Evaluation of carcass and muscle traits in Santa Ines female lambs finished with different agricultural products

[ Avaliação de características da carcaça e do músculo de cordeiras Santa Inês terminadas com diferentes produtos agrícolas]

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ABSTRACT

The aim of this study was to evaluate the effect of different agricultural products on quantitative aspects of carcass, body constituents, cooking loss, shear force and colorimetry of the Longissimus lumborum and Triceps brachii muscles in Santa Ines lambs. 24 Santa Ines female lambs received one of four diets which were isoproteic and isoenergetic with fixed levels of forage (60%) and concentrate (40%) of corn and soybean meal during 45 days. The forages per diet differed: coast-cross hay (HAY), cassava hay (CASS), dehydrated by-product of pea crop (PEA) and sugarcane (SC). The average weight of the lambs at the beginning of the experiment was 26.35kg. Animals were slaughtered in a federally certified abattoir. Initial and final pH, cooking losses, color using the CIELAB system, shear force and the quantity of sarcomeres per 100μm were measured. Hot carcass, cold and half carcass weights were affected by treatments (P<0.05). The sarcomere length of Triceps brachii muscle 24 hours after slaughter differed between diets and coast-cross hay had the lowest value. The sarcomere length differed significantly between diets and the dehydrated by-product of pea crop had the lowest number of sarcomeres immediately after slaughter compared to other diets. There was no influence of diet on colorimetry, cooking loss and shear force. The decrease in pH followed the development of the process of rigour mortis in the Longissimus lumborum and Triceps brachii muscles in the first hour and up to 24 hours after slaughter. Diets did not alter the pH, water holding capacity, colorimetry or shear force. The pea by-product and sugarcane can replace traditional sources of fodder without depreciation of meat characteristics.

Keywords: forage, Longissimus lumborum, quality, rigour mortis, Triceps brachii

RESUMO

O objetivo deste estudo foi avaliar o efeito de diferentes produtos agrícolas sobre aspectos quantitativos da carcaça, constituintes do corpo, perda por coção, força de cisalhamento e colorimetria dos músculos Longissimus lumborum e Triceps brachii em cordeiras Santa Inês. Vinte e quatro cordeiras Santa Inês receberam uma das quatro dietas, que foram isoproteicas e isoenergéticas, com níveis fixos de forragem (60%) e concentrado (40%) composto por milho e farelo de soja, durante 45 dias. As forragens diferiram entre as dietas: feno de coast cross (FENO), feno da parte aérea da mandioca (MAN), subproduto desidratado da cultura da ervilha (ERV) e cana-de-açúcar (CANA). O peso médio das cordeiras no início do experimento foi de 26,35kg. Os animais foram abatidos em frigorífico com inspeção sanitária federal. Foram avaliados o pH inicial e o final, as perdas por coção, a cor, por meio do sistema CIELAB, a força de cisalhamento e a quantidade de sarcômeros em 100μm. Os pesos de carcaça quente, de carcaça fria e de meia-carcaça foram afetados pelos tratamentos (P<0,05). O comprimento do sarcômero do músculo Triceps brachii 24 horas após o abate diferiu entre dietas e o feno coast cross apresentou o menor valor. O comprimento do sarcômero diferiu significativamente entre as dietas, e o subproduto da ervilha apresentou o menor número de sarcômeros imediatamente após o abate em comparação com as outras dietas. Não houve influência da dieta sobre as
INTRODUCTION

Farmers have become more aware of the importance of maximizing production efficiency with the goal of being able to produce high quality lean meat at the lowest possible cost. This includes producing meat with suitable characteristics for a healthy diet that is attractive to the consumer.

Since nutrition (mainly concentrate) is one of the major costs in lamb production (Paim et al., 2011), studies on animal feed have become high priority in sheep production systems (Peixoto et al., 2015). Therefore, the use of diets based on agricultural by-products can constitute an important alternative to reduce the production costs and increase efficiency and economy in sheep production (Inserra et al., 2014). It has also been shown that diet affects carcass weight, subcutaneous fat and other indicators of meat quality such as tenderness, flavour, colour and shelf-life (Ramírez-Retamal and Morales, 2014).

Colour, water-holding capacity and tenderness of meat are primary determinants of visual appeal and sensory acceptability (Hughes et al., 2014). Colour of the muscle is determined by the amount of myoglobin and the relative proportions of this pigment can be found in the reduced form as myoglobin (purple), oxymyoglobin (red) and metmyoglobin (brown colour) (Ramírez-Retamal and Morales, 2014). The proportion of these in the meat can determine its attractiveness to the consumer.

The water holding capacity on the other hand is a biophysical and chemical parameter which could be defined as the level of water in the actin-myosin muscle chain, that upon chewing translates into succulence (Osorio et al., 2009). Water holding capacity, weight loss during cooking, tenderness, colour and flavour may be influenced by final muscle pH (Hughes et al., 2014). The decrease in pH after death, caused by accumulation of lactic acid, resulting in changes in the transformation of muscle into meat, has important effects on the quality of meat and its products (Pearse et al., 2011).

The palatability of meat is based on three general criteria: tenderness, succulence and flavour. All these depend on the biochemical state of the muscle as it turns into meat. However, some studies have suggested that sarcomere length can also contribute to determining the tenderness and texture of meat, in particular by cold shortening due to the increased overlap between the thick and thin filaments (King et al., 2003). The functional units of the myofibril are sarcomeres and the outer sarcomere bands are called I band and contain actin filaments. The central band is a darker band “A”, whose ends are formed by actin and myosin. The lighter, H band, contains myosin. Post-mortem, when the muscle contracts, the I and H bands decrease in width, caused by the sliding of actin filaments over myosin, interfering with meat tenderness (Abrahão, 2007).

This study aimed to evaluate the effect of different agricultural by-products on the properties of carcass weight, cooking loss, shear force and colorimetry as well as the thickness of sarcomere of the Longissimus lumborum and Triceps brachii muscles of Santa Ines lambs.

MATERIAL AND METHODS

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the University of Brasilia, number 44568/2009.

Twenty-four six months old Santa Ines female lambs were used. The lambs were randomly divided into four groups of six animals each. The average weight of the lambs in the beginning of the experiment was 26.35±0.20 kilograms. They were housed in a shed with concrete floor, in individual pens (1.50m²) equipped with
individual feeders and water troughs. The animals were adapted to the diet for seven days before the beginning of the experiment that lasted 45 days.

The lambs were fed isonitrogenous and isocaloric diets, with fixed proportions of roughage (60%) and concentrate (40%). Concentrate was composed of corn and soybean as well as inorganic phosphate and the roughage differed between the experimental diets: HAY: coast-cross (Cynodon dactylon) hay; PEA: by-product of tillage (mulch) from pea (Pisums sativum); CASS: cassava (Manihot esculenta) foliage hay and SC: sugarcane (Saccharum spp.). Food and water were supplied twice a day, at the same time, with the diet balanced according to the National Research Council (NRC, 2007), calculated as 3% of the live weight of the animal.

The animals were weighed before and after being fasted for 12h to obtain final (FW) and fasting (FLW) live weights, respectively. Animals were slaughtered in a federally certified abattoir. They were desensitized, jugular veins cut and were bled out, and then skin, feet, head and internal organs were removed. Skin thickness (ST) was measured using calipers at the navel. The carcasses were weighed before (HCW) and after (CCW) chilling at 1º C for 24 hours and received a score for fat cover (FS) on a scale of 1 to 5, where 1 was very thin and 5 very fat. They were then sectioned longitudinally along the spinal cord to obtain half-carcass weight (HW). The pH (hydrogen point) was measured at slaughter (pHi) and 24 hours later (pHf) using a digital pH meter and the difference was calculated (DpH = pHi–pHf).

A 6.0cm long, 2.0cm wide and 1.0cm thick strip of the Longissimus lumborum and Triceps brachii muscles was collected from each carcass immediately after slaughter and after 24 hours of cooling. These samples were fixed, cleaved dehydrated, clarified and embedded in paraffin and sectioned with a thickness of five microns. Sections were stained with Gomori trichrome. The slides were analysed under optical microscope (Nikon microscope) with visible light using an immersion objective. With the aid of callipers, a 100μm of muscle fibre was measured and sarcomere characteristics were evaluated (Sloss and Kemp, 1978), including: sarcomere length of the Triceps brachii muscle at slaughter (T1), sarcomere length of the Triceps brachii muscle 24 hours after slaughter (T2), difference between T1 and T2 (DT), sarcomere length of the Longissimus lumborum muscle at the time of slaughter (L1), sarcomere length of the Longissimus lumborum muscle 24 hours after slaughter (L2) and difference between L1 and L2 (DL).

To calculate cooking losses, a gas oven was preheated to 170°C. Samples of raw meat of approximately 1cm³ were weighed and placed in trays and reweighed (SW). The samples remained in the oven until the thermocouple showed a temperature of 40°C. At this time, the sample was turned and remained in the oven until the internal temperature of the centre of the sample reached 70°C. The trays were removed from the oven and when cooled, they were weighed again (TW) to calculate the percentage of cooking loss (CL) by difference of weight.

The shear force (SFkg) was determined using the same samples used to determine loss of water during cooking. The samples were completely cooled and stored in bags in the refrigerator for 24 hours. After this procedure, they were cut with the aid of a stainless steel cutting cylinder and three cylinders of each sample were obtained. These were analysed in the transverse direction of muscle fibres with the aid of Warner Bratzler shear force instrument and the average was obtained (kgf/cm²).

Meat colour was assessed on the Longissimus lumborum with the aid of a Minolta colorimeter model Chrome®, which was calibrated to a standard white title and L*, a* and b* were determined, where (L*) is luminosity, (a*) is the red level and (b*) is the yellow content. Four assessments of colour were averaged for each sample.

The experimental design was completely randomized. Carcass weight, shear force, cooking loss, sarcomere length and colorimetry were analyzed using SAS version 9.3 (Statistical Analysis Institute, Cary, North Carolina) with the MIXED (variance analysis), CORR (correlation) and PRINCOMP (principal components) procedures. Averages were compared by Duncan test with a significance level of 5%. Traits with high coefficients of variation were transformed to the logarithmic scale.
RESULTS AND DISCUSSION

HCW, CCW and HW were influenced by the treatments (Table 1). HWC were higher for the PEA (14.36kg) and CASS (13.48kg) diets, compared to HAY (12.68kg) and SC (12.00kg) diets. Animals fed PEA and SC diets had heavier cold carcass (14.01 and 13.15kg, respectively) and half-carcass (7.25 and 6.68kg, respectively) weights compared to HAY and CASS diets (12.40 and 11.73; 6.41 and 5.91kg, respectively). This result may have been due to the slow rate of passage in the gastrointestinal tract of the CASS diet animals during the fasting period.

Table 1: Summary of analysis of variance in Santa Ines female lambs for carcass and meat characteristics in Santa Ines lambs fed different diets

<table>
<thead>
<tr>
<th>Traits</th>
<th>Treatments</th>
<th>R²</th>
<th>CV (%)</th>
<th>X</th>
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<td>PEA</td>
<td>CASS</td>
<td>SC</td>
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<tr>
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<td>27.20</td>
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<td>14.36a</td>
<td>13.48ab</td>
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<td>CCW</td>
<td>12.40bc</td>
<td>14.01a</td>
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<td>HW</td>
<td>6.41b</td>
<td>7.25a</td>
<td>5.91b</td>
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<td>43.33ab</td>
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<td>16.92</td>
<td>16.65</td>
<td>16.78</td>
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<tr>
<td>b*</td>
<td>7.46</td>
<td>8.56</td>
<td>8.08</td>
<td>8.01</td>
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</tbody>
</table>

Averages with different letters in a line differ according to Duncan test at 5% (P<0.05). HAY: coast-cross hay; PEA: by-product of pea crop; CASS: hay from cassava foliage; SC: sugarcane; IW: initial live weight; FW: final live weight; FLW: fasting live weight; HCW: hot carcass weight; CCW: cold carcass weight; HW: half carcass weight; pH: pH at slaughter; pHf: pH 24 hours after slaughter; SW: sample weight before cooking; SWC: sample weight after cooking; CL: cooking loss; SFkg: shear force in kgf/cm²; SFkgLog: log of shear force; T1: sarcomere length of Triceps brachii muscle at the time of slaughter; T2: sarcomere length of Triceps brachii muscle 24 hours after slaughter; DT: difference between T1 and T2; L1: sarcomere length of Longissimus lumborum muscle at the time of slaughter; L2: sarcomere length of the Longissimus lumborum muscle 24 hours after slaughter and DL: difference between L1 and L2.

Animals on CASS showed higher initial pH and SC showed higher final pH, but these were not significantly different between treatments. Oliveira et al. (2004), working with Santa Ines males and different cooling temperatures, found pHf values of 6.67 and 5.61 for the pHf in Longissimus lumborum but found no differences between young and adult animals. These authors
also reported the pH decline in the first 8 hours post-mortem and indicate that anaerobic glycolysis occurs faster at higher temperatures during the entire slaughtering and carcass cooling process due to the growth of microbes. Pre-slaughter stress in sheep results in a decrease of normal pH values (Ekiz et al., 2012).

The similarity of body weight associated with the similar slaughter ages probably contributed to the lack of differences between cooking loss (SW, SWC, CL), shear force (SF) and the colour of lamb meat (L*, a*, and b*) which were not influenced by the treatments. Colour is the indication of freshness and quality of meat that most influences the choice by the consumer (Gracia and Magistris, 2013). The average value found in this study for meat colour was considered attractive to the consumer and considered normal for sheep meat. Bressan et al. (2001), Bonagurio et al. (2003) and Dawson et al. (2002) found that the red intensity increases with the slaughter weight in sheep. Pinheiro et al. (2009) described that the flesh colour is influenced by the brightness and intensity of red, while the intensity of the yellow colour is the most significant for fat.

The quantity of sarcomeres (Fig. 1) in Triceps brachii muscle showed no treatment effect on T1. SC (46.00 units/100μm) had higher muscle contraction compared to HAY which had the lowest (38.66 units/100μm). This may be related to the deposition of carcass fat, and water retention may have occurred, as well as shortening of muscle fibers by cold. Pardi et al. (2007) observed that factors such as age at slaughter and the increasing number of cross-links of thermo-stable collagen, lower fat deposition in the carcass and also the low level of intramuscular fat flavour the faster cooling of muscle mass, causing a shortening of sarcomeres and hardening of the meat. Abrahão (2007) reported that in post-mortem, when the muscle contracts, the I and H bands decrease in width, which is caused by the sliding of actin filaments over myosin, interfering in meat tenderness.

Figure 1. Histological evaluation of Longissimus lumborum and Triceps brachii muscles of PEA treatment stained with Gomori trichrome, under optical microscopy (Nikon microscope) and visible light in immersion objective. A) L1: sarcomere length of Longissimus lumborum muscle at the time of slaughter; B) L2: sarcomere length of Longissimus lumborum muscle 24 hours after slaughter; C) T1: sarcomere length of Triceps brachii muscle at the time of slaughter and D) T2: sarcomere length of Triceps brachii muscle 24 hours after slaughter.
The sarcomere length L1 differed significantly between treatments and the PEA had the lowest number of sarcomeres compared to HAY, CASS and SC immediately after slaughter. The difference between treatments for DL was due to the difference found in L1. This relationship may be influenced by stress during transport and pre-slaughter and slaughter method, deposition of carcass fat, water retention, shortening of muscle fibres by cold and diet may have contributed to the difference found in Longissimus lumborum muscle sarcomeres, which did not happen with Triceps brachii.

The sarcomere length, the amount of connective tissue and proteolysis of myofibrillar proteins explain the variation among the different muscles analysed (Kooohmarai et al., 2002). Devine (2004) stated that slaughter using electric shock causes a rapid and controlled release of glycolysis induced by electrical stimulation, the pH decreases faster and rigor mortis occurs early, leading to cold shortening at low temperatures.

The DpH showed high correlations with pHi, and low and negative with pHf. This difference may be due to the decrease in the values of pHf (Table 2). The same was observed with DL and L1 (0.95), DL and L2 (-0.5), DT and T1 (0.83) and DT and T2 (-0.04). These correlations may be related to post-mortem factors. The characteristics that showed high and positive correlations were evaluated immediately after slaughter, and the characteristics that had low and negative correlations were measured 24 hours after slaughter and carcasses stored in a cold room leading to greater muscle contraction of L1 and T1.

Table 2. Correlations between carcass traits and meat in Santa Ines lambs fed different diets

| Trait | pH | pHi | DpH | FS | ST | SW | CL | SFkg | T1 | T2 | CST | CSST | CSL1 | CSL2 | DCSL | L* a* |
|-------|----|-----|-----|----|----|----|----|------|----|----|-----|-------|-------|------|------|------|------|
| pHf  | -0.11 -0.09 |     |     |    |    |    |    |      |    |    |     |       |       |      |      |      |      |
| DpHf | -0.14 -0.66 | -0.66 |     |    |    |    |    |      |    |    |     |       |       |      |      |      |      |
| FS   | 0.28 -0.27 | -0.27 -0.04 |     |    |    |    |    |      |    |    |     |       |       |      |      |      |      |
| ST   | 0.38 0.25 | 0.23 0.05 | 0.27 |     |    |    |    |      |    |    |     |       |       |      |      |      |      |
| SW   | 0.27 0.03 | 0.06 -0.01 | 0.05 0.23 |     |    |    |    |      |    |    |     |       |       |      |      |      |      |
| CL   | 0.23 0.04 | 0.02 0.02 | 0.07 0.20 | 1.00 |     |    |    |      |    |    |     |       |       |      |      |      |      |
| T1   | -0.38 -0.15 | -0.01 -0.12 | -0.03 -0.20 | -0.18 -0.18 | 0.08 0.06 | -0.16 |     |      |    |    |     |       |       |      |      |      |      |
| T2   | -0.15 -0.10 | -0.05 -0.04 | -0.14 -0.27 | -0.18 -0.18 | 0.01 0.04 | 0.53 |     |      |    |    |     |       |       |      |      |      |      |
| CST1 | -0.34 -0.21 | -0.02 -0.17 | 0.06 -0.06 | -0.09 -0.10 | 0.09 -0.22 | 0.83 0.04 |     |      |    |    |     |       |       |      |      |      |      |
| CST2 | 0.54 0.04 | 0.01 -0.03 | 0.04 0.23 | 0.11 0.12 | -0.09 0.16 | -0.97 -0.53 | -0.79 |     |    |    |     |       |       |      |      |      |      |
| DCSST| 0.32 0.12 | 0.17 0.04 | 0.10 0.15 | 0.32 0.21 | 0.21 0.20 | -0.03 -0.09 | -0.49 -0.09 | 0.08 0.51 |     |    |     |       |       |      |      |      |      |
| CST | 0.30 0.46 | 0.19 0.26 | -0.10 -0.08 | 0.10 -0.18 | 0.05 -0.28 | 0.54 0.40 | -0.90 0.56 | -0.43 |     |    |     |       |       |      |      |      |      |
| DCSL1| 0.24 0.40 | 0.30 0.24 | 0.14 0.14 | 0.04 -0.05 | -0.01 -0.20 | 0.14 0.14 | 0.15 0.25 | -0.23 0.10 | -0.33 |     |     |       |       |      |      |      |      |
| DCSL2| -0.02 -0.29 | 0.09 -0.27 | -0.20 -0.17 | -0.23 -0.27 | 0.25 -0.42 | 0.25 -0.09 | 0.26 -0.06 | -0.05 -0.27 | 0.21 0.26 | 0.24 | 0.25 |     |       |      |      |      |      |
| DL   | -0.24 0.01 | 0.26 -0.16 | 0.21 0.10 | 0.05 0.08 | -0.34 -0.07 | 0.17 0.05 | -0.24 0.25 | -0.01 0.28 | 0.95 -0.05 |     |      |     |       |      |      |      |      |
| CSL1 | 0.07 -0.51 | 0.24 -0.15 | -0.02 -0.02 | 0.02 0.00 | 0.20 -0.17 | 0.11 -0.14 | 0.22 -0.19 | 0.11 -0.11 | 0.59 -0.99 | -0.26 -0.05 | 0.24 | 0.25 |     |       |      |      |      |      |
| CSL2 | 0.02 0.30 | 0.06 0.27 | 0.19 0.16 | 0.23 -0.26 | 0.42 -0.25 | -0.06 -0.25 | -0.07 0.05 | 0.27 -0.21 | -0.25 -1.00 | 0.06 0.25 | 0.42 | 0.42 |     |       |      |      |      |      |
| DCST | 0.24 -0.05 | -0.29 0.13 | 0.23 0.08 | -0.07 -0.07 | 0.07 0.14 | -0.04 0.19 | -0.21 0.00 | 0.22 0.91 | 0.15 -0.25 | 0.99 -0.05 | 0.16 0.95 | 0.25 0.25 |     |       |      |      |      |      |

Animals: hot carcass weight; pHi: pH at the time of slaughter; pHf: pH 24 hours after slaughter; DpH: difference between pHi-pHf; HW: half carcass weight; FS: fat score measured on a scale of 1 to 5; ST: skin thickness; SW: sample weight before cooking; TW: sample weight after cooking; CL: cooking loss; SFkg: shear force in kgf/cm²; T1: sarcomere length of Triceps brachii muscle at the time of slaughter; T2: sarcomere length of Triceps brachii muscle 24 hours after slaughter; DT: difference between T1 and T2; L1: sarcomere length of the Longissimus lumborum muscle at the time of slaughter; DL: difference between T1 and L2; L*: luminosity; a*: red level; b*: yellow level; CST and CSL: the average length of a Triceps (T) and Longissimus (L) sarcomere; DCST and DCSL: difference between the average length of a Triceps (T) and Longissimus (L) sarcomere.

Autovector 1 explained 41% of the variance between traits (Fig. 2). An increase in pHi was accompanied by a decrease in pHf and vice versa. The rate of decrease in pH after slaughter, caused by accumulation of lactic acid, is one of the most significant factors in the transformation of muscle into meat and is important in meat quality (Pardi et al., 2007). Autovector 2 explained 24% of the variance analysed. In the 24 hours after slaughter there was a decrease in the number of Longissimus lumborum and Triceps brachii sarcomeres due to muscle contraction and relaxation that occurs in rigor mortis. The length of Longissimus lumborum
was higher immediately after slaughter. These data are consistent with the relaxation that occurs after actin separates from myosin.

The amount of Triceps brachii sarcomeres in the muscle 24 hours after slaughter was higher than in the first hour. This muscle has different characteristics than Longissimus lumborum. In these muscles, sarcomere length increased during the 15th to 30th hour post mortem (Abreu, 1984). T1 and T2 were evaluated in this study just 24 hours after slaughter.

Figure 2. First two principal components for carcass characteristics and meat in Santa Ines lambs fed with different diets.

HW: hot carcass weight; pH1: pH at the time of slaughter; pH2: pH 24 hours after slaughter; DpH: difference between pH1-pH2; HW: half carcass weight; FS: fat score measured on a scale of 1 to 5; ST: skin thickness; SW: sample weight before cooking; TW: sample weight after cooking; CL: cooking loss; SFkg: shear force in kgf/cm²; T1: sarcomere length of the Triceps brachii muscle at the time of slaughter; T2: sarcomere length of Triceps brachii muscle 24 hours after slaughter; DT: difference between T1 and T2; L1: sarcomere length of the Longissimus lumborum muscle at the time of slaughter; L2: sarcomere length of the Longissimus lumborum muscle 24 hours after slaughter; DL: difference between L1 and L2; L*: luminosity; a*: red level; b*: yellow level; CST and CSL: the average length of a Triceps (T) and Longissimus (L) sarcomere; DCST and DCSL: difference between the average length of a Triceps (T) and Longissimus (L) sarcomere.

CONCLUSIONS

The pea and sugarcane by-product can replace traditional sources of forage as an alternative source of food for the dry season without any reduction of meat quality traits. Maximum contraction during the process of rigor mortis occurred in both Longissimus lumborum and Triceps brachii muscles of Santa Ines lambs.

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