# The effect of feed restriction on the fat profile of Santa Inês lamb meat 

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#### Abstract

Consumers today are increasingly more demanding regarding their food，seeking healthier and better quality products，and in this context animal nutrition plays a key role．The meat composition can be altered by animal feed itself，being that lipid profile may directly contribute to consumer health， reducing the predisposition of developing cardiovascular diseases，main cause of mortality in the world． Thus，the aim of this study was to assess the effect of dietary feed restriction in Santa Inês lambs on their intramuscular，intermuscular，and subcutaneous fat profile，fat profile of the longissimus thoracis et lumborum（LTL）muscle，and the total meat lipids and cholesterol．Three groups of lambs were subjected to diets：without restriction（WR），and 30 and $60 \%$ feed restriction．Overall，stearic，palmitic，and oleic acids were the predominant and the lowest lipid and cholesterol levels were observed at the highest restriction level，presenting higher polyunsaturated：saturated（PUFA：SFA）and desirable（DFA）fatty acid ratios（ $p<0.05$ ）．Lambs subjected to $60 \%$ dietary feed restriction had a better quality meat with lower lipid and cholesterol contents，and profile favorable for human health due the presence of unsaturated fatty acids，that is important parameter the market demands to meet the consumers＇expectations．


Keywords：Brazilian Northeast；cholesterol；diet；fatty acids；longissimus thoracis et lumborum；nutrition．

## Introduction

Lipid components，especially fatty acids，are present in animal products，playing key roles in cell membrane structure and metabolic processes．The fat in the fatty deposits of ruminants is rich in triglycerides，with a predominance of saturated fatty acids（SFA）and lower ratios of polyunsaturated fatty acids（PUFA）．In some countries，this fat profile has accounted for the reduced intake of lamb meat and its derivatives，given the strong relation between dietary fat quality and human health（Kaić \＆Mioč，2016）．Studies have indicated the need for increasing dietary PUFA，especially those of the n－3 and n－6 classes．Higher conjugated linoleic acid（CLA）levels and PUFA：SFA ratios in the lipid fraction of ruminant meat are also sought，and a ratio of approximately 0.4 is recommended for foods characterized as healthier（Oliveira et al．，2012）．

Lamb meat is rich in SFA derived from the lipid digestion process specific to ruminants．Given the interest in improving meat quality，especially nutritional factors，new animal production strategies are being adopted to improve the fatty acid profile，rendering meat more appealing to the consumers＇health，as the occurrence of health problems has been associated with fat intake，especially saturated fat．Thus，PUFA intake and the dietary balance between unsaturated fatty acids（ $n-6: n-3$ ratio）and high linolenic acid（ $n-3$ ） and CLA levels may provide greater health benefits（Simopoulos，2016）．

Factors such as diet，age，sex，and breed may affect the composition of fatty acids deposited in ruminant meat．However，animal production systems and nutrition are the main modifying factors of carcass lipid profiles and lipid ratios（D＇Alessandro et al．，2012；Mushi，Thomassen，Kifaro，\＆Eik，2010；Park et al．， 2018）．Furthermore，ruminal metabolism also affects fat digestibility，promoting changes in the fatty acid profile，bioaccessibility，and biohydrogenation（Oliveira et al．，2013）．

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Diet is the main determining factor of possible variations in carcass tissue composition and, therefore, in commercial cuts, the production system and the concentrate and roughage ratios are the strongest determining factors (Oliveira et al., 2013). The dietary lipid composition is reflected in the carcass fat profile in most species (Arruda et al., 2012); however, in ruminants, lipids, particularly PUFA, are widely modified by ruminal microorganisms, affecting the fatty acid content and composition of skeletal muscles. Thus, the composition of meat fatty acids and fats strongly depends on diet and ruminal function.

A key factor for consumers when purchasing quality meat is the observation of meat characteristics such as color, fat ratio, and tenderness. The subcutaneous and intramuscular fat tissues are the most important fat deposits for meat quality. Carcasses should have minimal fat quantities in the subcutaneous tissue without a detrimental decrease in intramuscular fat (Costa et al., 2013). Double-bonded conjugated fatty acids are found in nature, and some are produced by ruminants, including CLA, which has been associated with the prevention of cardiovascular disease, diabetes, and obesity (den Hartigh, 2019). Another important fatty acid is $18: 1$ trans-vaccenic acid, the product of $18: 2 n-6$ biohydrogenation, which is converted into CLA (Dewanckele et al., 2018).

Several alternatives are used in ruminant production systems to reduce costs, including the use of roughage, restriction of qualitative dietary protein and fiber, exploitation of compensatory growth, and use of a single diet with quantitative restriction. However, importantly, the balance point of feed restriction must be established to avoid decreased carcass and meat quality (Costa et al., 2009; Leão et al., 2011; Yáñez et al., 2006).

Yáñez et al. (2006) reported that feed restriction leads to a decreased amount of animal adipose tissue, albeit without affecting the subcutaneous and intermuscular fat ratios. When the quantity of feed is restricted, the amount of feed supplied to reach the best adjustment of the intake portion should provide the greatest weight gain and reduce feed costs and fat quantity, thus rendering the meat healthier. The use of dietary restriction as a nutritional alternative can be an important tool for the application of meat with less fat deposition, because the restriction prioritizes metabolic activities of visceral tissues for body maintenance, without promoting decrease in animal productivity. With increased dietary restriction, the rumen receives a lower amount of unsaturated fatty acids from the diet. This smaller amount causes ruminal microorganisms to reduce the process of biohydrogenation, which refers to the transformation of unsaturated fatty acids into saturated. Thus, a greater amount of unsaturated fatty acids pass from the rumen to the intestine, where they are absorbed and stored in adipose tissue. Consequently, there is a linear increase in the ratio of unsaturated fatty acids to saturated fatty acids in the lipid profile of meat (Rodrigues et al., 2010).

The lambs that are usually reared under intensive farming and are fed concentrates and cereal straw until slaughter, within 90 days of age, produce meat with high $n-6$ concentrations (Brito, Ponnampalam, \& Hopkins, 2016). The same authors cited that the PUFA of lamb meat are affected by the dietary concentrate ratio, and according to Mushi et al. (2010), different dietary concentrate levels affect the fatty acid profile of the adipose tissue and increase the ratio of the desirable fatty acids (DFA) in the meat. This is justified probably because the marbling fat develops when the animal is gaining body weight and is the last fat to be deposited, but the first to be mobilized when the animal suffer a feed restriction.

In recent years, consumers have been showing great interest in general for the healthy foods and a higher requirement in relation to properties of their food, showing also a higher preference for meats with better nutritional and sensory quality. For this reason, the objective of the present study was to assess the effect of quantitative feed restriction on the lipid components of intramuscular fat, intermuscular fat, and subcutaneous fat of Santa Inês lambs subjected to different levels of feed restriction.

## Material and methods

## Site, Animals and Diet

The experiment was conducted at the Center for Rural Health and Technology (Centro de Saúde e Tecnologia Rural - CSTR) of the Federal University of Campina Grande (Universidade Federal de Campina Grande - UFCG), Patos Campus, Paraíba (PB), located in the Sertão Paraibano mesoregion, $7^{\circ} 01^{\prime} 28^{\prime \prime} \mathrm{S}$ latitude and $37^{\circ} 16^{\prime} 48^{\prime \prime}$ W longitude, at an altitude of 242 m above sea level. The regional climate is BSh (semi-arid), hot and dry, with winter rains and the annual rainfall ranges from 400 to 800 mm , with $28.5^{\circ} \mathrm{C}$ mean annual temperature; maximum and minimum temperatures of $37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, respectively and $61 \%$ mean annual relative air humidity.

Twenty-four intact Santa Inês lambs, aged 6 to 7 months, selected based on $30-\mathrm{kg}$ live weight, were used. The animals were divided into three groups of homogeneous weights (eight animals for each group), initially subjected to a 10-day dietary adaptation period, and subsequently fed without feed restriction (WR) or with 30 or $60 \%$ quantitative restriction. The dietary supply for animals under dietary restriction was determined based on the dry matter (DM) intake of the animals fed at will ( $0 \%$ restriction).

Dietary restriction should not interfere with the animal's energy metabolism and consequently protein metabolism, keeping parameters within the established range as normal for the species. Due this, the latter restriction level was based on the minimum maintenance requirements recommended by the National Research Council (NRC, 2007). According to this recommendation, the lambs were fed complete feed, which consisted of $45 \%$ elephant grass hay (Pennisetum purpureum) and $55 \%$ concentrate-soybean meal, cornmeal, calcitic limestone, dicalcium phosphate, and mineral salt (Table 1)-in order to meet the nutrient requirements for lambs from 30 to 45 kg , being that the experimental diet was formulated based on the gain requirements of $250 \mathrm{~g} \mathrm{day}^{-1}$ mean daily gain.

Table 1. Ingredients and chemical composition of Santa Inês lamb diet.

| Ingredients | $\mathrm{g} \mathrm{kg}^{-1} \mathrm{dry} \mathrm{matter}^{\prime}$ |
| :---: | :---: |
| Soybean meal | 235.00 |
| Cornmeal | 289.90 |
| Elephant grass hay (Pennisetum purpureum) | 450.00 |
| Calcitic limestone | 11.90 |
| Dicalcium phosphate | 3.20 |
| Commercial mineral salt ${ }^{1}$ | 10.00 |
| Chemical composition $\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | 928.70 |
| Dry matter | 80.10 |
| Mineral matter | 146.40 |
| Crude protein | 33.90 |
| Ether extract | 458.40 |
| Neutral detergent fiber ${ }^{2}$ | 339.60 |
| Acid detergent fiber | 308.90 |
| Nonfibrous carbohydrates | 1.89 |

${ }^{1}$ Composition: $147 \mathrm{~g} \mathrm{Na}, 120 \mathrm{~g} \mathrm{Ca}, 87 \mathrm{~g} \mathrm{P}, 18 \mathrm{~g} \mathrm{~S}, 3,800 \mathrm{mg} \mathrm{Zn}, 3500 \mathrm{mg} \mathrm{Fe}, 1,300 \mathrm{mg} \mathrm{Mn}, 870 \mathrm{mg}$ Fl, $590 \mathrm{mg} \mathrm{Cu}, 300 \mathrm{mg} \mathrm{Mo}, 80 \mathrm{mg}$ I, $40 \mathrm{mg} \mathrm{Co}, 20 \mathrm{mg} \mathrm{Cr}, 15 \mathrm{mg} \mathrm{Se}$, 250 mg Vitamin A (IU), 100 mg Vitamin D (IU), 500 mg Vitamin E (IU), ${ }^{2}$ Corrected for ash and protein, ${ }^{3}$ Estimated by metabolism assay (Pereira, 2011).

During the experimental period, the animals were weighed (initial weight - IW), and the provided feed leftovers were removed, weighed, sampled for dry matter determination and of the daily intake. The organic matter intake (OMI) was calculated by subtracting the mineral matter from the dry matter. After 90 days, the final weight (FW) of the animals was determined, which was used to calculate the performance. Furthermore, the mean weight gain of the animals was calculated based on the IW, which was related to the time in days, resulting in the average daily weight gain (ADWG) (Table 2).

Table 2. Performance of feedlot Santa Inês lambs fed diets with different levels of feed restriction (mean $\pm$ standard error).

|  | Treatments |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| Variable | Without restriction | $30 \%$ restriction | Significance |  |
| $\mathrm{IW}^{2}(\mathrm{~kg})$ | $31.84 \pm 1.21$ | $31.58 \pm 1.21$ |  | $\mathrm{NS}^{1}$ |
| $\mathrm{FW}^{3}(\mathrm{~kg})$ | $45.19 \pm 0.52$ | $39.31 \pm 0.60$ | $32.32 \pm 0.78$ | $*$ |
| $\mathrm{ADWG}^{4}(\mathrm{~g})$ | $248.00 \pm 12.42^{\mathrm{a}}$ | $133.00 \pm 13.25^{\mathrm{b}}$ | $20.00 \pm 5.59^{\mathrm{c}}$ | $* * *$ |
| $\mathrm{OMI}^{5}\left(\mathrm{~g} \mathrm{day}^{-1}\right)$ | $1350.00 \pm 52.50^{\mathrm{a}}$ | $928.00 \pm 34.79^{\mathrm{b}}$ | $543.00 \pm 39.74^{\mathrm{c}}$ | $* * *$ |
| $\mathrm{CCY}^{6}(\%)$ | $49.36 \pm 0.88$ | $47.87 \pm 0.81$ | $48.45 \pm 0.95$ | NS |
| $\mathrm{HCY}^{7}(\%)$ | $50.80 \pm 0.34$ | $49.30 \pm 0.30$ | $50.10 \pm 0.36$ | NS |

${ }^{\text {a,b,c }}$ Means followed by different lowercase letters in the same row indicate significant differences according to Tukey's test (5\%). ***p < 0.001; *p < 0.05 .
${ }^{1}$ Non significant, ${ }^{2}$ Initial weight, ${ }^{3}$ Final weight, ${ }^{4}$ Average daily weight gain, ${ }^{5}$ Organic matter intake, ${ }^{6}$ Cold carcass yield, ${ }^{7}$ Hot carcass yield.
The slaughter was made according Brazil (2008). After, the carcasses were immediately sent to cold storage ( $3 \pm 2^{\circ} \mathrm{C}$ for 24 hours) and dissected to obtain the longissimus thoracis et lumborum (LTL) muscle and the intermuscular fat and subcutaneous fat. The muscle and the fat were individually vacuum-packed in polyethylene bags, labeled and stored in a freezer at $-20^{\circ} \mathrm{C}$ for a maximum storage period of 4 months until analysis.

## Characterization of the LTL muscle and associated fats

The LTL muscle and fat were initially thawed at $4^{\circ} \mathrm{C}$ for 24 hours and then minced in a domestic multiprocessor until complete homogenization for analysis of the total lipid and cholesterol contents, fatty acid profile, and intermuscular and subcutaneous fat. The meat was subjected to lipid extraction, which represented the intramuscular fat that was tested. The meat was submitted to lipid extraction to obtain intramuscular fat (marbling), while the intermuscular (within the muscles groups) and subcutaneous (of cover) fat were separated from the meat for evaluation.

## Total lipids - Intramuscular fat

The total lipid content of the meat was determined by extraction in chloroform/methanol (2:1), followed by evaporation in an oven (Tecnal, TE397/4) at $105 \pm 2^{\circ} \mathrm{C}$ to constant weight, according to the method described by Folch, Lees, and Sloane Stanley (1957). The results were expressed as $\mathrm{g} 100 \mathrm{~g}^{-1}$ of sample.

## Total cholesterol

The cholesterol levels were measured according to the method of Bragagnolo and Rodriguez-Amaya (1997), and this measurement consisted of four steps: meat lipid extraction, saponification, unsaponifiable lipid extraction, and lipid extract injection. To determine the cholesterol levels, a high-performance liquid chromatograph was used (Waters 2690, Varian, Palo Alto, California, USA), and the identification was made in an ultraviolet-visible (UV-VIS) detector (photodiode array [PDA], 330) at 210 nm using standard curve between 0.04 to $1.00 \mathrm{mg} \mathrm{mL}^{-1}$. The results were expressed as $\mathrm{mg} 100 \mathrm{~g}^{-1}$ of sample.

## Fatty acid profile

Fatty acids were assessed using the previously prepared lipid extract, which was subjected to methylation as described by Hartman and Lago (1973). The fatty acid esters were identified and quantified by a gas chromatograph (Varian, 430-GC). Saturated and unsaturated fatty acids were identified by comparing the retention time with standards from a Supelco ME19 and ME14 Kits. The results were expressed as percentage of area (\%).

## Statistical analysis

A completely randomized design in three treatments (WR, $30 \%$ restriction, and $60 \%$ restriction) and eight replicates ( $3 \times 8$ ) was used to perform the meat analyses. For the other variables, the experimental groups consisted of a $3 \times 3$ factorial design (the restriction levels vs the types of fat). The results were subjected to analysis of variance (ANOVA), and in case of significant differences, the means were compared with Tukey's test at a 5\% significance level using Statistical Analysis System (SAS) software, version 9.3 (2011) and the general linear model (GLM):
$Y_{i j k}=\mu+D_{i}+G_{j}+D G_{i j}+e_{i j k}$
where, $Y_{i j k}=$ the observed value of each animal trait; $\mu=$ the overall mean effect; $D_{i}=$ the diet effect $(i=1,2$, $3)$; $\mathrm{G}_{\mathrm{j}}=$ the type of fat effect $(\mathrm{j}=1,2,3)$; $\mathrm{DG}_{\mathrm{ij}}=$ the diet x type of fat interaction effect, and $\mathrm{e}_{\mathrm{ij} \mathrm{k}}=$ the random error associated with each result.

The data for the LTL muscle fatty acids were subjected to principal component analysis (PCA) to identify the relations among these data, according to the variability between treatments.

The following mathematical model was used for the lipids and cholesterol:
$\mathrm{Y}_{\mathrm{ijk}}=\mu+\mathrm{D}_{\mathrm{ijk}}+\mathrm{e}_{\mathrm{ijk}}$
Where, $\mathrm{Y}_{\mathrm{ijk}}=$ the observed value of each animal trait; $\mu=$ the overall mean effect; $\mathrm{D}_{\mathrm{ij} \mathrm{k}}=$ the diet effect, and $\mathrm{e}_{\mathrm{ijk}}$ $=$ the random error.

## Results and discussion

## Assessment of lamb meat composition

Feed restriction led to trends toward decreased lipid and cholesterol levels in the lamb meat, with a similar reduction trend at the $60 \%$ restriction level compared with the WR treatment (Table 3), and these
decreased lipid and cholesterol levels were attributed to the greatest decrease in intramuscular fat deposition. Thus, according to the results, the meat lipid levels of the animals varied significantly ( $\mathrm{p}<0.05$ ), and the WR treatment showed the highest value, directly affecting cholesterol formation.

Table 3. Assessment of meat from lambs subjected to feed restriction (mean $\pm$ standard error).

| Parameter | Restriction levels |  |  | Standard Error | Significance |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | WR ${ }^{1}$ | 30\% | 60\% |  |  |
| Lipids ( $\mathrm{g} 100 \mathrm{~g}^{-1}$ ) | $5.16{ }^{\text {a }}$ | $4.59^{\text {a }}$ | $3.19^{\text {b }}$ | 0.25 | * |
| Cholesterol (mg $100 \mathrm{~g}^{-1}$ ) | $76.62^{\text {a }}$ | $48.56{ }^{\text {b }}$ | $51.75{ }^{\text {b }}$ | 3.94 | * |

The study of the fatty acid profile of lamb meat after intramuscular fat extraction identified 19 fatty acids, including seven SFA, five monounsaturated fatty acids (MUFA), and seven PUFA, as shown in Table 4, with a similar profile to the subsequently studied intermuscular fat and subcutaneous fat. In this case, the feed restriction did not significantly influence the relation between monounsaturated and saturated fatty acids, but increased the polyunsaturated:saturated ratio with increasing restriction level.

Table 4. Fatty acid profile (\% area) of the Longissimus thoracis et lumborum muscle of lambs subjected to quantitative feed restriction (mean $\pm$ standard error).

| Fatty acids | Without restriction | 30\% | 60\% | Significance |
| :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |
| C12:0 | $0.34 \pm 0.19$ | $0.18 \pm 0.02$ | $0.24 \pm 0.09$ | NS ${ }^{1}$ |
| C14:0 | $1.70 \pm 0.20$ | $2.00 \pm 0.08$ | $1.61 \pm 0.05$ | NS |
| C14:1 | $0.08 \pm 0.03^{\text {ab }}$ | $0.13 \pm 0.03^{\text {a }}$ | $0.03 \pm 0.01^{\text {b }}$ | * |
| C15:0 | $0.37 \pm 0.05$ | $0.41 \pm 0.05$ | $0.39 \pm 0.03$ | NS |
| C16:0 | $21.80 \pm 2.03$ | $24.42 \pm 0.32$ | $21.77 \pm 0.51$ | NS |
| C16:1 | $1.51 \pm 0.14$ | $1.82 \pm 0.08$ | $1.52 \pm 0.09$ | NS |
| C17:0 | $1.04 \pm 0.13$ | $1.06 \pm 0.10$ | $1.03 \pm 0.03$ | NS |
| C18:0 | $16.27 \pm 1.54$ | $16.63 \pm 0.99$ | $19.11 \pm 0.51$ | NS |
| C18:1n,9c | $38.51 \pm 3.56$ | $42.77 \pm 1.43$ | $39.34 \pm 1.15$ | NS |
| C18:1n,9t | $1.58 \pm 0.39$ | $1.77 \pm 0.40$ | $2.18 \pm 0.34$ | NS |
| C18:1n,11c | $0.52 \pm 0.21$ | $0.33 \pm 0.08$ | $0.40 \pm 0.06$ | NS |
| C18:2c9,t11 | $0.14 \pm 0.06$ | $0.05 \pm 0.01$ | $0.15 \pm 0.05$ | NS |
| C18:2c9, c11 | $0.25 \pm 0.07$ | $0.42 \pm 0.04$ | $0.36 \pm 0.06$ | NS |
| C18:2n-6c | $4.84 \pm 0.66^{\text {ab }}$ | $4.05 \pm 0.29^{\text {b }}$ | $6.11 \pm 0.46^{\text {a }}$ | , |
| C18:2n-6t | $0.03 \pm 0.01$ | $0.04 \pm 0.01$ | $0.05 \pm 0.01$ | NS |
| C18:3n-6 | $0.04 \pm 0.02$ | $0.06 \pm 0.01$ | $0.06 \pm 0.02$ | NS |
| C18:3n-3 | $0.28 \pm 0.06$ | $0.22 \pm 0.02$ | $0.28 \pm 0.02$ | NS |
| C20:0 | $0.30 \pm 0.09$ | $0.26 \pm 0.05$ | $0.40 \pm 0.06$ | NS |
| C20:4n-6 | $2.08 \pm 0.58$ | $1.55 \pm 0.27$ | $2.90 \pm 0.33$ | NS |
| SFA ${ }^{2}$ | $45.90 \pm 1.06$ | $45.43 \pm 1.07$ | $45.24 \pm 0.63$ | NS |
| UFA ${ }^{3}$ | $54.92 \pm 1.04$ | $55.31 \pm 0.96$ | $55.50 \pm 0.82$ | NS |
| MUFA ${ }^{4}$ | $46.56 \pm 1.56$ | $47.56 \pm 1.13$ | $44.31 \pm 1.26$ | NS |
| PUFA ${ }^{5}$ | $7.55 \pm 1.33^{\text {ab }}$ | $6.35 \pm 0.35^{\text {b }}$ | $10.45 \pm 0.33^{\text {a }}$ | * |
| n-6 | $7.07 \pm 1.42^{\text {ab }}$ | $6.38 \pm 0.69^{\text {b }}$ | $9.85 \pm 1.00^{\text {a }}$ | * |
| n-3 | $0.46 \pm 0.08$ | $0.45 \pm 0.06$ | $0.51 \pm 0.05$ | NS |
| $n-6: n-3$ | $15.79 \pm 0.86$ | $15.09 \pm 0.32$ | $15.92 \pm 0.38$ | NS |
| PUFA:SFA | $0.17 \pm 0.03^{\text {ab }}$ | $0.15 \pm 0.01^{\text {b }}$ | $0.23 \pm 0.02^{\text {a }}$ | * |
| MUFA:SFA | $1.02 \pm 0.05$ | $1.03 \pm 0.05$ | $0.98 \pm 0.04$ | NS |
| (C18:0+C18:1)/C16:0 | $2.59 \pm 0.04{ }^{\text {ab }}$ | $2.53 \pm 0.05^{\text {b }}$ | $2.82 \pm 0.11^{\text {a }}$ | * |
| $\mathrm{DFA}^{6}$ | $71.73 \pm 0.35^{\text {b }}$ | $71.17 \pm 0.39^{\text {b }}$ | $73.87 \pm 0.64{ }^{\text {a }}$ | * |
| $\mathrm{AI}^{7}$ | $0.33 \pm 0.01$ | $0.33 \pm 0.01$ | $0.30 \pm 0.01$ | NS |
| $\mathrm{TI}^{8}$ | $0.86 \pm 0.03$ | $0.86 \pm 0.04$ | $0.86 \pm 0.02$ | NS |

${ }^{\text {a,b }}$ Means followed by different lowercase letters in the same row indicate significant differences according to Tukey's test (5\%). " $p<0.05$. ${ }^{1}$ Non significant. ${ }^{2}$ Saturated fatty acids; ${ }^{3}$ Unsaturated fatty acids; ${ }^{4}$ Monounsaturated fatty acids; ${ }^{5}$ Polyunsaturated fatty acids; ${ }^{6}$ Desirable fatty acids (MUFA + PUFA + C18:0); ${ }^{7}$ Atherogenic Index $([(C 12: 0+(4 x C 14: 0)+C 16: 0)] / n 6+n 3+M U F A+C 18: 1) ;{ }^{8}$ Thrombogenic Index $(14: 0+16: 0+18: 0) /((0,5 \mathrm{x}(\mathrm{C} 18: 1+\mathrm{n} 6+\mathrm{MUFA}))+((3 \mathrm{xn} n-3)+(n-3 / n-6))$.

The results were subjected to PCA to assess the linearly independent variables, and the fatty acid profile corresponded to $95 \%$ according to the principal component analysis (Figure 1).

The findings showed that animals subjected to the highest restriction level, with significantly lower ( $\mathrm{p}<0.05$ ) OMI, had a greater decrease in the parameters tested and showed lower LWS, resulting from the low ADWG, as shown in Table 2.


Figure 1. Main component analysis of fatty acid ratios in meat from lambs subjected to quantitative feed restriction. ${ }^{1}$ Without restriction.

Studies on published nutritional aspects indicate that lamb meat has low total lipid levels, which may range from $2 \%$ to $8 \%$ (Jabbar \& Anjum, 2008; Madruga et al., 2006), in line with the levels found in the present study. The group subjected to $60 \%$ restriction showed a decrease in lipid levels, which may be associated with lipid synthesis by the animal. In a study characterizing Santa Inês lamb meat, Madruga, Sousa, Rosales, Cunha, and Ramos (2005), concluded that diet had a significant effect on cholesterol levels, indicating that feed may improve meat quality.

Irshad et al. (2012), using restriction levels of feed, cited that animals prioritize vital systems, for example, the nervous, respiratory, bone, and muscle systems, and ultimately lipid deposition. Another key aspect is the physiological maturity of each tissue, which differs according to the phase of development of the animal: bone tissue develops earlier, followed by muscle tissue and then adipose tissue. Furthermore, muscles grow faster in younger animals, and fat deposition is higher in older animals. These aspects account for the differences in the chemical composition found, even in carcasses from animals with similar muscle tissue ratios (Hausman, Bergen, Etherton, \& Smith, 2018) and initial weights, as observed in the present study.

Conversely, the decrease in cholesterol levels is related to lipid metabolism in the liver. Although dietary cholesterol has no significant effects on its serum levels in ruminants, biliary cholesterol is reabsorbed and synthesized from acetate in the intestinal mucosa (Zeng, Umar, Rust, Lazarova, \& Bordonaro, 2019). Thus, animals fed less feed ( 30 and $60 \%$ restriction) showed decreased cholesterol synthesis because biliary cholesterol may have been metabolized faster because of the decreased amount of feed absorbed. Arruda et al. (2012) reported cholesterol levels ranging from 21.74 to $54.06 \mathrm{mg} 100 \mathrm{~g}^{-1}$ in the longissimus dorsi muscle of Santa Inês lamb, which the authors considered a lean meat given the characteristics observed; those values are similar to those observed in muscle from the feed restriction treatments in the present study, not exceeding $51.05 \mathrm{mg} 100 \mathrm{~g}^{-1}$. Costa et al. (2009) assessed the fat profile of different lamb genotypes in the Brazilian Northeast and reported levels of $65.88 \mathrm{mg} 100 \mathrm{~g}^{-1}$ in the Santa Inês breed, lower than those of the WR of the present study, most likely resulting from differences in feed quantity and nutritional quality.

In this context, importantly, human dietary cholesterol intake should be lower than $300 \mathrm{mg}^{\text {day }}{ }^{-1}$ to help control cholesterolemia; cholesterol is a vital component of the body, essential for cell membrane synthesis and sex hormone, vitamin $D$ and digestive juice production, and plays a key role in nervous tissues and bile salts formation (Santos et al., 2013). Classical epidemiological studies show a strong association between high cholesterol intake and increased atherosclerosis incidence. Thus, consumers should seek to decrease cholesterol intake to avoid possible diseases related to fat accumulation in blood. Therefore, feed restriction could be recommended because it leads to lower cholesterol levels in the meat of these animals.

## Fatty acids evaluation

Dietary lipids are extensively metabolized through two processes: lipolysis and biohydrogenation. Enzymes (lipases) are able to hydrolyze "ester" type bonds and release free fatty acids through lipolysis,
which was not observed in animals under feed restriction, most likely because they showed the same quantity of microbial mass, despite the feed reduction at each level of restriction. Butyrivibrio fibrisolvens, Anaerovibrio lipolytica, and Propionibacterium bacteria are considered the main ruminal microorganisms responsible for lipolysis (Lourenço, Ramos-Morales, \& Wallace, 2010).

Meat is an important source of PUFA, including arachidonic acid (C20:4n-6), which is an essential fatty acid that reduces the risk of thrombosis (Christophersen \& Haug, 2011). Notably, the concentration of C20:4n-6 did not differ between the restriction levels. Considering that this fatty acid is produced through the ruminal processes of elongation and desaturation of the intermediate products of biohydrogenation (Tran, Malla, Kumar, \& Tyagi, 2017), feed restriction had no effect on its synthesis, most likely because the diet was the same qualitatively. That is, with the same nutrient levels and with only a set quantity available, the amount of nutrients ingested and absorbed was varied. Thus, the group with $60 \%$ restriction was favored because it showed a trend toward increased levels of C20:4n-6, with no difference from the control group (WR), in which C20:4n-6 was assimilated with the same efficiency. Thus, this pattern corroborates the fat profile assessment because it showed increased intramuscular fat deposition, as outlined in Table 5.

Studies suggest that cholesterol concentrations are affected by the dietary composition of fatty acids, wherein the levels of C18:1 decreased, C16:0 increased, and C18:0 caused no difference in blood serum cholesterol levels (Madruga et al., 2006). However, no significant differences in the levels of these fatty acids ( $p>0.05$ ) were observed that might explain the differences in cholesterol levels between the studied diets.

The percentage of CLA ( $\mathrm{C} 18: 2 c 9, c 11$ ) tends to increase with feed restriction, and this fatty acid modulates lipid metabolism, inhibiting the synthesis of fatty acids and the activity of lipogenic enzymes (Costa et al., 2018). Thus, the tissue concentration of CLA reflects the quantity available for absorption and, therefore, is directly affected by the quantity of dietary lipids, which will determine the ratio of muscle and adipose tissues. CLA acts as an intermediary during ruminal microbial biohydrogenation, under normal dietary conditions, and C18:0 (Buccioni, Decandia, Minieri, Molle, \& Cabiddu, 2012) which was one of the fatty acids quantified at the highest levels in meat and intermuscular and subcutaneous fat, is the main product.

It is important to emphasize that the excessive intake of SFA, importantly, may promote adipose tissue expansion and the release of inflammatory proteins such as cytokines and chemokines that induce inflammation and insulin resistance, thus increasing the risk for cardiovascular diseases and metabolic syndrome (Melo, Santos \& Ferreira, 2019). In this context, the feeding management in sheep farming that enables fat deposition with higher feed concentrations of desirable fatty acids, especially due to the presence of PUFA, becomes very important for nutrition because it ensures the intake of food beneficial to consumer health with anticarcinogenic and antioxidant properties, thereby preventing several chronic diseases (Berrighi et al., 2017).

In the present study, the percentage of DFA was higher ( $p<0.05$ ) at the $60 \%$ feed restriction, in both intramuscular and intermuscular fat and in meat, favoring the use of alternative diets when the objective is to obtain good quality carcasses. The DFA occurred at a maximum of $73.86 \%$, which was very close to the optimal value reported by Banskalieva, Sahlu, and Goetsch (2000) and other studies (Madruga et al., 2005). In the present study, the results suggest that the nutritional regime affects the percentage of fat that will be deposited, albeit without changing its composition, which may be considered an excellent factor in reducing production costs (Yáñez et al., 2006).

Thus, lamb meat has high levels of saturated fat, most likely resulting from ruminal biohydrogenation, wherein the dietary fatty acids are saturated and thus absorbed and incorporated into muscle tissue, which may affect its levels in intramuscular fat. No significant differences ( $p>0.05$ ) in these fatty acids were detected in the meat, despite the higher levels observed in the intramuscular and intermuscular fat.

The PUFA values were higher than those reported by Madruga et al. (2005), who studied the meat quality of Santa Inês lambs finished with different diets and observed levels ranging from 2.25 to $5.01 \%$.

In the present study, higher values ( $\mathrm{p}<0.05$ ) of these fatty acids were detected in the meat from the control treatment than in the meat from the highest feed restriction treatments, and this pattern is most likely explained by the high percentage of linoleic acid found in the treatments, resulting in an increased PUFA:SFA ratio, which is very important for consumer health (Wang, Chen, Luo, Liu, \& Liu, 2015).

However, the PUFA:SFA ratio of most treatments was considered low, despite the significantly higher value ( $p<0.05$ ) detected with $60 \%$ feed restriction, similar to the control diet and studies conducted by Komprda et al. (2012) and Yakan and Ünal (2010), who observed a ratio ranging from 0.13 to 0.23 when analyzing meat of the same species. In this context, most lamb meats may be classified as unfavorable when considering values ranging from 0.4 to 0.7 as the optimal parameter. (Nieto \& Ros, 2012). Despite this pattern, the levels of PUFA found in the meat from the $60 \%$ feed restriction were significantly higher ( $\mathrm{p}<0.05$ ) than that found in the meat from the $30 \%$ restriction, albeit similar to the control, which is relevant from a nutritional standpoint. Furthermore, lamb meat is affected by its own composition because it predominantly contains SFA, which keeps the values of this ratio low, and is rich in 16:0, 18:0, and 18:1 fatty acids (Fernandes, Trindade, Lorenzo, \& Melo, 2018).

## Composition of fatty acids in intramuscular, intermuscular and subcutaneous fats and influence of feed restriction

Given the increasing consumer awareness of healthy eating, the intramuscular fat composition of fatty acids is one of the most important traits (Wilches et al., 2011). Furthermore, regarding the beneficial effects of the different fatty acids found, the ( $\mathrm{C} 18: 0+\mathrm{C} 18: 1$ )/C16:0 ratio suggests that lamb meat, that is, intramuscular fat, has a good quality lipid fraction because it maintained values ranging from 2.10 to 2.80 , similar to those reported by Arruda et al. (2012) when assessing the longissimus dorsi muscle of Santa Inês lambs fed feeds with different levels of energy. Although C18:0 is saturated, its effect is neutral and has minor implications to the fat profile because it may be converted into oleic acid (C18:1) in the body (Banskalieva et al., 2000), with no significant differences ( $p>0.05$ ) among diets in the present study.

The results showed that the data from this experiment were similar to those found in the study by Terré, Nudda, Boe, Gaias, and Bach (2011), who evaluated the fatty acid profile of the longissimus dorsi muscle of lambs fed diets supplemented with different amounts of CLA and observed $n-6$ values ranging from 7 to $8 \%$, similar to those of the present study, which did not exceed 9.94\%.

According to the multivariate analysis, the first main component best explains the original data, given the high percentage found. Therefore, these variables are representative, based on linear combinations, corroborating Vainionpää et al. (2000). Thus, the PUFA, DFA, n-6 and C18:2n-6 ratios were higher, with greater variability, indicating their close relation to meats from animals subjected to $60 \%$ feed restriction.

The fat fatty acid profiles were significantly different ( $p<0.05$ ), and 17 fatty acids were identified-four SFA, five MUFA, and eight PUFA - and the latter were generally the least abundant (Table 5). An interaction effect was observed between the restriction levels and types of fat regarding the sum of fatty acids, and the intermuscular fat SFA, UFA, and MUFA levels were significantly affected by the diet ( $\mathrm{p}<0.05$ ), albeit with no difference between WR and the increased restriction level ( $p>0.05$ ). Conversely, the highest PUFA levels were observed in the intramuscular fat at $60 \%$ restriction, whereas the same variable showed lower levels than WR in the subcutaneous fat ( $\mathrm{p}<0.05$ ).

For all fatty acids tested, no interaction effect was observed between the restriction levels and the types of fat, except for lauric (C12:0) and elaidic (C18:1t) acid. Thus, no significant difference in intramuscular and intermuscular fat C14:0 was observed between WR and $30 \%$ restriction ( $p>0.05$ ), in contrast to the subcutaneous fat, wherein feed restriction caused a significant decrease in this fatty acid ( $\mathrm{p}<0.05$ ). A higher accumulation of subcutaneous fat than intramuscular fat occurred at the $60 \%$ restriction ( $\mathrm{p}<0.05$ ) level. Increased levels of C16:0 were detected in the intramuscular and intermuscular tissues ( $p<0.05$ ), and the content was similar between WR and $30 \%$ restriction ( $p<0.05$ ).

Regardless of the feed restriction level, stearic (C18:0), palmitic (16:0) and oleic (C18:1n-9c) acids were the fatty acids found at the highest concentrations, in descending order, similar to the profile trends of this type of meat found by other studies (Hajji et al., 2016). In the present study, these fatty acids accounted for approximately $80 \%$ of all fatty acids, particularly oleic acid, which contributed considerably to this ratio, showing higher levels ( $\mathrm{p}<0.05$ ) in subcutaneous fat in general.

Lopes et al. (2012) suggested that a possible explanation for the higher and lower animal fat values of oleic acid and palmitic acid, respectively, may be related to the increased conversion of palmitic acid into oleic acid through elongation and desaturation. Mushi et al. (2010), similar to the present study, detected no differences in palmitic acid when assessing the intramuscular fat composition of 5-month-old goats subjected to a similar feed restriction in an intensive production system.

Acta Scientiarum. Animal Sciences, v. 42, e4905, 2020

Table 5. Fatty acid profile (\% area) of lambs subjected to quantitative feed restriction ( ${ }^{1}$ Without restriction, 30\% and 60\%).

|  | Intramuscular |  |  | Intermuscular |  |  | Subcutaneous |  |  | SE | $\mathrm{LWS}_{\text {Ior }}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | WR ${ }^{1}$ | 30\% | 60\% | WR | 30\% | 60\% | WR | 30\% | 60\% |  | $\mathrm{p}_{\text {fat }}$ | $\mathrm{p}_{\text {restriction }}$ | interaction |
| C12:0 | $0.20{ }^{\text {Aa }}$ | $0.16{ }^{\text {Aa }}$ | $0.20{ }^{\text {Aa }}$ | $0.07{ }^{\text {Ab }}$ | $0.08{ }^{\text {Ab }}$ | $0.06{ }^{\text {Ab }}$ | $0.16{ }^{\text {Aa }}$ | $0.20{ }^{\text {Aa }}$ | $0.21{ }^{\text {Aa }}$ | 0.02 | <0.0001 | 0.4301 | 0.0540 |
| C14:0 | $1.88{ }^{\text {ABc }}$ | $2.00^{\text {Aa }}$ | $1.61{ }^{\text {Bb }}$ | $2.33{ }^{\text {Ab }}$ | $2.11{ }^{\text {ABa }}$ | $1.85{ }^{\text {Bab }}$ | $2.71{ }^{\text {Aa }}$ | $1.78{ }^{\text {Ba }}$ | $2.05^{\text {Ba }}$ | 0.14 | 0.0001 | <0.0001 | <0.0001 |
| C14:1 | $0.08{ }^{\text {Ac }}$ | $0.09{ }^{\text {Ab }}$ | $0.07{ }^{\text {Ab }}$ | $0.39^{\text {Ab }}$ | $0.35^{\text {ABab }}$ | $0.10^{\text {Bb }}$ | $1.52^{\mathrm{Aa}}$ | $0.52^{\text {Ba }}$ | $1.52{ }^{\text {Aa }}$ | 0.11 | <0.0001 | $<0.0001$ | <0.0001 |
| C16:0 | $23.77^{\text {Aa }}$ | $24.42{ }^{\text {Aa }}$ | $21.77{ }^{\text {Ba }}$ | $22.47{ }^{\text {Aa }}$ | $22.82^{\text {Aa }}$ | $21.31{ }^{\text {Aa }}$ | $19.08^{\text {Ab }}$ | $20.23{ }^{\text {Ab }}$ | $20.67^{\text {Aa }}$ | 0.76 | <0.0001 | 0.0227 | 0.0114 |
| C16:1 | 1.63 | $1.82^{\text {Ab }}$ | $1.52^{\text {Ab }}$ | 1.55 | $1.78{ }^{\text {Ab }}$ | 1.1 | $3.59{ }^{\text {Aa }}$ | $3.17{ }^{\text {Aa }}$ | 3.4 | 0.19 | <0.0001 | 0.0826 | 0.0121 |
| C18:0 | $17.62^{\text {Ab }}$ | $16.63{ }^{\text {Ab }}$ | $19.11^{\text {Ab }}$ | $26.48{ }^{\text {Ba }}$ | $23.49{ }^{\text {Ba }}$ | $31.37{ }^{\text {Aa }}$ | $9.57{ }^{\text {Ac }}$ | $11.87{ }^{\text {Ac }}$ | $10.50^{\text {Ac }}$ | 1.26 | <0.0001 | 0.0002 | <0.0001 |
| C18:1t | $2.18{ }^{\text {Ab }}$ | $2.37{ }^{\text {Ab }}$ | $2.52^{\text {Ab }}$ | $2.81{ }^{\text {Ba }}$ | $3.09{ }^{\text {ABa }}$ | $3.42{ }^{\text {Aa }}$ | $2.03{ }^{\text {Ab }}$ | $1.99{ }^{\text {Ab }}$ | $1.83{ }^{\text {Ac }}$ | 0.19 | <0.0001 | 0.0800 | 0.0577 |
| C18:1c9 | $41.76{ }^{\text {Aa }}$ | $42.77{ }^{\text {Aab }}$ | $39.34{ }^{\text {Ab }}$ | $35.97{ }^{\text {ABb }}$ | $38.87{ }^{\text {Ab }}$ | $32.81{ }^{\text {Bc }}$ | $40.59^{\text {Ba }}$ | $43.92{ }^{\text {ABa }}$ | $45.31{ }^{\text {Aa }}$ | 1.77 | <0.0001 | 0.0193 | 0.0091 |
| C18 | $0.44{ }^{\text {Ab }}$ | $0.45{ }^{\text {Ab }}$ | $0.46{ }^{\text {Ab }}$ | $0.61{ }^{\text {Ba }}$ | $0.61{ }^{\text {Ba }}$ | $0.82^{\text {Aa }}$ | $0.21{ }^{\text {Bc }}$ | $0.19{ }^{\text {Bc }}$ | $0.38{ }^{\text {Ab }}$ | 0.03 | <0.0001 | <0.0001 | . 0002 |
| C18:2c9,c11 | 0.36 | $0.42^{\text {Ab }}$ | 0. | 0.42 | 0.6 | $0.55^{\text {Ab }}$ | 0.5 | 0.60 | $0.77{ }^{\text {Aa }}$ | 0.04 | <0.0001 | $<0.0001$ | 0.0002 |
| C18:2c9,t11 | $0.32^{\text {Ab }}$ | $0.05^{\mathrm{Cab}}$ | $0.13^{\text {Ba }}$ | $0.50{ }^{\text {Aa }}$ | $0.00^{\text {Bb }}$ | $0.00^{\text {Bb }}$ | $0.24{ }^{\text {Ab }}$ | $0.11^{\mathrm{Ba}}$ | $0.19^{\text {Aa }}$ | 0.03 | 0.5426 | <0.0001 | <0.0001 |
| C18:2n-6c | $3.99^{\text {Ba }}$ | $4.05^{\text {Ba }}$ | $6.11{ }^{\text {Aa }}$ | $2.69{ }^{\text {Ab }}$ | $2.23{ }^{\text {Ab }}$ | $2.84{ }^{\text {Ab }}$ | $2.35{ }^{\text {Ab }}$ | $2.05{ }^{\text {Ab }}$ | $2.03{ }^{\text {Ac }}$ | 0.30 | <0.0001 | <0.0001 | <0.0001 |
| C18:2n-6t | $0.06{ }^{\text {Ab }}$ | $0.07{ }^{\text {Ab }}$ | $0.06{ }^{\text {ab }}$ | $0.08{ }^{\text {ABb }}$ | $0.10^{\text {Ab }}$ | $0.03{ }^{\text {Bb }}$ | $0.60{ }^{\text {Aa }}$ | $0.32{ }^{\text {Ba }}$ | $0.37{ }^{\text {Ba }}$ | 0.03 | <0.0001 | <0.0001 | <0.0001 |
| C18:3n-6 | $0.08^{\text {Ab }}$ | $0.09{ }^{\text {Ab }}$ | $0.09{ }^{\text {Ab }}$ | $0.05^{\text {Bb }}$ | $0.08{ }^{\text {ABb }}$ | $0.09^{\text {Ab }}$ | $0.16^{\text {Aa }}$ | $0.13{ }^{\text {Ba }}$ | $0.17{ }^{\text {Aa }}$ | 0.01 | <0.0001 | 0.0083 | 0.0177 |
| C18:3n-3 | $0.25^{\text {Aa }}$ | $0.22^{\text {Aa }}$ | $0.26{ }^{\text {Aa }}$ | $0.04{ }^{\text {Bb }}$ | $0.16{ }^{\text {Ab }}$ | $0.19{ }^{\text {Ab }}$ | $0.21{ }^{\text {Aa }}$ | $0.18{ }^{\text {Aab }}$ | $0.20{ }^{\text {Ab }}$ | 0.02 | <0.0001 | 0.0001 | <0.0001 |
| C20:4n-6 | $1.30{ }^{\text {Bb }}$ | $1.42{ }^{\text {Ba }}$ | $2.90{ }^{\text {Aa }}$ | $0.12^{\text {Ac }}$ | $0.07{ }^{\text {Ab }}$ | $0.09^{\text {Ab }}$ | $3.27{ }^{\text {Aa }}$ | $1.04{ }^{\text {Ba }}$ | $0.13{ }^{\text {cb }}$ | 0.20 | <0.0001 | <0.0001 | <0.0001 |
| C20:5n-3 | $0.07{ }^{\text {Bb }}$ | $0.25^{\text {Aa }}$ | $0.09{ }^{\text {Ba }}$ | $0.00^{\text {Ac }}$ | $0.00^{\text {Ab }}$ | $0.00^{\text {Ab }}$ | $0.19^{\text {Aa }}$ | $0.00^{\text {Bb }}$ | $0.00^{\text {Bb }}$ | 0.02 | <0.0001 | <0.0001 | <0.0001 |
| Others | $3.47{ }^{\text {Ab }}$ | $3.43{ }^{\text {Ab }}$ | $3.78{ }^{\text {Ab }}$ | $3.85{ }^{\text {Ab }}$ | $3.47{ }^{\text {Ab }}$ | $3.28{ }^{\text {Ab }}$ | $14.05^{\text {Aa }}$ | $13.31{ }^{\text {Aa }}$ | $7.80{ }^{\text {Ba }}$ | 0.79 | <0.0001 | <0.0001 | <0.0001 |
| $\Sigma$ SFA $^{2}$ | $43.47^{\text {Ab }}$ | $43.21^{\text {Ab }}$ | $42.70^{\text {Ab }}$ | $51.35{ }^{\text {ABa }}$ | $48.50^{\text {Ba }}$ | $54.58{ }^{\text {Aa }}$ | 31.53 Ac | $34.08^{\text {Ac }}$ | $33.43{ }^{\text {Ac }}$ | 1.42 | <0.0001 | 0.0992 | 0.0037 |
| $\Sigma \mathrm{UFA}^{3}$ | $52.43{ }^{\text {Aa }}$ | $53.98{ }^{\text {Aa }}$ | $53.91^{\text {Aa }}$ | $44.84{ }^{\text {ABb }}$ | $47.62{ }^{\text {Ab }}$ | $42.00^{\text {Bb }}$ | $53.95{ }^{\text {Aa }}$ | $53.70^{\text {Aa }}$ | $54.84{ }^{\text {Aa }}$ | 1.59 | <0.0001 | 0.2013 | 0.0367 |
| EMUFA ${ }^{4}$ | $46.01{ }^{\text {Aa }}$ | $47.41^{\text {Aab }}$ | $43.85{ }^{\text {ab }}$ | $40.94{ }^{\text {ABb }}$ | $44.35{ }^{\text {Ab }}$ | $38.21{ }^{\text {Bc }}$ | $46.42{ }^{\text {Ba }}$ | $49.27^{\text {ABa }}$ | $50.97{ }^{\text {Aa }}$ | 1.77 | <0.0001 | 0.0166 | 0.0108 |
| $\Sigma$ PUFA $^{5}$ | $6.42{ }^{\text {Ba }}$ | $6.57{ }^{\text {Ba }}$ | $10.06^{\text {Aa }}$ | $3.90{ }^{\text {Ab }}$ | $3.27{ }^{\text {Ab }}$ | $3.78{ }^{\text {Ab }}$ | $7.53^{\text {Aa }}$ | $4.43{ }^{\text {Bb }}$ | $3.86{ }^{\text {Bb }}$ | 0.49 | <0.0001 | $<0.0001$ | <0.0001 |
| $\Sigma$ DFA $^{6}$ | $70.04{ }^{\text {Aa }}$ | $70.61{ }^{\text {Aa }}$ | $73.01^{\text {Aa }}$ | $71.32^{\text {Aa }}$ | $71.10^{\text {Aa }}$ | $73.36{ }^{\text {Aa }}$ | $63.52^{\text {Ab }}$ | $65.57{ }^{\text {Ab }}$ | $65.33^{\text {Ab }}$ | 1.32 | <0.0001 | 0.0137 | 0.5661 |
| PUFA:SFA ${ }^{7}$ | $0.15{ }^{\text {Bb }}$ | $0.15{ }^{\text {Ba }}$ | $0.24{ }^{\text {Aa }}$ | $0.08{ }^{\text {Ac }}$ | $0.07{ }^{\text {Ab }}$ | $0.07{ }^{\text {Ac }}$ | $0.24{ }^{\text {Aa }}$ | $0.13{ }^{\text {Ba }}$ | $0.12^{\text {Bb }}$ | 0.01 | <0.0001 | <0.0001 | <0.0001 |
| MUFA:SFA ${ }^{8}$ | $1.06{ }^{\text {Ab }}$ | $1.10^{\text {Ab }}$ | $1.03{ }^{\text {Ab }}$ | $0.81{ }^{\text {ABc }}$ | $0.92{ }^{\text {Ac }}$ | $0.70^{\text {BC }}$ | $1.48{ }^{\text {Aa }}$ | $1.45{ }^{\text {Aa }}$ | $1.53{ }^{\text {Aa }}$ | 0.06 | <0.0001 | 0.1388 | 0.0183 |
| $\mathrm{AI}^{9}$ | $0.33^{\text {Ab }}$ | $0.33{ }^{\text {Aa }}$ | $0.30^{\text {Ab }}$ | $0.38{ }^{\text {Aa }}$ | $0.35{ }^{\text {Aa }}$ | $0.37{ }^{\text {Aa }}$ | $0.31{ }^{\text {Ab }}$ | $0.28{ }^{\text {Ab }}$ | $0.29{ }^{\text {Ab }}$ | 0.02 | <0.0001 | 0.0121 | 0.1719 |
| TI ${ }^{10}$ | $0.89{ }^{\text {Ab }}$ | $0.85{ }^{\text {Ab }}$ | $0.87{ }^{\text {Ab }}$ | $1.25{ }^{\text {Aa }}$ | $1.07{ }^{\text {Ba }}$ | $1.38{ }^{\text {Aa }}$ | $0.64{ }^{\text {Ac }}$ | $0.68{ }^{\text {Ac }}$ | $0.65{ }^{\text {Ac }}$ | 0.06 | <0.0001 | 0.0247 | 0.0025 |
| (C18:0+C18:1):C16:0 | $2.61{ }^{\text {ABb }}$ | $2.55{ }^{\text {Bb }}$ | $2.84{ }^{\text {Ab }}$ | $2.94{ }^{\text {Ba }}$ | $2.90{ }^{\text {Ba }}$ | $3.21{ }^{\text {Aa }}$ | $2.77{ }^{\text {Aab }}$ | $2.87{ }^{\text {Aa }}$ | $2.82{ }^{\text {Ab }}$ | 0.10 | <0.0001 | 0.0024 | 0.1103 |
| $n-6{ }^{11}$ | $5.42{ }^{\text {Ba }}$ | $5.63{ }^{\text {Ba }}$ | $9.17^{\text {Aa }}$ | $2.94{ }^{\text {Ab }}$ | $2.48{ }^{\text {Ab }}$ | $3.04{ }^{\text {Ab }}$ | $6.37{ }^{\text {Aa }}$ | $3.53{ }^{\text {Bb }}$ | $2.70^{\text {Bb }}$ | 0.47 | <0.0001 | 0.0002 | <0.0001 |
| $n-3^{12}$ | $0.32{ }^{\text {Bb }}$ | $0.48{ }^{\text {Aa }}$ | $0.35{ }^{\text {Ba }}$ | $0.04{ }^{\text {BC }}$ | $0.16{ }^{\text {Ab }}$ | $0.19^{\text {Ab }}$ | $0.40{ }^{\text {Aa }}$ | $0.18{ }^{\text {Bb }}$ | $0.20{ }^{\text {Bb }}$ | 0.02 | <0.0001 | 0.2362 | <0.0001 |
| $n-6: n-3^{13}$ | $17.69^{\text {Ab }}$ | $12.05^{\text {Aa }}$ | $25.73{ }^{\text {Aa }}$ | $16.29{ }^{\text {Aa }}$ | $16.04{ }^{\text {Aa }}$ | $15.90^{\text {Aa }}$ | $16.02{ }^{\text {Ab }}$ | $19.87^{\mathrm{Aa}}$ | $13.87{ }^{\text {Aa }}$ | 20.80 | 0.0001 | <0.0001 | <0.0001 |

*Means followed by the same uppercase letter in rows (between types of fats) and the same lowercase letter between rows (restriction levels) are not significantly different from each other according to Tukey's test (5\%). ${ }^{2}$ Saturated fatty acids; ${ }^{3}$ Unsaturated fatty acids; ${ }^{4}$ Monounsaturated fatty acids; ${ }^{5}$ Polyunsaturated fatty acids; ${ }^{6}$ Desirable fatty acids (MUFA+PUFA+C18:0); ${ }^{7}$ Ratio between polyunsaturated and saturated fatty acids; ${ }^{8}$ Ratio between monounsaturated and saturated fatty acids; ${ }^{9}$ Atherogenic index (C12:0+(4*C14:0) $\left.+\mathrm{C} 16: 0\right) /((n-6+n-3)+\mathrm{MUFA}+\mathrm{C} 18: 1) ;{ }^{10}$ Thrombogenic index $(14: 0+16: 0+18: 0) /\left(\left(0.5^{*}(\mathrm{C} 18: 1+n-6+\mathrm{MUFA})\right)+\left(\left(3^{*} n-3\right)+(n-3 / n-6)\right) ;{ }^{11}\right.$ Omega-6; ${ }^{12}$ Omega-3; ${ }^{13}$ Ratio between Omega- 6 and Omega- 3.

In general, the analysis of restriction levels with regard to the types of fat showed that the intermuscular C18:0 levels were significantly higher ( $\mathrm{p}<0.05$ ) in samples from animals fed diets with $60 \%$ feed restriction. These values were higher than those reported by Leão et al. (2011) when analyzing the longissimus dorsi muscle of lambs subjected to two levels of concentrate. However, no significant difference was observed in the other types of fat between the restriction levels, thus indicating that carcasses with the same muscle tissue ratio and fat levels were obtained even with different restriction levels, with a trend toward decreased fat cover (Irshad et al., 2012).

Stearic acid and oleic acid have a hypocholesterolemic function because they increase the plasma levels of high-density lipoprotein (HDL) and are able to absorb cholesterol crystals (Lopes et al., 2012). The importance of palmitic acid in feedlot lamb meat is reported because it is found in high amounts in meat fat and is also positively correlated with increased blood cholesterol, most likely resulting from the decreased activity of the low-density lipoprotein (LDL) receptor (Romero-Bernal, Almaraz, Ortega, Salas, \& GonzálezRonquillo, 2017). The analysis of the restriction levels shows homogeneity of stearic acid and oleic acid in intramuscular fat, most likely because animal feed restriction promoted improved efficacy of nutrient use by ruminal microbiota for fatty acid production (Rocha Júnior et al., 2015).

The $30 \%$ feed restriction promoted increased C18:1c9 accumulation in intermuscular fat and subcutaneous fat, similarly to the WR treatment ( $\mathrm{p}>0.05$ ), although this fatty acid was lower in intramuscular fat and intermuscular fat at the $60 \%$ restriction level, which may be explained by the late increase of these fats in relation to the subcutaneous fat in the same animal body region (Paulino et al., 2009). C18:2n-6c showed a higher value at $60 \%$ restriction ( $p<0.05$ ) in intramuscular fat, whereas C18:2n- $6 t$ showed higher levels in subcutaneous fat, although these levels were similar in the treatments with $30 \%$ and $60 \%$ restriction.

The ability to incorporate CLA into intramuscular fat is notable in sheep, facilitating the availability of this substance in the edible portion (Alves et al., 2012). The similarity ( $\mathrm{p}>0.05$ ) existing between the intramuscular fat $\mathrm{C} 18: 2 c 9, c 11$ levels at each restriction level was observed in the present study, with a pattern similar to $\mathrm{C} 18: 3 n-3$, which affects the tissue CLA content resulting from endogenous production, and both fatty acids are considered beneficial to health (Fuet et al., 2018).

## Relationship between fatty acids obtained and risk factors

Different ratios of fatty acids (SFA, UFA, MUFA, PUFA, DFA, PUFA:SFA, MUFA:SFA, AI, TI, (C18:0+C18:1)/C16:0, $n-6, n-3$, and $n-6: n-3$ ) in the human diet have been suggested as a way to evaluate dietary risk factors for increased blood cholesterol levels because SFA increases serum cholesterol (Costa et al., 2009), whereas UFA contributes to its reduction, thereby decreasing low-density lipoproteins (LDL, Pizzini et al., 2017). However, SFA are related to cardiovascular diseases, whereas PUFA, particularly $\alpha-$ linolenic acid and conjugated linoleic acid, decrease the risk for cancer, cardiovascular diseases, and type 2 diabetes and affect brain development and cerebral function (Ferguson et al., 2010).

In this context, the PUFA:SFA ratio is commonly used to analyze the nutritional value of oils and fats and indicates the cholesterolemic potential (Arruda et al., 2012). A significant interaction existed between the types of fat and the restriction levels; the highest PUFA:SFA ratios ( $\mathrm{p}<0.05$ ) were found in the subcutaneous fat WR and the intramuscular fat with $60 \%$ restriction, and the PUFA:SFA ratios were similar ( $p>0.05$ ) between WR and $30 \%$ restriction.

The atherogenic (AI) and thrombogenic (TI) indices are key factors, and despite the lack of significant effects regarding the restriction levels, the findings of the present study may be considered good results in the context of feed restriction because they did not differ ( $p>0.05$ ) from the control. The AI value found in the study was lower than that reported by Costa et al. (2009), who detected a low value (0.68) for this parameter in Santa Inês lamb meat. Thus, the meat of the animals subjected to feed restriction may be considered ideal for human consumption, both economically and because of the similar potential for health benefits, including the possible prevention of the onset of chronic and degenerative diseases due to the similar $(p>0.05)$ atherogenic and thrombogenic effects.

Fatty acids may promote or prevent the onset of atherosclerosis and coronary thrombosis, based on their effects on serum cholesterol and LDL cholesterol concentrations (Siri-Tarino, Chiu, Bergeron, \& Krauss, 2015). These authors report that the AI and TI highlight the importance of unsaturated lipids for addressing issues resulting from the excessive intake of saturated lipids. All UFA with one or several double bonds contribute to decreasing these indices, indicating the potential stimulation of platelet aggregation, that is, the lower these values are, the higher the amount of anti-atherogenic fatty acids present in a specific fat tissue and the lower the potential to prevent the onset of coronary heart disease (Aguiar et al., 2017; Sokoła-Wysoczanska, 2018). In the present study, the subcutaneous fat showed a significantly lower (p < 0.05 ) TI, followed by the intramuscular and intermuscular fat.

Among the 13 ratios assessed, no interaction effect was observed for DFA, AI, and (C18:0+C18:1)/C16:0. Therefore, the main factors (restriction levels and types of fat) were analyzed separately. In the present study, at $60 \%$ restriction, the intramuscular and subcutaneous fat showed the lowest AIs, whereas the percentage of DFA was higher ( $\mathrm{p}<0.05$ ) in the intramuscular and intermuscular fat regardless of the restriction.

The development of adipose tissue occurs by hyperplasia (increase in cell number) and hypertrophy resulting from fat accumulation in the cytoplasm, which increases the size of adipocytes. When animals reach the finishing phase, the fat deposits that develop earlier (intermuscular, perirenal, and mesenteric) have already completed their hyperplastic development and begin to deposit fat in adipocytes, whereas subcutaneous and intramuscular fat deposits continue to recruit new cells, while at the same time filling them with fat. This fat works as a thermal insulator, reducing the rate of carcass cooling and the risk for cold shortening during the meat maturation process (Paulino et al., 2009). However, the dietary lipid composition directly affects the carcass fat profile, and lipids, especially PUFA, are modified by ruminal microorganisms, affecting the skeletal muscle fatty acid content and composition (Arruda et al., 2012).

In this context, the PUFA:SFA ratio was higher in the intramuscular fat and subcutaneous fat, and interaction effects were observed between the restriction levels ( $p<0.05$ ). The increased in the PUFA:SFA ratio is important for reducing the risk for cardiovascular diseases, and this ratio is recommended to be 0.40 at most (Andreo et al., 2016; Lopez-Huertas, 2010). Thus, the PUFA:SFA ratio did not exceed 0.24, and a
lower ratio ( $\mathrm{p}<0.05$ ) was observed for the intermuscular fat, most likely resulting from unsaturated fatty acid biohydrogenation through ruminal microflora activity (Buccioni et al., 2012).

Higher levels of UFA and MUFA were observed in intramuscular and subcutaneous fat when the restriction levels were assessed, and a significant difference was observed for PUFA ( $p<0.05$ ), with increased intramuscular fat deposition in tissue from animals subjected to $60 \%$ feed restriction. At this restriction level, the animals were fed a strict diet, restricting the amount of feed supplied, wherein the maximum dietary nutrient absorption most likely occurred, thereby rendering the PUFA:SFA ratio nutritionally favorable. Feed restriction (60\%) in this group of animals showed improved nutrient assimilation, with trends toward a decreased percentage of MUFA and increased PUFA, resulting from the increased accessibility to ruminal microorganisms during the ruminal fermentation process before gastric and intestinal digestion. Thus, their tissue concentration is directly associated with the availability for absorption (Arruda et al., 2012).

The sum of $n-3$ fatty acids was significantly higher ( $p>0.05$ ) for intramuscular and intermuscular fat with $30 \%$ feed restriction, which favors the dietary intake of these fats. On average, SFA accounted for $43 \%$ of the total fatty acid profile in intramuscular fat, $52 \%$ in intermuscular fat, and $33 \%$ in subcutaneous fat, and intermuscular fat showed significantly higher levels ( $p<0.05$ ). The $60 \%$ feed restriction treatment, compared to $30 \%$ feed restriction, showed higher levels of intermuscular fat deposition, and these restriction levels may be considered detrimental to human health. Furthermore, the DFA levels in intramuscular and intermuscular fat were similar, with no significant difference between different diets ( p > 0.05 ). The values found were similar to those reported by Madruga et al. (2005), who analyzed Santa Inês lamb meat and observed values ranging from 70.27 to $72.48 \%$.

Fatty acids of the $n-6$ families are obtained from the diet or produced in the body from linoleic acid (C18:2n$6 c$ ) via the activity of the elongase enzyme (Monroig \& Kabeya, 2018). They are also prostaglandin precursors important for hormone metabolism regulation, including cholesterol synthesis, with pro-inflammatory activity (Anjo, 2004). In this context, this fatty acid showed increased intramuscular fat deposition with $60 \%$ feed restriction, confirming the results observed for $n-6$ percentage ( $p<0.05$ ). Thus, this showed excess of linoleic acid, most likely accounted for $n-6$ conversion and accumulation in intramuscular fat. Furthermore, a similar pattern was observed between intermuscular fat and subcutaneous fat, except in animals without restriction, wherein subcutaneous fat was $42 \%$ higher ( $\mathrm{p}<0.05$ ) than under the highest restriction level and compatible ( $\mathrm{p}>$ 0.05 ) with the intramuscular fat deposition. Mushi et al. (2010) also observed that the increase in restriction level caused an increased in the percentage of $n-6$ when assessing fat in goats.

## Conclusion

Feed restriction affects lipid and cholesterol levels and the profile of fatty acids deposited in different types of fat, favoring essential fatty acids deposition. Lambs subjected to $60 \%$ feed restriction had better nutritional quality meat with regard to the fat profile. Subcutaneous fat accumulated health-beneficial fatty acids, indicating that their intake should be further evaluated by nutritionists. Thus, feed restriction should be considered an alternative for sheep production in the Brazilian Northeast, especially in drought periods, and also should be used for economic purposes. However, should established a relation between quality and yield carcass, to increase revenue for producers.

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