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Corn ethanol coproduct in the feeding of semi-heavy laying hens in the initial laying phase

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ABSTRACT

The present study investigated the feasibility of using corn distillers dried grains with solubles (DDGS) as a substitute for soybean meal in the diet of semi-heavy laying hens in hot climates. Bird performance, egg quality, feed digestibility, and economic analysis were evaluated. There was no significant difference in the parameters of feed intake, weight gain, feed conversion, marketable egg production, individual weight, or laying age between groups with different levels of DDGS inclusion. Egg quality remained constant, including weight, albumen composition, yolk, shell, Haugh unit, and specific weight. Carotenoid content in the yolk also remained constant. Only yolk color, as measured using a yolk color fan, exhibited significant differences. In conclusion, DDGS can effectively replace soybean meal in laying hen diets without affecting performance or egg quality. Additionally, it improved yolk color. From an economic standpoint, including DDGS is viable up to 4.39%. Therefore, corn DDGS is an advantageous choice for laying hen producers, especially in warm climate regions.

Keywords: Yolk color; Corn DDGS; Egg production; Haugh unit.

Coproduto do etanol de milho na alimentação de galinhas poedeiras semipesadas na fase inicial de postura

RESUMO

O estudo investigou a viabilidade do uso de grão seco de destilaria de milho com solúveis (DDGS) como alternativa ao farelo de soja na dieta de galinhas poedeiras semi pesadas em clima quente. Avaliou-se o desempenho das aves, qualidade dos ovos, digestibilidade da ração e análise econômica. Os parâmetros de consumo de ração, ganho de peso, conversão alimentar, produção de ovos comercializáveis, peso individual e idade da postura não mostraram diferença significativa entre os grupos com diferentes níveis de inclusão de DDGS. A qualidade dos ovos, incluindo peso, composição de albúmen, gema, casca, unidade Haugh e peso específico, permaneceu constante, assim como o teor de carotenoides na gema. Apenas a coloração da gema, medida pelo leque colorimétrico, apresentou diferenças significativas entre os tratamentos. O estudo conclui que o DDGS pode ser uma alternativa eficaz ao farelo de soja na alimentação de galinhas poedeiras, não afetando o desempenho e a qualidade dos ovos. Além disso, melhorou a coloração da gema. Economicamente, a inclusão do DDGS é viável até 4,39%. Portanto, o DDGS de milho pode ser uma escolha vantajosa para produtores de galinhas poedeiras, especialmente em regiões de clima quente.

Palavras-chave: Coloração da gema; DDGS de milho; Produção de ovos; Unidade Haugh.

1. Introduction

Brazil stands out for its production of chicken eggs. In 2023, national production was 52.44 million units (most of which were destined for the domestic market, approximately 99.00% of the national total), per capita consumption in Brazil was 242 units/year, and the gross value of production (GVP) was R$24.52 billion (ABPA, 2024). Chicken eggs are a food with high biological value and are a source of protein that is essential for human nutrition (Freitas et al., 2011).

Corn and soybean meal are the main ingredients used in poultry feed (Torres et al., 2003). These products are utilized in various economic sectors, including the biofuel industries (biodiesel and ethanol) and the pharmaceutical sector (Procópio et al., 2022). The grain market is characterized by price instability, which contributes to increased production costs for feed in egg-laying poultry farming (Loureiro et al., 2007).

Competition with other economic sectors for the acquisition of corn and soybean meal has caused poultry farmers to seek alternative inputs to replace these raw materials in animal feed (Procópio et al., 2022). An alternative feed to soybean meal is corn distillers dried grains with solubles (DDGS). It is rich in protein, amino acids, energy, phosphorus, and fiber, but its variability and nutritional composition are the main challenges encountered during its use (Lumpkins et al., 2004). Owing to its low cost, the use of corn DDGS helps reduce waste disposal in the environment (Schöne et al., 2017).

The objective of this study was to evaluate the productive performance, egg quality, and economic analysis through the inclusion of different levels of corn DDGS in the diet of Hisex Brown semi-heavy laying hens raised in a floor system under hot climate conditions in the municipality of Santo Antônio do Leverger, state of Mato Grosso.

2. Material and Methods

The experiment was conducted at the Experimental Farm of the Federal University of Mato Grosso (UFMT), located in the municipality of Santo Antônio do Leverger, state of Mato Grosso. This research project was approved by the Ethics Committee for Animal Experimentation (protocol number 23108.194864/2017-37). The experiment involved 240 Hisex Brown semi-heavy laying hens aged 19–26 weeks, with an average initial weight of 1.412 kg (± 0.017 kg). In the laying phase, there were five levels of corn DDGS (0%, 2%, 4%, 6%, and 8%), six replicates, and eight birds per experimental unit, distributed in a completely randomized design.

The experimental diets (Table 1) were formulated based on corn and soybean meal, according to the nutritional recommendations of Rostagno et al. (2017), being isoenergetic and isoproteic. The amino acid content was based on Aminodat® 5.0 (Wiltafsky et al., 2010).

To mitigate the effects of heat, fans were turned on throughout the day, and sprinklers were turned on at scheduled times, maintaining a uniform temperature throughout the shed. Temperature and relative humidity (RH) were monitored twice a day, at 8 am and 4 pm, using a digital thermo-hygrometer placed in the center of the shed at the height of the birds' backs.

Hens were housed in boxes (experimental unit) measuring 1.76 x 1.53 m (length x width), providing an area of 0.336 m²/bird. They were equipped with tubular feeders, hanging drinkers, wooden nests for egg-laying, and a floor covered with rice straw. The variation in body weight was assessed by the difference in weight between the beginning and end of the birds' life stage, using a scale accurate to 0.001 g (grams). The viability of laying hens was determined by subtracting the total number of dead birds from the total number of live birds, with the values converted to a percentage at the end.

Feed intake was determined at the end of the experimental phase and the amount of feed consumed according to the number of birds in each treatment and the grams of feed consumed per bird/day, using a precision scale accurate to 0.001 g. Feed conversion per dozen eggs was calculated by dividing the total feed intake in kilograms by the dozen eggs produced (kg/dz), and feed conversion per egg mass was calculated by dividing the feed intake in kilograms by the total egg mass (kg/kg).

The average egg production during the period was obtained by calculating the number of eggs produced, including broken, cracked, and abnormal eggs (eggs with soft shells or without shells), and was expressed as a percentage of the average number of birds during the period (eggs/bird/day). To determine the production of marketable eggs, the number of broken, cracked, soft-shelled, and shell-less eggs was discounted from the total production, and the ratio of intact to total eggs produced during the experimental period was calculated. The start of laying was calculated in weeks, considering a production rate of 5% and 50% for all experimental units. At the end of the 26^{th} week of age, random eggs were collected from each replicate for three consecutive days, and the average egg quality was determined.

Eggs from each experimental unit were individually weighed. After weighing, the specific gravity was determined by immersing the eggs in saline solutions with densities ranging from 1.070 to 1.100 g/cm³, at an interval of 0.005 g/cm³, duly calibrated using a densimeter (OM-5565, Incoterm).

Table 1. List of ingredients and nutritional composition of treatments used for feeding Hisex Brown laying hens

Ingredients	**Corn DDGS levels (%)**				
	0.0	**2.0**	**4.0**	**6.0**	**8.0**
Ground corn	64.35	64.35	64.35	64.35	64.35
Soybean meal	21.25	19.25	17.25	15.25	13.25
Calcitic limestone	8.90	8.90	8.90	8.90	8.90
Dicalcium phosphate	1.10	1.10	1.10	1.10	1.10
Common salt	0.40	0.40	0.40	0.40	0.40
Laying mix [(1)]	1.87	1.87	1.87	1.87	1.87
L-lysine HCL	0.06	0.08	0.10	0.12	0.14
DL-Methionine	0.24	0.25	0.26	0.27	0.28
L-Threonine	0.03	0.05	0.07	0.09	0.11
Starch	0.80	0.75	0.70	0.65	0.60
Soybean oil	1.00	1.00	1.00	1.00	1.00
Corn DDGS	0.00	2.00	4.00	6.00	8.00
Calculated Nutritional Composition					
Metabolizable energy (kcal/kg)	2,850	2,850	2,850	2,850	2,850
Crude protein (%)	14.74	14.74	14.74	14.74	14.74
Crude fiber (%)	4.52	4.00	4.50	4.65	4.90
Digestible lysine (%)	0.736	0.736	0.736	0.736	0.736
Digestible methionine+cystine (%)	0.721	0.721	0.721	0.721	0.721
Digestible tryptophan (%)	0.169	0.169	0.169	0.169	0.169
Digestible threonine (%)	0.567	0.567	0.567	0.567	0.567
Calcium (%)	3.893	3.893	3.893	3.893	3.893
Available phosphorus (%)	0.318	0.318	0.318	0.318	0.318
Sodium (%)	0.179	0.179	0.179	0.179	0.179

[1]Mix composition: Calcium (min) 80 g/kg, Calcium (max) 100 g/kg Phosphorus (min) 37 g/kg, Sodium (min) 20 g/kg, Methionine (min) 21.5 g/kg, Lysine (min) 18 g/kg, Vitamin A (min) 125,000 IU/kg, Vitamin D3 (min) 25,000 IU/kg, Vitamin E (min) 312 IU/kg, Vitamin K3 (min) 20 mg/kg, Vitamin B1 (min) 20 mg/kg, Vitamin B2 (min) 62.5 mg/kg, Vitamin B6 (min) 37.5 mg/kg, Vitamin B 12 (min) 200 mcg/kg, Folic Acid (min) 6.25 mg/kg, Pantothenic Acid (min) 125 mg/kg, Biotin (min) 1.25 mg/kg, Choline (min) 1,700 mg/kg, Niacin (min) 312 mg/kg, Copper (min) 125 mg/kg, Iron (min) 680 mg/kg, Iodine (min) 8.75 mg/kg, Manganese (min) 937 mg/kg, Selenium (min) 3.75 mg/kg, Zinc (min) 500 mg/kg, Fluorine (max) 370 mg/kg.

Whole eggs were broken on a flat white surface, and the intensity of the yellow color of the yolk was assessed using the DSM-Firmenich YolkFan™. The color of fresh yolk was visually compared and classified using a yolk color fan (score from 1 to 15, ranging from light yellow to orange). This procedure was performed by the same judge on each day of assessment. The average of each treatment was calculated as the average of the pigmentation scores of the yolk of the eggs from each replicate. The three color parameters were assessed using a Konica Minolta CR-410 chroma meter. The a* value characterizes color in the region from red (+a*) to green (-a*), the b* value indicates color in the range from yellow (+b*) to blue (-b*), and the L value is lightness (Harder et al., 2008). Chroma is the relationship between the values a* and b*, which gives the real color of the analyzed egg.

The yolk was separated from the albumen and weighed. The weight of the albumen was obtained from the difference between the weight of the egg minus the weight of the yolk and the weight of the shell, which was obtained after washing the shell and subsequently drying it in air for 72 hours. With the eggs broken, the height of the dense albumen was measured using a digital caliper (DIGIMESSÒ) accurate to 0.01 mm, for later calculation of the Haugh unit [HU = 100 * log (h + 7.57 – 1.7 W 0.37)], where HU = Haugh unit, h = height of the albumen (mm) and W = weight of the egg (g).

The total carotenoid content of the yolk (expressed as zeaxanthin) was also assessed. These values were determined by modifying the method proposed by Rodriguez-Amaya (1999). Homogeneous yolk (13 g) was weighed on an analytical balance (accurate to 0.0001 g) and crushed with 8 g of Celite using a mortar and pestle. Cold acetone (30 mL) was added, and the mixture was left to stand for 5 minutes. Subsequently, vacuum filtration was performed with a sintered glass crucible coupled to a kitassato, repeating this process until the exhaustive extraction of carotenoids after washing with distilled water, which remained in the kitassato together with acetone.

For solvent partitioning, 20 mL of hexane was added to a separatory funnel, where acetone was separated and hexane remained with the carotenoid, and anhydrous sodium sulfate was added. The lower phase (water and acetone) was decanted, and the hexane phase was filtered and transferred to a 25 mL volumetric flask, which was made up to volume with hexane. The absorbance was immediately measured using a spectrophotometer at 449 nm. Carotenoid content (expressed as zeaxanthin) was calculated using Equation 1.

$$C\mu g/g = \frac{ABS\ x\ dilution\ x\ 10^4}{Molar\ Abs\ x\ m} \quad (1)$$

where C ($\mu g/g$) is the final concentration of total carotenoids (in zeaxanthin per g of yolk), ABS is the absorbance read on the spectrophotometer at 449 nm, dilution used for the sample (25 mL), Molar Abs is the absorption coefficient of zeaxanthin in hexane = 2,480, and m is the mass of the sample.

The feed digestibility and excreta of the birds were evaluated using the total collection method (Sakomura and Rostagno, 2016). A total of 120 birds were used, four birds per replicate, housed in cages with a tray for collecting excreta for 10 days, with five days of adaptation to the environment and five days of total excreta collection. Ferric oxide (2%) was used as a marker of excreta, indicating the beginning and end of collection.

After the adaptation period, excreta were collected twice a day (8 am and 8 pm), with a 12-hour interval to avoid fermentation and sample loss. During the collection phase, excreta were placed in plastic bags identified according to each repetition and stored in a freezer (-18 °C) until the end of the collection period. Finally, the samples were thawed, homogenized according to repetition and treatment, and weighed. A 550 g sample was taken from each repetition and dried in a forced ventilation oven at 60 °C to a constant weight.

After drying, the samples were weighed and ground in a knife mill using a 1-mm sieve for analysis of dry matter (DM), crude protein (CP), and crude fiber (CF). The gross energy (GE) values of the diets and excreta were quantified using a PARR calorimeter. Feed intake was evaluated during the 5 days of excreta collection according to the amount of feed consumed as a function of the number of birds in each repetition. To evaluate body weight, all birds were weighed at the beginning and end of the experimental phase, and weight gain per bird was determined. In addition, the apparent metabolizable energy (AME) (Equation 2) corrected for nitrogen balance (AMEn) (Equation 3), proposed by Matterson et al. (1965), was calculated.

$$AME = \frac{ingested\ GE - excreted\ GE}{ingested\ DM} \quad (2)$$

$$AMEn = \frac{ingested\ GE - excreted\ GE - 8.22\ x\ NB}{ingested\ DM} \quad (3)$$

where NB is Nitrogen Balance.

For economic analysis, the production cost of poultry feed was determined for different levels of corn DDGS (Table 2).

Table 2. List of inputs used in the experimental feed and market prices of the items.

Ingredients	Unit of Measurement	Cost (BRL)
Ground corn	60 kg bag	34.42
Soybean meal	60 kg bag	67.31
Calcitic limestone	1 kg	2.90
Dicalcium phosphate	1 kg	5.00
Common salt	1 kg	2.00
Mix (1)	20 kg bag	90.00
L-Lysine HCl	25 kg bag	260.00
DL-Methionine	25 kg bag	279.50
L-Threonine	25 kg bag	220.00
Starch	1 kg	4.57
Corn DDGS	1 kg	0.62

Source: Adapted from Agrolink (2020) and CONAB (2020).

Financial information was obtained for March 2020 according to the databases of Agrolink (2020) and the National Supply Company (CONAB, 2020).

After determining the cost of each type of feed (0%, 2%, 4%, 6%, and 8%), a production indicator was developed to verify the relationship between the feed cost and weight gain of the birds (Equation 4).

$$Productive\ indicator = \frac{weight\ gain\ of\ hens\ (kg)}{feeding\ cost\ (BRL)} \quad (4)$$

In the statistical analysis, analysis of variance was performed at a significance level of 5% (p<0.05) using Sisvar® software. Subsequently, the effects of the inclusion of corn DDGS were estimated using linear and quadratic regression models according to the best fit obtained for each variable. The contrasts were tested using Dunnett's test at a significance level of 5% (p<0.05), comparing the treatment without inclusion of corn DDGS (control) with the others (2%, 4%, 6%, and 8% of corn DDGS).

3. Results and Discussion

For laying hens to express their full genetic potential, it is important to provide them with balanced nutrition, health, and well-being in the environment in which they live. Birds are homeothermic animals; therefore, the temperature of the environment is an indicator of the thermal comfort zone, and it is important to consider thermoneutrality as a necessary parameter for producing and taking advantage of nutritional strategies (Silva et al., 2016).

The temperature and relative humidity of the air in the environment interfere with the raising of laying hens when they are outside the recommended range and can lead to discomfort and thermal stress, reducing productivity and causing a change in the behavior of the birds (Wishart et al., 2005). For adult birds, the thermal comfort zone is between 18 °C and 28 °C. When birds are subjected to temperatures above or below these limits, several physiological and behavioral responses are triggered to maintain a constant body temperature (41 °C) (Santana et al., 2017).

The state of Mato Grosso is characterized by high temperatures throughout the year, especially during the period in which the experiment was conducted (September and October). The maximum temperature recorded was 36.22 °C (± 2.49 °C), the minimum was 23.62 °C (± 1.49 °C), the maximum relative humidity was 74.88% (± 7.85%), and the minimum was 32.98% (± 11.87%) inside the shed. According to the Hisex Brown Management Guide (2021), during the laying phase, feed intake may decrease at temperatures above 24 °C and is extremely low at temperatures above 28 °C. In the present study, there was no significant difference (p<0.05) in feed intake between the treatments (Table 3).

Table 3. Productive performance of laying hens fed different levels of corn DDGS under hot weather conditions

Parameters	Corn DDGS levels (%)					CV (%)	***p*-value**
	0.0	2.0	4.0	6.0	8.0		
Feed intake (kg/bird/day)	0.104	0.102	0.102	0.99*	0.104	2.88	0.0794
Body weight (kg/bird)[1]	1.714	1.729	1.723	1.719	1.677	2.18	0.1551
Weight gain (g/bird)	0.307	0.323	0.302	0.293	0.261	14.73	0.1997
Feed conversion per dozen (kg/dz)	1.45	1.35	1.41	1.41	1.43	4.27	0.0868
Feed conversion per egg mass (kg/kg)	2.19	2.31	2.33	2.25	2.18	4.66	0.0643
Production/bird/day (%)	95.02	96.65	93.82	94.41	96.35	4.02	0.6599
Marketable eggs	94.12	96.35	93.37	93.52	95.83	4.14	0.5797
Age at 5% lay (weeks)	21.5	21.33	21.16	21.66	21.66	2.34	0.3701
Age at 50% lay (weeks)	23.00	23.00	23.50	23.50	23.33	1.79	0.0967
Viability	100	100	100	100	100	0.00	0.0000

CV – coefficient of variation; *Significant at 5% (p<0.05) by Dunnett test; [1]Body weight at 26 weeks of age.

However, in real terms, the guide confirms that feed intake was lower owing to high temperatures. Laying hens should consume approximately 112 g of feed per day. Owing to the high temperatures, the intake was lower than recommended.

Feed intake can change by approximately 1.72% for every 1 °C variation in ambient temperature between 18 and 32 °C, and can be even lower for the temperature range of 32–38 °C (5% for every 1 °C) (Plavnik, 2003). Low feed intake has a direct effect on the weight gain and feed efficiency of birds, as well as on egg size (Bertechini, 2012).

When the Dunnett test was applied, it was possible to observe that, when comparing the control feed (0%) with the other levels of inclusion of corn DDGS, the level of 6% presented a significance level of 5% (p<0.05), being the treatment that had the lowest feed intake per bird/day, 0.99 grams. Considering that all treatments presented intakes below the recommended level, this variation is justified by the heat stress they experienced during the experimental period. Lumpkins et al. (2005) found no significant difference (p<0.05) in feed intake and egg production of birds fed 15% corn DDGS. The hens consumed 95 g per day, which was justified by the heat stress that they suffered during the experimental period.

Heat stress also affects the reproductive systems of birds. At high temperatures, the endocrine profile of birds is altered, which occurs mainly because of reduced feed intake and, consequently, because of changes in the secretion of reproductive hormones (estradiol and progesterone), which are essential hormones for birds to produce eggs (Elnagar et al., 2010).

Due to low intake and body weight, the birds had a delayed onset of egg production. They reached 5% production with the inclusion of corn DDGS in their diet at approximately 21 weeks of age, and 50% production at 23 weeks of age. The strain guide predicted that the birds would reach peak production at 24 weeks of age. Body weight and weight gain did not differ between treatments (p<0.05) (Table 3). The weight of the birds at 26 weeks was lower than the ideal weight of 1.804 kg, according to the strain guide (Hisex Brown, 2021).

Egg production (birds/day) did not differ significantly (p<0.05) among the treatments analyzed (0%, 2%, 4%, 6%, and 8%), with peak egg production (± 95% production) reached at 26 weeks of age. The bird viability was 100%, and there were no deaths during the experimental period (Table 3).

Feed conversion per dozen (kg/dz) and egg mass (kg/kg) were not affected (p<0.05) by the inclusion of DDGS in the diet. The ideal feed conversion per dozen is 1.4 kg/dz according to the guide (Hisex Brown, 2021), a value close to that observed in the present study. And for feed conversion per mass, the recommended value is 2.17 kg/kg. This value was found here except in treatments with 2%, 4%, and 6% corn DDGS inclusion, which presented higher values. This can be explained by feed waste (Table 3).

Lumpkins et al. (2005) reported no significant difference in feed conversion per dozen when birds were fed 15% corn DDGS. However, they found that birds fed 0% corn DDGS had better feed conversion per dozen at 22 weeks of age. In an evaluation of Japanese quail, significant differences ($p<0.05$) were detected in feed intake and feed conversion per egg mass. The authors determined that 6.17% corn DDGS maximized feed intake and that 13.23% corn DDGS maximized feed conversion per egg mass (Bittencourt et al., 2019).

Scheideler et al. (2008) evaluated the use of corn DDGS in the diet of laying hens and observed that egg production, feed intake, and weight gain of the birds were not affected up to the maximum inclusion of 25% corn DDGS. However, egg weight decreased with the inclusion of 20% and 25% corn DDGS due to amino acid deficiency. Cheon et al. (2008), in turn, evaluated the effects of corn DDGS on chicken egg production and quality, testing 0%, 10%, 15%, and 20% inclusion levels. They observed that corn DDGS inclusion did not influence feed intake, egg production, egg mass, average egg weight, or feed conversion. However, yolk color increased significantly with increasing inclusion levels.

Similar to Cheon et al. (2008), a linear increase ($p<0.05$) in yolk color was observed when evaluated using a yolk color fan. The higher the level of corn DDGS included in the diet, the more yellow the egg yolk color appeared (Table 4). Using Dunnett's test to compare the 0% corn DDGS level with all other inclusion levels revealed statistical significance ($p<0.05$) for the 6% and 8% levels.

Table 4. Color and zeaxanthin content of egg yolk from laying hens fed different levels of corn DDGS.

Parameters	**Corn DDGS levels (%)**					**CV (%)**	***p*-value**
	0.0	**2.0**	**4.0**	**6.0**	**8.0**		
L*	65.76	64.98	66.25	65.41	65.55	2.06	0.5856
a*	4.67	4.62	4.89	4.27	4.49	8.15	0.0892
b*	47.02	48.31	48.10	49.60	49.60	4.62	0.2535
Chroma	47.26	48.53	45.50	49.79	47.26	10.06	0.6188
Yolk color fan[1]	4.33	4.79	4.75	4.91*	5.16*	6.83	0.0036
Zeaxanthin (ug/g)	4.45	5.06	5.43	4.35	5.02	40.04	0.8582

CV- coefficient of variation; [1]Linear effect ($p<0.05$); *Significant at 5% by Dunnett's test.

Egg yolk color is the result of the deposition of xanthophylls (carotenoid pigments), which can come from different sources, with the most common being corn (Garcia et al., 2002). Considering that DDGS are of corn origin, there was an increase in carotenoids in the diet, enabling an improvement in yolk color (Figure 1). Regarding the digital chroma meter, no significant differences were found ($p<0.05$) in the a*, b*, and L* parameters and chroma. The L and b values are color indicators that the human eye can distinguish. Lumpkins et al. (2005) also found no statistical difference ($p<0.05$) in the L and b parameters, finding only small differences in the indicator when the birds were given 15% corn DDGS.

No effect on yolk color was observed when birds were fed 10% corn DDGS (Roberts et al., 2007). Xanthophylls are sensitive to the presence of heat and light; therefore, different results and effects on yolk color may be found, especially with foods subjected to high temperatures, such as corn DDGS, which is subjected to heat during the drying process, leading to variation in the xanthophyll content of the co-product (Sauvant and Tran, 2004).

For zeaxanthin (ug/g), no significant difference ($p<0.05$) was observed between the levels of inclusion of corn DDGS in the diet of semi-heavy laying hens (Table 4), which may be related to yolk color. Cortés et al. (2015) evaluated different levels of corn DDGS for laying hens and total values of xanthophylls, zeaxanthin, and lutein and observed concentrations of 10–30 mg/g of lutein and zeaxanthin, which are higher than those found in the present study. The egg yolk color was linearly darker due to the presence of these xanthophylls in the diet and, consequently, present in the egg.

According to Silva et al. (2000), xanthophylls are fat-soluble and, when deposited in egg yolk, provide a yellowish color of varying intensity depending on the amount ingested by the bird.

Barbosa (2010) assessed the total carotenoid content by correlating it with the average pigmentation of egg yolks using the color fan and observed an increase in this content when a higher average color was presented using the color fan. Biscaro and Canniatti-Brazaca (2006) stated that pigmentation is not correlated with carotenoid content in egg yolk.

There are difficulties in measuring the carotenoid content due to the large quantity present in food, the qualitative and quantitative variation in food composition, the wide range of carotenoid concentrations in food, and their susceptibility to isomerization and oxidation during sample analysis and storage, as it is a very sensitive compound, especially to luminosity and temperature (Rodríguez-Amaya, 1999). Regarding egg quality, no statistical difference ($p<0.05$) was found for the evaluated parameters (Table 5).

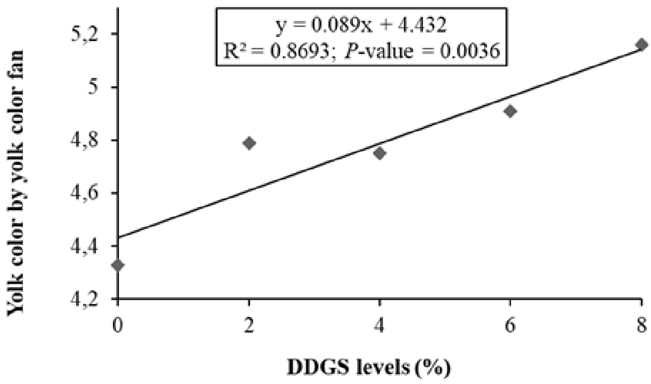

Figure 1. Yolk color analyzed by the yolk color fan.

Table 5. Egg quality of laying hens fed corn DDGS.

Parameters	Corn DDGS levels (%)					CV (%)	*p*-value
	0.0	**2.0**	**4.0**	**6.0**	**8.0**		
Egg weight (g)	56.68	56.60	57.96	56.15	56.32	3.80	0.6298
Yolk weight (g)	12.44	12.80	12.95	12.84	12.18	4.76	0.1816
Shell weight (g)	6.12	5.77	5.91	5.70	5.93	5.31	0.2005
Albumen weight (g)	38.11	38.05	39.09	38.67	38.20	5.65	0.9048
Haugh unit	82.19	84.81	86.70	86.80	86.73	4.49	0.1961
Specific gravity (g/cm^3)	1.095	1.095	1.094	1.092	1.095	0.24	0.1772

CV – coefficient of variation; g – grams.

As a consequence of low feed intake and the delay in the onset of egg laying, the weight of the eggs was lower than that recommended by the Hisex Brown management guide (2021) (59 g) when the birds reached 26 weeks of age. The average egg weight of the evaluated treatments was approximately 56 g (Table 5). Sexual maturity is extremely important because egg weight is directly related to body weight, and according to Leeson and Summers (2009), birds that lay small eggs at the beginning of the laying phase tend to produce eggs with lower weight for the rest of their lives.

El Hack et al. (2019) evaluated different levels of inclusion of corn DDGS and observed that the eggs of birds fed 6% and 12% DDGS had a significantly higher weight (68 grams, on average) compared to the control group (67 grams). However, with 18% DDGS, average egg weight decreased to 65 g. Furthermore, the birds that received 6% DDGS had a lower percentage of albumen and a higher percentage of yolk than the other groups. Cotrina et al. (2018) observed a better average egg weight result when 20% corn DDGS was included in the diet for semi-heavy laying hens, presenting a value of 63.7 g, while the control group (0% corn DDGS) presented an average weight of 59 g.

The weights of the yolk, shell, and albumen did not show significant differences ($p<0.05$) between the treatments evaluated with the inclusion of corn DDGS in the diet for laying hens. Sun et al. (2011) concluded that corn DDGS can positively affect the physical state of the egg albumen. It was possible to observe an increase in real values with the inclusion of 4%, 6%, and 8% corn DDGS (39.09 g, 38.67 g, and 38.20 g, respectively), compared to 0% corn DDGS (38.11 g), even though the diets are isonutritive.

Swiatkiewicz and Koreleski (2006) recommended the use of 15% corn DDGS without negatively affecting egg production or quality. Roberson et al. (2005) observed that as the level of corn DDGS increases in the diet of birds, it causes a linear decrease in egg production, egg weight, egg mass, and specific gravity. Therefore, they suggest that corn DDGS levels should not exceed 5% to avoid harming the availability of nutrients in the feed.

Bittencourt et al. (2019) detected no difference ($p<0.05$) in the weight of the yolk, albumen, or shell of quail eggs fed different levels of corn DDGS, indicating that the birds received isoenergetic and isoprotein diets. It is possible that the hens ingested adequate nutrients so that the weight of the eggs and their components (yolk and shell) remained stable when corn DDGS was added.

The Haugh unit (HU) was used to evaluate the internal quality of the eggs, and no significant differences were observed ($p<0.05$). According to the Egg Quality Control Program for consumption recommended by the United States Department of Agriculture (USDA), eggs considered to be of excellent quality (AA) must have HU values greater than 72 upon reaching the consumer. The average HU value of the analyzed treatments was 85 (Table 5), indicating excellent internal quality of the eggs. El-Hack et al. (2019) also found that increasing levels of DDGS in laying hen diets resulted in higher Haugh unit values than the control diet (0% DDGS). The highest value (89.97 HU) was obtained with 18% DDGS.

For specific gravity, there was no influence (p<0.05) with the inclusion of corn DDGS; ideally, this parameter should be above 1.080 g/cm^3 for eggs to be considered of good quality. The eggs from the analyzed treatments had an average specific gravity of 1.095 g/cm^3, indicating excellent quality. These results are similar to those of Lumpkins et al. (2005), who observed values higher than 1.080 g/cm^3 when 0% and 15% of corn DDGS were added to the diet of laying hens.

Roberson et al. (2005) found no difference in specific gravity with the inclusion of 0%, 5%, 10%, and 15% of corn DDGS; the treatments presented values of approximately 1.080 g/cm^3. No significant differences (p<0.05) were observed in apparent metabolizable energy (AME) or nitrogen balance (NB) across the evaluated corn DDGS levels (0%, 2%, 4%, 6%, and 8%) (Table 6).

Table 6. Apparent metabolizable energy (AME), apparent metabolizable energy corrected for nitrogen balance (AMEn) of corn DDGS.

Parameters	**Corn DDGS levels (%)**					**CV(%)**	***p*-value**
	0.0	**2.0**	**4.0**	**6.0**	**8.0**		
AME (kcal/g)	3,981.00	3,981.34	3,980.22	3,979.11	3,982.12	0.13	0.8913
AMEn (kcal/g)[1]	3,569.91	3,549.05	3,786.41	3,710.68	3,818.09	0.27	0.0000
NB	32.25	36.28	34.58	39.00	38.12	16.61	0.3195

CV: coefficient of variation. [1]quadratic effect.

The apparent metabolizable energy corrected for nitrogen balance revealed significant differences (p<0.05) between the corn DDGS levels evaluated, with the 3.05% level showing the greatest value (Figure 2). Rochell et al. (2011) reported a low correlation between AMEn and gross energy. In contrast, Meloche et al. (2013) observed a 69% correlation between these factors, which may be due to impaired digestibility caused by processing. Despite this correlation, there was no negative influence on the performance of these birds.

Rew et al. (2009) investigated the effect of including 0, 10, and 20% corn DDGS on the performance of laying hens using an economic analysis and observed satisfactory results when replacing soybean meal with the ethanol co-product. Corn DDGS was economically viable when used in laying hen diets (Table 7).

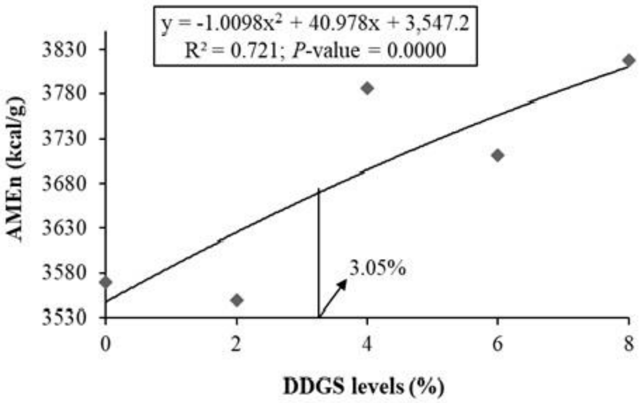

Figure 2. AMEn (Kcal/g) of corn DDGS (%).

Table 7. Feed cost (BRL) and production indicator (Kg/BRL) of experimental feeds with different levels of inclusion of corn DDGS

Parameters	**Corn DDGS levels (%)**					***p*-value**
	0	**2**	**4**	**6**	**8**	
Feed cost (BRL)[1]	4.96	4.82	4.73	4.55	4.66	0.0004
Production indicator (kg/BRL)	0.062	0.067	0.063	0.064	0.056	0.3658

[1]Quadratic effect.

Cheon et al. (2008) proposed using corn DDGS as an alternative to soybean meal and corn in laying hen feed due to its lower market value. Bittencourt et al. (2019) considered corn DDGS a strategic, economical feed for Japanese quail raised in hot climates. Among the analyzed treatments, the 5% corn DDGS level had the best cost-benefit ratio and the highest profit margin.

Feed cost was significantly affected (p<0.05) by the different levels of corn DDGS inclusion (Figure 3). Including 4.39% corn DDGS minimized feed costs for laying hens. There was no significant difference in the production indicator (p<0.05); however, the level with 8% corn DDGS had the lowest production indicator (0.56 kg/BRL) in absolute values.

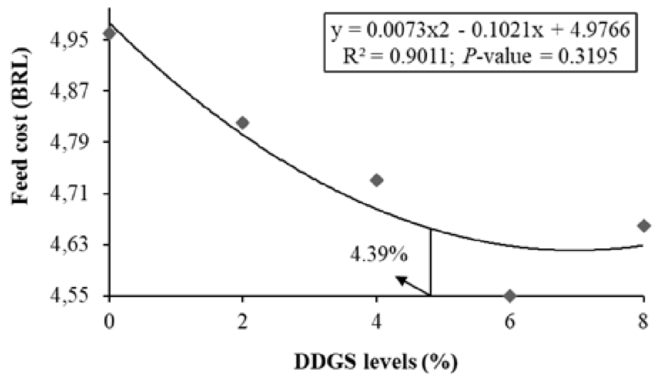

Figure 3. Cost of feed (BRL) in the laying phase**.**

The performance and egg quality results obtained here are below the values considered by the 2021 Hisex Brown management guide, but they are still acceptable. This highlights the importance of studies on alternative sources and substitute foods for conventional ones, which aim to reduce feed costs.

4. Conclusions

Corn DDGS is an alternative food to soybean meal. They can be used to feed semi-heavy laying hens raised in hot climates at peak laying. This does not negatively influence egg performance or quality. It also improves yolk color. Economically, it is viable when inclusion is up to 4.39%.

Authors' Contribution

Work extrated from the doctoral thesis of Tatiana Marques Bittencourt, supervised by Prof. Dr. Heder José D´Avila Lima; Caio Silva Quirino contributed to data collection, Isabelli Dias Brito Pereira contributed to data collection, and Elieverson Firmiani de Freitas Amaral contributed to data collection; Prof. Dr. Diego Pierotti Procópio contributed to the analysis of the economic information.

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