Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2026 / v.28 / n.1 / 001-009

http://dx.doi.org/10.1590/1806-9061-2025-2187

Original Article

Effect of Different Vegetable Oil Sources on Performance, Intestinal Histomorphometry, and Physiological Responses of Japanese Quails

■Author(s)

Valentim JK[I] https://orcid.org/0000-0001-8547-4149
Almeida AA[II] https://orcid.org/0000-0001-7313-4008
Serpa FC[II] https://orcid.org/0000-0002-3747-1688
Burbarelli MFC[II] https://orcid.org/0000-0002-8079-2890
Felix GA[III] https://orcid.org/0000-0002-7739-586X
Calderano AA[IV] https://orcid.org/0000-0002-2282-3580
Braz PH[V] https://orcid.org/0000-0002-5519-061X
Sgavioli S[VI] https://orcid.org/0000-0003-1156-2386
Lima PPABM[VII] https://orcid.org/0000-0003-1881-9551
Komiyama CM[II] https://orcid.org/0000-0001-8732-5745
Caldara FR[II] https://orcid.org/0000-0002-7564-6127
Garcia RG[II] https://orcid.org/0000-0002-4978-9386

*Affiliations

■Mail Address

Corresponding author e-mail address
Jean Kaique Valentim
Federal Rural University of Rio de Janeiro,
Department of Animal Production,
Seropedica, RJ, 23.897-000, Brazil.
Phone: +55 33 9808 7636
Email: kaique.tim@hotmail.com

■Keywords

Quail breeding, growing phase, vegetable oils, villi.

Section Editor: Tatiana Carlesso dos Santos

Submitted: 28/October/2025
Approved: 11/February/2026

ABSTRACT

This study evaluated the effects of different plant-based lipid sources on the performance, serum biochemical profile, meat physical traits, body temperature, and intestinal histomorphometry of Japanese quails during the rearing phase. A total of 400 Japanese quails were assigned to a completely randomized design with five dietary treatments consisting of soybean, corn, cottonseed, sunflower, or canola oil included at 25 g/kg, with ten replicates of eight birds each. Quails fed diets containing cottonseed, sunflower, or canola oil exhibited lower feed conversion ratios compared with those fed corn oil ($p<0.01$), without affecting body or surface temperature, meat quality traits, or serum biochemical parameters ($p>0.05$). In addition, birds receiving cottonseed oil showed a higher villus-to-crypt ratio in the ileum ($p<0.01$). Dietary inclusion of cottonseed oil at 25 g/kg improved feed efficiency and intestinal morphology in Japanese quails during the rearing phase, without inducing adverse changes in serum biochemical indicators related to lipid metabolism.

INTRODUCTION

The energy provided in the diet of poultry stands out for its functions in growth, metabolism, and animal health. One energy source used to meet the nutritional requirements of poultry is lipids, which can be provided through fatty acids derived from vegetable oils or animal fats (Reda *et al.*, 2020)

The performance of growing birds can be influenced by the fatty acid profile of the lipid source included in the diet. The differentiation of lipid sources is a decisive factor in their digestibility in animals, especially in their early years, before the full development of the enzymatic apparatus necessary for their complete digestion (Tancharoenra *et al.*, 2013)

In addition to bird health, the diet provided to poultry can also affect intestinal histomorphometry and microbiota composition (Lankelma *et al.*, 2015; Oriach *et al.* 2016). The intestinal histomorphometry of poultry plays a significant role in the health of the host, shaping the development of the immune system, and the metabolism of dietary nutrients, including fatty acids and glucose, the digestion of complex polysaccharides, and the synthesis of vitamins and bioactive molecules (Lankelma *et al.*, 2015; Oriach *et al.* 2016).

It is believed that different types and amounts of dietary fat can modify poultry performance (Liang *et al.*, 2015). Furthermore, it has been shown that the type (saturated and unsaturated) and amount of dietary fat can influence its metabolites (Agans *et al.*, 2018). Lipids provide several other benefits, such as improving nutrient digestibility by reducing the rate of food passage, which results in a longer exposure time of food with digestive enzymes to the intestinal epithelium (Reda *et al.*, 2020).

According to Musharaf *et al.* (1999), fiber and protein promote greater caloric increments than lipids, indicating that the former can contribute more markedly to heat production after feed intake. As defined by Blaxter (1989), the caloric increment corresponds to the increase in heat production associated with food consumption and nutrient metabolism. In this context, cloacal temperature is a reliable indicator of core body temperature and can be used to assess thermal comfort or stress in poultry (Brown–Brandt *et al.*, 2003). When birds experience thermal discomfort, physiological and productive disturbances may occur, including reduced growth rate, increased water intake, elevated heart rate, and alterations in feed conversion efficiency.

In addition to thermal responses, meat quality characteristics are important indicators of poultry health and well-being. Changes in feed intake, glycogen deposition, and subsequent reductions in meat pH may reflect compromised welfare, either under chronic or acute stress conditions, due to their close association with muscle glycogen metabolism (Gonçalves *et al.*, 2020). In this regard, the inclusion of lipid sources in poultry diets not only improves the nutritional profile of the feed, but may also positively influence pH and colorimetric traits of the meat, which are commonly associated with better physiological balance, improved well-being, and enhanced production efficiency (Barzegar *et al.*, 2020).

There are no previous studies investigating the effects of including different vegetable oil sources in the diet of quails from 21 to 42 days of age. We hypothesized that different plant oil sources would affect the performance and intestinal morphology of quails during rearing. Thus, the objective of this study was to evaluate the performance, biochemical profile, intestinal histomorphometry, physical analysis of the meat, and body temperature of Japanese quails (*Coturnix japonica*) supplemented with different lipid sources of vegetable origin in the diet during the rearing phase.

MATERIALS AND METHODS

Poultry Breeding and Experimental Design

All animal procedures were approved by the Institutional Committee for Animal Use (protocol no. 16/2020) and were conducted in accordance with the ethical guidelines and experimental protocols for the use of live birds established by the Brazilian College of Animal Experimentation.

Four hundred Japanese quails (female), aged 21 days, with an average weight of 82.6 ± 3.7 g were used. The birds were housed in galvanized wire cages measuring 25 × 50 cm, totaling 1,250 cm². The animal density per experimental unit was 156.3 cm²/bird. The Japanese quails were distributed in a completely randomized experiment with ten replications of eight birds in each experimental unit and five treatments, with the dietary inclusion of 25 g/kg of different oils: soybean; corn; cottonseed, sunflower, or canola. The birds were fed isonutritive diets formulated following the recommendations of the National Research Council (1994) for quails during the rearing phase, from 21-42 days (Table 1). Corn starch was used to replace part of the inert ingredient exclusively in cottonseed oil diets, in order to correct differences in metabolizable energy among lipid sources and ensure isoenergetic diets.

Table 1 – Ingredients and calculated nutritional composition of experimental diets for quails during the rearing phase (21--42 days).

Ingredient (%)	Lipid source				
	Soybean	Corn	Cottonseed	Sunflower	Canola
Ground corn	49.229	49.246	49.070	49.070	49.235
Soybean bran	43.351	43.351	43.351	43.351	43.351
Corn starch	0.000	0.000	0.900	0.000	0.000
Inert	2.000	1.99	1.260	2.160	1.990
Lipid source	2.500	2.500	2.500	2.500	2.500
Limestone	1.226	1.214	1.226	1.226	1.226
Dicalcium phosphate	0.893	0.893	0.893	0.893	0.893
Salt	0.320	0.320	0.320	0.320	0.320
DL-methionine	0.165	0.165	0.165	0.165	0.165
L-lysine	0.116	0.116	0.116	0.116	0.116
Mineral premix*	0.100	0.100	0.100	0.100	0.100
Vitamin premix*	0.100	0.100	0.100	0.100	0.100
Calculated nutritional composition					
Metabolizable Energy (Kcal/kg)	2,900.00	2,900.00	2,900.00	2,900.00	2,900.00
Crude protein (%)	24.00	24.00	24.00	24.00	24.00
Digestible lysine (%)	1.340	1.340	1.340	1.340	1.340
Methionine + Cystine (%)	0.894	0.894	0.894	0.894	0.894
Total methionine (%)	0.500	0.500	0.500	0.500	0.500
Calcium (%)	0.800	0.800	0.800	0.800	0.800
Available phosphorus (%)	0.300	0.300	0.300	0.300	0.300
Total phosphorus (%)	0.526	0.526	0.526	0.526	0.526
Sodium (%)	0.150	0.150	0.150	0.150	0.150

[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) 1,850,000.0 IU; vitamin E (Min) 4,500.0 IU; and vitamin K3 (Min) 918.0 mg. [2]Mineral supplement/ kg of diet: 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. *Values expressed per kg of product.

The birds were housed in a masonry shed, 6.0 m long, 2.5 m wide, and 3.5 m high, with a concrete floor and roof with fiber cement tiles, 0.60 m short walls, and a 0.50 m eave in length. It had yellow polyethylene external curtains with manual activation, and two air conditioners to control ambient temperature. The lighting program consisted of 24 hours of artificial light (with the same lamps used as a heating source) until the 21st day of birds' lives, and then a natural photoperiod was used until the 42nd day of age, so that the birds did not enter sexual maturity early.

The experimental rations were offered *ad libitum* three times a day in a trough-type galvanized sheet metal feeder that covered the entire length of the cages. Water was also provided *ad libitum* via a nipple drinker.

The experiment was carried out during rainy summers. The temperature and relative humidity were measured with two digital thermo-hygrometers (Instrutemp, ITHT 2250, São Paulo, Brazil) located at the height of the birds at equidistant points. The average of the maximum and minimum temperatures were measured, with recorded values of 29.8 ± 0.18°C and 17.21 ± 0.31°C, respectively. For the relative humidity, the average of the maximum and minimum were measured, with values of 82.0 ± 2.7% and 45.0 ± 1.2%, respectively.

Lipid Fatty Acid Profile of Experimental Oils

For the identification of lipid fatty acids, samples of the different experimental oil sources were collected, homogenized, and stored at −20 °C to preserve lipid integrity and prevent oxidative degradation. Lipid esterification was performed according to the methodology described by Hulan *et al.* (1989). The resulting fatty acid methyl esters were analyzed using a gas chromatograph (GC-17, Shimadzu®) equipped with a flame ionization detector and a capillary column (CARBOWAX), using hydrogen (H_2) as the carrier gas.

Fatty acids were identified by comparison of retention times with certified standards, and quantification was carried out by peak area integration, using the software connected to the detector. The total amounts of saturated, monounsaturated, and polyunsaturated fatty acids, as well as omega-3, omega-6, and omega-9 fatty acids, were calculated and expressed in grams. Vitamin E content was obtained according to manufacturer specifications.

Descriptive data regarding the energy value, lipid composition, and fatty acid profile of the different experimental oil sources are presented in Table 2.

Table 2 – Nutritional composition of lipid sources of soybean, corn cottonseed, sunflower and canola.

Composition	Lipid source				
	Soybean	**Corn**	**Cottonseed**	**Sunflower**	**Canola**
Total fats (g)	12.00	12.00	12.00	12.00	12.00
Saturated Fats (g)	1.90	1.80	3.40	1.40	1.00
Trans Fats (g)	0.00	0.00	0.00	0.00	0.00
Monounsaturated Fats (g)	2.80	4.40	2.160	4.00	7.50
Polyunsaturated fats (g)	7.30	5.80	6.44	6.60	3.50
Omega-9 (Oleic acid) (g)	0.00	0.00	2.40	0.00	7.50
Omega-3 (Linolenic Acid) (g)	0.70	0.01	0.40	0.05	0.80
Omega-6 (Linoleic acid) (g)	6. 40	5.70	5.52	6.60	0.10
Vitamin E (mg)	0.80	4.30	6.00	7.80	2.8 0

Performance

Weight gain, feed intake, and feed conversion ratio were evaluated from 21 to 42 days of age. Weight gain (WG; g/bird/day) was calculated based on body weights recorded at 21 and 42 days of age, always measured in the morning. Feed intake (FI) was determined as the difference between the amount of feed offered and the residual feed at the end of the experimental period, divided by the number of birds per experimental unit. Feed conversion ratio (FCR) was calculated as the ratio of FI to WG for each experimental unit.

Mortality was recorded daily, and FI and FCR were corrected accordingly by considering the body weight of the birds and the amount of feed present in the feeder on the day mortality occurred, following the methodology described by Sakomura and Rostagno (2015). Viability (%) was calculated for the period from 21 to 42 days of age, based on cumulative mortality. For performance analysis, each experimental unit was considered as one replicate (n=10).

Body Temperature

On the 35th and 42nd days of the study, data related to the temperature of the birds were collected. The evaluation of body temperature was carried out at 01:00 pm, using one bird from each experimental plot, totaling ten repetitions for each treatment at each age. The temperatures of the head, shin, chest, back, and wing were measured via an infrared thermometer (Digital Infrared Thermometer Mira Laser) with a laser sight, 15 cm away from the animal's skin. The cloacal temperature (CT) was determined via a digital clinical thermometer with a rigid tip (iColor- THGTH150B - White - G-Tech), which was introduced into the cloaca of the birds and emitted a sound signal when the temperature stabilized.

The collected data were subsequently used to calculate the average skin temperature (AST) and the average body temperature (ABT) of the birds according to the equation proposed by Richards (1971), considering the surface temperatures and the temperature at the bird's rectum:

ASK = (0.70 BT + 0.12 WT + 0.09 HT + 0.09 ST), where BT = back temperature (°C); WT = wing temperature (°C); HT = head temperature (°C); and ST = shin temperature (°C). ABT = 0.3 ASK + 0.7 CT, in °C, where CT = cloacal temperature (°C).

Serum Metabolites: Biochemical Profile of Blood

Blood was collected on the 42nd day via cardiac venipuncture, before measuring the birds' temperature, using one bird from each experimental plot, totaling ten repetitions for each treatment at each age. To carry out this process, the birds were placed in the supine position, and then a needle was inserted along the ventral floor of the thoracic inlet. The samples were centrifuged to separate the serum and then frozen at -20 °C until biochemical analysis. The levels of cholesterol, triglycerides, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were evaluated via commercial kits from Gold Analisa, according to the manufacturer's specifications. For reading, a COBAS C111®v spectrophotometer (Roche®, USA) was used, following the proper wavelength of each kit. Biochemical examinations and measurements were performed on an automatic analyzer. All the described analyzes were performed in duplicate.

Slaughter of Birds

At 42 days of age, the birds were fasted for 8 hours, with water provided *ad libitum*. After the fasting period, the quails were weighed and identified according to treatment, and 50 birds were selected, with one bird per replicate, totaling ten birds per treatment. Selection was based on a weight range of ±10% of the average body weight, with live weights averaging 146 ± 3.11 g.

The selected birds were humanely slaughtered in accordance with the guidelines of CONCEA. The procedure consisted of cervical dislocation followed by sectioning of the jugular veins and carotid arteries, allowing bleeding for three minutes. Subsequently, the carcasses were scalded by immersion in water at 60 °C for 30 s and manually plucked. After plucking, evisceration and removal of the feet and heads were performed manually.

The carcasses were then cooled with water for 12 min in a pre-chiller at 10–18 °C, followed by immersion in a chiller at 0–2 °C for 18 min. After chilling, samples of the breast muscle, duodenum, jejunum, and ileum were collected for further analyses.

Physical Analysis of the Meat

For the evaluation of physical analysis of the meat, the breast muscles (Pectoralis major) of the slaughtered birds were removed, kept in a cold chamber for 24 hours at 4 ± 1°C, and subsequently taken to the laboratory for analysis. The parameters evaluated were pH at 24 hours, luminosity (L*), red/green content (a*), and yellow/blue content (b*).

The determination of pH at 24 h was carried out following the methodology of Brossi *et al.* (2009), who used a glass body penetration electrode at four different points of the chest muscle, two at the top and two at the bottom. The device was a potentiometer (Oakton, pH 300, series 35618) with automatic temperature compensation.

For the analysis of luminosity (L*), red/green content (a*), and yellow/blue content (b*), a portable colorimeter (Minolta Chroma Meter, Model CR-400) was used. The parameters of the CIElab system were read with an illuminant source D65, calibrated in standard white porcelain (Y=93.7, x=0.3160, and y=0.3323), for the Pectoralis major muscle. The average of three readings obtained at different points of the muscle that were free of injuries was considered the final value for the ventral region, and two were taken each for the cranial and central regions, with the muscle on an opaque surface. This methodology was based on Brossi *et al.* (2009).

Intestinal Histomorphometry

Histological slides were made using 2.0-cm segments from the duodenum (distal portion of the duodenal loop), jejunum (2.0 cm before the ileal diverticulum), and ileum (2.0 cm after the ileal diverticulum), which were fixed in 10% buffered formaldehyde solution for 24 h. They were then stored in 70% alcohol, processed according to the methodology of Luna (1968), and stained through the Hematoxylin-Eosin method. Subsequently, 4-µm thick semi-serial sections were made with an electronic rotary microtome (model RM2255, Leica Biosystems, Buffalo Grove, Illinois, United States).

Images were obtained at fivefold magnification using an optical microscope (model DM4000B, Leica Microsystems, Wetzlar, Hessen, Germany) coupled to a microcomputer. The images were analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland, United States; freeware, https://imagej.net/Welcome). For each intestinal segment and replicate, 20 measurements of villus height, crypt depth, crypt diameter, villus width, and muscle layer thickness were obtained, totaling 160 measurements per segment for each treatment, and 800 measurements for each variable and segment.

Villus height was measured from the basal region of the villus to its apex, while crypt depth was measured from the base of the crypt to the villus–crypt transition region, as described by Fukayama *et al.* (2005). Crypt diameter was measured at the midpoint of the crypt, perpendicular to its longitudinal axis, as an indicator of crypt structural development. The villus height to crypt depth ratio was calculated by dividing villus height by crypt depth.

Statistical Analysis

The data were verified for normality of residues via the Shapiro-Wilk test, and homogeneity of variances via the Levene test. The data were subsequently subjected to an analysis of variance via the MIXED procedure of SAS (SAS 9.3), according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where Y_{ij} is the measured dependent variable, μ is the overall mean, α_i is the effect of the treatment, and ε_{ij} is the random error.

When there was a significant effect, comparisons of means were performed via the Tukey test at the 5% significance level.

RESULTS

During the total rearing period, from 21-42 days, the quails fed cottonseed oil presented greater weight gain (Table 3). However, there was no difference compared to the other treatments, except for the birds fed corn oil (p=0.015). There was no effect of the treatments on the feed intake of the birds (p=0.326). However, the feed conversion rate was lower in quails that received diets with cottonseed, sunflower, and canola oils than in those that received corn oil as a lipid source (p=0.004). The viability in all the treatment groups was also similar (p=0.544) (Table 3).

Table 3 – Performance of Japanese quails fed different lipid sources during the rearing phase (21--42 days).

Variable	Lipid source					SEM	*p*-value
	Soybean	Corn	Cottonseed	Sunflower	Canola		
WG (g/bird)	12.00	12.00	12.00	12.00	12.00	0.79	0.015
FI (g/bird)	1.90	1.80	3.40	1.40	1.00	0.16	0.326
FCR (g/g)	0.00	0.00	0.00	0.00	0.00	0.09	0.004
Viability (%)	2.80	4.40	2.160	4.00	7.50	0.32	0.544

Means followed by different letters in the rows differ statistically by Tukey's test at the 5% probability level. WG: weight gain; FI: feed intake; FCR: feed conversion rate. SEM: standard error of the mean.

There was no effect of different lipid sources on the body or surface temperature of the birds (Table 4), on the qualitative characteristics of the meat (Table 5), or on the serum metabolites of the birds (Table 6).

Table 4 – Body and surface temperature indices of Japanese quails fed different lipid sources.

Variable (°C)	Lipid source					SEM	*p*-value
	Soybean	Corn	Cottonseed	Sunflower	Canola		
Head	26.73	26.10	26.71	26.69	26.61	0.27	0.919
Shin	22.62	22.70	22.18	21.60	22.04	0.39	0.823
Dorsal region	27.09	27.15	27.21	27.15	27.31	0.22	0.996
Wing	27.90	27.15	27.93	27.81	28.09	0.33	0.831
Breast	27.94	27.80	28.65	27.657	28.20	0.33	0.752
Cloaca	39.19	39.14	39.23	39.40	39.73	0.21	0.781
ASK	28.02	28.01	28.21	28.25	28.22	0.22	0.980
ABT	35.84	35.81	35.93	36.26	35.89	0.21	0.885

ASK: average skin temperature. ABT: average body temperature. SEM: standard error of the mean.

Table 5 – Qualitative characteristics of Japanese quail meat with different lipid sources.

Variable	Lipid source					SEM	*p*-value
	Soybean	Corn	Cottonseed	Sunflower	Canola		
L*	47.87	47.52	48.14	47.80	46.72	0.39	0.833
A*	8.92	8.94	8.50	8.72	9.19	0.25	0.934
B*	3.01	2.69	3.13	2.03	2.71	0.22	0.281
pH 24 h	5.63	5.68	5.66	5.75	5.74	0.02	0.065

L*: brightness of black/white color; A*: intensity of green/red color; B*: intensity of blue/yellow color. SEM: standard error of the mean.

Table 6 – Biochemical profile of the blood of Japanese quails fed diets with different lipid sources during the rearing phase.

Variable	Lipid source					SEM	*p*-value
	Soybean	Corn	Cottonseed	Sunflower	Canola		
AST (UI/L)	280.51	366.36	262.070	336.10	328.13	34.04	0.201
ALT (UI/L)	2.21	2.35	2.14	2.47	2.30	0.30	0.125
Cholesterol (mmol/L)	117.30	105.70	114.10	115.80	119.90	11.80	0.932
Glucose (mg/dL)	284.20	282.40	285.30	324.60	287.40	25.32	0.737
Triglycerides (mmol/L)	334.40	368.50	369.70	405.20	421.40	55.42	0.822

AAST: aspartate aminotransferase; ALT: alanine aminotransferase; SEM: standard error of the mean.

Regarding the jejunal segment, the birds fed canola oil exhibited a greater crypt depth, without differing from the birds fed corn or cottonseed oil (*p*=0.009). The villus width and crypt diameter did not differ significantly between the experimental diets (Table 7).

When analyzing the ileum of the birds, there was greater villus height and crypt depth for the birds fed diets containing cottonseed, corn, or canola oil (*p*<0.001). Birds fed canola oil showed a greater villus width compared to the others (*p*<0.001). Regarding crypt diameter, higher values were observed for the birds fed corn or cottonseed oil, without differing from those fed soybean oil (*p*<0.001).

The muscle layer thickness was greater in the birds fed canola oil, without differing from those fed corn oil (*p*<0.001), and the highest villus:crypt ratio occurred in the birds that received the diet containing cottonseed oil, without differing from the other treatments, except for the soybean oil, which presented the lowest ratio (*p*=0.007) (Table 7).

Table 7 – Histological measurements of the ileum and jejunum of quails fed different lipid sources in the diet.

Variable	Lipid source					SEM	*p*-value
	Soybean	Corn	Cottonseed	Sunflower	Canola		
			Jejunum				
Villus width (μm)	158.29	154.71	158.28	161.38	163.95	3.92	0.971
Crypt Depth (μm)	162.68b	164.64ab	183.27ab	153.23b	204.03a	3.35	0.009
Crypt diameter (μm)	61.14	60.95	64.15	62.07	64.19	0.98	0.745
			Ileum				
Villus height (μm)	415.54b	582.22a	618.08a	473.46b	610.00a	2.71	<0.001
Villus width (μm)	83.79b	89.92b	89.40b	90.28b	101.82a	1.02	<0.001
Crypt Depth (μm)	86.83b	110.01a	105.90a	88.379b	104.43a	0.84	<0.001
Crypt diameter (μm)	50.65ab	52.97a	52.67a	48.31b	48.66b	0.34	<0.001
Muscle layer thickness (μm)	22.51d	29.05ab	26.45c	26.95bc	29.41a	0.28	<0.001
Villus: Crypt (μm)	4.88b	5.40ab	6.01a	5.35ab	5.72ab	0.10	0.007

Means followed by different letters in the rows differ statistically by Tukey's test at the 5% probability level. SEM: standard error of the mean.

DISCUSSION

The inclusion of cottonseed oil in the diets of quails resulted in better performance from 21 to 42 days of age when compared to corn oil. The hypothesis tested here relates to the difference found in the performance of birds with the addition of lipid sources, which is primarily related to the higher degree of unsaturation of the fatty acids of the used source. The higher the degree of unsaturation, the greater the potential for micelle formation in the intestinal lumen, thus improving the digestive process and consequently increasing energy values (Reda *et al.*, 2020). El Yamany *et al.* (2008) observed that supplementation with linseed, sunflower, and olive oils improved nutrient digestibility. Although digestibility was not assessed in the present study, these findings support the positive effect of oils with a higher degree of fatty acid unsaturation on nutrient absorption in birds, which can, in turn, influence performance.

The inclusion of lipids in the diet can alter the heat production of the birds, mainly through the caloric increase of each energy source (Rodrigues *et al.*, 2005). However, in the present study, there was no difference in the body temperature of the birds, meaning the tested sources can be used without interfering with heat production. For different handling situations, such as high-temperature environments, the partial replacement of carbohydrates with oils is recommended, as it leads to a reduction in the caloric increment of the feed, which is the metabolic heat generated during the animal's digestive process. This occurs because the caloric increase in lipids is the lowest among nutrients, favoring the thermal comfort of the birds (Macari & Maiorka, 2017).

In the present study, the pH of fresh breast meat varied between 5.63 and 5.74, which is within the normal range for breast pH and indicates good well-being conditions for the birds at slaughter, as previously noted by multiple studies (Genchev & Mihaylov, 2008; Gratta *et al.*, 2019; Mirshekar *et al.*, 2021). According to Mirshekar *et al.* (2021), fresh breast meat exhibited pH values ranging from 5.69 to 5.92, which are considered physiologically normal for breast muscle and are associated with satisfactory bird welfare, in agreement with the findings of Genchev & Mihaylov (2008). Furthermore, an ultimate pH between 5.94 and 6.10 is generally accepted as normal for broiler chicken meat, indicating that quality impairments are unlikely to occur. This finding shows that the addition of plant-based lipid sources to the diet of quails for extended periods does not have a detrimental effect on breast quality.

Several authors (Alagawany *et al.*, 2019; Al-Khalaifah, 2020; De Paula *et al.,* 2021) emphasize the importance of lipid sources in poultry diets, as these nutrients play a key role in the animal's immune defense and contain substances with antioxidant properties, such as vitamins A and E, particularly in canola and sunflower oils. These antioxidant substances neutralize oxidative stress generated during exercise, injuries, diseases, and allergens, blocking the oxidation of phospholipids and proteins essential to cell membranes. These changes may affect the quality of meat, such as luminosity, color, and pH (Abdel-Moneim *et al.*, 2020).

The biochemical parameters in the blood of birds are related to their health. Among these parameters, the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly used to detect tissue lesions in the body, especially liver and cardiac lesions (Schmidt *et al.*, 2007). These enzymes are released into the bloodstream after some modification or cellular damage. Therefore, when lesions occur, AST can be identified quickly in the blood, and ALT can be detected shortly after (Nelson & Cox, 2014). In this study, the concentrations of AST and ALT were within normal parameters, as observed by Yang *et al.* (2021) in a study with laying hens. However, according to these authors, the level of ALT in hens' serum was dropped by feeding cottonseed oil, which commonly indicates improved hepatic function. These findings suggest that lipid sources do not cause liver damage.

Fat intake, as reported by Valesco *et al.* (2010), affects blood concentrations of triglycerides, lipoproteins, and fatty acids, in addition to altering fat and meat composition. The levels of cholesterol, glucose, and triglycerides synthesized in the liver did not differ between treatments. There was no loss of liver function due to the addition of energy sources to the diet, which could result in overload of the organ from increased dietary fat and, possibly, hepatic steatosis (Musigwa *et al.*, 2021). Triglycerides and total cholesterol are generated by the high energy content of diets, and in this case, no changes were observed that could harm the birds (Griminger, 1986).

Although there is limited literature on quails, some studies have evaluated such parameters for broilers. A comparison of sunflower and olive oils in broiler diets and has shown that the total cholesterol level was lower in the group fed sunflower oil, which was not observed in this study (Crespo & Esteve-Garcia, 2023). Another study comparing lipid sources from corn, sunflower, soy, and tallow fat in chicken diets found that HDL and LDL cholesterol levels in blood serum differed, while triglyceride levels did not (Ozdogan *et al.*, 2003). This is important to evaluate because lipids can be transported via the bloodstream and deposited in muscle tissues, fat, and egg yolk. Finally, a study on diets containing corn, flaxseed, sunflower, and olive oil in broiler diets found no differences in triglyceride and HDL concentrations, corroborating the results of this study (El-Yamany *et al.*, 2008).

The performance and digestibility of nutrients depend on the maintenance of gastrointestinal tract structures, including both structural and functional integrity. The development of structures in the small intestine, such as villus height, crypt depth, and villus:crypt ratio, has been investigated due to its relationship with digestion and absorption processes (Boleli & Morita, 2017). The inclusion of cottonseed oil in the diet of Japanese quails during the rearing phase resulted in better intestinal histomorphometry outcomes, as it increased villus height and improved the villus:crypt ratio in the ileum.

Cyclopropenoid fatty acids, present in cottonseed oil, are a class of compounds containing a cyclopropene group, commonly referred to as malvalic and sterculic acids. These fatty acids have been noted to have adverse biological effects in laying hens (Davis *et al.*, 2002; Yang *et al.*, 2021). However, these studies typically use inclusion levels of between 4% and 6% in bird diets. In this study, the inclusion of 2.5% cottonseed oil in quail diets not only improved intestinal histomorphometry, but also did not compromise liver health, as evidenced by the absence of effects on AST and ALT enzymes.

Our hypothesis is that the intestinal histomorphometry improvements in quails are due to cottonseed oil being a source of polyunsaturated fatty acids, mainly rich in linoleic acid (C18:2), which has better absorption characteristics. This is mainly related to the unsaturation level of the fatty acids (Fernandes *et al.*, 2008).

CONCLUSION

Dietary inclusion of cottonseed oil at 25 g/kg improved feed efficiency and intestinal morphology in Japanese quails without adverse effects on serum biochemical indicators related to lipid metabolism.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (Finance Code 001), for their support. We also extend our heartfelt appreciation to the National Council for Scientific and Technological Development (CNPq) for the research grants (PQ 304806/2022-6) and the Early Career Postdoctoral Fellowship (PDJ) – Call No. 2021/2023 (Process: 150188/2022-6) and Call No. 32/2023 (Process: 177286/2023-7), which were instrumental in making this research possible. Also, we thank Fujikura farm – SP for donating the birds used in the experiment.

AUTHOR CONTRIBUTIONS

Valentim JK: formal analysis, investigation, methodology, project administration, writing – original draft preparation, visualization, data analysis; Almeida AA: formal analysis, investigation, methodology, project administration, writing – original draft preparation, visualization, data analysis; Serpa FC: formal analysis, writing – original draft preparation, visualization, data analysis; Burbarelli MFC: conceptualization,visualization, validation, writing – review and editing; Felix GA: conceptualization, visualization, validation, writing – review and editing; Calderano AA: conceptualization, visualization, validation, writing – review and editing; Braz PH: conceptualization, visualization, validation, writing – review and editing; Sgavioli S: conceptualization, visualization, validation, writing – review and editing; Lima PPABM: conceptualization, visualization, validation, writing – review and editing; Garcia RG: conceptualization, funding acquisition, supervision, visualization, validation, data analysis, writing – review and editing.

FUNDING

This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil – Finance Code 001).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER/PUBLISHER'S NOTE

*AFFILIATIONS

[I] Federal Rural University of Rio de Janeiro, Department of Animal Production, Seropedica, RJ, 23897 000, Brazil.
[II] Federal University of Grande Dourados, Faculty of Agricultural Sciences, Dourados, MS, 79823 501, Brazil.
[III] University Center of Grande Dourados, Department of Veterinary Medicine, Dourados, MS, 79823 501, Brazil.
[IV] Federal University of Viçosa, Department of Animal Science, Viçosa, MG, 36570 900, Brazil.
[V] Federal University of Fronteira do Sul, Department of Veterinary Medicine, Realeza, PR, 89815 899, Brazil.
[VI] Pontifícia Universidade Católica do Paraná, Curitiba, PR, 87013 250, Brazil.
[VII] Federal Rural University of Rio de Janeiro, Department of public health, Seropedica, RJ, 23897 000, Brazil.