



## Lipid sources and emulsifiers in Japanese quail diets as modulators of performance, egg quality and yolk fatty acid profile

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Using emulsifiers in poultry diets may increase digestibility of alternative lipid sources and enrich poultry egg yolks altering their fatty acid composition. This study aimed to evaluate performance, nutrient digestibility, egg quality, egg quality according to their storage period, and the fatty acid profile of Japanese quail egg yolk (*Coturnix coturnix japonica*). The design was completely randomized, in which 270 female quails were allocated in a 3x2 factorial diet arrangement: three lipid sources (soybean oil, poultry fat, and beef tallow), supplemented or not with an emulsifier, following two nutritional strategies – a diet formulated to meet the nutritional requirements proposed by INRA and a diet formulated with a reduction of 96 Kcal/kg of feed and added emulsifier. The effects of interactions between the lipid sources and the emulsifier were studied, their isolated effects when interactions were absent and the effect of storage time, and their interactions with the factors evaluated for egg quality variables. Feed intake was greater when beef tallow was added to

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the diets, while feed conversion was worse for birds fed diets with soybean oil and the emulsifier. Quails fed the diet with the emulsifier and soybean oil produced better quality eggs represented by the higher Haugh unit. Diets with beef tallow and poultry fat provided a higher percentage of palmitic and unsaturated fatty acids in the quail egg yolk. Alternative lipid sources such as beef tallow and poultry fat can be used as a substitute for soybean oil with added emulsifiers in diets for egg-laying quails without impairing performance and egg quality. The use of emulsifiers with alternative lipid sources to soybean oil can be considered a nutritional strategy in laying quail diets, but the reduction of energy in the diet must be adequate for the species.

**KEY WORDS:** egg yolk / soybean oil / poultry fat / beef tallow

The energy source in animal nutrition is one of the most valuable ingredients regarding financial costs. It can come from vegetable oils and/or animal fat, as slaughtering wastes [Santos *et al.* 2009]. Furthermore, the lipid source contributes to the fatty acid composition of meat and eggs, especially the polyunsaturated ones, resulting in a greater availability of these nutrients for human consumption [Bertipaglia *et al.* 2016].

Incorporation of oils and fats in poultry feed can increase dietary energy concentrations by up to 25%, improving their performance and production efficiency [Zamping *et al.* 2016]. The characteristics of lipid sources vary in their use by poultry due to the raw material quality, the way it is obtained, and the storage period, making it necessary to know its origin, processing, and quality [Dalla Costa *et al.* 2016].

The most widely used lipid source in poultry diets is soybean oil, which is considered a reference [Reda *et al.* 2020]. However, literature reports satisfactory results regarding the use of alternative sources to soybean oil, such as beef tallow [Oliveira *et al.* 2011] and poultry fat [Hu *et al.* 2019] in the diet of broilers and laying hens. On the other hand, in quail production these studies are scarcer.

Aimed at improving fat digestibility [Roy *et al.* 2010], the incorporation of emulsifiers in poultry diets has proven to be an interesting tool. Their use makes fat globules more available and promotes micelle formation. Furthermore, increasing the digestibility of alternative lipid sources can enrich poultry diets altering the fatty acid composition of the egg yolk [Santos *et al.* 2019]. According to Valentim *et al.* [2020], the inclusion of emulsifiers in poultry nutrition may also decrease manufacturing costs. When diets include emulsifiers, there is a reduction in the need for energy supply, which results in reduced costs for oil and fats.

Although the benefits of incorporation of emulsifiers in poultry diets are evident, some research gaps regarding their use in quail nutrition still remain. Thus, this study aimed to evaluate and compare different lipid sources with and without the inclusion of emulsifiers in the Japanese quails' diet, verify performance, nutrient digestibility, egg quality, and egg quality according to storage time, and evaluate the fatty acid profile of egg yolks.

## **Material and methods**

### **Location, animals, treatments, and performance**

The research was submitted and approved by the UFGD's Research Ethics Committee under protocol 16/2020. The experiment was conducted in the laying poultry and quail production sector of the School of Agricultural Sciences at the Universidade Federal da Grande Dourados.

The design was completely randomized with a factorial scheme of treatments, using three lipid sources and two energy levels, 2,800kcal/kg (Basal) and reduced AME 2,704kcal/kg + emulsifier (RE+Emul) totaling six treatments:

- basal feed with soybean oil (2,800 kcal/kg);
- basal feed beef with beef tallow (2,800 kcal/kg);
- basal feed with poultry fat (2,800 kcal/kg);
- reduced feed soybean oil + emulsifier (2,704 kcal/kg + 96 Kcal/kg);
- reduced feed beef tallow + emulsifier (2,704 kcal/kg + 96 Kcal/kg);
- reduced feed poultry fat + emulsifier (2,704 kcal/kg + 96 Kcal/kg).

The energy-reduced diet assumes that the Lipocel emulsifier at the inclusion of 100 g/ton provides 96kcal/kg during the laying phase of the birds.

There were 270 female quails (*Coturnix coturnix japonica*) with an average body weight of 201±7.3g in the laying phase, allocated in nine replications with five quails each. The birds were kept in galvanized wire cages with a 1,250cm<sup>2</sup> area.

The diets were isonutritive, differing only in the reduced energy + emulsifier and inclusion of lipid sources. They were formulated according to INRA [1999] recommendations, based on corn and soybean meal (Tab. 1), and were provided *ad libitum* twice a day in trough-type feeders. Water was supplied at will in *nipple*-type drinkers *ad libitum*.

The experiment lasted 84 days, divided into three experimental periods of 28 days, in which performance variables were evaluated at the end of each period (feed intake corrected for mortality, egg production/bird/day, commercial egg production, average egg weight, egg mass, feed conversion per mass and per dozen eggs, and viability).

### **Digestibility assay**

The digestibility assay was performed using the total excreta collection method at the end of the experimental period. The excreta were collected for four days twice daily, at 08:00 and 17:00, placed in plastic bags, and then taken to the freezer for storage at -18°C. The amount of feed consumed and the total amount of excreta produced were determined at the end of the collection period.

At the time of the analyses the samples were thawed, homogenized, and an aliquot was taken for pre-drying in an oven with forced ventilation at a controlled temperature of 55°C for 72 hours. The samples were ground in a knife mill with a 1mm sieve, stored in plastic bags and marked for laboratory analysis.

**Table 1.** Composition of formulated experimental diets

Food (g/kg)	Soybean	Tallow	Poultry Fat	Soybean + RE+Emul	Tallow + RE+Emul	Poultry Fat + RE+Emul
Corn, 7.88%	498.09	498.09	498.09	498.09	498.09	498.09
Soybean meal, 45%	331.87	331.87	331.87	331.87	331.87	331.87
Limestone	75.44	75.44	75.44	75.44	75.44	75.44
Soybean oil	40.00	-	-	29.07	-	-
Beef tallow	-	40.00	-	-	34.54	-
Poultry fat	-	-	4.00	-	-	29.44
Inert (Kaolin)*	35.00	19.25	33.77	45.81	40.35	45.45
Starch	-	15.76	1.24	-	-	-
Dicalcium Phosphate	10.65	10.65	10.65	10.65	10.65	10.65
Common salt (NaCl)	3.36	3.36	3.36	3.36	3.36	3.36
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine	1.57	1.5	1.57	1.57	1.57	1.57
Mineral premix	1.00	1.000	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00
Emulsifier (E)	-	-	-	0.12	0.12	0.12
Total	100.000	100.000	100.000	100.00	100.00	100.00
Meeting nutritional requirements - Natural Matter						
Nutrients	Soybean	Tallow	Poultry Fat	Soybean + RE+Emul	Tallow + RE+Emul	Poultry Fat + RE+Emul
Calcium, (g/kg)	32.00	32.00	32.00	32.00	32.00	32.00
AME Kcal/Kg (feed)	2800	2800	2800	2704*	2704*	2704*
AME Kcal/Kg (emulsifier)	-	-	-	96	96	96
AME Kcal/Kg (total)	2.800	2.800	2.800	2.800	2.800	2.800
Available phosphorus (g/kg)	2.99	2.99	2.99	2.99	2.99	2.99
Total Phosphorus (g/kg)	5.05	5.05	5.05	5.05	5.05	5.05
Total Lysine (g/kg)	11.63	11.63	11.63	11.63	11.63	11.63
Total Met+Cystine (g/kg)	7.87	7.872	7.87	7.872	7.872	7.87
Total Methionine (g/kg)	4.76	4.76	4.76	4.76	4.76	4.76
Crude protein (g/kg)	192.00	192.00	192.00	192.00	192.00	192.00
Sodium (g/kg)	1.50	1.50	1.50	1.50	1.50	1.50

<sup>1</sup>Vitamin supplement/Kg of diet – Folic acid (Min.) 145.4mg; Pantothenic acid (Min.) 5,931.6mg; Choline (Min.) 121.8g; Niacin (Min.) 12.9g; Selenium (Min.) 480.0mg; retinyl acetate (Min.) 1719.99 mg; cyanocobalamin (Min.) 6,500.0mcg; riboflavin (Min.) 2,000.0mg; pyridoxine (Min.) 250.0mg; cholecalciferol (Min.) 46.25 mg;  $\alpha$ -tocopherol acetate (Min.) 4,500.0 mg; menadione (Min.) 918.0mg. <sup>2</sup>Mineral supplement/Kg – Copper (Min.) 7,000.0mg; Iron (Min.) 50.0g; Iodine (Min.) 1,500.0mg; Manganese (Min.) 67.5g; Zinc (Min.) 45.6g; E-Emulsifier.\*Reduced AME diets in 96 Kcal/kg due to the use of emulsifier which releases 96 Kcal/kg of lipid sources. \*Vehicle for additive inclusion and substitution.

The moisture and nitrogen contents of the excreta and feed were assayed using the methodology applied and described by Silva and Queiroz [2002] – Appendix 1. The gross energy of the diets and excreta was determined using a calorimetric pump (IKA® model PARR 6200). The AME (apparent metabolizable energy) and AMEn (nitrogen-corrected apparent metabolizable energy) values were calculated using the equations described by Matterson *et al.* [1965].

#### Egg quality and storage

Four whole eggs were collected from each experimental unit on the last day of the three 28-day periods, with 216 eggs per period for egg quality analyses.

In the third 28-day period, in order to analyze the influence of storage periods on egg quality, 216 fresh eggs and two sets of 216 eggs collected in two subsequent days and subjected to 7 and 14 days of indoor storage at  $\pm 25^{\circ}\text{C}$  room temperature,

free from direct sunlight in a dry and ventilated place. The eggs were sent for quality analyses at the end of the storage period.

All collected eggs were properly marked and weighed. Their specific gravity was obtained by immersing the eggs in a saline solution with different densities, ranging from 1.065 to 1.100, in increments of 0.005, following the methodology cited by Castelló *et al.* [1989].

Egg yolk colorimetric determination was performed using a Minolta CR-400b colorimeter, duly calibrated with standards pre-established by Bible and Singha [1993]. The colorimeter reading was taken at three random points on the surface of the yolk, maintaining its integrity. The results recorded in the equipment ( $L^*$  and  $b^*$ ), by means of reflectance, indicated yolk color as  $b^*$ , ranging from yellow ( $+b^*$ ) to blue ( $-b^*$ ), in addition to the luminosity ( $L^*$ ) ranging from white ( $L = 100$ ) to black ( $L = 0$ ) – Harder [2007].

Heights and diameters were measured using a caliper and a tripod. The height of the yolk was measured in the central region, while the height of the albumen was measured at 4 mm from the yolk. Only one individual performed this analysis for greater data accuracy.

The yolk was separated from the albumen to be weighed individually on a digital scale. The weight of the albumen was obtained through the difference in the weight of the egg, the yolk, and the shell. The shells were washed to remove albumen and yolk residues and dried in an oven at 65°C for 72 hours. After the shells went through the washing and drying process, the shell thickness was measured using a Digimess 0.001mm precision caliper, with three readings taken at different points in the center-transverse region of the shell. The Haugh Unit (HU) – Haugh [1937] and the yolk index were determined.

#### **Lipid profile of diets and egg yolk**

In order to quantify the fatty acids in the egg yolk, the lipid fraction of four eggs was extracted following the methodology proposed by Bligh *et al.* [1959]. Sixty milligrams (60 mg) of this extracted fraction were weighed and then forwarded to the methylation process, according to Maia and Rodriguez-Amaya [1993], in preparation for the subsequent gas chromatography analysis.

A gas chromatograph equipped with a flame ionization detector, a split/splitless injector, and a fused silica capillary column containing polyethylene glycol as the stationary phase (DB-Wax, 30m x 0.25 mm, J&W Scientific) was used to perform the fatty acid methyl analysis under the following chromatographic conditions: injector temperature of 250°C; detector temperature of 260°C, carrier gas hydrogen at a flow rate of 1.0 mL/min, make-up gas nitrogen at 20mL/min, and injection volume of 1µL.

The retention times were compared with the methyl ester standards (Sigma-Aldrich) to identify the fatty acids. Meanwhile, quantification was performed by area normalization, expressing the result as a percentage of the area of each acid over the total area of fatty acids.

**Statistical analysis**

All data sets were verified for the statistical assumptions of normality of residuals and the homogeneity of subclass variances was verified using the Shapiro-Wilk test and the Levene test, respectively. Subsequently, they were subjected to analysis of variance using the MIXED procedure of the SAS (SAS 9.3). For performance data, the 28-day period effect was included in the linear model as a covariate.

The linear model used was:

$$y_{ijk} = \mu + \lambda(x_{ijk} - x) + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

$y_{ijk}$  – the  $ijk$ -th observation;

$\mu$  – the overall mean;

$\lambda$  – the partial linear regression coefficient between the covariate (X) and the response variable (y);

$x_{ijk}$  – the  $ijk$ -th observation of the covariate;

$x$  – the covariate mean;

$\alpha_i$  – the fixed effect of  $i$ -th level of lipid source;

$\beta_j$  – the fixed effect of  $j$ -th level of emulsifier;

$(\alpha\beta)_{ij}$  – the effect of the interaction of  $i$ -th level of factor  $\alpha$  and the  $j$ -th level of the factor  $\beta$ ;

$e_{ij}$  – the random error associated with  $ijk$ -th observation.

When the effects of interactions between lipid sources and the emulsifier were significant, interactions were then unfolded and measurements were compared by the Tukey test. When there were no significant interactions, the main effects were then evaluated by comparing means using the same test.

Egg storage data were submitted to the analysis of variance through the SAS MIXED procedure (SAS 9.3) using the REPEATED command, in which the evaluation days were considered repeated measures in time. The linear model used was:

$$y_{ijk} = \mu + \alpha_j + \beta_k + \pi_i + (\alpha\beta)_{jk} + (\alpha\pi)_{ji} + (\beta\pi)_{ki} + (\alpha\beta\pi)_{jki} + \varepsilon_{ijk}$$

$y_{ijk}$  – the  $ijk$ -th observation;

$\mu$  – the overall mean;

$\alpha_i$  – the fixed effect of  $i$ -th level of lipid source;

$\beta_k$  – the fixed effect of  $k$ -th level of emulsifier;

$\pi_i$  – the fixed effect associated with the  $j$ -th storage time;

$(\alpha\beta)_{jk}$  – represents the interaction effect of being in level  $j$  of lipid source and level  $k$  of emulsifier;

$(\alpha\pi)_{ji}$  – represents the interaction effect for the  $i$ -th storage time in of lipid source  $j$ ;

$(\beta\pi)_{ki}$  – represents the interaction effect for the  $i$ -th storage time in emulsifier  $k$ ;

$(\alpha\beta\pi)_{jki}$  – represents the three-way interaction effect for the  $i$ -th time in the  $j$ -th level of lipid source, and  $k$ -th level of emulsifier;

$\varepsilon_{ij}$  – the random error connected with  $ijk$ -th observation.

When the effects of interactions between lipid sources and time were significant, they were unfolded and evaluated through regressions using orthogonal polynomials. When evaluating the main effects of lipid sources for egg quality, the Tukey test was used to compare the means. When evaluating the main effects of emulsifier, the F test was used to compare the means. The significance level for all analyses performed was 0.05. All data were analyzed using the Statistical Analysis System (SAS 9.3) statistical package.

## Results and discussion

The percentage of each fatty acid found in the formulated diets and the total fatty acids found according to their treatment were shown in Table 2.

**Table 2.** Percentage of fatty acids in formulated diets

Feed	C16:0 <sup>1</sup>	C16:1 <sup>2</sup>	C18:0 <sup>3</sup>	C18:1w9 <sup>4</sup>	C18:2w6 <sup>5</sup>	C18:3w3 <sup>6</sup>	C20:4w6 <sup>7</sup>	C22:6w3 <sup>8</sup>	Total
Soybean oil	13.95	0.53	7.13	26.67	44.82	2.12	0.13	0.12	95.47
RE soybean oil + Emul.	14.45	4.03	7.41	25.42	42.28	1.18	0.11	0.11	94.99
Beef Tallow	23.16	2.93	27.34	36.67	5.13	0.12	0.11	0.12	95.58
RE beef tallow + Emul.	22.98	2.89	27.67	36.99	5.08	0.11	0.10	0.11	95.93
Poultry Fat	20.56	6.94	8.03	42.54	16.97	0.89	0.10	0.11	96.14
RE poultry fat + Emul.	20.56	6.67	7.98	42.58	16.91	0.85	0.11	0.11	95.77

RE – Reduced Energy Emul. – Emulsifier <sup>1</sup>Palmitic acid; <sup>2</sup>Palmitoleic acid; <sup>3</sup>Stearic acid; <sup>4</sup>Oleic acid (omega-9); <sup>5</sup>Linoleic acid (omega-6); <sup>6</sup>  $\alpha$ -linolenic acid (omega-3); <sup>7</sup>Arachidonic acid; <sup>8</sup>Docosahexaenoic acid.

Regarding performance of Japanese quails, there was an interaction between the lipid source and the energy of the diets for commercial eggs (%) and conversion per dozen eggs (g/g). Isolated effects of the diet source and emulsifier were also identified for the variables: feed intake, egg mass conversion, and viability. The other variables did not present significant effects (Tab. 3).

For commercial eggs, in both the RE+Emul diet and the basal diet, there was no difference between the lipid sources evaluated. Comparing RE+Emul and the basal diet between each lipid source only the diet containing beef tallow showed differences, with RE+Emul showing greater commercial egg production.

There was no difference between the lipid sources evaluated for the feed conversion per dozen eggs variable both in the RE+Emul and basal diets. Comparing RE+Emul and the basal diet between each lipid source, only the diet containing beef tallow showed differences, with RE+Emul showing worse feed conversion.

**Table 3.** Performance of Japanese quails fed on different oil sources with or without emulsifier added

Variable	Emulsifier	Lipid sources (S)			Mean	SEM <sup>1</sup>	P-values		
		Soybean	Tallow	Poultry Fat			Source	Emulsifier <sup>2</sup>	E*S
Intake (g)	RE+Emul	29.937	31.721	29.879	30.524 <sup>A</sup>	0.263	0.0450	0.0131	0.6359
	Basal	29.267	29.816	28.620	29.234 <sup>B</sup>				
	Mean	29.620 <sup>ab</sup>	30.768 <sup>a</sup>	29.250 <sup>b</sup>	29.879				
Egg production (%)	RE+Emul	91.865	89.909	91.400	91.058	0.474	0.8380	0.3405	0.1148
	Basal	91.390	93.710	90.853	91.984				
	Mean	91.126	91.627	91.809	91.532				
Commercial eggs (%)	RE+Emul	91.483	88.475 <sup>y</sup>	92.772	90.910	0.523	0.8308	0.4078	0.0088
	Basal	91.218	93.387 <sup>x</sup>	90.528	91.711				
	Mean	91.351	90.931	91.65	91.283				
Viability (%)	RE+Emul	97.777	91.851	97.777	95.802	0.69	0.0299	0.5762	0.2598
	Basal	96.296	95.555	97.777	96.543				
	Mean	97.037 <sup>ab</sup>	93.703 <sup>b</sup>	97.778 <sup>a</sup>	96.172				
Mass conversion (g/g)	RE+Emul	3.156	3.347	3.067	3.190 <sup>x</sup>	0.029	0.0792	0.0261	0.1105
	Basal	3.111	3.057	3.027	3.065 <sup>y</sup>				
	Mean	3.134	3.202	3.047	3.129				
Dozen conversion (g/g)	RE+Emul	2.813 <sup>a</sup>	3.14 <sup>3b</sup>	2.692 <sup>a</sup>	2.884	0.033	0.006	0.0535	0.0149
	Basal	2.808	2.762 <sup>x</sup>	2.714	2.761				
	Mean	2.81	2.955	2.703	2.822				
Egg mass (g)	RE+Emul	9.435	9.448	9.722	9.535	0.066	0.5232	0.8858	0.112
	Basal	9.463	9.779	9.416	9.553				
	Mean	9.449	9.613	9.569	9.544				
Egg weight (g)	RE+Emul	10.274	10.568	10.396	10.413	0.045	0.2972	0.9066	0.2433
	Basal	10.467	10.441	10.358	10.422				
	Mean	10.370	10.505	10.377	10.417				

x and y letters in the columns differ by the Tukey test at 5%. Different lowercase letters in the row differ by the Tukey test at 5% probability. Main means when significant were compared using the Tukey test (source) or the F test (emulsifier) at 5% probability. <sup>1</sup>Standard error of the mean; <sup>2</sup>Emulsifier+Reduced Energy 96 Kcal/kg.

Quails fed on diets formulated with beef tallow showed the highest feed intake, while birds fed on poultry fat showed the lowest feed intake. Regarding emulsifier additives, the RE+Emul diet showed a higher feed intake than in the basal diet.

The viability of the quails showed the worst results for those receiving the beef tallow diet, while those receiving the poultry fat showed the greatest viability. For the egg mass conversion variable, the RE+Emul diets provided worse feed conversion when compared to the basal diet.

When evaluating the variables obtained in the metabolism test it was found that only the metabolizable energy corrected for nitrogen (AMEn/Kcal/kg) was influenced by adding an emulsifier to the diets and that the diets with RE+Emul obtained lower values of AMEn when compared to the basal diet (Tab. 4).

Table 5 shows the egg quality analysis results. Only the Haugh unit showed an interaction effect between the lipid source and the inclusion of an emulsifier in the diets of laying quails.

In the RE+Emul diets, the quails that received the soybean oil diet showed a higher HU than those that received poultry fat. There was no difference in the lipid sources used in the basal diets. When comparing the basal diets with RE+Emul in each lipid source there was a difference only for poultry fat, with the basal diet showing a higher HU.



**Table 4.** Nitrogen-corrected metabolizable energy (AMEn/kcal/kg), metabolizable coefficient for dry matter (MCDM), metabolizable coefficient for crude protein (MCCP), metabolizable coefficient for mineral matter (MCMM), and metabolizable coefficient for ether extract (MCEE)

Variable	Emulsifier	Source			Mean	SEM <sup>1</sup>	P-values		
		Soybean	Tallow	Poultry Fat			Source	Emulsifier <sup>2</sup>	E*S
AMEn (Kcal/kg) <sup>3</sup>	RE+Emul	2.382	2.400	2.381	2.387 <sup>y</sup>	0.019	0.8509	0.0065	0.9372
	Basal	2.508	2.506	2.474	2.496 <sup>x</sup>				
	Mean	2.427	2.453	2.445	2.441				
MCDM (%) <sup>4</sup>	RE+Emul	81.468	80.344	81.648	81.154	0.575	0.8263	0.4243	0.4521
	Basal	82.669	82.959	80.687	82.105				
	Mean	82.069	81.652	81.168	81.629				
MCCP (%) <sup>5</sup>	RE+Emul	66.564	61.954	65.197	64.572	0.998	0.7993	0.8636	0.4728
	Basal	64.610	65.841	64.331	64.927				
	Mean	65.587	63.897	64.764	64.750				
MCMM (%) <sup>6</sup>	RE+Emul	46.847	49.604	42.526	46.326	0.952	0.4182	0.1496	0.1926
	Basal	49.885	47.982	49.604	49.067				
	Mean	48.366	48.793	45.930	47.696				
MCEE (%) <sup>7</sup>	RE+Emul	74.154	76.902	65.949	72.335	0.542	0.799	0.4802	0.0548
	Basal	73.189	75.573	57.602	68.788				
	Mean	73.672	76.238	61.775	70.872				

x and y letters in the columns differ by the Tukey test at 5%. Different lowercase letters in the row differ by the Tukey test at 5% probability. Main means when significant were compared using the Tukey test (source) or the F test (emulsifier) at 5% probability. <sup>1</sup>Standard error of the mean; <sup>2</sup>Emulsifier+Reduced Energy 96Kcal/kg; <sup>3</sup>Metabolizable energy corrected for nitrogen; <sup>4</sup>Metabolizable coefficient for dry matter; <sup>5</sup>Metabolizable coefficient for crude protein; <sup>6</sup>Metabolizable coefficient for mineral matter; <sup>7</sup>Metabolizable coefficient for ether extract.

The yolk weight showed an isolated effect of the lipid source, with the use of beef tallow in the diet of Japanese quails providing a higher yolk weight than poultry fat (Tab. 5), both with no difference compared to the use of soybean oil.

Regarding the “b\*” coloration, only the effect of the lipid source was observed. The beef tallow in the diets provided a higher value when compared to the color of the egg yolk of birds that received soybean oil, showing a more yellowish color.

The variables are egg weight, albumen weight, specific gravity, L\* value, albumen height, yolk height and diameter, shell weight and thickness, yolk index, yolk, shell, and albumen percentage, and effects of the adopted diets.

When evaluating the effect of storage of quail eggs produced by birds fed diets containing different lipid sources and the emulsifier in three periods (0, 7, and 14 days), no triple interactions were observed between storage time, lipid source, and use of the emulsifier for all variables analyzed (Tab. 6).

The eggs’ specific gravity showed a significant interaction between storage time and the use of emulsifiers, with both the RE+Emul and the basal diets showing a positive quadratic effect. The lowest specific gravity value (1.068) was found in the RE+Emul diet after the 10th day of storage. On the basal diet, eggs showed a reduction in specific gravity until day 15, at which point it stabilized at a gravity of 1.069 (Tab. 6).

Storage time alone influenced yolk weight and albumen weight, yolk percentage, shell percentage, and albumen percentage. The lipid source showed isolated effects for

**Table 5.** Egg quality of quails fed on different lipid sources with and without emulsifier in the diet

Variable	Emulsifier	Source (S)			Mean	SEM <sup>1</sup>	P-values		
		Soybean	Tallow	Poultry fat			Source	Emulsifier <sup>2</sup>	E*S
Egg weight (g)	RE+Emul	10.274	10.624	10.395	10.431	0.039	0.1584	0.9204	0.1453
	Basal	10.465	10.441	10.357	10.421				
	Mean	10.370	10.532	10.376	10.426				
Yolk weight (g)	RE+Emul	3.294	3.390	3.278	3.321	0.018	0.0265	0.3675	0.8805
	Basal	3.288	3.345	3.238	3.290				
	Mean	3.291 <sup>ab</sup>	3.368 <sup>a</sup>	3.258 <sup>b</sup>	3.330				
Albumen weight (g)	RE+Emul	6.15	6.426	6.556	6.377	0.043	0.1129	0.6386	0.1617
	Basal	6.393	6.462	6.397	6.418				
	Mean	6.272	6.444	6.476	6.397				
Gravity	RE+Emul	1.074	1.072	1.072	1.073	0.000	0.0621	0.4590	0.1647
	Basal	1.073	1.073	1.073	1.073				
	Mean	1.074	1.072	1.072	1.073				
L* <sup>2</sup> (yolk)	RE+Emul	55.444	56.024	55.566	55.678	0.124	0.2458	0.1532	0.8798
	Basal	55.263	55.571	55.123	55.319				
	Mean	55.353	55.798	55.344	55.498				
b* <sup>4</sup> (yolk)	RE+Emul	36.101	36.694	35.321	36.039	0.245	0.0196	0.9270	0.1848
	Basal	34.84	37.229	35.919	35.996				
	Mean	b35.47	a36.96	35.62 <sup>ab</sup>	36.01				
Albumen height (mm)	RE+Emul	4.054	3.841	3.726	3.873	0.039	0.2529	0.7536	0.0633
	Basal	3.851	3.933	3.900	3.895				
	Mean	3.952	3.887	3.813	3.884				
Yolk height (mm)	RE+Emul	10.258	10.423	10.125	10.269	0.037	0.1609	0.0501	0.3166
	Basal	10.118	10.151	10.117	10.129				
	Mean	10.188	10.287	10.121	10.199				
Yolk diameter (mm)	RE+Emul	22.526	22.993	22.743	22.754	0.072	0.3577	0.663	0.2948
	Basal	22.663	22.645	22.78	22.696				
	Mean	22.595	22.819	22.762	22.725				
Shell weight (g)	RE+Emul	0.886	0.888	0.874	0.883	0.004	0.2583	0.8435	0.805
	Basal	0.878	0.893	0.872	0.881				
	Mean	0.882	0.890	0.873	0.882				
Shell thickness (mm)	RE+Emul	0.222	0.217	0.217	0.219	0.001	0.2846	0.5823	0.9183
	Basal	0.223	0.220	0.218	0.22				
	Mean	0.222	0.218	0.218	0.219				
Haugh unit	RE+Emul	87.84 <sup>a</sup>	86.01 <sup>ab</sup>	84.05 <sup>xb</sup>	85.96	0.285	0.0292	0.3301	0.0025
	Basal	86.42	86.04	86.93 <sup>x</sup>	86.46				
	Mean	87.13	86.02	89.49	86.21				
Yolk index	RE+Emul	0.456	0.454	0.446	0.452	0.001	0.353	0.0957	0.6889
	Basal	0.448	0.445	0.444	0.446				
	Mean	0.452	0.449	0.445	0.449				
% Yolk	RE+Emul	31.855	30.941	29.649	30.815	0.26	0.4249	0.0839	0.0925
	Basal	29.64	29.915	30.202	29.919				
	Mean	30.748	30.428	29.925	30.367				
% Shell	RE+Emul	8.367	8.442	8.426	8.479	0.029	0.0939	0.6653	0.0757
	Basal	8.514	8.645	8.557	8.504				
	Mean	8.579	8.462	8.434	8.491				
% Albumen	RE+Emul	59.900	61.092	62.938	61.300	0.304	0.1465	0.3971	0.0943
	Basal	61.847	61.885	61.702	61.811				
	Mean	62.320	61.474	60.874	61.555				

x, y letters in the columns differ by the Tukey test at 5%. Different lowercase letters in the row differ by the Tukey test at 5% probability. Main means when significant were compared using the Tukey test (source) or the F test (emulsifier) at 5% probability. S; 2Emulsifier + Reduced Energy 96 Kcal/kg. <sup>1</sup>Standard error of the mean; <sup>2</sup>Ranging from white (L = 100) to black (L = 0); <sup>4</sup>Ranging from yellow (+b\*) to blue (-b\*).

**Table 6.** Egg quality variables of quails fed on different lipid sources with and without emulsifier in the litter at different storage periods

Variation sources	Variables							
	egg (g)	yolk (g)	albumen (g)	shell (g)	% yolk	% shell	% albumen	gravity
Poultry fat	10.116 <sup>y</sup>	3.434 <sup>xy</sup>	6.141 <sup>xy</sup>	0.845 <sup>y</sup>	31.098	8.413	59.699	1.068
Beef tallow	10.412 <sup>x</sup>	3.533 <sup>x</sup>	6.31 <sup>x</sup>	0.884 <sup>x</sup>	30.638	8.543	60.067	1.068
Soybean oil	10.012 <sup>y</sup>	3.402 <sup>y</sup>	5.885 <sup>y</sup>	0.850 <sup>y</sup>	32.047	8.513	58.73	1.068
RE+Emul	10.25	3.465	6.209 <sup>a</sup>	0.862	31.21	8.436	59.673	1.068
Basal	10.113	3.447	6.015 <sup>b</sup>	0.857	31.312	8.543	59.849	1.069
0 *	10.237	3.188	6.374	0.863	29.367	8.447	61.925	1.075
7	10.261	3.489	6.170	0.851	31.675	8.315	59.786	1.065
14	10.054	3.691	5.792	0.865	32.741	8.707	56.787	1.065
	P-values							
Storage	0.0585	<0.0001	<0.0001	0.3100	0.0036	<0.0001	<0.0001	<0.0001
Source	0.0001	0.0028	0.0200	0.0002	0.3642	0.1946	0.2082	0.4528
Emulsifier <sup>2</sup>	0.9211	0.5635	0.0466	0.4967	0.9014	0.0805	0.9709	0.0044
Sour*Stor	0.0821	0.5727	0.8872	0.6602	0.9186	0.5182	0.9709	0.6643
Emu*Stor	0.9404	0.8390	0.4295	0.2259	0.7703	0.3532	0.5843	0.0023
Emu*Sour	0.0919	0.0536	0.4861	0.3685	0.6688	0.2579	0.1556	0.6179
Stor*Emu*Sour	0.9649	0.6036	0.7477	0.5617	0.7799	0.9698	0.9522	0.7030
SEM <sup>1</sup>	0.041	0.023	0.052	0.004	0.412	0.032	0.9066	0.0004
	Polynomial regression							
Variable	P-value	Equations						r <sup>2</sup>
Yolk weight (g)	<0.0001	y=3.205+0.0359x						0.4947
Albumen weight (g)	<0.0001	y=6.400-0.0400x						0.1196
% yolk	0.0007	y=29.574+0.2409x						0.0693
% shell	<0.0001	y=8.447-0.055x+0.005x <sup>2</sup>						0.0654
% albumen	<0.0001	y=62.045-0.361x						0.224
Specific gravity	<0.0001	(Emul +) y=1.0745-0.0019x+0.00009x <sup>2</sup>						0.7972
	<0.0001	(Emul -) y=1.0772+0.00024x+0.000008x <sup>2</sup>						0.9052

<sup>1</sup>Standard error of the mean. <sup>2</sup>Emulsifier+Reduced Energy 96Kcal/kg. x,y,z letters in the columns represent difference for the lipid source. Different lowercase a,b, c letters in the columns represent difference for the inclusion of emulsifier both by the Tukey test at 5% probability. \*0, 7, 14 storage days.

the variables egg weight, yolk weight, albumen weight, and shell percentage. Isolated emulsifier addition influenced only albumen weight and specific gravity (Tab. 6).

The yolk weight and yolk percentage showed a positive linear effect. In other words, the yolks get heavier with the passing of the storage period. The albumen weight and percentage showed a negative linear effect. In other words, the longer the storage time, the lower the weight (Tab. 6). On the other hand, the shell percentage showed a positive quadratic effect, with a minimum of 8.315%.

The albumen weight of eggs from birds that received beef tallow was higher than those that received soybean oil. The RE+Emul diets resulted in higher albumen weight.

The highest egg, yolk, and albumen weights were obtained from quails fed on diets formulated with beef tallow as the lipid source compared to those fed on poultry fat and soybean oil. Regarding shell weight, it was possible to note that quails fed with diets based on beef tallow presented a higher weight of this variable. However, poultry fat and soybean oil did not provide significant differences between them (Tab. 6).

In the colorimetry of stored eggs, L\* value, there was an isolated effect of the storage time, presenting a positive linear equation, demonstrating that the yolks became lighter in color with time. The b\* value showed isolated effects of source and storage time. Regarding the fat source, beef tallow showed the highest b\* values (more yellowish coloration), while the b\* coloration of egg yolks from birds fed with

**Table 7.** Egg quality variables of quails fed on different lipid sources with and without emulsifier in the diet with different storage periods

Variation sources	Variables							
	L <sup>3</sup>	b <sup>4</sup>	shell thickness	albumen height	yolk height	yolk diameter	HU	YI
Poultry fat	57.756	39.504 <sup>y</sup>	0.205	3.052	8.346	25.695	80.424	0.337
Beef tallow	57.53	40.658 <sup>x</sup>	0.204	3.115	8.330	25.849	79.959	0.336
Soybean oil	57.59	39.595 <sup>xy</sup>	0.204	3.159	8.304	25.395	81.277	0.338
RE+Emul	57.619	39.715	0.203	3.118	8.346	25.688	80.390	0.338
Basal	57.638	40.122	0.206	3.099	8.289	25.604	80.714	0.336
0*	55.387	35.365	0.207	3.558	9.912	22.411	83.922	0.445
7	58.115	39.025	0.207	2.927	8.597	24.920	79.446	0.346
14	59.383	45.367	0.206	2.841	6.471	29.609	78.291	0.220
P-values								
Storage	<0.0001	<0.0001	0.9721	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Source	0.5912	0.0091	0.1985	0.3206	0.8516	0.0902	0.2763	0.8210
Emulsifier <sup>2</sup>	0.9216	0.4718	0.8791	0.7571	0.2167	0.6226	0.6368	0.7106
Sour*Stor	0.1676	0.7743	0.9271	0.4336	0.6520	0.6293	0.7585	0.9321
Emu*Stor	0.5759	0.2916	0.5237	0.0560	0.7902	0.7828	0.8122	0.5857
Emu*Sour	0.2392	0.1877	0.3932	0.1767	0.0686	0.4247	0.2336	0.1627
Stor*Emu*Sour	0.6476	0.6234	0.3305	0.7234	0.8521	0.6754	0.3943	0.6915
SEM <sup>1</sup>	0.163	0.3770	0.0001	0.038	0.115	0.249	0.384	0.007
Polynomial regression								
Variable	P-value	Equations						r <sup>2</sup>
L	<0.0001	y=55.627+0.2864x						0.6254
B	0.0011	y=35.352+0.334x+0.0272x <sup>2</sup>						0.751
Albumen height	<0.0001	y=3.558-0.128x+0.005x <sup>2</sup>						0.4288
Yolk height	<0.0001	y=9.910-0.129x-0.008x <sup>2</sup>						0.9352
Yolk diameter	<0.0001	y=22.410+0.202y+0.022x <sup>2</sup>						0.8871
Haugh unit	0.0210	y=83.922-0.876x+0.033x <sup>2</sup>						0.2475
Yolk index	0.0001	y=0.445-0.012x-0.0002x <sup>2</sup>						0.959

x,y,z letters in the columns represent difference for the lipid source. Different lowercase a, b and c letters in the columns represent difference for the inclusion of emulsifier both by the Tukey test at 5% probability.<sup>1</sup> Standard error of the mean; <sup>2</sup>Emulsifier+Reduced Energy 96Kcal/kg <sup>3</sup> ranging from white (L = 100) to black (L = 0);<sup>4</sup> ranging from yellow (+b\*) to blue (-b\*). \* 0,7,14 storage days.

the diet containing poultry fat had the lowest values. For storage time, a positive quadratic equation was found, where on the 6th day it showed a minimum reflectance point of 38.33 (Tab. 7).

Albumen height, yolk diameter, and the Haugh unit were influenced only by storage time, showing a positive quadratic behavior. In turn, the yolk index and height showed a negative quadratic effect of the storage time influence. Shell thickness was not influenced by diets and storage time and the interaction between these variables (Tab. 7).

For the total amount of fatty acids and palmitic acid (C16:0), there was an interaction between the lipid source and the use of emulsifiers (Tab. 8).

In the RE+emul diets the inclusion of poultry fat provided the highest total fatty acid values to the yolks, while the quails that received soybean oil obtained the lowest total values. However, in the basal diets the highest value of total fatty acids was recorded

**Table 8.** Fatty acids in egg yolk of quails fed on different lipid sources with or without emulsifying additive

Variable	Emulsifier (E)	Source (S)			Mean	SEM <sup>1</sup>	P-values		
		soybean	tallow	poultry fat			source	emulsifier <sup>10</sup>	E*S
C16:0 <sup>2</sup>	RE+Emul	25.995 <sup>3a</sup>	26.250 <sup>a</sup>	26.278 <sup>a</sup>	26.125	0.091	0.2971	0.0246	0.0020
	Basal	26.973 <sup>3a</sup>	26.461 <sup>ab</sup>	25.995 <sup>b</sup>	26.476				
	Mean	26.410	26.355	26.136	26.3				
C16:1 <sup>3</sup>	RE+Emul	1.586	1.568	1.591	1.582	0.004	0.6385	0.4879	0.1732
	Basal	1.575	1.600	1.591	1.588				
	Mean	1.580	1.584	1.591	1.585				
C18:0 <sup>4</sup>	RE+Emul	9.27	10.231	9.22	9.573	0.079	<0.0001	0.2578	0.8647
	Basal	9.305	10.242	9.263	9.603				
	Mean	9.287 <sup>b</sup>	10.236 <sup>a</sup>	9.241 <sup>b</sup>	9.588				
C18:1W9 <sup>5</sup>	RE+Emul	44.008	44.078	45.506	44.531	0.127	<0.0001	0.4218	0.1608
	Basal	44.316	43.811	45.07	44.399				
	Mean	44.162 <sup>b</sup>	43.945 <sup>b</sup>	45.288 <sup>a</sup>	44.465				
C18:2W6 <sup>6</sup>	RE+Emul	14.553	13.511	13.576	13.88	0.366	0.9954	0.3312	0.4112
	Basal	12.356	13.548	13.491	13.132				
	Mean	13.455	13.53	13.534	13.506				
C18:3W3 <sup>7</sup>	RE+Emul	0.176	0.18	0.168	0.175	0.001	0.008	0.2575	0.2521
	Basal	0.168	0.178	0.17	0.172				
	Mean	0.172 <sup>ab</sup>	0.179 <sup>a</sup>	0.169 <sup>b</sup>	0.173				
C20:4W6 <sup>8</sup>	RE+Emul	0.193	0.196	0.195	0.195	0.001	0.7123	0.0867	0.9522
	Basal	0.190	0.191	0.190	0.190				
	Mean	0.191	0.194	0.192	0.192				
C22:6W3 <sup>9</sup>	RE+Emul	0.205	0.205	0.21	0.206	0.001	0.5287	0.4517	0.6924
	Basal	0.2	0.206	0.205	0.203				
	Mean	0.202	0.205	0.207	0.204				
TOTAL	RE+Emul	95.840 <sup>3b</sup>	96.221 <sup>ab</sup>	96.785 <sup>a</sup>	96.282	0.113	0.2671	0.0911	<0.0001
	Basal	97.351 <sup>3a</sup>	96.343 <sup>b</sup>	95.976 <sup>b</sup>	96.557				
	Mean	96.595	96.282	96.38	96.419				

x and y letters in the columns differ by the Tukey test at 5%. Different lowercase letters in the row differ by the Tukey test at 5% probability. Main means when significant were compared using the Tukey test (source) or F test (emulsifier) at 5% probability. Main means when significant were compared using Tukey test (source) or F test (emulsifier) at 5% probability. <sup>1</sup>Standard mean error; <sup>2</sup>Palmitic acid; <sup>3</sup>Palmitoleic acid; <sup>4</sup>Stearic acid; <sup>5</sup>Oleic acid (omega-9); <sup>6</sup>Linoleic acid (omega-6); <sup>7</sup>α-linolenic acid (omega-3); <sup>8</sup>Arachidonic acid; <sup>9</sup>Docosahexaenoic acid. <sup>10</sup>Emulsifier+Reduced Energy 96Kcal/kg.

for the egg yolks of the birds that received the diet containing soybean oil. When comparing RE+emul and basal diets between lipid sources, there was a difference only for soybean oil, with the basal diets resulting in greater deposition (Tab. 8).

When the interaction for palmitic acid (C16:0) content was investigated in RE+emul diets, the lipid sources did not differ. Meanwhile, in the basal diets the inclusion of soybean oil provided a higher palmitic acid content in the yolks compared to poultry fat, but did not differ from beef tallow. When comparing RE+emul and basal diets between lipid sources, there was a difference only for soybean oil, with the basal diets resulting in greater deposition (Tab. 8).

For stearic acid (C18:0), oleic acid (C18:1w9), and  $\alpha$ -linolenic acid (C18:3w3), there was an isolated effect of lipid sources. For stearic acid (C18:0), beef tallow provided greater deposition of this fatty acid in quail egg yolks compared with the other sources, which did not differ in this respect. The concentration of omega-9 oleic acid (C18:1w9) was found in the egg yolks of the birds that received the poultry fat. Regarding  $\alpha$ -linolenic acid (C18:3w3), lipid sources had an isolated effect. In the egg yolks of quails that received beef tallow, there was a higher concentration when compared with the yolks of birds fed with poultry fat as a lipid source.

Based on the performance findings of this study, it was possible to reduce dietary energy and add emulsifiers increasing energy availability of the diet with a reduced effect on bird performance, regardless of the lipid source used. Considering the diets with soybean oil and poultry fat as a basis, there were no differences between the basal diets and those reduced in energy with the addition of an emulsifier, which demonstrates the ability of the emulsifier to provide a higher energy content from these sources. The same effect was not found for beef tallow.

The main effect of the emulsifier inclusion in the diets observed for feed intake may be related to the increased demand for feed to meet their energy demands. In order to compensate for this reduced AMEn in the diets, the birds had to consume more feed, even though the emulsifier was expected to provide enough energy. Similar to this study's findings, Souza *et al.* [2019] when using soybean gum added at 5% as an emulsifier in diets of commercial layers, also observed an increase in feed intake. In contrast, Roll *et al.* [2017] when working on the effect of lecithin in diets containing acid and degummed soybean oils, found no interactions between the types of lipid source and the presence or absence of lecithin as an emulsifier in the litter of Japanese quails and observed no significant changes for performance variables. Regarding the lipid sources used, the observed results are similar to those of Martins *et al.* [2017], who also found no differences in feed intake.

Higher feed intake directly contributes to a worse feed conversion rate per egg mass, which was verified with reduced energy and emulsifier inclusion. This fact was also observed by Hulan and Proudfoot [1981] in a study with laying hens fed with soybean gum inclusion, in which the authors found no significant effects on egg production, but observed an increase in the amount of feed needed to produce a dozen eggs and a worsening in feed conversion.

According to the emulsifier manufacturer, its inclusion would promote the release of 96 kcal/kg of the diet, substantiating the reduction determined for the diets used. When the energy reduction proposed in this study was evaluated, the mean values obtained in the metabolism trial showed that the diets provided less energy than expected and that the relative decrease between the two diets was greater than the 96 kcal/kg proposed.

These findings may explain the higher feed intake of birds fed on diets with an added emulsifier due to the lower energy availability. The birds increased their feed intake to meet their nutritional requirements [Morris 2004, Barreto *et al.* 2007]. The reduction proposed by the manufacturer is a more aggressive strategy than the quails can take advantage of the diets without increasing intake. Therefore, adopting lower energy reductions in the diets may be an alternative for better use of the nutrients without compromising consumption and feed conversion.

Barreto *et al.* [2007], when measuring levels of metabolizable energy in Japanese quails, reported that energy is the main nutritional component determining performance, mainly because about 20% of the energy consumed is for production. In other words, if the amount supplied is not sufficient, there will be a drop in production. This study did not observe these facts, possibly because the quails increased their feed intake, resulting in a worse feed conversion ratio. Barreto *et al.* [2007] recommended diets for Japanese quails with 2600 Kcal of EM/kg for higher production and egg weight and 2850 Kcal of EM/kg for better feed conversion.

Araújo *et al.* [2018], when working with metabolizable energy of different lipid sources rich in n-6 and n-3 lipids in laying hens, found different AMEn values among the lipid sources, unlike the results found in this study, where the different lipid sources did not show significant differences. This fact may be related to the absence of differences related to the permanence time of the food in the gastrointestinal tract. The different lipid sources did not differ in their passage rate, probably due to the similarity between the isonutritive diets used in this study [Penz Jr *et al.* 1999; Rabello 2002, Sakamoto *et al.* 2006, Santos *et al.* 2006].

The diets analyzed in this study influenced the Haugh unit, which is the most used parameter to express the albumen quality, considered a mathematical expression that correlates the weight of eggs with the height of dense albumen, so that the higher the Haugh unit value, the better the quality of the egg [Alleoni and Antunes 2001]. Despite the differences found, all the results characterize the excellent internal quality of the eggs, since values above 72 indicate good quality regarding freshness [USDA, 2000].

Unlike this study, Bertipaglia *et al.* [2016], when using soybean oil, poultry fat, fish waste, and grape seed oil, and Grobas *et al.* [2001], when using beef tallow and soybean oil, found no differences in the eggs' Haugh unit. The high values found, which demonstrate the maintenance of the high quality of quail eggs, may be related to the age of the birds. Young birds have a higher value for the Haugh unit when compared to old birds, regardless of the diet provided [Oliveira *et al.* 2010].



Grobas *et al.* [2001] observed no differences in yolk weight in layers fed on soybean oil and beef tallow, as in this study. However, they found that the treatment containing soybean oil provided higher values for egg weight, egg mass, albumen weight, and eggshell when compared to the same variables in those birds that received beef tallow, thus stating that these findings may indicate that the higher weight is attributed to the greater albumen weight provided by the linoleic acid. The synthesis of triglycerides and low-density lipoproteins in the liver and albumin synthesis in the oviduct occur under estradiol control due to the inclusion of fatty acids in the diet, especially linoleic acid, possibly influencing the increase in yolk and albumen weight [Whitehead *et al.* 1993].

A study by Bragg *et al.* [1973] evaluating four levels of beef tallow, soybean oil, sunflower oil, and rapeseed in laying hen feed found that the inclusion of 2% beef tallow provided an increase in yolk weight when compared to that obtained from soybean oil treatment, similar to that found in this study. According to the authors, increasing the energy intake improved the feed conversion and therefore obtained a higher egg and yolk weight. This study did not alter the feed conversion by including the lipid sources.

When analyzing the colorimetry variables of the yolks using reflectance [Harder 2007], the “b” variable ranges from yellow (+b\*) to blue (-b\*). According to Bittencourt *et al.* [2019], the intensity of yolk coloration is due to the deposition of natural pigments, among them the xanthophylls (group of carotenoid pigments), mainly lutein and zeaxanthin present in corn grain, the main component of poultry diet formulations. Alleoni and Antunes [2001] and Souza *et al.* [2019], when using emulsifiers in commercial layer diets, found a significant difference in yolk coloration, resulting in more intense pigmentation, i.e. results similar to those found in this study.

According to this study’s findings, the most important factor in maintaining the quality of eggs is storage time. Egg quality was significantly affected by storage time, and diets had little effect on preserving these characteristics. The changes due to time are biochemical and promote the liquefaction of the albumen and the release of carbon dioxide gas, which diffuses through the pores of the shell and is released to the environment [Rocha *et al.* 2013]. Refrigeration maintains the stability of egg coloration during storage and prevents chemical reactions from effecting physicochemical changes in eggs [Garcia *et al.* 2010, Santos *et al.* 2021]. In this study, eggs were stored at room temperature, simulating the Brazilian conditions of storing and retailing eggs to the consumer, contributing to egg quality degradation.

The quality and quantity of fatty acids in the egg yolk are modified according to the lipid sources in the diets formulated for hens, but must also consider the strain and age of the layers [Oliveira *et al.* 2010]. Thus, observing the lipid profiles of the diets offered to the quails in this study, we can evidence its influence on the lipid profile of the yolks. The sources of animal origin, beef tallow and poultry fat, provided a higher percentage of palmitic and unsaturated fatty acids, unlike the profile found with the addition of soybean oil in the diets, in which these same acids showed lower concentrations.



Mandarino *et al.* [1992] evaluated several lipid sources such as soybean, sunflower, corn, and coconut oil in the diets of laying hens, changing the concentrations of fatty acids, especially polyunsaturated fatty acids, related to the production of enriched eggs, which is similar to this study's findings. It was possible to modulate the concentration of omega-3 polyunsaturated fatty acids, especially  $\alpha$ -linolenic (C18:3w3), by changing the lipid source offered in quail diets.

According to Renner and Hill [1961], animal fats such as beef tallow have large amounts of saturated long-chain fatty acids such as palmitic and stearic acid. Similarly to this study's findings, egg yolks from quails fed on beef tallow had high contents of palmitic and stearic fatty acids. Mazalli *et al.* [2004] explained that vegetable oils added to poultry diets decrease the amount of C18:1 in the yolks, which is similar to this study's findings. It occurs because the C18:1 fatty acid is a precursor of the n-3 and n-6 fatty acids (linolenic and linoleic acid) [Oliveira *et al.* 2010]. Furthermore, the same authors pointed out that laying hens fed the control diet without oil inclusion obtained higher concentrations of oleic acid (C18:1) in the yolks compared to hens fed with vegetable oil, which is similar to this study's results, where the use of soybean oil was lower when compared to diets based on poultry fat.

It is possible to use alternative lipid sources, such as beef tallow and poultry fat, as a substitute for soybean oil with the addition of emulsifiers in diets for laying quails without impairing performance and egg quality, because the emulsifier was able to provide energy for the metabolism in birds. However, the reduction recommendation of 96kcal/kg proposed by the emulsifier manufacturer was high when considering its effects on feed intake and conversion, suggesting that more conservative strategies should be used. Storage time is a determining factor in egg quality reduction regardless of diet composition. It is possible to modulate the lipid profile of Japanese quail egg yolks by including different lipid sources in their diets regardless of the use of emulsifiers.

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