

## Effects of *Moringa oleifera* and *Brosimum alicastrum* partial feed substitution in intramuscular fat and adipose tissues and on the expression of lipogenic genes of Mexican hairless pigs

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**ABSTRACT.** The present study evaluated the effects of the inclusion of ground *Moringa oleifera* and *Brosimum alicastrum* leaf meal in the diet of Mexican hairless pigs (MHP) on the amount of intramuscular fat, subcutaneous fat, leg muscle (*Biceps femoris*) fat, loin muscle (*Longissimus dorsi*) fat, leg and back fat, and the expression of lipid metabolism genes. Hairless pigs are reared in the Mexican tropics and are characterised by their body and intramuscular fat accumulation. Eighteen male pigs fed for 82 d were randomly allotted to three experimental isoenergetic and isoproteic diets, where *M. oleifera* or *B. alicastrum* (six pigs per diet) replaced wheat bran. The diets used were a control diet, a diet with 10% *M. oleifera* leaf meal, and a diet with 10% ground *B. alicastrum* leaf meal. The *M. oleifera* diet decreased ( $P<0.05$ ) the fat ratio in the *Longissimus dorsi* muscles, back fat, rib fat, total carcass fat, and the carcass fat: meat ratio. The *B. alicastrum* diet only decreased fat in the *Biceps femoris* muscle, back fat, and rib fat. *Moringa oleifera* and *B. alicastrum* diets also promoted the overexpression of mRNA from the stearoyl-CoA desaturase (SCD), fatty acid synthase (FASN), acetyl-CoA carboxylase alpha (ACACA), sterol regulatory element-binding protein 1 (SREBP1) and acyl carrier protein (ACP) lipogenic genes in the *Biceps femoris* muscle and leg fat ( $P<0.001$ ). In addition, lower ACACA and SREBP1 mRNA expression in the *Longissimus dorsi* muscle and back fat ( $P<0.001$ ) were related to the lower amount of fat in pigs fed *M. oleifera* and *B. alicastrum*. The inclusion of *Moringa oleifera* and *Brosimum alicastrum* meals 10% in the diet of the pig MHP reduces fat, this is an important finding because fat is abundant in this type of pig.

*Key words:* *Brosimum alicastrum*, gene expression, meat quality, Mexican creole pig.

### INTRODUCTION

Mexican hairless pigs (MHP) have an obese phenotype because it tends to accumulate fat from an early physiological stage (Santos *et al* 2011). MHP are adapted to tropical rural environments in Mexico, where they have lived for more than 500 years. They are mainly fed unconventional food resources, but also consume typical forages of the region (Becerril *et al* 2009, Ramos-Canché *et al* 2020). Despite their social and economic importance in rural and backyard communities, these pigs are considered endangered (Hernández *et al* 2020). However, there is a growing demand for their products (Ramos-Canché *et al* 2020).

The body fat accumulation of MHP and the nutritional value of their meat have been previously studied (Dzib-Cauich *et al* 2020). They differ from commercial pigs in terms of performance and genetic background (Lemus-Flores *et al* 2001, Lemus-Flores *et al* 2020). According to Becerril *et al* (2009) and Aboagye *et al* (2019), their body fat accumulation can be attributed to genetic reasons.

Specifically, the expression of lipogenic genes in, for example, adipose and muscular tissues has been found to affect fat accumulation (Duran-Montgé *et al* 2009, Benítez *et al* 2016, Fernández *et al* 2017, Wang *et al* 2020). However, the fat content of the meat is also influenced by diet composition, as in other types of pigs (Duran-Montgé *et al* 2009, Mohan *et al* 2012, Benítez *et al* 2016, Wood and Enser 2017).

On the other hand, the increase in back and intramuscular fat is a characteristic of local pigs of Iberian origin, which is important for the elaboration of meat products of high economic value. In these pigs, fattening is generally promoted to improve the quality of fatty acids in meat. This is the reason why, these pigs are often fed with acorns or oils with oleic fatty acids (Fernández *et al* 2007, Benítez *et al* 2016). However, feeding MHP with conventional corn-soybean diets further increases back fat, and the carcass yield is not as high as other commercial pigs (Dzib-Cauich *et al* 2020). Therefore, different feeding approaches should be applied to commercial and local pigs to modulate the content and quality of fat (Mohan *et al* 2012, Albuquerque *et al* 2017, Aboagye *et al* 2019).

An alternative to commercial pig productions in rural areas is backyard or semi-intensive pig production systems. Such systems could reduce feed and production costs, while still produce good-quality meat products (Ramos-Canché *et al* 2020). When social, economic, and environmental challenges are considered, tree foliage and feeding patterns are clearly sustainable for animal husbandry such as pigs. Two forage sources in tropical and subtropical environments are *Moringa oleifera* and

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*Brosimum alicastrum* trees. Their use as forage for pigs has been previously studied (Moyo *et al* 2011, Dzib-Cauch *et al* 2016, Ly *et al* 2016, Zhang *et al* 2019). They are alternative protein sources in animal feeds because of the protein content of their leaves (Castro-González *et al* 2008, Teixeira *et al* 2014). In addition, they are rich sources of fatty acids, soluble fibre, and antioxidants (Moyo *et al* 2011). A limitation of forages in pigs feed is that high fibre content is associated with impaired nutrient utilisation and reduction of net energy values (Thacker and Haq, 2008). However, MHP can eat up to 40% *M. oleifera* leaf meal without effect on its productive response (Ortiz *et al* 2015); this could be associated with its large intestine and more active microflora. The present research was carried out to determine the effect of the inclusion of *M. oleifera* or *B. alicastrum* leaf meal in the diet of MHP on the amount of intramuscular fat, subcutaneous fat, leg muscle (*Biceps femoris*) fat, loin muscle (*Longissimus dorsi*) fat, leg and back fat, and on the expression of genes involved in lipid metabolism in tissues.

## MATERIAL AND METHODS

### ANIMALS AND DIETS

The present research was carried out at the Agricultural and Livestock Production and Research Unit of the “Instituto Tecnológico de Conkal” in Yucatan, Mexico (21° 05′ N and 89° 32′ W). The dominant climate of the region is warm sub-humid with rain mostly in summer. The ethics, care and welfare and handling of the pigs followed the guidelines of the official Mexican standards (NOM-062-ZOO-1999<sup>1</sup>, NOM-051-ZOO-1995<sup>2</sup> and NOM-033-SAG/ZOO-2014<sup>3</sup>). In addition, the experiment was carried out following the procedures related to the handling of animals approved by ITConkal in 2015. This study is also part of a report of doctoral studies.

*Moringa oleifera* and *Brosimum alicastrum* leaves were collected from a forage bank after 55 d of growth. The harvested material was dehydrated in a conventional oven<sup>4</sup> at 50 °C for 48 h. Subsequently, it was ground to a particle size of 1.0 mm. The meal obtained was stored

in hermetic containers until reaching 750 kg, and the chemical composition was calculated according to the standard method of the AOAC (1997). Each value was then a mean of three measures (table 1).

Eighteen castrated MHP males with an average initial live weight of 22 ± 1.11 kg were evaluated. They were distributed in a completely randomised design, each pig, representing an experimental unit. They were housed in 2.1 m x 1.1 m individual pens with slatted floor and were allowed *ad libitum* access to water and feed for 82 d. The animals were identified with ear tags. All pigs were slaughtered at the end of the experimental period, regardless of their weight. The animals fasted at least 16 h before slaughter. The animals were sacrificed at the slaughterhouse of the Autonomous University of Yucatán by exsanguination after electrical stunning.

The control diet was based on corn, soybean meal and wheat bran. The diet of *M. oleifera* or *B. alicastrum* contained corn, soybean meal, wheat bran and 10% flour *M. oleifera* or 10% *B. alicastrum* leaf meal. The diets were isoenergetic and isoproteic, where wheat bran was replaced with *M. oleifera* or *B. alicastrum* (table 2 and 3). Diets were formulated for pigs with a live weight of 22-50 kg according to N.R.C. (1998). Another article reported consumption and performance productive in growth.

Samples, fat measurement, and gene expression analysis

Forty-five min after slaughter, the carcasses (meat, fat, and bones without the skin, head, legs, and viscera) of each pig were weighed immediately. Back fat was measured at the height of the 10th rib (mm), and the primary pieces were weighed, separating the meat, bone, and fat to obtain the weight of the cold carcass (kg), performance of the carcass in relation to live weight (kg), and carcass fat: meat ratio (kg fat/kg meat). Rib weight (kg) and fat (kg) were also measured. Fifty grams of the *Longissimus dorsi* and *Biceps femoris* muscles were obtained to quantify intramuscular fat following the methodology described by the AOAC (1997) for lipids.

In addition, at the moment of slaughter, three 0.5 g samples of the *Longissimus dorsi* muscle, *Biceps femoris* muscle, back fat, and intramuscular fat of the leg muscles were taken for analysis of gene expression. The samples were collected in 2.0 mL cryotubes with a stabilising solution (DNA/RNA Shield, Zymo Research, USA), placed on ice and stored at -20 °C. Of the collected tissues, 75 mg were weighed and the Direct-zol™ RNA MiniPrep kit<sup>5</sup> was used for RNA extraction according to manufacturer instructions. The concentration and purity were quantified using Nanodrop spectrophotometry<sup>6</sup>. Subsequently, cDNA synthesis was carried out with 1000 ng of RNA from each

<sup>1</sup> Norma Oficial Mexicana NOM-062-ZOO 1999. 2001. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Available at: [https://www.dof.gob.mx/nota\\_detalle.php?codigo=762506&fecha=22/08/2001](https://www.dof.gob.mx/nota_detalle.php?codigo=762506&fecha=22/08/2001). Accessed 05.01.2019

<sup>2</sup> Norma Oficial Mexicana NOM-051-ZOO-1995. 1996. Tratamiento humanitario en la movilización de animales. Available at: <http://publico.senasica.gob.mx/?doc=531>. Accessed 05.01.2019

<sup>3</sup> Norma Oficial Mexicana NOM-033-SAG/ZOO-2014. 2015. Métodos para dar muerte a los animales domésticos y silvestres. Available at: [https://www.gob.mx/cms/uploads/attachment/file/567758/NOM-033-SAGZOO-2014\\_260815.pdf](https://www.gob.mx/cms/uploads/attachment/file/567758/NOM-033-SAGZOO-2014_260815.pdf). Accessed 05.01.2019.

<sup>4</sup> Terlab, (Jalisco, México)

<sup>5</sup> Zymo Research (California, USA)

<sup>6</sup> Thermo Fischer Scientific (Wilmington, USA)

**Table 1.** Chemical composition of ground *Moringa oleifera* and *Brosimum alicastrum* leaf meal (dry matter %), each value was mean of three measures.

Leaf meal	Fat	Protein	Ash	Moisture
<i>Brosimum alicastrum</i>	3.60±0.17	20.63±0.01	1.17±0.02	9.55±0.21
<i>Moringa oleifera</i>	4.97±0.14	23.68±0.37	1.12±0.01	7.97±0.09

**Table 2.** Ingredients and calculated composition in experimental diets (%).

Ingredients	Diet		
	CD	MOD	BAD
Corn	35.96	38.37	37.95
Soybean meal	11.52	11.28	11.73
Wheat bran	48.07	35.91	36.5
Sunflower oil	2	2	2
<i>Moringa oleifera</i> leaf meal	0	10	0
<i>Brosimum alicastrum</i> leaf meal	0	0	10
Calcium phosphate (monocalcium)	1.08	1.13	1.11
Calcium carbonate	0.75	0.72	0
Vitamin premix <sup>a</sup>	0.05	0.05	0.05
Mineral premix <sup>b</sup>	0.1	0.1	0.1
Lysine	0.22	0.16	0.28
Methionine	0	0.03	0.03
Salt	0.25	0.25	0.25

CD = control diet; MOD = *Moringa oleifera* diet; BAD = *Brosimum alicastrum* diet.

<sup>a</sup>Content in one kg: Vitamin A, 8 000 000 IU; Vitamin D3, 500 IU; Vitamin E, 35 000 IU; K3, 1.250 g; Thiamine, 500 mg, Riboflavin, 2 g; Piridoxin, 500 mg, Niacin, 10 g, pantotenic acid, 5 g, Antioxidant, 125 g, Vitamin B12, 7.5 mg, Biotin, 25 mg; Methionine was added on MOD and BAD diets to make it similar to CD diet.

<sup>b</sup>Content in one kg: Iron, 100 g; Manganese, 100 g; Zinc, 100 g; Copper, 10 g; Iodine, 300 mg; Selenium 200 mg; Cobalt, 100 mg.

**Table 3.** Calculated chemical composition of experimental diets (% of dry matter).

	Diet <sup>a</sup>		
	CD	MOD	BAD
Crude protein	16.00	16.00	16.00
ME <sup>b</sup> (Mcal kg <sup>-1</sup> of feed)	2.91	2.86	2.88
Ether extract	5.67	5.62	5.69
Neutral detergent fiber	24.71	22.90	23.63
Acid detergent fiber	7.88	8.95	8.95
Ca	0.60	0.60	0.62
P	0.50	0.50	0.50
Lysine	0.96	0.96	0.96
Methionine	0.26	0.26	0.26
Ca/P	1.20	1.22	1.23

<sup>a</sup>CD = control diet; MOD = *Moringa oleifera* diet; BAD = *Brosimum alicastrum* diet.

<sup>b</sup>ME = metabolizable energy.

**Table 4.** Sequence of primers used for gene expression analysis.

Genes	Primer sequence 5' → 3'	Amplicon length (bp)	Tm °C	Access number
ACACA	F- ATGTTTCGGCAGTCCCTGAT	133	62	AF175308
	R- TGTGGACCAGCTGACCTTGA			
FASN	F- CGTGGGCTACAGCATGATAG	108	64	AY954688
	R- GAGGAGCAGGCCGTGTCTAT			
ACP	F-CAGCAGGCCAGGTCAGCATT	236	60	XM_001924222
	R- GTCGACATGCCAACGCAGGA			
SCD	F- GCCGAGAAGCTGGTGATGTT	95	56	AY487829
	R-CAGCAATACCAGGGCAGCAT			
SREBP1	F- CGGACGGCTCACAATGC	114	64	NM_214157
	R-GACGGCGGATTTATTCAGCTT			
RNA S18	F-GGCCTCACTAAACCATCCAA	98	64	XM_012100710
	R-TAGAGGGACAAGTGGCGTTC			

SCD = stearyl-CoA desaturase; FASN = fatty acid synthase; ACACA = acetyl-CoA carboxylase alpha; SREBP1 = sterol regulatory element-binding protein 1; ACP = acyl carrier protein; F = forward primer; R = reverse primer; bp = base pair; Tm °C = melting temperature; RNA S18 = endogenous gene.

sample using the Maxima H Minus First Strand cDNA Synthesis Kit and dsDNase kit<sup>7</sup>.

Real-time polymerase chain reaction (PCR) was performed with a Step-One Plus Real-Time PCR kit<sup>8</sup> and the SYBR Green/ROX qPCR Master Mix (2x) kit<sup>9</sup>, with a final volume of 20 µL per reaction. The expression of the genes acetyl-CoA carboxylase alpha (ACACA), stearyl-CoA desaturase (SCD), sterol regulatory element-binding protein 1 (SREBP1), acyl carrier protein (ACP), and fatty acid synthase (FASN) were evaluated (table 4). These genes have been associated with lipid metabolism in pigs (Duran-Montgé *et al* 2009). The endogenous RNA18S gene was used to normalise gene expression. The triplicate real-time amplification of each sample was carried out in 40 cycles under the following conditions: initial denaturation at 95 °C for 8 min and cycling at 95 °C for 15 s and 60 °C for 30 s. The specificity of the amplification of each array was confirmed by the dissociation curve analysis and the temperature ramp of this analysis was 60 °C to 95 °C over 5 s. The reading of the dissociation curve yielded only one peak for each sample, confirming the amplification of each gene. No amplification was detected for the negative samples used as quality controls.

#### STATISTICAL ANALYSIS

The carcass and fat content variables were analysed using the model:  $y_{ij} = D_i + e_{ij}$ , where  $D_i$  is the  $i^{\text{th}}$  diet (control diet [CD], *M. oleifera* diet [MOD], and *B. alicastrum* diet [BAD]). Mean differences were tested using the Duncan test ( $P < 0.05$ ). Principal component analysis was carried out to associate the measured fat variables with the diets.

A statistical analysis of the gene expression data of each tissue was performed following the method of Steibel *et al* (2009) and Benítez *et al* (2016), which consists of the analysis of the cycle threshold value (Ct) of the target and endogenous genes. In addition, a linear mixed model with univariate analysis of the gene expression registered for the SCD, FASN, ACACA, SREBP1, and ACP genes was used:  $y_{gijk} = G_i + A_j + e_{ijk}$ , where  $y_{gijk} = -\log_2(E_g - Ct_{gijk})$ ,  $E_g$  is the PCR efficiency (E) of each gene (g),  $Ct_{gijk}$  is the value obtained from the thermal cycler software for the gene corresponding to the  $k^{\text{th}}$  repetition of the  $j^{\text{th}}$  animal belonging to the  $i^{\text{th}}$  group,  $G_i$  is the  $i^{\text{th}}$  group-specific effect on gene expression,  $A_j$  is the specific random effect on the quantitative polymerase chain reaction (qPCR) expression of the gene in the  $j^{\text{th}}$  pig, and  $e_{ijk}$  is the residual effect. Three different groups corresponding with the CD, MOD, and BOD diets were considered in the model. Differences were obtained between groups in the expression rate of the genes of interest (diffG = control group-test group) normalised by the endogenous gene. Contrasts were performed to obtain differences between groups for each gene (Steibel *et al* 2009). The adjusted  $P$ -values of the diffG were calculated using the Bonferroni correction method. The relative change values in gene expression (FC) were

<sup>7</sup> Thermo Fischer Scientific (Wilmington, USA)

<sup>8</sup> Applied Biosystems (Stockholm, Sweden)

<sup>9</sup> Fermentas Thermo Fischer Scientific (Wilmington, USA)

calculated from the estimated diffG values according to the following equation:  $FC = 2^{-diffG}$ . All calculations were performed in SPSS v. 20 (2011).

RESULTS

INTRAMUSCULAR AND SUBCUTANEOUS FAT

Carcass weight and performance were not affected by the experimental diets. The MOD diet decreased the ratio of intramuscular fat of the *Longissimus dorsi* muscle (IMFLD) and *Biceps femoris* muscle (IMFBF), back fat at the 10th rib, total carcass fat, carcass fat: meat ratio, and

rib fat (table 5). The MOD and BAD diets had a similar decreasing effect on IMFBF, back fat, and rib fat compared to the control diet. The multivariate analysis of the main components shows the association of higher amounts of fat with the control diet (figure 1).

GENE EXPRESSION IN LONGISSIMUS DORSI AND BICEPS FEMORIS MUSCLES, INTRAMUSCULAR FAT OF LEG MUSCLES, AND BACK FAT

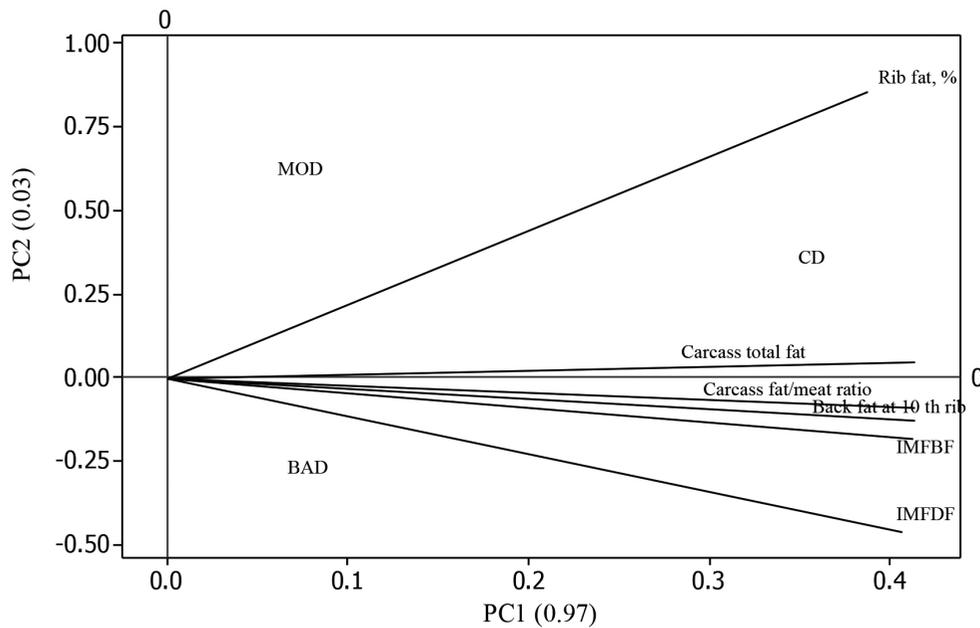
As shown in table 6, the FC of gene expression shows statistical differences between different tissue samples. In general, in the *Biceps femoris* muscle and the internal

**Table 5.** Differences mean in intramuscular and body fat of pigs fed *Moringa oleifera* and *Brosimum alicastrum*.

Variable	Diet			SEM	P<
	CD	MOD	BAD		
Slaughter weight, kg	51.18 <sup>a</sup>	51.80 <sup>a</sup>	51.04 <sup>a</sup>	0.63	Ns
IMFLD, %	13.45 <sup>a</sup>	11.02 <sup>b</sup>	11.92 <sup>ab</sup>	0.70	0.05
IMFBF, %	10.10 <sup>a</sup>	7.57 <sup>b</sup>	8.24 <sup>b</sup>	0.64	0.05
Back fat at 10th rib, mm	2.90 <sup>a</sup>	2.28 <sup>b</sup>	2.43 <sup>b</sup>	0.18	0.01
Cold carcass weight, kg	32.97 <sup>a</sup>	34.08 <sup>a</sup>	33.21 <sup>a</sup>	0.58	Ns
Carcass yield, %	64.4 <sup>a</sup>	65.79 <sup>a</sup>	65.07 <sup>a</sup>	0.51	Ns
Carcass total fat, kg	12.91 <sup>a</sup>	11.26 <sup>b</sup>	11.55 <sup>ab</sup>	0.51	0.09
Carcass fat: meat ratio, kg/kg	0.87 <sup>a</sup>	0.70 <sup>b</sup>	0.74 <sup>ab</sup>	0.05	0.05
Rib fat, %	35.36 <sup>a</sup>	30.14 <sup>b</sup>	28.94 <sup>b</sup>	1.89	0.01

<sup>a,b</sup>Values with different superscripts differ within a file ( $P < 0.05$ ).

CD = control diet; MOD = *Moringa oleifera* diet; BAD = *Brosimum alicastrum* diet; IMFLD = intramuscular fat of the *Longissimus dorsi* muscle; IMFBF = intramuscular fat of the *Biceps femoris* muscle.



**Figure 1.** Principle component analysis between diets and fat measurement variables. CD = control diet; MOD = *Moringa oleifera* diet; BAD = *Brosimum alicastrum* diet; IMFLD = intramuscular fat of the *Longissimus dorsi* muscle; IMFBF = intramuscular fat of the *Biceps femoris* muscle.

**Table 6.** Fold change ( $FC=2^{-\text{diffG}}$ ) of gene expression in different tissues between diets.

Contrast	Genes				
	ACACA	SCD	SREBP1	ACP	FASN
<i>Biceps femoris</i> muscle					
MOD-CD	1.10**	1.25**	0.91	1.09**	1.08**
BAD-CD	1.93**	1.12**	1.18*	1.75**	1.97**
SEM	0.01	0.02	0.08	0.01	0.03
Internal fat of leg muscles					
MOD-CD	1.21**	1.24**	1.55**	1.07**	1.07*
BAD-CD	1.49**	2.10**	1.44**	1.69**	1.34**
SEM	0.02	0.01	0.09	0.02	0.03
<i>Longissimus dorsi</i> muscle					
MOD-CD	0.60**	0.79**	0.34**	0.75**	0.84**
BAD-CD	0.87**	1.17**	0.51**	0.90**	1.10**
SEM	0.02	0.01	0.02	0.01	0.02
Back fat					
MOD-CD	0.58**	0.70**	0.42**	0.80**	0.74**
BAD-CD	0.61**	0.67**	0.47**	0.79**	0.74**
SEM	0.02	0.01	0.02	0.01	0.01

SCD = stearoyl-CoA desaturase; FASN = fatty acid synthase; ACACA = acetyl-CoA carboxylase alpha; SREBP1 = sterol regulatory element-binding protein 1; ACP = acyl carrier protein.

Values with different superscripts differ within a file \*= $P<0.05$ , \*\*= $P<0.001$

fat of the leg muscles, it was observed that the mRNA expression of lipogenic genes was more abundant compared to the *Longissimus dorsi* muscle and back fat. Meanwhile, in the *Biceps femoris* muscle, no difference in the expression of the SREBP1 gene was observed between the MOD and CD diets. In addition, the highest expression of SCD and FASN in the *Longissimus dorsi* muscle was in the BAD diet followed by the MOD diet, both with respect to the CD.

## DISCUSSION

These results agree with those of Mukumbo *et al* (2014), who found that the fat ratio of the *Longissimus thoracis* muscle decreased in pigs fed 7.5% *M. oleifera* leaves. Zhang *et al* (2019) did not observe an effect on the fat percentage of the *Longissimus thoracis* muscle in commercial pigs fed 3% to 9% *M. oleifera* for 45 d. However, the fatty acid content was modified, with an increase in monosaturated fatty acids and omega-3. These results are similar to those of Dzib-Cauich *et al* (2016) in MHP fed 20% to 40% *M. oleifera* in the diet. Pérez and García (2017) in Cuba fed 20% *M. oleifera* for 60 d and found a decrease in back fat without an effect on weight and carcass performance.

The inclusion of these forage species in animal feed is multipurpose because they have good bromatological characteristics, good palatability and digestibility, and a low amount of antinutritional compounds (Ly *et al* 2016,

Rojas-Schroeder *et al* 2017). In addition, *M. oleifera* and *B. alicastrum* forages are characterised by a large amount of bioactive compounds, including vitamins, carotenoids, and polyphenols (Moo-Huchin *et al* 2019). Thus, the consumption of their seeds and leaves has been shown to have beneficial effects (Moyo *et al* 2011, Rojas-Schroeder *et al* 2017). In addition, *M. oleifera* leaves reduced the levels of phospholipids, triglycerides, and cholesterol in the serum of growing pigs (Adegbenro *et al* 2016), suggesting a general reduction in lipid mobilisation and lipogenesis and confirming its relationship with a decrease in body fat. Adisakwattana and Chanathong (2011) evaluated *M. oleifera* leaf extract (in distilled water) *in vitro* and found that it led to a decrease in the enzymatic activity of pancreatic cholesterol esterase, which is related to lipid digestion and absorption. Specifically, the hydrolysis of cholesterol ester is catalysed by pancreatic cholesterol esterase, which releases cholesterol to the small intestine. Therefore, the inhibition of cholesterol esterase activity limits the absorption of cholesterol in the diet. Furthermore, phenolic compounds present in *M. oleifera* and *B. alicastrum*, such as phenolic acids and flavonoids (Moo-Huchin *et al* 2019), could contribute to the fat-reducing effects and play an important role in lipid regulation (Ezzat *et al* 2020). Zhang *et al* (2020) showed that the inclusion of 0.50% and 0.75% fermented *M. oleifera* leaves in the diet of white Peking ducks had a positive effect on the productive performance and characteristics of the carcass, reducing lipid deposition in liver and adipose tissues. This result

was associated with a reduction in serum adiponectin levels and an increase in leptin and insulin.

As shown here and confirmed by other studies, the incorporation of *M. oleifera* and *B. alicastrum* forage to the diet is one alternative for reducing body fat that does not affect weight or carcass performance.

The expression of lipogenic genes seems to differ according to tissue and diet, which affect lipid metabolism (Duran-Montgé *et al* 2009, Benítez *et al* 2016, Fernández *et al* 2017, Wang *et al* 2020). SCD is related to fatty acid esterification and ACP, ACACA, and FASN with lipogenesis, whereas, SREBP1 is a transcription factor. In the present study, the increase in the mRNA expression of lipogenic genes in pigs fed MOD and BAD diets in *Biceps femoris* muscle and internal fat of leg muscles was not related to a decrease in the amount of fat, indicating possible changes in novo fatty-acid synthesis, which was not studied here. However, MHP meat is known for its nutritional value, specifically, its high proportions of polyunsaturated fatty acids, omega-6, omega-3, docosahexaenoic acid. It also contains high proportions of palmitoleic acid/palmitic acid, oleic acid/stearic acid, linoleic acid/oleic acid, linoleic acid more linolenic acid/oleic acid, and lower values of saturated fatty acids and monounsaturated fatty acids (Dzib-Cauich *et al* 2020). Similar results were found in Iberian pigs (Benítez *et al* 2016, Fernández *et al* 2017). This effect could be explained through the SCD gene, which is the main enzyme involved in the synthesis of fatty acids. This enzyme is involved in the desaturation process necessary for MUFA biosynthesis, particularly for the synthesis of oleic acid from stearic and for the synthesis of palmitoleic from palmitic fatty acids (Fernández *et al* 2017).

Guillevic *et al* (2009) obtained identical results after feeding pigs sunflower or flaxseed oil. The carcass characteristics were not affected, and the lipid content of tissues did not increase, indicating that the enzymatic activity of lipid metabolism-related enzymes was poorly altered. However, they found that the fatty acid content was affected by the ingredients in the diet. In the present study, the decrease in the mRNA expression of the five lipogenic genes in the *Longissimus dorsi* muscle and back fat suggests an effect of the decrease in IMF<sub>LD</sub>, back fat at the 10th rib, total carcass fat, carcass fat: meat ratio and rib fat. According to Wang *et al* (2020), the low expression of FASN could be reflected in a lower intramuscular fat deposition because it is a predictor of the intramuscular fat content of the *Longissimus dorsi* muscle. The low expression of SREBP1, a transcription factor, is associated with the low expression of ACP, FASN, and SCD, as occurred in MOD (Mohan *et al* 2012). According to Mohan *et al* (2012) and Benítez *et al* (2018), a sunflower oil diet decreased the expression of FASN, SCD, and SREBP1. The decrease in SREBP1 could be due to an increase in the omega-6 polyunsaturated fatty acids, which in turn

would reduce the expression of FASN and SCD. Duran-Montgé *et al* (2009) observed that the expression of the FASN and SCD genes was reduced in the liver and muscle of pigs fed with polyunsaturated fatty acids, as similarly found here for MOD.

The expression of ACACA and SREBP1 could be related to the lower amount of fat present in the pigs fed the MOD and BAD diets. ACACA plays a fundamental role in the metabolism of fatty acids and acts as an intermediary in the de novo synthesis of long-chain fatty acids (Muñoz *et al* 2007), and the SREBP1 gene is a regulator of the action of other genes in lipid metabolism and accumulation (Chen *et al* 2008, Mohan *et al* 2012). The over-expression of SREBP1 in MHP has been related to an increase in the intramuscular fat of the *Longissimus dorsi* muscle compared to commercial pigs fed a corn-soy diet (Dzib-Cauich *et al* 2020). Also, in the present study, the SCD and FASN genes were overexpressed in the *Longissimus dorsi* muscle and back fat and showed differences between the BAD and MOD diets, probably in association with the decrease in the amount of fat.

Internal fat of leg muscles, *Biceps femoris* muscle, loin back and *Longissimus dorsi*, the expressions of the genes differed as previously reported, indicating that the lipogenic action varies according to tissue and diet (Duran-Montgé *et al* 2008, Duran-Montgé *et al* 2009, Benítez *et al* 2016). The present study demonstrates that the *M. oleifera* diet decreased the ratio of intramuscular fat of the *Longissimus dorsi* muscle, intramuscular fat of the *Biceps femoris* muscle, back fat, total carcass fat, carcass fat: meat ratio, and rib fat. The *B. alicastrum* diet only decreased IMF<sub>BF</sub>, back fat, and rib fat compared with the control diet. In addition, the inclusion of *M. oleifera* and *B. alicastrum* leaf meal in the diet increased the mRNA expression of the lipogenic genes (SCD, FASN, ACACA, SREBP1, and ACP) in the *Biceps femoris* muscle and in the intramuscular fat of the leg muscles and decreased gene expression in the *Longissimus dorsi* muscle and back fat. The low expression of ACACA, mRNA, and SREBP1 in the *Longissimus dorsi* muscle and back fat was associated with the lower amount of fat in these tissues in pigs fed both the *M. oleifera* and *B. alicastrum* diets.

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