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REGULAR ARTICLES

Effects of mango peel ethanolic extract as antioxidant in quail diets on performance, carcass traits, and meat lipid stability

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Abstract

The purpose of this study was to evaluate the inclusion of mango peel ethanolic extract (MPEE) as antioxidant in quail diets containing two lipid sources, on performance, carcass characteristics, and lipid stability of *in natura* and frozen meat. A total of 432 meat quails were used, males and females, from 7 to 42 days of age, distributed in a completely randomized design in a 3×2 factorial arrangement, with 3 levels of MPEE (0, 500, and 1000 mg/kg) and 2 lipid sources (soybean and sunflower oil), totaling 6 treatments with 6 replications of 12 birds. In order to evaluate the meat lipid stability, carcass samples were used in a 2×2×2×3 factorial arrangement, with 2 levels of MPEE (0 and 1000 mg/kg), 2 lipid sources (soybean and sunflower oil), 2 types of packaging (conventional and vacuum), and 3 storage times (0, 60, and 120 days), totaling 16 treatments with 6 replications. There was no effect of interaction ($P > 0.05$) between the factors on the performance variables and carcass characteristics. Oil types and MPEE levels did not influence ($P > 0.05$) performance. For carcass characteristics, it was found to be increased ($P < 0.05$) in breast meat yield due to the inclusion of MPEE. For the meat lipid stability, there was only an effect of interaction ($P < 0.05$) between type of packaging and storage time. The inclusion of 1000 mg/kg of MPEE provided greater meat lipid stability, enabling the use of common packaging for the storage of quail meat for up to 120 days.

Keywords Additive · Lipid oxidation · Mangiferin · Meat quality · Natural antioxidant · Vacuum packaging

Introduction

The use of fats and vegetable oils as lipid sources in the feeding of meat-type poultry is aimed at increasing the energy density of the diet and at providing a source of essential fatty acids, improving palatability, absorption of fat-soluble vitamins, and the consistency of the rations (Nogueira et al., 2014).

However, these lipid sources have in their composition high levels of unsaturated fatty acids that can increase the peroxidation of the feed, generating antinutritional and toxic compounds. Feed in advanced oxidative process can present changes in the nutritional quality, reducing the intake by the quails, and may change the lipid composition of the poultry meat, making it more susceptible to lipid oxidation, which can contribute to the reduction product quality (Guven et al., 2015; Mirshekar et al., 2021).

In order to interrupt or mitigate the effects of lipid oxidation in the feed and meat, antioxidants are used, which even at low concentrations help to preserve the feed nutrients and its energy values (Vasconcelos et al., 2014) and can also be transferred to the products, preserving them from oxidative

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damage and increasing their safety (Vargas-Sánchez et al., 2019). Currently, the most used antioxidants by the industry are synthetic, which has generated a lot of concern about their use, as certain products have restricted use in many countries due to the possibility of causing undesirable effects and because they are toxic to living organisms (Braga et al., 2016).

In this scenario, the importance of searching for natural and efficient compounds that act as antioxidants emerges. Freitas et al. (2012) and Freitas et al. (2015) used plant extracts from mango processing residues (*Mangifera indica* L.), as additives with antioxidant capacity in the diet of broilers, since different antioxidant sources are found in these residues, such as provitamin A, vitamin C, vitamin E, and the phenol compound glucosylxanthone in the form of the active ingredient mangiferin. In addition, the utilization of mango peel and seed for the production of natural antioxidants is an excellent alternative for the proper disposal of this waste, adding value to this material and contributing to the strengthening of the fruit production chain (Dorta et al., 2012; Huber et al., 2012).

Therefore, this study was conducted to evaluate the effect of the inclusion of mango peel ethanolic extract (MPEE) as antioxidant in quail diets containing two lipid sources, on performance, carcass traits, and lipid stability of *in natura* and frozen meat stored for 60 and 120 days in two types of packaging (conventional and vacuum).

Material and methods

Experimental design, diets, and quail management

A total of 432 European quails (*Coturnix coturnix coturnix*), males and females, from 7 to 42 days of age were used, being distributed in a completely randomized experimental design in a 3 × 2 factorial arrangement, where the studied factors were 3 levels of MPEE (0, 500, and 1000 mg/kg) and 2 lipid sources (soybean and sunflower oil), totaling 6 treatments, with 6 replications of 12 quails.

The soybean oil was obtained through the extrusion process of soybean grains, and the sunflower oil was obtained by cold pressing sunflower seeds with husk in a mechanical press (ERT 40-V1, Scott Tech, Valinhos, São Paulo, Brazil).

In the formulation of the diets containing different lipid sources and without the inclusion of the extract (Table 1), the composition values the feeds according to Rostagno et al. (2017) were considered, meeting the requirements proposed by Silva and Costa (2009) for quails. The other experimental diets were obtained by replacing the inert material (washed sand) according to the inclusion of the extract in the proportion of each treatment (500 and 1000 mg/kg). The experimental diets were formulated to be isonutrient and isoenergetic.

The diet was offered at will in tube feeders and the water was offered in pressure cup drinkers. For performance evaluation, the quails and diets were weighed at the beginning (7 days of age) and at the end of the experimental period (42 days of age), to obtain feed intake, weight gain, and feed conversion ratio. Daily mortality was recorded to correct feed intake and feed conversion ratio.

Preparation of the mango peel ethanolic extract

The ethanolic extract was prepared from the peel of ripe mango fruits *cv.* Coité and Jasmim, acquired in the natural form from a fruit pulp processing industry. The material was exposed to the sun on a nylon mesh for 48 h to pre-dry. Then, it was dried in a forced ventilation oven at 55 °C for approximately 72 h. During the entire drying period, the material was turned over twice a day, to facilitate dehydration and to prevent the appearance of fungi. Once the material was dry, it was ground.

The preparation of the natural extract of the mango peel was carried out by the cold extraction method, using organic solvents (hexane and ethanol), according to the methodology described by Freitas et al. (2015). Initially, the crushed residue was placed in a glass container, remaining submerged in hexane for a period of 7 days at room temperature. Then, the hexane was removed and subjected to evaporation in a rotary evaporator at 50 °C, with rotation of 60 rpm and reduced pressure to recover the solvent and to obtain the hexane extract. The recovered solvent was used for re-extraction twice, under the same conditions as the initial extraction. The hexane extracts obtained from the three extractions were discarded, and the residues were used for ethanol extraction in a similar way to those described for the hexane extraction. The obtained ethanolic extracts from the mango peel were placed in glass containers and identified for further use.

Determination of antioxidant potential, total phenolics, and total antioxidant activity of the mango peel ethanolic extract

Since the extract was in the gel form, after weighing, it was diluted in the lipid source to be mixed in each diet. Aliquots of the extract were taken to evaluate the antioxidant potential, total antioxidant activity, and determination of phenolic compounds (Table 2), where butylated hydroxytoluene (BHT) was used as a reference. The antioxidant potential of the extract was evaluated by the DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, according to the procedure described by Brand-Williams et al. (1995), with the results expressed as milligrams per liter, referring to half of the maximum inhibitory concentration (IC50). The total antioxidant activity (TAA) was determined through trial with the

Table 1 Proximate composition and calculated nutritional levels of experimental diets containing different lipid sources and without inclusion of extract

Ingredients	Lipid source	
	Soybean oil	Sunflower oil
Corn, grain	54.57	54.75
Soybean meal, 45% CP	39.69	39.66
Soybean oil	2.00	0.00
Sunflower oil	0.00	2.00
Washed sand (inert)	0.76	0.61
Limestone	1.06	1.06
Dicalcium phosphate	1.01	1.01
Common salt	0.37	0.37
Vitamin and mineral premix[1]	0.20	0.20
L-Lys HCl	0.02	0.02
DL-Met	0.27	0.27
Coxistac	0.05	0.05
Nutritional and energetic calculated composition		
Metabolizable energy (kcal/kg)	2950	
Dry matter	90.51	
Crude protein (%)	23.00	
Ether extract	4.56	
Calcium (%)	0.75	
Available phosphorus (%)	0.29	
Digestible lysine (%)	1.14	
Digestible methionine + cystine (%)	0.89	
Digestible threonine (%)	0.78	
Digestible tryptophan (%)	0.26	
Sodium (%)	0.16	
Chlorine (%)	0.29	
Potassium (%)	0.90	
Electrolyte balance (mEq/kg)	218.49	

[1]Composition per kilogram of diet: vitamin A (min), 5,500,000 IU; vitamin B_1 (min), 500 mg; vitamin B_{12} (min), 7500 mcg; vitamin B_2 (min), 2502 mg; vitamin B_6 (min), 750 mg; vitamin D_3 (min), 1,000,000 IU; vitamin E (min), 6500 IU; vitamin K_3 (min), 1250 mg; biotin (min), 25 mg; niacin (min), 17.5 g; folic acid (min), 251 mg; pantothenic acid (min), 6030 mg; cobalt (min), 50 mg; copper (min), 3000 mg; iron (min), 25 g; iodine (min), 500 mg; manganese (min), selenium (min), 100.05 mg; zinc (min), 22.49 g

Table 2 Antioxidant potential, total antioxidant activity, and phenolic compounds of the mango peel ethanolic extract

Antioxidants	DPPH[1] IC50 (mg/L)	ABTS[2] (µM TEAC/g)	Total phenolics[3] (mg GAEq/g)
BHT[4]	289.17	350.83	-
MPEE[5]	48.91	252.64	46.43

[1]2,2-Diphenyl-1-picrylhydrazyl; [2]2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical; [3]phenolic compounds; [4]butyl methyl phenol or butylated hydroxytoluene; [5]mango peel ethanolic extract

ABTS + (2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) free radical, and the result was expressed as micromolars of Trolox equivalent antioxidant capacity (TEAC) per gram of extract (Rufino et al., 2007). The analysis of phenolic compounds was performed through the Folin-Ciocalteu method, expressed as milligrams of gallic acid equivalent (GAEq) per gram of extract (Folin and Ciocalteu, 1927; Mueller-Harvey, 2001).

Carcass trait evaluation

For carcass evaluation, two quails from each experimental unit, one male and one female, were selected at 42 days of age according to the average weight of the plot, and after fasting for 6 h, the quails were weighed, stunned by electronarcosis, euthanized by bleeding, scalded, plucked, and eviscerated. After removing the head, neck, and feet, the carcass was weighed to determine the carcass yield using the weight of the fasting quail. Then, the entire breast, drumstick + thigh, abdominal fat, and liver were cut, separated, and weighed to calculate the cut yield. The yield of breast, drumstick + thigh, and abdominal fat was calculated in relation to the hot carcass weight. The relative weight of the

liver was obtained by the ratio of organ weight to the fasting quail weight.

Meat lipid stability evaluation

To analyze the effect of the lipid sources, antioxidant, and packaging on the meat lipid stability, the drumsticks + thighs and the back of the two quails of each plot were ground in an industrial meat grinder, divided into two sub-samples, identified, frozen in liquid nitrogen at − 80 °C, and kept under refrigeration (− 18 °C), with the first analysis performed after 1 day of storage and two others at 60 and 120 days of storage in conventional and vacuum packaging, for the determination of the concentration of thiobarbituric acid reactive substances (TBARS), as described by Kang et al. (2001). The calibration curve and sample preparation for the determination of meat lipid oxidation (TBARS) were performed using the aqueous acid extraction method according to the technique described by Kang et al. (2001). In a 15-mL tube, approximately 2 g of sample was weighed and homogenized with 6.75 mL of perchloric acid (3.86%) and 18.75 µL of BHT (4.5%). Then, 18 mL of 3.86% perchloric acid was added and the content was homogenized in a Terratec crusher (Tecnal, Piracicaba, SP) for 15 s at high speed. The homogenate was filtered and 0.75 mL was transferred to test tubes, together with 0.75 mL of 2-thiobarbituric acid (20 mM). The tubes were heated in a boiling water bath for 30 min. After cooling down to room temperature, the reading was carried out in a spectrophotometer at 531 nm. The blank used was prepared with 0.75 mL of perchloric acid and 0.75 mL of TBA solution. The number of TBARS in the sample was expressed as micrograms of malondialdehyde per gram of sample.

Statistical analysis

The statistical analysis of the data was performed through the GLM procedure of the SAS software (Version 8.2; SAS Inst. Inc., Cary, NC, US) according to a factorial model. Mean comparison was performed through the SNK test at 5% probability. In the analysis of the lipid stability data of the stored meat, the effect of storage time and types of packaging was added to the model.

Results

Performance evaluation

For the performance variables, no significant effect ($P > 0.05$) of interaction between lipid sources and levels of mango peel ethanolic extract was observed (Table 3). There were also no significant differences ($P > 0.05$) caused by the lipid sources or extract levels on the variables feed intake, weight gain, and feed conversion ratio of the quails.

Table 3 Performance of meat quails fed diets containing two lipid sources and three levels of inclusion of mango peel ethanolic extract from 7 to 42 days of age

Parameter	Feed intake (g/quail)	Weight gain (g/quail)	FCR[1]
Lipid source			
Soybean oil	905.98	242.66	3.74
Sunflower oil	896.06	240.05	3.73
SEM[2]	10.84	2.16	0.04
Levels of inclusion of MPEE[3] (mg/kg)			
0	891.09	240.15	3.71
500	884.22	241.96	3.65
1000	927.74	241.96	3.84
SEM[2]	11.11	1.89	0.04
P-value			
Lipid source	0.5264	0.4202	0.9432
MPEE	0.0625	0.8661	0.0920
Lipid source × MPEE	0.0955	0.8651	0.2270

[1]Feed conversion ratio; [2]standard error of the mean; [3]mango peel ethanolic extract

Carcass trait evaluation

In the evaluation of carcass traits (Table 4), it was found that there was no significant effect ($P > 0.05$) of interaction between the vegetable oils and extract levels on the yields of carcass, breast, and drumstick + thigh, nor on the proportion of fat and liver. There was also no significant effect ($P > 0.05$) of the vegetable oils on these variables. However, with the addition of MPEE, there was a significant difference ($P < 0.05$) for breast yield. Although not differing from each other, at both inclusion levels of 500 and 1000 mg/kg, the proportion of breast increased in comparison to the result obtained for the quails that received the control diet with 0 mg/kg of MPEE.

Meat lipid stability evaluation

In the assessment of meat lipid stability of the quails (Table 5), it was observed that only the interaction between the type of packaging and storage time was significant ($P < 0.05$). It was also found that the type of vegetable oil used did not interfere ($P > 0.05$) with lipid stability; however, the inclusion of the extract significantly influenced ($P < 0.05$) this variable, indicating that the addition of 1000 mg/kg of MPEE in the diet provided greater meat lipid stability.

Table 4 Carcass characteristics of meat quails fed diets containing two lipid sources and three levels of inclusion of mango peel ethanolic extract from 7 to 42 days of age

Parameter	Carcass (%)	Breast (%)	Drumstick + thigh (%)	Fat (%)	Liver (%)
Lipid source					
Soybean oil	71.34	40.97	22.84	2.55	2.75
Sunflower oil	70.82	41.52	23.60	2.29	2.66
SEM[1]	0.42	0.29	0.16	0.11	0.11
Levels of inclusion of MPEE[2] (mg/kg)					
0	71.94	40.36[b]	23.07	2.63	2.81
500	71.12	41.69[a]	23.94	2.38	2.53
1000	70.19	41.70[a]	22.65	2.26	2.78
SEM[1]	0.33	0.35	0.15	0.09	0.17
P-value					
Lipid source	0.4753	0.0646	0.1423	0.4282	0.4970
MPEE	0.1563	0.0007	0.1193	0.6506	0.1747
Lipid source × MPEE	0.5719	0.0838	0.2797	0.6976	0.5069

[1]Standard error of the mean; [2]mango peel ethanolic extract; [a, b]means followed by different letters in the column differ from each other by the SNK test (5%)

Interaction between packaging and storage time

When unfolding the effects of interaction between packaging × storage time (Table 6), it was observed that the storage time increased the meat lipid oxidation; however, in conventional packaging, the difference from first day already appeared at 60 days, while, for vacuum packaging, there was difference only at 120 days of storage, when the meat lipid oxidation was significantly higher than on first day.

Discussion

The use of vegetable oils in poultry feed is a routine practice to achieve the desired nutritional levels in the feed; however, this practice brings other benefits to the quails, such that a greater inclusion of oil benefits the performance of the quails. In addition, the differences between the nutritional value of one type of oil in comparison to another is in the influence that the lipid composition of each one may have on its energy metabolism, reducing or increasing its metabolizable energy value. In this context, the absence of significant influence of the lipid source on the performance variables can be associated with the fact that the same level of the different sources was included in the diet and the energetic contribution of each source to obtain isoenergetic rations was considered.

The results obtained for the effect of the lipid source in the diet on performance are similar to those found in the literature in which no significant influences of the type of vegetable oil in the ration are normally observed on performance, when the metabolizable energy value of the oil is correctly determined and considered for formulation and added in similar amounts. Mirshekar et al. (2021), found no significant difference in the performance between the use of sunflower and canola oil for quails up to 35 days of age.

The absence of a significant effect of the inclusion of the extract on the performance of the quails indicates that the addition of up to 1000 mg of MPEE/kg of diet, although not having a beneficial effect, does not bring any problems to the quails. Freitas et al. (2012) evaluated lower levels of inclusion and found no significant difference in the performance of broilers fed diets containing up to 400 mg of MPEE/kg of diet.

It should be mentioned that the use of plant extracts in poultry feeding has shown variable results on performance, due to the type of poultry, age of the poultry, sanitary challenge, and type and concentration of active compounds in the material used. Hussein et al. (2019) reported benefits in the performance of quails, since quails fed a diet containing 1.5-mL clove oil/kg of feed gained more weight when compared to quails of the control treatment. Dourado et al. (2020) verified that the addition of yerba mate extract, at a level of 1000 mg of extract/kg of feed, increased feed intake and weight gain without influencing the feed conversion of meat quails.

It has often been reported that changes in the relationship between energy and protein, or between energy and amino acids in the feed, can provide changes on carcass yield and even on carcass cuts (Mir et al., 2017). Thus, considering that the diets were calculated to be isoenergetic and isonutrient and that there was no significant change in feed intake, it can be inferred that the vegetable oils alone did not promote changes in the composition and use of nutrients in the diet that could alter these relationships and, consequently, influence the carcass traits. In addition, the improvement in

Table 5 Meat lipid oxidation of meat quails fed diets containing two lipid sources and two levels of inclusion of mango peel ethanolic extract from 7 to 42 days of age

Parameter	TBARS (mg of malondialdehyde/kg)
Lipid source	
Soybean oil	2.631
Sunflower oil	2.632
SEM[1]	0.013
MPEE[2] (mg/kg)	
0	2.650[a]
1000	2.610[b]
SEM[1]	0.008
Packaging	
Conventional	2.653
Vacuum	2.610
SEM[1]	0.009
Storage time (days)	
1	2.128
60	2.234
120	3.532
SEM[1]	0.115
Statistical effects	*P*-value
Lipid source	0.9151
Extract	0.0096
Packaging	0.0029
Storage time	< 0.0001
Lipid source × extract	0.4799
Lipid source × packaging	0.9614
Lipid source × storage time	0.0877
Extract × packaging	0.8086
Extract × storage time	0.6443
Packaging × storage time	0.0051
Lipid source × extract × packaging	0.5548
Lipid source × packaging × storage time	0.7283
Extract × packaging × storage time	0.8082
Lipid source × extract × packaging × storage time	0.8714

[1]Standard error of the mean; [2]mango peel ethanolic extract; [a, b]means followed by different letters in the column differ by the SNK test (5%)

Table 6 Effect of interaction between storage time and types of packaging in the meat conservation of meat quails fed diets containing two lipid sources and two levels of inclusion of mango peel ethanolic extract

Parameter	Storage time (days)		
Packaging	1	60	120
Conventional	2.128[Ac]	2.245[Ab]	3.587[Aa]
Vacuum	2.128[Ab]	2.224[Ab]	3.477[Ba]

Means followed by different capital letters in the columns and lowercase letters in the rows differ from each other by the SNK test (5%)

breast proportion with the use of the extract may be associated with its antioxidant action, contributing to the reduction of oxidative stress in the intestine that can compromise the digestion and absorption of nutrients (Mishra and Jha, 2019) and also reducing oxidative damage in protein synthesis. In some situations, the improvement in carcass traits has been associated with better digestion of dietary amino acids (Rizzo et al., 2010), with the use of plant extracts. According to Chudak et al. (2019), the addition of the extract (*Echinacea pallida*) was able to benefit the quality of the quail's meat, increasing protein deposition in the breast muscle.

It is worth mentioning that the results of some research on the effects of plant extracts on carcass traits have been variable, indicating a specific actions for each product tested, since the mode of action of the evaluated active principles is very diverse, in addition to great variation of experimental conditions, as the levels of the ingredient test, types of diets, and environmental and quail housing conditions. Thus, unlike what was observed for the quails in this research, Freitas et al. (2012) found no significant influence of MPEE on the carcass traits of broiler chickens. Likewise, Dourado et al. (2020) observed that the addition of yerba mate extract at a level of 1000 mg/kg did not influence the carcass traits of quails, although it increased weight gain.

The fatty acid composition of the quail carcass fat was influenced by the vegetable oil used in the feed, so that the intake of unsaturated fatty acids increases their concentration in the meat, making it more susceptible to oxidation (Guven et al., 2015). In this context, the lack of significant difference in the lipid oxidation of quail meat according to the types of oil used can be attributed to the similarity between the lipid composition of the oils, in which linoleic acid predominates (Rostagno et al., 2017). However, Mirshekar et al. (2021) reported that the use of canola oil promoted greater lipid oxidation of quail meat when compared to sunflower oil, which was related to a higher amount of polyunsaturated fatty acids in the carcass due to ingestion of canola oil, which is rich in linolenic acid in comparison to sunflower oil that is rich in linoleic acid.

The greater meat lipid stability of quails fed with MPEE might be due to the antioxidant properties of the compounds present in the mango peel and, consequently, in the extract (Freitas et al., 2013, 2015). Oliveira et al. (2011) cited the presence of phenolic compounds, vitamin C, and carotenoids with proven antioxidant capacity in mango. The antioxidant effect of the mango peel extract was also observed by Pereira et al. (2011), when they compared the effect of the BHT synthetic antioxidant to the ethanolic extract from mango seeds, which was higher by up to 0.2%.

The improvement in the meat lipid stability when using plant extracts has been one of the most reported effects in the literature. Vargas-Sánchez et al. (2019) reported that the use of essential oils from other plant species also used

for feeding resulted in improvement in the lipid stability of quail meat.

The increase in lipid oxidation of quail meat according to the storage time was also observed by other researchers. Sonale et al. (2014) obtained higher TBARS values of breast meat stored in low-density polyethylene bags in a freezer at – 18 °C after 60 days of storage, while Mirshekar et al. (2021) reported that there was greater oxidation of the quail meat after 90 days of storage in the freezer at – 20 C, with the magnitude of the increase in oxidation being related to the change in the carcass lipid composition caused by the oil used in the feed.

As for the type of packaging, the vacuum showed greater efficiency in controlling the meat lipid oxidation at 120 days of storage, being the most suitable for longer periods of storage. The magnitude of the increase in the TBARS value during the storage period was mainly attributed to the degree of oxygen permeability of the packaging material (Sonale et al., 2014). Thus, the removal of oxygen with vacuum and the better quality of the material used in the packaging reduce the lipid oxidation of fatty acids in the meat, as observed in the present research.

Considering that the interaction between the extract and the other factors was not significant and that there was a significant effect of the inclusion of the extract on lipid oxidation, it can be said that regardless of the type of vegetable oil, the type of packaging, and the storage time, the addition of 1000 mg of MPEE per kilogram of diet can benefit the lipid stability of the quail meat. Thus, if the difficulty of access and costs of the vacuum packaging system are taken into account, by reducing the effects of lipid oxidation during storage, the addition of the extract becomes a viable alternative to the use of conventional packaging to store the meat of these quails for up to 120 days.

According to the results obtained, it can be concluded that the use of soybean or sunflower oil in the diets of the meat-type quails did not influence the performance parameters, carcass traits, and meat lipid stability during storage. The mango peel ethanolic extract at a level of 1000 mg/kg, did not influence the performance of the quails; however, it increased the proportion of breast in the carcasses and delayed the meat lipid oxidation during storage, enabling the use of common packaging for the storage of quail meat for up to 120 days.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Davi Moreira Matos, Marcelle Craveiro Abreu de Melo, and Rafael Carlos Nepomuceno. The first draft of the manuscript was written by Davi Moreira Matos and Thalles Ribeiro Gomes; review and editing were performed by Thalles Ribeiro Gomes, Pedro Henrique Watanabe, and Germano Augusto Jerônimo do Nascimento; supervision was performed by Ednardo Rodrigues Freitas; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article; supplementary information can be accessed on reasonable request from the corresponding author.

Code availability Not applicable.

Declarations

Ethics approval That experiments were conducted in a manner that avoided unnecessary discomfort to the animals by use of proper management and laboratory techniques, and the experimental procedures were approved by the Ethics Committee on the Use of Animals, CEUA/UFC, under protocol No. 1729300919, according to the ethical principles adopted by the Brazilian Council for the Control of Animal Experimentation.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no conflict of interest.

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