Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2023v44n4p1541

Leucaena leaf meal as a feedstuff in laying hen diets from 32 to 44 weeks

Feno da folha de leucena na alimentação de poedeiras comerciais de 32 a 44 semanas

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Highlights

Leucaena leaf meal in laying hen diets provided more pigmented yolks.
The inclusion of Leucaena leaf meal at 8% affected egg production.
Diets with higher fiber level resulted in lower nutrient metabolisability.

Abstract

The objective of the study was to evaluate the effect of the inclusion of leucaena leaf meal in diets on nutrient metabolizability, performance and egg characteristics of commercial laying hens. A total of 216 laying hens of 32 weeks of age were used, being distributed in a completely randomised design with 6 treatments and 6 replicates of 6 birds each. Treatments consisted of a diet formulated with corn, a diet formulated with sorghum and another four formulated with sorghum containing 2, 4, 6 and 8% leucaena leaf meal. Linear reduction was observed for the inclusion levels of leucaena on metabolizable coefficients of dry matter, crude protein, gross energy and on the values of apparent metabolizable energy and apparent metabolizable energy corrected by the nitrogen balance. There was a linear reduction in egg production and apparent metabolizable energy corrected by the nitrogen balance ingestion as the leucaena leaf meal increased in diets. Hens fed diets containing 8% leucaena leaf meal presented significantly lower egg production and apparent metabolizable energy corrected by the nitrogen balance ingestion in relation to the birds fed with sorghum. The inclusion of 2% leucaena leaf

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Received: Mar. 02, 2022 Approved: Oct. 09, 2023

meal in the diet proportionate egg yolks had superior pigmentation to that obtained with diets containing corn. According to the results, leucaena leaf meal can be included, comprising up to 6% in laying hen diets formulated with sorghum.
Key words: Egg quality. Egg production. Leucaena. Metabolisability.

Resumo

O objetivo do estudo foi avaliar o efeito da inclusão do feno da folha de leucena em dietas sobre a metabolização de nutrientes, desempenho e características dos ovos de poedeiras comerciais. Foram utilizadas 216 poedeiras com 32 semanas de idade, distribuídas em delineamento inteiramente casualizado com 6 tratamentos, 6 repetições de 6 aves cada, durante 12 semanas. Os tratamentos consistiram em uma dieta formulada com milho, uma dieta formulada com sorgo e outras 4 formuladas com sorgo contendo 2, 4, 6 e 8% de feno da folha de leucena. Foi observada redução linear sobre os coeficientes de metabolizabilidade da matéria seca, proteína bruta, energia bruta e nos valores de energia metabolizável aparente e energia metabolizável aparente corrigida para balanço de nitrogênio. Observou-se redução linear na produção de ovos e na ingestão de energia metabolizável aparente corrigida para balanço de nitrogênio com o aumento da leucena nas dietas. Galinhas alimentadas com dietas contendo 8% de leucena apresentaram menor produção de ovos e ingestão de energia metabolizável aparente corrigida para balanço de nitrogênio em relação as aves alimentadas com o sorgo. A inclusão de 2% de feno da folha da leucena na dieta proporcionou gemas com pigmentação superior às obtidas com as dietas a base de milho. De acordo com os resultados, pode-se incluir até 6% de feno da folha de leucena em dietas de poedeiras comerciais formuladas com sorgo.
Palavras-chave: Leucena. Metabolizabilidade. Produção de ovos. Qualidade dos ovos.

Introduction

Many of the ingredients used in poultry farming are likely to be in short supply or unavailable in the coming years, mainly due to the climate challenges ahead. Poultry producers will have to look beyond maize, soybean meal and other cereal grains because of their low availability, cost, and inability to keep pace with increasing poultry production. Constant research on potential locally produced food resources is essential to meet this reality on farms.

In this context, forage plants tropical cultivated or exploited typically in the Brazilian northeast region may solve this demand. Forages can be sources of protein for birds, especially in regions where they are easily available and have low acquisition costs (Dal Bosco et al., 2014). Legumes have nutritional advantages due to their high protein levels and desirable amino acid profiles (De-Angelis et al., 2021; Lüscher et al., 2014).

Leucaena leucocephala (Lam.) of Wit., is native to Central America that has spread and/or naturalised all over the world and stands out in the north-eastern semi-arid region for adapting to the climatic conditions of the region (Pompeu et al., 2015). Recently, these leaf meals have been extensively used as feed supplements in poultry diets to improve productivity as well as product quality (Hien et al., 2017).

It is considered a source of protein for the poultry sector, comparable to alfalfa fodder (De-Angelis et al., 2021). The protein level ranges from 20 to 34%, and it is accompanied by an amino acid composition like that of soy meal and fishmeal, quite rich in essential amino acids such as isoleucine, leucine, phenylalanine, and histidine (Scapinello et al., 2000; Abou-Elezz et al., 2011; De-Angelis et al., 2021). Thanks to pigment content in these leaf meals, it proved that this content increased chicken's skin yellow darkness improved egg yolk color, and increased meat and egg palatability (Hien et al., 2016, 2017). Leucaena fodder still has good levels of vitamins and minerals (Abou-Elezz et al., 2011; Dumorné, 2018).

Leucaena can be an alternative to encourage the use of energy grains that presents low carotenoid pigment content (xantofil and carotenoid), such as sorghum (Sorghum bicolor L. Moench), which is responsible for yolk pigmentation. Until now, the low level of carotenoid pigments in sorghum was the main limiting factor in the ration of laying hens (Sriagtula et al., 2022). The supplementation of pigments in diets for commercial laying hens formulated based on sorghum is an important tool to adjust this colour to be similar and/or superior when using corn-based rations (Mendonça et al., 2018).

Sorghum presents adequate nutritional characteristics and is often used to replace corn, especially in semi-arid regions (Sriagtula et al., 2022). The chemical composition of sorghum is similar to corn, except for being deficient in lysine, methionine, and threonine (Rostagno et al., 2017). However, the digestibility of sorghum is 5% lower than corn, which may influence protein availability. The inclusion of leaf hay in sorghum-based diets for laying hens may be an important way to improve protein level of sorghum-based diets.

Although Leucaena it is a plant rich in nutrients, it contains tannins, trypsin inhibitors and toxic substances, such as the non-protein amino acid β-[N-(3-hydroxy-4-oxo pyridyl)]-α-aminopropionic (mimosine); its fibre level restricts its use by affecting the performance of laying hens according to the inclusion levels (Dumorné, 2018; Oliveira et al. 2014).

Leucaena leucocephala leaves are available and cheap in vast areas worldwide, so is expected to be sustainable resources for commercial poultry production in the tropics (Abou-Elezz et al., 2012). Given the above, the objective was to evaluate the effect of leucaena leaf meal (LLM) inclusion in corn and sorghum-based diets on nutrient metabolisability, productive performance and egg quality of laying hens.

Materials and Methods

The experiment was carried out in accordance with the ethical principles for testing on animals (Protocol No 23/2023 - CEUA) determined by the Council for Ethics in the Use of Animals (CEUA) of the Federal University of Ceará. The experiment was conducted in the poultry sector at the Federal University of Ceará, from July to October 2013. The temperature and relative humidity of the air during the experimental period were measured using a digital thermo-hygrometer and were 28.4°C and 85%, respectively. A total of 216 Hisex Brown commercial layers at

32 weeks of age were used, being distributed in a completely randomised design with 6 treatments and 6 replicates of 6 birds each. The birds were housed in galvanised wire cages provided with a cup feeder, a gutter-type trough and an egg collector at a density of two birds per cage.

Six diets were tested: one formulated with corn and the other five formulated with sorghum, containing 0, 2, 4, 6 and 8% of LLM. The experimental diets were formulated to contain the same amount of nutrients according to the nutritional requirements recommended by the strain manual. To calculate the diets, the chemical composition values of the LLM were determined in the laboratory and used together with the values presented by D'Mello and Acamovic (1989) (Table 1). The values presented by Rostagno et al. (2017) were considered for the other ingredients (Table 2).

The experimental period lasted 84 days and was divided into four 21-day periods. Throughout the experiment, the birds were fed feed and water ad libitum. The troughs were filled twice daily (at 08h00 and 16h00) to avoid feed wastage. The daily lighting programme adopted was 17 hours (natural and artificial), and egg collection was performed daily at 15h00.

The performance variables evaluated were egg production (EP) (%/bird/day), feed intake (FI) (g/bird/day), egg weight (EW) (g), egg mass (EM) (g/bird/day) and feed conversion ratio (FCR) (g/g). To assess the egg characteristics, once a week, after weighing, three eggs from each plot were selected to determine the specific density (g/cm^3), haugh units (HU) and yolk colour (DSM Yolk Color Fan).

The digestibility of the nutrients from the diets was determined in an assay using the total excreta collection method, performed after 30 days of bird feeding, during the second experimental period with 3 days of adaptation and 4 days of collection. Six birds from each experimental plot were housed in metallic cages, following the same experimental design, with plastic-lined aluminium trays. To delineate the beginning and end of the collection period, iron oxide was used as a marker and added to the experimental diets at a concentration of 1%. The excreta were collected twice daily, in the early morning and late afternoon, conditioned in plastic bags and frozen.

The excreta were dried in a forced-ventilation oven at 55°C for 72 hours and ground in a knife mill (1 mm mesh) and sent to the laboratory with the samples of experimental diets to determine the dry matter (DM), ethereal extract (EE) and nitrogen (N) contents, according to the methodology described by Silva and Queiroz (2006). Crude energy (CE) was also determined in an adiabatic calorimetry chamber (Model 1242, Parr Instruments Company, Illinois, EUA).

Based on the laboratory results, the dry matter metabolisability coefficient (DMMC), nitrogen metabolisability coefficient (NMC), ethereal extract metabolisability coefficient (EEMC), gross energy metabolisability coefficient (GEMC), apparent metabolisable energy (AME) and apparent metabolisable energy corrected by the nitrogen balance (AMEn) values of the diets were calculated. From the diet-composition data, FI by birds during the experiment and the AMEn values, the AMEn intake (AMEI) (kcal/bird/day) and CP intake (CPI) (g/bird/day) were then calculated.

Table 1
Composition and nutritional levels of leucaena leaf meal

Components	LLM[1]
Dry matter (%)	90.63
Gross energy (kcal/kg)[2]	4,600
AMEn[3] (kcal/kg)[4]	645
Crude protein (%)[2]	25.00
Crude fibre (%)[2]	12.31
Neutral detergent fibre (%)[2]	57.59
Acid detergent fibre (%)[2]	24.05
Ether extract (%)[4]	9.40
Mineral matter (%)[4]	4.20
Calcium (%)[4]	7.32
Total phosphorus (%)[4]	2.02
Sodium (%)[4]	0.21
Potassium (%)[4]	0.01
Lysine (%)[4]	1.25
Methionine (%)[4]	1.47
Methionine + Cystine (%)[4]	0.33
Tryptophan (%)[4]	0.51
Threonine (%)[4]	0.30
Tannin (%)	1.3 a 4.4
Mimosine (%)	1 a 12

[1] Leucaena leaf meal.
[2] Values obtained by the laboratory analysis and expressed as dry matter.
[3] AMEn, nitrogen-corrected apparent metabolizable energy determined with young chicks, according to D'Mello and Acamovic (1982).
[4] Values calculated based on the composition presented by D'Mello and Acamovic (1989).

Statistical analysis of the data was performed using the Statistical Analysis System (SAS) software. The data obtained for all diets were submitted to analysis of variance according to a completely randomised model followed by the comparison of means by the Dunnett test (5%) to compare the results obtained with the corn-based diet in relation to the sorghum-based diets containing 0, 2, 4, 6 and 8% LLM. Dunnett's test (5%) was also applied to the results obtained with diets containing 0, 2, 4, 6 and 8% LLM to verify the effect of adding different levels of LLM in the rations containing sorghum in relation to the ration containing sorghum without LLM. Subsequently, the results referring to the inclusion levels 2, 4, 6 and 8% of LLM in the diets were submitted to linear and quadratic regression analysis to establish the curve that best described the behaviour of the data.

Table 2
Percentage and nutritional composition of the experimental diets used for Hisex brown laying hens of 32 weeks of age

Ingredients	Experimental diets					
	Corn	Sorghum + 0% LLM	Sorghum + 2% LLM	Sorghum + 4% LLM	Sorghum + 6% LLM	Sorghum + 8% LLM
Corn	64.65	0.00	0.00	0.00	0.00	0.00
Low-tannin sorghum	0.00	62.96	60.96	58.98	57.08	55.15
Soybean meal	23.82	23.70	23.00	22.30	21.58	20.84
Leucaena leaf meal	0.00	0.00	2.00	4.00	6.00	8.00
Soy oil	0.00	1.74	2.52	3.30	4.03	4.78
Calcitic Limestone	9.10	9.10	9.00	8.90	8.79	8.69
Mono-dicalcium phosphate	1.56	1.54	1.56	1.56	1.57	1.58
Common salt	0.39	0.40	0.41	0.41	0.41	0.41
Vitamin supplement[1]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral supplement[2]	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.17	0.20	0.20	0.21	0.21	0.22
L-lysine HCl (78.5%)	0.02	0.06	0.05	0.04	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional and energetic calculated composition						
Crude protein (%)	16.50	16.50	16.50	16.50	16.50	16.50
Metabolizable energy (kcal/kg)	2719	2700	2700	2701	2700	2700
Dry matter (%)	87.16	87.16	87.29	87.45	87.60	87.30
Crude fibre (%)	2.67	2.80	2.87	2.93	2.99	3.05
Neutral detergent fibre (%)	8.89	7.60	8.40	9.21	10.02	10.83
Acid detergent fibre (%)	5.53	7.00	7.22	7.44	7.67	7.90
Calcium (%)	3.90	3.90	3.90	3.90	3.90	3.90
Available phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19
Lysine (%)	0.84	0.85	0.84	0.84	0.84	0.84
Methionine (%)	0.42	0.45	0.45	0.46	0.46	0.47
Methionine + cystine (%)	0.64	0.63	0.63	0.63	0.63	0.63
Threonine (%)	0.19	0.21	0.21	0.21	0.21	0.21

[1]Vitamin supplement (composition per kg): vitamin A - 3,500,000 UI; vitamin D3 - 750,000; vitamin E - 2000 mg; vitamin K3 - 1000 mg; vitamin B1 - 1000 mg; vitamin B2 - 1500 mg; vitamin B12 - 4000 mcg; niacin - 7500 mg; Ca pantothenate - 2500 mg; selenium - 150 mg; choline chloride - 250 g; antioxidant - 25 g; excipient q.s - 1,000 g.
[2] Mineral supplement (composition per kg): Mn - 65,000 mg; Zn - 50,000 mg; Fe - 50,000 mg; Cu - 12,000 mg; I - 1000 g; excipient q.s - 1000 g.

Results and Discussion

In comparison with the corn-based diet, a significantly lower metabolism of dry matter and nitrogen was observed for diets containing 4% or more LLM and gross energy from 6% LLM (P > 0.05). Ether extract metabolisation was significantly lower with diets containing sorghum without LLM or 2% LLM but did not differ in relation to the other LLM levels (P > 0.05). In addition, there was no significant difference between apparent and corrected apparent metabolisable energy (Table 3).

Table 3
Metabolisability coefficients and metabolisable energy values of Hisex brown laying hens of 32 weeks of age diets containing leucaena leaf meal

Diets	DMMC(%)	NMC(%)	EEMC(%)	GEMC(%)	AME (kcal/kg MS)	AMEn (kcal/kg MS)
Corn	75.83	51.86	88.47	77.30	2909	2770
Sorghum	74.69	49.34	82.59 º	78.65	2947	2818
Sorghum + 2% LLM	76.47	51.63	84.28 º	79.65	2908	2775
Sorghum + 4% LLM	73.21 º	45.97 º	86.89*	78.50	2863	2746
Sorghum + 6% LLM	72.95 º	42.74*º	88.69*	75.11*º	2861	2751
Sorghum + 8% LLM	72.24* º	43.00*º	87.63*	74.97*º	2809*º	2700*º
Mean	74.23	47.42	86.42	77.36	2883	2760
SEM	0.381	0.939	0.562	0.478	0.014	0.012
ANOVA			p-value			
Diets	0.0002	0.0003	0.0003	0.0003	0.0427	0.1173
LLM Levels	0.0010	0.0034	0.0009	0.0013	0.0419	0.0916
Regression			p-value			
Linear	0.0003[1]	0.0020[2]	0.0157[3]	<.0001[4]	0.0046[5]	0.0234[6]
Quadratic	0.2560	0.0846	0.0525	0.4411	0.8777	0.5971

[1] DMMC ($\hat{Y}$ = 76.95 - 0.65X,R2 = 0.63)
[2] NDC ($\hat{Y}$ = 53.11 - 1.46X,R2 = 0.50)
[3] EEMC ($\hat{Y}$ = 83.91 + 0.59X,R2 = 0.35)
[4] GEMC ($\hat{Y}$ = 81.41 - 0.87X,R2 = 0.73)
[5] AME MS ($\hat{Y}$ = 2.94 - 0.015X,R2 = 0.45)
[6] AMEn MS ($\hat{Y}$ = 2.80 - 0.011X,R2 = 0.32)
SEM, standard error of the mean
ANOVA, analysis of variance
*Significant statistical effect in relation to the sorghum-based treatment without the inclusion of LLM by Dunnett test (p<0.05).
ºSignificant statistical effect in relation to the corn-based treatment by Dunnett test (p<0.05).

Despite the lower metabolism of dry matter and nitrogen observed with 4% LLM and gross energy with 6% LLM inclusion, the reduction in AME and AMEn was identified only at the level of 8%. Thus, only 8% LLM inclusion reduced the energy available for laying hens' metabolism in comparison with the corn-based diets.

Compared to the sorghum-based feed without LLM, there was significantly less nitrogen metabolism and gross energy from 6% LLM and dry matter with 8% LLM (P > 0.05). This level also resulted in lower apparent and corrected apparent metabolisable energy. The metabolisation of the ether extract was significantly higher with 4% LLM.

According to the regression analysis, the inclusion of 2% or more LLM in the sorghum-based diet promoted a linear reduction in the coefficients of metabolisation of dry matter, nitrogen, gross energy, and apparent and corrected apparent metabolisable energy. However, a linear increase in the metabolisation coefficient of the ether extract was observed.

Abou-Elezz et al. (2012) fed hens at 0, 5, 10 and 15% LLM and reported lower digestibility of dry matter, crude protein, and gross energy. Arruda et al. (2010) also showed lower digestibility of crude protein and ether extract with 20% LLM in laying hens' diets at the rearing phase. In contrast with our results, Ayssiwede et al. (2010) reported feeding hens at 0, 7, 14 and 21% LLM and showed no significant effect on the utilization values of dry matter, crude protein or energy. However, they concluded that the controversy of their results with others was due to the fact that they treated the LLM with additive ferric sulphate to form a complex with mimosine, which led to the elimination of its toxicity.

The reduction in the digestibility can be associated with an increase in the fibrous fraction raising the pass rate and endogenous losses, combined with probable undesirable chelation with tannins that make elements unavailable and astringent action on the activity of intestinal proteolytic (Arruda et al., 2010; Leeson & Summers, 2001a; Oliveira et al. 2000; Ramos et al., 2007; Sakomura & Rostagno, 2007).

In the performance of laying hens, feed intake, protein intake, egg weight, egg mass and feed conversion were not significantly influenced by the treatments. However, compared to the corn-based diet, there was significantly less egg production and metabolisable energy ingestion with 8% LLM (P>0.05) (Table 4).

Table 4
Productive performance of Hisex brown laying hens of 32 weeks of age fed diets containing different levels of leucaena leaf meal

Diets	Feed Intake (g/bird/day)	Egg production (%/bird/day)	Ingested EMAn (g/bird/day)	Ingested CP (g/bird/day)	Egg weight (g)	Egg mass (g/bird/day)	Feed conversion ratio (g/g)
Corn	103.57	93.75	243.60	17.89	60.16	56.40	1.84
Sorghum	104.41	93.77	250.35	17.70	60.57	56.81	1.84
Sorghum + 2% LLM	106.31	94.09	249.26	17.65	61.08	57.46	1.85
Sorghum + 4% LLM	105.14	92.51	242.96	17.31	62.18	57.50	1.83
Sorghum + 6% LLM	105.47	91.90	246.69	17.45	62.33	57.25	1.83
Sorghum + 8% LLM	102.73	89.10*º	236.71*º	16.99	61.49	55.56	1.88
Mean	104.61	92.51	244.93	17.50	61.27	56.85	1.84
SEM	0.62	0.47	1.35	0.09	0.26	0.28	0.01
ANOVA				p-value			
DIETS	0.6279	0.0109	0.0324	0.0700	0.1072	0.3780	0.7281
Level	0.6842	0.0204	0.0381	0.2059	0.2778	0.3783	0.6816
Regression				p-value			
Linear	0.1789	0.00311	0.0290[2]	0.0777	0.6590	0.0788	0.5757
Quadratic	0.6518	0.5710	0.0583	0.7853	0.1463	0.2338	0.1913

[1] Egg production $\hat{Y}$ = (95.97 - 0.78X,R2 = 0.33)
[2] Ingested EMAn $\hat{Y}$ = (2.52 - 1.69X,R2 = 0.20)
SEM, standard error of the mean
ANOVA, analysis of variance
*Significant statistical effect in relation to the sorghum-based treatment without the inclusion of LLM by Dunnett test (p<0.05).
ºSignificant statistical effect in relation to the corn-based treatment by Dunnett test (p<0.05).

In comparison with the sorghum-based diets, a significantly lower egg production and metabolisable energy ingestion was observed with 8% LLM (P > 0.05).

According to the regression analysis, LLM inclusion resulted in a linear reduction in egg production and daily consumption of metabolizable energy by birds. The energy level of the diet is one of the factors that significantly affects FI in poultry. However, despite the reduction in AME and AMEn values, the birds did not have an increased FI. Several studies have been conducted to investigate the effects of dietary energy levels on the FI of laying hens, and it is generally accepted that hens adjust their FI to meet their energy requirements (De Persio et al., 2015; Pérez-Bonilla et al., 2012; Scappaticcio et al., 2021). According to Classen (2017), diet energy only accounts for 28% of the variation in FI, being highlighted by other important factors such as gender, age, breed, feed density and the nature of feed ingredients. It is likely that the inclusion of fibre in the diets increased the volume of the rations, reducing their density. Thus, the birds that ingested the diets that contained a higher level of fibre remained with a greater volume occupied in the gastrointestinal tract of the birds, causing a feeling of satiety, which is why there was no effect on FI.

This result is consistent with Abou-Elezz et al. (2012), who reported no effect on FI by laying hens when testing levels of 0, 5, 10 and 15% LLM. Ayssiwede et al. (2010) did not identify a reduction in FI with the levels of 0, 7, 14 and 21% of LLM in adult chickens. Oliveira et al. (2014) showed no influence of 0, 5 and 10% LLM on FI in laying hens at the growing phase. In contrast with our results, Lopes et al. (2014) identified a higher FI in laying hens fed with corn- and sorghum-based diets with 2% FFL. The variation in the results might be due to differences in botanical origin, animal species and animal age.

The reduction in the diets' AME contributed to the reduction of AMEI in birds. Birds that received the diet containing 8% LLM ingested 6.89 grams of AMEn less per day than birds that received corn-based feed, while the reduction of AMEI was 13 grams per day when comparing sorghum-based feed without LLM and the 8% LLM diet. This was also the main factor related to the reduction in EP of laying hens fed these diets, once they achieved a reduction of 4.65% in comparison with EP obtained with the corn-based diets. However, there was no significant reduction in CPI with the inclusion of LLM in the diet (P>0.05), although a tendency towards reduction was observed with increasing levels of hay inclusion.

Along with lower AMEI by the birds, egg production also decreased at the 8% LLM inclusion level (P<0.05). The energy of the diet is extremely influential on laying hens production. Once egg production begins, energy intake is a critical factor controlling the egg number. Studies by Harms et al. (2000) and Leeson et al. (2001b) showed that egg production is not affected by dietary energy level, but when energy intake is deficient, egg production declines.

Some previous studies reported that mimosine is responsible for some animal disorders by interfering with trypsin metabolism, resulting in goitre, loss of appetite and poor reproductive performance (Ndelekwute et al., 2018). In addition, the capacity of mimosine to form stable chelates with some mineral elements has been suggested, and it may deprive the animal of the much-required nutrient by interfering with its uptake and/or utilisation, thus resulting in reduced egg production (Atawodi et al., 2008).

Atawodi et al. (2008) observed that LLM supplementation at 5, 10 and 20% levels progressively decreased cumulative weekly average daily egg lay to 88.2, 68.7 and 53.4%, respectively, when compared to the standard commercial diet. Abou-Elezz et al. (2011) observed a quadratic effect on EP in red island hensat 36 weeks of age, which improved with 5% (57.25%) LLM compared to the control (57.10%); thereafter, it decreased gradually in 10 and 15% LLM (53.25% and 47.46%, respectively).

Despite the reduction in egg production with 8% LLM inclusion, there was no significant effect on EM or FCR (P>0.05). This probably occurred because it had no effect on FI and EW, variables used to obtain

EM and FCR data. Atawodi et al. (2008) observed no effect on EW up to the 20% inclusion level. According to Abou-Elezz et al. (2011), recommend the use of LLM in hen diets up to 10%, and higher levels markedly reduce EM and egg production.

Amino acid intake by laying hens has a direct effect on egg size (Alagawany & Abou-Kassem, 2014). Methionine and lysine supplementation normally increase EW (Kakhki et al., 2016; Macelline et al., 2021). As previously described, crude protein intake did not change among the treatments in the present study, and although NNC decreased from 6% LLM inclusion, it was not sufficient to influence EW.

Egg constituents as a percentage of yolk, albumen and shell, as well as Haugh units and specific egg density, were not affected by the inclusion of FFL. In comparison to the corn-based diet, a significant difference was observed in yolk colour (P > 0.05). All treatments containing LLM provided significantly more pigmented yolks than the corn-based diet. A significant difference for the Haugh unit was obtained for the ration without LLM and those rations with 6% or more LLM (P > 0.05) (Table 5).

Table 5
Egg quality of Hisex brown laying hens of 32 weeks of age fed diets with different levels of leucaena leaf meal

Diets	Yolk (%)	Albumen (%)	Shell (%)	Haugh units (HU)	Specific Density (g/cm³)	Yolk colour
Corn	22.98	67.11	9.91	85.45	1096	5.83
Sorghum	23.37	66.89	9.75	89.21	1079	1.59
Sorghum + 2% LLM	24.01	66.17	9.82	86.64	1106	6.43*º
Sorghum + 4% LLM	23.76	66.49	9.75	85.46	1077	7.95*º
Sorghum + 6% LLM	23.40	67.01	9.59	83.94*	1103	8.69*º
Sorghum + 8% LLM	23.81	66.53	9.66	84.82*	1105	9.13*º
Mean	23.55	66.70	9.74	85.97	1095	6.60
SEM	0.11	0.11	0.04	0.57	0.01	0.43
ANOVA				p-value		
Diets	0.0698	0.0839	0.2674	0.1181	0.2335	<.0001
Level	0.2967	0.1747	0.4755	0.0316	0.1974	<.0001
Regression				p-value		
Linear	0.3838	0.1810	0.1271	0.1990	0.6822	<.0001
Quadratic	0.1771	0.1254	0.4448	0.4111	0.2423	<.0001[1]

[1] Yolk colour Ŷ = (4.49 + 1.11X - 0.07X2,R2 = 0.98)
SEM, standard error of the mean
ANOVA, analysis of variance
*Significant statistical effect in relation to the sorghum-based treatment without the inclusion of LLM by Dunnett test (p<0.05).
ºSignificant statistical effect in relation to the corn-based treatment by Dunnett test (p<0.05).

In the comparison of the means of the treatments that contained LLM with the sorghum-based diet without LLM by the Dunnett test, it was observed that only yolk colour and Haugh units were affected by LLM inclusion in the diets. The yolk colour increased with 2% or more of hay inclusion, whereas the reduction in Haugh units was detected with 6% inclusion (P > 0.05) (Table 5).

In the regression analysis, a quadratic effect of the inclusion levels of LLM was observed (P<0.05) on yolk colour, indicating that it increased, reaching maximum colouration at 7.93% of inclusion. The inclusion of LLM from 2% in the sorghum-based diet promoted a quadratic effect on egg yolk colour, indicating a significant increase in this parameter until reaching the maximum value, with an estimated level of 7.93% LLM. However, the colour of the egg yolks obtained with 2% or more LLM was significantly higher than those obtained with the sorghum-based diet without LLM or in relation to the corn-based diet.

Yolk colour was better with 2% or more LLM in the diets compared with the diet with sorghum without LLM. All yolks obtained from the control group (0% leaf meal) were whiter than the lowest degree (6) of the yolk-coloured fan. The improvement in yolk colour is a result of the carotenoids present in *Leucaena* (P<0.05). The intense yellowish yolk colour recorded in our study for eggs produced from birds on diets containing LLM confirms its viability as a yolk-colouring agent, which can enhance the marketability of the eggs.

The results are in line with those observed by Abou-Elezz et al. (2011), who found that yolk colour values increased significantly and linearly with the inclusion of LLM. According to the authors, while corn-based diets without LLM obtained scores under 6.0, the levels of 5, 10 and 15% resulted in 7.5, 9 and 11.25 units at the fan score. Hien et al. (2017) identified a linear increase in yolk colour values with 6% LLM compared to the control group, which resulted in a difference of 5 units in the fan score (13.4 and 7.3, respectively).

Conclusion

According to the results, the inclusion of LLM in laying hens' diets with sorghum as the main source of energy can be performed at up to 6% without changes in laying performance or egg quality. This level provides the same laying performance obtained with corn-based diets, but with yolks with a much higher level of pigmentation, which proves that LLM can be successfully used as a natural pigment in sorghum-based diets to achieve the yolk pigmentation desired by consumers.

Statement of Animal Rights

"The project was approved by the Animal Research Ethics Committee of the Federal University of Ceará, by protocol 23/2013, dated 07/24/2013".

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