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Conjugated linoleic acid supplementation modified the body composition and serum leptin levels in weaning rats

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SUMMARY. Dietary supplementation with conjugated linoleic acid (CLA) may reduce body fat mass and increase lean body mass in various species. The objective of this study was to study the effects of conjugated linoleic acid (CLA) supplementation on body composition, serum leptin and triacylglycerol levels in Wistar rats. Rats received linoleic acid (group C) or conjugated linoleic acid (group AE, supplemented with AdvantEdge® CLA, and group CO, supplemented with CLA One®) in the concentrations of 2% of daily feed consumption. Serum leptin and triacylglycerol levels of rats were measured by means of commercial kits. After 42 days of supplementation, rats in the control group exhibited body fat contents of 53.94 ± 6.80 g, and those in groups AE and CO had 45.43 ± 4.86 g and 43.75 ± 1.93 g, respectively, corresponding to a mean body fat reduction of 18%. Water, whole body protein and ash contents of rats supplemented with CLA were statistically higher relative to control group content (corresponding to a mean increasing of 7.65%; 6.5% and 12.35%, respectively). Experimental groups AE and CO, which received CLA supplementation, had statistically lower serum leptin levels (3.45 ± 0.46 ng/mL and 3.08 ± 0.19 ng/mL, respectively) relative to the control group (4.21 ± 0.22 ng/mL) which received linoleic acid. Triacylglycerol levels did not change after CLA supplementation ($p > 0.05$). Supplementation with conjugated linoleic acid in the concentration of 2% of mean daily feed consumption was able to change body composition of rats after 42 days of experimentation.

Key words: Conjugated linoleic acid, supplementation, nutrition, body composition, leptin, lipid profile.

RESUMEN. La suplementación con ácido linoléico conjugado modificó la composición corporal y los niveles séricos de leptina en ratas recién destetadas. El objetivo de este trabajo fue estudiar los efectos del suplemento con ácido linoléico conjugado (CLA) sobre la composición corporal, leptina en suero y niveles de triacilglicerol en ratas Wistar. Las ratas recibieron ácido linoléico (grupo C) o ácido linoléico conjugado (grupo AE, suplementado con AdvantEdge® CLA, y grupo CO, suplementado con CLA One®) en una concentración de 2% del consumo diario de alimentación. Los niveles de leptina en suero y triacilglicerol de las ratas fue medido por medio de kits comerciales. Después de 42 días de suplementación, las ratas del grupo de control exhibieron contenidos de grasa corporal de 53.94 ± 6.80 g, y los de los grupos AE y CO tuvieron 45.43 ± 4.86 g y 43.75 ± 1.93 g, respectivamente, lo que corresponde a una reducción media de la grasa corporal del 18%. El agua, la proteína corporal total y el contenido de cenizas de las ratas suplementadas con CLA fueron estadísticamente superiores en relación al contenido del grupo control (lo que corresponde a un aumento medio de 7.65%; 6.5% y 12.35%, respectivamente). Los grupos experimentales AE y CO, que recibieron suplementación con CLA, tuvieron niveles de leptina en suero estadísticamente menores (3.45 ± 0.46 ng/mL y 3.08 ± 0.19 ng/mL, respectivamente) en relación al grupo control (4.21 ± 0.22 ng/mL) que recibió ácido linoléico. Los niveles de triacilglicerol no cambiaron después del suplemento con CLA ($p > 0,05$). La suplementación con ácido linoléico conjugado en una concentración de 2% del promedio del consumo diario de alimento fue capaz de cambiar la composición corporal de las ratas después de 42 días de experimentación.

Palabras clave: Acido linoléico conjugado, suplementación, nutrición, composición corporal, leptina, perfil lipídico.

INTRODUCTION

Conjugated linoleic acids (CLA), a compound naturally found in small amounts in a large variety of foods, is a group of geometrical and position isomers of linoleic acid with conjugated double bonds (1,2). CLA can be synthesized by ruminants by the biohydrogenation of unsaturated fatty acids by bacteria present in the rumen, in a way that the predominant isomer is *cis-9, trans-11* (1,3). The *cis-9, trans-11* CLA isomer can be produced in mammary glands by the delta 9 desaturase path. The *trans-10, cis-12* isomer is also believed to be one of the isomers synthesized in the rumen of polygastric animals (4). Thus, significant concentrations of CLA, especially *cis-9, trans-11* CLA, can be found in meats, milk, and their products.

Interest for conjugated linoleic acid (CLA) arose in 1979 when researchers found antimutagenic and anticarcinogenic substances, among them CLA, in grilled meats (5,6). Subse-

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quently, numerous studies associate CLA consumption with beneficial effects to human health, among them anticarcinogenesis, body composition change, atherosclerosis reduction, immune system modulation, and bone mineralization enhancement (7,8). The commercially available CLA mixtures are manufactured by alkali isomerization of linoleic acid or linoleic acid rich oils such as safflower oil and sunflower oil (9). These numerous mixtures of CLA and its isomers have been studied for their physiological effects. The *cis-9, trans-11* and the *trans-10, cis-12* isomers have distinct biological activities. It is believed that the isomer *trans10, cis12* CLA, with the most significant biological activity, is more closely related to lipid and glucose metabolism and body fat reduction (10), whereas the *cis-9, trans-11* isomer is associated to antioxidant and anticarcinogenic effects (11,12). CLA was first reported to influence body composition in a study with mice fed 0.5% CLA (50% *cis-9,trans-11* and 50% *trans-10, cis-12*), resulting in a decreased body fat mass and an increased lean body mass (13). Although CLA supplementation is able to increase lean mass and decrease body fat in different experimental models (13-17), mechanisms proposed to explain these changes are still controversial. CLA ingestion has been associated to a decrease in pre-adipocyte proliferation and differentiation, increase fatty acid oxidation (as evidenced by increased carnitine palmitoyltransferase activity), reduction of esterification of fatty acids into triacylglycerols, increase of energy expenditure, and changes in the activity of enzyme lipoprotein lipase, and the hormone leptin, among others (18, 19).

The objective of the present work was to assess the effect of conjugated linoleic acid supplementation on body composition, and serum leptin and triacylglycerol levels in Wistar rats.

MATERIALS AND METHODS

Supplements and reagents. Conjugated linoleic acid supplements used in the study were AdvantEdge® CLA 75% (EAS™, Golden, CO, USA) and CLA One® Free Fatty Acid Oil 75% (Pharmanutrients, Gurnee, IL, USA). Linoleic acid 60% supplement and all other reagents were obtained from Sigma-Aldrich (St. Louis, USA). Fatty acid composition of the linoleic acid supplement 60% Sigma (code L 1376) and the commercial conjugated linoleic acid mixtures 75% AdvantEdge®, CLA and 75% CLA One® Free Fatty Acid Oil 1CLA1-FFBL-KG, expressed in g/100 g of fatty acids, can be seen in Table 1.

Animals and diet. The animals were 30 healthy albino male recently-weaned Wistar rats aged 21 to 23 days, with mean weight 60.0 ± 3.51 g, from the Multidisciplinary Center for Biological Investigation (CEMIB/UNICAMP). The powdered diet was prepared according to American Institute of Nutrition (20), AIN-93G, with a protein concentration of 12% (21). This work has been approved by Animal Experiment Ethics Commission (CEEA – IB/UNICAMP, Protocol nº 564-1).

Experimental conditions. Animals were housed individually in steel cages and had free access to water and food at all times. Temperature and relative humidity were in the range of 22 ± 1°C and 60-70% respectively, and 12 h light:dark cycle. After 7 days adaptation period rats were divided into 3 groups of 10 animals each, in order to achieve body weight homogeneity within and across groups and supplemented for 42 days. Commercial mixtures of conjugated linoleic acid AdvantEdge® (EAS™) and CLA One® (Pharmanutrients) were fed to groups AE and CO respectively, and linoleic acid to the control group (C), in the concentration of 2% of daily feed consumption. During the assay, food and water consumption, as well as animal weight gain, were checked every two day. The choice of supplement concentration for the present study was based on results from a preliminary assay by the same research team with three CLA concentrations. The main objective of the previous assay was to standardize CLA supplementation conditions. Growing male Wistar rats were supplemented for 21 days with commercial conjugated linoleic acid mixture 75% AdvantEdge®, CLA (EAS™) at the concentrations of 1%, 2% and 4% of the daily feed consumption, having linoleic acid as a control. The concentration adopted for the present assay – 2% of feed consumption – was chosen on the basis of results and operational conditions of supplementation from the preliminary assay (22).

Supplementation. Animals were supplemented by orogastric intubation with 1 mL disposable syringes and *gavage* needles. The amount of supplement was calculated every other day on the basis of the average feed consumption of each group, so that supplementation followed normal feed ingestion. The amount of supplement varied from 0.25 a 0.49 mL and was calculated based on density. Supplements were aspirated with the syringe and kept away from light till the moment of administration. Rats were removed group by group from the experimental room, placed in plastic boxes, and taken to the supplementation room. This procedure was done daily during daytime and always at the same time, since rodent have nocturnal habits.

Determination of fatty acid profiles of linoleic acid and conjugated linoleic acid mixtures. Methylation followed the Christie method (23). About 50 g of each sample of conjugated linoleic acid supplements and linoleic acid were weighed in conic bottom graduated extraction tubes. Then 2 mL of 1% methanolic sulphuric acid were added. Tubes were capped and shaken in an electric agitator for 1 minute. Tubes remained in water bath at 37 ºC for 2 hours; 5 mL of sodium chloride 5% were added, followed by 1 minute shaking. 5 mL of

hexane were added, and tubes were again shaken for 30 seconds. The supernatant (hexane and methyl ester) was transferred to another extraction tube to which 4 mL of 5% potassium bicarbonate were added. Tubes were shaken in an electric agitator for 1 minute, and phase separation occurred rapidly. The new supernatant was transferred to another extraction tube containing 1 g of sodium sulphate. The hexane was evaporated with help of a nitrogen flux under water bath at 40 ºC. Finally, 2 mL of hexane were added, and the solution was transferred to a chromatography vial, labeled, and stored at -20 ºC. Fatty acid profile was determined by gas chromatography with a capillary silica column CP SIL 88 (0.25 mm x 0.2 ?m x 100 m) and a flame ionization detector (FID). A temperature gradient with initial temperature of 70 ºC was used for 4 minutes, followed by an increase at the rate of 13 ºC/min until 175 ºC; after 27 min the temperature was raised again at the rate of 4 ºC/min, to 240 ºC where it remained for 4 minutes, totalizing 70 min for the whole run. Injector and detector temperatures were 250 and 300 ºC respectively. Injection was in split mode with a ratio of 50:1. Drag gas was hydrogen with a flux of 1.8 mL/min, and pressure of 36.3 psi at the column head (24). Results were expressed in percenta-ge of total fatty acids. The standard adopted was CRM-164 (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium), which has certified values for 11 fatty acids; they were used to establish the correction factor for each certified fatty acid in order to transform the peak expressed in percentage of area into mass (mg/g total fatty acids).

TABLE 1
Fatty acid composition of linoleic acid and commercial conjugated linoleic acid mixtures

Fatty acids	Linoleic acid 60% Sigma	Conjugated linoleic acid 75% AdvantEdge® CLA (EAS™)	CLA One® Free Fatty Acid Oil 75% 1CLA1-FFBL-KG (Pharmanutrients)
	(g/100g of fatty acids)		
C8:0	-	0.02	0.06
C10:1	-	0.01	-
C12:0	-	0.01	-
C14:0	0.13	-	-
C16:0 iso	0.02	-	-
C16:0	2.89	2.15	3.86
C16:1 cis-9	0.17	0.01	-
C17:0	0.04	0.03	-
C17:1	0.07	0.01	-
C18:0	0.80	2.96	1.91
C18:1 cis-9	25.90	13.23	16.86
C18:1 cis-11	2.06	1.04	0.94
C18:2 cis-9. cis-12	59.64	0.75	0.93
C18:2 cis-9. trans-11 CLA	0.09	40.12	36.81
C18:2 trans-10. cis-12 CLA	0.08	39.15	36.27
C18:2 cis-11 trans-13 CLA	-	-	1.44
C18:2 trans-11 cis-15	0.82	0.21	0.47
C18:3	-	0.06	0.13
C20:0	1.01	-	-
C20:1	-	0.06	0.13
C20:2	6.18	0.01	-
C20:3	-	0.04	-
C20:4	0.01	-	-
C20:5	0.01	0.02	0.03
C22:0	-	0.04	0.07
Total	100.00	100.00	100.00

CLA: conjugated linoleic acid

Body composition analysis. After exsanguination, animals were killed by cervical displacement, and the whole gastrointestinal tract was removed, cleaned with physiological solution, and returned to the carcass (13). The latter was frozen in liquid nitrogen, sliced, lyophilized, chopped, ground, and stored at -80°C (Freezer Ultra Low REVCO). Water content, ashes and crude protein (N x 6.25) determinations were performed according to methods 930.15, 954.01 and 942.05 respectively, as described by AOAC (25), and fat (ether extract) according to Lees (26).

Serum triacylglycerol and leptin determinations. Blood from the animals was collected under anaesthesia (sodium pentobarbital, 46 mg/kg, Hypnol 3%), by cardiac puncture after a 12-hour fast at the end of the experiment. Blood samples were collected in tubes without anticlotter, remained in water bath at 37 °C for 30 minutes, and were centrifuged at 3000 rpm (Centrifuge Excelsa® II model 206 MP, FANEM) for 10 minutes. Serum was separated and stored at -80 °C until the moment of analyses. Analysis of serum triacylglycerol was done with the enzyme kit GPO-ANA-CAT.59 (Labtest Diagnóstica, Campinas, Brazil) and of serum leptin with the radioimmunoassay Rat Leptin Ria Kit RL-83K (Linco Research, Saint Charles, Missouri, USA).

Statistical analysis. Results are expressed as means ± SD. Data were analyzed statistically by ANOVA using Statistical Analysis System (SAS) (27). The Tukey test was used to test the differences among groups. Differences were considered significant at $P = 0.05$. The degree of association between leptin values and body fat was obtained by the Pearson linear correlation. The joint influence of independent variables on dependent variables was considered, and their forces were classified according to Levin (28), taking into account at least moderate correlation coefficients, both positive and negative, with r between 0.5 and 0.95, and between -0.5 and -0.95 respectively (29).

RESULTS

Supplements used in the present research contained 79.27 and 73.08% CLA for the brands AdvantEdge® CLA e CLA One®, respectively, and the proportion between the predominant isomers *cis-9, trans-11* and *trans-10, cis-12* was approximately 1:1 (Table 1).

Effect of conjugated linoleic acid supplementation on animal performance of rats is shown in Table 2. There were no significant effects of CLA supplementation on initial and final body weights, daily body weight gain, and food conversion efficiency between treatments; with exception of groups AE, which differed in the food intake ($p \leq 0.05$).

TABLE 2
Mean values ± standard deviation (n=10) of growth performance of experimental and control groups

	C	AE	CO
Initial body weight (g)	91.4 ± 2.8	96.3 ± 6.3	96.7 ± 4.9
Final body weight (g)	284.0 ± 21.7	299.8 ± 14.9	283.7 ± 12.9
Body weight gain (g/d)	5.3 ± 0.9	5.6 ± 0.5	5.6 ± 1.3
Food intake (g/d)	19.4 ± 1.1[a]	20.8 ± 1.3[b]	19.8 ± 1.1[a]
Food conversion efficiency	0.30 0.02	0.31 ± 0.01	0.30 ± 0.01

Values not sharing similar letter in the same line are different ($p \leq 0.05$) in the Tukey test.
C: control group; AE: group supplemented with AdvantEdge® CLA; CO: group supplemented with One® CLA

Table 3 shows the effect of CLA supplementation on body composition in these animals. Relative to control, the fat content (g) in experimental groups AE and CO was reduced by 16% and 19%, respectively. Hence, despite similar final body weights (Table 2), CLA supplementation resulted in significant less body fat ($p \leq 0.05$).

By contrast, the whole body protein, carcass water and ash (g) were significant enhanced for CLA-fed rats after 42 days (p 0.05) (Table 3).

TABLE 3
Mean values ± standard deviation of body composition on a wet basis of rats in control and experimental groups (n = 10) after 42 days of intervention

	C	AE	CO
Empty carcass weight (g)	254.12 ± 26.62	269.46 ± 17.02	262.3 ± 19.07
Water (g)	144.59 ± 1.92[a]	157.38 ± 1.07[b]	153.94 ± 1.67[c]
Protein (g)	49.62 ± 2.38[a]	53.27 ± 1.27[b]	52.73 ± 1.15[b]
Fat (g)	53.94 ± 6.80[a]	45.43 ± 4.86[b]	43.75 ± 1.93[b]
Ash (g)	8.04 ± 0.39[a]	9.25 ± 0.54[b]	8.82 ± 0.47[b]

Values not sharing similar letter in the same line are different ($p \leq 0.05$) in the Tukey test.
C: control group; AE: group supplemented with AdvantEdge® CLA; CO: group supplemented with One® CLA

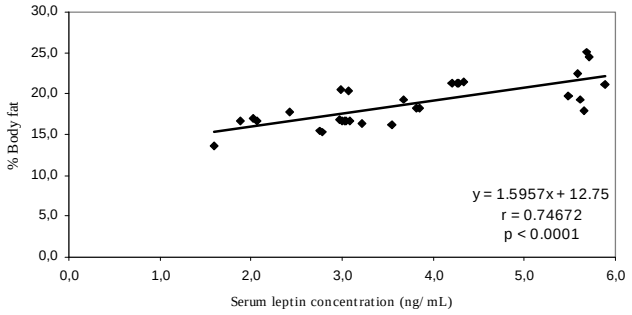
Serum levels of triacylglycerol (mg/dL) and leptin (ng/mL) of rats are presented in Table 4. Triacylglycerol values did not exhibit a statistically significant differences ($p > 0.05$). Leptin values of groups AE and CO were lower than the control group ($p = 0.05$). A moderate positive correlation was found between serum leptin values and body fat content ($r = 0.74672$, $p < 0.0001$) (Figure 1).

TABLE 4
Mean values ± standard deviation (n = 10) of serum leptin and triacylglycerols of control and experimental groups after 42 days of intervention

	C	AE	CO
Triacylglycerols (mg/dL)	142.95 ± 27.55	152.89 ± 44.53	168.39 ± 39.88
Leptin (ng/mL)	4.21 ± 0.22[a]	3.45 ± 0.46[b]	3.08 ± 0.19[b]

Values not sharing a similar letter in the same line are different ($p < 0.05$) in the Tukey test

FIGURE 1
Correlation between higher body fat percentage (%) and serum leptin concentration (ng/mL) of rats in control and experimental groups (n = 30) after 42 days of intervention



The present study investigated the effects after 42 days of CLA supplementation on serum triacylglycerol and leptin levels and body composition of rats. After this period of supplementation with two CLA isomer mixtures containing *cis*-9, *trans*-11 and *trans*-10, *cis*-12 in similar proportions, experimental groups had a body fat reduction and a whole body protein and ash content increase relative to the control group. The leptin concentrations were also suppressed in the supplemented groups. However, CLA supplementation did not change serum triacylglycerol levels.

Regarding animal growth performance, Table 2 shows that rats CLA-supplemented diet exhibited initial and final body weights that were similar from control ($p > 0.05$). By contrast, the data of AE group exhibit slightly enhanced food intake relative to control. In this study the weaning animals received water and food *ad libitum* and some it could result in small differences at some experimental time points. It is known that small differences in food intake do not affect body composition and that other physiological changes, in addition to decreases in leptin concentration, are required to mediate changes of appetite (30,31).

CLA has been found to be able to change body composition in different experimental models (18), promoting body fat reduction and lean mass increase. In the present study, the control group displayed body fat content of 53.94 ± 6.8 g, and groups AE and CO had 45.43 ± 4.86 g and 43.75 ± 1.93 g resulting in a reduction of 16 and 19%, respectively (Table 3). These data are similar to those of Delany and others (32) who reported a 30% reduction in body fat in mice after CLA supplementation for 39 days. Azain and coworkers (33) also observed a reduction of 13 to 30% in retroperitoneal fat in female rats supplemented with CLA for 35 days. Other works reported more pronounced reductions of body fat: mice lost about 60% of body fat when supplemented with 0.5% of CLA (10,13). It should be pointed out that experimental models used by these researchers exhibited visceral fat accumulation and a slight obesity, differently from animals in the present study. It is known that these experimental models are more responsive of supplementation with conjugated linoleic acid (34).

Being consistent with the reduction of fat deposition, overall body fat content has been shown to the reduced, while water, body protein and ash content was increased after CLA supplementation. Water represents such a large proportion of muscle, that minor increases in muscle synthesis could easily increase water content (35). In the present study, the carcass water content was significantly enhanced for CLA-fed rats compared with control. It was confirmed by the increased of whole body protein in the same groups, that suggest an enhanced lean body mass. The control group displayed a whole body protein of 49.62 ± 2.38 g, and groups AE and CO had 53.27 ± 1.27 g and 52.73 ± 1.15 g resulting in a increased by 7 and 6 %, respectively (Table 3). These data are similar to those of Park and others (13) who reported that a supplementation with 0.5% CLA in the diet increased protein and water contents in both male and female ICR mice. Supplementation of 0.25% CLA containing 79% *trans-10, cis-12* or 0.5% CLA containing 44% *trans-10, cis-12* and 41% *cis-9, trans-11* CLA isomers reduced body fat content and increased body protein, water and ash contents in weanling female ICR mice (10). The mechanism for the increased protein accumulation is unknown, but some authors speculates that the observed effect of CLA on body repartitioning is similar to that reported for carnitine and β-agonists that have been shown to depress fat deposition and stimulate protein accretion consistent with improved nitrogen retention through accelerated fat oxidation (36,37).

Ash content of rats supplemented with CLA were statistically higher ($p = 0.05$) (9.25 ± 0.54 g and 8.82 ± 0.47 g, for groups AE and CO respectively) relative to the control group (8.04 ± 0.39 g) (Table 3). Similar results have been reported by Park and others (13), when mice supplemented with 0.5% of CLA for 32 days displayed a significant increase in ash content. The same research team found a 14.5% increase in

ash content in mice fed with a 0.5% mixture of CLA isomers for 4 weeks (10). Some studies have suggested that CLA supplementation is capable of enhancing bone mineralization (13,38,39). Prostaglandin E_2 (PGE_2) is able to change synthesis of the insulin mediated growth factor, and consequently hamper bone formation. CLA competes with other polyunsaturated fatty acids, thus inhibiting the formation of PGE_2 via cyclooxygenase (38,39). Results obtained in the present work indirectly suggest that CLA supplementation for 42 days enhanced bone mineralization of animals of groups AE and CO, since they exhibited higher ash content than the control group.

There are several possible mechanisms for the reduced fat accumulation in response to CLA feeding, such as a decrease in pre-adipocyte proliferation and differentiation, increase fatty acid oxidation, as evidenced by increased carnitine palmitoyltransferase activity, increase of energy expenditure, reduction of esterification of fatty acids into triacylglycerols, and changes in the activity of enzyme lipoprotein lipase, and the hormone leptin (18,19,40). With regard to serum leptin concentration (ng/mL), it was found that experimental groups AE and CO, which received CLA supplementation, had statistically smaller values ($p = 0.05$) than the control group, which received linoleic acid (Table 4), corresponding to a reduction of 22.4%. Ours results showed that both commercial conjugated linoleic acid mixtures reduced body fat mass and serum leptin concentrations in Wistar rats ($p = 0.05$). Similar results were found by Rahman and collaborators (41), when Otsuka Long-Evans Tokushina Fatty (OLETF) rats fed AIN-93G which received 1% of a CLA isomer mixture in the form of triacylglycerol and in the form of free fatty acids, exhibited a significant reduction of serum leptin. Corroborating these results, Akahoshi et al. (42) found a significant reduction of serum leptin values in mice fed 1% of CLA for 8 weeks. More recently, Sprague-Dawley rats had a reduction in serum leptin values after supplementation with 1.5% of a CLA mixture for 3 weeks (43). The decreased levels of serum leptin and body fat mass might be coordinated responses. However, there might be reasons for the effects of CLA on serum leptin concentration. First, CLA incorporated into membrane phospholipids fractions might have effects on signal transducing pathways and modify leptin production (44). Second, CLA could activate peroxisome proliferator activated receptors γ (PPAR γ) might reduce leptin gene expression (45).

Leptin, a protein hormone produced by adipocytes, reflects the body fat content. Thus, obese individuals have higher serum leptin concentration than lean individuals, i.e., there is a positive correlation between serum leptin content and body fat (46). In the present work, animals supplemented for 42 days with 2% of CLA relative to the mean daily feed consumption displayed a moderate positive correlation between serum leptin values and body fat ($r = 0.74672$, $p < 0.0001$) (Figure 1). Akahoshi et al. (42) found the same positive correlation of $r = 0.74$, $p < 0.0001$ between fat content of epididymal adipose tissue and serum leptin.

Antiatherogenic properties were attributed to CLA and are believed to be a result, at least in part, of changes in lipoprotein metabolism (47,48). However, the reported effects of CLA on blood lipids are controversial. Serum triacylglycerol content (mg/dL) presented in Table 4 did not show a statistically significant difference ($p > 0.05$). Likewise, Azain et al. (33) did not observe differences in serum triacylglycerol content in Sprague-Dawley supplemented with 0.25 and 0.5% of CLA for 5 weeks. These results can be explained in terms of physiological effects of CLA on adipocytes. CLA is able to reduce lipoprotein lipase (LPL) activity and intracellular concentrations of triacylglycerol and glycerol. Therefore the unchanged triacylglycerol content in this study could have resulted from the lesser activity of the LPL, making that triacylglycerol remained in circulating lipoproteins (13,49,50).

In conclusion, the results obtained in the present study indicates that supplementation with conjugated linoleic acid in the concentration of 2% of mean daily feed consumption decreased content of body fat and adipose depots, but increased body protein content without growth performance changes, confirming the ability of CLA to modifies body composition in growing rats, when fat deposits are in formation. Metabolic changes that occurred seem to simultaneously promote reduction of lipogenesis and enhancement of lipolysis. Further research is need in order to verify the action mechanisms of CLA in young individuals, in a more profound studies.

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