R. Bras. Zootec., 54:e20240083, 2025
https://doi.org/10.37496/rbz5420240083

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effects of conjugated linoleic acid supplementation in broiler breeders and in their progeny pre-placement diet on embryo and broiler chicks development

Poliana Carneiro Martins[1]*, Itallo Conrado Sousa de Araújo[2], Januária Silva Santos[1], Genilson Bezerra de Carvalho[1], Helder Freitas de Oliveira[1], Fabyola Barros de Carvalho[1], Emmanuel Arnhold[1], José Henrique Stringhini[1]

[1] Universidade Federal de Goiás, Departamento de Zootecnia, Goiânia, GO, Brasil.
[2] Universidade Federal de Minas Gerais, Departamento de Zootecnia, Belo Horizonte, MG, Brasil.

ABSTRACT - This study aimed to evaluate the development of the gastrointestinal tract and organs in 18-days embryos (ED18) and newly hatched chicks from broiler breeders supplemented with conjugated linoleic acid (CLA), as well as the development of their progeny up to 7 days of age, which also received CLA supplementation in the pre-placement diet during the first 12 hours post-hatch. A total of 540 eggs from two Cobb500 breeder flocks, supplemented with either 0 or 0.025% CLA, were incubated, and after hatching, broiler chicks received diets containing either 0 or 0.025% CLA for 12 hours before placement. A total of 320 chicks were housed in a completely randomized 2 × 2 factorial design (breerder CLA × post-hatch CLA), resulting in four treatments with eight replicates of 10 birds each. Intestinal development and relative organ weight (RW) were assessed at 18 days of incubation, at hatching, at 12 hours of holding, and at 7 days post-hatch; performance from 1 to 7 days was also evaluated. Supplementation of CLA (both broiler breeders and pre-placement diet) increased body weight (BW) at 12 h of holding and reduced pancreas RW at 7 days ($P<0.05$). The RW of the stomach and liver were higher at 7 days when only one source of supplementation was provided ($P<0.05$). Post-hatch CLA supplementation increased the lengths of the total and small intestines, while breeder supplementation alone also increased small intestine length ($P<0.05$). Both total and small intestine RW were lowest in the absence of CLA supplementation ($P<0.05$). Breeder supplementation with CLA led to improved BW and weight gain by day 7. The data shows that CLA supplementation in broiler breeders and their progeny affects gastrointestinal tract and organ development, and supplementation of breeders is recommended to enhance early performance.

Keywords: breeder nutrition, early feeding, embryo development, initial performance, progeny

***Corresponding author:**
polianamartinsvet@hotmail.com

Received: June 25, 2024
Accepted: September 7, 2025

How to cite: Martins, P. C.; Araújo, I. C. S.; Santos, J. S.; Carvalho, G. B.; Oliveira, H. F.; Carvalho, F. B.; Arnhold, E. and Stringhini, J. H. 2025. Effects of conjugated linoleic acid supplementation in broiler breeders and in their progeny pre-placement diet on embryo and broiler chicks development. Revista Brasileira de Zootecnia 54:e20240083. https://doi.org/10.37496/rbz5420240083

Editors:
Ines Andretta
Wagner Azis Garcia de Araújo

1. Introduction

Broiler breeder nutrition plays a crucial role in determining the traits expressed by the progeny, and efforts should focus on producing eggs with the highest possible nutrient availability for the developing

embryo (Muniz and Diniz, 2014; Van Emous et al., 2015). The provision of feed during the holding period at the hatchery and throughout chick transport has been the subject of considerable discussion in recent years (Cardeal et al., 2020, 2021). While fasting for up to 48 hours is not known to harm chick development and performance (De Jong et al., 2017), evidence shows that providing immediate access to feed after hatching can enhance the development of gastrointestinal organs, improve first-week performance, immunity and promote better chick welfare (Panda et al., 2006; De Jong et al., 2017; Cardeal et al., 2020, 2021; Martins et al., 2023). Moreover, feed intake influences the development of the intestinal mucosa and associated structures, secretion of digestive enzymes, and the establishment of the local microbiota (Santin et al., 2001).

Post-hatch diets have been formulated to avoid the damage caused by post-hatch fasting to the gastrointestinal tract and the productive performance of chicks (Almeida et al., 2006). As a result, strategies focusing on the composition, form, and nutrient levels of pre-starter diets provided during the pre-placement phase have been developed (Cardeal et al., 2021; Sousa et al., 2021). The inclusion of specific ingredients, such as conjugated linoleic acid (CLA), may help support the early metabolic processes of newly hatched chicks (Martins et al., 2024).

Conjugated linoleic acid is a natural polyunsaturated fatty acid that comprises a group of positional and geometric isomers of linoleic acid, characterized by conjugated double bonds (Nicolosi et al., 1997; Preuss et al., 2013). It has been shown that CLA improve feed efficiency in various animal species (Kastelic and Kompan, 2006; Stanimirovic et al., 2012; Marcolla et al., 2017). In broiler breeders, CLA supplementation may positively affect egg quality and the metabolic development of their offspring (Martins et al., 2024).

A previous study in which CLA was provided through the diet has shown that it can be incorporated into fatty acids within cell membranes (Marcolla et al., 2017). As a result, it may influence membrane quality, including that of the intestinal epithelium by altering membrane composition and absorption capacity. This could support the early development of the gastrointestinal tract in newly hatched chicks, especially when maternal CLA supplementation is combined with pre-placement dietary supplementation for chicks.

This research was conducted to evaluate the effect of CLA supplementation in broiler breeder diets on the development of the gastrointestinal tract and organs in 18-day-old embryos (ED18) and newly hatched chicks, as well as the development of their progeny up to 7 days of age, which also received CLA supplementation during the first 12 hours post-hatch, prior to placement.

2. Material and methods

Research on animals was conducted according to the institutional committee on animal use (CEUA Protocol 011/2015).

2.1. Broiler breeder's diet

Two commercial flocks of Cobb500 broiler breeders aged 58 weeks were placed on a farm in Formosa, Goiás, Brazil (15° 32' 13" S, 47° 20' 9 W). Each flock was raised in a breeder house with 24,800 hens and 2,480 roosters. The stocking density used was 8 birds/m^2. Breeders were fed corn- and soybean meal-based diets, and one of the flock was supplemented with 0.042% of a commercial product (Lutalin® BASF), according to the manufacturer's recommendations, ensuring the supplementation of 0.025% CLA (*trans*-10, *cis*-12) in the diet for 26 days, the period necessary to induce changes in the yolk through the diet. CLA was provided for the experiment by the manufacturer itself. The breeders' diets were formulated according to the recommendations described by Rostagno et al. (2011) and prepared in the feed mill of the commercial farm where the birds were housed (Table 1).

Table 1 - Composition of the experimental diets for broiler breeders

Item	Treatment	
	Control	CLA
Ingredient (%)		
Corn	68.179	68.128
Soybean meal	17.416	17.425
Limestone	7.876	7.876
Kaolin	4.000	4.000
Meat and bone meal	1.500	1.500
Salt	0.223	0.223
Dicalcium phosphate	0.200	0.200
Sodium bicarbonate	0.150	0.150
Mineral premix[1]	0.150	0.150
Vitamin premix[2]	0.100	0.100
Choline	0.082	0.082
DL-Methionine	0.080	0.080
L-Threonine (%)	0.030	0.030
Antioxidants[3]	0.016	0.016
CLA[4]	0.000	0.042
Enzyme[5]	0.003	0.003
Calculated nutrient composition		
Metabolizable energy (kcal/kg)	2.736	2.734
Crude protein (%)	13.90	13.90
Digestible lysine (%)	0.60	0.60
Methionine + cystine (%)	0.47	0.46
Digestible threonine (%)	0.50	0.50
Calcium (%)	3.29	3.29
Available phosphorus (%)	0.20	0.20
Sodium (%)	0.34	0.34

[1] Mineral premix - guarantee levels per kilogram of product: manganese, 80,000 mg; zinc, 70,000 mg; iron, 40,000 mg; copper, 8,000 mg; iodine, 1,000 mg.
[2] Vitamin premix - guarantee levels per kilogram of product: vitamin E, 110,000 mg; vitamin A, 14,000 IU; vitamin D3, 3,000 IU; vitamin K3, 6,000 mg; vitamin B1, 3,000 mg; vitamin B2, 12,000 mg; vitamin B6, 6,000 mg; vitamin B12, 30 mg; niacin, 60,000 mg; pantothenic acid, 20,000 mg; folic acid, 4,000 mg; biotin, 300 mg.
[3] Ethoxyquim 66.6%, BHA 99% and citric acid 99.5%.
[4] Conjugated linoleic acid (Lutalin®).
[5] Enzyme phytase (Natuphos® 10,000).

2.2. Incubation procedures

A randomized block design was adopted considering the incubator used as a criterion for determining the blocks. For embryos at 18 days of development (ED18) and newly hatched chick analysis, two treatments were applied, consisting of the inclusion of 0.000 or 0.025% CLA in the breeders' diets. Eggs were randomly collected on the day of laying and underwent dry sanitation using paraformaldehyde for fumigation, and a total of 540 eggs were selected for incubation, considering the integrity of the shell, the elliptical shape, and the average weight of 70.30 ± 4.83 g. The eggs were sent to the university located in Goiânia, Goiás, Brazil (16° 40' S, 49° 15' W). The eggs were stored at 20 °C for 66 hours and then preheated to 26 °C for 6 hours, completing 72 hours of storage before the incubation.

After identification according to the treatments, the eggs were distributed in two incubators (model MA01DA – Gaiolas Almeida, Aparecida de Goiânia, GO, Brazil) with a capacity for 270 eggs, with three trays each and a capacity to accommodate 90 eggs per tray, half of each tray (45 eggs) being filled by each treatment. The incubators had a system for automatically turning eggs every two hours and a panel for controlling temperature and monitoring relative humidity. The eggs were kept at a constant

temperature of 37.7 °C and humidity around 60% until 18 days, constantly monitored by thermo-hygrometers.

2.3. Embryo and newly hatched chick analysis

Six eggs from each treatment (2 per tray) were candled and randomly selected on the 18th day of incubation, at the time of transferring the eggs to the hatching baskets, to perform a necropsy and to weigh (with and without the yolk sac) and the organs using a digital scale. The RW of the gizzard + proventriculus (stomach), liver, heart, and pancreas were calculated using the following formula: Relative weight (RW) = (100 × Organ weight) / Bird weight. The RW and length of the total and small intestine (SI) were also evaluated. Furthermore, fragments of the duodenum, jejunum, and ileum were collected and fixed in a 10% buffered formalin solution for subsequent production of histological slides.

All eggs containing live embryos were placed in hatching trays, with three trays allocated to each hatcher. The incubators used as hatchers were adjusted to hatching mode, set at a temperature of 36.5 °C, relative humidity of 55–60%, and constant ventilation. Chick hatching was monitored beginning at 456 h of incubation, with hatchers inspected every five hours until 523 h of incubation. At the hatching windows of 481, 486, 491, 496, and 501 h, one chick per treatment per tray was randomly selected (totaling 15 chicks per treatment). These chicks were euthanized via cervical dislocation. A necropsy was performed to record body weight (with and without the yolk sac), to assess the relative weight (RW) of the previously mentioned organs, measure the RW and length of the entire gastrointestinal tract and the small intestine, and to collect tissue samples from the duodenum, jejunum, and ileum for subsequent histological analysis.

2.4. Pre-placement feeding and broiler chick development until 7 days

After pulling, the first-class chicks were divided into two groups. Both groups received crushed post-hatch diets inside the transportation boxes (3 g per bird), with or without 0.025% CLA, to be consumed for 12 hours before housing. The diets were formulated based on corn and soybean meal (Table 2), in accordance with the recommendations established by Rostagno et al. (2011). In addition to feed, the chicks also received water *ad libitum* to avoid dehydration and quality impairment.

A completely randomized design in a 2 × 2 factorial scheme was adopted, with the addition of CLA to the breeders' diets and/or the pre-placement diet (breeder diet – 0.000 or 0.025% CLA × pre-placement diet – 0.000 or 0.025% CLA), totaling four treatments. The evaluated variables consisted of the same as those assessed for embryos and newly hatched chicks, and evaluations were conducted after the pre-placement supplementation period and at seven days of age. Moreover, the pre-starter performance of chicks was determined (1 to 7 days). For this, the chicks were weighed before the 12 hours of pre-placement, and both the chicks and diets were weighed before housing and at seven days to calculate the mean body weight, weight gain, feed intake, and feed conversion ratio. Mortality was checked daily, and viability was calculated.

After pre-placement time, five birds per treatment were euthanized and weighed with and without the yolk sac. The previously mentioned organs were weighed, the weight and length of the total and SI were determined, and fragments of the duodenum, jejunum, and ileum were collected. The chicks were then housed in galvanized steel cages containing linear-type feeding and drinking troughs, heated by a 100-volt incandescent lamp. A total of 240 birds were divided into four treatments and six replications of 10 chicks each, lighting program was 24h of light.

After housing, all chicks began to receive the common pre-starter diet, without CLA supplementation. The control post-hatch feed and the pre-starter feed had the same formulation, differing only in terms of physical form, with the post-hatch feed being crushed and the pre-starter feed mashed (Table 2).

At seven days, two chicks per replication were euthanized to verify BW, weight of organs and intestines, and intestine length and collect fragments of the duodenum, jejunum, and ileum.

Table 2 - Composition of the experimental diets for the progeny

Item	Treatment	
	Control	CLA
Ingredient (%)		
Maize	52.00	
Soybean meal	38.90	
Soybean oil	2.50	
Dicalcium phosphate	2.00	
Starch	1.54	1.50
Limestone	1.08	
Salt	0.57	
L-Lysine	0.40	
DL-Methionine	0.40	
L-Threonine	0.20	
Vitamin and mineral premix[1] (%)	0.40	
CLA[2] (%)	0.00	0.04
Calculated nutrient composition		
Crude protein (%)	22.46	
Metabolizable energy (kcal/kg)	2.95	
Calcium (%)	1.00	
Available phosphorus (%)	0.48	
Digestible lysine (%)	1.41	
Digestible methionine (%)	0.69	
Digestible methionine + cystine (%)	0.98	
Digestible threonine (%)	0.94	
Chloride (%)	0.39	
Potassium (%)	0.86	
Sodium (%)	0.24	
CLA[2] (%)	0.00	0.02

[1] Mineral and vitamin A premix (per 1 kg): vitamin A, 2.000,000 IU; vitamin D3, 600,000 IU; vitamin E, 5,000 IU; vitamin K3, 450 mg; vitamin B1, 500 mg; vitamin B2, 1,500 mg; vitamin B6, 700 mg; vitamin B12, 2,500 mcg; niacin, 9,000 mg; pantothenic acid, 3,500 mg; folic acid, 250 mg; biotin, 15 mg; choline, 80 g; copper, 2,500 mg; iron, 10 g; manganese, 20 g; iodine, 250 mg; zinc, 18 g; selenium, 75 mg; nicarbazin, 10 g; maduramycin, 937.50 mg; virginiamycin, 4,125 mg.
[2] Conjugated linoleic acid - Lutalin® (BASF).

2.5. Histological processing

Intestinal fragments collected from embryos, chicks at pulling, chicks after 12 h of placement time, and 7-day-old chicks were fixed in 10% buffered formalin, processed following the methodology described by Luna (1968), and stained with hematoxylin and eosin (H&E). A histological slide was prepared for each fragment collected. After this process, the images were obtained using a Leica DM550 microscope and digitized on a computer with a Leica ICC50 video camera. A 4X objective was used to capture images of the intestinal villi, a 10X objective for the crypts, and the Image J software was used to check the histomorphometrical variables (villus height and crypt depth). A total of 20 villi and 20 intestinal crypts were evaluated per slide.

2.6. Statistical analysis

The data were subjected to analysis of variance ($P<0.05$) to compare means, using the R software (R Development Core Team, 2014). The required packages in R were Epitools, Epicalc, Nortest, Agricolae, ExpDes and Easyanova.

The statistical analysis for each of the response variables in the incubation of the eggs and for embryo and newly hatched chick analyses was performed according to the following statistical model:

$$\gamma_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

in which γ is the observed value of the treatment i (i = 1,2) in the j block (j = 1,2); μ is the general constant; α_1 is the effect of the i (i = 1,2) treatment; β_j is the effect of the j (j = 1,2) block (incubator); and ε_{ij} is the random residual effect of each observation.

Post hatch trial variables, including hisyological processing, were analyzed according to the following statistical model:

$$\gamma_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

in which γ_{ijk} is the observated value for the i level of the α (i = 1,2) factor, j level of the β (j = 1,2) factor, in the k (k = 1,2,3...8) repetition; μ is the general constant; μ_i is the effect of i level of μ (i = 1,2) factor; β is the effect of j level of β (j = 1,2) factor; $(\alpha\beta)_{ij}$ is the effect of the interaction between the levels of α and β factors; and ε_{ijk} is the random effect of each observation.

The data was submitted to tests of normality of both means and residuals and homoscedasticity was verified by the Shapiro-Wilk test at P≤0.05. Data are expressed as LSMeans. Data that violated the principle of normality were analyzed by the Mann-Whitney test at P<0.05.

3. Results

3.1. Embryo and newly hatched chick analysis

At ED18, embryos from breeders supplemented with CLA showed lower crypt depth in the duodenum (P<0.05) and higher villus height in the jejunum (P<0.05) compared to the control group. No significant differences were observed in duodenal villus height, jejunal crypt depth, or ileal crypt depth (P>0.05). Ileal villus height was not affected by treatments (P>0.05). In newly hatched chicks, CLA supplementation increased crypt depth in the duodenum and jejunum, and decreased villus height in the ileum (P<0.05). No significant differences were detected in villus height in the duodenum and jejunum (P>0.05), or in ileal crypt depth (P>0.05). In addition, no effect of block was observed (P>0.05) (Tables 3 and 4).

Table 3 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets on intestinal biometry of chick embryos aged 18 days of incubation and newly hatched chicks

Treatment	Total intestine relative weight (%)	Small intestine relative weight (%)	Total intestine length (cm)	Small intestine length (cm)
		Embryo at 18 days of incubation		
Control	0.84	0.57	21.19	19.82
CLA	0.92	0.51	20.60	18.57
P-value	0.137	0.446	0.640	0.355
CV (%)	8.72	14.73	8.86	10.20
		Newly hatched chicks		
Control	2.56	2.38	34.48	31.74
CLA	2.57	2.31	34.71	31.88
P-value	0.934	0.516	0.755	0.837
CV (%)	21.60	18.69	9.23	9.41

CLA - conjugated linoleic acid; CV - coefficient of variation.
n = 10.

Table 4 - Effect of conjugated linoleic acid supplementation in broiler breeders' diet on villus height and crypt depth of the small intestine of chick embryos aged 18 days of incubation and newly hatched chicks

Treatment	Duodenum		Jejunum		Ileum	
	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)
			Embryo at 18 days of incubation			
Control	52	33a	49b	27	44b	21
CLA	48	27b	52a	26	68a	21
P-value	0.100	<0.001	<0.001	0.156	0.919	0.802
CV (%)	21.88	25.00	14.18	18.03	17.76	12.88
			Newly hatched chick			
Control	208a	61a	120	42a	81a	49
CLA	206b	66b	113	47b	72b	52
P-value	0.712	0.003	0.091	<0.001	0.003	0.068
CV (%)	10.96	14.45	16.40	15.26	20.62	14.64

CLA - conjugated linoleic acid; CV - coefficient of variation.
Means within a column with different letters differ according to ANOVA (P<0.05).
n = 10.

3.2. Broiler chick early post-hatch development of organs, intestinal histomorphometry and performance

An interaction between breeder diet and pre-placement diet was observed for body weight and relative pancreas weight of chicks after 12 h of placement time (P<0.05; Table 5). No interaction was observed for yolk-free body weight or for the relative weights of stomach, liver, or heart (P>0.05; Table 5). Breeder diet and pre-placement diet had no effect on yolk-free body weight or on the relative weights of stomach, liver, or heart (P>0.05; Table 5).

According to the interaction breakdown (Table 6), after 12 h of pre-placement, chicks from CLA-supplemented breeders that also received the CLA pre-placement diet showed the highest body weight, while the lowest value was observed in chicks from control breeders that received the control pre-placement diet (P<0.05). For relative pancreas weight, the highest value was recorded in chicks from CLA breeders that received the CLA pre-placement diet (P<0.05), and the lowest in chicks from control breeders that received the CLA pre-placement diet (P<0.05).

Table 5 - Effect of conjugated linoleic acid supplementation in broiler breeders' diet and pre-placement diet on relative weight (%) of the organs of chicks after 12 h of holding

Treatment	Body weight (g)	YFBW (g)	Stomach (%)	Liver (%)	Heart (%)	Pancreas (%)
Breeder diet						
Control	47.02	44.72	10.48	3.43	0.82	0.28
CLA	51.5	45.69	11.07	3.55	0.89	0.29
Pre-placement diet						
Control	47.11	45.74	10.46	3.40	0.88	0.27
CLA	51.42	44.68	11.09	3.58	0.83	0.29
P-value						
Breeder diet	<0.001	0.647	0.417	0.499	0.335	0.503
Pre-placement diet	<0.001	0.616	0.387	0.338	0.469	0.360
Breeder × Pre-placement	<0.001	0.376	0.351	0.974	0.433	0.026
CV (%)	1.00	9.99	14.35	11.67	16.56	14.44

YFBW - yolk free body weight; CLA - conjugated linoleic acid; CV - coefficient of variation.
n = 20.

Table 6 - Interaction between breeder diet and pre-placement diet on body weight and pancreas relative weight of broiler chicks after 12 hours of holding

Variable	Pre-placement diet	Breeder diet	
		Control	CLA
Body weight (g)	Control	43.47Bb	50.58Ba
	CLA	50.76Ab	52.27Aa
Pancreas relative weight (%)	Control	0.294Aa	0.262Ba
	CLA	0.256Ab	0.327Aa

CLA - conjugated linoleic acid.
Means followed by different uppercase letters in the columns and lowercase letters in the rows differ significantly by F Test (P<0.05).

No interaction was observed between CLA supplementation in the breeder diet and the progeny pre-placement diet on intestinal biometry after 12 h of placement (P>0.05; Table 7). Neither the breeder diet nor the pre-placement diet had a significant effect on the relative weights of the total and small intestines or on total and small intestine lengths (P>0.05).

An interaction between breeder diet and pre-placement diet was observed for crypt depth in the jejunum and ileum (P<0.05). No interaction was observed for villus height in any intestinal segment or for duodenal crypt depth (P>0.05; Table 8). The pre-placement diet affected crypt depth in the duodenum, jejunum, and ileum (P<0.05), with higher values observed in chicks that received the CLA-supplemented diet. Additionally, villus height in the ileum was reduced in chicks fed the CLA diet (P<0.05). No effect of pre-placement diet was observed on villus height in the duodenum or jejunum (P>0.05). The breeder diet had no effect on any of the evaluated intestinal variables (P>0.05; Table 8).

According to the interaction breakdown (Table 9), the highest jejunal crypt depth was observed in chicks from control breeders that received CLA in the pre-placement diet, while the lowest value occurred in chicks from CLA breeders that received CLA in the pre-placement diet (P<0.05). For ileal crypt depth, the highest value was found in chicks from control breeders receiving the control pre-placement diet, and the lowest in chicks from CLA breeders that received the control pre-placement diet (P<0.05).

Table 7 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets and pre-placement diet on intestinal biometry of chicks after 12 h of holding

Treatment	Total intestine relative weight (%)	Small intestine relative weight (%)	Total intestine length (cm)	Small intestine length (cm)
Breeder diet				
Control	6.39	4.34	53.05	49.51
CLA	6.68	4.99	50.70	47.65
Pre-placement diet				
Control	6.32	4.34	51.55	48.01
CLA	6.75	4.98	52.20	49.15
P-value				
Breeder diet	0.625	0.186	0.314	0.388
Pre-placement diet	0.464	0.192	0.777	0.594
Breeder × Pre-placement	0.291	0.787	0.476	0.445
CV (%)	19.80	22.54	9.75	9.65

CLA - conjugated linoleic acid; CV - coefficient of variation.
n = 20.

Table 8 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets and pre-placement diet on intestinal histomorphometry of chicks after 12 h of holding

Treatment	Duodenum		Jejunum		Ileum	
	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)
Breeder diet						
Control	420	84	177b	62	141	75
CLA	431	83	186a	61	146	75
Pre-placement diet						
Control	428	78b	182	66a	151a	73b
CLA	424	89a	181	58b	136b	77a
P-value						
Breeder diet	0.224	0.221	0.054	0.290	0.178	0.869
Pre-placement diet	0.647	<0.001	0.805	<0.001	<0.001	0.006
Breeder × Pre-placement	0.540	0.243	0.334	<0.001	0.623	<0.001
CV (%)	8.68	14.21	10.29	12.85	13.72	10.26

CLA - conjugated linoleic acid; CV - coefficient of variation.
Means within a column with different letters differ according to ANOVA (P<0.05).
n = 20.

Table 9 - Interaction between breeder diet and pre-placement diet on intestinal histomorphometry of chicks after 12 h of holding

Variable	Pre-placement diet	Breeder diet	
		Control	CLA
Jejunum crypt depth (µm)	Control	62Ba	63Aa
	CLA	70Aa	53Bb
Ileum crypt depth (µm)	Control	80Aa	71Bb
	CLA	74Ba	76Aa

CLA - conjugated linoleic acid.
Means followed by different uppercase letters in the columns and lowercase letters in the rows differ significantly by F Test (P<0.05).

An interaction between breeder diet and pre-placement diet was observed for the relative weights of stomach, liver, and pancreas (P<0.05; Table 10). No interaction was observed for the relative weight of the heart (P>0.05; Table 10), and breeder and pre-placement diets had no effect on this variable (P>0.05; Table 10). The breeder diet influenced the relative weights of stomach and liver (P<0.05), with higher values observed in the control group. No effects of the pre-placement diet were observed for any variable (P>0.05; Table 10).

According to the interaction breakdown (Table 11), the highest relative weight of the stomach was observed in chicks from control breeders receiving the control pre-placement diet, while the lowest value was observed in chicks from CLA breeders that also received CLA post-hatch (P<0.05). For liver relative weight, the highest value occurred in chicks from CLA breeders receiving the control pre-placement diet, and the lowest in chicks from CLA breeders that received the CLA pre-placement diet (P<0.05). Regarding pancreas relative weight, the highest value was observed in chicks from control breeders that received the control diet, and the lowest in chicks from CLA breeders supplemented with pre-placement diet (P<0.05).

An interaction between breeder diet and pre-placement diet was observed for the 7-day-old chicks' relative weights of the total and small intestines (P<0.05; Table 12). No interaction was observed for total and small intestine length (P>0.05; Table 12). The breeder diet affected small intestine length (P<0.05), with longer intestines observed in chicks from CLA breeders (P<0.05). Pre-placement diet increased the lengths of both the total and small intestines (P<0.05). No effect of breeder diet was detected on the relative weights of the total and small intestines or on total intestine length (P>0.05; Table 12).

Table 10 - Effect of conjugated linoleic acid supplementation in broiler breeders' diet and pre-placement diet on relative organs weight of seven-day-old broiler chicks

Treatment	Stomach (%)	Liver (%)	Heart (%)	Pancreas (%)
Breeder diet				
Control	8.00	3.77	1.000	0.453
CLA	7.42	3.57	0.966	0.465
Pre-placement diet				
Control	7.55	3.60	0.969	0.450
CLA	7.88	3.74	1.004	0.468
P-value				
Breeder diet	0.037	0.023	0.336	0.525
Pre-placement diet	0.229	0.104	0.419	0.327
Breeder × Pre-placement	<0.001	0.002	0.652	0.002
CV (%)	11.67	7.67	14.83	13.42

CLA - conjugated linoleic acid; CV - coefficient of variation.
n = 20.

Table 11 - Interaction between breeder' diets and pre-placement diet on relative stomach, liver and pancreas weights of seven-day-old broiler chicks

Variable	Pre-placement diet	Breeder diet	
		Control	CLA
Stomach (%)	Control	7.22Ab	8.78Aa
	CLA	7.88Aa	6.97Bb
Liver (%)	Control	3.57Ab	3.98Aa
	CLA	3.64Aa	3.51Ba
Pancreas (%)	Control	0.425Ab	0.505Aa
	CLA	0.475Aa	0.432Ba

CLA - conjugated linoleic acid.
Means followed by different uppercase letters in the columns and lowercase letters in the rows differ significantly by F Test (P<0.05).

Table 12 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets and pre-placement diet on intestinal biometry of seven-day-old chicks

Treatment	Total intestine relative weight (%)	Small intestine relative weight (%)	Total intestine length (cm)	Small intestine length (cm)
Breeder diet				
Control	8.19	6.20	88.91	83.05b
CLA	8.44	6.27	92.70	87.75a
Pre-placement diet				
Control	8.01	6.01	87.68b	82.61b
CLA	8.62	6.46	93.93a	88.18a
P-value				
Breeder diet	0.279	0.715	0.084	0.014
Pre-placement diet	0.010	0.017	0.005	0.004
Breeder × Pre-placement	<0.001	0.008	0.699	0.751
CV (%)	9.04	9.55	8.19	7.32

CLA - conjugated linoleic acid; CV - coefficient of variation.
Means within a column with different letters differ according to ANOVA (P<0.05).
n = 48.

According to the interaction breakdown (Table 13), the highest total intestine relative weight was observed in chicks from control breeders that received the CLA-supplemented pre-placement diet, while the lowest value was found in chicks from control breeders receiving the control diet. For small intestine relative weight, the highest value was recorded in chicks from CLA breeders that received the control pre-placement diet, and the lowest in chicks from control breeders that received the control diet.

An interaction between breeder diet and pre-placement diet was observed for crypt depth in the jejunum and villus height in the ileum (P<0.05; Table 14). The breeder diet affected crypt depth in the duodenum and jejunum (P<0.05), with greater values observed in chicks from the control group. Additionally, ileum villus height was increased and crypt depth reduced in chicks from CLA breeders (P<0.05). The pre-placement diet affected crypt depth in the jejunum and villus height in the ileum (P<0.05), with higher villus height and lower crypt depth observed in chicks that received the control pre-placement diet. No significant effects were found for villus height in the duodenum or jejunum, or for crypt depth in the ileum (P>0.05; Table 14).

According to the interaction breakdown (Table 15), the highest jejunal crypt depth was observed in chicks from control breeders that received the control pre-placement diet, and the lowest in chicks from CLA breeders that received the CLA diet. For ileum villus height, chicks from CLA breeders that received the CLA diet showed the highest values, while the lowest was found in chicks from CLA breeders fed the control diet.

Table 13 - Interaction between breeder' diets and pre-placement diet on relative weights of total intestine and small intestine of seven-day-old broiler chicks

Variable	Pre-placement diet	Breeder diet	
		Control	CLA
Total intestine (%)	Control	7.38Bb	9.00Aa
	CLA	8.64Aa	8.24Ba
Small intestine (%)	Control	5.72Bb	6.69Aa
	CLA	6.30Aa	6.24 Aa

CLA - conjugated linoleic acid.
Means followed by different uppercase letters in the columns and lowercase letters in the rows differ significantly by F Test (P<0.05).

Table 14 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets and pre-placement diet on intestinal histomorphometry of seven-day-old chicks

Treatment	Duodenum		Jejunum		Ileum	
	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)
Breeder diet						
Control	648	213	417	209	297a	170a
CLA	658	220	418	195	286b	183b
Pre-placement diet						
Control	656	223a	415	212	286b	175
CLA	650	210b	420	192	297a	178
P-value						
Breeder diet	0.393	0.112	0.834	<0.001	<0.001	0.001
Pre-placement diet	0.579	0.004	0.429	<0.001	<0.001	0.483
Breeder × Pre-placement	0.325	0.549	0.343	0.008	0.006	0.716
CV (%)	8.69	14.01	8.93	12.19	6.31	17.37

CLA - conjugated linoleic acid; CV - coefficient of variation.
Means within a column with different letters differ according to ANOVA (P≤0.05).
n = 48.

No interaction between breeder diet and pre-placement diet was observed for any of the performance variables (P>0.05; Table 16). The breeder diet affected body weight and weight gain at 7 days of age (P<0.05), with higher values recorded in chicks from CLA-supplemented breeders. No effect of breeder diet was observed for feed intake, feed conversion ratio, or viability (P>0.05). The pre-placement diet had no effect on any of the evaluated performance parameters (P>0.05; Table 16).

Table 15 - Interaction between breeders' diets and pre-placement diet on intestinal histomorphometry of seven-day-old broiler chicks

Variable	Pre-placement diet	Breeder diet	
		Control	CLA
Jejunum crypt depth (µm)	Control	223Aa	195Ab
	CLA	201Ba	189Ab
Ileum crypt depth (µm)	Control	294Aa	300Aa
	CLA	277Bb	295Aa

CLA - conjugated linoleic acid.
Means followed by different uppercase letters in the columns and lowercase letters in the rows differ significantly by F Test (P<0.05).

Table 16 - Effect of conjugated linoleic acid supplementation in broiler breeders' diets and chicks pre-placement diet on pre-start (1 to 7 days) broiler chick performance

Treatment	Body weight (g)	Feed intake (g)	Weight gain (g)	FCR (g:g)	Viability (%)
Breeder diet					
Control	164.6b	120.3	118.1b	1.013	100.0
CLA	172.6a	126.1	126.3a	0.979	100.0
Pre-placement diet					
Control	168.6	123.4	122.3	1.000	100.0
CLA	168.2	123.1	122.2	0.990	100.0
P-value					
Breeder diet	0.016	0.203	0.013	0.222	1.000
Pre-placement diet	0.892	0.941	0.975	0.662	1.000
Breeder × Pre-placement	0.817	0.624	0.743	0.984	1.000
CV (%)	4.66	8.02	6.03	6.71	0.00

CLA - conjugated linoleic acid; FCR - feed conversion ratio; CV - coefficient of variation.
Means within a column with different letters differ according to ANOVA (P≤0.05).
n = 6 cages.

4. Discussion

In the present study, the combination of CLA supplementation in both breeder and progeny diets led to the best results in terms of gastrointestinal development. This supports the hypothesis that early nutritional programming, especially through the maternal diet, can influence the physiological and morphological development of chicks. It has been shown that CLA can stimulate gastrointestinal tract development (Chaplin et al., 2015), a critical aspect in the early chick life, as it influences nutrient absorption and performance (Cardeal et al., 2020, 2021).

Martins et al. (2024) reported that supplementation of broiler breeders with CLA altered egg composition, increasing the proportion of unsaturated fatty acids and improving yolk lipid quality, which can directly impact embryo viability and early organ development. These improvements may contribute to enhanced immunological and digestive capacity in newly hatched chicks. Martins et al. (2023) also observed enhanced cellular immunity and greater transfer of immunoglobulins from the

yolk to the serum in chicks fed CLA post-hatch, which could contribute to better organ development. Additionally, Fu et al. (2022) demonstrated that maternal CLA supplementation altered hepatic lipid metabolism in progeny, indicating trans-generational metabolic programming effects.

Our findings showed that supplying CLA to breeders and chicks reduced the relative weight of the stomach. This reduction was also observed in unsupplemented chicks from unsupplemented breeders. While CLA is known to increase liver fat deposition (DeLany et al., 1999; An et al., 2003; Du and Ahn, 2003), our study did not find an increase in liver weight, suggesting that CLA might have exceeded metabolic processing capacity in young chicks, possibly due to overlapping supplementation. Supporting this, Martins et al. (2023, 2024) emphasized the importance of dose- and stage-specific effects of CLA on organ development and fat metabolism.

Maternal supplementation alone did not significantly affect intestinal biometry at pulling. However, its influence was evident at day seven, particularly in small intestine length. Pre-placement CLA supplementation had a more pronounced effect, improving all measured intestinal morphometric parameters by day seven. These results align with findings by Dierick et al. (2003), who observed increased villus height in piglets fed dietary fatty acids, and Nosrati et al. (2015), who reported similar effects in poultry. It has been suggested that CLA can protect the intestinal mucosa (Bergamo et al., 2011), modulate gut microbiota (Chanuwat et al., 2011; Chaplin et al., 2015), and regulate intestinal gene expression (Murphy et al., 2007), which supports its role in gut development. Furthermore, the work by Fu et al. (2022) demonstrated CLA incorporation into progeny tissues, showing how maternal dietary lipids influence organ metabolic function.

Importantly, CLA in the maternal diet can modify the yolk sac fatty acid profile, influencing embryonic development and potentially impairing intestinal maturation if not metabolically assimilated (Aydin and Cook, 2004; Martins et al., 2024). Martins et al. (2024) observed that CLA supplementation in breeder diets improved egg lipid composition, including higher monounsaturated and polyunsaturated fatty acids and reduced saturated fatty acids. These alterations may enhance embryo energy supply and cell membrane composition, potentially supporting intestinal and immune tissue development, however, in present study CLA in general did not affect embryo nor yolk sac weight and also intestinal histomorphology.

Lilburn and Loeffler (2015) noted that lipid absorption from the yolk is prioritized in early life, which may explain the limited effect of breeder-only supplementation. Egg quality and fatty acid composition are directly affected by maternal diet, as shown by Fu et al. (2022), who identified alterations in yolk fatty acid profile and hepatic expression patterns in chicks. Additionally, the study by Özlü et al. (2020) emphasized the critical role of early post-hatch nutrition, showing that while residual yolk reserves are sufficient up to a point, early access to feed and water supports better performance outcomes and body development. These findings align with the observed advantages of CLA supplementation in the early stages post-hatch. Moreover, Cardeal et al. (2021) emphasized the importance of early feeding in supporting intestinal development, especially in chicks from young breeders with limited yolk reserves. This aligns with our findings, in which post-hatch supplementation proved more effective in promoting histological and biometric intestinal improvements.

Regarding performance, chicks from CLA-supplemented breeders showed higher body weight and weight gain, in agreement with Leone et al. (2009). However, other studies have reported contrasting findings. An et al. (2003) and Nosrati et al. (2015) reported no significant effect of CLA on weight gain or feed intake. Despite the improvements in intestinal development observed in this study, these benefits were not fully translated into better early performance when CLA was supplied only post-hatch. Although breeder supplementation positively affected weight gain and final body weight at seven days, progeny supplementation alone was insufficient to promote significant performance gains. This suggests that structural and functional improvements in the intestine may require longer adaptation periods or may be more effectively utilized under challenging conditions, such as environmental stress or disease pressure. These findings raise important considerations regarding the timing, duration, and combination of CLA supplementation strategies to optimize both gut development and growth performance in broiler chicks.

5. Conclusions

Supplementing broiler breeders and their progeny with conjugated linoleic acid (CLA) enhanced intestinal development and improved early weight gain in chicks. However, improvements in gut morphology alone were not sufficient to significantly enhance overall early performance. These results suggest that combined supplementation in both maternal and post-hatch diets may be necessary to fully leverage the benefits of CLA for broiler development.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Martins, P. C.; Santos, J. S.; Carvalho, F. B. and Stringhini, J. H. **Data curation:** Martins, P. C.; Araújo, I. C. S.; Santos, J. S.; Carvalho, G. B.; Oliveira, H. F.; Arnhold, E. and Stringhini, J. H. **Formal analysis:** Martins, P. C.; Oliveira, H. F.; Carvalho, F. B.; Arnhold, E. and Stringhini, J. H. **Funding acquisition:** Stringhini, J. H. **Methodology:** Martins, P. C.; Santos, J. S.; Carvalho, G. B.; Oliveira, H. F. and Stringhini, J. H. **Project administration:** Martins, P. C.; Carvalho, G. B.; Carvalho, F. B. and Stringhini, J. H. **Software:** Carvalho, F. B. and Arnhold, E. **Supervision:** Martins, P. C.; Santos, J. S.; Carvalho, F. B. and Stringhini, J. H. **Validation:** Martins, P. C. **Visualization:** Araújo, I. C. S. and Santos, J. S. **Writing – original draft:** Martins, P. C. and Araújo, I. C. S. **Writing – review & editing:** Martins, P. C.; Araújo, I. C. S.; Carvalho, F. B.; Arnhold, E. and Stringhini, J. H.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank the Brazilian research agencies CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for scholarship, the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for funding (project No. 421043/2016-4), and BASF and Asa Alimentos for supporting the development of this research.

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