Effect of housing environment and hen strain on egg production and egg quality as well as cloacal and eggshell microbiology in laying hens

Milan K. Sharma,[*] Christopher D. McDaniel,[*] Aaron S. Kiess,[*] Robert E. Loar II[†] and Pratima Adhikari[*],[1]

[*]*Department of Poultry Science, Mississippi State University, Mississippi State, MS, USA; and* [†]*Cal-Maine Foods Inc., Jackson, MS, USA*

ABSTRACT This study was conducted to determine the effect of housing environment and laying hen strain on performance, egg quality, and microbiology of the cloaca and eggshell. A total of 1,152 Hy-Line Brown (**HB**) and Hy-Line W-36 White Leghorn (**W-36**) hens were used. All hens were kept in conventional cages (**CC**) from 18 to 32 wk of age and then moved to either enriched colony cages (**EC**) or free-range (**FR**) pens or continued in CC. Hens were randomly allocated into a 2 × 3 factorial arrangement of 2 laying hen strains (brown and white) and 3 housing environments (CC, EC, and FR) in a split plot in time (hen age) design. The experiment was conducted from 32 to 85 wk of age. The experiment was divided into 2 phases: early phase (32 −51 wk of age) and late phase (52−85 wk of age). A 3-way interaction was observed for hen day egg production (**HDEP**) among housing environments, hen strain, and bird age in the early phase ($P = 0.004$) as well as in the late phase ($P < 0.0001$). In both of the phases, HDEP was higher in CC and FR than in EC. Hy-Line W-36 hens raised in EC had the lowest HDEP compared to other treatments. A 3-way interaction was observed for feed intake (**FI**; $P = 0.017$) and feed conversion ratio (**FCR**) in the late phase ($P < 0.0001$). The lowest FI and highest FCR were observed in EC for W-36 hens. Free-range hens performed in-between for eggshell quality when compared to CC and EC while HB had better egg quality than W-36. Free-range hens had higher cloacal bacterial counts for aerobes, anaerobes, and coliforms than CC and EC. Higher eggshell bacterial contamination was observed in eggs from FR versus eggs from CC and EC. These results indicate that both housing environment and laying hen strain affect performance and egg quality as well as cloacal and eggshell microbiology. Further studies should be conducted to determine food safety and economic impacts when using different hen strains and housing environments.

Key words: egg production, egg quality, eggshell and cloacal microbiology, housing environment, laying hen strain

INTRODUCTION

Over the past 60 yr, the egg industry has changed from small to large scale, producing a greater number of eggs with increased integration from free-range or semi-intensive to intensive farming. During that period, several studies have been conducted regarding housing environment, and those studies have explored several aspects of production performance, food safety, bird health, and management. Conventional cages (**CC**) have been extensively used in the United States laying hen industry since the 1960s, and research in the fields of genetics and breeding has led to the development of new hen strains that were capable of exhibiting optimal performance in that environment (Brambell, 1965). Although there are benefits to CC such as a reduction in labor with greater automaton, improvement in bird health, and food safety, concerns have arisen for potentially compromising the laying hen's welfare for increased productivity (Brambell, 1965; Mench et al., 2011).

Consumer emphasis on the welfare of laying hens and eggs produced from hens housed in a welfare-friendly production system with more space per hen has influenced the United States egg industry to explore alternative housing environments. Currently, consumer demands are oriented toward healthy eggs that do not have any food safety concerns and are produced under the enhanced welfare standards of laying hens (Ferrante et al., 2009). With increasing concerns regarding the welfare of the laying hens, the European Union

banned the use of CC (European Union, 1999), which has led to the development of alternative housing systems such as aviaries, enriched colony cages, or free-range systems. The increasing demand for eggs from alternative housing environments may be due to the perception that healthy eggs are produced by hens raised in alternative housing environments with minimal stress (Miao et al., 2005). Previous findings indicated that many US consumers are concerned about the welfare of animals, and around 59% are willing to pay more for animal products from welfare-friendly systems (Spain et al., 2018; Lusk, 2019; Ochs et al., 2019). As a result, major United States retailers and food manufacturers have pledged to purchase only cage-free eggs by 2025 (UEP, 2017). In an effort to meet this demand, producers have started to modify conventional cage systems to comply with alternative housing environments. This transition from traditional CC to alternative housing environments may impact the production performance as the housing environment is an external factor that influences the production and quality of eggs (Mench et al., 2011). Several studies have shown that the hen day egg production (HDEP) was higher from layers raised in the CC than the alternative housing systems such as aviary, litter, free-range or organic (Tauson, 1999; Leyendecker et al., 2001; Van Den Brand et al., 2004; Singh et al., 2009; Küçükyilmaz et al., 2012). However, the results for the hen day egg production from their studies were contradictory to each other. Pohle and Cheng (2009) reported higher production in the EC in comparison to CC; however, Yilmaz Dikmen et al. (2016) reported higher production in the CF system as compared to the CC and EC.

Apart from that, different laying hen strains could behave differently under different management conditions in alternative housing environments. Previous studies by Tauson et al. (1999) and Singh et al. (2009) observed the differences between laying hen strains for HDEP and egg quality parameters. Therefore, it is necessary to understand how alternative housing environments can be used to meet the maximum genetic potential of a specific hen strain. Therefore, production performance indices such as egg production, feed intake, feed conversion ratio, egg quality, and hen livability need to be investigated before shifting the production system completely to an alternative housing environment.

In the United States, more than 75% of table eggs are produced by birds housed in CC. Concurrently, the eggs from CC are considered superior in hygienic standards with less bacterial contamination (Jones and Anderson, 2013; Englmaierová et al., 2014; Galvão et al., 2018). Due to the dynamic nature of the microbes, both housing environment and laying hen strain might influence microbial growth on the eggshell (Holt et al., 2011), which is related to food safety in the egg industry. Previous studies have shown that eggs from alternative housing systems such as aviaries, litter, free-range or organic systems have a higher eggshell bacterial load than those from conventional cages (De Reu et al., 2005;

Buhr et al., 2009; Singh et al., 2009; Holt et al., 2011; Englmaierová et al., 2014; Jones et al., 2015a; Galvão et al., 2018; Vlčková et al., 2018). Similarly, Jones and Anderson (2013) observed that eggshell microbial load was affected by laying hen strains in different housing environments where the eggshell aerobic bacterial load was different between 2 laying hen strains, Hy-Line Brown and Hy-line silver Brown raised in CC. It has also been observed that eggshell bacterial contamination at the time of collection affects the final products causing foodborne illness (Petrak et al., 1999). Additionally, the total number of microorganisms penetrating the eggshell into the shell membrane and albumen were observed to be higher in FR compared to EC (Vlčková et al., 2018). The cloaca, which is a common opening for a bird's digestive, urinary and reproductive tract, may harbor different pathogenic bacteria such as *Salmonella* Spp., *Staphylococci* spp., *Escherichia Coli, or Campylobacter spp.* These bacteria may contaminate the eggshell during egg passage through the cloaca (Pesavento et al., 2017). Therefore, in addition to performance, bacterial load in the cloaca and on the eggshells from different housing environments for different laying hen strains needs to be compared.

Most of the studies regarding alternative housing systems were conducted in Europe and very few studies have been done in the United States using aviaries as a cage-free system. However, European results regarding performance and eggshell quality as well as cloacal microbiology might not be relevant in the United States because of environmental and strain variation. Therefore, the objectives of the present study were to evaluate performance, egg quality, and microbiological load of the cloaca and eggshell for 2 laying hen strains (Hy-Line Brown [**HB**] and Hy-Line W-36 White Leghorn [**W-36**]), housed in 3 housing environments (conventional cage [**CC**], enriched colony cage [**EC**] and free-range [**FR**]) in the United States.

MATERIAL AND METHODS

Birds, Diets, and Management

The experiment was conducted at the Poultry Research Unit at Mississippi State University (Mississippi State, MS) utilizing the limited resources of the University. The experiment was approved by the Institutional Animal Care and Use Committee of Mississippi State University (IACUC# 17-554). A total of 1,152 laying hens of 2 strains (HB and W-36 at 30 wk of age) were weighed and randomly placed in CC, EC, or FR environments. Hens of both strains were reared until the age of 18 wk in floor pens and then moved to conventional cages until allocated to the respective treatments. Hens were allowed to acclimate to their environments and feed for 2 wk. The trial began when the birds reached 32 wk of age (32−85 wk of age, from July 2018 to July 2019). At the beginning of the trial, birds were weighed and randomly allocated to treatment. The experimental design was completely randomized with a 3 × 2 factorial

arrangement for 3 housing environments (CC, EC, and FR) and 2 laying hen strain (HB and W-36) and a split-plot over hen age.

A total of 192 hens of each strain were kept in the following treatment groups: Hy-Line Brown in conventional cages (**CCHB**), Hy-Line W-36 White Leghorns in conventional cages (**CCW-36**), Hy-Line Brown in enriched colony cages (**ECHB**), Hy-Line W-36 White Leghorns in enriched colony cages (**ECW-36**), Hy-Line Brown in free-range (**FRHB**) and Hy-Line W-36 White Leghorns in free-range (**FRW-36**). In all 3 housing environments, each treatment group consisted of 6 replicates, and each replicate was alternated between the brown and white hens when placed. For the hens in CC, 8 adjacent cages were considered as one replicate, and each cage had 4 hens giving a total of 32 hens. In the EC and FR, each pen was considered a replicate, and there were 32 hens in each pen.

Diets were formulated based on the recommendation of the management guidelines for both Hy-Line Brown and White Leghorns hens according to phase feeding (Hy-Line International, 2016). The feed formulation and composition are shown in Tables 1 and 2. All diets were formulated to meet or exceed the nutritional recommendation for both HB and W-36 hens. The ratio of coarse to fine calcium was changed with the phases. In all the housing environments, birds were fed manually, and water was provided via nipple drinkers. All housing environments followed the lighting regime of 16L:8D, and the temperature was maintained at similar levels throughout the experimental period between housing environments. Feed and water were provided ad libitum.

Housing Environments

Conventional cage, EC, and FR houses were located at the Poultry Research Unit at Mississippi State University, where the FR was located approximately 250 m away from the CC and EC housing environments. Conventional and EC cages were installed within the same open-sided layer house. The CC was in a 3-tier A-frame arrangement with manure shields, and the EC was in a 2-tier arrangement with manure belts under each tier. Both CC and EC consisted of galvanized wire cages with a galvanized trough-type feeder. The feeder space in the CC was 15 cm/bird, whereas the feeder space in the EC was 22.5 cm/bird. The CC contained 2 nipple drinkers per cage, and the EC had 8 nipple drinkers per pen. The floor space in CC was 772 cm^2/bird, whereas it was 1505 cm^2/bird in the EC. The EC was also installed with a dark nesting area covered by non-transparent plastic curtains, perches running parallel to the cage with of 22.5 cm/bird of perching space, and a scratchpad.

The free-range system had an indoor and an open outdoor area (range), and all birds had access to the range. The indoor area was equally divided into 12 pens, with each pen having an area of 5.57 m^2. The outdoor range was also equally divided into 12 pens, with each pen having an area of 11.6 m^2. The indoor and outdoor areas of the pen were secured with galvanized wire so that pens were separated from each other. The indoor area and outdoor range of a pen were connected via a window. The litter material for the indoor floor area was pine shavings. One circular plastic feeder with a feeder space of 3.5 cm/bird and 3 nipple drinkers were provided in the indoor area. Two wooden perches (18.5 cm/bird) and a 2-tier nest box (each tier containing 5 nest boxes (30.5 cm × 30.5 cm × 30.5 cm)) were fitted in the indoor area of each pen. A total of 1,742 cm^2 of floor space/bird was provided in the indoor pen, and 3,484 cm^2 of floor space/bird was provided in the range. No pasture was maintained in the outdoor range for consumption except for natural vegetation. The windows were opened at least 7 h per d, giving hens access to the outdoor range throughout the experimental period.

Table 1. Composition and nutrient content of Hy-Line W-36 diets from 30 to 85 wk of age.

Ingredients	30−37 wk	38−48 wk	49−62 wk	63−76 wk	77−85 wk
Corn	50.02	61.20	57.00	57.00	56.00
Soybean meal	27.00	20.49	21.79	21.79	20.00
Limestone	10.40	10.40	10.39	11.06	12.85
Distiller's dried grains with solubles	3.00	3.00	5.00	5.00	4.50
Poultry fat	2.50	2.50	3.25	2.84	3.50
Dicalcium phosphate	1.72	1.72	1.71	1.44	2.20
Salt	0.30	0.30	0.30	0.30	0.40
Vitamin mineral premix[1]	0.26	0.26	0.26	0.26	0.25
DL-Methionine	0.12	0.12	0.23	0.23	0.10
Lysine HCL	0.02	0.02	0.09	0.09	0.09
Calculated composition					
ME (kcal/kg)	2,840.00	2,840.00	2,822.00	2,800.00	2,778.00
Crude protein (%)	16.00	15.50	15.25	15.00	14.75
Calcium (%)	4.10	4.30	4.40	4.60	4.75
Calcium particle size (fine:coarse%)	50:50	45:55	40:60	35:65	35:65
Available P (%)	0.48	0.47	0.45	0.40	0.38
dLys (%)	0.80	0.75	0.71	0.70	0.68
dMet (%)	0.39	0.37	0.35	0.33	0.33
dCys (%)	0.28	0.26	0.25	0.24	0.23

[1]Contains minimum of: Manganese, 4%; zinc, 4%; Iron, 2%; copper, 4,500 ppm; iodine, 600 ppm; selenium, 60 ppm; vit. A, 1,400,000 IU/lb; vit. D3, 500,000 ICU/lb; vit.E, 3,000 IU/lb; vit. B1, 2 mg/lb; menadione, 150 mg/lb; riboflavin, 1,200 mg/lb, D-pantothenic acid, 1,200 mg/lb; niacin, 5,000 mg/lb; choline, 70,000 mg/lb; folic acid, 125 mg/lb; pyridoxine, 250 mg/lb; thiamine, 200 mg/lb; biotin, 6 mg/lb.

Table 2. Composition and nutrient content of Hy-Line Brown diets from 30 to 85 wk of age.

Ingredients	30−37 wk	38−48 wk	49−62 wk	63−76 wk	77−85 wk
Corn	56.79	61.20	57.00	57.00	56.00
Soybean meal	23.00	20.49	22.78	21.79	20.00
Limestone	9.71	10.40	10.21	11.80	12.85
Distiller's dried grains with solubles	2.31	3.00	5.00	5.00	4.50
Poultry fat	5.00	2.50	2.60	3.10	3.90
Dicalcium phosphate	1.80	1.72	1.54	1.44	2.20
Salt	0.30	0.30	0.30	0.30	0.40
Vitamin mineral premix[1]	0.26	0.26	0.26	0.26	0.25
DL-Methionine	0.20	0.12	0.23	0.23	0.10
Lysine HCL	0.20	0.02	0.09	0.09	0.09
Calculated composition					
ME (kcal/kg)	2,970.00	2,925.00	2,925.00	2,830.00	2,830.00
Crude protein (%)	16.00	15.80	15.10	14.60	14.20
Total Calcium (%)	4.00	4.20	4.35	4.50	4.75
Calcium particle size (fine:coarse, %)	50:50	45:55	40:60	35:65	35:65
Available P (%)	0.41	0.38	0.34	0.33	0.31
dLys (%)	0.78	0.76	0.74	0.72	0.71
dMet (%)	0.40	0.38	0.36	0.34	0.33
dCys (%)	0.29	0.27	0.26	0.25	0.24

[1]Contains minimum of: Manganese, 4%; zinc, 4%; iron, 2%; copper, 4,500 ppm; iodine, 600 ppm; selenium, 60 ppm; vit. A, 1,400,000 IU/lb; vit. D3, 500,000 ICU/lb; vit.E, 3,000 IU/lb; vit. B1, 2 mg/lb; menadione, 150 mg/lb; riboflavin, 1,200 mg/lb, D-pantothenic acid, 1,200 mg/lb; niacin, 5,000 mg/lb; choline, 70,000 mg/lb; folic acid, 125 mg/lb; pyridoxine, 250 mg/lb; thiamine, 200 mg/lb; biotin, 6 mg/lb.

Layer Performance and Egg Quality

Egg production and mortality were recorded twice a day for both cages and FR rearing systems. Body weight of the hens was recorded at the beginning of the trial (32 wk) and every 6 wk from 52 wk onward. For body weight measurement, 25% of the hens in each system were weighed as a sample body weight. Hen day egg production (**HDEP**) data was calculated bi-monthly from the total number of eggs laid in 2 wk, divided by the hen days during that period. Feed intake (**FI**) and feed conversion ratio (**FCR**; kg feed per dozen eggs) were calculated by replicate pen at 5 different time points in the early phase (32−51 wk of age). In the late phase (52−85 wk of age), feed offered was weighed at the beginning, and feed retained was weighted at the end of every week to calculate weekly feed consumption. Data for FI and FCR were presented bi-monthly. Hen day egg production, FI, and FCR were adjusted for mortalities throughout the early and late phase experimental period.

Egg quality (external and internal) was analyzed at 6-wk intervals from the beginning until the end of the trial. A total of 216 fresh eggs were collected randomly (6 eggs from each replicate) to measure egg quality parameters. External egg quality was determined by evaluating specific gravity, eggshell breaking strength, eggshell thickness, and eggshell percentage. The specific gravity was measured by dipping the eggs in a prepared saltwater solution with a specific gravity ranging from 1.060 to 1.100 (Peebles and McDaniel, 2004). Eggshell breaking strength was measured using the Instron Universal Testing Machine model 3345 (Instron Inc., Norwood, MA). For the eggshell breaking strength, another 6 eggs per replicate were collected on each sampling day, and the analysis was carried out on the same day at room temperature. Eggshell breaking strength was performed using a constant crosshead speed of 20 mm/min using a 100 N load cell and a 35 mm probe

as a compression device (Clerici et al., 2006; Sharma et al., 2020). Once the egg was compressed with the probe, breaking strength in kilogram-force (**KgF**) was recorded using Bluehill Software (Instron Inc., Norwood, MA). Total egg weight was measured in grams and then the egg was broken onto a flat surface. The eggshell was rinsed with tap water to remove the shell membrane and remnants of the albumen. Eggshells were dried for 2 d at room temperature, and eggshell weight was recorded (grams). Eggshell percentage was calculated by dividing the eggshell weight by egg weight and then multiplying the result by 100. Eggshell thickness was measured without the shell membrane at 3 different areas of the shell (top, equator, and bottom) using the Ames micrometer (B. C. Ames Incorporated, MA) and an average score of the 3 points was calculated (Sharma et al., 2020).

Internal egg quality, albumen height, Haugh unit, albumen percentage, and yolk percentage were measured. Samples for internal egg quality were collected on 2 consecutive days and performed on their respective days of collection. Once the egg was broken onto a flat surface, albumen height was measured using the TSS QCD apparatus (Technical Services and Supplies Ltd, York, England), and later Haugh unit was calculated using the method of Haugh (Haugh, 1937). After measuring the Haugh unit, the yolk was separated from the albumen, and yolk weight was measured (grams). Albumen weight was calculated by subtracting the yolk weight and eggshell weight from egg weight. Albumen percentage and yolk percentage were calculated by dividing their absolute weight by the egg weight.

Cloacal and Eggshell Microbiology

Total aerobic, anaerobic, and coliform bacterial counts were evaluated in cloacal and eggshell samples. Cloacal

samples were collected every 6 wk (8 collection time points total) utilizing the same bird from each replicate pen. At each time point, 36 cloacal swab samples were collected. Each cloacal swab sample was collected using a sterile cotton swab (Puritan Medical Products, Guilford, ME), which was then aseptically placed into a sterile 15 mL empty tube (Fisher Scientific, Hampton, NH) and kept on the ice until further processing. Ten mL of a sterile phosphate buffer saline (PBS, Fischer Scientific, Hampton, NH)) was added to each tube containing the cloacal swab and then vortexed for 30 s. The obtained solution was 10-fold serially diluted and then spread onto the agar plates. For eggshell microbiology, one egg from each replicate pen (total 36 eggs) within each housing environment (CC, EC, and FR) was collected aseptically and placed into a sterile whirl-pak bag (Nasco Whirl-Pak, Fort Atkinson, WI). Fifty mL of sterile PBS solution was added to each bag containing the egg, and each egg sample was massaged for 30 s before diluting (Jones and Anderson, 2013). Each egg sample was 10-fold serially diluted prior to spreading onto the agar plates.

For the cloacal and eggshell samples, 100 μl of the appropriate serial dilution was spread onto each respective agar plate in duplicate (3 dilutions plated in duplicate, 6 total plates per sample). Tryptic Soy agar (TSA, Millipore Sigma, St. Louis, MO) was utilized to obtain total aerobic and total anaerobic bacterial counts. Total coliforms were enumerated using Eosin Methylene Blue agar (EMB, Millipore Sigma, St. Louis, MO). Plates for total anaerobes were placed in anaerobic canisters (Advanced Instruments, Norwood, MA) and subjected to anaerobic conditions (0% O_2, 10% CO_2, 5% H_2, 85% N_2) using an Anoxomat Mark II CTS (Advanced Instruments, Norwood, MA). The anaerobic plates were incubated at 37°C for 48 h. The plates for total aerobes and total coliforms were incubated aerobically at 37°C for 24 h (VWR International, 1535 incubator, Cornelius, OR). After incubation, visible colonies were counted and reported as total aerobic, anaerobic, or coliform microorganisms. The counts were then log transformed prior to the analysis.

Statistical Analysis

Statistical analyses were performed using PROC GLM of SAS 9.4 (SAS Institute Inc., Cary, NC) for all the performance and microbiology data, as a split-plot over time. Bacterial counts were log-transformed before analyzing the data. Plate counts with no growth were converted to zero after log-transformation. Correlation analysis among the eggshell properties were conducted using PROC CORR of SAS 9.4 (SAS Institute Inc., Cary, NC). The number of cages or pens (the replicate of each housing environment) was considered as a random factor, and the age of the birds was considered as a fixed factor. Data were analyzed by phases: early phase (32−51 wk of age) and late phase (52−85 wk of age). A P-value ≤ 0.05 was considered significant for all analyses. Tukey HSD was used to separate the means among treatments (Steel and Torrie, 1980).

RESULTS

Early Phase (32−51 Wk of Age)

Performance Data At the beginning of the trial, there were no differences between body weights of hens among housing environments for both HB and W-36 birds (P = 0.844), but among the laying hen strain, HB (2.03 kg) had a higher body weight than the W-36 (1.65 kg; P < 0.0001). The effect of the housing environment, hen strain, and bird age on HDEP in the early phase of
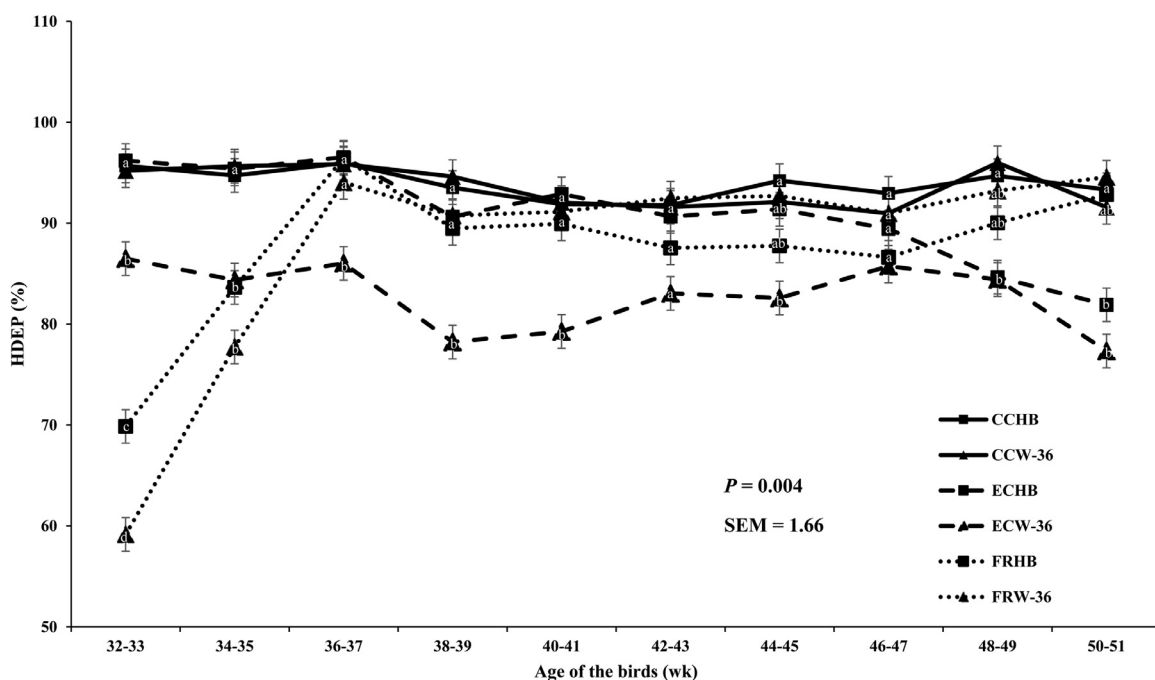

Figure 1. Effect of housing environment, hen strain, and age of the birds on hen day egg production (HDEP) in early phase of production (32−51 wk of age). The superscripts are only making comparisons within a week for treatments and not between weeks.

production is shown in Figure 1. A 3-way interaction between housing environment, hen strain, and bird age was observed for the HDEP ($P = 0.004$). Egg production was higher in the CC (93.7%) compared to FR (87.6%) and EC (86.9%). However, HDEP for FRHB and FRW-36 were lowest at 32 to 33 and 34 to 35 wk of age but became similar to the CCHB, CCW-36, and ECHB by wk 36 to 37. Hen day egg production for ECW-36 was lowest among all treatment groups from wk 36 until wk 51. The HDEP for the ECHB became lower than the other treatments from 46 to 47 wk of age and onward.

A 2-way interaction was observed for FI among housing environments and bird age ($P < 0.0001$; Table 3). Overall, the highest FI was observed in the free-range birds (129.8 g) when compared to CC (124.8 g) and EC (124.4 g). Feed intake of the hens was lower in FR at 38 to 40 wk of age when compared to CC and EC pens; however, at 41 to 42 wk of age, CC and FR hens had higher FI than the EC. In contrast, FR had higher FI than other housing environments from 43-44 wk onward. A 2-way interaction of hen strain and bird age was also significant for FI ($P = 0.006$). Overall, in this phase, FI was higher in HB (130.5 g) compared to W-36 (122.4 g). Feed intake was higher in the HB than the W-36 for all weeks except for wk 43 to 44, where FI was not different among strains. A 2-way interaction of housing environment and bird age was significant for FCR ($P < 0.0001$). Overall, FCR was higher in FR (1.71 kg/dozen of egg) and EC (1.71 kg/dozen of egg) compared to that of CC (1.61 kg/dozen of egg). Feed conversion ratio of hens raised in EC was higher at 38 to 40 wk of age compared to that of CC and FR; however, at 43 to 44 wk, EC and FR had higher FCR compared to CC. Feed conversion ratio was higher in FR compared to EC and CC from 43 to 44 wk onward. Similarly, a main effect of laying hen strain was observed for FCR ($P = 0.013$), where W-36 (1.61 kg/dozen of egg) had a lower FCR than HB (1.71 kg/dozen of egg).

Egg Quality The effect of housing environment, hen strain, and bird age on egg quality parameters in the early phase of production are shown in Table 4. Egg weights were highest in CC (62.8 g) and lowest in FR (61.6 g; $P = 0.018$). Hy-Line brown had a higher egg weight (62.8 g) compared to W-36 (62.0 g; $P = 0.046$). Egg weights were also significantly affected by bird age where egg weight increased as the hens aged ($P < 0.0001$).

A housing environment by hen strain interaction was observed for specific gravity ($P = 0.031$). The specific gravity was higher in both CC and EC compared to FR, and within each housing environment, HB had higher specific gravity than the W-36. A main effect of bird age was observed for specific gravity ($P < 0.0001$), which increased as the age of the birds progressed. A 3-way interaction between housing environment, hen strain, and bird age was observed for Haugh unit ($P = 0.034$). Haugh unit increased from wk 32 to 38 in all treatment groups and was similar in all treatment groups at 38 wk of age. At 45 wk of age, Haugh unit decreased in Hy-Line brown hens reared in EC and for both of the strains in FR; whereas for other treatments, it remained similar to that of wk 38.

Table 3. Effect of housing environment, hen strain, and age of the birds on feed intake (FI) and feed conversion ratio (FCR) in early phase of production (32−51 wk of age).

Treatments	FI (g)	FCR (kg of feed/ dozen of egg)
Environment		
CC	124.8[b]	1.61[b]
EC	124.4[b]	1.71[a]
FR	129.8[a]	1.71[a]
SEM	1.23	0.020
Strain		
HB	130.5[a]	1.71[a]
W-36	122.4[b]	1.64[b]
SEM	1.00	0.016
Age		
38−40 wk	123.2[b]	1.62[cd]
41−42 wk	131.0[a]	1.70[b]
43−44 wk	118.4[c]	1.59[d]
45−49 wk	127.2[ab]	1.68[bc]
50−51 wk	130.5[a]	1.78[a]
SEM	1.15	0.018
Environment × Strain		
CC × HB	130.2	1.68[a]
CC × W−36	119.1	1.55[b]
EC × HB	128.3	1.71[a]
EC × W-36	121.0	1.71[a]
FR × HB	132.6	1.75[a]
FR × W-36	127.0	1.68[a]
SEM	1.75	0.028
Environment × Age		
CC × 38−40 wk	126.3[b-e]	1.62[cde]
CC × 41−42 wk	123.9[c-f]	1.60[cde]
CC × 43−44 wk	114.9[f]	1.50[e]
CC × 45−49 wk	126.5[b-e]	1.61[cde]
CC × 50−51 wk	131.4[bc]	1.74[abc]
EC × 38−40 wk	130.1[bcd]	1.70[abc]
EC × 41−42 wk	134.2[b]	1.75[abc]
EC × 43−44 wk	112.1[f]	1.59[cde]
EC × 45−49 wk	121.1[def]	1.69[bc]
EC × 50−51 wk	117.4[ef]	1.77[ab]
FR × 38−40 wk	113.2[f]	1.53[de]
FR × 41−42 wk	134.7[b]	1.76[abc]
FR × 43−44 wk	124.3[c−f]	1.67[bcd]
FR × 45−49 wk	134.1[b]	1.76[abc]
FR × 50−51 wk	145.1[a]	1.85[a]
SEM	2.02	0.031
Strain × Age		
HB × 38−40 wk	127.2[bcd]	1.66
HB × 41−42 wk	137.2[a]	1.75
HB × 43−44 wk	118.4[e]	1.59
HB × 45−49 wk	132.1[abc]	1.74
HB × 50−51 wk	134.3[ab]	1.80
W-36 × 38−40 wk	119.2[e]	1.56
W-36 × 41−42 wk	124.7[de]	1.65
W-36 × 43−44 wk	118.5[e]	1.59
W-36 × 45−49 wk	122.4[de]	1.63
W-36 × 50−51 wk	126.7[cd]	1.76
SEM	1.63	0.030
P-value		
Environment	0.0006	0.001
Strain	0.003	0.412
Age	<0.0001	<0.0001
Environment × Strain	0.131	0.019
Environment × Age	<0.0001	<0.0001
Strain × Age	0.006	0.072
Environment × Strain × Age	0.515	0.456

Abbreviations: CC, conventional cage; EC, enriched colony cage; FCR, feed conversion ratio; FI, feed intake; FR, free-range; HB, Hy-Line Brown; W-36, Hy-Line W-36.

[a-h]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Two-way interactions between housing environment and bird age ($P = 0.004$) as well as hen strain and bird age ($P = 0.0004$) were observed for albumen percentage. Albumen percentage was lower in FR eggs at 32 wk of

Table 4. Effect of housing environment, hen strain, and age of the birds on egg quality parameters in early phase of production (32−51 wk of age).

Treatments	EW (g)	SG	HU	AP (%)	YP (%)	SW (g)	SP (%)	ST (0.01 mm)
Environment								
CC	62.8[a]	1.084[a]	89.0[a]	65.4	25.4	5.77[a]	9.20[a]	39.3[a]
EF	62.7[ab]	1.083[b]	88.1[a]	65.4	25.4	5.76[a]	9.21[a]	38.9[a]
FR	61.6[b]	1.080[c]	85.6[b]	65.3	25.5	5.48[b]	8.96[b]	38.0[b]
SEM	0.33	0.0004	0.65	0.21	0.17	0.042	0.054	0.18
Strain								
HB	62.8[a]	1.085[a]	87.7	65.6[a]	24.9[b]	5.82[a]	9.32[a]	39.6[a]
W-36	62.0[b]	1.080[b]	87.4	65.1[b]	26.0[a]	5.51[b]	8.93[b]	37.8[b]
SEM	0.27	0.000	0.53	0.17	0.14	0.035	0.044	0.14
Age								
32 wk	59.6[c]	1.079[b]	79.0[b]	64.8[b]	25.7[a]	5.49[c]	9.24[b]	37.3[b]
38 wk	61.7[b]	1.084[a]	92.6[a]	65.8[a]	24.7[b]	5.84[a]	9.48[a]	39.5[a]
45 wk	65.8[a]	1.084[a]	91.2[a]	65.5[ab]	25.9[a]	5.68[b]	8.65[c]	39.4[a]
SEM	0.30	0.0004	0.50	0.20	0.12	0.033	0.057	0.22
Environment × Strain								
CC × HB	63.3	1.086[a]	90.3	66.0	24.6	5.93[a]	9.38	40.1
CC × W-36	62.4	1.082[bc]	87.7	64.9	26.1	5.61[c]	9.02	38.5
EC × HB	62.9	1.085[ab]	87.7	65.6	25.0	5.87[ab]	9.36	39.7
EC × W-36	62.5	1.081[c]	88.4	65.2	25.8	5.65[bc]	9.05	38.1
FR × HB	62.1	1.083[b]	85.1	65.3	25.0	5.67[abc]	9.21	39.1
FR × W-36	61.0	1.077[d]	86.2	65.3	26.1	5.29[d]	8.71	36.9
SEM	0.46	0.001	0.91	0.30	0.24	0.060	0.076	0.25
Environment × Age								
CC × 32 wk	60.3	1.082	80.1[c]	65.5[a]	25.3[b-e]	5.51	9.14	38.4[ab]
CC × 38 wk	62.2	1.086	92.1[a]	65.5[a]	24.9[de]	6.00	9.65	40.0[a]
CC × 45 wk	66.0	1.085	94.7[a]	65.3[a]	25.9[abc]	5.81	8.82	39.4[a]
EC × 32 wk	60.5	1.078	80.2[c]	65.3[a]	25.3[b-e]	5.67	9.39	37.5[bc]
EC × 38 wk	61.8	1.085	92.9[a]	65.5[a]	24.9[c-e]	5.88	9.53	39.4[a]
EC × 45 wk	65.8	1.085	91.1[ab]	65.3[a]	26.0[ab]	5.73	8.70	39.9[a]
FR × 32 wk	58.1	1.077	76.7[d]	63.6[b]	26.5[a]	5.30	9.20	35.9[c]
FR × 38 wk	61.1	1.083	92.70[a]	66.4[a]	24.4[e]	5.64	9.26	39.3[a]
FR × 45 wk	65.5	1.081	87.6[b]	65.8[a]	25.78[a-d]	5.50	8.42	38.8[ab]
SEM	0.51	0.001	0.86	0.35	0.2	0.057	0.10	0.38
Strain × Age								
HB × 32 wk	60.3	1.082	79.6	64.6[b]	25.5[bc]	5.69	9.48	38.
HB × 38 wk	61.9	1.087	93.1	66.1[a]	24.2[d]	5.98	9.66	40.4
HB × 45 wk	66.0	1.086	90.4	66.2[a]	25.0[c]	5.81	8.81	40.1
W-36 × 32 wk	58.9	1.076	78.4	65.1[ab]	25.9[b]	5.30	9.01	36.1
W-36 × 38 wk	61.5	1.082	92.0	65.5[ab]	25.3[bc]	5.70	9.29	38.7
W-36 × 45 wk	65.5	1.081	91.9	64.7[b]	26.8[a]	5.55	8.48	38.6
SEM	0.42	0.001	0.71	0.29	0.17	0.046	0.081	0.31
Environment × Strain × Age								
CC × HB × 32 wk	61.1	1.083	83.5[de]	65.9	24.8	5.69	9.33	39.3[a-e]
CC × HB × 38 wk	62.9	1.088	93.1[abc]	66.0	24.1	6.19	9.84	41.0[a]
CC × HB × 45 wk	65.8	1.087	94.3[a]	66.2	24.9	5.90	8.98	39.8[a-d]
CC × W-36 × 32 wk	59.4	1.080	76.7[f]	65.22	25.8	5.32	8.95	37.4[de]
CC × W-36 × 38 wk	61.5	1.084	91.2[abc]	64.9	25.7	5.80	9.45	38.9[a-e]
CC × W-36 × 45 wk	66.2	1.083	95.2[a]	64.4	26.9	5.72	8.66	39.0[a-e]
EC × HB × 32 wk	61.1	1.080	79.4[ef]	65.3	25.1	5.82	9.55	38.2[b-e]
EC × HB × 38 wk	61.8	1.087	94.1[ab]	65.7	24.6	6.01	9.74	40.5[ab]
EC × HB × 45 wk	65.9	1.087	89.5[a-d]	65.7	25.5	5.79	8.80	40.4[abc]
EC × W-36 × 32 wk	59.9	1.076	81.0[ef]	65.3	25.5	5.53	9.24	36.9[e]
EC × W-36 × 38 wk	61.9	1.082	91.6[abc]	65.4	25.3	5.76	9.31	38.2[b-e]
EC × W-36 × 45 wk	65.7	1.084	92.7[abc]	64.8	26.6	5.66	8.61	39.3[a-e]
FR × HB × 32 wk	58.8	1.082	75.8[f]	62.5	26.5	5.54	9.56	37.7[cde]
FR × HB × 38 wk	61.1	1.084	92.2[abc]	66.7	23.9	5.74	9.41	39.6[a-e]
FR × HB × 45 wk	66.4	1.084	87.3[cd]	66.6	24.6	5.73	8.66	40.1[a-d]
FR × W-36 × 32 wk	57.4	1.072	77.5[ef]	64.7	26.5	5.06	8.84	34.0[f]
FR × W-36 × 38 wk	61.1	1.081	93.2[abc]	66.1	24.8	5.54	9.10	39.02[a-e]
FR × W-36 × 45 wk	64.6	1.078	87.9[bcd]	65.0	26.9	5.26	8.18	37.6[de]
SEM								
***P*-value**								
Environment	0.018	<0.0001	0.003	0.846	0.736	<0.0001	0.004	<0.0001
Strain	0.046	<0.0001	0.728	0.037	<0.0001	<0.0001	<0.0001	<0.0001
Age	<0.0001	<0.0001	<0.0001	0.004	<0.0001	<0.0001	<0.0001	<0.0001
Environment × Strain	0.777	0.031	0.100	0.167	0.276	0.130	0.437	0.338
Environment × Age	0.289	0.282	0.0009	0.0004	0.0004	0.358	0.114	0.043
Strain × Age	0.420	0.507	0.094	0.004	0.001	0.293	0.689	0.375
Environment × Strain × Age	0.542	0.071	0.034	0.199	0.443	0.269	0.699	0.046

Abbreviations: AP, albumen percentage; CC, conventional cage; EC, enriched colony cage; EW, egg weights; FR, free-range; HB, Hy-Line Brown; HU, Haugh unit; SG, specific gravity; SP; eggshell percentage; ST, eggshell thickness; SW, eggshell weight; W-36, Hy-Line W-36; YP, yolk percentage.

[a-f]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

age compared to that of CC and EC. There was no difference between CC, EC, and FR eggs for albumen percentage at 38 and 45 wk of age. The albumen percentage was higher in HB than W-36 at wk 45; however, there was no difference at 32 and 38 wk of age. Similarly, interactions between housing environment and bird age ($P = 0.0004$) as well as hen strain and bird age ($P = 0.001$) were observed for yolk percentage. At 32 wk of age, yolk percentage was higher in FR when compared to CC and EC; however, from 38 wk onward, it was similar in all 3 housing environments. There was no difference between W-36 and HB eggs for YP on wk 32, but it increased in W-36 eggs and decreased in HB eggs such that W-36 had the highest yolk percentage on wk 45.

A 3-way interaction among housing environment, hen strain, and bird age ($P = 0.046$) was detected for eggshell thickness. At 32 wk of age, eggshell thickness was highest for CCHB (0.393 mm) and lowest for FRW-36 (0.340 mm). Shell thickness increased in all treatment groups from 32 to 38 wk of age with CCHB (0.410 mm) having the highest and ECW-36 (0.382 mm) having the lowest, and again on wk 45, FRW-36 (0.376 mm) had the lowest and ECHB (0.404 mm) had the highest eggshell thickness. The main effects of housing environment ($P = 0.004$), hen strain ($P < 0.0001$) and bird age ($P < 0.0001$) were observed for eggshell percentage. Eggshell percentage was higher in CC and EC (9.20% and 9.21%, respectively) when compared to the FR (8.96%). Between the 2 laying hen strains, HB (9.32%) had a higher eggshell percentage compared to W-36 (8.93%). Eggshell percentage was highest at wk 38 (9.48%) and lowest at wk 45 (8.65%).

Cloacal and Eggshell Microbiology The effects of housing environments, hen strain, and bird age on cloacal and eggshell microbiology during the early phase of production are shown in Table 5. A 2-way interaction between housing environment and bird age was observed for total cloacal aerobes ($P = 0.006$). Total aerobic bacterial counts were higher in FR than CC and EC on wk 38 but were not significantly different between the housing environments at 45 wk of age. A main effect of hen strain was observed for total cloacal aerobes ($P = 0.027$), where HB (3.896 log CFU/mL) had higher bacterial counts than W-36 (2.840 log CFU/mL). Total cloacal anaerobic bacterial counts were affected by housing environment and hen strain ($P < 0.0001$). Total anaerobic bacterial counts were higher in HB hens housed in CC compared to W-36; whereas in EC, HB had lower counts compared to W-36. An interaction (hen strain and bird age) was detected for total cloacal anaerobic bacterial counts ($P = 0.039$). At 38 wk of age, the bacterial counts for anaerobes were higher in HB than W-36, but there were not any differences at 45 wk of age. There was an interaction observed between housing environment and bird age for total coliform bacterial counts ($P = 0.0004$). The counts of total coliforms were higher for FR at 38 wk of age than for CC and EC, but there were no differences detected between environments at 45 wk of age.

An interaction between housing environment and hen strain was observed for eggshell aerobic bacterial counts ($P = 0.034$). Aerobic bacterial counts were higher in FR when compared to CC and EC at both 38 and 45 wk of age. A main effect of housing environment was observed for total eggshell anaerobic bacteria ($P < 0.0001$), which were higher in FR (3.864 log CFU/mL) compared to CC (1.497 log CFU/mL) and EC (0.990 log CFU/mL). An interaction between housing environment and hen strain was observed for total eggshell coliform bacterial counts ($P = 0.001$) where the counts were higher in FR than in EC and CC at 45 wk of age.

Late Phase (52−85 Wk of Age)

Performance Data The effects of housing environment, hen strain, and age of the birds on HDEP is shown in Figure 2, and performance parameters such as FI and FCR are shown in Figures 3 and 4, and Table 6. Overall, higher body weight was observed for hens raised in CC (2.09 kg) and EC (2.07 kg) compared to FR (2.00 kg; $P = 0.0003$). Similarly, HB had higher body weight (2.29 kg) compared to W-36 (1.81 kg; $P < 0.0001$). An interaction among the housing environment, hen strain, and bird age was observed for HDEP ($P < 0.0001$), FI ($P = 0.017$), and FCR ($P < 0.0001$). Overall, HDEP was higher in FR (87.3%) than in CC (85.5%) and EC (71.8%). Hen day egg production for the FR and CC were similar throughout the period; however, both strains of hens in EC had lower production consistently from the start of the late phase compared to rest of the groups.

Overall, there was no difference for FI between CC (124.4 g) and FR (123.3 g), but it was significantly lower in the EC (112.0 g; $P < 0.0001$). Feed intake for the W-36 hens housed in EC was lower than the rest of the treatments throughout the period, while FI for CCHB was higher for most of the weeks. During 62 to 63 wk of age, FRW-36 had higher FI compared to other treatments. Overall FCR was highest in the EC (1.81 kg/dozen of eggs) than the CC (1.74 kg/dozen of eggs) and FR (1.72 kg/dozen of eggs; $P < 0.0006$). The feed conversion ratio was higher for most of the weeks in hens of both strains housed in EC, but from 66 to 67 wk onward, FCR decreased and became lower compared to those of CCHB and FRHB.

Egg Quality The effects of housing environment, hen strain, and bird age for egg quality are shown in Table 7. Interactions between housing environment and bird age ($P = 0.039$) as well as hen strain and bird age ($P = 0.0002$) were observed for egg weight. Overall, egg weights were higher in CC (67.20 g) and EC (66.7 g) than in FR (65.4 g), but there were no significant differences between the hen strains. Egg weights were higher in CC for most of the weeks except for wk 72, where the CC, EC, and FR had similar egg weights. Between the 2 strains, egg weights were similar for most of the weeks except for wk 79, where W-36 had higher egg weights than HB.

Table 5. Effect of housing environment, hen strain, and age of the birds on eggshell and cloacal microbiology (log CFU/mL) in early phase of production (32−51 wk of age).

Treatments	Aerobes cloaca	Aerobes egg	Anaerobes cloaca	Anaerobes egg	Coliform cloaca	Coliforms egg
Environment						
CC	2.45[b]	1.55[b]	3.91[a]	1.50[b]	0.87[a,b]	0.00[b]
EC	2.97[b]	1.45[b]	2.79[b]	0.99[b]	0.34[b]	0.00[b]
FR	4.68[a]	4.90[a]	4.39[a]	3.86[a]	1.53[a]	1.29[a]
SEM	0.394	0.332	0.212	0.408	0.271	0.269
Strain						
HB	3.9[a]	2.40	3.88	1.90	1.15	0.37
W-36	2.84[b]	2.87	3.49	2.34	0.68	0.49
SEM	0.322	0.271	0.173	0.333	0.221	0.220
Age						
38 wk	2.82[b]	2.42	2.90[b]	2.14	1.50[a]	0.10[b]
45 wk	3.92[a]	2.85	4.46[a]	2.10	0.33[b]	0.76[a]
SEM	0.210	0.279	0.311	0.228	0.214	0.162
Environment × Strain						
CC × HB	3.49	0.59[b]	5.12[a]	0.74	1.46	0.00
CC × W-36	1.42	2.52[b]	2.71[c]	2.26	0.29	0.00
EC × HB	3.58	1.72[b]	2.20[c]	1.25	0.68	0.00
EC × W-36	2.36	1.19[b]	3.39[bc]	0.73	0.00	0.00
FR × HB	4.62	4.90[a]	4.34[ab]	3.70	1.32	1.11
FR × W-36	4.75	4.91[a]	4.46[ab]	4.02	1.75	1.47
SEM	0.557	0.469	0.300	0.577	0.383	0.380
Environment × Age						
CC × 38 wk	1.64[d]	0.95	2.83	1.59	1.15[b]	0.00[b]
CC × 45 wk	3.26[bc]	2.16	4.99	1.41	0.59[b]	0.00[b]
EC × 38 wk	1.96[cd]	1.42	1.99	0.89	0.28[b]	0.00[b]
EC × 45 wk	3.98[ab]	1.49	3.60	1.09	0.40[b]	0.00[b]
FR × 38 wk	4.86[a]	4.90	3.97	3.93	3.07[a]	0.29[b]
FR × 45 wk	4.50[ab]	4.91	4.79	3.80	0.00[b]	2.29[a]
SEM	0.363	0.483	0.539	0.394	0.370	0.281
Strain × Age						
HB × 38 wk	3.49	2.15	3.60[ab]	1.96	1.83	0.00
HB × 45 wk	4.31	2.66	4.17[a]	1.84	0.47	0.74
W-36 × 38 wk	2.16	2.70	2.16[b]	2.31	1.16	0.19
W-36 × 45 wk	3.52	3.05	4.75[a]	2.36	0.19	0.79
SEM	0.297	0.394	0.440	0.322	0.302	0.230
Environment × Strain × Age						
CC × HB × 38 wk	3.28	0.00	4.91	0.86	2.31	0.00
CC × HB × 45 wk	3.69	1.18	5.33	0.61	0.61	0.00
CC × W-36 × 38 wk	0.00	1.90	0.76	2.32	0.00	0.00
CC × W-36 × 45 wk	2.84	3.14	4.66	2.20	0.58	0.00
EC × HB × 38 wk	2.31	1.61	2.10	1.19	0.55	0.00
EC × HB × 45 wk	4.85	1.84	2.29	1.32	0.80	0.00
EC × W-36 × 38 wk	1.61	1.23	1.87	0.60	0.00	0.00
EC × W-36 × 45 wk	3.11	1.14	4.90	0.85	0.00	0.00
FR × HB × 38 wk	4.86	4.83	3.79	3.84	2.64	0.00
FR × HB × 45 wk	4.38	4.96	4.88	3.57	0.00	2.21
FR × W-36 × 38 wk	4.86	4.97	4.18	4.02	3.49	0.57
FR × W-36 × 45 wk	4.63	4.85	4.69	4.03	0.00	2.37
SEM	0.514	0.683	0.763	0.558	0.523	0.398
P-value						
Environment	0.001	<0.0001	<0.0001	<0.0001	0.015	0.002
Strain	0.027	0.228	0.137	0.361	0.140	0.698
Age	0.0009	0.286	0.002	0.906	0.0006	0.007
Environment × Strain	0.156	0.034	<0.0001	0.220	0.120	0.858
Environment × Age	0.006	0.386	0.473	0.874	0.0004	0.001
Strain × Age	0.365	0.836	0.039	0.789	0.525	0.764
Environment × Strain × Age	0.070	0.978	0.156	0.994	0.098	0.913

Abbreviations: CC, conventional cage; EC, enriched colony cage; FR, free-range; HB, Hy-Line Brown; W-36, Hy-Line W-36.

[a-d]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Specific gravity was only affected by hen strain ($P = 0.037$), where HB had a higher specific gravity (1.085) than W-36 (1.081). The main effects of housing environment ($P = 0.036$) and bird age ($P < 0.0001$) were observed for Haugh unit. Higher Haugh units were observed in eggs from the CC (96.8) and EC (96.2) than those of FR (95.2). Haugh units increased as hens aged with the highest value at 85 wk of age (104.1) and the lowest at 52 wk of age (90.5). Significant interactions between housing environment and bird age ($P < 0.0001$)

as well as hen strain and bird age ($P = 0.008$) were observed for albumen percentage. At 52 wk of age, albumen percentage was higher in the FR than in the CC and EC, but on wk 59, it decreased and became the lowest. Among the 2 strains, HB had a higher albumen percentage than W-36 for all weeks except for at 65 wk of age.

There was an interaction between housing environment and bird age for yolk percentage ($P = 0.0006$), where a higher yolk percentage was observed at 59 wk of
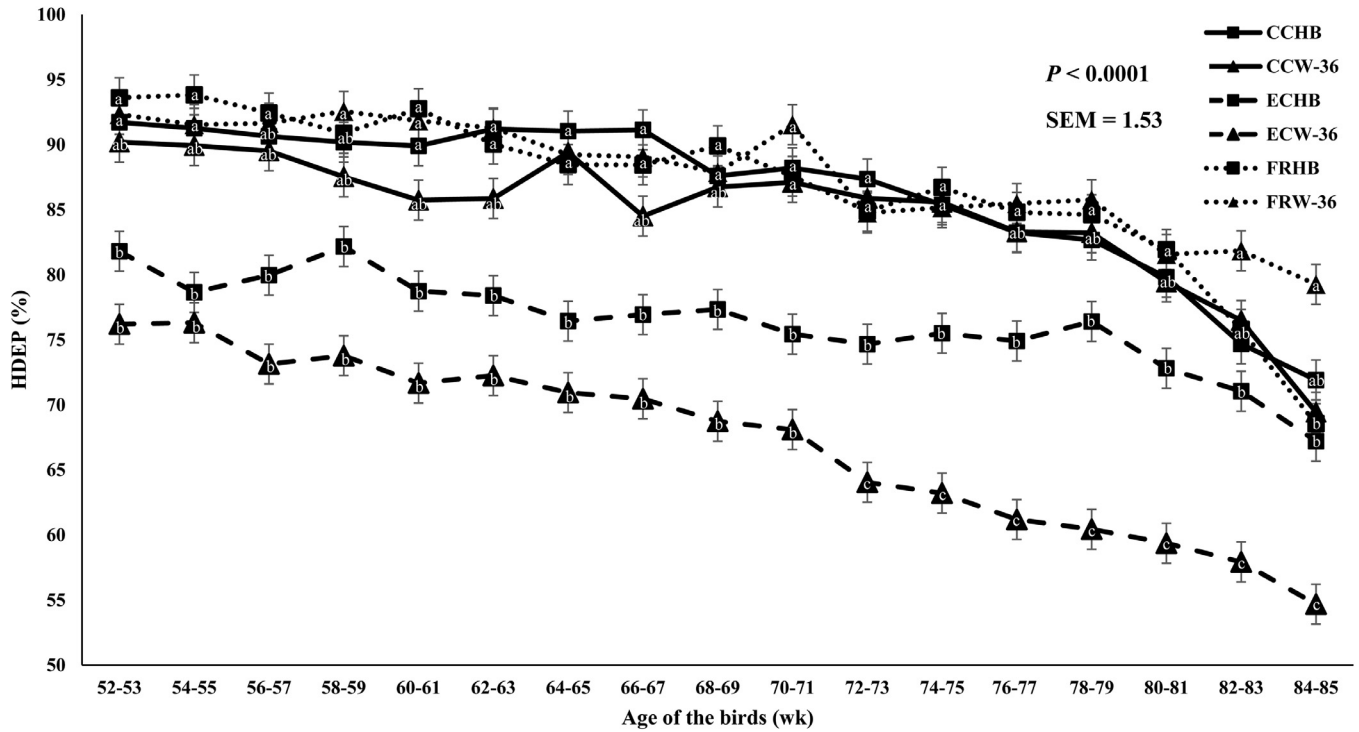
Figure 2. Effect of housing environment, hen strain, and age of the birds on hen day egg production (HDEP) in late phase of production (52−85 wk of age). The superscripts are only making comparisons within a week for treatments and not between weeks.

age in the FR compared to the CC and EC. However, for the rest of the weeks, yolk percentage was similar in all housing environments. A main effect of laying hen strain was also observed for yolk percentage ($P < 0.0001$), where W-36 had a higher yolk percentage (27.0%) compared to HB (25.5%).

An interaction between housing environment and bird age was observed for eggshell percentage ($P = 0.0009$). Eggshell percentage was similar for almost all weeks except for wk 85, where eggs from FR had a higher egg-shell percentage than those from EC. A main effect of laying hen strain ($P < 0.0001$) was also observed where

eggs laid by HB had higher eggshell percentage (9.38%) than those from W-36 (8.77%). A negative correlation was observed between egg weights and eggshell percentage ($P < 0.0001$, R = -0.405; Table 8) where eggshell percentage decreased with an increase in egg weights.

Interactions between housing environment and hen strain ($P = 0.006$), housing environment and bird age ($P < 0.0001$), as well as hen strain and bird age ($P = 0.030$) were observed for eggshell thickness. Hy-Line Brown hens in FR had the highest eggshell thickness (0.391 mm) compared to those raised in the EC (0.385 mm) and CC (0.384 mm). However, for W-36
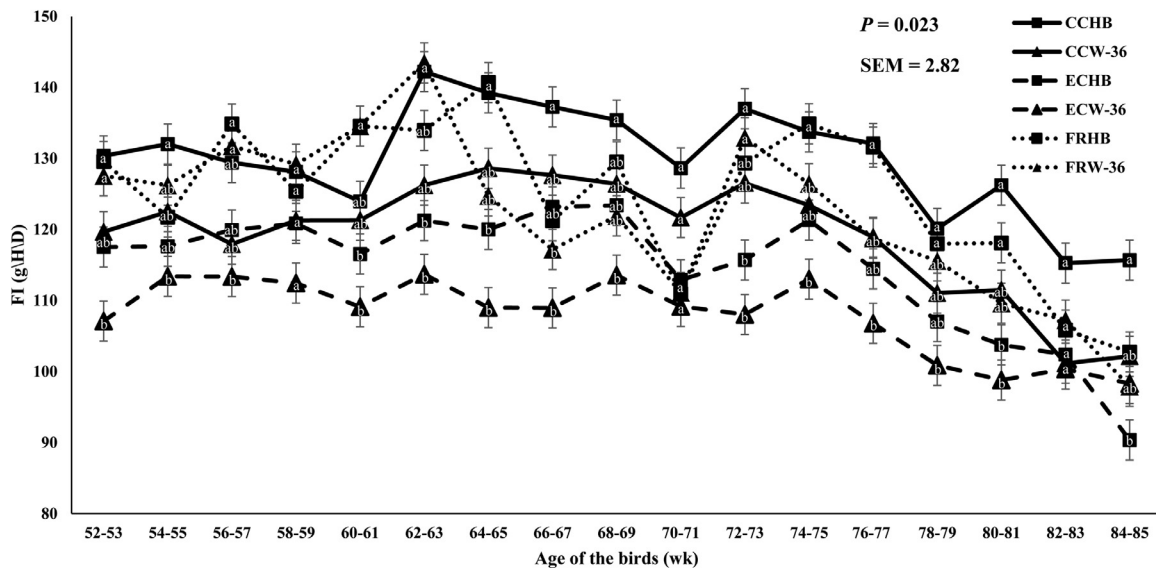

Figure 3. Effect of housing environment, hen strain, and age of the birds on feed intake (FI) in late phase of production (52−85 wk of age). The superscripts are only making comparisons within a week for treatments and not between weeks.
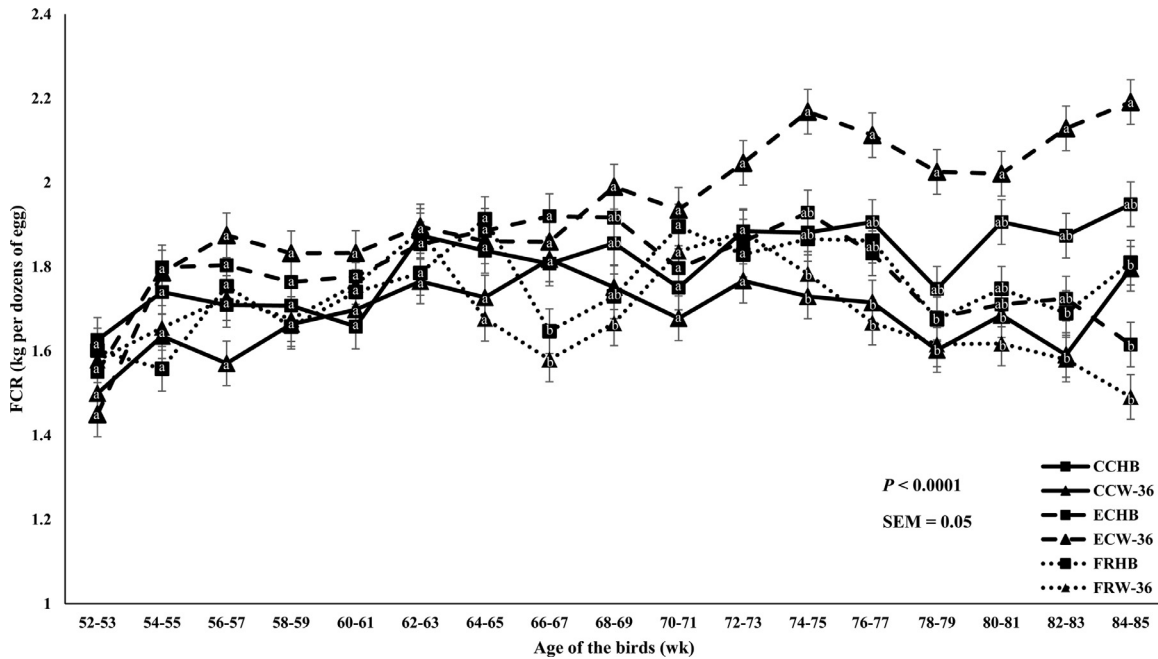
Figure 4. Effect of housing environment, hen strain, and age of the birds on feed conversion ratio (FCR) in late phase of production (52−85 wk of age). The superscripts are only making comparisons within a week for treatments and not between weeks.

hens, eggshell thickness was higher in CC (0.376 mm) than in EC (0.371 mm) and FR (0.368 mm). At 52 wk of age, eggshell thickness was higher in the CC and EC than in the FR; but at 65 wk of age, both FR and EC had higher eggshell thickness compared to the CC. Further, eggshell thickness decreased continuously from 72 to 85 wk of age in all housing environments. Eggshell thickness was higher in HB hens on 65, 79, and 85 wk of age compared to W-36 hens. A negative correlation was found between egg weight and eggshell thickness ($P = 0.0006$, R = -0.232; Table 8) where eggshell thickness decreased with an increase in egg weights.

The main effects of housing environment, hen strain, and age of the birds were observed for eggshell breaking strength (all $P < 0.0001$). Eggshell breaking strength was higher in eggs from CC (3.28 KgF) than those from FR (3.15 KgF) and EC (2.99 KgF). Between the strains, HB hens had a higher eggshell breaking strength (3.27 KgF) compared to W-36 (3.00 KgF). Eggshell breaking strength decreased as the hens aged. A negative correlation was observed between egg weight and eggshell breaking strength ($P < 0.0001$, R = -0.298) while positive correlations were detected between eggshell thickness and eggshell breaking strength ($P < 0.0001$, R = 0.590) and eggshell percentage and eggshell breaking strength ($P < 0.0001$, R = 0.594; Table 8). Eggshell breaking strength increased with an increase in eggshell thickness and percentage but decreased with an increase in egg weights.

Cloacal and Eggshell Microbiology The effects of housing environment, hen strain, and bird age on cloacal and eggshell microbiology in the late phase of production are shown in Table 9. There was a significant interaction between housing environment and bird age for total cloacal aerobic bacteria ($P = 0.0002$). Overall, the highest

bacterial counts were observed in FR (4.408 log CFU/mL) compared to EC (3.036 log CFU/mL) and CC (2.306 log CFU/mL). Until 65 wk of age, the bacterial counts were higher in FR than in EC and CC. There was no significant difference in the aerobic bacterial counts between FR and EC at 72 and 79 wk of age, but the numbers were still higher than CC. The bacterial counts for aerobes were similar among the housing environments at 85 wk of age.

Significant main effects of housing environment ($P = 0.003$), hen strain ($P = 0.021$), and bird age ($P < 0.0001$) were observed for total cloacal anaerobic bacterial counts. The bacterial counts were higher in FR (4.622 log CFU/mL) compared to CC (3.756 log CFU/mL) and EC (3.527 log CFU/mL). Similarly, HB hens had higher bacterial counts (4.214 log CFU/mL) compared to W-36 (3.731 log CFU/mL), with the highest counts observed at 79 wk of age (5.531 log CFU/mL).

There was an interaction between the housing environment and bird age for total cloacal coliform bacterial counts ($P = 0.0003$). Overall, bacterial counts were higher in FR (2.949 log CFU/mL) compared to CC (0.891 log CFU/mL) and EC (0.597 log CFU/mL). Coliform bacterial counts were similar between the houses at 52 wk of age, but as hens aged, the counts increased in the FR. The coliform counts were higher in FR and EC than CC at 72 wk, but on wk 79, they were similar in all 3 housing environments. At 85 wk of age, coliform bacterial counts for EC decreased whereas they remained constant for FR.

An interaction among housing environment and bird age was observed for total aerobic bacterial counts on the eggshell ($P = 0.028$). Overall, aerobic bacterial load was higher in FR (5.079 log CFU/mL) compared to CC (2.518 log CFU/mL) and EC (2.102 log CFU/mL).

Table 6. Effect of housing environment, hen strain, and age of the birds on hen day egg production (HDEP), feed intake (FI) and feed conversion ratio (FCR) in late phase of production (52−85 wk of age).

Treatments	HDEP (%)	FI (g)	FCR (kg of feed/ dozen of egg)
Environment			
CC	85.5[a]	124.6[a]	1.75[b]
EC	71.8[b]	111.3[b]	1.87[a]
FR	87.3[a]	123.5[a]	1.72[b]
SEM	0.97	1.40	0.027
Strain			
HB	83.2[a]	123.0[a]	1.78
W-36	79.9[b]	116.5[b]	1.77
SEM	0.79	1.14	0.022
Age			
52−53 wk	87.6[a]	122.0[bc]	1.55[f]
54−55 wk	86.9[ab]	122.3[bc]	1.70[e]
56−57 wk	86.2[abc]	124.7[abc]	1.76[cde]
58−59 wk	86.2[abc]	122.9[bc]	1.72[de]
60−61wk	85.1[a-d]	123.3[bc]	1.74[cde]
62−63 wk	84.8[a-d]	130.1[a]	1.84[abc]
64−65 wk	84.3[bcd]	127.0[ab]	1.82[a-d]
66−67 wk	83.4[cd]	122.6[bc]	1.77[cde]
68−69 wk	83.0[de]	125.0[abc]	1.82[a-d]
70−71 wk	83.0[de]	115.7[de]	1.82[a-d]
72−73 wk	80.3[ef]	124.9[abc]	1.88[ab]
74−75 wk	80.3[ef]	125.5[abc]	1.89[a]
76−77 wk	78.8[fg]	120.4[cd]	1.85[abc]
78−79 wk	78.9[fg]	112.1[e]	1.72[de]
80−81 wk	75.8[gh]	111.3[e]	1.78[b-e]
82−83 wk	73.0[h]	105.4[f]	1.74[cde]
84−85 wk	68.5[i]	101.2[f]	1.81[a-d]
SEM	0.63	1.15	0.022
P-value			
Environment	<0.0001	<0.0001	0.001
Strain	0.006	0.0003	0.727
Age	<0.0001	<0.0001	<0.0001
Environment × Strain	0.002	0.158	0.003
Environment × Age	0.213	<0.0001	<0.0001
Strain × Age	0.612	0.038	0.343
Environment × Strain × Age	<0.0001	0.023	<0.0001

Abbreviations: CC, conventional cage; EC, enriched colony cage; FR, free-range; HB, Hy-Line Brown; W-36, Hy-Line W-36.

[a-i]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Bacterial counts were higher in the FR environment than CC and EC, except at 85 wk of age where the counts were similar between all 3 housing environments. For eggshell anaerobic bacterial counts, an interaction between the housing environment and bird age was significant ($P = 0.0002$). Overall, the bacterial load was higher in FR (3.912 log CFU/mL) followed by CC (2.847 log CFU/mL) and then EC (1.143 log CFU/mL). The anaerobic bacterial counts were always lower in the EC. Total anaerobic bacterial counts for FR were higher at wk 52, 72, and 79 than for CC. An interaction among housing environment and bird age was observed for total eggshell coliform bacterial counts ($P < 0.0002$). The total eggshell coliform bacteria were higher in FR (2.949 log CFU/mL) than CC (0.891 log CFU/mL) or EC (0.597 log CFU/mL). The coliform counts were similar between all 3 housing environments at 52 and 59 wk, and the counts were higher in FR compared to CC and EC from 65 wk onward until the end of the phase. Laying hen strain did not have any effect on eggshell bacterial counts for aerobes, anaerobes, or coliforms ($P > 0.05$).

DISCUSSION

The United States egg industry is transitioning from the conventional cage system to alternative housing systems in response to consumer demand for cage-free eggs in addition to legislation. More than 75% of United States consumers are concerned about the welfare of animals (Spain et al., 2018) and are willing to pay more premium for eggs from welfare-friendly systems (Lusk, 2019, Ochs et al., 2019), and egg producers are looking for alternative housing environments to raise laying hens to embrace consumer demands. Housing environments such as aviary, enriched colony cages, or free-range systems might be alternatives that could improve the welfare status of laying hens (Appleby and Hughes, 1991; Tactacan et al., 2009, Yilmaz Dikmen et al., 2016).

Previous studies have reported that egg production was similar between CC, EC and cage-free systems such as aviaries or barns (Tactacan et al., 2009; Neijat et al., 2011; Ahammed et al., 2014), whereas others reported higher HDEP in CC compared to cage-free, or free-range

Table 7. Effect of housing environment, hen strain, and age of the birds on measured egg quality parameters in late phase of production (52−85 wk of age).

Treatments	EW (g)	SG	HU	AP (%)	YP (%)	SP (%)	ST (.01 mm)	EBS (KgF)
Environment								
CC	67.2[a]	1.082	96.8[a]	64.8	26.2	9.06	38.0	3.28[a]
EC	66.7[a]	1.083	96.2[ab]	64.7	26.3	9.05	37.8	2.99[c]
FR	65.4[b]	1.084	95.2[b]	64.6	26.3	9.10	38.0	3.15[b]
SEM	0.27	0.0001	0.42	0.13	0.11	0.041	0.15	0.036
Strain								
HB	66.3	1.085[a]	95.9	65.2[a]	25.5[b]	9.38[a]	38.7[a]	3.27[a]
W-36	66.5	1.081[b]	96.2	64.3[b]	27.0[a]	8.77[b]	37.2[b]	3.00[b]
SEM	0.22	0.0001	0.34	0.10	0.09	0.033	0.12	0.029
Age								
52 wk	62.5[c]	1.085	90.5[c]	65.0[a]	25.5[c]	9.57[a]	39.9[a]	3.55[a]
59 wk	66.4[b]	1.083	91.9[c]	64.8[a]	26.1[b]	9.22[b]	38.3[c]	3.22[b]
65 wk	67.4[ab]	1.080	96.5[b]	64.6[a]	26.3[ab]	9.11[b]	39.2[ab]	3.27[b]
72 wk	67.5[ab]	1.084	96.1[b]	64.6[a]	26.3[ab]	9.16[b]	38.8[bc]	3.19[b]
79 wk	68.0[a]	1.084	97.0[b]	64.4[a]	26.7[a]	8.84[c]	36.9[d]	2.86[c]
85 wk	66.9[ab]	1.081	104.1[a]	64.9[a]	26.6[a]	8.53[d]	34.5[e]	2.73[c]
SEM	0.30	0.002	0.56	0.14	0.12	0.056	0.19	0.042
Environment × Strain								
CC × HB	67.2	1.082	96.3	65.4	25.3	9.30	38.4[ab]	3.42
CC × W-36	67.2	1.082	97.2	64.3	27.0	8.81	37.6[bc]	3.14
EC × HB	66.6	1.086	96.2	65.0	25.71	9.37	38.5[a]	3.07
EC × W-36	66.7	1.081	96.1	64.5	26.9	8.74	37.1[c]	2.90
FR × HB	65.1	1.086	95.2	65.1	25.5	9.46	39.1[a]	3.32
FR × W-36	65.6	1.081	95.1	64.2	27.1	8.75	36.8[c]	2.97
SEM	0.38	0.002	0.59	0.18	0.16	0.058	0.21	0.051
Environment × Age								
CC × 52 wk	63.2[fg]	1.085	91.2	64.9[abc]	25.6[ef]	9.56[ab]	40.7[a]	3.70
CC × 59 wk	67.4[a-e]	1.083	92.4	65.4[ab]	25.8[c-f]	9.04[c-f]	38.3[c-f]	3.37
CC × 65 wk	68.0[a-d]	1.071	96.3	64.7[abc]	26.2[a−e]	9.15[b-f]	38.3[c-f]	3.39
CC × 72 wk	68.0[abc]	1.085	96.5	64.6[abc]	26.2[a-e]	9.26[b-e]	38.8[bcd]	3.35
CC × 79 wk	69.1[a]	1.085	97.8	64.5[abc]	26.7[a-d]	8.80[efg]	36.8[ef]	2.97
CC × 85 wk	67.5[a-e]	1.082	106.2	65.0[abc]	26.5[a-e]	8.53[h]	35.1[gh]	2.88
EC × 52 wk	61.7[g]	1.084	89.3	64.6[abc]	25.8[b-f]	9.75[a]	40.2[ab]	3.38
EC × 59 wk	66.3[b-e]	1.083	91.5	65.3[ab]	25.7[def]	9.16[b-f]	37.6[def]	3.04
EC × 65 wk	68.4[ab]	1.084	98.3	64.4[abc]	26.6[a-d]	9.09[b-f]	39.4[abc]	3.09
EC × 72 wk	67.3[a-e]	1.084	97.0	64.2[bc]	26.7[a-d]	9.12[b-f]	38.4[cde]	3.09
EC × 79 wk	68.7[ab]	1.085	96.3	64.4[abc]	26.7[a-d]	8.92[d-g]	37.1[ef]	2.70
EC × 85 wk	67.7[a-e]	1.081	104.5	65.5[a]	26.3[a-e]	8.28[h]	34.0[h]	2.62
FR × 52 wk	62.5[g]	1.086	90.9	65.6[a]	25.1[f]	9.39[a-d]	38.9[bcd]	3.57
FR × 59 wk	65.3[ef]	1.084	91.8	63.8[c]	26.8[abc]	9.46[abc]	39.0[a-d]	3.26
FR × 65 wk	65.6[c-f]	1.084	94.9	64.7[abc]	26.3[a-e]	9.09[b-f]	39.7[abc]	3.34
FR × 72 wk	67.2[a-e]	1.083	94.9	65.0[abc]	26.0[a-f]	9.10[b-f]	39.1[a-d]	3.12
FR × 79 wk	66.3[b-e]	1.083	97.0	64.4[abc]	26.8[ab]	8.81[efg]	36.7[fg]	2.90
FR × 85 wk	65.4[def]	1.080	101.4	64.4[abc]	26.9[a]	8.77[fg]	34.4[h]	2.68
SEM	0.52	0.003	0.97	0.25	0.21	0.096	0.34	0.072
Strain × Age								
HB × 52 wk	62.7[d]	1.087	90.3	65.8[a]	24.6	9.78	40.5[a]	3.74
HB × 59 wk	67.0[bc]	1.085	92.6	65.1[abc]	25.4	9.47	38.9[bcd]	3.36
HB × 65 wk	67.0[bc]	1.078	95.9	64.7[cd]	25.9	9.46	39.8[ab]	3.38
HB × 72 wk	67.1[bc]	1.087	96.1	65.1[abc]	25.5	9.47	39.2[bc]	3.34
HB × 79 wk	66.8[bc]	1.087	96.2	64.7[bcd]	26.0	9.26	37.9[d]	2.96
HB × 85 wk	67.3[abc]	1.084	104.2	65.6[ab]	25.6	8.80	35.8[e]	2.83
W-36 × 52 wk	62.2[d]	1.083	90.7	64.3[cd]	26.4	9.36	39.4[abc]	3.36
W-36 × 59 wk	65.7[c]	1.081	91.2	64.6[cd]	26.7	8.97	37.7[d]	3.09
W-36 × 65 wk	67.8[ab]	1.082	97.0	64.4[cd]	26.8	8.75	38.5[cd]	3.16
W-36 × 72 wk	67.9[ab]	1.082	96.2	64.1[d]	27.1	8.85	38.4[cd]	3.03
W-36 × 79 wk	69.2[a]	1.081	97.9	64.2[cd]	27.4	8.42	35.9[e]	2.75
W-36 × 85 wk	66.4[bc]	1.078	103.9	64.3[cd]	27.5	8.25	33.3[f]	2.62
SEM	0.42	0.003	0.79	0.20	0.17	0.079	0.27	0.059
P-value								
Environment	0.0002	0.698	0.036	0.570	0.597	0.608	0.534	<0.0001
Strain	0.492	0.037	0.588	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age	<0.0001	0.340	<0.0001	0.022	<0.0001	<0.0001	<0.0001	<0.0001
Environment × Strain	0.805	0.209	0.583	0.232	0.233	0.168	0.006	0.222
Environment × Age	0.039	0.434	0.079	<0.0001	0.0006	0.0009	<0.0001	0.938
Strain × Age	0.0002	0.388	0.461	0.008	0.072	0.110	0.030	0.629
Environment × Strain × Age	0.650	0.465	0.310	0.856	0.478	0.377	0.379	0.559

Abbreviations: AP, albumen percentage; CC, conventional cage; EC, enriched colony cage; EW, egg weights; FR, free-range; HB, Hy-Line Brown; HU, Haugh unit; SG, specific gravity; SP; eggshell percentage; ST, eggshell thickness; SW, eggshell weight; W-36, Hy-Line W-36; YP, yolk percentage.
[a-h]Values within columns not sharing the superscripts are significantly different at $P \le 0.05$.

Table 8. Correlation between egg weights, eggshell thickness, eggshell percentage, and eggshell breaking strength.

Measured parameters	EW	ST	SP
ST	-0.232[***]		
SP	-0.405[****]	0.751[****]	
EBS	-0.298[****]	0.590[****]	0.594[****]

Abbreviations: EBS, eggshell breaking strength; EW, egg weights; SP, eggshell percentage; ST, eggshell thickness.
[***]$P < 0.001$.
[****]$P < 0.0001$.

systems (Tauson et al., 1999; Leyendecker et al., 2001, Englmaierová et al., 2014). These contradictory results regarding HDEP might be due to differences in the environments in which they were reared, housing systems (cage/aviary/enriched colony system from different manufacturer), flock management, or laying hen strains used. Yilmaz Dikmen et al., 2016 reported that HDEP was higher in FR than EC, whereas in our study FR and CC had higher production compared to EC. Lower egg production in the FR at the beginning of our study may have been due to stress while shifting the birds from one environment to another and the fact that hens were not completely adapted to the new environment. Between the strains, HB had similar HDEP compared to W-36 in the CC and FR; however, there was a considerable difference in egg production between strains in the EC. The similar HDEP in HB and W-36 hens in CC and FR may be due to intense genetic selection for maximum egg production (Singh et al., 2009). However, the lower egg production in the EC in our study might be due to the hyperactive nature of the W-36 birds leading to injury and stress, thus decreasing egg production. Previously, it has been reported that limited space provided in the EC might not be enough for birds to move freely and they might collide with surrounding wires or perches injuring themselves (Blatchford et al., 2016; Regmi et al., 2016). Blatchford et al. (2016) observed higher keel bone injuries in aviaries and EC than in CC, and Regmi et al. (2016) observed similar keel bone injuries in range and CC. An association between keel bone fracture and egg production was noted previously, where increased physiological stress due to keel bone fracture reduced egg production (Rufener et al., 2019). Küçükyilmaz et al. (2012) reported that laying hen genotype, not housing environment, affects egg production. However, in our study, both housing environment and laying hen genotype influenced egg production.

In this study, the lowest FI was observed in the EC during the late phase of production when compared to CC and FR, which is similar to previous studies (Elson and Croxall, 2006; Neijat et al., 2011). The lower FI in EC may be due to the perches. Utilization of perches in this environment increases roosting behavior, thus decreasing motor activities and increasing resting time (Tauson, 1998; Matsui et al., 2004). Enriched colony cages had greater feeder space than CC and FR, which may have contributed to non-aggressive and non-competitive feeding behavior (Thogerson et al., 2009) and may have contributed to lower FI as well as lower feed waste. Also, an increase in FI for the FR may be due to higher motor activities such as foraging, wing flapping, or jumping to the perches, whereas CC birds may spend more time eating due to fewer motor activities and lower feeder space. Additionally, higher FI in CC might be due to lower stocking density (772 cm^2/bird), which allowed easier access to the feed (Appleby and Hughes, 1991; Jalal et al., 2006). Higher FI observed in HB hens compared to the W-36 in both phases of production in our study can be explained by differences in body weight and egg production (Lacin et al., 2008). In addition, Harms et al. (1982) observed a linear relationship between body weight of Deklab XL hens and FI, where FI increased as body weight increased. The lower FI toward the end of the study might be due to the drop in egg production as energy requirement is directly related with production.

In the early phase of production, FCR was higher in EC and FR compared to that of CC; however, in the late phase of production, CC and FR had lower FCR than that of EC. Feed intake, as well as egg production, is the major factors modulating FCR. Although FI was the same in CC and EC in the early phase of production, egg production was significantly lower in EC, affecting FCR. However, lower egg production and higher FI was observed in FR compared to CC, which may be the reason for differences in FCR during the early phase among treatments. In the late phase of production, FI and egg production was significantly lower in EC as compared to CC and FR, thus yielding the highest FCR for EC. In our study, W-36 had lower FCR compared to HB in the early phase of production, which is similar to the previous finding by Singh et al. (2009), who observed differences in FCR between Lohmann White, Lohmann Brown, H&N White and a non-commercial cross. The differences in FCR among laying hen strains might be due to variation in FI among laying hen strains (Lacin et al., 2008). Although W-36 had lower FI compared to HB, there was no significant difference in FCR in the late phase of production, which might be due to the drastic decrease in egg production by W-36 hens. In all of the housing environments in our study, FI and FCR were higher in both of the laying strains compared to breeder standards (Hy- Line International, 2016). In our research, the reason for higher FI and FCR compared to breeder standards might be due to body weight. Harms et al. (1982) reported that for each 100g increase in body weight, birds might consume 6.5 to 6.8 g more feed per day. In our study, average body weight of both W-36 and HB hens were higher (100−300 g) compared to the guidelines in both phases, which might have yielded increased feed intake. In the present study, it is important to note that the performance of laying hens

Table 9. Effect of housing environment, hen strain, and age of the birds on cloacal and eggshell microbiology (log CFU/mL) in late phase of production (52−85 wk of age).

Treatments	Aerobes cloaca	Aerobes egg	Anaerobes cloaca	Anaerobes egg	Coliforms cloaca	Coliform eggs
Environment						
CC	2.31[b]	2.52[b]	3.76[b]	2.85[b]	0.93[b]	0.89[b]
EC	3.04[b]	2.10[b]	3.53[b]	1.14[c]	1.50[b]	0.60[b]
FR	4.41[a]	5.08[a]	4.62[a]	3.91[a]	2.44[a]	2.95[a]
SEM	0.220	0.224	0.173	0.193	0.230	0.169
Strain						
HB	3.38	3.16	4.21[a]	2.45	1.76	1.51
W-36	3.16	3.33	3.73[b]	2.83	1.49	1.45
SEM	0.180	0.182	0.141	0.157	0.188	0.138
Age						
52 wk	2.92[bcd]	2.92	3.80[bcd]	2.03[b]	0.50[c]	0.00[c]
59 wk	2.71[cd]	2.99	3.08[cd]	2.43[ab]	0.84[c]	0.29[c]
65 wk	2.58[d]	3.40	2.95[d]	3.20[a]	1.22[bc]	2.05[ab]
72 wk	3.65[abc]	2.89	4.31[b]	2.87[ab]	2.23[ab]	1.74[b]
79 wk	4.11[a]	3.76	5.53[a]	2.70[a,b]	2.74[a]	2.10[ab]
85 wk	3.76[ab]	3.51	4.14[bc]	2.59[ab]	2.23[b]	2.73[a]
SEM	0.245	0.251	0.282	0.247	0.257	0.215
Environment × Strain						
CC × HB	2.75	2.50	4.32	2.80	1.30	0.97
CC × W-36	1.88	2.54	3.24	2.90	0.55	0.81
EC × HB	4.40	4.91	4.68	3.63	2.46	3.04
EC × W-36	4.42	5.24	4.56	4.19	2.42	2.86
FR × HB	2.97	2.03	3.66	0.93	1.52	0.53
FR × W-36	3.11	2.17	3.40	1.37	1.49	0.66
SEM	0.311	0.316	0.245	0.273	0.326	0.179
Environment × Age						
CC × 52 wk	1.98[cd]	1.76[cd]	4.09	1.47[d-g]	0.58[c]	0.00[d]
CC × 59 wk	1.88[cd]	2.31[bcd]	2.34	2.96[a-e]	0.00[c]	0.56[cd]
CC × 65 wk	1.64[d]	3.26[a-d]	2.98	3.98[abc]	0.58[c]	1.46[cd]
CC × 72 wk	1.84[cd]	1.15[d]	3.39	2.52[b-f]	0.38[c]	0.28[c]
CC × 79 wk	2.85[a-d]	3.21[a-d]	5.55	2.64[b-e]	2.02[abc]	0.83[cd]
CC × 85 wk	3.98[abc]	3.42[abc]	4.48	3.51[a-d]	1.99[abc]	2.22[bc]
EC × 52 wk	2.48[bcd]	1.76[cd]	3.20	0.31[g]	0.30[c]	0.00[d]
EC × 59 wk	1.96[cd]	1.56[cd]	2.78	0.45[fg]	0.97[bc]	0.00[d]
EC × 65 wk	1.65[d]	1.58[cd]	1.99	1.32[efg]	0.00[c]	0.62[cd]
EC × 72 wk	4.23[ab]	2.14[cd]	4.26	1.23[efg]	3.08[ab]	0.56[cd]
EC × 79 wk	4.81[a]	2.84[bcd]	5.26	1.53[d-g]	3.21[ab]	0.76[cd]
EC × 85 wk	3.09[a-d]	2.66[bcd]	3.67	1.95[c-g]	1.47[abc]	1.67[cd]
FR × 52 wk	4.29[ab]	5.23[a]	4.12	4.18[ab]	0.61[c]	0.00[d]
FR × 59 wk	4.31[ab]	5.11[a]	4.13	3.88[abc]	1.54[abc]	0.32[d]
FR × 65 wk	4.45[ab]	5.21[a]	3.96	4.30[ab]	3.07[ab]	4.09[ab]
FR × 72 wk	4.97[a]	5.25[a]	5.28	4.85[a]	3.22[a]	4.39[a]
FR × 79 wk	4.26[ab]	5.23[a]	5.78	3.94[abc]	2.98[ab]	4.60[a]
FR × 85 wk	4.22[ab]	4.46[ab]	4.41	2.32[b-g]	3.23[a]	4.30[a]
SEM	0.425	0.436	0.491	0.428	0.446	0.373
Strain × Age						
HB × 52 wk	3.26	2.71	4.01	1.82	0.99	0.00
HB × 59 wk	2.94	3.02	3.59	2.34	0.84	0.19
HB × 65 wk	3.05	3.85	3.60	3.10	1.27	2.64
HB × 72 wk	3.56	2.69	4.21	2.69	2.46	1.80
HB × 79 wk	3.92	3.76	5.53	2.45	2.43	1.82
HB × 85 wk	3.63	3.05	4.40	2.31	2.57	2.64
W-36 × 52 wk	2.57	3.13	3.60	2.25	0.00	0.00
W-36 × 59 wk	2.49	2.96	2.58	2.52	0.83	0.40
W-36 × 65 wk	2.11	3.03	2.26	3.31	1.16	1.47
W-36 × 72 wk	3.73	3.08	4.41	3.05	2.00	1.68
W-36 × 79 wk	4.30	3.76	5.54	2.95	3.05	2.40
W-36 × 85 wk	3.88	3.98	3.93	2.88	1.89	2.83
SEM	0.346	0.356	0.399	0.350	0.364	0.305
P-value						
Housing environment	<0.001	<0.0001	0.0003	<0.0001	0.0003	<0.0001
Strain	0.371	0.502	0.021	0.113	0.320	0.742
Age	<0.0001	0.044	<0.0001	0.024	<0.0001	<0.0001
Environment × Strain	0.269	0.901	0.141	0.686	0.462	0.757
Environment × Age	0.0002	0.028	0.337	0.0002	0.0003	<0.0001
Strain × Age	0.315	0.261	0.418	0.992	0.306	0.115
Environment × Strain × Age	0.213	0.251	0.507	0.597	0.655	0.391

Abbreviations: CC, conventional cage; EC, enriched colony cage; FR, free-range; HB, Hy-Line Brown; W-36, Hy-Line W-36.
[a-g]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

raised in CC and FR were similar. However, between the laying hen strains, W-36 performed better compared to HB except for those raised in EC.

In both phases, egg weights were higher in CC than FR, which increased as the birds aged regardless of strain. The results of this study are similar to previous studies where CC had higher egg weights than cage-free aviary or barn systems (Englmaierová et al., 2014). Tumová and Ebeid (2005) and Tůmová et al. (2007) reported that the time of oviposition affects egg weights where eggs laid in the morning are heavier than those laid in the afternoon and egg mass declines with oviposition time. In our study, egg laying patterns were different in different housing environments where CC and EC laid more eggs early in the morning, but FR laid late in the morning (data not shown). Also, HB had higher egg weights compared to W-36 in the early phase of production, but the significant difference in egg weights disappeared in the later phase. The heavier egg in CC may be linked to higher hen body weight in the HB strain.

In the early and late phases of production, albumen percentage was higher in the HB when compared to W-36, whereas yolk percentage was higher in W-36 when compared to HB. The previous study by Leyendecker et al. (2001) observed a difference in albumen percentage and yolk percentage between white and brown hens. In both strains, albumen weight was a major factor determining egg weights. In our study, external egg quality parameters such as specific gravity, eggshell percentage, eggshell thickness, and eggshell breaking strength were affected by both hen strain and housing environment during the late phase of production. Specific gravity, eggshell thickness, eggshell percentage, and eggshell breaking strength were improved in HB when compared to W-36. Previous studies have shown that between the strains, HB have higher calcium intake due to higher FI, thus increasing the rate of calcium deposition leading to better eggshell parameters (Taylor and Martin, 1928; Lichovníková and Zeman, 2008). Similar influences on external egg quality were observed for housing environments. Although eggshell thickness and eggshell percentage were identical between EC and FR, eggshell breaking strength was higher in FR than EC. Ledvinka et al. (2012) reported that housing environment affects the microstructure of the eggshell for higher eggshell breaking strength. Also, Solomon (2010) reported that stress of hens has a negative impact on eggshell thickness and eggshell breaking strength. In our case, despite having the same eggshell thickness between FR and EC, FR had higher eggshell breaking strength which might be due to better utilization and effective metabolism of calcium and phosphorus (Van Den Brand et al., 2004; Lichovníková and Zeman, 2008) . Regardless of housing environment and hen strain, eggshell breaking strength decreased with age of the birds. Rodriguez-Navarro et al. (2002) reported that microstructural crystal orientation changes as hens age, thus negatively affecting eggshell breaking strength. The negative correlation found between egg weight and eggshell breaking strength might have been due to a decrease in eggshell thickness, eggshell percentage, calcium deposition or change in shell ultrastructure as eggs increased in size (Roland et al., 1975; Rodriguez-Navarro et al., 2002). Zita et al. (2009) reported that eggshell quality indices were affected by the genotype of the hens and observed negative correlations between egg weight and shell quality parameters such as eggshell percentage (R = -0.4; P < 0.0001) and eggshell breaking strength (R = -1; P < 0.05).

In the current study, a higher Haugh unit was observed in CC and EC than in FR. The higher Haugh unit from the caged birds may be due to eggs experiencing less exposure to ammonia than FR due to the litter (Benton and Brake, 2000; Robert, 2004; Singh et al., 2009; Ledvinka et al., 2012). Among laying hen strains, there was no significant difference for HU, which was contrary to previous studies by Silversides and Scott (2001), Singh et al. (2009), and Küçükyilmaz et al. (2012). They observed a higher Haugh unit in white egg layers than brown. Also, Haugh unit increased with bird age regardless of the housing environments or hen strain, and these results are similar to Zita et al. (2009). However, previous studies by Doyon et al. (1986), Singh et al. (2009), and Ledvinka et al. (2012) observed that the Haugh unit decreases as age progresses. Williams (1992) reported that albumen quality decreases with increasing age, thus reducing the Haugh unit; but in the present study, we did not observe that trend. The main reason for increasing the HU toward the end of the lay is when hens are gone through molting (Williams, 1992; Robert, 2004). In our case, HDEP was comparatively lower toward the end of the trial, and maybe some birds have already gone through the molt at that age, resulting in increased HU (Williams 1992; Robert, 2004).

In our study, the reason behind higher aerobic cloacal bacteria in the FR housing environment may be because the birds in the FR were allowed in the range every day. The birds in FR may consume soil, forage, or litter, which could change cloacal microflora. Previous studies by Zhao et al. (2013) and Jones and Anderson (2013) found that environmental bacterial load in CC and EC is lower than that of the cage-free aviary, which may alter the hen's microflora. Between hen strains, HB had higher aerobic counts than W-36 in the early phase of the production and higher anaerobic counts in the late phase of production. The difference in the strains may be due to foraging behavior where we observed a larger number of HB hens on the range compared to W-36 hens.

Similar results were observed for eggshell bacterial counts. The bacterial count was mainly affected by housing environment and not by strain. The highest total aerobic, anaerobic, and coliform eggshell bacterial counts were observed in FR. Several previous studies on alternative housing environments observed that cage-free hens had higher eggshell bacterial loads than CC and EC (Buhr et al., 2009; Singh et al., 2009; Holt et al., 2011; Englmaierová et al., 2014). Higher eggshell

bacterial contamination in FR may be due to exposure of eggs to litter. A positive correlation between bacterial concentration in the air and eggshell contamination has been observed (De Reu et al., 2005). Previous studies have observed higher microbial load in the air of cage-free than that of CC and EC, which may increase eggshell microbial load (De Reu et al., 2008; Jones et al., 2015b). The current study did not find any significant differences among the laying hen strains for eggshell microbial load; however, the microbial load was significantly altered by housing environments.

CONCLUSION

In conclusion, both housing environment and laying hen strain have effects on performance, egg quality indices, and cloacal and eggshell microbiology. Hens housed in CC and FR had higher egg production and lower FCR than hens housed in EC. However, most of the measured egg quality parameters for FR were intermediate between CC and EC housing environments. In addition, higher eggshell bacterial contamination was observed in FR compared to CC and EC. Hy-Line Brown hens outperformed W-36 in both egg production and egg quality indices in EC. It can be concluded that the laying hen strain should be considered when deciding on a housing environment to use. Our findings challenge us to further investigate food safety and economic benefits, which are the main factors that need to be considered when deciding on a hen housing environment.

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DISCLOSURES

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