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Effect of oil supplementation extracted from nontoxic purging nut (*Jatropha curcas* L) on carcass traits, tissue composition, muscle CLA concentration, and visceral mass of feedlot lambs

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ABSTRACT. The effects of purging nut (*Jatropha curcas*, JCO) supplementation (0, 2, 4, and 6%, DM basis of diet) on carcass traits, tissue composition and conjugated linoleic acids (CLA) concentration in muscle was evaluated in twenty intact male lambs fed a finishing diet during 56 d. The linoleic acid proportion in JCO was 50%. Lambs were harvested at a final weight of 54.03±2.9 kg. There were no treatment effects on hot carcass weight (HCW), *longissimus* muscle (LM) area nor kidney-pelvic fat. However, as JCO supplementation increased, dressing percentage was decreased and fat thickness was increased. Increasing JCO in diet decreases the proportion of muscle and increases the proportion of fat in whole shoulder clod. Content of stearic acid (C18:0) in LM was not affected by JCO. However, JCO linearly increased total CLA, and hence, the CLA:C18:0 ratio. Empty body or visceral mass were not affected by JCO. Increasing JCO in diet increases visceral fat mainly through increased mesenteric fat. It is concluded that supplemental JCO does not negatively affect HCW and LM area, and represents a viable alternative for increasing CLA concentration in meat of finishing feedlot lambs.

Key words: *Jatropha curcas*, supplemental oil, lambs, carcass, conjugated linoleic acid.

RESUMEN. Los efectos de la suplementación (0, 2, 4, y 6%, en base seca de la dieta) de aceite de nuez purgante (*Jatropha curcas*, JCO) sobre las características de la canal, la composición tisular y la concentración de ácidos linoleicos conjugados (CLA) en músculo se evaluó en veinte corderos machos intactos, alimentados con una dieta de finalización durante un periodo de 56 días. La proporción de ácido linoleico en JCO fue de 50%. Los corderos fueron faenados con un peso final de 54.03 ± 2.9 kg. No hubo efecto de los tratamientos en el peso de la canal caliente (PCC), el área del músculo *longissimus* (ML) o la grasa pélvica-renal. Sin embargo, a medida que aumentó la suplementación de JCO, se disminuyó el rendimiento de la canal y aumentó el espesor de grasa dorsal. El aumentar JCO en la dieta disminuyó la proporción del músculo y aumentó la proporción de grasa en la paleta. El contenido de ácido esteárico (C18:0) en ML no se vio afectado por JCO. Sin embargo, JCO aumentó linealmente el total de CLA en ML, y por tanto, la proporción CLA:C18:0. El peso corporal vacío o la masa visceral no fueron afectados por JCO. El incrementar JCO en la dieta aumentó la grasa visceral por el aumento de la grasa mesentérica. Se concluye que la suplementación con JCO no afecta negativamente el PCC o el área de ML y representa una alternativa viable para aumentar la concentración total de CLA en la carne de corderos en finalización.

Palabras clave: *Jatropha curcas*, aceite, corderos, canal, ácido linoleico conjugado.

INTRODUCTION

Conventional supplemental fats fed to feedlot lambs are largely comprised of vegetable oils (i.e. yellow grease) with a high proportion of unsaturated fatty acids (30:70 saturate:unsaturate fatty acid ratio). The major unsaturated fatty acids in conventional feed fats is oleic acid (C18:1), which is largely hydrogenated in the rumen. Consequently,

stearic acid (C18:0) is the major fatty acid entering to the small intestine and subsequently deposited, mainly in muscle (Jenkins 2008). According to the Food and Agriculture Organization (FAO 2010), the consumption of saturated fatty acids represents a risk to the human health. Although biohydrogenation of unsaturated FA decreases with high concentrate diets (Jenkins 1994), the processes of ruminal biohydrogenation are nevertheless extensive (65%; Plascencia *et al* 1999). Fat sources with high concentration of linoleic acid (C18:2) promote greater flow of trans-fatty acids of biological importance (T11-C18:1 and C9 T11-C18:2) to the small intestine, as they lend to increased tissue conjugated linoleic acid (CLA) concentration. The presence of CLA isomers in meat and milk products may have important health benefits including: anti-carcinogenesis, decreased blood cholesterol and reduced body fat accumulation (Drackley 2000). This effect increases along with the levels of oil supplementation (~7%; Kucuk *et al* 2004). Thus, it is expected that supplementation of finishing lambs with fat sources high in C18:3 will have a positive impact on

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CLA concentrations in meat. The offer of consumption of a more healthy meat product would mean significant economic enhancement for the ruminant meat industry¹. Soy oil, corn oil, and sesame oil are good sources of C18:2 (~45%), but due to greater cost, are seldom used as feed ingredient in ruminant diets. Oil extracted from purging nut (*Jatropha curcas* L.) seed is rich in C18:2 (45-55%). However, as long as *Jatropha* oil is used mainly for fuel industry (largely produced for use as a biofuel, Rashid *et al* 2010), its cost is affordable as a supplemental energy source for livestock (King *et al* 2009). The purging nut seed cake (from a Mexican non-toxic variety) derivative from the process to extract *Jatropha* crude oil is a suitable feedstock for finishing lambs (Félix-Bernal *et al* 2014). However, *Jatropha* oil has not been evaluated as ingredient for ruminant diets. The objective of this experiment was to evaluate effects of *Jatropha curcas* L. oil supplementation on carcass traits, tissue composition, tissue CLA concentration, and visceral organ mass of finishing lambs.

MATERIAL AND METHODS

DIETS, ANIMALS AND EXPERIMENTAL DESIGN

This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in the Culiacán, México (24° 46' 13" N and 107° 21' 14" W). Culiacán is about 55 m above sea level, and has a tropical climate. All animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care. Twenty intact male lambs (¼Pelibuey × ¾Kathadin, 40.7 ± 3 kg initial BW) were used. Lambs were dewormed 30 days before initiation of the experiment. Upon initiation of the experiment, lambs were weighed individually prior to the morning meal (electronic scale; TORREY TIL/S: 107 2691, TORREY electronics Inc., Houston, TX, USA), and blocked by weight into five uniform weight groups and assigned within weight group to 20 pens (1 lamb/pen). Individual pens were 6 m² with overhead shade, automatic waterers and 1 m fence-line feed bunks. During a 15 d adaptation period before initiation of the experiment, all lambs received the basal diet (no JCO supplementation, table 1). Dietary treatments consisted in a dry rolled corn-based finishing diet supplemented with either 0, 2, 4, or 6% JCO (DM basis). Supplemental JCO replaced dry rolled corn in the basal diet. Diets were maintained isonitrogenous by the addition of supplemental urea (table 1). The JCO was obtained by mechanically pressing using a German screw press (Type Komet DD 85 G; IBGMonforts, Oekototec, GmbH & Co. KG, An der Waldesruh 23

Mönchengladbach Nordrhein-Westfalen, Germany) whole seed from a nontoxic variety (*Jatropha curcas*) harvested in San Ignacio, Sinaloa, México. Butylated hydroxytoluene (BHT, 0.02%, wt/vol) was added to prevent oxidation. Corn (white corn variety) was prepared by passing whole corn through rollers (46 × 61 cm rolls, 5.5 corrugations/cm; Memco, Mills Rolls, Mill Engineering & Machinery Co., Oklahoma, CA) that had been adjusted to provide an approximate rolled-grain density (as-is basis) of 0.62 kg/L. Sudan grass hay was ground in a hammer mill (Bear Cat #1A-S, Westerns Land and Roller Co., Hastings, NE) with a 2.7 cm screen, before incorporation into complete mixed diets. The physicochemical composition of dry corn (DRC) replaced by JCO are shown in the footnote of table 1. Dietary treatments were randomly assigned to lambs within weight groupings. Treatments were evaluated during a 56 day finishing period. Lambs were individually weighed. All lambs were fasted from feed (drinking water was not withdrawn) for 18 h before recording the final BW. Lambs were allowed *ad libitum* access to dietary treatments. Feed refusals were collected, and weighed prior to the morning feeding. Dry matter intake was determined on a daily basis.

CARCASS AND VISCERAL MASS DATA

All lambs were harvested on the same day following the specifications of humanitarian sacrifice for domestic and wild animals (NOM-033-ZOO-1995). Hide and gastrointestinal organs were separated and weighed. Carcasses (with kidney pelvic and heart fat included) were chilled at -0.5 °C for 48 h. Subsequently, the following measurements were obtained: 1) fat thickness perpendicular to the *m. longissimus thoracis* (LM), measured over the center of the ribeye between the 12th and 13th rib; 2) LM surface area, measured using a grid reading of the cross sectional area of the LM between 12th and 13th rib, and 3) kidney and pelvic fat (KP). The KP was removed manually from the carcass, weighed, and is reported as a percentage of the cold carcass weight (USDA 1982). Each carcass was split along the vertebrae into two halves. Shoulders were obtained from the forequarter. Shoulder weight was recorded, and composition was assessed using physical dissection (Luaces *et al* 2008).

All tissue weights were reported on a fresh basis. Previous data suggests that there is very little variation among fresh and dry weights for visceral organs (Neville *et al* 2008). Organ mass was expressed as grams of fresh tissue per kilogram of final EBW. Final EBW represents the final full BW minus the total digesta weight. Full visceral mass was calculated by the summation of all visceral components (stomach complex + small intestine + large intestine + liver + lungs + heart), including digesta. The stomach complex was calculated as the digesta-free sum of the weight of the rumen, reticulum, omasum and abomasum.

¹ McGinley S. 2003. Improving meat quality with CLA. Agricultural Experiment Station Research Report. https://cals.arizona.edu/pubs/general/resrpt2003/article3_2003.html. Accessed 20 June 2016.

Table 1. Composition of experimental diets.

Item	<i>Jatropha</i> crude oil level (%)			
	0	2	4	6
Ingredient composition (%)				
Dry-rolled corn ^a	72.00	70.00	68.00	66.00
<i>Jatropha</i> crude oil	---	2.00	4.00	6.00
Soybean meal	5.50	5.50	5.50	5.50
Sudan grass hay	12.00	12.00	12.00	12.00
Molasses cane	8.00	7.93	7.86	7.79
Urea	---	0.07	0.14	0.21
Trace mineral salt (agromix) ^a	2.50	2.50	2.50	2.50
Chemical composition ^b , (DM basis)				
Crude protein (%)	12.15	12.15	12.14	12.14
Ether extract (%)	3.23	5.10	6.42	9.52
NDF (%)	16.51	16.30	16.08	15.87
Calculated net energy (Mcal/kg)				
Maintenance	2.00	2.08	2.16	2.24
Gain	1.35	1.42	1.49	1.56

^aComposition and density of dry-rolled corn were (%): DM, 89.1; OM, 96.8; CP, 8.8; NDF, 103.0; ADF, 4.1; starch, 69.4; ether extract, 3.8.; bulk density (g/L), 600.

^bMineral premix contained: CP, 50%; Calcium, 28%; Phosphorous, 0.55%; Magnesium, 0.58%; Potassium, 0.65%; NaCl, 15%; vitamin A, 1,100 IU/kg; vitamin E, 11 UI/kg.

^cDietary composition was determined by analysing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other

^dBased on tabular net energy (NE) values for individual feed ingredients (NRC2007) .

SAMPLE ANALYSIS

Corn grain, JCO, and complete mixed diets were subjected to all or part of the following analyses: Dry matter (DM, oven drying at 105 °C until no further weight loss; method 930.15, AOAC 2000); crude protein (CP, N× 6.25, method 984.13, AOAC 2000); ash (method 942.05, AOAC 2000); aNDFom [Van Soest *et al* 1991, corrected for NDF-ash, incorporating heat stable α -amylase (Ankom Technology, Macedon, NY) at 1 mL per 100 mL of NDF solution (Midland Scientific, Omaha, NE)]; and ether extract (method 920.39, AOAC 2000). Additionally, phorbol (highly toxic diterpene esters found in some plant oils) content of JCO was assayed according to Makkar *et al* (2007). Fatty acids of composition of JCO and CLA in LM muscle were sampled and determined using the techniques and methods described by Lorezen *et al* (2007) and by Sosa-Segura *et al* (2014). Dry matter content (method 930.15, AOAC 2000) of feed and feed refusal was determined daily.

STATISTICAL ANALYSIS

Carcass data, shoulder composition, and FA and in LM muscle were analysed as a randomised complete block design, with the individual lamb being the experimental unit. The MIXED procedure of SAS (SAS Inst. Inc., Cary,

NC) was used to analyse the variables. Treatment effects were tested for linear, quadratic and cubic components of the JCO supplementation level. Orthogonal polynomials were considered significant when the *P* value was ≤ 0.05 , and tendencies were identified when the *P* value was > 0.05 and ≤ 0.10 .

RESULTS

Cubic effects were not significant ($P \geq 0.10$). Thus, the *P* values for this component are not present in the tables.

Composition of supplemental JCO is shown in table 2. The moisture, impurity, and unsaponifiables (MIU) content of JCO was 1.42%, indicative of very high degree oil purity. No phorbol esters were detected in the supplemental JCO. Linoleic acid content (50.3%) is comparable to that of corn, cottonseed, soybean and sunflower seed oil.

Since there were no treatment effects on daily DM intake (averaging 1.288 ± 0.085 kg), daily intakes of JCO averaged 24.7, 51.1, and 77.3 g /day, or 0.57, 1.08 and 1.62 g/kg LW for levels 2, 4 and 6% of supplementation, respectively.

Treatment effects on carcass characteristics, composition of shoulder muscle and total CLA concentration in *L.* muscle are shown in table 3. There were no treatment effects ($P > 0.20$) on HCW, LM area and KP. However,

Table 2. Composition of supplemental *Jatropha* crude oil.

Item	<i>Jatropha</i> crude oil
Free fatty acids, %	6.58
Fatty acid, %	
C16:0	13.96
C16:1	0.49
C18:0	8.28
C18:1	26.00
C18:2	50.32
Others	0.95
Iodine value, g iodine/100 g fat ^a	82.77
Moisture, %	0.30
Impurities, %	0.50
Unsaponifiable matter, %	0.62
Phorbols sters	ND

JCO supplementation decreased (linear effect, $P=0.03$) dressing percentage and increased (linear effect, $P<0.01$) fat thickness. Supplemental JCO inclusion decreased (linear effect $P<0.04$) the proportion (g tissue/100 g of shoulder weight) of muscle and increased (linear effect, $P=0.02$) the proportion of fat in whole shoulder clod. The average concentration of stearic acid in LM was not affected ($P\geq 0.47$). However, JCO increased (linear effect, $P<0.01$) muscle CLA. Hence, JCO increased (linear effect, $P=0.03$) the CLA:stearic acid ratio.

Treatment effects on visceral organ mass are shown in table 4. Replacing corn with JCO did not affect empty body weight (EBW, as percentage of full weight) or the organ weights as a proportion of EBW (g/kg EBW). Supplemental JCO did not affect omental fat, but increased (linear effect, $P=0.03$) visceral fat mainly due to increased (linear effect, $P<0.01$) mesenteric fat.

Table 3. Treatment effects on carcass characteristics, chemical composition of shoulder muscle, and C18:0 and CLA concentrations in *longissimus* muscle.

Item	<i>Jatropha</i> crude oil level (%)					P^a value	
	0	2	4	6	SEM ^b	L	Q
Hot carcass weight (kg)	32.80	32.13	32.23	32.30	0.51	0.55	0.49
Dressing percentage	60.99	60.10	59.60	58.64	0.66	0.03	0.96
Cold carcass weight (kg)	32.31	31.90	32.06	32.01	0.56	0.88	0.51
LM ^c area (cm ²)	17.32	16.40	16.44	16.38	0.55	0.25	0.56
Fat thickness (cm)	0.25	0.30	0.36	0.39	0.020	<0.01	0.61
Kidney pelvic and heart fat (%)	3.16	3.38	3.54	3.57	0.26	0.24	0.72
Shoulder clod composition							
Total weight (kg)	2.432	2.375	2.364	2.320	0.072	0.31	0.94
Muscle (kg)	1.540	1.508	1.454	1.405	0.055	0.09	0.88
Fat (kg)	0.437	0.426	0.466	0.468	0.023	0.22	0.76
Bone (kg)	0.454	0.441	0.444	0.447	0.013	0.74	0.56
Shoulder composition (%)							
Muscle	63.35	63.54	61.32	60.48	0.993	0.04	0.62
Fat	17.97	17.91	19.78	20.19	0.675	0.02	0.73
Bone	18.68	18.55	18.91	19.40	0.73	0.46	0.68
Muscle to fat ratio	3.53	3.60	3.12	3.03	0.17	0.03	0.62
Muscle to bone ratio	3.40	3.43	3.29	3.15	0.16	0.26	0.62
<i>Longissimus</i> muscle							
Stearic acid (%)	14.67	13.81	14.09	14.11	0.58	0.59	0.47
CLA (%)	5.81	5.78	6.10	8.61	0.44	<0.01	0.02
CLA-to-C18:0 ratio	0.394	0.429	0.432	0.602	0.058	0.03	0.27

^a P = observed significance level for linear, quadratic and cubic effect of supplementation level of JSC. Since cubic effects were not significant ($P>0.10$) the P -values for those components are not presented in the tables.

^b SEM, standard error of mean.

Table 4. Treatment effects on visceral organ weight.

Item	<i>Jatropha</i> crude oil level (%)					<i>P</i> ^a value	
	0	2	4	6	SEM ^b	L	Q
GIT ^c fill (kg)	4.17	4.24	4.37	4.33	0.26	0.59	0.83
Empty body weight, kg	49.63	49.23	49.74	50.43	0.69	0.36	0.44
Empty body weight (% of full weight)	92.28	92.10	91.89	92.05	0.47	0.68	0.73
Full viscera (kg)	10.01	10.30	10.40	10.57	0.32	0.26	0.89
Organs (g/kg, empty body weight)							
Stomach complex	31.32	32.57	31.79	31.78	0.80	0.87	0.45
Intestines	42.46	45.10	45.02	45.62	1.22	0.11	0.42
Liver/spleen	21.42	22.86	21.24	22.73	1.08	0.64	0.98
Kidney	2.76	2.63	2.85	2.79	0.112	0.55	0.78
Heart/lungs	22.76	22.51	22.79	23.54	0.86	0.51	0.57
Omental fat	26.11	27.95	27.80	28.34	1.11	0.21	0.57
Mesenteric fat	4.02	5.56	6.20	7.11	0.49	<0.01	<0.53
Visceral fat	30.14	33.52	34.01	35.46	1.40	0.03	0.50

^a*P* = observed significance level for linear, quadratic and cubic effect of supplementation level of JSC. Since cubic effects were not significant (*P*>0.10) the *P*-values for those components are not presented in the tables.

^bSEM, standard error of mean.

^cGIT, gastrointestinal tract.

DISCUSSION

Jatropha curcas (non-toxic variety) is a plant native to Mexico, its wide distribution makes it a resource highly available with a low cost of production (Escoto *et al* 2014). Therefore, the diversification of their products (oil and meal) is appropriate.

The absence of phorbol esters in the JCO used in the present experiment is consistent with the findings of Goel *et al* (2007), who reported very low (> 0.27 mg/mg) or non-detectable levels of phorbol esters in oil extracted from nontoxic varieties of *Jatropha curcas*, a species native to tropical regions of Mexico and Central America. The FA composition of JCO used in this study is consistent with previous reports for the species (Rodríguez-Acosta *et al* 2010). The major fatty acids in JCO were C18:1 (26%) and C18:2 (50%). The ratio between the two may vary (more C81:1 than C18:2) depending on the region where the plant grows (Martínez-Herrera *et al* 2006, Ovando-Medina *et al* 2011). The JCO used in the present study was harvested in the Mexican state of Sinaloa. In a previous study, Soto-León *et al* (2014) observed that the FA composition of JCO harvested in Sinaloa was of 8.7, 6.4, 34.0, and 50.8% for C16:0, C18:0, C18:1 and C18:2, respectively, in close agreement with the composition of JCO used in the present experiment.

Carcass weight was not affected by treatments averaging 32.37±1.65 kg. Final carcass weight is consistent with other feedlot finishing studies using similar lamb breeds (Estrada *et al* 2013, Castro-Pérez *et al* 2014). The effects

of supplemental fat on carcass characteristics of cattle have been variable. In some studies, fat supplementation increased HCW and dressing percentage (Zinn 1989, Zinn *et al* 2000), whereas in others (Quinn *et al* 2008, Donicht *et al* 2011) there were no effects of supplemental fat on carcass characteristics. In feedlot lambs, the effects of fat supplementation of carcass characteristics was minimal (Dutta *et al* 2008, Bhatt *et al* 2011). However, a greater fat thickness in lambs fed with supplemental fat had been previously observed (Solomon *et al* 1992, Popova *et al* 2011). The negative linear effect of supplemental JCO on dressing percentage observed in the present experiment is uncertain. Much of the inconsistency in carcass characteristics response to supplemental fat may be more related to total lipid intake rather than to percentage supplemental fat (Zinn 1994), and by degree of maturity at time of harvest (McPhee *et al* 2008). As mentioned below, the effects of JCO on visceral fat and weight of intestines are contributing factors to the lower dressing percentage.

Consistent with the present study, Popova *et al* (2011) also observed a decrease in the muscle:fat ratio of shoulder clods of lambs fed supplemental coconut oil. Likewise, Bhatt *et al* (2011) noted an 18% increase in carcass fat in finishing lambs supplemented with 5% of coconut oil. Increased carcass fat due to fat supplementation had also been observed in feedlot cattle (Zinn 1988, 1989). In contrast, Ferreira *et al* (2014) observed that the addition of a combination of soybean oil and fish oil up to 7.5% of diet DM did not affect carcass fat deposition in feedlot lambs.

In the last decades, the amounts of saturated fatty acids in the animal products have become in an important concerns of consumers and government institutions (Dilzer and Park 2012). However, the presence of FA isomers like CLA, may have important health benefits including anti-carcinogenesis, decreased blood cholesterol, and reduced body fat accumulation (Drackley 2000). Dietary fat sources fed to ruminants that are high in linoleic acid (C18:2) promote greater flows to small intestine of vaccenic acid (t11 C18:1) and conjugated linoleic acid. Following endogenous desaturation (Δ^9 desaturase), t11 C18:1 is converted to c9, t11- C18:2 (Dhiman *et al* 2005, Bauman *et al* 2006). Due to anticipated health benefits of CLA, research efforts have been directed at evaluating this additional contribution of feedstuffs that are high in linoleic acid, including forages (Khanal and Olson 2004, Ortega-Pérez *et al* 2010) and as well as supplemental fats (Jenkins *et al* 2008, Wang and Lee 2015). Coupling both production efficiencies and the consumption of a more healthful meat product would mean significant enhancement economically for the ruminant meat industry¹. Fat sources such as corn oil, soy oil and sesame oil, are good sources of C18:2 (~ 45%), but due to their higher cost are seldom used as feed ingredient in ruminants diets. In the present experiment, increased CLA in LM muscle as result of JCO supplementation confirms that trans-isomers and CLA formed during ruminal biohydrogenation of C18:2 result in great CLA incorporation into meat.

Kott *et al* (2010) also observed increased LM CLA concentration with no change in C18:0 concentration in lambs fed diets high in linoleic acid (with 21% of safflower seed). Likewise, CLA concentration in meat increased linearly in lambs fed diets supplemented with soy oil, fish oil, and canola oil (fat sources that have a C18:2 concentration similar to that of JCO used in the present experiment; Ferreira *et al* 2014, Adeyemi *et al* 2015).

Results of the effect of vegetable oils on non-carcass components are limited in the literature. Supplementation of 2% soybean oil to Texel × Santa Inês lambs increased small intestinal weight as a percentage of total carcass weight. Likewise, in our study, intestines weight (g/g of EBW) tended to increase along with dietary JCO. Dávila-Ramírez *et al* (2014) observed that inclusion of 6% supplemental soybean oil decreased lung and liver weight in Dorper × Pelibuey lambs. Consistent with our findings, Soares *et al* (2012) observed a 23% of increase in mesenteric fat, in Texel × Santa Inês lambs supplemented with 2% of soybean oil during an 87-day feeding period, and they did not observe differences on omental fat deposits. In feedlot cattle, increased visceral fat has been a consistent response to increasing levels of fat supplementation (Zinn 1988, Plascencia *et al* 1999).

It is concluded that purging nut (*Jatropha curcas*, a Mexican non-toxic variety) oil supplementation at levels of up to 6% of diet dry matter does not negatively affect HCW and LM area, and represents a viable alternative

for increasing CLA concentration in meat in finishing feedlot lambs.

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