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Physicochemical and sensory characteristics of meat from lambs fed diets containing mulberry hay

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ABSTRACT

The aim of this study was to evaluate the physicochemical and sensory characteristics of the of meat lamb fed diets containing 0%, 12.5%, and 25.0% mulberry hay (total diet). Twenty-four Île-de-France lambs in the feedlot at an average age of 60 days and 15 kg body weight were allocated to individual stalls and slaughtered at 32 kg BW. Mulberry hay inclusion in the diet did not affect the meat pH 45 min (6.45) and 24 h (5.70) after slaughter. Average luminosity (L*), red intensity (a*), and yellow intensity (b*) values in the meat 24 h after slaughter were 40.85, 14.51, and 4.17, respectively. No differences were observed for water-holding capacity (58.03%), cooking loss (41.95%), shear force (2.80 kgf/cm²), sarcomere length (1.60 µm), or the sensory traits appearance (7.29), taste (7.23), tenderness (7.51), and overall acceptance (7.48). Inclusion of up to 25% mulberry hay in a concentrate-based diet did not compromise the physicochemical and sensory traits of the meat, indicating that this ingredient is as a good alternative to provide nutrients in the diet of feedlot lambs.

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Introduction

The last decade was characterised by important changes in the feeding habits of meat consumers. The search for healthier foods and the higher demands in terms of product quality has led part of the market niche to consume meats of better nutritional and sensory quality (Costa et al. 2008). The sheep meat is one of the available options for consumers which are willing to pay for a high-quality product; however, it fails in gaining market space due to the lack of standardisation and quality when it reaches the consumer.

Feedlotting is an alternative for finishing lambs where the animals reach the adequate conformation and fat cover for slaughter in less time, providing high-quality standardised carcasses. In this rearing model, one of the obstacles are the expenses with feed, which often greatly elevate the costs of a production system. Conventional sources of protein and

energy in concentrates, such as soybean meal and corn grain, greatly increase the feeding costs, and thus alternative sources are an interesting option to the production chain as long as they do not compromise the animal performance or the end product's quality.

The use of mulberry in ruminant feeding has been evaluated in several studies (Liu et al. 2001; Okamoto et al. 2008; Vu et al. 2011). However, most of them address the animal performance without approaching the qualitative aspects of sheep meat, which is a necessity, considering the marked influence of the diet on these traits (Madruga et al. 2008; Moreno et al. 2015; Campos et al. 2017). Mulberry (*Morus sp.*) is used as a forage in ruminant feeding because of its grass-related characteristics such as green mass production of 25–30 t/ha/y; high acceptability; crude protein content of 18–28%; total digestible nutrients content of 76%; dry matter digestibility of 75–85%; and high

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percentages of omega-3 fatty acids (50.43%) (Sanchez 2002; Ba et al. 2005; Cirne 2013). All of these positive aspects define this plant as an important alternative feedstuff.

Given the importance of nutrition on the production and general aspects of the meat, research should be carried out to investigate the use of alternative feedstuffs that do not change the features indicative of sheep meat quality. In this regard, the present study was conducted to evaluate the physicochemical and sensory traits of the meat from lambs fed diets containing mulberry hay.

Materials and methods

Study location

Experimental procedures complied with the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee in Animal Use (CEUA) of the Faculty of Agricultural and Veterinary Sciences (FCAV) at Unesp (no. 014105/11).

Animal housing, feeding, and experimental materials

The experiment was developed in the Sheep Farming Unit at the Department of Animal Science at FCAV - Unesp, Jaboticabal Campus, SP, Brazil, starting in May 2011. Twenty-four newly weaned Île-de-France lambs at approximately 60 days of age and an initial body weight of 15.48 ± 0.07 kg were housed in individual stalls measuring approximately 1.0 m^2 , with slatted suspended floor, equipped with individual feeders and drinkers. The stalls were located in a covered shed, where the animals remained approximately 66 days. At the beginning of the experiment, lambs were identified, dewormed, vaccinated with a polyvalent vaccine against Clostridial diseases, supplemented with vitamins A, D, and E, and distributed at random at eight animals per treatment.

Experimental diets were formulated so as to meet the requirements recommended by the NRC (2007) for weaned lambs with an average daily weight gain of 300 g. Treatments (T; diets) were composed as follows: T1 – sugarcane + concentrate without mulberry hay; T2 – sugarcane + concentrate with 12.5% mulberry hay; and T3 – sugarcane + concentrate with 25.0% mulberry hay. The sugarcane adopted was forage variety IAC 86-2480, which was chopped to a particle size of 1.0 cm and provided fresh. Mulberry branches from the silkworm facility in the Sericulture Section of

FCAV – Unesp were cut at 60 days of regrowth and later dried in the sun until reaching the hay point below 20% moisture (Costa and Resende 2006). After hayed, this material was ground using a 0.8-mm-screen sieve to facilitate the uniformity of ingredients and concentrates and prevent selectivity by the animals. The ground corn and soybean meal were mixed with the ground mulberry hay and stored in drums with the respective identification of the treatments; upon being provided to the animals, they were previously mixed with the ground sugarcane. The total diet had a concentrate (with or without mulberry hay):sugarcane ratio of 50:50. The chemical composition of the ingredients is described in Table 1, and the centesimal composition of the ingredients and chemical composition of the diets are given in Table 2.

Feed was supplied at 7 h and 17 h, allowing for at least 10% asorts. The weight of supplied feed and asorts were recorded daily to determine the DM intake. Lambs were weighed every 14 days, on which occasion haemonchosis was monitored by the Famacha® method (Van Wyk and Bath 2002). Upon reaching a body weight (BW) of 32.20 ± 0.49 kg, the animals were weighed at an appropriate location at the very Sheep Farming Unit of the University, deprived of solid feeds for 16 h, and then stunned by a 220-V electric shock held for 2 s, followed by bleeding by sectioning the jugular and carotid vessels. After skinning, evisceration, and removal of head and limbs, measured the pH ($\text{pH}_{45\text{min}}$) and the temperature ($T_{45\text{min}}$, in °C) were measured in triplicate in different points of the *Longissimus lumborum* muscle (left side) using a TESTO 205 digital pH metre coupled to a penetration electrode. The average of the triplicate corresponded to each sample per animal.

Carcasses were transferred to a cold room in the Sheep Farming Unit, at 6°C, where they remained over 24 h hung by the tendons of the *Gastrocnemius* muscle on appropriate hooks, spaced 17 cm apart. At the end of this period, the pH ($\text{pH}_{24\text{h}}$) and temperature ($T_{24\text{h}}$, in °C) were measured in triplicate at distinct points in the same muscle. Meat colour ($\text{colour}_{24\text{h}}$) was evaluated using a Minolta CR-200 colorimeter operating in the CIE (L^* , a^* , and b^*) system, in which L^* represented lightness; a^* the intensity of the colour red; and b^* the intensity of the colour yellow. The colorimeter was calibrated to a white standard. Before analysis, samples were exposed to room temperature for 30 min for the formation of oxymyoglobin, the main pigment responsible for the meat bright red colour (Cañeque and Sañudo 2000). After this time, and as described by Miltenburg et al. (1992), the L^* , a^* , and b^* coordinates were measured in three distinct points

Table 1. Chemical composition of the ingredients in the experimental diets.

Nutrient	Sugarcane	Mulberry hay	Ground corn	Soybean meal
Dry matter	27.12	89.36	89.12	89.05
Organic matter ^a	97.70	89.45	97.79	92.37
Mineral matter ^a	2.30	10.55	2.21	7.63
Crude protein ^a	2.92	20.92	14.26	44.28
Ether extract ^a	.38	2.64	6.75	1.74
Lignin ^a	2.28	2.69	1.40	2.40
Neutral detergent fiber ^a	33.72	21.99	17.75	12.42
Acid detergent fiber ^a	22.75	15.47	8.95	12.53
Total carbohydrates ^a	92.80	65.89	76.78	46.35
Non-fibrous carbohydrates ^a	59.08	43.90	59.03	33.93
Gross energy, Mcal/kg DM	4.29	4.54	4.65	4.73

^aPercentage of DM.

of the internal muscle surface, and the average of the triplicates of each coordinate was calculated per animal sample.

Carcasses were subsequently divided lengthwise into two half carcasses. The left half was sectioned into five anatomical regions: neck, shoulder, ribs, loin, and leg, as described by Silva Sobrinho (2001). The *Longissimus lumborum* muscles, according to the nomenclature proposed by Schaller (1999), were identified individually, vacuum-packed, and stored at -18°C until the beginning of analyses. In the preparation of the samples for analyses, the loins were thawed inside plastic bags in a B.O.D. incubator at 10°C for 12 h and dissected using a scalpel and a knife. Samples were extracted from the *Longissimus lumborum* muscle to determine the water-holding capacity, cooking loss, shear force, and sarcomere length and for sensory analysis.

Analytical procedures

For the determination of the water-holding capacity, we adopted the methodology described by Hamm, cited by Silva Sobrinho (1999), according to which meat samples weighing 500 ± 20 mg were placed in the transverse direction of the fibres onto filter paper between two acrylic plates, over which a 10-kg weight was placed for 5 min. Subsequently, the samples were weighed, and the lost water was calculated as the difference. The result was expressed as a percentage of exuded water in relation to the initial weight of the sample.

To determine the cooking losses, the samples were weighed and cooked in an industrial oven preheated to 170°C until the internal temperature of the samples reached 75°C , which was measured with an Incoterm 6132 stick thermometer; thereafter, they were removed from the oven and weighed again for the calculation in percentage terms. Next, to determine the shear force, the eight cooked samples from each treatment were

Table 2. Centesimal composition of ingredients and chemical composition of experimental diets.

	Mulberry hay, %		
	0	12.5	25.0
Ingredient, % DM			
Sugarcane	50.00	50.00	50.00
Mulberry hay	–	12.50	25.00
Soybean meal	28.49	24.60	21.33
Ground corn	17.80	9.00	.00
Soybean oil	1.00	1.00	1.00
Urea	.80	.80	.80
Mineral–vitamin supplement ^a	.50	.50	.50
Calcitic limestone	.47	.43	.25
Dicalcium phosphate	.94	1.17	1.12
Chemical composition			
Dry matter	58.44	56.94	57.18
Organic matter ^b	93.63	92.56	91.92
Mineral matter ^b	4.17	4.99	5.87
Crude protein ^b	18.88	18.51	18.40
Ether extract ^b	2.88	2.55	2.21
Lignin ^b	2.07	2.19	2.32
Neutral detergent fiber ^b	23.56	24.26	25.01
Acid detergent fiber ^b	16.54	17.20	17.92
Total carbohydrates ^b	73.27	72.95	72.76
Non-fibrous carbohydrates ^b	49.71	48.68	47.75
Metabolisable energy, Mcal/kg DM	2.99	2.95	2.55

^aProvides per kg: calcium 120 g, chlorine 90 g, cobalt 10 mg, copper 50 mg, sulphur 34 g, iron 1064 mg, phosphorus 50 g, iodine 25 mg, magnesium 54 g, manganese 1500 mg, selenium 20 mg, sodium 62 g, zinc 1600 mg, fluorine (max) 0.73 g, vitamin A 100,000 U, vitamin D₃ 40,000 U, and vitamin E 600 U.

^bPercentage of DM.

cut into three $1.5\text{-cm} \times 1.5\text{-cm}$ cubes to calculate the area in square metres. These cubes were then cut in the transverse direction of the muscle fibres at a speed of approximately 3.3 mm/s by a texture analyser (Brookfield engineering) coupled to a Warner–Bratzler blade. Values were expressed as kgf/cm^2 (Ramos and Gomide 2012), with the average of each evaluated triplicate corresponding to each sample per animal.

Five-gram samples of the *Longissimus lumborum* muscle were obtained to measure the sarcomere length. These samples were placed in wide-mouth plastic jars containing 10% buffered formalin (50 mL). After fixation, the samples were cleaved, dehydrated, cleared, paraffin-embedded, and sectioned in an $8\text{-}\mu\text{m}$ (Pika-Seiko). Histological cuts were stained with

Mallory's phosphotungstic acid-haematoxylin, following the technique recommended by Behmer et al. (1976). Slides were read using an Olympus BX51 microscope coupled to an Olympus DP72 camera; the method was based on the count of 10 sarcomeres of five different myofibrils, with the average of the five evaluated myofibrils corresponding to each sample per animal. Morphometric analyses of sarcomere length were performed using the ImageJ software.

Sensory evaluation

In the sensory analysis, the samples of the *Longissimus lumborum* muscle were roasted in an oven at a temperature of 175 °C until their geometric centre reached 75 °C, as measured by a digital thermometer. After chilling, the samples were cut into cubes measuring approximately 4 cm² (2 × 2 cm²), wrapped in aluminium foil, and kept in a water bath at a temperature of approximately 60 °C until being served to a panel of 30 consumers. The consumers evaluated three samples corresponding to the three treatments in individual booths equipped with a red light to avoid possible influences on the appearance of the samples, which were served in plastic containers coded with three random digits. Along with the sample, the tasters were offered water (to sip in between the tasting of each sample) and a spreadsheet in which they assessed the attributes colour, flavour, tenderness, and overall acceptance on a nine-point hedonic scale that consisted of: (1) dislike extremely, (2) dislike very much, (3) dislike moderately, (4) dislike slightly, (5) neither like nor dislike, (6) like slightly, (7) like moderately, (8) like very much, and (9) like extremely (Moraes 1993; Nassu 2009).

Statistical analysis

A completely randomised design with three treatments and eight replicates was adopted, totalling 24 experimental units. However, for the sensory analysis, a randomised block design with three treatments and 30 replicates was adopted. Results were evaluated by analyses of variance and regression, with the degrees of freedom decomposed into linear or quadratic effects, according to the percentages of mulberry hay. The significance of regressions was obtained by the *F*-test at the 1% or 5% probability levels, using Sisvar and AgroEstat Software (Ferreira 2011; Barbosa and Maldonado 2012).

Results and discussion

The temperature and pH of the *Longissimus lumborum* muscle in feedlot lambs assessed at 45 min and 24 h

Table 3. Temperature (T), pH, and colour of the *Longissimus lumborum* muscle of lambs fed diets containing mulberry hay.

Item	Mulberry hay, %			<i>p</i> Value ^a		CV, % ^b
	0	12.5	25.0	L	Q	
<i>T</i> (45 _{min} , °C)	36.12	36.11	35.74	0.684	0.824	4.29
<i>T</i> (24 _h , °C)	7.37	8.55	8.28	0.219	0.254	14.78
pH (45 _{min})	6.45	6.50	6.42	0.785	0.543	2.81
pH (24 _h)	5.68	5.76	5.66	0.824	0.288	2.96
Colour (24 _h)						
<i>L</i> [*]	40.12	39.99	42.46	0.214	0.415	6.48
<i>a</i> [*]	14.42	14.66	14.47	0.967	0.857	13.76
<i>b</i> [*]	4.17	4.20	4.13	0.919	0.883	26.64

^aEffect: L: linear; Q: quadratic.

^bCV: coefficient of variation.

after slaughter were not influenced ($p > .05$) by the percentages of mulberry hay (Table 3). The numerically observed decline of pH from 6.45 to 5.70 at 24 h after slaughter respectively, are near the range considered normal for sheep meat, which, as stated by Sañudo et al. (1992), is from 6.56 to 6.69 at 45 min and from 5.50 to 5.80 for 24 h post-slaughter, indicating normal development of rigour mortis and inexistence of pre-slaughter stress (Lawrie 2005; Della Malva et al. 2016).

The process of transformation of muscle into meat is related to several factors, one of which is temperature (Aberle et al. 2012). From the values of these parameters, we could infer about the end-product quality, because the final pH and the temperature of the carcass during chilling are correlated with the meat physical and organoleptic properties (Bonagurio et al. 2003). Because the pH was not changed ($p > .05$) by the inclusion of mulberry hay in the animal diets and the results remained satisfactory for normal meats, the present values for colour, water-holding capacity, cooking loss, tenderness, and sensory attributes are considered acceptable.

Madrugá et al. (2008) assessed the inclusion (16.7, 33.3, and 50%) of silk-flower hay (*Calotropis procera* Sw) in the diet of feedlot-finished Santa Inês lambs and observed changes ($p < .05$) in the pH measured in the *Longissimus dorsi* muscle, which was 6.20 at 24 h after slaughter in the treatment with 50% inclusion. The pH values reported in the current study (Table 3) are consistent with the 6.27 observed by Moreno et al. (2015) at 45 min and 5.45 at 24 h after slaughter, measured in the *Longissimus lumborum* of Santa Inês lambs fed diets containing 30%, 40%, 50%, and 60% saltbush hay. The present values also agree with Endo et al. (2014) and Leão et al. (2012), who obtained 6.65 and 6.58 for pH at 45 min and 5.69 and 5.60 for pH at 24 h, respectively, after slaughter, in the *Longissimus lumborum* of feedlot-finished Île-de-France lambs fed diets with sugarcane and concentrate,

suggesting that mulberry hay is an alternative source of nutrients in the diet of feedlot lambs.

The meat colour is the qualitative trait that most influences the choice of the consumer, arousing their interest to purchase or reject the product (Ramos and Gomide 2012). Regardless of the percentage inclusion of mulberry hay in the diet, the coordinates that indicate the lightness (L^*), redness (a^*), and yellowness (b^*) of meat 24 h after slaughter did not differ ($p > .05$), with respective values of 40.86, 14.52, and 4.17. According to Sañudo et al. (2000), Warris (2003), and Souza et al. (2004), sheep meat usually presents L^* values from 30.03 to 49.47, a^* from 8.24 to 23.53, and b^* from 3.38 to 11.10. The values found in this study are thus similar to those described in the literature. Costa et al. (2011) observed no changes in meat colour using percentages (15, 30, and 45%) of silk-flower hay replacing the concentrate in the diet of Morada Nova lambs finished in the feedlot. Similarly, Moreno et al. (2015) reported no changes in meat colour when evaluating saltbush hay (*Atriplex nummularia*) in finishing diets for Santa Ines lambs in the feedlot.

Water-holding capacity (WHC), cooking loss (CL), shear force (SF), and sarcomere length (SL) (Table 4) were also not changed ($p > .05$) by the inclusion of mulberry hay in the diet. The similarity between results is likely related to the adequate development of rigour mortis and the pH decline in the carcasses after slaughter.

Water-holding capacity has a marked influence on the development and appreciation of sensory traits and on the nutritional and commercial value of the meat (Ordóñez 2005), in addition to determining its juiciness (Sañudo et al. 1992). Cooking losses occur during the process of preparation of the meat for consumption, as the cell membrane is ruptured and alterations in the structure of proteins are responsible for these losses (Moreno 2011). These losses are a measure of quality associated with the yield of the meat at consumption, influenced mainly by WHC, with higher WHC leading to lower CL.

The WHC, CL, and SF results of 58.03%, 41.95%, and 2.59 kgf/cm², respectively, were close to those reported by Zeola et al. (2011) (55.27%, 44.23%, and 2.74 kgf/cm²), Endo et al. (2014) (57.08%, 43.51%, and 3.12 kgf/cm²), and Moreno et al. (2015) (60.99%, 38.04%, and 2.58 kgf/cm²). Based on the SF results, the meat from the lambs of this study can be considered tender, since, according to Tatum et al. (2000), *Longissimus* muscle in sheep presenting a shear force of up to 5 kgf/cm² is considered so. The low values for shear force may be related to the fact that animals

Table 4. Water-holding capacity (WHC), cooking loss (CL), shear force (SF), and sarcomere length (SL) in the *Longissimus lumborum* muscle of lambs fed diets containing mulberry hay.

Item	Mulberry hay, %			p Value ^a		CV, % ^b
	0	12.5	25.0	L	Q	
WHC, %	57.61	60.45	56.05	0.550	0.124	7.38
CL, %	42.38	39.55	43.94	0.550	0.124	10.16
SF, kgf/cm ²	2.57	2.43	2.76	0.768	0.669	32.10
CS, μ m	1.58	1.57	1.66	0.087	0.178	3.36

^aEffect: L: linear; Q: quadratic.

^bCV: coefficient of variation.

Table 5. Sensory analysis of the *Longissimus lumborum* muscle of lambs fed diets containing mulberry hay.

Item	Mulberry hay, %			p Value ^a		CV, % ^b
	0	12.5	25.0	L	Q	
Appearance	7.06	7.36	7.46	0.060	0.583	11.09
Flavour	7.23	7.26	7.20	0.908	0.843	15.53
Tenderness	7.60	7.63	7.30	0.278	0.442	14.12
Overall acceptance	7.30	7.63	7.53	0.210	0.180	9.53

^aEffect: L: linear; Q: quadratic.

^bCV: coefficient of variation.

were slaughtered young, at approximately 130 days of age, and finished in the feedlot.

The sarcomere length of 1.60 mM observed in this study (Table 4) was close to the 1.72 mM mentioned by Borghi et al. (2016), who evaluated the *Longissimus lumborum* of feedlot-finished Île-de-France lambs; to the 1.70 mM reported by Oliveira et al. (2004) with Santa Inês lambs and sheep; and to the 1.67 mM observed by Silva Sobrinho et al. (2005) in Romney $\times$ East Friesian $\times$ Poll Dorset lambs. Sarcomeres are the myofibril units responsible for the muscular contraction. The results obtained in the present study for meat SF (Table 4) are in line with the positive correlation between meat tenderness and the length of the sarcomeres reported by other authors (Veiseth et al. 2004). This explains the results observed for SF, since, as stated by Wheeler et al. (2000), lower sarcomere lengths mean tougher meats.

No differences were observed ($p > .05$) between the treatments for the scores assigned to the sensory traits appearance (7.29), flavour (7.23), tenderness (7.51), and overall acceptance (7.48) of the meats (Table 5). These results on meat sensory evaluation reflect the absence of effects of the dietary treatments on muscle pH, colour, WHC, CL, and SF, which are quality parameters closely related to the meat sensory attributes.

Contrarily to what it was observed in the present study, several authors have reported that the inclusion of alternative forage sources in the diets of lambs may influence meat sensory quality (Madruga et al. 2005; Costa et al. 2011; Moreno et al. 2015). Madruga et al. (2005) observed differences in the meat from Santa

Ines lambs fed diets containing four different types of forages; Costa et al. (2011) reported the influence of silk flower hay and Moreno et al. (2015) reported the effect of saltbush hay on meat sensory quality of Santa Ines lambs. Scores assigned by the judges on a scale of 1 (dislike extremely) to 9 (like extremely) indicate that meat from lambs fed diets containing mulberry hay had good acceptability, what supports the potential use of this alternative source of nutrients in lamb's feedlot diet without modifying the meat sensory attributes.

Conclusions

Inclusion of mulberry hay at up to 25% in the total diet did not affect the meat physicochemical and sensory characteristics, allowing the incorporation of this ingredient as an alternative forage source in diets of feedlot lambs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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