

Final Report for United Sorghum Checkoff Program (USCP)

USCP Contract Number: RG002-21

Project Title: EFFECTS OF REPLACING CORN WITH SORGHUM IN LAYING HEN DIET ON THEIR PERFORMANCE, BEHAVIOR, AND WELFARE

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Project Duration: 2 years (2021-2023)

Technical Objectives: *The long-term goal of the proposed study is to support the sustainability of the grain sorghum market through expanding feed sources for the poultry industry, particularly in value-added markets such as those for organic and G.M. free products. The proposed study will support the poultry industry's sustainability by enhancing the marketing opportunities for grain sorghum varieties by better understanding the nutritional and economic benefits for use and possible impacts in egg production enterprises.*

To date, no study has examined the possible influences and impacts of partial replacement of corn with sorghum with or without supplementation of commercial canthaxanthin-like substance on hen production, health, behavior, and welfare. To help close this gap, our research will scientifically and practically address the possibility of incorporating sorghum in laying hens' diets and possible direct effects to develop solutions that are practical for commercial producers.

- **Objective 1:** Determine the impact of partial replacement of corn with modern sorghum varieties (high and low protein sorghum), either with or without supplementation of commercial canthaxanthin-like substance on pullet (0-17 weeks of age) health and productivity.
 - **1a.** Compare pullets' performance and mortality under different diets
 - **1b.** Use body-worn sensors to record pullets' activity and behavior and assess welfare in response to dietary manipulation.
 - **1c.** Assess the influence of dietary manipulation on pullets' bone health and total blood calcium levels.
- **Objective 2:** Determine the impact of partial replacement of corn with modern sorghum varieties (high and low protein sorghum), either with or without supplementation of commercial canthaxanthin-like substance, on laying hens (18 - 40 weeks of age) health and productivity.
 - **2a.** Determine the influence of dietary interventions on hens' performance, production, health, and mortality.
 - **2b.** Track hens' activity and behavior through body-worn sensors and assess their welfare in response to dietary manipulation.
 - **2c.** Test the influence of dietary interventions on hens' bone and total blood calcium levels.
 - **2d.** Test external and internal egg quality and total antioxidant capacity under different diets.
- **Objective 3:** Determine the economic impact of pullets and laying hens fed selected modern varieties (low protein and high protein U.S. No. 2 yellow) of grain sorghum compared to a standard corn-based diet.

Accomplishments:

Birds, Housing, and Dietary Treatment: A total of 900 HyLine Brown day-old chicks were obtained from a commercial HyLine hatchery, transported, tested, and housed in an environmentally controlled house, set up to include whole-house heating in the Morgan Poultry farm at Clemson University. On the first day, birds were provided with diets containing **sorghum** (50% of the dietary corn was replaced with sorghum) of **2 different protein levels** (**L.S.** "U.S. No. 2 sorghum": low protein content (less than 11%), **H.S.:** high protein content (more than 11%)), and corn-based diet. Sorghum substitutions were combined with **two patterns of canthaxanthin pigment** (**Carophyll®**, **DSM international**) **supplementation (- or +)**, and a **control group (C)** received a corn-soybean meal-based diet either with or without pigment supplementation.

This dietary manipulation resulted in a total of 6 different dietary treatment combinations, as follows; Low Protein Sorghum diets (L.S.), hens received diets containing 50% of corn replaced with **low** protein sorghum either with (**LS+**) or without (**LS**) pigment supplementation. High Protein Sorghum diets (H.S.), hens received diets containing 50% corn replaced with **high** protein sorghum either with (**HS+**), or without (**HS**) pigment supplementation. Corn or Control diets (C), hens received corn-soybean meal-based diets either with (**C+**) or without (**C**) pigment supplementation. Birds were allotted to treatments via a completely randomized design for a 2×2×2 factorial arrangement of treatments with five pen replicates/treatment.

Birds were housed in identical testing pens (~ 3.2 m²) at an initial stocking rate of 30 hens/pen. Each pen contained a commercial tube feeder, a trough drinker, and 5 cm of wood shavings on the floor as litter, **as shown in picture 1**. Each treatment included five replicates (pens), the 30 experimental pens used in this study were divided into five blocks based on location within the test house, with each treatment combination appearing at least once in each block. Chicks will be used for 40 weeks in two phases. A first phase (i.e., **Pullet phase**; 0 to 17 weeks of age [WOA]) and a second phase (i.e., **Laying phase**; 18 to 40 WOA). Whereas during the pullet phase, birds are growing and just beginning to lay eggs, the birds during the laying phase reach maximal egg production, coinciding with an increased nutrient requirement.

Pullet Feeding and lighting regimen: For the first three weeks, the heat was provided by a focal electric brooder per pen in addition to a gas-fired brooder for the entire house. The temperature was initially set at 95-97°F at day 0, then progressively reduced by 4-6°F every week until 3 weeks, as focal brooders were removed at this time. Temperature was reduced weekly until 6 weeks of age to 70 °F, then maintained to the end of the rearing phase (17 weeks), following the standard breed guidelines (Hy-Line, 2022). Feed and water were provided adlibitum. From 0 to 3 weeks, the feed was provided in tube feeders, and water was in gallon drinkers. For the first week of life, supplementary feed trays were provided. After 3 weeks, feed was provided in circular hanging feeders, and water was available in automatic cup drinkers.

The diets were formulated to meet or exceed requirements, following the standard breed guidelines (Hy-Line, 2022-please refer to the research proposal). The hens were fed a mash diet throughout the experiment. The starter 1 diet was given from 0-3 weeks old, and the starter 2 diet was given from 4-6 weeks old, the grower diet was given from 7-15 weeks old, and the pre-lay diet from 15-17 weeks of age (Table L-Appendix). During the pre-lay and pre-peak periods, the hens were fed a "Peak Feed" lay diet. Post-peak feed type was provided based on the average consumption, egg production, and egg weight over the previous four-week period of strain. The diets were formulated to provide the following daily intakes of nutrients at the following stages of production at the various consumption levels (please refer to layer dietary regimen, Table M-Appendix).

Throughout the rearing phase, pullets were provided varying lighting schedules following the recommended lighting protocol to achieve optimum reproduction and growth following the standard HyLine Brown breed guidelines. The light was provided by a single 60-watt incandescent overhead lightbulb per pen, and pens were kept on a decreasing lighting schedule starting at 20L:4D cycle at 1 week old and decreased by increments of either 1.5 or 2 hours until 10L:14D from 7 to 17 weeks old and remained at that ratio for the rest of the trial (40 weeks of age-Table N-appendix).

Data collection:

Performance and production: Body weight (**BW**) and average daily feed intake per bird (**ADFI**) were calculated weekly at weeks 1, 3, 5, 7, 9, 11, 13, 15, and week 17. Feed offered and refused were recorded weekly, and ADFI was calculated; similarly, the body weight of birds was used to calculate average daily body weight gain per bird (**ADWG**) using the following formulas. Pullets per each unit were weighted individually, and the coefficient of variation (CV%) of the body weight (B.W.) of the birds within each unit was used to determine B.W. uniformity. (*Status; performance data obtained and analyzed*).

$$ADFI = \frac{\text{Feed offered} - \text{feed refused}}{\#days \times \#birds} \quad ADWG = \frac{\text{Finish weight} - \text{start weight}}{\#days}$$

Laying phase: Feed offered and refused was recorded at 24, 30, 36, and 40 weeks old for calculation of average daily feed intake (ADFI); moreover, the number of eggs collected was recorded daily. Feed conversion ratio (FCR) and hen-day egg production (HDEP) was calculated using the following formulas.

$$HDEP = \frac{\text{total number of eggs produced in a day}}{\text{total number of hens present}} \times 100\% \quad FCR = \frac{\text{feed intake (g)}}{\text{egg weight (g)}} \times 100$$

Health: On the last day of weeks 11 and 17 for the rearing phase and weeks 36 and 40 for the laying phase, blood samples (1.5 ml) were obtained (**Picture 2**) from the brachial vein of 10 randomly selected hens per treatment (2 hens/unit). Blood samples were centrifuged at 3,000 rpm, at 4°C for 10 minutes. Samples were prepared, and serum glucose, lipid profile, liver and kidney function indicators, and total antioxidant capacity were analyzed (*Status; blood samples obtained and analyzed*).

To determine the influence of the variable feed elements such as fiber and fat content in the implemented dietary intervention, gastrointestinal tract (GIT) traits are currently examined as follows; at 11 and 17 weeks of age for the rearing phase and 40 weeks of age for the laying phase, 2 birds/pen replicate were randomly selected, weighed, and euthanized by cervical dislocation, and the GIT (from the post-crop esophagus to the cloaca, including digesta content, liver, pancreas, and spleen) were removed and weighed. After GIT removal, the pullets were weighed again, and carcass yield (% B.W., including the head, neck, and feet) was calculated. Then, the liver, full proventriculus, and gizzard were carefully excised and weighed. The pH of the gizzard contents was measured in duplicate in situ using a digital pH meter (**Picture 3**). The proventriculus and gizzard were emptied from any digesta, cleaned, dried with desiccant paper, and weighed again, and the fresh digesta content of the 2 organs was calculated as the difference between the full and empty organ weight and expressed relative (i.e., percent) to full organ weight. In addition, the length of the small intestine (duodenum, jejunum, and ileum) from the gizzard to the ileocecal junction and of the 2 ceca (from the ostium to the tip of the ceca) were measured on a glass surface using a flexible tape and expressed relative length to full B.W. (*Status; birds were euthanized, dissected and analysis was conducted*).

Behavior tracking: The behavior of the birds was video recorded for 72 continuous hours per week during weeks 5, 8, 11, 14, and 17 for the pullet phase and weeks 24, 30, 36, and 40 for the laying phase using ceiling-mounted cameras (**Picture 4**). Digital video recordings were coded using instantaneous scan sampling at 15 min time intervals. Behavioral time budgets (i.e., eat, drink, walk, preen, forage, dust bathe, agonistic interactions, stand, sit, and rest) for each treatment were obtained by calculating the percentage representing the average frequency of each behavior performed per 15-min interval, which will be averaged over the 23-hour of photoperiod per day, and then over the 72-hour of video recording (*Status; video recording obtained, and decoding was finalized*).

Bird kinematic activity: bird activity was measured during behavior observations; fifteen hens per treatment (3 hens/pen) were fitted with activity monitors (HOBO Pendant G; **Picture 5**), (*Status; activity data obtained, and analysis was conducted*).

Hen Welfare: at the end of each behavior observation period, 10% of hens within each unit (including the individually tracked birds) were assessed for basic physical health and well-being parameters following a modified version of the Welfare Quality® scoring system (*Status; Welfare data obtained, and analysis is ongoing*).

In-vivo digital radiography: on the last day of weeks 11 and 17 for the pullet phase, lateral digital radiographs of the keel bone and femora were acquired for 10 randomly selected hens per treatment (2 hens/unit; **Picture 6**). Radiographs were evaluated using image analysis freeware (<https://horosproject.org/>): number of keel bone and femoral fractures, and the proportion of deviated keel bone area (*Status; radiographs obtained and analyzed*).

Bone computed tomography: Immediately after euthanasia, birds were scanned by an imaging technician under the supervision of a board-certified veterinary radiologist. Scans were acquired using a multi-slice C.T. scanner at the Godley Snell Research Center (Toshiba Aquilion 16-slice, Universal Medical Systems). A calibration phantom with known density values was included in the scans (**Picture 7**). Volumetric scan data were acquired for each specimen using a 0.625 mm slice thickness and a standard algorithm. The DICOM format images for each scan will be analyzed in CoPi: Jones' research laboratory using the lab's established methodologies, and the following values will be measured for each bone specimen using a standardized image analysis protocol; Total bone length of the tibia, cortical thickness and density, values will be measured in triplicate, and inter-observer repeatability will be determined for C.T. measures (*Status; scans obtained, and analyzed*).

Bone mechanical properties: bone samples were collected from a total of 10 birds per treatment of the previously mentioned euthanized birds for GIT traits testing. Euthanized hens will be dissected, and pectoralis muscles containing keel bone, both legs including the thigh region, and drumsticks containing femora and tibiae will be amputated, labeled, and frozen at -20°C for further analysis. Tibia length will be measured using a digital caliper, and weight was obtained on an electronic scale. Fracture resistance and deformity will be analyzed using a 3-point bending setup (**Picture 8**; *Status; samples were obtained, and analysis was conducted*).

Bone mineral analysis: Left tibiae were placed in soxhlet for ether extraction of lipids. Ether-extracted bones were further dried at 105°C for 24 h in a forced-air oven and then weighed. Finally, bones were placed into an ash oven at 600°C for 6 h, and ash weights were determined (*Status; analysis was conducted*).

Egg Quality: The quality of egg components was evaluated once a week at weeks 18, 24, 30, 36, and 40 of the bird's age. During these weeks, all eggs from each treatment will be collected, identified, and weighed on an electronic scale. From these, 50 eggs will be selected per treatment for assessment of quality and characteristics (avoiding broken, cracked, or dirty eggs). Eggs were collected and immediately transported to the lab on flats labeled according to pen (**Picture 9**). Eggs were sorted and candled for cracks and dirt spots. Each egg was first weighed using the scale on the EggAnalyzer® (EA27432021, ORKA Food Technology, West Bountiful, Utah, USA). The egg was placed on a spring cradle and measured for breaking strength on the Egg Force Reader (EF06762021, ORKA Food Technology, West Bountiful, Utah, USA). The egg was cracked open, and internal contents were placed on the circular tray in the Egg Analyzer. The Egg Analyzer measured albumin weight, yolk color, and Haugh units. Egg yolks were extracted and weighed separately. Eggshells were placed on labeled flats to dry for approximately 48 hours, and 3 separate locations were measured for weight and thickness using a thickness gauge (Mitutoyo 547-500S, Mitutoyo Corp., Kawasaki, Kanagawa, Japan).

Data analysis:

Data from performance, blood analyses, behavioral observations, radiographs, C.T. scans, and bone health were analyzed using GLMM with treatment and week of age as fixed effects and unit, day of observation, and individual birds as random effects (α set at 0.05) with R software. Statistically significant effects in all models were further analyzed with Tukey's honestly significant difference (HSD) multiple comparison procedures. On the other hand, acceleration/activity data were post-processed using MATLAB (MATLAB and Statistics Toolbox Release 2012, The MathWorks, Inc., Natick, MA). Data smoothing included passing of the raw acceleration values through

an asymmetrical 3-point-moving average low-pass filter and through a step function to define thresholds used to remove minor fluctuations (threshold values of minor fluctuations, i.e., between 0.001 to 0.043 g).

Outcomes:

I. Rearing phase:

In the rearing phase, we fully investigated the possible influences and impacts of partial replacement of corn with sorghum, either with or without supplementation of commercial canthaxanthin-like substance, on pullet production, health, behavior, and activity.

Performance: Birds across treatments showed no statistically significant differences in the average bird body weight, average daily weight gain, and average daily feed intake (Table 1-3, Figures 1-3). Moreover, by comparing bird's performance (B.W.- ADFI) across weeks and dietary treatments to the standard breed guidelines (Hy-Line Brown -2022), birds across treatments and weeks were either within or exceeded the expected standard breed ranges of B.W. and ADFI (Tables 1-3). Finally, Uniformity was calculated at 6, 8, 10, 12, and 14 weeks of age, and no significant differences were observed in flock uniformity for any of the ages evaluated ($P > 0.05$; for more details, please refer to the Appendix Table X - shows raw data of individual bodyweight and coefficient of variation% grouped by dietary treatment/pen).

Health: Results of antioxidant capacity in serum are presented in Table 4. At 11 and 17 weeks old, TAC levels were significantly higher in the HS/HS+ and LS+, and C+ groups ($p=0.01$) compared to C hens. MDA levels were significantly lower in the HS/HS+, LS/LS+, and C+ groups compared to the C group. However, no significant differences were recorded in blood cholesterol, triglycerides, or HDL between treatment and/or across weeks of treatments (Table 4). Although no significant differences were recorded between treatments and across weeks in blood levels of glucose, calcium, urea, and creatinine, blood proteins were significantly ($p=0.01$) higher in HS/HS+ treatments compared to other diets across weeks (Table 5). Finally, concentrations of ALT and AST were significantly lower in canthaxanthin treatments (C+, LS+, HS+) than in C, HS, and LS groups at both ages ($p=0.01$; Table 5). Upon comparing the gastrointestinal traits across dietary treatments and weeks of the trial, no significant differences were recorded in the entire length of GIT, SI, ceca, or gizzard pH (Table 6), and no significant differences were recorded in the weight of GIT, liver, spleen, proventriculus and gizzard across treatments as shown in table 7.

Bone Health: The cortical bone mineral densities in HS/HS+ and LS/LS+ treatments at week 17 were significantly higher than in control groups ($p=0.01$; Table 8), while no significant differences were recorded for the total and medullary Tibiotarsal cross-sectional area and mineral density between treatments and across weeks of treatments (Table 8). Similarly, at week 17, tibiotarsae of HS/HS+ and LS/LS+ birds showed more strength and required heavier loads (N) to break compared to other CON treatments, while no significant differences were observed in bone stiffness, bending points, and ash weight across treatments and weeks (Table 9).

Behavior and Activity: Replacing corn with sorghum did not impact the bird's behavior or activity throughout the weeks of testing in the rearing phase. Pullets across treatments and weeks showed a stable and uniform pattern of instinct behaviors such as eating, drinking, dustbathing, and resting (Table 10), compared to the previously reported behavioral repertoire of the genetic strain. Similarly, patterns of daily activity recorded via triaxial accelerometers showed consistent levels of activity between treatments and weeks of age (Table 11).

Conclusion: Birds fed either low or high-protein sorghum-based diets (50% replacement of dietary corn content) showed similar or even higher performance parameters (body weight and feed intake), higher blood proteins and antioxidant levels, and better Tibiotarsal bone when compared to birds fed corn-based diets across the 17 weeks of the rearing phase). Based on the current findings, the partial replacement of corn with sorghum, either with or without supplementation of canthaxanthin, did not adversely impact growth performance, health, bone, behavior, and welfare of brown egg-type pullets.

II. Laying phase

In the rearing phase, we fully investigated the possible influences and impacts of partial replacement of corn with sorghum, either with or without supplementation of commercial canthaxanthin-like substance, on hen's egg production, health, behavior, and activity.

Performance and Egg Quality: Hens across treatments and weeks (Weeks 24, 30, 36, and 40) showed no statistically significant differences in the average daily feed intake (g/hen), FCR (g feed/g egg), and the Hen-Day egg production (Table 11). However, both HS and LS treatments with or without pigment improved egg quality by significantly increasing the egg albumen weight and Haugh unit ($P<0.05$; Table 12), compared to C treatments across all weeks of the lay phase (24 - 40). Yolk color was significantly impacted by replacing corn with sorghum (HS and LS) compared to C treatment ($P<0.05$; Table 12); however, yolk color was significantly improved by pigment inclusion HS+, LS+, and C+ compared to C, HS, and LS ($P<0.05$). For external egg quality, the dietary interventions did not affect shell thickness, eggshell weight, and shell-breaking strength (Table 13).

Health: Results of antioxidant capacity in serum are presented in Table 14. At 36 and 40 weeks old, TAC levels were significantly higher in the HS+, LS+, and C+ groups ($p=0.01$) compared to C, HS, and LS hens. MDA levels were significantly lower in the HS/HS+, LS+, and C+ groups compared to the C, and LS groups. However, no significant differences were recorded in blood cholesterol, triglycerides, or HDL between treatment and/or across weeks of treatments (Table 14). Although no significant differences were recorded between treatments and across weeks in blood levels of glucose, calcium, urea, and creatinine, blood proteins were significantly ($p=0.01$) higher in HS/HS+ and LS/LS+ treatments compared to Control diets across weeks (Table 15). Finally, no significant differences were recorded in the ALT and AST concentrations (Table 15). Upon comparing the gastrointestinal traits across dietary treatments and weeks of the trial, no significant differences were recorded in the entire length of GIT, SI, ceca, or gizzard pH (Table 16), and no significant differences were recorded in the weight of GIT, liver, spleen, proventriculus and gizzard across treatments as shown in table 17.

Bone Health: The total and cortical bone mineral densities in HS/HS+ and LS/LS+ treatments at week 40 were significantly higher than in control groups ($p=0.01$; Table 18), while no significant differences were recorded for the total and medullary Tibiotarsal cross-sectional area and the medullary mineral density between treatments (Table 18). Similarly, at week 17, tibiotarsae of HS/HS+ and LS/LS+ birds showed more stiffness (N/mm) and required heavier loads (N) to break compared to other CON treatments, while no significant differences were observed in the maximum bending points, and ash weight across treatments (Table 19).

Behavior and Activity: Replacing corn with sorghum did not impact the bird's behavior or activity throughout the weeks of testing during the lay phase. Hens across treatments and weeks showed a stable and uniform pattern of instinct behaviors such as eating, drinking, dustbathing, and resting (Table 20), compared to the previously reported behavioral repertoire of the genetic strain. Similarly, patterns of daily activity recorded via triaxial accelerometers showed consistent levels of activity between treatments and weeks of age (Table 21).

Conclusion: Birds fed either low or high-protein sorghum-based diets (50% replacement of dietary corn content) showed similar performance parameters (egg production, feed conversion ratio, and average daily feed intake), better internal egg quality in terms of albumen weight, and haugh unit, higher blood proteins and antioxidant levels, and better Tibiotarsal bone health when compared to birds fed corn-based diets across the lay phase 17-40 weeks of age). Although replacing corn with sorghum impacted the yolk color compared to the corn-based diet, the inclusion of the Canthaxanthin-like pigment significantly improved the yolk color to exceed the yolk-color quality of the C group. Based on the current findings, the partial replacement of corn with sorghum, either with or without supplementation of canthaxanthin, did not adversely impact growth performance, health, bone, behavior, and welfare of brown egg-type pullets.

Impacts: Outcomes of this project offered to the egg industry a practical, applicable, and integrated solution for improving nutritional strategies of both pullets, and hens (HyLine Brown) during the rearing and lay phases through testing dietary implementation strategies of modern sorghum varieties without impacting production, health, and welfare. This trial helps in supporting the sustainability of the grain sorghum market by expanding feed sources for the poultry industry, particularly in value-added markets such as those for organic and G.M. free products. The outcomes of this project have been discussed with the advisory committee. The outcomes of this project will be presented in poultry-focusing scientific meetings and will be submitted for publishing in specialty peer-reviewed journals.

Alexa Johnson, Gracie Anderson, Santiago Sasia, Mireille Arguelles-Ramos, Ahmed Ali: Effect of the inclusion of U.S. grain sorghum varieties and canthaxanthin on growth performance of brown egg-type pullets. Poultry Science Association Meeting, 2022.

Santiago Sasia, Alexa Johnson, Gracie Anderson, Mireille Arguelles-Ramos, Ahmed Ali: Effect of the inclusion of U.S. grain sorghum varieties and canthaxanthin on growth performance of brown egg-type pullets. Journal of Applied Poultry Science (submitted).

Alexa Johnson, Gracie Anderson, Mireille Arguelles-Ramos, Ahmed Ali: Effect of the inclusion of U.S. grain sorghum varieties and canthaxanthin on egg production and bone health of brown egg-type hens (in-prep).

Leveraged Funding

- DSM has generously donated two 50-kg packs of Carophyll yellow and Red to support pigment inclusion in the current trial.
- Godly Snell, Clemson University, agreed to support the current trial by conducting whole-body computed tomography at no cost for ~230 birds.
- A complete Egg quality assessment device set was purchased through a recently funded USDA project (P.I.: Ali) and was used for the automatic assessment of egg quality for the Sorghum trial.
- HyLine has generously donated 950 day-old chicks (HyLine Brown) to support the trail.

Visuals (Figures and Tables) - Rearing Phase

I. The influence of dietary treatments on pullet performance.

I.1. Dietary treatments and average bird body weight across weeks of the rearing phase (Fig. 1).

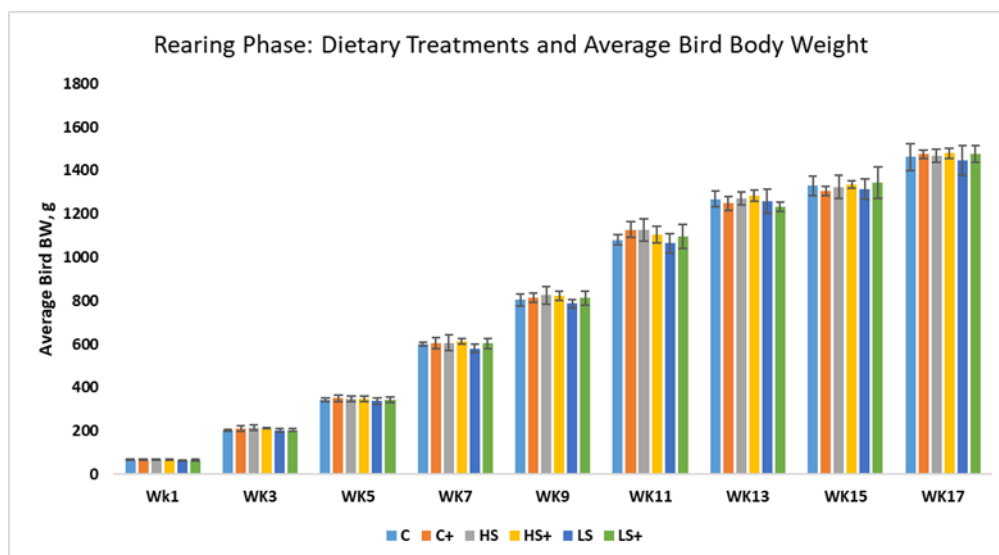


Figure 1: Average Bird Body Weight (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). All parameters are expressed as Mean $\pm$ SEM. Different superscripts if any, indicate statistically significant differences ($P < 0.05$) among different treatments

I.1. Dietary treatments and average bird body weight across weeks of rearing phase in comparison with standard breed bodyweight recommendation (Table 1).

	Wk1	WK3	WK5	WK7	WK9	WK11	WK13	WK15	WK17
C	66.75	202.93	344.27	599.43	803.09	1,080.38	1,268.30	1,329.54	1,461.72
C+	67.57	212.08	350.55	603.64	812.12	1,127.39	1,247.89	1,305.18	1,474.10
HS	67.43	216.87	347.14	605.82	825.47	1,125.83	1,271.43	1,324.27	1,466.21
HS+	67.23	212.98	346.65	612.33	821.94	1,103.47	1,282.51	1,335.45	1,478.91
LS	64.09	201.56	338.68	580.71	785.98	1,064.61	1,257.82	1,314.71	1,445.70
LS+	65.16	203.83	344.73	602.63	812.69	1,096.50	1,232.32	1,343.75	1,476.26
Min*	68	170	330	520	730	940	1,110	1,260	1,400
Max*	72	190	360	560	780	960	1,165	1,300	1,480

Table 1: Average Bird Body Weight (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). *Min and Max, indicate the minimum and maximum expected ranges of bird body weight per week of age as per the standard breed guidelines (Hy-Line Brown; https://hylinena.com/wp-content/uploads/2019/10/Brown_Alt_English.pdf).

I.2. Dietary treatments and average daily weight gain across weeks of the rearing phase (Figure 2, and Table 2).

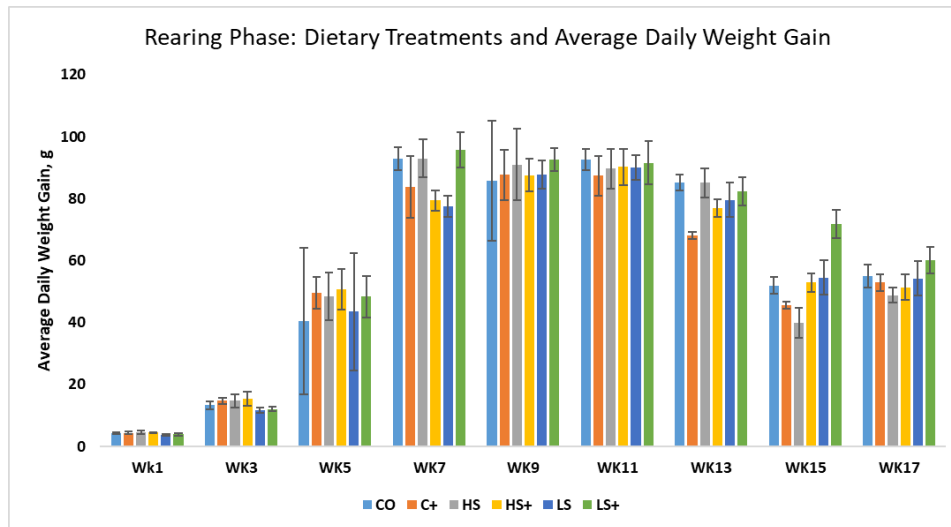


Figure 2:: Average Daily Weight Gain (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). All parameters are expressed as Mean $\pm$ SEM. Different superscripts if any, indicate statistically significant differences ($P < 0.05$) among different treatments.

	WK1	WK3	WK5	WK7	WK9	WK11	WK13	WK15	WK17
CO	4.23	13.38	40.52	93.01	85.66	92.68	85.16	52.01	55.09
C+	4.42	14.78	49.59	83.71	87.68	87.42	68.14	45.58	52.89
HS	4.57	14.70	48.38	93.01	90.95	89.63	85.08	39.84	48.82
HS+	4.49	15.46	50.80	79.43	87.58	90.29	76.89	52.93	51.41
LS	3.82	11.74	43.54	77.43	87.67	90.03	79.63	54.56	54.21
LS+	3.93	12.02	48.38	95.78	92.62	91.57	82.34	71.83	60.10

Table 2: Average Daily Weight Gain (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

I.3. Dietary treatments and average daily feed intake/bird across weeks of the rearing phase (Fig. 3).

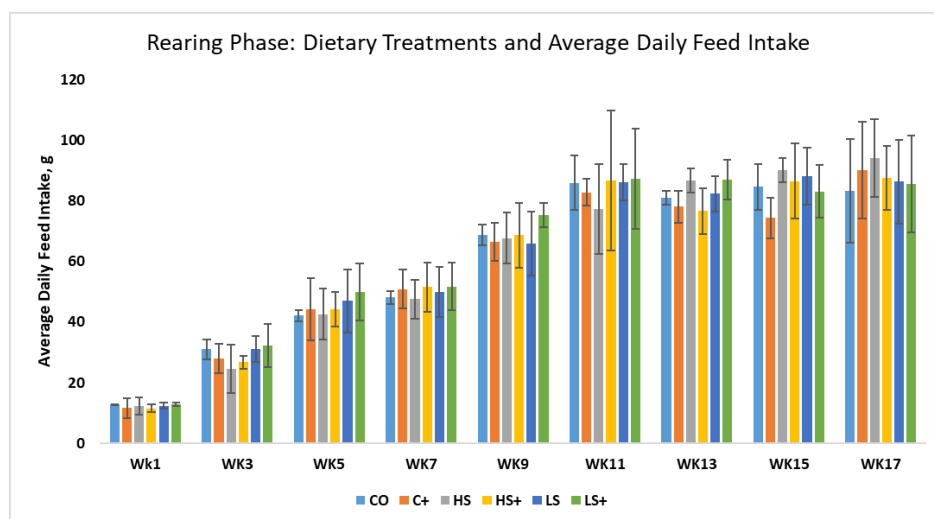


Figure 3: Average Daily Feed Intake (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). All parameters are expressed as Mean $\pm$ SEM. Different superscripts if any, indicate statistically significant differences ($P < 0.05$) among different treatments.

I.3. Dietary treatments and average daily feed intake/bird (g) across weeks of rearing phase in comparison with standard average daily feed intake/bird (g) recommendation (Table 3).

	Wk1	Wk3	Wk5	Wk7	Wk9	Wk11	Wk13	Wk15	Wk17
CO	12.76	30.97	42.11	48.14	68.65	85.93	80.96	84.61	83.25
C+	11.61	28.04	44.26	50.86	66.44	82.73	78.03	74.33	90.08
HS	12.31	24.63	42.63	47.55	67.71	77.30	86.67	90.11	93.97
HS+	11.60	26.70	44.14	51.52	68.66	86.63	76.56	86.43	87.53
LS	12.42	31.16	47.03	49.84	65.78	86.09	82.25	88.04	86.29
LS+	12.87	32.37	49.89	51.74	75.34	87.14	86.98	83.07	85.57
Min*	14	23	34	41	49	58	67	72	78
Max*	15	25	36	43	53	62	71	76	82

Table 3: Average Daily Feed Intake/Bird (g) across weeks (week 1 to 17), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). *Min and Max, indicate the minimum and maximum expected ranges of Average Daily Feed Intake/Bird per week of age as per the standard breed guidelines (Hy-Line Brown; https://hylinena.com/wp-content/uploads/2019/10/Brown_Alt_English.pdf).

II. The influence of dietary treatments on pullet health.

II.1. The influence of dietary treatments on blood lipids and antioxidant capacity indicators (Table 4).

		TAC (mmol/l)	MDA (nmol/mg)	SOD (U/mg)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL (mmol/L)
CO	WK11	7.1 ± 0.52 ^b	19.8 ± 1.23 ^a	260.1 ± 25.66 ^b	4.3 ± 0.56	35.1 ± 4.63	1.3 ± 0.12
	WK17	6.9 ± 0.16 ^b	21.3 ± 1.63 ^a	274.2 ± 36.52 ^b	4.9 ± 0.69	39.6 ± 6.63	1.2 ± 0.15
C+	WK11	14.6 ± 1.23 ^a	11.6 ± 1.03 ^b	289.9 ± 36.96 ^a	3.9 ± 0.96	23.4 ± 3.96	3.0 ± 0.69
	WK17	13.8 ± 1.63 ^a	13.5 ± 1.1 ^b	291.6 ± 43.95 ^a	4.3 ± 1.02	25.7 ± 2.85	2.6 ± 0.52
HS	WK11	13.6 ± 2.36 ^a	12.6 ± 1.69 ^b	299.8 ± 33.63 ^a	3.8 ± 0.36	24.6 ± 1.6	2.8 ± 0.36
	WK17	15.3 ± 2.63 ^b	11.6 ± 1.89 ^b	297.5 ± 56.36 ^a	4.1 ± 0.88	26.5 ± 1.66	3.1 ± 0.52
HS+	WK11	19.9 ± 2.03 ^a	7.5 ± 1.02 ^c	322.2 ± 45.32 ^a	3.6 ± 0.55	22.4 ± 1.03	3.2 ± 0.89
	WK17	20.5 ± 2.01 ^a	7.1 ± 1.11 ^c	316.5 ± 55.56 ^a	3.7 ± 0.78	20.5 ± 2.08	3.7 ± 0.58
LS	WK11	9.6 ± 1.06 ^b	14.6 ± 1.99 ^b	296.5 ± 63.69 ^a	4.1 ± 0.96	33.5 ± 2.25	1.9 ± 0.16
	WK17	9.8 ± 2.63 ^b	13.5 ± 2.85 ^b	289.5 ± 56.89 ^a	4.7 ± 1.03	36.8 ± 3.89	1.6 ± 0.22
LS+	WK11	14.6 ± 1.88 ^a	12.6 ± 2.03 ^b	311.6 ± 45.69 ^a	3.8 ± 0.85	31.6 ± 3.58	2.1 ± 0.15
	WK17	16.6 ± 1.98 ^a	9.9 ± 1.68 ^b	309.8 ± 43.85 ^a	3.9 ± 0.78	30.8 ± 2.89	2.3 ± 0.19

Table 4: The influence of dietary treatments on blood lipids and antioxidant capacity indicators treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). Antioxidant capacity parameters found in serum: TAC; total antioxidant capacity, MDA; malondialdehyde, SOD; superoxide dismutase, GSH-Px; glutathione peroxidase, HDL; High-Density Lipoproteins. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$)

II.2. The influence of dietary treatments on blood metabolites, liver and kidney function indicators (Table 5)

		Glucose mg/dL	Protein g/dL	Calcium mmol/L	Urea mmol/L	Creatinine	ALT (IU)	AST (IU)
CO	WK11	137.5 ± 22.52	3.5 ± 0.85 ^c	3.7 ± 0.75	5.4 ± 0.89	0.4 ± 0.06	39.9 ± 3.69 ^a	122.6 ± 19.63 ^a
	WK17	184.2 ± 32.63	3.6 ± 0.52 ^b	4.2 ± 0.78	5.7 ± 0.96	0.5 ± 0.07	33.6 ± 5.63 ^a	122.6 ± 21.06 ^a
C+	WK11	146.3 ± 36.52	3.6 ± 0.78 ^b	3.6 ± 0.45	4.3 ± 0.76	0.4 ± 0.09	20.4 ± 4.5 ^b	86.1 ± 12.85 ^b
	WK17	176.9 ± 34.52	3.7 ± 0.78 ^b	3.9 ± 0.26	5.3 ± 0.89	0.4 ± 0.09	22.8 ± 3.96 ^b	60.3 ± 30.25 ^b
HS	WK11	175.6 ± 33.56	6.9 ± 1.2 ^a	4.1 ± 0.85	5.3 ± 0.78	0.4 ± 0.03	37.9 ± 2.36 ^a	176.9 ± 22.3 ^a
	WK17	198.5 ± 42.36	7.8 ± 1.03 ^a	4.3 ± 0.96	5.6 ± 0.96	0.5 ± 0.04	36.8 ± 3.6 ^a	181.9 ± 16.98 ^a
HS+	WK11	169.9 ± 32.36	7.1 ± 1.8 ^a	4.2 ± 0.69	4.9 ± 0.85	0.4 ± 0.09	15.6 ± 1.6 ^b	66.9 ± 13.63 ^b
	WK17	201.8 ± 22.96	7.6 ± 1.06 ^a	4.1 ± 0.75	4.8 ± 0.85	0.4 ± 0.05	14.8 ± 1.03 ^b	71.9 ± 14.52 ^b
LS	WK11	133.5 ± 30.23	3.5 ± 1.03 ^b	4.0 ± 0.63	5.6 ± 1.03	0.4 ± 0.03	33.2 ± 3.36 ^a	101.6 ± 32.3 ^a
	WK17	188.5 ± 22.52	3.9 ± 1.06 ^b	3.9 ± 0.26	5.9 ± 0.96	0.5 ± 0.05	41.9 ± 3.99 ^a	143.6 ± 33.69 ^a
LS+	WK11	141.4 ± 55.63	3.5 ± 1.08 ^b	3.8 ± 0.16	5.2 ± 0.91	0.4 ± 0.06	16.8 ± 2.6 ^b	80.6 ± 22.68 ^b
	WK17	191.8 ± 44.56	3.5 ± 0.99 ^b	3.7 ± 0.22	5.1 ± 0.75	0.4 ± 0.09	13.5 ± 4.52 ^b	78.4 ± 16.63 ^b

Table 5: The influence of dietary treatments on blood metabolites, liver and kidney function indicators (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). ALT; alanine transferase, AST; aspartate transferase. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$).

III.1. Gastrointestinal traits of pullets' organ lengths at 11 and 17 weeks of age (Table 6).

		GIT (cm)	%GIT	SI (cm)	%SI	μ ceca (cm)	%Ceca	μ Gizzard pH
CO	WK11	143.1 $\pm$ 1.6	12.1 $\pm$ 1.5	127.6 $\pm$ 3.3	11.2 $\pm$ 1.3	13.3 $\pm$ 1.6	1.2 $\pm$ 0.3	4.8 $\pm$ 1.2
	WK17	158.6 $\pm$ 2.3	10.6 $\pm$ 1.2	141.6 $\pm$ 2.6	9.3 $\pm$ 1.1	15.6 $\pm$ 1.5	1.1 $\pm$ 0.1	5.4 $\pm$ 1.0
C+	WK11	141.2 $\pm$ 1.9	12.8 $\pm$ 1.0	129.9 $\pm$ 4.5	11.6 $\pm$ 1.3	13.6 $\pm$ 1.5	1.2 $\pm$ 0.6	4.3 $\pm$ 1.0
	WK17	159.9 $\pm$ 1.0	11.2 $\pm$ 1.0	145.9 $\pm$ 3.9	9.1 $\pm$ 0.9	14.9 $\pm$ 1.8	1.1 $\pm$ 0.5	5.3 $\pm$ 1.2
HS	WK11	144.5 $\pm$ 1.9	13.5 $\pm$ 2.0	130.8 $\pm$ 2.9	12.1 $\pm$ 1.2	12.9 $\pm$ 1.6	1.3 $\pm$ 0.5	4.8 $\pm$ 1.0
	WK17	155.3 $\pm$ 1.8	12.8 $\pm$ 2.0	140.8 $\pm$ 5.6	9.6 $\pm$ 1.1	15.2 $\pm$ 1.8	1.2 $\pm$ 0.2	5.4 $\pm$ 1.0
HS+	WK11	144.2 $\pm$ 1.0	14.1 $\pm$ 1.8	127.8 $\pm$ 4.6	11.9 $\pm$ 1.3	13.5 $\pm$ 1.8	1.4 $\pm$ 0.5	4.7 $\pm$ 1.1
	WK17	151.6 $\pm$ 1.0	13.3 $\pm$ 1.5	148.9 $\pm$ 3.9	9.7 $\pm$ 1.01	14.9 $\pm$ 1.5	1.2 $\pm$ 0.1	5.7 $\pm$ 1.0
LS	WK11	141.8 $\pm$ 1.1	11.8 $\pm$ 1.8	129.8 $\pm$ 1.8	12.0 $\pm$ 1.8	12.7 $\pm$ 1.2	1.3 $\pm$ 0.5	4.1 $\pm$ 1.9
	WK17	152.6 $\pm$ 1.8	12.8 $\pm$ 1.0	139.8 $\pm$ 2.9	9.5 $\pm$ 1.0	14.5 $\pm$ 1.6	1.2 $\pm$ 0.2	5.3 $\pm$ 1.0
LS+	WK11	146.6 $\pm$ 2.8	12.8 $\pm$ 1.8	130.7 $\pm$ 3.9	11.8 $\pm$ 1.1	13.9 $\pm$ 1.8	1.3 $\pm$ 0.5	4.1 $\pm$ 0.9
	WK17	158.8 $\pm$ 3.0	10.8 $\pm$ 1.8	147.2 $\pm$ 2.7	9.1 $\pm$ 1.1	15.9 $\pm$ 1.8	1.2 $\pm$ 0.2	5.0 $\pm$ 1.3

Table 6: Gastrointestinal traits of organ lengths. Results presented as mean $\pm$ standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). Percentages expressed as cm of organ per gram of body weight. GIT, gastrointestinal tract; SI, small intestine. μ = average. (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

III.2. Gastrointestinal traits of pullets' organ weights at 11 and 17 weeks of age (Table 7).

		Entire GIT (g)	% Entire GIT	Liver (g)	% Liver	Spleen (g)	% Spleen	PROV empty (g)	% PROV empty	Gizzard empty (g)	% Gizzard empty
CO	WK11	107.1 $\pm$ 28.6	13.1 $\pm$ 0.4	27.9 $\pm$ 2.1	2.6 $\pm$ 0.4	3.8 $\pm$ 0.5	0.34 $\pm$ 0.03	6.4 $\pm$ 0.6	0.56 $\pm$ 0.04	42.6 $\pm$ 2.4	3.7 $\pm$ 0.2
	WK17	207.1 $\pm$ 36.5	13.7 $\pm$ 0.3	36.5 $\pm$ 1.9	2.4 $\pm$ 0.2	4.3 $\pm$ 0.6	0.28 $\pm$ 0.01	9.1 $\pm$ 0.8	0.58 $\pm$ 0.03	49.6 $\pm$ 2.6	3.8 $\pm$ 0.3
C+	WK11	108.5 $\pm$ 31.2	13.2 $\pm$ 0.2	27.6 $\pm$ 1.5	2.5 $\pm$ 0.3	3.9 $\pm$ 0.5	0.31 $\pm$ 0.02	6.5 $\pm$ 1.3	0.55 $\pm$ 0.03	43.5 $\pm$ 2.9	3.6 $\pm$ 0.2
	WK17	210.5 $\pm$ 37.8	13.6 $\pm$ 0.5	37.2 $\pm$ 1.6	2.4 $\pm$ 0.1	4.2 $\pm$ 0.2	0.29 $\pm$ 0.01	9.6 $\pm$ 0.9	0.59 $\pm$ 0.06	49.8 $\pm$ 2.6	3.9 $\pm$ 0.6
HS	WK11	111.3 $\pm$ 36.6	13.4 $\pm$ 0.5	26.9 $\pm$ 1.3	2.5 $\pm$ 0.4	3.7 $\pm$ 0.3	0.32 $\pm$ 0.03	6.5 $\pm$ 0.8	0.54 $\pm$ 0.01	45.8 $\pm$ 2.8	3.6 $\pm$ 0.4
	WK17	216.8 $\pm$ 52.6	13.8 $\pm$ 0.4	34.8 $\pm$ 1.9	2.5 $\pm$ 0.2	4.6 $\pm$ 0.6	0.26 $\pm$ 0.02	9.5 $\pm$ 0.8	0.59 $\pm$ 0.05	48.8 $\pm$ 3.1	3.8 $\pm$ 0.6
HS+	WK11	115.8 $\pm$ 34.5	13.1 $\pm$ 0.2	27.2 $\pm$ 1.8	2.4 $\pm$ 0.1	3.6 $\pm$ 0.4	0.36 $\pm$ 0.06	6.9 $\pm$ 0.8	0.54 $\pm$ 0.03	46.8 $\pm$ 2.7	3.7 $\pm$ 0.5
	WK17	209.8 $\pm$ 54.8	13.7 $\pm$ 0.5	36.8 $\pm$ 1.5	2.6 $\pm$ 0.4	4.5 $\pm$ 0.3	0.27 $\pm$ 0.03	9.4 $\pm$ 0.2	0.58 $\pm$ 0.06	46.8 $\pm$ 3.8	4.0 $\pm$ 0.6
LS	WK11	109 $\pm$ 38.9	13.2 $\pm$ 0.1	28.1 $\pm$ 1.3	2.3 $\pm$ 0.5	3.5 $\pm$ 0.5	0.35 $\pm$ 0.05	6.7 $\pm$ 0.6	0.56 $\pm$ 0.06	46.8 $\pm$ 2.6	3.7 $\pm$ 0.2
	WK17	212.5 $\pm$ 52.6	13.8 $\pm$ 0.2	37.5 $\pm$ 1.6	2.6 $\pm$ 0.2	4.7 $\pm$ 0.6	0.28 $\pm$ 0.08	9.3 $\pm$ 0.8	0.59 $\pm$ 0.09	47.8 $\pm$ 3.5	3.8 $\pm$ 0.3
LS+	WK11	113.5 $\pm$ 37.8	13.3 $\pm$ 0.3	27.6 $\pm$ 1.1	2.4 $\pm$ 0.6	3.6 $\pm$ 0.2	0.38 $\pm$ 0.06	6.8 $\pm$ 0.4	0.58 $\pm$ 0.06	47.8 $\pm$ 2.7	3.8 $\pm$ 0.2
	WK17	218.5 $\pm$ 45.5	13.9 $\pm$ 0.5	36.9 $\pm$ 1.9	2.6 $\pm$ 0.5	4.8 $\pm$ 0.9	0.29 $\pm$ 0.02	9.7 $\pm$ 0.9	0.58 $\pm$ 0.08	50.8 $\pm$ 3.9	3.7 $\pm$ 0.5

Table 7: Gastrointestinal traits of organ weights. Results presented as mean $\pm$ standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). Percentages expressed as grams of organ per gram of body weight. GIT, gastrointestinal tract; PROV, proventriculus.

IV. The influence of dietary treatments on pullet bone health

IV.1. The influence of dietary treatments on pullet Tibiotarsal bone area and density at 11 and 17 weeks of age (Table 8).

		Total Tibiotarsal		Medullary Tibiotarsal		Cortical Tibiotarsal	
		BCSA (mm ²)	BMD (mg/cm ³)	BCSA (mm ²)	BMD (mg/cm ³)	BCSA (mm ²)	BMD (mg/cm ³)
CO	WK11	45.3 ± 1.9	321.8 ± 19.8	16.9 ± 2.3	49.2 ± 5.6	28.1 ± 2.3	383.3 ± 33.5 ^b
	WK17	46.1 ± 2.0	761.3 ± 22.3	12.1 ± 1.3	127.8 ± 22.5	33.6 ± 3.2	424.2 ± 65.8 ^b
C+	WK11	44.6 ± 1.8	333.2 ± 20.5	17.5 ± 1.3	51.8 ± 7.5	29.6 ± 2.3	369.9 ± 63.6 ^b
	WK17	46.5 ± 1.7	725.6 ± 33.3	13.5 ± 1.0	122.8 ± 33.6	30.2 ± 2.3	452.6 ± 63.2 ^b
HS	WK11	49.8 ± 1.2	545.6 ± 33.5	15.5 ± 1.3	68.5 ± 11.3	27.6 ± 3.2	545.3 ± 56.6 ^b
	WK17	48.8 ± 1.6	999.6 ± 36.6	12.5 ± 1.1	198.5 ± 22.6	31.2 ± 2.3	1112.3 ± 96.6 ^a
HS+	WK11	48 ± 1.8	563.5 ± 45.5	14.2 ± 1.6	67.8 ± 9.9	27.2 ± 2.3	601.3 ± 69.6 ^b
	WK17	49.8 ± 1.9	886.6 ± 44.2	12.5 ± 1.8	189.6 ± 21.3	30.3 ± 3.2	1128 ± 93.6 ^a
LS	WK11	46.8 ± 1.2	521.6 ± 54.6	15.8 ± 1.2	69.6 ± 8.5	28.3 ± 1.3	599.6 ± 85.6 ^b
	WK17	49.8 ± 1.8	901.5 ± 52.3	11.9 ± 1.1	200.3 ± 23.5	31.3 ± 2.3	1111.3 ± 75.8 ^a
LS+	WK11	46.8 ± 1.8	586.6 ± 45.6	16.5 ± 1.5	70.2 ± 4.36	29.6 ± 3.3	587.6 ± 45.6 ^b
	WK17	48.8 ± 1.1	945.3 ± 45.6	11.3 ± 1.1	199.9 ± 45.6	30.2 ± 2.3	1069 ± 63.8 ^a

Table 8: the influence of dietary treatments on bone Tibiotarsal bone sectional area and density (BCSA; Bone Cross Sectional area, BMD; Bone Mineral Density). Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

IV.2. The influence of dietary treatments on pullet Tibiotarsal bone breaking strength and ash content at 11 and 17 weeks of age (Table 9).

		Failure Load (N)	Stiffness (N/mm)	Max Bending Moment (N/m)	Ash weight (g)
CO	WK11	153.5 ± 3.5 ^b	178.6 ± 12.3	437.7 ± 22.3	2.1 ± 0.16
	WK17	220.1 ± 5.5 ^b	240.5 ± 11.3	660.1 ± 23.3	2.3 ± 0.19
C+	WK11	155.6 ± 5.6 ^b	177.8 ± 10.5	412.6 ± 23.6	2.1 ± 0.13
	WK17	221.8 ± 8.5 ^b	250.8 ± 16.6	642.5 ± 33.3	2.4 ± 0.16
HS	WK11	185.6 ± 9.3 ^b	201.5 ± 16.6	498.8 ± 36.9	2.3 ± 0.12
	WK17	312.8 ± 6.8 ^a	281.5 ± 14.5	750.3 ± 56.6	2.5 ± 0.15
HS+	WK11	178.6 ± 6.6 ^b	203.5 ± 14.5	478.9 ± 45.6	2.4 ± 0.21
	WK17	325.8 ± 9.9 ^a	291.8 ± 17.8	710.6 ± 25.8	2.4 ± 0.12
LS	WK11	174.6 ± 8.6 ^b	199.9 ± 6.9	489 ± 96.6	2.2 ± 0.14
	WK17	301.9 ± 10.8 ^a	288.8 ± 18.6	721.5 ± 56.6	2.5 ± 0.14
LS+	WK11	177.5 ± 7.6 ^b	198.8 ± 8.9	485.6 ± 23.6	2.3 ± 0.13
	WK17	356.2 ± 8.9 ^a	298.8 ± 16.9	706.6 ± 52.6	2.4 ± 0.12

Table 9: The influence of dietary treatments on pullet Tibiotarsal bone breaking strength and ash content at 11 and 17 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

V. The influence of dietary treatments on pullet behavior, activity, and welfare

V.1. The influence of dietary treatments on pullet behavior at 11 and 17 weeks of age (Table 10).

		Eating	Drinking	Preening	Dustbathing	Locomotion	Resting
CO	WK5	42.6 ± 21.3	0.6 ± 0.03	1.4 ± 0.2	4.6 ± 0.5	13.0 ± 2.3	37.8 ± 10.3
	WK8	36.9 ± 12.3	0.9 ± 0.06	1.5 ± 0.3	5.3 ± 0.6	10.3 ± 1.3	45.1 ± 11.3
	WK11	41.3 ± 15.6	0.7 ± 0.05	1.0 ± 0.2	4.6 ± 0.6	11.5 ± 1.3	40.9 ± 23.3
	WK14	34.8 ± 14.6	0.6 ± 0.1	1.6 ± 0.3	3.9 ± 0.5	13.5 ± 1.6	45.6 ± 15.6
	WK17	43.6 ± 15.3	0.9 ± 0.08	1.9 ± 0.2	5.2 ± 0.7	16.6 ± 2.3	31.9 ± 16.3
C+	WK5	35.9 ± 22.0	0.6 ± 0.06	1.6 ± 0.5	4.8 ± 0.6	11.3 ± 2.5	45.7 ± 14.5
	WK8	41.6 ± 21.5	1.0 ± 0.03	1.0 ± 0.1	5.6 ± 0.3	12.5 ± 3.6	38.3 ± 15.6
	WK11	36.8 ± 13.6	0.5 ± 0.05	1.9 ± 0.1	5.9 ± 0.5	15.5 ± 4.3	39.4 ± 17.6
	WK14	45.8 ± 16.8	0.6 ± 0.05	1.9 ± 0.2	4.6 ± 0.6	16.3 ± 3.6	30.9 ± 16.6
	WK17	34.8 ± 16.5	0.7 ± 0.01	1.6 ± 0.3	5.6 ± 0.8	12.2 ± 4.3	45.2 ± 15.8
HS	WK5	46.9 ± 17.8	0.8 ± 0.04	1.7 ± 0.3	5.8 ± 0.9	13.6 ± 3.5	31.2 ± 14.6
	WK8	33.9 ± 17.9	0.6 ± 0.03	1.9 ± 0.8	4.8 ± 0.5	14.2 ± 4.6	44.7 ± 16.8
	WK11	37.3 ± 20.3	0.6 ± 0.06	1.9 ± 0.1	4.9 ± 0.6	13.3 ± 1.3	41.9 ± 19.9
	WK14	42.5 ± 17.5	0.9 ± 0.04	1.7 ± 0.3	5.8 ± 0.8	10.5 ± 1.8	38.6 ± 14.6
	WK17	35.5 ± 18.5	0.7 ± 0.04	1.0 ± 0.3	6.1 ± 0.7	11.6 ± 6.3	45.0 ± 16.3
HS+	WK5	44.0 ± 22.3	0.6 ± 0.03	1.9 ± 0.1	4.8 ± 0.6	13.9 ± 2.3	34.8 ± 17.8
	WK8	37.0 ± 14.3	0.9 ± 0.5	1.9 ± 0.1	5.8 ± 0.6	17.0 ± 3.6	37.4 ± 14.6
	WK11	42.7 ± 16.3	0.7 ± 0.06	1.6 ± 0.3	6.0 ± 0.4	11.6 ± 1.3	37.4 ± 13.9
	WK14	37.8 ± 15.6	1.0 ± 0.04	1.7 ± 0.5	5.0 ± 0.6	12.9 ± 1.9	41.5 ± 21.3
	WK17	47.2 ± 17.5	0.5 ± 0.03	1.9 ± 0.4	5.2 ± 0.6	16.0 ± 5.6	29.2 ± 19.8
LS	WK5	35.9 ± 17.5	0.6 ± 0.03	2.0 ± 0.2	6.0 ± 0.6	16.9 ± 4.6	38.6 ± 14.5
	WK8	48.5 ± 16.8	0.7 ± 0.04	1.7 ± 0.3	6.4 ± 0.7	12.6 ± 1.9	30.0 ± 13.3
	WK11	35.1 ± 14.2	0.9 ± 0.06	1.1 ± 0.1	5.0 ± 0.6	14.1 ± 4.6	43.8 ± 15.6
	WK14	38.7 ± 13.5	0.6 ± 0.06	2.0 ± 0.3	6.1 ± 0.6	14.8 ± 3.9	37.8 ± 14.3
	WK17	44.2 ± 14.3	0.7 ± 0.05	2.0 ± 0.4	5.9 ± 0.5	13.9 ± 4.3	33.3 ± 14.9
LS+	WK5	37.0 ± 14.2	0.9 ± 0.08	1.7 ± 0.3	6.2 ± 1.1	11.0 ± 2.6	43.3 ± 14.3
	WK8	46.0 ± 20.3	0.7 ± 0.09	1.8 ± 0.2	5.1 ± 0.8	12.1 ± 4.3	34.2 ± 16.3
	WK11	38.7 ± 17.8	0.6 ± 0.05	2.0 ± 0.2	5.2 ± 0.9	14.6 ± 4.3	38.9 ± 10.3
	WK14	44.7 ± 14.6	0.5 ± 0.06	2.1 ± 0.3	6.2 ± 1.2	17.9 ± 3.6	29.1 ± 11.3
	WK17	39.7 ± 10.3	0.6 ± 0.08	1.8 ± .5	6.6 ± 1.3	12.2 ± 1.3	39.7 ± 12.3

Table 10: The influence of dietary treatments on pullet behavior at 5, 8, 11, 14 and 17 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

V.2. The influence of dietary treatments on pullet activity at 11 and 17 weeks of age (Table 11).

		Overall Activity (g)	Vertical Activity (g)	Horizontal Activity (g)
CO	WK5	1.65 ± 0.2	0.15 ± 0.02	1.50 ± 0.3
	WK11	1.38 ± 0.3	0.16 ± 0.03	1.22 ± 0.2
	WK17	1.29 ± 0.1	0.11 ± 0.03	1.18 ± 0.1
C+	WK5	1.55 ± 0.3	0.12 ± 0.01	1.43 ± 0.1
	WK11	1.45 ± 0.2	0.13 ± 0.01	1.31 ± 0.3
	WK17	1.32 ± 0.3	0.12 ± 0.03	1.12 ± 0.2
HS	WK5	1.42 ± 0.1	0.13 ± 0.02	1.13 ± 0.1
	WK11	1.52 ± 0.2	0.14 ± 0.03	1.23 ± 0.1
	WK17	1.63 ± 0.3	0.16 ± 0.03	1.16 ± 0.2
HS+	WK5	1.35 ± 0.1	0.12 ± 0.02	1.03 ± 0.3
	WK11	1.25 ± 0.3	0.13 ± 0.01	1.12 ± 0.3
	WK17	1.56 ± 0.1	0.16 ± 0.01	1.21 ± 0.2
LS	WK5	1.52 ± 0.2	0.13 ± 0.03	1.13 ± 0.2
	WK11	1.45 ± 0.2	0.16 ± 0.02	1.13 ± 0.1
	WK17	1.36 ± 0.3	0.14 ± 0.02	1.15 ± 0.1
LS+	WK5	1.52 ± 0.1	0.16 ± 0.03	1.03 ± 0.2
	WK11	1.36 ± 0.3	0.12 ± 0.03	1.2 ± 0.3
	WK17	1.58 ± 0.2	0.13 ± 0.02	1.03 ± 0.3

Table 10: The influence of dietary treatments on pullet activity at 5, 11 and 17 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

Visuals (Figures and Tables) – Laying Phase

I. The influence of dietary treatments on laying hen performance.

I.1. Dietary treatments and average daily feed intake, feed conversion rate, and egg production across weeks of lay phase (Table 11).

		WK24	WK30	WK36	WK40
C	ADFI (g/hen)	108.9 ± 10.3	107.6 ± 11.3	108.6 ± 10.6	106.6 ± 11.8
	FCR (g feed/g egg)	2.1 ± 0.65	2.1 ± 0.96	2.1 ± 1.03	2.1 ± 0.32
	HDEP	94.8 ± 5.23	95.9 ± 2.84	96.9 ± 3.73	98.04 ± 2.09
C+	ADFI (g/hen)	116.2 ± 11.6	114.3 ± 14.2	112.1 ± 11.5	109.9 ± 10.5
	FCR (g feed/g egg)	2.0 ± 0.63	2.0 ± 0.85	2.1 ± 1.01	2.1 ± 0.36
	HDEP	94.9 ± 3.28	95.7 ± 3.41	96.4 ± 3.48	98.0 ± 1.79
HS	ADFI (g/hen)	105.9 ± 11.5	102.6 ± 10.8	104.3 ± 11.5	103.8 ± 10.6
	FCR (g feed/g egg)	1.94 ± 0.23	1.99 ± 0.42	2.03 ± 0.45	1.89 ± 0.52
	HDEP	95.1 ± 4.30	96.3 ± 4.36	96.2 ± 4.63	98.6 ± 1.31
HS+	ADFI (g/hen)	106.2 ± 11.8	103.3 ± 10.8	104.8 ± 11.8	104.39 ± 11.7
	FCR (g feed/g egg)	1.9 ± 0.42	2.0 ± 0.56	2.0 ± 0.45	1.9 ± 0.85
	HDEP	95.7 ± 3.63	96.6 ± 3.56	96.4 ± 3.52	98.7 ± 1.90
LS	ADFI (g/hen)	115.8 ± 11.5	113.6 ± 14.2	111.5 ± 12.5	109.4 ± 11.8
	FCR (g feed/g egg)	2.0 ± 0.23	2.0 ± 0.36	2.1 ± 0.53	2.1 ± 0.23
	HDEP	94.1 ± 4.56	95.2 ± 4.63	96.2 ± 3.58	97.9 ± 1.60
LS+	ADFI (g/hen)	109.3 ± 10.5	108.2 ± 11.8	109.2 ± 14.6	107.1 ± 12.7
	FCR (g feed/g egg)	2.1 ± 0.63	2.1 ± 0.30	2.1 ± 0.32	2.1 ± 0.26
	HDEP	95.6 ± 2.88	96.4 ± 2.78	97.1 ± 1.61	98.1 ± 1.41

Table 15: Performance of laying hen; FCR= feed conversion ratio; ADFI= average daily feed intake; HDEP= hen-day egg production across weeks (week 24, 30, 36, 40), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). Values within the same week with unlike letters indicate significant differences ($p < 0.05$).

I.2. Dietary treatments and internal egg quality across weeks of lay phase (Table 12).

		WK24	WK30	WK36	WK40
C	Egg weight (g)	56.9 ± 5.63	62.36 ± 6.36	62.25 ± 6.66	63.03 ± 3.69
	Albumin weight(g)	39.8 ± 2.31 ^b	39.85 ± 3.63 ^b	40.69 ± 7.52 ^b	41.25 ± 4.58 ^b
	Yolk weight (g)	13.0 ± 1.03	13.96 ± 1.52	13.85 ± 4.52	14.56 ± 1.52
	Yolk color	10.6 ± 1.69 ^b	10.36 ± 1.66 ^b	10.85 ± 3.36 ^b	10.96 ± 1.25 ^b
	Haugh unit	74.8 ± 2.3 ^b	73.96 ± 6.99	75.58 ± 8.99	74.03 ± 9.87
C+	Egg weight (g)	57.3 ± 5.69	62.96 ± 8.63	62.59 ± 7.85	63.88 ± 7.96
	Albumin weight(g)	41.0 ± 4.25 ^b	41.69 ± 3.65	40.99 ± 3.66	41.69 ± 6.98
	Yolk weight (g)	12.5 ± 1.25	13.03 ± 1.25	14.52 ± 4.25	14.69 ± 1.85
	Yolk color	14.23 ± 1.03 ^a	13.96 ± 1.52 ^a	14.03 ± 1.25 ^a	14.09 ± 8.39 ^a
	Haugh unit	75.23 ± 6.69 ^b	76.69 ± 6.69 ^b	77.26 ± 4.25 ^b	76.69 ± 3.69 ^b
HS	Egg weight (g)	57.2 ± 3.63	63.03 ± 6.58	62.85 ± 3.36	62.85 ± 4.63
	Albumin weight(g)	46.36 ± 4.52 ^a	49.85 ± 4.52 ^a	48.3 ± 4.58 ^a	49.01 ± 3.88 ^a
	Yolk weight (g)	12.58 ± 3.36	12.85 ± 1.25	13.58 ± 1.36	13.99 ± 1.85
	Yolk color	6.85 ± 1.52 ^c	6.85 ± 1.63 ^c	7.52 ± 4.85 ^c	7.63 ± 1.69 ^c
	Haugh unit	86.9 ± 6.63 ^a	88.96 ± 6.69 ^a	89.63 ± 4.96 ^a	90.03 ± 4.36 ^a
HS+	Egg weight (g)	56.23 ± 4.25	62.03 ± 8.99	62.58 ± 9.69	62.96 ± 3.69
	Albumin weight(g)	48.56 ± 3.63 ^a	49.96 ± 3.69 ^a	48.59 ± 4.58 ^a	48.56 ± 4.63 ^a
	Yolk weight (g)	14.03 ± 1.63	14.52 ± 1.25	14.23 ± 1.85	14.63 ± 1.8
	Yolk color	15.23 ± 1.02 ^a	14.99 ± 1.55 ^a	15.56 ± 1.2 ^a	15.23 ± 1.02 ^a
	Haugh unit	85.58 ± 6.69 ^a	85.63 ± 6.66 ^a	84.03 ± 9.99 ^a	83.96 ± 9.98 ^a
LS	Egg weight (g)	56.23 ± 4.85	62.03 ± 5.58	62.58 ± 4.58	62.9 ± 9.86
	Albumin weight(g)	43.56 ± 3.31	37.96 ± 4.63	38.59 ± 6.69	38.56 ± 4.85
	Yolk weight (g)	74.03 ± 1.26	14.52 ± 1.25	14.23 ± 3.65	14.63 ± 1.96
	Yolk color	5.23 ± 1.03 ^c	4.99 ± 1.03 ^c	5.56 ± 4.52 ^c	5.23 ± 1.88 ^c
	Haugh unit	83.58 ± 4.63 ^a	84.63 ± 4.52 ^a	83.03 ± 3.69 ^a	89.96 ± 8.93 ^a
LS+	Egg weight (g)	55.89 ± 3.58	52.03 ± 8.69	62.57 ± 4.36	52.88 ± 9.88
	Albumin weight(g)	40.96 ± 2.36	38.52 ± 4.25	37.58 ± 3.69	38.85 ± 7.58
	Yolk weight (g)	14.12 ± 1.69	14.63 ± 3.69	14.85 ± 4.58	14.66 ± 1.63
	Yolk color	15.85 ± 1.52 ^a	15.69 ± 1.25 ^a	15.28 ± 1.25 ^a	15.99 ± 1.78 ^a
	Haugh unit	82.52 ± 9.63 ^a	82.33 ± 6.69 ^a	81.55 ± 9.87 ^a	80.56 ± 9.89 ^a

Table 12: Egg quality; Egg weight, Albumen weight, yolk weight and color and Haugh unit across weeks (week 24, 30, 36, 40), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). Values within the same week and across treatments with unlike letters indicate significant differences ($p < 0.05$).

I.3. Dietary treatments and external egg quality across weeks of lay phase (Table 13).

		WK24	WK30	WK36	WK40
C	Shell thickness (mm)	0.46 ± 0.03	0.32 ± 0.06	0.38 ± 0.05	0.39 ± 0.06
	Shell breaking strength N	40.36 ± 1.36	36.56 ± 1.03	35.63 ± 1.52	36.12 ± 3.63
	Eggshell weight (g)	7.03 ± 0.06	7.25 ± 0.52	7.63 ± 0.06	7.85 ± 0.08
C+	Shell thickness (mm)	0.45 ± 0.06	0.34 ± 0.04	0.34 ± 0.06	0.36 ± 0.09
	Shell breaking strength N	40.2 ± 4.52	39.52 ± 3.32	34.68 ± 1.25	37.52 ± 3.63
	Eggshell weight (g)	7.12 ± 0.52	7.52 ± 0.63	7.55 ± 0.36	7.78 ± 0.07
HS	Shell thickness (mm)	0.51 ± 0.06	0.52 ± 0.04	0.53 ± 0.03	0.56 ± 0.06
	Shell breaking strength N	46.63 ± 4.25	47.85 ± 3.52	46.96 ± 4.52	46.21 ± 1.36
	Eggshell weight (g)	8.66 ± 0.52	8.89 ± 0.52	8.56 ± 0.63	8.69 ± 0.52
HS+	Shell thickness (mm)	0.49 ± 0.06	0.51 ± 0.06	0.51 ± 0.36	0.53 ± 0.06
	Shell breaking strength N	45.25 ± 5.58	46.52 ± 4.52	46.21 ± 3.63	46.85 ± 2.63
	Eggshell weight (g)	8.63 ± 0.63	8.78 ± 0.25	8.02 ± 0.63	8.86 ± 0.07
LS	Shell thickness (mm)	0.48 ± 0.06	0.49 ± 0.06	0.46 ± 0.05	0.48 ± 0.63
	Shell breaking strength N	41.33 ± 3.52	43.36 ± 4.52	41.2 ± 2.36	42.63 ± 2.36
	Eggshell weight (g)	7.89 ± 0.45	7.93 ± 0.52	8.03 ± 0.32	8.13 ± 0.8
LS+	Shell thickness (mm)	0.45 ± 0.6	0.46 ± 0.05	0.47 ± 0.05	0.49 ± 0.06
	Shell breaking strength N	42.02 ± 4.58	42.66 ± 5.58	42.12 ± 2.36	42.11 ± 2.25
	Eggshell weight (g)	7.55 ± 0.52	7.25 ± 0.14	7.86 ± 0.25	7.96 ± 0.85

Table 13: Egg quality; eggshell weight, shell thickness and breaking strength across weeks (week 24, 30, 36, 40), and dietary treatments (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation). Values within the same week and across treatments with unlike letters indicate significant differences (p<0.05).

II. The influence of dietary treatments on laying hen health.

II.1. The influence of dietary treatments on blood lipids and antioxidant capacity indicators (Table 14).

		TAC (mmol/l)	MDA (nmol/mg)	SOD (U/mg)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL (mmol/L)
CO	WK36	8.3 ± 0.85 ^b	21.8 ± 0.23 ^a	245.3 ± 35.25	4.5 ± 0.33	39.6 ± 3.63	1.2 ± 0.14
	WK40	7.1 ± 0.25 ^b	23.3 ± 0.63 ^a	223.3 ± 26.63	4.3 ± 0.46	33.2 ± 5.23	1.1 ± 0.13
C+	WK36	15.1 ± 2.41 ^a	10.2 ± 0.13 ^b	252.3 ± 43.50	4.3 ± 0.65	26.3 ± 2.63	2.01 ± 0.52
	WK40	14.5 ± 0.85 ^a	11.2 ± 2.1 ^b	245.6 ± 31.95	4.6 ± 1.83	26.9 ± 3.66	2.2 ± 0.46
HS	WK36	10.8 ± 1.25 ^b	10.3 ± 1.69 ^b	287.8 ± 25.20	3.9 ± 0.64	29.8 ± 1.69	2.1 ± 0.23
	WK40	11.8 ± 1.25 ^b	12.2 ± 1.29 ^b	282.5 ± 46.3	4.3 ± 0.66	23.5 ± 2.33	3.0 ± 0.43
HS+	WK36	20.8 ± 1.50 ^a	9.2 ± 2.02 ^b	332.2 ± 63.22	3.9 ± 0.64	21.3 ± 2.03	3.4 ± 0.14
	WK40	19.8 ± 0.89 ^a	8.3 ± 0.11 ^b	356.2 ± 54.52	3.9 ± 0.89	23.8 ± 3.2	3.1 ± 0.53
LS	WK36	7.6 ± 1.02 ^b	18.4 ± 3.39 ^a	283.2 ± 53.63	4.5 ± 0.34	39.9 ± 3.03	1.2 ± 0.1
	WK40	11.2 ± 3.53 ^b	19.3 ± 2.85 ^a	276.3 ± 65.82	4.9 ± 1.66	39.6 ± 2.69	1.3 ± 0.30
LS+	WK36	16.6 ± 2.5 ^a	11.2 ± 1.03 ^b	336.6 ± 25.63	4.6 ± 0.53	36.5 ± 3.25	2.3 ± 0.22
	WK40	14.6 ± 1.23 ^a	10.1 ± 1.28 ^b	326.1 ± 35.85	4.3 ± 0.39	34.3 ± 4.03	2.5 ± 0.31

Table 16: The influence of dietary treatments on blood lipids and antioxidant capacity indicators. Treatments; Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation. Antioxidant capacity parameters found in serum: TAC; total antioxidant capacity, MDA; malondialdehyde, SOD; superoxide dismutase, GSH-Px; glutathione peroxidase, HDL; High Density Lipoproteins. Results presented as mean ± standard error. Values within the same column, and across treatments not sharing the same letter are significantly different ($p \leq 0.05$)

II.2. The influence of dietary treatments on blood metabolites, liver and kidney function indicators (Table 15)

		Glucose mg/dL	Protein g/dL	Calcium mmol/L	Urea mmol/L	Creatinine	ALT (IU)	AST (IU)
CO	WK36	143.6 ± 23.52	3.2 ± 0.66 ^b	3.5 ± 0.36	5.2 ± 0.77	0.5 ± 0.02	33.6 ± 5.69	89.85 ± 25.85
	WK40	176.6 ± 29.63	3.2 ± 0.36 ^b	4.6 ± 0.63	5.3 ± 0.85	0.4 ± 0.09	33.8 ± 4.83	163.6 ± 43.58
C+	WK36	134.3 ± 26.52	3.3 ± 0.82 ^b	3.3 ± 0.46	4.1 ± 0.63	0.5 ± 0.09	21.8 ± 3.85	101.1 ± 25.69
	WK40	153.3 ± 26.85	3.4 ± 0.99 ^b	3.3 ± 0.36	5.6 ± 0.69	0.4 ± 0.08	26.6 ± 4.26	89.6 ± 26.85
HS	WK36	163.5 ± 33.52	5.9 ± 0.36 ^a	4.6 ± 0.56	5.9 ± 0.56	0.5 ± 0.06	19.9 ± 3.86	83.8 ± 23.85
	WK40	183.6 ± 40.23	6.8 ± 0.26 ^a	4.6 ± 0.85	5.5 ± 0.36	0.4 ± 0.06	18.9 ± 5.69	59.6 ± 14.85
HS+	WK36	173.6 ± 35.85	7.6 ± 0.46 ^a	4.5 ± 0.63	4.3 ± 0.96	0.5 ± 0.06	18.5 ± 1.46	101.3 ± 42.25
	WK40	223.6 ± 26.52	7.5 ± 0.23 ^a	4.3 ± 0.55	4.4 ± 0.63	0.4 ± 0.03	13.9 ± 1.63	89.7 ± 29.89
LS	WK36	123.8 ± 29.85	5.6 ± 0.16 ^a	4.2 ± 0.23	4.6 ± 1.25	0.5 ± 0.01	32.8 ± 3.85	112.9 ± 45.85
	WK40	163.8 ± 23.23	5.4 ± 0.23 ^a	3.3 ± 0.63	4.9 ± 0.9	0.4 ± 0.09	43.5 ± 3.20	123.8 ± 39.85
LS+	WK36	132.5 ± 56.33	5.3 ± 0.26 ^a	3.6 ± 0.32	4.6 ± 0.36	0.5 ± 0.06	23.8 ± 2.89	101.9 ± 35.85
	WK40	189.6 ± 44.26	5.4 ± 0.85 ^a	3.5 ± 0.36	5.6 ± 0.52	0.4 ± 0.06	35.8 ± 4.69	126.6 ± 16.59

Table 15 : The influence of dietary treatments on blood metabolites, liver and kidney function indicators. Treatments; Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation. ALT; alanine transferase, AST; aspartate transferase. Results presented as mean ± standard error. Values within the same column, and across treatments not sharing the same letter are significantly different ($p \leq 0.05$)

III.1. Gastrointestinal traits of hens' organ lengths at 40 weeks of age (Table 16).

	GIT (cm)	%GIT	SI (cm)	%SI	μ ceca (cm)	%Ceca	μ Gizzard pH
CO	136.5 $\pm$ 2.1	11.3 $\pm$ 1.3	135.9 $\pm$ 3.3	10.6 $\pm$ 1.3	13.5 $\pm$ 1.6	1.2 $\pm$ 0.2	5.6 $\pm$ 1.2
C+	133.6 $\pm$ 1.3	12.3 $\pm$ 1.1	136.9 $\pm$ 3.3	10.6 $\pm$ 0.6	13.9 $\pm$ 1.3	1.2 $\pm$ 0.2	5.6 $\pm$ 1.3
HS	136.9 $\pm$ 1.2	12.5 $\pm$ 1.6	146.8 $\pm$ 2.2	10.3 $\pm$ 1.3	14.6 $\pm$ 1.3	1.1 $\pm$ 0.2	5.6 $\pm$ 1.2
HS+	133.8 $\pm$ 1.2	12.5 $\pm$ 1.2	149.8 $\pm$ 2.2	10.6 $\pm$ 1.2	16.5 $\pm$ 1.3	1.3 $\pm$ 0.2	5.3 $\pm$ 1.2
LS	139.8 $\pm$ 1.3	11.2 $\pm$ 1.1	142.6 $\pm$ 3.3	10.6 $\pm$ 1.3	15.5 $\pm$ 1.3	1.3 $\pm$ 0.3	5.3 $\pm$ 1.3
LS+	136.8 $\pm$ 3.2	11.6 $\pm$ 1.3	143.8 $\pm$ 2.3	10.8 $\pm$ 1.3	13.3 $\pm$ 1.3	1.6 $\pm$ 0.1	5.3 $\pm$ 1.2

Table 16: Gastrointestinal traits of organ lengths. Results presented as mean $\pm$ standard error. Percentages are expressed as cm of organ per gram of body weight. GIT, gastrointestinal tract; SI, small intestine. μ = average. Treatments; Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation. Values within the same column, and across treatments not sharing the same letter are significantly different ($p \leq 0.05$).

III.2. Gastrointestinal traits of hens' organ weights at 40 weeks of age (Table 17).

	Entire GIT (g)	% Entire GIT	Liver (g)	% Liver	Spleen (g)	% Spleen	PROV empty (g)	% PROV empty	Gizzard empty (g)	% Gizzard empty
CO	217.8 $\pm$ 39.8	14.3 $\pm$ 0.2	39.3 $\pm$ 1.2	2.3 $\pm$ 0.3	4.2 $\pm$ 0.5	0.23 $\pm$ 0.06	9.3 $\pm$ 0.5	0.53 $\pm$ 0.09	52.6 $\pm$ 3.3	4.1 $\pm$ 0.2
C+	212.5 $\pm$ 41.8	14.2 $\pm$ 0.3	36.2 $\pm$ 1.3	2.3 $\pm$ 0.3	4.3 $\pm$ 0.6	0.26 $\pm$ 0.06	9.6 $\pm$ 0.6	0.56 $\pm$ 0.06	53.6 $\pm$ 3.2	4.2 $\pm$ 0.6
HS	219.8 $\pm$ 55.9	14.3 $\pm$ 0.3	35.3 $\pm$ 1.2	2.6 $\pm$ 0.2	4.5 $\pm$ 0.3	0.29 $\pm$ 0.06	9.6 $\pm$ 0.6	0.56 $\pm$ 0.06	51.9 $\pm$ 3.6	4.3 $\pm$ 0.6
HS+	210.8 $\pm$ 60.5	14.3 $\pm$ 0.2	33.3 $\pm$ 1.2	2.5 $\pm$ 0.3	4.6 $\pm$ 0.5	0.29 $\pm$ 0.05	9.6 $\pm$ 0.6	0.53 $\pm$ 0.05	52.9 $\pm$ 2.9	4.3 $\pm$ 0.5
LS	219.3 $\pm$ 45.8	14.2 $\pm$ 0.5	33.6 $\pm$ 1.2	2.3 $\pm$ 0.3	4.6 $\pm$ 0.6	0.29 $\pm$ 0.05	9.9 $\pm$ 0.6	0.53 $\pm$ 0.03	53.3 $\pm$ 3.6	4.2 $\pm$ 0.5
LS+	209.6 $\pm$ 43.5	14.3 $\pm$ 0.3	35.3 $\pm$ 1.3	2.5 $\pm$ 0.4	4.6 $\pm$ 0.3	0.28 $\pm$ 0.03	9.9 $\pm$ 0.6	0.53 $\pm$ 0.03	51.3 $\pm$ 2.9	4.3 $\pm$ 0.3

Table 17: Gastrointestinal traits of organ weights. Results presented as mean $\pm$ standard error. Percentages expressed as grams of organ per gram of body weight. GIT, gastrointestinal tract; PROV, proventriculus. Treatments; Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation. Values within the same column, and across treatments not sharing the same letter are significantly different ($p \leq 0.05$).

IV. The influence of dietary treatments on hen bone health

IV.1. The influence of dietary treatments on hen Tibiotarsal bone area and density at 40 weeks of age (Table 18).

	Total Tibiotarsal		Medullary Tibiotarsal		Cortical Tibiotarsal	
	BCSA (mm ²)	BMD (mg/cm ³)	BCSA (mm ²)	BMD (mg/cm ³)	BCSA (mm ²)	BMD (mg/cm ³)
CO	48.6 ± 2.0	712.9 ± 69.6 ^b	13.6 ± 1.9	125.8 ± 31.9	34.6 ± 3.1	458.6 ± 34.9 ^b
C+	48.9 ± 1.8	598.9 ± 56.8 ^b	15.6 ± 1.6	122.6 ± 19.9	32.6 ± 2.3	342.99 ± 28.9 ^b
HS	49.4 ± 1.6	907.8 ± 43.8 ^a	13.6 ± 1.5	156.9 ± 21.5	33.6 ± 2.1	798.6 ± 31.6 ^a
HS+	48.9 ± 1.9	898.6 ± 65.8 ^a	15.9 ± 1.3	199.9 ± 32.6	33.6 ± 3.2	807.8 ± 30.9 ^a
LS	48.5 ± 1.8	756.9 ± 45.8 ^a	13.9 ± 1.2	223.6 ± 52.6	32.5 ± 2.3	705.6 ± 32.9 ^a
LS+	49.6 ± 1.1	856.9 ± 52.9 ^a	12.6 ± 1.3	212.6 ± 65.9	31.6 ± 2.2	711.9 ± 29.8 ^a

Table 18: the influence of dietary treatments on bone Tibiotarsal bone sectional area and density (BCSA; Bone Cross Sectional area, BMD; Bone Cross Sectional area). Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

IV.2. The influence of dietary treatments on hen Tibiotarsal bone breaking strength and ash content at 40 weeks of age (Table 19).

	Failure Load (N)	Stiffness (N/mm)	Max Bending Moment (N/m)	Ash weight (g)
CO	185.2 ± 6.9 ^b	205.6 ± 15.9 ^b	610.9 ± 45.9	2.1 ± 0.11
C+	175.2 ± 8.9 ^b	215.9 ± 17.9 ^b	598.9 ± 36.9	2.1 ± 0.11
HS	235.1 ± 7.9 ^a	286.9 ± 16.9 ^a	690.9 ± 41.9	2.2 ± 0.12
HS+	245.8 ± 8.9 ^a	295.9 ± 15.9 ^a	689.9 ± 36.9	2.2 ± 0.11
LS	241.6 ± 9.8 ^a	279.9 ± 16.9 ^a	626.5 ± 42.9	2.1 ± 0.10
LS+	236.9 ± 7.9 ^a	289.8 ± 17.9 ^a	619.96 ± 45.9	2.1 ± 0.11

Table 19: The influence of dietary treatments on pullet Tibiotarsal bone breaking strength and ash content at 11 and 17 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

V. The influence of dietary treatments on hen behavior, activity, and welfare

V.1. The influence of dietary treatments on hen behavior (Table 20).

		Eating	Drinking	Preening	Dustbathing	Locomotion	Resting
CO	WK24	39.8 ± 16.9	0.5 ± 0.05	1.3 ± 0.2	5.9 ± 0.6	12.0 ± 3.6	41.6 ± 16.9
	WK30	33.9 ± 19.8	0.4 ± 0.06	1.4 ± 0.3	6.2 ± 0.5	11.3 ± 5.9	49.1 ± 15.8
	WK36	36.9 ± 13.9	0.5 ± 0.03	1.2 ± 0.2	6.9 ± 0.6	9.5 ± 3.6	46.9 ± 16.89
	WK40	33.8 ± 15.8	0.4 ± 0.4	1.8 ± 0.3	5.9 ± 0.9	9.5 ± 2.9	49.6 ± 15.6
C+	WK24	36.9 ± 15.9	0.4 ± 0.03	1.8 ± 0.5	6.9 ± 0.6	10.3 ± 3.9	46.7 ± 11.8
	WK30	35.8 ± 18.9	0.5 ± 0.04	1.7 ± 0.1	5.9 ± 0.3	10.5 ± 2.9	41.6 ± 15.9
	WK36	32.6 ± 13.6	0.6 ± 0.05	1.9 ± 0.1	6.9 ± 0.9	10.5 ± 2.9	42.9 ± 15.8
	WK40	38.9 ± 17.9	0.6 ± 0.06	1.8 ± 0.2	6.8 ± 0.6	10.3 ± 2.6	42.9 ± 7.9
HS	WK24	35.9 ± 16.9	0.7 ± 0.03	1.8 ± 0.3	5.9 ± 0.8	11.6 ± 3.9	42.9 ± 19.8
	WK30	36.9 ± 18.9	0.6 ± 0.05	1.7 ± 0.8	6.9 ± 0.9	12.2 ± 4.5	45.9 ± 15.8
	WK36	35.8 ± 21.8	0.8 ± 0.06	1.9 ± 0.1	6.9 ± 0.6	12.3 ± 1.3	46.9 ± 19.9
	WK40	36.8 ± 19.8	0.5 ± 0.04	1.9 ± 0.3	6.5 ± 0.6	9.5 ± 2.6	42.9 ± 12.8
HS+	WK24	37.9 ± 22.3	0.6 ± 0.05	1.9 ± 0.1	5.9 ± 0.6	10.9 ± 3.6	46.9 ± 15.8
	WK30	33.8 ± 19.8	0.5 ± 0.06	1.8 ± 0.1	6.2 ± 0.5	11.0 ± 2.6	41.6 ± 12.9
	WK36	34.8 ± 12.9	0.7 ± 0.06	1.6 ± 0.3	6.8 ± 0.5	12.6 ± 3.6	46.9 ± 15.8
	WK40	36.8 ± 15.6	0.9 ± 0.04	1.8 ± 0.5	5.9 ± 0.6	12.9 ± 2.6	43.5 ± 21.3
LS	WK24	34.8 ± 14.8	0.6 ± 0.06	1.0 ± 0.2	6.9 ± 0.3	11.9 ± 4.9	46.9 ± 16.9
	WK30	37.8 ± 13.9	0.5 ± 0.07	1.9 ± 0.3	6.2 ± 0.6	10.6 ± 1.9	42.9 ± 16.9
	WK36	34.8 ± 12.8	0.5 ± 0.08	1.7 ± 0.1	6.3 ± 0.6	10.1 ± 3.6	43.9 ± 19.9
	WK40	33.8 ± 13.5	0.6 ± 0.06	1.7 ± 0.3	6.9 ± 0.3	9.8 ± 3.6	46.9 ± 16.9
LS+	WK24	34.8 ± 13.8	0.9 ± 0.03	1.8 ± 0.3	5.9 ± 1.3	10.0 ± 2.9	46.9 ± 13.9
	WK30	32.8 ± 19.8	0.6 ± 0.09	1.8 ± 0.2	6.9 ± 0.5	10.1 ± 3.6	45.9 ± 16.9
	WK36	33.8 ± 14.8	0.6 ± 0.05	2.0 ± 0.2	5.9 ± 0.6	10.6 ± 5.6	41.9 ± 10.5
	WK40	35.8 ± 13.6	0.5 ± 0.03	2.1 ± 0.3	5.9 ± 1.3	10.9 ± 5.6	45.9 ± 11.2

Table 20: The influence of dietary treatments on pullet behavior at 24, 30, 36, and 40 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

V.2. The influence of dietary treatments on hen activity at 24, 36 and 40 weeks of age (Table 21).

		Overall Activity (g)	Vertical Activity (g)	Horizontal Activity (g)
CO	WK24	1.55 ± 0.1	0.14 ± 0.03	1.40 ± 0.2
	WK36	1.41 ± 0.2	0.14 ± 0.02	1.32 ± 0.1
	WK40	1.31 ± 0.2	0.14 ± 0.02	1.38 ± 0.3
C+	WK24	1.45 ± 0.3	0.11 ± 0.03	1.33 ± 0.3
	WK36	1.36 ± 0.2	0.12 ± 0.02	1.31 ± 0.2
	WK40	1.31 ± 0.2	0.13 ± 0.02	1.22 ± 0.2
HS	WK24	1.38 ± 0.2	0.13 ± 0.03	1.23 ± 0.2
	WK36	1.41 ± 0.3	0.13 ± 0.02	1.22 ± 0.2
	WK40	1.45 ± 0.2	0.15 ± 0.02	1.10 ± 0.3
HS+	WK24	1.32 ± 0.2	0.14 ± 0.06	1.11 ± 0.2
	WK36	1.21 ± 0.3	0.13 ± 0.03	1.02 ± 0.1
	WK40	1.41 ± 0.2	0.15 ± 0.03	1.13 ± 0.3
LS	WK24	1.41 ± 0.3	0.14 ± 0.02	1.02 ± 0.2
	WK36	1.45 ± 0.2	0.15 ± 0.03	1.05 ± 0.1
	WK40	1.31 ± 0.2	0.16 ± 0.02	1.02 ± 0.3
LS+	WK24	1.14 ± 0.6	0.14 ± 0.02	1.07 ± 0.4
	WK36	1.34 ± 0.3	0.14 ± 0.02	1.06 ± 0.3
	WK40	1.42 ± 0.2	0.15 ± 0.03	1.13 ± 0.2

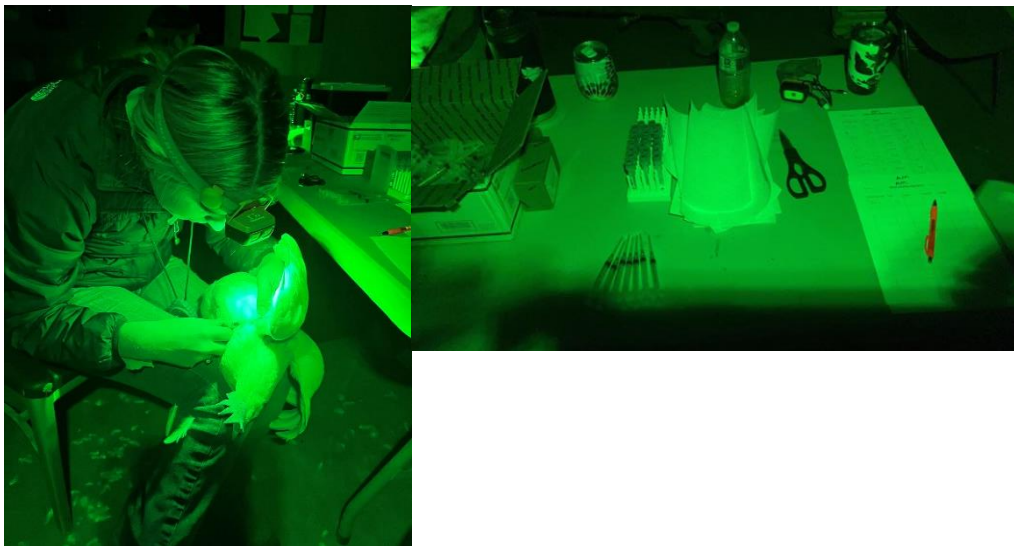
Table 21: The influence of dietary treatments on pullet activity at 24, 36 and 40 weeks of age. Results presented as mean ± standard error. Rows within the same column not sharing the same letter are significantly different ($p \leq 0.05$). (Low Protein Sorghum diets (LS), hens received diets containing 50% of corn replaced with low protein sorghum either with (LS+), or without (LS) pigment supplementation; High Protein Sorghum diets (HS), hens received diets containing 50% of corn replaced with high protein sorghum either with (HS+), or without (HS) pigment supplementation; Corn or Control diets (C), hens received corn-soybean meal-based diets either with (C+), or without (C) pigment supplementation).

Pictures:

1. **Picture 1.** Shows a testing pen containing a commercial tube feeder, a trough drinker, and 5 cm of wood shavings on the floor as litter. Moreover, additional feed was offered to the birds during the first couple of weeks of the rearing phase.



2. **Picture 2.** Shows the blood sampling process. All procedures were conducted during the dark phase, using green headlights to avoid disturbing the sleep cycle, 24 gauge syringes, and 2 ml EDTA coated vacutainers.



3. **Picture 3.** Testing bird's GIT (from the post-crop esophagus to the cloaca, including digesta content, liver, pancreas, and spleen) were removed and weighed (Bottom left picture), and the length of the GIT was measured (Top picture). After GIT removal, the pullets were weighed again, and carcass yield (% B.W., including the head, neck, and feet) was calculated. Then, the liver and the full proventriculus and gizzard were carefully excised and weighed (Liver; bottom middle picture). The pH of the gizzard contents was measured in duplicate in situ using a digital pH meter (Bottom right picture).



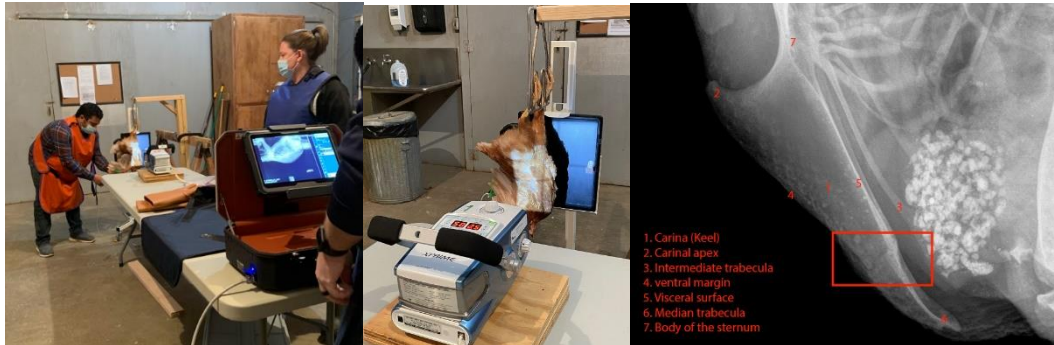
4. **Picture 4.** Shows video recordings of the behavior of birds (3 weeks of age (left) and 17 weeks of age (right)) for 72 continuous hours per week.



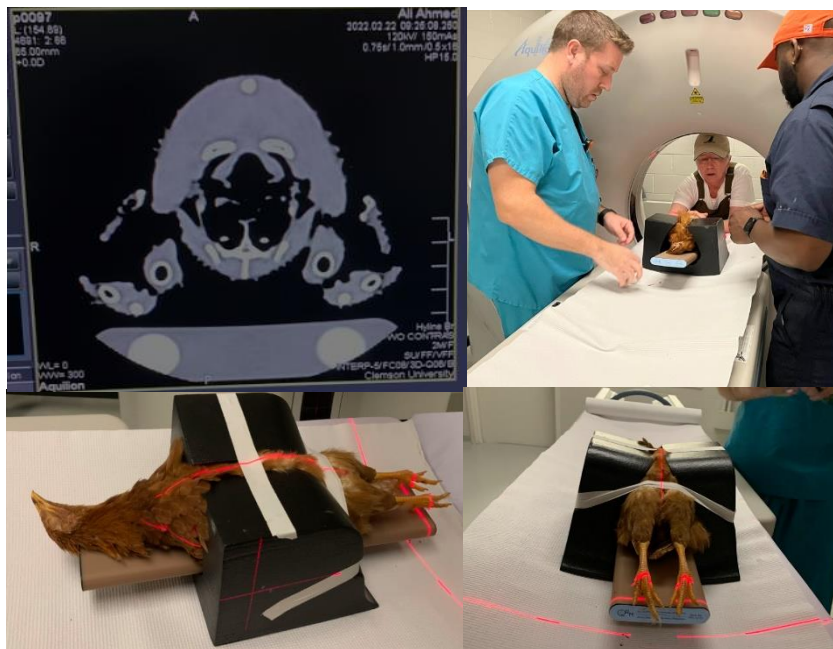
5. **Picture 5.** Bird activity (left) was measured during behavior observations using activity monitors (HOBO Pendant G; top right) and predesigned backpacks (bottom right).



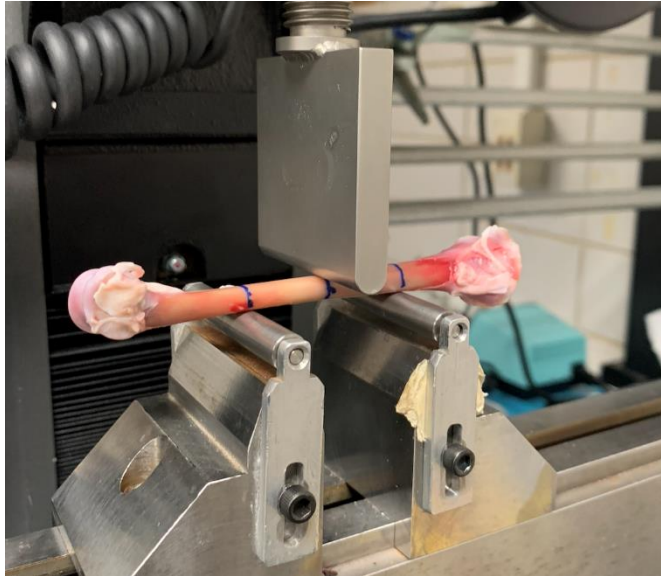
6. **Picture 6.** Lateral digital radiographs of the keel bone was conducted at week 11 and week 17.



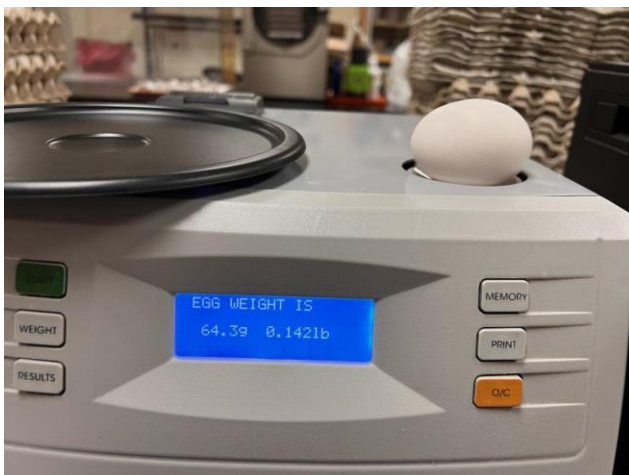
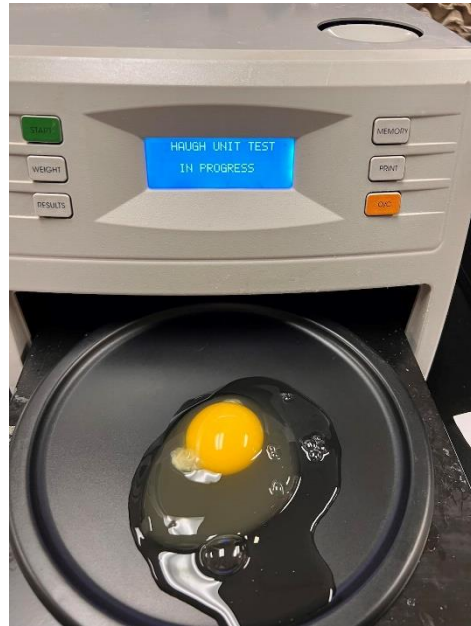
7. **Picture 7.** Computed tomography of euthanized birds was conducted. Scans were acquired using a multi-slice CT scanner at the Godley Snell Research Center (Toshiba Aquilion 16-slice, Universal Medical Systems).



8. **Picture 8.** Instron configuration with rounded supports and breaking blade machined according to ANSI standards.



9. **Picture 9.** Shows the process of egg quality assessment using ORKA egg analyzer and egg force reader, and a digital caliber to measure the eggshell thickness.



Appendix:**Table L.** Ingredient percentage and calculated nutrient analysis of 4 basal diets (Control) used in this experiment.

Ingredient	Starter 1 (%)	Starter 2 (%)	Grower (%)	Pre-lay (%)
Corn	58.5	61.9	61.9	61.8
45% Soybean Meal	35.7	27.8	22.0	21.7
Mono-dicalcium Phosphate	1.45	1.41	1.38	2.19
Wheat Middlings	1.31	6.42	12.4	9.68
Calcium Carbonate	1.24	1.26	1.36	2.88
Soybean Oil	0.50	0.00	0.00	0.00
Salt	0.45	0.45	0.45	0.45
Choline chloride 60%	0.45	0.40	0.40	0.40
DL-Methionine	0.27	0.22	0.19	0.19
Vitamin/Mineral Premix*	0.15	0.15	0.15	0.15
L-Threonine	0.04	0.04	0.05	0.06
L-Lysine	0.00	0.03	0.05	0.53
Calculated analysis				
Crude Protein	20.0	18.3	17.5	16.5
Crude Fat	1.89	1.66	1.90	1.95
Crude fiber	4.04	3.87	2.91	2.65
Calcium	1.05	1.00	0.95	2.50
Phosphorus	0.35	0.34	0.74	0.81
Metabolizable Energy (kcal/kg)	2926	2906	2882	2893

Samples of all diets were analyzed to confirm nutrient composition. For LS and HS diets, 50% of the corn was replaced by either LS or HS based on the applied treatment. *Provimi Corporate Layer 2 with phytase (Lewisburg, OH, USA) composed of: selenium 255 ppm, zinc 6.5%, vitamin A 8294000 IU/kg, phytase activity 399166.2 FTU/kg.

Table M. Ingredient percentage and calculated nutrient analysis of peak lay diet

Ingredient	Peak-Lay (%)
Corn	55.03
SBM	28.11
Calcium carbonate	9.09
Soybean oil	2.66
Wheat middlings	2.00
Mono-dicalcium phosphorus	1.41
Salt	0.44
Choline chloride	0.36
DL-Methionine	0.34
L-Threonine	0.12
Valine	0.10
Isoleucine	0.10
Vitamin/Mineral Premix*	0.05
Calculated analysis	
Crude protein	17.82
Crude fat	3.96
Crude fiber	2.18
Calcium	3.87
Phosphorus	0.67
Metabolizable Energy (kcal/kg)	2843.93

Samples of all diets were analyzed to confirm nutrient composition. For LS and HS diets, 50% of the corn was replaced by either LS or HS based on the applied treatment. *Provimi Corporate Layer 2 with phytase (Lewisburg, OH, USA) composed of selenium 255 ppm, zinc 6.5%, vitamin A 8294000 IU/kg, phytase activity 399166.2 FTU/kg.

Table N. Lighting schedule used during the rearing and laying phase.

Hours light: hours dark	Age of birds (weeks)
20:4	0
18:6	2
16.5:7.5	3
15:9	4
13.5:10.5	5
12:12	6
10:14	7-17 to 40 WOA

Adapted from standard guidelines for Hy-Line Brown hens

Table P. Flock uniformity showed as the coefficient of variation% grouped by dietary treatment/pen across week of age.

Grain	Pigment	Pen	BLK	Age, wk	birds, n	meanBW, g	BW+10%, g	BW-10%, g	Birds out of range, n	Uniformity, %	CV%
C	Y	3	1	6	29	399	439	359	13	55	21.3
C	Y	3	1	8	29	790	869	711	3	90	8.3
C	Y	3	1	10	29	949	1044	854	2	93	7.3
C	Y	3	1	12	28	1153	1269	1038	3	89	5.8
C	Y	3	1	14	28	1317	1449	1185	2	93	5.8
HS	Y	4	1	6	29	440	483	396	7	76	8.8
HS	Y	4	1	8	29	845	929	760	8	72	9.0
HS	Y	4	1	10	29	1051	1156	946	10	66	12.1
HS	Y	4	1	12	28	1212	1333	1091	5	82	7.5
HS	Y	4	1	14	28	1393	1532	1254	4	86	6.3
LS	N	5	1	6	29	399	439	359	12	59	11.5
LS	N	5	1	8	29	943	1037	849	8	72	8.1
LS	N	5	1	10	29	782	860	704	13	55	9.3
LS	N	5	1	12	28	1163	1279	1047	2	93	6.7
LS	N	5	1	14	27	1332	1465	1199	3	89	6.9
LS	Y	6	1	6	29	411	452	370	13	55	8.3
LS	Y	6	1	8	29	795	874	715	5	83	8.7
LS	Y	6	1	10	29	960	1056	864	7	76	7.4
LS	Y	6	1	12	28	1139	1253	1025	8	71	7.7
LS	Y	6	1	14	28	1333	1467	1200	2	93	5.9
HS	N	9	1	6	32	393	432	353	9	72	11.2
HS	N	9	1	8	29	768	845	691	10	66	9.1
HS	N	9	1	10	29	923	1015	831	6	79	8.0
HS	N	9	1	12	28	1126	1238	1013	5	82	6.9
HS	N	9	1	14	28	1314	1445	1182	4	86	6.0
C	N	10	1	6	32	395	435	356	9	72	10.2
C	N	10	1	8	31	787	866	708	2	94	6.2
C	N	10	1	10	30	942	1036	848	3	90	6.4
C	N	10	1	12	29	1168	1285	1052	2	93	5.6
C	N	10	1	14	29	1375	1512	1237	2	93	4.9
HS	Y	11	1	6	30	425	467	382	3	90	7.1
HS	Y	11	1	8	30	815	896	733	6	80	8.2
HS	Y	11	1	10	30	987	1086	889	7	77	8.8
HS	Y	11	1	12	29	1182	1301	1064	3	90	6.5
HS	Y	11	1	14	29	1378	1516	1240	5	83	6.5
C	Y	12	1	6	30	835	918	751	5	83	7.9
C	Y	12	1	8	30	815	896	733	6	80	8.2
C	Y	12	1	10	30	998	1098	898	6	80	7.4
C	Y	12	1	12	29	1211	1332	1090	7	76	7.0
C	Y	12	1	14	29	1401	1542	1261	6	79	7.9
LS	N	13	1	6	30	404	444	363	15	50	9.9
LS	N	13	1	8	30	800	880	720	4	87	8.6
LS	N	13	1	10	30	972	1069	875	5	83	7.5
LS	N	13	1	12	29	1178	1296	1060	4	86	7.7
LS	N	13	1	14	29	1400	1540	1260	6	79	7.0
LS	Y	14	1	6	31	421	464	379	6	81	10.1
LS	Y	14	1	8	31	828	911	745	6	81	8.5
LS	Y	14	1	10	31	982	1080	884	6	81	8.6
LS	Y	14	1	12	29	1172	1289	1054	6	79	8.8
LS	Y	14	1	14	29	1401	1542	1261	3	90	6.7
LS	Y	17	1	6	29	388	427	349	5	83	9.2
LS	Y	17	1	8	29	791	871	712	5	83	8.3
LS	Y	17	1	10	29	951	1046	856	0	100	7.2
LS	Y	17	1	12	28	1163	1279	1047	8	71	7.4
LS	Y	17	1	14	28	1383	1522	1245	5	82	7.0
LS	N	18	1	6	31	402	443	362	5	84	8.6
LS	N	18	1	8	30	804	885	724	3	90	7.4
LS	N	18	1	10	30	974	1071	876	7	77	8.3
LS	N	18	1	12	29	1195	1314	1075	3	90	6.7
LS	N	18	1	14	29	1409	1550	1268	3	90	5.6

C	Y	19	1	6	30	410	451	369	14	53	9.9
C	Y	19	1	8	30	794	873	714	4	87	7.9
C	Y	19	1	10	30	946	1041	852	4	87	6.9
C	Y	19	1	12	29	1153	1268	1037	5	83	6.4
C	Y	19	1	14	28	1362	1499	1226	4	86	6.7
HS	Y	20	1	6	30	411	452	370	14	53	9.1
HS	Y	20	1	8	30	812	893	731	10	67	9.0
HS	Y	20	1	10	30	968	1064	871	9	70	8.6
HS	Y	20	1	12	29	1172	1289	1054	6	79	8.0
HS	Y	20	1	14	29	1369	1505	1232	2	93	6.4
C	N	21	1	6	30	423	466	381	2	93	6.5
C	N	21	1	8	30	819	901	738	8	73	7.4
C	N	21	1	10	30	972	1069	875	6	80	7.0
C	N	21	1	12	29	1162	1278	1046	3	90	6.1
C	N	21	1	14	29	1361	1497	1225	1	97	5.8
HS	N	22	1	6	30	429	472	386	2	93	6.6
HS	N	22	1	8	29	841	926	757	3	90	7.0
HS	N	22	1	10	29	985	1084	887	3	90	5.9
HS	N	22	1	12	28	1174	1292	1057	3	89	6.4
HS	N	22	1	14	28	1387	1525	1248	2	93	5.3
HS	N	29	2	6	29	418	459	376	6	79	9.4
HS	N	29	2	8	29	860	946	774	6	79	11.4
HS	N	29	2	10	29	1006	1106	905	1	97	5.5
HS	N	29	2	12	28	1234	1358	1111	3	89	5.5
HS	N	29	2	14	28	1379	1516	1241	3	89	5.9
C	N	30	2	6	30	416	457	374	6	80	11.1
C	N	30	2	8	30	842	926	758	5	83	10.2
C	N	30	2	10	30	1022	1124	920	6	80	9.3
C	N	30	2	12	29	1231	1354	1108	4	86	8.6
C	N	30	2	14	29	1423	1566	1281	4	86	8.3
C	N	31	2	6	27	403	444	363	13	52	7.9
C	N	31	2	8	27	800	880	720	2	93	7.1
C	N	31	2	10	27	986	1085	888	5	81	6.8
C	N	31	2	12	26	1176	1293	1058	3	88	6.4
C	N	31	2	14	26	1373	1510	1236	5	81	7.0
HS	N	32	2	6	31	420	462	378	9	71	11.5
HS	N	32	2	8	31	833	916	749	6	81	11.0
HS	N	32	2	10	30	1016	1118	914	5	83	10.5
HS	N	32	2	12	28	1199	1319	1079	2	93	5.5
HS	N	32	2	14	28	1345	1479	1210	1	96	5.5
LS	N	33	2	6	29	375	413	338	6	79	13.7
LS	N	33	2	8	29	773	850	695	8	72	10.2
LS	N	33	2	10	29	928	1020	835	7	76	8.6
LS	N	33	2	12	28	1139	1253	1025	7	75	9.0
LS	N	33	2	14	28	1340	1474	1206	2	93	6.2
HS	Y	34	2	6	29	419	461	377	5	83	8.0
HS	Y	34	2	8	29	829	912	746	2	93	6.4
HS	Y	34	2	10	29	982	1080	884	6	79	9.5
HS	Y	34	2	12	28	1212	1333	1091	3	89	5.6
HS	Y	34	2	14	28	1369	1506	1232	0	100	4.9
HS	Y	35	2	6	30	417	459	376	10	67	10.8
HS	Y	35	2	8	30	859	945	773	12	60	9.0
HS	Y	35	2	10	29	1032	1136	929	5	83	8.2
HS	Y	35	2	12	28	1251	1376	1126	6	79	8.2
HS	Y	35	2	14	28	1408	1549	1267	3	89	7.2
LS	N	36	2	6	31	413	454	371	8	74	10.8
LS	N	36	2	8	30	821	903	739	11	63	11.5
LS	N	36	2	10	30	1001	1101	901	8	73	11.0
LS	N	36	2	12	29	1228	1351	1105	7	76	9.3
LS	N	36	2	14	29	1433	1576	1289	8	72	8.1
C	Y	39	2	6	29	419	461	377	5	83	8.0
C	Y	39	2	8	29	841	926	757	3	90	12.8
C	Y	39	2	10	29	993	1093	894	2	93	6.9
C	Y	39	2	12	28	1202	1322	1082	2	93	6.5
C	Y	39	2	14	28	1380	1518	1242	2	93	5.5
LS	Y	40	2	6	31	432	475	388	4	87	7.6

LS	Y	40	2	8	31	868	954	781	6	81	7.7
LS	Y	40	2	10	29	1046	1151	942	4	86	7.3
LS	Y	40	2	12	28	1251	1376	1126	2	93	6.5
LS	Y	40	2	14	28	1429	1572	1286	4	86	6.0
LS	Y	41	2	6	29	410	451	369	20	31	11.3
LS	Y	41	2	8	29	832	915	749	7	76	9.2
LS	Y	41	2	10	29	996	1096	897	5	83	8.7
LS	Y	41	2	12	28	1223	1345	1101	7	75	8.2
LS	Y	41	2	14	28	1395	1534	1255	7	75	7.2
C	Y	42	2	6	29	411	452	370	13	55	8.3
C	Y	42	2	8	29	851	936	766	4	86	7.1
C	Y	42	2	10	29	1004	1105	904	1	97	6.0
C	Y	42	2	12	28	1215	1336	1093	3	89	6.6
C	Y	42	2	14	28	1388	1527	1249	3	89	6.0
HS	N	43	2	6	30	423	466	381	12	60	12.4
HS	N	43	2	8	30	868	955	781	7	77	8.7
HS	N	43	2	10	30	1060	1166	954	13	57	9.0
HS	N	43	2	12	29	1286	1414	1157	5	83	8.4
HS	N	43	2	14	29	1445	1590	1301	4	86	6.9
C	N	44	2	6	30	375	412	337	5	83	8.9
C	N	44	2	8	30	818	900	736	7	77	7.5
C	N	44	2	10	30	990	1089	891	2	93	7.5
C	N	44	2	12	29	1223	1345	1101	4	86	7.2
C	N	44	2	14	28	1417	1559	1276	4	86	7.0