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Original Article

Emulsifier in Diets with Different Alternative Lipid Sources: Effects on the Health of Japanese Quails

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Bovine tallow, intestinal morphometry, poultry fat, soybean oil, serum biochemical profile.

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ABSTRACT

The present research aims to evaluate the inclusion of an emulsifying additive based on mono- and diglycerides of fatty acids in diets with different lipid sources, studying its effects on duodenum histology, biometry of the digestive and reproductive tract, and blood biochemical profile of Japanese quails. The study was carried out in the experimental aviary of the Federal University of Grande Dourados, Mato Grosso do Sul, Brazil. The experimental design was completely randomized, with diets in a 3x2 factorial design with three different lipid sources (soybean oil, poultry fat, and beef tallow), either supplemented or not with an emulsifier. The birds that received a diet composed of poultry fat and an emulsifying additive had a larger gizzard. Animals that received a diet containing an emulsifier, regardless of the lipid source, had greater heart weight. The duodenum of animals that received bovine tallow in their diet showed a greater height and villus:crypt ratio. Biochemical parameters were not affected by either the lipid source or the inclusion of emulsifier in quail diets. The biochemical blood profile of birds in treatments did not show significant changes among all diets used. The use of soybean oil, bovine tallow, and poultry fat with or without the inclusion of an emulsifier does not show changes in the health and development of the quails' organs, which also indicates that the health of Japanese quails in the laying phase is kept in good standards.

INTRODUCTION

The use of vegetable oils and/or animal fat is essential in formulating poultry diets, as these sources help to achieve ideal levels of metabolizable energy (Villanueva-Lopez *et al.*, 2020). Due to the high demand and diversified use of vegetable oils, not only for animal but also human use, the world market has faced difficulties in producing high quantities to supply these chains. In addition to soybean oil, the main lipid source used in animal diets, oils have high added value. Therefore, the poultry industry has been looking for alternatives to replace this ingredient in poultry laying for another that equally meets the energy needs of birds. As alternatives, we may mention palm oil, corn, sunflower, poultry fat, and beef tallow (Roll *et al.*, 2018).

In addition to providing energy, lipids are sources of fatty acids, which act in various metabolic functions of the organism and in the health of birds (Fonseca *et al.*, 2018). The use of oils in the diets provided to birds provides an increase of up to 25% in the energy of the diet, thus leading to better bird performance and improving productive efficiency, leading to economic benefits in the production chain (Zampiga *et al.*, 2016).

According to Reda *et al.* (2020), diets are formulated according to recommendations to meet the requirements of each of the productive phases of birds. For laying hens, energy requirements are related to body

weight, weight gain, egg mass production, ambient temperature, level of feathering, body composition, and egg composition (Baron *et al.*, 2020).

The best use of nutrients and energy from lipid sources is one of the important objectives in poultry nutrition. This is especially true of laying quails, since these birds show limitations regarding the digestibility of nutrients as compared to laying hens and poultry, due to inferior advances in genetic improvement. Roy *et al.* (2010) report that the use of an emulsifier improves the digestibility of fat in birds and increases the availability of fat globules that help in the formation of micelles. Micelles help fatty constituents become more soluble and able to move better in the aqueous intestinal environment. They also improve bile and pancreatic secretions, promoting greater digestion, absorption, and gastrointestinal peristalsis.

Productive performance, intestinal physiology, organ development, and blood biochemical profile are evidences of a greater diet nutrient use in Japanese quails. Therefore, the present research aims to evaluate the use of different lipid sources and the inclusion of an emulsifying additive in diets, with a reduction in the energy level by 96 Kcal/Kg, on the intestinal histology (duodenum), biometry of the digestive and reproductive tract, and the biochemical blood profile of birds.

MATERIAL AND METHODS

This research was carried out in the poultry and quail farming sector of the Faculty of Agricultural Sciences of the Federal University of Grande Dourados. It lasted 84 days, which were divided into three periods of 28 days. This experiment protocol was submitted to and approved by the Research Ethics Committee of UFGD under protocol no. 16/2020.

The experiment was completely randomized. The factorial design consisted of three lipid sources and two energy levels 2,800 kcal/kg (Basal) and 2,704 kcal/kg + emulsifier (RE+Emul), totaling six treatments (Soy oil basal ration, bovine tallow basal ration, poultry fat basal ration, soybean oil reduced ration + emulsifier, bovine tallow reduced ration + emulsifier, and poultry fat reduced ration + emulsifier). The energy-reduced diet presupposes that the Lipocel emulsifier at a 100 g/ton rate of inclusion provides 96 kcal/kg during the laying phase of birds, the dose recommended by the manufacturer. 270 quails (*Coturnix coturnix japonica*) in the laying phase were allocated in nine replications with five quails each.

The groups of five quails were housed in galvanized wire cages, and the density used was one bird/250 cm² per experimental unit. According to the Köppen classification, the climate of the region is Cwa (humid mesothermic), with rainy summers and dry winters, average annual rainfalls of 1,500 mm, and average annual temperature of 22°C.

Experimental rations were provided *ad libitum*, twice a day, in trough-type feeders; water was supplied *ad libitum* in nipple-type drinkers. Rations were formulated based on corn bran and soybean bran. The nutritional requirements were those recommended by INRA (1999). Diets were isonutritive except for metabolizable energy, which was reduced in diets to which the emulsifier was added, considering that its inclusion should provide 96 kcal/kg, making it isoenergetic in relation to the others (Table 1).

The light program used was 16 hours plus artificial light with LED lamps. At the end of the experimental period (84 days), 54 birds within a ± 10% range of the average weight were selected (one bird per experimental unit) for blood collection by cardiac puncture, and organ biometrics. After blood collection, the selected birds were sacrificed by cervical dislocation followed by section of jugular veins and carotid arteries, and bled for three minutes.

The birds then went through the scalding process, followed by manual plucking of carcasses. Evisceration and removal of feet and heads was performed manually. Eviscerated carcasses free of head and feet were weighed. The organs (liver, heart, gizzard, proventriculus, ovaries, oviducts, duodenum, jejunum+ileum, and cecum) were individualized and later weighed for morphometric evaluation and collection of fragments for intestinal histological evaluation (duodenum).

After the slaughter and evisceration of carcasses, each organ (duodenum, jejunum, jejunum+ileum, ceca, ovaries, and oviduct) was evaluated for biometrics. A 90-cm graduated tape measure was used for the measurements (with a precision of 0.1 mm) of all analyzed segments. To obtain relative length values, the measurements of each segment were divided by the total length of the organ, and the result was then multiplied by 100.

After biometry, 2.0 cm segments of the duodenum were collected and fixed in a buffered 10% formaldehyde solution, according to the methodology proposed by Tolosa *et al.* (2003). The slides were stained with Hematoxylin and Eosin and analyzed in an optical microscope with five times magnification,

Table 1 – Percentage and calculated composition of experimental diets.

Ingredient	Diet					
	Soybean Oil (OS)	Beef Tallow (BT)	Poultry fat (PF)	SO + E[2]	BT + E[2]	PF + E[2]
Corn 7.88%	49.809	49.809	49.809	49.809	49.809	49.809
Soybean meal 45%	33.188	33.188	33.188	33.188	33.188	33.188
Limestone	7.544	7.544	7.544	7.544	7.5440	7.5440
Soybean oil	4.000	-	-	2.908	-	-
Beef tallow	-	4.000	-	-	3.454	-
Poultry fat	-	-	4.000	-	-	2.944
Inert (sand)	3.500	1.925	3.378	4.581	4.035	4.545
Starch	-	1.576	0.124	-	-	-
Dicalcium phosphate	1.065	1.065	1.065	1.065	1.065	1.065
Salt	0.336	0.336	0.336	0.336	0.336	0.336
DL-Methionine	0.200	0.200	0.200	0.200	0.200	0.200
L-Lysine	0.157	0.157	0.157	0.157	0.157	0.157
Min-Birds	0.100	0.100	0.100	0.100	0.100	0.100
Vit-Birds	0.100	0.100	0.100	0.100	0.100	0.100
Emulsifier (E)	-	-	-	0.012	0.012	0.012
Total	100.000	100.000	100.000	100.00	100.00	100.00
Meeting nutritional requirements – Natural Matter						
Nutrient	Soybean Oil	Beef Tallow	Poultry fat	SO + E	BT + E	PF + E
Calcium %	3.20	3.20	3.20	3.20	3.20	3.20
EM Poultry Kcal/kg	2800.00	2800.00	2800.00	2704.00	2704.00	2704.00
Available Phosphorus %	0.30	0.30	0.30	0.30	0.30	0.30
Total Phosphorus %	0.51	0.51	0.51	0.51	0.51	0.51
Total lysine %	1.16	1.16	1.16	1.16	1.16	1.16
MET+Total Cystine %	0.79	0.79	0.79	0.79	0.79	0.79
Total Methionine %	0.48	0.48	0.48	0.48	0.48	0.48
Crude protein %	19.20	19.20	19.20	19.20	19.20	19.20
Sodium %	0.15	0.15	0.15	0.15	0.15	0.15
Reduction 96 Kcal/Kg	-	-	-	96	96	96

[1]Vitamin supplement/kg of diet: Folic Acid (Min.) 145.4 mg; Pantothenic Acid (Min.) 5,931.6 mg; Choline (Min.) 121.8 g; Niacin (Min.) 12.9 g; Selenium (Min.) 480.0 mg; Vitamin A (Min.) 5,000,000.0 IU; Vitamin B12 (Min.) 6,500.0 mcg; Vitamin B2 (Min.) 2,000.0 mg; Vitamin B6 (Min.) 250.0 mg; Vitamin D3 (Min.) 1,850,000 IU; Vitamin E (Min.) 4,500 IU; Vitamin K3 (Min.) 918.0 mg. [2]Mineral supplement/kg: Copper (Min.) 7,000.0 mg; Iron (Min.) 50.0 g; Iodine (Min.) 1,500.0 mg; Manganese (Min.) 67.5 g; Zinc (Min.) 45.6 g; [2] Emulsifier.

model Leica DM 4000B, coupled to a microcomputer. Images were analyzed using the ImageJ software. Villus height, villus width, crypt depth, and villus:crypt muscle layer thickness were determined.

For the biochemical profile, cardiac puncture was performed using 3-ml syringes coupled with 25 x 0.8 mm needles without anticoagulants. The birds were placed in a supine position and the needle was inserted along the ventral floor of the thoracic inlet up to the heart. Samples were immediately centrifuged to separate the serum, and then frozen at -20 °C until the time of biochemical analysis. Cholesterol, triglycerides, AST, ALT, and glucose were evaluated.

Biochemical tests were processed by spectrometry (BioPlus 200), as indicated by the manufacturer of commercial kits (GoldAnalisa®). Blood samples without anticoagulants were used for the lipidogram (cholesterol and triglycerides), being kept in Eppendorf plastic bottles for one hour at room temperature and centrifuged at 3,500 rpm and 4 °C for 15 minutes to separate the serum. The doses were obtained by using commercial kits, and the samples were prepared and analyzed according to the manufacturer's specifications. Three readings were performed in a spectrophotometer (Beckman Coulter, DU-800) using a wavelength of 500 nm.

All data were analyzed using the statistical package Statistical Analysis System (SAS 9.3). First, the statistical assumptions of normality of residuals and homogeneity of variances were 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). When the effects of interactions between lipid sources and emulsifier were significant, interactions were then broken down and measurements were compared by Tukey test. When there were no significant interactions, the main effects were then evaluated by comparing means using the same test. In all analyses performed, the level of significance adopted was 5%.

RESULTS

For the gizzard, duodenum, jejunum+ileum, and complete intestine weight variables, there was an interaction between the lipid sources and the inclusion or not of an emulsifying additive. The relative weight of the heart, and size of the duodenum (cm) variables showed significant differences only for the use of emulsifier (Table 2).

Regarding the weight of the gizzard (g/g live weight - LW) per lipid source of the animals that received Re+Emul diets, bovine tallow yielded lower values, while quails fed on poultry fat obtained the highest values. In basal diets, there were no differences between lipid sources. Comparing RE+Emul and the basal diet of each source, there were no differences either (Table 2).

For the weight of the duodenum, comparing the different lipid sources within the RE+Emul and basal diets, there were no differences. When confronting RE+Emul and basal diets in each of the different sources, there was a difference only for soybean oil, with smaller results in animals that received diet without emulsifier (Table 2).

As for the weight of the jejunum+ileum and the whole intestine, comparing the different lipid sources

Table 2 – Relative weight and organ size of Japanese quails using different lipid sources in diet with and without inclusion of emulsifier.

Variable	Emul. (E[2])	Source (F)			Mean	SEM[1]	Probability		
		Soybean oil	Beef tallow	Poultry fat			Source	Emuls+Red.Energy[3]	E[2]*F[4]
Full carcass (g/gLW[7])	[5]With	87.314	88.311	86.819	87.481	0.226	0.1885	0.4179	0.5869
	[6]Without	87.517	88.207	87.830	87.851				
	Mean	87.415	88.259	87.324	87.666				
Liver (g/gLW)	With	2.889	2.948	2.835	2.891	0.072	0.6166	0.3259	0.7736
	Without	2.589	2.881	2.757	2.742				
	Mean	2.739	2.915	2.796	2.816				
Heart (g/gLW)	With	0.885	0.876	0.937	0.900B	0.015	0.3925	0.0433	0.8186
	Without	0.976	0.936	0.981	0.964A				
	Mean	0.931	0.906	0.959	0.932				
Gizzard (g/gLW)	With	2.792Aab	2.412Ab	3.050Aa	2.751	0.061	0.0116	0.3068	0.0336
	Without	2.902Aa	2.499Aa	2.511Aa	2.637				
	Mean	2.847	2.455	2.781	2.687				
Proventriculus (g/gLW)	With	0.551	0.471	0.525	0.516	0.103	0.1959	0.8909	0.3778
	Without	0.535	0.518	0.503	0.519				
	Mean	0.543	0.495	0.514	0.517				
Duodenum (g/gLW)	With	1.466Aa	1.276Aa	1.390Aa	1.377	0.034	0.7916	0.0194	0.0283
	Without	1.156Ba	1.367Aa	1.153Aa	1.226				
	Mean	1.311	1.322	1.271	1.312				
Jejunum + Ileum (g/gLW)	With	2.138Aa	1.884Aa	2.224Aa	2.082	0.050	0.7173	0.0180	0.0472
	Without	1.892Aa	1.957Aa	1.706Ba	1.852				
	Mean	1.201	1.921	1.965	1.967				
Cecum (g/gLW)	With	0.742	0.801	0.828	0.790	0.027	0.4849	0.2732	0.0584
	Without	0.958	0.773	0.790	0.849				
	Mean	0.863	0.787	0.809	0.820				
Whole Intestine (g/gLW)	With	5.035Aa	4.521Aa	5.074Aa	4.877	0.092	0.5606	0.0378	0.0469
	Without	4.612Aa	4.721Aa	4.188Ba	4.507				
	Mean	4.823	4.621	4.631	4.691				
Duodenum (cm)	With	10.244	10.388	10.066	10.233A	0.168	0.1823	0.0280	0.4183
	Without	9.011	10.200	9.311	9.507B				
	Mean	9.627	10.294	9.688	9.870				
Jejunum+ileum (cm)	With	41.555	41.333	42.411	41.766	0.500	0.8426	0.1389	0.9484
	Without	40.222	40.055	40.400	40.225				
	Mean	41.405	40.694	40.888	40.996				
Whole intestine (cm)	With	56.511	56.887	54.700	56.032	0.587	0.7809	0.0569	0.4675
	Without	52.800	54.066	54.388	53.751				
	Mean	54.655	55.477	54.544	54.854				

Different uppercase letters in the columns indicate significant differences, and different lowercase letters on the lines indicate statistical differences by Tukey test at 5% probability.
[1]Mean standard error; [2]Emulsifier; [3]Emulsifier+96 kcal/kg reduction; [4]Lipid source; [5]Inclusion of emulsifier additive; [6]No emulsifying additive [7]Live weight.

within the RE+Emul and basal diets, there were no differences. When confronting the RE+Emul and basal diets for each lipid source, there was a difference only for animals that received the diet composed of poultry fat, with smaller values in the absence of the emulsifier (Table 2).

There was an isolated effect of the use of emulsifier in diets with reduced energy on heart weight and duodenum size, with diets with the additive making for lower relative weight of heart and greater length of duodenum (Table 2).

Regarding the variables of reproductive tract biometry, only the ovary showed a significant difference (p<0.05) depending on the source of oil used. The use of bovine tallow-based diets led to a greater relative weight for this variable as compared to poultry fat. The other variables, such as oviduct weight, reproductive completeness, and oviduct size did not differ between treatments (Table 3).

Table 3 – Relative weight and size of the reproductive tract of Japanese quails fed on different lipid sources with and without the inclusion of an emulsifier in the formulations.

Variable	Emul.(E[2])	Source			Mean	SEM[1]	Probability		
		Soybean oil	Beef tallow	Poultry fat			Source	Emuls+Red. Energy[3]	E[2]*F[4]
Oviduct (g/gLW[7])	[5]With	4.081	3.884	3.418	3.794	0.116	0.8825	0.1890	0.1609
	[6]Without	3.260	3.501	3.700	3.487				
	Mean	3.670	3.692	3.559	3.645				
Ovary (g/gLW)	With	2.726	3.463	2.130	2.773	0.160	0.0198	0.1915	0.5351
	Without	2.006	2.910	2.201	2.372				
	Mean	2.366a	3.186a	2.165b	2.572				
Whole reproductive (g/gLW)	With	6.798	7.338	5.633	6.590	0.244	0.0540	0.2333	0.2936
	Without	5.278	6.906	5.897	6.027				
	Mean	6.038	7.122	5.765	6.310				
Oviduct (cm)	With	30.777	25.611	27.25	27.879	0.616	0.1001	0.3376	0.3229
	Without	27.055	25.833	27.267	26.718				
	Mean	28.916	25.722	27.258	27.300				

Different uppercase letters in the columns indicate significant differences, and different lowercase letters on the lines indicate statistical differences by Tukey test at 5% probability. [1]Mean standard error; [2]Emulsifier; [3]Emulsifier+96 kcal/kg reduction; [4]Lipid source; [5]Inclusion of emulsifier additive; [6]No emulsifying additive [7]Live weight.

Regarding the histology of the intestine, we can observe that there were isolated effects of lipid sources on villus height and villus:crypt ratio (Table 4); for the other variables, there were no significant results.

For biochemical parameters, ALT and AST did not show significant differences between treatments, which was also true for cholesterol (mg/dL) and triglycerides (mg/dL) (Table 5).

DISCUSSION

Evaluating the relative weight, morphology and histology of organs as well as the serum biochemical profile of laying quails may be considered a good tool, that provides valuable indicators of their health. In terms of making use of diets and expressing the genetic potential of the species, it is essential that these structures are maintained and their perfect functioning is preserved. Excessive energy consumption in quails during the egg production period results in increased abdominal fat and greater deposition in some organs, especially the reproductive system and the liver (Leksrisompong, 2014). However, this study did not have results related to liver weight and full carcass weight, which may indicate that the nutritional strategies adopted here were adequate to meet the demands of quails without providing a surplus of energy to be transformed into fat.

When using diets with different energy levels and emulsifying additives, Upadhaya *et al.* (2018) and Zhao & Kim (2017) did not observe interactions or a significant difference between treatments when evaluating the weight of organs (liver, gizzard, abdominal fat, and breast). In the present study, we observed similar results by comparing organ weights, which reinforces the possibility of using emulsifiers in energy-reduced diets without exceeding the energy demands of birds.

Working with chickens, Duarte *et al.* (2012) reported that foods containing higher levels of energy obtained by a greater inclusion of lipid sources can increase intestinal development, especially of the duodenum. This may indicate a better use of energy from diets. The present study shows that a greater weight of the

Table 4 – Histological measurements of the duodenal segment of quails fed on different lipid sources with and without the inclusion of an emulsifier.

Variable (duodenum)	Emul.(E²)	Source			Mean	SEM¹	Probability		
		Soybean oil	Beef tallow	Poultry fat			Source	Emuls+Red. Energy³	E²*F⁴
Villus height (µm)	⁵With	1,044.76	1,485.81	750.62	1093.73	2.143	0.0037	0.7954	0.1091
	⁶Without	1,010.47	1,190.06	999.92	1066.81				
	Mean	1,027.61ab	1,337.93a	875.27b	1182.78				
Villus width (µm)	With	197.08	246.44	224.01	222.51	0.614	0.2543	0.3272	0.3651
	Without	207.79	211.66	208.70	209.40				
	Mean	202.43	229.05	216.38	215.96				
Crypt Depth (µm)	With	189.98	190.08	186.45	188.84	0.148	0.3593	0.569	0.312
	Without	149.47	195.22	196.60	180.43				
	Mean	169.72	192.65	191.52	184.63				
Crypt diameter (µm)	With	84.6	91.78	82.00	86.15	1.67	0.2093	0.1297	0.7689
	Without	81.267	83.27	78.50	81.02				
	Mean	82.96	87.53	80.25	83.58				
Villus:Crypt	With	5.97	8.02	3.98	5.99	0.422	0.0292	0.9411	0.0666
	Without	7.43	5.67	5.03	6.05				
	Mean	6.71ab	6.84a	4.51b	6.02				

Different uppercase letters in the columns indicate significant differences, and different lowercase letters on the lines indicate statistical differences by Tukey test at 5% probability. ¹Mean standard error; ²Emulsifier; ³Emulsifier+96 kcal/kg reduction; ⁴Lipid source; ⁵Inclusion of emulsifier additive; ⁶No emulsifying additive.

Table 5 – Biochemical parameters of quails fed on different lipid sources with and without the inclusion of an emulsifier.

Variable	Emul.(E²)	Source			Mean	SEM¹	Probability		
		Soybean oil	Beef tallow	Poultry fat			Source	Emuls+Red. Energy³	E²*F⁴
ALT (U/L)⁵	With⁷	9.52	8.51	5.04	7.69	0.630	0.4386	0.7624	0.1462
	Without⁸	6.75	9.00	8.47	8.07				
	Mean	8.14	8.75	6.75	7.88				
AST (U/L)⁶	With	338.78	449.33	340.67	376.26	2.591	0.4759	0.2375	0.1738
	Without	348.11	321.38	342.00	337.16				
	Mean	343.44	385.35	341.33	356.71				
Cholesterol (mg/dL)	With	110.67	124.00	205.25	146.60	4.386	0.1279	0.2104	0.1720
	Without	188.44	154.22	180.29	174.32				
	Mean	149.56	139.11	192.77	160.47				
Triglycerides (mg/dL)	With	471.11	576.11	488.33	511.85	3.016	0.4169	0.7681	0.7971
	Without	424.11	603.20	587.00	538.12				
	Mean	447.61	589.68	537.67	524.28				

Different uppercase letters in the columns indicate significant differences, and different lowercase letters on the lines indicate statistical differences by Tukey test at 5% probability ¹Mean standard error; ²Emulsifier; ³Emulsifier+96 kcal/kg reduction; ⁴Lipid source; ⁵Alanine aminotransferase; ⁶Aspartate aminotransferase; ⁷Inclusion of emulsifying additive; ⁸No emulsifying additive included.

duodenum could be observed with the use of the emulsifier.

Ribeiro *et al.* (2002) related the size of the gizzard and its muscle mass with a greater development when birds are fed on diets with greater digestion complexity. Poultry fat probably caused a similar effect, requiring greater mechanical work and consequently leading to greater size of this organ.

Aydin *et al.* (2006) reported in their work that there was an increase in the reproductive characteristics of quails supplemented with linseed oil, relating this finding to hormonal metabolism, especially estrogen, and a greater supply of essential fatty acids. The bovine tallow used in the present study is also an important source of essential fatty acids. It may have positively influenced the weight of ovaries, meeting the high

demands of this organ for yolk development. The weight of the eggs can be related to the weight of the ovaries, since yolks are formed in these structures. Gobras *et al.* (2001) observed a larger size of eggs when lipid sources were added to the diet. This indicates that the addition was sufficient to increase the weight of eggs.

In addition to evaluating the organs macroscopically, it is necessary to assess the integrity of their structures at the cellular level; thus, evaluations of histological measurements of the intestine of quails are important to verify the intestinal health, and the digestive and absorptive capacity. Vieira (2002) reports that, from a digestive-absorptive point of view, the duodenum is a place of intense mixing of food with digestive and alkaline secretions.

The inclusion of bovine tallow in diets used during bird laying in the production phase provided better intestinal histology results. It did not differ from the most commonly used source, soybean oil, with a higher villus height and higher villus:crypt ratio. This represents a greater development of these structures and consequently a greater surface area for absorbing and harnessing energy from the litter (Santin *et al.*, 2001; Baurhoo *et al.*, 2007; Cunningham *et al.*, 2004). Bavaresco *et al.* (2019) observed that the inclusion of 1% lecithin in the diet did not affect the height of the villi. These results are similar to those of the present research, in which the use of emulsifier did not change intestinal histology, while there were significant effects when using different lipid sources.

The biochemical parameters of the blood of birds are indicators of the physiological health and metabolism of animals, and nutrition may directly affect them. According to Lumeij (1997), enzymatic evaluations can help diagnose abnormal conditions in birds. The main enzymes in the cytoplasm are aspartate aminotransferase (AST) and alanine aminotransferase (ALT); they are considered liver markers, as they are released into the bloodstream after some modifications or even cell damage (Nelson & Cox, 2014).

Lumeij (1997) attributes to most bird species a variation of 19 to 50 IU/L of ALT, and the results found in this study are below these thresholds. In general, AST values greater than 350 IU/L represent moderate increases in the enzyme. 800 IU/L and above are considered highly suggestive of severe hepatocellular damage disorders, even more so when there is presence of biliverdinuria or biliverdinemia (Capitelli & Crosta, 2013). Thus, we can observe in the present study that AST values were within the expected normality thresholds.

A report by Lightfoot (2006) warns that the high rate of lipid peroxidation in formulations rich in polyunsaturated lipid sources may result in a great formation of fatty acid hydroperoxides and consequently tissue damage, with AST being an indicator of this physiological alteration. The data obtained in this study show a maintenance of the AST enzyme within normal limits. This indicates steady liver health in quails fed on different lipid sources with and without energy reduction and use of emulsifiers.

Cholesterol and triglyceride levels, which in turn are synthesized in the liver, did not show significant differences between treatments, corroborating the findings for ALT and AST in the present study, with no loss of liver function caused by the addition of dietary energy sources.

Adding fat to diets may affect blood concentrations of triglycerides, fatty acids, and lipoproteins, in addition to altering fat and meat composition (Velasco *et al.*, 2010). The assessment of cholesterol and triglyceride levels is of interest for laying hens because there is a possibility that lipids are transported via bloodstream and deposited in muscle tissues, fat, and egg yolks. When working with quails, there is a lack of cholesterol and triglycerides assessments. However, when using lipid sources such as corn oil, soybean oil, sunflower oil, and bovine tallow in diets formulated for chicken, Ozdogan & Aksit (2003) found the cutoff values meant significant differences for HDL and LDL cholesterol in the blood serum of broiler chickens, while triglycerides did not show such a difference. Lipid sources, together with emulsifiers, are capable of altering the morphometric characteristics of organs and the architecture of intestinal cells and blood parameters of Japanese quails in the laying phase without compromising the health of birds.

The findings for quail organ morphometry, intestinal histology, and liver physiology of the use of diets formulated with a reduction of 96 Kcal/kg of feed plus an emulsifier based on mono- and diglycerides of fatty acids, together with soybean oil, bovine tallow or poultry fat, indicate that the health of Japanese quails in the laying phase is kept in good standards. Therefore, these sources can be used as a nutritional strategy of interest for this species.

REFERENCES

Aydin R, Karaman M, Toprak HHC, *et al.* The effect of long-term feeding of conjugated linoleic acid on fertility in Japanese quail. South African Journal of Animal Science 2006;36(2):99-104. http://dx.doi.org/10.4314/sajas.v36i2.3991

Baron LF, Pazinatto R, Baron CP. Oxidação de lipídeos e as implicações na nutrição e saúde de animais de produção. Cadernos de Ciencia e Tecnologia 2020;37(1):26597. DOI:http://dx.doi.org/10.35977/0104-1096.cct2020.v37.26597

Baurhoo B, Phillip L, Ruiz-feria CAA. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poultry Science 2007;86:1070-8. https://doi.org/10.1093/ps/86.6.1070

Bavaresco C, Nunes AP, Forgiarini J, *et al.* Intestinal morphometry and bone quality of Japanese quails fed for a prolonged period with soybean oil by-products. Archives of Veterinary Science 2019;24(1):72-82.

Capitelli R, Crosta L. Overview of psittacine blood analysis and comparative retrospective study of clinical diagnosis, hematology and blood chemistry in selected psittacine species. Veterinary Clinics Exotic Animal Practices 2013;16(1):71-120. https://doi.org/10.1016/j.cvex.2012.10.002

Cunningham JG. Tratado de fisiologia veterinária. Rio de Janeiro: Guanabara Koogan; 2004.

Duarte KFO, Junqueira OM, Borges LL, *et al.* Desempenho e morfometria duodenal de frangos de corte submetidos a diferentes níveis de energia e programas de alimentação de 42 a 57 dias de idade. Ciencia Animal Brasileira 2012;13(2):197-204.

Fonseca SS, Silva VC, Valentim JK, Geraldo A. Efeito da adição de diferentes emulsificantes na dieta sobre o rendimento de carcaça de frangos de corte. Revista da Academia de Ciencia Animal 2018;16:1-13. https://doi.org/10.7213/1981-4178.2018.16010

Grobas SJ, Méndez J, Lázaro R, *et al.* Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poultry Science 2001;80:1171-9. https://doi.org/10.1093/ps/80.8.1171

INRA - Institut National de la Recherche Agronomique. Alimentação dos animais monogástricos: suínos, coelhos e aves. 2nd ed. São Paulo: Roca; 1999. p.245.

Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. San Diego: Academic Press; 2008.

Leksrisompong N, Romero-Sanchez H, Oviedo-Rondón EO, *et al.* Effect of feeder space during the growing and laying periods and the rate of feed increase at the onset of lay on broiler breeder female reproductive function. Poultry Science 2014;93(7):1599-607. https://doi.org/10.3382/ps.2013-03277

Lightfoot TJ, Skibola CF, Smith AG, *et al.* Polymorphisms in the oxidative stress genes, superoxide dismutase, glutathione peroxidase and catalase and risk of non-Hodgkin's lymphoma. Haematologica 2006;91(9):1222-7.

Lumeij JT. Avian clinical biochemistry. Clinical biochemistry of domestic animals. San Diego: Academic Press; 1997. p.857-83.

Nelson LD, Cox M. Princípios de bioquímica de Lehninger. 6th ed. São Paulo: Sarvier; 2014. p.1336.

Özdoğan M, Akşit M. Effects of feeds containing different fats on carcass and blood parameters of broilers. Journal of Applied Poultry Research 2003;12(3):251-6. https://doi.org/10.1093/japr/12.3.251

Reda FM, El-Kholy MS, Abd El-Hack ME, *et al.* Does the use of different oil sources in quail diets impact their productive and reproductive performance, egg quality, and blood constituents?. Poultry Science 2020;99(7):3511-8. https://doi.org/10.1016/j.psj.2020.03.054

Ribeiro AML, Magro N, Penz Jr AM. Granulometria do milho em rações de crescimento de frangos de corte e seu efeito no desempenho e metabolismo. Brazilian Journal of Poultry Science 2002;4(1). https://doi.org/10.1590/S1516-635X2002000100006

Roll AAP, Forgiarini J, Bavaresco C, *et al.* Desempenho e metabolizabilidade de dietas em codornas alimentadas com níveis crescentes de óleo ácido de soja. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2018;70(4):1282-92. https://doi.org/10.1590/1678-4162-9185

Roy A, Haldar S, Mondal S, *et al*. Effects of supplemental exogenous emulsifier on performance, nutrient metabolism, and serum lipid profile in broiler chickens. Veterinary Medicine International 2010;2010:26204. https://doi.org/10.4061/2010/262604

Santin E, Maiorka A, Macari M, *et al.* Performance and intestinal mucosa development of broiler chickens fed diets containing saccharomyces cerevisiae cell wall. Journal of Applied Poultry Research 2001;10:236-44. https://doi.org/10.1093/japr/10.3.236

Tolosa EMC, Rodrigues CJ, Behmer OA, *et al.* Manual of techniques for normal and pathological histology, 2nd. São Paulo: Manole; 2003. 341 p.

Upadhaya SD, Lee JS, Jung KJ, *et al.* Influence of emulsifier blends having different hydrophilic-lipophilic balance value on growth performance, nutrient digestibility, serum lipid profiles, and meat quality of broilers. Poultry Science 2018;97:255-61. https://doi.org/10.3382/ps/pex303

Upadhaya SD, Park JW, Park JH, *et al.* Efficacy of 1, 3-diacylglycerol as a fat emulsifier in low-density diet for broilers. Poultry Science 2017;9(6):1672-8. https://doi.org/10.3382/ps/pew425

Velasco S, Ortiz LT, Alzueta C, *et al.* Effect of inulin supplementation and dietary fat source on performance, blood sérum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broilerchickens. Poultry Science 2010;89(8):1651-62. https://doi.org/10.3382/ps.2010-00687

Vieira SL. Carboidratos: digestão e absorção. In: Macari, Furlan, Rl, Gonzales E. Fisiologia aviária aplicada a frangos de corte. 2nd ed. Jaboticabal: Funep/Unesp; 2002. p.125-33. ISBN: 85-87632-48-5

Villanueva-Lopez DA, Infante-Rodríguez F, Nájera-Pedraza OG, *et al.* Effect of dietary frying fat, vegetable oil and calcium soaps of palm oil on the productive behavior and carcass yield of broiler chickens. Brazilian Journal of Science 2020;22:4. https://doi.org/10.1590/1806-9061-2020-1310

Zapinga M, Meluzzi A, Sirri F. Effect of dietary supplementation of lysophospholipids on productive performance, nutrient digestibility and carcass quality traits of broiler chickens. Italian Journal of Animal Science 2016;15(3):521-8. https://doi.org/10.1080/1828051X.2016.1192965

Zhao PY, Kim IH. Effect of diets with different energy and lysophospholipids levels on performance, nutrient metabolism, and body composition in broilers. Poultry Science 2017;96(5):1341-7. https://doi.org/10.3382/ps/pew469.