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# Fatty acid profile in dorsal fat and Longissimus dorsi muscle of post-weaned hairless piglets fed with arboreal leaves meal

Perfil de ácidos grasos en la grasa dorsal y en el músculo Longisimus dorsi de lechones Pelones post-destetados alimentados con hojas de arbóreas

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#### **ABSTRACT**

In the present investigation, the inclusion of flours from the arboreal Cnidoscolus aconitifolius (Chaya), Morus alba (Mulberry) and Moringa oleifera (Moringa) in the conventional Corn-Soy diet was compared to evaluate the lipid profile in the Longisimus dorsi (LD) and in the back fat (DF) of the postweaned Pelon pigs. A sample of 28 animals, 24 days old, postweaned, was used. These were divided into four experimental groups, homogenized by sex and weight and subjected to a seven-day adaptation period. They were subsequently fed ad libitum for 28 days and slaughtered after 59 days. For the statistical analysis of the variables associated with the fatty acid profile (saturated, monounsaturated and polyunsaturated), a randomized block design statistical model was used, with the aim of determining whether there were statistically significant differences (P<0,05) between the variables associated with the fatty acid profile and the diets. It was shown that the SFA variable in LD and GD was higher in piglets fed with Mulberry, the MUFAs in LD and GD were higher in the animals of the Control group and the values of the PUFAs in LD and GD increased in the piglets supplemented with Moringa and Chaya, allowing to conclude that Mulberry increases the values of SFA in LD and GD of Hairless pigs; on the other hand, Chaya and Moringa increase the content of the PUFAs in LD and GD of these animals.

Key words: Lipids; meat; forage plants; Pelon pigs

#### RESUMEN

En la presente investigación se comparó la inclusión de harinas de las arbóreas Cnidoscolus aconitifolius (Chaya), Morus alba (Morera) y *Moringa oleifera* (Moringa) en la dieta convencional Maíz–Soya, para evaluar el perfil lipídico en el *Longisimus* dorsi (LD) y en la grasa dorsal (GD) de cerdos Pelones post destetados. Se utilizó una muestra de 28 animales, de 24 días de nacidos en etapa post destete, los cuales fueron divididos en cuatro grupos experimentales, agrupados por sexo y peso y sometidos a un período de siete días de adaptación. Posteriormente fueron alimentados ad libitum durante 28 días y sacrificados a los 59 días. Para el análisis estadístico de las variables asociadas al perfil de ácidos grasos (SFA, MUFA, PUFA) se empleó un modelo estadístico de diseño de bloques al azar, con el objetivo de determinar si existían diferencias estadísticamente significativas (P<0,05) entre las variables asociadas al perfil de ácidos grasos y las dietas. Se demostró que la variable SFA en LD y en GD fue mayor en los lechones alimentados con Morera, los MUFAs en LD y GD resultaron ser más altos en los animales del grupo Control y los valores de los PUFAs en LD y GD incrementaron en los lechones suplementados con Moringa y Chaya, permitiendo concluir que Morera incrementa los valores de SFA en LD y GD de cerdos Pelones; por otro lado, la Chaya y la Moringa aumentan el contenido de los PUFAs en LD y GD de estos animales.

Palabras clave: Lípidos; carne; plantas forrageras; lechones Pelones



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#### INTRODUCTION

The swine market has undergone several changes in recent years, due to the interest of consumers in eating healthier, longer-lasting and optimal quality meats, which has led to the production of meats with these qualities [1,2,3]. The researchers Mertínez-Aispuro et al. [4] affirm that the quantity and quality of fat in meats consumed by humans can affect their health, so an adequate balance in fatty acid intake (lower SFA content, higher MUFA content and balanced PUFA) is sought to improve aspects of human health. Therefore, it is important that food consumption allows for balance and improvement of dietary fatty acid profiles of people.

The increase in polyunsaturated fatty acids in meat can affect its flavor and shelf life, due to the reduction in oxidative stability that it produces, which leads to the oxidation of lipids and proteins, thus affecting the quality meat [5]. Some authors [6,7] suggest that in non-ruminant animals such as pigs (Sus scrofa domesticus), it is possible to modify lipid content based on the type of food they consume, and in this way take care of the health the swine-consuming community, offering them a better quality product.

In this sense, the use of the leaves of arboreal *Morus alba* (Mulberry), *Moringa oleifera* (Moringa) and *Cnidoscolus aconitifolius* (Chaya) in pig feed is proposed, which in Mexico have an adequate distribution, making them easy to acquire for producers, in addition to standing out for their important contribution and content of protein, minerals, fiber compounds, metabolizable energy and amount of amino acids [8, 9, 10].

This study allows us to evaluate the fatty acid profile in the back fat (DF) and *Longissimus dorsi* muscle of post-weaning hairless piglets fed with arboreal leaf meal.

### **MATERIALS AND METHODS**

### Location

The animals were raised in the swine post of the Nutritional Physiology and Experimental Surgery Laboratory, located in the Academic Unit Agriculture of Autonomous University of Nayarit (21° 26′ N, 104° 54′ W). Lipid analyses of the back fat (DF) and Longissimus dorsi (LD) of post-weaned hairless piglets were performed in the Instrumental Analytical Laboratory Unit of Instituto Tecnológico Superior to Calkiní, Campeche (ITESCAM) (20° 20′ N, 90° 2′ W).

# **Animals**

A sample consisted of 28 post-weaned piglets, 24 days old (weaning weight of  $5 \pm 0.3$  kg), divided into four experimental groups, each containing seven hairless pigs grouped by weight and sex (14 females and 14 males) from different litters. The minimum sample size was determined using the sample size calculation method to estimate a mean [11].

After weaning, the animals were transferred from the Academic Unit of Veterinary Medicine and Animal Husbandry, Compostela, Nayarit (21.23164° W 21° 13′ 54″ N) to Experimental Pig Shed of the Academic Unit of Agriculture (UAA), Xalisco, Nayarit (transfer distance: approximately 35 km). The transfer was carried out in accordance with NOM-062-

ZOO-1999 of technical specifications for production, care and use of laboratory animals [12].

Upon arrival at the Academic Unit of Agriculture, the pigs were housed in individual pens for 7 days (adaptation period) in compliance with the recommendations of the Mexican Official Standard (NOM-051-ZOO-1995) [13]. Subsequently, they were fed with the treatments ad libitum for 28 experimental days. The animals were sacrificed at 59 days of age, in accordance with the guidelines of the Mexican Official Standard NOM-033-SAG / ZOO-2014, on methods for the slaughter of domestic and wild animals [14].

## **Experimental design**

Four experimental groups were formed, where a base diet (Corn-Soy Paste) was used, with the inclusion of different arboreal leaf flours, which were: Control group with base diet (Corn-Soy Paste), Mulberry group, with the inclusion of 10% of Mulberry leaf flour, Moringa group with the inclusion of 10% of Moringa leaf flour and Chaya group with the inclusion of 10% of Chaya leaf flour.

The formulation of the diets (TABLE I) was carried out taking into account the nutritional requirements for piglets in post-weaning stage, referenced in the Brazilian tables for poultry (*Gallus gallus domesticus*) and pigs [15]. The nutritional content of the diets was calculated based on the nutritional values of the ingredients and metabolizable energy was calculated using McDowell *et al.* [16] methodology (TABLE I). The ingredients were mixed homogeneously and pelletized (PELLET MACHINE, model KL150B/C, made in China), with a particle size of 2 cm.

TABLE I  Nutritional composition of balanced diets and nutritional content						
Ingredients (%)	Control	Chaya	Mulberry	Moringa		
Inclusion level	0	10	10	10		
Ground corn	63.20	58.30	59.70	59.00		
Soybean meal	31.70	26.40	25.00	25.90		
L –Lysine	0.60	0.80	0.80	0.60		
CaCO <sub>4</sub> / ortophosphate 1:1	2.00	2.00	2.00	2,00		
Vitamin and mineral premix	0.20	0.20	0.20	0.20		
Canola Oil	2.30	2.30	2.30	2.30		
Calculated analysis						
Crude protein (CP)	20.03	20.04	20.05	20.04		
Metabolizable energy (Mcal)	3.38	3.38	3.35	3.37		
Lysine	1.55	1.54	1.50	1.51		
Lysin/ ME Ratio	4.57	4.54	4.47	4.49		
Crude Fiber	3.22	3.72	3.63	3.45		

ME: Metabolizable Energy; CP: Crude Protein; CaCO\_Calcium Carbonate

#### Measured variables

#### Lipid profile in back fat and the Longissimus dorsi muscle

For lipid extraction from the *Longissimus dorsi* muscle (LD) and back fat (DF), 500 mg of the sample was taken following the procedure of Hanson and Olley [17].









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Approximately 100 mg of the extracted fat from each sample was converted into fatty acid methyl esters using potassium hydroxide (KOH) methanol and boron trifluoride (BF3) [18].

For chromatographic analysis, a Gas Chromatography SCIENTIFIC, equipment (brand: Thermo model: K2432B730100000, manufactured in China) was used. The chromatographic system was coupled to a flame detector, set to a temperature of 260 °C, with a helium (He) gas flow of 35 mL/ min, an air flow of 350 mL/min and an injector temperature of 250 °C. The separation of the analytes was carried out with a HP-Innowax capillary column of 60 µm x 0.32 mm internal diameter and 0.25 µm particle thickness, using He as a carrier gas with a flow of 3 mL/min. The separation of fatty acid methyl esters (FAME) was achieved with a temperature ramp starting at 60 °C for 3.5 min, increasing 10 °C/min until reaching 200 °C and this temperature was maintained for 15 min, then another increase of 1 °C/min until reaching a temperature of 225 °C, and another of 2 °C/min up to 240 °C which was maintained for 12.5 min.

Fatty acid methyl ester samples were identified by comparing the retention times of the FAME peaks of the 37-component standard (Supelco 37 Component FAME Mix 47885-U Sigma-Aldrich) and the results were expressed as percentages.

# Indexes associated with the lipid profile of dorsal fat and Longisimus dorsi muscle

Hypocholesterolemic / hypercholesterolemic index (h/H)= ((C18:1+C18:2n6+C18:3n6+C20:3n6+C20:4n6+C20:5n3+C22:6n3)/(C14:0+C16:0))

Atherogenic index (AI)= ((C12:0 + 4\* (C14:0 + C16:0)) / (MUFA + Omega-n6 + Omega-n3)

Thrombogenic index (TI)= ((C14:0 + C16:0 + C18:0) / ((0.5 \* MUFA) + (0.5 \* Omega-n6) + (3 \* Omega-n3) + (Omega-n3 / Omega-n6)

lodine index (IV)= ((C16:1 \* 0.95) + (C18:1 \* 0.86) + (C18:2n6 \* 1.372) + (C18:3n3 \* 2.616) + (C20:1 \* 0.785) + (C22:1 \* 0.723))

#### Statistical analysis

For the statistical analysis of variables associated with the fatty acid profile (saturated, monounsaturated, and polyunsaturated), a completely randomized block design was used to determine whether there were differences (P < 0.05) between the means of the variables associated with the fatty acid profile and the treatments. SPSS version 20 (2011) was used for this purpose.

 $Y = \mu + T + B + e$ 

The statistical model consists of the variable  $\mu$ , which is the overall mean; T: the number of treatments according to the different diets; B: the block; and e: the experimental random error, which were related to litter at birth. Principal components analysis was performed in MINITAB15 to group the variables analyzed (indicators associated with the fatty acid profile) for each treatment.

### **RESULTS AND DISCUSSION**

TABLE II shows the composition of fatty acids that make up the lipid profile of the different treatments with which the hairless piglets were supplemented post-weaning.

#### TABLE II

Percentage composition of fatty acids in 4 diets for pigs: the Control and those supplemented with flours from the arboreal Cnidoscolus aconitifolius (Chaya), Morus alba (Mulberry) and Moringa oleifera

(ivioringa)							
	Fatty acid	Control	Mulberry	Moringa	Chaya		
C14	Myristric	1.86	7.76	6.41	4.69		
C16	Palmitic	5.42	4.58	3.49	2.83		
C18	Stearic	6.44	13.85	11.78	11.35		
C20	Arachidic	1.81	0.35	0.15	0.09		
	SFA	15.53	26.54	21.83	18.96		
C14:1	Myristoleic	2.37	6.56	7.05	5.17		
C16:1	Palmitoleic	7.67	5.71	6.73	4.84		
C17:1	Margaric Cis-10	0.05	0.25	0.47	0.38		
C18:1	Oleic	47.38	35.7	31.93	38.2		
C20:1	Cis eicoseneate	0.16	0.33	0.54	0.44		
C22:1	Burp	0.82	0.61	1.01	0.63		
	MUFA	58.45	49.16	47.73	49.66		
C18:2n6	Cis (alpha) linoleic	11.12	15.29	18.59	18.32		
C18:3n6	Gamma linoleic	2.32	4.00	4.33	4.85		
C20:3n6	Cis-8 Eicosadienoic	0.04	0.82	1.35	2.07		
C20:4n6	Arachidonic	0.2	0.32	0.54	0.63		
	Total Omega - n6	13.68	20.43	24.81	25.87		
C18:3n3	Linolenic	5.37	3.71	5.44	5.29		
C20:5n3	Eicosapentanoic (EPA)	3.14	0.02	0.05	0.06		
C22:6n3	Docosahexaenoic (DHA)	3.84	0.15	0.15	0.16		
	Total Omega - n3	12.35	3.88	5.64	5.51		
	PUFA	26.03	24.31	30.45	31.38		

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

The results in TABLE II indicate that Mulberry diet presented the highest percentages of SFA, Control treatment, highest MUFA values, and Chaya diet, the highest PUFA results.

In TABLE III, an analysis was performed of the variables associated with fatty acid profile of *Longissimus dorsi* muscle in post-weaned piglets of Pelón breed, fed with the treatments: Control, Mulberry, Moringa and Chaya, to determine if the diets caused statistical differences (P< 0.05) in terms of fatty acid content of the LD of these pigs.









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Variables assoc	iated with the fatty acid profile in Longissim	TABLE III us dorsi from pigs fed	four diets: The Co	ontrol and those	supplemente	d with	
Variables associated with the fatty acid profile in Longissimus dorsi from pigs fed four diets: The Control and those supplemented with arboreal leaf meal							
	Fatty acid	Control	Mulberry	Moringa	Chaya	EE	
C14	Myristric	2.26ª	1.59 <sup>b</sup>	1.59 <sup>b</sup>	1.33°	0.03*	
C16	Palmitic	19.92 <sup>d</sup>	23.35ª	21.96 <sup>b</sup>	20.25°	0.08*	
C18	Stearic	5.65 <sup>d</sup>	21.68ª	19.80 <sup>b</sup>	18.75°	0.13*	
C20	Arachidic	0.59⁵	0.79aª	0.58 <sup>b</sup>	0.50°	0.01*	
	SFA	28.42 <sup>d</sup>	47.40°	43.94b	40.83°	0.15	
C14:1	Myristoleic	1.58ª	0.25°	0.39 <sup>b</sup>	0.47 <sup>b</sup>	0.023	
C16:1	Palmitoleic	5.42°	4.68 <sup>b</sup>	5.39°	5.29°	0.11	
C17:1	Margaric Cis-10	0.74ª	0.28°	0.38 <sup>b</sup>	0.43 <sup>b</sup>	0.02*	
C18:1	Oleic	47.67ª	33.76 <sup>d</sup>	34.8°	37.27 <sup>b</sup>	0.1*	
C20:1	Cis eicoseneate	0.47ª	0.15 <sup>b</sup>	0.18 <sup>b</sup>	0.18 b	0.01*	
C22:1	Burp	1.57ª	0.18 <sup>b</sup>	0.09b	0.09 b	0.07	
	MUFA	57.49ª	39.31 <sup>d</sup>	41.24°	43.73 b	0.09	
C18:2n6	Cis (alpha) linoleic	10.11°	10.64 <sup>b</sup>	11.97ª	12.21 a	0.12	
C18:3n6	Gamma linoleic	0.27ª	0.24°	0.25 <sup>bc</sup>	0.26ab	0.01	
C20:3n6	Cis-8 Eicosadienoic	0.44ª	0.06°	0.07°	0.08b	0.003	
C20:4n6	Arachidonic	1.20ª	0.24 <sup>b</sup>	0.25 <sup>b</sup>	0.26b	0.01	
	Total Omega - n6	12.10 <sup>b</sup>	11.18°	12.54ab	13.06ª	0.18	
C18:3n3	Linolenic	0.60°	0.56⁵	0.57 <sup>b</sup>	0.61ª	0.005	
C20:3n3	Eicosatrienoic	0.90°	0.20 °	0.23 <sup>b</sup>	0.23 <sup>b</sup>	0.01	
C20:5n3	Eicosapentaenoic (EPA)	0.07 <sup>d</sup>	0.14 <sup>c</sup>	0.16 <sup>b</sup>	0.18ª	0.004	
C22:6n3	docosahexaenoic (DHA)	0.49°	1.31 b	1.32ab	1.33ª	0.01	
	Total Omega - n3	2.00°	2.20 b	2.28ab	2.36ª	0.03	
	PUFA	14.10 <sup>b</sup>	13.39°	14.82ª	15.42ª	0.17	
	PUFA/SFA	0.50ª	0.28 d	0.34°	0.38 <sup>b</sup>	0.01	
	PUFA/MUFA	0.25c	0.34 <sup>b</sup>	0.36°	0.35 <sup>ab</sup>	0.004	
	SFA/MUFA+PUFA	0.4d	0.90°	0.78 <sup>b</sup>	0.69°	0.004	
	C16:1/C16:0	0.27ª	0.20°	0.25 <sup>b</sup>	0.26 ab	0.005	
	C18:1/C18:0	8.5°	1.56 b	1.76 <sup>b</sup>	1.99b	0.16	
	C18:2/C18:1	0.21 <sup>d</sup>	0.32 °	0.34ª	0.33 <sup>b</sup>	0.003	
	(C18:2+C18:3)/C18:1	0.22 <sup>d</sup>	0.32 °	0.35°	0.33 <sup>b</sup>	0.003	
	n6/n3	6.08 ª	5.07 <sup>b</sup>	5.50 <sup>b</sup>	5.53b	0.15	
	lodine Index	62.91ª	49.79 <sup>d</sup>	53.16°	55.64b	0.14	
	h/H	2.72 ª	1.86 <sup>d</sup>	2.07°	2.40 <sup>b</sup>	0.02	
	Atherogenic Index	1.24 <sup>d</sup>	1.90°	1.69 <sup>b</sup>	1.46°	0.01	
	Thrombogenic Indiex	0.68 <sup>d</sup>	1.45°	1.28 <sup>b</sup>	1.13°	0.01	

a,b,c,d Different letters within each variable that indicate significant difference indicate (\*P < 0.05); EE: experimental error; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; h/H: hypocholesterolemic/hypercholesterolemic ratio

The results in TABLE III show that in means of the values for each treatment there were significant statistical differences (P< 0.05), where the Mulberry diet is attributed to the most harmful due to its content of saturated fatty acids (SFA), which are related to development of cardiovascular diseases, diabetes and high blood pressure [19, 20].

On the other hand, the Chaya diet turned out to present higher percentages of PUFA, which, together with an adequate content of antioxidants such as phenolic compounds, promote the balance of the oxidation of polyunsaturated fatty acids in the meat, resulting in an increase in its quality, in addition to the fact that Omega-3 (EPA and DHA) significantly decrease triglyceride









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levels in the blood, which reduces the risk of plaque formation in the arteries and the incidence of cardiovascular diseases [4].

The reports in TABLE III also showed that animals supplemented with Morera treatment had higher SFA content (P < 0.05) in the *Longissimus dorsi* (LD) muscle compared to the values reported for pigs fed the Control, Moringa and Chaya diets; however, some researchers have shown that SFA: palmitic (C16:0) and stearic (C18:0) have functional importance in counteracting brain injury related to cardiopulmonary arrest [21].

Table III also shows that pigs fed the Control and Chaya diets had higher MUFAS values (P < 0.05) in DF compared to animals supplemented with Mulberry and Moringa leaf flour. Although gene expression studies were not performed in the present study in animals supplemented with arboreal nutrients, the results obtained, associated with fat desaturation in DF when feeding pigs with arboreal nutrients, could be attributed to an increase in gene expression of the enzyme sterol regulatory element-binding protein (SREBP1) stearoyl CoA desaturase (SCD), which catalyzes the formation of double bonds between carbon atoms 9 and 10 of saturated fatty acids, converting them into monounsaturated fatty acids [22,23].

Similarly, the above result coincides with the research reports of authors Dzib-Cauich et al. [24], who demonstrated that when feeding Pelon pigs with diets supplemented with arboreal leaf flour, an increase in the expression of the SCD gene was observed, causing an increase in fat desaturation in the LD of animals supplemented with tree plants with forage potential.

In relation to the previous result, other authors have mentioned that this gene participates in the synthesis of C16:00 (palmitic) to transform it into C16:1 (palmitoleic), which can cause an increase in monounsaturated fatty acids of the lipids profile in meat of the animals fed with the inclusion of flours made with arboreal leaves in conventional diets [25].

Animals treated with the Chaya diet had the highest values of eicosapentaenoic acid (C20:5n3) and docosahexaenoic acid (C22:6n3), indicating that this diet had a greater effect on modifying the fatty acids profile in the *Longissimus dorsi* of pigs supplemented with this treatment, which is supported by Wood *et al.* [6] and Quiles and Hevia [7], researchers have shown that the fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can only be incorporated into pig's body when they consume foods containing linoleic and linolenic acid (essential fatty acids). These are deposited in subcutaneous and intramuscular fat, and through the metabolic pathways of desaturation and elongation, are converted into EPA and DHA.

The reports in TABLE III indicate that piglets supplemented with Moringa and Chaya treatments had the highest percentages of PUFA, Omega-n6 and Omega-n3, which coincides with the nutritional content of these arboreals according to the reports of some researchers [25]. These results were explained by Dzib-Cauich et al. [24], scientists who demonstrated in their research results that, by supplementing Pelons pigs with arboreal leaf flour, the lipid profile of *Bicep femoris* muscle of the animals was modified, resembling the fatty acid profiles of the diets.

Regarding the previous result, some authors [25,26] reported that Moringa and Chaya leaf flours are rich in phenolic compounds, which act as hydrogen donors that neutralize free radicals, interrupting the chain reactions of peroxidation, thus protecting essential PUFA, avoiding degradation of Omega-3 and Omega-6, which are related to the reduction of cardiovascular diseases [4].

The PUFA/SFA ratios, 18:1/18:0, n6/n3, iodine index (IV) and h/h were found to be higher in piglets fed with the Control diet, indicating that these animals had a higher percentage of PUFA, which can be associated with low quality meats if there is no balance with the antioxidant content, since PUFA in meat can increase the production of free radicals, which causes a reduction in its oxidative stability, resulting in an increase in the oxidation of lipids and proteins, decreasing its sensory characteristics and shelf life [27].

The C16:1/C16:0 ratio was highest in piglets supplemented with the Control and Chaya diets; the C18:2/C18:1 ratio (C18:2/C18:3)/C18:1 were highest in piglets fed Moringa, and the PUFA/MUFA ratio was highest in the LD of piglets fed the Moringa and Chaya treatments. These results can be attributed to the effect of arboreal when used as food for pigs, since some scientists [28] showed that including arboreal plants with forage potential in the diet of Pelones piglets increases the expression of the SDC gene, an enzyme responsible for synthesizing fatty acids and is involved in the desaturation process, necessary for the biosynthesis of MUFA, particularly to synthesize oleic acid (C18:1) from stearic acid (C18:00) and palmitoleic acid (C16:1) from palmitic fatty acid (C16:00) [29].

Regarding the percentage of atherogenic index (AI) and thrombogenic index (IT), these were higher in the *Longissimus dorsi* of piglets fed with the Morera diet, indicating that these meats contain a higher percentage of saturated fatty acids which promote inflammation, cellular and vascular damage, promoting the tendency to form clots in the blood vessels, which is supported by Dzib-Cauich *et al.* [27], authors who refer to AI and IT as a relationship between saturated and unsaturated fatty acids, where the higher the atherogenic and thrombogenic index, the higher the percentage of saturated fatty acids, indicating that it is a poor quality meat with less demand [3,4].

The results related to the lipid profile of *Longissimus dorsi* (LD) from piglets supplemented with arborea, during this research reported values where SFA ranged from 40.83 to 47.40%, MUFA from 39.31 to 43.7% and PUFA from 13.39 to 15.42%, in accordance to the research results of Teye *et al.* [30], researchers who reported the following percentages in the fatty acid profile in the LD of crossbred pigs (50% Duroc, 25% Large White and 25% Landrace) fed with soybean oil in their diet: polyunsaturated (17.70%), monounsaturated (42.88%) and saturated (39.42%) [29], which shows that feeding pelones piglets with arboreal leaf meal increases the quality of the fatty acid profile of the LD of these animals, bringing them closer to the quality of commercial pig meat.

In TABLE IV, the variables associated with the fatty acid profile in the dorsal fat (DF) in post-weaned piglets of the Pelón breed, fed with the treatments: Control, Morera, Moringa and Chaya, were analyzed to determine if the treatments cause differences (P < 0.05) in the fatty acid profile of the DF of these pigs.









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Variable	TABLE IV  Variables associated with the fatty acid profile in dorsal fat from pigs fed four diets: the Control and those supplemented						
with arboreal leaf meal							
	Fatty acid	Control	Mulberry	Moringa	Chaya	EE	
C14	Myristric	1.61ª	1.67ª	1.64ª	1.14 <sup>b</sup>	0.02*	
C16	Palmitic	20.83 <sup>d</sup>	24.4ª	23.5 <sup>b</sup>	21.98°	0.03*	
C18	Stearic	9.76 <sup>d</sup>	22.62ª	21.48 <sup>b</sup>	20.20 <sup>c</sup>	0.04*	
C20	Arachidic	0.41 <sup>b</sup>	0.69ª	0.57⁵	0.43°	0.005*	
	SFA	32.61 <sup>d</sup>	49.38ª	47.17 <sup>b</sup>	43.78°	0.04*	
C14:1	Myristoleic	0.02 <sup>d</sup>	0.03°	0.05ª	0.05 <sup>b</sup>	0.001*	
C16:1	Palmitoleic	3.9 <sup>d</sup>	4.80°	5.14 <sup>b</sup>	5.98ª	0.01*	
C17:1	Margaric Cis-10	0.47ª	0.29 d	0.37°	0.39 <sup>b</sup>	0.004*	
C18:1	Oleic	55.45ª	35.72 <sup>d</sup>	36.53°	37.78 <sup>b</sup>	0.04*	
C20:1	Cis eicoseneate	0.33 d	0.90°	1.05 <sup>b</sup>	1.77ª	0.01*	
C22:1	Burp	1.03ª	0.06°	0.09b	0.08 <sup>b</sup>	0.004*	
	MUFA	61.21ª	41.82 <sup>d</sup>	43.22°	46.05 <sup>b</sup>	0.05*	
C18:2n6	Cis (alpha) Linoleic	4.12°	5.79b	6.21ª	6.08ª	0.04*	
C18:3n6	Gamma linoleic	0.20 <sup>b</sup>	0.23ª	0.24ª	0.23ª	0.003	
C20:3n6	Cis-8 Eicosadienoic	0.31 <sup>c</sup>	0.77b	0.82b	1.53ª	0.02*	
C20:4n6	Arachidonic	0.34ª	0.14 <sup>d</sup>	0.15°	0.33 <sup>b</sup>	0.001*	
	Omega - n6	4.96 <sup>d</sup>	6.92°	7.42 <sup>b</sup>	8.17ª	0.03*	
C18:3n3	Linolenic	0.25 <sup>d</sup>	0.49°	0.55ª	0.53 <sup>b</sup>	0.002*	
C20:3n3	Eicosatrienoic	0.63ª	0.17 <sup>d</sup>	0.19 <sup>b</sup>	0.20 <sup>b</sup>	0.002	
C20:5n3	Eicosapentanoic (EPA)	0.05°	0.13 <sup>b</sup>	0.15ª	0.16ª	0.002	
C22:6n3	Docosahexaenoic (DHA)	0.35 <sup>d</sup>	1.08°	1.29ª	1.15 <sup>b</sup>	0.01*	
	Omega - n3	1.28 <sup>d</sup>	1.88°	2.18ª	2.004 <sup>b</sup>	0.02*	
	PUFA	6.23 <sup>d</sup>	8.80°	9.60b	10.20ª	0.02*	
	PUFA/SFA	0.19°	0.18 <sup>d</sup>	0.20°	0.23ª	0.001	
	PUFA/MUFA	0.10°	0.21 <sup>b</sup>	0.22ª	0.22ª	0.001	
	SFA/MUFA+PUFA	0.48 <sup>d</sup>	0.98ª	0.89 <sup>b</sup>	0.78°	0.002	
	C16:1/C16:0	0.19 <sup>d</sup>	0.20°	0.22 <sup>b</sup>	0.27ª	0.001	
	C18:1/C18:0	5.68ª	1.58 <sup>d</sup>	1.70°	1.87 <sup>b</sup>	0.004	
	C18:2/C18:1	0.07°	0.16 <sup>b</sup>	0.17°	0.16 <sup>b</sup>	0.001	
	(C18:2+C:18:3)/C18:1	0.07°	0.16°	0.17°	0.16°	0.001	
			3.68 <sup>b</sup>			0.001	
	n6/n3	3.88ª		3.41° 47.13°	3.98ª		
	lodine Index	58.69ª	45.28 <sup>d</sup>		49.38 <sup>b</sup>	0.06*	
	h/H	2.70ª	1.68 <sup>d</sup>	1.81°	2.04 <sup>b</sup>	0.01*	
	Atherogenic Index	1.34 <sup>d</sup>	2.07ª	1.91 <sup>b</sup>	1.65°	0.004	
	Índice Thrombogenic Index	0.87 <sup>d</sup>	1.61ª	1.45°	1.29°	0.003	

a.b.c.d Different letters within each variable that indicate significant difference indicate (\*P < 0.05); EE: experimental error; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; h/H: hypocholesterolemic/hypercholesterolemic ratio.









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The results obtained in TABLE IV indicate that all the values associated with the fatty acid profile of dorsal fat (DF) of the Pelon pigs fed with the Control, Mulberry, Moringa and Chaya treatments, presented significant statistical differences (P < 0.05).

The animals supplemented with Mulberry had a higher percentage of saturated fatty acids (C14:00, C16:00, C18:00, C20:00 and SFA) in the dorsal fat (DF), a characteristic that influences the decrease in the demand for this meat in the market, due to the health problems that come with the consumption of foods with a high content of saturated fatty acids, which is supported by the research results of Martínez et al. [31], who demonstrated that the high content of saturated fatty acids in breed can cause high blood pressure, elevated triglyceride levels, and obesity in humans when consumed. This is because these fatty acids increase basal lipogenesis, with a decrease in catecholamine-stimulated lipolysis; they also coexist with adipocyte insensitivity to the actions of insulin, increasing lipid storage in the subjects.

Regarding monounsaturated fatty acids, the Pelon breed supplemented with Moringa presented higher values of C14:1 and C20:1, those fed with the Chaya diet of C16:1 and those supplemented with the Control of C17:1, C18:1, C22:1 and MUFA. These results demonstrate that, when using the arboreal Moringa and Chaya as a food supplement in the diets of Pelon pigs, the fatty acid profile is modified, both in the *Longissimus dorsi* and in the dorsal fat of these animals, due to the increase in the expression of the SDC gene, converting saturated fatty acids into monounsaturated ones [24].

The results in TABLE IV, referring to the polyunsaturated fatty acids profile of dorsal fat of animals fed with the experimental diets, indicated that the piglets supplemented with the Chaya treatment presented the highest values of PUFA in DF. Related to the above, some researchers [25] reported that Chaya leaves have a high content of phenolic compounds which act as antioxidants, which allows delaying the oxidation process of meats, increasing their juiciness and tenderness, in addition to being associated with good quality meat [26].

Regarding the previous result, several authors [25,26] demonstrated that arboreal plants with forage potential have a high antioxidant potential, so they could be sources to protect meat from oxidative problems; in addition, due to their nutritional content, their flours could be used in the diet of the Pelon pig to improve the quality of its meat.

Similarly, the results obtained from the analysis of polyunsaturated fatty acids in dorsal fat (DF) showed that pigs fed the Chaya diet had a higher percentage of Omega-6 and those supplemented with Moringa had a higher content of Omega-3.

The SFA/MUFA+PUFA, IA and IT index in DF of piglets fed the Mulberry diet were higher, indicating that these animals are more prone to increased triglyceride synthesis, which leads to a higher probability of suffering from cardiovascular diseases [4], in addition to decreasing the quality of the meat and its market demand [24].

In animals assigned to Control treatment, the highest values were observed in C18:1/C18:0, IV and h/H indexes in their dorsal

fat, resulting in a lower content of saturated fatty acids and a higher proportion of monounsaturated fatty acids, which leads to higher quality meat.

The PUFA, PUFA/SFA, C16:1/C16:0 and n6/n3 indexes were higher in DF of piglets supplemented with Chaya, the PUFA/MUFA ratio was higher in animals treated with Chaya and Moringa, and the C18:2/C18:1(C18:2+C18:3)/C18:1 value was higher in piglets fed with Moringa, confirming that arboreal species Moringa and Chaya produce an increase in content of polyunsaturated fatty acids in dorsal fat of bald piglets when incorporated into their diet.

The analysis of TABLE IV shows that Chaya and Moringa treatments modified fatty acid composition of the dorsal fat Pelones piglets, decreasing their saturated fatty acid content.

The analyses in TABLES III and IV corroborate some research results [2], which showed that the diet to which pigs are subjected can modify their dorsal and intramuscular fat composition, and in turn modify the expression of their genes.

Similarly, researchers Pérez-Palacios et al. [32] studied the fatty acids profile of Longissimus dorsi and dorsal fat of Iberian pigs fed on forage in montaneras, where they reported that fatty acids composition of the subcutaneous fat of two batches animals at weaning presented a percentage of palmitic acid (C16: 0) of 17.32%, stearic acid (C18: 0) of 10.04%, oleic acid (C18: 1 n-9) of 56.21% and linoleic acid (C18: 2 n-6) of 9.32%, which is similar to fatty acids content of the DF of the Pelon piglets used for the experiment, meaning that with the inclusion of arboreal in feed of these animals, it is possible to raise the quality of Pelon pig's fat, bringing it closer to the quality of Iberian meat.

In FIG. 1, the indicators related to the fatty acids profile in *Longissimus dorsi* (LD) muscle of Pelones pigs fed with the treatments: Control, Mulberry, Moringa and Chaya were analyzed.

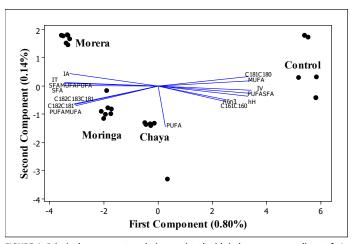


FIGURE 1. Principal component analysis associated with indexes corresponding to fatty acid profile in Longissimus dorsi (LD) muscle for each treatment. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; IV: iodine index; IA: atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic/hypercholesterolemic index









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In FIG. 1, the IA and IT relationships in *Longissimus dorsi* (LD) were closer to Mulberry diet, which is related to meats that contain a higher percentage of saturated fatty acids, which promote the development of inflammatory processes and the formation of clots in blood vessels [3, 4].

In the LD, the C16:1/C16:0 index showed a greater association with Control and Chaya diets, C18:2/C18:1 and (C18:2/C18:3)/C18:1 ratios were closer to Moringa treatment and the PUFA/MUFA to treatments Moringa and Chaya, which is related to effect of the areboreal when used as food in pigs, which increase the expression of the SCD gene, which participates in the synthesis of C16:00 (palmitic) to transform it into C16:1, which is associated with the desaturation of fatty acids in LD, improving not only the organoleptic properties of meat, but also offering benefits for human health, since unsaturated fatty acids are considered healthier than saturated ones [24, 25].

The results in LD fatty acids profile, shown in FIG. 1, can be related to research of Fernández et al. [29] and Dzib et al. [24], authors who demonstrated that, by including arboreals plants in pig feed, the percentage of polyunsaturated fatty acids in their lipid profile increases. These acids have a positive effect on humans, such as the maintenance of cell membranes, antioxidant activity and hypocholesterolemic effect [32,33].

With the analysis of the principal components associated with fatty acids profile in *Longissimus dorsi* (LD) muscle of Pelones piglets fed with the different treatments (Control, Mulberry, Moringa and Chaya), the results obtained from TABLE III are corroborated, where the PUFA/SFA, 18:1/18:0, n6/n3, IV and h/h ratios present a greater association with Control, which indicates that these animals presented a higher percentage of monounsaturated fatty acids in LD.

In FIG. 2, the indicators related to fatty acid profile in dorsal fat (DF) of Pelones pigs fed with the treatments: Control, Mulberry, Moringa and Chaya were analyzed.

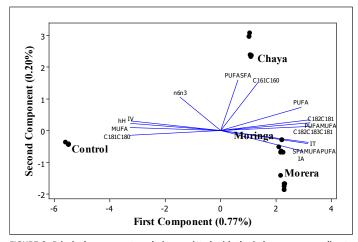


FIGURE 2. Principal component analysis associated with the indexes corresponding to fatty acids profile in dorsal fat (DF) for each treatment. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; IV: iodine value; IA: atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic/hypercholesterolemic index

The SFA/MUFA+PUFA, IA and IT indexes in dorsal fat (DF) showed a higher relationship with Mulberry diet, indicating that animals fed with this treatment had the highest SFA values, compared to rest of diets, which could be attributed to high protein and carbohydrate contained in *Morus alba* flour compared to other flours from the arboreals in question [34].

Related to above, saturated fatty acids: myristic, palmitic, and stearic have been shown to be influenced by dietary intake (i.e., exogenous sources). However, they are also synthesized endogenously through de novo lipogenesis, a process by which excess carbohydrates and proteins are converted into fatty acids [35,36].

The C18:1/C18:0, IA, and h/H ratios in DF were closer to control treatment, which is related to a fat with a lower content of saturated fatty acids and a higher proportion of monounsaturated fatty acids. Meats with these characteristics are in high market demand, as the low amount of SFA present in their lipid profile indicates that these meats are the healthiest for humans.

In relation with the above, several researchers mentioned that a diet rich in saturated fatty acids (SFA) has been suspected for decades as a causal factor contributing to risk of atherosclerotic cardiovascular disease (ASCVD), largely because a higher intake of 12- to 16-carbon SFA increases circulating low-density lipoprotein cholesterol (LDL-C) concentration compared to carbohydrates or unsaturated fatty acids [19, 20].

The principal components analysis performed in FIG. 2 supports the results obtained in TABLE IV which shows that Chaya and Moringa treatments are capable of modifying the fatty acids composition in dorsal fat (DF) of animals supplemented with these treatments, decreasing their saturated fatty acids content.

With the analysis of principal components associated with the profile of fatty acids in DF of the Pelones piglets fed with the different treatments (Control, Mulberry, Moringa and Chaya), it is observed that PUFA, PUFA / SFA, C16:1 / C16: 0 and n6 / n3 ratios, were closer with Chaya diet, the PUFA / MUFA ratio with Chaya and Moringa treatments, and the C18: 2 / C18: 1 (C18: 2 + C18: 3) / C18: 1 with Moringa, which is associated with an increase in the content of polyunsaturated fatty acids in DF of the animals supplemented with these arboreals, which means that by including these vegetal species in the diet of Pelones pigs, the saturation of fatty acids in their DF decreases.

### **CONCLUSIONS**

The inclusion of Mulberry results in high levels of saturated fatty acids in *Longissimus dorsi* (LD) and back fat (DF) of hairless piglets. The Control treatment with traditional diet, increase the monounsaturated fatty acids content in LD and DF. The inclusion of Moringa and Chaya increases the percentage of polyunsaturated fatty acids in DF and DF.

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#### **Conflict of interest**

The authors declare no conflict of interest

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