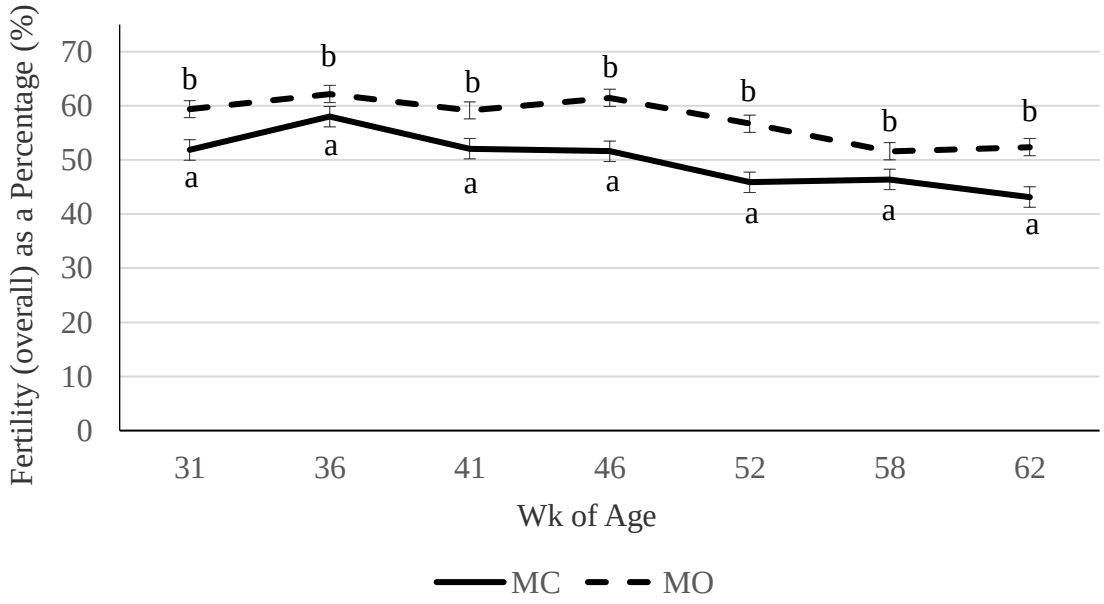


Figure 6. Glutathione Peroxidase enzyme activity as affected by the different dietary treatments: MC = male control diet **(—)**, MO = male diet with organic Se (- -). Each value represents the mean (U/L) of total GPX enzyme activity produced within each dietary treatment. There were no significant differences (P<0.05) between the dietary treatments.

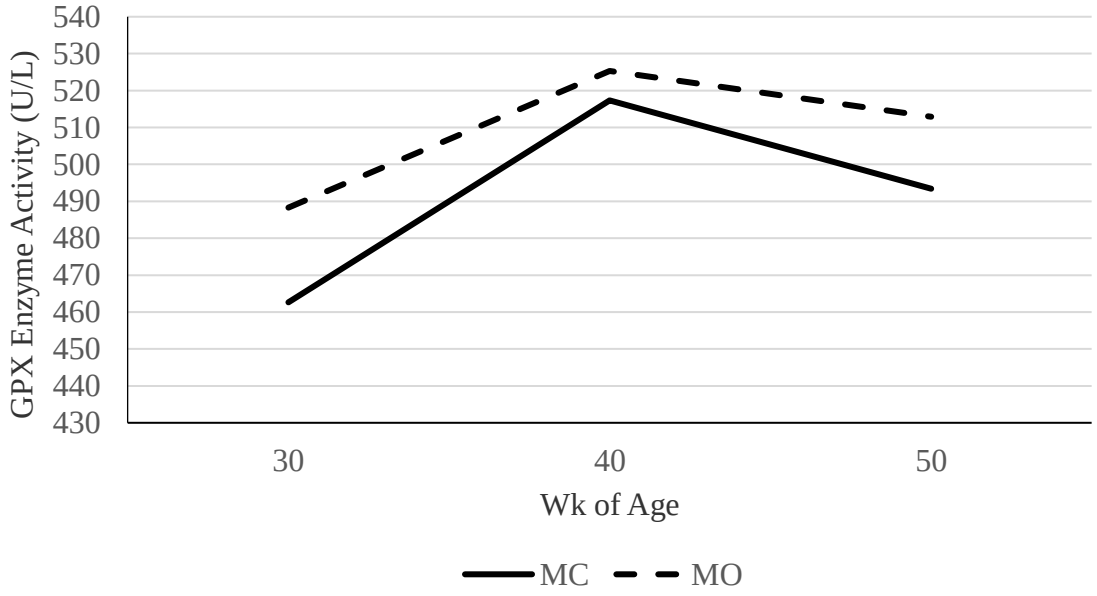
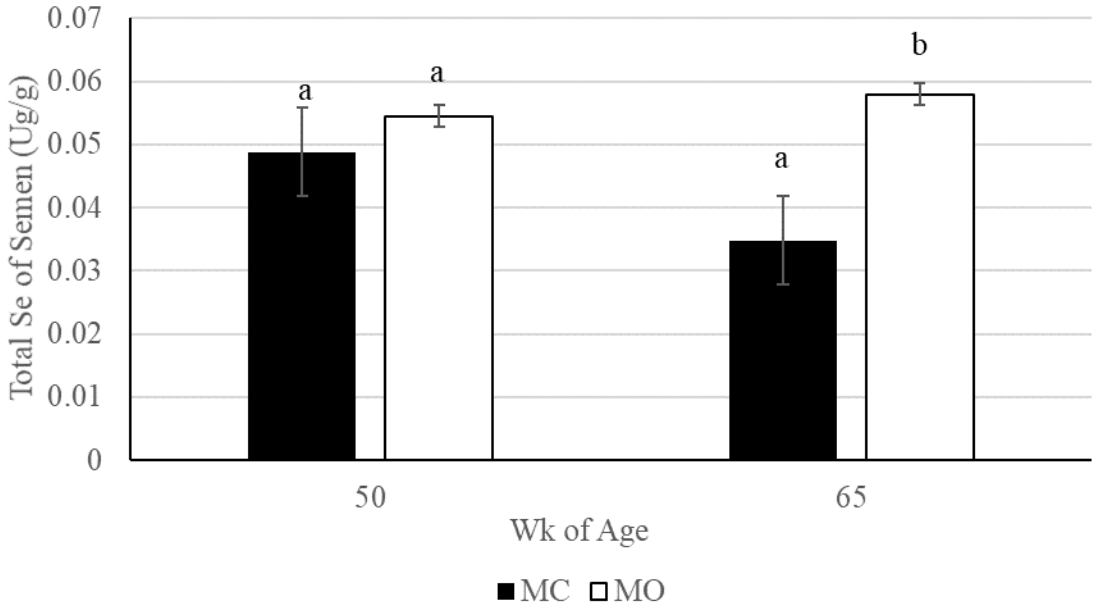


Figure 7. Total Selenium content as affected by the different dietary treatments: MC = male control diet **(—)**, MO = male diet with organic Se (- -). Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. Each value represents the mean (Ug/g) of total Se in semen within each dietary treatment. There were significant differences (P<0.05) between the dietary treatments at 65 wk.



CHAPTER 6

COMPARISON OF A STANDARD MALE DIET TO A DOCOSAHEXAENOIC ACID (DHA) SUPPLEMENTED MALE DIET ON ROOSTER SEMEN QUALITY AND REPRODUCTIVE PERFORMANCE

[1]Sweeney, K. M., L. P. Avila, B. P. Hudson, and J. L. Wilson. To be submitted to *Poultry Science*.

ABSTRACT

In the US, broiler breeder males are typically fed a female diet out of convenience, but hen diets have too high a level of calcium and protein for roosters. In this study, a basal diet with 2720 kcal/kg energy, 12 % crude protein and 0.74 % calcium were mixed according to primary breeder guidelines specific to the roosters' needs. The basal was split into two diets with the male control (MC) diet and male DHA (MD) diet containing 0.28 % Docosahexaenoic acid (DHA). The objective of this study was focused on evaluating the dietary inclusion of DHA would improve rooster semen production, quality, and fertility when compared to a traditional male diet. All treatment groups were fed equal amounts of feed from 16 to 65 wk. A total of 60 Ross Yield Plus roosters were assigned to each dietary treatment (8 replicates, 10 roosters per replicate). During the production phase, approximately 25 to 30 % of males in each treatment were weighed weekly, with groups rotating over time. Percentage of roosters producing semen, semen volume, sperm concentration, and sperm mobility were measured every 5 wk from 25 to 65 wk. Individually caged Ross 708 hens were artificial inseminated with 0.05 mL pooled and diluted semen from a replicate group of males. Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 days' post-insemination and incubated for fertility analysis. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was $P \leq 0.05$. None of the diets had significant influence on body weight, semen volume, or semen concentration. Sperm mobility was significantly impacted from week 34-65 wk ($P<0.05$) with the males fed the MD diet having greater sperm mobility. The dietary supplementations of significant impacts on fertility through 65 wk. DHA increased fertility 1 wk and 2 wk post-insemination as well as increased overall fertility at 31, 36, 41, 52, and 62 wk of age.

Key words: Rooster diet, semen quality, fertility, omega 3 fatty acid, DHA

INTRODUCTION

In the US, broiler breeder males are typically fed a hen diet out of convenience. These diets are formulated for a hen's nutrition needs with approximately 15 % CP and 3 % Ca compared to the males' nutritional needs of 12 % CP and 0.70 % Ca according to the Aviagen Parent Stock Nutrition Specifications (2018). It is also speculated that a hen diet fortified with all the protein, vitamins and minerals needed to produce a high-quality chick is over formulated for roosters, especially in protein level. The excess of amino acids can make managing male body weight (BW) and breast fleshing difficult. According to Wilson et al. (1987) feeding males a lower protein diet assisted in maintaining lower BW while not impacting semen concentration. Hen diets also have 3-3.5 % calcium to provide the 2.2 g of calcium carbonate needed for good eggshell quality (Butcher and Miles, 2019). Since the rooster does not make eggshells, the level in the hen diet is excess for his needs. An excess of Ca in broilers can cause a malfunction in the kidneys which leads to an increase in mortality (Riccardi et al., 1995). Managing male BW is critical for maintaining physical and reproductive soundness in broiler breeders. Using a low protein diet can assist in BW management. While utilizing a male diet assists in male body weight management it does not dramatically improve late in life flock fertility, suggesting that broiler breeder male nutrition needs further investigation.

To improve fertility several feed additives have been investigated that potentially influence spermatogenesis, reduce lipid peroxidation, and improve reproductive tract development with some success, but some are impractical to use. Menhaden oil has been proven to increase the number of fertile eggs, spermatozoa numbers, and improve sperm motility (Surai et al., 2000; Hudson and Wilson, 2003; Cerolini et al., 2005). However, fish oil usage in poultry diets is limited due to potential issues with product stability Fish oil is high in polyunsaturated

fatty acids (n-3 PUFAs) that are found in avian spermatozoa and seminal plasma specifically docosatetraenoic acid (C22:4n-6) (Darin-Bennett et al., 1974; Kelso et al., 1996; Cerolini et al., 1997). This fatty acid content of the spermatozoa makes them very susceptible to lipid peroxidation. Lipids are essential for membrane flexibility, flagella movement, and ease of membrane penetration for fertilization (Cerolini et al., 1997).

DHA has been shown to improve fertility, soft tissue development, and tumor cell proliferation, but has typically been fed as liquid products, such as tuna oil (Horrocks and Yeo, 1999; Surai and Sparks, 2000). However, these products are sensitive to heat and have been shown to negatively impact bird productivity when degraded (Tang et al., 2018). Dried algae products offer very similar concentrations of DHA when compared to fish oils. These products are typically in a powdered form that is shelf stable and easy to handle. For this project a powdered feed grade algae product was added to the feed to investigate the impact of a shelf stable algae product on rooster semen quality and reproductive performance. Based on previous research we hypothesize that this product will improve semen quality, flock fertility, as well as be easier to handle in a feed mill. The objective was to evaluate semen quality and flock fertility of roosters fed a standard male diet compared to those fed a standard male diet with powdered DHA supplementation.

METHODS

A total of 200 Ross Yield Plus cockerels were obtained for a commercial integrator at 8 wk and were divided equally among four floor pens (3.5 m x 3 m) with new pine shavings in an environmentally controlled solid side walled poultry house. These cockerels were fed a common developer diet on a traditional skip-a-day basis. The four pens were housed in the same room separated by a chain-link partition and cockerels were fed from a standard pan feeder (5

cm/opening with 14 openings) with water provided *ad libitum* by nipple drinker line (3 m with 20 nipples). All birds were wing banded at 10 wk of age to track growth rate and assign treatments. A group of 300 Ross 708 pullets of the same age were also reared in the same manner for artificial insemination during the production period. The pullets were reared using the male control diet which was a standard developer diet. At 5 % egg production the pullets were switched to a standard hen ration based on the Aviagen guidelines (Aviagen, 2018). At 16 wk the four pens of cockerels were divided into two treatments with two reps per treatment (n=100 birds/trt). The same basal developer was used but split into two treatments, the male control (MC) (2820 kcal/kg, 14.9 % CP) and the male with DHA (MD) (2820 kcal/kg, 14.9 % CP, 0.25 % DHA) (Table 1). The photoperiod to 22 wk of age was 23 h of light:1 h of darkness (23 L:1 D) for the first 3 d, followed by an 8 L:16 D pattern until 22 wk of age. The photoperiod was increased to 15.25 L:8.75 D at 22 wk of age and remained constant until the end of the study at 65 wk of age.

At 22 wk 160 cockerels were transferred to individual rooster cages (59.7×58.4× 35.5 cm; 1 rooster per cage). The facility was enclosed with forced air heaters and evaporative cooling to keep the environment comfortable for the birds. Water was supplied free choice by a nipple drinker. Each treatment was represented by 8 replicate groups of 10 roosters. The body weight (BW) and variation of BW across all treatments were similar at the start of the dietary treatments. To prevent feed from moving within the feed trough, an extra rooster was positioned between the feed treatments that created a physical barrier to prevent roosters within feed treatment consuming the incorrect feed.

At 22 wk of age the males were switched to a restricted amount of feed on an every-day basis. The cockerels were switched from a developer diet to a rooster diet. The treatments for the

production period were as follows: MC (2720 kcal/kg, 12.1 % CP, 0.74 % Ca), MD (2720 kcal/kg, 12.1 % CP, 0.74 % Ca, 0.28 % DHA) (Table 2). Based on the formulation for the MD diet, roosters were receiving 75 mg of DHA/feed day. Once the roosters were in their cages, their daily intake of feed was limited to 113 g/day. This was to keep the roosters from gaining excessive weight due to inactivity.

Body Weight and Uniformity

A sample BW (25 % of the birds from each treatment) was taken weekly and all birds were weighed at 8 and 20 wk of age to divide them equally into their respective treatment. Once photostimulated, 25 % of the birds from each treatment were weighed weekly through 65 wk of age. Coefficient of variation (CV) for BW were calculated on a per pen basis during rearing as a measure of flock uniformity. Post photostimulation, BW for each treatment was calculated on a treatment basis (n=10 birds per rep, 80 birds per treatment and total of 160 roosters).

Malondialdehyde analysis using Thiobarbituric Acid Test

Semen was collected free of contaminants using abdominal massage from one replicate of roosters at a time (10 roosters). Immediately after collecting semen, 200 µL of semen from each male per replicate was pipetted into a 16 x 100 mm test tube. Semen samples were brought up to an equivalent volume of 4 mL with ice cold phosphate buffered saline solution (PBS) with 1mM of EDTA. Sperm were washed with PBS (w/EDTA) at 1500 x g 4° C for 20 min. A Lowery assay was performed on a portion of the lysate to equilibrate total protein among samples and the remainder of the sample was stored at -80° C. Each replicate group was analyzed for lipid peroxidation by a TBARS analysis using a QuantiChrom TBARS Assay Kit (Bioassay Systems, Hayward, CA).

Semen Production and Sperm Mobility Index

At 25 wk of age, all roosters were trained to the semen collection process using the abdominal massage method (Burrows and Quinn, 1937). Semen was collected weekly through 65 wk of age. The percentage of males producing semen was calculated as a percentage of the live males at each collection period everting the phallus and the presence of semen.

A 6 % (wt/vol) Accudenz® solution was prepared along with a 320 mmol/kg mobility buffer as described by Froman and McLean (1996). Spermatozoa mobility and semen concentration was determined for individual rooster using a visible spectrophotometer Genesys 40 (Thermo Fisher Scientific, Madison, WI).

Fertility Determination

Individually caged Ross 708 hens that were of equivalent age to the roosters were placed on a standard hen diet (Table 3). The hens were artificially inseminated with 0.05 mL of pooled and diluted semen from a replicate group of 10 roosters at 31, 36, 41, 46, 52, 58 and 62 wk of age. Hens were artificially inseminated once with semen from the same assigned group of males at each time period. If ejaculates were less than 50 µL, they were discarded. Otherwise, semen from each male within a replicate of 10 males was pooled then diluted accordingly. Semen was diluted using Beltsville Chicken Extender II (Continental Plastic Corp, Delavan, WI) to a minimal dose of 7.5×10^7 sperm concentration. Semen from each replicate was used to inseminate a maximum of 12 hens. Eggs were collected for 14 d (day 2 - day 14) post a single insemination and incubated. Eggs were collected 3 to 4 times per d by individual cage. Eggs were sorted and incubated by replicate group. Eggs were stored for no more than 4 d prior to incubation. From each day after insemination all eggs were incubated in a Natureform incubator (Natureform Inc.,

Jacksonville, FL) at 37.5° C and 54 % relative humidity for 5-7 d. Fertility was determined by gross evaluation after opening the incubated eggs.

Fatty Acid Determination

Semen was collected free of contaminants and prepared for fatty acid analysis as described by Hudson and Wilson (2003). Fatty acids were determined by converting fatty acids to fatty acid methyl esters and analyzed by gas chromatography using methods as described by Cruz-Hernandez et al. (2006). Methyl esters were evaluated using a 5890 GC (Conquer Scientific, Poway, CA) with a split/split less injector and FID detector. A Phenomenex 7HG-G033-10 column was used (Phenomenex Inc., Torrance, CA). The standards used were a FAME mix CRM 47885 from Sigma as well as individual standards from Nu-Chek Prep (Nu-Chek Prep INC, Elysian, MN) for fatty acids not found in the sigma FAME standard.

Semen Volume and Testicle Analysis

Semen volume was measured once every 5 wk from 25 to 65 wk of age. Semen was collected from individual males and free of contaminants using the abdominal massage method. Volume was measured indirectly by weight (1 mL =1 g) (Brillard and de Reviers, 1981). Semen was collected into a pre-weighed and labeled 16 x 100 mm glass test tube. The exterior of each tube was wiped clean and weighed again for the collective weight of semen and tube. The initial weight of the tube was subtracted from the tube weight with semen to determine semen volume (*initial tube wt- tube wt with semen = semen volume*).

At 65 wk of age all roosters were weighed and euthanized for necropsy using CO_2 gas and cervical dislocation. Testicles were removed whole and weighed to calculate their relative percentage of overall body weight. Post weighing, a 2 cm midsection containing a portion of the epididymis was placed in 10 % formalin. The testicles underwent routine tissue processing, embedding, sectioning, and staining of slides was performed for measuring the diameter of the

seminiferous tubules and epithelium thickness as described by Wilson et al. (2018) and de Melo et al. (2013). A Keyence BZ-X800 microscope (Keyence Corporation of America, Itasca, IL) was used to take pictures of the histology slide and imaging processing software, ImageJ, was used to take the measurements.

Statistical Analysis

Body weight, semen quality measurements, TBAR, and fertility were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Slice analysis specifies the effect to test for differences between interactions LS-mean, to produce tests of simple effects (Winer, 1971). This method evaluates the effect of the treatment and minimizing the impact of time (week). Differences were deemed to be significant when the P-value ≤ to 0.05.

Results

Body weight, Uniformity, and Semen Volume

Body weight was not significantly impacted by the dietary treatments during rearing nor during the production phase of the project (data not shown). Uniformity was also unaffected by the dietary treatments (data not shown).

Semen volume was used as an indicator of testicle size. For this study there were no significant differences in semen volume for the duration of the production period (data not shown) as well as there were no differences in testicle weight as measured at 65 wk (data not shown).

Semen Production and Sperm Mobility Index

Semen production is critical for the longevity of broiler breeder flocks. Fertility is dependent on the production of quality semen from males. In this study percentage of roosters in semen production was significantly different ($P<0.001$) between the two dietary treatments at 25

wk (MC 88.1 % and MD 95.0 %, respectively) (Figure 1). There were no significant differences in percentage of roosters in semen production for the remainder of the study (27 to 65 wk).

In this study, semen concentration was significantly different ($P<0.05$) at 48 and 56 wk of age with those males fed the MC diet having higher semen concentration than those males fed the MD diet (Figure 2). Sperm mobility was significantly different at 33, 38, 48, and 62 wk of age with those males fed the MC diet having lower mobility scores than males fed the MD diet (Figure 3).

Fertility analysis

Fertility was analyzed 1 wk post the minimal single dose insemination (2-7 d), 2 wk (8-14 d) post the minimal single dose, and overall (2-14 d) post the single minimal insemination dose. Fertility was significantly increased ($P<0.05$) during the wk 1 analysis at 31, 36, 41, 52, and 62 wk of age with those males fed the MD diet having better fertility than those males fed the MC diet (Figure 4). Fertility was also significantly improved ($P<0.05$) during the wk 2 analysis (31, 36, 41, 46, and 62 wk) as well as the overall fertility analysis (31, 36, 41, 52, and 62 wk) (Figures 5 and 6).

Testicle Analysis

Seminiferous tubule diameter and germinal epithelium were measured as an indicator of the potential for normal spermatogenesis late in life (65 wk). There were no significant differences between the two dietary treatments in seminiferous tubular diameter or germinal epithelium (data not shown).

Fatty Acid Determination

Feeding the MD diet with the DHA product expressed a markedly lower n6-to-n3 fatty acid ratio as well as a higher C22:6 n3 (DHA) content compared to the MC diet (Table 4). Similar results were also noted in the testicle analysis for fatty acids (Table 5).

Malondialdehyde

The oxidative degradation of cells by reactive oxygen species (ROS) was evaluated using a TBAR test and measuring the end product malondialdehyde (MDA). An increased level of MDA would indicate that those cells (sperm cells) had a greater level of oxidative stress/damage (Partyka et al., 2012). The only significant difference ($P<0.05$) in MDA levels was at 65 wk. Males on the MD diet had lower MDA level than those on the MC diet (MC 0.484 µM and MD 0.162 µM, respectively).

DISCUSSION

Body Weight, Uniformity, and Semen Volume

Since all males were being fed an isocaloric and isonitrogenous diet, it was expected that there would be no significant differences in BW, uniformity, and semen volume. Using a low protein male diet is advantageous for many reasons. Wilson et al. 1987 found that feeding males a lower protein diet did not impact semen production and volume. Also, feeding males a high protein diet can increase breast fleshing which can negatively impact fertility. These high levels of proteins are typically found in a hen diet that is fed to males out of convenience. Protein sources such as soybean meal and meat and bone meal can increase the cost dramatically when making diets. If we can feed these males a lower protein diet and still achieve quality semen production and limit breast fleshing it would be advantageous from a monetary and bird management perspective. Diets formulated for the rooster's nutritional needs should be further

researched to determine their effects on breast fleshing, livability, and fertility in a floor pen setting.

Semen Production and Sperm Mobility Index

Assuring that males reach sexual maturity is essential for overall flock fertility. Males must be of proper age and BW before photostimulation to ensure that maximum testicle development is reached for optimum semen production. In this study, males on the MD diet had more males in semen production at an earlier age than those on the MC diet. As previously mentioned, DHA can significantly impact the development of tissues such as that in the reproductive tract (i.e. testicles). Perhaps this is the reason we observed that the males on the DHA diet achieved a higher number of roosters in semen production at an early age. Due to a limited number of males, we were unable to euthanized males at multiple time points to evaluate testicle size and morphology, which would have provided further information on testicular development and maintenance.

Semen quality is essential for maximum flock fertility. One of the methods used in this study to measure semen quality was to determine semen concentration in billions of spermatozoa per mL as well as sperm mobility. Semen concentration is important to ensure that spermatozoa levels are normal. Sperm mobility allows for an indirect measurement for normal structural development of the spermatozoa. Sperm mobility is essential for fertility. If spermatozoa are unable to swim to the infundibulum of the hen to fertilize the follicle, fertility will be negatively impacted. While research is limited on the effects of DHA on sperm mobility of broiler breeder males at multiple ages, it has been shown to increase sperm mobility in young breeder roosters (Feng et al., 2015), bulls (Gholami et al., 2010), rams (Alizadeh et al., 2014), and boars (Estienne et al., 2008). The findings of this study agree with those previously mentioned that feeding DHA

to broiler breeder males significantly improves sperm mobility. Froman and McLean (1996) showed that an improvement in sperm mobility improves fertility.

Fertility analysis

When fertility decreases, several measures can be taken to assist in reviving the flock's fertility such as adding additional younger males to the flock (spiking) or if the flock is old enough simply selling the flock. However, adding younger roosters increases the risk of introducing diseases and external parasites to the hen flock as the added roosters will come from other farms and flock sources. The other option of selling a flock early has negative financial consequences to both farmer and integrator and can lead to a fertile egg shortage.

The overall goal of a broiler breeder flock is to produce quality fertile eggs. This cannot be achieved without reproductively and structurally fit broiler breeder males. As mentioned earlier feeding males a diet tailored to their reproductive needs then supplemented with a DHA product assists in keeping the males reproductively fit (sperm mobility). In this study, DHA supplementation improved fertility throughout the production period, and these findings agrees with Hudson and Wilson (2003), Kelso et al. (1997a), and Zanussi et al. (2019) that the supplementation of a PUFA such as DHA improves fertility. Because lipids such as PUFA's are important for flagella movement of sperm and increased fusing with membranes such as the perivitelline membrane of the ovum, an increase in fertile eggs and acrosome reaction has been reported (Cerolini et al., 1997).

Fatty Acid

Finding a potential nutritional enhancement for a male diet that could improve flock fertility was the ultimate goal of this study. Feeding a male diet supplemented with DHA improved flock fertility. These findings agree with previous research (Blesbois et al., 1997;

Kelso et al., 1997b; Casanovas, 1999; Hudson and Wilson, 2003). The different levels of dietary fatty acid influenced the fatty acid profiles of the spermatozoa as well as the testicles as expected (Tables 4 and 5). In this study there was a 5x difference in the n-6 to n-3 fatty acid ratio in the semen and testicles of these roosters when fed the DHA supplemented diet having a lower ratio. Safari et al. (2018) found that lower ratios of n-6 to n-3 fatty acids improved sperm motility, membrane functionality, and viability significantly in roosters. These lower n-6 to n-3 ratios have also been proven to improve bone health, bone mineral density, and bone mineral content (Liu et al., 2003, 2004) which has the potential to also influence broiler breeder fertility and should be evaluated. Perhaps the differences in fertility during artificial inseminations may be related to the previous suggestions that polyunsaturated fatty acids improve flagella movement seen in the improvement in mobility scores as well as fatty acid profiles.

Malondialdehyde

When lipid peroxidation increases it can negatively impact fertility (Lin et al., 2005 and Bazyar et al., 2019). In this study roosters fed the MD diet had lower MDA levels than males fed the MC diet at ($P<0.05$) at 65 wk (data not shown), but this difference was not apparent at the other observation points. This finding suggests that feeding a DHA supplemented diet could improve or maintain fertility as broiler breeder males age preventing dramatically low flock fertility observed in many commercial flocks as they age. Many forms of stress such as heat stress, high stocking density, feeding frustrations, and contaminated feed can cause cellular damage. Perhaps the evaluation of MDA can be used in commercial flocks to evaluate stress levels that could correlate to a drop in fertility or perhaps even egg production. Reducing this cellular stress through feed additives has proven to reduce MDA in egg yolks which is highly correlated with decreased embryonic mortality (Xia et al., 2020). Further research should be

conducted to find a better understanding of the cellular damage occurring in spermatozoa due to oxidative stress and how PUFA can impact that damage.

CONCLUSION

Finding potential solutions for the perils of broiler breeder fertility is important for the future success of these breeders and broiler meat production. In this study we found significant improvements in sperm mobility and more importantly flock fertility by feeding broiler breeder males a diet supplemented with DHA. While these findings are important and useful, it should be noted that in the US the majority of poultry integrators do not have the equipment to mix, deliver, and feed broiler breeder males a separate diet. However, due to research like this and many others the benefits of feeding male tailored diets can be seen. To help integrators see the value of increased flock fertility, a monetary evaluation should include the livability of males under commercial conditions when fed a male diet.

REFERENCES

Alizadeh, A., V. Esmaeili, A. Shahverdi, and L. Rashidi. 2014. Dietary fish oil can change sperm parameters and fatty acid profiles of ram sperm during oil consumption period and after removal of oil source. Cell J. 16:289.

Aviagen. 2018. Ross 708 Aviagen Parent Stock Nutrient Specifications. Aviagen Inc., Huntsville, AL.

Bazyar, M., M. Sharafi, and A. Shahverdi. 2019. Changes in seminal parameters and hormonal profile with use of aromatase inhibitor in management of aging broiler breeder roosters. Poult. Sci. 98:6100-6107.

Blesbois, E., M. Lessire, I. Grasseau, J. M. Hallouis, and D. Hermier. 1997. Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. Biol. Reprod. 56:1216–1220.

Brillard, J. P., and M. de Reviers. 1981. Testis development and daily sperm output in guinea-fowl raised under constant daily photoperiods. Reprod. Nutr. Develop. 21(6b):1105-1112.

Burrows, W. H., and J. P. Quinn. 1937. The collection of spermatozoa from the domestic turkey. Poult. Sci. 16:19-24.

Butcher, G. D, and R. Miles. 2019. Concepts of eggshell quality. University of Florida Extension Publication. College of Veterinary Medicine.

Casanovas, P. 1999. Methods of alleviating the age-related decline in fertility of broiler breeder males. M.S. Thesis, University of Georgia, Athens, GA.

Cerolini, S., K. A. Kelso, R. C. Noble, B. K. Speake, F. Pizzi, and L. G. Cavalchini. 1997. Relationship between the spermatozoa lipid composition and fertility during aging of chickens. Biol. Reprod. 57:976-980.

Cerolini, S., P. F. Surai, B. K. Speake, and N. H. C. Sparks. 2005. Dietary fish and evening primrose oil with vitamin E effects on semen variables in cockerels. Br. Poult. Sci. 46:214-222.

Cruz-Hernandez, C., J. K. G. Kramer, J. Kraft, V. Santercole, M. Or-Rashid, Z. Deng, and M. E. R. Dugan. 2006. Systematic analysis of trans and conjugated linoleic acids in the milk and meat of ruminants. In Advances in Conjugated Linoleic Acid Research. 3:45–93. Ed. by M. P. Yuramecz. Taylor & Francis, Abingdon, UK.

Darin-Bennett, A., A. Poulos, and I. G. White. 1974. The phospholipids and phospholipid-bound fatty acids and aldehydes of dog and fowl spermatozoa. J. Reprod. Fertil. 41:471-474.

de Melo, F. C. S. A., T. P. de Sousa, K. L. C. Costa, S. L. P. da Matta, F. R. de Melo, and R. de Mattos Santa-Rita. 2013. Descriptive morphology and stereology of the tubular compartment in the wild rodent *Hylaeamys megacephalus* (Rodentia:Cricetidae) from central Brazil. Anim. Reprod. Sci. 138:110-117.

Estienne, M. J., A. F. Harper, and R. J. Crawford. 2008. Dietary supplementation with a source of omega-3 fatty acids increases sperm number and the duration of ejaculation in boars**.** Theriogenology. 70:70-76.

Feng, Y., Y. Ding, J. Liu, Y. Tian, Y. Yang, S. Guan, and C. Zhang. 2015. Effects of dietary omega-3/omega-6 fatty acid ratio on reproduction in the young breeder rooster. BMC Vet. Res. 11:13-18.

Froman, D. P., and D. J. McLean. 1996. Objective measurement of sperm mobility based upon sperm penetration of Accudenz ®. Poult. Sci. 75:776-784.

Gholami, H., M. Chamani, A. Towhidi, and M. H. Fazeli. 2010. Effect of feeding a docosahexaenoic acid-enriched nutraceutical on the quality of fresh and frozen-thawed semen in Holstein bulls. Theriogenology. 74:1548-1558.

Horrocks, L. A., and Y. K. Yeo. 1999. Health benefits of docosahexaenoic (DHA). Pharmacological Research. 40:211-225.

Hudson, B. P., and J. L. Wilson. 2003. Effects of dietary menhaden oil on fertility and sperm quality of broiler breeder males. J. Appl. Poult. Res. 12:341-347.

Kelso K. A., S. Cerolini, R. C. Noble, N. H. Sparks, and B. K. Speake. 1997b. The effects of dietary supplementation with docosahexaenoic acid on the phospholipid fatty acid composition of avian spermatozoa. Comp. Biochem. Physiol. Biochem Mol. Biol. 118:65–69.

Kelso, K. A., A. Redpath, R. C. Noble, and B. K. Speake. 1997a. Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. J. Reprod. Fertil. 109:1–6.

Kelso, K. A., S. Cerolini, R. C. Noble, N. H. Sparks, and B. K. Speake. 1996. Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. J. Reprod. Fertil. 106:201-206.

Lin, Y. F., S. J. Chang, J. R. Wang, Y. P. Lee, and A. L. Hsu. 2005. Effects of supplemental vitamin E during the mature period on the reproductive performance of Taiwan native chicken cockerels. Br. Poult. Sci. 46:366-373.

Liu, D., H. P. Veit, and D. M. Denbow. 2004. Effects of long-term dietary lipids on mature bone mineral content, collagen, crosslinks, and prostaglandin E_2 production in Japanese quail. Poult. Sci. 83:1876-1883.

Liu, D., H. P. Veit, J. H. Wilson, and D. M. Denbow. 2003. Long-term supplementation of various dietary lipids alters bone mineral content, mechanical properties and histological characteristics of Japanese quail. Poult. Sci. 82:831-839.

Partyka, A., E. Łukaszewicz, and W. Niżański. 2012. Efffect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. Poult. Sci. 77:1497-1504.

Riccardi, D., J. Park, W. S. Lee, G. Gamba, E. M. Brown, and S. C. Hebert. 1995. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. Proceedings of the National Academy of Sciences. 92:131-135.

Safari, R. A, F. Shariatmadari, M. Sharafi, M. A. Karimi Torshizi, and A. Shahverdi. 2018. Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Ross breeder roosters fed a diet supplemented with a moderate ration of n-3:n-6 fatty acids. Poul. Sci. 97:4113-4121.

Surai, P. F. 2000. Phospholipid fatty acid composition, vitamin E content and susceptibility to lipid peroxidation of duck spermatozoa. Theriogenology. 53:1025-1039.

Surai, P. F., and N. H. C. Sparks. 2000. Tissue-specific fatty acid and α-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci. 79:1132-1142.

Tang, S., Y. Bin, J. Xu, and E. Bao. 2018. Rosemary reduces heat stress by inducing CRYAB and HSP70 expression in broiler chickens. Oxidative Medicine and Cellular longevity. 2018: 7014126.

Wilson, F. D., D. I. Johnson, D. L. Magee, and F. J. Hoerr. 2018. Testicular histomorphometries including Sertoli cell quantitation for evaluating hatchability and fertility in commercial broiler-breeder roosters. Poult. Sci. 97:1738-1747.

Wilson, J. L., G. R. McDaniel, and C. D. Sutton, 1987. Dietary protein levels for broiler breeder males. Poultry Sci. 66: 237-242.

Winer, B. J. 1971. Statistical Principles in Experimental Design. Second Edition, New York: McGraw-Hill Book Co.

Xia, W. G., W. Chen, K. F. M. Abouelezz, D. Ruan, S. Wang, Y. N. Zhang, A. M. Fouad, K. C. Li, X. B. Huang, and C. T. Zheng. 2020. The effect of dietary Se on productive and reproductive performance, tibial quality, and antioxidant capacity in laying duck breeders. Poult. Sci. 99:3971-3978.

Zanussi, H. P., F. Shariatmadari, M. Sharafi, and H. Ahmadi. 2019. Dietary supplementation with flaxseed oil as source of omega-3 fatty acids improves seminal quality and reproductive performance in aged broiler breeder roosters. Theriogenology. 130:40-48.

Table 1. Composition of broiler breeder grower diets.

	Treatment	
Ingredients**, %**	MC	MD
Corn, coarse ground	58.40	58.15
Wheat middling's	2.00	2.00
Soybean meal	16.76	16.76
Soy hulls	4.00	4.00
Oats, whole	13.00	13.00
Mono-dicalcium phosphate	2.13	2.13
Limestone (fine)	1.48	1.48
Soybean Oil	0.56	0.56
Salt	0.39	0.39
Sodium bicarbonate	0.12	0.12
Trace mineral premix[1]	0.11	0.11
Vitamin premix[2]	0.67	0.67
L-Lysine HCL	0.04	0.04
DL-methionine	0.22	0.22
L-Threonine	0.12	0.12
DHA blend[3]	-	0.25
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	14.99	14.99
Ca, %	1.00	1.00
P, %	0.81	0.81
Available P, %	0.44	0.44
Metabolizable energy, kcal per kg of diet	2,820.0	2,820.0
Digestible lysine, %	0.61	0.61
Digestible methionine, %	0.41	0.41
Docosahexaenoic acid (DHA), mg per kg of diet	-	600.0

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Zn, 110 mg as Zn sulfate; Fe, 40 mg as ferrous sulfate; Cu, 16 mg as copper sulfate; I, 0.8 mg as calcium iodate and 0.5 as ethylenediamine dihydroiodide; Se, 0.3 mg as sodium selenite.
[2] Supplied per kg of diet: vitamin A, 14,300 IU; vitamin D3, 2,866 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5.7 mg; riboflavin, 11.5 mg; menadione, 2.9 mg; vitamin B6, 5.7 mg; niacin, 115 mg; pantothenic acid, 29 mg; folic acid, 1.4 mg; biotin, 0.29 mg.
[3]Supplied per kg of diet: DHA, 600 mg as docosahexaenoic acid.

Table 2. Composition of broiler breeder rooster diets.

	Treatment	
Ingredients**, %**	MC	MD
Corn, coarse ground	62.69	62.41
Wheat middlings	15.00	15.00
Soybean meal	7.60	7.60
Soy hulls	8.00	8.00
Oats, whole	2.30	2.30
Mono-dicalcium phosphate	1.49	1.49
Limestone (fine)	1.05	1.05
Soybean Oil	0.35	0.35
Salt	0.31	0.31
Sodium bicarbonate	0.17	0.17
Trace mineral premix[1]	0.10	0.10
Vitamin premix[2]	0.67	0.67
L-Lysine HCL	0.04	0.04
DL-methionine	0.11	0.11
L-Threonine	0.04	0.04
Choline Cl. 60%	0.08	0.08
DHA blend[3]	-	0.28
Total	100.00	100.00
Calculated Analysis		
Crude protein, %	12.1	12.1
Ca, %	0.74	0.74
P, %	0.71	0.71
Available P, %	0.35	0.35
Metabolizable energy, kcal per kg of diet	2,720.0	2,720.0
Digestible lysine, %	0.44	0.44
Digestible methionine, %	0.26	0.26
Docosahexaenoic acid (DHA), mg per kg of diet	-	670.0

[1] Supplied per kg of diet: Mn, 120 mg as Mn sulfate; Zn, 110 mg as Zn sulfate; Fe, 40 mg as ferrous sulfate; Cu, 16 mg as copper sulfate; I, 0.8 mg as calcium iodate and 0.5 as ethylenediamine dihydroiodide; 0.3 mg as sodium selenite.
[2] Supplied per kg of diet: vitamin A, 14,300 IU; vitamin D3, 2,866 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5.7 mg; riboflavin, 11.5 mg; menadione, 2.9 mg; vitamin B6, 5.7 mg; niacin, 115 mg; pantothenic acid, 29 mg; folic acid, 1.4 mg; biotin, 0.29 mg.
[3]Supplied per kg of diet: DHA, 670 mg as docosahexaenoic acid.

Table 3. Composition of broiler breeder hen diets.

	Treatment
Ingredients**, %**	Hen
Corn, coarse ground	57.22
Wheat middlings	4.00
Soybean meal	17.50
Soy hulls	2.50
Oats, whole	6.75
Mono-dicalcium phosphate	1.63
Limestone (fine)	3.65
Oyster Shell	3.65
Soybean Oil	1.57
Salt	0.23
Sodium bicarbonate	0.20
Trace mineral premix[1]	0.09
Vitamin premix[2]	0.59
L-Lysine HCL	0.04
DL-methionine	0.21
L-Threonine	0.17
Total	100.00
Calculated Analysis	
Crude protein, %	14.43
Ca, %	3.1
P, %	0.68
Available P, %	0.34
Metabolizable energy, kcal per kg of diet	2,750.0
Digestible lysine, %	0.61
Digestible methionine, %	0.40

[1] Supplied per kg of diet: Mn, 100 mg as Mn sulfate; Zn, 100 mg as Zn sulfate; Fe, 20 mg as ferrous sulfate; Cu, 3 mg as basic copper chloride; I, 0.75 mg as calcium iodate; Se, 0.3 mg as sodium selenite.

[2] Supplied per kg of diet: vitamin A, 12,500 IU; vitamin D3, 2,505 IU; vitamin E, 90 mg; vitamin B12, 0.03 mg; thiamine, 5 mg; riboflavin, 10 mg; menadione, 2.5 mg; vitamin B6, 5 mg; niacin, 100 mg; pantothenic acid, 25.1 mg; folic acid, 1.3 mg; biotin, 0.25 mg.

Table 4. Fatty acid profiles of sperm collected at 65 wk of age, from male consuming the control diet (MC) and males consuming the diet supplemented with DHA Natur (MD)[a].

	Diet[b]	
Fatty acid	MC (%)	MD (%)
C14:0	0.31	0.30
C16:0	17.24	14.95
C16:1 n7	3.20	2.79
C18:0	9.67	8.65
C18:1 n9	0.03	0.01
C18:2 n6	2.44	2.25
C20:0	0.39	0.42
C20:1 n6	1.03	0.95
C20:4 n6	13.19	10.91
C21:0	0.52	1.22
C22:4 n6	19.56	14.36
C22:6 n3	0.64	4.39
Total PUFA[c]	9.43	9.44
n6-to-n3 ratio	17.07	3.00

[a]Semen was collected and pooled by replicate and treatment at 65 wk of age.
[b]Values are given as a percentage of total fatty acids.
[c]Polyunsaturated Fatty Acid.

Table 5. Fatty acid profiles of whole testicles collected at 65 wk of age, from male consuming the control diet (MC) and males consuming the diet supplemented with DHA Natur (MD)[a].

	Diet[b]	
Fatty acid	MC (%)	MD (%)
C14:0	0.36	0.35
C16:0	21.58	21.65
C16:1 n7	0.67	0.71
C18:0	13.75	14.04
C18:1 n9	8.58	9.44
C18:2 n6	2.84	2.88
C20:0	0.84	0.79
C20:1 n6	2.08	2.07
C20:4 n6	17.56	16.41
C21:0	0.23	0.21
C22:4 n6	12.91	11.82
C22:6 n3	0.26	2.27
Total PUFA[c]	34.85	37.06
n6-to-n3 ratio	37.20	7.04

[a]Testicles were collected individually by replicate and treatment at 65 wk of age.
[b]Values are given as a percentage of total fatty acids.
[c]Polyunsaturated Fatty Acid.

Figure 1. Semen production (%) as affected by the different dietary treatments: MC = male control diet **(**—), MD = male diet with DHA (- -). Each value represents the mean percentage of males producing semen within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 25 wk age.

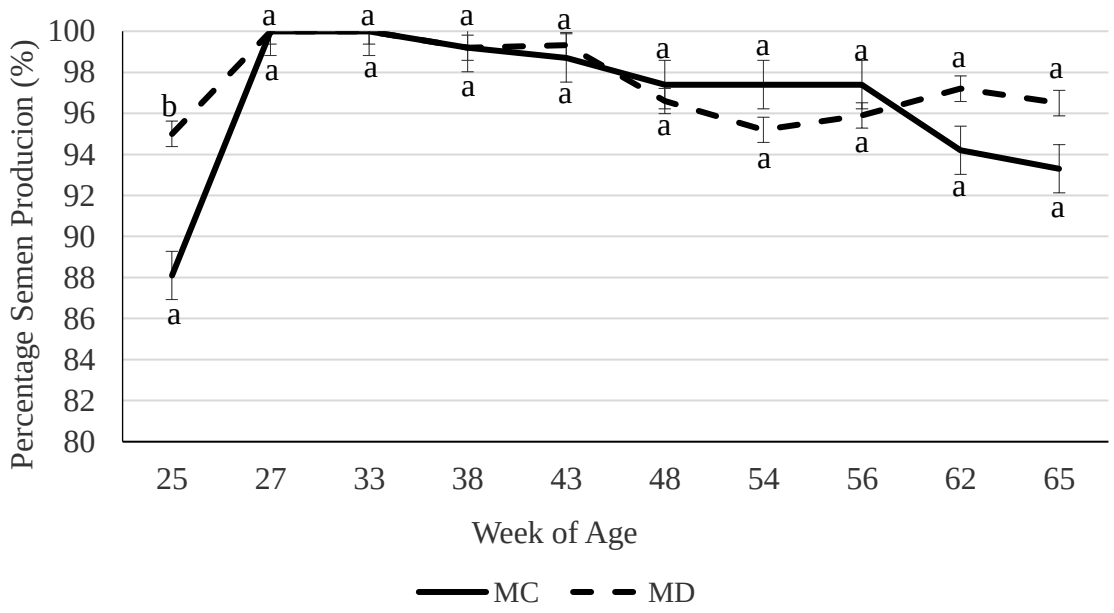


Figure 2. Semen concentration as affected by the different dietary treatments: MC = male control diet (■), MD = male diet with DHA (□). Each value represents the mean absorbance of neat semen within each dietary treatment. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 48 and 56 wk age.

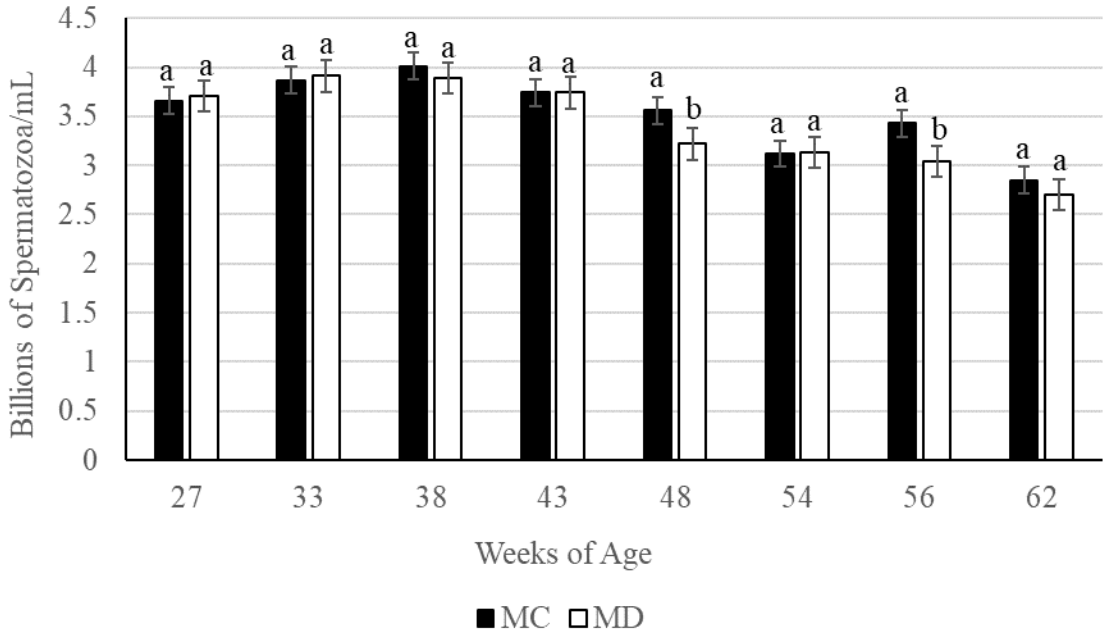


Figure 3. Semen mobility as affected by the different dietary treatments: MC = male control diet **(■)**, MD = male diet with DHA (□). Each value represents the mean absorbance of diluted semen within each dietary treatment. Mean for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 33, 38, 48, and 62 wk of age.

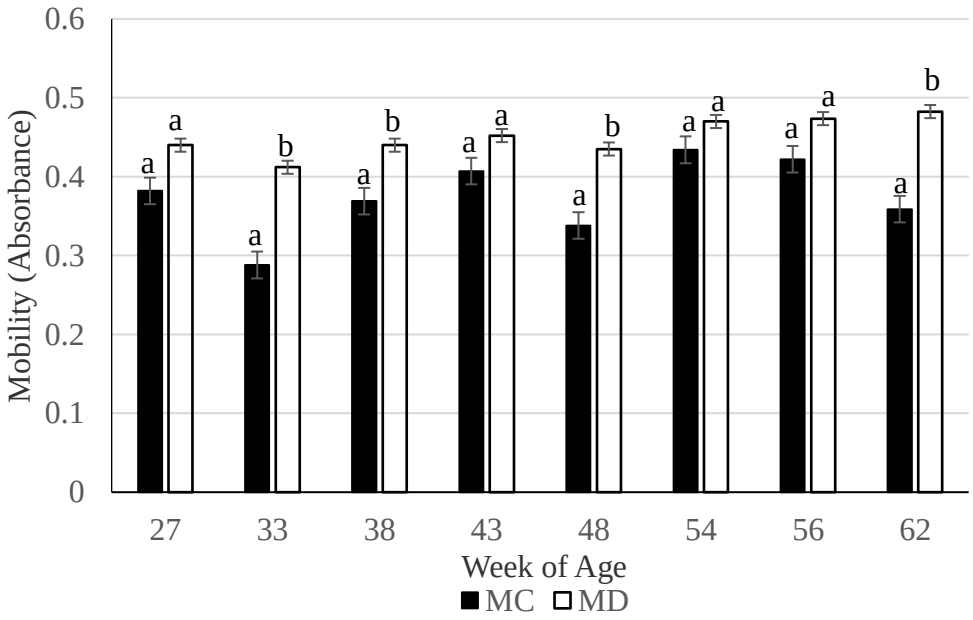


Figure 4. Overall fertility for wk 1 post insemination as affected by the different dietary treatments: MC = male control diet **(—)**, MD = male diet with DHA (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 31, 36, 41, 52, and 62 wk of age.

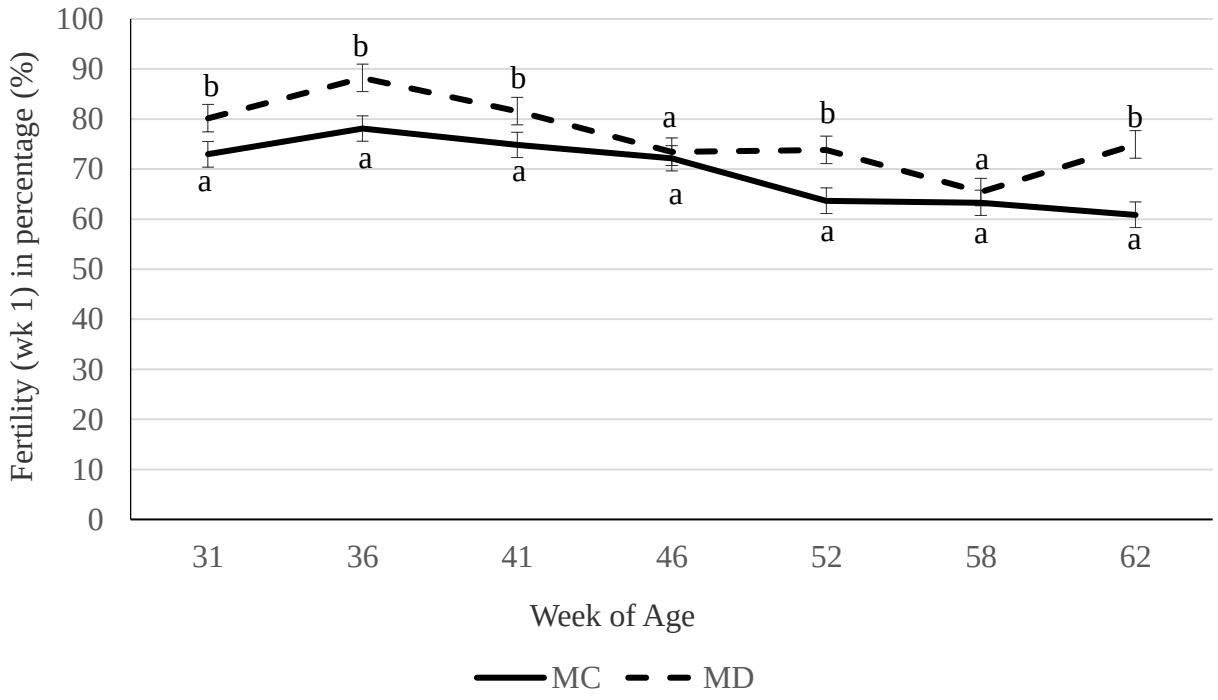


Figure 5. Overall fertility for wk 2 post insemination as affected by the different dietary treatments. Dietary treatments were as follows: MC = male control diet **(**—), MD = male diet with DHA (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 31, 36, 41, 46, and 62 wk of age.

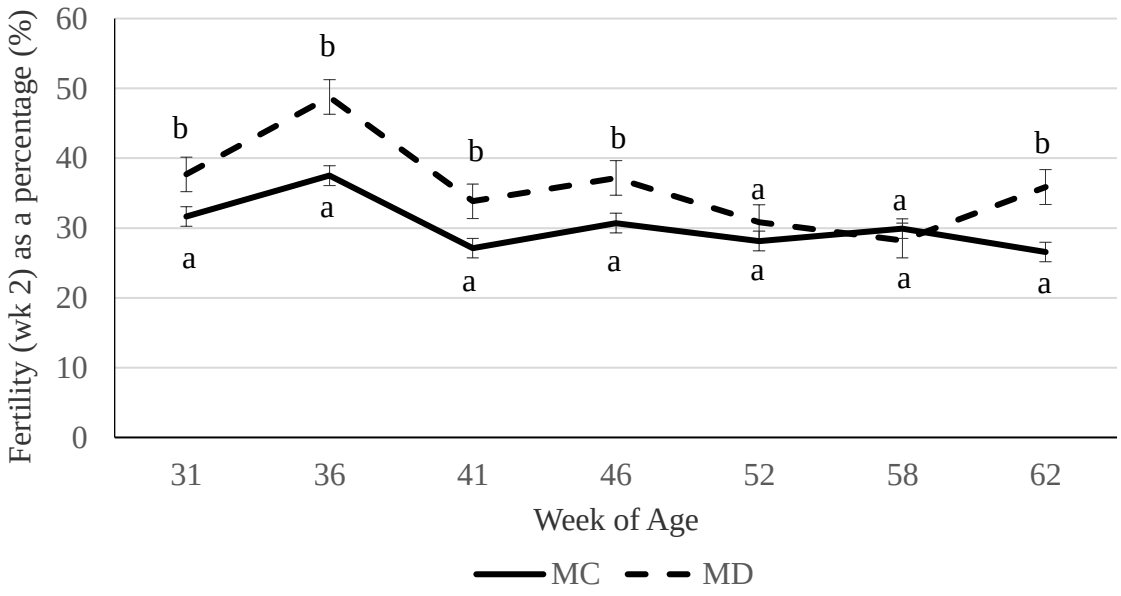
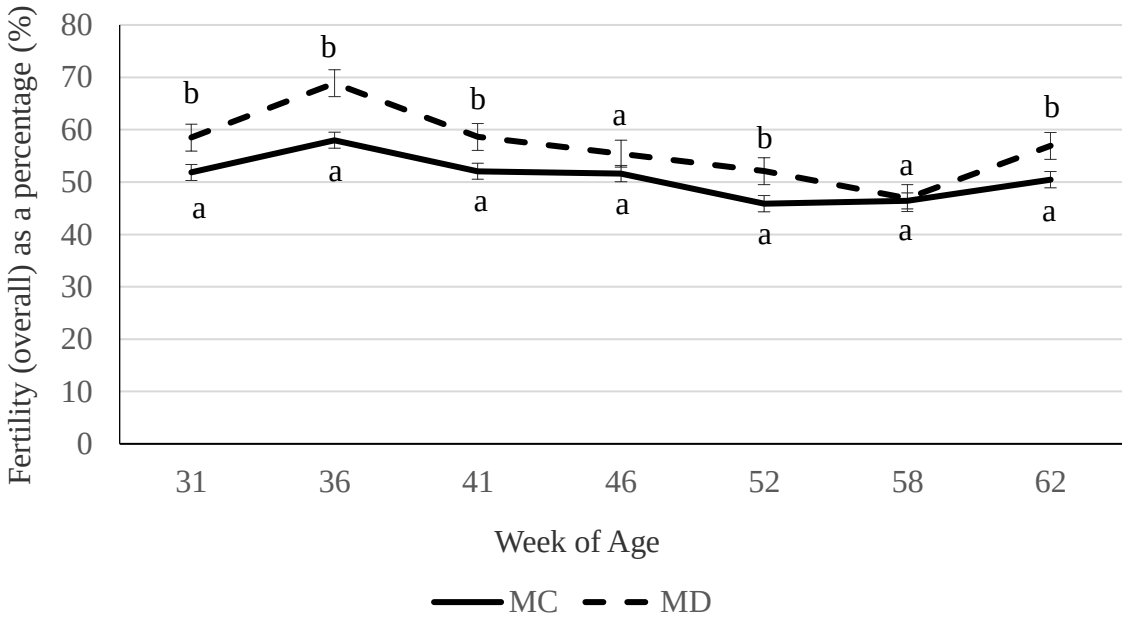


Figure 6. Overall fertility (wk 1 and wk 2) post insemination as affected by the different dietary treatments: MC = male control diet **(—)**, MD = male diet with DHA (- -). Each value represents the mean percentage of total fertile eggs produced within each dietary treatment. Percentage for different treatments with no common superscript (a-b) are significantly different (P<0.05). No superscript means no significant differences among treatments. There were significant differences (P<0.05) between the dietary treatments at 31, 36, 41, 52, and 62 wk of age.



CHAPTER 7

CONCLUSION

Due to the genetic improvement for increased growth and meat yield in broilers has increased the appetite for broiler breeders. This makes feed restriction programs an essential management tool to limit excessive body weight, poor flock uniformity, lameness, and poor reproductive performance. Qualitative feed restriction with high fiber and large particle size feedstuffs has become a positive alternative for quantitative feed restriction where birds consume smaller amounts of a concentrated diet. This method allows for increased volume to alleviate negative behaviors (frustration, boredom, pacing) associated with the high levels of feed restrictions as well as provide a sense of satiety. In our every-day (ED) versus skip-a-day (SAD) feeding study, we showed and improvement in growth and production parameters in those pullets fed ED. The use of a high fiber and bulky diet fed ED increases the body weight and body weight uniformity when compared to those pullets fed the same diet on a SAD basis. These pullets also achieving sexual maturity earlier (i.e., first egg) as well as improved overall eggshell quality and egg weight when in lay pullets were fed ED during rearing. We further studied the effect of SAD feeding of broiler breeder pullets compared to ED feeding on intestinal development via villus height and crypt depth. Where we found that ED feeding encouraged longer, and more robust villi compared to the SAD fed pullets. Perhaps the differences in

production, body weight and body weight uniformity between the two rearing feeding programs could be attributed to increased stress on the off-feed day for the SAD fed pullets and the ability to mobilize and utilize nutrients for the ED fed pullets.

Fertility and the ability to manage broiler breeder males to optimize flock fertility is of concern for broiler breeder managers. Changes in energy, protein, and calcium can impact semen quality, fertility, and testicle and kidney morphology. In our studies we evaluated the effect of diets tailored to a male's nutritional needs and supplemented with organic selenium (Se) or docosahexaenoic acid (DHA) compared to the effects of a standard hen diet, or a hen diet supplemented with organic zinc (Zn) fed to males since feeding males a hen diet is the most common practice in the US. The use of a male diet showed histological differences in lesions in the testicles and kidneys as well as an increase the number of roosters in semen production early in life when compared to males fed a hen diet that is higher protein and calcium. We also observed that when fed a diet tailored to a male's nutritional needs then supplemented with organic Se or DHA increased semen mobility and flock fertility. Future research should be conducted to evaluate the impacts of male diets on body composition, body weight management, and ability to maintain flock fertility in a floor pen setting. In addition to that research, future research should also be conducted to determine the impact of feeding DHA and organic Se on growing and developing broiler breeder males.

APPENDIX A. DETERMINING SPERM MOBILITY USING SPECTROPHOTOMETER

1. Turn on spectrophotometer and set wavelength to 550 nm. Allow 10-15 minutes for machine to warm up.

2. Prior to beginning the assay, place 3% NaCl solution, mobility buffer solution, and Accudenz® in a hot water bath maintained at 41°C.

3. Pipet 2.5 mL of 3% NaCl into a standard cuvet (blank), place into the spectrophotometer and zero the blank.

4. Add 10 uL of neat semen into the standard cuvet containing the NaCl solution, mix by inversion, and place into the machine to determine semen concentration.

5. Mix the amount of determined prewarmed mobility buffer with 50uL of raw semen.

6. Overlay 60 uL of the semen/buffer mixture over 600 uL of prewarmed 6% Accudenz® solution.

7. Incubate for 5 minutes at 41°C in a hot water bath.

8. Dry off cuvet and place on a flat surface for 1 min.

9. Place dry cuvet into spectrophotometer and read for sperm mobility determination.

APPENDIX B. MOBILITY BUFFER (100 mL)

Add the following to deionized H_2O:

1. 0.65 g NaCl (111mM NaCl)
2. 0.45 g Glucose (25 mM Glucose)
3. 0.0444 g $CaCl_2$ pellets (4 mM $CaCl_2$)
4. 1.15 g TES (50 mM TES)
5. Adjust pH to 7.4 with 1 M NaOH
6. Store at 4° C

APPENDIX C. KCl + TES SOLUTION (100 mL) AND 30 % (wt/vol) ACCUDENZ® STOCK SOLUTION AND 6 % (wt/vol) WORKING SOLUTION

Add the following to deionized H_2O:

1. 0.0224 g of KCl (3 mM)
2. 0.115 g of TES (5 mM TES)
3. Adjust pH to 7.4 with 1 M NaOH
4. Store at 4° C
5.

Add 3.0 g per 10 mL of the 3 mM KCL + 5 mM TES solution (30 % stock solution).
Add 1 mL of the 30 % stock Accudenz® solution to 4 mL of mobility buffer.