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Effects of Dietary Conjugated Linoleic Acid on Broiler Performance and Carcass Characteristics

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Abstract

The effects of three levels of conjugated linoleic acid dietary inclusion on the carcass characteristics and performance of broilers were evaluated. A total of 405 chickens were raised from 1 until 42 days of age, housed in a room with water and food ad libitum. The experimental design was completely randomized, with three treatments (0.0, 0.5 and 1% CLA) and nine replications (pen) to performance analysis, 18 replications (two birds per pen) to carcass composition, and five replications (left legs) to lipid profile. Performance was determined weekly and after 42 days, 18 birds per treatment were slaughtered to quantify breast and leg yield. Protein and fat was quantified in the leg and breast, as well as the detailed lipid profile of the leg. Data were analyzed by ANOVA and means compared by LS means. From 1 to 21 days chickens with 0% supplementation of CLA performed better compared to those receiving 0.5 and 1% CLA ($P < 0.05$), however, these differences were no longer significant from 21 to 42 days or for the overall study period ($P > 0.05$). Conjugated linoleic acid inclusion did not influence leg, breast and carcass yield, and leg and breast content of protein and fat. Both levels of CLA changed the leg lipid profile: there was an increased accumulation of CLA in meat, increased levels of saturated fatty acids and reduction of polyunsaturated fatty acids. Conjugated linoleic acid supplementation increased n-6:n-3 ratio. CLA supplementation in broiler feed is effective to produce meat enriched with its isomers and change lipid profile.

Keywords: broiler, fatty acid, functional food, meat quality, performance

1. Introduction

Conjugated linoleic acid (CLA) is a polyunsaturated fatty acid and represents a mixture of positional and geometric isomers with conjugated double bonds of octadecadienoic acid (C18: 2) (Gouvêa, Franco, Marques, & Pereira Netto, 2012) and was originally isolated from beef extracts (Pariza & Hargraves, 1985). It has subsequentaly become the subject of research, particularly when CLA was topically applied to the dorsal skin of mice and tumor incidence was lowered by about 50% compared with control mice (Ha, Grimm, & Pariza, 1987). Among the several isomers of CLA, emphasis has been given to cis-9, trans-11 and trans-10, cis-12 due to the beneficial biological effects identified and associated with them (Kennedy et al., 2010). CLA has an effect in reducing fat and increasing lean mass in humans (Chen et al., 2012) and studies in animal models have confirmed that CLA has anticarcinogenic effects and can act at various stages of development of cancer (Padilha & Pinheiro, 2004). CLA is a modulator of PPAR alpha (Miranda et al., 2011) and PPAR gamma (Yuan, Chen, & Li, 2015), whereas the PPAR gamma induces apoptosis of cancer cells and inhibits the proliferation of prostate, breast and colon cancer in humans (Mueller et al., 2000; Sarraf et al., 1999; Tontonoz & Spiegelman, 2008). When studied separately, the CLA isomers presented different effects. Trans-10, cis-12 isomer is involved in the reduction of body fat, whereas cis-9, trans-11 isomer did not show the same effect (Wang & Jones, 2004). In another research, Lavillonniere et al. (2003) supplementing 1% CLA in the rats diets, observed that only the trans-10, cis-12 isomer increased the number of adenomas in colon tumors. Broilers are highly sensitive to changes in the level and type of lipid in the diet, thus it is possible to incorporate CLA isomers in commercial

breeds (Suksombat, Boonmee, & Lounglawan, 2007). When supplemented in broiler diets, CLA influences the elongation and desaturation of various fatty acids, modifying the ratio among them (Javadi et al., 2007; Buccioni et al., 2009; Halle, Jahreis, Henning, Kohler, & Danicke, 2012). CLA influenced feed intake, weight gain and feed conversion of broilers, and demonstrated ability to alter the amount of fat and protein and carcass yield (Suksombat, Boonmee, & Lounglawan, 2007; Zhang et al., 2008; Halle, Jahreis, Henning, Kohler, & Danicke, 2012; Jiang, Nie, Qu, Bi, & Shan, 2014; Moraes, Ribeiro, Santin, & Klasing, 2015). The results demonstrated by previous studies have shown that the CLA results depend on the supplemental dose, but it is still unclear which dosage has a positive effect on the productive variables and carcass characteristics. Consumers are increasingly interested in functional foods with nutritional characteristics that can provide beneficial substances to health, so it is interesting to enrich chicken meat with CLA, while maintaining the production levels. Therefore, the aim of this study was to evaluate the effect of dietary supplementation of CLA at different levels on broiler performance, carcass yield and lipid composition in the main commercialized cuts.

2. Method

2.1 The Material Studied

The experiment consisted of three treatments arranged as a dose-response method, with either 0.0, 0.5 and 1% dietary inclusion of conjugated linoleic acid (CLA). The oil rich in CLA was obtained from BASF S/A, São Paulo, Brazil, and consisted of 50% of *cis-9*, *trans-11* and 50% of *10-trans*, *12-cis* CLA isomers, which is produced from alkaline isomerization of oils rich in linoleic acid.

2.2 Area Descriptions

The broilers were housed in a building with 27 pens of 1 m^2 with wood shavings, equipped with two nipples drinker and one tubular feeder. Environmental temperatures were managed with heaters to maintain birds in thermoneutral conditions during the experimental period.

2.3 Methodology

All procedures used in this experiment were approved by the Ethics Committee on Animal Use of Federal University of Rio Grande do Sul, under protocol number 20669 and follow the legislation for the protection of animals used for scientific purposes. A total of 405 Cobb 500 male broiler chickens (±40 g) were used, which were obtained directly from the hatchery (Languiru, Teutonia city, Rio Grande do Sul, Brazil). On the first day of life, 15 bird groups were weighed and distributed in pens, ensuring the variation in weight among groups of birds did not exceed 3%. At 35 days, the capacity was adjusted to 10 animals per pen and birds were reared until 42 days. Each pen represented an experimental unit. The feed supply and water were *ad libitum*, and the animals were kept in thermal confort.

A three phase feeding program was used—starter (1 to 7 days), grower (8 to 21 days) and finisher (22-42 days) —formulated with nutritional levels recommended by the Poultry and Swine Brazilian Tables (Rostagno et al., 2011), differing only in the addition levels of CLA isomers, replacing soybean oil (Table 1).

Table 1. Ingredient formulas and chemical composition of experimental diets fed to broilers receiving different inclusion levels (0.0, 0.5 or 1.0%) of conjugated linoleic acid as-fed basis

Item	Initial	Growth	Finish
Ingredients, %			
Corn	53.80	53.94	61.99
Soybean Meal, 45% Crude Protein	39.33	38.53	30.47
Vegetal Oil[1]	2.53	3.83	4.17
Dicalcium Phosphate	1.90	1.56	1.25
Limestone	1.00	1.00	0.88
Salt	0.51	0.48	0.46
L-Lys HCl	0.27	0.16	0.25
DL-Met	0.36	0.29	0.28
L-Tre	0.10	0.04	0.07
Mineral Premix[2]	0.10	0.08	0.08
Vitaminic Premix[2]	0.05	0.04	0.04
Choline Chloride 60%	0.05	0.05	0.05
Nutricional Values (calculated)			
ME, Mcal/kg	2.96	3.05	3.17
Crude Protein, %	22.55	22.01	19.10
Crude Fiber, %	3.02	2.98	2.69
Fat, %	5.15	6.43	6.94
Dry Matter, %	87.56	87.85	87.66
Nutricional Values (calculated), %			
Lys dig.	1.32	1.22	1.10
Met dig.	0.65	0.58	0.54
Met + Cys dig.	0.95	0.88	0.80

Note. [1] In diets containing 0% CLA all vegetable oil used was soybean oil. Treatment with 0.5% CLA, used 0.83% CLA and 0.87, 3.0 and 3.34% of soybean oil for starting, grower and finisher diets, respectively. Treatment with 1% CLA, used 1.67% CLA and 0.86, 2.16 and 2.5% of soybean oil for starting, grower and finisher diets, respectively.

[2] Composition (content per kg of premix): 150000 mg of Mn, 100000 mg of Zn, 80000 mg of Fe, 15000 mg of Cu, 1200 mg of I, 700 mg of Se, 23200000 UI of A vitamin, 5600000 UI of D vitamin, 52000 mg of K vitamin, 6000 mg of B1vitamin, 18000 mg of B2vitamin, 9000 mg of B6vitamin, 132000 mg of niacin, 44000 mg of panthotenic acid, 2400 mg of folic, 200000 μg of biotin, 40000 μg of B12 vitamin.

Body weight (BW), feed intake (FI), weight gain (WG) and feed conversion (FC) were measured weekly to evaluate growth performance. At 42 days of age, 18 birds per treatment (2 birds per pen) were slaughtered to evaluate legs fat and protein content and carcass, breast and leg (thigh + drumstick) yield. Animals were slaughtered by cervical dislocation and subsequent bleeding. Birds went through scalding, plucking of the feathers and evisceration. Carcasses were weighed without head, feet and viscera and sectioned in commercial cuts by a competent person; legs and breast were weighed to calculate the yield of each cut, and the legs were designated for nutritional composition analysis. Analysis of dry matter (method number 930.15), ash (method number 923.03), crude protein (method number 976.05) and gross energy (isoperibolic calorimetric bomb model C2000 - IKA Werke GmbH & Co. KG, Staufen, Germany) were conducted under the rules of AOAC (1996). The crude fat (CF) was calculated using the formula:

$$CF = [\text{Crude Energy} - (\text{Crude Protein} \times 56.6)]/94 \quad (1)$$

Fatty acid profile analyses of the leg were made in five samples per treatment, each one with a total of 50g of thigh and drumstick sub-samples. The total lipid extraction and quantification was made according the technique proposed by Hara et al. (1978) using isopropanol, while the esterification was made by methanolic boron trifluoride. Fatty acids quantification was measured according to Christie (1989). The fatty acid profile was determined using a gas chromatograph (Agilent 45813-01, USA), equipped with an ionization detector (FID) and a capillary column of fused silica 100 m length × 250 μm diameter (Supelco 2560). Nitrogen was used as carrier gas with a flow of 1 mL per min and sample injection volume of 1 uL in 1/50 split mode, with injection and detection temperature of 250 °C. The fatty acids were identified by comparison between standards of known

methyl esters retention time (Sigma: Supelco Mix 37 Components FAME; Linoleic Acid Methyl Ester Mix (cis/trans), trans-11-Octadecenoic Methyl Ester; Linoleic Acid Conjugated methyl Ester), and the samples esterified.

The experimental design was completely randomized, with three levels of dietary inclusion of CLA oil (0.0, 0.5 and 1% CLA) and nine replications (pen) to performance analysis were used. Eighteen replications (two birds per pen) were used to obtain legs nutritional composition and carcass, breast and leg yield, and five left legs per treatment were used to analyse lipid profile. All responses were analyzed by ANOVA and in the presence of a significant F, the means were compared by LSmeans by SAS statistical program ($P < 0.05$).

3. Results

3.1 Growth Performance and Body Composition

The chickens supplemented with 0% CLA obtained better body weight (BW), weight gain (WG) and feed conversion (FC) than the chickens fed 0.5 and 1% of CLA during the 1-21 days old (d) period ($P < 0.05$). However, differences on WG and FC observed in the initial period were not maintained during 22-42 d, nor in the 1-42 d period (Table 2).

Table 2. Effect of different dietary levels of conjugated linoleic acid (CLA) on broiler performance reared from1 to 42 days of age

Variable	CLA Levels			SEM	P
	0%	0.5%	1%		
1-21 d					
Live Weight 21 (g)	920 [a]	897 [ab]	873 [b]	10	0.010
Feed Intake (g)	1145	1121	1150	15	0.376
Weight Gain (g)	874 [a]	856 [ab]	833 [b]	11	0.033
Feed Conversion	1.30 [a]	1.31 [b]	1.38 [b]	0.02	0.030
22-42 d					
Live Weight 42 (g)	2763	2694	2687	34	0.251
Feed Intake (g)	3036	2916	2820	73	0.147
Weight Gain (g)	1844	1791	1831	25	0.311
Feed Conversion	1.64	1.62	1.54	0.04	0.108
1-42 d					
Live Weight 42 (g)	2763	2694	2687	34	0.251
Feed Intake (g)	4182	4023	4078	44	0.058
Weight Gain (g)	2715	2636	2669	25	0.102
Feed Conversion	1.54	1.53	1.53	0.01	0.729

Note. [a,b] Means followed by different letters in the same row are different ($P < 0.05$).

There was no effect of different CLA levels on carcass, breast and leg yield and the amount of protein and fat in the leg and breast ($P > 0.05$) (Table 3).

Table 3. Effect of different dietary levels of conjugated linoleic acid (CLA) on carcass yield and composition of comercial cuts at 42 days of age

Item	CLA, %			SEM	*P*
	0	0.5	1		
Yield, %					
Carcass	77.0	76.4	77.5	0.2	0.112
Breast	37.2	36.6	37.4	0.4	0.414
Leg[1]	31.1	30.8	30.6	0.3	0.537
Leg composition[2], %					
Crude Fat	29.8	28.6	29.7	0.9	0.531
Crude Protein	65.4	66.9	65.6	1.0	0.368

Note. [1] Leg = thigh + drumstick; [2] Leg composition on Dry Matter.

3.2 Fatty Acid Profile

The profile of chicken leg fatty acids was changed significantly ($P < 0.05$) by the dietary inclusion of CLA (Table 4). As expected, incorporation of CLA in tissues increased according to increased levels of supplementation, chickens supplemented with 0.5% and 1.0% CLA had deposition 54.77 and 63.47% higher than the treatment without supplementation ($P < 0.05$). The *cis-9*, *trans-11* isomer was found in greater extent, representing approximately 60% of total CLA, 20% more uptake than the isomer *trans-10*, *cis-12*, in both supplementation levels. When CLA was supplemented, there was an increase in the level of saturated fatty acids (SFA) myristic, palmitic, stearic and arachidic, and reduction of polyunsaturated fatty acids (PUFA) linoleic, alpha-linoleic, and at level of 1% supplementation, arachidonic, DPA and DHA were reduced. There was a decrease of 32.4 and 35.9% ($P < 0.05$) in ratio PUFA:SFA when broilers received 0.5 and 1.0% of CLA. The changes in PUFA levels increased ($P < 0.05$) the n-6:n-3 ratio, broiler supplemented with 0.5% and 1.0% CLA had ratio 7.57 and 18.09% higher than the treatment without CLA supplementation.

Table 4. Effect of different dietary levels of conjugated linolenic acid (CLA) on the leg[1] fatty acid profile of broiler at 42 days of age

Fatty Acid, g/100 g FAME[2]	CLA inclusion, %			*SEM*	*P*
	0	0.5	1		
Saturated					
C12:0	0.07	0.09	0.08	0.12	0.673
C14:0	0.54^{b}	0.80^{a}	0.86^{a}	0.04	0.000
C15:0	0.08	0.09	0.09	0.00	0.069
C16:0	20.31^{b}	24.31^{a}	25.24^{a}	0.44	0.000
C17:0	0.16	0.20	0.27	0.03	0.081
C18:0	8.29^{b}	12.28^{a}	12.71^{a}	0.23	0.000
C20:0	0.07^{b}	0.09^{a}	0.10^{a}	0.00	0.000
Monounsaturated					
C14:1	0.09	0.07	0.11	0.03	0.505
C16:1	2.97^{a}	1.67^{b}	1.22^{b}	0.15	0.000
C18:1 trans-9	0.13^{b}	0.18^{a}	0.17^{a}	0.01	0.001
C18:1 trans-11	0.06^{c}	0.24^{b}	0.35^{a}	0.02	0.000
C18:1	27.57^{a}	21.09^{b}	20.21^{b}	0.53	0.000
C20:1	0.31^{a}	0.26^{b}	0.24^{b}	0.01	0.000
Polyunsaturated					
C18:2	30.92^{a}	28.26^{b}	27.07^{b}	0.56	0.001
C18:3 n-6	0.20	0.20	0.18	0.02	0.697
C18:3 n-3	2.31^{a}	1.82^{b}	1.80^{b}	0.07	0.000
C18:2 cis-9 trans-11, CLA	0.11^{c}	1.77^{b}	3.75^{a}	0.11	0.000
C18:2 trans-10 cis-12, CLA	0.07^{c}	1.07^{b}	2.28^{a}	0.07	0.000
C20:2	0.46^{ab}	0.53^{a}	0.36^{b}	0.04	0.032
C20:3 n-6	0.56^{a}	0.48^{a}	0.26^{b}	0.04	0.000
C20:3 n-3	0.06	0.12	0.05	0.04	0.332
C20:4	3.37^{a}	3.05^{a}	1.76^{b}	0.24	0.001
C20:5	0.13	0.19	0.14	0.02	0.221
C22:5	0.63^{a}	0.71^{a}	0.41^{b}	0.06	0.006
C22:6	0.44^{a}	0.37^{a}	0.20^{b}	0.04	0.001
Fatty acids ratio					
n-6:n-3	9.65^{c}	10.44^{b}	11.78^{a}	0.06	0.001
PUFA:SFA	1.42^{a}	0.96^{b}	0.91^{b}	0.19	0.001

Note. ^{a,b} Means followed by different letters in the same row are different ($P < 0.05$). [1] Leg = thigh + drumstick; [2] FAME = Fatty Acid Methyl Ester.

4. Discussion

4.1 Growth Performance and Body Composition

Previous studies observed similar effect on performance over the total rearing period when broilers were also supplemented up to 1% CLA (Zhang, Guo, Tian, & Yuan, 2008; Halle, Jahreis, Henning, Kohler, & Danicke, 2012; Jiang, Nie, Qu, Bi, & Shan, 2014). However, Suksombat, Boonmee, and Lounglawan (2007) supplemented the broilers diets from 21 to 42 d with up to 1.5% CLA and observed approximately 12% less average daily gain when compared to 1% CLA supplementation. In the present study, CLA exerted a negative effect on weight gain and feed conversion in the initial rearing period (1-21 d). Moraes, Ribeiro, Santin, Klasing (2015) found similar results when broilers were fed diets containing 1 and 2% CLA. West et al. (1998) observed a performance reduction in mice fed CLA and attributed this result to increased energy expenditure, suggesting increase in energy demand for maintenance. The mechanism by which CLA affects energy metabolism is still unknown, but some studies suggest that it is by increasing oxygen consumption (Choi et al., 2004) and increased expression of uncoupling proteins (Park & Song, 2004). According to Moraes, Ribeiro, Santin, and Klasing (2015) if broiler performance is altered by changes in adipose tissue, CLA should have a greater impact on performance at the end of rearing period (22-42 d) when there is greater deposition of fat, and not in the initial phase (1-22 d), when protein deposition rates are higher. Kang, Liu, Albright, Park, and Pariza (2003) reported that trans-10, cis-12 isomer inhibits the differentiation of 3T3-L1 adipocytes and decreases the receptor activated by peroxisome proliferator gamma expression, suggesting that fat reduction can be linked in part to the inhibition of pre-adipocyte differentiation (Zhou, Sun, Liu, & Zhao, 2008; Yuan, Chen, & Li, 2015). Due to the complex metabolism of CLA, more studies are necessary to define its association with loss of performance in broiler starter phase.

Suksombat, Boonmee, and Lounglawan (2007), supplementing broilers with up to 1.0% CLA, observed no differences in yield or in the amount of protein and fat in breast, thighs and drumsticks. But when 1.5% CLA was supplemented, there was a reduction of fat amount in the thigh. Such benefit may be associated to CLA reducing body fat by decreasing adipose cell mass or cell number. This can be achieved in part by increasing lipoprotein lipase at adipose cells, enhancing apoptosis of preadipocytes and adipocytes, and modulating lipolysis (Hargrave et al., 2004, Storkson, Park, Cook, & Pariza, 2005). Contrarily to our findings, Szymczyk, Pisulewski, Szczurek, and Hanczakowski (2001) observed an increase in leg yield and reduction of abdominal fat content when the level of CLA supplementation was 1%. In contrast, Duh and Ahn (2002) fed broilers with 0.5% CLA during 3 weeks from 21 days old and observed an increase in abdominal fat content. The differences in the results found in these studies may be related to differences in diet ingredients, supplementation period, age and strain of the birds (Pariza, Park, & Cook 2001; Suksombat, Boonmee, & Lounglawan, 2007). Another important factors are the isomers used and the ratio between them, because studies show that isomers have different results on performance. The trans-10, cis-12 isomer has been associated with a fat reduction effect, while the cis-9, trans-11 isomer has been reported to have no effect (Wang & Jones, 2004; Martorell, 2012).

4.2 Fatty Acid Profile

Broilers are highly sensitive to changes in the level and type of lipid in the diet, so was expected changes in fatty acid profile. The incorporation of CLA in tissues increased according to increased levels of supplementation, demonstrating that dietary CLA were efficient transferred. But the concentration of individual CLA isomers in muscle does not reflect those in the diet, the cis-9, trans-11 isomer was found in greater extent, 20% more uptake than the isomer trans-10, cis-12, in both supplementation levels, and the percentage of cis-9, trans-11 and trans-10, cis-12 in the CLA source were 50 and 50%. Similar results were found by Suksombat, Boonmee, and Lounglawan (2007) and Buccioni et al. (2009). It is not fully understood why this isomer is incorporated in greater quantity. However, it may be connected to the fact that some isomers have better mobilization efficiency than others (Zang et al., 2007), and has a preference for deposition.

Conjugated linoleic acid has the capacity to interfere in lipid metabolism, using the oleic acid metabolic pathway to accumulate in the tissues, or metabolize in the same pathway of linoleic acid by influencing the desaturation and elongation of other fatty acids (Carta et al., 2002). When CLA was supplemented, there was an increase in the level of SFA and reduction of PUFA. The reduction of arachidonic acid, DHA, EPA and its precursor, alpha-linoleic and linoleic acids, may have occurred because CLA also competes by the enzyme Δ 6 desaturase (Duarte et al., 2010). The linoleic acid reduction was likely associated with the substitution of soybean oil, which contains 55% of this fatty acid (Smith et al., 2006). The increasing levels of SFA and reduction of other PUFA is linked to the inhibition of the enzyme Δ 9 desaturase in the liver, caused by greater levels of CLA (Suksombat, Boonmee, & Lounglawan, 2007) and impaired conversion of stearic acid to oleic acid by reduction of the

enzyme stearoyl-CoA desaturase in the liver. As result of these changes there was a decrease ($P < 0.05$) in ratio PUFA:SFA: 1.42; 0.96 and 0.91 to 0.0; 0.5 and 1.0% CLA. Although there was a reduction of this relationship, it is kept above 0.45, recommended as beneficial by the Brazilian Society of Cardiology (Santos et al., 2013). The changes in PUFA levels increased ($P < 0.05$) the n-6:n-3 ratio: 9.65; 10.44 and 11.78 to 0.0; 0.5 and 1.0% CLA. Food and Agriculture Organization of the United Nations (2010) considered beneficial n-6:n-3 ratios in the range of 4.0 to 5.0. High intake of SFA coupled with low intake of MUFA, together with a low n-6:n-3 ratio in human diet has been linked to the production of thromboxanes, leukotrienes and C-reactive protein, which are related to various diseases such as diabetes, cancer, obesity, rheumatoid arthritis and asthma (Simopoulos, 2004). Enrichment of chicken meat with CLA must be analyzed not only for the beneficial effects of CLA per se, but also by changes in profile of other fatty acids.

5. Conclusions

Dietary CLA supplementation at 0.5 and 1.0% levels did not exert a negative effect on growth performance in the total period of broiler rearing, yield of relevant cuts of meat and their nutritional composition, but decreased performance in the initial growth period.

Conjugated linolenic acid inclusion in poultry diet is an effective way to produce meat enriched with its isomers. CLA also reduced PUFA content and increase n-6:n-3 ratio in leg meat, with a concomitante increase in SFA levels.

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