ABSTRACT

RAHIMI, AMIN. The Effect of Housing System, Nutrition, and Photoperiod on Welfare and Production Parameters on Commercial White Egg Layers. (Under the direction of Dr. Prafulla Regmi).

The housing system and nutrition are the two most important factors that affect the welfare and performance of the egg-laying hens. Conventional cage housing was widely adopted in the 1960s for providing high-quality eggs. In this system, hens are housed in stacked rows of cages. Each cage gives the bird continual access to water and food. The cage has wire mesh floors that allow manure to drop through to a belt below, which keeps manure away from the birds, their eggs, food, and water. After a hen lays an egg, it gently rolls off the slightly sloped mesh flooring onto an egg-collection belt. The belt moves the egg to process, where it is checked for imperfections, cleaned, and packaged. In alternative housing systems such as cage-free systems, eggs are primarily laid in the nest boxes but can be laid on the litter as well. Therefore, fecal contamination can compromise the quality of the egg. Cage- free housing provides greater space and can enhance birds' welfare by promoting the expression of natural activities such as wing flapping, dust bathing, and perching. In contrast, these activities are limited in the conventional cage housing system.

Nutrition in the layer industry has a key role in the performance of the bird and the cost of production for the producers. Providing good nutrition in the layer feed is crucial for egg production and preventing diseases. One of the main nutritional components that is costlyin the layer diet

is energy. Corn is one of the sources of energy in the layer's diet and is becoming an expensive ingredient in the layer's feed due to producing ethanol for the automobile industry. Chickens kept in the cage-free housing system require more energy due to walk around, dust bathe, and jump on the perches than conventional cage housing systems. Therefore, it is important to know how much energy the hens will need for their performance (weight gain, egg production) with respect to their housing environment.

In the first study, I investigated whether the birds on the floor system required more energy due to their activities or not. A total number of 180 hens were reared in the conventional cages. At 36 weeks of age 90 hens were transferred to the cage-free housing system. Hens were fed with two different levels of metabolizable energy in their diet in each housing system. I observed that cage-free hens that were provided with 7.8 ft^2 /bird with a total of 90 hens produced 3.5% more eggs than hens reared in conventional cages with an area of 234 in^2/bird with the same number of birds as the cage-free system. However, hens in the cage-free system also consumed more feed and gained 4% more body weight than the hens in the conventional cages. The egg production rate of cage-free hens was 3% greater than the conventional cage hens, but the feed conversion ratio (FCR) of the conventional cage hens was 9% better than the cage-free hens. The energy intake of hens fed with low metabolizable energy in the diet was significantly greater than the hens fed with high metabolizable energy in their diet. The egg production was significantly greater for the hens with a high level of

metabolizable energy in their diet than hens with low metabolizable energy. In this experiment, I found that the level of energy in the diet is a significant factor that controls FCR and egg production rate, and the floor system can increase energy intake and, hence, the performance of the hens.

The layer industry often relies on the manipulation of photoperiod and the intensity of light in the poultry house to achieve optimum performance. Studies show that the layers' activities, growth rate, and egg performance are related to the intensity and light and duration of hours of light (photoperiod). The sexual maturation of the hens can be accelerated by increasing the photoperiod. In my second study, I investigated the effect of different ages at photo-stimulation (increasing the hours of light for many hours in a specific day) of two strains of the hens (Hy-line W36 and 1940 leghorns) on their egg performance, egg quality as well as the quality of their bones. Pullets were photo-stimulated at 15 and 20 weeks of age. Delay in photo-stimulation (photo-stimulation at 20 weeks of age) increased the strength of the tibia and humerus, and the onset of sexual maturity was similar as early photo- stimulated hens, and quality characteristics of the eggs, as well as hen-day egg production, were the same as those hens that were photo-stimulated at 15 weeks of age. The egg production rate and quality of the egg were better in Hy-line W36, but the tibia's diameter was wider than Hy-line W36. Also, the strength of the humerus was better than Hy-line W36.

The Effect of Housing system and Photoperiod on Welfare and Production Parameters on Commercial White Egg Layers

by
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BIOGRAPHY

Amin was born in East Lansing, Michigan, on December 30, 1988. Amin and his family moved to Iran (Tehran), and Amin grew up in Tehran. He attended his major of study in biology in high school. He graduated from high school in August 2008. Amin's father graduated from the college of veterinary medicine in Tehran, and after his graduation, he and his family moved to the United States. Amin's father started his master's in poultry science at the University of Michigan. Amin's father and his family moved back to Tehran, and Amin grew up in Tehran. Amin became more interested in the poultry field, and he worked with his father at the farm while he was in Tehran. Amin's father and his family moved again to the United States for one year of sabbatical at North Carolina State University. His father began to work with Dr. JesseGrimes during that time.

Amin took a course named poultry science and production with Dr. Carm Parkhurst that year. After his father's sabbatical year, they were moved to Tehran, and Amin decided to continue his academic area in veterinary medicine. He attended Islamic Azad University in Tehran in the college of veterinary medicine. His thesis for his doctorate was about the serological monitoring of three viral diseases (Avian Influenza, Infectious Bronchitis, and Newcastle disease) in Tehran province. He graduated in the summer of 2016. Amin got married after his graduation, and he decided to move to the United States after his graduation and bring his wife with him to the United States. He moved to California in the summer of 2016, and he started to work for many

years to bring his wife to the United States. Amin applied for admission to North Carolina State University for a Master's in poultry science. Amin was accepted for the master's program, and he decided to move to North Carolina. After living in North Carolina for three months, Amin's wife received her green card and moved to the United States. Amin was pursuing his education in poultry science under the supervision of Dr. Regmi, and he was a research assistant for his master's program. Dr. Regmi's field of study was about the commercial egg layers and welfare in the poultry industry. Amin became more interested in the layer's field and started his research with commercial egg layers. He understands the different aspects of welfare in the layer's industry and the factors affecting thewelfare and performance of the egg-laying hens.

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LITERATURE REVIEW

The first cage system for laying hens was introduced in California in 1926 and, since then, the egg industry has changed dramatically. By the 1990s, 75% of all commercial layers in the world and95% in the United States were kept in cages. However, the European Union council directive resulted in a ban on conventional battery cages since 2012 throughout the European Union, primarily due to the welfare issues of hens in conventional cages. At this point, United Egg Producers (UEP Certified) indicates more than 90% of eggs worldwide come from cage layers. Therefore, in this review, we want to explore the welfare of the egg-laying hens in cage-free and conventional battery cages and the performance of hens in each housing system. We have also picked two major points of discussion – energy use and skeletal issues of laying hens housed in the cage-free system, as these issues are quite important for the sustainability of the egg production system.

The conventional cage housing system for layers

The conventional cages were developed to increase the number of birds with limited space for hens to increase egg production and reduce labor costs. The advent of conventional cages reduced mortality rate, egg losses, feather pecking, and the exposure of eggs to environmental pathogens. Still, this system has been criticized for limiting the welfare of hens by limiting the space allowances for each hen. Hen's welfare means providing a good environment for hen's physical and mental needs, including physical activities such as nesting, perching, dust bathing, wing-

stretching. Conventional caged hens are not able to perform any of these activities. The public concern for the welfare of the hen in this system is increasing every day that has led many states in the United States, such as California, Massachusetts, Washington, Michigan, Ohio, and Rhode Island, to ban the use of battery cages since March 2020. The ability of hen to perform physical activities like jumping and wing flapping can significantly increase the demand for hen for more feed intake. Eventually, producers need to give more feed to the cage-free hens than conventional cage hens. Also, more space allowances for hens in this system compared to the conventional cages would potentially increase the feed costs per dozen eggs (Matthews and Sumner. 2015). Therefore, it is important to compare the cost of production and welfare issues in two different housing systems (cage-free versus conventional cages) and find a practical way to increase hens' welfare and productivity in each housing system.

Advantages of conventional cage system

The quality of the eggs has been reported to be better in the conventional system from the food-safety standpoint since there are likely to be fewer eggs contaminated due to fecal droppings (Weitzenbuger *et al.,* 2006). Since the birds rarely come into direct contact with their excreta, the risk of roundworms and coccidiosis is less in cages than the cage-free system. Cages also protect hens from predators and other wildlife and reduce the proliferation of injurious pecking and subsequent mortalities. Hens housed in cage housing systems are at a lower risk of infectious

disease than hens housed in cage-free systems (Fossum *et al.,* 2009). Conventional caging also allows the opportunity to monitor individual birds' health and well-being. There are many studies that have shown that better performance of layers is achieved in conventional cage systems, including more eggs, improved feed conversion ratios, and lower mortality (Tauson *et al.,* 1998; Leyendecker *et al.,* 2001, Tumova *et al*.,2003; Hulzebosch *et al.,* 2006; Voslarova *et al.,* 2006; Valkonen *et al.,* 2010). The eggs produced in cages often have higher egg quality indices when albumen, and yolk indices (Anderson *et al.,* 1994; Tumova *et al.,* 2003). Overall, the conventional cages are more hygienic, contribute to a lower incidence of infectious diseases, allow easier management, and eggs from conventional eggs are less costly than non-cage systems (Patterson et al., 2001; Summer et el., 2008).

The metabolizable energy (ME) use of hens can be monitored better in this housing system compared to the cage-free system. The ME is the amount of gross energy consumed minus the gross energy in the feces and urine. Because of the limited physical activity in cages, more dietary energy goes toward egg production. Therefore, the energy expenditure of hens in this system can be monitored by assessing body weight and egg production. Since cage-free hens have more space to perform their activity, it is challenging to track what portion of the ME is used for physical activity in addition to the energy used in egg production and maintenance.

Disadvantages of conventional cage system

The limited space allowances can restrict the hen's natural behaviors such as walking, foraging, and dust bathing (Albentosa et al., 2004, Mollenhorst et al., 2005). Nesting and roosting are two highly conserved behaviors in chickens that the hens are not able to perform in conventional caging systems. Hens show signs of unrest when they are deprived of the opportunity to perch at night and experience frustration and reduced welfare if perching is impossible (Olsson *et al.,* 2002; Fraser *et al*., 2013). Hens prefer to lay eggs in a nest rather than on a sloping wire floor, and the lack of a nest may reduce welfare (Hughes *et al*., 1989; Lay Jr *et al*., 2011). Furthermore, the lack of movement of hens in the cage system increases the risk of osteoporosis that leads to bone fragility and eventually breakage of the skeleton at the end of the laying cycle (Fleming et al., 1994; Whitehead *et al.,* 2004; Regmi et al., 2017). Hens kept in cages are reported to have high incidences of fatty liver hemorrhagic syndrome (FLHS), which is a noninfectious disease characterized by excessive accumulation of fat in the liver and abdominal cavity (Weitzenbürger *et al.,* 2005; Kaufman-Bart *et al.,* 2009; Lay Jr *et al*., 2011; Widowski *et al*., 2013). This condition is observed in females with the initiation of egg production, when estrogen levels in the serum increase and are associated with high energy intake by the bird and limited movement activity (Crespo. 2019). Layers with FLHS have increased estrogen, osteocalcin, and leptin-like protein, which seems to upregulate the bone turnover, which causes calcium depletion out of the

skeleton, which laying hen relies on the substantial daily flux of calcium in and out of the skeleton (Crespo. 2019). Affected birds have enlarged liver, putty color, and friable, with varying degrees of hemorrhage in the liver, and eventually, FLHS causes blood loss and reduced egg production (Crespo. 2019). In an epidemiologic survey, 40 percent of caged hen mortality was associated with FLHS. Hens in affected flocks are generally obese (overweight by 20 percent or more) and experience a sudden drop in egg production (Dinev, 2010). The excess energy in the diet is deposited as fat in the liver, making the liver yellow and friable. Other Cage-related welfare problems are trapping the feet between wires or experiencing foot damage secondary to overgrown claws. However, improved cage designs and the use of abrasive strips have largely eliminated these occurrences (Tauson *et al.,* 1986; Albentosa *et al.,* 2004).

Advantages of the cage-free system

The cage-free system allows hens with opportunities to express their normal behavior along with the ability to exercise compared to the cages (Blokhuis *et al.,* 2006). The physical activity of hens in this system increases musculoskeletal strength and can potentially decrease the incidence of osteoporosis and fractures during depopulation. Rodenburg et al. (2008) investigated the effect of cage-free and furnished cage systems on hen's bone strength and reported cage-free hens had stronger wings and keel bones than hens reared in furnished cages. Similarly, Fleming et al. (1994) found considerable improvements in a wide range of bone morphometric in

aviaries compared to the conventional cages, including greater humerus strength in the aviaries. Transferring the hens from cages to an aviary increased the tibiae strength within 20 days by increasing the bone formation rather than inhibition of bone resorption (Newman and Leeson, 1998). A lower incidence of bone breakage has been found in birds depopulated from aviary or free- range systems compared to battery cages (Gregory et al., 1990; Van Niekerk and Reuvekamp, 1994).

Disadvantages of the cage-free system

The cage-free housing system has been associated with higher experience a higher incidence of cannibalism than the conventional cage (Shini et al., 2019). Data from the housing system survey revealed that most hens kept in the free-range died from cannibalism (77%), and only 5% of examined hens in conventional cages were pecked and died due to cannibalism (Shini et al., 2019). Literature showed hens mortality rates are higher in the cage-free system than conventional cages (Kreinenbrock et al. 2004; Lay Jr. et al., 2011) and furnished cages (Michel and Huonnic, 2003; Rodenburg et al., 2008). From an air quality point of view, ammonia and dust levels were found higher than in the conventional cages (Nimmermark *et al.,* 2009). Increased ammonia levels can cause keratoconjunctivitis and have harmful effects on the respiratory tracts of birds (Nimmermark *et al*., 2009). A cage-free system also requires greater labor resources and attention to ensure that litter is of suitable quality. Dustbathing in a wet litter is associated with a greater prevalence of footpad lesions (Wang et al.,

1998). In addition, a large group size in this system increases the risk of feather pecking and cannibalism. Bacterial infections were the most common cause of mortality in birds on floor-based systems and included erysipelas, colibacillosis, and pasteurellosis (Fossum et al., 2009). Histomoniasis is generally associated with soil contact and has been reported in free-range laying hens (Esquenet et al., 2003). Also, there is a higher incidence of viral diseases (lymphoid leucosis, Marek's disease, and Newcastle disease), coccidiosis, and red mites (Dermanyssus gallinae), in the cage-free and free-range than in the cage system (Lay Jr et al., 2011; Rodenburg et al., 2008; Fossum et al., 2009; Widowski et al., 2013). In general, birds in cage-free systems are at greater risk of contamination with soil, litter, feces, and other vectors, including rodents and insects that would increase the risk of infectious diseases. The presence of keel bone fractures is another welfare issue in the aviary system. Fracture prevalence in the aviary and other cage-free systems has been higher compared to the furnished cages. Traumatic collisions of hens due to unsuccessful landing and collision with perches and other structures such as the lower tiers, feeders, and drinkers have been hypothesized to cause higher keel fractures in the aviary system than in the furnished cages (Rodenburg et al., 2008). The prevalence of keel bone fracture in two housing systems (cage-free versus conventional cages) showed hens in cage-free systems can experience more fractures during the laying period than those in cage systems (Widowski *et al*., 2013; Lay Jr *et al.,* 2011; Fraser *et al*., 2013).

Finally, energy-demanding activities such as jumping, flying, and dust bathing is the most influential behavioral trait for chickens' feed intake (Braastad et al., 1989; Morrison et al., 1978). All these activities increase the heat, which results in energy loss and could result in an increase in the cost of production.

The importance of hen's welfare on marketing

Majority of the United States egg industry shifted to the conventional cage system during the 1920s and 30s to reduce diseases such as coccidiosis and roundworms and injuries due to pecking. Throughout the decades of egg production in cages, some consumers were interested in cage-free and organic eggs due to the welfare concern of the animal. The cage-free system began to resurge recently, and egg producers are transitioning from conventional cages to cage-free systems (United egg producers. 2021). At the end of 2020, 28% of all hens in the United States were in cage-free production (United egg producers. 2021). USDA's Agricultural Marketing Service estimated 66% of all hens in the United States will be in cage-free environments by 2026. Over 70% of United States citizens surveyed reported 'concern' for farm animal welfare (Lusk and Norwood, 2011). Similarly, Zhang and Goddard (2010) carried out a survey of United States citizens, finding 72% of the surveyed people believed free-range to be 'more natural, free from antibiotics (45%), and tasting better (44%)'. The demands for cage-free eggs have risen in other parts of the world as well. For example, in a study conducted by the University of Adelaide, consumers chose to buy

free-range or cage-free eggs because they believed that these eggs tasted better and were of better quality than eggs from caged layers. Despite participants describing conventional cage production as “cruel,” they did not emphasize welfare reasons as critical for their purchases. The authors note that the findings suggest that consumers think about animal welfare in a much broader way than previously thought. They believe that better welfare is connected to a better-quality product.

Providing larger space for hens, such as using a cage-free system, can increase the loss of energy by heat loss. Therefore, farmers need to give more feed to the hens to achieve

good production from those hens. Increasing the amount of feed can directly increase the egg price, making it hard to buy cage-free eggs for some people. Therefore, it is important to compare the energy needs of the hens in two housing (cage and cage-free) to provide a good diet based on the formulation that meets the energy demands of each housing system that improves hen welfare and reduces cost.

Importance of energy level in the diet

Dietary energy is the largest proportion of the poultry diet, which contributes to the cost of the diet. A good energy level in the diet can directly affect feed efficiency and the productive parameters of the flocks. Dietary energy, derived from carbohydrates, lipids, and protein, is the largest proportion of poultry diets and contributes importantly to diet cost. It also interacts with other nutrients such as amino acids to promote high and

efficient production in chickens. (Classen *et al*., 2020). The energy requirements for production are primarily for daily egg production and body mass increase between the onset of sexual maturity and the attainment of mature body weight (BW), including feather growth (Tauson *et al*., 1980). The energy content of the diet affects egg size, number, and quality of the egg (Leeson *et al.,* 2001). Egg production is directly related to the amount of energy level in the diet and declines when energy intake is deficient (Harms *et al.,* 2000; Leeson *et al*., 2001). Harms *et al.* (2000) also observed that hens fed high-energy diets (containing around 6 % oil) produce heavier eggs. Colvara *et al*. (2002) verified that increases in dietary energy levels (2,700, 2,800, 2,900, and 3,000 kcal of ME/kg) increase egg weight.

The Dietary Energy Level and Feed Intake

The amount of feed consumed by an animal determines the number of nutrients that are available for the hens for their maintenance and production. Dietary energy is one of the most important ingredients in a layer's feed due to its effect on the utilization of other nutrients. This ability helps the hen regulate its feed intake. The poultry feed needs to be formulated with an optimum energy level with a lower feed cost. The literature shows the dietary energy level controls feed intake such that high energy levels decrease feed intake independent of bird age and, consequently, improve feed conversion without changing performance parameters or egg quality traits (Summers *et al*., 1983; Sell *et al.,* 1987; Keshavarz *et al.,* 1995; Wu *et al*., 2005; Kang *et al.,* 2018). However, Jalal

et al. (2006) reported no change in feed intake with an increase in dietary energy from 2800 to 2900 kcal/kg. Klasing *et al*. (2015) reported a high energy level diet must contain a proportionally higher number of amino acids, vitamins, and minerals. Dietary energy accounted for 28% of the variation in feed intake (Klasing et al., 2015). Some other factors such as feed density, feed nutrient composition, environmental temperature, body weight, and hen's age also accounted for the variation in feed intake (Korver et al., 1998; Liu et al., 2015; Leeson et al., 2005). The amount of feed in the gut or other physiological limitations in the hen's body can also change feed intake (Ahiwe *et al*., 2018). Still, among all these factors, dietary energy is the most influential predictor in feed efficiency (decreasing the feed cost with increasing the production rate) in the egg-laying hens (Classen, 2017). Furthermore, the relation between the nutrient concentrations to dietary energy level seems to have the greatest practical application for the production performance of leghorn chickens that are generally fed diets of low to moderate energy content (Baldini *et al*., 1995). Energy intake by birds can be summarized as the sum of metabolized energy (ME) and energy ingested (feces, urine) and is expressed as kcal per unit of watts (W). Ingested energy is measured as gross energy intake (GEI), the product of the mass of food ingested multiplied by the heat of combustion of the food materials. Excretory energy (EXE) is the energy content of the urine and fecal materials. ME is the total cost of basal metabolic processes, thermoregulation, specific dynamic action, physical activity, and production

and is obtained by subtracting EXE from GEI. A comprehensive model of avian energy balance must include consideration of rates of energy intake minus several additive layers of expenditures, including basal metabolism (is the rate of energy use by birds at rest within the zone of thermoneutrality), the cost of thermoregulation, physical activity, molt, growth, lipid-deposition, reproduction, and energy lost in digestion and assimilation.

Furthermore, these measurements may be affected by body composition, sex, and ambient temperature. The use of specific concentrations of protein/amino acid to dietary energy ratios in formulating poultry diets must be carefully evaluated.

The Effect of Dietary Energy Level on Egg Performance

Hen-day egg production can be influenced by dietary energy content. For example, hen-day egg production increased as dietary apparent metabolizable energy (AME_n) increased from 2,750 to 3,050 kcal AME_n/kg (Kang *et al*., 2018). In the same study, the number of broken eggs, egg weight, and egg mass were not affected by the amount of energy in the diet; instead, the excess energy resulted primarily in body weight gain (Kang *et al*., 2018). Also, Perez-Bonilla et al. (2012) investigated the effect of four different dietary energy on the egg performance of Hy-line brown. They observed that as dietary energy increased, the egg production, egg mass, and energy efficiency (Kcal of AMEn/g of egg) increased. Wu *et al*. (2005) reported increasing AME_n level to Dekalb white and Bowan White increased

egg production, egg mass, body weight, and feed conversion ratio. In contrast, many studies reported no change in egg quality with increasing dietary energy (Colvara *et al.,* 2002; Junqueira *et al*., 2006; Almeida *et al*., 2012; Silva *et al*., 2012). Similarly, many other studies reported no change in egg production with increasing dietary energy levels (Costa et al., 2004; Wu *et al.,* 2007; Jalal *et al*., 2006; Piozzi da silva *et al.,* 2012). On the other hand, Araújo & Peixoto (2005) observed a reduction in egg production ($P < 0.05$) as the dietary energy level increased.

The Impact of Housing Systems on Energy Requirement of Laying Hens

The housing system can dictate energy requirements. Other variable factors, including hen's activity, ambient temperature, and plumage, can also influence the energy requirements (Peguri *et al.,* 1991; Peguri *et al*., 1993). Energy spent in jumping, running, walking, and short-distance flights can potentially comprise a significant portion of the energy budget in hens housed in floor pens. Hens in the cage-free system or free-range system probably entailed higher energy expenditure for the chicken due to higher locomotor activity (Riek *et al*., 2021). Still, there is no published study that measured the energy expenditure in unrestrained, freely moving chickens.

Tiller (2001) has previously suggested that the maintenance energy required for hens housed in the barn and free-range conditions is higher than for caged hens. While several studies have determined that hens kept in non-cage systems, such as an organic, barn, or free-range production, require an additional 10–15% more energy, and this much extra energy may

compromise egg production (Tiller et al., 2001; Aerni *et al*., 2005; Leenstra *et al*., 2012; Leinonen *et al*., 2012; MacLeod *et al*., 2013).

The Impact of Housing Systems on Egg Performance and Quality of the Eggs

The housing system could be one of the factors that can significantly affect egg quality and egg performance. Many studies showed that egg performance (number of eggs and quality of the eggs) was improved in the CC system (Tauson *et al*., 1999; Tumova *et al*., 2003; Hulzebosch *et al.,* 2006; Voslarova *et al.,* 2006; Valkonen *et al.,* 2008; Jin *et al*., 1988). The housing systems can influence the productivity and health of birds and the quality of their eggs (Abrahamsson *et al*., 1988; Tauson *et al*., 2005). Jin *et al*. (1988) compared the egg performance of hens reared in conventional cages and cage-free and reported that the egg production rate was increased by 6% for the hens reared in the cages. Also, shell thickness was greater for the hens reared in cages. Contrastingly, a study by Abrahamsson *et al.* (1998) reported that the layer's performance in an aviary system equipped with multi tiers was greater than the performance of layers in a cage system. Englmaierova et al. (2014) reported conventional cage hens had higher albumen than cage- free hens, whereas cage-free hens had a higher yolk index than the caged-hens. The authors also reported higher microbial contamination in eggs from the cage-free hens to compare to the caged-hens. Conflicting reports were published by others where greater albumen height and Haugh unit were reported in cage-free than cage hens (Dukic-Stocic *et al*., 2009; Van Den Brand *et al*., 2004). Lewko et al. (2011) investigated the effect of three housing systems (cage,

floor-pens, and free-range) on the egg performance of the hens where the heaviest eggs were reported from birds in cages.

The role of Corn in the poultry diet

Dietary carbohydrates are important sources of energy for poultry. Cereal grains such as corn, grain sorghum, and wheat barely contribute most carbohydrates to poultry diets (Nutrient Requirements of Poultry. 1994). Among the cereal grains, corn (maize) is the largest proportion of the layer's diet in the world due to its high energy content. Corn is particularly advantageous because its fatty acids are mostly unsaturated and usually constitute a large portion of a feed (Nutrient Requirements of Poultry. 1994).

A sustained rise in grain prices driven by ethanol feedstock demand could lead to higher food prices (Yacobucci *et al*., 2007). In 2002, the US government increased the production of domestically produced energy, encouraging the production of ethanol and other biofuels. Most ethanol produced in the United States is made from corn. Ethanol processing plants have been in existence in the United States for decades, but the production costs and the lack of economic value of ethanol previously kept production at small but stable levels. The United States government developed the Renewable Fuel Mandate through subsidies, tax incentives, and fuel mixing mandates to encourage investment in new ethanol plants. This program exceeded expectations as ethanol production levels increased substantially, fueling additional demand for the corn which set the stage for the increase in animal feed costs beginning in late 2006. In October of 2006, the average

price of corn fed to chickens was

$2.60 per bushel, and soybean meal was $170 per ton. By October of 2007, corn had increased to $4.20 per bushel (61%), and soybean meal had increased (53%) to $260 per ton (Donohue *et al*., 2009). Increasing the share of the United States corn harvest to ethanol production will likely lead to higher prices for all grains and oilseeds that compete in the same land to use for the poultry feed, which results in higher feed costs for poultry producers (Yacobucci et al., 2007). The increase of 20 to 40 % rise in corn prices for the poultry feed would increase 1 to 2 % rise in the retail price of grocery food items in the US (Condon *et al*., 2013). Babcock (2010) asserts that corn's price is one of the most critical factors in determining the cost of livestock products because it serves as a reference price for other key carbohydrate sources, such as barley and wheat. Another relevant assumption is the treatment of ethanol co-products. Distillers' dried grains (DDGs) are a joint output of the corn ethanol production process that can be used as animal feed, mitigating some of the effects of higher grain prices on the livestock industry (Condon *et al.,* 2013).

The Impact of Photo-stimulation on the Sexual Maturity of Egg-laying Hens

The photoperiod is the most important environmental factor that can change the onset of sexual maturity. Photoreceptors detect the circadian rhythm within the brain. Photo-stimulation in birds increases the gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus, and

then GnRH stimulates the anterior pituitary to release luteinizing hormone (LH) and follicular stimulating hormone (FSH). The birds' ovulation releases the progesterone from the granulosa cells of the F1 follicle and by the theca cells of the F2 follicle by the effect on LH and release of progesterone and estradiol by the theca cells (steroidogenesis) by the influences of the FSH. The estradiol develops the left oviduct and affects the yolk synthesis. The surge of estrogen at the onset of sexual maturity also affects the bone. Estrogen changes the function of osteoblasts to begin forming medullary bone that serves as a labile source of calcium for impending egg production. Medullary bone develops to serve the eggshell for the calcium need. The other hormone that supports the shell for calcium is the parathyroid hormone (PTH). Parathyroid hormone stimulates calcium transfer by osteoblast and osteocytes for the formation of the eggshell during the period when an egg is in the shell gland. The photo-stimulation (increasing the lighting hours) initiates estrogen secretion from the gonad. The estrogen hormone changes the bone structure to the medullary bone. The medullary bone with less intrinsic strength influences the mechanical strength than the cortical bone (Knott *et al.,* 1995; Fleming *et al.,* 1998). The medullary bone is present during the reproductive cycle, which provides the calcium for the shell formation. The medullary bone is a secondary source of calcium for eggshell formation after dietary calcium.

The Effect of Photoperiod on the Overall Performance of Egg-laying Hens

The age of the birds at the onset of photo-stimulation can determine the influence on the onset of their sexual maturity. Pishnamazi *et al*. (2014) investigated the effect of age at photo-stimulation on sexual maturation. Pullets were photo-stimulated at four different ages (17, 19, 21, and 23 weeks of age). They found that those birds that were photo- stimulated in the later ages became sexually mature later. They observed 42 days differenceon the age at first egg between birds that were photo- stimulated at 17 weeks of age versus the ones at 23 weeks of age. Pishnamazi *et al*. (2014) also reported that delayed photo- stimulation resulted in heavier body weight in the hens. Birds with delayed photo- stimulation were observed to have well-developed reproductive tract. Also, late photo- stimulated birds demonstrated heavier birds compared to early photo-stimulated birds. Furthermore, photo-stimulation at older ages would decrease the duration between photo- stimulation to oviposition (Pishnamazi *et al*., 2014; Yuan *et al.,* 1994; Robinson et al., 1996). Robinson *et al.* (1996) reported that birds reached sexual maturity more uniformly by delaying photo-stimulation than birds that were photo-stimulated in early ages. Like their study, Renema *et al*. (2007) found that more uniformity in sexual maturity was observed for those birds that were photo-stimulated at 22 weeks of age of age compared to those that were photo-stimulated at 18 weeks of age of age.

A delay in photo-stimulation by two weeks increased the egg size of the hens compared to early photo-stimulated hens (Pishnamazi et al., 2014). In another study, the effect of three lighting regimens before 17 weeks of

hen's age on their skeletal integrity on three strains of the hens were investigated; three steps down lighting regimen (fast, moderate, and slow) before 17 weeks at an age to delay the sexual maturity by this method so the pullet's skeleton will have more opportunity to develop structural bone more fully before the onset of egg-laying (Hester et al., 2011); they observed that those birds that received a fast lighting program (reducing 4 hours of light weekly from 0 – 4 weeks of age) showed heavier body weight and higher bone mineral density at 66 weeks of age than moderate lighting regimen (decrease in hours of light 1 hour weekly from 2 – 17 weeks of age) and slight lighting regimen (decrease in light gradually from 20 hours to 10 hours of light by 17 weeks of age) indicating fast lighting regimen hens had less demand for bone calcium for egg formation at 66 weeks of age compared to other lighting regimens. The authors also reported a strain-related response to photo-stimulation. Hy-line Brown was reported to have a denser tibia than Hy-line W-98. The width and the area of the bones were also greater in Hy-Line Brown than in Hy-line W-98, whereas Hy-line W-98 had a higher bone mineral density of the ulna (Hester et al., 2011). In another study by Silversides *et al.* (2006), three strains of hens (Babcock B-300, ISA-Brown, and Unselected Brown Leghorn) were reared in 2 different ages at photo-stimulation (18 and 20 weeks of age) were investigated. They found that photo-stimulation at 20 weeks of age of age increased the bone area of radius at 50 weeks of age, and greater bone density was observed at 74 weeks of age compared to 18 weeks of age hens.

An increase in humerus breaking strength was also observed for hens that were photo-stimulated at 20 weeks of age of age (Silversides et al., 2006). Silversides *et al.* (2006) also reported a 1-week delay on the onset of sexual maturity for the brown layer than the other two strains. Therefore, finding an appropriate time at photo-stimulation in different strains of the birds is a crucial factor that can influence on the hen's body weight, the size of the egg, and the quality of the bones.

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Chapter 1

Effects of dietary metabolizable energy levels and housing system on the production performance and egg quality of white leghorn hens

ABSTRACT

This study aimed to determine whether the hens in the cage-free floor housing have different energy needs for their maintenance and productive performance than those in conventional cages due to their opportunities to perform more physical activities. Commercial white leghorn hens were housed in conventional cages (CCs) and cage-free (CF) from 36 to 52 weeks of age. Dietary metabolizable energy (ME) consisted of low ME and high ME (2,750 and 2,950 ME kcal/kg, respectively) and were given with a limited feeding amount (97 g/hen/day) from 39 weeks of age. Hens were housed individually in CCs and 15 cages on the same row comprised a single replicate for a total of 3 replicates each for housing by diet combinations. Floor pens equipped with nest boxes and perches housed 15 hens/pens with three replicates per treatment combination. Hen-day egg production and the quality of the eggs (egg weight, shell strength, vitelline strength, and Haugh unit) were recorded. The average body weight and the average feed intake were measured during the study. Mixed- model ANOVA with main effects of different ME levels in the diet in two different housing systems and interaction between housing and ME levels were incorporated into the statistical model to assess production parameters' impact. There was a significant ($P < 0.05$) interaction between the housing and feed intake for egg

production. Hens with low ME and raised in CF environments had a significantly higher egg production rate than hens at CCs with high ME in their diet. Also, egg production was 3.42% greater for hens housed in CF than hens reared in CC ($P < 0.05$). Average body weight was greater for hens reared in CF systems than hens reared in the CCs ($P < 0.05$). Hens housed in CF housing had 9.19% more feed intake than hens reared in CC ($P < 0.05$). Housing and diet did not affect albumen height and Haugh unit of the eggs. CF hens had a 3.4 % more egg production rate than the CC hens, but the feed conversion ratio of CF was 10% higher than CCs. The feed consumption of CF was significantly ($P < 0.05$) 9% higher than CCs. It is evident that more space in the CF environment significantly increases the feed demand. Decreasing space allowance per hen by cage system improved the efficiency of the hens (less amount of feed for high egg production rate).

INTRODUCTION

The composition, formulation, and amount of feed is an important factor that controls the maintenance and productivity of a poultry flock. Energy is one of the major factors required to maintain vital body functions, such as physical movement, body temperature regulation, and organic tissue synthesis. The maintenance of energy homeostasis in an animal is a highly complex phenomenon and is the summation of a wide range of external and internal stimuli that, in turn, affect the intake of nutrients (Classen, 2017). In laying hens, energy is either used for maintenance, directed towards egg

production, or stored (National research council, 1994). As a result, the energy requirement of laying hens is often considered based on the maintenance of body weight for egg production. Optimum body weight (and fat reserves) is essential for puberty as body mass is known to influence the onset of sexual maturity in layers (Leeson and Summers, 2005). An increase in the body weight around peak egg production is an important indicator that implies hens are getting enough nutrients, and loss of weight towards the peak often results in decreased egg production. The research on the metabolizable energy (ME) requirements of commercial layers, however, has produced conflicting results. For instance, literature reports suggest a negative influence of increasing dietary ME content on egg quality (Colvara *et al*., 2002; Junqueira *et al*., 2006; Almeida *et al.,* 2012; Silva *et al.,* 2012). Ribeiro *et al.* (2014) investigated the effect of 5 different energy levels on the performance and quality of the eggs. They found that increasing the apparent metabolizable energy corrected to zero nitrogen retention (AMEn) negatively affected egg production and mass but did not influence body weight and egg weight. The concurrent increase in feed conversion ratio and feed intake was also observed after increasing the AMEn in the feed (Ribeiro *et al.*, 2014). Experiments related to energy requirement in laying hens are often conducted in cages. The result from such experiments fails to account for environments where hens are freely moving and perform a comparatively higher level of energy-demanding physical activity than in the cages. Physical activity has been reported to be the most influential

factor for increasing the feed intake in chickens (Braastad et al., 1989; Morrison et al., 1978; Riek *et al*., 2021).

The influence of the housing environment on the energy content of the hen's diet has become quite significant in recent years as commercial egg production transitions from conventional cages to cage-free housing systems. Conventional cages were banned in the EU in 2012, and since then, hens have been primarily reared in free-range or barn systems. Similar changes are expected in the United States, with about 23% of 340 million laying hens currently reared in cage-free systems. Birds in cage-free systems perform activities like walking, jumping, wing-flapping, dust bathing, etc. that can increase the hen's metabolic rate leading to heat production and, ultimately, energy loss (Ning et al.,2012; Van Kampen, 1976a, 1976b). Many studies have reported that egg performance (number of eggs and quality of the eggs) was improved in the conventional cage (CC) system compared with deep litter cage-free (CF) systems (Tauson *et al*., 1999; Voslarova *et al*., 2006). In contrast, (Jin and Craig, 1988; Anderson and Adams. 1994) reported the hen-day egg production in laying hens that were reared in two different rearing systems (CF and CC) was not affected by rearing conditions. In another study by Jalal *et al.* (2006), four different cage spaces (342, 413, 516, and 690 cm^2/hen) for three different levels of dietary ME (2,800, 2,850, and 2,900 ME kcal/kg) in a 4 ×3 factorial arrangement were investigated. Jalal et al., 2006 observed that as cage space increased, the metabolizable energy intake increased, and hens that were

housed in 690 cm^2/hen cages had the greatest feed intake and metabolizable energy intake. The same group of hens had the highest egg production rate compared to other cage space allowances. Furthermore, an imbalance in dietary energy content can predispose hens to health issues. High energy and low-protein diet with an imbalance in amino acid compositions can increase the high energy intake more than they need for production and maintenance, resulting in excess fat in the liver. The fat oxidation in the liver can cause lethal hepatic hemorrhage, a condition known as fatty liver hemorrhagic syndrome. The fatty liver hemorrhagic syndrome occurs most commonly in layers that are housed in confined cage housing (Weitzenbürger *et al.,* 2005; Kaufman-Bart *et al.,* 2009; Lay Jr *et al*., 2011; Widowski *et al*., 2013) when hens are fed high energy diets and suffer from a low level of exercise (Butler, 1976).

The need to precisely incorporate energy in the diets of laying hens with respect to their housing environment also stems from the fact that energy is the primary determinant of feed cost. Poultry diets used in the United States are primarily corn and soy-based diets. As such, corn is one of the most critical factors in determining the cost of livestock and poultry products because it serves as a reference price for other key carbohydrate sources, such as barley and wheat (Babcock, 2010). Corn is also used in the production of ethanol, and ethanol serves as a primary fuel component in the United States. The increase in demand for corn as a source for ethanol and biofuel production has been a significant factor in increased feed

ingredient costs for the US poultry industries (Donohue et al., 2009). A sustained rise in grain prices driven by ethanol feedstock demand could lead to higher United States and world food prices. The feed-price effect will first translate into higher prices for poultry and hogs, which cannot use alternate feedstuffs (Yacobucci *et al*., 2007). Although distillers' dried grains (DDGs), a byproduct of the corn ethanol production process, can be used as animal feed (Condon *et al*., 2013), corn remains the primary source of energy in the poultry diet. Therefore, our study aimed to investigate energy requirements in Hy-line W36 laying hens housed in cages and cage-free floor systems based on their average daily feed intake, body weight gain, egg production, and egg quality.

MATERIALS AND METHODS

All procedures within this trial were reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee.

Bird Housing and Management

White Leghorn hens (Hy-line W36) reared in conventional cages were used in the experiment. At 36 weeks of age, 90 pullets were transferred to a cage-free floor (CF) system, whereas another 90 hens were housed individually in conventional layer cages (CCs). The CF pens were 9.3ft wide, 12.6ft long, and 10.7ft high with an area of 117.18 ft^2 and housed 15 hens/pen. Additionally, each pen was fitted with a perch and four nest

boxes with dimensions of 12in wide, 12in long, and 13in high. The conventional cages were 13in wide, 18in deep, and 23.5in high, with an area of 234 in^2. Hens were housed individually in each cage. Fifteen consecutive cages within the same row comprised a replicate in the CCs, and each floor pen was considered a replicate in the CF. The lighting schedule was based on the recommendation of Hy-line W36 management guide (https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/W36). Birds' diet formulation is present in Table 1.1 that met or exceeded the breeder's recommendation prior to the experimental diet.

Experimental Design

The experimental treatments were arranged in a 2 x 2 factorial design with 2 levels of housing environment and 2 levels of dietary energy. The housing environment consisted of CCs and CF, whereas the dietary treatments consisted of low metabolizable energy (LME; 2750 Kcal/Kg) and high metabolizable energy (HME; 2950 Kcal/Kg). The composition of experimental diets is presented in Table 1.1. There were 3 replicates each of the housing by diet combination. The experimental diets were started at 39 weeks of age (after 3 weeks of acclimation), and the hens were fed 97 grams/day to avoid compensatory feeding within the treatment groups. The experiment was terminated when the hens were 51 weeks of age.

Production parameters

The baseline body weight measurement was taken three weeks prior to the introduction of the experimental diet. After 39 weeks of age, body weight was measured every two weeks until the end of the experiment. The feed intake was calculated weekly from 36 to 51 weeks of age based on an average daily feed intake. The feed conversion ratio (FCR) was calculated based on the formula below. FCR (per kg egg mass) = Kg of feed consumed/ Kg of egg produced Eggs were collected daily, and hen-day egg production was calculated every week until the end of the experiment. Egg quality parameters were measured once before introducing the experimental diets and then bi- weekly until the end of the experiment. The egg quality parameters consisted of egg weight, shell strength, albumen height, Haugh unit, and vitelline strength.

Statistical Analyses

Statistical analyses were performed using two-way ANOVA, using the PROC GLM procedure of SAS (version 9.1, 2019, SAS Institute Inc., Cary, NC). The main effects of housing environment and dietary energy level, their interaction, and residual error were included in the model. Results were considered statistically significant at $P < 0.05$. The data were presented as least square means with respective standard error of the mean.

RESULTS

This study indicates that laying hens housed in CF weighed approximately 4.5% heavier than the hens in CCs. The body weight of CCs

was significantly lower than the body weight of CF (Figure 1.1). The body weight of CCs was dropped from 39 – 41 weeks of age, but for CF, body weight dropped from 39 – 43 weeks of age (Figure 1.1).

Interestingly, egg production for hens housed in cages decreased sharply after 45 weeks of age until the end of the experiment (Figure 1.2). For the first 2 weeks of the trial, egg production of CF was lower than the later weeks (Figure 1.2). The average hen day egg production of CF with LME was significantly greater than other housing systems and other treatments (Figure 1.3). The feed intake of CF was greatly more than the feed intake of CC at different ages (Figure 1.4). However, the feed intake continued to increase during the production period in the CC system. Also, the feed conversion ratio of CF was significantly higher than CC at different ages throughout the experiment (Figure 1.5).

In general, hens kept in CC had lower body weight regardless of feed intake (Figure 1.1). As expected, hens under HME diet weighed more than LME (Table 1.1). Dietary energy level did not influence feed intake in this study. A housing-by-diet interaction was observed for hen day egg production. Cage-free (CF) hens that were in LME diet produced 7% more eggs compared to CCs hens in LME diet, which was statistically significant. Regardless of the housing type, hens under HME diet had similar hen-day egg production. An interaction between the housing system and the dietary energy level was observed for egg weight as well. Hens fed LME diet and housed in CF produced lighter eggs than CCs under LME (Table 1.5; $P <$

0.05). Overall, hens fed HME produced heavier eggs than the hens that received low ME (Table 1.5).

The egg quality parameters are presented in Table 1.6. Albumen height and Haugh unit were not affected by the housing system or dietary energy level (Table 1.6). Shell strength was influenced by the housing system, with CF hens having stronger eggshells than the CCs hens (Table 1.6). On the other hand, vitelline membrane strength was significantly greater for hens fed LME diet than HME diet regardless of the housing system (Table 1.6).

DISCUSSION

The housing system and the nutrient composition of the diet play a significant role in the egg industry for the maintenance and production of the hens. Pullets are grown under a range of environmental conditions and housing systems, which can influence energy and nutrient needs. The average feed intake and energy consumption can differ among housing systems. The result of this study corroborates the findings of previous studies (Dikmen et al.; 2016 Singh et al., 2009; Soomro et al., 2019) where feed intake was reported to be greater for hens housed in a cage-free system than for hens in cages and enriched colony cages. Further, hens that received the low-energy diet consumed more feed and produced fewer eggs per day than the birds fed the high-energy diet (Valkonen *et al*., 2008; Kang *et al.,* 2018). Feed intake was also observed to increase for the hens that were fed a low-energy diet than the hens fed with a high-energy diet in our study.

Routine behaviors such as wing flapping, jumping, running, and dust bathing are more commonly observed in CF compared to CCs because of the availability of more usable space for the hen. As a result, hens in CF are more likely to consume more feed to meet their energy needs (Ning et al., 2013; Van Kampen, 1976a, 1976b).

In the current study, body weight was observed to be 4.5 % greater for the hens raised in the CF housing than the hens raised in the CC. The feed intake data showed that the hens in CF consumed9% more feed than the hens raised in the CCs. Higher feed intake most likely resulted in the difference in hens' body weight between the systems. Hens that received high ME had 2.2 % more body weight gain than the hens that received low ME in their diet. Bonilla *et al.* (2012) compared diets with 2,650, 2,750, 2,850, and 2,950 kcal of available ME kcal/kg and reported body weight increase in hens corresponding to increasing dietary energy level. In another study, hens that received the high energy diet gained the most weight, and the hens who received the low energy diet gained the least weight (Harms *et al*. 2000b). In contrast, Jalal *et al.* (2006) observed similar body weight in Hy-line W36 hens when apparent metabolizable energy in the diet increased from 2800 to 2900 kcal/kg at 21 weeks of age. As the birds used in our study were older, the difference in results between the studies suggests that stage of production could affect the body weight response of hens to dietary energy content.

Hen-day egg production of CF was observed to be lower than CCs at 39 weeks of age. of age, and this difference in egg production could have

resulted from the change in the housing environment at the beginning of the experiment. At 39 weeks of age (2 weeks after hens were transferred from cages to the floor for the trial), hens may not have been completely acclimated to the floor housing. Litter materials were found in the intestine upon necropsy of the early mortality cases from the floor system. We also observed an egg-eating phenomenon in one replicate in the CF system fed with LME from 45 weeks of age to the end of the experiment. Although eggs were collected at least twice daily, egg-eating could have confounded the results of egg production. Overall, we observed an interaction effect between the housing system and feed intake for egg production. Contrary to our hypothesis, when hens were fed LME, egg production was different between the housing systems, with CF hens producing more eggs than CCs. This study is the first to our knowledge to compare different dietary energy levels in cage and floor systems. Unlike the results of this study, hens ate more and produced fewer eggs under LME diet (2342 to 2414 kcal/kg) compared to HME diet (2581 to 2629 kcal/kg) regardless of whether they were housed in conventional cages or furnished cages (Valkonen *et al.* 2008). There are conflicting reports in the published literature on the influence of the housing system on egg production. Some studies have reported similar egg production (Neijat *et al*., 2011; Ahammed *et al.,* 2014), while others have reported higher hen-day egg production for the hens raised in the CCs than CF (Tauson *et al.,* 1999; Leyendecker *et al*., 2001; Dikmen *et al*. 2016). Carew *et al.* (1980) reported that egg

production did not change significantly as dietary energy increased, whereas Kang *et al*. (2018) reported increases in egg production as dietary energy levels increased within one housing system.

In this study, we observed that dietary energy significantly impacted egg weight in CF but not in CC. Hens fed HME diet produced heavier eggs in the cage-free floor system. Others have reported an increase in egg weight with an increase in dietary energy level (Grobas *et al*. 1999(b), Bouvarel *et al*. 2010, Ciftci *et al*. 2003, Granchelli *et al*. 2019, and Wu *et al*. 2005; Harms *et al*. 2000b). In contrast, Valkonen *et al*. (2008), with comparing dietary energy from (2,342 kcal/kg to 2,629 kcal/kg), and Jalal *et al*. (2006), with AMEn levels of 2800, 2850, 2900 kcal/kg, reported that dietary energy had no significant effects on egg weight. Like the results of this study, Carew *et al*. (1980) found dietary energy level did not affect the Haugh unit. On the other hand, the housing system influenced shell strength, with floor hens having stronger eggshells than caged hens (Soomro et al., 2019; Lewko *et al*., 2011; Van den brand *et al*. 2004). Surprisingly, other studies have reported higher incidences of cracked eggs in cage-free systems (Mertens *et al*. 2006; Valkonen *et al*. 2008; Abrahamsson and Tauson, 1997; Guedson and Faure, 2004). The feed conversion ratio was increased for the hens housed in the CC system; the higher value of FCR in the CF system suggests that hens in this system consumed more feed due to compensating for greater physical activity than hens housed in CCs for egg weight that they produced. Similar reports of greater FCR have been reported in an

aviary or floor system in other studies (Tauson *et al*., 2005; Tauson *et al*., 1999; Michel and Huonnic, 2003; Stewart et al., 2006).

CONCLUSIONS

In this study, the energy intake was 9.2 % greater for the CF than the CCs. We also observed hens in the CF were more activities such as jumping, walking, and wing flapping compared to the hens in the CC. Therefore, we conclude that the hens in the CF consumed more energy to compensate for the energy losses. Although the egg production rate in the CF system was 3.4 % greater than the CCs, the FCR of the CCs was 10.5% better than the CF. Therefore, this study showed that the hens in the CC system are more efficient within the same amount of energy levels in the diet than the CF.

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TABLES AND FIGURES

Table 1.1 Feeding program of diets and formulation according to the age of the hens

	STARTER (0 – 6 weeks of age)	GROWER (6 – 12 weeks of age)	DEVELOPER (12 – 17 weeks of age)	LAYER (18 – 39 weeks of age)
Metabolizable energy, kcal/kg	2977–3087	2930–3087	2880-3050	2844-2955
Lysine, %	0.98 / 1.07	0.88 / 0.96	0.76/0.83	0.84
Methionine, %	0.44 / 0.47	0.40 / 0.44	0.36/0.38	0.44
Methionine Cysteine, %	0.74 / 0.83	0.67 / 0.75	0.59/0.67	0.77
Threonine, %	0.66 / 0.77	0.60 / 0.70	0.52/0.62	0.59
Tryptophan, %	0.18 / 0.21	0.17 / 0.20	0.15/0.18	0.18
Arginine, %	1.05 / 1.13	0.94 / 1.01	0.81/0.87	0.90
Isolucine, %	0.71 / 0.76	0.65 / 0.70	0.57/0.61	0.67
Valine, %	0.73 / 0.80	0.69 / 0.76	0.61/0.67	0.74
Crude protein, %	18.25	17.50	16.00	17.58
Calcium, %	1.00	1.00	1.00	4.37
Phosphorus, %	0.49	0.47	0.45	0.51
Sodium, %	0.17	0.17	0.18	0.19
Chloride, %	0.17	0.17	0.18	0.19
Linoleic acid %	1.20	1.20	1.20	2.11
Choline, mg/kg	1,800	1,800	1,500	1,895

	Growing period	Laying period
Vitamin A, IU	10,000,000	8,000,000
Vitamin D3, IU	3,300,000	3,300,000
Vitamin E, g	25	20
Vitamin K (menadione), g	3.5	2.5
Thiamin (B1), g	2.2	2.5
Riboflavin (B2), g	6.6	5.5
Niacin (B3), g	40	30
Pantothenic acid (B5), g	10	8
Pyridoxine (B6), g	4.5	4
Biotin (B7), mg	100	75
Folic acid (B9), g	1	0.9
Cobalamin (B12), mg	23	23
Manganese, g	90	90
Zinc, g	85	80
Iron, g	30	40
Copper, g	15	8
Iodine, g	1.5	1.2

Table 1.2 High metabolizable energy diet formulation (HME – 2950 ME Kg/Kcal) given to the hens from 39 – 52 weeks of age:

Ingredient name	Amount
Corn, Yellow	44.453 %
Corn, Distiller	2 %
Fat, Vegetable	7.456 %
Poultry By-Pro	1.322 %
Soybean meal	20.97 %
Crude protein	17.13 %
Crude Fat %	10 %

Table 1.3 Low metabolizable energy diet formulation (LME – 2750 ME Kg/Kcal) given to the hens from 39 – 52 weeks of age:

Ingredient name	Amount
Corn, Yellow	49.262 %
Corn, Distiller	3 %
Fat, Vegetable	3.526 %
Poultry By-Pro	5.839 %
Soybean meal	12.5 %
Crude protein	17.13 %
Crude Fat %	7.01 %

Table 1.4 Diet. Ingredient composition and nutrient content of two experimental diets(fed 39 weeks of age – 52 weeks of age, High ME, and low ME kcal/kg)

	Diet treatments	
Nutrient	High energy diet	Low energy diet
Crude protein (%)	17.84	18.19
Calcium (%)	3.10	3.46
Phosphorus (%)	0.73	0.79
Gross energy (Kcal/kg)	4,188	4,146
Ash (%)	11.85	11.96
Dry matter (%)	89.91	89.47

Table 1.5 Effect of dietary ME and housing system on Layer performance: egg production, egg weight, food consumption ratio (FCR), and body weight

Dietary energy level	[1]High ME		[2]Low ME		*Level of significance (α)*	
Housing systems	Cage	CF	Cage	CF	Housing	ME
Body weight (kg)	1.33±0.01[c]	1.39±0.03[a]	1.30±0.03[d]	1.36±0.01[b]	<0.0001	<0.0001
[4]Feed Intake	87.57±4.90[b]	95.53±3.10[a]	88.08±6.64[b]	96.27±1.89[a]	<0.0001	0.52
[5]FCR	1.51±0.02[b]	1.62±0.02[a]	1.51±0.02[b]	1.72±0.02[a]	<0.0001	0.06
Hen-day egg production (%)	86.22±4.03[a]	84.94±7.38[ab]	80.43±5.72[b]	87.41±5.03[a]	0.03	0.22
Egg Weight (g)	57.62±1.37[a]	58.48±1.74[a]	57.94±1.74[a]	55.93±1.62[b]	0.10	0.002
[3]Egg mass (%)	49.45±0.85[a]	49.37±0.85[a]	46.30±0.85[a]	48.39±0.85[a]	0.25	0.02

[1]High ME diet (2950 ME kcal/kg)

[2]Low ME diet (2750 ME kcal/kg)

[3]Egg mass is defined as the percentage of the hen day egg production × average egg weight. [4]Feed Intake is the average amount of feed that hens consumed daily in two levels of dietary energy (g/h/day).

[5]FCR in here is the average amount of feed intake divided by the average weight of the eggs.

Data are presented as means ± SE

[a-b-c] letters within the same rows with no common superscripts differ significantly ($P < 0.05$).

Table 1.6 The effect of the housing system and dietary ME on egg quality parameters

Dietary energy level	High ME		Low ME		*Level of significance (α)*		
Housing systems	Cage	CF	Cage	CF	Housing	ME	Housing× ME
Albumen Height (mm)	8.41±0.69	10.82±10.75	8.54±1.40	8.97±0.95	0.23	0.40	0.46
Haugh Unit	91.67±3.52	87.56±19.10	92.25±6.49	94.73±4.33	0.72	0.09	0.15
Shell Strength (Kg of force to crack)	3.65±0.5^{b}	4.10±0.8^{ab}	3.66±0.9^{b}	4.20±0.5^{a}	0.003	0.74	0.79
Vitelline strength (of force puncture)	1.70±0.13^{b}	1.76±0.19^{b}	1.91±0.21^{a}	1.81±0.26^{ab}	0.61	0.003	0.07

Data are presented as means ± SD

a-b Means within rows with no common superscripts differ significantly *(*$P < 0.05$*)*.

Figure 1.1 The average body weight of the hens in two housing systems (CCs and CF) atdifferent ages. Data are presented as mean ± SEM

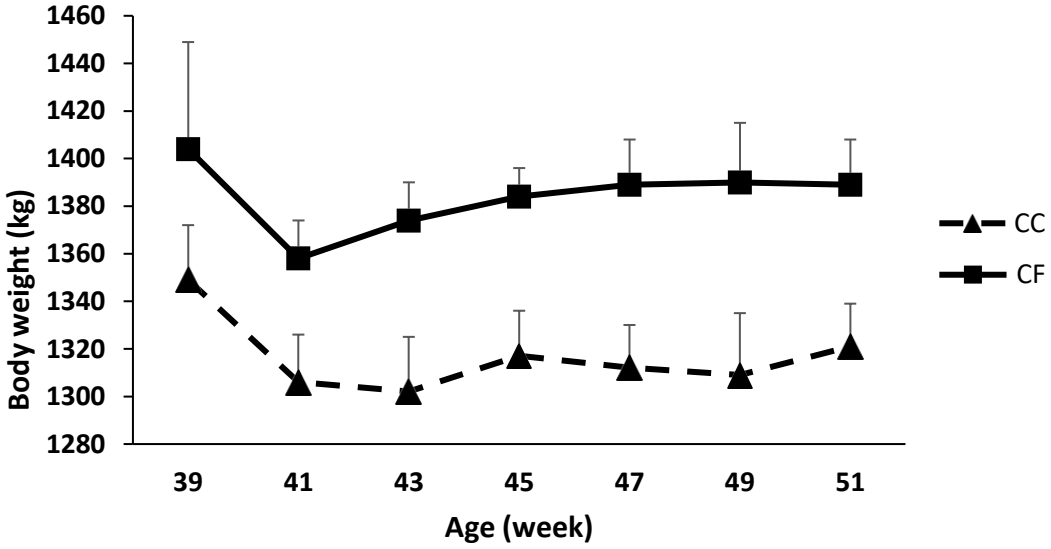


Figure 1.2 Hen-day egg production of CF and CCs at different ages. Data are presented as mean ± SEM

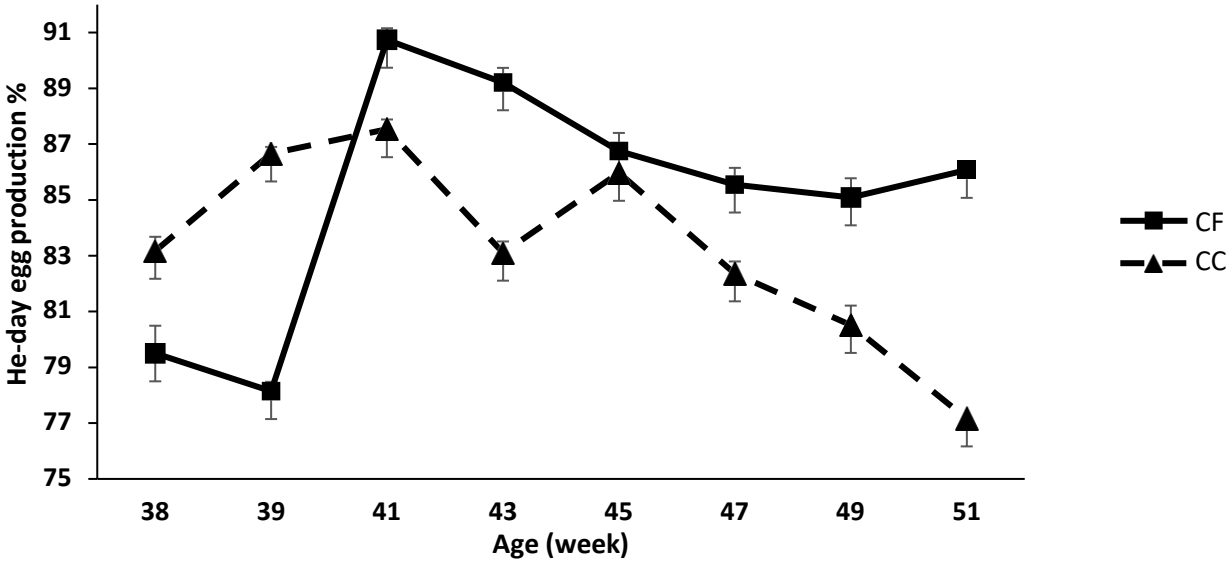


Figure 1.3 Hen-day egg production of CCs VS. CF in two different dietary energy levels (HME and LME) at different ages. Data are presented as mean ± SEM

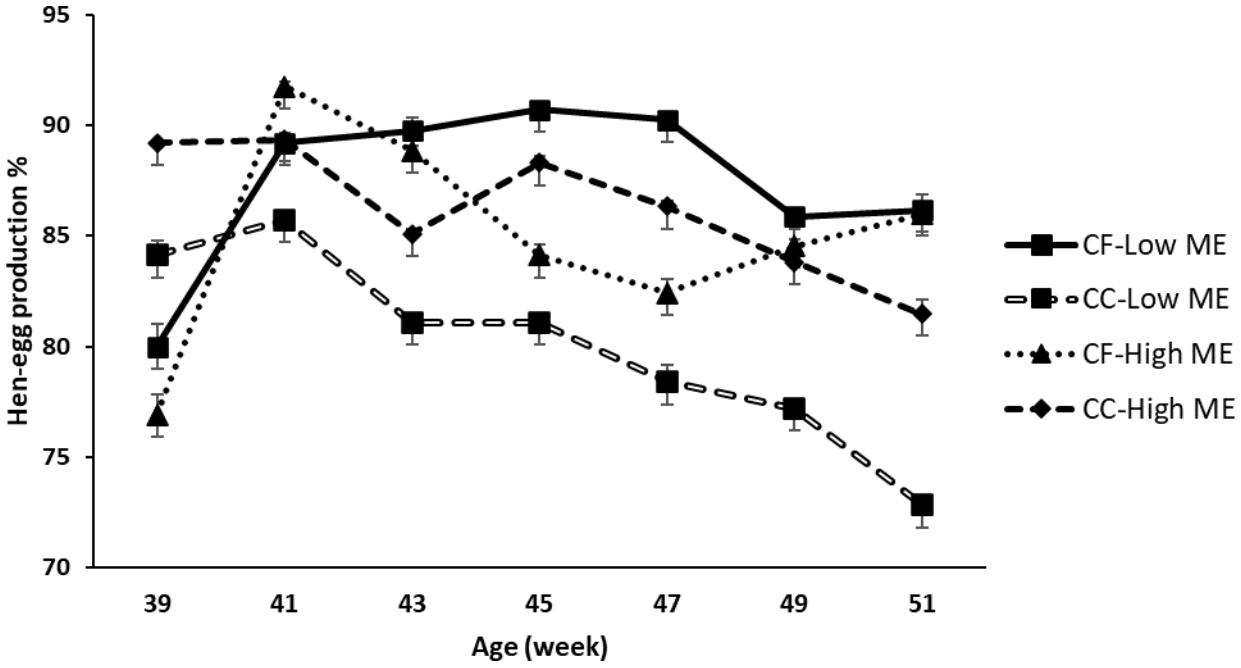


Figure 1.4 Average feed intake of CF VS. CCs at different ages. Data are presented as mean ± SEM

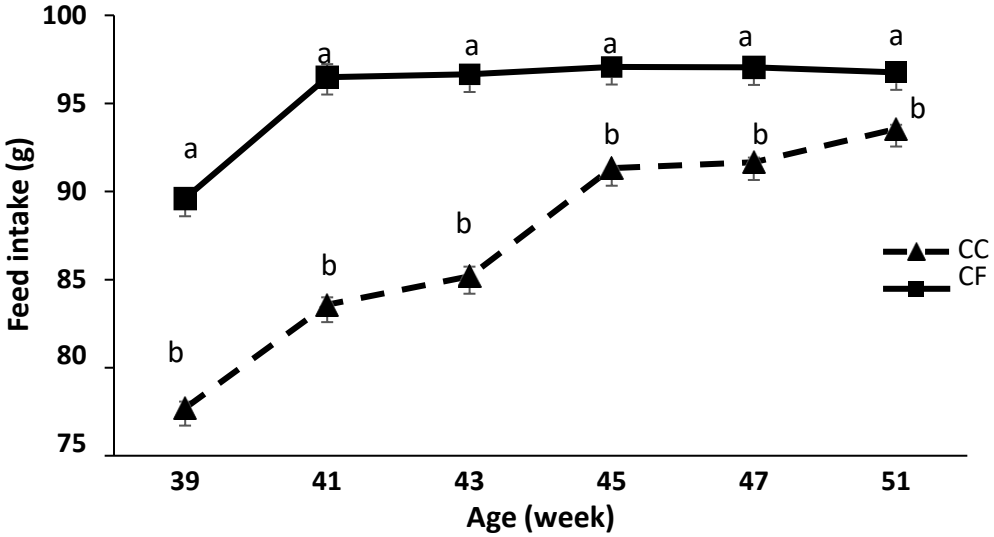
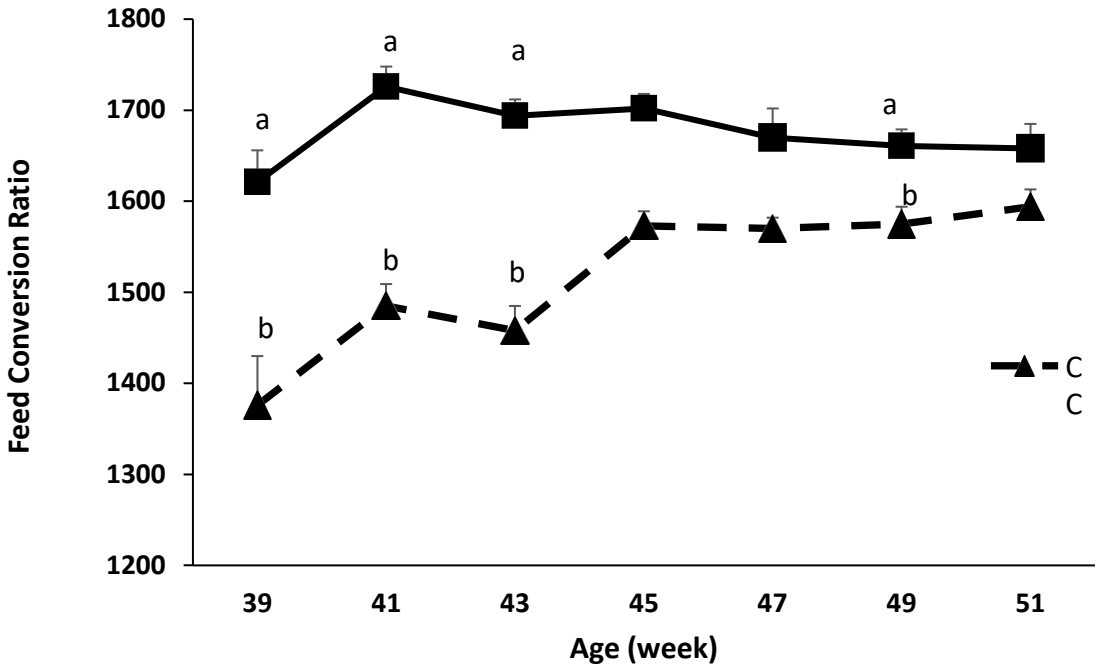


Figure 1.5 Feed conversion ratio compared in CF VS. CCs in different ages. Data are presented as mean ± SEM



Chapter 2

Effect of age at photo-stimulation on performance, egg quality, reproductive performance, and skeletal properties of laying hens

ABSTRACT

The risk of bone fractures is an important welfare concern in commercial egg layer flocks. The inactivity of the pullets in conventional cages has potentially increased the risk of bone loss and susceptibility to fracture. This study aimed to investigate the delay in photo- stimulation on bone quality and egg performance of laying hens. A total number of 133 pullets of two strains of hens (Hy-line W36 and 1940 leghorn) were housed in conventional cages at nine weeks of age. Hens were separated into two rooms at 14 weeks of age. The first room assigned for photo-stimulation at 15 weeks of age (PS15) contained 61 hens, and the other room assigned for photo-stimulation at 20 weeks of age (PS20) and contained 72 hens. Body weight, egg production, egg quality tests, bone quality tests for tibia, humerus, femur, keel, and plasma parathyroid hormone (PTH), and estradiol hormone, and serum calcium and phosphorus were measured throughout the study. At 8, 12, 15, 20, and 34 weeks of age, serum concentrations of ionized calcium and phosphorus along with plasma concentrations of estradiol hormone and parathyroid hormone were measured. At 15, 19, and 34 weeks of age, hens were euthanized to sample the tibia, femur, humerus, and keel. Tibia cortical thickness was measured at each sampling time point. The breaking strength of the tibia, femur, and humerus was measured. The calcium and phosphorus

content of the bones were measured. Tibiae cortical thickness was significantly ($P < 0.05$) higher in 1940 leghorn than Hy-line W36 hens. The anterior, posterior, medial, and lateral dimensions of the tibia cortex from PS15 were significantly ($P < 0.05$) thicker than the tibia cortex of PS20. There was no effect of different ages at photo-stimulation on egg production rate. The Hy-line W36 hens had significantly ($P < 0.05$) better egg production rate, heavier eggs, higher Haugh unit, higher vitelline strength, and higher shell strength than 1940 leghorn hens. The Hy-line W36 from PS15 had significantly ($P < 0.05$) higher calcium content in their bones than PS20 lighting regimen. The 1940s from PS20 had significantly ($P < 0.05$) higher calcium and phosphorus content in their bones than PS15 lighting regimen.

INTRODUCTION

The sexual maturation of pullets begins with photo-stimulation after eight weeks of age. The photons of the light stimulate the hypothalamus-pituitary-gonadal axis in the brain (Wang et al., 2019), which stimulates the secretion of the GnRH (Gonadotropin-Releasing Hormone), in turn, stimulates the anterior pituitary to release Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (L.H.) (Dittami et al., 1985; Dawson et al., 2001). These two hormones are involved in the development of the oviduct and make the ovary functional. The surge of L.H. and FSH stimulates the release of progesterone from the corpus luteum of the ovary and results in the

maturation of the ovary (Richards et al., 1987). The L.H. surge also influences the theca layer of the ovary to release estrogen (estradiol). The estradiol develops the left oviduct and affects the yolk synthesis (vitellogenesis) via its action on the liver (Johnson, 1986; Bacon et al., 1980).

The other hormone that supports shell formation is the parathyroid hormone (PTH). Parathyroid hormone stimulates calcium transfer by osteoblast and osteocytes for the formation of the eggshell. The photo-stimulation (increasing the lighting hours) initiates estrogen secretion from the gonads. The surge in estrogen production changes the function of osteoblast to begin forming medullary bone, which this type of bone serves as a labile source of calcium for impending egg production (Whitehead, 2004). The medullary bone has less intrinsic strength and, as such, influences the mechanical strength of the bone to a lesser extent than the cortical bone (Knott *et al.,* 1995; Flemming *et al*., 1998). Once the structural bone formation ceases, the high demand for calcium for the formation of the eggshell can develop structural bone loss and eventually result in bone fractures (Cransberg *et al.,* 2001). Many factors can increase bone fragilities like nutrition, housing environment, and genetics (Whitehead, 2004). Delay in photo-stimulation can increase the degree of infilling the bone with the structural bone and make them more resistant to fracture (Whitehead. 2004). Several factors need to be considered during photo-stimulation, such as adequate age, body weight and uniformity in body weight, and the frame

size of the pullets of a specific strain of the pullet. Pullets that are photo-stimulated before reaching the optimum body weight will exhibit lower egg production (Robinson, 1991; Melnychuk *et al.,* 2004). The different hen strains (based on their frame size and body weight) require different ages at photo- stimulation for optimum egg production. Silversides *et al*. (2006) previously reported that the unselected brown leghorn line reached sexual maturity one week later than Babcock B- 300 and ISA Brown hens. Pullet body weight at photo-stimulation can determine the onset of sexual maturity in laying hens as well as egg weight (McDaniel *et al.,* 1981). The study by Klein et al. (2018) and Pishnamazi et al. (2014) showed that hens with lower body weight came into the lay later than hens with higher body weight at the same age at photo- stimulation. Therefore, delay in photo-stimulation could be one of the external factors that can significantly affect the layer's performance and welfare. In this study, we hypothesized that the structural bone component would have a greater chance of becoming mature by delaying photo-stimulation in pullets. The bone fragility and incidence of bone breakage in caged hens will decrease. Moreover, calcium storage in the bones would better support the shell gland for eggshell formation.

MATERIALS AND METHOD

All procedures within this trial were reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee.

Bird Housing and Management

The experiment used two strains of white leghorn hen (Hy-line W36 and a strain from the 1940s). Pullets were reared on a floor system from day-old to 9 weeks of age. At 9 weeks of age, 133 pullets were transferred to cages in two light-tight rooms. The cages were 12" wide x 18" long x 18" high at the front and 16" at the back, with each cage assigned with 3 random hens. Six consecutive cages within the same row comprised a replicate with 2 replicates for each strain for each room. The first room consisted of 36 Hy-line W36 pullets and 25 1940s pullets. The other room contained 36 Hy-line W36 and 36 1940s pullets. Treatment arrangements are presented in Table 2.1 and Table 2.2. Low hatchability and lack of enough fertile eggs at the experiment resulted in a lower number of 1940s birds recruited for the experiment. The lighting schedule from the first day of rearing to 15 weeks was based on the Hy-line W36 management guide (https://www.hyline.com/filesimages/Hy-Line-Products/Hy- Line-Product-PDFs/W36). Birds were fed with a limited feeding regimen based on the daily requirement guideline set by Hy-line W36 management guide. Feed was formulated based on NCSU layer diet formulation with four different types of feed for different phases of the hen's cycle (Table 2.3). The pullets were fed starter diet until 6 weeks of age,

followed by grower diet until 12 weeks of age. From 12 weeks to 17 weeks of age, all strains were provided with developer diet. Birds were fed layer diet from 18 - 34 weeks of age. Water was provided ad libitum throughout the experiment.

Experimental design

The experiment was arranged in a 2 x 2 factorial design with 2 different strains of hen (Hy- line W36 and 1940s) and two different ages at photo-stimulation (15 weeks and 20 weeks of age). The results of feed analysis for different feeding phases are presented in Table 2.4.

At 15 weeks of age, birds in Room 1 were photo-stimulated (PS15), whereas photo- stimulation was delayed until 20 weeks of age (PS20) in birds kept in Room 2. The lighting program with photoperiod schedule is presented in Table 2.5.

General performance and egg quality parameters

The baseline body weight measurement was taken at 8 weeks of age when pullets were in the floor system. Body weight measurements were taken every 4 weeks until the end of the experiment. Feed intake was calculated every 3 days from 9 – 18 weeks of age. After 18 weeks of age the limited feed was given to the hens based on the Hy-line W36 management guide (https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/W36). Also, welfare assessment was conducted in hens for parameters such as keel bone deviation, keel bone fracture, footpad condition, feather scores, and skin lesions according to the Welfare Quality protocol.

Eggs were collected daily, and hen-day egg production was calculated every week until the end of the experiment. Egg quality parameters were measured at 28, 30, and 32 weeks of age. Egg quality parameters measured included egg weight, shell strength, albumen height, Haugh unit, and vitelline membrane strength.

Skeletal parameters

A total of 840 bones were collected during the experiment. Bones were sampled at three different ages at 15, 19, and 34 weeks. The keel bone, both humerus, femur, and tibia were sampled. Once excised, adhering soft tissues were removed from the bones. Tibiae: Anterior-posterior (A-P) and medial-lateral (M-L) outer dimensions of the bones were measured by a digital caliper at the midpoint of the bone (diaphysis) along with total length. Tibiae were then weighed and loaded until failure with the lateral surface in tension (Regmi et al., 2015) in a TA.H. D plus C Texture Analyzer (Stable Microsystems, Surrey, UK) to measure bone strength. A loading cell weight of 250 kg was used in the machine for breaking the tibia, humerus, and femur. A span length of 8.5 cm was used in the machine for breaking the tibiae. The internal cortical thickness at the fracture site of the tibiae along four orientations (anterior-posterior and medial-lateral) was measured by a digital caliper.

Femur and Humerus: The length of the left and right bones was measured. Bones were weighed and then underwent a 3-point bending test with the posterior surface in tension like tibiae explained above. A span length

diameter of 3.5 cm was used in the machine for the femur and humerus.

Keel: The pectoralis muscles were removed, and the keel bone was separated from the ribs, the coracoid, and the clavicle. The total length, along with the length of the cartilaginous portion of the keel, was measured. The width and height of the keel were measured as well. After morphometric measurements, keel bones were scored for deviation and fractures on a binary scale ('Yes' or 'No') at each sampling point.

Bone ash: Bone ash was measured using the dry ash technique. In this procedure, all the adhering tissues (muscles, cartilage caps) were stripped, and the bones were weighed. The bones were then put into the crucible and placed into a muffle furnace. The furnace was set at a temperature of 600°C for 10 hours. This procedure removed the organic compounds of the bone and, subsequently, bone ash was measured. The ash samples were further used for measuring calcium and phosphorus content using atomic spectroscopy.

Analysis of calcium and phosphorus in the ash

For this procedure, 1 g of the ash sample from each bone was placed into a beaker with 10 ml of 6 M hydrochloric acid (HCL). The beaker was then placed on a hot plate for 5 minutes, and another 5 ml of hydrochloric acid was added. The HCL solution with 50 ml of distilled water was added to a volumetric flask. In the next step, 200 ml of distilled water was added to the volumetric flask. After shaking the flask, 15 ml of this solution was

poured into a 15 ml conical tube. Atomic Absorption Spectroscopy analyzed the samples in the conical tubes to measure the amount of calcium and phosphorus in each sample. A total of 276 samples (6 hens/each treatment/sampling point) of bone samples (tibia, femur, humerus) from one side of the body along with the keel bone were selected for analysis of calcium and phosphorus in the bone's ash.

Hormone analysis

Blood samples (5 ml/hen) were collected at 8, 12, 15, 19, 24, and 34 weeks of age from the wing vein for analysis of plasma estradiol and parathormone (PTH) concentrations. Blood samples were also used to measure serum calcium and phosphorus content. Uncoagulated and coagulated blood samples were centrifuged at 2000 RPM/616 G for 20 minutes for separation of plasma and serum, respectively. Plasma and serum samples were stored at -20°C until further analysis. For estradiol assay, plasma samples were extracted with methylene chloride and isopropanol under the nitrogen gas. The 300 µl assay buffer was placed in the glass tubes to obtain the extracted plasma for the assay. Three ELISA kits (Cayman Chemical Company) were used for the estradiol samples. The coefficient of variation (CV) within the assays was 14.65%, whereas the CV between the assays was 13.59%. The parathyroid hormone ELISA assay was performed with unstripped plasma for all samples. A total of three ELISA kits were used for plasma PTH. The within assay CV was 6.16%, whereas the between assay CV was 7.86%. The Commercially available PTH ELISA kit (Cusabio

Technology LLC) was used for the analysis.

Statistical Analysis

Data were analyzed using the multivariate mixed model ANOVA. The statistical model included the main effects of lighting regimen and strain of the hens, and the interaction between lighting regimens and strain of the birds for their performance parameters were analyzed. All analyses were performed using JMP (version 15). Differences between means were tested using Tukey HSD, where a significant difference was detected. Significance was defined at ($P < 0.05$). Values were represented as least- square means with their respective standard error of the mean.

RESULTS

Body weight and feed intake data are presented in Table 2.6. Body weight of the hens was affected by age at photo-stimulation and genetic strain, whereas interaction effects were not observed between the two factors (Figure 2.1). Delaying the photo-stimulation affected the overall body weight of the birds, with PS20 weighing heavier greater than PS15 (Table 2.6). Similarly, body weight at 5% egg production of PS20 was 20% greater than PS15 (Table2.6). Body weight of Hy-line W36 was approximately 11% greater than 1940s (Table2.6). Age at photo-stimulation also influenced feed intake in the birds. The average feed intake was 15% more in PS15 than PS20 (Table2.6).

Internal egg quality parameters such as the Haugh unit, albumen height, vitelline strength, and shell strength of Hy-line W36 were also greater than 1940s leghorns (Table 2.7). Hy-line W36 from PS20 had greater albumen height than Hy-line W36 from PS15 (Table2.7). As expected, a delay in photo-stimulation did not influence any egg quality parameters except vitelline membrane strength, which was greater in PS20 compared to PS15(Table2.7).

The PS20 significantly delayed the age at 5% hen-day egg production of Hy-line W36 and 1940s with 3.6 and 11 days respectively and also delayed 50% hen-day egg production of Hy-line W36 and 1940s with 7 and 8 days respectively; also the PS20 delayed the age at 90% hen-day egg

production of 1940s for 16 days, but the age at 90% hen-day egg production of Hy-line W36 was not delayed by delay in photo-stimulation (Table 2.8 and Table 2.9).

The strain of the hen significantly affected the hen-day egg production; Hy-line W36 had greater egg production than 1940s at different ages ($P < 0.05$; Figure 2.2). Length of tibia, femur, humerus, and keel was not affected by age at photo-stimulation (Figure 2.3). On the other hand, Hy-line W36 had longer keel and tibia than 1940 leghorns (Figure 2.4) significantly. An interaction effect of age at photo-stimulation and the genetic strain was observed for bone strength of the femur. Hy-line W36 from PS15 had significantly stronger femur than PS20, whereas an opposite effect was observed in the 1940s birds (Table 2.10). The opposite strain effect was observed for the tibia's anterior-posterior outer diameter, which was wider in 1940 leghorns (Table 2.11). Delay in photo-stimulation had different results in different strains of hens. The tibia's posterior, medial, and lateral cortical thickness was greater in PS15 than PS20 (Table 2.11). This study also observed that 1940 leghorns from PS20 had higher calcium and phosphorus content in the tibia, femur, humerus, and keel than PS15 (Table 2.12). Contrastingly, Hy-line W36 with PS15 had greater bone calcium content than PS20, whereas the phosphorus content was not affected (Table 2.12). Age at photo-stimulation did not change the bone ash content (Table 2.13). The humerus of Hy-line W36 had greater bone ash content than 1940s (Table 2.13). The results of hormone assays are presented in Tables 2.14 and 2.15.

Plasma estradiol concentration was greater in PS20 than PS15 before any photo-stimulation was induced (Table 2.14). An increase in estradiol concentration was observed in both PS15 and PS20 post-photo-stimulation at 15 and 20 weeks, respectively (Figure 2.5). Overall, plasma estradiol level was greater in PS15 than PS20 at 20 and 32 weeks of age (Table 2.14). Different ages throughout this study did not influence parathyroid plasma hormone at photo- stimulation (Table 2.15). Plasma parathyroid hormone was at the lowest level at 20 weeks of age compared to other ages and sharply increased from 20 to 24 weeks (Figure 2.6). There was no significant difference in serum calcium level between the two strains of the hen. Hy-line W36 at 22 weeks of age had significantly higher calcium concentration (mmol/L) than other lighting regimens and other strains of the hen (Figure 2.7). Overall, circulating levels of phosphorus were approximately 17% greater in Hy-line W36 than in 1940 leghorns. Delayed photo-stimulation resulted in increased phosphorus concentration in the serum (21% higher in PS20 than PS15; Figure 2.8). Like calcium results, Hy-line W36 from PS20 had significantly higher phosphorus concentration at 22 weeks of age compared to other lighting regimens and other strains of the hen (Figure 2.8).

DISCUSSION

In the present study, we expected to delay the onset of egg-laying in white leghorns by delaying the photo-stimulation towards the end of the

rearing period. Delaying photo- stimulation from 15 to 20 weeks of age delayed the onset of age at 5% egg production for Hy- line W26 and 1940s. Pishnamazi *et al*. (2014) compared photo-stimulation at 17 weeks of age and 23 weeks of age and reported a similar result in which 25-day delay in sexual maturity in the latter group. Silversides et al. (2006) reported the opposite results when photo-stimulation was delayed from 18 to 20 weeks of age, the age at 5% hen-day egg production was not delayed. Later photo-stimulation, however, delayed the age at 50% egg production in Hy-line W36 and 1940s with 7 days and 8 days, respectfully. Shi *et al*. (2019) reported a similar result with photo-stimulation at 16 and 22 weeks of age, delaying the age at 50% of hen-day egg production; others have reported a longer delay in the onset of egg lay because of delayed photo-stimulation.

Changes in feed intake, body weight, vitelline membrane strength, and skeletal parameters were affected by age at photo-stimulation. In the current study, the body weight of PS20 at sexual maturity was greater than PS15. Similarly, Pishnamazi et al. (2014) reported that the PS23 were heavier at sexual maturity than PS17. Photo-stimulation stimulates the hypothalamic-pituitary-gonadal axis to release more estrogen toward the formation and development of the ovary, oviduct, and medullary bones. Therefore, it was not a surprise that the plasma estradiol level of both PS15 and PS20 increased sharply after photo-stimulation. Shi *et al.* (2019) had similar findings in which serum estradiol levels increased from 0 – 6 weeks after photo-stimulation at 16, 18, 20, and 22 weeks of age. Delay in photo-stimulation did

not change hen-day egg production. However, unlike the results of our study, a decrease in egg production has been reported when photo-stimulation was delayed to 22 weeks of age compared to 16 weeks of age (Shi et al., 2019). The authors also reported increased incidences of broken eggs and abnormal eggs from hens that were photo-stimulated at 16 weeks of age than 22 weeks of age (Shi et al., 2019). Age at photo-stimulation did not alter egg quality properties either except vitelline membrane strength. Silversides *et al.* (2006) reported that

later photo-stimulation resulted in heavier eggs. The differences between the studies could have been a result of the age and body weight of the birds when the photo-stimulation occurred, and the extent of delay achieved before the onset of lay.

The present study demonstrated that age at photo-stimulation affects skeletal properties which may be dependent on the strain of the hens. In this study, early photo- stimulation increased the diameter of the internal surface of the cortex as well as the phosphorus content in circulation. The phosphorus content of the bones was greater in PS20 birds from 1940s line only. In a study conducted in laying ducks, Cui *et al*. (2019) did not observe any changes on bone ash phosphorus content with different photoperiods. However, in their study the photoperiod treatment was applied when the ducks were already into lay. The result of bone calcium content was interesting as the effect of photo-stimulation was opposite in Hy-line W36 and 1940s leghorns. In 1940s leghorns, delayed the photo- stimulation (and the onset of egg-lay) resulted in

greater calcium content of the bones, unlike the modern hybrid. The changes observed in bone mineral composition between the treatments were reflected in the mechanical strength of the femur. Delayed photo-stimulation resulted in the femur with greater strength but only in the 1940s birds. The exact mechanism for these changes was unclear. In our study, the parathyroid plasma level of PS15 and PS20 increased sharply to 24 weeks of age, indicating that hens demanded more calcium from the bone to support the eggshell while achieving peak production. The parathyroid hormone transfers calcium from the bones to the shell gland when the eggshell requires more calcium for eggshell formation.

In the current study and as we expected, egg weight, shell strength, Haugh unit were greater in Hy-line W36 hens. Similarly, Hy-line W36 had greater albumen height compared to 1940s. Silversides *et al.* (2006) reported strain differences in the albumen height of commercial brown layers. The 1940s consumed less feed compared to Hy-line W36 regardless of the age at photo-stimulation. The body weight at sexual maturity of Hy-line W36 was significantly greater than that of 1940 hens. The higher egg production and heavier eggs of Hy-line W36 might be because of the higher body weight than in the 1940s. Since the Hy- line W36 is a modern hybrid that is genetically more developed than 1940s, they reached the body weight for sexual maturity earlier than 1940s. In this study, we found that some aspects of the skeletal properties of the 1940s were better than Hy-line W36. The 1940s showed a wider cortical diameter and increased cortical thickness of tibia than Hy-line W36

CONCLUSIONS

This study showed delayed photo-stimulation for five weeks, delayed the age at 50%, and 90% egg production for 5 and 7 days, respectively. A delay in the onset of egg production was expected to be beneficial for hens to use the bone's minerals for eggshell formation later in their laying cycle. However, we found that delay in photo-stimulation did not significantly improve the structural properties of the bones in modern layers, whereas some positive traits were observed in the 1940s birds. We believe that a delay in the age at 50% and 90% hen-day egg production was not enough to bring major changes in the skeletal properties of contemporary layers. Additionally, our results indicate that the significant differences were between the two strains of hens rather than the two different photo-stimulation regimens. The commercial strain of hens (W36) had significantly better performance in their egg production, quality characteristics of the eggs, and bone quality than 1940s leghorn.

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TABLES AND FIGURES

Table 2.1 Pullets assigned in two separate rooms at 12 weeks of age to receive different lighting regimens (Room# 1- Photo-stimulation at 15 weeks of age)

Room # 1	Photo-stimulation at 15 weeks of age			
Total	24 Cages/Room	2 Rep/Strain	6 Cages/Rep	61 Hens
Hy-line W36	2 Reps@ 72 in^2 /Hen	3 Hens/Cage	18 Hens/Rep	36 Hens
1940 leghorn	2 Reps@ 72 in^2 /Hen	3 Hens/Cage	18 Hens/1st.Rep. 7 Hens/2nd.Rep	25 Hens

Table 2.2 Pullets assigned in two separate rooms at 12 weeks of age to receive different lighting regimens (Room# 2- Photo-stimulation at 20 weeks of age)

Room # 2	Photo-stimulation at 20 weeks of age			
Total	24 Cages/Room	2 Rep/Strain	6 Cages/Rep	72 Hens
Hy-line W36	2 Reps@ 72 in /Hen	3 Hens/Cage	18 Hens/Rep	36 Hens
1940 leghorn	2 Reps@ 72 in^2 /Hen	3 Hens/Cage	18 Hens/Rep	36 Hens

Table 2.3 Feeding program of diets and formulation according to the age of the hens

	STARTER (0 – 6 wks. of age)	GROWER (6 –12wks.of age)	DEVELOPER (12 – 17 Wks. of age)	LAYER (18 –34 Wks. of age)
Metabolizable energy, kcal/kg	2977– 3087	2930– 3087	2880- 3050	2844 -2955
Lysine, %	0.98 / 1.07	0.88 / 0.96	0.76/0.83	0.84
Methionine, %	0.44 / 0.47	0.40 / 0.44	0.36/0.38	0.44
Methionine+ Cysteine, %	0.74 / 0.83	0.67 / 0.75	0.59/0.67	0.77
Threonine, %	0.66 / 0.77	0.60 / 0.70	0.52/0.62	0.59
Tryptophan, %	0.18 / 0.21	0.17 / 0.20	0.15/0.18	0.18
Arginine, %	1.05 / 1.13	0.94 / 1.01	0.81/0.87	0.90
Isoleucine, %	0.71 / 0.76	0.65 / 0.70	0.57/0.61	0.67
Valine, %	0.73 / 0.80	0.69 / 0.76	0.61/0.67	0.74
Crude protein, %	18.25	17.50	16.00	17.58
Calcium, %	1.00	1.00	1.00	4.37
Phosphorus, %	0.49	0.47	0.45	0.51
Sodium, %	0.17	0.17	0.18	0.19
Chloride, %	0.17	0.17	0.18	0.19
Linoleic acid %	1.20	1.20	1.20	2.11
Choline, mg/kg	1,800	1,800	1,500	1,895

	Growing period	Laying period
Vitamin A, (IU)	10,000,000	8,000,000
Vitamin D3, (IU)	3,300,000	3,300,000
Vitamin E, g	25	20
Vitamin K (menadione), g	3.5	2.5
Thiamin (B1), g	2.2	2.5
Riboflavin (B2), g	6.6	5.5
Niacin (B3), g	40	30
Pantothenic acid (B5), g	10	8
Pyridoxine (B6), g	4.5	4
Biotin (B7), mg	100	75
Folic acid (B9), g	1	0.9
Cobalamin (B12), mg	23	23
Manganese, g	90	90
Zinc, g	85	80
Iron, g	30	40
Copper, g	15	8
Iodine, g	1.5	1.2

Table 2.4 Results of feed analysis of four different feeding phases after the experiment

	Period (weeks of age)			
	Starter	Grower	Developer	Layer
Nutrient	0 – 6	6 - 12	12 - 18	18 – 34
Crude protein (%)	21.80	21.54	17.50	18.21
Calcium (%)	1.15	1.11	2.34	0.70
Phosphorus (%)	0.67	0.72	0.82	0.67
Gross energy (Kcal/kg)	3,756	4,466	4,014	4,350
Ash (%)	5.88	5.37	8.61	11.31
Dry matter (%)	87.34	87.12	88.88	89.2 0

Table 2.5 Lighting schedule of two different programs of photo-stimulation (PS15 and PS20)

Age	Light Intensity (lux)	_PS15 (Hours)	PS20 (Hours)
Day 1	10 Foot – Candle (ft-c.) :(100 lux)	24	24
Day 3	1 ft-c. (10 lux)	23	23
Week 1	1 to 0.5 ft-c. (10 to 5 lux)	22	22
Week 2	1 to 0.5 ft-c. (10 to 5 lux)	20	20
Day 17	1 to 0.5 ft-c. (10 to 5 lux)	18	20
Day 20	1 to 0.5 ft-c. (10 to 5 lux)	16	16
Day 23	1 to 0.5 ft-c. (10 to 5 lux)	14	14
Day 26	1 to 0.5 ft-c. (10 to 5 lux)	12	12
Week 4	1 to 0.5 ft-c. (10 to 5 lux)	10	10
Week 5	1 to 0.5 ft-c. (10 to 5 lux)	10	10
Week 6	1 to 0.5 ft-c. (10 to 5 lux)	10	10
Week 7	1 to 0.5 ft-c. (10 to 5 lux)	10	10
Week 8	1 to 0.5 ft-c. (10 to 5 lux)	10	10
Week 9 to 15	1 to 0.5 ft-c. (10 to 5 lux)	8	-
Week 9 to 20	1 to 0.5 ft-c. (10 to 5 lux)	-	8
Week 16	1 to 0.5 ft-c. (10 to 5 lux)	10	8
Week 17	1 to 0.5 ft-c. (10 to 5 lux)	10	8
Week 18	1 to 0.5 ft-c. (10 to 5 lux)	11	8

Week 19	1 to 0.5 ft-c. (10 to 5 lux)	12	8
Week 20	1 to 0.5 ft-c. (10 to 5 lux)	13	10
Week 21	1 to 0.5 ft-c. (10 to 5 lux)	13.30	11
Week 22	1 to 0.5 ft-c. (10 to 5 lux)	14	12
Week 23	1 to 0.5 ft-c. (10 to 5 lux)	14.30	13
Week 24	1 to 0.5 ft-c. (10 to 5 lux)	15	13.30
Week 25	1 to 0.5 ft-c. (10 to 5 lux)	15.30	14
Week 26	1 to 0.5 ft-c. (10 to 5 lux)	15.55	14.30
Week 27	1 to 0.5 ft-c. (10 to 5 lux)	16	15
Week 28	1 to 0.5 ft-c. (10 to 5 lux)	16	15.30
Week 29	1 to 0.5 ft-c. (10 to 5 lux)	16	15.55
Week 30-34	1 to 0.5 ft-c. (10 to 5 lux)	16	16

Table 2.6 The effect of age at photo-stimulation and strain of the hen's total average body weight (g), the body weight at 5% egg production, and the average feed intake

	Age at photo-stimulation (wk.)				The strain of the hens			
	15	20	SEM	*P*-value	W36	1940	SEM	*P*-value
Total body weight (kg)	1.057[b]	1.111[a]	11.71	0.021	1153[a]	1016[b]	11.71	<.0001
Body weight at 5% egg production	998.4[b]	1200.6[a]	10.82	<.0001	1160.8[a]	1038.2[b]	12.78	<.0001
Feed Intake (g/hen/day)	54.67[a]	47.30[b]	0.25	<.0001	51.76[a]	51.90[a]	0.34	0.84

[a-b] Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.7 Effect of age at photo-stimulation and strain of the pullets on egg performance

	Age at photo-stimulation (wk.)						
	15		20			*P*-value	
The strain of the hens	W36	1940	W36	1940	SEM	Treatment	Strain
Egg production (%)	76.79^{a}	56.75^{bc}	68.17^{ab}	50.52^{c}	1.88	0.05	<0.0001
Egg weight (g)	56.45^{a}	36.34^{b}	56.75^{a}	36.21^{b}	0.21	0.85	<0.0001
Haugh unit	93.78^{a}	86.44^{b}	95.37^{a}	84.86^{b}	0.37	0.99	<0.0001
Albumen height (mm)	8.78^{a}	6.15^{b}	9.12^{a}	6.15^{b}	0.06	0.70	<0.0001
Vitelline strength (g of force to puncture)	1.74^{b}	1.64^{b}	1.97^{a}	1.72^{b}	0.02	0.004	0.001
Shell strength (kg of force to crack)	4.44^{a}	3.91^{b}	4.79^{a}	3.84^{b}	0.6	0.25	<0.0001

$^{a\text{-}c}$ Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.8 Effect of different ages at photo-stimulation at the age of 5%, 50%, and 90% egg production of Hy-line W36

	Age at photo-stimulation (wk.)			
	15	20	SEM	P-value
Age at 5% egg production (D)[1]	147.4[b]	151[a]	0.74	0.03
Age at 50% egg production (D)	157[b]	164[a]	0.57	<0.0001
Age at 90% egg production (D)	171[a]	171[a]	0.00	1.0

[a-b] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Day of age at egg production.

Table 2.9 Effect of different ages at photo-stimulation at the age of 5%, 50%, and 90% egg production of 1940s

	Age at photo-stimulation (wk.)			
	15	20	SEM	P-value
Age at 5% egg production (D)	159[b]	170[a]	0.57	<0.0001
Age at 50% egg production (D)	171[b]	179[a]	0.57	<0.0001
Age at 90% egg production (D)	199[b]	215[a]	0.57	<0.0001

[a-b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 2.10 Effect of age at photo-stimulation and different strains of the henson the strength of the bones (newton force)

The strain of the hens	W36				1940			
Age at photo-stimulation (wk.)	15	20	SEM	P-value	15	20	SEM	P-value
Tibia	148.37 [a]	149.45[a]	1.96	0.79	145.92[a]	152.10[a]	2.35	0.19
Humerus	98.94 [a]	106.20[a]	3.33	0.28	124.54[a]	137.39[a]	4.02	0.11
Femur	161.22[a]	146.02[b]	3.72	0.047	157.09[b]	160.14[a]	3.04	0.037

[a-b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 2.11 Effect of age at photo-stimulation on the thickness of the outer and internal portion of the tibia cortex

	Age at photo-stimulation						
	15		20				
	W36	1940	W36	1940	SEM	Treatment	Strain
A-P[1] outer dimension (mm)	5.29[c]	5.47[ab]	5.36[bc]	5.53[a]	0.02	0.16	0.0001
M-L[2] outer dimension (mm)	6.29[a]	6.39[a]	6.27[a]	6.41[a]	0.03	0.91	0.05
Anterior (mm)	0.72[a]	0.71[a]	0.67[a]	0.61[a]	0.01	0.017	0.21
Posterior (mm)	0.64[b]	0.74[a]	0.62[b]	0.63[b]	0.009	0.0004	0.001
Medial (mm)	0.70[ab]	0.76[a]	0.68[b]	0.65[b]	0.01	0.0009	0.37
Lateral (mm)	0.82[a]	0.76[a]	0.77[a]	0.65[b]	0.01	<0.0001	<0.0001

[a-c] Value within a row with different superscripts differ significantly at $P < 0.05$.
[1]A-P (Antero-posterior)
[2]M-L (Medio-Lateral)

Table 2.12 Effect of age at photo-stimulation and two strains of the hens on bones' calcium and phosphorus contents.

The strain of the hens	W36				1940			
Age at photo-stimulation (wk.)	15	20	SEM	P-value	15	20	SEM	P-value
Calcium (mg/g)	402.7[a]	399.70[b]	1.53	0.0449	400.07[b]	406.3[a]	1.34	0.021
Phosphorus (mg/dl)	188.27[a]	189.17[a]	1.0	0.65	187.46[b]	191.27[a]	0.76	0.014

a-b Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.13 Effect of age at photo-stimulation on bone ash percentage in two strains of the hens

	Age at photo-stimulation (wk.)				The strain of the hen			
	15	20	SEM	P-value	W36	1940	SEM	P-value
Tibia	41.57[a]	40.70[a]	2.23	0.84	42.25[a]	41.63[b]	2.01	0.87
Humerus	42.95[a]	42.26[a]	1.25	0.88	45.48[a]	40.28[b]	1.03	0.025
Femur	30.80[a]	30.21[a]	0.86	0.73	30.83[a]	30.19[a]	0.86	0.71
Keel	28.84[a]	28.32[a]	1.67	0.87	28.62[a]	28.54[a]	1.67	0.98

a-b Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.14 Effect of age at photo-stimulation on plasma estradiol hormone at different of hen's age

	Age at photo-stimulation (wk.)			
Hen's age (wk.)	15	20	SEM	P-value
12	277.22[b]	436.56[a]	62.48	0.026
15	158.57[b]	405.94[a]	62.48	0.001
20	660.10[a]	276.69[b]	61.15	<0.0001
24	496.56[a]	529.74[a]	62.48	0.50
32	818.39[a]	663.94[b]	65.02	<0.0001

a-b Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.15 Effect of age at photo-stimulation on plasma parathyroid hormone atdifferent of hen's age

	Age at photo-stimulation (wk.)			
Hen's age(wk.)	15	20	SEM	P-value
12	11.43[a]	11.92[a]	1.03	0.96
15	9.59[a]	8.98[a]	1.03	0.38
20	6.93[a]	6.73[a]	1.18	0.61
24	10.19[a]	12.66[a]	1.03	0.29
32	9.13[a]	10.26[a]	1.06	0.75

a-b Values within a row with different superscripts differ significantly at $p < 0.05$.

Figure 2.1 Effect of age at photo-stimulation on body weight of two strains of hens(W36 and 1940s) at different ages. Data are presented as mean ± SEM

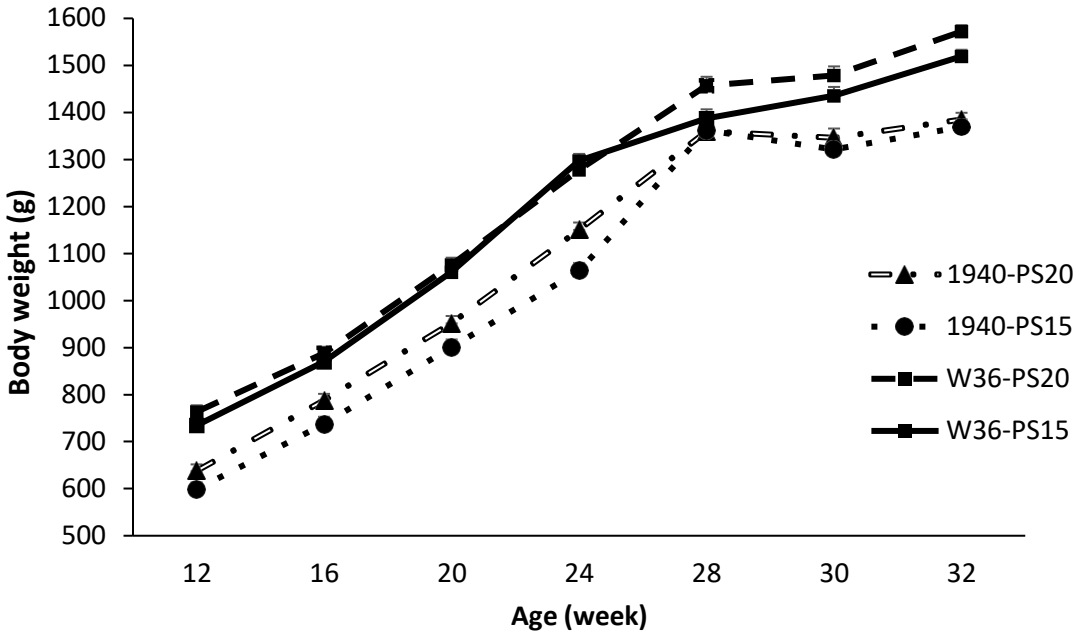


Figure 2.2 Effect of age at photo-stimulation on hen-egg production in two strains of hens(W36 and1940s) at different ages. Data are presented as mean ± SEM

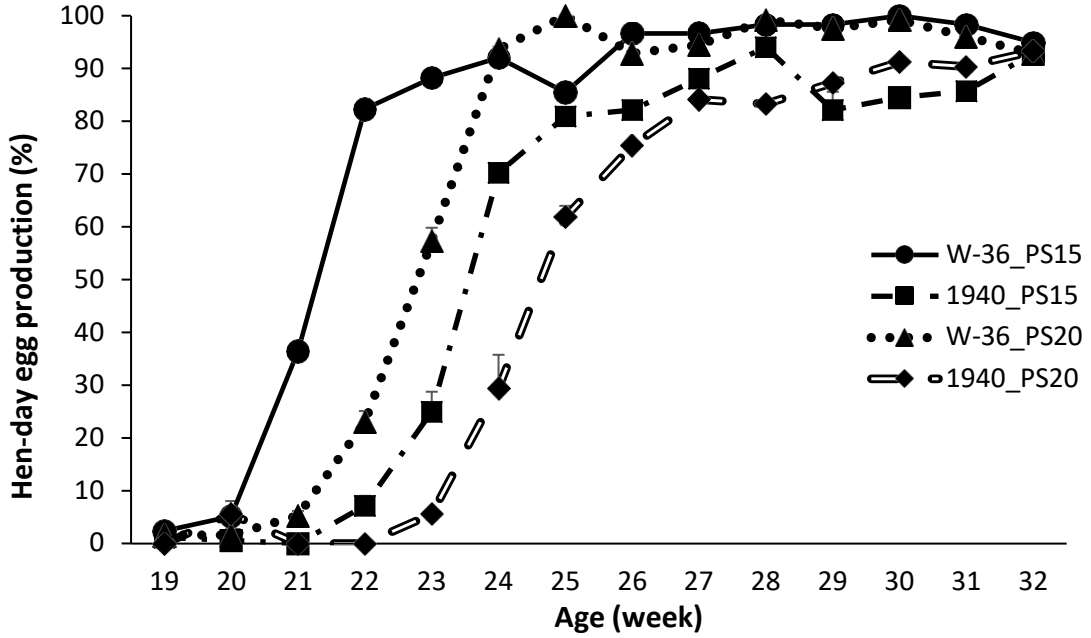


Figure 2.3 Effect of age at photo-stimulation on length of the bones (keel, tibia, femur, humerus). Data are presented as mean ± SEM

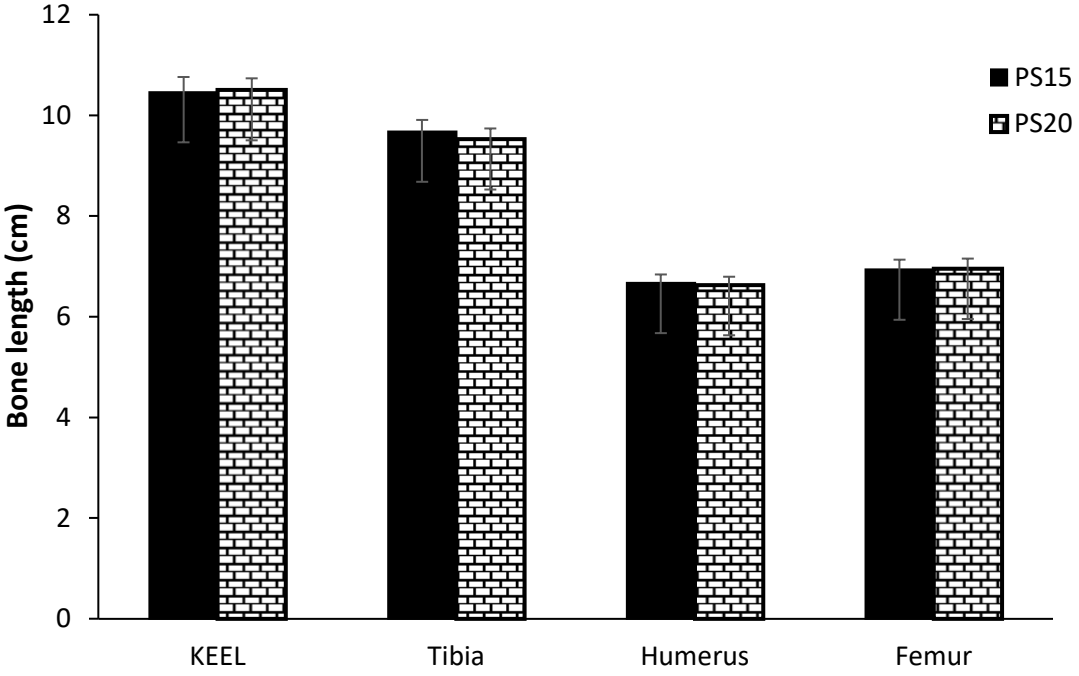


Figure 2.4 Length of the bones (keel, tibia, femur, humerus) in two strains of hens (W36 and 1940s). Data are presented as mean ± SEM

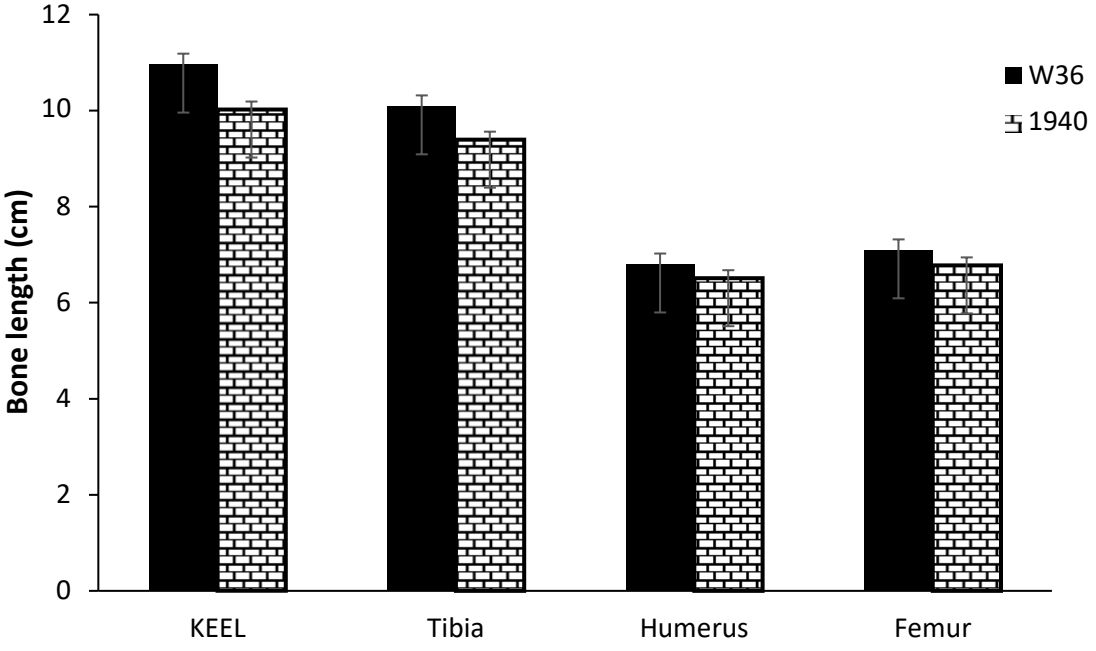


Figure 2.5 Effect of photo-stimulation (PS15 and PS20) on plasma estradiol hormone in two strains of the hen in different ages. Data are presented as mean ± SEM

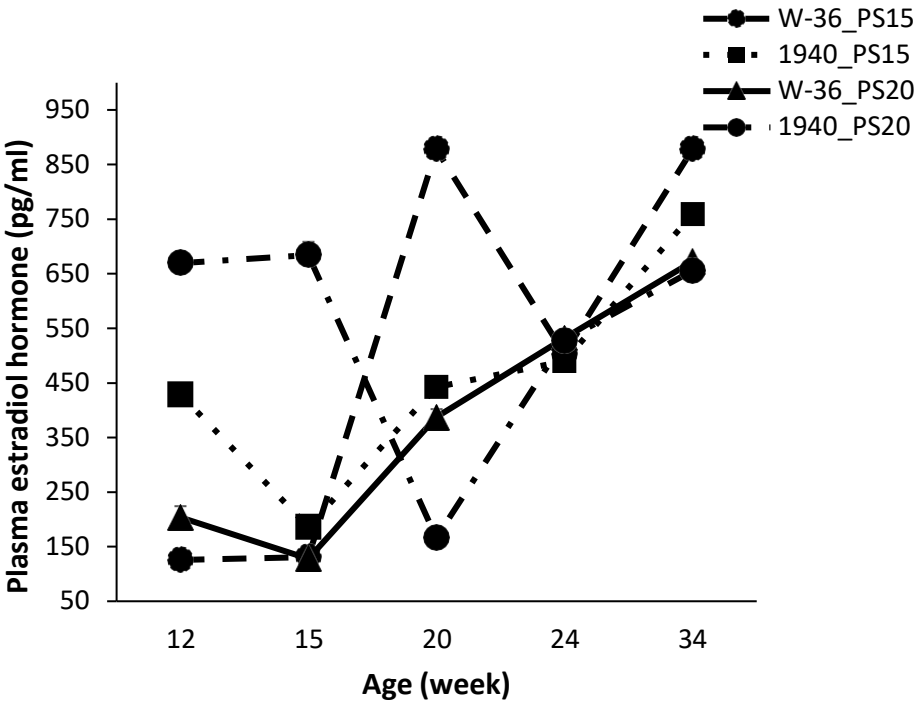


Figure 2.6 Effect of photo-stimulation (PS15 and PS20) on plasma parathyroid hormone in two strains of the hen in different ages. Data are presented as mean ± SEM

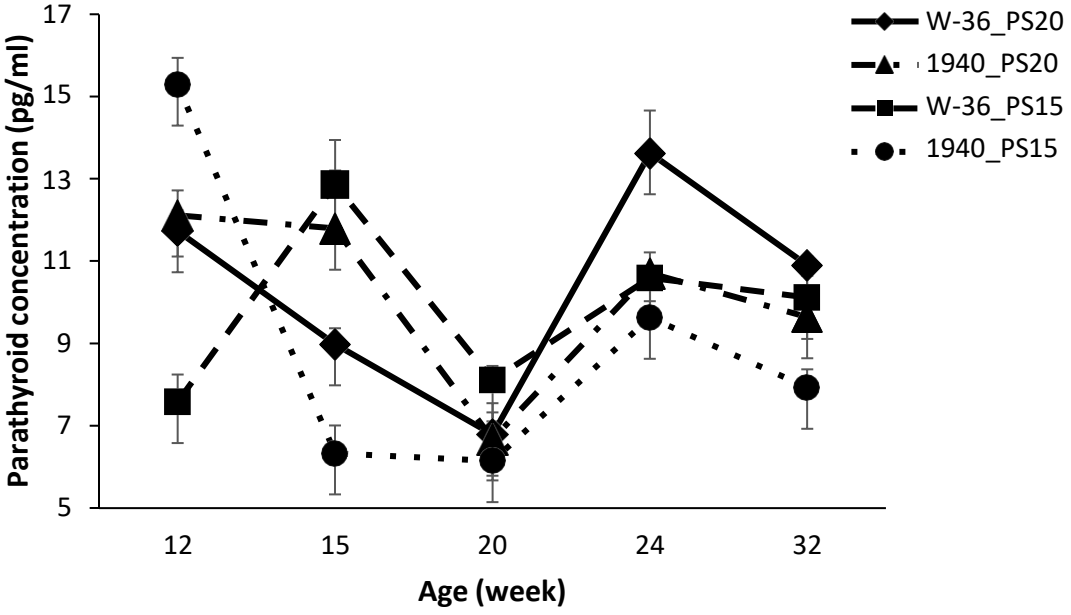


Figure 2.7 Effect of photo-stimulation (PS15 and PS20) on serum calcium concentration (mmol/L) in two strains of the hen in different ages. Data are presented as mean ± SEM

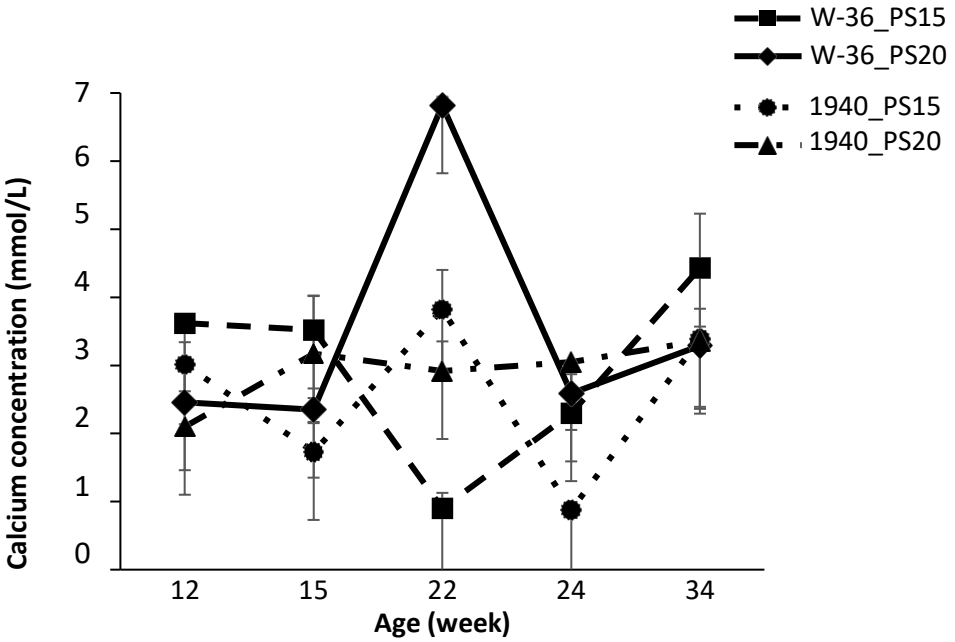
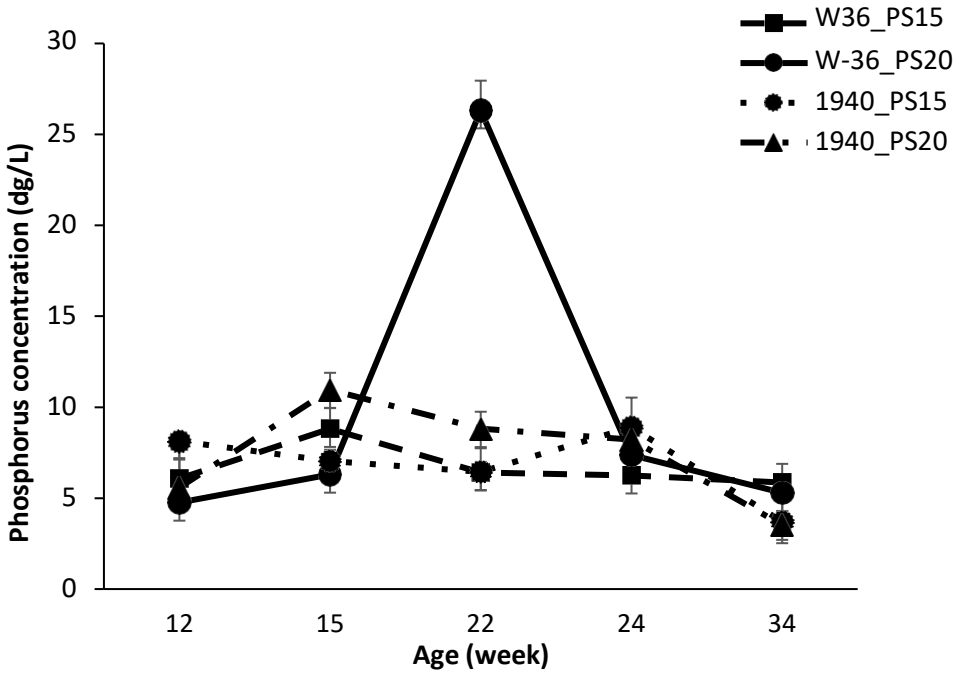


Figure 2.8 Effect of photo-stimulation (PS15 and PS20) on serum phosphorus concentration (dg/L) in two strains of the hen in different ages. Data are presented as mean ± SEM



FINAL CONCLUSIONS

The modern housing system, such as battery cages, was developed to reduce labor costs and increase the flock size to increase the production rate within smaller housing spaces compared to the cage-free housing system. Limited space per hen in this system restricted hens to exercise, spread their wings, and perform nesting behavior (Fleming and Whitehead, 2000), resulting in brittle bones that would cause bone breakage at the end of the laying cycle (Fleming et al., 1994). Due to these welfare issues, most food-service retailers, chain restaurants, grocers are pledged to buy only cage-free eggs by 2025. Based on our results, the feed conversion ratio was better in the caged-hens than in the cage-free hens; this means that cage-free hens demand more feed for their maintenance and production. As a result, the producers need to feed the cage-free hens, which eventually increases the producers' feed cost, and the price of the eggs will increase. Therefore, the transition of hens from cage to cage-free would increase a significant investment. Besides those factors mentioned earlier, microbial contamination of the eggs and the higher potential of infectious diseases such as coccidiosis, bacteria, and fungi in cage-free systems need to be considered. Studies show that egg production and quality of the eggs tend to be better in the cage system and have fewer egg losses than in the cage-free system. Therefore, we need to consider the cost of the products that should be affordable for the customers and find ways to improve the welfare of the caged hens by some management approach. In this study, we compared the

energy requirements of hens housed in cages and cage-free housing systems. In my first study, I investigated whether the hens in the cage-free system require more energy due to their activities for their productive performance compared to caged-hens or not for egg production, egg quality, and feed conversion ratio. The results indicated that cage-free hens consumed significantly higher (9%) more feed with (10%) higher feed conversion ratio than caged-hens to produce (3%) more eggs than the caged-hens.

In the second experiment, I manipulated the age at photo-stimulation to delay sexual maturity and reduce the broken bones later in the laying cycle. The objective of this study was to evaluate the productive performance characteristics (hen-day egg production, egg quality) and the bone quality of two strains of hens in two different ages at photo-stimulation (photo-stimulation at 15 weeks of age, photo-stimulation at 20 weeks of age). This study aimed to investigate if photo-stimulation at 20 weeks of age (PS20) can increase the strength and quality of the bones by postponing the sexual maturity of this group compared to those photo-stimulated at 15 weeks of age (PS15). This study indicated that the age at 5%, 50%, and 90% egg production was delayed in the PS20 group for both strains of the hens, and only the age at 90% hen-day egg production of Hy-line W36 was not affected by the delay in photo-stimulation.

In comparison, the quality characteristics of the egg did not change, except for the vitelline strength improvement in the PS20 hens. Also, the

bone's length was not affected by different ages at photo-stimulation, which indicates the growth rate of hens in PS20 was not delayed by delay in photo-stimulation. A longer study time for this experiment (more than 34 weeks of hen's age) can be a practical way to see the differences in bone quality properties in two different lighting regimens.