Original Research Article

Effect of alpha-lipoic acid on productive parameters and ruminal VFA profile in sheep

ABSTRACT

Aims: The objective of this study was to evaluate the effect of alpha-lipoic acid (ALA) on feed over the productive parameters of sheep, as well as volatile fatty acids (VFA) in the rumen and blood glucose.
Place and Duration of Study: The experiment was conducted at the Centro de Enseñanza, Investigación y Extensión en Producción Animal del Altiplano (CEIEPAA), located in Tequisquiapan, Querétaro-México.
Methodology: A total of 64 sheep (Pelibuey x Dorset), two months old (32 males and 32 females with an average weight of 20.7 ± 1.3 kg) were used. Animals were housed in 16 pens, 4 animals per pen, and each pen was assigned to one of the 4 ALA inclusion treatments (0, 40, 80, 120 ppm). ALA was mixed with 50 g of ground corn to ensure its consumption; the animals of treatment 0 also received 50 g of ground corn without ALA.
Results: Differences in weight gain were observed from day 56 in the animals supplemented with 80 ppm of ALA ($P < .0001$) and an improvement in daily weight gain (246 g) and feed conversion ($P = .05$) was shown. Animals treated with 40 ppm of ALA observed lower blood glucose without affecting the productive parameters ($P = .05$). A lower concentration of VFA was found in the treatment 120 ppm on day 84 (334.2 ± 26.5 vs 260.0 ± 15.7; $P = .05$).
Conclusion: The inclusion of 80 ppm of ALA in the diet of sheep could improve the productive parameters.

Keywords: Alpha-lipoic Acid, Sheep, Productive Parameters, Volatile Fatty Acids

1. INTRODUCTION

Alpha-lipoic acid (ALA) is an organosulfur fatty acid derived from octanoic acid, composed of eight carbons, and it is an integral component of mitochondria that can regulate energy metabolism [1,2]. ALA has an asymmetric carbon atom and two isomers, the R enantiomer and the S enantiomer. Only the R isomer is endogenously synthesized in cells. ALA binds proteins, and the complex formed between ALA and lysine (lipoamide) participates as a cofactor of important enzyme complexes in the Krebs cycle [3], such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase [4].

Due to its chemical structure, the biological form of ALA is known as lipoate, this molecule acts as a regulator in the oxidation-reduction reaction, which is why it is considered an antioxidant [5, 6, 7], this means that it neutralizes naturally occurring but harmful substances known as free radicals. It can regenerate vitamins soluble in water and fat (such as C and E vitamins), it is the endogenous antioxidant responsible for the elimination of free radicals in all types of cells [8], it is also has shown that it can be used as an additive in animal feed since it has been seen that the use of antioxidants in animal diets improves the productive parameters in sheep production systems [9].

Sheep farming is a very widespread productive activity in the world. The consumption of sheep meat is part of the population's diet and some nations produce a large volume of meat and there is an increase in the imports of sheep meat [10], it is expected an increase in the price due to the increase in demand, especially from developing countries [11].

Trends in the world context indicate that lamb production will remain stable, however, in recent years the small ruminants sector is experiencing economics, structural, technical, market, and financial challenges; the lamb production sector must find solutions to problems like the low technological advances in the lamb productions, poor structures, low profitability compared with other agricultural enterprises, poor management training, and lack of innovation culture [12]. Therefore, it is necessary to introduce biotechnological advances that allow achieving products with excellent quality and that offer greater opportunities to increase their productivity and competitiveness both in the national and international markets [11].

In previous studies, the antioxidant effect of ALA on meat quality was tested, but there are few reports on the effect of ALA on productive parameters in ruminants [8, 13]. Therefore, in the present work, the effect of ALA on the productive parameters of sheep is studied, to look for new ways to improve the production efficiency in the productive units.

2. MATERIAL AND METHODS

The experiment was conducted at the Centro de Enseñanza, Investigación y Extensión en Producción Animal del Altiplano (CEIEPAA), located in Tequisquiapan, Querétaro-México.

A total of 64 sheep (Pelibuey x Dorset), two months old (32 males and 32 females with an average weight of 20.7 ± 1.3 kg) were used. They were housed in 16 pens, 4 animals per pen, and each pen was assigned to one of the 4 ALA inclusion treatments (0, 40, 80, 120 ppm). ALA was mixed with 50 g of ground corn to ensure its consumption; the animals of treatment 0 also received 50 g of ground corn without ALA. The animals were fed with native grass hay, alfalfa, corn silage, and concentrated feed (Nulamb®), following the same feeding scheme that was regularly used in the production unit (Table 1). ALA was offered daily and individually to each animal in the morning.

The experiment lasted 84 days, every 28 days (on two consecutive days) productive parameters were evaluated. Blood samples and ruminal fluid were taken for the quantification of glucose and volatile fatty acids (VFA) concentration respectively.

Table 1. Diet ingredients and composition of the experimental animals [Also at the end].

Diet ingredients	Dry Matter, %	Metabolizable energy, Mcal	Protein, g
Alfalfa	89	2.1	190
Corn silage	34	2.6	80
Native forage	91	1.0	70
Concentrate Nulamb®, (Nutec)	88	2.5	15

2.1 Blood glucose quantification

Blood was taken from the jugular vein, following aseptic procedures to obtain the sample, it was collected in Vacutainer® tubes without anticoagulant; the blood sample was centrifuged at 1500 rpm for 15 min to obtain the serum to measure glucose concentration.

For the quantification of glucose, the glucose-oxidase method was used [14]. The reaction mixture was prepared with phosphate buffer glycerol, enzymes (glucose oxidase and peroxidase), and orthodianisidine; then 50 µL of blood serum were added to 300 µL to the mixture reaction. These were incubated for 30 min at 37 °C with constant shaking at 900 rpm. Subsequently, 1mL of 4N HCl was added to stop the reaction; finally, the absorbance was read in a spectrophotometer (Hewlett Packard 8453) at 540nm.

In the reaction, the released peroxide stoichiometrically oxidizes orthodianisidine (which acts as a chromogen) by peroxidase. Finally, orthodianisidine oxidized in an acid medium acquires a pink color whose absorbance can be measured [15].

2.2 Ruminal fluid extraction and VFA quantification

Ruminal fluid was obtained using the probing technique [16, 17, 18]. At least 15 mL of ruminal fluid was taken per animal. To preserve and avoid volatilization of VFA, 2 mL 6N HCl was added to the ruminal fluid sample.

For the preparation of the samples, 1.5 mL of ruminal fluid was taken and centrifuged at 5000 rpm for 10 min at 4 °C; 1200 µL of supernatant were recovered in a new 1.5 mL tube, after that, 240 µL of 25% metaphosphoric acid were added to obtain a 1:5 ratio. The samples were incubated on ice for 30 min for proteins precipitation and there were centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were finally filtered with glass fiber membranes and stored in a 1:5 dilution at –20 °C until their analysis [19].

An Agilent 7890B gas chromatograph was used for the quantification of VFAs. The conditions were: oven 170 °C, injector 190 °C, detector 210 °C, the column with a flow of 2.5 mL min-1, and FID detector (Flame Ionization Detector).

2.3 Statistical analysis

To evaluate the effect of ALA on weight gain, daily weight gain, feed conversion, circulating blood glucose and VFA a completely randomized model was used. The SAS GLM procedure (SAS 9.3) was used.

3. RESULTS AND DISCUSSION

3.1 Effect of dietary supplementation of ALA on body weight, daily weight gain and feed conversion:

Differences in weight gain were observed since day 56 in the animals that were supplemented with 80 ppm of ALA ($P < .0001$), showing an improvement in weight gain of 8% compared to the other feeding treatments (Table 2); also an improvement in the daily weight gain (DWG, Table 3) of 246 g and an effect on the feed conversion of the animals supplemented with 80 ppm of ALA ($P = .05$) was shown (Table 3).

Table 2. Effect of dietary supplementation of ALA on body weight of lambs during 84-days [Also at the end].

ALA (ppm)	Days	Body-Weight gain (kg)	SE
0	1	20.6 [a]	1.3
	28	22.3 [a]	1.3
	56	28.4 [a]	1.3
	84	33.4 [a]	1.3
X̄		26.2[a]	1.3
40	1	20.4 [a]	1.3
	28	22.4 [a]	1.3
	56	29.8 [a]	1.3
	85	34.5 [a]	1.3
X̄		26.8[a]	1.3
80	1	21.6 [a]	1.3
	28	23.9 [a]	1.3
	56	30.4 [b]	1.3
	84	37.3 [b]	1.3
X̄		28.4 [b]	1.3
120	1	20.5 [a]	1.3
	28	23.0 [a]	1.3
	56	27.8 [a]	1.3
	84	33.7 [a]	1.3
X̄		26.3[a]	1.3

[a,b] Different literals between the same column indicate difference ($P < .0001$, n=4).
ALA: Alpha Lipoic Acid; ppm: parts per million; X̄: media; SE: Standard Error

Table 3. Effect of dietary supplementation of ALA on feed conversion and daily weight gain in lambs during 84-day [Also at the end].

ALA (ppm)	Feed conversion (Total feed consumed / Total weight gain)	SE	DWG (g)	SE
0	7.75[a]	1.2	178[a]	1.3
40	8.85[b]	1.2	168[b]	1.3
80	6.01[c]	1.2	246[c]	1.3
120	7.09[d]	1.2	210[d]	1.3

[a,b,c,d] Different literals between the same column indicate difference (P = .05, n=4).
ALA: Alpha Lipoic Acid; ppm: parts per million; SE: Standard Error; DWG: Daily Weight Gain

Concerning the effects of ALA on weight gain (Table 2), in a study conducted by Scmith et al. [13], steers were supplemented with different doses of ALA (0, 8, 16 mg kg-1), non-effect was observed on final weight gain or daily weight gain per the addition of ALA between the different treatments, and even the control animals had a greater weight gain at the end of the experiment compared to the group that was given 16 mg kg-1 of ALA.

Wang et al. [20] tested three different doses of ALA (0, 300, 600 mg Kg-1) on the productive parameters of Hainan Black goats, there was no differences in the body weight and feed intake, but animals supplemented with concentrated plus 600 mg kg-1 of ALA had a higher daily weight gain (69 g d-1 vs 77 g d-1) (P < .05) and a better feed conversion ratio (6.00 vs 5.29) (P < .05) than those that were not administered ALA.

The doses of ALA supplementation in the diet changed between the different studies performed in ruminants, which can range from 8mg/Kg to 600mg/Kg, many of which have been based on tests carried out in human medicine [13]; Also it is reported that the use of 400 mg/kg to 1200 mg/kg ALA in the diet-inhibited mRNA expression of COL3A1 gene in muscle and decreased muscle glycolysis early post-mortem in sheep's [20]. The present study use doses of 23.3, 50.4, 70 mg/Kg (comparative conversions corresponding to the doses used of 40, 80, 120 ppm respectively) which places them within the range of inclusion of ALA, with the addition of 50.4mg/Kg shows positive responses in the productive parameters in daily weight gain, final weight and feed conversion in lambs, however, these effects are not maintained when the dose of ALA is increased or decreased.
The results of this work agree with previous studies that show that the addition of ALA in the diets had different effects on the productive parameters and the quality of the meat, however, an optimal dose of ALA utilization in ruminants has not been found. This may be attributed to the fact that the structure of ALA can change during digestion and metabolism in ruminants, and then structural changes can cause changes in antioxidant status [20]. ALA digestion and metabolism in ruminants need further investigation to more fully describe the dose-response curve [13].

Few are the studies carried out regarding the effect of ALA on the productive parameters in ruminants. However, in studies developed in non-ruminants, a positive effect of ALA on weight gain has been observed in broilers. [21], used different doses of ALA (0, 10, 20, 40 ppm) and measured its effect on the productive variables. Animals supplemented with 40 ppm of ALA during 7 weeks significantly improved feed conversion, decreased general mortality and mortality attributable to ascites syndrome, lowered thiobarbituric acid reactive substances and hydroxyl radicals in the liver, and increased total glutathione, showing that the use ALA has action in broilers with high risk to develop ascites syndrome due to oxygen availability limitation and improving productive parameters.

Alvarez et al. [1] carried out a test on broilers that were supplemented with 40 ppm of ALA during the 7 weeks of the fattening period. Their results show improvements in birds that consumed ALA, these show an increase in weight gain of 101 g for males and 30 g for females at the time of slaughter (P < .010), as well as an improvement in feed conversion and a reduction in mortality.

Hamano et al. [22] indicated that dietary ALA (50 ppm) did not affect growth rates (body weight) in broilers, even though, the metabolic effect of ALA on energy distribution depended on chicken age, a dietary level of more than given may be necessary to improve growth performance. Yasin et al. [23] reported that the lower concentration of ALA (25 ppm) in feed

improved the growth performance of broilers, and the higher concentration of ALA (150 ppm) suppressed the growth rate in chickens. This is consistent with the studies by Sigler et al. (2015) and El-Senousey et al. [24] where he reports that an increase in ALA doses affects the consumption of animals, affecting growth and weight gain.

As we mentioned before, this behavior is also observed in this study where the increase in the ALA dose from 80 ppm to 120 ppm reduces weight gain (Table 2) and VFA production (Table 5). Some authors suggest that the effects of ALA act in a dose-dependent manner depending on the age and productive stage of the animal [13, 22].

ALA is considered the universal antioxidant, antioxidants have shown a positive effect on the productive parameters of slaughter animals and its effect has been seen on the quality, performance, and the carcass, the tenderness of the meat, and the display characteristics such as the coloration of the beef that reaches the market [8, 13].

ALA and vitamin E act in synergy since the latter is regenerated through lipoate. ALA requires at least two intermediate metabolic steps (glutathione and ascorbate pathway) to regenerate alpha-tocopherol. Although independently of the aforementioned pathways, it has been proposed that the ubiquinol pathway may itself contribute to the recycling of Vitamin E. It is possible that other metabolic processes are involved in the synergism of ALA and vitamin E. Varied mechanisms such as elevated catalase activity and increases in SOD, specifically manganese-dependent SOD (MnSOD) have been involved with the synergism of ALA and Vitamin E [25].

3.2 EFFECT OF DIETARY SUPPLEMENTATION OF ALA ON BLOOD GLUCOSE LEVES

A lower amount of glucose in the blood can be observed for the animals treated with 40 ppm of ALA (Table 4), however, this decrease did not affect the productive parameters or the health of the animal ($P < .05$).

Table 4. Effect of dietary supplementation of ALA on blood glucose levels [Also at the end].

ALA (ppm)	Glucose (mg dL^{-1})	SE
0	81.10[a]	2.35
40	71.50[b]	2.35
80	78.78[a]	2.35
120	79.85[a]	2.35

[a,b] Different literals between columns indicate differences ($P = .05$, n=4)
ALA: Alpha Lipoic Acid; ppm: parts per million; SE: Standard Error

Galván et al. [26] show that metabolites such as glucose are affected by environmental conditions like the time of sampling and the sex of the animals where the average values found were: glucose 75.57 ± 27.5 mg dL-1 in females and 83.70 ± 37.7 mg dL-1 in males, it is mentioned that this may be related to the preparation of the organism in females for their next production stage, results that are consistent with the values obtain in this study were vary between 71.50 -81.10 mg dL-1 , also Ulbrich et al. [27], reported that blood glucose decrease with age, going from 100 mg dL-1 in lactating animals to 71 mg dL-1 in weaned animals.

Samad et al. [28] studied the effect of the inclusion of ALA (32 mg kg-1), and its effect on parameters such as body temperature, heart rate, and respiratory rate in buffaloes. Their results showed that animals supplemented with ALA and subjected to conditions of high environmental temperatures improved in body temperature and respiratory rate after 8 days of treatment, this could indicate better thermoregulatory mechanisms and avoiding conditions of heat stress when there is chronic exposure to heat, improving non-evaporative heat losses in the group supplemented with ALA.

Investigations in muscle and fat cell lines have shown that ALA stimulates glucose receptors and has a positive effect on insulin-stimulated glucose uptake [29]. In the case of ruminants, these animals are usually hypoglycemic, so the main route by which glucose is synthetized is gluconeogenesis, even so, when comparing the glucose need for the central

nervous system, pregnancy, and lactation, with the glucose needs of monogastric, it is found that it is not lower, but even higher, as in the case of high-producing cows [30, 31].

Fiore et al. [32] tested the combined effect of cyanocobalamin and ALA on the hepatic metabolism of high-producing cows, they found that the use of these can increase glucose, and reduce triglycerides and urea which seems to indicate that this type of treatment positively influences hepatic metabolism and reduces the risk of hyperketonemia and hepatic lipidosis during the weeks after calving in high-yielding cows. It is well known that glucose metabolism becomes more important in ruminants during early lactation. The lactating mammary gland requires major adjustments in glucose production and utilization [32] therefore; glucose synthesis during early lactation must be increased to meet the demands. An alternative to meet these demands is the use of ALA, and its enhanced effect on glucose and consequently on the energy metabolism of lactating.

3.3 EFFECT OF DIETARY SUPPLEMENTATION OF ALA ON VOLATILE FATTY ACIDS (VFA) CONCENTRATION IN THE RUMINAL FLUID

Non effect on VFA concentration was found in ruminal fluid on day 0. A lower concentration of VFA was found in the treatment 120 ppm on day 84, there were no differences between treatments 0, 40, 80 ppm contrary to what was expected, considering the increase in weight gain corresponding to the 80 ppm treatment (Table 5).

Table 5. Effect of different levels of ALA supplementation in the diet of sheep, on VFA concentration at the ruminal level in an 84-day trial [Also placed at last].

ALA (ppm)	Days	Acetate (mmol L^{-1})	SE	Propionate (mmol L^{-1})	SE	Butyrate (mmol L^{-1})	SE
0	1	367.7	26.5	101	7.4	66.1	4.7
	84	334.0 [a]	15.7	81.1 [ab]	4.0	70.7 [a]	3.4
40	1	299.0	26.5	78.2	7.4	52.3	4.7
	84	355.9 [a]	15.7	90.8 [a]	4.0	73.6 [a]	3.4
80	1	336.7	26.5	89.3	7.4	58.5	4.7
	84	322.8 [a]	15.7	85.0 [ab]	4.0	75.2 [a]	3.4
120	1	334.2	26.5	91.2	7.4	57.5	4.7
	84	260.0 [b]	15.7	70.4 [b]	4.0	52.5 [b]	3.4

[a,b] Different literals between day 84 columns indicate differences (P = .05, n=4)
ALA: Alpha Lipoic Acid; ppm: parts per million; SE: Standard Error

Animal performance is the most direct measure in evaluating feed quality. However, performance data is often insufficient to determine the possible interactions that may take place in the ruminal environment, which is why it is necessary to measure the VFA production [33, 34], however, our study found that supplementation with higher amounts of ALA could reduce the VFA production in sheep's, however, is necessary more studies due to our work is one of the few studies who analyzes the effects of ALA in the VFA production.

One of the alternatives that have been investigated to combat losses as methane at the ruminal level and increase acetate, propionate and butyrate production are antioxidants in the form of organic acids or essential oils [35, 36, 37], these oils have been used as regulators of the action of ruminal microorganisms, altering the permeability and stability of bacterial membranes, modifying ruminal fermentation patterns, and increasing the production of VFA [36, 37].

Wang et al. [38], Tekeli et al. [35], Bañuelos et al. [36] studied the effects of three different extracts on nutrient digestibility and ruminal fermentation. Quiao et al. [39] used herbs as an antioxidant supplement in sheep, improving the antioxidant function, increasing the total concentration of VFA, as well as the propionic fraction. Zhong et al. [40]; Wang et al. [41] report similar results with the use of roots of Astragalus membranaceus on the growth of lambs where total VFA were affected by an interaction between treatment and feeding time.

This same effect is shown in works such as Salinas et al. [42] and Sgorlong et al. [43] where ingredients with antioxidant properties, such as coffee pulp, are included in diets, and differences in ruminal fermentation patterns are seen.

ALA had effect on rumen microorganisms, it is known that lipoate metabolism can be found in most bacterial, fungal, and protozoan. These organisms can acquire lipoate through de novo synthesis or scavenging from the environment and a variety of lipoylation strategies are employed by microorganisms in response to their adaptation to the environments [44], for these reasons will therefore be very important to study in depth the effect of ALA at the rumen level and under different feeding conditions.

4. CONCLUSION

The inclusion of 80 ppm of ALA in the diet of growing and fattening sheep could help to improve productive parameters. However, more research is needed to evaluate the effect of ALA on the ruminal microbiome and its physiology.

ETHICAL APPROVAL

Sheep were housed and used at Universidad Nacional Autónoma de México (UNAM) according to regulations of the Mexican government (CICUAL-UNAM, NOM-062-ZOO-1999 4.2.2, #065).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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