



Biscuit Residue in the Nutrition of Laying Hens: Effects on Animal Health, Performance and Egg Quality

Roger Rocha Gebert¹, João Henrique dos Reis¹, Bruno Fernando Fortuoso¹, Gabriela Miotto Galli², Marcel Manente Boiago^{1,2}, Diovani Paiano^{1,2}, Aniela Pinto Kempka³, Matheus Dellaméa Baldissera⁴ & Aleksandro Schafer Da Silva^{1,2}

ABSTRACT

Background: Corn and soybean meal are common ingredients used in poultry feed in order to supply the demand for energy and protein, respectively. Also, these ingredients directly influence the final price of the diets, and consequently, the final cost of production. A major problem is associated to the oscillation of these grains in some months of the year. Therefore, there is a need to search for nutritionally and economically viable alternatives to mitigate this problem. The aim was to evaluate whether the addition of biscuit residue could partially replace the use of corn in the feed of laying hens in order to obtain economic viability without impairment on production, egg quality and animal health.

Materials, Methods & Results: The experiment was completely randomized and each repetition was considered one experimental unity. One hundred Hy-line brown 48 week-old chickens were allocated in cages and divided into five groups with four repetitions each, allocating five animals per cage. The group T0 was composed by animals that received the basal diet, i.e. without biscuit residue. The other groups were composed by increasing levels of biscuit residue to substitute corn i.e., group T7 (7% of biscuit residue), T14 (14% of biscuit residue), T21 (21% of biscuit residue) and T28 (28% of biscuit residue). Productive performance of the animals, egg quality, as well as analysis of blood parameters related to lipid metabolism, carbohydrates and proteins of birds were measured at the beginning of the experiment (day 0) and at the end of each cycle (days 21, 42 and 63 of experiment). Among the performance variables, only feed conversion was altered by biscuit residue, i.e., the feed conversion was lower in the groups that received the residue when compared to the control. The replacement of corn by biscuit residue did not affect laying rate ($P > 0.05$). Regarding egg quality, a less intense coloration was observed in the eggs of the treatments that received biscuit residue compared to the control group ($P < 0.05$), but without difference between treatments. Seric levels of uric acid were higher in the treatments that received biscuit residue in the days 21 and 42 compared to the control group, effect not seen in the third cycle (day 63). The cost (\$) of feed reduced as the levels of inclusion of the biscuit residue in the diet were increased, with the control diet costing \$ 0.244/kg, while 28% of biscuit inclusion lower its cost to \$ 0.189/kg (22.3% lower). The final cost to produce a dozen eggs from each cycle was on average of \$ 0.524, 0.415, 0.441, 0.397 and 0.332 for 0, 7, 14, 21 and 28% inclusion, respectively.

Discussion: Residues of biscuit is widely used to feed swine, with positive results as an ingredient to attend the demand for energy. Even though the Brazilian Tables for Poultry and Swine include biscuit residue as a possible ingredient, there is a lack of scientific research in laying hens. These study was verified that the inclusion of biscuit residue (18.936%) is feasible, since it favors the productive performance of the animals, without adverse effects on their health; in addition, the inclusion of biscuit residue reduces dietary costs, as the cost of egg production. Whereas animal feed accounts for a large part of the production cost, the reduction in feed costs without impairment on animal productivity, is a key factor in animal farming. At certain times of the year, food can account for 80% of the production costs and consequently it influences the final price of the product in the market. In our study, we observed a significant reduction on total costs of diets that received biscuit residue, which occurred because a part of the energetic portion of the diet was supplied by the biscuit residue, and not by corn, which influenced the final price.

Keywords: egg quality, biscuit residue, economic viability, nutrition.

DOI: 10.22456/1679-9216.101586

Received: 14 February 2020

Accepted: 15 May 2020

Published: 10 June 2020

¹Programa de Pós-graduação em Zootecnia & ²Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. ³Graduate Program in Food Science and Technology - UDESC, Pinhalzinho, SC. ⁴Department of Physiology and Pharmacology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. CORRESPONDENCE: A.S. Da Silva [aleksandro_ss@yahoo.com.br]. Departamento de Zootecnia - UDESC. Rua Beloni Trombeta Zanini n° 680. CEP 89815-630 Chapecó, SC, Brazil.

INTRODUCTION

Brazilian egg and broiler meat production have representativeness importance, placing the country at the seventh position in the world. The overall Brazilian egg production is destined to the domestic market, since its consumption tends to increase over the years [9]. The egg is a good nutritionally complete with high biological value, exerting several benefits for human health when consumed at necessary quantities [5]. Despite this, it is a proteic food of lower market value, and due to this reason its production must be extremely efficient in order to maintain market competitiveness [16]. In order to achieve high productivity, it is important that have all production variables well aligned, and among them the animal nutrition is the main one, which directly influences the animal production and health.

Corn and soybean meal are common ingredients used in poultry feed in order to supply the demand for energy and protein, respectively [7]. Therefore, there is a need to search for nutritionally and economically viable alternatives to mitigate this problem [22]. According to the literature, biscuit residue provides a large portion of the energetic fraction of the diet, providing also protein and amino acids, as lysine at values very similar to that found in corn. Thus, this residue was studied to understand the mechanism of utilization and exploitation of the nutrients of this by-product [2]. In this sense, Corassa et al. [6] revealed that biscuit residue (15%) could improve the performance of piglets, enhancing body weight and feed consumption, which could be related to better palatability. Therefore, the objective of this study was to evaluate whether the inclusion of biscuit residue in the nutrition of laying hens as a replacement of corn could cause a positive effect on animal production and health, as well as economic viability.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in an experimental poultry farm at the Universidade do Estado de Santa Catarina (UDESC), located in Chapecó city, Southern of Brazil during three production cycles of 21 days each.

Biscuit residue

Biscuit residue was donated and increasing amounts of it were used according to its chemical

composition, in addition to other ingredients, as cited in the Brazilian Table for Poultry and Swine [19].

Experimental design

The experiment was completely randomized and each repetition was considered one experimental unity. One hundred Hy-line brown 48 week-old chickens were allocated in cages and divided into five groups with four repetitions each, allocating 5 animals per cage. The group T0 was composed by animals that received the basal diet, i.e. without biscuit residue. The other groups were composed by increasing levels of biscuit residue to substitute corn i.e., group T7 (7% of biscuit residue), T14 (14% of biscuit residue), T21 (21% of biscuit residue) and T28 (28% of biscuit residue). The diets were formulated according to the specific nutritional requirements as shown in Table 1 [19].

Analysis of biscuit residue and animal diets

Nutritional composition

Analyses of dry matter (DM), mineral matter (MM), ethereal extract (EE), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed following the method described by Silva and Queiroz [21] in biscuit residue and feed samples.

Determination of total phenolic compounds (TPC) and antioxidant activity by the elimination of radicals by DPPH

For extraction, 0.5 g of each sample was dissolved in 50 mL of distilled water. The mixture was placed in an ultrasonic bath (70 W) for 3 h and remained in the dark for 3 more hours. The supernatant was filtered with quantitative filter paper ('Whatman' # 40) and stored in a 100 mL volumetric flask wrapped in aluminum foil. After extraction, extracts were stored in Eppendorf tubes and maintained at - 80 °C until analysis [1,12]. Quantification of TPC was performed by Folin-Ciocalteu colorimetric method modified by Bonoli *et al.* [3]. An aliquot of each diluted sample was mixed with 0.5 mL of Folin-Ciocalteu reagent and was stirred for 1 min. Next, 2 mL of sodium carbonate (20 %) was added into the mixture and stirred for 30 s. After 2 h of incubation, the absorbance was read at 750 nm in relation to the blank. The standard curve was prepared by solutions of gallic acid in methanol. The concentration of TPC was obtained using an

equation derived from the standard curve of gallic acid (expressed in mg of gallic acid equivalent per g of dry sample; mg EGA/g). Data were shown as the mean \pm SD of triplicates. Evaluation of free radical scavenging activity in the extracts was determined by the antioxidant reduction capacity of DPPH radical according to Brand-Williams *et al.* [4]. Five dilutions of each extract were prepared in test tubes and 0.3 mL of each diluted extract was added to 2.7 mL of DPPH radical (40 μ g/mL). After incubation for 1 h in the dark, the absorbance was read at 515 nm Free radical scavenging activity was reported as the IC 50 (μ g/mL), which is defined as the antioxidant concentration required to eliminate 50% of the DPPH present in the test solution. All tests were performed in triplicate and IC 50 values were reported as mean \pm SD of triplicates.

Animal performance

Body weight

Two animals per repetition were individually weighted in the beginning of the experiment and at the end of the productive cycle using a digital balance.

Egg production (%)

Daily, the eggs were collected and counted from each replicate of the treatments. Thus, at the end of each cycle, it was possible to calculate egg production (%).

Egg mass (g/bird/day)

At the end of each productive cycle, the eggs were identified and individually weighted. Based in this data, the egg mass was obtained by the formula: Egg mass = mean weight (g) x percentage of egg production (%).

Feed consumption (g/bird/day)

Experimental diets were weighted and provided to the animals using specific feeders and the amount of feed consumed in each period of 21 days was recorded. These results reflect the average of consumed feed for both cycles.

Feed conversion (kg/kg and kg/dp)

The feed conversion (FC) per kg of egg was obtained by the average of feed intake in the experimental period divided by the egg mass, i.e., the amount of feed consumed to produce one kilogram of egg. To calculate feed conversion per dozen eggs produced, the

quantity of food consumed in the period was divided by quantity of eggs produced multiplied by 12.

Analysis of egg quality

At the end of each cycle, fresh eggs were collected to analyze: egg weight, specific gravity [11]. Shell resistance was evaluated using the equipment TA.XT. PLUS¹, and results were expressed in Kgf. Shell thickness was evaluated after washing and drying during 48 h using a digital pachymeter, and the results were expressed as the average of the three-point measure in the egg (low, middle and high). Also, it was calculated the Haugh unit, yolk index and quantity of albumen in relation to egg weight and bark weight, as well as percentage of albumen and yolk [10]. Brightness (L), intensity of red (a) and intensity of yellow (b) were performed using an electronic colorimeter. Yolk and albumen pH were determined by using a specific equipment (Testo 205)², and the intensity of yolk color was evaluated using a 16-color fan.

Blood collection and serum biochemistry

Blood collection was performed in two animals per repetition, totalizing eight animals per treatment at four moments: the beginning of the experiment (day 0) and at the end of each cycle of 21 days each (days 21, 42 and 63 of the experiment). The animals were contained manually, and the blood was collected from ulnar vein using insulin syringe. The blood was stored into tubes without anticoagulant to obtain serum after centrifugation (850 g during 10 min) and stored at -20°C until analysis.

Serum levels of alkaline phosphatase, triglycerides, cholesterol, uric acid, total protein, glucose and albumin were measured using colorimetric commercial kits (Analisa)³ in the semi-automated (BioPlus 2000)⁴. Levels of globulin were obtained by the formula: total protein - albumin.

Economic analysis

The economic analysis of the inclusion of increasing amounts of biscuit residue in the animals' diet involve the final price per kg of formulated feed, where the average price of raw materials in the month of November 2018 was used. The cost of each feed ingredient was: corn - \$ 0.19/kg, soybean meal - \$ 0.30/kg, wheat bran - \$ 0.17/kg, biscuit residue - \$ 0.038/kg, D-L methionine - \$ 2.75/kg, L-lysine \$ 1.66/kg, threonine - \$ 1.79/kg, tryptophan - \$ 14.32/kg,

calcium - \$ 0.05/kg, soy oil - \$ 0.78/kg, dicalcium phosphate - \$ 0.58/kg, premix - 5.19/kg, common salt - \$ 0.05/kg, and sodium bicarbonate - \$ 0.51/kg. Also, the cost of feed per kg of egg produced (RC/kg) was calculated by the formula: $RC/Kg = FC \text{ per kg} * (\text{diet cost}/100)$ and per dozen produced (RC/Dp), $RC/Dp = FC \text{ per dozen} * (\text{diet cost}/100)$, the final cost was adjusted for three cycles.

Statistical analysis

The data were submitted to descriptive analysis, followed by the evaluation of the normality of the data by the Shapiro Wilk test. Data without normal distribution (biochemical variables) were transformed to log. The data were submitted to analysis of variance (ANOVA) followed by Tukey test. A regression analysis was performed to determine the appropriate inclusion levels of biscuit residue. It was considered significant when $P < 0.05$.

RESULTS

Feed composition

Biscuit residue has high values of crude protein and ethereal extract (Table 1), which becomes adequate to compose the energetic and protein portions in the animal diet. Also, it was evident that increasing amounts of biscuit residue also increased ethereal extract levels.

Total phenolic compounds (TPC) of biscuit residue was 0.50 mg/100 g of DM (dry matter). An increase on TPC was observed in the treatments T7 (0.047), T14 (0.079), T21 (0.102) and T28 (0.20) mg of TPC/100 g of DM when compared to T0 (0.0). Antioxidant activity against the DPPH - IC_{50} radical was not observed in the biscuit residue and in the diets, that is, the analyzed samples showed no IC_{50} .

Performance

Average egg weight, CA/Dp, egg mass, and percentage of egg production did not significant differ between groups ($P > 0.05$; Table 2). Feed conversion per kg of produced egg (CA/Kg) was lower in the groups that received biscuit residue compared to the control group ($P < 0.05$). Considering the values of CA/Kg, the regression analyses indicated that the ideal value of biscuit residue in the diet is 18.936%. No significant difference was observed between groups regarding body weight ($P > 0.05$). However, the weight

gain was higher in the period of 0 to 63 days in the treatment T21 and T28 compared to T0 (Table 3).

Egg quality

The results for egg quality were showed in Table 3. The values of egg color related to red intensity ("a") was lower in the groups T21 and T28 (first to third cycle) compared to T0, while the same result was found in the third cycle for the group T14. The percentage of yolk in relation to egg weight was higher in the groups that received biscuit residue compared to the control group ($P < 0.05$). Calculated gravity, bark resistance, bark thickness, L and b intensity, yolk and albumen pH, Haugh unit, yolk index, bark %, yolk % and albumen % did not differ between groups in three cycles ($P > 0.05$; Table 3).

Serum biochemistry

Results of serum biochemistry were showed in Table 4. Serum levels of uric acid was higher in the groups T21 and T28 compared to the control group in the two first cycles ($P < 0.05$). There was no significant difference between groups regarding serum alkaline phosphatase, glucose, triglycerides, cholesterol, total proteins, albumin and globulins ($P > 0.05$; Table 4).

Economic analysis

Feed cost was considerably lower when biscuit residue was added, as shown in Table 5. Also, a reduction in the cost of feed per kg of egg produced and per dozen of eggs was observed, contributing to lowering production costs, leading to a difference in the cost of feed per dozen produced of \$ 0.50 (T0%) for \$ 0.33 (T28%), which represents a reduction on 22.3 % of the total cost of productions.

DISCUSSION

Residues of biscuit is widely used to feed swine, with positive results as an ingredient to attend the demand for energy [13]. In our study, the productive efficiency of laying hens was positively affected when biscuit residue was used, since a significant reduction on feed conversion per kg of egg was observed. Therefore, its inclusion has applicability on poultry nutrition once it improved feed conversion and did not affect egg production.

The weight gain was higher during all experimental period in laying hens that received 21 and 28% of biscuit residue in the diet compared to the control

Table 1. Composition of the experimental diets: ingredients, and calculated and analyzed compositions.

Ingredient (g/kg of natural matter)	Control	7%	14%	21%	28%
Corn	667.72	589.91	512.11	434.30	356.50
Soybean meal	199.53	195.04	190.55	186.06	181.56
Biscuit residue ¹	0.00	70.00	140.00	210.00	280.00
Wheat bran	0.00	16.28	32.56	48.84	65.12
Methionine	3.10	3.14	3.18	3.22	3.26
Lysine	1.30	1.40	1.50	1.60	1.70
Threonine	0.70	0.83	0.96	1.08	1.21
Tryptophan	0.15	0.14	0.13	0.12	0.11
Limestone	91.00	91.04	91.08	91.12	91.16
Soybean oil	15.01	11.26	7.50	3.75	0.00
Dicalcium phosphate	13.02	12.83	12.65	12.46	12.28
Premix ²	3.00	3.00	3.00	3.00	3.00
Common salt	1.58	1.34	1.09	0.85	0.60
Bicarbonate	3.90	3.80	3.70	3.60	3.50
Nutritional calculated composition					
Crude protein (g/kg)	146.65	147.29	147.94	148.59	149.24
Metabolizable Energy (Kcal/kg)	2894	2897	2901	2905	2909
Calcium (g/kg)	38.94	38.94	38.94	38.93	38.93
Sodium (g/kg)	1.79	1.79	1.79	1.80	1.80
Chlorine (g/kg)	1.64	1.64	1.65	1.65	1.65
Total phosphorus (g/kg)	5.11	5.13	5.15	5.17	5.19
Disponibile phosphorus (g/kg)	3.19	3.19	3.19	3.19	3.19
Lysine (g/kg)	7.35	7.35	7.34	7.34	7.33
Methionine + Cysteine (g/kg)	7.24	7.24	7.24	7.24	7.24
Tryptophan (g/kg)	1.68	1.69	1.69	1.69	1.69
Threonine (g/kg)	5.68	5.68	5.68	5.68	5.69
Nutritional analyzed composition					
Dry matter (%)	89.31	89.63	90.05	90.09	90.51
Crude protein (%)	16.23	16.86	16.21	16.87	16.33
Ethereal extract (%)	4.67	4.58	5.36	5.18	5.75
Mineral matter (%)	13.50	13.05	14.10	14.30	14.23
NDF (%)	7.12	nd	7.56	nd	7.51
ADF (%)	2.53	7.16	2.65	12.46	nd

¹Composition of dry matter: 93.54%, crude protein: 12.58%, ethereal extract: 14.04%, mineral matter: 2.85%, NDF: 4.83%; nd: not-detected. ²Pre-mixture¹ vitaminic and minerals per kg of feed: folic acid (200 mg/kg); pantothen iodine (min 4,33 mg/kg); copper (min 2.66 mg/kg); colina (min 78.12 mg/kg); iron (min 16.7mg/kg); phytase (min 166.66 ftu/kg); iodine (min 400.0 mg/kg); manganese (min 23.3 g/kg); niacin (min 10.0 g/kg); selenium (min 66.7 mg/kg); vitamin A (min 2.333.333 UI kg); vitamin B1 (min 666.7 mg/kg); vitamin B12 (min 3.333 mcg/kg); vitamin B2 (min 1.666 mg/kg); vitamin B6 (min 1000 mg/kg); vitamin D3 (min 733333 UI/kg); vitamin E (min 3.666 UI/kg); vitamin K3 (min 533.33 mg/kg); zinc (min 16.7 g/kg); celestine sulfate (min 3.333 mg/kg).

Table 2. Performance of laying hens during three production cycles (mean) fed with increasing levels of biscuit residue.

Level	PEP	MEW	EW	FC/KG	FC/DZ
0	81.1	62.4	54.3	2.7	2.0
7	91.6	63.1	57.8	2.3	1.8
14	87.6	65.4	57.4	2.2	1.8
21	85.9	64.4	54.7	2.5	1.9
28	88.4	63.4	56.1	2.3	1.7
Analysis of variance considering qualitative treatments					
P	0.280	0.517	0.622	0.031*	0.274
CV	7.471	4.006	6.836	8.174	9.978
Analysis of variance considering quantitative treatments					
Linear	0.427	0.401	0.921	0.072	0.268
Quadratic	0.425	0.262	0.655	0.056	0.442
Coefficient for equations					
A	-	-	-	2.645	-
B	-	-	-	-0.038	-
C	-	-	-	0.001	-
R ²	-	-	-	0.287	-
Derived	-	-	-	18.936	-

* $P < 0.05$ indicates a significant difference between groups; PEP: % of egg production; MEW: mean egg weight; EW: Egg weight; FC/kg: Feed conversion per kilogram of egg; FC/DZ: Feed conversion per dozen eggs.

Table 3. Body weight (days 0, 21, 42 and 63), weight gain (0-21; 0-42; and 0-63) and egg quality (days 21, 42 and 63) of laying hens fed with increasing levels of biscuit residue. (continua...)

Variable	Day	T0	T7	T14	T21	T28	P	CV
Body Weight (kg)	0	2.04	1.94	2.03	1.97	1.92	> 0.05	11.3
	21	2.13	2.02	2.06	2.08	2.08	> 0.05	14.2
	42	2.09	2.08	2.12	2.16	2.15	> 0.05	8.1
	63	2.08	2.07	2.08	2.16	2.17	> 0.05	7.9
Weight gain (kg)	0 - 21	0.09	0.08	0.03	0.11	0.16	> 0.05	10.6
	0 - 42	0.05 ^c	0.14 ^{ab}	0.09 ^{bc}	0.19 ^{ab}	0.23 ^a	< 0.05	6.9
	0 - 63	0.04 ^c	0.13 ^{ab}	0.05 ^{bc}	0.19 ^{ab}	0.25 ^a	< 0.05	6.3
Calculated gravity	21	1.08	1.08	1.08	1.08	1.08	> 0.05	4.6
	42	1.08	1.08	1.08	1.08	1.08	> 0.05	5.1
	63	1.08	1.07	1.08	1.08	1.08	> 0.05	4.8
Bark resistance	21	4346.86	4032.4	4984.11	4186.67	4258.08	> 0.05	10.5
	42	3794.5	7522.86	4764.43	4179.13	3994.82	> 0.05	17.2
	63	4437.0	3497.3	3848.2	38.46.2	3989.7	> 0.05	8.8
Color (sub)	21	7.5 ^a	7.12 ^a	7.28 ^a	5.85 ^b	5.75 ^b	< 0.05*	7.5
	42	6.87 ^a	6.12 ^{ab}	6.37 ^{ab}	6.25 ^{ab}	5.75 ^b	< 0.05*	8.3
	63	7.57 ^a	6.57 ^{ab}	6.00 ^{bc}	6.14 ^{bc}	4.75 ^c	< 0.05*	7.4
L	21	49.75	52.93	47.25	52.85	53.53	> 0.05	9.7
	42	48.08	42.91	42.41	47.69	46.55	> 0.05	10.8
...								

Table 3. Body weight (days 0, 21, 42 and 63), weight gain (0-21; 0-42; and 0-63) and egg quality (days 21, 42 and 63) of laying hens fed with increasing levels of biscuit residue. (...conclusão)

Variable	Day	T0	T7	T14	T21	T28	P	CV
A	63	47.9	51.3	53.7	50.6	52.4	> 0.05	9.5
	21	-3.00 ^b	-4.05 ^{ab}	-3.26 ^b	-5.25 ^a	-5.24 ^a	< 0.05*	8.3
	42	-2.39 ^c	-3.05 ^{bc}	-2.55 ^{bc}	-3.24 ^{ab}	-3.83 ^a	< 0.05*	12.3
B	63	-3.64 ^c	-4.73 ^{bc}	-5.27 ^{ab}	-5.03 ^{ab}	-5.88 ^a	< 0.05*	8.4
	21	38.91	40.46	34.93	38.93	37.66	> 0.05	14.8
	42	35.51	32.55	32.81	36.96	33.37	> 0.05	7.6
Shell thickness	63	39.4	39.99	40.98	40.35	37.7	> 0.05	8.5
	21	0.34	0.33	0.36	0.37	0.35	> 0.05	9.0
	42	0.37	0.35	0.36	0.36	0.35	> 0.05	6.8
(mm)	63	0.35	0.31	0.32	0.34	0.33	> 0.05	8.1
	21	5.99	5.97	6.00	6.00	6.07	> 0.05	8.9
	42	5.93	5.90	5.92	5.92	5.94	> 0.05	9.4
pH yolk	63	5.92	5.94	5.91	6.06	5.92	> 0.05	5.5
	21	8.34	8.58	8.36	8.33	8.36	> 0.05	5.8
	42	8.21	8.23	8.26	8.27	8.43	> 0.05	4.4
pH albumen	63	7.72	8.01	7.95	7.78	7.97	> 0.05	3.9
	21	92.09	88.16	92.13	93.94	90.90	> 0.05	11.3
	42	87.71	90.64	93.9	93.53	90.36	> 0.05	20.4
Haugh unit	63	92.9	94.1	95.6	95.2	94.9	> 0.05	8.4
	21	5.00	4.85	4.91	5.00	4.84	> 0.05	17.3
	42	4.99	4.87	4.90	4.90	4.72	> 0.05	13.4
Yolk index	63	4.76	4.81	4.98	4.87	4.98	> 0.05	10.1
	21	25.34 ^b	27.92 ^{ab}	25.87 ^{ab}	24.53 ^b	28.85 ^a	< 0.05*	14.3
	42	25.20	27.54	26.05	28.68	27.14	> 0.05	6.7
Yolk (%)	63	23.6 ^b	28.3 ^a	28.7 ^a	27.8 ^a	27.7 ^a	< 0.05*	7.1
	21	65.42	62.99	64.79	66.26	62.37	> 0.05	10.3
	42	63.11	62.88	64.31	61.76	63.51	> 0.05	11.3
Albumen (%)	63	66.9	66.6	62.6	63.3	63.3	> 0.05	8.4
	21	9.23	9.07	9.32	9.19	9.37	> 0.05	6.7
	42	9.67	9.56	9.63	9.54	9.33	> 0.05	5.5
Shell (%)	63	9.38	8.57	8.56	8.79	8.89	> 0.05	5.8

*P < 0.05 and different letters (a, b) on the same line show significant difference between groups.

Table 4. Serum biochemistry of laying hens fed with increasing levels of biscuit residue on days 0, 21 and 42 of the experiment. (continua...)

Variable	Day	T0	T7	T14	T21	T28	P	CV
Glucose (mg/dL)	0	203.7	180.8	192.5	209.6	187.4	> 0.05	23.1
	21	239.1	245.6	246.0	236.6	247.1	> 0.05	18.1
	42	211.2	211.2	223.7	206.3	216.5	> 0.05	10.5
	63	249.2	232.0	221.1	208.0	228.2	> 0.05	19.4

Table 4. Serum biochemistry of laying hens fed with increasing levels of biscuit residue on days 0, 21 and 42 of the experiment. (...conclusão)

Variable	Day	T0	T7	T14	T21	T28	P	CV
Alkaline phosphatase (UI/L)	0	532.6	515.8	471.12	482	628.00	> 0.05	30.4
	21	721.1	922.9	709.57	598.5	759.83	> 0.05	30.4
	42	669.0	741.2	549.14	747.25	585.13	> 0.05	29.8
	63	666.7	529.6	588.4	631.2	584.4	> 0.05	24.1
Total proteins (g/dL)	0	5.46	4.83	4.71	4.68	4.38	> 0.05	10.2
	21	4.75	4.45	4.76	4.53	4.75	> 0.05	10.2
	42	4.80	5.55	4.96	4.92	5.25	> 0.05	9.54
	63	4.46	4.50	4.35	3.77	4.47	> 0.05	11.8
Triglycerides (mg/dL)	0	868.0	961.6	973.1	853.0	797.5	> 0.05	23.8
	21	934.2	727.1	695.1	951.6	834.1	> 0.05	27.6
	42	1332.5	1188.1	1128.5	1140.0	1308.6	> 0.05	19.6
	63	885.2	955.5	807.6	882.7	943.0	> 0.05	15.0
Cholesterol (mg/dL)	0	118.2	92.1	87.8	73.1	68.5	> 0.05	31.4
	21	90.6	52.7	54.0	62.8	69.2	> 0.05	35.1
	42	64.6	80.0	69.8	63.3	82.7	> 0.05	20.8
	63	52.2	66.0	52.1	61.8	60.7	> 0.05	27.1
Uric acid (mg/dL)	0	6.85	4.3	4.51	5.00	4.88	> 0.05	10.1
	21	2.97 ^c	3.55 ^{bc}	2.97 ^c	4.2 ^{ab}	4.62 ^a	< 0.05*	8.4
	42	3.8 ^b	5.11 ^a	4.81 ^a	4.65 ^{ab}	5.25 ^a	< 0.05*	7.3
	63	4.06	3.65	2.98	3.94	3.24	> 0.05	14.1
Albumin (g/dL)	0	2.46	2.16	2.21	2.45	2.26	> 0.05	6.7
	21	1.72	1.38	1.55	1.6	1.85	> 0.05	6.8
	42	1.97	1.85	1.98	1.77	1.92	> 0.05	5.1
	63	1.56	1.80	1.73	1.71	1.72	> 0.05	7.2
Globulins (g/dL)	0	3.00	2.66	2.5	2.23	2.11	> 0.05	9.6
	21	3.02	3.06	3.21	2.93	3.12	> 0.05	11.3
	42	2.80	3.70	2.97	3.15	3.32	> 0.05	9.1
	63	2.90	2.70	2.61	2.27	2.74	> 0.05	7.4

*P < 0.05 and different letters (a, b) on the same line show significant difference between groups.

Table 5. Cost (US\$) of the experimental diets and costs related to animal production. (continua...)

Variable	T0	T7	T14	T21	T28
Cost/kg of ration	0.24	0.23	0.21	0.20	0.18
First cycle of production					
RC/kg (\$)	0.62	0.54	0.45	0.52	0.45
RC/Dozen produced (\$)	0.48	0.41	0.37	0.42	0.35
Second cycle of production					
RC/kg (\$)	0.83	0.55	0.66	0.48	0.42
RC/Dozen produced (\$)	0.56	0.41	0.51	0.37	0.31

...

Table 5. Cost (US\$) of the experimental diets and costs related to animal production. (...conclusão)

Variable	T0	T7	T14	T21	T28
Third cycle of production					
RC/kg (\$)	0.43	0.65	0.55	0.52	0.52
RC/Dozen produced (\$)	0.48	0.41	0.40	0.40	0.33
Average of three cycles					
RC/kg (\$)	0.70	0.55	0.55	0.51	0.43
RC/Dozen produced (\$)	0.50	0.41	0.42	0.40	0.33

RC/kg: ration cost per kg of produced egg; RC/Dz: ration cost per dozens of eggs.

group, which can be direct related to increased ethereal extract content in the diets with biscuit residue. The weight gain represents the efficiency in the use of energy from the diet by the animals, i.e., the animals deposited more energy in the corporal tissues [20]; however, it did not cause impairment on egg production. Despite that, we have to consider the possibility that, at in the long term, this effect could bring losses to animal production.

Regarding egg quality, the egg yolk coloration altered with the presence of biscuit residue, i.e., the red intensity lower (lighter yolk). The yolk coloration is dependent on the carotenoid content, and corn has a significant content of carotenoids that provides good yolk coloring [17]. This fact explains the reduction on yellow coloration of the yolk of treatments with crescent levels of biscuit residue in the diet, since the amount of corn decreased. It is important to highlight that one of the ingredients of the biscuit is wheat flour, a major component, which shows a different profile of carotenoids. Thus, in many cases it is necessary the incorporation of pigments in the diet in order to suppress this coloring effect and provide better acceptance by consumers, since them mistakenly associated yolk coloration with higher nutritional egg value [5].

The reason for higher yolk production in relation to egg weight found only in the group T28 can be explained by the elevated EE content in the biscuit residue. According to Peebles *et al.* [14], sources and levels of fat in the diet can affect the egg fatty content. These authors revealed that increased dietary energy decrease the percentage of albumin, and the use of corn oil instead the animal fat (poultry), decreases egg shell percentage.

The serum uric acid levels increased in the groups that received 21 and 28% of biscuit residue compared to the control group, which can be related to an imbalance on levels of amino acids. According to Ross *et al.* [18], the acceptable values for uric acid

in brown Leghorn chickens ranged from 0.27 to 4.93, which are lower when compared to laying hens that received biscuit residue in the first two productive cycles. Higher levels of protein in the diet leads to higher amino acid imbalance, and consequently, lower use of the protein ingested, as well as higher elimination of uric acid in the environment [15]. Thus, it is necessary to maintain the nutritional balance of diets using ingredients of known bromatological composition, especially when alternative ingredients are used, as is the case of biscuit residue.

Whereas animal feed accounts for a large part of the production cost, the reduction in feed costs without impairment on animal productivity, is a key factor in animal farming. At certain times of the year, food can account for 80% of the production costs [8] and consequently it influences the final price of the product in the market. In our study, we observed a significant reduction on total costs of diets that received biscuit residue, which occurred because a part of the energetic portion of the diet was supplied by the biscuit residue, and not by corn, which influenced the final price. Thus, the presence of biscuit residue reduced feed costs without impacting productivity and animal performance. Regarding the control diet (without wholemeal biscuit) there was a reduction in the diet cost of 5.3; 10.7; 17.1; and 22.3% for the diets of the treatments T7, T14, T21 and T28, respectively. The decrease in production cost involved with feed directly reflected in feed costs to produce one kilogram of egg and one dozen of eggs, as well on feed conversion, which was lower in these groups. The potential of cost reduction from the inclusion of biscuit residue in the laying hens diet can be considered a nutritionally viable alternative, as well as economically advantageous. However, more research is needed to prove the use of biscuit residue in other ages and raising conditions, since this practice is empirically carried out in the field.

CONCLUSION

The inclusion of 18.936% of biscuit residue in laying hens is feasible, since it favors the productive performance of these animals without adverse effect on bird health. In addition, the inclusion of biscuit residue reduces dietary costs, and thus the cost of egg production.

MANUFACTURERS

¹Stable Micro Systems Products. Frederick, MD, USA.

²Testo Brasil. Campinas, SP, Brazil.

³Gold Analisa Diagnóstica. Belo Horizonte, MG, Brazil.

⁴Bioplus Produtos para Laboratórios Ltda. Barueri, SP, Brazil.

Acknowledgements. We thank CAPES and CNPq for their technical support. We also thank the Company Casaredo for the donation of the biscuit residue used in this study.

Ethical approval. This study was approved by the Committee on Ethics of Animal Welfare (CEUA), protocol number 4987040418.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

- 1 Bertoletti L.L., Skoronski E., Schittler L. & Kempka A.P. 2018. Extracts of Leaves of *Ficus auriculata* Lour.: antioxidant, antimicrobial and phytotoxic activity. *Agriculturae Conspectus Scientificus*. 83(2): 321-328.
- 2 Boggess M., Stein H.H. & De Rouchey J. 2008. Alternative feed ingredients in swine diets. *National Pork Board*. 56: 1-3.
- 3 Bonoli M., Verardo V., Marconi E. & Caboni M.F. 2004. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *Journal of Agricultural and Food Chemistry*. 52(10): 5195-5200.
- 4 Brand-Williams W., Cuvelier M.E. & Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology* 28(1): 25-30.
- 5 Galli G.M., Da Silva A.S., Ourique A.F., Santos C.G., Lopes L.S., Baldissera M.D. & Stefani L.M. 2018. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. *Research Veterinary Science*. 118(1): 101-106.
- 6 Corassa A., Ballerini K., Komiyama C.M., Santos Pina D., Ballerini N. & Dal Magro T.R. 2013. Farelo de biscoito em rações para leitões na fase inicial. *Comunicata Scientia*. 4(2): 231-237.
- 7 Dahlke F., Ribeiro A.M.L., Kessler A.D.M. & Lima A.R. 2001. Tamanho da partícula do milho e forma física da ração e seus efeitos sobre o desempenho e rendimento de carcaça de frangos de corte. *Revista brasileira de ciência avícola. Brazilian Journal of Poultry Science*. 3(3): 241-248.
- 8 De Souza-Loureiro R.R., Bôa-Viagem Rabello C., Ludke J.V., Moreira Dutra Júnior W., De Souza Guimarães A.A. & Vilar da Silva J.H. 2007. Farelo de tomate (*Lycopersicon esculentum* Mill.) na alimentação de poedeiras comerciais. *Acta Scientiarum: Animal Sciences*. 29(4): 387-394.
- 9 Figueiredo G.O., Bertechini A.G., Fassani E.J., Rodrigues P.B., Brito J.Á. & Castro S.F. 2012. Performance and egg quality of laying hens fed with dietary levels of digestible lysine and threonine. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 64(3): 743-750.
- 10 Freitas E.R., Silva Borges Â., Trevisan M.T.S., Cunha A.L., Melo Braz N., Watanabe P.H. & Nascimento G.A.J. 2013. Extratos etanólicos de manga como antioxidantes na alimentação de poedeiras. *Pesquisa Agropecuária Brasileira*. 48(5): 714-721.
- 11 Freitas E.R., Sakomura N.K., Gonzalez M.M. & Barbosa N.A.A. 2004. Comparação de métodos de determinação da gravidade específica de ovos de poedeiras comerciais. *Pesquisa Agropecuária Brasileira*. 39(4): 509-512.
- 12 Larrauri J.A., Rupérez P. & Sauracalixto F. 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *Journal of Agricultural and Food Chemistry*. 45(11): 1390-1393.
- 13 Oliveira M.D.S. & Lancanova J.A.C. 2001. Efeito da substituição do milho pelo resíduo de biscoito na digestibilidade *in vitro* da matéria seca, da proteína bruta e da energia bruta. *ARS Veterinária*. 17(3): 249-253.
- 14 Peebles E.D., Zumwalt C.D., Doyle S.M., Gerard P.D., Latour M.A., Boyle C.R. & Smith T.W. 2000. Effects of breeder age and dietary fat source and level on broiler hatching egg characteristics. *Poultry Science*. 79(5): 698-704.
- 15 Pereira A.A., Junqueira O.M., Andrade P., Griep Júnior D. & Praes M. 2011. Utilização de rações de poedeiras comerciais formuladas com fitase e níveis de proteína bruta sobre a excreção de fósforo, nitrogênio e cálcio. *ARS Veterinaria*. 26(2): 178-183.

- 16 **Rodrigues K.R.M. & Sala E. 2001.** Atitudes de granjeiros, atacadistas, varejistas e consumidores em relação à qualidade sanitária do ovo de galinha in natura. *Revista de Nutrição*. 14(3): 185-193.
- 17 **Rodriguez-Amaya D.B. 1985.** Os carotenóides como precursores de vitamina A. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*. 19(2): 227-242.
- 18 **Ross J.G., G. Christie, W.G. Halliday & R.M. Jones. 1978.** Haematological and blood chemistry” comparison values” for clinical pathology in poultry. *The Veterinary Record*. 102(1): 29-31.
- 19 **Rostagno H.S., Albino L.F.T., Donzele J.L., Gomes P.C., Oliveira R.F.M., Lopes D.C., Ferreira A.S., Barreto S.L.T. & Euclides R.F. 2017.** *Tabelas Brasileiras Para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais*. Viçosa: Editora UFV, pp.451-488.
- 20 **Sakomura N.K., Basaglia R., Sá-Fortes C.M. & Fernandes J.B.K. 2005.** Modelos para estimar as exigências de energia metabolizável para poedeiras. *Revista Brasileira de Zootecnia*. 34(2): 575-583.
- 21 **Silva D.J. & Queiroz A.C. 2002.** *Análise de alimentos: métodos químicos e biológicos*. Viçosa: UFV 3, 253p.
- 22 **Strada E.S.D.O., Abreu R.D., Oliveira G.J.C.D., Carvalho G.J.L.D., Franca A.S. & Azevedo J.L.M.D. 2005.** Uso de enzimas na alimentação de frangos de corte. *Revista Brasileira de Zootecnia*. 34(6): 2369-2375.