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Effect of conjugated linoleic acid on quality of rabbit meat

Efecto del ácido linoleico conjugado sobre la calidad de la carne de conejas

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ABSTRACT

The use of fatty acids in food animal supplements is to improve animal health and decrease the use of medications. CLA is a bioactive compound and can be incorporated into the diet of non-ruminant animals. The objective of this experiment was to evaluate the effect of incorporating CLA into the diet of New Zealand rabbits on meat quality. The variables evaluated were the microbiological quality of cecotrophs: microbial load of LAB, *enterobacteria* and *Clostridium* spp., zootechnical parameters and physicochemical quality of meat from rabbits at slaughter. The results obtained showed the presence of LAB and *Clostridium* and the absence of Enterobacteriaceae during the study period. Proximal analysis of rabbit meat showed no difference ($p > 0.05$) in live weight (LW), weight gain (WG), feed efficiency (FE) and feed conversion ratio (FCR) in the treatments and the control. But WRC and color showed difference ($p \leq 0.05$) between LD and BF muscles. The FA profile showed the presence of CLA in the thigh of rabbits.

Keywords: CLA, rabbits, WRC, cecotrophs, pH, color.

RESUMEN

El uso de ácidos grasos en suplementos para animales de consumo humano es para mejorar la salud del animal y disminuir el uso de medicamentos. El CLA es un compuesto bioactivo y se puede incorporar en la dieta de animales no rumiantes. El objetivo de este experimento fue evaluar el efecto de la incorporación de CLA en la dieta de conejas Nueva Zelanda, sobre la calidad de la carne. Las variables evaluadas fueron la calidad microbiológica de cecótrofos: carga microbiana de BAL, enterobacterias y clostridium spp., los parámetros zootécnicos y la calidad fisicoquímica de la carne de conejas al sacrificio. Los resultados obtenidos mostraron presencia de BAL y Clostridium y ausencia de Enterobacterias durante el tiempo del estudio. El análisis proximal de la carne de conejas no mostró diferencia ($p > 0.05$) en el peso vivo (PV), ganancia de peso (GP), eficiencia alimenticia (EA) y conversión alimenticia (CA) en los tratamientos y el testigo. Pero la CRA y el color mostraron diferencia ($p \leq 0.05$) entre los músculos LD y BF. El perfil de AG mostró la presencia de CLA en el muslo de las conejas.

Palabras clave: CLA, conejas, CRA, cecótrofos, pH, color.

INTRODUCTION

The term CLA describes the geometric and positional isomers of C18:2 linoleic acid containing double bonds in *cis* or *trans* configuration along the 18-carbon chain. The result of microbial biohydrogenation of C18:2 and C18:3 linolenic is the formation of 28 possible isomers, where cis-9, trans-11 and trans-10, cis-12 isomers are the most active in cancer, obesity, inflammation, diabetes and atherosclerosis (Castro-Webb *et al*., 2012; Yang *et al*., 2015; Jelińska e*t al*., 2017). Agricultural feeds with CLA are milk, meat and by-products derived from these; production efficiency and meat quality can be improved by including CLA in the diet of livestock species. This concentration depends on *ante*- and *post-mortem* factors, i.e., breed, climate, intestinal health, type of production, type of muscle and raw materials.

Rabbit meat is considered a functional food (Dalle Zotte & Szendrő, 2011), because regular consumption can provide bioactive compounds: vitamins and minerals, antioxidants and FAs such as CLA. Rabbits are a small species with advantages such as breeding techniques, biological factors such as age and weight, genetic and breed effects and technological processes for meat processing that larger species such as poultry, pigs and cattle do not have. The objective of this research was to evaluate the effect of CLA in the diet of Nueva Zelanda rabbits on meat quality.

MATERIAL AND METHODS

Experimental conditions

Nueva Zelanda rabbits (n=15) were acquired from the UNAM rabbit farm, Mexico, and transported by land to the zootechnical post of the University of Papaloapan, campus Loma Bonita, Oaxaca. The study was divided into two stages: the probiotic stage and the CLA stage. Newly weaned females (30 d) presented similar birth date and weight (762 ± 50.9 g). The population was divided into two experimental groups: control (T) and probiotic treatment (P), housed in double cages of galvanized mesh with dimensions of 90x60x40 cm. The two groups: T and P were fed *ad libitum* with commercial base feed (Conejina Turbo^{MR}, Purina, Mexico) but the P group was administered probiotic BioPlus 2B® (Chr. Hansen BioSystems A/S, Denmark), the probiotic stage lasted six weeks. Then, in the CLA stage (seventh week), the population was divided into three groups: control (T), treatment 1 (T1) and treatment 2 (T2), with five rabbits each. The females were distributed to control experimental noise and to allow the coincidence of characteristics such as age, live weight, genotype, origin (same farm) and management.

Experimental diets

The three groups were fed a diet based on cereals, minerals and essential oils (Table 1), but with different CLA content (Corino *et al*., 2003).Diets were prepared by hand, without pelleting or extrusion.

Table 1. Ingredients used in diets for rabbits

Ingredient (g)	T	T_1	T_2
Alfalfa hay	52	52	52
Ground corn	18	18	18
Soybean meal	12	12	12
Oats	9	9	9
Wheat bran	5	5	5
Salts and minerals	1.5	1.5	1.5
Canola oil	1.5	1.1	0.7
Synthetic methionine	0.5	0.5	0.5
CLA	0	0.4	0.8

T= without CLA; T1= 0.4 g CLA kg^{-1}; T2 = 0.8 g CLA kg^{-1}. Protein: 20.5±0.9 %, EE: 2.8±0.1%, Ash: 6±0.1% and Crude fiber: 19±0.8%.

CLA was purchased from the supplier GNC® presentation in a bottle of 90 capsules of 1.6 g, each containing CLA 55 mg. Each ration contained CLA administered in aqueous dilution with 20 mL of distilled water. The application was after preparing the complete ration and by manual atomization. The second stage lasted six weeks and the total time was 12 weeks.

Microbiological quality

Soft feces (cecotrophs) were analyzed as part of normal feeding behavior in T and P. The microbiological study included the counting of three microbial groups: *lactic acid bacteria* (LAB), *enterobacteria* and *clostridium*. The procedure for sample collection, handling, transport, preparation and dilution was carried out according to Linaje *et al*., (2004). The identification of LAB was performed on MRS Agar, *enterobacteria* on MacConkey Agar and *clostridium* on Reinforced Clostridial Agar. The culture media were prepared according to specifications of the supplier Fluka Analytical©, Switzerland. During the collection of the cecotrophs, sterile forceps, gloves and test tubes were used, transferred to the chemical analysis laboratory and stored frozen at -18 °C.

The 1g sample treatment of cecotrophs was transferred to sterile Stomacher bags, sterile 0.1% pepton water (1g peptone, 8.5 g NaCl in 1000 mL water) was added to a 1:10 dilution, the mixture was homogenized for 1-2 min. Appropriate aliquots of dilutions were

taken from test tubes with 0.1% sterile pepton water. The analysis was performed in triplicate and colonies were counted after the incubation time. The criterion used in the selection of the boxes was 30 and 300 colonies. Counts obtained were expressed as Log10 CFU g^{-1}.

Zootechnical parameters

Live weight (LW) of rabbits was recorded weekly in the mornings, during the duration of the experiment and before feeding the experimental diet. Feed efficiency (FE) was determined with expression 1. In practice, the inverse of this ratio is known as feed conversion (FC) calculated with expression 2 and is used in animal husbandry to refer to the conversion of feed to weight gain. In all cases the weight was obtained with a Torrey©, Mexico, model LPCR multifunctional scale.

$$EA = (average\ daily\ weight\ gain/average\ daily\ feedings)\ (1)$$

$$FC = (1/FE)\ \ (2)$$

These expressions are indicators of nutrition, the former measures biological efficiency, while the latter is an economic measure of feeding. Animals with a low FC value tend to be more efficient, (Gaillard *et al*., 2020), however, they are different depending on the species, genetics, health status, feeding practices and environmental conditions. On the other hand, the rabbits were desensitized and slaughtered according to NOM-033-SSA1, inside the chemical-biological laboratory of the university. Rabbit carcasses were frozen after slaughter at 5 °C until further analysis. Live weight (LW) was recorded before slaughter; cold carcass weight (CC) and offal (O) corresponding to viscera, feet, head and tail were obtained after slaughter. Weight gain was obtained by difference, while perirenal fat (PRF) and interscapular fat (IF) were obtained from the kidney area and external vertebral section, respectively. For all the determinations, an Ohaus© model CS-5000 granatary balance was used.

Physicochemical quality

Physicochemical quality was analyzed after slaughter and at 24 h postmortem on carcasses from two different muscles; *Longissimus dorsis* (LD) and *Biceps femoris* (BF). Proximal analysis of dry matter (DM), moisture (M), ash (As), ethereal extract (EE) and crude protein (CP) (AOAC, 1990) was carried out in triplicate. Hydrogen potential (pH) was analyzed with an Orion Star A211 pH meter, Thermo Scientific, according to NOM-317-S-1978. Color saturation (*a, *b and *L), was analyzed using an UltraScan® Vis colorimeter (HunterLab, H unter Associates Laboratory Inc., Hills Road, Reston, Virginia USA). The color reading was determined in both muscles, in the visible zone from (400 to 700 nm).

Water retention capacity (WRC), was analyzed using 0.6 M NaCl as an added solution, the sample was allowed to stand for 30 min and centrifuged at 3000 rpm for 15 min. The supernatant was quantified as percentage of water retained in the sample. The FA profile, was quantified with a solid-liquid extraction and a 2:1 v/v chloroform-methanol mixture, (Figueiredo *et al.*, 2016). The solution was analyzed by gas chromatography (GC) with an HP chromatograph equipped with flame ionization detector and a SupelcoWax-10 capillary column of 100 m x 0.25 mm diameter and a film thickness of 0.2µm. The results obtained were expressed as % FA.

Statistical analysis

The data obtained were normalized and analyzed by analysis of variance and Tukey's mean comparison with a significance level $p \leq 0.05$.

Microbiological quality

The average BAL count during the duration of the experiment was 8.27 + 0.72 CFU g^{-1} and 8.37 + 0.62 CFU g^{-1} for T and P, respectively. Figure 1 shows the behavior of BAL and indicates that there is a difference between the two groups.

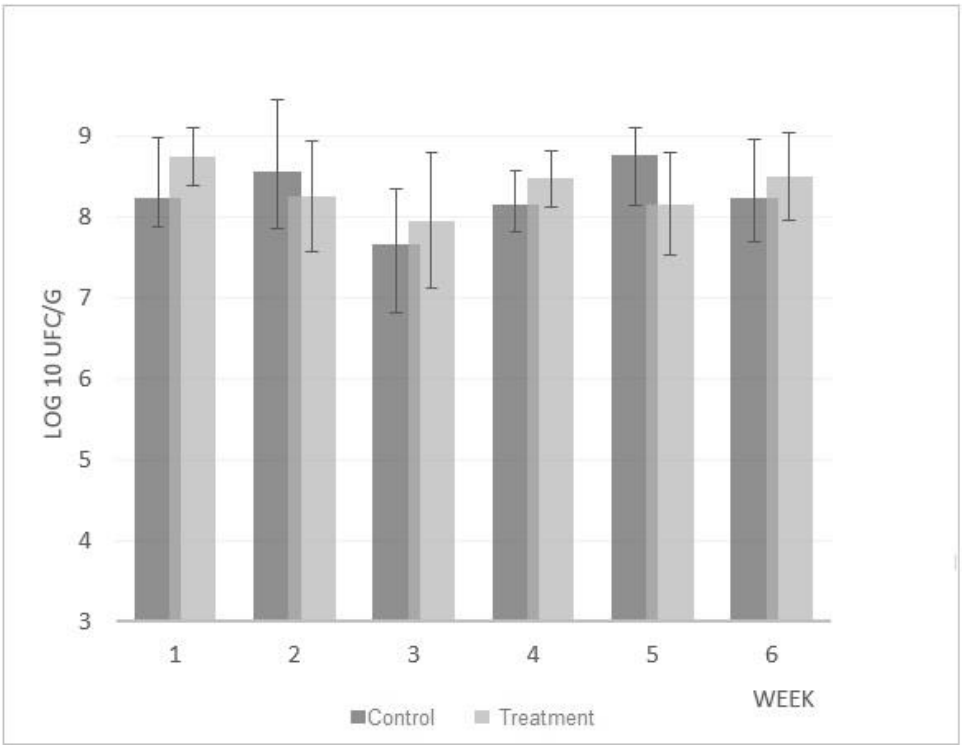

Figure 1. Quantification of BAL in rabbit caecotrophs for 6 weeks

The average of *enterobacteria* in T was 6.64 ± 1.85 Log10 CFU g^{-1} and for P there was no growth of this bacterial group, even from the first sampling. Finally, the *Clostridium* spp. counts were 8.31±0.87 CFU g^{-1} and 8.39±0.60 CFU g^{-1} for T and P, respectively.

Zootechnical parameters

The LW of rabbits is higher in T compared to T1 and T2 even though WG is the same for all groups (Table 2). CC weight is obtained after slaughter and it was observed that T1>T>T>T2 and is consistent with D where it is shown that T1 and T2 represent a higher amount of offal (skin, feet and tail), which can also be utilized as by-products (local handicrafts). Viscera, blood and organs can be transformed into meal, an ingredient for animal feed. The FE ratio is the same at T1 and T2 and FC has a value of 2.2 and 2.1, respectively.

Table 2. Zootechnical parameters of rabbits supplemented with CLA

Variable	T	T_1	T_2	±
LW (kg)	2.1	2.4	2.3	1.4
WG, (kg)	1.1	1.1	1.1	0.01
CC (kg)	1.2	1.3	1.0	0.05
O (kg)	0.9	1.1	1.3	0.05
FE	0.3	0.2	0.2	0.01
FC	2.0	2.2	2.1	0.03
PRF (g)	4.8	8.8	9.5	2.5
IF (g)	1.0	2.8	2.2	0.9

T=without CLA, T1=0.4 g CLA kg^{-1}, T2=0.8 g CLA kg^{-1}. LW=live weight, CC=cold carcass, O=offal, FC=feed conversion, FE=feeding efficiency, WG=weight gain, PRF=perirenal fat, IF=interscapular fat, SD=standard deviation.

Finally, PRF values are different in both treatments and T and this behavior is present in IF values.

Physicochemical quality

The proximal chemical composition is shown in Table 3, the H content is 74-73%, similar to that reported by FAO, which reports 74.9% in rabbit meat. DM, therefore, presented the highest content in proximal analysis (25-26%). The C content represents the minerals, that is, the essential ions for humans; it was different only when compared by treatment (Table 3).

Table 3. Proximal composition in rabbit meat supplemented with CLA.

%	T	T_1	T_2	SEM	Prob	LD	BF	SEM	Prob
DM	25.66	26.33	25.84	0.410	NS	25.94	25.95	0.335	NS
P	22.44	23.07	23.06	0.396	NS	22.51	22.52	0.373	NS
C	1.37ab	1.29a	1.47b	0.038	0.005	1.38	1.39	0.031	NS
EE	1.27	1.30	1.17	0.208	NS	1.23	1.24	0.170	NS

Control =no CLA, T_1=0.4 gCLA Kg^{-1}, T_2=0.8 gCLA Kg^{-1}. LD= *Longissimus dorsis*, BF= *Biceps femoris*. Values present mean ±SEM. abcd, different letters in each column indicate significant difference ($p \leq 0.05$). SEM=standard error of the mean, NS=not significant.

The technological quality parameters of the meat are shown in Table 4. WRC changes during the *posmorten* period and decreases as the meat matures. It was observed that there is a statistical difference between treatments (T_1 and T_2) and muscles (LD and BF) after 1 and 24 h after slaughter. WRC is a parameter that depends on pH, which causes loss of moisture, change in texture, increase in tenderness and difference in color. The change in pH towards acid values is associated with the irreversible process of muscle contraction at the level of the actin-myosin chains and the production of lactic acid, which lowers the pH to 5.4. The pH value in this study shows significant changes only between treatments (T_1 and T_2) after 1 and 24 h of sacrifice. Factors associated with WRC changes and pH are the production of lactic acid and therefore the decrease in pH, the consumption of glycogen in the muscle, the decrease in energy compounds (ADP and ATP) that initiate cadaveric stiffness and, therefore, protein denaturation.

Color is associated with physical (b*), chemical (a*) and hemoglobin content (L*). In all cases the parameter L>b>a (Table 4). The parameter L* associated with hemoglobin content was the only parameter that did not show a significant difference when the results were analyzed by treatments (T_1 and T_2) and by muscle type (LD and BF). This is important because color is not affected by the diets tested and also when this analysis is performed on two different commercial muscles. The parameter b* was statistically different when compared by treatments (T_1 and T_2) while a* was not different when the results were evaluated in both conditions.

Table 4. Technological quality parameters of meat from rabbits supplemented with CLA

%	T	T_1	T_2	SEM	Prob	LD	BF	SEM	Prob
WRC_{1h}	18.65ab	19.08b	17.53a	0.501	0.04	17.79	19.04	0.409	0.05
WRC_{24h}	9.36b	8.88a	8.86a	0.150	0.04	8.43	9.64	0.123	0.01
pH_{1h}	6.42	6.42	6.52	0.050	NS	6.84	6.07	0.041	0.01
pH_{24h}	5.94	5.98	5.96	0.065	NS	6.29	5.66	0.053	0.01
$Color_{24h}$									
L*	62.44	60.36	62.19	0.976	NS	60.96	62.36	0.797	NS
b*	12.30	12.37	11.86	0.664	NS	10.31	14.04	0.542	0.01
a*	-0.05a	-0.28ab	-0.57b	0.176	0.05	-2.82	2.22	0.144	0.01

Control =no CLA, T1=0.4 gCLA Kg^{-1}, T2=0.8 gCLA Kg^{-1}. LD= *Longissimus dorsis*, BF= *Biceps femoris*. WRC =water retention capacity, L*=luminosity, a*=red, b*=yellow. Values present mean ±SEM. abcd, different letters in each column indicate significant difference (p≤0.05). NS= not significant.

Fatty acid profile

On the other hand, the FA profile is shown in Table 5, it is observed that the composition in rabbit meat from LD muscle was 18:1>C14:0>C16:0>FAPI, presenting a significant difference only in the FA C18:1 among the treatments tested. While the BF muscle composition was C18:2>C18:1>C16:0>C18:2, c_9t_{11}>C18:2, $c_{12}t_{10}$>C14:0.

Table 5. FA profile in meat from rabbits supplemented with CLA

	Longissimus dorsis			*Biceps femoris*			
	T	T_1	T_2	T	T_1	T_2	SEM
C14:0	21.4b	23.91c	23.50c	sd	sd	sd	0.55
C16:0	8.49a	9.12a	8.73a	11.38b	21.79d	20.10c	0.41
C18:1	27.16de	24.40bc	28.13e	13.92a	25.78cd	24.08b	0.52
C18:2	nd	nd	nd	16.51b	25.84c	26.94c	0.62
C18:2, $_{c9t11}$	nd	nd	nd	nd	0.487b	0.710c	0.03
C18:2, $_{c12t10}$	nd	nd	nd	5.3b	nd	nd	0.06

Control=no CLA, T1=0.4 gCLA kg^{-1}, T2=0.8 gCLA kg^{-1}. Values present mean ±SEM, abcd, different letters in each column indicate significant difference (p ≤ 0.05), nd=not detected.

DISCUSSION

Latorre *et al*., (2014) evaluated the persistence of *Bacillus subtilis* spores, a type of LAB, through the gastrointestinal tract (GIT), the results showed that approximately 90% of the spores germinate in the GIT. Changes related to different physiological, nutritional and microbiological conditions can be compared with LAB, which have the ability to germinate

at the GIT level and survive in the tested environment, as shown in Figure 1. The increase of LAB in this study during weeks 4 and 6 allows inferring a possible increase in the production of antimicrobial substances that help the health of the rabbit's GIT. The administration of probiotic has the function of preventing or delaying the onset of gastrointestinal diseases and rabbits that consumed probiotic in the diet showed adequate health condition (Gaggia *et al.*, 2010). *E. coli* bacteria belong to the enterobacteria family and their presence is associated with diarrhea in newly weaned animals and the substitution of antibiotics in the diet. Zootechnical characteristics can be significantly improved due to the development and maintenance of the cecal microbiota that promotes the health status of the animals. The probiotics *Saccharomyces cerevisiae* and *Enterococcus faecilum* were administered orally for two weeks in healthy rabbits; the treatment did not affect fecal levels of bacteroides species, *fibrobacter succinogenes* or *clostridium spiroforme*, (Summa & Brandao, 2017).

Meat technological quality parameters, pH, WRC and color showed expected changes while zootechnical parameters were low to those reported by Palma & Hurtado (2009), they fed male young rabbits for 60 d with a combined diet of commercial feed (CFE) and fresh Creole mango (CM), the CA was from 2.41 to 2.13 in diets with 60 and 40 g of CFE and 60 and 80 g of CM. In another study, (Corino *et al.,* 2003) found that animals supplemented with CLA did not obtain significant differences in productive parameters. Pérez-Martínez *et. al*., (2018) evaluated productive parameters in rabbits fed with different parts of the *Tithonia tubaeformis* plant, they found no significant difference in the measured variables, however, the CFE was from 3.18 to 3.41 such results are similar to what was reported by Meineri *et al*., (2010) y Dalle Zotte & Szendro (2011).

Malavé (2013), reported that dietary supplementation of different animals did not significantly affect proximal chemical composition, results consistent with this study where difference was observed only in T content (1.47-1.29%).
These results indicate the presence of CLA, cis-9, trans-11, in meat from BF muscle in both treatments and it is higher in T2, that is, when rabbits are fed 0.8 gCLA Kg^{-1}. Corino *et al*., (2003) determined the FA profile in *Longissimus lumborum* in rabbits supplemented with 0.5% CLA and obtained 20% of C18:1, 29% of C18:2n6 and 25% of C16:0. In another study, the relative proportion of different types of FA in LD was reported, reaching 38% saturated FA, 28% monounsaturated FA and 32% polyunsaturated FA. These proportions of FA allow comparing rabbit meat with other species such as pork, chicken, veal and beef (Dalle Zotte & Szendrő, 2011). However, rabbit meat has functional characteristics such as low cholesterol content, high content of protein and essential amino acids; especially in LD, high digestibility and high levels of polyunsaturated FA that allow it to be considered as a functional food with nutritional and dietary properties (Dalle & Szendrő, 2011).

CONCLUSIONS

Meat quality depends on several parameters, in this study the CLA-enriched diet was analyzed on microbiological, productive and physicochemical quality. The microbiological quality indicated that LAB are present during the time of the study (six weeks) and it is sufficient to inhibit *enterobacteria*. This behavior is important considering that in the first weeks of life immunity is acquired in the presence of various pathogens that damage the animal's health. The commercial weight was reached in 80 d of feeding with a low FC and a carcass yield according to expectations. The productive quality is not affected when two different diets are fed, however, the evaluation of the physicochemical quality of the meat indicated that the WRC is the parameter affected in the evaluation and is reflected in the physicochemical state of hemoglobin. The FA profile showed that SFAs do not change by muscle type and the presence of CLA was demonstrated at least in BF muscle. This can be used to consider BF or thigh meat as an alternative source of CLA.

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