



# High-energy diet does not overcome the negative impact of conjugated linoleic acid on young broiler performance

Kátia Maria Cardinal<sup>1\*</sup>, Julia Guazzelli Pezzali<sup>2</sup>, Lucas de Marques Vilella<sup>1</sup>, Priscila de Oliveira Moraes<sup>3</sup> and Andréa Machado Leal Ribeiro<sup>1</sup>

<sup>1</sup>Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 7712, 91540-000, Porto Alegre, Rio Grande do Sul, Brazil.

<sup>2</sup>Animal Biosciences Department, University of Guelph, Ontario, Canada. <sup>3</sup>Departamento de Ciência Animal e de Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. \*Author for correspondence. Email: katia.zootecnia@hotmail.com

**ABSTRACT.** The aim of this study was to evaluate the effect of conjugated linoleic acid (CLA) supplementation in diets with different energy levels in broiler performance. Birds were offered a starter (1-21 d), grower (22-35 d) and finisher (36-42 d) diets; wherein soybean oil was replaced by CLA. The study consisted of a 3 × 2 factorial arrangement with two CLA levels (0 and 1%) and three energy levels (3050, 3100 and 3150 ME kg<sup>-1</sup> diet). During the grower and finisher periods, birds were fed diets with same energy level and CLA supplementation was maintained the same. Growth performance was assessed weekly, and carcass and cuts yield were assessed at 42d. Interaction effect of CLA by energy level was observed in broiler performance and carcass yield throughout the study ( $p > 0.05$ ). During the overall period (1-42 d) broiler performance was not affected by CLA ( $p > 0.05$ ). However, CLA supplementation (1%) decreased weight gain ( $p < 0.05$ ) at 21d, regardless of energy level, with no effects on feed intake and feed conversion rate ( $p > 0.05$ ). The increase in dietary energy was not able to compensate the negative effect on growth performance of broilers supplemented with 1% CLA at the starter period.

**Keywords:** animal nutrition; metabolism; fatty acid; supplementation.

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## Introduction

Diet is known for having a significant impact on human health, and specific nutrients can even influence the initiation and progression of some cancers (Doll, 1992). Thus, the search for ingredients with nutraceutical properties is unremitting within the human food industry and the agriculture community. Conjugated linoleic acid (CLA) has been claimed as a nutraceutical fatty acid with a potential impact on several chronic diseases, including some cancers, atherosclerosis, obesity, bone density loss, and diabetes (Tontonoz & Spiegelman, 2008; Yuan, Chen, & Li., 2015).

CLA consists of a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Among all the CLA isomers, cis-9, trans-11, trans-10, and cis-12 stand out due to their biological properties (Kennedy et al., 2010; Kim, Kim, Kim, & Park, 2016). These fatty acids are produced through the ruminal biohydrogenation of linoleic acid, and thus are naturally found in beef and dairy products. Although meat from monogastric species is not considered a rich source of CLA, it can be incorporated in animal tissue lipids by dietary supplementation (Halle, Jahreis, Henning, Köhler, & Dänicke, 2012; Cardinal et al., 2017). The production of chicken meat enriched with CLA would provide value-added healthy animal products for human consumption and would create a new market opportunity for the broiler industry. However, CLA supplementation may impair broiler performance; thus, it may not be a viable supplement from an economic standpoint.

The effects of CLA on broiler performance are still controversial. While some studies have not observed differences in the performance of broilers supplemented with CLA at 0.5 and 1.0% (Suksombat, Boonmee, & Lounglawan, 2007; Zhang, Guo, & Yuan, 2005; Halle et al., 2012; Jiang, Nie, Qu, Bi, & Shan, 2014), other authors have shown a negative effect (Javadi et al., 2007; Szymczyk, Pisulewski, Szczurek, & Hanczakowski, 2001; Badinga, Selberg, Dinges, Corner, & Miles, 2003). Furthermore, Cardinal et al. (2017) and Moraes, Ribeiro, Santin, and Klasing (2016) reported a negative impact of 1.0% CLA inclusion on performance of

chicks during the initial phase (1-21 d). CLA supplementation may increase the energy expenditure by an increase in oxygen consumption (Choi, Jung, Park, & Song, 2004) and the up regulation of uncoupling proteins (Ryder et al., 2001), explaining its negative effect on performance. It was hypothesized that a high energy diet would supply the increase in energy expenditure caused by CLA supplementation, overcoming its detrimental effect on broiler performance. The aim of this study was to evaluate this hypothesis, studying the effects of CLA supplementation on the performance of broilers fed diets with different energy levels during the starter period.

## Material and methods

All experimental procedures were approved by the Ethics Committee on Animal under protocol number 20669. The present study was conducted in the Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul – Brazil. A total of 360 one-day-old Cobb 500 (male) broiler chicks ( $42.3 \pm 1.3$  g) were obtained from a local commercial hatchery. Before the start of experiment, the chicks were weighed and distributed into 12-bird groups, targeting a weight variation within groups at a maximum of 3%. Each bird group, which represented an experimental unit, were housed in a 1 m<sup>2</sup> pen, equipped with two nipple drinkers and one tubular feeder located in a controlled-temperature room. Food and water were provided ad libitum throughout the experimental period.

Birds were offered a starter (1 to 21 days), a grower (22 to 35 days), and a finisher (36 to 42 days) diet formulated with nutritional levels recommended by the Poultry and Swine Brazilian Tables (Rostagno, 2011; Table 1), in which CLA was added by replacing vegetable oil. The oil rich in CLA, obtained from BASF S/A, São Paulo, Brazil, consisted of 50% of cis-9, trans-11 and 50% of trans-10, cis-12 CLA isomers, which are produced from alkaline isomerization of oils rich in linoleic acid. The product shows 60% purity, such that 1.0% of CLA isomers added to the feed constituted 16.7 g kg<sup>-1</sup> of the CLA supplement.

**Table 1.** Composition of experimental diets as fed basis.

Item	Starter			Grower	Finisher
	3050	3100	3150		
Energy Levels (kcal kg <sup>-1</sup> )					
Ingredient (g kg <sup>-1</sup> as fed)					
Corn	583.9	573.4	561.6	603.8	601.4
Soybean Meal, 45%	346.3	347.9	350.1	321.0	321.4
Vegetable Oil <sup>1</sup>	30.8	39.7	49.4	41.5	48.7
CLA <sup>†</sup>	0.0/16.7	0.0/16.7	0.0/16.7	0.0/16.7	0.0/16.7
Dicalcium Phosphate	16.7	16.7	16.7	13.1	11
Limestone	9.3	9.3	9.3	9.1	7.8
Salt	5.1	5.1	5.1	4.6	4.6
DL-Methionine	3.2	3.2	3.2	2.4	1.4
L-Lysine HCl	2.7	2.8	2.7	2.8	2.3
L-Threonine	0.8	0.8	0.8	0.5	0.1
Mineral Premix <sup>‡</sup>	0.7	0.7	0.7	0.7	0.7
Vitamin Premix <sup>§</sup>	0.4	0.4	0.4	0.5	0.5
Choline chloride	0.1	0.1	0.1	0.2	0.2
Chemical analyzed composition					
Metabolizable Energy (kcal kg <sup>-1</sup> )	3050	3100	3150	3150	3200
Crude Protein (g kg <sup>-1</sup> )	207.4	207.4	207.4	196.8	195.4
Ca (g kg <sup>-1</sup> )	8.3	8.3	8.3	7.4	6.4
Available P (g kg <sup>-1</sup> )	4.2	4.2	4.2	3.5	3.1
Sodium (g kg <sup>-1</sup> )	2.2	2.2	2.2	2	2.0
Lys dig* (g kg <sup>-1</sup> )	12.2	12.2	12.2	11.3	10.5
Met + Cys dig. (g kg <sup>-1</sup> )	8.7	8.7	8.7	8.1	7.6
Arg dig. (g kg <sup>-1</sup> )	13.0	13.0	13.0	12.2	12.2
Trip dig. (g kg <sup>-1</sup> )	2.3	2.3	2.3	2.2	2.2
Treo dig. (g kg <sup>-1</sup> )	7.8	7.8	7.8	7.2	6.8
Choline (mg kg <sup>-1</sup> )	1374	1372	1317	1316	1361

<sup>†</sup>Diets with 0.0 g kg<sup>-1</sup> CLA had soybean oil as the only vegetable oil source. Treatments with 1.0% of CLA isomers had added 16.7 g kg<sup>-1</sup> CLA product, and soybean oil was included in each of these diets to reach the vegetable oil percentage reported. <sup>‡</sup>Mineral premix (content per kg premix): 150.000 mg Mn, 100.000 mg Zn, 80.000 mg Fe, 15.000 mg Cu, 1.200 mg I, 700 mg Se, 25.200.000. <sup>§</sup>Vitamin premix (content per kg premix): IU vitamin A, 5.600.000 IU vitamin D, 52.000 mg vitamin K, 6.000 mg vitamin B1, 18.000 mg vitamin B2, 9.000 mg vitamin B6, 13.2000 mg niacin, 44.000 mg pantothenic acid, 2.400 mg folic acid, 200.000 µg biotin, 40.000 µg vitamin B12. \*Dig.: Digestible.

The experiment was developed as a completely randomized design. Five replications (pen) per treatment were used to evaluate the growth performance. The experiment, at the starter period, consisted of a 2 × 3 factorial treatment arrangement with two CLA doses (0 and 1%) and three energy levels (3050, 3100, and 3150 ME kg<sup>-1</sup> diet), totaling six treatments. The increase in dietary energy was achieved by a higher inclusion of vegetable oil. During the grower and finisher periods, birds were fed experimental diets with the same energy level, yet the inclusion of CLA was maintained the same in each treatment as reported in the starter period.

To evaluate the yield of commercial cuts (breast, thigh and drumstick), 5 male birds per treatment were slaughtered at 42 day. The slaughter was performed by cervical dislocation and bleeding. The birds went through scalding, feather plucking and evisceration. The carcasses were weighed without head, feet and viscera and separated into commercial cuts by one trained person; breast and drumstick were weighed to calculate the yield of each cut relative to the carcass weight.

The experiment was developed as a completely randomized design. Five replications (pen) per treatment were used to evaluate the growth performance, weight gain (WG), and feed conversion ratio (FCR), which were determined based on weekly measurements of body weight (BW) and feed intake (FI). The effect of energy level and CLA supplementation, and their interaction was examined by analysis of variance using the GLM procedure of SAS (9.4 SAS Institute Inc. Cary, NC, 2013). In the presence of a significant F, the means were compared by *Tukey* test. Results were considered significant at  $p < 0.05$  and marginally significant between  $p \geq 0.05$  and  $p \leq 0.10$ .

## Results

Broilers consumed feed and gained weight according to the expected performance of the genotype throughout the trial. During the experimental period, no health problems were observed, and mortality was below 3%.

There was no significant interaction between the dietary energy level and CLA supplementation with respect to FI, WG, and FCR during the experimental periods (1 – 21; 21 – 42; 1 – 42 days of age; Table 2). From 1 to 21 days of age, the supplementation of CLA at 1% decreased WG ( $p < 0.05$ ) regardless the dietary energy level. However, FI and FCR were not affected by CLA supplementation during the initial phase. A negative effect of dietary CLA supplementation on FCR was observed in broilers from 7 – 14 days of age (1.35 vs 1.41;  $p < 0.05$ ). In addition, it worsened WG from 14-21 days of age ( $p < 0.05$ ). Neither dietary energy level nor CLA supplementation in the starter period affected FI, WG, and FCR in the grower period (21 to 42 days) ( $p > 0.05$ ). For the overall study period, from 1 – 42 days of age, there was no significant effect of CLA supplementation on FI, WG, and FCR of broilers ( $p > 0.05$ ). On the other hand, birds fed the highest dietary energy level (3150 kcal kg<sup>-1</sup> diet) had a decrease in FI ( $p < 0.06$ ) and an increase WG ( $p < 0.05$ ) compared to those fed 3050 kcal kg<sup>-1</sup> diet. Consequently, an improvement in the FCR was observed ( $p < 0.05$ ).

No interaction between CLA and energy levels was observed for carcass, breast, drumstick and thigh yield. Similarly, CLA supplementation did not affect these responses ( $p > 0.05$ ; Table 3). The energy levels affected the breast ( $p < 0.06$ ) and thigh ( $p < 0.05$ ) yields, and the 3150 kcal kg<sup>-1</sup> diet showed the best results; it was observed that as the dietary energy level increased, both breast and thigh yields were higher.

**Table 2.** Effects of conjugated linoleic acid (CLA) supplementation with different dietary energy levels on feed intake, weight gain and feed conversion of broiler chickens during the starter period.

Dietary treatment	Feed intake (g)			Weight gain (g)			Feed conversion (g*g)		
	1 – 21d	21 – 42d <sup>a</sup>	1- 42d	1-21d	21 – 42d <sup>a</sup>	1- 42d	1-21d	21 – 42d <sup>a</sup>	1- 42d
Effect of energy level									
3050	1254	3397	4645 <sup>b</sup>	846 <sup>b</sup>	1985	2831 <sup>b</sup>	1.48 <sup>a</sup>	1.68	1.60 <sup>a</sup>
3100	1242	3362	4599 <sup>ab</sup>	835 <sup>b</sup>	2014	2850 <sup>b</sup>	1.49 <sup>a</sup>	1.62	1.58 <sup>ab</sup>
3150	1203	3375	4529 <sup>a</sup>	917 <sup>a</sup>	2064	2981 <sup>a</sup>	1.31 <sup>b</sup>	1.64	1.52 <sup>b</sup>
Effect of CLA level									
0.0	1228	3405	4633	884 <sup>a</sup>	2038	2922	1.39	1.67	1.58
1.0	1225	3257	4482	849 <sup>b</sup>	2004	2853	1.44	1.63	1.57
p- value									
Energy*CLA	0.642	0.614	0.668	0.904	0.199	0.829	0.834	0.482	0.402
CLA	0.934	0.181	0.178	0.039	0.355	0.092	0.154	0.389	0.705
Energy	0.135	1.188	0.057	0.001	0.199	0.015	0.031	0.543	0.05
Measure of dispersion									
CV <sup>†</sup> , %	6.58	8.74	6.45	5.05	4.74	3.91	6.89	7.87	5.11

<sup>ab</sup>Means followed by different letters in the same row are different ( $p < 0.05$ ). <sup>a</sup>From 21-42d, animals were fed isoenergetic diets while the inclusion of CLA was maintained the same as the initial period for each treatment. <sup>†</sup>CV: coefficient of variation.

**Table 3.** Yield carcass and cuts of broiler chickens fed control diets supplemented with 0.0 or 1.0% of CLA and tree energy levels at 42 day of life

Treatments	Yield (%)			
	Carcass	Breast	Drumstick	Tight
Energy (Kcal kg <sup>-1</sup> )				
3050	72.78	42.25 <sup>c</sup>	12.57	17.77 <sup>b</sup>
3100	72.58	42.52 <sup>b</sup>	12.82	17.64 <sup>b</sup>
3150	72.93	42.89 <sup>a</sup>	12.66	18.96 <sup>a</sup>
CLA (%)				
0.0	72.98	42.44	12.81	18.01
1.0	72.54	42.00	12.55	18.24
p value				
Energy*CLA	0.904	0.649	0.261	0.653
Energy	0.860	0.055	0.573	0.014
CLA	0.394	0.649	0.178	0.334
Measure of dispersion (%)				
CV <sup>†</sup>	2.66	5.30	5.82	5.45

<sup>a,b</sup>Means followed by different letters in the same row are different ( $p < 0.05$ ); <sup>†</sup>CV(%): coefficient of variation

## Discussion

To the best of our knowledge, this is the first study to determine the effects of CLA supplementation on the performance of broilers fed diets differing in energy content. The results herein showed that the supplementation of CLA at 1% to the starter diets impaired weight gain, and the increment in energy content was not able to overcome this decrease in weight gain. Previous studies also found a negative effect of CLA supplementation on broiler performance (Moraes et al., 2016), especially in early stages (Javadi et al., 2007; Zhang, Guo, Tian, & Yuan, 2008; Jiang et al., 2014; Cardinal et al., 2017). As CLA may have a detrimental effect on broiler performance due to an increase in energy expenditure, we were expecting an improvement in the performance of broilers fed the high energy diet. However, this was not observed in this study, since CLA supplementation reduced broiler performance during the starter period (1-21 days) regardless of energy level. The high energy level used in this study (3150 ME kcal<sup>-1</sup>) may not be enough to provide the additional energy required as a result of an increase in the maintenance energy demand.

Many studies have demonstrated that dietary CLA decreases body fat content and adipose tissue weight and increases fatty acid oxidation and energy expenditure (West et al., 1998; Thom, Wadstein, & Gudmundsen, 2001). Choi et al. (2004) reported an increase in acyl coenzyme A oxidase (ACO) and uncoupling protein 2 (UCP-2) mRNA in the liver and muscle of rats fed a diet containing 9 *Cis*-11 *Trans* CLA, which may lead to a decrease in body weight gain. In the same study, the level of peroxisome proliferator-activated receptor  $\alpha$  (PPAR  $\alpha$ ) was not increased by CLA, although it may act on energy expenditure through the activation of PPAR- $\alpha$ . The lower weight gain observed in this study, with CLA supplementation during the starter period (Table 1), may be explained by its association with UCP and PPARs. PPARs are nuclear transcription factors that play a central role in the storage and catabolism of fatty acids. CLA is a known ligand for the PPARs and forms a co-activator complex with PPAR, which is associated with the transcription of genes related to the differentiation of adipocytes, lipolysis ( $\beta$ -oxidation), mitochondrial biogenesis, and insulin sensitivity (Tavares, Hirata, & Hirata, 2007; Liu, Tang, Yang, & Li, 2017).

The UCPs are proteins found in the inner mitochondrial membrane that allow proton flow from the intermembrane space to the mitochondrial matrix. However, the return of protons into the mitochondrial matrix does not lead to energy storage in the form of ATP; thereby resulting in the production of heat (Busiello, Savarese, & Lombardi, 2015). UCP-1 (thermogenin) often speeds up this process. If UCP gene expression is increased by CLA, the authors hypothesized that it may negatively affect weight gain. The metabolic effects of CLA are complex, and it may be affecting broiler performance by other unknown mechanisms.

Currently, there are no reports in literature explaining the detrimental effects of CLA, especially in the starter period. It is possible that the increase in energy expenditure promoted by CLA becomes more evident as a result of the increased physical activity of broilers in the early stage (Kristensen et al., 2007). The negative effect of CLA on performance was also observed when it was fed to mice (West et al., 1998). The authors attributed this result to an increase in energy expenditure possibly due to an enhanced maintenance energy demand. As expected, a better performance was observed in broilers fed the high energy diet in the starter (1-21 days) phase, and its effect was maintained throughout the experimental period (1-42 days).

This can be attributed to the extra-caloric and extra-metabolic effect of fats. These lead to an increase in the nutrient availability of feed ingredients and to an improvement in energy efficiency by increasing the net energy content of the feed (Baião & Lara, 2005; Latshaw, 2008). Although for many years a low energy diet approach was used in the starter phases by the industry, early nutrient and energy supply for chicks is essential to increase the intestinal mechanical activity, hasten intestinal development, promote a greater assimilation of feed, and also assist in the development of immunity, thereby contributing to an overall improvement in growth performance (Panda, Bhanja, & Sunder, 2015; Prabakar, Pavulraj, Shanmuganathan, Kirubakaran, & Mohana, 2016).

In our study, the energy level was increased by the higher inclusion of vegetable oil. This high energy diet probably led to an increase in fat deposition, which resulted in the increased weight gain in broilers during the starter (1-21 days) phase, and as such this effect lasted throughout the experimental period (1-42 days). This may be associated with the fact that the energy consumed was not spent by the chicken, because inside the 1 m<sup>2</sup> pens the chickens make little movement, consequently there is little energy expenditure. During the initial phase there is a greater demand for protein for muscle deposition, and over time the greater demand becomes for energy, which was provided throughout the study. Meza et al. (2015) evaluated the performance and carcass quality of broilers fed diets with different energy levels for 42 days, and a greater fat content was observed in the carcass of broilers fed the highest level of metabolizable energy. In this study, broilers fed the high energy diet, only in the starter phase, presented high breast and thigh yield, which may be a consequence of high fat deposition.

## Conclusion

Dietary CLA supplementation at 1% had a negative effect on the performance of broilers reared at the starter period regardless of the dietary energy level. However, the utilization of CLA resulted in no differences throughout the experimental period. The feeding of high energy diets to broilers in the starter phase positively affected the performance in the subsequent phases. Further studies investigating the effects of CLA on metabolism in young broilers are needed in order to promote its dietary supplementation without impairing the production rates during the initial period of breeding.

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