



## ANIMAL SCIENCE

# Inclusion of industrial egg residue in the feed of laying hens to replace limestone: digestibility, productive performance and egg quality

MAURICIO BARRETA, MARCEL M. BOIAGO, ALINE ZAMPAR, BRUNO F. FORTUOSO, ROGER R. GEBERT, EDUARDO ROSCAMP, ROSILENE C. OLIVEIRA, JÉSSICA D. DALIANE, MARINDIA KOLM, GABRIELA M. GALLI & ALEKSANDRO S. DA SILVA

**Abstract:** Our objective was to determine whether inclusion of industrial egg residue (IER) in the diets of laying hens would replace calcitic limestone without interfering with productive efficiency, egg quality or digestibility. In a first study (Experiment I), we used 30% IER in the diets of laying hens and found that the apparent digestibility coefficients were 51.6%, 42.8%, 51.6% and 17.8% for dry matter, crude protein, calcium and phosphorus, respectively. In the second study (Experiment II), we compared a control diet containing calcitic limestone with four diets containing increasing levels of IER, in proportions of 25%, 50%, 75% and 100%. During the first cycle (day 1–28), there was no difference between treatments in terms of productive performance or egg quality. During the second production cycle (day 29–56), we observed less food consumption by birds that ingested the highest levels of IER (100% substitution) than in controls. During the third cycle (day 57–84), we found that the inclusion of IER negatively affected performance, particularly lower production numbers, lower egg mass and higher feed conversion. Finally, during the third cycle, chickens broke and ingested their eggs shortly after laying. We conclude that the use of industrial egg residue cannot replace limestone in the feed of commercial laying hens, because it reduces performance and affects egg quality.

**Key words:** Animal behavior, calcium, eating, phosphorus.

## INTRODUCTION

Industrial egg production is becoming increasingly common worldwide. These processes generate large volumes of waste, most of which is currently used as organic fertilizer or is discarded in landfills. This material consists primarily of shells and residual albumen. The shell is composed primarily of calcium carbonate (Murakami et al. 2007) and the albumen remnants, matrix and shell film contribute to the protein and energetic composition of the residue. Its bromatological composition makes animal waste useful for feed

formulations (Garcia 2010), possibly contributing to the sustainability of production systems as well as lowering production costs; according to Marinho et al. (2010), these costs can reach 70% of the total.

Alternative sources of calcium have been explored; nevertheless, industrial egg residue (IER) has not yet been tested in commercial laying hens in terms of its composition, digestibility or effects on production. Lima (2016) tested eggshell flour as an alternative source of calcium and found a bromatological composition distinct from that of the residue. Our research group conducted a pilot study with

IER in Japanese quail feed and found that the substitution did not affect variables related to egg production or quality (unpublished data); nevertheless, we measured only metabolizable energy and did not evaluate IER nutrient retention. Therefore, in the present study, we determined whether replacement of limestone by IER at various levels would interfere with production, quality and efficiency of egg laying in hens; we also measured various effects on digestibility indexes.

## MATERIALS AND METHODS

### Experimental location and ethical approval

The experiment was carried out in an experimental poultry house in southern Brazil. The study took place in during the Brazilian winter, where temperatures ranged between 1.2 °C and 29 °C, with humidity ranging from 62% to 95%. The project was approved by the committee of animal use in the research of the State University of Santa Catarina, protocol number 5326030418.

### Industrial egg residue (IER)

The IER was obtained from an industrial egg pasteurization plant located in the municipality of Chapecó, SC and was processed (dehydrated and ground) in the animal nutrition laboratory of UDESC. The residue was dehydrated in a forced-air chamber at 55 °C until constant weight was obtained. Subsequently, it was ground in a hammer mill with 1-mm pores. To measure the contents of dry matter, mineral matter, crude protein, ethereal extract, calcium and phosphorus, we used methodologies described by Silva & Queiroz (2006). Crude energy was measured using a calorimetric pump (Model IKA C200®).

Total bacterial counts (TBC), total coliforms (TC), *Salmonella* spp. and *Escherichia coli* were

measured in fresh eggshells and IER. The total bacterial counts were performed according to the Normative Instruction (Number 62 of 26 August 2003) from the Ministry of Agriculture, Livestock and Supply (Brazil). Quantifications of *Salmonella*, *E. coli* and total coliforms were made using 3M TM Petrifilm Plates. The numbers of colony forming unit (CFU) were evaluated after 48 h incubation of samples at controlled temperature.

In the microbiological analysis of fresh (without processing) eggshells, we found  $8.4 \times 10^5$  CFU/g,  $5.4 \times 10^4$  CFU/g and  $2.1 \times 10^4$  CFU/g of TBC, TC and *E. coli*, respectively. In the IER, TC and *E. coli* were not found; however, the TBC count was  $4.6 \times 10^2$  CFU/g. *Salmonella* spp. were not identified in any samples.

### Experiment I: digestibility assay

#### Animals, experimental design and sample collection

For the digestibility assay, we used 30 Hy-Line Brown hens in laying phase at 28 weeks. The hens were housed in metabolic cages (3 hens/cage) made of galvanized steel fitted with trough-type feeders and nipple drinkers. The birds were allocated into groups receiving one of two diets (reference and control), with five replicates for each group.

The experimental diets consisted of a reference diet (Table I), based on corn and soybean meal, formulated according to chemical composition and energy values proposed by Rostagno et al. (2017) and a test diet, composed of 70% of the reference diet and 30% of IER (Sakomura & Rostagno 2007).

The experiment lasted eight days: four days for adaptation to experimental diets and four days for total excreta collection, following a model by Matterson et al. (1965). After the

**Table I. Ingredients and calculated chemical composition of the diet base and reference to be used in the Experiment I of digestibility, following recommendations of the Brazilian table of poultry nutrition (Rostagno et al. 2017).**

Ingredients	%
Corn	65.7
Soybean meal (45%)	21.8
Calcitic limestone	8.90
Soybean oil	1.10
Dicalcium phosphate	1.50
DL-Methionine (98%)	0.20
Sodium chloride (NaCl)	0.50
Vit. and mineral premix*	0.30
TOTAL	100.00
Calculated centesimal composition	
Metabolizable energy (Kcal/Kg)	2.84
Crude protein (%)	15.6
Calcium (%)	3.87
Available phosphorus (%)	0.37
Digestible lysine (%)	0.68
Digestible methionine (%)	0.42
Methionine + dig. cysteine (%)	0.65
Sodium	0.23

\* Product composition (kg): vit. A 7,000,000 IU; vit. D3 4,000,000 IU; vit. E 5000 mg; vit. K 1200 mg; vit. B1 360 mg; vit. B2 2000 mg; vit. B6 700 mg; vit. B12 7000 mcg; niacin 7500 mg; biotin 30 mg; pantothenic acid 6000 mg; folic acid 300 mg; choline 200 mg; iron 1 1000 mg; copper 3000 mg; iodide 204 mg; chloride 360 mg; growth promotion efficiency. Feed 20 mg; coccidiostatics 100 g; antifungals 2000 mg; antioxidants 10 mg; magnesium 50 g; sulfur 40 g; energy and protein vehicle (q.s.p.) 1,000 g.

adaptation period, the excreta were collected, using ferric oxide (2%) in the diet as a marker of the beginning and end of the collection. During the entire experimental period, excreta collection occupied an interval of twelve hours. The excreta were packaged, identified and frozen until the end of collection.

### Analysis of samples and data

Subsequently, the excreta were thawed, homogenized, weighed and dried in a forced-air chamber at 55 °C for 72 hours. After drying, the samples were ground to determine dry matter (DM), mineral matter (MM), crude energy (CE), crude protein (CP), calcium (Ca) and phosphorus (P). We calculated the following parameters: apparent metabolizable energy (MEa); coefficient of apparent dry matter digestibility (CDMDa), coefficient of apparent crude protein digestibility (CCPDa); coefficient of apparent mineral matter digestibility (CMMDa); coefficient of apparent calcium digestibility (CACaD); and coefficient of apparent phosphorus digestibility (CPDa). Calculation of MEa followed the model recommended by Sakomura & Rostagno (2007), using the following formulae:

$$\text{MEa Reference feed} = (\text{ME ing reference feed} - \text{CE exc}) / \text{DM ing};$$

$$\text{MEa Test feed} = (\text{CE ing} - \text{CE exc}) / \text{DM ing};$$

$$\text{MEa food} = \text{MEa ref} + (\text{MEa test} - \text{MEa ref}) / \text{g of ingredients per g of feed.}$$

where MEa = apparent metabolizable energy; CE ing = Crude energy intake; CE exc. = Crude energy excreted; DM ing = Dry matter ingested; MEa ref = apparent metabolizable energy of reference feed; MEa test = apparent metabolizable energy of test feed.

For the determination of the apparent digestibility coefficients of the other nutrients, the model recommended by Costa (2009) was used, according to the following formulae:

$$\text{CDCP}_{\text{basal feed}} = (\text{CP ing} - \text{CP exc}) / \text{CP ing.}$$

$$\text{CDCP}_{\text{ingredient}} = \text{CDCP}_{\text{basal feed}} + (\text{CDCP}_{\text{test feed}} - \text{CDCP}_{\text{basal feed}}) / \% \text{ ing.}$$

where CDCP = coefficient of digestibility of crude protein; CP ing. = crude protein ingested; CP exc. = excreted crude protein; % ing. = Percentage of ingredient inclusion.

Note that the other nutrients were calculated following the same methodology and the %-inclusion values of the ingredients were calculated considering the actual proportions of inclusion.

## **Experiment II: productive performance and egg quality**

### ***Animals and experimental design***

To perform egg quality and performance tests, we used 125 36-week-old commercial hens of the Hy-Line Brown lineage, receiving 16 light hours per day, allocated in metallic cages equipped with individual trough-type feeders and nipples drinkers.

The hens were distributed in a completely randomized design with five treatments and five replicates (cages with five hens each). The treatments consisted of increasing levels of calcium (0, 25, 50, 75 and 100%) to replace limestone, based on the nutritional requirements for calcium. The IER was milled to an average particle size of 1.50 mm, and when the inclusion of IER in the diets began, it replaced fine limestone. The experimental diets (Table II) were formulated based on corn and soybean meal and the nutritional requirements and nutritional composition of the foods were based on Brazilian tables for poultry and swine (Rostagno et al. 2017).

The experiment lasted 84 days, divided into three cycles of 28 days each, during which time hens consumed the same experimental diet. Egg collection was performed daily at noon, where the amounts in each experimental plot were recorded. At the beginning and end of each cycle, the feed was weighed so as to calculate the consumption per plot and other performance variables.

### ***Zootechnical design***

The performance variables evaluated were egg production (%), feed intake (g/bird/day), feed conversion (kg feed/kg of eggs and kg of feed/dozen), egg weight (g) and egg mass (g/bird/day). To obtain the mass of eggs, in the final 3 days of each experimental cycle, all eggs of each repetition were weighed, and the average was multiplied by the percentage of production of the respective repetition.

### ***Levels of blood calcium***

Blood calcium levels were measured at the beginning of the experiment (day 0) and at the end of each experimental cycle (days 28, 56 and 84). Blood was collected from one bird per cage using an insulin syringe. Blood was stored in microtubes without anticoagulant, and centrifuged at 5500 g for 10 min to obtain the serum that was frozen ( $-20\text{ }^{\circ}\text{C}$ ) until analysis. Calcium levels were measured using a specific commercial kit (ANALISA<sup>®</sup>) and read on semi-automatic equipment (BIOPLUS 2000<sup>®</sup>), following the manufacturer's recommendations.

### ***Egg quality***

Egg quality was evaluated on the final of each experimental cycle (Days 28, 56 and 84). Two eggs were collected per experimental unit (cage), totaling 10 eggs per treatment, to perform analyses as follows: specific gravity (Freitas et al. 2004), and shell resistance (kgf) using a texturometer (TAXT Plus<sup>®</sup>) coupled to a probe P75. Haugh units (Haugh 1937), yolk index, yolk color (measured using a DSM YolkFan<sup>™</sup>), brightness (L), intensity of red ( $a^*$ ) and intensity of yellow ( $b^*$ ) were measured using a Minolta colorimeter (CR-400<sup>®</sup>); percentage of yolk, albumen and shell, thickness of the shell, pH of yolk and albumen were measured using

**Table II. Ingredients, diet cost and chemical composition calculated from each experimental diet used in this study with different levels of replacement of industrial egg residue with calcic limestone: Experiment II.**

<b>Ingredient (%)</b>	<b>T0</b>	<b>T25</b>	<b>T50</b>	<b>T75</b>	<b>T100</b>
Corn (7.88%)	65.84	65.72	65.55	65.40	65.25
Soybean meal (44 %)	20.62	20.04	19.46	18.88	18.53
Industrial egg residue <sup>1</sup>	0.00	3.14	6.28	9.42	11.38
Calcitic Limestone	9.36	6.76	4.18	1.60	0.00
Soybean oil	1.68	1.80	1.94	2.06	2.16
Dicalcium phosphate	1.26	1.26	1.27	1.28	1.28
Sodium chloride (NaCl)	0.43	0.43	0.43	0.43	0.43
DL-Methionine	0.30	0.31	0.32	0.33	0.33
L-Lysine	0.13	0.15	0.16	0.18	0.20
L-Threonine	0.07	0.08	0.09	0.10	0.11
L-Tryptophan	0.01	0.01	0.02	0.02	0.03
Vit. and mineral premix <sup>2</sup>	0.30	0.30	0.30	0.30	0.30
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Price R\$ /kg</b>	<b>1.07</b>	<b>1.06</b>	<b>1.05</b>	<b>1.04</b>	<b>1.03</b>
<b>Calculated chemical composition</b>					
CP (%)	14.74	14.74	14.74	14.74	14.74
ME (kcal/kg)	2850	2850	2850	2850	2850
Ca (%)	3.90	3.90	3.90	3.90	3.90
Linoleic acid (%)	2.28	2.28	2.28	2.28	2.28
Available phosphorus (%)	0.32	0.32	0.32	0.32	0.32
Digestible lysine (%)	0.74	0.74	0.74	0.74	0.74
Digestible methionine (%)	0.52	0.52	0.52	0.52	0.52
Digestible Met.+ Cyst. (%)	0.72	0.72	0.72	0.72	0.72
Digestible threonine (%)	0.57	0.57	0.57	0.57	0.57
Digestible tryptophan (%)	0.17	0.17	0.17	0.17	0.17
Sodium (%)	0.18	0.18	0.18	0.18	0.18
Chloride (%)	0.32	0.32	0.32	0.32	0.32

Note: <sup>1</sup> residue industrial egg production, including: 98.4% dry matter, 7.5% crude protein, 31% calcium, 0.16% phosphorus, 1.85% of ethereal extract and the crude energy was 635 Kcal/kg.

<sup>2</sup> Premix composition: folic acid (200 mg/kg); pantothenic acid (min 4.33 mg/kg); copper (min 2.66 mg/kg); choline (min 78.12 mg/kg); iron (min 16.7 mg/kg); phytase (min 166.66 ftu/kg); iodide (min 400 mg/kg); manganese (min 23.3 g/kg); niacin (min 10 g/kg); selenium (min 66.7 mg/kg); vitamin A (min 2,333,333 IU/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 733.333 IU/kg); vitamin E (min 3,666 IU / kg); vitamin K3 (min 533.33 mg/kg); zinc (min 16.7 g/kg); colistin sulfate (min 3.333 mg/kg).

digital pH meter (Testo 205<sup>®</sup>) and mean egg weight. To calculate the shell percentage and shell thickness, eggs were broken and washed to remove excess albumen and dried in forced air for 24 hours at 55 °C. After drying, the shells were weighed and the thickness was measured in the basal and equatorial portions using a

digital pachymeter to later obtain the average of the two measurements.

### **Statistical analysis**

A completely randomized design with five treatments and five replicates each was used.

The treatments consisted of the increasing substitution of the calcitic limestone by IER at 0, 25, 50, 75, and 100%. The obtained data were subjected to analysis of normality of distribution and then analysis of variance. In cases of significant differences, the means were subjected to polynomial regression and compared using the Tukey test (5%). For blood calcium concentrations, we used a design of measures repeated over time and Tukey test.

## RESULTS AND DISCUSSION

The bromatological composition of IER stands is characterized by its levels of calcium, crude protein and ethereal extract (Data in the footer of Table II). When analyzing the calcium levels, we observed that the residue contained approximately 82% of the level of calcium found in calcitic limestone, the main source of calcium used in animal nutrition. However, IER presented a considerable concentration of crude protein that does not exist in limestone. The results obtained for crude protein and ethereal extract are explained by the fact that the residue has a certain quantity of albumen and yolk, structures rich in proteins and fats, respectively.

The metabolizability of crude energy was 33%, generating apparent metabolizable energy of 209.95 kcal (Table III). This low efficiency is explained by the low digestibility of dry matter and crude protein, probably because of the high mineral concentration in the product. Products with lower concentrations of mineral matter such as meat-and-bone meal (48%) and soybean meal have, according to Rostagno et al. (2017) crude protein digestibility of 80.4% and 91%, respectively. The coefficient of digestibility of calcium was lower than that of calcitic limestone. Sá et al. (2004) and Leão (2018) found values of 84.67% and 81.57% for

**Table III. Coefficient of apparent dry matter digestibility (CDMDa), coefficient of apparent digestibility of crude protein (CDCPa), coefficient of apparent digestibility of calcium (CDCa), coefficient of apparent phosphorus digestibility (CPDa) and apparent metabolizable energy (MEa).**

Variable	%
CDMDa	51.66
CDCPa	42.81
CDCa	51.66
CPDa	17.81
MEa (Kcal/Kg)	209.95

calcitic limestone, respectively. Low phosphorus retention was observed, due to the fact that the nutrient is present at low levels in the feed.

Table IV shows that the treatments did not substantially influence the amount of calcium in the blood of the hens, except when comparing the control treatment (T0) with T75, because T0 presented a significantly smaller amount of calcium. We observed that serum calcium levels significantly decreased with advancing age of hens. According to Vieira (2009), for the absorption of this mineral to take place, calcium binding proteins are required, and these show diminished activity with the passage of time (Costa et al. 2010).

No significant differences were observed in terms of performance results (Table 5) or in the qualitative aspects of the eggs (Table VI) during the first and second production cycles, suggesting that total replacement of limestone by IER did not affect the hens. Nevertheless, in the second cycle for the feed consumption variable, there was a linear decrease as the inclusion of the residue increased (Figure 1). This behavior was also observed by Reis et al. (2012), when evaluating the total and partial replacement of calcitic limestone in Japanese quail diets; that is, there was a linear and numerical reduction



**Table IV. Blood calcium levels (mg/dL) of the hens submitted to the treatments at the different collection periods, that is, at the beginning of the experiment (day 0) and at the end of each production cycle (days 28, 56 and 84).**

	Treatment (T)
T0	28.20 B
T25	33.24 AB
T50	30.73 AB
T75	34.39 A
T100	31.20 AB
P-value	0.043
Days of collection (DC)	
0	44.44 A
28	33.04 B
56	25.13 C
84	23.59 C
P- value for T x DC	0.118
P-value for collection	<0.001
CV (%)	16.51

<sup>A, B</sup> Different letters in the same column indicate significant difference by the Tukey test (P<0.05). CV = coefficient of variation.

in feed consumption by the hens. Because IER was included in the diet there was a withdrawal of thick calcitic limestone, and this difference in granulometry may have interfered by increasing the amount of soluble calcium. In these cases, there is rapid absorption in the intestine (Bronner 1998), and consequent elevation of ionic calcium in the blood, inhibiting the hen's appetites (Lobaugh et al. 1981).

In the third production cycle, we observed significant effects of experimental diets on a larger number of variables, both productive (Table V) and qualitative variables (Table VI). Figure 2 shows that, as the limestone was replaced by the IER, the productive indexes became lower, except for feed consumption. In this cycle, there was a decrease in egg production (Table V), occurring as a possible result of

nutrient deficiency during the experimental period (mainly crude protein) because as IER was added, the amount of soybean meal decreased linearly. Another observation suggesting nutritional deficiency was consumption of eggs by hens, because this behavior occurred in all experimental treatments that included IER in feed composition.

In the first productive cycle, there were no alterations in egg quality variables; in the second cycle, two variables differed (Table VI); that is, we observed significant differences in the indices of coloration (L and range) and for pH of the albumen. For coloration evaluated using the colorimetric range (L) we obtained a quadratic equation ( $Y = 6.05 + 0.0389X - 0.000222X^2$ ,  $R^2 = 0.51$ ). For the value of L, we observed linear behavior ( $Y = 58.11 + 0.0218X$ ,  $R^2 = 0.15$ ), suggesting that the increase in dietary IER caused egg yolk to become brighter. Nevertheless, the aspect that showed the greatest relationship with the experimental diets was the pH of the albumen, showing a positive linear regression ( $Y = 8.19 + 0.00256X$ ,  $R^2 = 0.28$ ); that is, as the amount of IER in the diet increased, the pH of the albumen became higher. This observation may be explained by the inverse relationship of food intake and pH of the albumen, because, when eating less food, the bird ingests lower amounts of crude protein, causing lower deposition of amino acids in the albumen and consequent alkalization of egg pH. Cupertino et al. (2009) reported that hens ingesting lower levels of methionine and cystine presented both lower egg production and lower egg weight than those receiving adequate doses of these amino acids.

In the third production cycle, three other egg quality variables differed between treatments (Table VI); that is, the colorimetric range that increased with elevation of substitution levels, as well as shell percentage and shell thickness that decreased with higher IER levels. The linear reduction in both shell thickness and percentage

**Table V.** Production percentage averages (PP), feed intake (FI, g/hen/day), egg mass (EM, g/hen/day) and feed conversion (FC, Kg/dz and kg/kg) of the hens that received the different levels of industrial egg residue in the diet to replace calcitic limestone in the first, second and third production cycles.

Treatment	PP	FI	EM	FC (Kg/dz)	FC (Kg/Kg)
<b>First cycle of production</b>					
T0	91.42	113.96	57.65	1.61	1.92
T25	90.71	112.21	58.66	1.67	1.91
T50	92.14	109.06	54.61	1.67	2.08
T75	92.71	110.04	60.44	1.57	1.82
T100	88.75	107.78	57.02	1.58	1.92
P	0.44	0.28	0.152	0.48	0.19
CV (%)	3.80	4.24	6.41	6.84	8.26
<b>Second cycle of production</b>					
T0	93.39	131.75 A	59.44	1.66	2.17
T25	91.85	127.06 AB	58.87	1.68	2.08
T50	88.92	125.86 AB	56.47	1.67	2.23
T75	93.03	124.77 AB	61.06	1.58	2.04
T100	87.28	119.42 B	56.00	1.64	2.14
P	0.388	0.026*	0.16	0.793	0.13
CV	5.86	5.39	6.89	6.54	7.01
<b>Third cycle of production</b>					
T0	90.41 A	136.16AB	58.60 A	1.73 B	2.45 AB
T25	87.50 AB	144.34A	58.27 A	1.99 A	2.32 B
T50	77.50 BC	132.30AB	50.95 B	2.13 A	2.62 A
T75	76.66 C	135.28AB	52.54 B	1.94 AB	2.66 A
T100	72.16 C	128.53B	46.65 B	2.11 A	2.67 A
P	<0.001*	<0.001*	<0.001*	<0.001*	0.017*
CV	6.82	6.41	6.55	6.37	6.69

**Note:** CV = Coefficient of variation; \* P<0.05 indicates the difference between treatments, the differences being shown by equal letters on the same line (Tukey test).

may be directly related to blood calcium levels in the third production cycle; this is because older hens tended to have lower intestinal calcium absorption capacity, attributable to a reduction in activity of calcium transporter proteins (Costa et al. 2010).

Intake of IER for long-term commercial laying hens is not recommended; however, it may be used occasionally for short periods. This is because over the long term (i.e., 3<sup>rd</sup> cycle) the birds engaged in egg ingestion behavior shortly

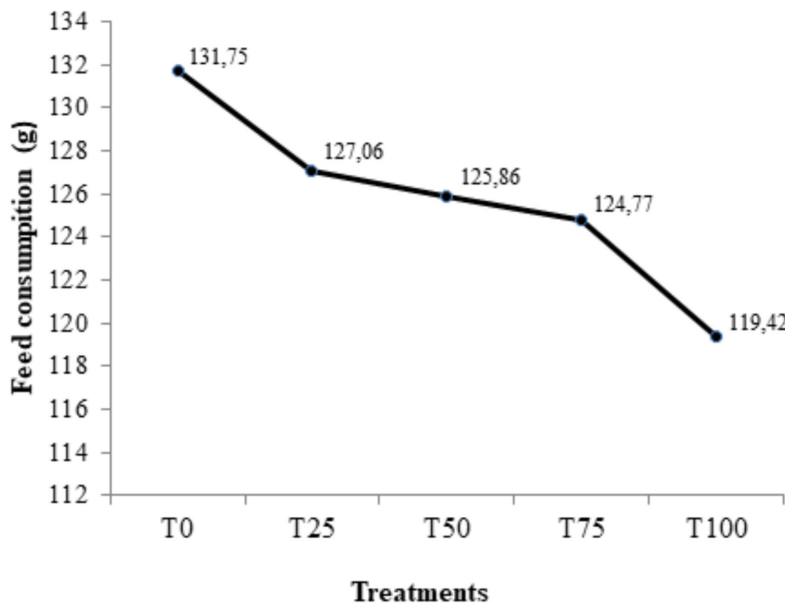
after oviposition; there was also a combined nutritional deficiency of low IER digestibility and a significantly reduced laying rate. Further studies with the cost-effectiveness of this by-product are needed to confirm these results, as well as to associate with other ingredients, which may have other positive effects on laying birds. We believe that laying hens consumed eggs because of calcium deficiency; nevertheless, this hypothesis should also be investigated.



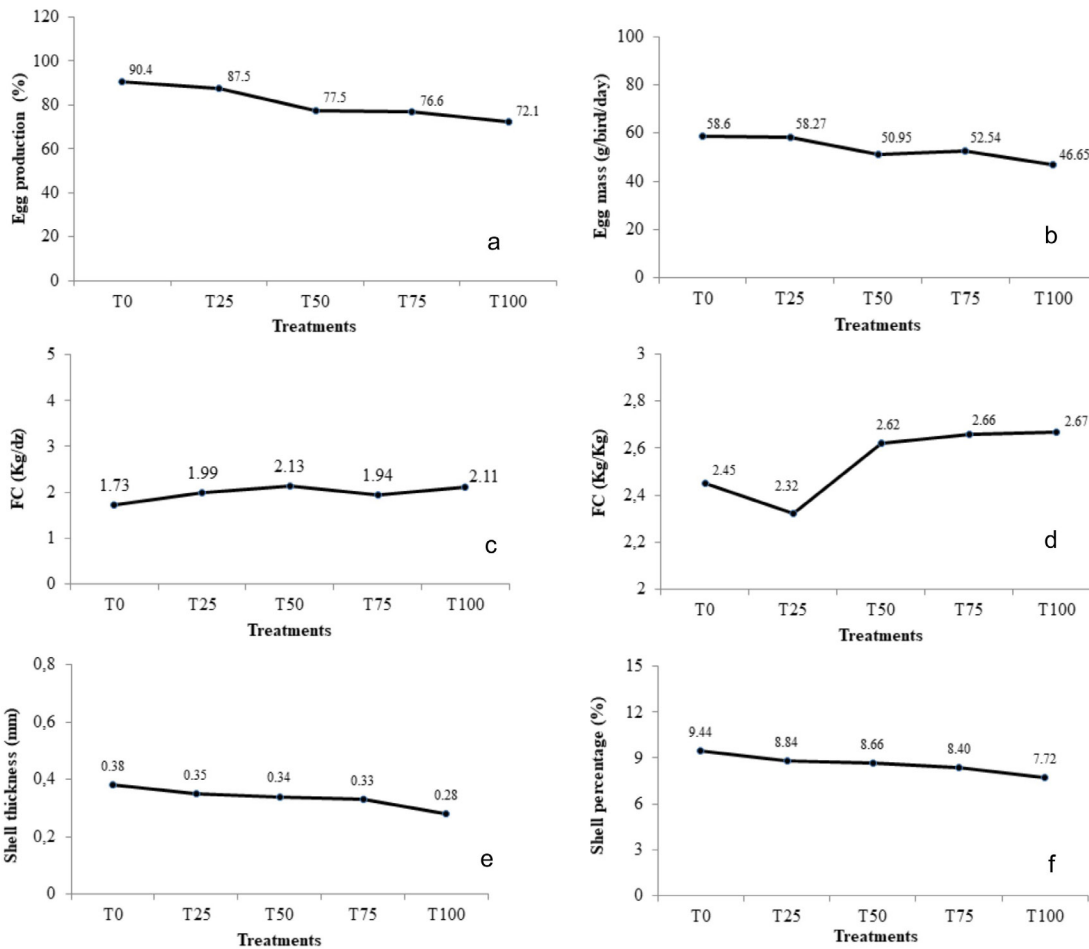
**Table VI. Egg quality: Haugh units (HU), Yolk index (YI), yolk coloring using the colorimetric DSM range (range), luminosity (L), yellow intensity (a\*), yellow intensity (b\*), yolk percentage (YP), Shell percentage (SP), albumen percentage (PA), shell thickness (ST, mm), yolk pH (Y pH), albumen pH (A pH), specific gravity (SG) and shell resistance (SR, Kgf) of eggs from the first, second and third production cycles.**

Variable	Treatment					P	CV (%)
	T0	T25	T50	T75	T100		
<b>1<sup>o</sup> cycle of production</b>							
HU	92.19	93.11	89.34	92.38	91.64	0.203	2.69
YI	0.49	0.5	0.47	0.51	0.53	0.086	6.26
Range	5.40	5.20	5.10	5.00	4.70	0.129	7.87
L	62.30	60.02	61.23	60.38	61.58	0.328	3.00
a*	-6.80	-6.15	-6.63	-7.08	-6.61	0.122	7.79
b*	48.22	46.86	49.11	46.34	47.54	0.534	5.63
YP	26.42	25.87	26.02	25.81	25.85	0.902	4.26
SP	9.84	9.49	9.35	9.58	10.05	0.230	5.18
PA	63.57	64.72	64.66	64.61	64.5	0.742	2.36
ST	0.36	0.35	0.35	0.36	0.37	0.077	4.44
Y pH	6.13	6.14	6.3	6.02	5.94	0.564	5.78
A pH	8.27	8.15	8.3	8.33	8.25	0.721	2.49
SG	1.084	1.092	1.09	1.089	1.077	0.822	2.02
SR	5.411	5.197	5.192	5.841	5.562	0.233	8.97
<b>2<sup>o</sup> cycle of production</b>							
HU	92.29	93.15	88.62	92.68	91.27	0.081	2.77
YI	0.49	0.50	0.47	0.51	0.53	0.080	6.36
Range	6.10 B	6.70 AB	7.70 A	7.60 A	6.90 AB	0.001*	7.95
L	57.45 B	59.51 AB	59.89 AB	58.42 AB	60.91 A	0.032*	2.79
a*	- 4.63	- 4.89	- 5.04	- 5.22	- 5.15	0.595	12.69
b*	45.69	45.74	47.83	44.16	48.06	0.328	7.11
YP	25.58	24.94	26.54	26.64	26.24	0.267	5.17
SP	9.68	9.54	9.61	9.87	9.83	0.679	4.26
PA	64.74	65.43	63.84	63.48	63.92	0.168	2.01
ST	0.37	0.4	0.38	0.41	0.4	0.331	8.43
Y pH	5.99	5.91	5.92	5.92	5.98	0.311	1.44
A pH	8.13 B	8.31 AB	8.42 A	8.23 B	8.50 A	<0.001*	1.97
SG	1.089	1.087	1.087	1.085	1.09	0.252	0.31
SR	4.871	4.974	5.399	5.298	5.283	0.544	11.09
<b>3<sup>o</sup> cycle of production</b>							
HU	97.41	91.10	85.35	95.14	94.45	0.374	7.07
YI	0.48	0.47	0.45	0.48	0.47	0.092	4.06
Range	6.70 B	7.80 A	8.00 A	8.30 A	8.50 A	<b>0.001*</b>	7.41
L	27.78	26.42	26.54	28.17	28.02	0.109	4.57
a*	9.44 A	8.84 AB	8.66 AB	8.40 AB	7.72 B	0.012*	7.46
b*	62.76	64.73	64.80	63.42	64.25	0.122	2.12
YP	0.38A	0.35 AB	0.34 AB	0.33 AB	0.28 B	0.023*	8.88
SP	6.13	5.98	6.00	6.06	6.10	0.757	5.98
PA	7.99	7.99	8.02	8.15	8.28	0.211	2.62
ST	1.084	1.082	1.115	1.077	1.086	0.456	2.07
Y pH	4.171	3.955	3.802	4.015	3.381	0.572	18.66

**Note:** CV = Coefficient of variation; \* P<0.05 indicates the difference between treatments, the differences being shown by equal letters on the same line (Tukey test).



**Figure 1.** Feed consumption (g/bird/day) of hens in the second cycle of production (days 29 to 56 of experiment).



**Figure 2.** Eggs production (a); Egg mass (b); Feed conversion kg/dz (c); Feed conversion kg/kg (d); shell thickness (e) and egg shell percentage (f) third cycle of production (day 57 to 84 of experiment).

## CONCLUSION

The use of industrial egg residue cannot replace limestone in the feed of commercial laying hens, because it reduces performance and affects egg quality. The metabolizability of crude energy was lower with the inclusion of IER owing to the low digestibility of dry matter and crude protein, probably because of the high mineral concentration in the by-product. In the long run, the shell percentage and shell thickness of eggs decreased with higher IER levels, negatively affecting egg quality. IER in the diet also caused laying hens to break and ingest the eggs shortly after laying.

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#### MAURICIO BARRETA<sup>1</sup>

<https://orcid.org/0000-0001-6261-2182>

#### MARCEL M. BOIAGO<sup>1,2</sup>

<https://orcid.org/0000-0002-0950-4577>

#### ALINE ZAMPAR<sup>1,2</sup>

<https://orcid.org/0000-0002-2269-7932>

#### BRUNO F. FORTUOSO<sup>2</sup>

<https://orcid.org/0000-0001-6090-6590>

#### ROGER R. GEBERT<sup>2</sup>

<https://orcid.org/0000-0001-5581-8860>

#### EDUARDO ROSCAMP<sup>2</sup>

<https://orcid.org/0000-0002-4770-7578>

#### ROSILENE C. OLIVEIRA<sup>2</sup>

<https://orcid.org/0000-0003-1147-3076>

#### JÉSSICA D. DALIANE<sup>2</sup>

<https://orcid.org/0000-0002-2830-3930>

#### MARINDIA KOLM<sup>3</sup>

<https://orcid.org/0000-0002-1246-4107>

#### GABRIELA M. GALLI<sup>1</sup>

<https://orcid.org/0000-0001-6734-8659>

#### ALEKSANDRO S. DA SILVA<sup>1,2</sup>

<https://orcid.org/0000-0002-9860-1933>

<sup>1</sup>Programa de Pós-Graduação em Zootecnia, Universidade do Estado de Santa Catarina (UDESC), Rua Beloni Trombeta Zanin, 680 E, Santo Antônio, 89815-630 Chapecó, SC, Brazil

<sup>2</sup>Departamento de Zootecnia, UDESC, Rua Beloni Trombeta Zanin, 680 E, Santo Antônio, 89815-630 Chapecó, SC, Brazil

<sup>3</sup>Cooperativa Central Aurora Alimentos, Rua João Martins, 219 D, São Cristóvão, 89803-040 Chapecó, SC, Brazil

Correspondence to: **Marcel M. Boiago, Aleksandro S. da Silva**  
E-mails: [mmboiago@gmail.com](mailto:mmboiago@gmail.com); [aleksandro\\_ss@yahoo.com.br](mailto:aleksandro_ss@yahoo.com.br)

#### Author contributions

Mauricio Barreta: Master's student in Animal Science, responsible for preparing and executing the project and writing the manuscript. Marcel M. Boiago and Aleksandro S. da Silva: Professors. Responsible for writing the project, monitoring, supervising and guiding the students. Aline Zampar: Responsible for designing and performing statistical analysis of the experiment, in addition to assisting in the discussions of the results. Bruno F. Fortuoso and Roger R. Gebert: They helped with blood collections, performed blood calcium analysis and contributed to writing the manuscript. Eduardo Roscamp, Rosilene C. Oliveira and Jéssica D. Daliane: Scientific initiation students. They helped in the implementation and conduction of the experiment I (digestibility assay), in addition to helping to calculate digestibility values. Maríndia Kolm and Gabriela M. Galli: They helped to prepare the rations and with the daily handling of the birds of the experiment II. They also performed the egg quality analysis.

