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Original Article

Relationship of Linoleic and Alpha-Linolenic Acids on the Productive Performance and Serum Biochemical Profile of Japanese Quail Breeders

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ABSTRACT

Linoleic (LA, 18:2) and alpha-linolenic (ALA, 18:3) acids are omega-6 and omega-3 polyunsaturated fatty acids considered essential to human and animal health. The optimum LA/ALA ratio for the production of fertile eggs in Japanese quail breeders has been little investigated. Thus, this study aimed to assess the effects of LA/ALA ratio on productive performance, egg quality, serum biochemical profile, body composition, and organ weight in Japanese quail. A completely randomized design was used. Birds were distributed into five treatments consisting of different LA/ALA ratios (1.48:1, 4.57:1, 7.63:1, 10.69:1, and 13.75:1), obtained by adding soybean (LA) and flaxseed (ALA) oils to the feed. Productive performance and egg quality were assessed in three cycles of 28 days each. At the end of the experiment, the birds were slaughtered and evaluated for biochemical profile, genital organ weights, and body composition. Data were subjected to analysis of variance and regression ($p<0.05$). LA/ALA ratio had no effect on productive performance or egg quality. Blood cholesterol in females and males and total triglycerides in females showed a quadratic response. Breeders fed the highest level of flaxseed oil (1.48:1) had the best serum levels of cholesterol and total triglycerides. Live weight and relative organ weights were not influenced by LA/ALA ratio. Differences in ash and crude protein levels were observed between groups, with LA increasing the deposition of these nutrients. It was concluded that LA/ALA ratio didn't affect productive performance or egg quality. However, based on the results of body composition, serum triglycerides, and cholesterol, it is recommended to use an LA/ALA ratio of 1.48:1 in diets.

INTRODUCTION

Fatty acids have several physiological functions in birds. These compounds can be divided into essential and non-essential depending on their functionality and the body's ability to synthesize them. In birds, alpha-linolenic acid (ALA) (omega-3) and linoleic acid (LA) (omega-6) are essential fatty acids with important roles in growth and development during embryonic and adult stages. ALA is a precursor of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are necessary for development of the central nervous system, particularly the brain and retinal element. EPA and DHA are crucial in the early stages of fetal development. LA is a precursor of arachidonic acid, which serves as a substrate in the biosynthesis of prostaglandins, thromboxanes, and leukotrienes (Innis, 2008).

For fatty acids to be used by the embryo, they need to be incorporated into the egg yolk, which is only possible when breeders are fed diets containing adequate levels of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). Interestingly, the lipid profile of the yolk is closely related to that of the female breeder diet (Santana, 2018). Yolk fatty acids modulate the lipid metabolism of progeny. Omega-3 fatty acids are mobilized from the yolk sac and incorporated into cell membrane phospholipids during embryonic and postnatal development. Omega-3 and -6 fatty acids also participate in immune system development (Koppenol *et al.*, 2015).

Oils and fats containing essential fatty acids are added to bird diets as a strategy to improve egg quality. Examples of omega-3-rich oils used in animal feed include flaxseed, canola, and fish oils, and the most commonly used omega-6-rich oils are soybean, corn, and rapeseed oils (Bourre, 2005). Several studies investigated the effects of different sources of omega-3 and the optimum omega-6/omega-3 ratio to improve the performance of laying hens and egg quality (Gonzalez-Esquerra & Leeson, 2000; Gadkowski *et al.*, 2011; Fraeye *et al.*, 2012). Supplementation of quail diets with 5% flaxseed oil resulted in an omega-3 content of 3.07% in egg yolk, of which 1.93% was ALA and 0.20% was DHA (Silva *et al.*, 2009).

Another important effect of the omega-6/omega-3 ratio is improving fertile egg production, fertility rate, hatchability, and chick quality in quail. In laying quail, the dietary requirement for LA is 1.046% (Rostagno *et al.*, 2017). However, there is no consensus on dietary requirements for other fatty acids or optimum omega-6/omega-3 ratios. There is a shortage of research particularly on quail breeders. In view of this, this study aimed to assess the effects of the LA/ALA ratio on the productive performance, egg quality, serum biochemical profile, body composition, and organ weight of male and female Japanese quail breeders.

MATERIAL AND METHODS

All experimental procedures were approved by the Animal Research Ethics Committee at the State University of Maringá, Paraná State, Brazil (protocol number 5544170220).

Animals, housing, and handling

The study used 360 Japanese quail aged 15 weeks, with average egg production of 90%, mean female weight of 160 g, and mean male weight of 125 g. Birds were selected by age, batch, egg production. Birds were housed in a conventional laying shed in galvanized wire cages (25 × 39 cm) equipped with nipple drinkers and trough feeders. Feed and water were provided *ad libitum*. The lighting program was 17 h light (natural + artificial) throughout the entire experimental period, which lasted 84 days, divided into three cycles of 28 days each.

Temperature and relative air humidity were measured daily by using a thermohygrometer. Mean maximum (35.63 °C) and minimum (21.2 °C) temperatures and maximum (70.8%) and minimum (38.6%) relative humidity were recorded.

Design and experimental diets

The design was completely randomized, with 5 treatments (diets) and 12 replications (cages) of 6 birds each (4 females and 2 males). Each cage was considered an experimental unit. Productive performance was calculated as a mixed batch, given that cages contained males and females. Treatments consisted of experimental diets containing different LA/ALA ratios (1.48:1, 4.57:1, 7.63:1, 10.69:1, and 13.75:1), obtained by using different proportions of LA-rich (flaxseed) and ALA-rich (soybean) oils, the fatty acid composition of oils table 1. Diets were based on corn and soybean meal (Table 2) the values presented by Rostagno *et al.* (2017) were used as reference for the formulation of the experimental rations).

Table 1 – Fatty acids composition (g/100 g of fatty acids) of soybean and linseed oil.

Fatty acid	Soybean oil	Linseed oil
14:00	0.5	1.1
16:00	16.5	6.8
18:00	11.2	10.9
20:00	0.6	0.4
20:1 n-3	0.05	0.2
18:3 n-3	6.89	62.0
18:3 n-6	52.00	14.8
20:4 n-6	0.6	nd
20:5 n-3	nd	0.1
22:6 n-3	nd	nd
SFA[1]	28.9	19.2
MUFA[2]	30.00	30.5
PUFA[3]	59.49	76.9
n-6	52.6	14.8
n-3	6.94	62.1
n-6/n-3	7.57	0.24

[1]SFA = saturated fatty acid. [2]MUFA = monounsaturated fatty acid. [3]PUFA = polyunsaturated fatty acid. nd = non-detected during fatty acids analysis.

Productive performance and egg quality

Before the beginning of the experiment, birds were allowed 7 days for dietary adaptation. The experimental period lasted 84 days, divided into three cycles of 28 days each. Birds were weighed at the beginning and end of the experiment. The following variables were assessed in each cycle: daily feed intake (g/bird), egg

Table 2 – Ingredient and chemical composition of experimental diets formulated to contain different ratios of linoleic acid (LA) to alpha-linolenic acid (ALA).

Ingredient[1] (%)	LA/ALA ratio				
	1.48:1	4.57:1	7.63:1	10.69:1	13.75:1
Corn	57.445	57.445	57.445	57.445	57.445
Soybean meal (45% protein)	30.806	30.806	30.806	30.806	30.806
Limestone	6.729	6.729	6.729	6.729	6.729
Dicalcium phosphate	1.167	1.167	1.167	1.167	1.167
Soybean oil	0.000	1.105	1.391	1.524	1.600
Linseed oil	1.600	0.495	0.209	0.076	0.000
Common salt	0.301	0.301	0.301	0.301	0.301
L-Lysine (76.5%)	0.260	0.260	0.260	0.260	0.260
DL-Methionine (98.5%)	0.436	0.436	0.436	0.436	0.436
Vitamin–mineral premix[2]	0.400	0.400	0.400	0.400	0.400
Inert (kaolin)	0.853	0.853	0.853	0.853	0.853
Estimated composition					
Metabolizable energy (kcal/kg)	2,810	2,810	2,810	2,810	2,810
Crude protein (%)	19.00	19.00	19.00	19.00	19.00
Calcium (%)	2.99	2.99	2.99	2.99	2.99
Available phosphorus (%)	0.31	0.31	0.31	0.31	0.31
Ether extract (%)	4.21	4.21	4.21	4.21	4.21
Digestible lysine	1.149	1.149	1.149	1.149	1.149
Digestible methionine + cysteine	0.942	0.942	0.942	0.942	0.942
Chlorine (%)	0.24	0.24	0.24	0.24	0.24
Sodium (%)	0.147	0.147	0.147	0.147	0.147
Electrolyte balance (mEq/kg)	150.05	150.05	150.05	150.05	150.05
Omega -3[3]	8.58	3.56	2.25	1.65	1.64
Omega -6	10.89	15.53	16.77	17.54	23.08

[1]All diets were also added with antioxidant Banox® (1 mg/kg diet).

[2]Provided per kilogram of premix: vitamin A. 10.000 IU; vitamin D3. 2.000 IU; vitamin E. 25 mg; vitamin B1. 2.5 mg; vitamin B2. 6 mg; vitamin B6. 5 mg; vitamin B12. 20 mg; vitamin K3. 3 mg; pantothenic acid. 12 mg; niacin. 24 mg; folic acid. 250 mg; biotin. 1 mg; choline. 0.3 g; butylated hydroxytoluene.0.8 mg; zinc. 52 mg; iron. 1 mg; manganese. 60 mg; copper. 0.2 mg; cobalt.0.2 mg; iodine. 1 mg; selenium. 0.25 mg.

[3]Values obtained by gas chromatography (n=4).

mass (g), feed conversion per egg weight (g/g) and per dozen eggs (g/dozen). For correction of feed intake and conversion, dead birds and feed leftovers were weighed and the results were calculated according to the method described by Sakomura & Rostagno (2016).

On the last 3 days of each cycle, 3 eggs were selected per experimental unit based on mean weight, totaling 36 eggs per treatment. The following variables were assessed: mean egg weight (g), specific gravity (g/mL), yolk index (%), Haugh unit (%), shell percentage, yolk percentage, albumen percentage, shell thickness (mm), and yolk color. After collection, all eggs were identified according to treatment and replication. Specific gravity was estimated by immersing the eggs in saline solutions with densities ranging from 1.060 to 1.090 g/cm³, with a 0.05 g/cm³ density interval between solutions, according to the method of Hamilton (1982). Egg data were used to calculate the Haugh unit (HU), as expressed by the equation $HU = 100 \log (h_a + 7.57 - 1.7w^{0.37})$, where h_a is the albumen height (mm) and w is the egg weight (g). The yolk index (YI) was calculated as $YI = (h_y/d_y) \times 100$, where h_y is the yolk height (mm) and d_y is the yolk diameter (mm). Yolk color was measured using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Americas Inc., New Jersey, USA), and results are expressed in CIELab coordinates (L^*, lightness; a^*, redness–greenness; and b^*, yellowness–blueness).

Shells were washed and dried in a forced-air oven at 65 °C for 72 h. After drying, shells were weighed and measured using a digital micrometer (Mitutoyo Co, Model &00s, Kawasaki, JP) to obtain the shell thickness.

Blood collection and determination of viscera weight

At the end of the experimental period, 10 birds per treatment were randomly selected from different experimental units. Birds were weighed, and the maximum volume of blood was collected by venipuncture. Blood samples were subsequently centrifuged to obtain the serum. Serum samples (n =

10 per sex and diet) were subjected to determination of albumin, total cholesterol, and triglycerides. Commercial kits were used (Gold Analisa Diagnóstica Ltd., São Paulo, Brazil) according to the manufacturer's recommendations.

The same females used for blood collection (n = 10) were sacrificed by cervical dislocation following loss of consciousness due to hypovolemia. The liver, heart, spleen, and genital organs (ovary and oviduct) were excised and weighed to the nearest 0.01 g on a semi-analytic digital scale. Viscera weights were calculated by the equation Relative organ weight = (Organ weight ÷ Live bird weight) × 100.

Ovarian follicle diameter (mm) was measured using a digital caliper, and oviduct length (mm) was determined using a tape measure.

Body composition analysis

Determination of body composition was performed on three birds selected according to final mean weight ± 5%. Birds were anesthetized with an inhaled anesthetic (3% isoflurane) and, after loss of consciousness and reflexes, sacrificed by cervical dislocation and frozen whole (with feathers, viscera, feet, and head). After thawing, carcasses were weighed, pre-ground in an industrial mill, and homogenized. Then, samples were dried in a forced-air oven at 55 °C for 72 h and ground again.

Dry matter (925-09), mineral matter (923-03), crude protein (920-87), and ether extract (920-85) were determined according to AOAC methods (2005).

Statistical analysis

Data were subjected to analysis of variance using SAS software (SAS, 2010). Differences were considered significant at $p<0.05$. Regression analysis was conducted using the GLM procedure to determine optimal LA/ALA ratios.

RESULTS

Productive performance

LA/ALA ratio had no effect ($p>0.05$) on egg production, daily feed intake, feed conversion per egg weight, feed conversion per dozen eggs, or egg mass (Table 3). Egg production remained above 90% in all treatments throughout the entire experimental period (15–27 weeks).

Table 3 – Productive performance, egg quality and egg yolk color of Japanese quail fed diets containing different ratios of linoleic acid (LA) to alpha-linolenic acid (ALA).

Variable	LA/ALA ratio					Mean	CV (%)	SEM	*p*-value	
	1.48:1	4.57:1	7.63:1	10.69:1	13.75:1				L	Q
Productive performance										
Egg production rate (%)	90.93	90.29	91.10	91.23	90.84	90.88	0.40	0.163	0.706	0.937
Feed intake (g/day)	24.30	23.86	23.49	24.30	24.15	24.02	1.44	0.155	0.896	0.134
Egg mass (g/day)	9.53	9.45	9.52	9.52	9.62	9.53	0.64	0.027	0.582	0.604
FCR_1 (g feed/g egg)	2.56	2.54	2.48	2.56	2.52	2.53	1.32	0.015	0.640	0.548
FCR_2 (g feed/dozen eggs)	322.29	317.44	311.07	318.97	317.68	317.48	1.28	1.815	0.597	0.212
Egg quality										
Egg weight (g)	10.46	10.52	10.57	10.40	10.61	10.51	0.80	0.027	0.688	0.899
Eggshell (%)	7.65	7.50	7.65	7.45	7.52	7.55	1.20	0.042	0.201	0.775
Yolk (%)	31.12	30.36	30.90	31.34	30.28	30.80	1.51	0.201	0.477	0.552
Albumen (%)	61.24	62.16	61.45	61.21	62.83	61.65	0.79	0.254	0.334	0.624
Shell thickness (mm)	0.207	0.209	0.210	0.210	0.210	0.209	0.00	0.022	0.543	0.744
Specific gravity (g/L)	1067.32	1066.48	1068.08	1066.42	1066.88	1067.03	0.06	0.302	0.603	0.758
Haugh unit	87.46	88.06	87.52	87.62	87.83	87.70	0.28	0.115	0.815	0.925
Yolk index	0.46	0.47	0.47	0.47	0.47	0.47	1.17	0.015	0.570	0.379
Egg yolk color										
Lightness	53.07	53.20	52.83	52.82	53.22	53.03	0.36	0.081	0.966	0.658
Redness–greenness	–1.62	–1.78	–1.86	–1.79	–1.77	–1.76	4.98	0.028	0.279	0.136
Yellowness–blueness	32.79	32.92	32.29	31.65	32.81	32.49	1.62	0.234	0.581	0.441

Abbreviations: FCR_1. feed conversion ratio per egg weight; FCR_2. feed conversion ratio per dozen eggs; CV. coefficient of variation; SEM. standard error of mean; L. linear regression; Q. quadratic regression.

Egg quality and yolk color

There was no effect of LA/ALA ratio on egg weight, yolk percentage, shell percentage, albumen percentage, shell thickness, specific gravity, Haugh unit, yolk index, or yolk color (Table 3). The mean values of these variables were 10.51 g (egg weight), 7.55% (shell percentage),

Table 4 – Serum biochemical variables of 24-week-old Japanese quail breeders fed diets containing different ratios of linoleic acid (LA) to alpha-linolenic acid (ALA).

Variable	LA/ALA ratio					Mean	CV (%)	SEM	*p*-value	
	1.48:1	4.57:1	7.63:1	10.69:1	13.75:1				L	Q
Females										
Cholesterol (mg/dL)	417.33	265.56	304.80	263.00	635.10	377.16	41.68	70.30	0.012	<0.001
Albumin (mg/dL)	148.44	149.33	152.22	173.00	152.29	155.05	6.56	4.55	0.231	0.287
Triglycerides (mg/dL)	1353	1759	0.817	1583	2552	1612	39.28	0.28	0.016	0.001
Males										
Cholesterol (mg/dL)	329.99	265.10	333.50	319.00	605.20	370.56	36.16	59.93	<0.001	<0.001
Albumin (mg/dL)	135.40	150.00	132.60	138.80	155.60	148.48	6.92	4.41	0.270	0.370
Regression equations								R^2		Vertex $\hat{y}$[1]
Cholesterol (females)	$\hat{y} = 0.5589 - 0.09803LA/ALA + 0.0074(LA/ALA)^2$							0.86		6.62
Triglycerides (females)	$\hat{y} = 1.9064 - 0.2563LA/ALA + 0.0216(LA/ALA)^2$							0.52		5.93
Cholesterol (males)	$\hat{y} = 0.4059 - 0.0522LA/ALA + 0.00472(LA/ALA)^2$							0.66		5.52

Abbreviations: CV. coefficient of variation; SEM. standard error of mean; L. linear regression; Q. quadratic regression.

[1] Maximum or minimum $\hat{y}$ value.

30.80% (yolk percentage), 61.65% (albumen percentage), 0.21 mm (shell thickness), 1067.03 g/mL, 87.70 (Haugh unit), and 0.47 (yolk index).

Serum biochemistry

At the end of the experiment, biochemical analyses were performed on serum samples from male and female breeders. For both sexes, LA/ALA ratio did not influence the albumin level. A quadratic effect was observed on total cholesterol and triglycerides. The optimal LA/ALA ratio for total cholesterol was estimated as 5.52 for males and 6.62 for females. The optimum LA/ALA ratio for serum triglycerides in females was 5.93 (Table 4).

Table 5 – Live weight. relative organ weights. and reproductive organ parameters of 28-week-old Japanese quail breeders fed diets containing different ratios of linoleic acid (LA) to alpha-linolenic acid (ALA).

Variable	LA/ALA ratio					Mean	CV (%)	SEM	*p*-value	
	1.48:1	4.57:1	7.63:1	10.69:1	13.75:1				L	Q
Live weight (g)	176.97	168.85	163.68	169.21	169.10	169.56	0.02	2.12	0.082	0.239
Liver (%)	2.86	3.34	3.29	2.91	3.35	3.51	7.73	0.11	0.155	0.572
Heart (%)	0.80	0.85	0.76	0.80	0.81	0.8	3.99	0.01	0.796	0.872
Spleen (%)	0.05	0.07	0.06	0.07	0.06	0.06	13.50	0.004	0.735	0.247
Ovary (%)	3.66	3.73	3.89	3.79	3.56	3.73	3.37	0.06	0.883	0.353
Oviduct (%)	4.35	4.32	4.61	3.74	4.02	4.21	8.17	0.15	0.106	0.505
Ovarian follicle weight (%)										
F1	2.02	2.22	2.11	2.02	1.84	2.042	6.84	0.06	0.149	0.147
F2	1.22	0.98	1.21	1.08	1.09	1.116	8.97	0.05	0.738	0.875
F3	0.50	0.46	0.6	0.54	0.43	0.506	13.23	0.03	0.793	0.259
F4	0.13	0.12	0.2	0.13	0.12	0.14	24.22	0.02	0.811	0.175
Follicle diameter (mm)										
F1	13.14	12.57	13.29	13.43	13.28	13.14	2.56	0.15	0.628	0.917
F2	9.14	8.43	9.86	10.29	8.71	9.29	8.38	0.35	0.688	0.364
F3	5.14	4.71	3.57	6.86	4.28	4.91	25.12	0.55	0.851	0.955
F4	2.42	1.85	3.29	2.29	2.29	2.43	21.75	0.24	0.925	0.483
Oviduct length (mm)	23.77	21.93	24.27	22.85	24.25	23.33	4.24	0.44	0.162	0.390

Abbreviations: CV. coefficient of variation; SEM. standard error of mean; L. linear regression; Q. quadratic regression.

Relative organ weights

The relative weights of the liver, heart, spleen, ovary, and oviduct of females are shown in Table 5. There were no differences in relative organ weights between treatments (p>0.05), but live weight was influenced by LA/ALA ratio (p>0.010). Increasing flaxseed oil concentrations exerted linear decreasing effects on live weight.

Body composition

LA/ALA ratio had a decreasing linear effect on ash content and a quadratic effect on crude protein

Table 6 – Body composition of Japanese quail fed diets containing different ratios of linoleic acid (LA) to alpha-linolenic acid (ALA).

Variable	LA/ALA ratio					Mean	CV (%)	SEM	*p*-value	
	1.48:1	4.57:1	7.63:1	10.69:1	13.75:1				L	Q
DM (%)	90.53	90.06	91.88	91.40	90.50	90.87	0.008	0.332	0.138	0.470
Ash (%)	9.91	8.85	9.01	8.54	8.48	8.96	5.475	0.246	0.002	0.261
CP (%)	66.89	64.14	61.40	64.86	56.80	62.81	6.007	1.679	0.050	0.007
EE (%)	15.25	18.46	20.54	17.96	20.54	18.55	11.394	0.935	0.733	0.611

Regression equations		R^2	Vertex $\hat{y}$[1]
Ash (%)	$\hat{y} = 8.3177 + 0.0882LA/ALA$	0.76	-
CP (%)	$\hat{y} = 61.9217 + 1.1404LA/ALA - 0.1057(LA/ALA)^2$	0.61	5.39

Abbreviations: DM. dry matter; CP. crude protein; EE. ether extract; CV. coefficient of variation; SEM. standard error of mean; L. linear regression; Q. quadratic regression.

[1] Maximum or minimum $\hat{y}$ value.

content (Table 6). No significant differences in ether extract or dry matter contents were observed.

DISCUSSION

Productive performance

LA/ALA ratio did not influence egg production or the productive performance of Japanese quail breeders. These results corroborate those of Khatibjoo *et al.* (2018), who investigated the effects of different omega-6/omega-3 ratios (4:1, 6:1, 8:1, and 16:1) on Ross broiler breeders and did not observe differences in egg laying rate between 30 and 32 weeks. For broilers aged 33 to 35 weeks, the authors observed an effect of fatty acid ratio and age on laying rate, with a decrease in egg production.

In Japanese quail breeders, the intake of different oil sources from the beginning of laying onward may influence laying rate and egg mass. In a study by Al-Daraji *et al.* (2010b), dietary intake of 3% flaxseed and fish oils resulted in higher laying rate, egg mass, and feed conversion in Japanese quail aged 7 to 20 weeks than intake of corn and sunflower oils. The authors used ratios of 251.23:1 (sunflower oil), 0.21:1 (flaxseed oil), 42.94:1 (corn oil), and 0.08:1 (fish oil), and diets were not isoenergetic. In the current study, all experimental diets were isoenergetic and contained 1.6% oil. Here, no differences in laying rate or egg mass were observed between breeders aged 15 to 27 weeks. This might have occurred because oil concentrations were nearly half the values used by Al-Daraji *et al.* (2010b). Thus, it is likely that the amount of oil used here was insufficient to influence performance.

Feed conversion was similar between the treatments. A study on laying hens and quail also reported no differences in feed conversion with supplementation of soybean oil, flaxseed oil, or their mixture (Mendonça, 2013). Feed intake was not influenced by LA/ALA ratio because experimental diets were isoenergetic. Given that intake is controlled by energy levels and that diets did not differ in energy, no effects on feed intake were observed.

As a result, use of different fatty acid ratios did not influence feed intake or palatability. Inclusion of 4% oil or more may affect feed palatability (Costa *et al.*, 2008), but, because lower values were used, this factor was not considered in this study.

The vegetable oils used in experimental diets have high PUFA contents, being highly susceptible to oxidation. Lipid oxidation is the main cause of loss of feed and feed quality. This process affects color, taste, texture, and aroma, leading to the production of toxic compounds and decreasing the nutritional value of feed (Scott *et al.*, 1982). Here, lipid oxidation of fatty acids was probably prevented by the use of antioxidants and adequate production and storage practices. Therefore, feed palatability was not affected by the LA/ALA ratios used in this study.

Egg quality and yolk color

Addition of omega-6 and -3 fatty acids to the feed of Japanese quail breeders did not result in significant effects on external or internal egg quality. In a study by Radwan *et al.* (2012), supplementation of broiler breeder diets with LA/ALA at ratios of 2:1, 4:1, 6:1, 8:1, and 10:1 also did not lead to differences in internal or external egg quality.

Egg weight was not affected by a decrease in dietary LA. However, it is known that egg weight may be influenced by LA content, as well as by dietary proteins and amino acids (Lesson & Summers, 1997). In the present study, given that diets had the same metabolizable energy, crude protein, mineral, and amino acid contents, the decrease in LA was not sufficient to reduce the mean egg weight, as the other nutrients remained unchanged.

In a previous study, Japanese quail breeders aged 8 to 16 weeks were fed 1.5% corn, flaxseed, peanut, sunflower, and soybean oils (Reda *et al.*, 2020). It was observed that peanut and soybean oils afforded the highest egg weight because of their influence on feed conversion rate and intake (Reda *et al.*, 2020). Here, no differences in egg weight were observed, probably because feed intake and conversion were not influenced. Similarly, Santos (2005) observed no decrease in egg weight in laying birds fed commercial diets without oil addition, having corn and soybean bran as exclusive sources of LA and ALA. Changes in egg weight depend on the source and amount of oil added to diets.

Shell weight and thickness were not affected by LA/ALA ratio. One hypothesis is that the lipid content was not sufficiently high to result in bonds with calcium and other minerals, which may lead to the formation of insoluble soaps that prevent nutrient absorption (Burgalli *et al.*, 1999). Here, the same amount of oil was used in all treatments, with no changes in lipid content. The focus of the study was to modify the fatty acid profile by altering proportions of soybean and flaxseed oils.

Egg albumen content is influenced by high-fat diets, which decrease intestinal transit rate, thereby improving nutrient absorption and enhancing the formation of albumen proteins (Keshavarz & Nakajima, 1995). Given the isoenergetic nature and the same oil content of diets, there were no differences in intestinal transit.

In laying hens, supplementation of up to 2% flaxseed oil is not sufficient to modify yolk percentage (Souza, 2007). Accordingly, yolk percentage and yolk index did not differ between treatments, attributed to the lack of changes in lipid deposition in yolk. Although different types of oils were used, this was not sufficient to modify lipid metabolism.

Yolk color also did not differ between treatments. According to Faitarone (2010), differences in yolk color occur with the addition of 5% flaxseed oil. Given that the highest flaxseed oil concentration was 1.6%, no effects on yolk pigmentation were observed.

Inclusion of vegetable oils rich in LA and ALA in quail diets promotes economic gains without causing adverse effects on productive performance, in addition to improving physiological parameters (El-Yamany *et al.*, 2008). Overall, the findings show that all experimental diets were suitable for promoting the egg quality and productive performance of Japanese quail breeders.

Serum biochemistry

A reduction in cholesterol and triglyceride levels was observed with increasing LA/ALA ratios, attributed to the presence of long- and medium-chain fatty acids at high concentrations. These fatty acids influence serum triglyceride and total cholesterol levels, reducing their concentrations in blood. Regardless of the chain size, metabolism is affected (Saeidi *et al.*, 2016). High LA intake promotes reductions in cholesterol and triglycerides. Omega-3 PUFAs (EPA and DHA) have high affinity for the pathways used by triglycerides, causing a decrease in plasma lipid levels. Similar results were found by Radwan *et al.* (2012), who observed that, among Dandarawi chicken breeders fed diets containing different LA/ALA ratios, those supplemented with 4:1 LA/ALA had the lowest cholesterol and triglyceride levels, whereas those fed 10:1 LA/ALA had the highest levels. An increase in dietary omega-3 levels may inhibit lipid synthesis and reduce blood cholesterol and triglycerides (Clarke, 2000; Sanz *et al.*, 2000; Crespo & Esteve-Garcia, 2003).

This shows that the flaxseed oil concentration was sufficient to reduce cholesterol levels in birds, attributed to the presence of dietary omega-3 fatty acids, which inhibit the activity of enzymes responsible for cholesterol synthesis in the liver, such as 7-α-hydroxylase and β-hydroxy β-methylglutaryl-CoA. The former enzyme converts cholesterol into bile acids, whereas the latter participates in cholesterol synthesis by converting mevalonate into squalene (Lehninger *et al.*, 2014).

The reduction in cholesterol levels as a function of low LA/ALA ratios is consistent with reports of laying hens and quail fed diets containing lipid sources rich in unsaturated fatty acids (omega-3) (Al-Daraji *et al.*, 2010; Al-Fadhlee, 2011). This effect occurs because omega-3 and omega-6 fatty acids have hypocholesterolemic action, thereby reducing the levels of low-density lipoproteins (LDL) and modifying the composition of cell membranes and lipoproteins. In addition, high levels of omega-3 fatty acids can increase bile and fecal excretions of cholesterol, reducing the synthesis of very low-density lipoproteins (VLDL) in the liver, lowering the amount of cholesterol in the blood of birds (British Nutrition Foundation, 1994). Females require higher dietary cholesterol levels than males because cholesterol is used for egg formation.

Triglyceride values can be affected by diet, especially fatty acid contents, given that triglycerides are synthesized in the intestinal mucosa and liver through digestion and absorption of fatty acids (Hochleithner, 1994). Addition of omega-3 fatty acids to diets reduces blood VLDL levels, thereby reducing circulating LDL levels and the rate of triglyceride synthesis in the liver (Crespo & Esteve-Garcia, 2003).

Relative organ weight

A linear decreasing effect on quail weight was observed with increasing LA/ALA ratio. This might have occurred because of the high PUFA content of flaxseed oil, which decreases fat mass gain and increases that of lean mass. Studies on mice show that high levels of omega-3 fatty acids provide effective control of peroxisome proliferator-activated receptors, modulating genes involved in fat metabolism (Madsen *et al.*, 2010). No significant effects of treatments on relative organ weights were observed. These results are consistent with those of Katcha *et al.* (2014), who did not find differences in the relative weight of organs of broilers fed different omega-3/omega-6 ratios (1:1, 1:3, 1:5, 1:7; 1:9, and 1:11).

Despite the important contributions of omega-3 and omega-6 fatty acids to the immune system, the relative weight of the immune system organ was not affected. Opposite results were reported by Wang *et al.* (2002). The authors found that hens fed PUFAs had greater spleen weight; such a result might be due to the high oil concentration used (5% sunflower, flaxseed, or fish). Here, a low flaxseed oil concentration was used (1.6%), explaining the differences between studies. It is likely that the oil concentration was insufficient to increase organ weight.

No differences in ovary weight, oviduct weight, follicle weight, or follicle diameter were observed between treatments, showing that flaxseed contents were not sufficient to modify these variables. Egg weight and egg quality variables were also not influenced by treatments, demonstrating that all experimental diets met the needs of birds for the production of fertile eggs.

Body composition

Ash content decreased with increasing dietary LA/ALA ratio, in agreement with the findings of Laurin *et al.* (1985). The authors found that diets containing corn oil (high PUFA concentration) led to a reduction in the ash content of broiler chickens of different ages. High PUFA contents may impair mineral absorption and/or deposition, leading to the formation of insoluble mineral soaps in the intestinal lumen (Duarte *et al.*, 2013).

In this study, LA/ALA ratios modified crude protein and ash deposition. Bruxel (2016) found no differences in ether extract, ash, or crude protein in 42-day-old laying quail, although animals were fed diets with different levels of metabolizable energy provided by different ratios of soybean oil and digestible lysine.

The differences in ash and crude protein contents observed here demonstrate that lipid content is inverse to mineral matter and crude protein contents. Body composition is associated with performance; elucidation of this relationship is crucial for a better understanding of nutritional aspects, such as how nutrients are interrelated and how they behave in relation to deposition (Toledo, 2015).

One of the main factors for improving the productive performance of birds is ensuring adequate protein and fat deposition (Neme, 2006). In this study, a 10% variation in protein content was observed, with the lowest content estimated to be achieved using an LA/ALA ratio of 5.34 and the highest protein content obtained with the diet containing flaxseed oil only. This result may be attributed to the fact that flaxseed oil can reduce fat deposition.

Despite being rich in omega-3 fatty acids, flaxseed oil decreased fat content because of the high oxidation capacity of these acids. The main form of omega-3 fatty acid deposition is via membrane phospholipids, differing from saturated fatty acids, which are deposited as triglycerides in tissues (Pannampalam *et al.*, 2001). In rats, an increase in dietary omega-3/omega-6 ratio led to an increase in peroxisomes and mitochondria, both originating from beta-oxidation of LA. The increase in these organelles may promote the production of carnitine acyl transferase I, which reduces lipid levels and increases lean mass (Moussavi *et al.*, 2010).

CONCLUSIONS

Supplementation of Japanese quail breeders with LA/ALA at a ratio of 1.48 did not have a negative effect on productive performance, egg quality, or relative organ weights (liver, heart, spleen, or genital organs). Treatment improved cholesterol, serum triglyceride, protein, and body ash contents. Therefore, it is recommended to supplement quail breeders with LA/ALA at a ratio of 1.48.

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