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Sunflower cake in the diets of lightweight laying pullets: Effects on the growth phase and the beginning of production cycle

Torta de girassol em dietas para frangas poedeiras leves: Efeitos na fase de crescimento e no início do ciclo de produção

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ABSTRACT

The use of sunflower cake (SC) in animal feed has been studied mainly due to the characteristics that are given to them, since this by-product when incorporated in the feed can increase the economic viability and the nutritional value of the diet. The objective of this study was to evaluate the inclusion of sunflower cake in lightweight pullet diets and check their nutrient digestibility and metabolizable energy for several parameters, including diet, performance, body composition, nutrient retention, energy metabolism at the growth phase, performance of egg production, sexual maturity, and egg quality at the beginning of the laying phase. We distributed a total of 648 pullets in our study, which was a completely randomized design including six treatments with six replicates of 18 pullets each. The treatments included diets with 0, 5, 10, 15, 20, and 25% SC. Our study results concluded that pullets fed with diets containing SC showed a reduction ($P<0.01$) in nutrient digestibility and metabolizable energy compared to those fed with the control diet. Pullets fed with 25% SC showed an increase ($P<0.01$) in feed intake and feed conversion ratio. However, dietary inclusion of SC in pullet diets did not influence ($P>0.05$) their body composition, nutrient retention, energy metabolism, sexual maturity, egg-laying performance, and egg quality of laying hens between 18 and 35 weeks of their age. Therefore, up to 25% of SC could be included in the diet of lightweight laying pullets in the growth phase since it exhibited no residual effect on their productive performance at the beginning of the laying phase.

Index terms: Egg quality; energy metabolism; *Helianthus annuus*; laying hens; laying performance.

RESUMO

A utilização da torta de girassol (TG) na alimentação animal vem sendo estudada principalmente devido às características que lhes são conferidas, pois esse subproduto quando incorporado na ração pode aumentar a viabilidade econômica e o valor nutricional da dieta. O objetivo deste trabalho foi avaliar a inclusão da torta de girassol (TG) em dietas para frangas leves sobre a digestibilidade dos nutrientes e energia metabolizável das dietas, desempenho, composição corporal, retenção de nutrientes, metabolismo energético na fase de crescimento e desempenho na produção de ovos, maturidade sexual e qualidade dos ovos no início da fase de postura. Um total de 648 frangas foram distribuídas em um delineamento inteiramente casualizado com 6 tratamentos e 6 repetições de 18 frangas cada. Os tratamentos consistiram em dietas contendo níveis de inclusão de 0, 5, 10, 15, 20 e 25% TG. Os autores concluíram que as frangas alimentadas com dietas contendo TG apresentaram redução (P<0,01) na digestibilidade dos nutrientes e da energia metabolizável em comparação com aquelas alimentadas com a dieta controle. As frangas alimentadas com 25% TG apresentaram aumento (P<0,01) no consumo de ração e na conversão alimentar. A inclusão de TG nas dietas não influenciou (*P*>0,05) a composição corporal, a retenção de nutrientes e o metabolismo energético, bem como a maturidade sexual, desempenho produtivo e qualidade dos ovos das poedeiras de 18 a 35 semanas de idade. A TG pode ser incluída até 25% na dieta de frangas leves na fase de crescimento, sem efeito residual no desempenho produtivo no início da fase de postura.

Termos para indexação: Qualidade dos ovos; metabolismo energético; *Helianthus annuus*; galinhas poedeiras; desempenho de postura.

INTRODUCTION

The sunflower (*Helianthus annuus*) crop is found widespread throughout the world and is responsible for supplying sunflower oil. Sunflower oil is the main product of the oilseed chain and is produced through the following two distinct industrial processes: the first process exhibits high efficiency and uses solvent (hexane associated with high temperature) to produce a by-product known as a sunflower meal. In the second process, seeds are cold-pressed in a mechanical process to produce the by-product called sunflower cake (SC) (Costa et al., 2005; Oliveira et al., 2012; Berwanger et al., 2014).

Despite its lower efficiency, the mechanical process of extracting sunflower oil is more affordable and an economically viable option (Costa et al., 2005). This method has been widely adopted in both small and medium enterprises since it provides a considerable amount of by-products, i.e., 34% of oil yield generates 66% of SC (San Juan; Villamide, 2001).

The SC is considered a protein source (22.46 to 25.80%) involving a higher value of ether extract (22.00 to 24.83%) (Pinheiro et al., 2013; Berwanger et al., 2014; Kargopoulos et al., 2017). However, factors inherent to cultivation and grain processing (Liu et al., 2015), low lysine amounts (Antoszkiewicz; Tywonczuc; Matusevicius, 2004), and the presence of antinutritional factors, including high fiber content and presence of phytates and chlorogenic acid (Berwanger et al., 2014; Berwanger et al., 2017; Kargopoulos et al., 2017) can cause variations in chemical and energy compositions, limiting the usage of this feedstuff in poultry diets. Simultaneously, SC also reduces production costs and increases the financial return of the agricultural systems, enabling ecologically and economically favorable usage of such by-products in animal feed (Souza et al., 2020).

Several studies have been conducted on the usage of SC in poultry diets of broilers at the growth phase (Oliveira et al., 2012; Berwanger et al., 2014; Berwanger et al., 2017). The growth phase is considered a critical phase within the production system (Freitas et al., 2014), where fibrous feed is provided to control the broilers' excessive weight gain (Braz et al., 2011). Some authors proposed the inclusion of SC in laying pullets (Kargopoulos et al., 2017; Alencar et al., 2019; Souza et al., 2020; Alencar et al., 2021) and demonstrated the possibility of additional studies with SC in white layers' diets. Considering the differences in strains, greater practical application, and a better understanding of the obtained results, adjustments were made to achieve optimal levels without compromising the health and performance of pullets. Moderate levels of fibers show beneficial effects on the development of the digestive tract and feed intake, favoring the beginning of the production phase. Hence, the poultry tends to ingest a reduced amount of feed, directly impacting their egg production (Tierzucht, 2016). Based on the above considerations, we aimed to evaluate the inclusion of sunflower cake in diets of lightweight laying pullets for the nutrient digestibility and metabolizable energy in the diets, performance, body composition, nutrient retention, energy metabolism at the growth phase (7 to 17 weeks of age), sexual maturity, productive performance, and egg quality at the beginning of the laying period.

MATERIAL AND METHODS

Poultry, housing, and experimental design during the growth phase

All experimental procedures were approved by the Ethics Committee on Animal Research (Federal University of Ceará, Fortaleza, Brazil) as per the ethical principles of the Brazilian Animal Experimentation Control Council.

We purchased a total of 900 one-day-old pullets (Hy-Line W36, Hy-Line of Brazil Ltda., São José do Rio Preto, São Paulo, Brazil) and housed them in a conventional shed. The pullets were managed according to the management guide of lineage (Hy-Line International, 2017). At the end of the sixth week, pullets were weighed individually (average initial weight=451±11.95 g), and the ones with homogeneous mean weights were distributed in the experimental groups (Sakomura; Rostagno, 2016). In the growth phase experiment, i.e., between 7 and 17 weeks of age, 648 selected pullets were housed in a conventional shed with galvanized wire cages (50 cm long × 50 cm wide × 45 cm high) equipped with galvanized trough feeders and nipple-type drinkers. The stocking density was 416 cm^2/pullet, and the pullets were exposed to natural light for approximately 12 h per day.

The experiment had a completely randomized design, including six treatments with six replicates of 18 pullets each. The treatments included diets containing 0, 5, 10, 15, 20, and 25% SC.

Feeding program and experimental diets during the growth phase

The growth phase was divided into the following two phases: breeding (7 to 12 weeks of age) and rearing (13 to 17 weeks of age). According to the nutritional requirements of lineage (Hy-Line International, 2017) and feedstuffs composition values proposed by Rostagno et al. (2017), the experimental diets were formulated to be iso-energetic, and iso-nutrient (Tables 1 and 2), except for SC, and were prepared at the Experimental Feed Factory (Animal Science Department of the Federal University of Ceará, Fortaleza, Brazil).

The mechanical pressing method of the dry whole seeds with hulls (black-striped husk variety) used for oil removal was also used to obtain the sunflower cake. A mechanical press (Scott Tech, model ERT 40-V1, Valinhos, São Paulo, Brazil) with a power of 0.75 KW and a seed processing capacity of 6 kg/h was used. The obtained sunflower meal for grounding was packed in appropriate bags and included in the experimental diets later. Each kg of processed seed provided 29.20% of oil and 70.80% of SC. Previously, the nutritional and energy composition of

SC (in the natural matter) was determined using the method described by Silva and Queiroz (2002) at the Animal Nutrition Laboratory (Federal University of Ceará, Fortaleza, Brazil) while the metabolizable energy from the total collection of excreta was determined using a previous metabolic assay (Sakomura; Rostagno, 2016). Similarly, calcium, available phosphorus, sodium, chlorine, potassium, digestible lysine, digestible methionine, digestible methionine+cystine, digestible threonine, and digestible tryptophan were estimated based on the (Fundación Espanola Desarrollo Nutrición Animal - FEDNA, 2010) tables. The above-mentioned nutritional values are shown in Table 3.

Table 1: Composition and nutritional and energetic levels of experimental diets fed to lightweight laying pullets between 7 and 12 weeks of age.

Ingredients	Sunflower cake level (%)					
	0.0	5.0	10.0	15.0	20.0	25.0
Corn, grain	65.42	65.07	61.16	57.24	53.32	49.41
Soybean meal (45% crude protein)	26.97	24.82	23.32	21.82	20.32	18.82
Sunflower cake	0.00	5.00	10.00	15.00	20.00	25.00
Soybean oil	1.90	1.11	1.53	1.95	2.36	2.78
Calcitic limestone	1.12	1.10	1.07	1.04	1.02	0.99
Dicalcium phosphate	2.01	2.01	2.01	2.02	2.03	2.03
Mineral and vitamin premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Common salt	0.38	0.38	0.38	0.38	0.38	0.38
DL-Methionine	0.19	0.18	0.17	0.17	0.16	0.16
L-Lysine HCl	0.09	0.13	0.15	0.17	0.19	0.21
L-Threonine	0.00	0.00	0.01	0.01	0.02	0.02
Washed sand (inert)	1.72	0.00	0.00	0.00	0.00	0.00
Calculated nutritional and energy composition						
Metabolizable energy (kcal/kg)	3000.00	3000.00	3000.00	3000.00	3000.00	3000.00
Dry matter (%)	88.73	88.70	88.99	89.29	89.59	89.89
Crude protein (%)	17.55	17.55	17.55	17.55	17.55	17.55
Acid detergent fiber (%)	4.39	6.18	7.91	9.64	11.37	13.10
Neutral detergent fiber (%)	11.52	14.33	16.81	19.28	21.75	24.23
Ether extract (%)	4.91	4.84	5.86	6.88	7.88	8.90
Ca (%)	1.00	1.00	1.00	1.00	1.00	1.00
Available P (%)	0.47	0.47	0.47	0.47	0.47	0.47
Na (%)	0.17	0.17	0.17	0.17	0.17	0.17
Cl (%)	0.28	0.28	0.28	0.28	0.28	0.28
Digestible lysine (%)	0.89	0.89	0.89	0.89	0.89	0.89
Digestible methionine (%)	0.68	0.68	0.68	0.68	0.68	0.68
Digestible Met + Cys (%)	0.43	0.43	0.43	0.43	0.43	0.43
Digestible threonine (%)	0.60	0.60	0.60	0.60	0.60	0.60
Digestible tryptophan (%)	0.19	0.19	0.19	0.19	0.19	0.19

[1]Provided per kilogram of diet: cobalt (mim) 50 mg; copper (mim) 3,000 mg; iron (mim) 25 g; iodine (mim) 500 mg; manganese (mim) 32.5 g; selenium (mim) 100.05 mg; zinc (mim) 22.49 g; vitamin A (mim) 5,500,000 IU; vitamin B1 (mim) 500 mg; vitamin B12 (mim) 7,500 mcg; vitamin B2 (mim) 2.502 mg; vitamin B6 (mim) 750 mg; vitamin D3 (mim) 1,000,000 IU; vitamin E (mim) 6,500 IU; vitamin K3 (mim) 1,250 mg; biotin (mim) 25 mg; niacin (mim) 17.5 g; folic acid (mim) 251 mg; pantothenic acid (mim) 6.030 mg.

Table 2: Composition and nutritional and energetic levels of experimental diets fed to lightweight laying pullets between 13 and 17 weeks of age.

Ingredients	Sunflower cake level (%)					
	0.0	5.0	10.0	15.0	20.0	25.0
Corn, grain	71.94	69.28	66.59	63.43	59.51	55.59
Soybean meal (45% crude protein)	22.59	20.87	19.15	17.52	16.03	14.54
Sunflower cake	0.00	5.00	10.00	15.00	20.00	25.00
Soybean oil	0.00	0.00	0.00	0.16	0.58	1.00
Calcitic limestone	1.20	1.17	1.14	1.12	1.09	1.06
Dicalcium phosphate	1.93	1.93	1.94	1.94	1.95	1.95
Mineral and vitamin supplement[1]	0.20	0.20	0.20	0.20	0.20	0.20
Common salt	0.41	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.13	0.12	0.12	0.11	0.10	0.10
L-Lysine HCL	0.04	0.07	0.09	0.12	0.14	0.16
Washed sand (inert)	1.56	0.96	0.37	0.00	0.00	0.00
Calculated nutritional and energy composition						
Metabolizable energy (kcal/kg)	2950.00	2950.00	2950.00	2950.00	2950.00	2950.00
Dry matter (%)	88.42	88.60	88.78	89.01	89.31	89.60
Crude protein (%)	16.00	16.00	16.00	16.00	16.00	16.00
Acid detergent fiber (%)	4.26	6.00	7.76	9.50	11.23	12.96
Neutral detergent fiber (%)	11.70	14.29	16.88	19.43	21.90	24.38
Ether extract (%)	3.18	3.82	4.46	5.25	6.26	7.28
Ca (%)	1.00	1.00	1.00	1.00	1.00	1.00
Available P (%)	0.45	0.45	0.45	0.45	0.45	0.45
Na (%)	0.18	0.18	0.18	0.18	0.18	0.18
Cl (%)	0.30	0.30	0.30	0.30	0.30	0.30
Digestible lysine (%)	0.75	0.75	0.75	0.75	0.75	0.75
Digestible methionine (%)	0.59	0.59	0.59	0.59	0.59	0.59
Digestible Met + Cys (%)	0.36	0.36	0.36	0.36	0.36	0.36
Digestible threonine (%)	0.55	0.55	0.54	0.54	0.53	0.53
Digestible tryptophan (%)	0.17	0.17	0.17	0.17	0.17	0.17

[1]Provided per kilogram of diet: cobalt (mim) 50 mg; copper (mim) 3,000 mg; iron (mim) 25 g; iodine (mim) 500 mg; manganese (mim) 32.5 g; selenium (mim) 100.05 mg; zinc (mim) 22.49 g; vitamin A (mim) 5,500,000 IU; vitamin B1 (mim) 500 mg; vitamin B12 (mim) 7,500 mcg; vitamin B2 (mim) 2.502 mg; vitamin B6 (mim) 750 mg; vitamin D3 (mim) 1,000,000 IU; vitamin E (mim) 6,500 IU; vitamin K3 (mim) 1,250 mg; biotin (mim) 25 mg; niacin (mim) 17.5 g; folic acid (mim) 251 mg; pantothenic acid (mim) 6.030 mg.

Evaluation of nutrient digestibility and metabolizable energy of the diet

The total excreta collection method was used to perform two metabolic assays. The first assay was performed between the 10th and 11th weeks of age, while the second was performed between the 13th and 14th weeks. Aluminum trays were installed under the cages to collect the excreta and avoid content losses. On the first and last day of collection, 1% ferric oxide was added to the diets to identify the excreta. The excreta not marked on the first and last collections were discarded. Daily collection of excreta was performed at 08 00 h and 16 00 h. Next, the total excreta were weighed, packed in plastic bags, identified by replicates, and then frozen. The feed intake and total excreta produced during the collection period were determined at the end of each experiment.

Table 3: Nutritional and energy composition of the sunflower cake.

Nutrients and energy	Sunflower cake
Analyzed composition[1]	
AME_n[2] (kcal/kg)	2774.00
Dry matter (%)	92.81
Crude protein (%)	19.35
Neutral detergent fiber (%)	43.95
Acid detergent fiber (%)	28.96
Ether extract (%)	15.52
Ash (%)	2.66
Reported composition[3]	
Ca (%)	0.28
Available P (%)	0.09
Na (%)	0.03
Cl (%)	0.08
K (%)	1.16
Digestible lysine (%)	0.59
Digestible methionine (%)	0.59
Digestible Met + Cys (%)	0.68
Digestible threonine (%)	0.61
Digestible tryptophan (%)	0.22

[1]Values obtained by the laboratory analysis; [2]AME_n, nitrogen-corrected apparent metabolizable energy; [3]Estimated based on FEDNA (2010) tables.

According to (Association of Official Agricultural Chemists – AOAC, 2005), feed and excreta samples were dried and analyzed for dry matter (DM) and nitrogen (N). Gross energy (GE) was determined using an oxygen bomb calorimeter (IKA C200, Staufen, Germany). Based on the results and equations proposed by Matterson, Potter, and Stutz (1965), digestibility coefficients (%) of dry matter (DCDM), nitrogen (DCN), gross energy (DCGE), values of apparent metabolizable energy (AME), and apparent metabolizable energy corrected by the nitrogen balance (AMEn) in diets (kcal/kg DM) were calculated.

Performance evaluation during the growth phase

Considering the feed intake, initial and final weights at the growth phase (breeding time: 7 to 12 weeks; rearing time: 3 to 17 weeks) and performance variables for feed intake, weight gain, feed conversion ratio, and uniformity were evaluated.

Evaluation of body composition, nutrient retention, and energy metabolism

Considering the average weight of the pullets in each plot, three groups of two 7-week-old pullets were selected for the experiment. The pullets were placed separately in cages and were left to fast for 24 h. After fasting, they were weighed, euthanized, plucked, and weighed again to determine the fasting weight without feathers. The same procedure was performed again at 17 weeks, with two pullets selected per plot based on the average weight of pullets in each plot.

At both ages, carcasses (whole fowl without feathers) were frozen and ground in a 15-hp industrial meat grinder. According to AOAC (2005) and GE in an oxygen calorimeter bomb (IKA C200, Staufen, Germany), one sample from each carcass was dried for 72 h and processed in a ball mill to determine dry matter (DM), crude protein (CP), ethereal extract (EE), and mineral matter (MM).

After analyzing EE, CP, MM, and GE of carcasses at 7 and 17 weeks, the comparative slaughter method (Farrel, 1974) was used to calculate nutrient retention using the differences in retained CP (RCP), retained EE (REE), retained MM (RMM), retained body energy (RE), and heat production (HP) between both ages. HP was calculated using the following equation: HP = IME – RE, considering AMEn to be determined for each diet in the metabolism assay, IME = AMEn ingested, and RE = retained body energy.

Effect of SC in pullet diets during the pullet-layer transition period

At the end of 17 weeks, the remaining 432 pullets were transferred to a laying house, and the same experimental design (as in the growth phase) was maintained. However, the replications now had 12 pullets and were distributed to galvanized wire cages (25 cm long × 45 cm wide × 40 cm high) with galvanized trough feeders and nipple drinkers. Each cage housed two pullets. After transfer to the laying shed, the lighting was provided for 14 h /day, which was increased to 15 min /day until it reached 16 h of light/day. After this, the light was kept constant until the end of the experiment.

Here, the experimental period lasted until 35 weeks of pullet's age, which was divided into six periods of 21 days each. According to the nutritional requirements of lineage (Hy-Line International, 2017) and the feedstuff composition values proposed by Rostagno et al. (2017), all pullets were fed *ad libitum* with a laying diet (Table 4), which was based on corn and soybean meal.

Table 4: Composition and nutritional and energetic levels of experimental diets fed to lightweight laying hens between 18 and 35 weeks of age.

Composition	Diet
	18 to 35 weeks
Corn, grain	60.50
Soybean meal (45% crude protein)	24.35
Soybean oil	3.30
Calcitic limestone	8.88
Dicalcium phosphate	2.14
Mineral and vitamin supplement[1]	0.10
Common salt	0.41
DL-Methionine	0.24
L-Lysine HCL	0.08
Calculated nutritional and energy composition	
Metabolizable energy (kcal/kg)	2900.00
Dry matter (%)	89.67
Crude protein (%)	16.00
Acid detergent fiber (%)	4.01
Neutral detergent fiber (%)	10.58
Ether extract (%)	6.07
Ca (%)	4.15
Available P (%)	0.49
Na (%)	0.18
Cl (%)	0.30
Digestible lysine (%)	0.81
Digestible methionine (%)	0.68
Digestible Met+Cys (%)	0.46
Digestible threonine (%)	0.55
Digestible tryptophan (%)	0.17

[1] Per kilogram of diet: cobalt (min) 100 mg, copper (min) 6,000 mg, iron (min) 50 g, iodine (min) 1,000 mg, manganese (min) 50 g, selenium (min) 200 mg, zinc (min) 50 g, vitamin A (min) 8,000,000 IU, vitamin B1 (min) 1,000 mg, vitamin B12 (min) 6,000 mcg, vitamin B2 (min) 3,000 mg, vitamin B6 (min) 1,000 mg, vitamin D3 (min) 2,198,214 IU, vitamin E (min) 8,000 IU, vitamin K3 (min) 2,000 mg, biotin (min) 20 mg, niacin (min) 20 g, folic acid (min) 200 mg, pantothenic acid (min) 9,280 mg, *Bacillus subtilis* 150 × 10e^9 CFU.

The evaluated variables for sexual maturity included mean age at 1st egg (MA 1st), the mean age for 50% of pullets to lay eggs (MA 50%), the mean age for 100% of pullets to lay eggs (MA 100%), and the mean weight of the 1st egg (MW 1st). Similarly, the evaluated variables for performance included egg production, feed intake, egg weight, egg mass, and feed conversion/egg mass. The egg quality was evaluated based on the percentage of albumen, yolk, shell, specific gravity, Haugh units, and shell thickness.

The egg produced from each replicate was recorded daily to calculate the variables. For every repetition, the diets and leftovers in each period were weighed. Furthermore, once a week, the eggs from each plot were collected, identified, and weighed on an electronic scale with a precision of 0.01 g (ARA520, Ohaus Corporation, Pine Brook, NJ, US).

To assess the quality (specific density in g/cm^3 and Haugh units) and characteristics (percentages of yolk, eggshell, and albumen) of the eggs, three eggs were selected from each plot (avoiding broken, cracked, or dirty eggs) according to their average weight. Specific density was determined as per the weighing apparatus of the egg in water. The quality of albumen was assessed by determining the Haugh unit. The values of egg weight in the air and albumen height were used for the following calculations: $HU=100\times\log(H + 7.57-1.7 \times W^{0.37})$, where HU=Haugh units; H=albumen height in mm, and W=egg weight in grams. To determine the percentage of yolk to the total egg weight, the albumen was separated, and the yolk was weighed. To calculate the percentage of the shell, eggs were broken, and shells were separated, which were washed, and dried in an oven with air circulation at 55 °C for 48 h. After drying, the percentage of the shell to the total egg weight was calculated by weighing the shells on a semi-analytical scale, with a sensitivity of 0.01 g. The percentage of albumen was obtained by the following formula: % albumen = 100 – (% yolk + % shell). Next, the shells were weighed, and the thickness of the eggshell (mm) was determined using a micrometer with divisions of 0.01 mm, where the average values of the poles, the thickness of the greater region, lesser region, and equatorial region of the shell were measured.

Ambient temperature and humidity

Portable data loggers (HOBO U12–012, Onset Computer Corporation, Bourne, MA, US) were used to record ambient temperature and relative humidity in the sheds during the whole experimental period every 10 min. The average values of temperature and humidity were 28.59±2.23 °C and 59.79±12.59% for the growth phase and 29.50±2.49 °C and 62.07±14.41% for the pullet-layer transition period, respectively.

Statistical Analysis

The GLM procedure of SAS (Version 8.2; SAS Inst. Inc., Cary, NC, US) was used for statistical analyses. Data were subjected to analysis of variance according to the completely randomized model, which was as follows:

$$Yij = \mu + Ti + eij$$

where, Yij = observation j of the experimental unit subjected to treatments Ti; μ = overall mean; Ti = the effect of experimental diet including 0, 5, 10, 15, 20 and 25% SC; eij = random error associated with each observation. To determine the performance, body composition, retention of nutrients, and energy metabolism in the growth phase, each pen with 18 pullets was distributed to three cages, where six pullets were assigned per cage. Subsequently, to determine sexual maturity, egg characteristics, and egg quality in the production phase, each pen with 12 pullets was distributed to six cages, with two pullets maintained per cage.

To establish the best curve for the data behavior and to determine the best dietary percentage of SC, the degrees of freedom referring to the levels of inclusion of SC but excluding the control diet (0% of inclusion) were submitted to the polynomial regression. Additionally, Dunnett's test was used to compare the dietary inclusion levels of SC (5, 10, 15, 20, and 25%) with the control diet (0% of SC).

RESULTS AND DISCUSSION

Nutrient digestibility

According to the metabolic assay, between 7 and 12, and 13 and 17 weeks of age (Table 5), the treatments showed significant differences among the DCDM, DCN, DCGE, AME, and AMEn values ($P<0.05$). Furthermore, compared to the pullets given a diet without the feedstuff, pullets fed with a diet starting at 5% SC showed a reduction ($P<0.05$) in DCDM, DCN, DCCE, AME, and AMEn values.

The inclusion of SC promoted a linear reduction ($P<0.01$) in the metabolizable coefficients and metabolizable energy of the diets. Between 7 and 12 weeks of age, a linear reduction was observed in DCDM (Y=81.63–0.46X, R^2=0.96), DCN (Y=74.08–0.49X, R^2=0.85), DCGE (Y=84.63–0.38X, R^2 = 0.98), AME (Y=3,634–4.16X, R^2=0.99), and AMEn values (Y=3,421–2.18X, R^2=0.96). Similarly, between 13 and 17 weeks of age, linear reduction ($P<0.05$) was observed in DCDM (Y=78.83–0.38X, R^2=0.97), DCN (Y=57.98–0.22X, R^2=0.95), DCCE (Y=82.90–0.34X, R^2=0.97), AME (Y=3,415–2.04X, R^2=0.97), and AMEn values (Y=3,272–1.62X, R^2=0.96).

The metabolic assay results suggested that these effects were associated with the antinutritional factors present in SC, especially the increased fiber content in the diets. SC has a high concentration of non-starch polysaccharides, especially cellulose and lignin, which are not degraded by endogenous enzymes (Berwanger et al., 2017). Thus, it acts as a barrier in the digestive system of poultry, leading to damaged enzymatic action and increased endogenous loss of nutrients. Consequently, the availability of dietary energy and the nutrient availability for absorption is reduced, leading to increased peristaltic movements of the intestinal tract, motility of the food bolus, less maintenance of the food in the gastrointestinal tract, and reduced enzymatic action on the substrate (Pascoal; Watanabe, 2014).

Berwanger et al. (2014) determined the metabolizable energy of this feedstuff in broilers between 21 and 31 days of age by substituting 10, 20, 30, and 40% SC levels from the reference diet using the total excreta collection method and observed a decrease in the AMEn values according to the SC replacement level. They attributed this effect to the high fiber content in the feedstuff.

Performance evaluation during the growth phase

At the growth phase (Table 6), pullets fed with diets including 25% SC showed an increase in FI and FCR compared to those fed with the control diet. The inclusion of SC from 5% onward promoted a linear increase in FI (Y=3,383.5+8.077X, R^2=0.97) and FCR (Y=4.684+0.0088X, R^2=0.85) ($P<0.01$). However, it did not influence weight gain (WG) and uniformity (UN).

The effect of dietary SC on FI and FCR of pullets at the growth phase could be mainly related to the fiber content of the feedstuff (47.36% NDF and 31.20% ADF). This effect on the performance corroborates with the lower nutrient digestibility and energy metabolism in diets presented in our study (Table 5). Roberts et al. (2007) reported that an increase in dietary fiber content may lead to the dilution of feed energy, which leads to a compensatory increase in the FI of pullets, achieving the energy levels required for desirable growth, development, and production. Thus, the pullets fed with a diet containing 25% SC presented an increase in FI to meet energy and nutritional requirements, which was evidenced by the maintenance of WG. Contrastingly, the observed increase in FI of these pullets increased the FCR, which was consistent with the results obtained by Souza et al. (2020), who tested the inclusion of SC in the growth of brown-egg pullets.

Table 5: Digestibility of the nutrients and metabolizable energy values in diets including SC in lightweight laying pullets between 7 and 12, and 13 and 17 weeks of age.

Levels of SC (%)	DCDM[1] (%)	DCN[2] (%)	DCGE[3] (%)	AME[4] (kcal/kg/DM[6])	AMEn[5] (kcal/kg DM)
7 to 12 weeks of age					
0	80.95	77.99	85.10	3,693	3,480
5	78.71*	70.50*	82.38*	3,616*	3,413*
10	77.12*	69.03*	80.81*	3,591*	3,399*
15	75.58*	68.20*	79.32*	3,570*	3,385*
20	73.13*	66.39*	77.36*	3,547*	3,374*
25	69.27*	59.60*	74.51*	3,534*	3,371*
SEM[7]	0.72	1.03	0.64	0.01	0.01
Linear *p* – value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Quadratic *p* – value	0.30	0.42	0.21	0.65	0.60
13 to 17 weeks of age					
0	79.16	61.23	83.11	3,562	3,405
5	76.98*	57.24*	81.45*	3,403*	3,265*
10	74.50*	55.76*	78.93*	3,393*	3,255*
15	73.96*	54.16*	78.25*	3,387*	3,246*
20	70.63*	53.43*	75.55*	3,377*	3,244*
25	69.34*	52.93*	74.56*	3,360*	3,230*
SEM	0.64	0.55	0.58	0.01	0.01
Linear *p* – value	< 0.01	< 0.01	< 0.01	< 0.05	< 0.05
Quadratic *p* – value	0.79	0.085	0.49	0.75	0.98

[1]DCDM-Digestibility coefficient of dry matter; [2]DCN-Digestibility coefficient of nitrogen; [3]DCGE-Digestibility coefficient of gross energy; [4]AME-Apparent metabolizable energy; [5]AMEn-Apparent metabolizable energy corrected by the nitrogen balance; [6]DM-Dry matter; [7]SEM-Standard error of the mean; *Mean differs from the control treatment by Dunnett's test (P < 0.05).

Table 6: Performance of lightweight laying pullets fed with diets containing SC between 7 and 17 weeks of age.

Levels of SC (%)	FI[1] (g)	WG[2] (g)	FCR[3]	UN[4] (%)
0	3429.82	726.45	4.72	86.67
5	3424.40	728.67	4.70	90.57
10	3459.32	717.04	4.82	88.00
15	3518.34	732.04	4.81	87.62
20	3530.83	730.47	4.84	90.00
25	3590.57*	731.19	4.91*	85.56
SEM[5]	16.22	3.27	0.02	1.16
Linear *p* – value	< 0.01	0.39	< 0.01	0.39
Quadratic *p* – value	0.97	0.75	0.69	0.93

[1]FI-Feed intake; [2]WG-Weight gain; [3]FCR-Feed conversion ratio; [4]UN-Uniformity; [5]SEM-Standard error of the mean; *Mean differs from the control treatment by Dunnett's test (P < 0.05).

Although the studies using SC for pullets are limited in literature, the results obtained are similar to the results found in our study. Pinheiro et al. (2013) evaluated increasing levels of SC (0, 7, 14, and 21%) in brown-egg pullet diets from 10 to 16 weeks of age and concluded that the highest level of SC also did not affect the performance of pullets. Similarly, Kargopoulos et al. (2017) evaluated 0, 6.25, and 12.5% SC in brown-egg layer pullets' diet from 1 to 20 weeks of age and observed no adverse effects on pullets at this phase. Souza et al. (2020) showed that up to 300 g/kg of sunflower cake could be included in the feed of brown-egg laying pullets between 7 and 17 weeks of age without exhibiting any negative influence on their performance, which also corroborated with the results of our study.

Body composition, nutrient retention, and energy metabolism

Body composition (Table 7), nutrient retention (Table 8), and energy metabolism (Table 9) of the pullets were not influenced (P>0.05) by the treatments, which indicated that up to 25% of SC did not affect these variables. The no-effect of dietary SC on body composition and nutrient retention of pullets can be attributed to the fact that the negative nutritional effects of this feedstuff were compensated by an increase in FI by the pullets, which was an attempt to maintain an adequate quantity of nutrients and energy to meet the body demands. This compensatory effect on FI was observed by the maintenance of WG (Table 6). Consequently, the similar final mean weight among the pullets at the end of the growth phase was 1175.80 g, which was as per the recommendations by the lineage management guide (Hy-Line International, 2017) for this phase.

Hetland and Svihus (2001) and González-Alvarado et al. (2007) have demonstrated that the inclusion of moderate levels of fiber in poultry diets was beneficial for increasing the transit time of feed in the digestive tract, improving the development and functioning of gizzard and other digestive tract sites. Moreover, dietary fiber stimulates the production of hydrochloric and bile acids to enhance the activity of amylase, generating significant benefits in the digestion process and the absorption of nutrients (Mateos et al., 2012).

According to Noblet and Van Milgen (2004), an increase in the concentration of fiber in the diet led to an increase in caloric increment, reducing the energy efficiency of the diet. However, according to our results, the energy metabolism of pullets was not influenced by the inclusion of SC. The amount of oil present in the ingredient (15.52% EE) increased the oil content of the diet, which may have counterbalanced the caloric increment and the energy efficiency of the diet. This may be due to the increase in the energy efficiency of the diet with the addition of fat, leading to extra-caloric effects (Aardsma; Mitchell; Parsons, 2017), but the decrease in the energy efficiency with the addition of fiber. According to Noblet and Van Milgen (2004), fiber has an efficiency of using 60% of metabolizable energy while fat has 90% efficiency. Hence, despite having a high fiber content, the addition of up to 25% SC did not compromise the energy metabolism of the pullets up to 17 weeks of age.

Pullet-layer transition period

During the pullet-layer transition period, dietary SC did not affect the variables of MA 1st, MA 50%, MA 100%, and MW 1st in the pullet diet (Table 10). The sexual maturity of laying hens was directly related to the WG, UN, and body composition of pullets at the end of the growth phase. Since the treatments did not influence (*P*>0.05) these variables up to 17 weeks of age, and the UN was very close to those recommended by the lineage management guide (Hy-Line International, 2017), no effect was considered on sexual maturity as well.

The dietary inclusion of SC in lightweight pullets did not promote any residual effect (*P*>0.05) on the feed intake (FI), egg weight (EW), egg production (EP), egg mass (EM), and feed conversion/egg mass (FCEM) of pullets at the beginning of the laying phase (Table 11). Besides influencing the pullet-layer transition period, the characteristics of pullets at the end of the growth phase also exerted an influence on the performance during the laying period. Thus, the results obtained for FMW, UN, and body composition at the end of the growth phase also ensured a similar performance among the pullets given different treatments at the laying phase.

None of the egg quality variables were influenced (*P*>0.05) by the SC levels in the feed of laying hens during the growth phase (Table 12).

To support proper body development and formation of the reproductive system along with the accumulation of energy reserves, pullets need to have an adequate intake of nutrients and energy balance, which are the most critical steps during the production phase. Thus, at the end of the growth phase, future laying hens must present ideal weight and body conditions to fulfill their productive potential (Braz et al., 2011; Freitas et al., 2014).

Table 7: Body composition of 17-weeks-old lightweight laying pullets fed with diets containing SC between 7 and 17 weeks of age.

Levels of SC (%)	DM[1] (%)	CP[2] (% DM)	EE[3] (% DM)	MM[4] (% DM)
0	37.39	20.43	18.44	2.77
5	37.66	20.38	18.37	2.74
10	37.74	20.32	18.40	2.70
15	37.56	20.39	18.36	2.71
20	37.33	20.33	18.44	2.69
25	37.37	20.28	18.35	2.63
SEM[5]	0.26	0.09	0.21	0.03
Linear *p* – value	0.66	0.80	0.99	0.28
Quadratic *p* – value	0.96	0.89	0.96	0.80

[1]DM-Dry Matter; [2]CP-Crude protein; [3]EE-Ethereal extract; [4]MM-Mineral matter; [5]SEM-Standard error of the mean.

Table 8: Retention of carcass nutrients in lightweight laying pullets fed with diets containing SC between 7 and 17 weeks of age.

Levels of SC (%)	RCP[1] ($g/kg^{0,75}$/day)	REE[2] ($g/kg^{0,75}$/day)	RMM[3] ($g/kg^{0,75}$/day)
0	2.56	3.07	0.30
5	2.53	3.03	0.30
10	2.51	3.04	0.29
15	2.53	3.03	0.29
20	2.53	3.05	0.29
25	2.51	3.03	0.28
SEM[4]	0.04	0.02	0.01
Linear *p* – value	0.95	0.99	0.50
Quadratic *p* – value	0.92	0.96	0.97

[1]RCP-Retained crude protein; [2]REE-Retained ethereal extract; [3]RMM-Retained mineral matter; [4]SEM-Standard error of the mean.

Table 9: Energy metabolism of lightweight laying pullets fed with diets containing SC between 7 and 17 weeks.

Levels of SC (%)	IME[1] (kcal/ $kg^{0,75}$/d)	RE[2] (kcal/$kg^{0,75}$/d)	HP[3] (kcal/$kg^{0,75}$/d)	RE/IME (%)	HP/IME (%)
0	195.68	37.46	158.22	19.15	80.85
5	195.64	37.26	158.38	19.04	80.96
10	195.65	37.24	158.40	19.05	80.95
15	195.64	37.22	158.42	19.03	80.97
20	195.60	37.19	158.41	19.01	80.99
25	195.56	37.10	158.46	18.98	81.02
SEM[4]	0.45	0.27	0.49	0.14	0.14
Linear *p* – value	0.32	0.40	0.40	0.82	0.32
Quadratic *p* – value	0.95	0.96	0.96	0.80	0.28

[1]IME-Ingested metabolizable energy; [2]RE-Retained energy; [3]HP-Heat production; [4]SEM-Standard error of the mean.

Table 10: Sexual maturity of lightweight laying pullets fed with diets containing SC between 7 and 17 weeks of age.

Levels of SC (%)	MA 1st[1] (d)	MA 50%[2] (d)	MA 100%[3] (d)	MW 1st[4] (g)
0	125.83	137.67	149.00	41.68
5	123.83	137.00	147.67	38.78
10	126.50	136.50	146.17	37.24
15	124.67	135.17	148.33	38.41
20	125.83	136.83	147.17	37.50
25	125.83	136.17	150.33	40.32
SEM[5]	0.64	0.35	0.59	0.59
Linear *p* – value	0.47	0.61	0.22	0.38
Quadratic *p* – value	0.67	0.39	0.32	0.13

[1]MA 1st-Mean age at 1st egg; [2]MA 50%-Mean age for 50% of pullets to lay eggs; [3]MA 100%-Mean age for 100% of pullets to lay eggs; [4]MW 1st-Mean weight of 1st egg; [5]SEM-Standard error of the mean.

Table 11: Performance of lightweight laying hens (18 to 35 weeks of age) fed with SC during the growth phase.

Levels of SC (%)	FI[1] (g/pullet/d)	EP[2] (%)	EW[3] (%)	EM[4] (g/pullet/d)	FCEM[5] (g/g)
0	80.18	92.70	54.01	50.11	1.60
5	80.78	92.93	53.96	50.18	1.61
10	80.64	93.33	53.95	50.38	1.60
15	79.96	93.14	53.39	49.69	1.61
20	80.44	93.54	53.68	50.22	1.60
25	82.10	92.91	54.19	50.37	1.63
SEM[6]	0.24	0.13	0.23	0.14	0.01
Linear *p* – value	0.11	0.57	0.87	0.81	0.19
Quadratic *p* – value	0.14	0.41	0.18	0.60	0.41

[1]FI-Feed intake; [2]EP-Egg production; [3]EW-Egg weight; [4]EM-Egg mass; [5]FCEM-Feed conversion/egg mass; [6]SEM-Standard error of the mean.

Table 12: Egg quality of lightweight laying hens (18 to 35 weeks of age) fed with SC during the growth phase.

Levels of SC (%)	Albumin (%)	Egg Yolk (%)	Eggshell (%)	SG[1] (g/cm^3)	HU[2]	ST[3] (mm)
0	67.87	22.43	9.71	1.094	97.26	0.42
5	67.77	22.58	9.65	1.092	97.65	0.42
10	67.84	22.39	9.77	1.092	97.94	0.42
15	67.81	22.47	9.74	1.090	97.91	0.42
20	67.69	22.55	9.76	1.088	96.56	0.43
25	67.79	22.45	9.77	1.090	96.92	0.42
SEM[4]	0.04	0.04	0.02	0.01	0.19	0.01
Linear *p* – value	0.44	0.90	0.22	0.15	0.19	0.13
Quadratic *p* – value	0.82	0.83	0.97	0.12	0.11	0.30

[1]SG-Specific gravity; [2]HU-Haugh Unit; [3]ST-Shell thickness; [4]SEM-Standard error of the mean.

According to Leeson and Summers (1997), egg weight is closely related to the body weight of the pullet and also its sexual maturity. Moreover, both the egg weight and composition are strongly influenced by the laying nutrition, including the concentration of protein, amino acids, and dietary linoleic acid. This research proved that it was unlikely for SC dietary inclusion to exert any residual effect in pullets regarding egg characteristics and egg quality since the pullets presented similar body weights at 17 weeks of age and similar sexual maturity parameters across the treatments. Between 18 and 35 weeks, the laying hens were fed the same experimental diet, which was formulated to meet the nutritional and energy recommendations as per the strain manual (Hy-Line International, 2017). Also, the diets did not exhibit any difference in FI.

Braz et al. (2011) evaluated the residual effect of dietary NDF levels (14.5, 16.5, and 18.5%) in the lightweight and semi-heavy pullets between 7 and 17 weeks of age and found no influence on sexual maturity, performance, and egg quality of laying hens between 18 and 35 weeks of age.

However, Abdallah, Beshara, and Ibrahim (2016) observed that dietary levels of sunflower cake in pullets between 11 and 19 weeks of age showed a reduction in the age of sexual maturity from 137 days (0% SC) to 131.33 (7% SC) and 130.67 days (14% SC) along with an increase in the performance during the laying phase (20 to 40 weeks of age). The use of adequate levels of fiber during the growth phase has shown beneficial results in several breeding situations in many countries. Therefore, when the feed consumption is generally not enough to supply the nutritional demand of pullets, fiber may act positively on the development of the digestive tract of pullets and their feed intake at the beginning of egg production (Tierzucht, 2016).

CONCLUSIONS

The sunflower cake, up to a level of 25%, can be included in the diet of lightweight laying pullets between 7 and 17 weeks of age without any prejudice on the performance during the growth phase and also at the beginning of the production cycle.

AUTHOR CONTRIBUTION

Conceptual idea: Souza, DH; Gomes, TR; Nepomuceno, RC; Freitas, ER; Methodology design: Gomes, TR; Nepomuceno, RC; Freitas, ER; Data collect: Souza, DH; Alencar, AVO; Costa, MKO; Data analysis and interpretation: Freitas, ER; Souza, DH; Gomes, TR; Nepomuceno, RC; Writing and editing: Souza, DH; Gomes, TR; Nepomuceno, RC; Freitas, ER.

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