Association of gene expression with blood metabolites and fatty acid profile in lambs

Asociación de la expresión de genes con metabolitos sanguíneos y perfil de ácidos grasos en corderos

Associação da expressão gênica com metabólitos sanguíneos e perfil de ácidos graxos em cordeiros

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Abstract

Some agricultural byproducts can be used as viable alternatives in animal feed. Therefore, the effect of the inclusion of avocado meal and sunflower oil on the profile of fatty acids, blood metabolites and the expression of genes associated with lipid metabolism in lambs was evaluated. Three treatments were evaluated: 0 % control, 10 % avocado meal and 10 % sunflower oil for 84 days. It was found that including avocado meal in the diet increased the amount of blood glucose and also globulin, but decreased creatinine and Glutamic Oxaloacetic Transaminase, compared to the addition of sunflower oil where the content of cholesterol, triglycerides, and VLDL decreased. and R A/G. There was no difference for muscle fat percentage. The concentration of fatty acids C20:0, C20:1 and C23:0 was lower with avocado meal, without affecting the MUFA and UFA values, the UFA/SFA ratio and the saturation index. The inclusion of avocado meal caused a change in the expression of the ACACA, FASN, SCD, FASBP3, PPARG and SREBF1 genes in the *Longisimus thoracis* muscle, there was a positive association between glucose and the FASBP3 gene, also of HDL with the PPARG gene, MUFA, proteins and indices of desaturation of fatty acids were associated with genes such as SCD, FASN, SREBF1 and ACACA The inclusion of avocado meal is an alternative to modify genetic expression, also to reduce very low density cholesterol values in the blood.

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Resumen

Algunos subproductos agrícolas pueden ser utilizados como alternativas viables en la alimentación animal. Por lo tanto, se evaluó el efecto de la inclusión de harina de aguacate y de aceite de girasol sobre el perfil de ácidos grasos, metabolitos sanguíneos y la expresión de genes asociados al metabolismo lipídico en corderos. Se evaluaron tres tratamientos: 0 % control, 10 % harina de aguacate y 10 % de aceite de girasol durante 84 días. Se encontró que al incluir harina de aguacate en la alimentación aumentó la cantidad de glucosa en sangre y también la globulina, pero disminuyó creatinina y Transaminasa Glutámica Oxalacética, en comparación con la adición de aceite de girasol donde disminuyó el contenido de colesterol, triglicéridos, VLDL y R A/G. No hubo diferencia para el porcentaje de grasa muscular. La concentración de los ácidos grasos C20:0, C20:1 y C23:0 fue menor con harina de aguacate, sin afectar los valores de MUFA y UFA, la relación UFA/SFA y el índice de saturación, la inclusión harina de aguacate causó un cambio en la expresión de los genes ACACA, FASN, SCD, FASBP3, PPARG y SREBF1 en el músculo *Longisimus thoracis*, existió una asociación positiva entre glucosa y el gen FASBP3, también de HDL con el gen PPARG, MUFA, proteínas e índices de desaturación de ácidos grasos se asociaron a genes como SCD, FASN, SREBF1 y ACACA La inclusión harina de aguacate es una alternativa para modificar la expresión genética, también para disminuir los valores del colesterol de muy baja densidad en sangre.

Palabras clave: expresión génica, asociación de genes, metabolismo lipídico, cordero.

Resumo

Alguns subprodutos agrícolas podem ser utilizados como alternativas viáveis na alimentação animal. Portanto, foi avaliado o efeito da inclusão de farinha de abacate e óleo de girassol no perfil de ácidos graxos, metabólitos sanguíneos e na expressão de genes associados ao metabolismo lipídico em cordeiros. Foram avaliados três tratamentos: 0 % controle, 10 % farinha de abacate e 10 % óleo de girassol por 84 dias. Verificou-se que a inclusão da farinha de abacate na dieta aumentou a quantidade de glicemia e também de globulina, mas diminuiu a creatinina e a Transaminase Glutâmica Oxaloacética, em comparação à adição de óleo de girassol onde diminuiu o teor de colesterol, triglicerídeos e VLDL. G. Não houve diferença para o percentual de gordura muscular. A concentração dos ácidos graxos C20:0, C20:1 e C23:0 foi menor com a farinha de abacate, sem afetar os valores de MUFA e UFA, a relação AGL/SFA e o índice de saturação. A inclusão da farinha de abacate causou alteração no expressão dos genes ACACA, FASN, SCD, FASBP3, PPARG e SREBF1 no músculo *Longisimus thoracis*, houve associação positiva entre glicose e o gene FASBP3, também de HDL com o gene PPARG, MUFA, proteínas e índices de dessaturação de gordura ácidos foram associados a genes como SCD, FASN, SREBF1 e ACACA. A inclusão da farinha de abacate é uma alternativa para modificar a expressão genética, também para reduzir os valores de colesterol de densidade muito baixa no sangue.

Palavras-chave: expressão gênica, associação gênica, metabolismo lipídico, cabrito.

Introduction

Currently, there are agricultural by-products that can be used in animal fattening, for example the avocado fruit (*Persea americana* Mill.), a product whose high production and export standards have an impact on the discarding of many fruits due to their small size or physical damage; however, since the nutritional value of discarded avocados is not affected, they can be included in rations for sheep (Bugarín *et al*., 2021; Lemus-Flores *et al*., 2020), as it presents high levels of unsaturated fatty acids, vitamins, antioxidants and minerals, a high fat content with values ranging from 10 to 30 % (Araujo *et al*., 2018), avocado fruits are highly digestible (Ly *et al*. 2021) contain mainly fatty acids such as oleic and linoleic, which decrease saturated fats and the harmful effects of animal fats (Hernández-López *et al*., 2016).

Other alternatives in supplementation for domestic animals are oilseed oils, which are used in ruminant feed (Mapiye *et al*., 2013; Zsédely *et al*., 2012). Several studies indicate that sunflower oil (SO) in ruminants and avocado meal (AM) in pigs are modulators of intramuscular fat and blood metabolites, so they can be used as viable alternatives in animal feed (González-Jiménez *et al*., 2021; Lemus-Avalos *et al*., 2020; Wang *et al*., 2019).

However, it is necessary to know the effect that these nutritional sources have on lipid metabolism, so this study was proposed with the objective of evaluating the effect of avocado meal and sunflower oil on the expression of lipid metabolism genes, and to associate it with the metabolites in blood and lipids in the *Longissimus thoracis* muscle in lambs at the finishing stage.

Materials and Methods

Ethics Committee

This project was registered at the Universidad Autónoma de Nayarit under number SIP15-65, sheep were handled according to the Mexican Official Standards for the use, care (NOM-062-ZOO-1999) and humane slaughter of domestic and wild animals (NOM-033-SAG/ZOO-2014).

Location

The trial was conducted at the Academic Unit of Agriculture of the Universidad Autónoma de Nayarit, 9 km Tepic-Puerto Vallarta highway, Mexico.

Elaboration of avocado whole fruit paste meal

To prepare the feed rations, Hass avocados were used, from packing houses in the municipality of Xalisco, Nayarit, Mexico, following the recommendations of Hernández-López *et al*. (2016). The avocado meal and the ration were prepared according to the recommendations of Lemus-Flores *et al*. (2020), the vegetable oil used was sunflower oil of the commercial brand "Cristal®", of which the total amount to be used for the trial was purchased.

Diets and animals

Eight Pelibuey-Dorper male sheep of 24.3 ± 2.7 kg initial BW were randomly assigned to each of the three experimental diets: 0 % control, 10 % avocado meal and 10 % sunflower oil, for a period of 84 days. To determine the inclusion values, we considered what was reported by Bugarín *et al*. (2021) and Lemus-Flores *et al*. (2020), who previously worked with the inclusion of avocado paste in sheep feed; table 1 shows the proximal content of the rations, which had a forage:concentrate ratio of 40:60, the ingredients used were grain sorghum, soybean paste, canola paste, molasses, urea and minerals;

for daily consumption, 3.5 % of their BW was considered. Prior to the trial, an adaptation period was considered for the management and feeding of the sheep.

Table 1. Proximal chemical composition (dry matter basis) of the experimental rations.

Variable	AM	SO	CO
Total Energy (Mcal)	2.97	3.16	2.58
Crude Protein (CP, %)	14.00	14.00	14.00
Ether Extract (E.E., %)	6.92	12.11	2.39
Crude Fiber (CF, %)	12.43	13.20	13.29
Calcium (Ca, %)	0.75	0.87	0.85
Phosphorus (P, %)	0.30	0.27	0.28

AM: Avocado meal. SO: Sunflower oil. CO: Control.

Metabolites in blood

When the sheep were slaughtered, a blood sample was taken from each one to obtain the blood serum for biochemical analysis, determined with Byosistem A160 spectrophotometry equipment. The following were determined: Glucose in $mg.dL^{-1}$, Urea in $mg.dL^{-1}$, Creatinine in $mg.dL^{-1}$, Uric Acid in $mg.dL^{-1}$. For the lipid profile: total cholesterol in $mg.dL^{-1}$, triglycerides in $mg.dL^{-1}$, high density cholesterol (HDL) in $mg.dL^{-1}$, low density cholesterol (LDL) in $mg.dL^{-1}$, very low density cholesterol (VLDL) in $mg.dL^{-1}$ were quantified. The liver profile was also evaluated: total protein in $g.dL^{-1}$, Albumin in $g.dL^{-1}$, Globulin in $g.dL^{-1}$ and Albumin/Globulin Ratio (R A/G), also Glutamic Oxaloacetic Transaminase (GOT) in $mg.dL^{-1}$ and Glutamic Pyruvic Transaminase (GPT) in $mg.dL^{-1}$.

Profile of fatty acids in muscle

Intramuscular fat expressed in percentage was quantified in the samples from *L. thoracis* muscle, using solvents such as Chloroform-methanol, according to the method proposed by Folch *et al*. (1957). Fatty acid profiles were determined in a 30 mg cold methylated sample in a Bruker Scion 456-GC gas chromatograph with CombiPAL autosampler, equipped with a programmed temperature vaporization (PTV) injector, a flame ionization detector (FID) and a Select FAME capillary column, Agilent J&W CP7420 (100 m x 0.250 mm i.d. x 0.25 µm). The individual identification of the fatty acids was in comparison with the Supelco 37 FAME Mix standard (CRM47885), Pentadecane was used as internal standard. The data obtained were expressed as the percentage of the total fatty acids contained.

Analysis of gene expression in the *L. thoracis* muscle

At the time of slaughter of the sheep, 0.5 g samples were taken from the interior of the *L. thoracis* muscle at the level of the tenth rib, using cryotubes with a capacity of 2.0 mL, which had a stabilizing solution for nucleic acids (DNA/RNA Shield, Zymo Research, USA) and were stored at -20 ºC.

With 75 mg of tissue in the Direct-zol TM RNA MiniPrep nucleic acid extraction kit (Zymo Research, USA), RNA extraction was performed and the concentration and purity were quantified by Thermo Scientific NanoDrop 2000c spectrophotometry. For cDNA synthesis, 1000 ng of RNA from each sample was used with the Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase (Thermo Scientific, USA). Quantitative real-time polymerase chain reaction (qPCR) was performed with the Rotor-Gene Q 5plex HRM Qiagen, with final volume of 20 uL per reaction containing: cDNA 6 µL (30 $ng.\mu L^{-1}$ of cDNA), 3 µL nucleic acid-free water, 10 µL of SYBR Green/ROX qPCR Master Mix (2x) (Fermentas, USA) and 1 µL of sense and antisense oligonucleotide for each target gene. The specific sequence was used for the genes described in table 2. For real-time amplification was performed for 40 cycles was considered: initial denaturation (95 °C for 5 minutes), cycling (95 °C for 15 seconds and finally 62 °C for 30 seconds). To confirm the specificity of the amplification in each array, the denaturation curve analysis (melting) was used, the temperature ramp of this process was from 62 to 95 °C for 5 seconds.

Table 2. Sequence of the primers used for gene expression analysis.

Genes	Forward and Reverse Primers (5' → 3´)	Tm°C	Access no.
1ACACA	F- CATGGAAATGTACGCGGACC R- GGTGGTAGATGGGAAGGAGG	58	NM_001009256
1FASN	F- ACAGCCTCTTCCTGTTTGACG R- CTCTGCACGATCAGCTCGAC	60	DQ223929
1FAPB3	F- CCTCTCCTTCCACTGACTGC R- TTGACCTCAGAGCACCCTTT	58	BT021486
2PPARG	F- CATTTCTGCTCCGCACTAC R- GGAACCCTGACGCTTT	60	NM_001100921.1
2SCD	F- TTCTCTTTCTCCTCATTGCCCC R-TCGGCTTTGGAAGCTGGAA	60	NM_001100921.1
1SREBP1	F- CCAGCTGACAGCTCCATTGA R- TGCGCGCCACAAGGA	60	TC263657
RNA S18	F-GGCCTCACTAAACCATCCAA R-TAGAGGGACAAGTGGCGTTC	56-64	XM_012100710

ACACA: Acetyl-CoA carboxylase α. FASN: Fatty acid synthase. FAPB3: Fatty acid transport protein. PPARG: Peroxisome proliferator-activated receptor gamma. SCD: Stearoyl-CoA desaturase. SREBP1: Sterol Regulatory Element Binding Protein-1. RNA S18: endogenous gene.

Statistical analysis

With the variables of blood metabolites and fatty acid profile in *L. thoracis*, an analysis of variance was performed with a linear mixed model, where the fixed effect corresponds to the diet and each animal was included as a random effect. The comparison of means for the effect of diets was performed with Duncan's test ($p<0.05$) in the Statistical Product and Service Solutions Software (SPSS, 2011). For statistical analyses of gene expression in *L. thoracis* the method of Steibel *et al*. (2009) was used, Ct values for target and endogenous genes were considered with the use of a linear mixed model. For this, a univariate analysis model was used for each gene in the recorded gene expression readings. To evaluate dietary differences in expression rate in genes of interest (diffT) normalized by endogenous gene, AM vs CO, SO vs CO, and AM vs SO contrasts were performed. Adjusted probability (P) values were calculated by using the Bonferroni correction method. To obtain the fold change (FC) values from the estimated diffT values between the contrasts, the equation: $FC = 2^{-diffT}$ was used. The calculations were performed with the Statistical Product and Service Solutions Software (SPSS, 2011).

Concentration values were calculated for each sample for each diet and gene, following the software equation on the Rotor-Gene Q 5plex HRM Qiagen computer (concentration=10^((Ct-B0)/B1); where 10^ is the 10 power to normalize the log10 Ct, B0 the intercept value of the regression equation and B1 the slope value.

The association between values of concentrations calculated as log10 normalized quantitative expression with the variables blood metabolites and fatty acid profile was evaluated with Minitab 15

Statistical Software (Minitab, 2007), in a principal component analysis, using the correlations with significant probability and the relationships between the variables and the diets were plotted.

Results and discussion

When comparing rations with AM and SO in relation to blood metabolites (table 3), it was observed that AM (P<0.05) increased in almost 15 mg.dL^{-1} the amount of glucose in blood compared to the control diet and the amount of globulin of the AM diet (4.12) increased 0.67 g.dL^{-1} compared to the SO (3.45), in addition, creatinine (1.09) and TGO (63.86) decreased compared to the SO diet (1.45 and 91.57, respectively); the inclusion of AM compared to SO decreased cholesterol, triglycerides, VLDL and R A/G, which could be a tendency in the modification of these variables by the inclusion of AM (P<0.10). The increase in blood glucose due to the inclusion of AM reflects a greater disposition of energy, associated to a greater production of propionic acid available for muscle production, as reported by de Castro *et al*. (2023) and Rodrigues *et al*. (2022).

Globulin is associated with greater immunological response, creatinine with less renal effects and TGO with less hepatic damage, so a positive effect is observed with AM on animal health, a situation similar to that reported by Avellanet *et al*. (2007). The inclusion of SO presented an increase of cholesterol (72.00) in blood with respect to the control diet (61.83), this is associated with the increase of triglycerides and VLDL, as a result of a greater absorption of lipids in the intestine, due to the existence of a greater availability of lipids in the diet containing SO. The effect of cholesterol lowering with AM coincides with de Castro *et al*. (2023) when using *Theobroma grandiflorum* in sheep, similar when using pomegranate peel extract in cows (Abarghuei *et al*., 2014) and moringa foliage used as feed in sheep (Astuti *et al*., 2011).

According to Rodrigues *et al*. (2022) the higher R A/G in the diet with SO indicates higher microbial protein available in the intestine. According to what was obtained with the inclusion of AM, it is possible that this is more metabolized in the rumen and does not exceed a greater amount of lipids and proteins compared to the inclusion of SO, as happened to Abarghuei *et al*. (2014) when they used pomegranate extract in the feeding of cows. Total protein and albumin were similar between diets because they were isoproteic and the protein content of avocado paste is low (5 %). Substituting starches for fats as energy sources can affect ruminal fermentation depending on the level of unsaturation in fatty acids or that of the fats used, which should be 2 and 6 % inclusion (Santana and Correa, 2016). A higher than recommended level of sunflower oil could influence TGO concentrations, R A/G, globulin, creatinine and glucose. There were no statistical differences in the rest of the variables.

The fat content and fatty acid profile of *L. thoracis* muscle are presented in table 4. The percentage of fat was statistically similar between treatments (P>0.05). The C20:0 and C20:1 fatty acid contents were lower with the inclusion of AM than with the inclusion of SO, with no statistical differences (P>0.05), when CO treatment was compared with SO treatment and CO treatment with AM treatment. The C23:0 content was the same (0.25 %) for the SO and CO treatments, which were higher than that of the AM treatment with 0.14 %. The values of saturated (SFA) and polyunsaturated (PUFA) fatty acids were statistically equal with the inclusion of AM, while the monounsaturated (MUFA), unsaturated (UFA), unsaturated/saturated (UFA/SFA) and desaturation index showed a slight increase with the inclusion of AM, which was not sufficient to present significant statistical differences. Miltko *et al*. (2019) achieved a decrease in SFA when flaxseed oil was included in sheep feed and pointed out that this result may be a reflection of incomplete biohydrogenation due to the high consumption of unprotected fats rich in linolenic acid.

On the other hand, the MUFA content of 51.9 % in avocado paste was reflected in the UFA content and UFA/SFA ratio in *L. thoracis* muscle. High SFA content in food for human consumption is undesirable because it influences the increase of LDL (Kostik *et al*., 2013). The fatty acid profile in *L. thoracis* with AM is within that reported for some breeds of sheep from different countries (Díaz *et al*., 2005). Romero-Bernal *et al*. (2017) indicate that the inclusion of fish meal in sheep increases intramuscular fat in *L. thoracis* muscle, contrary to the inclusion of soybean meal where no statistically significant decrease in the percentage of fat was observed. A similar situation occurred for PUFA values and in the AM treatment there was an increase in MUFA content but this was not statistically significant. This slight increase in the modification of fatty acids in *L. dorsi* when AM was included in the animal feed is important and could perhaps improve with a higher consumption of AM, as happened in pigs (Hernández-López *et al*., 2016), also in sheep without affecting productive behavior (Bugarín-Prado *et al*., 2021; Lemus-Flores *et al*., 2020), due to the digestibility of its lipids and the availability of MUFA (Ly *et al*., 2021). However, high levels of AM can improve

Table 3. Blood metabolite concentrations observed with the experimental diets.

Metabolites	AM	CO	SO	eem	P Value
Glucose, mg.dL^{-1}	126.14^{a}	111.29^{b}	114.57^{ab}	2.66	0.05
Creatinine, mg.dL^{-1}	1.09^{b}	1.31^{ab}	1.45^{a}	0.06	0.04
Total cholesterol, mg.dL^{-1}	66.75^{ab}	61.83^{b}	72.00^{a}	2.04	0.09
Triglycerides, mg.dL^{-1}	50.13^{b}	64.50^{a}	56.75^{ab}	2.95	0.09
VLDL. Very Low Density Cholesterol, mg.dL^{-1}	10.03^{b}	12.90^{a}	11.35^{ab}	0.59	0.09
Total protein, g.dL^{-1}	6.75	6.38	6.25	0.13	0.30
Albumin, g.dL^{-1}	2.37	2.39	2.67	0.08	0.25
Globulin, g.dL^{-1}	4.12^{a}	4.13^{a}	3.45^{b}	0.13	0.03
R A/G. Albumin/Globulin ratio	0.56^{b}	0.55^{b}	0.72^{a}	0.03	0.10
TGO. Glutamic oxaloacetic transaminase, mg.dL^{-1}	63.86^{c}	76.75^{b}	91.57^{a}	3.03	0.01

AM: Avocado meal. CO: Control. SO: sunflower oil. ^{abc}Different literals between columns indicate significant statistical difference. **P<0.05 *P<0.10. NS: Not significant. eem: Standard error of the mean.

meat quality, without affecting the amount of fat, but affect ration consumption, as has been reported in monogastrics and ruminants (de Evan *et al*., 2020; Fránquez *et al*., 2017; Ruíz-Dimas *et al*., 2022).

Table 4. Fat percentage and lipid profile of *Longissimus thoracis* with the experimental diets.

	AM	CO	SO	eem	Valor P
Fat %	4.05	4.09	3.06	0.82	0.59
C20:0	0.08[b]	0.10[ab]	0.13[a]	0.01	0.01
C20:1	0.11[b]	0.13[ab]	0.16[a]	0.01	0.03
C23:0	0.14[b]	0.25[a]	0.25[a]	0.03	0.03
% SFA	47.16	52.93	51.63	2.98	0.73
% MUFA	46.61	40.31	40.36	3.37	0.71
% PUFA	6.16	6.68	7.62	0.53	0.56
% UFA	52.77	41.99	47.98	3.25	0.42
UFA/SFA	1.13	0.99	1.05	0.09	0.81
Desaturase index	0.52	0.45	0.46	0.06	0.70

AM: Avocado meal. CO: Control. SO: Sunflower oil. sEm: Standard error of the mean. [a, b, c]Different letters indicate statistical difference significant at P<0.05.

Table 5 shows the different changes in gene expression (FC) in *L. thoracis* muscle for the diet comparisons. When AM was included, it was observed that in relation to the CO diet there was an increase in the expression of the genes analyzed (ACACA, FASN, SCD, FASBP3, PPARG and SREBF1). When the diet included SO and was compared with the CO treatment, there were two genes (FASN and SCD) that did not show significant differences. When the inclusion of AM vs. SO was compared only in the expression of the PPARG gene, the AM treatment had higher expression in five genes (ACACA, FASN, SCD, FASBP3 and SREBF1). Feeding the sheep with AM presented higher gene expression compared to CO and SO, which indicates physiological effects on lipid modulation in *L. thoracis*, in this sense Lemus-Avalos *et al*. (2020), mention that gene expression associated with lipid metabolism is different in each animal species and according to each tissue. The increase in the PUFA ratio in *L. thoracis* muscle reduced the expression of FASN, SCD and SREBP1 genes when sunflower oil was used, similar to that reported by Mohan *et al*. (2012), considering that the diet with GA tended to present a higher amount of PUFA than the diet with AM, the effect was similar when decreasing the expression of these genes in GA and comparing them with AM, a similar situation occurred in an evaluation in lambs where soybean, cotton and rapeseed meals were added to the diet with a decrease in PUFA in thoracic muscle and an increase in the expression of lipogenic genes (Wang *et al*., 2019).

In figure 1, with principal component analysis 1 (PC1), 13.3 % variability and 2 (PC2) with 86.7 % variability through the correlation matrix, it was observed in the right quadrants (PC1) that all the genes (ACACA, FASN, SCD and FASBP3 are lipogenic and PPARG and SREBF1 adipogenic) were related to the inclusion of 10 % AM. In this same principal component analysis, when correlating the log10 normalized quantitative expression with the variables blood metabolites and fatty acid profile, it was also observed that with the inclusion of 10 % AM there is an association with the expression of the genes studied (right quadrant CP1): an association of glucose with the FASBP3 gene, also HDL with the PPARG gene. Associations of MUFA, proteins and fatty acid desaturation indices with the SCD, FASN, SREBF1 and ACACA genes. In the left quadrant of CP1, it was appreciated that in CO diets and with inclusion of SO at 10 %, there is association with SFA, PUFA, VLDL and LDL. The amount of fat in *L. thoracis*, being in the middle of the quadrants of the principal components as shown in figure 1, was not associated with gene expression or with the diets tested.

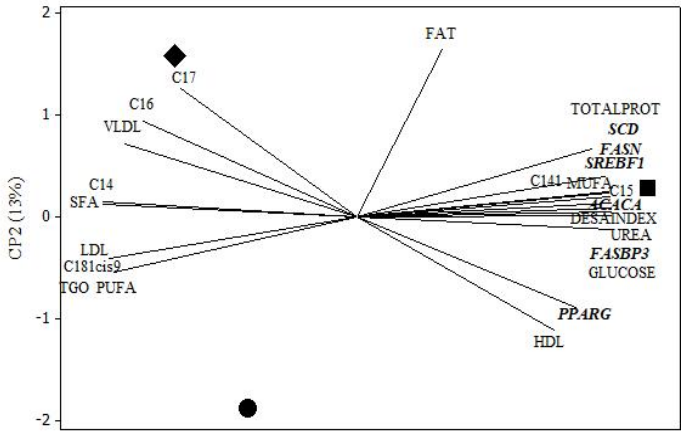

Figure 1. Association of genes through principal components according to the dietary rations used.

In a study conducted by Wang *et al*. (2019) where they supplemented the diet for sheep by increasing the amount of protein and ether extract, an increase in the expression of FAS, SREBF1 and PPARG genes was observed, similar to what happened here when AM

Table 5. Changes in gene expression (FC) in *L. thoracis* for diet comparisons.

Contrast	AM vs CO			SO vs CO			AM vs SO		
	FC	eem	P <	FC	eem	P <	FC	eem	P <
ACACA	1.14	0.005	0.0001	1.04	0.005	0.0001	1.10	0.005	0.0001
FASN	1.15	0.004	0.0001	1.00	0.004	1.0000	1.14	0.004	0.0001
FASBP3	1.15	0.004	0.0001	1.06	0.004	0.0001	1.09	0.004	0.0001
PPARG	1.11	0.055	0.05	1.21	0.055	0.001	0.92	0.055	0.10
SCD	1.05	0.009	0.001	0.99	0.009	0.09	1.07	0.009	0.001
SREBF1	1.34	0.01	0.001	1.07	0.01	0.001	1.25	0.01	0.001

AM: avocado meal. CO: Control. SO: avocado oil. FC: gene expression. eem: Standard error of the mean. ACACA: Acetyl-CoA carboxylase α.FASN: Fatty acid synthase. FAPB3: Fatty acid transporter protein. PPARG: Peroxisome proliferator-activated receptor gamma. SCD: Stearoyl-CoA desaturase. SREBP1: Sterol Regulatory Element

was added at 10 %, with an increase in MUFA. In this sense Yue *et al*. (2016), mention that the expression of lipogenic genes is involved for the increase of fatty acid desaturation, which is associated with the increase of MUFA by SFA. An association such as glucose with the FASBP3 gene could mean a greater development of muscle tissue according to Castro *et al*. (2023) and Rodrigues *et al*., (2022).

Conclusion

The inclusion of 10 % avocado meal caused changes in the expression of ACACA, FASN, SCD, FASBP3, PPARG and SREBF1 genes in *Longissimus thoracis* muscle in lambs, there was a correlation of ACACA, FASN, SCD and FASBP3 genes which are lipogenic and PPARG and SREBF1 genes with adipogenic action, FASN, SCD and FASBP3, which have lipogenic action, and PPARG and SREBF1, which have adipogenic action, were correlated with the inclusion of 10 % AM, when correlating the variables of blood metabolites and the fatty acid profile, it was observed that with the inclusion of 10 % AM there is an association with the expression of the FASBP3 genes with glucose, the PPARG gene with HDL, the SCD, FASN, SREBF1 and ACACA genes with proteins and fatty acid desaturation indexes, without this expression being associated with increases in muscle fat, finally a decrease in the values of very low density cholesterol in blood was observed.

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