



## AGRARIAN SCIENCES

# Performance, metabolic efficiency and egg quality in Japanese quails fed with acidulated soybean soapstock and lecithin for a prolonged period

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**Abstract:** This study aimed to evaluate the use of acidulated soybean soapstock in association with lecithin on productive performance, metabolic efficiency in the utilization of nutrients, and the egg quality of Japanese quails. A total of 192 quails were used, distributed randomly in a 2×2 factorial scheme that included two types of oils, two levels of supplementation (4% and 8%) and two levels of lecithin (1% or 0%). At the end of the six-month experimental period, some double significant interactions were shown between the level of oil and lecithin for the performance variables (egg weight  $p=0.04$ , feed intake  $p<0.01$  and feed conversion rate  $p=0.04$ ). The feed conversion rate also was influenced by a double significant interaction between the type of oil and the level of oil ( $p<0.01$ ). The nutrient digestibility showed that different interactions affected the results. The evaluation of egg quality, was verified that the use of acidulated soybean soapstock did not affect most variables of internal quality. The results showed that it is possible to use 8% acidulated soybean soapstock in combination with 1% lecithin in the diets of Japanese quails for a period of up to six months without a reduction in performance.

**Key words:** Digestibility, emulsifier, energy, oils, poultry.

## INTRODUCTION

Quail production is an activity with global relevance, with China, Japan, Brazil and France the largest global producers of quail eggs (Bertechini 2012). Compared with chickens, quails have reduced space requirements, greater disease resistance and faster sexual maturation (six weeks) making breeding them attractive (Randall & Bolla 2006). In broiler production, laying hens or quails, the fats and oils represent important sources of energy in the diet. The addition of these ingredients confer several benefits in the nutrition of poultry, including a reduction in pulverulence and a decrease

in the segregation of particles, facilitating the improvement of liposoluble and vitamins and contributing fatty acids essential for poultry (Ravindran et al. 2016).

In poultry feeding, degummed soybean oil (DSO) is the main source of fat for energy supplementation. Producers, however, have employed alternative energy sources in the form of by-products of the soybean oil refining process (Peña et al. 2014), such as crude lecithin (LEC) and acidulated soybean soapstock (ASS), compounds that are generally cheaper than DSO.

ASS is obtained from the acidification sludge that results from stage degumming/

neutralization of soybean crude oil (Raber et al. 2009). There is, however, a limitation on the use of this by-product: the higher quantity of free fatty acids (FFA), reducing the digestibility, because of their low proportion of monoglycerides and diglycerides, compounds responsible for 50–70% of fat absorption in poultry (Leeson & Summers 2001).

On the other hand, crude LEC is extracted in the degumming stage and is considered an emulsifier due to its composition, which consists of a mixture of phospholipids, triglycerides, and glycolipids (Mandalawi et al. 2015). Cho et al. (2008) indicated that LEC is can to promote the incorporation of fatty acids in the micelles, facilitating their absorption. Those studies that do exist have been conducted mainly on broilers and laying hens, there is a need to work with quails.

Peña et al. (2014) report that it is unusual to add these ingredients simultaneously to poultry diets. It is known that studying fat supplementation is very complex, due to factors that can influence the poultry results, such as the degree of oil saturation, length chains, and free fatty acid composition as well as the level of fat included in the diet (Ravindran et al. 2016). Therefore, to know the possible interactions among factors that can influence fat digestion, this work also evaluated two oil levels in diets.

As the productive life of Japanese quails is generally around one year, this study aimed was to evaluate over six months the association of LEC with the oils ASS or DSO, as well as the effect of the level of oil included in the diet on the productive performance, nutrient metabolic efficiency and egg quality of Japanese quails.

## MATERIALS AND METHODS

The methods and protocols for this experiment were approved by the Ethics Commission in Animal Experimentation (CEEAA) of the Federal University of Pelotas, Brazil, under protocol number 3772.

### Acidulated soybean soapstock oil, degummed soybean oil and crude lecithin

The 4% of oil supplementation in experimental diets is according to Rostagno et al. (2011). The level of 8% of acidulated soybean soapstock oil was included to potentiate the negative effects of this treatment and to evaluate the capacity of lecithin to ameliorate this condition. The values of acidity in oleic acid for the oils were 64.68% ASS and 0.28% DSO; the acidity index of LEC was 22.89 mg of KOH/g. The unsaturated (U) and saturated (S) quantity found in the oils was 71.22% and 24.55% for ASS and 82.25% and 16.06% for DSO. For lecithin the values of reference were 69% and 21% for unsaturated and saturated, respectively (Mateos et al. 2012).

The quantity of metabolizable energy (ME) considered in the nutrient matrix for the formulation of diets was 7,913 kcal/kg for ASS (Freitas et al. 2005), 8,790 kcal/kg for DSO and 6,036 kcal/kg for LEC (Rostagno et al. 2011).

### Experimental design, animals and diets

The experiment was performed at the Poultry Section of the Laboratory of Teaching and Animal Experimentation (LEEZO) of the Federal University of Pelotas. One hundred and ninety-two Japanese quails (*Coturnix coturnix japonica*), 54 days old, were divided into pairs in 96 metal cages equipped with nipple waterers and manual feeders.

The quails were distributed in a 2×2×2 factorial scheme in a completely randomized design that included two types and levels of

oils and the presence or absence of lecithin. The experimental unit was the cage with two birds, totaling 12 replicates per treatment. The experimental diets were formulated based on corn and soybean meal to meet the nutritional requirements of Japanese quails, according to Rostagno et al. (2011), as shown in Table I.

The quails were fed for 168 days with experimental diets containing either ASS or DSO, either 4% or 8% of oil, and either with or without the supplementation of 1% lecithin, resulting in the following treatments: T1 – diet with 4% ASS; T2 – diet with 4% ASS + 1% LEC; T3 - diet with 8% ASS; T4 - diet with 8% ASS + 1% LEC; T5 - diet with

**Table I. Ingredient composition and calculated chemical analysis of the experimental diets.**

Ingredient (kg)	Diets <sup>9</sup>							
	T1	T2	T3	T4	T5	T6	T7	T8
Maize	42.60	40.68	32.37	30.36	41.50	39.53	30.08	28.14
SBM (45%) <sup>1</sup>	35.70	36.00	37.55	37.66	35.83	36.20	37.98	38.33
Inert <sup>2</sup>	6.35	6.97	10.71	11.36	7.31	7.92	12.62	13.21
PVM <sup>3,4</sup>	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	4.50	4.50	4.40	4.45	4.50	4.50	4.45	4.45
Dicalcium phosphate	1.17	1.17	1.30	1.20	1.17	1.17	1.20	1.20
BHT <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL- metionine	0.37	0.37	0.38	0.38	0.37	0.37	0.38	0.38
L- lysine	0.11	0.11	0.09	0.09	0.12	0.11	0.09	0.09
ASS <sup>6</sup>	4.00	4.00	8.00	8.00	-	-	-	-
DSO <sup>7</sup>	-	-	-	-	4.00	4.00	8.00	8.00
LEC <sup>8</sup>	-	1.00	-	1.00	-	1.00	-	1.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis								
AME <sub>n</sub> (kcal/kg)	2800	2800	2800	2800	2800	2800	2800	2800
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Calcium (%)	3.10	3.10	3.09	3.09	3.10	3.10	3.09	3.09
Av <sup>10</sup> . Phosphorus(%)	0.32	0.33	0.32	0.33	0.32	0.33	0.32	0.33
Av. Met+Cy(%) <sup>11</sup>	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Av.lysine (%)	1.08	1.08	1.10	1.10	1.09	1.09	1.10	1.10
Av. threonine (%)	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19

<sup>1</sup>Soybean meal; <sup>2</sup>Sand; <sup>3</sup>Vitamin and mineral premix <sup>4</sup>Composition per kg of product: vitamin A, 207 000 IU; vitamin D3, 43 200 IU; vitamin E, 540 mg; vitamin K3 51.5mg; vitamin B1, 40 mg; vitamin B2, 120 mg; vitamin B6, 54 mg; vitamin B12, 430 mg; niacin, 840 mg; folic acid, 16.7 mg; pantothenic acid, 204.6 mg; choline, 42 mg; biotin, 1.4 mg; methionine, 11 g; manganese, 1485 mg; zinc, 1535 mg; iron, 1695 mg; copper, 244 mg; iodine, 29 mg; selenium, 3.2 mg; zinc bacitracin, 600 mg; BHT, 700 mg; calcium, 197.5 g; cobalt, 5.1 mg; fluorine (maximum), 400 mg; phosphorus, 50 g; sodium, 36 g. <sup>5</sup>Butylated hydroxytoluene. <sup>6</sup>Acidulated soybean soapstock oil; <sup>7</sup>Degummed soybean oil; <sup>8</sup>Lecithin; <sup>9</sup>Diets: T1: 4% ASS; T2: 4% ASS+1% LEC; T3: 8% ASS; T4: 8% ASS+1% LEC; T5: 4% DSO; T6:4% DSO+1% LEC; T7: 8% DSO; T8: 8% DSO + 1% LEC. <sup>10</sup>Available; <sup>11</sup> methionine + cystine.

4% DSO; T6 - diet with 4% DSO + 1% LEC; T7 - diet with 8% DSO; T8 - diet with 8% DSO + 1% LEC.

The birds had *ad libitum* access to feed and water throughout the whole experiment. The daily lighting program had 17 hours of light followed by 7 hours of darkness.

### Performance and egg quality

All eggs produced were collected every day during six periods of 28 days each for determination of total production and average weight. The egg production percentage was calculated as the number of eggs produced divided by the number of birds in each replicate. The egg mass was obtained as the percentage of eggs produced (bird d<sup>-1</sup>) multiplied by the average egg weight for each replicate, multiplied by 100. Daily feed intake was calculated as the difference between the total feed supplied and the amount leftover at the end of the cycle, divided by the number of days and the number of birds in the replicate. Feed conversion per egg mass was calculated as the daily feed intake divided by the egg mass (g g<sup>-1</sup>). To verify the internal quality of the eggs, all the eggs produced in the last two days of each cycle were collected. The following variables were analyzed: albumen height, albumen percentage, yolk color, yolk percentage, and Haugh unit. A specific rule (FHK trademark) was used to determine the albumen height (mm). Yolk color was obtained using a colorimetric fan (DSM). The yolk percentage was determined by weighing each yolk on a digital scale (Marte, model AS 5500C, accurate to 0.1g), multiplying the respective weight by 100 and dividing it by the egg weight. The albumen percentage was determined by the sum of the weight of yolk and shell from which was subtracted the egg weight. The Haugh unit was obtained from egg weight and albumen height according to the formula: HU = 100 log (H + 7.57 - 1.7 W<sup>0.37</sup>), in which H is albumen height and W is egg weight.

External egg quality was determined according to the following variables: specific gravity (g cm<sup>-3</sup>), shell thickness (mm), and percentage of the shell (%). To obtain the specific gravity, all eggs were placed in a perforated plastic basket and immersed in plastic buckets containing NaCl solutions of concentrations ranging from 1.050 to 1.098 g cm<sup>-3</sup> at intervals of 0.004 g cm<sup>-3</sup>. The eggs were removed from the basket when they floated. The eggshells were identified, washed, and dried at room temperature in order to measure their weight and thickness. To determine the percentage of shells, the shells were individually weighed with an analytical digital scale (UniBloc, AU-220, accurate to 0.1 mg). The shell weight was multiplied by 100 and divided by the egg weight. The shell thickness (mm) was measured at the central ring of each egg using a digital micrometer (Starrett brand) with a precision of 0.01 mm.

### Nutrient digestibility

Nutrient digestibility was evaluated from 138 to 144 days of age. During this period, the birds were housed in metallic cages with a grid floor and a metallic collector tray. An indigestible marker (2% ferric oxide) was added to the feed to establish the beginning and end of the period of the total collection of excreta. Excreta were collected once a day, packaged, identified, and frozen to prevent fermentation until further processing (Sakomura & Rostagno 2007). Excreta samples were collected from five out of the twelve replicates from each treatment.

At the end of the collection period, samples were thawed, weighed and homogenised. The samples were dried in an air forced oven at 60°C for 72 h for further analysis and calculation of nutrient digestibility of dry matter (DM), crude fat (CF) and gross energy (GE). For the

determination of DM, the samples were placed in a dry oven (105°C) for 16 h.

The GE was determined with a calorimeter (Leco AC 500) following the manufacturer's recommendations, using the isoperibol method at 25°C for approximately 8 minutes. For this method, 1 g ( $\pm 0.0001$ ) of the sample was initially weighed in a metal crucible, which was later introduced into a decomposition vessel. The sample was combusted in an O<sub>2</sub>-rich atmosphere (400 psi) and the temperature variation ( $\Delta T$ ) of the system was then determined, with the resulting GE expressed in kcal kg<sup>-1</sup>.

During this period (138 to 144 d of age), samples of the diets were also collected for the analysis of the nutrient digestibility coefficient. The following formula was used: ADC (%) = [(NC - NEx)/NC] × 100, in which: ADC = apparent nutrient digestibility coefficient (%); NC = amount of the nutrient consumed; and NEx = amount of the nutrient excreted. The apparent nutrient digestibility coefficients of DM, CF, and CE were calculated.

### Statistical analysis

The mathematical model used for statistical analysis of the results was:  $Y_{ijk} = \mu + O_i + N_j + E_k + (ONE)_{ijk} + e_{ijk}$ , in which:  $\mu$  = general mean;  $O_i$  = effect of oil type ( $i = 1,2$ );  $N_j$  = effect of levels of oil supplementation ( $j = 1,2$ );  $E_k$  = Effect of Lecithin ( $k = 1,2$ );  $(ONE)_{ijk}$  = effect of interaction ONE at level  $i, j, k$ ; and  $e_{ijk}$  = random error. Data were analysed by ANOVA with O, N and E as fixed factors followed by tukey post hoc test ( $p < 0.05$ ).

The correlations between the egg weight, relative weights of albumen, yolk, and shell, yolk color, Haugh Unit and Shell thickness and productive performance (egg production, feed intake, and egg mass), were evaluated based on the Pearson correlation coefficient ( $p < 0.05$ ). For all the tests the statistical package R (R CORE TEAM 2017) was used.

## RESULTS

### Performance of birds

All performance data can be observed in Table II.

At the end of six months of data collection, a significant double interaction ( $p = 0.04$ ) was verified between the level of oil and the presence or absence of lecithin on egg weight (Figure 1).

Poultry fed with 1% LEC associated with 8% oil produced higher-weight eggs ( $11.87 \pm 0.09$  g) in comparison to birds fed with 8% oil without the emulsifier ( $11.54 \pm 0.14$  g). For poultry fed with diets supplemented with 4% oil, this difference did not appear, therefore, the supplementation of lecithin did not influence egg weight when the diets were supplemented with the lower level of oil.

The evaluation of egg production and egg mass did not show significant interaction or isolated effects of the factors studied, but the feed intake was affected by treatments and a significant double interaction ( $p < 0.01$ ) between the level of oil and LEC was observed (Figure 2).

The highest level of oil used in diets without the association with LEC reduced the feed intake ( $27.88 \pm 0.33$  g/day). When the diets were supplemented with an emulsifier, however, this difference was not identified, whereas only when using the 8% level of oil, the supplementation of the LEC increased the feed intake ( $29.14 \pm 0.29$  g/day).

For the feed conversion rate (FCR)/egg mass, significant double interactions were observed between the level and the type of oil ( $p < 0.01$ ) and the level of oil and lecithin ( $p = 0.04$ ). According to Figure 3a, it is possibly observed that quails fed with a higher level of ASS presented better FCR/egg mass ( $2.65 \pm 0.03$  g/g) compared to poultry that received diets with 4% ASS ( $2.85 \pm 0.05$  g/g) and 8% DSO ( $2.77 \pm 0.04$  g/g). In Figure 3b it can be observed that quails fed with 8% oil without

**Table II.** Influence of acidulated soybean oil (ASS) and lecithin fed to Japanese quails during six productive periods on productive performance (mean  $\pm$  standard error).

Treatments	Egg weight (g)	Egg production (%)	Feed intake (g/day)	Egg mass (g.bird <sup>-1</sup> .d <sup>1</sup> )	FCR (g.g <sup>-1</sup> )	Body weight (g)
T1	12.01 $\pm$ 0.15	88.32 $\pm$ 2.72	30.30 $\pm$ 0.52	10.59 $\pm$ 0.27	2.89 $\pm$ 0.10	174.48 $\pm$ 1.85
T2	11.54 $\pm$ 0.20	91.58 $\pm$ 1.44	28.59 $\pm$ 0.75	10.65 $\pm$ 0.21	2.82 $\pm$ 0.05	175.55 $\pm$ 2.66
T3	11.63 $\pm$ 0.19	90.89 $\pm$ 1.65	27.67 $\pm$ 0.53	10.56 $\pm$ 0.18	2.66 $\pm$ 0.06	176.75 $\pm$ 1.97
T4	12.00 $\pm$ 0.11	91.68 $\pm$ 1.09	28.93 $\pm$ 0.38	11.00 $\pm$ 0.16	2.65 $\pm$ 0.04	179.00 $\pm$ 2.83
T5	11.57 $\pm$ 0.22	90.37 $\pm$ 1.51	28.60 $\pm$ 0.65	10.46 $\pm$ 0.25	2.77 $\pm$ 0.07	179.84 $\pm$ 3.03
T6	11.72 $\pm$ 0.21	91.25 $\pm$ 1.41	28.86 $\pm$ 0.34	10.67 $\pm$ 0.18	2.73 $\pm$ 0.03	176.77 $\pm$ 2.06
T7	11.45 $\pm$ 0.15	93.11 $\pm$ 0.76	28.11 $\pm$ 0.43	10.65 $\pm$ 0.13	2.65 $\pm$ 0.04	181.09 $\pm$ 2.23
T8	11.76 $\pm$ 0.16	88.46 $\pm$ 1.65	29.36 $\pm$ 0.46	10.30 $\pm$ 0.19	2.90 $\pm$ 0.06	180.15 $\pm$ 2.27
Factors						
ASS	11.79 $\pm$ 0.59	90.61 $\pm$ 6.27	28.87 $\pm$ 2.10	10.69 $\pm$ 0.71	2.75 $\pm$ 0.24	176.44 $\pm$ 8.10
DSO	11.62 $\pm$ 0.63	90.79 $\pm$ 4.91	28.73 $\pm$ 1.68	10.52 $\pm$ 0.66	2.76 $\pm$ 0.19	179.46 $\pm$ 8.28
4%	11.70 $\pm$ 0.68	90.37 $\pm$ 6.33	29.08 $\pm$ 2.08	10.59 $\pm$ 0.77	2.80 $\pm$ 0.23	176.65 $\pm$ 8.43
8%	11.71 $\pm$ 0.55	91.03 $\pm$ 4.81	28.51 $\pm$ 1.66	10.62 $\pm$ 0.60	2.71 $\pm$ 0.20	179.24 $\pm$ 8.03
0%	11.66 $\pm$ 0.63	90.67 $\pm$ 6.27	28.66 $\pm$ 2.06	10.56 $\pm$ 0.72	2.74 $\pm$ 0.25	178.03 $\pm$ 8.18
1%	11.75 $\pm$ 0.60	90.74 $\pm$ 4.91	28.93 $\pm$ 1.72	10.65 $\pm$ 0.66	2.77 $\pm$ 0.18	177.86 $\pm$ 8.48
Effects	p value					
Oil	0.17	0.87	0.70	0.20	0.84	0.07
Level	0.98	0.56	0.12	0.78	0.04	0.13
Lecithin	0.47	0.95	0.47	0.53	0.42	0.91
Oil*Level	0.75	0.55	0.12	0.38	<0.01	0.87
Oil*Lecithin	0.26	0.09	0.18	0.27	0.09	0.28
Level*Lecithin	0.04	0.08	<0.01	0.74	0.04	0.62
Oil*Level*Lecithin	0.17	0.50	0.18	0.09	0.16	0.89

\* means the interaction between the factors evaluated.

the inclusion of emulsifier presented better FRC/egg mass (2.65 $\pm$ 0.03 g/g) than the birds that received diets with 4% oil (2.82 $\pm$ 0.05 g/g) or 8% oil with associated LEC (2.77 $\pm$ 0.03 g/g).

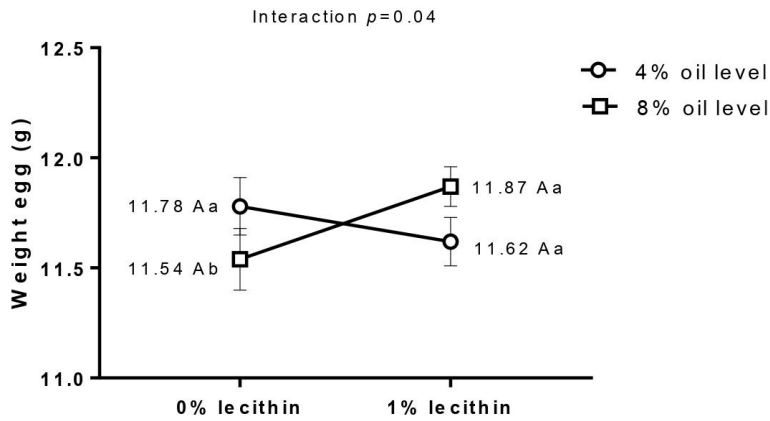
### Nutrient digestibility

According to Figure 4a, there is possible a significant double interaction between the level of oil and the type of oil on the digestibility of dry matter ( $p=0.04$ ). In an effect of the type of oil when the quails were fed with 4 (55.77% $\pm$ 1.42) or 8% (52.95% $\pm$ 1.21) of DSO oil, there was a reduction in the digestibility of this nutrient.

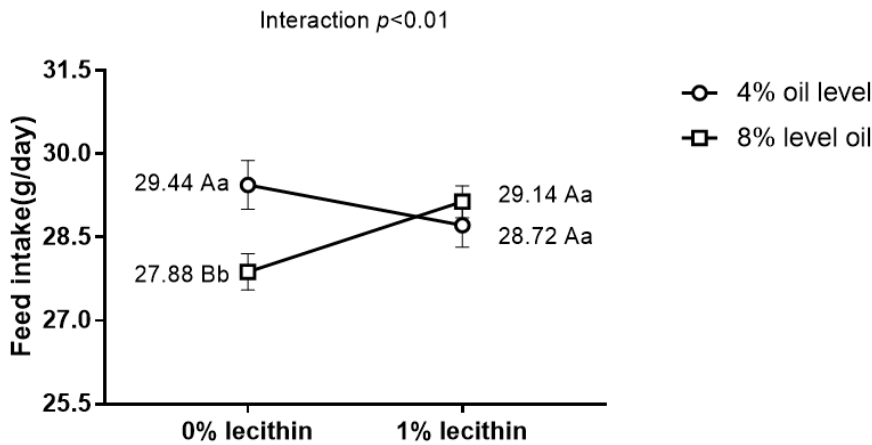
About digestibility, crude fat (CF) was observed to have a significant double interaction

between the level of oil and LEC ( $p=0.03$ ). When added to LEC in diets with 4% oil, there was a reduction in the use of CF (84.51% $\pm$ 3.25) in comparison with diets 4% oil without lecithin (87.81% $\pm$ 2.62) (Figure 4b).

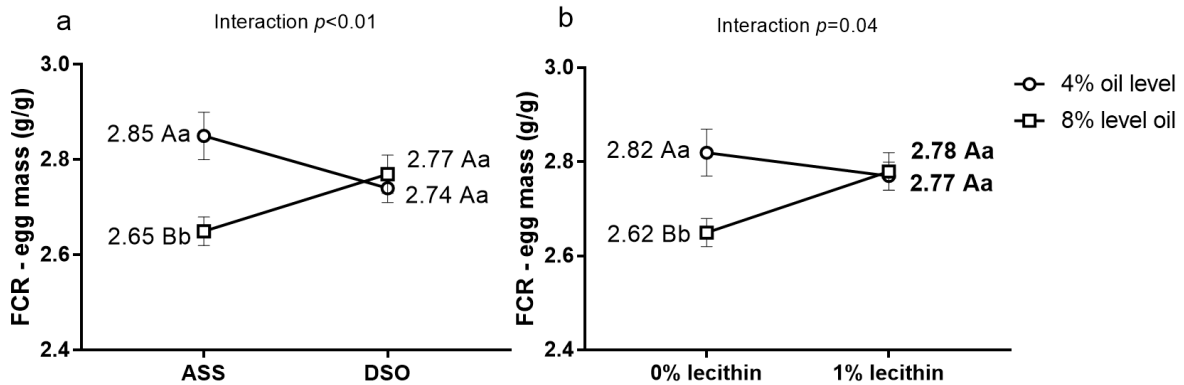
Similarly to what has been verified for the digestibility of dry matter, a significant double interaction between the type and level of oil for crude energy digestibility was observed ( $p=0.01$ , Figure 4c). At the level of 8% of oil inclusion there was no difference in energy digestibility, regardless of the type of oil used. Using 4% of oil, however, the use of ASS reduced this variable (73.66% $\pm$ 1.28) in relation at higher level this oil (75.66% $\pm$ 0.77).



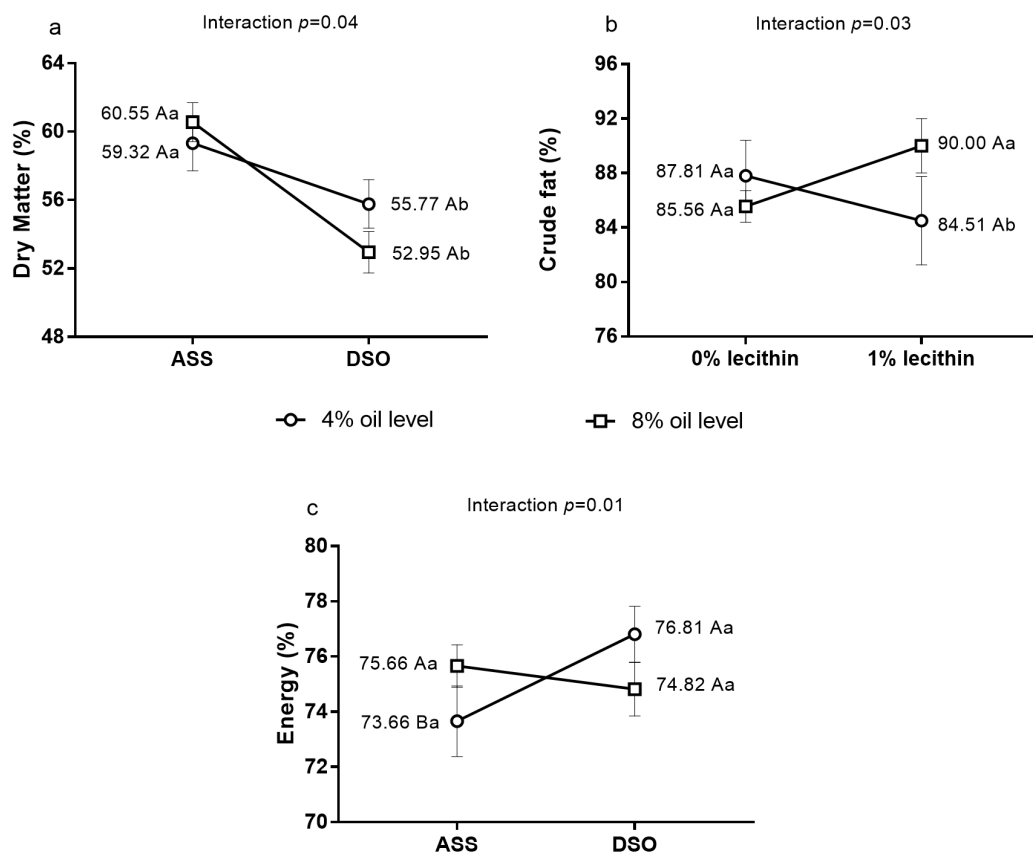
**Figure 1.** Effect of the interaction between level oil and presence or absence of lecithin on weight eggs of Japanese quails. Different lowercase letters are significantly different between presence or absence of lecithin; Different capital letters are significantly different between oil level.



**Figure 2.** Effect of the interaction between level oil and the presence or absence of the lecithin on feed intake of Japanese quails. Different lowercase letters are significantly different between presence or absence of lecithin; Different capital letters are significantly different between oil level.



**Figure 3.** Effect of interaction between type of oil and level oil on FCR (3a) and interaction between presence or absence of lecithin and level oil on FCR (3b) of Japanese quails. (a): Different lowercase letters are significantly different between type of oil; Different capital letters are significantly different between level oil. (b): Different lowercase letters are significantly different between presence or absence of lecithin; Different capital letters are significantly different between level oil.



**Figure 4.** Effect of interaction between level of supplementation and type of oil, oil level and lecithin on digestibility of dry matter (4a), crude fat (4b) and energy (4c). (a) and (c): Different lowercase letters are significantly different between type oil; Different capital letters are significantly different between oil levels; (b): Different lowercase letters are significantly different between presence or absence of the lecithin; Different capital letters are significantly different between oil levels.

**Quality of eggs**

All data of egg quality can be observed in Table III.

The relative weight of the albumen and yolk were not affected by the factors studied. A significant double interaction between the type and level of oil was verified for yolk colour ( $p<0.0001$ ).

Figure 5 possible shows no significant difference in yolk colour between the levels of oil (4 and 8%) when ASS was used. When DSO was used at 8%, however, a significant reduction of the yolk colour ( $4.12\pm0.06$ ) in comparison with DSO included at a level of 4% was observed ( $4.67\pm0.06$ ), and in comparison with ASS included at a level of 8% ( $4.77\pm0.07$ ).

An effect was verified of the type of oil on the variable Haugh unity ( $p=0.03$ ). When the quails were fed with diets containing ASS, they showed greater Haugh unity averages compared to the ones that received diets with DSO.

Evaluating the external quality of eggs, it was verified that the birds fed with DSO produced eggs with a greater relative weight of the shell ( $p=0.01$ ) and shell thickness ( $p=0.02$ ) than quails that received diets with ASS.

**Correlation between egg quality and productive performance**

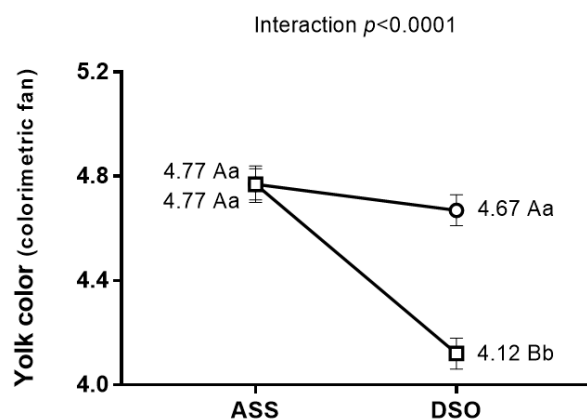
The results show only one significant correlation ( $r=0.869$ ;  $p<0.01$ ) between feed intake and yolk (%).



**Table III. Influence of acidulated soybean oil (ASS) and lecithin fed to Japanese quails during six productive periods on egg quality (mean  $\pm$  standard error).**

Treatments	Relative weights (% of the egg)			Yolk color	Haugh Unit	Shell thickness (mm)
	Albumen	Yolk	Shell			
T1	59.18 $\pm$ 0.47	32.43 $\pm$ 0.40	8.39 $\pm$ 0.11	4.65 $\pm$ 0.09	91.91 $\pm$ 0.44	0.263 $\pm$ 0.004
T2	59.58 $\pm$ 0.56	31.84 $\pm$ 0.49	8.58 $\pm$ 0.12	4.90 $\pm$ 0.10	91.97 $\pm$ 0.41	0.267 $\pm$ 0.005
T3	59.56 $\pm$ 0.38	31.77 $\pm$ 0.41	8.67 $\pm$ 0.08	4.76 $\pm$ 0.08	92.11 $\pm$ 0.30	0.268 $\pm$ 0.002
T4	59.47 $\pm$ 0.32	31.91 $\pm$ 0.33	8.62 $\pm$ 0.10	4.79 $\pm$ 0.11	91.34 $\pm$ 0.40	0.268 $\pm$ 0.003
T5	59.56 $\pm$ 0.43	31.68 $\pm$ 0.47	8.76 $\pm$ 0.11	4.70 $\pm$ 0.09	91.56 $\pm$ 0.50	0.273 $\pm$ 0.005
T6	59.76 $\pm$ 0.41	31.63 $\pm$ 0.31	8.61 $\pm$ 0.15	4.64 $\pm$ 0.09	91.23 $\pm$ 0.43	0.274 $\pm$ 0.005
T7	59.72 $\pm$ 0.37	31.52 $\pm$ 0.36	8.76 $\pm$ 0.12	4.18 $\pm$ 0.08	90.38 $\pm$ 0.69	0.269 $\pm$ 0.004
T8	59.14 $\pm$ 0.26	31.96 $\pm$ 0.25	8.90 $\pm$ 0.10	4.06 $\pm$ 0.08	91.35 $\pm$ 0.43	0.277 $\pm$ 0.005
<b>Factors</b>						
ASS	59.45 $\pm$ 1.49	31.99 $\pm$ 1.39	8.56 $\pm$ 0.35b	4.77 $\pm$ 0.33	91.83 $\pm$ 1.34a	0.266 $\pm$ 0.01b
DSO	59.55 $\pm$ 1.27	31.70 $\pm$ 1.20	8.75 $\pm$ 0.41a	4.39 $\pm$ 0.40	91.13 $\pm$ 1.80b	0.273 $\pm$ 0.01a
4%	59.68 $\pm$ 1.59	31.72 $\pm$ 1.45	8.60 $\pm$ 0.42	4.72 $\pm$ 0.33	91.66 $\pm$ 1.52	0.269 $\pm$ 0.01
8%	59.47 $\pm$ 1.15	31.80 $\pm$ 1.15	8.73 $\pm$ 0.34	4.44 $\pm$ 0.45	91.29 $\pm$ 1.71	0.270 $\pm$ 0.01
0%	59.50 $\pm$ 1.40	31.86 $\pm$ 1.42	8.64 $\pm$ 0.37	4.57 $\pm$ 0.37	91.48 $\pm$ 1.80	0.268 $\pm$ 0.01
1%	59.48 $\pm$ 1.37	31.83 $\pm$ 1.19	8.68 $\pm$ 0.41	4.59 $\pm$ 0.46	91.47 $\pm$ 1.43	0.271 $\pm$ 0.01
<b>Effects</b>						
	<b>p value</b>					
Oil	0.73	0.28	0.01	<0.0001	0.03	0.02
Level	0.87	0.70	0.06	<0.0001	0.26	0.64
Lecithin	0.94	0.95	0.63	0.70	0.96	0.30
Oil*Level	0.53	0.48	0.97	<0.0001	0.62	0.53
Oil*Lecithin	0.54	0.44	0.62	0.08	0.30	0.75
Level*Lecithin	0.28	0.26	0.95	0.29	0.72	0.77
Oil*Level*Lecithin	0.80	0.82	0.08	0.54	0.10	0.39

<sup>a, b</sup> Means followed by different lowercase letters are significantly different. \* means the interaction between the factors evaluated.



**Figure 5.** Effect of interaction between type of oil and level of oil on yolk color of the eggs of Japanese quails. Different lowercase letters are significantly different between type of oil; Different capital letters are significantly different between level oil.

## DISCUSSION

### Performance of birds

The main factors affecting the weight of the egg are related to the methionine quantity, linoleic acid (LA) and fat level in diets (Safaa et al. 2008). Crude lecithins are a complex mixture of various species of phospholipids and do not have a standardised composition, but rather are natural mixtures of several components (Mandalawi et al. 2015). LEC supplementation in diets with 8% oil may have contributed considerably to LA, beyond promoting better absorption of nutrients, since LEC has phospholipids in its composition. They correspond to the 24.4–31.6% of the weight of total lipids of a hen's egg yolk, made up to 69–77% lecithin, 17–24% cephalin, 1.04–2.3% sphingomyelin, 2.2–3% lysolecithin, 2.73% phosphatidylserine and 3.24% diphosphatidylglycerol (Burley & Vadehra 1989).

These results correspond to what Mandalawi et al. (2015), working with laying hens, concluded: that LEC can be used in a diet between 23 and 51 weeks of age, as a strategy for increasing egg size and intensifying the yolk colour.

The benefits of adding fats to poultry diets are well established; its addition may cause various changes in the feeding and digestive behaviour of animals. According to Baião &

Lara (2005), the use of fat in poultry feed can reduce the passage rate of the digest in the gastrointestinal tract. Thus, in the present study, the animals fed without LEC adjusted their feed intake according to the addition of fat in the diets, as expected. When the quails received diets with LEC, however, this difference was no longer detected, demonstrating the effect of the emulsifier on this variable. Mandalawi et al. (2015) affirmed that lecithin could increase the efficiency of egg production and feed consumption through the emulsifying action of phospholipids.

In the evaluation of FCR, it is evident that there is a positive influence of the emulsifier in the diets with the highest level of ASS (a higher quantity of FFA), making it possible to maintain satisfactory indices on the performance of Japanese quails. This improvement caused by the use of LEC results from the emulsifying action of phospholipids, which can be increased through the supplementation of phospholipids in the diet (Liu & Ma 2011) because the process of hydrolysis of fat is affected by molecules like bile salts, phospholipids, lysophospholipids, and proteins present at the interface (Delorme et al. 2011).

Another factor that may influence the use of fat is the ratio of the saturated (S)

and unsaturated (U) fatty acids within the components of diets. Leeson & Summers (2005) have suggested that a U:S ratio of 3:1 is suitable for maintaining satisfactory digestibility for birds of all ages, and that higher ratios of these fatty acids do not necessarily indicate superior results. In studies conducted by Powles et al. (1993) and Wiseman et al. (1990) a significant increase in fat utilisation up to a ratio of 2.08 (U:S) was observed. The use of higher rates had little impact on this response.

The rates (U:S) found in the analysis of the oils were 2.90 and 5.71 for ASS and DSO, respectively, suggesting that the diets containing ASS presented the most adequate U:S rates, and that these results may have influenced the productive responses, improving some variables of animal performance.

### **Nutrient digestibility**

Vieira et al. (2002) and Raber et al. (2009) verified that in broilers the digestibility of dry matter increased with a higher level of oil in diets (8%). Those results can be explained by the additive effect of oil on the digestibility of other nutrients and reduction in the feed passage rate (Sibbald & Kramer 1978). In this study, however, which may have occasioned the increase of digestibility of dry matter in diets with ASS (4 or 8%), there was a larger quantity of corn and soybean meal because of the lowest metabolizable energy considered for ASS in relation to DSO.

The use of LEC affected the response of the digestibility of CF, and numerically, the birds fed the treatments containing 8% of oil with LEC showed greater use of the crude fat in the diets. Mandalawi et al. (2015), assessing different lipid sources and their levels in the diets of laying hens, verified that with the increased supplementation of emulsifier in diets a progressive increase in the digestibility of CF occurred.

When by-products like ASS are considered for use in poultry diets, it is necessary to consider their lower quality in comparison to the original ingredient (in this case DSO). This fact can explain the lower digestibility of the CE for diets with 4% ASS, which can be related to the quantity of FFA found in this oil (68%), suggesting that the lower level of oil ASS in diets did not provide an adequate supply of nutrients.

### **Quality of eggs**

The several stages of oil refining aim to improve the appearance, odor and taste of products by removal various substances, among them the carotenoids (Mandarino et al. 2015). Similarly to this, by going through fewer steps of refining, ASS contains a higher quantity of pigment compounds in comparison to DSO.

With the necessity of keeping diets isoenergetic, there occurred a reduction of corn in diets with larger levels of oil, which decreased the quantities of pigments supplied. Corn is a great source of pigments, and when present in the diets of poultry it confers a higher level of coloration in the yolk of the eggs. The main pigments found in the corn are xanthophylls (lutein, beta-cryptoxanthin and zeaxanthin) and carotenes (beta-carotene, alfa-carotene and beta-zeacarotene) (Janick et al. 1999).

The birds fed with 8% DSO showed a difference in yolk color that can be attributed to the smaller quantity of carotenoids in DSO, in diets with 8% ASS, however, the reduction in the quantity of pigments was compensated by the pigments present in ASS.

The type of oil also influenced the external quality of eggs. The use of products with high quantities of free fatty acids in the feeding of poultry, like ASS, may result in a reaction between the acid group of the FFA and the ionized minerals, such as calcium, forming soap (Bregendahl 2006). If these soaps are insoluble,

they make fatty acids and minerals unavailable for the poultry and therefore may adversely affect the energy value of the fat, the mineral retention and consequently the shell quality of the eggs.

### Correlation between egg quality and productive performance

We found a strong association between feed intake and yolk percentage ( $r=0.86$ ), regardless of the treatments being tested. The quality of eggs is affected by a several of factors, among them the nutritional level of diets (Ledvika et al. 2012). Possibly animals that have higher feed intake will show higher fatty acids deposition in yolk eggs, increasing their percentage in the whole egg.

### CONCLUSIONS

For most variables of the performance and internal egg quality, no negative effects were detected from the use of ASS when used at the higher level (8%), nor when the lecithin was associated with this oil in an 8% supplementation. This demonstrated a positive performance and improved productive responses. ASS reduced the external egg quality, however, by reducing the shell quality. For the variables of nutrient digestibility the emulsifier acted only on crude fat, improving this response when the diets were supplemented with 8% oil. For the other variables of digestibility (dry matter and crude energy) the use of 8% ASS was determinant for the improvement of these parameters.

In general, the results showed that is possible to use 8% of acidulated soapstock in combination with 1% of lecithin in the feeding of Japanese quails for up to six months without a reduction in productive performance. The

different interactions acting on the responses evaluated in the present study, however, make it difficult to clarify their relationships, showing that further studies are needed on the association of the co-products of soybean oil in the diet of quails.

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### **Author contributions**

Project developed by the master's degree student Caroline Bavaresco together with her advisor, Victor Roll. Student responsible for the organization of all experimental stages, data tabulation, interpretation of statistical analysis and writing the article. Suelen Silva and Renata Dias are graduate students, assisted in field activities, laboratory analysis and article writing. Débora Lopes and Eduardo Xavier are co-advisors, assisted in the formulation of experimental diets, Xavier assisted in field activities and both, in monitoring the results obtained and in correcting the article.

