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Sources of conjugated linoleic acid and lauric acid inoculated into the eggs of quails and its effects on immunity

Fontes de ácido linoléico conjugado e ácido láurico inoculados em ovos de codornas e seus efeitos sobre a imunidade

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Highlights

CLA 240 mg, at 21 days, decreases the total cholesterol content in the blood.
Levels of total cholesterol and AST increases with the inoculations of CLA and AL.
Inoculation of AL confers higher total cholesterol levels than inoculation with CLA.
CLA can benefit bird health by expressing lower blood cholesterol.
In nutrition *in ovo* can be a technique to improve the immune status of quails.

Abstract

The objective of this study is to evaluate the effects of in-egg inoculation with sources of conjugated linoleic acid (CLA) and lauric acid (AL), going by the weight of lymphoid organs and the biochemical profile of the blood of cut quails, from one to 35 days of age. We used 360 quails distributed in a completely randomized design, in six treatments and six replicates of 10 birds per plot. Corn oil (OM) was used to dilute the CLA and AL. The experimental treatments were: healthy eggs (control); eggs inoculated with OM thinner; eggs inoculated with CLA 120 mg / 50 mL OM; eggs inoculated with CLA 240 mg / 50 mL OM; eggs inoculated

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with AL 60 mg / 50 mL OM and eggs inoculated with AL 90 mg / 50 mL OM. The results were analyzed through Program R at the level of 5% significance. To verify the normal distribution of errors, the Shapiro-Wilk normality test was applied. At 21 days, an effect of $p < 0.05$ was observed for the total blood cholesterol content, inoculations with CLA reduced, even as AL increased, regardless of the level. At 35 days, it was found ($p < 0.05$) that the levels of total cholesterol and AST in the blood decreased with inoculations of CLA and AL at the lower levels, compared to those that did not receive the inoculations. The CLA and AL levels supplemented via egg inoculations did not interfere with the weight of the lymphoid organs (thymus, Fabricius bursa) of the birds, at both ages. Supplementation in eggs of cut quails with CLA 240 mg reduced the total blood cholesterol content compared to CLA 120 mg, at 21 days of age. At 35 days of age, higher concentrations of CLA and AL increased the total cholesterol and AST in the blood of birds and AL had a greater effect on the increase than CLA.
Key words: Fatty acids. Coturniculture. Immunology. Nutrition *in ovo.* Inoleic acid. AL: Acid lauric.

Resumo

O objetivo deste trabalho foi avaliar os efeitos da inoculação in ovo com fontes de ácido linoleico conjugado (CLA) e ácido láurico (AL), sobre os órgãos linfoides e o perfil bioquímico do sangue de codornas de corte de um a 35 dias de idade. Foram utilizadas 360 codornas distribuídas em delineamento inteiramente casualizado, em seis tratamentos e seis repetições de 10 aves por parcela. Os tratamentos experimentais foram: ovos íntegros (controle); ovos inoculados com diluidor OM; ovos inoculados com CLA 120 mg/50mL OM; ovos inoculados com CLA 240 mg/50mL OM; ovos inoculados com AL 60 mg/50mL OM e ovos inoculados com AL 90 mg/50mL OM. Aos 21 dias observou-se efeito ($P<0,05$) para teor de colesterol total no sangue, as inoculações com CLA reduzem, enquanto o AL aumenta independentemente do nível. Os resultados foram analisados pelo programa R no nível de significância de 5%. Para verificar a distribuição normal dos erros, foi aplicado o teste de normalidade Shapiro-Wilk. Aos 21 dias, observou-se efeito ($P < 0,05$) para o teor total de colesterol no sangue, inoculações com CLA reduzida, enquanto a AL aumenta independentemente do nível. Aos 35 dias, verificou-se ($P < 0,05$) que os níveis de colesterol total e AST no sangue diminuíram com as inoculações de CLA e AL em níveis mais baixos, comparados àqueles que não receberam inoculações. Os níveis de CLA e AL suplementados por inoculações de ovos não interferiram no peso dos órgãos linfoides (timo, bursa de Fabricius) de aves em ambas as idades. A suplementação em ovos de codornas de corte com CLA 240 mg reduz o conteúdo total de colesterol no sangue em comparação com o CLA 120 mg aos 21 dias de idade. Aos 35 dias de idade, concentrações mais altas de CLA e AL aumentam o colesterol total e AST no sangue das aves e o AL tem um efeito maior em aumentar o CLA sanguíneo.
Palavras-chave: Ácidos graxos. Coturnicultura. Imunologia. *Nutrição in ovo.*

Introduction

The use of immuno-stimulants is one of the courses used to improve the immunity of animals, to decrease their susceptibility to infectious diseases (Coelho et al., 2010). The immune response of birds can be modulated by the characteristics of their diet, in which small changes in the nutritional levels or ingredients used can make the bird more or less susceptible to diseases, as in the case of some potential fatty acids, such as conjugated

linoleic acid (CLA) and lauric acid (LA) (Barbosa et al., 2010).

Conjugated linoleic acid derives from linoleic acid, being an isomerady compound, present in large quantities in milk lipids and ruminant muscle tissue (Preuss et al., 2013). It is able to stimulate the synthesis of immunoglobulins and reduce the production of pro-inflammatory cytokines, thus modulating the inflammatory response (Dilzer & Park, 2012). Lauric acid, in turn, is abundantly found in coconut oil and has immunological activities, which are described in several studies as antiviral, antibacterial, and anti-protozoan properties (Donaldson, Madziva, & Erlwanger 2016). The nutritional management of a diet can act as a modulator to produce specific responses, naturally strengthen immunity, and thus improve the performance of birds in the face of health challenges present in the breeding system (Damasceno et al., 2017). According to Pinto et al. (2014), the current interests revolve around n-3 fatty acids that can act by inhibiting the synthesis of inflammatory mediators derived from achydodon acid. This tool can be used not only against immune-depressive pathogens, but also to maintain the health status of high-production birds, without overloading the defense system (Groff-Urayama et al., 2019). The ability of the immune system of birds can be evaluated by measuring the development of some organs, in which their weight reflects the body's ability to produce defense cells during a challenge, and their increase occurs in the presence of the antigenic stimulus and in stressful situations (Adedokun & Olojede, 2019).

Usually, stress conditions manifest with different degrees of involution of the lymphoreticular system, and the release of corticosteroids can cause lymphoid tissue involution and suppression of humoral and cellular immunity (Verma, Yadav, & Haldar, 2017).

Thus, the verification of lymphoid organs such as the spleen, Fabricius bursa, and thymus, are of fundamental importance, because the maturation process of B and T lymphocytes occurs in their compartments, (Agina, Ezema, & Iwuoha, 2017). The present study aims to evaluate the development of organs involved in the immune activity and the blood biochemical profile of quails at 21 and 35 days of age, after supplementation of the egg with CLA and AL.

Material and Methods

The experiment was carried out in the Incubator and in the Laboratory of Monogastric Animal Research, both belonging to the Animal Science Department of the Federal University of Jequitinhonha and Mucuri (UFVM) Valleys, Diamantina, MG, Brazil. The research was approved by the Commission of Ethics in the Use of Animals (CEUA) of UFVJM, with protocol No. 026/2017. The experimental periods consisted of the incubation phase, with duration of 18 days, and growth phase of the birds, which were evaluated from 1 to 21 and 22 to 35 days of age.

Incubation of eggs: Eight hundred and thrity-nine fertile quail eggs were obtained from the 29-week-old batch of European lineage from the "Fujikura quail farm", located in Suzano, SP - Brazil. The individual weight of each egg was computed to calculate the yield of the quail chick. An incubator, brand COPEMARQ, model Labo 13, previously disinfected and equipped with automatic controls for temperature control, humidity, and turning of the eggs, was

used. For CLA supplementation, the product Lipo-6 CLA, commercial brand, from Nutrex Research, with a concentration of 1000 mg was used, and for the supplementation of LA, extra virgin coconut oil of commercial origin was used, with the packaging of 200 mL, belonging to the brand Copra Coco.

On the seventh day of incubation the eggs were inoculated with the experimental treatments. After disinfection of the injection site with ethanol, the eggs were punctured and then injected with 0.05 mL of the supplement, using 1 mL disposable syringes, in the albumin region, approximately 3 mm below the shell. The duration of the inoculation procedure for each treatment was approximately 40 minutes. Thus, to ensure that all treatments were subjected to the same period of time outside the incubator, the intact eggs that were comprised in this treatment were also removed from the machine and exposed to the same environment, to standardize withdrawal of the incubator in all treatments. At day 15 of incubation, all eggs were transferred to the hatchers where they remained until hatching, for another two or three days.

At birth, the selected birds were housed in galvanized wire cages, containing a trough-type trough, a drinking cup-type water cooler, and 100 watt incandescent lamps for heating. In this phase, 360 quails were used in a completely randomized design, in six treatments, with six replicates of ten quails per plot, applying the same treatments defined in the incubation phase. The supply of water and feed to the quails was at ease.

For the composition of the treatments with supplement of fatty acids, the commercial oils were weighed in the proportions needed and diluted in 50 mL of the diluent (corn oil (CO)). The experimental treatment followed the following pattern: T1: Control group, without inoculation; T2: Eggs inoculated with diluents CO; T3: Eggs inoculated with CLA 120 mg / 50 mL CO; T4: Eggs inoculated with CLA 240 mg / 50 mL CO; T5: Eggs inoculated with LA 60 mg / 50 mL CO, and T6: Eggs inoculated with LA 90 mg / 50 mL CO. The rations were deveoped based on the conventional diets of the species, meeting the nutritional requirements recommended by Silva & Costa (2009), both for the initial breeding phase (1 to 21 days) with composition of 25% crude protein, 2,900 kcal kg^{-1}, calcium 0.85, nonphytase *p* 0.32%, sodium 0.17%, digestible lysine, metionine + cystine, threonine, and tryptophan, 1.37, 1.04, 1.04, and 0.27, respectively. For the growth stage (22 to 35 days) a composition of 22% crude protein, 3,000 kcal kg^{-1}, calcium 0.70, nonphytate *p* 0.27%, sodium 0.15%, digestible lysine, metionine + cystine, threonine, and tryptophan, 1.08, 0.80, 0.78, 0.24, respectively.

As a form of a sanitary challenge to stimulate the immune system, it was decided not to regularly clean the drinking fountains and the floor of the cages (lined with newspaper), and the lack of execution of the schedule of vaccines indicated for the species.

The variables evaluated were weight of the lymphoid organs (thymus, spleen and bursa of Fabricius) and blood biochemical factors: alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), triglycerides, total cholesterol, and high density lipoproteins (HDL). For the measurement of weight of the lymphoid organs and blood biochemical factors at 21 and 35 days of age, two birds were removed from each experimental unit. The birds were identified, weighed individually, and submitted to a six-hour food fast. Soon after, they were slaughtered by cervical detachment,

the cuts and separation of lymphoid organs were done, and expressed in grams. All organs were weighed and discarded later.

For the blood collection procedure, a 3 mL disposable syringe was used. The quails were contained by immobilization, lightly punctured in the neck to identify the best positioning of the judging vein, and approximately 1.5 mL of blood was collected from each animal. The collected blood was stored in eppendorf tubes, identified according to the bird and the plot, and then packed in support for the rest of the period, at room temperature for about 30 minutes, in order to form the serum. After this period, the samples in the eppendorf tube were centrifuged at 3000 rpm for 10 minutes and finally brought to freezing point at -20ºC, and stored until the moment of analysis.

The enzymatic-colorimetric method (Trinder) was applied, using Labtest reagent kits, for quantititative determination of the serum. In the analysis of the variables ALT and AST, the kinetic-UV method was used. The kits used for all analyses were belonged to the brand Gold Analisa Diagnóstica Ltda, and labor-to-labor procedures on the package leaflet's instructions, according to the manufacturer, were followed. All samples were read in the semi-automatic biochemical analyzer model PW-3000M, from the Pioway Medical Lab. All procedures of the laboratory analysis were supervised by pharmaceutical technicians.

The results were analyzed through the program R (R Core Team [R], 2017) at a level of 5% significance. To verify the normal distribution of errors, the Shapiro-Wilk normality test was applied. The homoscedasticity was evaluated by the Bartlett test and the independence of the errors was evaluated by the Durbin-Watson test. The nonparametric Kruskal-Wallis test was used for variables that did not satisfy the assumptions. The data were subjected to analysis of variance (ANOVA), between the treatments, as well as the orthogonal contrasts, to compare the results obtained from the birds that received the control treatments, with each of the levels of supplementation of the tested fatty acids (CLA and LA) inoculated in the eggs.

Results and Discussion

The absolute weight of the lymphoid organs (thymus, spleen, and bursa of Fabricius) of the birds that received in-egg inoculation with diluent (CO), CLA, and AL, were compared with those that did not receive them (control treatment, T1), and they showed no significant effect ($p > 0.05$) at 21 and 35 days of age (Table 1).

Table 1
Means and standard deviation of lymphoid organs of cut quails supplemented in egg with CLA and LA at 21 and 35 days of age

	21 days		
	Thymus (g)	Spleen (g)	Bursa of Fabricius (g)
Control (with out diluent)	0.31±0.09	0.08±0.01	0.15±0.01
CO	0.32±0.09	0.10±0.02	0.19±0.05
CLA 120 mg	0.37±0.16	0.09±0.02	0.16±0.04
CLA 240 mg	0.29±0.13	0.08±0.01	0.15±0.04
LA 60 mg	0.33±0.07	0.09±0.03	0.18±0.04
LA 90 mg	0.33±0.13	0.10±0.02	0.17±0.02
P value	0.924	0.346	0.572
CV (%)	36.32	25.99	24.00
	35 d		
	Thymus (g)	Spleen (g)	Bursa of Fabricius (g)
Control (with out diluent)	0.44±0.16	0.13±0.03	0.30±0.03
CO	0.32±0.18	0.11±0.04	0.35±0.12
CLA 120 mg	0.37±0.16	0.14±0.03	0.31±0.05
CLA 240 mg	0.43±0.14	0.12±0.02	0.31±0.07
AL 60 mg	0.49±0.11	0.13±0.02	0.34±0.02
AL 90 mg	0.55±0.13	0.12±0.02	0.34±0.03
P valor	0.228	0.596	0.608*
CV (%)	36.06	36.06	23.83

CO: Corn oil; CLA: Conjugated linoleic acid; AL: Lauric acid; CV: Coefficient of variation (%).*Kruskal-Wallis nonparametric test.

There was no significant effect ($p \geq 0.05$) on the biochemical profile of quails who received in-egg inoculation treatments with CLA and LA or diluent (OM), and birds without inoculations (control treatment), except for the total cholesterol level at 21 days, and total cholesterol and AST at 35 days of age (Table 2).

The weight of the lymphoid organs is often used as a parameter to indicate the immune status of birds and its increase occurs in the presence of antigenic stimulation and stress (Bloy et al., 2017). However, the lack of significant effects on the weight of the lymphoid organs of the quails in the present study, which received CLA and LA inoculations in the eggs, can possibly be attributed to the sanitary challenge applied in the breeding environment, which was not sufficient interference for such effects to be expressed, or, the levels of inoculations with CLA and LA were insufficient to trigger considerable responses in the development of the immune system of birds.

Table 2
Biochemical serum profile of cut quails supplemented in egg with CLA and LA at different ages

	21 days				
	Cholesterol (mg dl)	HDL (mg dl)	TRI (mg dl)	AL (mg/dl)	AST (mg dl)
Control (with out diluent)	171.26±24.78*	58.07±19.16	116.32±31.72	14.51±7.73	162.81±21.06
CO	159.04±16.62	43.12±30.67	127.40±33.22	10.46±4.52	159.90±35.76
CLA 120 mg	180.98±36.60	63.19±17.45	105.59±18.75	11.12±2.72	178.59±21.44
CLA 240 mg	147.37±29.94*	52.02±27.19	120.13±25.88	8.93±1.41	173.42±17.04
AL 60 mg	148.46±13.94	54.72±18.06	101.61±16.80	10.82±3.47	184.76±31.48
AL 90 mg	167.53±35.50	53.64±22.39	117.39±21.78	9.69±1.78	167.13±28.37
P value	0.0261**	0.4440	0.1506	0.3224	0.2145
CV (%)	16.00	42.31	22.19	38.64	15.75
	35 days				
	Cholesterol (mg dl)	HDL (mg dl)	TRI (mg dl)	ALT (mg dl)	AST (mg dl)
Control (with out diluent)	181.34±21.70*	76.81±25.04	102.31±15.19	10.60±3.03	203.63±44.49*
OM	173.31±23.51	80.02±21.42	117.31±24.44	9.58±1.91	202.51±19.55
CLA 120 mg	163.60±20.80	63.13±18.14	102.86±27.51	10.47±2.09	149.58±33.23
CLA 240 mg	180.18±17.33	60.21±24.70	99.31±14.29	10.53±1.60	185.43±25.73*
AL 60 mg	189.03±36.02	69.47±19.06	112.74±20.52	11.42±2.25	176.40±34.50
AL 90 mg	218.83±27.75*	73.72±19.98	134.93±39.99	11.34±1.86	209.42±30.75*
P valor	0.0005	0.1992	0.0686	0.2406	0.0003
CV (%)	24.00	30.57	22.00	20.54	17,15

CO: Corn oil; CLA: Conjugated linoleic acid; AL: Lauric acid; HDL: High Density Lipoproteins; TRI: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase. *Significant at the level of 5%, by the F test (P<0.05). **Kruskal-Wallis test; CV: Coefficient of variation (%).

Table 3
F test of orthogonal contrasts of total cholesterol and AST of the serum of quails supplemented in egg with CLA and LA at 35 days of age

	Total cholesterol (mg dl)	AST (mg dl)
Control x LA 90	0.0006	0.6693
Injected CO x LA 90	0.0001	0.6100
CLA 120 x CLA 240	0.0005	0.0195
LA 60 x LA 90	0.005	0.0195
CLA 120; 240 x LA 60; 90	0.0001	0.0105
Control x CLA 120	0.0992	0.0002
Control x LA 60	0.9128	0.0470
Injected CO x CLA 120	0.3637	0.0002

CO: Corn oil; CLA: Conjugated linoleic acid; AL: Ácido lauric.

Dilzer and Park (2012) state that the specific use of CLA has the potential to stimulate the development of lymphoid organs, such as bursa and thymus, as this fatty acid influences lymphocytic proliferation and consequently increases antibody productivity, corroborating with the study by Białek, Stawarska, Bodecka, Białek and Tokarz (2017), who described a spleen increase in rats that had a supplementation of CLA in their diet. Mehr, Hassanabadi, Mirghelenj and Kermanshasi (2014) verified an increase in the relative weight of Fabricius bursa and the thymus in broilers at 21 and 42 days of age when they studied the inoculation of CLA in eggs, at levels of 150 and 300 mg, and suggested that CLA could influence the mediation of immunity cells in broilers. The lymphoid organs did not show any difference between the treatments tested, but in contrast, Taves, Hamden and Soma, K. K. (2017) emphasized that chronic stress could lead to increased serum concentrations of corticosteroids, which could cause atrophy of the thymus and bursa, by a mechanism known as apoptosis or programmed death of the lymphoid cells.

On the other hand, Saeidi, Shokrollahi, Karimi and Amiri-Andi (2016), evaluating the effects of medium-chain fatty acids (caproic acid < 3%, caprylic acid = 30%, cupric acid = 56%, lauric acid = 10%, other fatty acids = 0.03%) added to diets of Japanese quails at 42 days of age, with the inclusion of 0%, 1%, 2%, and 4% substitution of soybean oil in the diet, did not observe differences in the weight of the liver, spleen, and bursa of Fabricius.

It was observed that the quails that received in-egg inoculations with CLA 240 mg had a lower content (147.37 mg dl) of total cholesterol in the blood compared to those inoculated with CLA 120 mg (180.98 mg dl), at 21 days of age. This result could be justified by the effect that CLA had on the animal organism. Among other roles, it reduced the lipid levels through its potential for alteration in the gene expression of lipogenic enzymes in lipid metabolism. Alvarez-Curto and Milligan (2016), reported that polyunsaturated fatty acids played a relevant role in the immune system, and pointed out that Arachidonic acid-derived eicosanoids modulate inflammatory and immune responses.

Even though the mechanism of action is not yet clearly elucidated, CLA has been shown to regulate lipids in metabolism through the binding and activation of PPAR-α, which is a receptor protein, during transcription processes, and it is a significant regulator of lipid metabolism in the liver, leading to reduced concentrations of triacylglycerol and cholesterol (Chen et al., 2019). The assumptions associated with CLA and lipids in their metabolic activity are, the decrease in the proliferation and differentiation of preadipocytes, decreased esterification of fatty acids in triacylglycerols, increased energy expenditure, increased lipolysis, altered activity of carnitine palmitoyltransferase and lipoprotein lipase enzymes, and leptin hormone concentration, among others (Lei, Xiaoyi, & Fuchang, 2017).

Lower total circulating cholesterol concentrations were also verified by Mehr et al. (2014) when conducting in-egg inoculation of CLA (150 and 300 ppm) in broilers, and the authors reported a decrease in the level of serum LDL with 300 ppm. Mean plasma levels of total cholesterol (199.25 ± 17.55 mg dl), triglycerides (192.50 ± 55.59 mg dl), and HDL (110 mg dl) were observed by Ali et al. (2012),

in adult Japanese quails, and he reported a range of variations, from 186.00 to 225.00 mg / dl of total cholesterol. The quails in the present study, even when not inoculated, presented a mean of total cholesterol below that estimated by these authors, but corroborating with the range described.

In other studies, the authors Krupakaran (2013) and Kabir (2013), observed lower levels of total cholesterol in the blood of Japanese quails in adulthood, as obtained in this research, with values of 105 ± 4.45 mg dl; 144 ± 2.58 mg dl, and 116.00 mg dl, respectively. Agina et al. (2017), observed a mean value of 78.57 ± 2.95 mg dl in laying quails. Such divergent measures indicate that not only CLA, but LA and OM also generate influences on serum lipid readings, and also on the breeding model. Krupakaran (2013) points out that for adult Japanese quails, triglyceride values ranged from 153.00 to 273.00 mg / dl. The measured means of triglycerides in all groups of birds sampled in this research presented values above that estimated by the authors.

It was observed that there was a difference ($p < 0.05$) in diluent (CO) and AL 90 inoculation, and AL increased the total cholesterol levels. Saeidi et al. (2016) obtained beneficial effects by using a mixture of medium-chain fatty acids, (among them lauric acid) in the diet of Japanese quail, at 42 days of age, and observed that low-density lipoprotein, triglycerides, and total cholesterol concentrations were reduced and high-density lipoprotein was increased with the inclusion of these fatty acids, compared to the control group (soybean oil only).

Among the treatments that received the same fatty acid, an effect was also observed as a function of the level of inoculation ($p < 0.05$), and higher concentrations of AL and CLA resulted in increased total cholesterol levels. When comparing the effect between AL and CLA on total cholesterol, AL inoculation conferred higher serum levels than when CLA was inoculated (189 and 218 versus 163 and 180 mg dl, respectively). It is known that CLA participates effectively in lipid metabolism and may influence a decrease in blood cholesterol levels, which is attributed as a beneficial effect when provided to birds.

With advancing age, at 35 days, it can be noticed that an increase in cholesterol values measured in birds that were inoculated with CLA 240 mg, had a reverse effect, when compared to the same group of birds at 21 days, which led to a consideration that the shelf life of CLA supplementation in the body had a limited effect. The beneficial activities of AL, commonly introduced by coconut oil, have been reported in several studies, however, a similar behavior was not verified in this study, which led to the need to explore higher levels of supplementation in order to obtain perceptible responses in the body.

According to the results obtained by Donaldson et al. (2016), for serum metabolic health parameters (uric acid, triglycerides, cholesterol, total protein, albumin, aspartate transaminase, and bilirubin), constant supplementation of a hyperlipidemic diet enriched with 22% coconut oil, for 12 weeks, for Japanese quails, was not significantly different from those birds that received a standard diet. The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed in the present study, to determine the possible liver damage of birds.

There was an effect ($p < 0.05$) between treatments, for AST present in the blood of

quails, when evaluated at 35 days. It presented a reduction in the contents of this enzyme with the inoculation of CLA 120 mg and AL 60 mg, compared to healthy eggs and compared to treatment containing only the dilutor (OM) with CLA 120 mg. Birds from non-inoculated eggs (control treatment) presented higher means of AST (203.63 ± 44.49 mg dl) compared to CLA 120 and AL 60, with mean values of 149.58 ± 33.23 and 176.40 ± 34.50 mg dl, respectively. This may suggest the interference of CLA and LA is a modulator of immune activities, acting on inflammatory responses in the body, according to reports by Krinski et al. (2010). High levels of AST indicate liver or muscle damage, however, the absence of the measurement of creatinine kinase activity makes it difficult to complete the origin of the damage (Dolan, Gualano, & Rawson, 2019). However, for Japanese quail, Agina et al. (2017) reported that the level of ALT found was 20.85 IU / L and of AST was 59.99 IU / L. The levels were lower than those obtained in the present study. It was observed that the inoculated fatty acids also showed a difference between the groups (CLA versus AL) in terms of dosages. Birds born from eggs inoculated with CLA reduced the AST content in the blood, when compared to those that were inoculated with AL.

Inoculating CLA 120 mg can be perceived as a reduction in AST content when compared to CLA 240 mg, and with respect to AL, the highest concentration (90 mg) promoted the increase of AST in the blood. Therefore, to avoid the occurrence of deleterious effects on the liver, it is suggested to use the lowest levels of CLA and AL in in-egg inoculations, although, it is also necessary to measure creatinine kinase to complete the origin of the damage.

According to Dourado et al. (2017), high cholesterol levels, compared to other values found in the literature, were associated with an increase in AST, which could indicate metabolic impairments and hepathological pathologies. The toxic effect of high concentrations of AL and CLA was reported when they were offered in inadequate proportions in the diet of Japanese quails and broilers (Longato, Meineri, & Peiretti, 2017). The data established as reference values for the cut-off of the serum profile of quails was scarce, thus making it difficult to compare results and diagnose organ health. Much of the existing research considers the biochemical parameters under different experimental conditions, usually varying the diet offered or the breeding environment, which can naturally influence the results.

The health challenge performed in this study aimed to obtain the physiological responses of birds by the activation of the immune system, due to unfavorable sanitary conditions of the environment. However, it was possible that it was insufficient, because it did not modify the weight of the lymphoid organs, even with the inoculation of fatty acids (CLA and LA).

The proposal to provide such nutrients early is to advance resources to combat the adversities of the breeding systems and promote better living conditions for animals. However, further studies are needed to verify whether the level of CLA and AL supplementation has the same potential action when offered in-egg, and whether or not they are related to the increased immune response in quails which can be better elucidated by reading blood immunoglobulins.

Conclusion

The CLA and AL levels supplemented via egg inoculations do not interfere with the weight of the lymphoid organs (thymus, Fabricius bursa) of birds, at both ages. Supplementation in-eggs of cut quails, with CLA 240 mg, reduces the total blood cholesterol content compared to CLA 120 mg at 21 days of age. At 35 days of age, higher concentrations of CLA and AL increase the total cholesterol and AST in the blood of birds and AL has a greater effect on the increase than CLA.

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