DOI: 10.5433/1679-0359.2019v40n2p793

Physicochemical characteristics and gene expression of meat from does fed with dried carnauba wax palm fruit

Características físico-químicas e expressão gênica da carne de cabras alimentadas com fruto da carnaúba desidratado

Aline Maia Silva[1]; Cláudio Henrique de Almeida Oliveira[1]; César Carneiro Linhares Fernandes[2]; Stephen Rathinaraj Benjamin[3]; Maria Izabel Florindo Guedes[4]; Davide Rondina[5*]

Abstract

This trial was carried out with the objective to evaluate the effect of feeding dried carnauba wax palm fruit on the quality of goat meat. Initially, the animals were divided into two groups: a control group, fed with ground corn-based concentrate, and a treatment group, fed with dried carnauba wax palm fruit included in the concentrate. After 28 days of feeding, the animals were slaughtered, and different muscles (*Longissimus lumborum* and *semimembranosus*) were evaluated for physicochemical characteristics, fatty acid profile, and expression of some genes related to meat quality. The diet with carnauba wax palm fruit does not interfere in the chemical composition of the meat in terms of moisture content, protein, fat, pH, and meat color. On the other hand, palmitic (C16:0) and linoleic (C18:2) fatty acids presented differences between muscles in the group fed carnauba wax palm fruit. The treatment with carnauba wax palm fruit reduces the expression of genes related to lipogenesis and cholesterol metabolism, suggesting the production of a tender meat with lower cholesterol content. The inclusion of carnauba wax palm fruit in the diet of does maintained the physicochemical characteristics of the loin and leg, and it reduced the expression of the analyzed genes, suggesting improvement in meat quality.
Key words: Fatty acids. Genes. Goat. Meat. Quality.

Resumo

O experimento foi conduzido com o objetivo de avaliar o efeito da inclusão do fruto da carnaúba desidratado na alimentação de cabras sobre a qualidade da carne. Inicialmente, os animais foram divididos em dois grupos: um grupo controle, alimentado com concentrado tendo o milho como base alimentar, e um grupo tratamento, no qual se incluiu o fruto da carnaúba desidratado em substituição ao milho. Após 28 dias de alimentação, os animais foram abatidos e diferentes músculos (*Longissimus lumborum* e *Semimembranoso*) foram avaliados quanto às características físico-químicas, perfil de ácidos graxos e expressão de alguns genes relacionados à qualidade da carne. Observou-se que a

[1] Profs. Drs., Faculdades Terra Nordeste, FATENE, Caucaia, CE, Brasil. E-mail: maias.aline@gmail.com; claudiohao@hotmail.com
[2] Pós-Doutor, Programa de Pós-Graduação em Ciências Veterinárias, Universidade Estadual do Ceará, UECE, Fortaleza, CE, Brasil. E-mail: caancesar@gmail.com
[3] Pós-Doutor, Programa de Doutorado em Biotecnologia da Rede Nordeste de Biotecnologia, RENORBIO, Fortaleza, CE, Brasil. E-mail: biotechstephen@gmail.com
[4] Profª Drª, Programa de Doutorado em Biotecnologia, RENORBIO, Fortaleza, CE, Brasil. E-mail: florinfg@uol.com.br
[5] Prof. Dr., Programa de Pós-Graduação em Ciências Veterinárias, UECE, Fortaleza, CE, Brasil. E-mail: davide.rondina@uece.br
[*] Author for correspondence

Received: Apr. 03, 2018 - Approved: Oct. 29, 2018

inclusão do fruto da carnaúba desidratado não interferiu na composição química da carne em termos de umidade, proteína, gordura, pH e coloração. Por outro lado, os níveis de ácido palmítico (C16:0) e linoleico (C18:2) apresentaram diferenças entre os músculos do grupo alimentado com o fruto da carnaúba desidratada. O tratamento com fruto da carnaúba desidratado reduziu a expressão de genes relacionados com os processos de lipogênese e metabolismo do colesterol, sugerindo a produção de uma carne tenra e com menor teor de colesterol. A inclusão de fruto da carnaúba desidratado na dieta de cabras manteve as características físico-químicas dos músculos avaliados e reduziu a expressão dos genes analisados, sugerindo uma melhora na qualidade da carne.
Palavras-chaves: Ácidos graxos. Cabras. Carne. GENES. Qualidade.

Introduction

Global meat production is projected to grow moderately in the coming years, while world meat consumption continues to have one of the highest growth rates among major agricultural products.[1] However, market analysis projects a growing change in the type of meat consumed, with an overall increase in the consumption of white meat such as chicken (2.7%) in relation to beef (1.98%) or pork (1.79%) (MAPA, 2012). This market trend in Brazil, as in other countries, has been the result of two changes in recent years in the consumer profile. Between 2009 and 2011, there was an increase in the elderly population in Brazil of 7.6%. In 2012, 11.5% of the total population was aged 60 and over, and that group is projected to reach 22% of the global population by 2050 (MAPA, 2012). Parallel to the growth in the elderly population, there is also an increase in the incidence of obesity. Based on the report of the World Health Organization (WHO), at least 41 million children in the world under the age of five are obese or overweight (IBGE, 2013).

The evolution of the population's consuming habits, as well as increased consumer awareness about food safety and health, have leveraged a progressive demand for quality meat, nutritional functionality, and product traceability. In this context, goat meat, among red meat, has gained popularity, especially in developed countries, mainly because of its lower lipid, cholesterol, and saturated fatty acids contents (ANAETO et al., 2010), in addition to its essential amino acids, such as lysine, threonine, and tryptophan. At the same time, in developing countries such as Brazil, goat meat has also been increasingly consumed, and it is traditionally one of the main sources of animal protein in the diet of small farmers, mainly in the semi-arid region of the Northeast. Due to the prominent characteristics of goat production, the market in the Northeast and other regions of Brazil is focused on the slaughter and commercialization of meat from adult animals without distinction of sex, which may present distinct meat physicochemical characteristics compared to younger animals, which are commonly more appreciated. The breeding system predominantly adopted uses feed from natural forest resources, which may be the determining factor for the occurrence of differential characteristics of the meat.

One way to characterize meat products and to verify the difference in its commercial value is through the study of physicochemical properties such as color, conjugated linoleic acid (CLA) and polyunsaturated fatty acids contents (ATTI et al., 2006), and pH (KANNAN et al., 2006). Among these, gene expression evaluation is currently used as a traceability tool, providing information about the origin of the animal as well as the feed provided, since gene expression can be influenced by the diet (DERVISHI et al., 2011; WANG et al., 2015).

The use of alternative regional feed has been the subject of recent research seeking to qualify such foods and to determine optimum levels to the diets of ruminants, reaching satisfactory animal productivity and, preferably, producing quality products. Among the regional resources present in the Northeast region of Brazil is carnauba (*Copernicia prunifera*), which is widely used in extractive activity in the

region. The fruit was used to investigate antioxidant capacity and bioactive components (RUFINO et al., 2010; SILVA et al., 2005) and to verify the quality for fresh consumption and processing (RUFINO et al., 2009). Regarding its use in animal feeding, in addition to the research already cited using carnauba wax, Silva et al. (2015) used the whole fruit milled for feeding does in the postpartum period. Recently, Paim et al. (2017) used aqueous fruit pulp extract administered in hyperlipidemic mice reduced hypercholesterolemia, showing a potential preventive effect against cardiovascular diseases.

Thus, the objective of this study was to analyze physicochemical characteristics of the prime cuts (loin and legs) using two muscles (*Longissimus lumborum* and *semimembranosus*) as well as to evaluate the expression of genes relevant to the quality of meat from does fed dried carnauba wax palm fruit.

Materials and Methods

Carnauba wax palm fruit

As described by Silva et al. (2015), the carnauba wax palm fruit was harvested prior to maturation, when they were predominantly colored green. The crop was harvested from April to May in Aracati, Ceará, and the fruit was transported to the experimental farm, dried outdoors, and revolved several times a day to ensure uniform dehydration. Thereafter, the fruit was milled and stored in polyethylene bags until use.

Animals and feed treatment

All procedures were previously approved by the Ethics Committee from the State University of Ceará for the experimental use of animals (protocol no. 12780187-1) respecting the standards established by the National Council for Animal Experiments Control by the regulations of Law No. 11.794 (2008).

The study was conducted at the experimental Dr Esaú Accyoli de Vasconcelos farm located in Guaiuba-Ceará at 4°2'23'' S and 38°38'14'' W during 2012. A total of 15 adult multiparous crossbreed does were used, with an average age of 40.4 ± 5.8 months, mean body weight of 35.5 ± 7.4 kg, and body condition score of 2.6 ± 0.4 (mean ± SD). The animals were divided into two feeding groups, with eight animals in the control group (WWP) and seven in the treatment group (WP). The WWP was fed chopped Bermuda grass hay and ground corn-based concentrate (838.2 g kg DM^{-1} corn, 106.4 g kg DM^{-1} soybean meal, and 55.4 g kg DM^{-1} minerals, on a dry matter basis). The WP received a diet based on chopped Bermuda grass hay and a concentrate including carnauba wax palm fruit (798.7 g kg DM^{-1} carnauba fruit, 148.1 g kg DM^{-1} soybean meal, and 53.2 g kg DM^{-1} minerals, on a dry matter basis) for 28 days. The dietary ingredients' chemical composition is shown in Table 1 and percent composition in Table 2. Diets contained a 40:60 (concentrate:roughage) ratio, and both were offered twice a day (07h00 and 15h00). The animals received mineral salt and water *ad libitum*. Both diets were formulated to meet 1.3 times the maintenance requirements for non-dairy mature does (NRC, 2007). Dry matter, organic matter, crude protein, ether extract, and ash contents of the diets were analyzed using the procedures described by AOAC (1990), and the neutral detergent fiber, acid detergent fiber, and lignin contents were analyzed according to Van Soest et al. (1991).

Plasma cholesterol, triglycerides, and total lipids analysis

For analysis of total cholesterol (TC) and total triglycerides (TT), blood plasma was used that was obtained by jugular venipuncture using heparinized tubes (BD Vacutainer®, São Paulo, Brazil), with the puncture site previously cleaned with 70% alcohol. Samples were collected weekly during the experimental period. To obtain the plasma,

samples were centrifuged at 600 g for 15 min, and the reserved supernatant was frozen and kept at -20° C until analysis. Specific commercial kits for each metabolite (Bioclin®, Quibasa - Minas Gerais, Brazil) and an automated biochemical device (Mindray BS-120, Shenzhen, China) were used, which were previously calibrated and supervised prior to all analyses. The estimation of total lipids (TL) was calculated as follows: 2 x (TC + TT) x 1.1.

Table 1. Chemical composition of the dietary ingredients.

Based on g kg DM^{-1}	BH	DWPF	CCD	CWPD
Dry Matter	888.1	887.8	862.1	868.9
Organic Matter	928.3	947.7	954.5	885.5
Crude Protein	105.7	67.8	158.9	126.5
Ether Extract	25.3	42.2	47.7	52.3
Neutral detergent fiber	729.2	538.9	162.0	468.9
Acid detergent fiber	350.3	381.1	29.9	348.8
Mineral matter	72.1	53.1	46.6	115.6
Lignin	185.0	161.2	-	-

DM = dry matter, BH = Bermudagrass hay, DWPF = dehydrated wax palm fruit, CCD = concentrated control diet, CWPD = concentrated wax palm diet.

Table 2. Ingredients and chemical appearance of dietary.

	Diet	
	Control	Carnauba
Ingredients g kg DM^{-1}		
Tifton Hay	600.0	600.0
Corn	334.0	-
Dehydrated carnauba fruit	-	319.3
Soybean meal	43.4	58.3
Mineral	22.6	22.4
Composition g kg DM^{-1} [a]		
Dry matter	888.8	890.0
Crude protein (CP)	158.1	130.1
Ether extract (EE)	41.6	38.8
Neutral detergent fiber (NDF)	215.3	635.2
Acid detergent fiber	81.7	381.3
Mineral (M)	47.4	47.4
Non-fibrous carbohydrates (NFC) [a]	537.6	148.5
Total Carbohydrates (TC) [b]	752.9	783.7

[a] NFC = TC - NDF (VAN SOEST et al., 1991). [b] TC = 100 - (%CP + %EE + %M) (SNIFFEN et al., 1992).

Slaughter and meat sampling

Before slaughter, the animals were subjected to *ante-mortem* inspection and kept fasting from solids and water around 12 to 16 h for reducing gastric contents to facilitate evisceration and minimize carcass contamination. After fasting, the animals were weighed and then slaughtered. The slaughter followed the instructions of the Regulation on Industrial and Sanitary Inspection of Products of Animal Origin (legitimized by Decree No. 30,691/1952).

After skinning, evisceration, and removal of the head and extremities, the loin and leg were weighed and collected in triplicates by sampling *Longissimus lumborum* (LL) and *semimembranosus* (SM) muscles, using a scalpel, surgical scissors, and surgical tweezers, washed with distilled water, and stored in 2-mL cryotubes. For analysis of fatty acids, samples were stored within 10 min after slaughter at -20º C. For gene expression analysis, samples were immediately stored at -196º C (liquid nitrogen) and maintained at -80º C.

Evaluation of meat pH and color

After slaughter, the carcasses were stored at 2° C for 24 h for maturation. The pH and muscle color of the loin (LL), between the 9th and 13th lumbar ribs, and leg (SM) were determined. In each evaluation, the equipment was properly cleaned with distilled water and 70% alcohol. The pH was determined at 45 min post-slaughter (pH-45 min), 12 h (pH-12 h), and 24 h (pH-24 h) using a portable pH meter (Testo SE & Co. KGaA®, model 205, Lenzkirch, Germany). The color was determined by two measurements at 1 h and 24 h after slaughter using a spectrophotometer (model CM-2500d, Konica Minolta®, Osaka, Japan) and a CIELAB evaluation system, with L* corresponding to lightness, a* to redness, and b* to yellowness.

Analysis of fatty acids from loin and leg

TL were extracted from approximately 5 mg of each muscle with chloroform/methanol (2/1) according to Folch et al. (1957). The lipids were methylated by the method of O'Keefe et al. (1968) with sodium methylate. Methyl esters were analyzed using gas chromatography-mass spectrometry (model GCMS-QP2010S, SHIMADZU®, Kyoto, Japan) in a 30-m long capillary column RTX-5 with an internal diameter of 0.25 mm and 1 µm thickness. A sample was injected into a splitless injector at 250º C, with a total flow of 78.1 mL/min and column flow of 2.52 mL/min, totaling 50 min. A quadrupole analyzer with 70 eV and 33-750 daltons was used. The eight main fatty acids were identified by comparing the retention time of the methyl esters of the samples with fatty acid esters standards (Merck®, New Jersey, USA).

Gene expression analysis

Total cellular RNA was isolated from the muscle tissue of LL using the RNeasy® Mini kit (Qiagen, Hilden, Germany). RNA isolation was performed according to the manufacturer's protocol, with adaptations. Briefly, the tissue was macerated using liquid nitrogen, and about 30 mg of the obtained powder was transferred to a tube. Thereafter, RLT and proteinase K buffer (Invitrogen™, California, USA) were added and then incubated at 55° C for 10 min. Total RNA was eluted from the matrix with 40 µl of RNase-free water. Residual genomic DNA was removed by incubating the RNA solution with 1 µl of RNase-free DNase I - Amplification Grade (Life Technologies, USA) for 10 min at 37°C. To check the quality and quantity of total RNA, a NanoDrop® spectrophotometer was used (Thermo Scientific, NanoDrop 2000, USA). After the quantification of the extracted RNA, the integrity of the material was analyzed on agarose gel 1% with GelRed™ (Biotium, Inc., Fremont, California, USA). This process was accomplished through

the presence of bands corresponding to 18S and 28S ribosomal RNAs after electrophoresis (2100 Bioanalyzer, Agilent Technologies, USA).

The reverse transcription reactions contained equivalent amounts of total RNA. The reactions were incubated at 65°C for 5 min and then at 25°C for 10 min, 42ºC for 50 min, and 75ºC for 15 min. The cDNA samples were aliquoted and stored at -80°C. The analyzed genes were stearoyl-CoA desaturase (SCD), calpastatin (CAST), insulin-like growth factor 1 receptor (IGF1-r), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA-r), apolipoprotein A-1 binding protein (APOA-1 bp), and TATA-Box binding protein (TBP; Table 3). PCR reactions were performed with cDNA equivalents using a thermocycler (Swift™ MaxPro Termal Cycler, Esco Healthcare Pte Ltd, Singapore) under the following conditions: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55ºC for 30 s, 72°C for 40 s, and 72ºC for 10 min. Samples were placed in agarose gel (1%) x TAE buffer (40 mM Tris-acetate and 1 mM EDTA) containing GelRed™. Afterward, the samples were subjected to electrophoresis at 80W for 40 min and subsequently analyzed in transilluminator UV - 365 nm (Herolab, GmbH Laborgeräte, Ludwig-Wagner-Straße), and the image was captured using photodocumentation (Microfoc, Bio-Rad, California, EUA). Band intensity was quantified using ImageJ® software (Image J, National Institutes of Health, Millersville, USA). The relative amount of the mRNA of interest was calculated by dividing the intensity of the band for each gene of interest by the intensity of the constitutive gene (TBP).

Table 3. Sequences of the PCR primers used.

Gene	Access number in GenBank	Primers [forward (F) and reverse (R)]	Products bp)
SCD	GU947654.1	F: CAGAGAAAAGGGTGCTACGC R: GATGAAGCACAACAGCAGGA	106
CAST	GU944861.1	F: CCTCCTCCAGACTCCTCGAA R: AGCAGCACTTCTGATCACCC	168
IGF1-r	JN200823.1	F: GGCTCAACCCAGGGAACTAC R: ATGGGCAGAGCGATCATCAG	142
HMG-CoA-r	NM_001105613.1	F: ACCCATGAGCGAGGTGTATC R: GCTCCCATCACCAAGGAGTA	116
APOA-1 BP	AY528250.1	F: CTGGTGGTGGATGCCATCTT R: TCTCCACGTCCCATCCTGAT	136
TBP	NM_001075742	F: CCTAAAGACCATTGCACTTCG R: CTTCACTCTTGGCTCCTGTG	146

Statistical analysis

A completely randomized design was used for the statistical analyses, with two diets and 15 total replicates. Data were submitted to analyses of variance (ANOVA) using the GLM procedure. For daily weight gain and plasmatic concentrations of metabolites, the main factors were dietetic group (WWP, WP), time (interval of assessment used), and interaction dietetic group *vs*. time. For chemical composition and fatty acids profile effect, the dietetic group, muscle (LL, SM), and interaction dietetic group *vs*. muscle were tested. For pH and color parameters, the factors used were dietetic group, time, muscle and dietetic group interaction *vs*.

time, and dietetic group *vs*. muscle. Other weights and gene expressions were tested for dietetic group effect only. Data of gene expression and metabolites were transformed into log 10 values. Means were compared using the Duncan test.

Results and Discussion

The qualitative and quantitative evaluation of carcass components of small ruminants can be influenced by several factors, such as breed, age, sex class, and dietary factors. There was no effect of diet ($P > 0.05$) for total weight gain, slaughter weight, and cold carcass weight, as well as for the weight of LL and SM muscles (Table 4). This can be explained by the maintenance diet offered to both groups, in addition to being adult animals, since feed conversion is inversely proportional to age.

Table 4. Treatments effect on weight gain, body weight, muscles weight and plasmatic parameters. Values are expressed as mea ± standard error of the mean.

Parameters	Group		P-value		
	WWP	WP	Group	Time	G x T
Total weight gain (kg)	2.2 ± 0.8	2.7 ± 0.6	0.667	-	-
Slaughter body weight (kg)	34.7 ± 2.4	33.1 ± 2.7	0.649	-	-
Dress cold weight (kg)	13.3 ± 0.9	12.6 ± 1.1	0.546	-	-
	Weights of cut muscles				
Longissimus lumborum (g)	333.6 ± 20.2	402.2 ± 25.7	0.322	-	-
Semimembranosus (g)	99.6 ± 10.1	99.0 ± 11.4	0.968	-	-
	Plasma				
Triglycerides (mg dL^{-1})	12.1 ± 1.5	15.0 ± 1.6	0.165	0.018	0.945
Cholesterol (mg dL^{-1})	61.2 ± 2.1	68.2 ± 3.4	0.097	0.134	0.605
Total Lipids (mg dL^{-1})	161.2 ± 5.8	183.0 ± 9.5	0.054	0.028	0.691

WWP = without wax palm fruit, WP = wax palm fruit, G = group, T= time.

The plasma lipid profile of ruminants faithfully reflects the metabolic status of the tissues, allowing the assessment of organ dysfunction and adaptation to nutritional and physiological challenges and specific metabolic or nutritional imbalances (COTE; HOFF, 1991). Considering that plasma lipid concentrations are influenced by the fatty acid composition of the diet, no effect was observed ($P > 0.05$) in relation to plasma triglycerides, cholesterol, and total lipid levels (Table 4). Despite the effect of time observed for triglycerides and TL, the plasma concentrations of triglycerides, cholesterol, and TL are in accordance with the reference values for goats recommended by Smith and Sherman (2009).

Knowledge of the meat's chemical composition is essential for the elucidation of its nutritional values, as well as to determine adequate diets according to different population groups. The data related to the meat chemical composition can be observed in Table 5. Effect of diet was observed only for ash content of the LL ($P < 0.05$), with higher values for the WWP (11.9 ± 0.2 g kg^{-1}) compared to the WP (11.2 ± 0.2 g kg^{-1}). This may be related to the different ingredients present in the diet, as mentioned by Qwele et al. (2013). Despite the differences between the LL and SM, the results were similar to the literature for goats in relation to moisture (708.0 to 802.5 g kg^{-1}), ash (7.9 to

16.8 g kg $^{-1}$), protein (185.0 to 233.9 g kg $^{-1}$), and lipid values (15 to 130 g kg $^{-1}$) (BESERRA et al., 2004; DHANDA et al., 1999; MADRUGA, 1999; OLIVEIRA et al., 2013).

Regarding the fatty acid profile (Table 5), there was no effect (P > 0.05) of diet or muscle type, with the exception of palmitic fatty acid (C16:0), which showed higher values (P < 0.05) in the LL than in the SM for the WP (34.9 ± 0.4 *vs*. 33.4 ± 1.1, P = 0.03). In addition, linoleic fatty acid (C18:2) was affected (P < 0.05) by muscle type, with higher values for the SM in relation to the LL for the WP (2.52 ± 0.5 *vs*. 1.42 ± 0.2; P = 0.04). Although there was no statistical difference between the WP and WWP, higher values were observed in the SM for linoleic fatty acid. This result is relevant, since linoleic acid is characterized as essential because the human organism does not produce it, so its ingestion through food is necessary. It is a fatty acid precursor of CLA, which has important biological effects, including its anti-carcinogenic activity and its nutrient delivery property (CORL et al., 2003). This change may be a reflection of the alteration of lipogenesis in the WP (see expression of evaluated genes), considering that the fatty acid composition of the diets was different due to the use of different ingredients (carnauba wax palm fruit). However, probably because of the feeding period, the change did not present different values between the groups.

Table 5. Composition and fatty acids profiles of the *Longissimus lumborum* (Loin) and *Semimembranosus* (Leg) muscles of goats fed with wax palm fruit. Values are expressed as mean ± standard error of the mean.

Item	Group				P-value		
	WWP		WP				
	LL	SM	LL	SM	Group	Muscle	G x M
Moisture (g kg $^{-1}$)	759.1 ± 3.7a	780.1 ± 7.2b	760.8 ± 3.1a	784.5 ± 5.6b	0.683	0.001	0.942
Ash (g kg $^{-1}$)	11.9 ± 0.2Aa	19.7 ± 0.6b	11.2 ± 0.2Ba	18.1 ± 1.6b	0.035	0.001	0.410
Protein (g kg $^{-1}$)	223.1 ± 3.9a	195.0 ± 3.6b	225.6 ± 3.5a	190.3 ± 2.3b	0.816	0.001	0.450
Fat (g kg $^{-1}$)	15.5 ± 0.8	15.6 ± 0.6	17.7 ± 0.6	14.4 ± 0.5	0.560	0.903	0.061
Fatty acids profile (%)							
Myristic C14:0	2.38 ± 0.14	1.37 ± 0.51	1.94 ± 0.73	1.89 ± 0.36	0.884	0.103	0.138
Palmitic C16:0	34.7 ± 0.8	31.1 ± 1.7	34.9 ± 0.4a	33.4 ± 1.1b	0.268	0.034	0.319
Margaric C17:0	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	0.966	0.807	0.436
Stearic C18:0	18.9 ± 1.8	21.6 ± 0.5	19.2 ± 1.1	19.8 ± 0.8	0.511	0.206	0.420
Palmitoleic C16:1	1.4 ± 0.1	1.3 ± 0.1	1.7 ± 0.3	1.5 ± 0.1	0.278	0.420	0.828
Oleic C18:1 (n-9)	36.7 ± 1.5	38.2 ± 2.0	36.6 ± 0.3	36.2 ± 0.9	0.402	0.681	0.480
Vaccenic C18:1 (n-11)	2.8 ± 0.3	3.2 ± 0.4	2.8 ± 0.3	3.3 ± 0.5	0.919	0.227	0.859
Linoleic C18:2	1.5 ± 0.1	1.72 ± 0.3	1.42 ± 0.2a	2.52 ± 0.5b	0.229	0.044	0.156

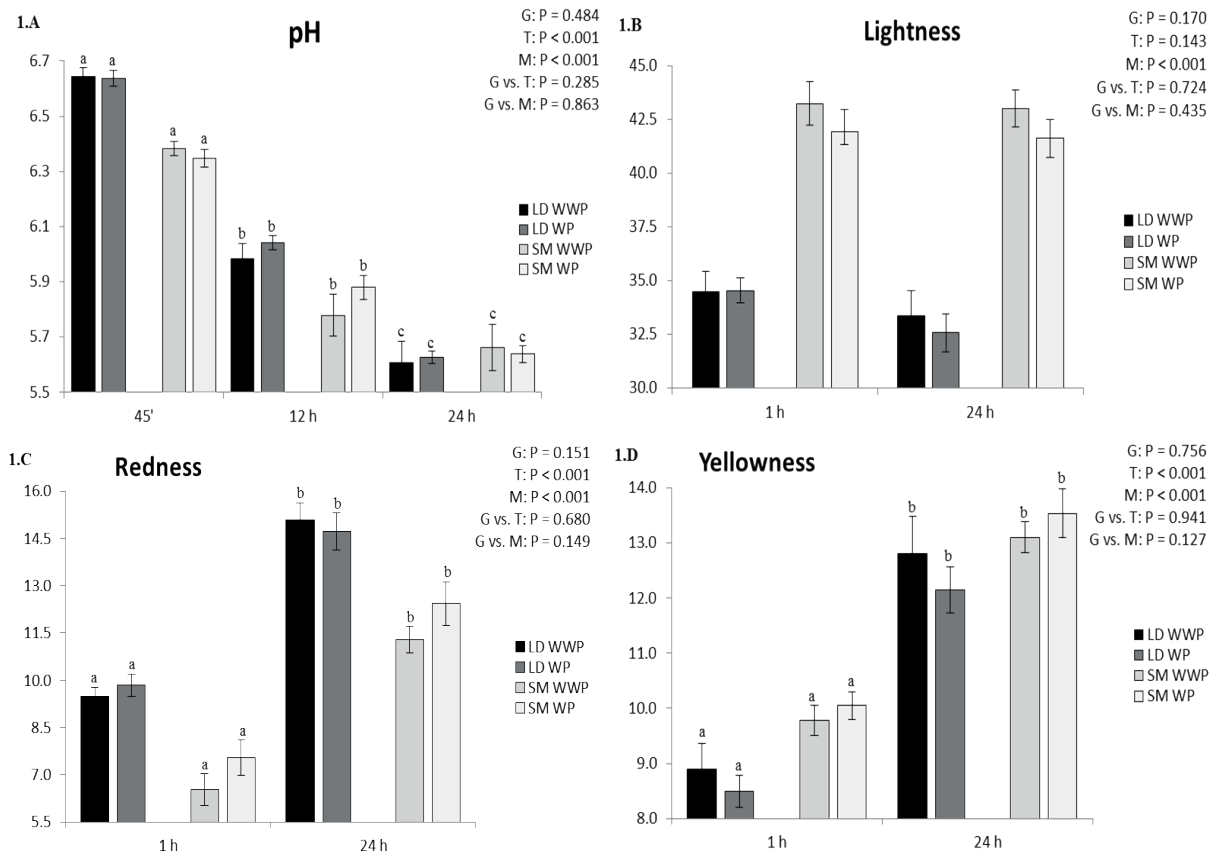
[a,b] P < 0.05, Comparison between muscles in the same group; [A,B] P < 0.05, Comparison between groups in the same muscle. WWP = without wax palm fruit, WP = wax palm fruit, LL = *longissimus lumborum*, SM = *semimembranosus*, G = group, M = muscle.

The intake of carnauba wax palm fruit did not affect the pH parameters (initial and after 12 and 24 h) in both muscles (P > 0.05), but a reduction of pH over time in both groups (P < 0.05) and muscle effect (P < 0.001) were observed, with higher values for the LL compared to the SM (6.09 ± 0.04 *vs*. 5.95 ± 0.03; P < 0.001), as demonstrated in Figure 1. The reduction of pH is an expected change, due to the accumulation of glycogen in the *ante-mortem* period, which causes the transformation into lactic

acid, caused by the absence of oxygen in the cells, resulting in biochemical reactions *post mortem* and generating the transformation of muscle in meat (PINHEIRO et al., 2009). The difference in values between the LL and SM can be explained by considering that the SM is more active, with a consequently lower reserve of glycogen, leading to lower pH values, or even because this muscle is more exposed to the reduction of temperature due to lower fat coverage.

The results concerning the color components evaluated in the present study can be seen in Figure 1. The intake of carnauba wax palm fruit did not affect the color of the LL and SM muscles ($P > 0.05$), indicating no difference in myoglobin concentrations in the muscle of the does. The values found in the present study were higher than those reported by Oliveira et al. (2015), who provided co-products of the biodiesel chain for young goats.

Figure 1. Means and standard error of pH (1.A), lightness (1.B), redness (1.C), yellowness (1.D) changes over time of *Longissimus lumborum* (Loin) and *semimembranosus* (Leg) muscles of goats fed carnauba wax palm fruit. LL = *longissimus lumborum*, SM = *semimembranosus*, WWP = without wap palm fruit, WP = wax palm fruit. ANOVA results for the effects of group (G), time (T), muscle (M) and the interaction group *vs.* time and group *vs.* muscle.



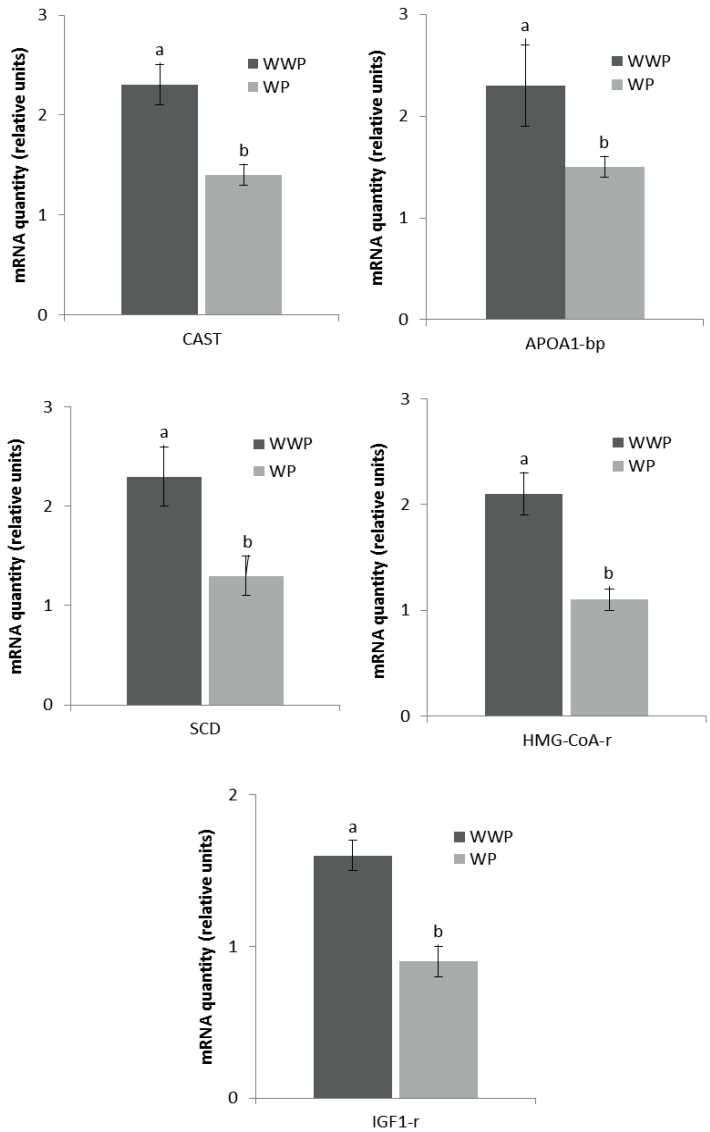
[a,b]: Letters were referred to comparison between the interval of assessment (Time effect) for the same muscle.

In order to analyze the effect of diet on the molecular quality of the meat, the expression of some genes, such as CAST, IGF1-r, SCD, HMG-CoA-r, and APOA-1 bp, were analyzed (Figure 2). Animals fed carnauba wax palm fruit (WP) had a significant reduction in the expression of all

evaluated genes ($P < 0.05$) in relation to the WWP. This result is interesting, since these genes are directly involved in the quality of the meat.

The reduction of the CAST gene suggests the production of a tender meat, since it encodes an endogenous calpain inhibitor (calcium-dependent cysteine protease), playing a key role in *post mortem* meat softening. There is considerable evidence that, in different species, calpastatin activity in the *post mortem* muscle is highly related to meat tenderness through inhibition of endogenous cysteine peptidases, the calpain. Therefore, there is a direct relationship between this gene and the shear force (REARDON et al., 2010) and meat hardness (GIUSTI et al., 2013). The IGF1-r gene encodes the type 1 insulin-like growth factor receptor, which plays a crucial role in signaling, which is important for cell proliferation and survival (SZEWCZUK et al., 2013). It is directly related to the increase in the diameter of the muscle fiber and the shear force in the muscle, and, consequently, to the tenderness of the meat (SU et al., 2014).

Figure 2. Means and standard errors of gene expression (arbitrary units) in *longissimus lumborum* (Loin) from goats (n = 15) fed with wax palm fruit. WWP = without wax palm fruit, WP = wax palm fruit. [a,b]: $P < 0.05$



Regarding the genes related to lipogenesis and cholesterol metabolism, HMGCoA is the rate-limiting enzyme in cholesterol synthesis (BROWN; GOLDSTEIN, 1980). The APOA-1 bp gene encodes apolipoprotein A-1, the main protein component of high-density lipoprotein in the plasma. SCD encodes an enzyme involved in fatty acid biosynthesis, contributing to the biosynthesis of membrane phospholipids, cholesterol esters, and triglycerides. SCD plays a key role in denaturation of saturated fatty acids to monounsaturated fatty acids. Studies have demonstrated a relationship between SCD expression and fatty acid composition (TANIGUCHI et al., 2004), improving organoleptic characteristics by balancing n-6 and n-3 fatty acids (SEVANE et al., 2013), and a relation to other color characteristics (REARDON et al., 2010) significantly modulated by the feed system (DERVISHI et al., 2011). Although cholesterol reduction was not observed in the meat, the use of carnauba wax palm fruit influenced the reduction of the expression of genes related to lipogenesis and cholesterol metabolism, especially the HMG-CoA gene. This indicates a possible hypolipidemic action, suggesting the production of meat with a lower cholesterol content.

The inclusion of dried carnauba wax palm fruit (*Copernicia prunifera*) for 28 days in diets for does maintains the physicochemical aspects of the loin and leg evaluated using two muscles (LL and SM) of great commercial interest, despite altering its constitution at the molecular level. Therefore, it is suggested that other studies using carnauba wax palm fruit for goats should be performed.

Acknowledgements

The Public Notice 06/2011 - CAPES/FUNCAP (ref. n º. 23039.007957/2012-05) Programa Áreas Estratégicas, supported this study. Aline Silva Maia is a doctoral student in the graduate program Northeast Biotechnology Network (RENORBIO), Brazil. Davide Rondina is a senior investigator of CNPq/Brazil.

References

ANAETO, M.; ADEYEYE, A. J.; CHIOMA, O. G.; OLARINMOYE, O. A.; TAYO, O. G. Goat products: meeting the challenges of human health and nutrition. *Agriculture and Biology Journal of North America*, Milford, v. 1, n. 6, p. 1231-1236, 2010.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official methods of Analysis. 15th ed. Washington: AOAC, 1990.

ATTI, N.; MAHOUACHI, M.; ROUISSI, H. The effect of cactus (*Opuntia ficus-indica* f. *inermis*) supplementation on growth, carcass, meat quality and fatty acid composition of male goat kids. *Meat Science*, Cowra, v. 73, n. 2, p. 229-235, 2006.

BESERRA, F. J.; MADRUGA, M. S.; LEITE, A. M.; SILVA, E. M. C.; MAIA, E. L. Effect of age at slaughter on chemical composition of meat from Moxotó goats and their crosses. *Small Ruminant Research*, Oxford, v. 55, n. 1, p. 177-181, 2004.

BROWN, M. S.; GOLDSTEIN, J. L. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *Journal of Lipid Research*, Bethesda, v. 21, n. 5, p. 505-517, 1980.

CORL, B.; BARBANO, D. M.; BAUMAN, D. E.; IP, C. Cis-9, trans-11 CLA derived endogenously from trans-11 18:1 reduces cancer risk in rats. *Journal of Nutrition*, Oxford, v. 133, n. 9, p. 2893-2900, 2003.

COTE, J. F.; HOFF, B. Interpretation of blood profiles in problem dairy herds. In: COTE, J. F.; HOFF, B. *The bovine practitioner.* Stillwater: American Association of Bovine Practitioners, v. 26, p. 7-11, 1991.

DERVISHI, E.; SERRANO, C.; JOY, M.; SERRANO, M.; RODELLAR, C.; CALVO, J. H. The effect of feeding system in the expression of genes related with fat metabolism in semitendinous muscle in sheep. *Meat Science*, Cowra, v. 89, n. 1, p. 91-97, 2011.

DHANDA, J. S.; TAYLOR, D. G.; MURRAY, P. J.; MCCOSKER, J. E. The influence of goat genotype on the production of Capretto and Chevon carcasses. 4. Chemical composition of muscle and fatty acid profiles of adipose tissue. *Meat Science*, Cowra, v. 52, n. 4, p. 375-379, 1999.

FOLCH, J.; LEES, M.; STANLEY, G. H. S. A. A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, Rockville, v. 226, n. 1, p. 497-509, 1957.

GIUSTI, J.; CASTAN, E.; PAI, M. D.; ARRIGONI, M. B.; BALDIN, S. R.; OLIVEIRA, H. N. Expression of

genes related to quality of *Longissimus lumborum* muscle meat in Nellore (Bos indicus) and Canchim (5/8 Bos taurus ×3/8 Bos indicus) cattle. *Meat Science*, Cowra, v. 94, n. 2, p. 247-252, 2013.

INSTITUTO BRASILEIRO DE GEOGRAFA E ESTATÍSTICA - IBGE. Pesquisa Nacional de Saúde (PNS). Percepção do estado de saúde, estilos de vida e doenças crônicas. Rio de Janeiro: IBGE, 2013.

KANNAN, G.; GADIYARAM, K. M.; GALIPALLI, S.; CARMICHAEL, A.; KOUAKOU, B.; PRINGLE, T. D.; MCMILLINC, K. W.; GELAYEA, S. Meat quality in goats as influenced by dietary protein and energy levels, and post-mortem aging. *Small Ruminant Research*, Oxford, v. 61, n. 1, p. 45-52, 2006.

MADRUGA, M. S. Carne caprina: verdades e mitos à luz da ciência. *Revista Nacional da Carne*, Rio de Janeiro, v. 264, n. 23, p. 34-40, 1999.

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO - MAPA. Projeções do Agronegócio - Brasil 2010/11 a 2020/21. Brasília: MAPA, 2012.

NATIONAL RESEARCH COUNCIL - NRC. Nutrient requirements of small ruminants. Washington: National Academy Press, 2007. 362 p.

O'KEEFE, P. W.; WELLINGTON, G. H.; MATTICK, L. R.; STOUFFER, J. R. Composition of bovine muscle lipids at various carcass locations. *Journal of Food Science*, North Carolina, v. 33, n. 2, p. 188-192, 1968.

OLIVEIRA, C. H. A.; SILVA, A. M.; SILVA, L. M.; VAN TILBURG, M. F.; FERNANDES, C. C. L.; MOURA, A. A.; MORENO, F. B. M. B.; MONTEIRO-MOREIRA, A. C. O.; MOREIRA, R. A.; BEZERRA, F. J.; RONDINA, D. Meat quality assessment from young goats fed for long periods with castor de-oiled cake. *Meat Science*, Cowra, v. 106, p. 16-24, 2015.

OLIVEIRA, C. H. A.; SILVA, L. M.; RODRIGUES, F. V.; SILVA, A. M.; FERNANDES, C. C. L.; GOES, K. L. S.; DUARTE, S. S.; RODRIGUES, F. V.; BERTOLINI, L. R.; BEZERRA, F. J.; GOMES FILHO, M. A.; RONDINA, D. Anatomic and tissue characteristics in goats fed for extended periods with residue of castor biodiesel production. *Semina: Ciências Agrárias*, Londrina, v. 34, n. 6, p. 2865-2878, 2013.

PAIM, R. T. T.; BENJAMIN, S. R.; RONDINA, D.; MARQUES, M. M. M.; VIANA, D. A.; GONZAGA, M. L. C.; VIEIRA, I. G. P.; MENDES, F. N. P.; GUEDES, M. I. F. Antihypercholesterolemic effects of fruit aqueous extract of *Copernicia prunifera* (Miller) H. E. moore in mice diet-induced hypercholesterolemia. *Evidence-Based Complementary and Alternative Medicine*, Berlin, v. 2017, p. 1-15, 2017.

PINHEIRO, R. S. B.; SOBRINHO, A. G. S.; SOUZA, H. B. A.; YAMAMOTO, S. M. Qualidade de carnes provenientes de cortes da carcaça de cordeiros e de ovinos adultos. *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 38, n. 9, p. 1790-1796, 2009.

QWELE, K.; HUGO, A.; OYEDEMI, S. O.; MOYO, B.; MASIKA, P. J.; MUCHENJE, V. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (Moringa oleifera) leaves sunflower cake and grass hay. *Meat Science*, Cowra, v. 93, n. 3, p. 455-462, 2013.

REARDON, W.; MULLEN, A. M.; SWEENEY, T.; HAMILL, R. M. Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. *Meat Science*, Cowra, v. 86, n. 2, p. 270-275, 2010.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; PÉREZ-JIMÉNEZ, J.; SAURACALIXTO, F.; MANCINI-FILHO, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, Norwich, v. 121, n. 4, p. 996-1002, 2010.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; SILVEIRA, M. R. S.; MOURA, C. F. H. Quality for fresh consumption and processing of some non-traditional tropical fruits from Brazil. *Fruits*, Cambridge, v. 64, n. 6, p. 361-370, 2009.

SEVANE, N.; ARMSTRONG, E.; CORTÉS, O.; WIENER, P.; PONG WONG, R.; DUNNER, S.; CONSORTIUM, G. Association of bovine meat quality traits with genes included in the PPARG and PPARGC1A networks. *Meat Science*, Cowra, v. 94, n. 3, p. 328-335, 2013.

SILVA, A. M.; OLIVEIRA, C. H. A.; FERNANDES, C. C. L.; SILVA, L. M.; NUNES-PINHEIRO, D. C.; FERNANDES, A. A. O.; LIMA, I. M. T.; SILVA, C. M. G.; RONDINA, D. Post-partum reproductive activity and estrus synchronization responsiveness in anglonubian x sprd fed with dried carnauba wax palm fruit (*Copernicia prunifera*) long term. *Semina: Ciências Agrárias*, Londrina, v. 36, n. 4, p. 2619-2632, 2015.

SILVA, C. G.; HERDEIRO, R. S.; MATHIAS, C. J.; PANEK, A. D.; SILVEIRA, C. S.; RODRIGUES, V. P.; RENNÓ, M. N.; FALCÃO, D. Q.; CERQUEIRA, D. M.; MINTO, A. B. M.; NOGUEIRA, F. L. P.; QUARESMA, C. H.; SILVA, J. F. M.; MENEZES, F. S.; ELEUTHERIO, E. C. A. Evaluation of antioxidant activity of Brazilian plants. *Pharmacological Research*, Milano, v. 52, n. 3, p. 229-233, 2005.

SMITH, M. C.; SHERMAN, D. M. *Goat medicine.* 2th ed. Iowa: Wiley-Blackwell, 2009. 871 p.

SNIFFEN, C. J.; O'CONNOR, J. D.; VAN SOEST, P. J.; FOX, D. G.; RUSSEL, J. D. A net carbohydrate and protein system for evaluation catlle diets. II. Carbohydrate and protein availability. *Journal of Animal Science*, v. 70, n. 11, p. 3562-3577, 1992.

SU, R.; SUN, W.; LI, D.; WANG, Q. Z.; LV, X. Y.; MUSA, H. H.; CHEN, L.; ZHANG, Y. F.; WU, W. Z. Association between DLK1 and IGF-I gene expression and meat quality in sheep. *Genetics and Molecular Research*, Ribeirão Preto, v. 13, n. 4, p. 10308-10319, 2014.

SZEWCZUK, M.; ZYCH, S.; WÓJCIK, J.; CZERNIAWSKA-PIĄTKOWSKA, E. Association of two SNPs in the coding region of the insulin-like growth factor 1 receptor (IGF1R) gene with growth-related traits in Angus cattle. *Journal of Applied Genetics*, Poznan, v. 54, n. 3, p. 305-308, 2013.

TANIGUCHI, M.; UTSUGI, T.; OYAMA, K.; MANNEN, H.; KOBAYASHI, M.; TANABE, Y.; OGINO, A.; TSUJI, S. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, v. 15, n. 2, p. 142-148, 2004.

VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. A. Methods for dietary fiber, neutral detergent fiber, and non starch polyssacarides in relation to animal nutrition. *Journal of Dairy Science*, Champaign, v. 74, n. 10, p. 3583-3597, 1991.

WANG, D.; ZHOU, L.; ZHOU, H.; HOU, G.; SHI, L.; LI, M.; HUANG, X.; GUAN, S. Effects of nutritional level of concentrate-based diets on meat quality and expression levels of genes related to meat quality in Hainan black goats. *Animal Science Journal*, Sendai, v. 86, n. 2, p. 166-173, 2015.