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Effect of guanidinoacetic acid on performance, egg quality, yolk fatty acid composition, and nutrient digestibility of aged laying hens fed diets with varying substitution levels of corn with low-tannin sorghum

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ABSTRACT A study was conducted to evaluate the efficiency of guanidinoacetic acid (**GAA**) in diets containing varying levels of corn replacement with low-tannin sorghum (**LTS**) for laying hens in the later stage of production. In a 12-wk study, a total of 288 laying hens at 52 wk of age were divided into 6 treatment groups. Each treatment group had 8 replicates, each of which consisted of 6 hens. A 2 × 3 factorial design was used to investigate the impact of substituting corn with LTS at 3 levels (100% corn, 50% LTS, and 100% LTS) with 2 doses of GAA supplementation (0 and 0.6 g/kg). The results indicate that there were interaction effects ($P < 0.05$) between diet type and GAA supplementation on protein digestibility and AMEn, with the GAA supplement being more effective in the 100% LTS group. Replacing corn with LTS at both levels had no negative effects on performance and metabolic profile. In contrast, the 100% LTS diet increased monounsaturated fatty acids in the yolk ($P < 0.05$), but decreased the yolk color index, the ratio of polyunsaturated fatty acids (**PUFA**) to saturated fatty acids (**SFA**) in the yolk, ileal digestibility of energy, and AMEn when compared to the 100% corn diet ($P < 0.05$). Regardless of the diet, dietary supplementation with GAA resulted in increases ($P < 0.05$) in shell-breaking strength, the PUFA to SFA ratio in egg yolk, and concentrations of creatine and nitric oxide in serum. There was also a decrease ($P < 0.05$) in serum malondialdehyde concentration with GAA supplementation. In conclusion, the positive effects of GAA on protein digestibility and AMEn were found to be more pronounced when corn was completely replaced with LTS. However, the positive effects of GAA on egg-laying performance, eggshell quality, antioxidant status, and yolk fatty acid composition remained consistent regardless of the extent to which corn was substituted with LTS.

KEY WORDS: digestibility, laying hen, metabolic profile, sorghum-based diet, yolk composition

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is considered a promising alternative to corn because of its nutritional compatibility (Bonilla et al., 2017). A previous study has shown that sorghum possesses nutritional properties that are slightly inferior to those of corn (Douglas et al., 1990). Sorghum's capacity to cultivate in challenging agricultural conditions underscores its potential as a resilient and sustainable crop for regions experiencing water scarcity and salinity issues, such as central and southern Iran (Pour-Reza and Edriss, 1997). Tannin plays a crucial role in determining the energy content of sorghum when it is consumed by poultry species. In a previous investigation, it was found that the inclusion of high-tannin sorghum in the diet of laying hens had a negative impact on their productivity (Sell et al., 1983; Ambula et al., 2003). However, the utilization of low-tannin sorghum (**LTS**) varieties in poultry feeds has increased due to their development (Arroyo et al., 2016; Xu et al., 2017). Previous research has indicated that although sorghum exhibits a higher protein content, the digestibility of essential amino acids such as lysine, methionine, and threonine is relatively low when compared to corn (Ebadi et al., 2011; Puntigam et al., 2020). According to Taylor et al. (1984), it has been observed that kafirin, the main protein component comprising around 48% of the total protein content in sorghum, undergoes digestion at a comparatively slower rate. The low bioavailability of essential amino acids in kafirin can also have detrimental effects on the digestibility of amino acids, primarily due to kafirin's resistance to proteolysis (Selle et al., 2010). Additionally, it has been

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noted that kafirin has the potential to hinder the process of starch digestion through physical and chemical interactions (Duodu et al., 2003), which could potentially lead to a reduction in energy efficiency. Hence, it is imperative to acquire knowledge concerning the nutrient composition of LTS and its nutritional accessibility. This knowledge is crucial for developing diets that effectively fulfill the dietary requirements of animals and contribute to the advancement of economic and ecological sustainability in the poultry egg production sector.

Guanidinoacetic acid (**GAA**) has gained significant attention as a feed supplement due to its notable stability and high bioavailability. The primary function of this substance is to optimize energy utilization across a wide range of applications. One key factor in achieving this function is the efficient conversion of creatine within the body (Tossenberger et al., 2016; Ale Saheb Fosoul et al., 2018). In the context of avian organisms, it has been observed that the endogenous synthesis of creatine may not be sufficient to meet the requirements of commercially raised poultry that exhibit accelerated growth or enhanced productivity (Majdeddin et al., 2019; Khajali et al., 2020). However, it is important to highlight that the use of creatine has certain limitations that should be taken into account. The limitations primarily revolve around concerns related to stability and the financial implications associated with supplementation, particularly when compared to the use of GAA (Baker, 2009). The existing body of literature suggests that the supplementation of GAA may be a promising strategy for improving the overall performance and metabolic efficiency of broiler chickens (Mousavi et al., 2013; Ahmadipour et al., 2018; Miri et al., 2022). In a recent study conducted by Salah et al. (2020), it was discovered that the inclusion of GAA in the diet led to enhanced performance of commercial layers in the later stages of production.

The utilization of GAA in various scientific studies has produced promising results in boosting the levels of creatine and arginine, 2 key components that play a vital role in mitigating the harmful effects of free radicals and supporting the body's ability to combat oxidative stress (Zhao et al., 2021; Cui et al., 2022). A previous study (Amiri et al., 2019) demonstrated that the inclusion of GAA in the diet of broiler chickens resulted in a significant increase in the activities of antioxidant enzymes, along with a reduction in the levels of malondialdehyde (**MDA**) content. Thus, by incorporating GAA into broiler diets, farmers and producers may potentially enhance the overall health and well-being of their flocks. However, a noticeable gap exists that warrants attention regarding the investigation into the potential effects of GAA supplementation on the antioxidant status and egg fatty acid composition in laying hens. Previous studies have also provided evidence suggesting that the administration of exogenous GAA can enhance nutrient digestibility in laying Japanese quails (Raei et al., 2020) and energy retention as fat in broiler chickens (Ale Saheb Fosoul et al., 2018). In light of the existing data, it was hypothesized that the effects of GAA on the nutritional and physiological responses of aged laying hens may be influenced by the type of cereal included in their diet. To date, there is a lack of literature documenting the utilization of GAA in layer diets incorporating sorghum. Hence, the current investigation was undertaken to examine the impacts of GAA on performance, egg quality, metabolic profile, yolk fatty acid profile, and nutrient retention in laying hens that were fed diets with 50% and 100% substitution levels of corn with LTS.

MATERIALS AND METHODS

Diets and Experimental Design

The experimental techniques utilized in this study were approved by the Institutional Animal Care and Use Committee of Arak University (contract number 1401-8685). A total of 288 Super Nick white laying hens at 50 wk of age were selected from a larger population of approximately 3,000 birds. The selected hens were uniform in terms of body weight (**BW**) and laying rate. After a 14-d period of acclimation, the hens were weighed and then divided into 6 treatment groups. In the present study, a 3 × 2 factorial arrangement was used to examine the effects of 3 different substitution levels of corn with LTS (100% corn, 50% LTS, and 100% LTS), along with 2 concentrations of GAA (0 and 0.6 g/kg of diet). The optimal level of GAA was chosen based on the preliminary experiment conducted in our research farm, as well as the previously reported dosage in broiler chickens (Majdeddin et al., 2019), laying Japanese quails (Raei et al., 2020), and aged broiler breeder hens (Sharideh et al., 2016). The present study was conducted for a duration of 12 wk, from the 52nd wk to the 64th wk of age. The experimental setup consisted of cages, each measuring 75 cm in length, 54 cm in width, and 45 cm in height. These cages housed 6 hens per cage, resulting in a stocking density of approximately 675 cm^2 per hen. A total of 48 cages were used, each of which was equipped with linear feeders and nipple drinkers. The hens were housed in a controlled-environment facility, where the ambient temperature was carefully regulated. The average daily minimum and maximum temperatures were set at 20°C and 24°C, respectively. The experiment used the Fouman variety of red/bronze grain sorghum, which is a low-tannin animal feed-grade type sourced from the central desert of Iran. The GAA supplementation utilized in the current study was GuanAMINO (Evonik Degussa GmbH, Hanau-Wolfgang, Germany), with a documented purity exceeding 96% GAA. In order to establish optimal lighting conditions, the experimental setup was designed to provide a consistent duration of 16 h of light per d. The experimental diets administered to the laying hens in this study were provided in a mashed and homogenized form. These diets were formulated to align with the nutrient recommendations specified in the Super Nick Nutrition Management Guide (Super Nick H&N, 2020). The chemical composition of the corn, LTS, and soybean

Table 1. Determined chemical composition of corn, low-tannin sorghum (LTS), and soybean meal samples used in the experimental diets.

Item[1] (%, unless stated otherwise)	Corn	LTS	Soybean meal
Dry matter	89.5	91.2	90.7
Crude protein	8.2	9.5	44.4
Ether extract	3.7	2.9	2.1
Crude fiber	2.1	2.3	3.6
Total ash	1.1	1.9	6.5
Calcium	0.04	0.03	0.33
Total phosphorous	0.24	0.30	0.62
Gross energy, Kcal/kg	3,874	3,930	4,143
Total tannin[2]	0.18	0.51	0.05
Amino acid profile			
Total lysine	0.18	0.22	2.65
Total methionine	0.16	0.21	0.59
Total cysteine	0.15	0.24	0.61
Total sulfur amino acids	0.31	0.43	1.20
Total threonine	0.22	0.32	1.72
Total valine	0.31	0.45	2.20
Total tryptophan	0.05	0.08	0.60
Total arginine	0.31	0.57	3.23
Total glycine	0.24	0.41	1.88
Leucine	0.75	1.03	3.39
Isoleucine	0.23	0.34	1.98
Histidine	0.20	0.27	1.16
Fatty acid profile, % of total fatty acids			
C16:0	10.5	14.3	11.2
C18:0	1.8	1.7	3.8
C18:1n-9	26.1	38.2	23.1
C18:2n-6	59.5	42.2	54.0
C18:3n-3	1.3	1.8	7.2

[1]Mean of 3 samples.
[2]Tannin content expressed as catechin equivalents.

meal used in this study is shown in Table 1. The ingredient composition and nutrient contents of the experimental diets are presented in Table 2.

Chemical Analysis

The nutritional composition, i.e., dry matter, crude fat, crude protein, crude fiber, crude ash, calcium, total phosphorus, and nitrogen-free extract, of the main feed ingredients (corn, LTS, and soybean meal) and experimental diets was determined in accordance with the guidelines provided by the AOAC (2007). The estimation of crude protein content was done by determining the nitrogen content of samples and subsequently multiplying it by a factor of 6.25. The gross energy determination was performed using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). The calorimeter was calibrated using benzoic acid as the standard substance. The tannin content of corn, LTS, and soybean meal samples was determined according to the method described by Maxson and Rooney (1972). Samples from both feedstuffs and diets were analyzed for their total amino acid profile using an automatic amino acid analyzer (L-8800, Hitachi, Tokyo, Japan). Before conducting the analysis, the samples were subjected to hydrolysis using a 6M hydrochloric acid solution at 110° C for 24 h. Performic acid oxidation was also performed to determine sulfur amino acids. The coefficients for amino acid digestibility and apparent metabolizable energy corrected for nitrogen (**AMEn**) values of different feedstuffs were obtained from the AminoDat (2015) dataset.

Productive Performance

The daily feed intake per bird was calculated by dividing the total feed consumption for each replication during the 12-wk feeding period by the number of d within this period. The daily number and weight of eggs laid were also determined. The hen-day egg production was measured by counting the number of eggs laid in each group. The egg mass was determined by considering both the daily egg production and the average weight of the eggs. The feed conversion ratio was calculated per cage by dividing the feed intake by the total egg mass. The final BW of the hens was also recorded at the end of the experiment.

Egg Quality Parameters

To evaluate the indicators of egg quality, a total of 32 eggs were randomly selected from each treatment group (4 eggs from each replicate) at the end of the experiment. Measurements were conducted within the first 24 h after egg collection. The eggshell breaking strength (kg/cm^2) was measured using an eggshell strength tester (OSK 13473, Ogawa Seiki Co., Ltd., Tokyo, Japan). In accordance with the procedure previously described (Abbasi Arabshahi et al., 2021), the eggs were weighed and then cracked open to separate the albumen and yolk components. After removing the chalazae and any adhering albumen from the yolks, the weight of the yolks was measured using a digital scale with a precision of 0.01 g. The yolk color scores were evaluated using the Roche color fan scale (Roche Ltd., Basel, Switzerland). The scale used in this study ranged from 15 (representing a dark orange color) to 1 (indicating a light pale color). The height of the albumen was measured using a digital caliper with a precision of 0.01 mm. The Haugh unit was calculated using the formula established by Eisen et al. (1962). The eggshells underwent a 48-h air-drying process at room temperature, and then their weights were recorded. The measurement of eggshell thickness was conducted using a caliper with a precision of 0.01 mm at 3 specific points on each egg: the air cell, equator, and sharp end. The mean value of the measurements was computed to represent the eggshell thickness.

Serum Parameters

To evaluate serum parameters, 5 mL of blood samples were collected from the wing vein of 2 randomly chosen hens in each replicate (n = 16 hens per treatment group) at 64 wk of age. The serum was obtained by centrifuging blood samples at 3,000 g for 10 min at 4°C, followed by a 30-min incubation at room temperature. The isolated serum was stored at −80°C in a 2-mL tube until further analysis. The measurement of MDA concentration was

Table 2. Composition of corn- and low-tannin sorghum (LTS)-based basal diets, and their calculated and determined analysis (as-fed basis), fed to laying hens (weeks 52 to 64).

	Basal diet				Basal diet		
Item, (%, unless stated otherwise)	100% corn	50% LTS	100% LTS	Analyzed nutritive value[4], g/kg	100% corn	50% LTS	100% LTS
Corn	60.18	30.08	–	Total energy, kcal/kg	3,475	3,509	3,544
Sorghum	–	31.41	62.83	Crude protein	16.89	16.46	16.02
Soybean meal	26.21	24.62	23.02	Ether extract	3.46	3.48	3.52
Soybean oil	0.82	1.07	1.31	Crude fiber	3.03	3.00	2.96
Dicalcium phosphate	1.48	1.51	1.53	Total ash	13.92	14.10	14.26
Limestone	5.25	5.23	5.21	Total phosphorous	0.56	0.58	0.59
Oyster shell	5.00	5.00	5.00	Calcium	3.97	3.97	3.96
Salt (NaCl)	0.25	0.25	0.24	Amino acid profile			
Sodium bicarbonate	0.03	0.08	0.14	Total lysine	0.85	0.86	0.88
Vitamin and mineral premix[1]	0.50	0.50	0.50	Total methionine	0.39	0.41	0.44
DL-methionine	0.18	0.15	0.12	Total cysteine	0.31	0.31	0.30
L-lysine HCl	0.06	0.08	0.10	TSAA	0.70	0.72	0.74
L-threonine	0.04	0.02	–	Total threonine	0.65	0.62	0.60
Calculated nutritive value				Total valine	0.78	0.79	0.79
AMEn, kcal/kg	2,700	2,700	2,700	Total tryptophan	0.19	0.19	0.19
Crude protein	16.70	16.70	16.70	Total arginine	0.95	0.97	1.00
Calcium	4.30	4.30	4.30	Total tannin[5]	0.12	0.23	0.32
Nonphytate phosphorous	0.38	0.38	0.38	Fatty acid profile, % of total fatty acids			
Digestible TSAA[2]	0.67	0.67	0.67	C16:0	10.31	11.61	12.89
Digestible lysine	0.75	0.75	0.75	C18:0	2.37	2.29	2.21
Digestible threonine	0.52	0.52	0.52	C18:1n-9	24.18	28.39	32.59
Sodium	0.16	0.16	0.16	C18:2n-6	55.43	49.53	43.62
DEB,[3] mEq/kg	220	220	220	C18:3n-3	3.00	3.09	3.17

[1]Supplied per kg of diet: all-trans-retinyl acetate, 8800 IU; cholecalciferol, 2500 IU; α-tocopherol acetate, 6.6 mg; menadione sodium bisulfite, 2.5 mg; thiamine mononitrate, 1.5 mg; riboflavin, 4.4 mg; nicotinic acid, 20 mg; calcium D-pantothenate, 8 mg; pyridoxine, 2.5 mg; folic acid, 1.1 mg; cyanocobalamine, 0.08 mg; biotin, 0.15 mg; choline chloride, 400 mg, Mn (from manganese sulfate), 60 mg; Fe (from ferrous sulfate), 30 mg; Zn (from zinc sulfate), 66 mg; Cu (from copper sulfate), 6 mg; I (from potassium iodate), 0.8 mg; Se (from sodium selenite), 0.2 mg.

[2]Total sulfur amino acids.

[3]DEB (dietary electrolyte balance) = (Na^+, mEq/kg + K^+, mEq/kg) − CL^-, mEq/kg.

[4]Mean of 3 samples per diet. In the 100% corn diets, the analyzed concentrations of GAA were <1 and 612 mg/kg for the diets without GAA supplementation and with 0.6 g/kg GAA supplementation, respectively. The analyzed GAA values for 50% LTS diets were <1 and 595 mg/kg, and for 100% LTS diets, the analyzed GAA values were <1 and 618 mg/kg, respectively.

[5]Tannin content expressed as catechin equivalents.

performed using the 2-thiobarbituric acid assay at a wavelength of 532 nm, as outlined in the study by Jensen et al. (1997). The serum total antioxidant capacity (**TAC**) was assessed using the ABTS assay, following the standard protocol provided by the commercial kit (Randox kit, Pars Azmun, Tehran, Iran). The serum concentrations of creatine and creatinine were measured using commercially available spectrophotometric kits (Sigma Aldrich Company Ltd., Poole, Dorset, United Kingdom). The serum nitric oxide (**NO**) concentration was determined using the methodology described by Haddad et al. (1995). The experimental protocol consisted of combining 100 μL of the serum sample with an equivalent volume of the Griess reagent. The absorbance of the resulting mixture was measured at a wavelength of 540 nm, as determined by a spectrophotometer (Corning 480, Corning Inc., New York, NY).

Fatty Acid Profile Measurement

After conducting an assessment of the quality traits of eggs, the yolks from each replicate (32 egg yolks per treatment group) were individually separated. The yolks were pooled within each replicate, homogenized, and subsequently stored at −20°C for further analysis of their fatty acid profiles. The fatty acids in diets and egg yolks were separated using gas chromatography (Model 4600, Unicam, Cambridge, United Kingdom). The experimental setup consisted of a flame ionization detector and a BPX70 fused silica capillary column. The column was characterized by its dimensions: a length of 30 meters, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm. The fatty acids were separated and detected using the setup that was described. As an internal standard, pentadecanoic acid (Sigma, St. Louis, MO) was used. The determinations were conducted under controlled experimental conditions. The gas carrier used was helium, with a flow rate of 1 mL/min. The column flux was set at a constant rate of 1.00 mL/min, and the linear velocity was consistently maintained at 24 cm/s. The injector temperature was maintained at 240°C, and the detector temperature was set at 280°C. The oven temperature was set to initiate at 50°C and maintained for 10 min. Subsequently, a gradual increase in temperature to 180°C was executed over a period of 2 min. The temperature was increased gradually from 180 to 240°C at a constant rate of 5°C per min. The volume of the injected sample into the split injector was 1 μL. The identification of individual fatty acid peaks was achieved by comparing their retention times with those of corresponding standards, as outlined in the study conducted by Ghasemi et al. (2022). The quantification of individual fatty acids was conducted by analyzing the peak area and expressing the results as a percentage relative to the total fatty acids. The levels of

saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**), and polyunsaturated fatty acids (**PUFA**) were determined by calculating the mean values for each fatty acid.

Nutrient Digestibility

The apparent ileal digestibility (**AID**) of nutrients was assessed using the acid insoluble ash (**AIA**) marker, following the methodology described by Abbasi Arabshahi et al. (2021). All diets were supplemented with celite at a rate of 5 g/kg for a period of 4 d prior to the conclusion of the study. At the end of the study, a random selection was made of 2 laying hens from each replicate cage. The hens were euthanized by cervical dislocation, and their ileal contents (from the vitelline diverticulum to the ileocecal junction) were collected. The contents were pooled per replicate cage, freeze-dried, and subsequently pulverized using a hammer mill (Model 5543 GEN, Isfahan Dasht, Isfahan, Iran) to obtain particles that could pass through a 0.5-mm screen prior to undergoing chemical analysis. The chemical analysis of the ileal digesta was performed using the same methodologies as those used for analyzing feedstuffs and diets. The levels of AIA were quantified in diet and ileal digesta samples, following the methodology described by McCarthy et al. (1974). The determination of the AID of nutrients in the diets was conducted using the following equation:

$$\text{AID}\ (\%) = \left[1 - \left(\text{AIA}_{\text{feed}}/\text{AIA}_{\text{digesta}}\right) \times \left(\text{nutrient}_{\text{digesta}}/\text{nutrient}_{\text{feed}}\right)\right] \times 100$$

Where, AIA $_{\text{feed}}$ and nutrient $_{\text{feed}}$ are the concentrations of AIA and nutrient in the feed (%) and AIA $_{\text{digesta}}$ and nutrient $_{\text{digesta}}$ represent the concentrations of the same AIA and nutrient in the ileal digesta (%).

For the measurement of AMEn, excreta samples were collected (n = 8 per treatment). The equation used to calculate the AMEn value was as follows:

$$\text{AMEn (kcal/kg of feed)} = \text{GE}_{\text{feed}} - [(\text{GE}_{\text{excreta}} \times \text{IF}) + 8.22 \times (\text{N}_{\text{feed}} - \text{N}_{\text{excreta}} \times \text{IF})]$$

Where, GE $_{\text{feed}}$ represents gross energy content in feed (kcal/kg), $\text{GE}_{\text{excreta}}$ represents gross energy content in excreta (kcal/kg), IF represents the indigestibility factor ($\text{AIA}_{\text{feed}}/\text{AIA}_{\text{excreta}}$), N_{feed} represents nitrogen content in feed (%), $\text{N}_{\text{excreta}}$ represents nitrogen content in excreta (%), and 8.22 represents the energy equivalent (kcal/g) of uric acid.

Statistical Analysis

The data collected from the experiment were analyzed using a 2-way analysis of variance with a 2 × 3 factorial design to investigate the effects of diet type and GAA level, as well as their interactions. The analysis was performed using the proc mixed procedure of the SAS 9.3 package (SAS, 2010). The experimental unit was varied according to the specific parameters being measured. The cage served as the experimental unit for evaluating performance traits and egg quality parameters. However, individual data from laying hens was used as an experimental unit for other measurements. The normality of the data was assessed by conducting the Shapiro-Wilk test, while the homogeneity of variances was evaluated through Levene's test. A Tukey's post hoc analysis was conducted to assess significant differences at a significance level of $P < 0.05$. The study findings were reported as means, accompanied by their corresponding standard errors of the means (**SEM**).

RESULTS

Productive Performance

Table 3 presents the effects of different diet types and GAA supplementation on the productive performance of laying hens. During the experimental period (52−64 wk of age), the inclusion of GAA in the diet resulted in increases in egg production ($P = 0.051$) and egg mass ($P = 0.004$), while also leading to a decrease in the feed conversion ratio ($P = 0.010$). In contrast, no significant effects ($P > 0.05$) of GAA on egg weight, feed intake, or final BW were observed. Moreover, there were no significant effects of diet type and its interaction with GAA supplementation on any of the measured productive parameters in this study ($P > 0.05$).

Egg Quality

The results of egg quality characteristics, including the proportion of egg components, albumen height, Haugh unit, yolk color index, shell thickness, and shell breaking strength, are presented in Table 4. Supplementation with GAA resulted in an increase in albumin height ($P = 0.081$), Haugh unit ($P = 0.053$), shell thickness ($P = 0.061$), and shell breaking strength ($P = 0.019$). However, no significant changes were observed ($P > 0.05$) in other egg qualitative metrics as a result of GAA supplementation. The diet type had no significant effect on egg quality indicators, except for the egg yolk color index. The yolk color index decreased in the 100% LTS diet compared to the 100% corn diet ($P = 0.004$), while the 50% LTS group showed intermediate results and did not significantly differ from the other treatments ($P > 0.05$). The results also indicate that no significant interaction effect was observed between diet type and GAA supplement on any of the measured egg quality parameters ($P > 0.05$).

Serum Biochemical Parameters

According to Table 5, GAA supplementation resulted in increased ($P < 0.001$) concentrations of creatine and nitric oxide while also causing a decrease ($P = 0.019$) in serum MDA concentration (Table 5). In addition, the inclusion of GAA in the diet exhibited a tendency to

Table 3. Effects of guanidinoacetic acid (GAA) on productive performance in laying hens fed different substitution levels of corn with low-tannin sorghum (LTS) at 52 to 64 wk of age.

Item[1]		Egg production %	Egg weight g	Egg mass g/hen/d	Feed intake g/hen/d	Feed conversion ratio	Body weight g
Diet type							
100% corn		85.92	63.70	54.73	104.69	1.92	1,677
50% LTS		85.42	63.82	54.50	104.98	1.93	1,665
100% LTS		85.55	63.58	54.40	104.47	1.92	1,668
SEM		0.809	0.457	0.628	0.636	0.024	13.1
GAA level (g/kg diet)							
0		84.63[b]	63.34	53.60[b]	104.72	1.96[a]	1,668
0.6		86.63[a]	64.06	55.48[a]	104.70	1.89[b]	1,673
SEM		0.660	0.347	0.463	0.549	0.020	10.3
Diet type	GAA level (g/kg diet)						
100% corn	0	85.14	63.59	54.13	104.75	1.94	1,670
100% corn	0.6	86.71	63.80	55.32	104.62	1.90	1,684
50% LTS	0	84.56	63.62	53.79	105.19	1.96	1,669
50% LTS	0.6	86.28	64.03	55.21	104.76	1.90	1,661
100% LTS	0	84.20	62.82	52.88	104.22	1.98	1,663
100% LTS	0.6	86.90	64.35	55.92	104.71	1.87	1,673
SEM		1.144	0.601	0.802	0.951	0.035	17.8
Significance							
Diet type		0.894	0.932	0.929	0.851	0.943	0.795
GAA level		0.051	0.128	0.004	0.980	0.010	0.723
Diet × GAA		0.872	0.449	0.379	0.897	0.585	0.792

[a,b]Means within each column with no common superscript differ ($P < 0.05$).
[1]Data represent means from 8 replicates (i.e., cages) per treatment.

elevate serum creatinine levels ($P = 0.091$). However, dietary GAA supplementation did not have an impact on serum TAC concentrations ($P > 0.05$). Moreover, neither the type of diet nor its interaction with GAA supplementation had an impact on the blood parameters examined ($P > 0.05$).

Egg Yolk Fatty Acid Profile

The results regarding the effects of different types of diet and GAA supplementation on the total percentage composition of SFA, MUFA, PUFA, n-6 PUFA, n-3 PUFA, and total PUFA in the egg yolks are presented in Table 6. Additionally, Supplementary Table S1 presents the detailed fatty acid composition. The results showed that feeding laying hens with the 100% LTS diet resulted in a significant increase ($P < 0.05$) in oleic acid (C18:1n-9) and MUFA, while there was a significant decrease ($P < 0.05$) in arachidonic acid (C20:4n-6) and the ratio of n-6/n-3 PUFA when compared to the 100% corn diet. The levels of linoleic acid (C18:2n-6), n-6 PUFA, total PUFA, and PUFA/SFA ratio were reduced ($P < 0.05$) by both 100% LTS and 50% LTS diets, with the lowest values observed in laying hens fed

Table 4. Effects of guanidinoacetic acid (GAA) on egg quality in laying hens fed different substitution levels of corn with low-tannin sorghum (LTS) at 64 wk of age.

Item[1]		% of egg components			Albumin height mm	Haugh unit	Yolk color	Shell thickness mm	Shell breaking strength. kg/cm^2
		Yolk	Albumen	Shell					
Diet type									
100% corn		28.84	61.45	9.72	5.96	84.11	8.31[a]	0.346	3.310
50% LTS		29.23	61.26	9.40	6.00	83.56	7.56[a,b]	0.339	3.276
100% LTS		29.43	60.84	9.72	6.07	83.24	6.88[b]	0.343	3.267
SEM		0.220	0.265	0.137	0.107	0.736	0.268	0.0049	0.058
GAA level (g/kg diet)									
0		29.27	61.14	9.51	5.92	82.93	7.50	0.337[b]	3.194[b]
0.6		29.06	61.23	9.72	6.10	84.34	7.67	0.349[a]	3.375[a]
SEM		0.192	0.218	0.117	0.080	0.546	0.215	0.0043	0.049
Diet type	GAA level (g/kg diet)								
100% corn	0	28.79	61.58	9.64	5.89	83.41	8.25	0.340	3.219
100% corn	0.6	28.89	61.31	9.80	6.03	84.82	8.38	0.352	3.401
50% LTS	0	29.40	61.11	9.26	5.90	82.89	7.50	0.333	3.194
50% LTS	0.6	29.05	61.41	9.54	6.09	84.24	7.63	0.346	3.358
100% LTS	0	29.62	60.73	9.64	5.95	82.51	6.75	0.337	3.169
100% LTS	0.6	29.24	60.96	9.81	6.19	83.96	7.00	0.349	3.366
SEM		0.332	0.377	0.202	0.139	0.945	0.373	0.0074	0.085
Significance									
Diet type		0.178	0.280	0.171	0.765	0.698	0.004	0.621	0.861
GAA level		0.467	0.790	0.258	0.081	0.053	0.584	0.061	0.019
Diet × GAA		0.743	0.728	0.948	0.932	0.998	0.981	0.996	0.983

[a,b]Means within each column with no common superscript differ ($P < 0.05$).
[1]Data represent means from 8 replicates (i.e., cages) per treatment.

Table 5. Effects of guanidinoacetic acid (GAA) on serum parameters in laying hens fed different substitution levels of corn with low-tannin sorghum (LTS) at 64 wk of age.

Item[1]		Creatine μmol/L	Creatinine μg/mL	Nitric oxide μmol/L	Malondialdehyde nmol/mL	Total antioxidant capacity mmol/L
Diet type						
100% corn		22.93	0.440	29.27	2.24	1.57
50% LTS		22.86	0.417	29.21	2.22	1.55
100% LTS		23.75	0.429	28.55	2.28	1.54
SEM		0.814	0.0185	0.850	0.064	0.059
GAA level (g/kg diet)						
0		17.77[b]	0.407	25.70[b]	2.37[a]	1.50
0.6		28.59[a]	0.450	32.31[a]	2.13[b]	1.61
SEM		0.712	0.0161	0.826	0.059	0.046
Diet type	GAA level (g/kg diet)					
100% corn	0	18.04	0.419	25.28	2.38	1.49
100% corn	0.6	27.82	0.461	33.26	2.11	1.64
50% LTS	0	16.67	0.395	26.91	2.30	1.53
50% LTS	0.6	29.05	0.438	31.50	2.15	1.58
100% LTS	0	18.59	0.408	24.92	2.42	1.49
100% LTS	0.6	28.90	0.451	32.18	2.15	1.60
SEM		1.233	0.0279	1.430	0.102	0.079
Significance						
Diet type		0.694	0.669	0.805	0.813	0.962
GAA level		<0.001	0.091	<0.001	0.019	0.100
Diet × GAA		0.592	0.999	0.558	0.828	0.839

[a,b]Means within each column with no common superscript differ ($P < 0.05$).
[1]Data represent means from 8 replicates (i.e., cages) per treatment.

the 100% LTS diet. The addition of the GAA supplement resulted in an increase in linoleic acid, n-6 PUFA, total PUFA, and the PUFA/SFA ratio in egg yolk ($P < 0.05$). However, the diet type or GAA supplementation in the diet did not significantly affect the levels of SFA and n-3 PUFA ($P > 0.05$). The results also indicate that the concentrations of fatty acids in egg yolk were not affected ($P > 0.05$) by the interaction of diet type and GAA supplementation.

Nutrient Digestibility

The results presented in Table 7 indicate that the AID of energy was reduced ($P = 0.012$) in the 100% LTS group when compared to the 50% LTS and 100% corn groups. The complete substitution of corn with LTS also resulted in a decrease ($P = 0.024$) in the AMEn value. The inclusion of GAA in the diet resulted in increases in the AID of dry matter ($P = 0.061$) and crude protein ($P = 0.010$). Furthermore, a significant increase in the

Table 6. Effects of guanidinoacetic acid (GAA) on the fatty acid composition of egg yolk in laying hens fed different substitution levels of corn with low-tannin sorghum (LTS) at 64 wk of age.

Item[1]		SFA[2]	MUFA[3]	n-6 PUFA[4]	n-36 PUFA[5]	Total PUFA[6]	PUFA/SFA	n-6/n-3 PUFA
Diet type								
100% corn		33.04	45.27[b]	20.54[a]	1.14	21.69[a]	0.657[a]	18.24[a]
50% LTS		33.35	46.34[a,b]	19.13[b]	1.17	20.30[b]	0.610[b]	16.50[a,b]
100% LTS		33.56	47.17[a]	18.07[c]	1.20	19.27[c]	0.575[c]	15.26[b]
SEM		0.307	0.397	0.203	0.034	0.195	0.0078	0.593
GAA level (g/kg diet)								
0		33.79	46.11	18.94[b]	1.16	20.11[b]	0.596[b]	16.54
0.6		32.85	46.42	19.56[a]	1.18	20.73[a]	0.632[a]	16.79
SEM		0.233	0.300	0.171	0.026	0.168	0.0065	0.441
Diet type	GAA level (g/kg diet)							
100% corn	0	33.48	45.05	20.32	1.15	21.47	0.642	18.10
100% corn	0.6	32.60	45.49	20.77	1.14	21.90	0.672	18.38
50% LTS	0	33.81	46.19	18.86	1.14	20.00	0.592	16.71
50% LTS	0.6	32.89	46.50	19.41	1.20	20.61	0.627	16.29
100% LTS	0	34.07	47.08	17.65	1.20	18.85	0.553	14.82
100% LTS	0.6	33.06	47.26	18.49	1.20	19.68	0.596	15.71
SEM		0.404	0.519	0.297	0.045	0.291	0.0113	0.765
Significance								
Diet type		0.489	0.006	<0.001	0.565	<0.001	<0.001	0.007
GAA level		0.079	0.958	0.024	0.723	0.021	0.006	0.663
Diet × GAA		0.983	0.989	0.807	0.663	0.805	0.824	0.634

[a–c]Means within each column with no common superscript differ ($P < 0.05$).
[1]Data represent means from 8 replicates (i.e., cages) per treatment.
[2]Saturated fatty acids (SFA) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0.
[3]Monounsaturated fatty acids (MUFA) = C16:1 + C18:1 + C20:1.
[4]n-6 polyunsaturated fatty acids (PUFA) = C18:2 + C20:2 + C20:3 + C20:4.
[5]n-3 PUFA = C18:3 + C20:5 + C22:6.
[6]Total PUFA = n-6 PUFA + n-3 PUFA.

Table 7. Effects of guanidinoacetic acid (GAA) on apparent ileal digestibility of nutrients and apparent metabolizable energy corrected for nitrogen (AMEn) in laying hens fed different substitution levels of corn with low-tannin sorghum (LTS) at 64 wk of age.

Item		Dry matter	Crude protein	Crude fat	Ash	Energy	AMEn
		%					kcal/kg
Diet type							
100% corn		73.91	67.29	77.54	51.62	72.24	2,673
50% LTS		72.86	66.75	77.97	50.85	71.71	2,667
100% LTS		72.60	66.56	78.17	49.27	70.39	2,659
SEM		0.563	0.353	0.546	0.757	0.404	3.9
GAA level (g/kg diet)							
0		72.41[b]	66.35[b]	77.69	50.26	70.97	2,658[b]
0.6		73.83[a]	67.38[a]	78.10	50.90	71.93	2,675[a]
SEM		0.460	0.274	0.400	0.663	0.500	3.2
Diet type	GAA level (g/kg diet)						
100% corn	0	73.18	67.17a	77.25	51.33	71.67	2,671[a]
100% corn	0.6	74.63	67.42[a]	77.83	51.91	72.82	2,675[a]
50% LTS	0	72.60	66.56[a]	77.74	50.73	71.44	2,661[a]
50% LTS	0.6	73.12	66.94[a]	78.20	50.96	71.99	2,673[a]
100% LTS	0	71.46	65.32[b]	78.08	48.72	69.80	2,642[b]
100% LTS	0.6	73.73	67.79[a]	78.26	49.82	70.97	2,676[a]
SEM		0.796	0.474	0.693	1.148	0.866	5.6
Significance							
Diet type		0.178	0.334	0.705	0.105	0.012	0.024
GAA level		0.061	0.010	0.422	0.534	0.291	0.004
Diet × GAA		0.601	0.041	0.952	0.939	0.948	0.021

[a,b]Means within each column with no common superscript differ ($P < 0.05$).[1]Data represent means from 8 replicates (i.e., cages) per treatment.

AMEn value ($P < 0.004$) was observed with the supplementation of GAA in the diet. The results also demonstrated a significant interaction between diet type and GAA supplementation regarding protein digestibility ($P = 0.041$) and AMEn ($P = 0.021$), with the GAA supplementation showing greater effectiveness in the 100% LTS group. However, there was no significant effect of diet type on the AID of dry matter, crude protein, crude fat, ash, and energy ($P > 0.05$).

DISCUSSION

The current study aimed to assess the effects of substituting corn with LTS at different proportions, with or without GAA supplementation, on various production parameters. The results revealed that replacing corn with LTS at both the 50% and 100% ratios did not exhibit any negative impact on any of the production parameters under investigation. This suggests that the substitution of corn with LTS can be successfully implemented in the layer diet without compromising the overall production capacity. The findings of this experiment align with a previous study that suggests a partial or total replacement of corn by LTS is practicable without any negative effects on the performance of broiler chickens throughout the entire trial period (Puntigam et al., 2020). The potential negative impact of sorghum on performance has been suggested to be associated with its high tannin levels. Previous research has indicated that the presence of a high quantity of tannin in sorghum (1−3%) has been associated with a decrease in the digestibility of both starch and protein (Mahmood et al., 2014). In a study conducted by Ebadi et al. (2005), it was proposed that the presence of tannins in the grain at medium (1.9%) or high (3.7%) levels resulted in a reduction of up to 23%, 44%, 32%, 54%, and 75% in the bioavailability of methionine, cysteine, lysine, arginine, and proline, respectively. This reduction in nutrient digestibility may have the potential to negatively affect the overall productive performance of avian species. The sorghum grain variety used in this study had an average tannin concentration of 0.51 ± 0.03% (as catechin equivalent), suggesting that it belongs to the category of sorghum with low tannin content.

According to the current investigation, the positive effects of GAA on egg production rate, egg mass, and feed conversion ratio were observed irrespective of the type of diet used, whether it was based on corn, corn + LTS, or LTS diets. The effects of incorporating GAA into the diet of laying poultry have been investigated, revealing inconsistencies in terms of their productive performance. In a study conducted by Salah et al. (2020), it was observed that the inclusion of either 1.0 or 1.5 g/kg of GAA in the diet of aged laying hens resulted in significant improvements in their laying performance. Raei et al. (2020) also suggest that the inclusion of different dietary levels of GAA (0, 0.6, 1.2, and 1.8 g/kg) in the diet can positively impact the reproductive performance of quails, particularly in terms of laying rate, egg weight, and egg mass. In a recent investigation examining the laying hens during the latter stage of production, Pimenta et al. (2023) discovered that the favorable feed conversion per dozen eggs was achieved through the incorporation of 0.6 and 1.2 g/kg of GAA into their diets. In contrast to previous investigations, the inclusion of GAA in the diet of laying hens did not result in a significant enhancement in their overall performance (Khakran et al., 2018). The positive impact of GAA on productive performance in the current study can be

attributed to its essential role as a precursor to creatine. Creatine, in the form of phosphocreatine, plays an important role as an energy carrier in various physiological processes, specifically in the reproductive system (Wyss and Kaddurah-Daouk, 2000; Khajali et al., 2020). The increase in serum NO levels resulting from the dietary supplementation with GAA in the current study (Table 5) is also an important finding that provides insight into the potential mechanisms responsible for the observed improvements in laying performance. According to a study conducted by Manwar et al. (2006), it is believed that the increased synthesis of NO may potentially contribute to the promotion of follicular growth. This may, in turn, result in improved ovulation and increased production of eggs. In recent studies, Uyanga et al. (2022) have proposed that NO may be an important intermediate in creating a favorable environment for optimal reproductive performance. This is suggested due to its regulatory effects on blood flow and hormone secretion (Gladwin et al., 2006).

The results of the study revealed that the complete substitution of corn with sorghum led to a decrease in the yolk color index. Consistent with the findings of our investigation, previous studies have shown that the inclusion of sorghum in diets led to a reduction in yolk color when compared to diets containing corn (Imik et al., 2006; Garcia et al., 2010). The correlation between the consumption of corn-based diets and the alteration of Roche color fan values in egg yolks has been attributed to the high levels of carotenoid pigments, particularly carotenes and xanthophyll, found in yellow corn varieties (Abbasi Arabshahi et al., 2021). Therefore, the addition of pigments, either natural or artificial, should be implemented in sorghum-based diets in order to achieve the desired yolk color.

The decline in eggshell quality towards the end of a hen's laying cycle has been a subject of concern for poultry farmers (Sirri et al., 2018). The results of this study revealed a significant increase in shell-breaking strength and a tendency toward higher shell thickness in the group of hens that received GAA supplementation. The mechanism behind the observed effects of GAA on eggshell quality has not yet been fully understood. However, it is hypothesized that GAA may induce this effect through its sparing effect on the dietary arginine intake of laying hens. Previous studies on rats have provided evidence supporting the clinical recommendation of arginine as a potential remedy for metabolic disruptions affecting calcium absorption (Fiore et al., 2000; Yaman et al., 2016). Additionally, a significant increase in eggshell proportion was observed when the commercial laying hens were provided with L-arginine-enriched diets (Lieboldt et al., 2016). The tendency towards higher albumin height and Haugh units, as found in this study, also suggests that including GAA in the hens' diet may improve egg quality. Salah et al. (2020) found that dietary supplementation with GAA improved various aspects of internal egg quality, including the albumen ratio, yolk index, and Haugh unit, in laying hens during the later stages of production. The potential impact of GAA on the Haugh index may be attributed to its role in enhancing methionine usage efficiency within the body. Dinesh et al. (2018) hypothesized that creatine and GAA supplements can spare and optimize the usage of dietary methionine. Reda et al. (2020) found that feeding the diet supplemented with DL-methionine at a dosage of 1.5 g/kg enhanced albumin percentage, whereas supplementing at 3.5 g/kg improved Haugh unit and shell thickness.

The present study yielded results indicating that the specific diet type employed did not exert a statistically significant effect on the measured blood metabolite concentrations. However, incorporating GAA into the diet of laying hens during the latter stage of their production cycle resulted in an increase in serum creatine levels. Additionally, there were observed trends towards increased creatinine concentrations. In the study conducted by Córdova-Noboa et al. (2018), it was observed that the administration of GAA resulted in elevated levels of creatine and creatinine in the serum of broiler chickens. In a study conducted by Wyss and Kaddurah-Daouk (2000), it was discovered that approximately 1.7% of the overall creatine and phospho-creatine pool undergoes a permanent transformation into creatinine on a daily basis. The converted form of creatinine is subsequently excreted from the body through urine excretion.

The current study also provides evidence that the administration of GAA results in a decrease in MDA levels and a numerical increase in TAC value. A reduction in MDA levels has been associated with a potential decrease in oxidative damage (Li et al., 2022), suggesting that dietary GAA at a level of 0.6 g/kg is effective in mitigating oxidative stress. Previous studies have also highlighted the potential antioxidant effects of supplemental GAA in low to moderate quantities (Amiri et al., 2019; Cui et al., 2022). In contrast, Ostojic (2015) demonstrated that an excess of GAA in the body or an abnormal metabolic process involving this compound can result in a pro-oxidant effect, which leads to the generation of oxidative stress. Therefore, it can be stated that the administration of GAA had a notable impact on the oxidant-antioxidant system, functioning as both an antioxidant and a pro-oxidant agent. Hiramatsu (2003) observed that the generation of superoxide anion, a widely recognized indicator of oxidative stress, occurs when pure GAA donates an electron from its conjugate base. This suggests that GAA may potentially have a direct pro-oxidant role. In contrast, it was proposed that, although GAA may not possess inherent antioxidant properties, there exists the possibility of obtaining indirect antioxidant advantages through its related metabolites, specifically arginine and creatine (Dao and Swick, 2021). In a recent study conducted by Zhao et al. (2021), it was reported that arginine and creatine have the potential to inhibit or neutralize free radical reactions.

Previous studies have highlighted the significant role of dietary fatty acid composition in influencing the fatty acid composition of egg yolk in laying hens (Beheshti

Moghadam et al., 2020; Mens et al., 2022). The findings of our study contribute to the existing body of evidence regarding the impact of a sorghum-based diet on the composition of egg yolk in hens. Our results indicate that such a diet leads to an increase in the concentration of MUFA while simultaneously causing a decrease in the concentration of PUFA and the ratio of PUFA to SFA in the egg yolk. It seems that the lower levels of linoleic acid and n-6 PUFA in the sorghum-based diet, coupled with higher levels of MUFA, may have contributed to a similar fatty acid profile in the egg yolk when compared to a corn-based diet. Similarly, it has been noted that a decreased PUFA content in the diet of laying hens results in a corresponding reduction in PUFA levels within the egg yolk (Batkowska et al., 2021; Arbabi-Motlagh et al., 2022).

The present investigation yielded findings suggesting that the incorporation of GAA into the diet of laying hens potentially induces a favorable modification in the fatty acid profile of egg yolk. It is worth noting that the ratio of PUFA to SFA, which serves as a significant marker of lipid quality (Sinanoglou et al., 2011), demonstrated a significant increase following the inclusion of GAA supplementation in the dietary regimen. In a recent investigation conducted by Cui et al. (2022), it was found that GAA supplementation led to an increase in the concentrations of linolenic acid (C18:3n-3), arachidonic acid, and total PUFA in the *Longissimus thoracis* muscle in Tibetan pigs. Our findings suggest that the observed increase in the levels of C18:2n-6 in egg yolk can potentially be attributed to the enhanced efficacy of GAA in facilitating the transportation and deposition of this fatty acid within the yolk. The potential antioxidant properties of GAA (Amiri et al., 2019; Zhao et al., 2021) can be considered as an additional hypothesis to explain the observed effect. Therefore, it is plausible that this particular mechanism could potentially result in an increase in the levels of PUFA in the specific tissue being targeted.

The results indicate that the substitution of corn with LTS, whether partially or fully, did not yield a significant impact on the AID of nutrients. However, the complete substitution of corn with LTS resulted in a partial decrease in the AMEn value. The effect of LTS substitution levels has been found to vary across different studies. The results of a previous study indicated that feeding LTS diets did not have a significant effect on the digestibility coefficients of crude protein and ash, as well as ME, in broiler chickens (Saleh et al., 2019). However, Pan et al. (2021) found that replacing corn with LTS in nursery pig diets led to a decrease in the apparent digestibility of crude protein, while the apparent digestibility of dry matter, organic matter, and energy remained unaffected by this replacement. In a study conducted by Moritz et al. (2023), it was observed that certain polyphenolic compounds found in red grain sorghum varieties possess antinutritional factors. These factors were discovered as potentially impeding the energy digestibility and absorption processes, thereby compromising the overall energy digestibility and AMEn of the sorghum. Khoddami et al. (2015) also suggested that the presence of hydroxycoumarin, a polyphenol compound linked to coumaric acid, in red/bronze grain sorghum could potentially have a negative effect on energy utilization.

The results of the present study indicate that there were interaction effects between LTS substitution level and GAA supplementation on the AID of protein and the AMEn. The results of this study indicate that supplementing GAA at a level of 0.6 g/kg in the LTS-based diet can lead to improved protein utilization and increased AMEn. However, when added to corn-based diets, supplementation with GAA may not yield any additional benefits in terms of nutrient digestibility. The previous investigation conducted by Raei et al. (2020) on laying Japanese quails found that dietary supplementation with GAA had both linear and quadratic effects on the ileal digestibility of dry matter, crude protein, ether extract, and ash. Our findings suggest that the addition of dietary GAA promotes nutrient digestion within the intestinal tract. The study conducted by Wallimann et al. (2011) suggests that the inclusion of GAA in the diet may potentially enhance creatine production and energy metabolism in intestinal cells of various species, including humans, animals, chickens, and fish. Therefore, it can be deduced that the addition of GAA may have a positive impact on the absorption rate and metabolism of energy and protein.

CONCLUSIONS

Based on the findings of this study, it can be concluded that substituting corn with LTS, either partially or entirely, is a practical approach for formulating diets for aged laying hens without any detrimental effects on the hens' productivity or nutritional responses, except for a decrease in yolk color index and a partial reduction in AMEn. In addition, supplementing GAA at a dietary inclusion level of 0.6 g/kg improves protein digestibility and AMEn in aged laying hens, but only when they are fed a sorghum-based diet. Our findings also indicate that supplementing with GAA may improve egg-laying performance, eggshell quality, yolk fatty acid profile, and antioxidant status in laying hens, regardless of the type of diet they were fed (corn- or sorghum-based). However, additional research is needed to explore the long-term effects and optimal dosage of GAA supplementation in laying hens that are fed with various types of diets.

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DISCLOSURES

The authors assert that the study was carried out without any financial or commercial relationships that could be construed as a potential source of conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2023.103297.

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