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# Different nutritional systems influence the tenderness and lipid oxidation of ewe lamb meat without altering gene expression

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**Abstract:** Feeding is a determining factor in the various characteristics of sheep meat and animal performance, the objectives were to evaluate the effect of supplementation of ewe lambs finished in different nutritional planes on the gene expression of CASP3, CAPN1, CAPN2 and CAST and its possible association with meat quality. Samples of the *Longissimus lumborum* muscle of 24 ewe lambs were used, distributed in 3 groups (n=8): P (pasture), PS (pasture and supplement) and F (feedlot). Physicochemical analyses were performed for centesimal analysis, pH, lipid oxidation, Warner-Bratzler shear force and RT-qPCR for the analysis of relative gene expression of the following genes: CASP3, CAPN1, CAPN2 and CAST. There is an increase in daily weight gain and ethereal extract values in the meat of confined animals, due to the greater energy intake in the nutrition of these animals. Animals kept only on pasture have lower lipid oxidation in meat than other treatments because of the lower percentage of lipids. The Warner-Bratzler shear force is considerably higher in the meat of animals kept only on pasture but is still considered tender. The different nutritional systems do not interfere with the gene expression of CASP3, CAPN1, CAPN2 and CAST in ewe lambs.

**Key words:** µ-Calpain, calpastatin, caspase-3, *Ovis aries*, RT-qPCR.

## INTRODUCTION

Sheep farming is performed on virtually all continents; the wide use of sheep is mainly a result of animal adaptability and the lack of ethnic or religious restrictions on the consumption of its various products (Garcia et al. 2000, Viana 2008).

Thus, for successful rearing, it is necessary that the systems of breeding and feeding enhance the meat quality, and animals fed higher proportions of certain nutrients have higher fat content, which in turn increases meat succulence and tenderness (Moreno et al. 2015).

Regarding the concept of different diets, Medeiros & Ferreira (2018) affirmed that food can promote direct changes in gene transcription, since nutritional factors may be responsible for activating genes. Thus, there is great interest in exploring dietary sources that can improve specific characteristics, such as meat quality.

The different nutritional compositions of the feeding systems used in lamb's breeding can influence the physiology and expression of various genes, this statement is since genes interact with each other in a way that reveals a more complex regulation of transcription, recovering functional information of the tissue analyzed at the molecular level (Flora et al. 2017, Hudson et al. 2012, Fuller et al. 2007).

The enzyme caspase-3 (CASP3) is responsible for the proteolytic cleavage of several proteins postmortem (Anwar et al. 2004), and the main enzymes involved in this process are calpains and their inhibitor, calpastatin. Calpains degrade myofibrillar proteins at certain internal points of the molecules according to the extracellular calcium reserve, improve the tenderness of meat, and are inhibited by calpastatin (Koohmaraie & Geesink 2006).

The objectives of this study were to analyze the relative gene expression of the target genes caspase-3, μ-calpain, m-calpain, and calpastatin and their possible relationship with meat tenderness and to evaluate lipid oxidation, pH, and centesimal composition in lambs subjected to different nutritional systems.

## MATERIALS AND METHODS

### Development site

The study was approved by the Ethics Committee on the Use of Animals (ECUA) of the institution of origin, under protocol number 4610, and carried out at the University of Western São Paulo - UNOESTE, Campus II, in the city of Presidente Prudente-SP, latitude 22°07'21.06" north and longitude 51°23'17.71", tropical climate, starting in July 2018 and ending in January 2021.

### Nutritional management

Twenty-four ewe lambs (7/8 Dorper), with initial live weight (ILW) of 27.5 ± 3.08 kg to pasture group (P), 27.8 ± 3.67 to pasture with supplement group (PS) and 29.12 ± 1.4 to feedlot group (F) and initial age between 6 and 7 months, and born after fixed-time artificial insemination (TAI) with semen of the same ram, were used. At the beginning of the experiment, all females were weighed, and feces were collected for examination of fecal egg count (FEC), which was repeated monthly until the end of the field experiment. An antihelminthic was applied according to the FEC value.

The Nutrient Requirements of Small Ruminants (NRC 2007) recommends 11.3% crude protein, 73% dry matter, and 58% TDN for this type of sheep, with predicted increases of 0.200 kg/day.

In this way, the females were divided into three groups at random: P, where the ewe lambs consumed between 70 and 80% of the nutritional requirement advised by the NRC (2007); PS, where the females received between 100% and 110% of the nutritional requirement, with supplementary intake as needed based on the period's bromatological analysis and the group; and pasture with no supplement (P). F, where the females were fed separately and received a balanced meal that met 140% of their nutritional needs (NRC 2007). The animals in the feedlot were placed in separate stalls with slatted and suspended floors and had access to limitless water during the trial. Reading was used to manage the feed, resulting in feed leftovers of about 10%.

The methodology of food systems was based on the research of Carvalho et al. (2006), because the different sheep production systems in Brazil presented disuniformity in animal performance and, consequently, the age of animals at slaughter when compared animals in fattening system with grazing, grazing and supplement and feedlot (Ribeiro et al. 2009).

The pasture underwent three bromatological analyses at the beginning, middle, and end of the trial. During the beginning, middle, and last thirds of the experiment, dry matter (DM), ether extract (EE), ash content, crude protein (CP), total digestible nutrients (TDN), neutral detergent fiber (NDF), and acid detergent fiber (ADF), were assessed (Table I). According to Capelle et al. (2001), the analyses were carried out using the methods recommended by Silva & Queiroz

**Table I. Chemical composition of pasture and commercial feed used as feed for the ewe lambs during the experiment.**

| | PASTURE (%) | | | Comercial feed® (%) |
|---|---|---|---|---|
| | Beginning (D 0 – D 60) | Middle (D 61 – D 120) | End (D 121 – D 180) | |
| DM | 26,22 | 45,12 | 25,11 | 88,0 |
| EE | 1,32 | 1,29 | 1,49 | 3,3 |
| Ashes | 11,38 | 9,38 | 11,05 | 5,0 |
| CP | 6,31 | 7,55 | 9,39 | 16,0 |
| TDN | 53,24 | 48,58 | 49,51 | 72,0 |
| NDF | 75,81 | 79,33 | 80,45 | 10,0 |
| ADF | 45,78 | 51,76 | 50,56 | 7,0 |

**DM: dry matter/ EE: ether extract / ashes/ CP: crude protein/ TDN: total digestible nutrients/ NDF: neutral detergent fiber / ADF: acid detergent fiber.**

(2002), total digestible nutrient (TDN) values (2001). The fiber in acid detergent (ADF) contents according to the methodology obtained by the method of van Soest (1963). Commercial feed® (FORT OVINOS 16, FORTSAL®) was utilized as a supplement and a complete diet for the PS and F groups, respectively, the nutritional experiment lasted 180 days and the bromatological analyses were performed in three periods: the beginning between day 0 (D 0) and day 60 (D 60), in the middle of the experiment between days 61 (D 61) and days 120 (D 120) and at the end of the experiment between days 121 (D 121) and days 180 (D 180) (Table I).

Thus, the different dietary systems of the experimental groups were initially established according to the worst grazing of Brazilian pasture. Currently, many Brazilian pastures are insufficient, and the animals remain in a nutritional deficit. The animals in group P (n=8) were maintained exclusively on pasture (*Panicum maximum* cv. Tanzânia), with access to water and mineral salt *ad libitum*. The PS group (n=8) was also maintained on pasture (*Panicum maximum* cv. Tanzânia), but animals also received 1.5% of their live weight in commercial feed® (FORT OVINOS 16, FORTSAL®) containing 16% crude protein and 72% TDN.

The P and PS ewe lambs were kept on pasture in a rotation module, previously sealed for 150 days, for helminth control and quality and grass availability, totaling an area of 0.8 hectares. This was subdivided into 4 padocks of approximately 200 $m^2$, where the animals remained for approximately 9 days in each padock, with a 27-day grass rest period (Hegarty et al. 2007).

Feedlot ewe lambs (n=8) were confined throughout the experimental period and were administered a diet with a forage/concentrate ratio of 20:80, composed of Tifton hay and commercial feed. The average intake was 4.5% of the live weight, according to trough reading (Parente et al. 2009, Rogério et al. 2007). Throughout the experiment, the ewe lambs in groups PS and F were fed twice a day at eight o'clock in the morning and four o'clock in the afternoon and were weighed every 15 days to adjust the diet according to live weight.

## Slaughter schedules as a function of live weight

Upon reaching an average weight of 37.26 ± 3.25 kg/BW, the ewe lambs were slaughtered after fasting for 18h, due to the kg/BW requirement, the animals in the F group reached slaughter

weight before the animals in the PS and P groups due to the different nutritional intakes offered. Table II shows the slaughter order of the ewe lambs according to the sequence in which they reached the pre-stipulated weight.

The slaughter took place in a commercial slaughterhouse, following the rules of ORDINANCE No. 62, OF MAY 10, 2018, published by the Ministry of Agriculture, Livestock and Supply (MAPA).

## Physicochemical analyses of meat

Centesimal analyses of the *Longissimus lumborum* muscle (% moisture, % crude protein, % ethereal extract, and % mineral matter) were performed according to AOAC (2005) methodology.

The pH of the meat was determined using a portable potentiometer (peagameter) with a penetration electrode (Hanna Instruments, Brazil), 1 cm deep in the slice muscle (Gomide et al. 2013). The lipid oxidation index was determined using the T'bars test, with a spectrophotometer reading at 538 nm (Pikul et al. 1989).

The carcasses of each animal were kept in cold storage for the proper measurement of rigor mortis and pH drop, and the samples were prepared for the evaluation of the shear force (WBSF) the following day. They were then placed into an electric oven that was preheated to 180 °C. The steaks were roasted until the internal temperature reached 71 °C (Pikul et al. 1989). The samples were collected using a cylindrical sampler.

Six readings per animal were obtained from each of the three samples that were used, each of which had two subsamples that were each roughly 1.25 cm thick (Koohmaraie et al. 2002). A 3mm thick Warner-Bratzler blade and a Brookfield® CT3 Texture Analyzer (Brookfield engineering, Middleboro, MA) were used to evaluate the shear force objectively (Ramos & Gomide 2007).

For the oxidative analysis of the meat, the Thiobarbituric Acid Assay (TBA) was performed as described by Pikul et al. (1989). Samples were collected during slaughter, frozen and stored in a freezer at -80 °C until processing, and evaluated one week after the last slaughter, in which 10g of lamb meat with 50 mL of trichloroacetic acid 7.5% was homogenized for 1 minute in turrax. Then the mixture was filtered, and 5 mL of the extract was transferred to a tube containing 5 ml of 2-thiobarbituric acid 0.02 M. The tubes were heated in a boiling water bath for 40 minutes and cooled in running water for 10 minutes,

**Table II. Order, date, quantity, group belonging and age per slaughter (days after initial experiment) to the slaughtered ewe lambs and average weight per slaughter of the groups.**

| Order of slaughter | Slaughter days | P (grass) | PS (grass + supplement) | F (feedlot) |
|---|---|---|---|---|
| 1st | 27/09/2018 | 01 | 01 | 04 |
| 2nd | 11/10/2018 | - | 02 | 04 |
| 3rd | 29/10/2018 | 02 | 03 | - |
| 4th | 06/12/2018 | 03 | 01 | - |
| 5th | 10/12/2018 | 02 | 01 | - |
| **Age per slaughter (days after initial experiment)** | - | 145.66±16.6 | 116.28±27.16 | 86.28±8.87 |
| **Average weight per slaughter experimental group** | - | 34.37±1.13 | 35.84±2.64 | 40.9±1.32 |

for the measurement of reactive substances to 2-thiobarbituric acid (TBARS) malonaldehyde quantification was estimated by comparing absorbance values of samples and standards at 538 nm. The values were expressed in mg of malonaldehyde (MDA)/kg.

## Gene expression

During slaughter, *Longissimus lumborum* muscle samples were collected for RNA extraction for gene expression analysis. Muscle samples were collected immediately after slaughter, deposited in liquid nitrogen (-196 °C), and stored in a freezer at -80 °C until processing for RT-qPCR. These fragments (~40 mg) were crushed in a tissue homogenizer and subjected to the TRIzol® (Thermo Fisher Scientific®) extraction protocol for total extraction.

The total RNA concentration recovered and the 260/280 ratio were measured using a NanoDrop® (Thermo Fisher Scientific, Brazil). All total RNA samples were treated with DNAse before being submitted to RT-qPCR, according to the instructions of the DNAse I - Amplification Grade (Invitrogen) protocol®.

Reverse transcription was performed using the High-Capacity protocol (Applied Biosystems™, Thermo Fisher Scientific, Brazil) following the manufacturer's protocol. qPCR was performed for quantitative analysis of relative gene expression. The primers and probes of target genes correlated with meat quality were for CASP3 (Oa0481763_m1), CAPN1 (Oa04658113_g1), CAPN2 (Oa04659692_m1), and CAST (Oa0456608_m1). Three endogenous genes were used as internal controls for real-time qPCR reactions in real time, and 3 endogenous genes were used: B2M (Oa04818291_m1), HMBS (Oa04838105_g1), and TBP (Oa04666642_m1) (Table III).

For data normalization, a combination of HMBS and B2M was used, which was shown to be more stable by the NormFinder software® program (MOMA, Denmark), to normalize the results obtained for the target gene. Primers for endogenous and target genes and their respective probes were obtained from standardized TaqMan® assays (Applied Biosystems®, Foster, USA).

## Statistical analyses

The initial experiment consisted of three completely randomized treatments, with eight replications. For initial weight, average daily gain, slaughter weight, centesimal analysis: moisture, crude protein (CP), ashes, and ethereal extract (EE), pH, WBSF, and lipid oxidation, homogeneity of variance was tested using the Bartlett test ($p > 0.05$), and residue normality was verified using the Shapiro-Wilk test. The data were submitted to variance analysis and when significant, were submitted to the Tukey test at a level of 5%. All

**Table III. Primers, genes, TaqMan® assays, reference sequence and products used in qPCR.**

| Primers | Gene | TaqMan® Assays | Seq. of reference | Product |
|---|---|---|---|---|
| CASP3 | Alvo | Oa0481763_m1 | XM_015104559.2 | 77 pb |
| CAPN1 | Alvo | Oa04658113_g1 | NM_001127267.1 | 53 pb |
| CAPN2 | Alvo | Oa04659692_m1 | NM_001112817.1 | 63 pb |
| CAST | Alvo | Oa0465608_m1 | NM_001009788.1 | 56 pb |
| B2M | Endógeno | Oa04900279_mH | XM_027971438.1 | 84 pb |
| HMBS | Endógeno | Oa04704013_g1 | XM_004016091.4 | 62 pb |
| TBP | Endógeno | Oa04818075_m1 | XM_015097549.2 | 66 pb |

statistical evaluations were performed using the RStudio Software.

The qPCRs were conducted in duplicate for each sample, and expression was determined by quantification in relation to the endogenous gene. The Pfaffl (2001) method was used for relative quantification of amplifications. Gene expression data were analyzed for Shapiro-Wilk normality and then subjected to variance analysis (ANOVA). Differences were considered statistically significant at $p < 0.05$.

## RESULTS

The average daily gain (ADG) was higher in the confined animals ($p < 0.05$) and lower in the animals of the PS and P groups. The lowest weight gain in the PS and P groups occurred because of the food system to which the animals were subjected.

Consequently, as expected for ADG, there was a difference in the total weight gain ($p < 0.05$) with an increase in concentrate, according to the nutritional plan (Table III). The values found in the centesimal analysis of lamb meat showed no statistical difference between treatments for most variables, except ether extract (EE), which showed a linear increase as a function of dietary system (Table IV).

The pH remained within the expected pattern for ewe lamb meat, and there was no significant difference between treatments, with an average value of 5.54.

In relation to lipid oxidation, there was a statistical difference between treatment groups, and the group where the animals remained on pasture presented the lowest values, with an average of 1.1998 mg MDA/kg of meat (Figure 1).

For WBSF, there was a significant difference between the three treatment groups, and group F presented a value of 27.06 N, followed by the PS group (31.28 N), which showed a lower WBSF than that in treatment group P (50.40 N) (Figure 2).

With the results duly corrected for qPCR efficiency, there was no statistical difference in any of the genes evaluated in the relative gene expression analysis ($p > 0.05$) (Figure 3a-d).

## DISCUSSION

When breeding animals exclusively using grazing feeding systems, the nutritional quality often does not meet the requirements of production or reproduction, especially in genetically modified animals, and there is a need to seek alternatives to address this problem, such as supplementation (Borton et al. 2005).

**Table IV. Initial live weight, daily average gain, mean values (%) for moisture, CP, ash, EE and pH of ewe lamb meat, in three groups with different feeding systems.**

| Variables | TREATAMENTS | | | Average | Standard error | p value* |
|---|---|---|---|---|---|---|
| | P | PS | F | | | |
| ILW | 22.24 | 22.56 | 20.14 | 21.64 | 1.264 | 0.4611 |
| ADG | 0.0539c | 0.178b | 0.222a | 0.151 | 0.0083 | 2.1231 |
| Moisture | 71.76 | 71.57 | 68.93 | 70.91 | 0.71 | 0.2328 |
| CP | 23.74 | 23.20 | 23.52 | 23.50 | 0.58 | 0.9345 |
| Ash | 1.25 | 1.03 | 1.18 | 1.16 | 0.04 | 0.1309 |
| EE | 1.77a | 3.56b | 5.10c | 3.29 | 0.39 | <0.001 |
| pH | 5.62 | 5.47 | 5.54 | 5.54 | 0.03 | 0.05 |

**P = pasture / PS = pasture and supplement / F = feedlot / ILW = initial live weight / ADG = average daily gain / CP = crude protein / Ash = ash content / EE = ether extract.**
***(p < 0.05).***

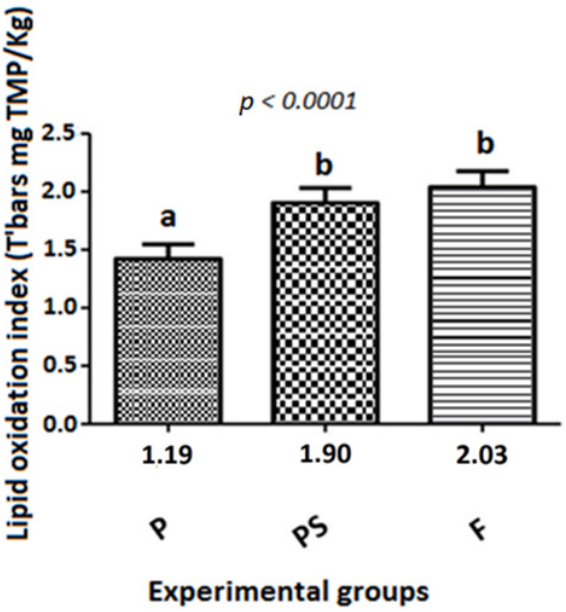


**Figure 1.** Lipid oxidation in the meat of ewe lambs fed with different nutritional systems: P (pasture, in which lambs received between 70 and 80% of the nutritional requirement [NRC 2007], n=8); PS (pasture and supplementation, in which females received 100 to 110% of the nutritional requirement (NRC 2007), n=8); and F (feedlot, in which the lambs were fed in an individual trough and provided 140% of the nutritional requirement [NRC 2007], n=8).

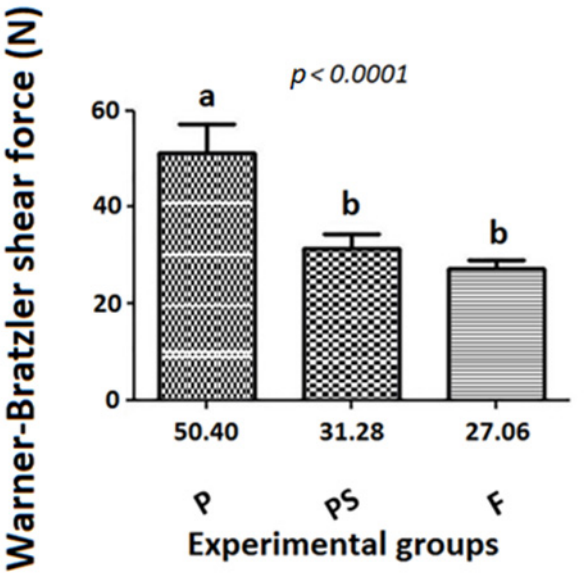


**Figure 2.** WBSF of the meat of ewe lambs fed with different nutritional systems: P (pasture, in which ewe lambs received between 70 and 80% of the nutritional requirement [NRC 2007], n=8); PS (pasture and supplementation, in which females received 100 to 110% of the nutritional requirement (NRC 2007), n=8); and F (feedlot, in which the lambs were fed in an individual trough and provided 140% of the nutritional requirement [NRC 2007], n=8).

In relation to the performance of ewe lambs in the feedlot fattening system, as expected, they reached slaughter weight in a shorter time because of the nutritional contribution provided by this system, and the animals also likely reached their optimum health status (Pelegrini et al. 2008). According to Gomes et al. (2018), feedlots show better results because, although the costs of feeding are higher than those in grazing alone, the higher production of body weight and reduction in the slaughter age dilute these costs, and the decision to use the confinement system offers the possibility of achieving more cycles in the year, since the conversion into animal weight is optimized (Berwanger & Amorim 2022).

According to Guimarães et al. (2020, 2021), the average centesimal composition of the meat of lambs consuming pastures supplemented with different energy sources is 73% to 76% moisture, 20% crude protein, 1.1% to 4.9% ethereal extract, and 1% to 1.6% mineral matter, which may be influenced by feeding and type of termination.

Ewe sheep fed more energy-dense diets had increased values of ethereal extract in their meat. According to Gois et al. (2017) and Oliveira et al. (2020), the mean value for this variable was 3% to 3.75% in the meat of confined lambs.

Araújo et al. (2014) compared animals on pasture with and without supplementation and observed higher lipid values in the meat of supplemented animals, with values of 3.67% and 2.02%, respectively. Furthermore, Santos (2019) found lipid values of 2.2% in the meat of animals fed pasture and supplement and 3.44% for confined animals, corroborating the results of the present study.

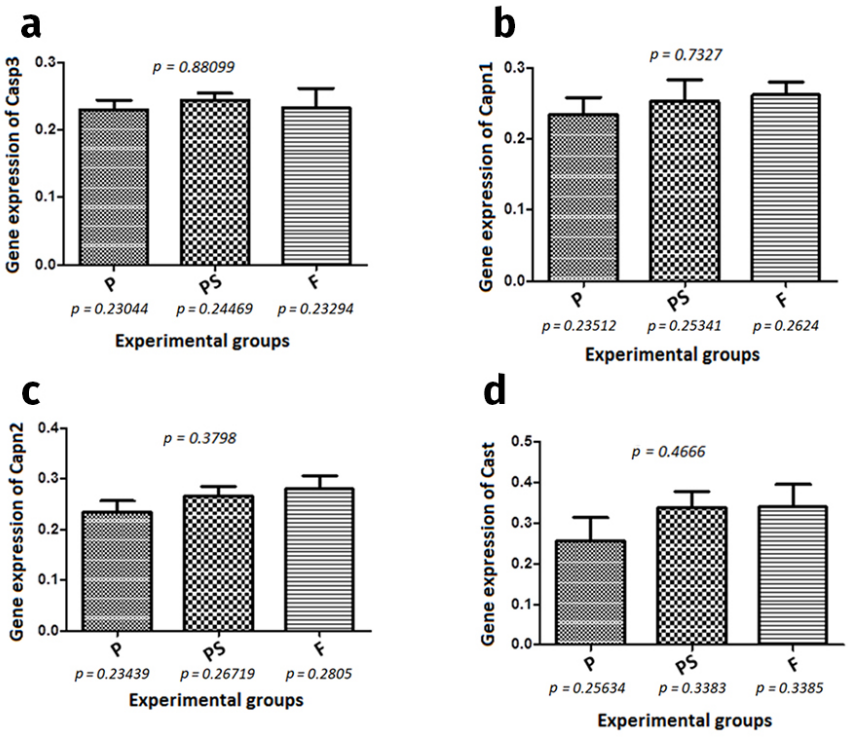


**Figure 3. Relative gene expression of CASP3 (a), CAPN1 (b), CAPN2 (c), and CAST (d) genes evaluated in the *Longissimus lumborum* ewe lamb muscle, using the mean endogenous HMBS and B2M levels as normalizers, in three groups with different feeding systems: P (pasture, in which the lambs received between 70 and 80% of the nutritional requirement [NRC 2007]; PS (pasture and supplementation, in which females received 100 to 110% of the nutritional requirement [NRC 2007]; and C (confinement, in which the ewe lambs were fed in an individual trough and provided 140% of the nutritional requirement [NRC 2007]).**

According to Souza et al. (2009), diets with a higher lipid content lead to an improvement in the metabolic efficiency of anabolic reactions in adipose tissue, with this fatty acids readily available for deposition causing a reduction in the energy cost of fat synthesis from short-chain fatty acids.

For sheep meat, the final pH is expected to be between 5.5 and 5.8, which is important for the quality of the product, especially tenderness, because it is related to the transformation of muscle into meat by the enzymatic degradation that occurs in the myofibrillar structure of the muscle (Silva Sobrinho et al. 2005). The observed pH values corroborate recent data for the meat of sheep and lambs and demonstrate that slaughter was carried out within the animal welfare standards, allowing correct rigor-mortis (Valadez-Garcia et al. 2021).

The average lipid oxidation value observed in the meat of the animals in treatment group P (1.19 mg of malonaldehyde $kg^{-1}$) was within the desired range, because results below 1.59 mg of malonaldehyde $kg^{-1}$ are considered acceptable and safe for the consumer (Torres & Okani 1997). On the other hand, the animals in groups PS and F presented higher than desired averages.

This may be related to the diet of the animals, correlating with Yamamoto's (2006) findings that showed increasing ethereal extract values as a function of concentrate levels in the diets of confined ewe lambs (3.41% and 3.96%). In other words, the lower concentration of malonaldehyde detected in animals on pasture may also be related to the lower proportion of fat found in these animals, since the TBARS methodology analyzes lipid oxidation, and the lower percentage of fat in these (Girgih et al. 2015). Concomitantly, the average lipid oxidation also increased (0.9 to 1.71 mg MDA/kg).

In ruminant meat reared in the pasture system, pasture with supplementation, and feedlot, other authors also found lower values of lipid oxidation for animals fed only with feed from pasture (pasture: 0.09 mg MDA/kg; confined: 0.28 mg MDA/kg), which present, consequently, lower

fat content in meat (Daley et al. 2010, Descalzo et al. 2005, Santé-Lhoutellier et al. 2008).

Although lipid oxidation is dependent on polyunsaturated fatty acids, which tends to be higher per g of tissue in animals in fattening system with grazing, Luciano et al. (2012) emphasized that lamb meat in a fattening system with grazing presented a higher amount of unsaturated fatty acids, lower lipid oxidation and greater color stability when compared to the meat of confined lambs. Also in the same study, it can be concluded that the diet affected the fat content of the meat, because it expressed the classes of fatty acids as percentages of total fat and this fact may not be particularly relevant in the discussion of the susceptibility of the muscle to lipid oxidation, because the animals that received concentrate had a higher content of ether extract and, therefore, a higher absolute content of easily oxidisable fatty acids.

In the same way that Insani et al. (2008) found that lipid oxidation was higher for confined animals, in addition, the results showed that the high level and synergistic action of α-tocooferol and β-carotene found in animals in a fattening system with grazing contributed to oxidative stability and meat color.

The significant difference in SF may be explained physiologically by the fact that grazing animals move more in search of food, consequently exercising more, producing more collagen, which brings greater resistance to the meat causing increased hardness (Field 1996). Another factor is that confined lambs were butchered with a higher percentage of fat, since fat is responsible for the sensation of tenderness of the meat favoring juiciness, in addition to the fact that confined animals have less intramuscular collagen (Hocquette et al. 2010, Listrat et al. 2016, Lorenzo 2019).

According to the SF, Boleman et al. (1997) categorized the meat's tenderness as follows: highly tender (22.55 to 35.30 N), moderately tender (40.20 to 52.95 N), and not very tender (57.85 to 70.6 N). The meat from treatment P may be categorized as tender using the values indicated previously, whereas the meat from treatments PS and F can be categorized as very tender. For lambs and ewe lambs, values between 34.9 N and 57.8 N were close to those reported in the literature (Valadez-Garcia et al. 2021, Zhao et al. 2017).

Regarding factors that affect tenderness, gene expression has been the target of many studies (Lonergan et al. 2010, Chung & Davis 2012, Picard et al. 2015). Souza (2012) used Nelore bulls in his experiment to track genes related to meat quality, and all animals were reared in confinement until the time of slaughter. Hence, there was no influence of different feeds on the abundance levels of genes of interest, showing that different nutritional plans can alter gene expression levels.

The CASP3 gene was investigated because it is involved in the process of muscle proteolysis; that is, when there is an increase in the level of its expression, it is possible to verify an increase in the abundance of CAPN1 and CAPN2 RNA, which are responsible for the breakdown of muscle myofibrils, leading to a higher percentage of tenderness (Ouali et al. 2006). However, in this study, no alterations were observed in the expression of this gene in the different groups.

In the evaluation of the tenderness of the meat of discarded cows, there was no association of shear force with the activity of caspase 3. Despite being related to phenotype, this fact demonstrates that even with its proper action on proteolysis, caspase 3 does not influence the tenderness of the meat (Underwood et al. 2008).

According to Guillemin et al. (2011), deprivation of nutrients and oxygen in cells and tissues after slaughter presumably involves muscle cells in the process of cell death via apoptosis rather

than necrosis. The response to this stimulus has a 12% interaction with caspase 3; that is, this mechanism of action stimulus is defined as a change in the state or activity of an organism (in terms of secretion, enzyme production, or gene expression).

For the CAPN1 and CAPN2 genes, the lack of difference in expression in the different systems may be because the animals were from the same genetic and contemporary group, corroborating Ferraz (2009), and the fact that they remained on different diets for a short period of time. In beef cattle, when examining the relative gene expression of these calpains between distinct breeds (Nelore and Angus) fed with two nutritional sources, a statistical difference was observed.

Bagatoli et al. (2013) evaluated sheep and found greater expression of the CAST gene in the muscle samples of Santa Inês lambs than that in the meat of crossbred White Dorper × Santa Inês and Dorper × Santa Inês. The authors suggested that the expression of this gene in the Santa Inês breed leads to a higher production of the calpastatin enzyme and consequent inhibition of calpain, resulting in lower meat tenderness.

In another study using goats with the same racial pattern and different ages, there was no difference ($p > 0.05$) in the expression of the CAPN1 gene in the meat of post-pubescent goats when compared to that in the meat of younger goats (Saccà et al. 2019). The low age difference between the animals at slaughter in the present study was not a determining factor, so the results of gene expression could be manifested.

## CONCLUSIONS

The confined animals had higher average daily weight gain and shorter slaughter time than animals raised exclusively on pasture and pasture with supplementation. There was an increase in the ethereal extract in the meat of confined animals because greater energy intake increases this variable. Animals kept only on pasture had lower lipid oxidation in meat compared with that in other treatment groups because of the lower ethereal extract content. The WBSF was considerably higher in the meat of animals kept only on pasture, but the meat was still considered tender. The nutrition of ewe lambs did not interfere with the expression of CASP3, CAPN1, CAPN2, and CAST. More research should be performed in different sheep breeds using longer experimental periods.

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