ABSTRACT

BOOT, EMMILLIE. The Effect of Fish Meal on Broiler Breeder and Red Jungle Fowl Rooster Semen Characteristics and the Impact of Water Delivery Systems on Broiler Breeder Semen Characteristics, Egg Production, and Fertility. (Under the direction of Dr. Ramon D. Malheiros).

In the previous decade, hatchability of broiler chicks in the United States has experienced a noticeable decline from approximately 85% to 80%. This trend can be attributed to many factors as broiler breeders are unique in their role. The elite stocks from which they have descended have been genetically selected for growth parameters such as feed conversion ratio and body weight gain. However, broiler breeders also must be managed to maximize their reproductive ability to create broiler chicks. Although nutrition is important for broiler production, different nutritional strategies are also key components to managing reproductive performance.

In this thesis, the impact of fish meal added to the diets of broiler breeder and Red Jungle Fowl roosters on semen quality parameters was evaluated. Two trials were done; both utilized two treatment diets, a control diet and a diet containing 3.2% added fish meal. Six broiler breeders and 5 Red Jungle Fowl were allocated to each treatment diet in the first trial. Semen was collected four times between 37 and 43 weeks of age. Semen concentration and sperm motility did not differ between the two dietary treatments. However, there was a larger volume of semen collected from roosters of both breeds when on the fish meal treatment diet. Additionally, broiler breeder roosters had significantly greater semen volume and total number of sperm cells per ejaculation compared to Red Jungle Fowl.

Two separate concurrent experiments comprised the second trial. The first experiment examined the semen of roosters fed diets containing fish meal, The second experiment examined the possibility that incorporating fish meal into the rooster's diet would improve the percentage

of fertile eggs and hatchability. In the first experiment, 5 roosters were fed the control diet and 5 were given a 3.2% fish meal diet. To collect samples for evaluation of semen volume, concentration, and motility, roosters were housed in floor pens without hens. The second experiment consisted of 6 floor pens with 16 hens and two roosters in each pen. Pens were randomly assigned to either fish meal or control treatments; only roosters received the experimental diets. Hens were given a commercial broiler breeder hen feed. No significant differences occurred in sperm parameters, egg fertility, or hatchability between the control and fish meal treated groups throughout the full production cycle.

The third trial compared egg production, egg fertility, and rooster semen quality between broiler breeders with access to either bell drinkers or gender specific nipple drinkers from 29 to 64 weeks of age. Thirty-two floor pens containing 34 hens and 6 roosters each were divided equally to create 16 replicate floor pens per treatment. Eggs were collected twice daily throughout the study. Fertility was assessed three times during the 35-week trial period. Males were separated from the hens at 61 weeks of age and semen was collected from 12 roosters per treatment 4 times between 63 and 64 weeks of age to assess semen quality. Roosters with access to gender specific nipple drinkers had higher sperm motility, while roosters in pens with bell drinkers had higher semen volume. Egg production and fertility did not differ between the two water delivery treatments. .

In conclusion, these studies on rooster fertility found that the modern broiler breeder rooster has similar semen characteristics as the Red Jungle Fowl, which supports the idea that semen quality has not improved through current methods of genetic selection. Broiler breeder roosters produced greater semen volume compared to Red Jungle Fowl roosters, but this may be due to the heavier weight of broiler breeders. As the concentration of spermatozoa did not differ

between the two types of roosters, total spermatozoa produced by broiler breeders compared to Red Jungle Fowl can be attributed to the greater volume of semen produced by broiler breeder roosters. This research found that adding fish meal to the diet increased the volume of semen produced from both breeds. Fish meal may be a useful feed additive for producers to incorporate into their rooster diets when their reproductive capabilities naturally decline with age. Lastly, broiler breeder hens' egg production, and fertility was not affected by the water delivery system. Roosters did have higher sperm motility when they had access to nipple drinkers, but roosters with access to bell drinkers had higher semen volume. Although both characteristics are important for egg fertilization, improving sperm motility is essential to ensure sperm can travel through the hens' reproductive tract to fertilize the egg. Producers need to evaluate how gender-specific nipple drinkers could be used in their broiler breeder operations to improve sperm motility.

The Effect of Fish Meal on Broiler Breeder and Red Jungle Fowl Rooster Semen Characteristics and the Impact of Water Delivery Systems on Broiler Breeder Semen Characteristics, Egg Production, and Fertility

by
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DEDICATION

To my husband, who is the light of my life and my best friend. I would not be where I am without you and wouldn't want you to be anywhere else than by my side.

BIOGRAPHY

Emmillie Boot was born and raised in her home state of Michigan to Reverends Rochelle and Nelson Ray. Coming from a long lineage of agriculturalists, she began her first chicken flock with two lavender cochin chicks at the age of 6. Being the youngest of four children, she was regularly involved in any activity her older siblings were in. This of course included FFA and 4-H, where she served in multiple officer positions, earned an American FFA Degree, and learned to love agriculture and research.

After high school, she attended Michigan State University as a fourth generation Spartan, and she married her high school sweetheart. She then earned her bachelor's in animal science with a concentration in poultry, while taking advantage of every opportunity to learn more about poultry that she could find. After completing her degree, she proceeded to work as production manager of a 2-million hen layer operation for 8 months, before taking the role she adored at Zoetis as an animal research technician. After enjoying her work at Zoetis for two years, she and her husband decided to move to Raleigh, NC for her to begin her Master's program under the direction of Dr. Ramon D. Malheiros. At North Carolina State University, her work focused on rooster reproduction, and physiology and management of broiler breeders. After graduation, Emmillie plans to pursue her PhD remaining under the direction of Dr. Ramon D. Malheiros.

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TABLE OF CONTENTS

LIST OF TABLES vii
LIST OF FIGURES viii

Chapter 1: Literature Review 1
Rooster Fertility 1
Fish Meal and Fish Oil 6
Polyunsaturated Fatty Acids 8
Drinker Types 10
References 14

Chapter 2: Comparison of Broiler Breeders and Red Jungle Fowl Semen Characteristics and the Influence of Fish Meal in their Diet 23
Abstract 23
Introduction 25
Materials and Methods 26
Housing and Management 26
Semen Quality Analysis 27
Statistical Analysis 28
Results and Discussion 28
References 30
Tables and Figures 32

Chapter 3: The Effect of Fish Meal in the Diet of Broiler Breeder Roosters on Semen Characteristics, and Subsequent Impact on Egg Fertility and Hatchability, and Semen Quality 34
Abstract 34
Introduction 36
Materials and Methods 37
Housing and Management 37
Semen Quality Analysis 38
Egg Fertility and Hatchability 39
Statistical Analysis 39
Results and Discussion 40
References 43
Tables and Figures 45

Chapter 4: The Comparison of Bell Drinkers or Nipple Drinkers on the Egg Production, Egg Fertility and Rooster Semen Characteristics of Broiler Breeders 49
Abstract 49
Introduction 51
Materials and Methods 52
Housing and Management 52
Egg Production and Fertility 53
Semen Quality Analysis 54
Statistical Analysis 54

Results and Discussion 55
References 58
Tables and Figures 60

LIST OF TABLES

Chapter 2: Comparison of Broiler Breeders and Red Jungle Fowl Semen Quality and the Influence of Fish Meal in their Diet

Table 1.1 Composition of formulated rooster diets and calculated analysis 32

Table 1.2 Main and interaction effects on semen quality parameters of roosters 33

Chapter 3: The Effect of Fish Meal in the Diet of Broiler Breeder Roosters on Egg Fertility, Hatchability, and Semen Quality

Table 2.1 Composition of formulated rooster diets and calculated analysis 45

Table 2.2 Chemical and fatty acid analysis of experimental broiler breeder rooster diets..... 46

Table 2.3 Semen quality parameters of broiler breeder roosters fed a control or fish meal containing diet 47

Table 2.4 Fertility and hatchability percentage of eggs collected from pens where broiler breeder roosters were fed a control or fish meal containing diet 48

Chapter 4: The Comparison of Bell Drinkers or Nipple Drinkers on the Egg Production, Egg Fertility and Rooster Semen Quality of Broiler Breeders

Table 3.1 Composition of formulated diets and calculated analysis 60

Table 3.2 Semen quality parameters of roosters at 63 and 64 weeks of age with access to either nipple drinkers or bell drinkers..... 61

Table 3.3 Egg production and fertility of broiler breeder hens with access to nipple or bell drinkers 62

LIST OF FIGURES

Chapter 3: The Comparison of Bell Drinkers or Nipple Drinkers on the Egg Production, Egg Fertility and Rooster Semen Quality of Broiler Breeders

Figure 3.1 Hen-day egg production by week of age and treatment 63

CHAPTER 1

Literature Review

The US Broiler industry had $50 billion total sales in 2022 (USDA NASS, 2023a). On October 1st of 2023 there were 63.4 million broiler breeders in the US (USDA NASS, 2023b). The success of the industry depends on the success of broiler breeder farms and the hatchability of the eggs produced by broiler breeder hens. In 2022 hatchability of broiler breeder eggs was approximately 80%, which is 4% lower than in 2016 (USDA NASS, 2023c). Due to decreased hatchability, the broiler industry must place more broiler breeder flocks to provide chicks for broiler production. This leads to increased laying of broiler breeder eggs, which requires the company to expand their hatcheries to provide more incubator space simply to obtain the same number of chicks. This, combined with the steady increase in consumer consumption of broiler meat over the past decade, means that poultry integrators have a need for feed and management practices that will support broiler breeder producers in increasing egg hatchability.

Rooster Fertility

Although many factors contribute to broiler breeder egg hatchability, one of crucial importance is egg fertility. Fertility of broiler breeder eggs declines after peak egg production and continues to decline as the birds age (Kirk et al., 1980). This decline could be attributed to the rooster as they yield millions of gametes per ejaculation compared to a hen that produces only one blastoderm a day. Due to the 1:10 rooster to hen ratio commonly used in the industry, the impact that a rooster has on egg fertility is immense. That ratio has been studied and commonly accepted within the industry to create a balance between putting too much stress on hens from excessive mating and optimizing flock fertility rates (Leeson and Summers, 2010).

Multiple factors contribute to a rooster's sperm quality including environment, nutrition, genetics, and age.

Since genetic companies select their broiler breeding stock through growth performance characteristics, such as feed conversion ratio and total body weight gain, their fertility characteristics have suffered (Hocking, 2010). To overcome some of the decline in fertility at the pedigree level, primary genetics companies commonly use artificial insemination. This allows the companies to apply more selective pressure on growth traits, which cannot be achieved through natural selection. By doing this, roosters that would be unsuccessful at mating naturally, due to their size or body conformation, are still able to pass on their genetics to the next generation which will ultimately be passed down to the parent flocks (Pollock, 1999). In 1973, a study was done comparing sperm quality between two different lines of White Rock chickens, one selected for low-weight and one for high-weight at 6 weeks of age. The high-weight line had significantly higher rates of abnormal spermatozoa, while the low-weight line had a higher percentage of live sperm cells (Edens et al., 1973). This showed that the consequence of 11 generations of selection for higher body weight, resulted in roosters with higher body weights and poorer sperm quality. More recently, researchers determined that there was a higher frequency of unsuccessful matings in heavier roosters (Bilcik et al. 2005). In some strains of primary broiler breeders, the dorsal pelvic width of the rooster was found to be negatively correlated with egg fertility (McGary et al., 2003). Alteration in the width of the pelvis may have had an impact upon semen transfer occurring during mating. Collectively, these studies show that traits associated with larger physical size in roosters also contribute to negative reproductive outcomes. However, it is possible to improve semen quality through selection. Traits such as semen volume, motility, and percent deformity have been found to be highly heritable (Hu et al.,

2013). Because artificial insemination is common practice at the pedigree level, elite roosters that may be mediocre at natural mating will continue to produce the offspring for the next generation, which could have an unintentional negative effect on rooster reproductive function. There have been incredible genetic advancements of terminal traits in broilers, but due to the negative correlation of reproductive traits and performance traits, fertility and hatchability potential of broiler breeders needs to carry more weight during the selection process.

Managers of broiler breeder flocks need ways to quickly identify roosters within their flocks that can mate successfully with hens and produce an adequate amount of quality sperm. The simplest method for producers to choose which roosters to retain in their flock would be based on phenotypic traits. In 2012, a correlation was made that larger combs were linked to low sperm viability, while more vibrantly red colored combs were linked with high sperm viability (Navara et al. 2012). Red Jungle Fowl hens choose to mate with roosters with larger combs given the choice, and they will be much slower to mate with roosters with smaller combs or will not mate at all (Zuk et al., 1990). These studies suggest that producers would be able to sort through roosters based on size and color of their combs. In another study, roosters were divided into three groups based on phenotype, group 1 labeled “fertile”, group 2 labeled “sub-fertile”, and group 3 “moderate”. Labels were based solely on physical characteristics such as vibrancy in color of the comb and cloaca. The roosters in group 1 had higher sperm motility, sperm count, testis weight, and Sertoli cell index than those of group 2 but was not different compared to group 3 (Talebi et al. 2018). This showed that roosters with phenotypic traits of those labeled “sub-fertile” could be successfully culled from a flock, but that “moderate” labeled roosters can perform at the same level as roosters that appeared to be “fertile”. Another study performed with two different strains of primary broiler breeders determined that Strain A had a strong correlation between fertility,

testicular weight, and comb area, but comb area could not be correlated with fertility for Strain B (McGary et al., 2002). This showed that even within strains of primary broiler breeders, phenotypic traits do not always correlate with fertility traits. Although phenotypes may provide indications of poor reproductive capabilities, they cannot be used to accurately determine ranking among a group of roosters or whether they are effectively mating and producing quality sperm. Determining which roosters are best to keep in the breeder flock is especially important as broiler breeder flocks age.

Another crucial factor among all biological species is the fertility window. As broiler breeder flocks age, the fertility and hatchability of their eggs decline, generally occurring after 45 weeks of age (Kirk et al., 1980; Walsh and Brake, 1997). However, this decline, has not been clearly attributed to either the hen or the rooster. To address this uncertainty, researchers have sought methods to identify out-of-production roosters. One study found that laparoscopy could be used on live roosters to determine if testicular atrophy, common as roosters age, is present, allowing for removal of affected roosters (Lagares et al., 2017). In a further study examining late cycle roosters, it was discovered that those weighing under 3800g exhibited low levels of testosterone, high levels of corticosterone, and were categorized as infertile or sub-fertile. (Sarabia Fragoso et al., 2013). These studies emphasize the importance of monitoring roosters throughout the production cycle and the lack of ways producers can make culling decisions for roosters in their flock. Although there is a natural decline in fertility as roosters age, one way producers can support their roosters is through providing nutrients that specifically support their fertility.

Nutrition of the rooster is one component that has a significant impact on rooster reproduction. In the US, broiler breeders are typically reared on skip-a-day feeding programs as

opposed to every day feeding programs. Everyday feeding programs involve feeding all birds in a house simultaneously at the same time each day. As the birds grow, their feed allowances are gradually increased to align with a growth curve recommended by the breed management guide. As these birds are not allowed to eat freely after 3 weeks of age but still have appetites comparable to broilers, their diets are deliberately formulated to be less nutrient-dense. Fiber is added to increase the volume of food they consume, helping to satisfy their appetite. In both every day and skip-a-day feeding programs, birds are fed *ad libitum* through weeks 0-3. Then skip-a-day programs may start to incorporate a day of fasting every few weeks to eventually reach a schedule where the birds are fed double their allowed daily intake every other day. These skip-a-day programs have been developed to support broiler breeders when they are being reared, by extending daily feed times, which gives more birds more time to eat at the feeder resulting in higher flock uniformity compared to every day feeding (Sweeney et al., 2022). To maintain a healthy body weight for the roosters, each of these feeding plans maintains feed restriction of the rooster which is accompanied by lower nutrient uptake according to the varying breed management guides (Renema et al., 2007). Introducing feed supplements to the diet of broiler breeders can complement restricted feed programs to which broiler breeder roosters are exposed and can improve their reproductive characteristics. For example, addition of 50mg/kg of camphor to the diet of roosters improved the fertility rate compared to the control diet while also increasing testosterone concentrations (Raei et al., 2021). Feeding 200 µL of Moringa extract/kg of body weight to roosters increased sperm concentration, motility, percentage of live sperm, and increased fertility and hatchability rates (Ghadimi et al., 2024). These studies show the variety of nutrients that can impact rooster sperm quality and fertility. While extensive research has been

conducted on many key nutrients in poultry diets, there are others, like fish meal, that have not been studied as thoroughly and deserve further consideration.

Fish Meal and Fish Oil

Fish meal has been used as a feed ingredient for many years as a protein source for swine and poultry and demand continues to increase as fish meal is being used in a higher percentage of aquatic diets. In the past, fish meal was a cost-effective feed ingredient as was produced as by-product of the fishing industry. Approximately 70% of fish meal and oil is refined from small, open-ocean fish, including anchovies, herring, menhaden, and sardines. The remaining 30% comes from scraps produced during the processing of fish for human consumption (NOAA, 2024). It is nutrient dense as it normally contains between 60-70% crude protein by weight. It is also high in energy and rich in calcium, phosphorous, selenium, iodine, choline, biotin, and vitamins B_{12}, A, D, and E (Cho and Kim, 2011). Additionally, selenium and vitamin E in the diet of roosters has been shown to reduces oxidation of sperm cells resulting in better sperm quality (Khan, 2011). Because poultry sperm contains high levels of polyunsaturated fatty acids, diets that contain higher levels of n-3 fatty acids also improve sperm motility and viability (Safari Asl et al., 2018). Pike (1999) reviewed studies that support using fish meal or fish oil in other animal feeds as well as in poultry, and it has been shown to positively affect growth parameters along with reproduction in poultry, swine, and cattle. For these reasons, fish meal is an attractive feed ingredient.

Researchers have examined using fish meal in poultry diets over the previous 60 years, although few specifically evaluate its effect on breeder fertility. In one study conducted with breeders, adding 2% and 4% fish meal to the diet of White Plymouth Rock chickens increased

both fertility and hatchability compared to the control diet (Opstvedt and Gjefsen, 1975). In broilers, a 5% inclusion rate of fishmeal in the diet increased feed consumption and body weight gain (Karimi, 2006). From these studies, including fish meal in poultry diets can benefit both terminal and reproductive traits. However, as prices have risen, and availability can be scarce, researchers have tried to find suitable substitutes. Soybean meal and duckweed were not suitable for replacing fish meal at a 12% rate in broiler diets; the fishmeal treatment performed better in terms of livability, feed conversion ratio, and body weight gain (Islam et al., 1997). A study in Shaver broilers found that replacing fish meal with *Leucaena* leaf protein concentrate did not maintain the same feed conversion ratio and body weight gain as the control diet that contained 5% fish meal (Agbede, 2003). In contrast, partial replacement of fish meal with fermented soybean meal was effective in improving feed conversion ratios while maintaining similar levels of meat quality in broilers (Premathilaka et al., 2020). Another study provided evidence that broilers had higher body weights, but similar feed conversion ratios in diets using soybean meal compared to fish meal (Frempong et al., 2019). In all these studies, fish meal had an impact on production parameters, which could not be matched by other ingredients presumably because of unidentified growth factors in fish meal.

Although adding fish meal offers many growth and reproductive benefits to poultry producers, there are also limits to how much should be included in the diet. White Sussex broilers fed diets containing 9% or 25% fish meal confirmed that at high rates of inclusion, fish meal was responsible for causing gizzard erosion and proventricular abnormalities (Harry et al., 1975). Components in fish meal can contribute to negative gut health for broilers, especially at high inclusion rates. A later study found a diet containing 20% fishmeal resulted in similar gizzard erosions and proventricular alterations in broilers (Itakura et al., 1982). Due to these

negative effects, it is common for producers to limit the amount of fish meal in broiler diets to no more than 10%. Due to the high nutrient density of fish meal, it is challenging to include more than 5% in broiler breeder diets while keeping the overall calorie and protein levels as low as required to maintain rooster body weights. Although fish meal has been researched in broiler diets, there are few studies that examine its use in broiler breeders or the reproductive impact that it has on roosters.

Polyunsaturated Fatty Acids

One main benefit of feeding fish meal or fish oil in poultry diets is the ability to balance n-6:n-3 fatty acid ratios. As dietary n-6 fatty acids such as linoleic acid and arachidonic acid and n-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid are absorbed using the same receptors they compete for uptake into the body. Balanced n-6:n-3 ratios in the diets of many species of animals have been associated with positive health outcomes for growth and fertility. Corn and soybeans, which make up most of the diet for poultry in the US, contain higher levels of n-6 fatty acids which means most poultry diets contain high ratios of n-6:n-3 fatty acids.

Fish meal and oil have a high content of n-3 long-chain polyunsaturated fatty acids (PUFA) (Sargent and Henderson, 1995). Salmon oil provides significantly higher egg fertility rates over corn oil at the same 5% inclusion rate and the proportion of n-3 fatty acids in the spermatozoa fatty acid profile was higher in the salmon oil treatment while n-6 fatty acids were lower (Blesbois et al., 1997). During an *Eimeria tenella* challenge, parasite invasion and development were reduced in broilers fed diets high in n-3 fatty acids from fish oil (Allen et al., 1996). Broilers supplemented with the n-3 fatty acids, eicosapentaenoic and docosahexaenoic

acids, were more effective at limiting cecal coccidia (Allen and Danforth, 1998). In Hy-line Silver-Browns aged 69-77 weeks, diet that included fish oil had the lowest egg weight and fertility, but it had the highest hatchability (Olubowale et al., 2014). Developing embryos absorb docosahexaenoic acid preferentially from the yolk sac, indicating the importance of the n-3 fatty acid in embryo development (Cherian and Sim, 1992). The addition of fish oil to the diets of broilers improved growth performance and decreased inflammatory responses (Korver and Klasing, 1997; Korver et al., 1998). Adding 2% sunflower oil or flaxseed oil to diets decreased late embryonic deaths and increased hatchability, which were attributed to the oils containing high amounts of n-3 PUFAs (Saber and Kutlu, 2020). Fish oil supplemented with vitamin E sustains sperm production per ejaculation in aging roosters (Surai et al., 2000). Clearly, the benefits of n-3 fatty acids in the diet of poultry are numerous, but the mechanism(s) by which they improve fertility has yet to be clearly identified.

Increasing n-3 fatty acids in the diets of people has stimulated research on ways for them to consume a greater balance in the n-6:n-3 ratio. Including fish meal or fish oil in the diets of chickens, the meat and eggs they produce are fortified with eicosapentaenoic acid and docosahexaenoic acid (Ganesan et al., 2014). In the process of fortifying poultry products with n-3 fatty acids, consumers identified a “fishy” flavor in eggs and poultry meat from birds fed diets containing fish meal or fish oil (Carrick and Hauge, 1926; Marble et al., 1938; Vondell, 1948). Leskanich and Noble (1997) showed that it is possible for poultry meat to be fortified with n-3 fatty acids from fish meal or fish oil without the “fishy” taste by limiting the percentage of fish by-products in the feed and by using high quality by-products. In a blind taste test of eggs from chickens fed diets containing fish oil, participants could not distinguish fortified from conventional eggs. Participants who consumed one fortified egg daily had PUFA n-6:n-3 ratios

in blood plasma that were significantly reduced from 12.2:1 to 6.5–7.7:1 (Farrell, 1998) Although the definitive cause of the fishy flavor in meat and egg products has not been discovered, it can be mitigated through managing the lipid autoxidation of the PUFAs in the diet and subsequently the meat and egg products (Venkateshwarlu et al., 2004). Including fish oil or fish meal in the diets of poultry not only benefits the birds, but also results in egg and meat products that contain more n-3 fatty acids.

Drinker Types

One of the most important nutrients for poultry is water. In general, water systems within poultry houses can be classified as either open or closed source. Examples of open sources are troughs, Plasson bell drinkers (Plasson USA, Sugarland, TX, USA), or supplemental drinkers used for chicks. They allow contact with the air, shavings, and birds within the barn. Chickens utilize gravity for drinking and eating in order to facilitate movement of material into the crop. This requires the birds to dip their beaks into the water and then lift their head up so that the water can be swallowed. Closed sources of water, include systems that use nipple drinkers that work by being triggered by the beak of the bird. Typically, the bird must lift its neck and peck the nipple to trigger the release of water from the water line, which makes it an easier motion for chickens to complete. Many poultry producers have converted to closed source systems, specifically nipple drinkers in their operations. Benefits to closed source systems, which make them an attractive option for producers, include decreased bacterial contamination in the water, and they are significantly easier to sanitize.

Microbial load of drinker lines is especially important for producers to monitor for their flocks as many viral and bacterial pathogens that affect poultry can be transmitted by water (Amaral, 2004). Pathogens such as *Pseudomonas* spp*., Stenotrophomonas* spp., and

Ochrobactrum spp*.,* have been isolated from broiler drinking lines. They exceeded the maximum limit of 4.0 $\log_{10}$ CFU/mL even after a chlorine dioxide (ClO_2) water treatment (Mustedanagic et al., 2023). However, in another study, sanitizing the water lines daily with chlorine kept microbial loads below 1,000 cfu/ml (Maharjan et al., 2016). Broilers performed better with access to nipple drinkers compared to bell drinkers, and the bell drinkers regularly contained higher amounts of microbiological contaminates (Valias and Silva, 2001). Together *E. coli* and nitrogen in the drinking water of broiler breeders resulted in decreased egg production with lower fertile egg hatchability (Grizzle et al., 1997). In a field trial, as the drinking water quality decreased so did broiler breeder performance (Zaghari et al., 2011).

Productivity of poultry provided water via nipple drinkers compared to troughs or bell drinkers is essential to understand, as the poultry industry continues to produce meat for consumers. In broilers, no difference was found in body weight gain or feed consumption between trough and nipple watering systems or bell and nipple systems (McMasters et al., 1971; Fernandes et al., 2002). However, when nipple lines with and without cups, broilers getting water from nipple lines without cups had lower body weights at 7 weeks (Andrews et al., 1993). When given the choice, broilers were found to consume more water from bell drinkers compared to nipple drinkers, but only when temperatures were 32 or 35 degrees Celsius (May et al., 1997). In general, there is no consensus on the optimal type of water drinker for broiler growth; however, nipple drinkers are more commonly used because they also help reduce water wastage and improve litter conditions.

Nipple drinkers can significantly help water demand and usage in poultry egg production. For instance, Pekin breeder ducks with access to open water troughs experienced higher rates of foot lesions, used more water, had increased litter moisture, and had troughs with higher

microbial loads, resulting in fewer eggs laid compared to ducks using nipple drinkers (Lowman et al., 2016). Similarly, in laying hens, use of nipple drinkers resulted in greater egg production and improved feed conversion compared to drinker cups (Togashi et al., 2008). By reducing litter moisture, nipple drinkers help keep birds cleaner, make litter management easier, and facilitate better control of temperature and humidity, all of which contribute to more efficient egg production.

Chickens tend to drink soon after they eat, which means water consumption patterns of broilers and broiler breeders differ compared to layers. However, regardless of the feed schedule of breeders, total water consumption did not vary, just consumption patterns (Bennett and Leeson, 1989). Nipple drinkers have become the primary water delivery system in layer houses. Nipple drinkers also were found to be an appropriate substitute for bell drinkers during the production cycle of broiler breeders as there was no difference in flock mortality or egg production between water sources (Colvero et al., 2014).

Water delivery is also essential for mitigating heat stress in broiler breeders, as heat stress causes a reduction in reproductive parameters. When roosters were under active heat stress, semen from them had decreased sperm penetration of the germinal disc (McDaniel et al., 1995). Ensuring birds have adequate access to drinking water can mitigate some of the effects of higher temperatures. In the summer months, there were no differences in egg production of semi-heavy hens when water sources were nipple or bell drinkers (Silverio Klosowisk et al., 2009). These papers suggest that ensuring broiler breeders have adequate access to water through either drinker type is essential. However limited research showing if either type has a benefit to broiler breeders is limited.

In summary, the success of the US broiler industry hinges on improving the hatchability and fertility of broiler breeder eggs. As the industry faces challenges such as declining hatchability rates and the rise in demand for broilers, addressing the factors that influence egg fertility becomes crucial. Rooster fertility, influenced by genetics, age, and nutrition, plays a pivotal role in optimizing reproductive outcomes. Strategic nutritional interventions, including the use of supplements and carefully managed feeding programs, can enhance sperm quality and overall fertility. Additionally, incorporating fish meal into diets has shown promise in improving reproductive traits, though careful management of inclusion rates is necessary to avoid negative effects. Water delivery systems, particularly closed sources like nipple drinkers, contribute to better management of water wastage and litter quality. To support the continued growth of the broiler industry, poultry integrators need to focus on integrated approaches that encompass nutritional strategies, and effective management practices, to enhance the reproductive performance of broiler breeders and maintain industry sustainability.

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CHAPTER 2

Comparison of Broiler Breeders and Red Jungle Fowl Semen Characteristics and the Influence of Fish Meal in their Diet

ABSTRACT

The hatchability of broiler chicks has decreased in recent years, resulting in the need for improvements in reproduction in both the broiler breeder hen and rooster The rooster is responsible for providing millions of viable sperm to hens for eggs to be fertilized, whereas hens will only contribute a germinal disk associated with one egg per day at most. These two components determine whether it is fertilized or not. Thus, improving or sustaining sperm quality parameters is essential to improving egg fertility. The objective of this study was to determine if the addition of 3.2% inclusion rate of fish meal to the diet of broiler breeder roosters (*Gallus domesticus*) and Red Jungle Fowl (*Gallus gallus*) would improve sperm quality parameters. Twelve broiler breeder roosters (6 replicates per treatment) and 10 Red Jungle Fowl (5 replicates per treatment) were assigned to either a fish meal dietary treatment or a control diet (industry standard). All broiler breeders used were fed 140 grams of their respective diets daily while all Red Jungle Fowl were fed *ad libitum*. A total of 88 semen sample (broiler breeder and Red Jungle Fowl) collection attempts were made over a 6-week period. Sample collection resulted in 61 semen samples with a volume high enough for analysis. Immediately after collection, 100 microliters of each sample were diluted in 2mL of semen extender and kept at 40°C until testing was completed using a SQA-Vt™ Automated Semen Quality Analyzer (Medical Electronic Systems, Encino, CA, USA). Semen volume, sperm concentration, motile sperm concentration and sperm motility were measured by the SQA-Vt™, and data was analyzed via one-way ANOVA in JMP 17.2 (SAS, Cary, NC, USA), with a significance level of $P \leq 0.05$.

When comparing the semen quality parameters between the broiler breeder and Red Jungle Fowl roosters, it was found that broiler breeders had a higher volume of semen at each collection ($P<0.0001$) and had a higher total amount of sperm cells produced per ejaculation ($P=0.0008$). There was also a statistical difference in the volume of semen collected between the dietary treatments, where roosters on the fish meal treatments produced more semen than the control treatment roosters regardless of breed ($P=0.0006$). There were not any statistical differences between dietary treatments on the semen quality of the roosters. The results of this study suggest that the addition of fish meal to the diet of broiler breeder roosters and other chicken breeds needs further investigation as a viable additive to increase semen quality, as it did increase semen volume.

INTRODUCTION

The hatch rate for fertile broiler eggs has been steadily decreasing over the last decade. Due to this decline, new ways of increasing the reproductive ability of broiler breeders need to be evaluated. The rooster plays an essential part in egg fertility and in the commercial setting is responsible for regularly mating with at least ten or more hens throughout the lay cycle. The ability of the rooster to successfully mate with the hen can be hindered by many factors, including their diet. There have been numerous studies which support the addition of fish meal or other feed ingredients that are high in n-3 polyunsaturated fatty acids which result in better reproductive outcomes (Opstvedt and Gjefsen, 1975; Pike, 1999). However, there are limited research studies on the effects of fish meal associated with the reproductive ability of modern broiler breeder roosters.

Red Jungle Fowl, are readily accepted as the ancestor of the modern chicken, and as such have been used as a model for comparing physiological adaptations that have been made through the process of genetic selection. One study was done that compared semen quality of Red Jungle Fowl to the "domestic chicken" and showed that the Red Jungle Fowl had higher sperm motility, concentration and semen volume, however what the researchers used as the "domestic chicken" strain was not revealed (Malik et al., 2013). Because of the large physiological differences between modern broiler breeder roosters and Red Jungle Fowl, and how often Red Jungle Fowl are used in studies related to mating, mate preferences, and semen preservation, the value in understanding how these two breeds vary in terms of semen quality would be very impactful for the scientific community going forward.

This research evaluates the impact of fish meal on the quality of semen from both broiler breeder and Red Jungle Fowl roosters. By evaluating semen quality parameters such as volume,

sperm concentration, and sperm motility, this research seeks to determine the efficacy of fish meal as a beneficial dietary additive. The findings could provide valuable insight to enhance the reproductive performance in not just broiler breeders, but other strains of chickens as well.

MATERIALS AND METHODS

Housing and Management

All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee under protocol #22-280. The roosters used in this study were reared in a close sided, temperature-controlled poultry house in floor pens with access to litter. At 35 weeks of age, 12 Ross 708 (Aviagen, Huntsville, AL, USA) broiler breeder roosters and 10 Red Jungle Fowl roosters were transferred to individual wire floor cages. The set temperature for the room was 21°C and the light cycle was 16L:8D. At the time of move, roosters were randomly assigned to either a fish meal or control diet treatment (Table 1.1), and all roosters were on their diets for two weeks to allow the effects of the treatment diet to take effect. All Red Jungle Fowl were fed *ad libitum,* with 5 Red Jungle Fowl being fed the fish meal diet and 5 being fed the control diet. The broiler breeders were divided into two treatments, with 6 being fed the fish meal diet and another 6 being fed the control diet. Red Jungle fowl were fed *ad libitum* to emulate their foraging pattern in their natural habit (Klasing, 2005). The broiler breeder roosters were fed 140 grams daily to emulate commercial production feeding habits which strive to maintain their body weight and minimize weight gain over the 8 total weeks they were in their individual cages. Sample collection for analysis began when the roosters were 37 weeks of age. All roosters were trained to the dorso-abdominal massage technique (Burrows and Quinn, 1937) three times during the two week acclimation

period before the first experimental semen collection took place, to allow the roosters to adjust to the personnel handling them.

Semen Quality Analysis

Semen analysis was performed immediately after collection for all samples. The semen samples were collected in 4mL microcentrifuge tubes and volume was measured by eye according to the total amount of fluid captured in the tube. The microcentrifuge tube volume gradations were previously tested using a calibrated micropipette to ensure volume accuracy. Then, 100µL of semen was gently mixed into 2mL of SQA-Vt™ turkey semen diluent which had been warmed to 40°C. An aliquot of this sample was drawn into a pre-warmed 1mL slip tip syringe and immediately dispensed through the SQA-Vt™ capillary. The capillary was then inserted into the SQA-Vt™ Semen Analyzer for sample analysis. Sperm concentration, motile sperm concentration and percent motility data was generated from the SQA-Vt™ Semen Analyzer for each sample analyzed.

Volume was calculated based on semen volume collected at each attempt from each rooster. The rest of the semen quality parameters were analyzed according to the semen samples that were able to be collected as not every rooster produced semen with every attempt. Sperm concentration (TSC) is presented as billions of sperm cells/mL of semen. Motile sperm concentration (MSC) is presented as billions of motile sperm cells/mL of semen and accounts for any dead sperm or nonmotile sperm cells within the sample. Motility percentage is calculated by (motile sperm concentration)/(total sperm concentration)*100. The total sperm cells produced is calculated by (total sperm concentration)*(volume)*1000.

Statistical analysis

All data were analyzed as a 2x2 factorial randomized design with each rooster serving as a replicate within the treatment group. Data was analyzed by two-way ANOVA using JMP 17.2 and the means were then statistically distinguished using Student's t-test. The main effects and interaction effects were considered significant at $P \leq 0.05$.

RESULTS and DISCUSSION

There have been limited studies that have examined the differences in semen quality between Red Jungle Fowl and modern broiler breeder roosters. Analysis between broiler breeder roosters and Red Jungle Fowl roosters displayed in Table 1.2 show that broiler breeders have a significantly higher volume of semen produced on average than the Red Jungle Fowl ($P<0.0001$). This resulted in the total number of sperm cells produced being significantly higher from the broiler breeders as well ($P<0.0008$). This difference may be attributed to the body weight and size differences between the two breeds. This agrees with other research in roosters that showed that there is a correlation between testicular weight and volume of semen and number of sperm cells produced (Sarabia Fragoso et al., 2013). Our results disagreed with the results of Malik et al. (2013) who determined that Red Jungle Fowl had a higher volume of semen when compared to the "domestic chicken". This difference may be due to the feed requirements of the broiler breeder changing as it has been selected over time for faster growth, larger body weight, and better feed conversion.

This trial did not show any statistical differences between broiler breeders and Red Jungle Fowl roosters in total sperm concentration, motile sperm concentration, or percentage motility. This suggests that the modern broiler breeder rooster at peak flock age has comparable

semen concentration and motility to their ancestral counterpart as very little selection pressure has been placed on broiler breeder roosters for reproductive ability.

When comparing the semen quality between the fish meal and control dietary treatments, it was determined that roosters being fed the fish meal diet did have a higher volume of semen produced whether in broiler breeder or jungle fowl roosters ($P<0.006$). This suggests that a component of the fish meal does support the production of seminal fluid which makes up the majority of semen. Selenium which, is found abundantly in fish meal, also contributes to the production of glutathione peroxidase that is known to decrease the oxidation of sperm cells (Khan, 2011). The suspected additional selenium in the fish meal diet is likely to have improved the ability of the roosters to produce the seminal fluid which facilitates the ejaculation of sperm cells from the testes.

This trial did not support our hypothesis that the addition of fish meal would have a positive effect on the semen quality parameters of roosters as there were no statistical differences in semen quality parameters between dietary treatments although in broiler breeders there was a 1.2 billion/mL increase in total sperm concentration and motile sperm concentration between the dietary treatments. This study showed no significant interaction effects among treatments. These results are in opposition to the research conducted by many other researchers which showed that the inclusion of fish meal improved growth and reproductive parameters in poultry (Opstvedt and Gjefsen, 1975; Pike, 1999; Agbede, 2003; Premathilaka et al., 2020). However, there is further research needed in this area, as very little literature is published on the effect of fish meal on the semen quality of roosters. The role of fish meal and its unidentified growth factor need to be evaluated as the value of broiler chicks continue to rise, and hatchability continues to decline in the US.

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Table 1.1 Composition of formulated rooster diets and calculated analysis.

Ingredients[1]	**Control**	**Fish Meal**
Corn	69.00	58.00
Soybean Meal	5.00	1.04
Wheat Bran	21.00	20.00
[2]Fish Meal	0.00	3.20
Filler Sand	0.00	8.80
Limestone Fine	1.26	1.00
Mono-Dicalcium Phosphate	1.33	1.00
Sodium Chloride	0.25	0.25
Sodium Bicarbonate	0.25	0.25
L- Lysine	0.058	0.064
DL- Methionine	0.120	0.104
L-Threonine	0.356	0.382
[3]Mineral Premix	0.20	0.20
[4]Vitamin Premix	0.05	0.05
[5]Selenium Premix	0.05	0.05
[6]Optiphos 6000 PF	0.05	0.05
Poultry Fat	1.00	5.66
Calculated Analysis		
Crude Protein	11.00	11.00
Metabolizable Energy (Kcal/Kg)	1300	1300
Ca	0.752	0.750
P	0.702	0.701
Available P	0.400	0.400
Lysine	0.490	0.490
Methionine	0.310	0.310

[1]Ingredients presented as percent of diet.

[2]Special Select™-menhaden fish meal, stabilized with 0.06% ethoxyquin

[3]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[4]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[5]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

[6]Optiphos 6000 PF, phytase enzyme purchased from Huvepharma, Sofia, Bulgaria.

Table 1.2 Main and interaction effects on semen quality parameters of roosters.

Interaction		TSC[2]	MSC[3]	Motility[4]	Volume[5]	Total Cells[6]
Rooster Type	Diet[1]	(Billions/mL)	(Billions/mL)	(%)	(μL)	(Billions)
Broiler Breeder	Control	4.461	1.173	43.94	279.17b	1.98ab
	Fish Meal	5.640	2.358	38.22	450.00a	2.70a
Red Jungle Fowl	Control	4.500	1.344	29.10	40.00c	0.72b
	Fish Meal	4.502	1.941	45.22	175.00b	1.02b
SEM		0.38	0.25	3.66	21.44	0.20
P-Value		0.5259	0.9766	0.1431	0.6774	0.6010
Main effects (P-Value)						
Rooster Type		0.4155	0.4235	0.5955	**<0.0001**	**0.0008**
Diet		0.5228	0.2250	0.4815	**0.0006**	0.2201

[1]Dietary treatments: Control=conventional diet containing soybean meal and corn; Fish Meal=diet containing soybean meal, corn and 3.2% fish meal.
[2]TSC=Total sperm concentration
[3]MSC=Motile sperm concentration
[4]Motility=Percentage of motile sperm
[5]Volume=Amount of semen collected at each handling
[6]Total Cells=Calculation of total sperm cells found by TSC x Volume for each sample

CHAPTER 3

The Effect of Fish Meal in the Diet of Broiler Breeder Roosters on Semen Characteristics, and Subsequent Impact on Egg Fertility and Hatchability

ABSTRACT

In the poultry industry, enhancing reproductive performance of broiler breeders is crucial for sustaining the broiler supply in the US. As the demand for broiler meat continues to rise worldwide, producers need solutions to reduce the impact of age on reproductive parameters in broiler breeders. The objective of this study was to determine if a 3.2% inclusion of fish meal in the diet of broiler breeder roosters would improve sperm quality parameters and, in response, improve the fertility and hatchability of broiler breeder eggs. To evaluate semen quality, 10 total broiler breeder roosters were randomly assigned to a diet with or without fish meal and placed into floor pens. Semen was collected eight times from each rooster between 34 and 65 weeks of age for a total of 80 semen collection attempts and 65 usable samples. Immediately after collection, 100 microliters of each semen sample was diluted in 2mL of semen extender and kept at 40°C until testing was complete using the SQA-Vt™ Automated Semen Quality Analyzer (Medical Electronic Systems, Encino, CA, USA). Semen volume, sperm concentration, and sperm motility were measured at a standard cells/ml. To investigate egg fertility and hatchability, six floor pens were used, with 16 hens and two roosters randomly assigned to each pen. All hens were fed a commercial diet according to the breed management guide. Eggs were collected and incubated to assess egg fertility fifteen times and hatchability seven times over an entire flock production cycle. Egg hatchability and fertility were determined by setting all eggs collected over two days of lay and candling them at 10 days of incubation to remove any eggs that appeared to not be developing. These eggs were then opened and visually inspected to determine

the fertilization status of the germinal disc. At day of hatch, residue was also broken out to confirm fertility in eggs that did not hatch. Data was analyzed via two-sample t-test in JMP 17.2 (SAS, Cary, NC, USA), with a significance level of $P \leq 0.05$. All sperm quality parameters were not significantly different between treatments. Neither egg fertility nor hatchability was statistically different between the two treatments.

INTRODUCTION

As broiler breeder egg hatchability has fallen to approximately 80% in the US, poultry integrators need to have solutions that they can use on the farm to maintain higher egg fertility and hatchability from their birds (USDA NASS, 2023c). In many broiler breeder operations, the rooster is constricted to eating the same diet as the hens, that has not been formulated specifically for them. One potential avenue for improvement is through nutrition, specifically for the rooster since they are often overlooked in production houses especially when many houses don't have bin space for rooster diets.

Since many diets are not formulated with the ratio of polyunsaturated fatty acids in mind, this study explores the potential impact of fish meal being added to the diet of broiler breeders to balance the ratio. By increasing the amount of n-3 fatty acids in the diet, a previous study has shown an increase in egg fertility and that the concentration of n-3 fatty acids in semen samples were higher (Blesbois et al., 1997). N-3 fatty acids coupled with vitamin E added to the diet also appears to maintain fertility of aging roosters (Surai et al., 2000). In a layer study, where fish oil was compared to other feed oils, the fish oil treatment provided the highest egg hatchability (Olubowale et al., 2014). All of these studies point to the ability of n-3 fatty acids to improve reproductive outcomes in poultry.

Fish meal is a common ingredient in poultry diets, and is also known to be high in protein, minerals, and energy (Cho and Kim, 2011). When fish meal was used in 1975 in the diets of White Plymouth Rock chickens, the fertility and hatchability of the eggs were higher than in the control diets (Opstvedt and Gjefsen). Despite its potential, fish meal has not been thoroughly investigated as an ingredient in the diet of broiler breeder roosters for the purpose of improving rooster reproductive parameters. One of the objectives of this project was to address

the gaps in previous research by focusing on the modern broiler breeder rooster. Effects of feeding fish meal on semen quality parameters, egg fertility and hatchability will provide an analysis on the potential benefits to the current poultry industry and may allow for a rise in hatchability overall in the US.

MATERIALS AND METHODS

Housing and Management

All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee under protocol #22-385. This study used Ross 708 parent stock (Aviagen, Huntsville, AL, USA) that were reared in a temperature-controlled poultry house in floor pens with access to litter. They were transferred at 25 weeks of age to a curtain sided house for the lay period. The high and low ambient temperature ranged from 15 to 30°C. At 25 weeks of age, 10 roosters used for semen quality analysis were randomly allocated to a control or fish meal treatment floor pen. Five broiler breeder roosters were assigned to the fish meal diet treatment pen, and 5 roosters were assigned to be in the control diet treatment pen. The treatment diets for all the roosters in this trial are shown in Table 2.1. In the same poultry house, there were a total of 96 hens and 12 additional roosters that were randomly allocated to either the control or fish meal diet to collect eggs to measure egg fertility and hatchability. There were three replicate pens for both the fish meal and control diet treatments, with 16 hens and two roosters assigned to each pen. All broiler breeder roosters were allocated the same amount of their respective diet, while all hens were allocated the same amount of a commercial broiler breeder hen diet containing 15% crude protein, 1270

kcals/kg, 3% calcium, and 0.7% phosphorus. All feed amount changes followed the Ross 708 parent stock guidelines.

All roosters were trained to the dorso-abdominal massage method (Burrows and Quinn, 1937) three times before their first experimental semen collection took place, this was to allow the roosters to adjust to the personnel handling them. There were eight semen collection dates throughout the study beginning at week 34 of age with the last collection at 65 weeks of age for a total of 80 samples collection attempts which produced 65 samples total for quality analysis.

Eggs were hand collected twice daily and recorded and labeled for each pen. Eggs were collected and incubated for 10 days from 28 weeks of age to 56 weeks of age to assess egg fertility 15 times and returned to the incubator until hatch seven times. Each fertility or hatchability test contained at least 12 eggs from each pen, for a total of 1,080 eggs set. All groups of eggs to be set were collected over a two-day period. Once collected, eggs immediately went into a fertile egg cooler kept at 13°C and 55% humidity until they were incubated.

Semen Quality Analysis

Semen analysis was performed immediately after collection of all the samples. Samples were collected in 4mL microcentrifuge tubes and volume was measured according to the total amount of fluid captured in the tube. Then, 100µL of semen was gently mixed into 2mL of SQA-Vt™ turkey semen diluent which had been warmed to 40°C. An aliquot of this sample was drawn into a pre-warmed 1mL slip tip syringe and immediately dispensed through the SQA-Vt™ capillary. The capillary was then inserted into the SQA-Vt™ Semen Analyzer for sample analysis.

Volume was calculated based on how much semen is collected at each attempt from each rooster. The rest of the semen quality parameters were analyzed according to the semen samples

that were able to be collected as not every rooster produced semen with every attempt. Sperm concentration (TSC) is presented as billions of sperm cells/mL of semen. Motile sperm concentration (MSC) is presented as billions of motile sperm cells/mL of semen and accounts for any dead sperm or nonmotile sperm cells within the sample. Motility percentage is calculated by (motile sperm concentration)/(total sperm concentration)*100.

Egg Fertility and Hatchability

After egg collection, all eggs were labeled with their corresponding pen number and stored in an egg cooler maintained at 14°C and 55% humidity for up to two days. Eggs were inspected for cracks, checks, and shell abnormalities and sorted by pen. Then they were placed in trays and incubated in the same GQF 1200 series Sportsman incubator (Savannah, GA, USA) at 37.5 °C and 55% humidity. At 10 days of incubation all eggs were candled for fertility. If there was no development observed through candling, the eggs were broken open and examined by eye to determine if fertilization had occurred at the germinal disc. The eggs that had the appropriate development at candling were returned to the incubator to continue incubating. At 18 days of incubation, the eggs were candled again to remove any embryos that had died and were transferred to hatch baskets in pedigree bags to separate chicks by pen. The embryos were all hatched in the same GQF 1200 series Sportsman incubator at 36.9°C and 65% humidity. On day 21 of incubation, hatch trays were pulled and hatched chicks were counted and recorded based on pen.

Statistical analysis

All semen quality data were analyzed by treatment with each rooster serving as a replicate within each treatment group. Egg fertility and hatchability data used the pen as the experimental unit with three replicate pens for each treatment. Data was analyzed by two-sample

t-test using JMP 17.2 and the means were then statistically distinguished using Student's t-test. The treatment effects were considered significant at $P \leq 0.05$.

RESULTS and DISCUSSION

The impact of a 3.2% inclusion of fish meal in the diet of broiler breeder roosters on semen quality parameters indicated there were no significant differences in any semen quality parameter between the two dietary treatments. Even though the feed was formulated to be isocaloric and isonitrogenous, the feed analysis results showed that the treatment diets from Table 2.2 had different nutrient values. The crude protein was higher in the control diet by 1%, and the control diet contained 400 Kcal/Kg more gross energy. The total fat content of the fish meal diet was 2% higher than that of the control. When examining the fatty acid profile of the diets the control diet was found to contain a higher percentage of n-3 fatty acids at 23.06% compared with 2.28% in the fish meal diet. This may explain why the control treatment had higher averages for all semen quality parameters measured (Table 2.3). Although the diets were not formulated according to fatty acids, we suspected from a previous preliminary trial that incorporating fish meal had improved rooster reproduction due to the high amount of n-3 fatty acids that are contained in fish meal, which may have provided a more balanced fatty acid ratio. The diet was recreated for this trial and sent for fatty acid profile analysis at ATC Scientific (Little Rock, AR, USA). The results of the analysis between the diets showed opposite result. The n-6:n-3 fatty acid ratio in the control diet was very low, whereas the fatty acid ratio of the fish meal diet was approximately 12:1.

A few factors may have contributed to these results, such as the variability in fish meal products. Variability in protein, mineral, and fatty acid content has been noted in research since

the 1950s (MacIntyre, 1957). The fatty acid ratio of fish meal also varies based on what species of fish is being used to produce the fish meal (Giogios et al., 2009). Another factor is that the polyunsaturated fatty acids within the fish meal were likely decomposing over the time that the trial occurred. Due to the low number of roosters on trial, new feed was mixed every three months, which is very long compared to the industry that typically has new feed delivered every week. N-3 fatty acids in fish meal don't degrade as rapidly as in fish oil, but they do degrade linearly over time (Lin et al., 2021). The lack of n-3 fatty acids in the fish meal diet shows just how variable fish meal can be as a feed ingredient and the importance of identifying the origin of fish meal along with acquiring an ingredient analysis before formulating diets.

The analysis of the semen quality data showed that there were no significant differences between treatments for any of the parameters we examined. This supports the idea that a balance of n-6:n-3 fatty acids are essential to rooster fertility, since neither diet contained an ideal 1:1 fatty acid ratio (Alagawany et al., 2019).

There was no statistical difference between treatments in terms of egg fertility or hatchability either (Table 2.4). The fertility of the control treatment eggs was 91.84% and hatchability was 76.11%, which shows a 15% embryo mortality rate. The fish meal treatment eggs had a fertility of 89.69% with a hatchability rate of 82.90%, resulting in a 6% embryo mortality rate. These results were also unexpected as previous literature has shown that the addition of fish meal in the diets of poultry increase growth and reproductive parameters (Opstvedt and Gjefsen, 1975; Pike, 1999). However, many researchers have attributed this benefit to the lower n-6:n-3 fatty acid ratio that fish meal provides (Sargent and Henderson, 1995; Blesbois et al., 1997; Olubowale et al., 2014). Due to our fish meal diet not containing the

appropriate n-3 fatty acid levels, the result of no significant differences in this trial in egg fertility and hatchability was likely.

Overall, this research could not support the idea that fish meal improves the reproductive ability of commercial broiler breeder roosters. Future research should focus on exploring the optimal inclusion rate of fish meal in the diets of broiler breeders, with special attention to diet formulation, quality ingredient acquisition and balanced fatty acid ratios. Additionally, investigating the influence of fish meal in the diets of hens and its effect on egg production, fertility and hatchability, should be considered.

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Table 2.1 Composition of formulated rooster diets and calculated analysis.

Ingredients[1]	**Control**	**Fish Meal**
Corn	69.00	58.00
Soybean Meal	5.00	1.04
Wheat Bran	21.00	20.00
[2]Fish Meal	0.00	3.20
Filler Sand	0.00	8.80
Limestone Fine	1.26	1.00
Mono-Dicalcium Phosphate	1.33	1.00
Sodium Chloride	0.25	0.25
Sodium Bicarbonate	0.25	0.25
L- Lysine	0.058	0.064
DL- Methionine	0.120	0.104
L-Threonine	0.356	0.382
[3]Mineral Premix	0.20	0.20
[4]Vitamin Premix	0.05	0.05
[5]Selenium Premix	0.05	0.05
[6]Optiphos 6000 PF	0.05	0.05
Poultry Fat	1.00	5.66
Calculated Analysis		
Crude Protein	11.00	11.00
Metabolizable Energy (Kcal/Kg)	1300	1300
Ca	0.752	0.750
P	0.702	0.701
Available P	0.400	0.400
Lysine	0.490	0.490
Methionine	0.310	0.310

[1]Ingredients presented as percent of diet.

[2]Special Select^{TM}-menhaden fish meal, stabilized with 0.06% ethoxyquin

[3]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[4]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[5]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

[6]Optiphos 6000 PF, phytase enzyme purchased from Huvepharma, Sofia, Bulgaria.

Table 2.2 Chemical and fatty acid analysis of experimental broiler breeder rooster diets.[1]

Nutrient[2]	**Control**	**Fish Meal**
Protein	11.61	10.04
Gross Energy (Kcal/Kg)	3722	3323
Total Fat	4.22	6.81
Saturated Fat[3]	46.83	25.95
Trans Fat[3]	0.01	0.31
Omega 3 Fatty Acids[3]	23.06	2.38
Omega 6 Fatty Acids[3]	1.67	29.41
Omega 9 Fatty Acids[3]	13.24	36.80

[1]Dietary treatments were analyzed by an AOAC-certified laboratory, (ATC Scientific, Little Rock, AR, USA) using standard AOAC-approved methods. Dietary treatments: Control=conventional diet containing soybean meal and corn; Fish Meal=diet containing soybean meal, corn and 3.2% fish meal.
[2]Nutrients are presented as percentage of diet unless otherwise noted.
[3]Represents the percent of nutrient out of the total fat.

Table 2.3 Semen quality parameters of broiler breeder roosters fed a control or fish meal containing diet.[1]

Treatment	TSC[2] (Billions/mL)	MSC[3] (Billions/mL)	Motility[4] (%)	Volume[5] (μL)
Control	4.703 ± 0.34	3.160 ± 0.27	68.06 ± 3.66	280 ± 29
Fish Meal	4.177 ± 0.35	2.702 ± 0.28	67.55 ± 3.47	225 ± 29
P-Value	0.2878	0.2478	0.9234	0.1889

[1]Dietary treatments: Control=conventional diet containing soybean meal and corn; Fish Meal=diet containing soybean meal, corn and 3.2% fish meal. Data expressed as (mean ± SEM).
[2]TSC=Total sperm concentration
[3]MSC=Motile sperm concentration
[4]Motility=Percentage of motile sperm
[5]Volume=Amount of semen collected at each handling

Table 2.4 Fertility and hatchability percentage of eggs collected from pens where broiler breeder roosters were fed a control or fish meal containing diet.

Treatment[1]	Fertility[2] (%)	Hatchability[3] (%)
Control	91.84 ± 1.50	76.11 ± 4.01
Fish Meal	89.69 ± 1.41	82.90 ± 3.91
P-Value	0.6969	0.2331

[1]Dietary treatments: Control=conventional diet containing soybean meal and corn; Fish Meal=diet containing soybean meal, corn and 3.2% fish meal. Data expressed as (mean ± SEM).
[2]Fertility is calculated as (total number of fertile eggs/total number of eggs set)*100
[3]Hatchability is calculated as (total number of hatched chicks/total number of eggs set)*100

CHAPTER 4

The Comparison of Bell Drinkers or Nipple Drinkers on the Egg Production, Egg Fertility and Rooster Semen Characteristics of Broiler Breeders

ABSTRACT

Hydration in broiler breeder flocks can be an issue depending on the type of drinking devices the flock is provided. However, since hens and roosters have differing consumption needs the different drinking devices can determine if the roosters are getting enough water. The objective of this study was to compare the fertility of broiler breeders with access to either bell drinkers or gender specific nipple-type drinkers. A total of 1088 hens and 192 roosters at 26 weeks of age, were randomly allocated to one of 32 pens with either bell drinkers or gender specific Big Z drinkers for the entire production cycle of the flock. Each pen contained 34 hens and 6 roosters at the beginning of the study. Eggs were hand collected twice daily and recorded to evaluate the average number of eggs laid per hen/replicate throughout the trial. All birds were provided equal amounts of feed daily. At 45, 52, and 64 weeks of age, 12 eggs were set from each pen for a total of 1,152 eggs and incubated for 10 days, then candled, to determine egg fertility. In weeks 63 and 64, semen was also collected four times from 24 roosters (12 replicates per treatment) and analyzed immediately using the SQA-Vt™ Automated Semen Quality Analyzer (Medical Electronic Systems, Encino, CA, USA). All data was analyzed via two-sample t-test in JMP 17.2 (SAS, Cary, NC, USA) with a significance level of $P \leq 0.05$. Egg production was not different between the two treatments throughout the study. Egg fertility percentage between treatments was also not significant. Sperm motility was higher from roosters with access to nipple drinkers compared to roosters housed with bell drinkers ($P<0.0001$). Whereas semen volume was higher from roosters with exposure to bell drinkers ($P<0.0001$).

These results show that the water delivery system in broiler breeder houses can have an impact on rooster fertility parameters, which are paramount to producing fertilized eggs from breeder flocks.

INTRODUCTION

The efficiency of broiler breeder operations is crucial for the poultry industry, particularly in the context of reproductive performance as hatchability is continuing to decrease in the US over time. Although broiler breeders are fed once a day, compared to broilers or egg layers which always have access to feed, their total water consumption does not vary based on feed schedule (Bennett and Leeson, 1989). In broiler breeder production, studies have shown that as drinker water quality decreased so does performance, and if there is a high microbial load detected in the water a lower hatchability rate can result. (Grizzle et al., 1997; Zaghari et al., 2011). In terms of egg production, studies conducted in layer hens demonstrated that birds with access to nipple drinkers had higher egg production and better feed conversion (Togashi et al., 2008). Since water is one of the most important nutrients that poultry receive, identifying the best delivery route that works well on farm is of great importance.

The two main types of water systems in modern poultry houses are Plasson bell drinkers (Plasson USA, Sugarland, TX, USA), which are an open-source system, and a nipple drinker system which is considered to be a closed source system. In an open-source system, birds will need to dip their beaks in the water, and then lift their heads to drink out of the water source, which allows dirt, wood shavings, feed, and other particles to fall into the water source. In a closed system using a nipple line, the birds trigger the nipple with their beak to release water from the waterline that is typically positioned at or near the bird's eye level. Because of their design, closed systems are easier to sanitize which keep microbial levels low (Maharjan et al., 2016). They also reduce water wastage improve litter quality. Since broiler breeders are commonly kept in barns with some access to bedding, nipple drinkers have been used to reduce foot lesions, and improve air quality, which both impact reproduction and welfare. In general,

very little research has been done with water delivery in modern broiler breeders houses in the US.

This study aims to compare the fertility outcomes of broiler breeder eggs and rooster semen quality when provided bell or nipple type drinker systems. By measuring egg production, egg fertility, sperm concentration, sperm motility, and semen volume this research may provide valuable insight into the best management practices that support reproductive health in broiler breeder houses.

MATERIALS AND METHODS

Housing and Management

All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee under protocol #22-385. The trial contained a total of 1,088 Ross 708 hens and 192 roosters (Aviagen, Huntsville, AL, USA) with 36 hens and 6 roosters per pen. At 26 weeks of age, all birds were randomly allocated to one of 32 pens which contained either bell drinkers or gender specific nipple drinkers to represent 16 replicate pens per treatment. The two water delivery treatments that were used for this study are the Plasson Breeder Drinker from Plasson USA (Sugarland, TX, USA) and the Ziggity Big Z system (Middlebury, Indiana, USA) which does not require the use of catch/drip cups. The bell drinker and nipple drinker height was adjusted regularly to ensure they were in the optimal drinking position for the birds. Bell drinkers were managed so that the water level would be even with the backs of the hens, as the bell drinkers are not gender-specific. The nipple drinkers were adjusted so that the hen line was in a position that the hens could trigger the nipple at a 45° angle, and the rooster line which was in the scratch area was adjusted so that the roosters could trigger it at a 45° angle as well. The birds used in this study were reared in a black-out

temperature-controlled poultry house in floor pens with access to litter. The production house is a curtain sided raised slat house, where the litter area was 25% of the total floor space. The high and low ambient temperature ranged from 14 to 30°C, and the lighting schedule was set for 16 hours of light and 8 hours of darkness throughout the study. All birds were allocated the same amount of feed daily, and the rooster and hen diets were separately formulated to contain the nutrient content that was provided in accordance with the Ross 708 breed management guide (Table 3.1). Eggs were hand collected twice daily and recorded to evaluate the egg production throughout the trial. At 45, 52, and 64 weeks of age, 12 eggs were set from each pen for a total of 1,152 eggs which were then incubated for 10 days before being candled to determine egg fertility.

All roosters were trained to the dorso-abdominal massage method (Burrows and Quinn, 1937) three times before their first semen analysis took place, this was to allow the roosters to adjust to the personnel handling them. In weeks 63 and 64 of age, semen was also collected four times from 24 roosters (12 replicates per treatment) for a total of 96 attempts and 65 total semen samples collected which were analyzed immediately after collection, using the SQA-Vt™ Automated Semen Quality Analyzer (Medical Electronic Systems, Encino, CA, USA).

Egg Production and Fertility

Egg production was measured as both hen housed production and hen-day production. Hen housed production is the percentage of eggs produced based on how many hens were originally housed and is calculated as follows: (eggs produced)/(hens housed)*100. Hen-day production is adjusted for mortality during the trial and is calculated as follows: (eggs produced)/(hen day)*100.

The fertility status of each egg was determined by candling at 10 days of incubation. At which point any eggs that were found to not be developing were broken open to examine the germinal disc for its fertility. Egg fertility is calculated as: (total number of fertile eggs/total number of eggs set)*100.

Semen Quality Analysis

Semen analysis was performed immediately after collection of all the samples. Samples were collected in 4mL microcentrifuge tubes and volume was measured according to the total amount of fluid captured in the tube. Then, 100µL of semen was gently mixed into 2mL of SQA-Vt™ turkey semen diluent which had been warmed to 40°C. An aliquot of this sample was drawn into a pre-warmed 1mL slip tip syringe and immediately dispensed through the SQA-Vt™ capillary. The capillary was then inserted into the SQA-Vt™ Semen Analyzer for sample analysis.

Volume was calculated based on how much semen is collected at each attempt from each rooster. The rest of the semen quality parameters were analyzed according to the semen samples that were able to be collected as not every rooster produced semen with every attempt. Sperm concentration (TSC) is presented as billions of sperm cells/mL of semen. Motile sperm concentration (MSC) is presented as billions of motile sperm cells/mL of semen and accounts for any dead sperm or nonmotile sperm cells within the sample. Motility percentage is calculated by (motile sperm concentration)/(total sperm concentration)*100.

Statistical analysis

All semen quality data were analyzed by treatment with each rooster serving as a replicate within each treatment group. Egg production and fertility data used the pen as the experimental unit. Data was analyzed by two-sample t-test using JMP 17.2 and the means were

then statistically distinguished using Student's t-test. The treatment group effects were considered significant at $P \leq 0.05$.

RESULTS and DISCUSSION

The accessibility to drinking water for roosters in broiler breeder housing is extremely important for reproduction. Gender specific nipple drinkers which were used in this study provide roosters with the ability to drink from a nipple line that is at the correct height for them to make the motion of drinking water even easier than bell drinkers. This easier motion may have contributed to the high sperm motility that the nipple drinker treatment had. When comparing semen quality parameters our results displayed in Table 3.2 showed that the roosters in the nipple drinker treatment did perform better when evaluated based upon the sperm motility percentage ($P<0.0001$), and the motile sperm concentration was trending higher in the nipple drinker treatment although it was not statistically significant ($P=0.0627$). The location of the rooster specific nipple drinkers may also have influenced the sperm motility as they are positioned approximately 2 feet from the rooster feeder. In the bell drinker pens, the bell drinker is approximately 5 feet away from the feeder. This may make the delay from eating to drinking a shorter duration to improve digestibility. However, bell drinker treatment had produced a higher semen volume ($P<0.0001$) compared to the nipple drinker treatment. One consideration for why the volume was higher in the bell drinker treatment may be associated with water waste that would affect that litter quality in this trial. Other researchers have identified that as foot pad lesion scores increase, Sertoli cell concentration in the testes decrease (Wilson et al., 2018). Since the bell drinkers were only positioned over the slats in the production house, that may have allowed for the litter to stay drier in the scratch area where the rooster feeder is, as opposed to the nipple drinker treatment that had the rooster specific nipple drinkers positioned over the litter.

Overall, because the impact of sperm motility is so important to the avian reproductive tract, the nipple drinker treatment proves to be the better water delivery system for rooster semen quality. This result is favorable for the broiler breeder industry, as many broiler breeder houses have been converted to nipple drinkers due to the many benefits in general husbandry that nipple drinkers provide. A literature search found no research showing similar results in broiler breeder roosters. Further research should be conducted to understand the mechanism behind the benefit of using gender-specific nipple lines on rooster semen quality while exploring the benefit of bell drinkers on the volume of semen produced. Potential future research should examine water intake, blood chemistry, and blood hematocrit samples to identify if there is a difference of hydration level of the rooster. This study shows that there is in fact an impact of drinker type on semen quality parameters. Due to the potential for fertility improvement in broiler breeders, water delivery systems should be further evaluated. Understanding the many physiological impacts that are made through various management practices is the key to improving sustainability, health, and productivity of broiler breeder operations. In terms of egg production there were no differences between the nipple or bell drinker treatments in either hen housed or hen-day egg percentages (Table 3.3). Figure 3.1 displays the production curve by week for the bell and nipple treatments from weeks 29-61. There were differences between treatments in weeks 33, 35, and 57 ($P<0.0001$). This production curve does mimic a traditional egg production curve and is expected to be seen during any egg production flock cycle. Because the statistics showed that the type of drinker had no impact on total egg production, the results are in agreement with studies that were done with broiler breeders and Pekin ducks (Silverio Klosowisk et al., 2009; Colvero et al., 2014; Lowman et al., 2016).

The percentage of fertile eggs was not significantly different between treatments although the eggs from the nipple drinker treatment had 2% higher fertility than the bell drinker treatments. Although this difference was not statistically significant, with the vast size of the broiler breeder sector it suggests there is value in further research to identify if the increase can be completely contributed to the increase in sperm motility from the rooster, or if there is some physiological benefit to the hen as well. Hatchability rates, while not included in this specific analysis, could also benefit from the improved semen quality associated with nipple drinkers.

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Table 3.1 Composition of formulated diets and calculated analysis.

Feed Ingredients[1]	**Hen Breeder 1**	**Hen Breeder 2**	**Rooster**
Corn	44.07	66.76	69.00
Soybean Meal	14.84	17.83	5.00
Wheat Bran	24.29	5.32	21.00
Calcium Carbonate	7.08	7.21	6.30
Limestone Fine	0.00	0.00	1.26
Mono-Dicalcium Phosphate	1.33	1.15	1.33
Sodium Chloride	0.25	0.25	0.25
Sodium Bicarbonate	0.25	0.25	0.25
L-Lysine	0.00	0.00	0.058
DL-Methionine	0.120	0.153	0.120
L-Threonine	0.356	0.173	0.356
[2]Mineral Premix	0.200	0.200	0.200
[3]Vitamin Premix	0.050	0.050	0.050
[4]Selenium Premix	0.050	0.050	0.250
[5]Optiphos 6000 PF	0.000	0.000	0.050
Poultry Fat	6.940	0.600	1.000
Calculated Analysis			
Crude Protein	15	15	11
Metabolizable Energy (Kcal/Kg)	1270	1300	1300
Ca	3.00	3.00	0.752
P	0.778	0.602	0.702
Available P	0.400	0.350	0.400
Lysine	0.700	0.734	0.490
Methionine	0.400	0.410	0.310

[1]Ingredients presented as percent of diet.

[2]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[3]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[4]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

[5]Optiphos 6000 PF, phytase enzyme purchased from Huvepharma, Sofia, Bulgaria.

Table 3.2 Semen quality parameters of roosters at 63 and 64 weeks of age with access to either nipple drinkers or bell drinkers.[1]

Treatment	**TSC[2]** (Billions/mL)	**MSC[3]** (Billions/mL)	**Motility[4]** (%)	**Volume[5]** (µL)
Bell	5.571 ± 0.29	2.908 ± 0.24	51.22 ± 3.00b	401.56 ± 28.26a
Nipple	5.070 ± 0.36	3.632 ± 0.30	72.15 ± 3.85a	205.20 ± 28.26b
P-Value	0.2846	0.0627	**<0.0001**	**<0.0001**

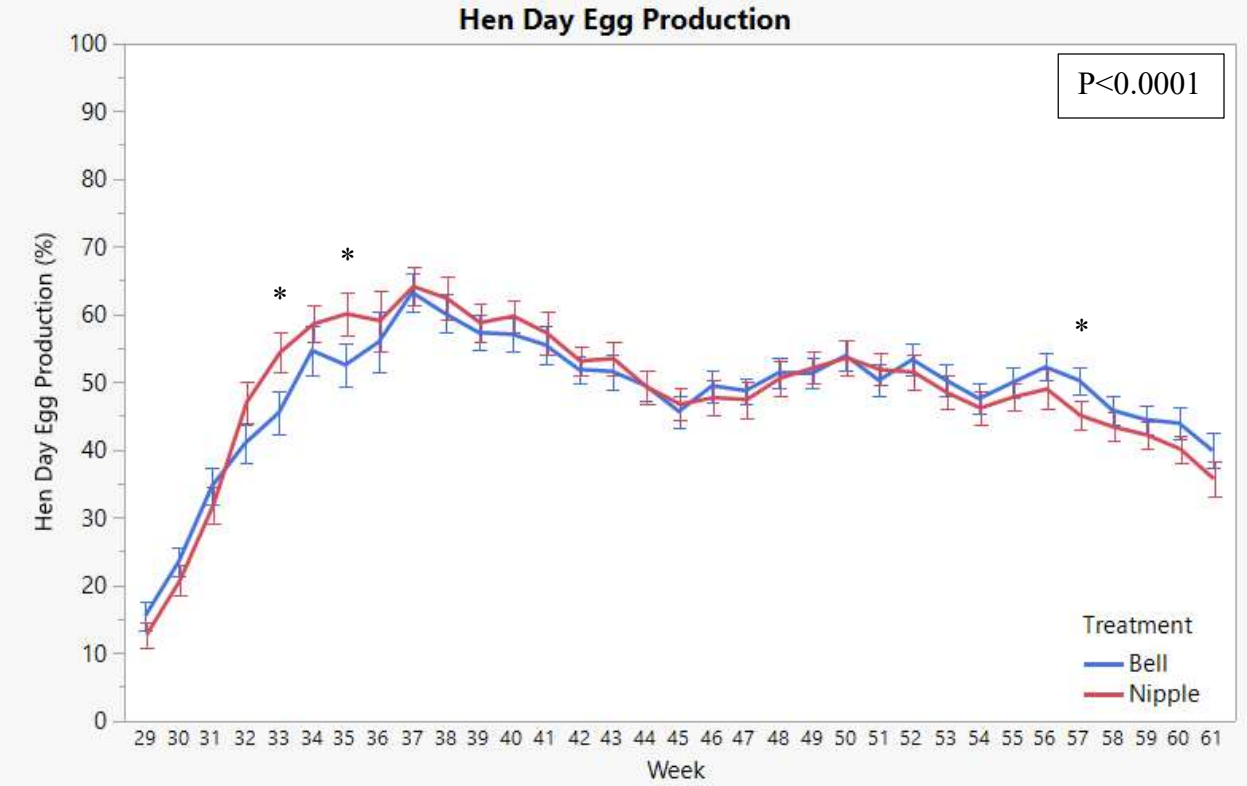
[1]Data expressed as (mean ± SEM)
[2]TSC=Total sperm concentration
[3]MSC=Motile sperm concentration
[4]Motility=Percentage of motile sperm
[5]Volume=Amount of semen collected at each handling
[a,b]Means within a column differed at $P \leq 0.05$.

Table 3.3 Egg production and fertility of broiler breeder hens with access to nipple or bell drinkers.

Treatment[1]	Hen Housed Prod. [2] (%)	Hen Day Prod[3]. (%)	Egg Fertility [4] (%)
Bell	48.08	48.41	91.62
Nipple	47.49	48.55	93.53
SEM	0.385	0.384	1.290
P-Value	0.2804	0.7176	0.2916

[1]Hens either had access to bell or nipple drinkers through the life of the flock.
[2]Hen housed production is calculated by (eggs produced/number of hens placed at the beginning of the trial)*100
[3]Hen day production is calculated by (total number of eggs laid/total number of hen days)*100
[4]Fertility is calculated as (total number of fertile eggs/total number of eggs set)*100

Figure 3.1 Hen day egg production by week of age and treatment.[1]



[1]Hen day egg production by week of age and treatment when broiler breeders are provided access to either bell or nipple drinkers over the life of the flock. Red line represents the nipple drinker treatment and blue line represents the bell drinker treatment. Error bars represent 95% confidence of difference between treatment interval and those that do not overlap denote a difference between treatments.