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Original Article

Ratio of Linoleic and Alpha-Linolenic Acids and Vitamin E: The Productive Performance, Serum Biochemical Profile, and Yolk Composition of Japanese Quail Breeders

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ABSTRACT

Vitamin E in poultry has beneficial effects on productive and reproductive performance. It also acts as an antioxidant, protecting polyunsaturated fatty acids from the omega-3 group, such as alpha-linolenic acid (ALA) and linoleic acid (LA). This paper aims to assess the effects of vitamin E (vitE) in diets containing two LA/ALA ratios on the productive performance, egg quality, egg composition, and biochemical parameters of Japanese quail breeders. A completely randomized design was used in a 2×3 factorial design with two LA/ALA ratios (9.29:1 and 13.75:1) × three vitamin E levels (25, 200, and 250 mg) with 10 replications of 6 females + 2 males. Data was submitted for analysis of variance in a factorial scheme using the Tukey test. There was no significant effect on performance and egg quality with the interactions. Eggs from females fed with an LA/ALA ratio of 9.29 had the lowest percentage of yolk, the highest percentage of albumen, and the highest luminosity. Females and males fed with the 13.75 ratio had higher levels of triglycerides and serum cholesterol. An interaction effect was observed for serum HDL and LDL levels in females. Breeders fed the diet with the 9.29 ratio and 200 mg of vitamin E has increased HDL levels and reduced LDL levels. The treatments had no effect on the bromatological composition of the yolk. In conclusion, the LA/ALA ratio of 9.29 with 200 mg of vitamin E supplementation can be fed to Japanese quail breeders without affecting productive performance. This ratio also can improve the biochemical composition of blood and yolk.

INTRODUCTION

The enrichment of poultry diets with polyunsaturated fatty acids (PUFA), especially alpha-linolenic (ALA) and docosahexaenoic (DHA) fatty acids, has been studied for its potential benefits for productive and reproductive performance, as well as for the enrichment of meat and eggs with ALA. However, these fatty acids are susceptible to oxidation throughout the production process due to the high number of double bonds in their structure. The main plant source used for supplementation with ALA is linseed oil (Mousa *et al*., 2017).

Linseed (*Linum usitatissimum*) has been tested as a source of ALA in the diet of laying hens and quails. Such use may be possible through the conversion of ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Coorey *et al*., 2015; Keum *et al*., 2018; Panaite *et al*., 2021). However, there are some disadvantages to using linseed as a source of ALA to enrich eggs, such as its high susceptibility to oxidation. Thus, diets rich in PUFA increase the need for antioxidant substances. Increased oxidation in ALA-enriched eggs reduces nutritional value and results in an undesirable flavor (Panaite *et al*., 2021). Furthermore, oxidative processes affect their conversion to EPA and DHA in tissues in general. This leads to low supplementation of these acids in the egg, which can affect embryonic development (Shinn *et al*., 2018).

Using natural antioxidants is an alternative to increase the protection of PUFAs, thereby guaranteeing the ability to convert ALA into EPA and DHA by reducing the oxidation of these fatty acids (Njembe *et al*., 2021; Vlaicu *et al*., 2021). Moreover, such use improves antioxidant status (Wen *et al*., 2019) and meets consumer concerns about the safety and toxicity of synthetic antioxidants (Kavtarashvili *et al*., 2018). Vitamin E is a natural antioxidant that has been widely used and is an effective antioxidant for preventing lipid peroxidation in eggs and tissues (Kavtarashvili *et al*., 2018; Untea *et al*., 2020). The antioxidant properties of vitamin E contribute to reducing oxidative stress by protecting and maintaining the oxidation-reduction balance in tissues (Han *et al*., 2019).

Lipid oxidation can occur in meat, tissues, and egg yolks, which causes losses in nutritional value. Furthermore, such oxidation causes the formation of potentially toxic compounds, which compromise product quality, as well as the productive and reproductive performance in poultry (Elwan *et al*., 2019). Natural antioxidants inhibit free radicals from attacking lipids, proteins, and the double bonds of polyunsaturated fatty acids. Thus, they prevent tissue damage and integrity loss in products such as eggs (Alagawany *et al*., 2018).

Supplementing the diet of Japanese quail breeders with *Tagetes erecta* flower extract (3 g/kg) combined with high levels of vitamin E (up to 250 mg/kg) significantly improves yolk oxidative status. The maternal diet increased α-tocopherol and total carotenoid concentrations in the fresh yolk and reduced lipid peroxidation in embryonic and hepatic tissues (Roza *et al*., 2023). These results support the effectiveness of natural antioxidants as a nutritional strategy to promote embryonic health and early progeny quality, even without affecting the post-hatch productive performance of the chicks.

Previous studies have determined that the LA/ALA ratio of 9.29:1 does not affect productive performance and egg quality in quail breeders but improves incubation parameters and chick quality (Silva *et al*., 2024). Nevertheless, there is little information in the literature about the influence of vitamin E in diets containing low LA/ALA ratios for quail breeders. Therefore, this study hypothesized that vitamin E combined with low omega-3/omega-6 ratios could improve performance and reduce lipid oxidation. Therefore, this research investigated the effects of vitamin E in diets containing two LA/ALA ratios on productive performance, egg quality, egg composition, and biochemical parameters of Japanese quail breeders.

MATERIALS AND METHODS

The Ethics Committee for the Use of Animals in Experimentation of the State University of Maringá, Maringá - Paraná state approved the project under protocol number 312406021.

Animals and Handling

A total of 480 Japanese quails (360 females + 120 males) aged 14 weeks were used, being selected by weight (160±1.10 females and 125±1.50g males) and egg laying (90%±5%). The poultry were placed in a laying house equipped with galvanized iron cages (25 × 39 cm) containing a nipple drinker and a feeding trough. The lighting program comprised 17 hours of light (natural + artificial) to maintain stimulation of oviposition in poultry. Feed and water were provided *ad libitum* throughout the experiment.

Experimental Design and Diets

The experimental design used was completely randomized, in a 2 × 3 factorial scheme, with two LA/ALA ratios (9.29:1 and 13.23:1) and three vitamin E levels (25, 200, and 250 mg), with 10 replications with 8 birds each (6 females + 2 males). The source of vitamin E used was tocopherol acetate (500 g/kg of product, Basf®). Refined soybean oil was used as the source of LA, and golden linseed oil (cold-pressed oil from Cisbra) was used as the source of ALA. The diets were formulated based on corn and soybean meal (Table 1), according to the Brazilian poultry and swine table for the composition of ingredients and nutritional requirements for quails in the laying phase (Rostagno *et al*. 2017). Table 2 describes fatty acid content in feed and oils. Vitamin E requirement in the basal diet followed the NRC (1994) recommendation of 25 mg/kg of feed. Table 3 describes the amount of vitamin E supplied in the diet for the level tested.

Table 1 – Composition of experimental diets for laying quails.

Ingredients	LA/ALA ratios	
	9.29	**13.75**
Grain corn	57.45	57.45
Soybean meal (45%)	30.81	30.81
Limestone	6.73	6.73
Dicalcium phosphate	1.17	1.17
Soybean oil	1.49	1.60
Linseed oil	0.11	0.00
Salt	0.30	0.30
L-Lysine (78%)	0.26	0.26
DL-Methionine (98%)	0.44	0.44
Vitamin and Mineral Supplement [1]	0.40	0.40
Calculated composition		
Metabolizable Energy (Mcal/kg)	2.810	2.810
Protein (%)	19.00	19.00
Calcium (%)	2.99	2.99
Available Phosphorus (%)	0.31	0.31
Ether extract (%)	4.21	4.21
Digestible Lysine	1.149	1.149
Digestible methionine + cystine	0.942	0.942
Digestible threonine	0.701	0.701
Digestible tryptophan	0.242	0.242
Digestible isoleucine	0.747	0.747
Chlorine (%)	0.24	0.24
Sodium (%)	0.147	0.147
Electrolyte balance (mEq/kg)	150.05	150.05

[1] Amount per kg of feed - Vitamin A 10,000 IU; Vitamin D3: 2. 000 IU; Vitamin E: 25 mg; Vitamin K3: 3 mg; Vitamin B1: 2.5 mg; Vitamin B2: 6 mg; Vitamin B6: 5 mg; Vitamin B12: 20 mg; Calcium pantothenate: 12 mg; Niacin: 24 mg; Folic acid: 1 mg; Biotin: 0.2 mg; Choline: 300 mg; Zinc: 52mg; Iron: 52mg; Manganese: 60 mg; Copper: 24 mg; Iodine: 1 mg; Cobalt: 0.2 mg; Se: 0.252 mg; Etoxim: 0.1 mg.

Table 2 – Composition of the fatty acid profile analyzed in soybean and linseed oil and in the experimental diets.

Fatty Acid	Soybean Oil	Linseed oil	LA/ALA 9.29:1	LA/ALA 13.75:1
Linoleic acid (C18:2 n-6)	52.6	17.41	12.54	13.58
Alpha linolenic acid (C18:3 n3)	6.94	46.15	1.10	0.90
Arachidonic acid (C20 4)	0.150	0.074	1.25	1.01
Eicosapentaenoic acid (C20:5 n3)	0.003	0.05	0.06	0.05
Docosahexaenoic acid (C22:6n3)	0.005	0.04	0.30	0.10
LA/ALA	7.59	2.61	9.44	13.89

Table 3 – Vitamin E content in the products used in the experimental diets.

Ingredient	Vitamin E mg/kg		
	25	**200**	**250**
Vitamin premix (tocopherol acetate)	0.025	0.025	0.025
Tocopherol acetate (Basf)	-	0.011	0.020
Inert (Kaolin)	0.853	0.842	0.833

Egg Performance and Quality

The quails were evaluated after a 7-day adaptation period to the diets in 3 production cycles of 21 days each. The total experimental period was 63 days. Production performance assessed egg production (%/bird/day), average feed consumption (g/bird/day), feed conversion (FC g/g and g/dozen), and egg mass (g/bird/day). Production performance considered both male and female consumption in the calculations, since there were both males and females in the same cage.

To evaluate egg quality, three eggs from each replication were collected in the last three days of each cycle. The eggs were randomly collected, then identified and weighed on a digital scale with a precision of 0.01 g. Specific gravity (SG) was estimated by immersing each egg in a saline solution with a density ranging from 1.055 to 1.090 g of salt/cm³ of water. To this end, a petroleum densimeter was used according to the method described by Hamilton (1982). The eggs were then cracked on a flat glass surface to measure albumen and yolk height (mm) and yolk diameter using a digital caliper. The Haugh Unit (HU) was determined using equations proposed by Haugh (1937), in which: HU = Haugh Unit, H = height of the thick albumen (mm), and W = egg weight (g).

The yolk index was determined by dividing the yolk height and diameter. Yolk and shell weights were obtained from a digital scale (0.01 g). Shells were previously washed, dried in a forced-air oven for 72 h, and then weighed. Shell thickness (ST) was obtained at 3 spots in the middle of the egg, using a digital micrometer (Mitutoyo Co., Model 700s, Kawasaki, JP). Albumen weight was obtained from the difference between egg weight and yolk + shell weight in each egg. Relative weights of each egg component (yolk, albumen, and shell) were obtained by dividing the components by the egg weight.

The coloration was obtained using a Konica Minolta CR-400 colorimeter, previously calibrated on a white base according to pre-established standards. Coloration was determined directly on the yolk surface, which was placed in a container so that it could be in contact with the equipment without moving, thus maintaining the integrity of the yolk. Yolk color was assessed using the Cielab system for: L* (luminosity), a* (red content), and b* (yellow content).

Serum Biochemistry

At the end of the experiment, blood was collected from the wing vein of 10 females and 10 males per treatment. Blood samples were centrifuged at 3000 rpm (15 min), and serum was aliquoted and frozen for biochemical analyses. Serum levels of cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol, and total triglycerides were determined using a Bioplus biochemical analyzer and reagent kits, following the manufacturer's instructions.

Yolk Composition Analysis

The chemical composition of the yolk was determined in 10 pools of 5 yolks/treatment. Yolk content was isolated from the perivitelline membrane, homogenized, frozen in an ultra-freezer at - 80C, and lyophilized. The methodologies used followed AOAC guidelines (2005), and the contents of dry matter (925-09), mineral matter (923-03), crude protein (920-87), and ether extract (920-85) were determined.

Fatty Acids Analysis

The analyses of yolk, feed, and oil FA composition were conducted qualitatively. Two pools of yolk content were used, and 2 feed samples from each treatment were frozen in an ultra-freezer at - 80C. Soybean and linseed oil samples were analyzed in duplicate. Lipid extracts were obtained from each sample of feed or yolk content by adapting the extraction method described by Bligh & Dyer (1959). 3g of sample was used and distilled water (12ml), methanol (30ml), chloroform (30ml), and another 15 ml of distilled water were added. The solution was stirred for 12 minutes, vacuum filtered in a Buchner funnel, and then the contents were transferred to a separating funnel. Next, the samples were drained and the bottom phase containing chloroform was evaporated. Subsequently, the samples were transesterified in n-heptane and KOH/methanol solution, according to ISO method 5509 (1978). The fatty acid esters were separated in a gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column. Fatty acids were identified by comparing their retention times with Sigma standards, and concentrations were determined by integration of peak areas.

Statistical Analysis

The data were subjected to the Shapiro-Wilks normality test and analysis of variance using the Statistical Analysis System (SAS®) program. The means were analyzed in a 2 x 3 factorial scheme, with two ratios (9.29:1 and 13.75:1) and with 3 levels of vitamin E in the diet (0, 200, and 250 mg/kg), and their interaction. The means of each ratio and vitamin E levels were compared with each other using the Tukey test.

RESULTS

Production Performance and Egg Quality

There was no significant effect of the interaction between LA/ALA ratios and vitE levels or isolated effects on egg-laying rate, feed intake, and feed conversion (Table 4). Regarding egg mass, there was a significant effect on vitamin E levels (p=0.0095), and the 250 mg supplementation presented the lowest egg mass (10.23).

Table 4 – Productive performance of quail breeders fed with different LA/ALA ratios associated with vitamin E concentrations at 14 to 23 weeks (n=10).

LA/ALA ratios	Egg-laying (%)	Feed intake (g/day)	Egg Mass (g)	FCR_1 (g feed/g egg)	FCR_2 (g feed/dozen eggs)
9.29	93.52	24.56	10.51	2.34	315.89
13.75	93.63	24.89	10.42	2.39	319.34
Vit E					
25	93.40	25.05	10.57[a]	2.37	322.21
200	94.33	24.69	10.60[a]	2.33	314.37
250	93.00	24.42	10.23[b]	2.39	315.51
Mean	93.58	24.72	10.47	2.36	317.37
CV %	2.19	3.52	3.89	5.20	3.89
SEM	2.09	0.89	0.43	0.12	12.60
ANOVA			***p*-value**		
Ratio	0.843	0.142	0.402	0.120	0.220
Vit E	0.118	0.076	0.009	0.297	0.106
Interaction	0.232	0.864	0.713	0.897	0.567

[a,b] Different letters differ statistically by Tukey's test (p<0.05); FCR_1 (g feed/g egg) - feed conversion ratio per egg weight; FCR_2 (g feed/dozen eggs) feed conversion ratio per dozen eggs.

As for the variables of internal and external egg quality and egg-yolk color, no significant interactions were observed between LA/ALA and vitE or their isolated effects on egg weight, shell percentage, shell thickness, HU, gravity, and yolk index (p>0.05) (Table 5). In terms of yolk percentage (p=0.005) and albumen (p=0.004), there was an effect of the LA/ALA ratios, whereby there was a lower yolk percentage and a higher albumen percentage for the ratio of 9.29.

Serum Biochemistry

The LA/ALA ratio significantly affected triglycerides and cholesterol levels in the blood of the analyzed Japanese quail breeders. Females fed with the 13.75 ratio had higher triglyceride and cholesterol levels (600.80 and 202.00 mg/dL, respectively) compared to the ones fed with the 9.29 ratio (261.13 and 179.93 mg/dL, respectively) (Table 6).

Table 5 – Internal and external egg quality of Japanese quail breeders fed with different LA/ALA ratios associated with vitamin E concentrations at 14 to 23 weeks (n=10).

	Weight Egg (g)	Yolk (%)	Albumen (%)	Shell (%)	ST (μm)	SG (g/L)	HU	YI	Yolk Color		
									L*	a*	b*
LA/ALA ratios											
9.29	11.24	30.27^{b}	61.68^{a}	8.05	0.221	1070.58	92.53	0.47	54.36	-1.77	33.27
13.75	11.13	30.98^{a}	60.96^{b}	8.06	0.231	1070.25	92.23	0.47	53.84	-1.72	33.31
Vit E											
25	11.32	30.56	61.39	8.06	0.211	1070.74	92.17	0.47	54.21	-1.75	33.03
200	11.14	30.45	61.41	8.14	0.240	1070.23	92.11	0.47	54.09	-1.77	33.59
250	11.10	30.88	61.16	7.95	0.220	1070.28	92.85	0.46	53.99	-1.72	33.24
Mean	11.21	30.62	61.38	8.06	0.220	1070.42	92.38	0.47	54.07	-1.74	33.29
CV %	2.39	3.07	1.52	3.03	4.09	0.16	1.37	3.49	1.73	-10.21	2.24
SEM	0.30	0.99	0.97	0.25	0.01	1.64	1.27	0.02	0.94	0.18	0.77
ANOVA						***p*-value**					
Ratio	0.212	0.005	0.004	0.897	0.414	0.453	0.372	0.948	0.095	0.306	0.823
Vit E	0.088	0.353	0.647	0.062	0.366	0.573	0.131	0.418	0.795	0.689	0.071
Interaction	0.111	0.876	0.980	0.551	0.439	0.636	0.856	0.617	0.721	0.394	0.206

^{a,b} Different letters differ statistically by Tukey's test ($p<0.05$); ST - shell thickness, SG - specific gravity, HU - Haug unit, YI - yolk index, L* - lightness, a* - yellow, b* - blue.

Table 6 – Serum biochemical variables of Japanese quail breeders fed with different LA/ALA ratios associated with vitamin E concentrations.

				Female			
LA/ALA	**Vitamin E**			**Mean**	**ANOVA**		
	25	**200**	**250**		**LA/ALA**	**Vit E**	**Interaction**
				Triglycerides (mg/dL)			
9.29	233.60	292.40	257.40	261.13^{B}	<0.0001	0.0913	0.6902
13.75	550.60	626.00	625.80	600.80^{A}			
Mean	392.1	459.2	441.6				
				Cholesterol (mg/dL)			
9.29	183.40	177.20	179.20	179.93^{B}	<0.0001	0.131	0.8564
13.75	207.60	199.20	199.20	202.00^{A}			
	195.5	188.2	189.2				
				HDL (mg/dL)			
9.29	98.60^{bB}	123.00^{aA}	96.60^{bB}	106.07	<0.0001	<0.0001	0.0196
13.75	84.00^{cC}	93.20^{bB}	85.80^{cC}	87.67			
Mean	91.30	108.10	91.20				
				LDL (mg/dL)			
9.29	84.80^{cC}	54.22^{dC}	82.60^{cD}	73.87	<0.0001	<0.0001	0.0325
13.75	123.60^{aA}	106.00^{bB}	113.40^{aAB}	114.33			
Mean	104.2	80.11	98.00				

^{a,b} Different letters differ statistically by Tukey's test ($p<0.05$); Upper case letters in the column differ between ratios, and Lower case letters in the row differ among vit E levels.

LA/ALA ratios and vitamin E levels had a significant interaction regarding serum HDL and LDL levels. For serum HDL, the unfolding of the interaction showed that the breeders fed the 9.29 ratio and supplemented with 200 mg vitamin E had the highest HDL concentrations. Meanwhile, the breeders fed the 13.75 ratio and supplemented with 25 and 200 mg had lower concentrations. For serum HDL, the unfolding of the interaction demonstrated that the breeders that received the 9.29 ratio feed and vitE 200 mg supplementation had the highest HDL concentrations, while the breeders with 13.75 and 25 and 200 mg supplementation had lower concentrations. The opposite effect was observed in the interaction regarding LDL, in which the mean values decreased in the 9.29 ratio group with 200 mg of vitamin E and increased in the 13.75 ratio group at all supplementation levels (Table 6).

As for the serum biochemistry of the blood of the males (Table 7), a significant effect was observed for triglycerides, cholesterol, and LDL. These parameters had the highest values in quails that received the 9.29 ratio. Vitamin E supplementation affected triglyceride levels, which had the lowest mean at 200 mg and the highest at 250 mg. LDL values also had significant differences, whereby the highest value was with 200 mg and the lowest with 250 mg.

Table 7 – Serum biochemical variables of Japanese quail breeders fed with different LA/ALA ratios associated with vitamin E levels.

Male							
LA/ALA	Vitamin E			Mean	ANOVA		
	25	200	250		LA/ALA	Vit E	Interaction
			Triglycerides (mg/dL)				
9.29	88.60	84.80	97.40	90.26^{B}	<0.0001	0.0002	0.1399
13.75	123.60	106.00	132.80	120.80^{A}			
Mean	106.10^{ab}	95.40^{b}	115.10^{a}				
			Cholesterol (mg/dL)				
9.29	153.20	147.00	157.40	152.53^{B}	<0.0001	0.4917	0.1027
13.75	250.60	250.40	227.40	242.87^{A}			
	201.90	198.70	192.50				
			HDL (mg/dL)				
9.29	95.60	86.00	101.60	94.40	0.8514	0.3840	0.2556
13.75	101.00	94.20	90.80	95.33			
Mean	98.30	90.10	96.20				
			LDL (mg/dL)				
9.29	57.60	61.00	55.80	58.13^{B}	<0.0001	0.0108	0.1583
13.75	149.60	153.20	136.80	147.53^{A}			
Mean	103.60^{ab}	108.60^{a}	96.30^{b}				

a,b Different letters differ statistically by Tukey's test (p<0.05); capital letters in the column differ between ratios, and lower-case letters in the line differ between vitamin E concentrations.

Yolk Composition

The fatty acid levels were analyzed qualitatively. The 9.29 ratio demonstrated an increase in the content of alpha-linolenic acids, DHA, and EPA. For the 13.75 ratio, the highest contents were those of the fatty acids, linoleic acid, and AA, presenting an LA/ALA ratio of 9.66 and 13.70 respectively (Table 8).

For the DM, MM, and CP contents, no significant interaction was observed between the LA/ALA ratios and the vitamin E levels. Regarding the bromatological composition of the yolk, it was observed that the 9.29 ratio decreased the EE content and that the vitamin E supplementation affected the crude protein content of the yolk, in which the highest concentration was found in the 250 mg treatment (Table 9).

Table 8 – Qualitative description of the fatty acid profile of egg yolks from Japanese quails fed with different LA/ALA ratios associated with vitamin E concentrations (n=2/pools).

Fatty Acid	LA/ALA	
	9.29:1	13.75:1
Linoleic acid (C18:2 n-6)	16.93	18.25
Alpha linolenic acid (C18:3 n3)	0.89	0.79
Arachidonic acid (C20:4)	0.37	0.24
Eicosapentaenoic acid (C20:5 n3)	0.07	0.02
Docosahexaenoic acid (C22:6n3)	0.83	0.54
Polyunsaturated	19.09	19.84
N6	17.30	18.49
N3	1.79	1.35
N6/N3	9.66	13.70

Table 9 – Productive performance of quail breeders fed with different LA/ALA ratios associated with vitamin E concentrations at 14 to 23 weeks (n=10).

	DM %	MM%	EE%	CP%
LA/ALA ratios				
9.29	52.14	3.15	56.22^{a}	25.03
13.75	51.60	3.02	54.43^{b}	26.38
Vit E				
25	52.65	3.00	55.83	24.54^{b}
200	51.08	3.05	55.19	24.72^{b}
250	51.88	3.22	54.95	27.84^{a}
Mean	51.87	3.09	55.33	25.70
CV %	5.17	8.87	3.37	8.86
SEM	0.52	0.05	0.46	0.57
ANOVA		***p*-value**		
Ratio	0.6270	0.2533	0.0307	0.1654
Vit E	0.5184	0.2721	0.6261	0.0153
Interaction	0.4610	0.2640	0.288	0.1277

a,b Different letters differ statistically by Tukey's test (p<0.05); DM - dry matter. MM - mineral matter. EE - ether extract. CP - crude protein.

DISCUSSION

Production Performance and Egg Quality

In general. the ratios and levels of vitamin E in the diet of quail breeders did not affect production performance. In line with the present study. Abadi *et al*. (2016) observed that supplementation of up to 240 mg of vitamin E did not affect feed intake and feed conversion. The ratios used did not affect egg-laying rate. daily feed intake. feed conversion (g/g and g/dozen) or egg mass (Table 4). These results are aligned with those by Castro *et al*. (2020), who observed no difference in feed intake when using three ratios of 1; 4; and 10:1 LA/ALA for quail breeders.

The 9.29 LA/ALA ratio decreased yolk weight and increased the albumen weight. Egg yolk is composed of approximately 30% lipids. whose composition varies depending on the age and diet of the hen (Sahan *et al*., 2014). Castro *et al*. (2020) suggest that increasing n-3 PUFA in the diet of laying hens may have adverse effects on yolk weight. since ALA fatty acids are more used to form cell membranes. bone tissue. and eicosanoids. The fat deposition decreases with increased ALA absorption. As a result. the yolk may have a lower concentration of lipids and a higher protein content. thus reducing its weight (Castro *et al*., 2020).

The findings of the present study corroborate the data of Roll *et al*. (2014), who used canola oil and organic selenium in layers and found no significant interaction for any of the performance and egg quality parameters. Therefore, the association between vitamin E and different ratios can be used without affecting the production performance and egg quality of Japanese quail breeders.

Feed intake is regulated by physiological mechanisms, such as intestinal hormone signaling and satiety regulation (Pitargue *et al*., 2019). The LA/ALA ratio and vitamin E levels did not significantly alter the energy density or lipid profile of the diet to a degree that would influence these signals. As a result, feed intake and feed conversion were not affected. Moreover, vitamin E does not have a direct effect on central energy metabolism, which may explain the lack of impact on feed conversion and intake (Prastiya, 2021).

Despite variations in the LA/ALA ratio and vitamin E levels, the metabolism of the quails was able to adjust the synthesis and utilization of essential fatty acids without directly affecting productive performance. The avian liver plays a crucial role in modulating lipid metabolism and can compensate for dietary differences without altering performance parameters. If the variation in the LA/ALA ratio and vitamin E levels did not compromise the bioavailability of lipids and antioxidants for yolk and shell synthesis, the laying rate would not be affected (Sumiati *et al*., 2022).

The reduction in egg mass in response to supplementation with 250 mg of vitamin E may be related to different physiological mechanisms, such as alterations in oxidative balance. Vitamin E possesses antioxidant properties that help protect cell membranes against oxidative stress (Lee *et al*., 2021). However, at high doses, it may exhibit a pro-oxidant effect, impairing cellular metabolism and affecting essential physiological processes such as nutrient deposition in the yolk and albumen. In addition, vitamin E plays a role in regulating the synthesis and secretion of reproductive hormones such as estradiol, which influences egg formation. High doses may lead to hormonal imbalances, reducing the deposition of proteins and lipids in the yolk and albumen (Sumiati *et al*., 2022).

Serum Biochemistry

Triglyceride and cholesterol levels were affected by the change in the LA/ALA ratio in the diet. The decrease in the LA/ALA ratio increases the concentrations of alpha-linolenic acid, DHA, and EPA in the diet of Japanese quails and interferes with the serum concentrations of triglycerides and total cholesterol. In this study. as the concentration of ALA in the diet increased with the inclusion of linseed oil. lipid metabolism was affected due to the increase in the number of carbon bonds in the fatty acids. This is because these fatty acids have a greater affinity for the pathways of triglycerides and cholesterol. Therefore, serum triglyceride. cholesterol. and LDL levels decreased and HDL levels increased for the 9.29 ratio. Saeidi *et al*. (2016) also observed a decrease in the levels of triglycerides, cholesterol, and LDL, as well as an increase in HDL when providing 4 levels of medium-chain fatty acids to Japanese quails.

The change in cholesterol content observed in the present study was probably due to the biochemical action of ALA, which can inhibit the activity of enzymes involved with cholesterol synthesis in the liver. The main liver enzymes that aid cholesterol synthesis are 7 alpha-hydroxylase and beta-hydroxymethylglutaryl-CoA, which transform mevalonate into squalene to produce cholesterol. The supplementation of ALA leads to a decrease in the production of these enzymes and, thereby, a decrease in cholesterol synthesis (Lehninger *et al*., 2014).

ALA can also have hypocholesterolemic effects on LDL production, reducing the activity of hepatic enzymes associated with fatty acid synthesis and therefore the synthesis of very low-density lipoproteins (VLDL) decreases. There is also a decrease in low-density lipoprotein (LDL) levels due to increased biliary and fecal cholesterol excretion; thus decreasing the cholesterol content and the rate of triglyceride synthesis in the liver (Santos, 2001). Such metabolic effect probably occurred in the quails in this study due to the increased concentration of omega-3 due to the inclusion of linseed oil in the diet.

In the present study. LDL levels decreased and serum HDL increased in females supplemented with vitamin E. These results occurred because tocopherols are bound to HDL and LDL lipoproteins in the plasma in order to be transported and absorbed in the body. High levels of LDL can cause several cardiovascular diseases, and its oxidation in the body can increase its toxicity and atherogenicity. Vitamin E is not only transported by this cholesterol, but also helps protect against oxidation and protects arterial walls (Martínez *et al*., 2002).

LDL and HDL levels are influenced by liver activity, whereby polyunsaturated fatty acids in the diet increase the activity related to fatty acid oxidation. The enzyme responsible for increased oxidation is the hepatic enzyme carnitine palmitoyltransferase. In the interaction between the ratios and vitamin E, it was observed that the 9.29 ratio with 200 mg vitamin E presented the best values for LDL and HDL. This may be due to the activation of the enzyme causing a decrease in cholesterol levels derived from omega-3s and a decrease in oxidative activity due to vitamin E (Martínez *et al*., 2002).

In the liver, triglyceride synthesis depends on factors such as the availability of fatty acids and the activity of regulatory enzymes like glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT), which are responsible for forming triglycerides from fatty acids and glycerol (Pitargue *et al*., 2019). Vitamin E can modulate the expression of these genes, reducing hepatic triglyceride synthesis and, consequently, the secretion of very low-density lipoprotein (VLDL), which transports triglycerides into the circulation (Mariana *et al*., 2018).

The vitamin E may increase the expression of genes related to lipid oxidation such as peroxisome proliferator-activated receptor alpha (PPAR-α), which regulates the activity of mitochondrial enzymes responsible for fatty acid breakdown for energy production (Huang *et al*., 2020). With increased β-oxidation, more fatty acids are directed to the mitochondria and degraded, reducing their availability for triglyceride synthesis. This may contribute to lower plasma triglyceride levels and improved metabolic efficiency in birds (Sumiati *et al*., 2022).

Vitamin E and ALA (alpha-linolenic acid) fatty acids have anti-inflammatory properties that directly impact lipid regulation. Chronic inflammation is an important factor in increasing triglyceride and cholesterol levels, as well as in lipoprotein dysfunction (Huang *et al*., 2020). ALA, by reducing inflammatory mediators such as pro-inflammatory cytokines (TNF-α, IL-6), helps improve the lipid profile by reducing hepatic lipid production and enhancing hepatic lipoprotein uptake (Ganeco *et al*., 2020). Vitamin E, by reducing oxidative stress, complements this anti-inflammatory action by helping regulate inflammatory processes that can disrupt lipid production in the body (Pitargue *et al*., 2019). The reduction in inflammation results in a healthier balance of blood lipoproteins, favoring lower LDL and triglyceride levels while promoting an increase in HDL.

When vitamin E is combined with ALA fatty acids in poultry diets, these nutrients work synergistically to improve lipid metabolism in several ways (Sumiati *et al*., 2022). While ALA reduces the production of triglycerides and LDL, vitamin E ensures that these lipids are not oxidized, preserving lipoprotein functionality and improving the efficiency of cholesterol and triglyceride transport (Al Khalaifah *et al*., 2020). This interaction not only results in a reduction in triglyceride and LDL levels, but also promotes an increase in HDL, contributing to a healthier lipid profile (Sumiati *et al*., 2022).

Yolk Composition

The fat content of the yolk was higher in the 9.29 ratio, but no statistical difference was observed for the protein content. Lee *et al*. (2021) found the same results when they fed linseed oil to laying hens and observed a linear increase in the ether extract in the eggs after 2 weeks of the diet. The authors reported that the fat in the diet can be efficiently transferred to eggs, thus replacing crude protein levels in egg composition as the dietary ratio decreases (Lee *et al*., 2021). The same situation may have occurred in the present study.

The crude protein content of the egg yolk increased with the inclusion of vitamin E. Such an increase in protein content may have occurred due to the antioxidant function of vitamin E improving protein quality and stability (Lehninger *et al*., 2014). The stability of lipids and proteins can be affected by the peroxide radical, modifying their biological structure and function (Barbosa *et al*., 2010). Vitamin E improves the intestinal absorption of proteins and lipids in the diet, which can be transferred more easily to the egg yolk (Philippi, 2014).

In the present study, the ratio of fatty acids in the egg yolk was similar to that provided in the diet. This occurred since almost all the fatty acids are transferred to the egg yolk. The yolks from the 9.29 ratio had higher levels of EPA and DHA due to a higher ALA content. Studies using different PUFAS ratios found that as the ratio decreased from 16:1 to 4:1, the levels of DHA, EPA, and ALA and total n-3 increased, in addition to a decrease in the n-6 content in the egg yolk (Khatibjoo *et al*., 2018). Similar to our results, Koppenol *et al*. (2015) included fish oil in the diet of breeders to obtain different ratios of EPA and DHA (1:1. 1:2. or 2:1). The authors reported that all diets enriched with n-3 had lower n-6 concentrations and higher n-3 concentrations in the yolk, resulting in a low LA/ALA ratio in the yolk. In turn, EPA concentrations were similar to dietary EPA supplementation.

Tadesse *et al*. (2023) also observed this increase in ALA, EPA, and DHA after feeding breeders linseed and different natural antioxidants including vitamin E. This increase in the sum of n-3 PUFAs (LNA + EPA + DPA + DHA) in egg yolks is one of the most desirable functions of enriched eggs, which makes them more favorable than regular eggs for human nutrition and for the embryo. This is because in the last week of incubation and on the first day of life there is a proportional increase in LA, ALA, AA, DHA, and total polyunsaturated fatty acids for the embryo to complete its development (Khatibjoo *et al*., 2018; Kralik *et al*. 2019; Shetty *et al*., 2020 *et al*.).

The breeder feed with the 9.29:1 ratio and supplementation of 200 mg of vitamin E can therefore be used, as it improves blood parameters and yolk enrichment without affecting production. Thus, we suggest that these values can be used for heavy breeders and laying hens to improve egg quality.

CONCLUSION

The use of linseed oil to obtain the LA/ALA ratio of 9.29 with the supplementation of 200 mg of vitamin E can be used in Japanese quails without affecting production performance, improving the serum lipid profile and the composition of fatty acids in the egg yolk, especially the polyunsaturated fatty acids of the omega –3 group.

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AUTHOR CONTRIBUTIONS

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DATA AVAILABILITY STATEMENT

The data will be made available upon request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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