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Meat quality of culled adult goats finished with increased feeding plans

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Abstract

This study evaluated the effect of increasing the nutritional plane in finishing diets on meat quality traits of culled adult goats. Forty-one crossbred does were divided into four groups: baseline diet formulated to meet 100% of the nutritional requirements (BD); BD + 30% (BD30); + 60% (BD60); + 90% (BD90) of the nutritional level of BD. The goats received the experimental diets for 28 days. The protein profile of the *longissimus lumborum* muscle was evaluated using 1D SDS–PAGE and mass spectrometry. Cholesterol and albumin concentrations were highest in the BD group. There were no alterations in loin proximate composition or tissue composition. However, there was a significant reduction in the PUFA content in groups BD60 and BD90 compared with the group fed the baseline diet. Of 37 protein bands identified, 35 showed lower intensities in group BD90 than in BD and 22 bands showed lesser expression in groups BD60 and BD90 than in BD. The multivariate model identified 14 band proteins involved in group-diet segregation. Therefore, increasing the nutrient supply for finishing goats did not have marked positive effects on carcass. A lower expression of these proteins can have a profound impact on the qualitative aspects of the meat product.

Keywords: goats; meat quality; finishing diet; protein expression.

Practical Application: High-energy diets interfere with structural and metabolic muscle proteome.

1 Introduction

Among the domestic species destined for meat production, there has been a considerable expansion in goat farming in recent years, as driven by the Chinese and Australian markets. According to data from the Food and Agriculture Organization of the United Nations, there was a 1.5% increase in production of the species in the year 2018, generating more than 83,079,226 t of goat meat (Food and Agriculture Organization, 2019). The proportion of females in the total number of slaughtered ruminants has increased in the last years as a result of the renewal of dairy herds, since the discarded animals are destined for slaughter and their meat sold. According to FAO data, 217 million of the approximately 633 million head of animals slaughtered in 2018 consisted of adult goats (Food and Agriculture Organization, 2020). Due to their low feed conversion efficiency, these animals require diets with a higher energy content to ensure heavier slaughter weights and better carcass quality (Assan, 2015). Thus, several studies have investigated the effect of increasing the energy density in nutritional and finishing planes for small ruminants and shown positive effects on weight gain in different breeds of goat and sheep (Aguayo-Ulloa et al., 2013).

Skeletal muscle quality is directly determined by the diet composition, since it induces changes in the main proteins that make up the contractile system, e.g., myosin, actin, troponin and tropomyosin. In ruminants, myoblasts are formed in the fetal period and undergo myofibrillar differentiation between the 4th and 8th weeks after birth. However, as the body develops, the muscular structure does not undergo major changes, since adult individuals have higher levels of factors that inhibit cell division such as Sprouty 1, which inhibits fibroblast growth factor (FGF) signaling, besides exhibiting loss and/or aging of satellite cells (Sousa-Victor et al., 2015).

The study of the protein profile proves to be a more accurate strategy, as it provides a better understanding of the biological processes that determine the phenotypes as well as the explanation for and prediction of variations in meat quality (Picard et al., 2014). Of the molecular tools of expression, allows a correlation of protein abundance with meat quality (Lametsch & Bendixen, 2001), indicating the expression of proteins that interfere with parameters such as tenderness, juiciness, flavor and color, which are considered the most important intrinsic traits in meat quality from a consumer perspective (Miller et al., 2001).

Research results show that there is a differential protein expression in the muscle in response to various events, e.g. increase in nicotinamide adenine dinucleotide dehydrogenase (NADH)

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and heat-shock protein 70 (HSP70) in the muscle protein post-mortem, which alters myofibrillar stability (Bjarnadóttir et al., 2010); increased fragmentation of myosin light chain 1 (MYL1), troponin C and desmin, which help to predict meat tenderness (Zapata et al., 2009); as well as negative regulation of carbonic anhydrase 2 (CA2) and myosin light chain 3 (MYL3) during the deposition of intramuscular fat (Zhang et al., 2010). However, information is still lacking on changes in the meat protein profile of goat in response to dietary changes.

At the intracellular level, the increase in energy availability can lead to disturbances of homeostatic balance, triggering an inflammation in the endoplasmic reticulum, a sarcoplasmic organelle, in response to metabolic stress (Deldicque et al., 2010). This stress can induce defense mechanisms such as unfolded protein response (UPR) (Deldicque et al., 2010), whose action promotes changes in the formation of the tertiary and quaternary structure of proteins by altering the expression of chaperone proteins (Xu et al., 2005). These, in turn, can compromise muscle protein composition and, consequently, the quality of meat products.

Nevertheless, conflicting information is found in the literature regarding the diet effect on the meat composition of adult ruminants, since nutritional stimuli can no longer change the chemical composition of the meat once the animal body reaches maturity. Rather, these stimuli can improve carcass yield as a result of increased lipid deposition (Fruet et al., 2016). In goats, fat deposition occurs later, and prominently in the omental tissue through adipocyte hypertrophy with triglycerides, which are generated from the degradation of dietary carbohydrates and drained into the tissues by the hepatic portal system (Cianzio et al., 1985).

A large part of the existing studies in ruminants, especially in the goat species, are carried out with young animals, underestimating the importance of the production of meat from adult or cull animals, whose consumption is a market reality and a practice strongly associated with local food traditions.

In view of this dearth of information, research is warranted to clarify the impact of the nutritional supply on the molecular aspects of meat from adult animals. Thus, our objective was to examine, under experimental conditions, the effect of different finishing diets on the main organs; chemical and tissue composition and fatty acid profile of the loin; as well as the protein profile of the *longissimus lumborum* (LL) muscle of culled adult goats.

2 Materials and methods

The experiment was conducted at School of Veterinary of the Ceará State University – UECE, Brazil and all procedures used in this study were approved by the Ethics Committee in Animal Experimentation of UECE (n°. 12066667-7/18, CEUA - UECE). The present study represents an effort to investigate how diets influence adult animal meat quality, following a primary evaluation focused on effects on reproduction and proteome of oviduct and uterus of goats (Fernandes et al., 2018a, b).

2.1 Animals and carcass samples

The animals used in this experiment were 41 Anglo-Nubian crossbred nonpregnant and nonlactating goats with age of 2.8 ± 0.7 (overall mean ± SD) years and live weight of 30.0 ± 2.9 kg. They were fed twice a day (07:00 and 15:00 hr) with a baseline diet (BD) composed of chopped Bermuda grass hay (11.1% CP), milled corn (9.6% CP), wheat bran (16.5% CP), and soybean meal (43.9% CP). A BD was administrated during 28 days to four groups-treatments. Goats in Group BD (n = 11) were fed with a BD to meet 100% of their nutritional requirements (National Research Council, 2007) and maintain live weight. In the other three groups diet was furnished in quantity to promote a weight gain providing respectively, + 30% (Group BD30; n = 10), + 60% (Group BD60; n = 10), + 90% (Group BD90; n = 10) than nutrient level of Group BD. Goats were kept in open shed pens modified to allow the control of individual intake during the feeding administration. Orts were collected daily and weighed to determine the intake and the extent of acceptance of the diet by the animals. Water and mineral salts were provided ad libitum to the animals.

After 4 weeks of feeding, the animals were weighed and then subjected to solid and liquid fasting for 16 hours and then were weighed again and slaughtered according to Brasil (1952). After skinning, evisceration and removal of the head and extremities, the anatomical components (the lungs, heart, spleen, liver, kidneys, pancreas, empty stomach, empty intestines, omentum adipose tissues) were weighed. The carcasses were then stored at 4 °C for 24 h. Next, carcasses were then longitudinally cut into two half-carcasses and the loin were collected from the left half-carcass, and then were identified, wrapped using parafilm and stored in a freezer at -20 °C for further tissue composition analyses.

For the *longissimus lumborum* (LL) proteomic analysis, fractions of approximately 10g of the muscle were collected between the second and fourth lumbar vertebrae, around 10 minutes after slaughter of each animal, which were placed in sterile tubes, then frozen in liquid nitrogen and stored at -80 ° C until the subsequent analysis. The stored LL muscle samples were lyophilized for 24 h, and then macerated to a very fine powder, which was stored in sterile tubes at -20 °C until the time of protein extraction.

Moreover, the loin (complete portion between the first and sixth lumbar vertebra) of all slaughtered goats were collected, identified, vacuum packed and stored in a freezer at -20 °C for further tissue composition analyses.

2.2 Loin tissue dissection and proximate composition

The complete loin was thawed inside plastic bags in a refrigerator at 10 °C for 20 h for the tissue analyses according Oliveira et al. (2015). Briefly, the muscle, adipose and bone tissues were separated using a scalpel, knife and anatomical clamp. The muscle tissue consisted of all the muscles dissected after the complete removal of the attached subcutaneous and intermuscular fat. The adipose tissue consisted of the external (located below the skin) and the intermuscular (located below the deep fascia, associated with the muscle) fat. The bone tissue was obtained after the complete removal of all the attached muscle and fat. After the dissection, each tissue was weighed.

The proximate composition of the muscle was determined according to the Association of Official Analytical Chemists

(1990), and the moisture was obtained by drying a sample in an oven at 105 °C until it reached a constant weight. The nitrogen content was determined by the Kjeldahl method and converted to crude protein using a factor of 6.25. The fixed mineral residue was determined by incineration at 550 °C. The total lipids were determined by a hot extraction process using an organic solvent (he xane) at 120 °C.

2.3 Loin fatty acid composition

The lipids were extracted from 10g muscle of loin samples according Folch et al. (1957) method and the conversion to fatty acid methyl esters was performed according to Hartman & Lago (1973).

The extracted lipids were directly analysed by chromatographic analysis using a Thermo Scientific™ TRACE GC Ultra chromatograph coupled with a Thermo Scientific™ ISQ mass spectrometer equipped with a SP®-2560 fused Sigma-Aldrich® silica capillary column (100 m x 0.25 mm x 0.2 µm film thickness) with helium as the carrier gas, with flow rate 1 ml.min^{-1}. The temperature of the injector and ion source were programmed at 260 °C and 270 °C, respectively. The initial column temperature of 90 °C was held for 1 min and warmed to 180 °C at 4 °C/min, held for 1 min, then warmed 3 °C/min to 220 °C for 10 min. A commercial standard mixture (ref. FAME mix 37, 10 mg/mL components from Supelco Inc., Bellefont, PA, USA) was used as internal standard for FA methyl esters quantification.

2.4 Protein analysis of the longissimus lumborum muscle

Protein extraction

For protein extraction, the procedure was made according to Sanchez et al. (2001), with adaptations. Briefly, 5 miligrams of lyophilized sample were put into a microtube and 100 µL MiliQ water (Direct-Q, Millipore®) with 1% X-100 Triton and incubated for 1 hour at 4 ° C. After this time, 400 µl of buffet (7 M urea, 2M thiourea, 4% CHAPS, 40mM DTT [ditiotreitol] - GE Healthcare) were added and subsequently sonicated on ice. Then, treated samples was centrifuged for 30 min at 5000 g at 4 °C and the supernatant was reserved. Proteins were then resuspended in ultrapure water and protein concentration was determined in triplicates according to Bradford's method (Bradford, 1976), using Eppendorf BioSpectrometer®.

1D SDS-PAGE gel electrophoresis

All the protein samples were adjusted to 50 µg/µL and mixed (1:1) with the sample buffer (1,5 M TRIS-HCL at pH 8.8, 4% sodium dodecyl sulfate [SDS], 20% (v/v) glycerol, 0,2 M dithiothreitol [DTT] and 0.02% bromophenol blue). Then, the samples were heated for 90 seconds at 100 °C prior electrophoresis. The separation of the proteins were by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% polyacrylamide gel (Amersham ECL, GE Healthcare®, USA) at 500 V, 90 W e 25 mA/gel, with 10 µL of 225-12 kDa molecular weight standart mix (GE Healthcare, Piscataway, NJ, USA). The gel was stained with Coomassie Brilliant Blue R-250 (GE Healthcare®, USA) for 12 h, and destained ater several washes in a solution containing methanol and acetic acid (40%:7%). After destaining, the gel was scanned at 300 dpi (ImageScanner III, GE Healthcare®, USA) and evaluated using QuantityOne® Software, version 4.6.3 (Bio-rad, Rockville, MD, USA). The bands were identified and matched among the animals to determine of their molecular weights and intensity.

Protein identification by electrospray ionization quadrupole time-of-flight (ESI-Q-ToF) mass spectrometry

Thirty-seven bands obtained were excised from the gel, sliced into small pieces (~1 mm^3), and subjected to digestion by trypsin (De Lazari et al., 2019). Briefly, the bands were destained with 25 mM ammonium bicarbonate ph 8.0 in 50% acetonitrile solution, dehydrated by acetonitrile for 5 minutes and denatured under 10 mM DTT solution. Then the peptides were alkylated (iodoacetamide 50 mM) and digested with trypsin at 37 °C, were recovered after 18 h, extracted in 5% formic acid and 50% acetonitrile and dried in Speed Vac concentrator. Then, the products of trypsin digestion were injected in a nanoAcquityTM system (Waters Corp., Milford, MA, USA), using an BEH300 C18 column (100 µm x 100 mm). And eluted at 600 µl/min with acetonitrile gradient (5–85%) containing 0.1% formic acid. The liquid chromatography system was connected to a nanoelectrospray mass spectrometer source (SYNAPT HDMS system, Waters Corp., Milford, MA, USA). The mass spectrometer was operated in positive mode using a source temperature of 90 °C and capillary voltage of 3.5 kV. The instrument was calibrated with fragments of the double protonated ion [Glu1]-fibrinopeptide B (m/z 785.84), and the Lock mass used during the acquisition was the intact ion. The LC–MS/MS procedure was performed according to the data-dependent acquisition (DDA) method, selecting MS/MS doubly or triply charged precursor ions. Ions were fragmented by collision-induced dissociation using argon as the collision gas and ramp collision energy that varied according to the charge state of the selected precursor ion. Data acquisition was performed at an m/z range of 300–2100 for the MS survey (1 scan/sec) and at an m/z range of 50–2500 for MS/MS. Data were collected with MassLynx 4.1 software and processed using the Protein Lynx Global Server 2.4 (Waters Corp.) and were converted to peak list text files (.pkl) for database searching. For peptides identification, MS/MS ion searches were performed through the MASCOT server (Matrix Science Inc., London, UK, v.2.6) to search the NCBIprot and SwissProt databases. Searches were made given there was maximum one missed trypsin cleavage, that peptides were monoisotopic (with +1, +2 and +3 charge) and using partially oxidized methionine residues and carbamidomethylated cysteine residues. However, candidate peptide IDs were only accepted if the m/z values were observed within 0.1 Da (typically less than 0.05 Da) of the theoretical mass of the candidate ID, as determined when manually reviewing MASCOT search results.

Relationship proteins-meat quality and Gene Ontology

Protein information obtained by MASCOT was analysed using the freely accessible UniProtKB databases for searching Gene Ontology (GO) terms associated with proteins in a proteomics

results identification list. In addition, proteins were analyzed for possible effects on the physical chemical parameters of meat by bibliography research.

Protein interaction network analysis

Protein–protein interaction (PPI) networks were retrieved from STRING version 10.0 database, which consists of known and predicted protein interactions collected from direct (physical) and indirect (functional) associations. Such database quantitatively integrates interaction data from four sources—genomic context, high throughput experiments, coexpression and previous knowledge from research publications. Network analysis was only evaluated for proteins differently expressed between the diets tested. STRING software was set to show no more than 10 interactions and medium confidence. Pathways not described for *Capra hircus* were analysed for *Bos taurus* species.

2.5 Blood sampling, metabolites and glutathione peroxidase assays

Blood samples were taken at the beginning and end of the experimental feeding period by through jugular venipuncture using heparinized vacutainer tubes (Labor import, Wei Hai, China), which was always performed prior to the morning feeding. The blood samples were centrifuged at 600 g for 15 min, and the plasma obtained was stored −20 °C for subsequent quantification of the metabolites. Plasma concentrations of total protein, albumin, glucose and cholesterol were determined using an automated biochemical analyzer (Mindray BS 120, Mindray®) with commercial kits (Bioclin®, Quibasa — Minas Gerais, Brazil). The sensitivity of the assay kit was 0.043 g/dL for protein, 0.327 g/dL for albumin, 1.31mg/dL for glucose and 1.472 mg/dL for cholesterol. Glutathione peroxidase (GPx) were analyzed before the starving period using a semi-automated biochemical analyzer (Randox RX Monza TM, Randox Laboratories®, Crumlin, UK), by commercial kits (Randox Laboratories®, Crumlin, UK) with 75U/l sensitivity.

2.6 Statistical analysis

Anova and means comparisons

Data were subjected to analysis of variance (ANOVA) using the GLM procedures (Statistica v. 13.4.0.14, TIBCO Software, Inc., Palo Alto, CA, USA). Diet treatment (BD, BD30, BD60, and BD90), Time length of diets supplementation (Time) and interaction Diet vs. Time were the main effects tested for the measurements of dry matter intake, weight gain and metabolites' plasma composition. For the parameters of anatomical records, tissue dissection, proximate composition and fatty acid concentration, factor used was the Diet treatment. For all parameters the polynomial contrasts to test linear and quadratic effects of increasing levels of diet were computed.

Data from intensity of protein gel band was transformed into log10x and subjected to ANOVA using R program (R Core Team, 2018), with main effect the diet treatment.

Pairwise comparisons were performed by the Newman-Keuls test except for protein gel bands where means comparisons were performed using Tukey's multiple test.

Multivariate analysis

Protein bands intensities were also subjected to multivariate analysis using the mixOmics package of R program (R Core Team, 2018) for principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). Variable importance in the projection (VIP) values obtained from supervised statistical PLS-DA was used to quantify the contribution of each variable in a PLS-DA model. According to the criteria of a VIP value >1.0 in the PLS-DA model (Gosselin et al., 2010).

3 Results

3.1 In vivo performance and metabolites

Table 1 shows the dry matter intake, live weight gain and plasma metabolite values measured during the experimental period. Dry matter intake increased quadratically with the feeding levels and differed between the treatment groups. Live weight gain was similar between groups BD60 and BD90 and higher in these animals when compared with groups BD and BD30.

Plasma glucose, total protein and glutathione peroxidase concentrations did not differ between the nutritional planes. The highest cholesterol and albumin values were found in the BD group. Cholesterol decreases the concentrations in a quadratic way in relation to the dietary levels of the diets.

3.2 Anatomical records

The weights of kidney and omental adipose tissue were highest in the animals of group BD90 (Table 2). Differences were also found for liver weight between groups BD and BD90, in favor of the latter, and for pancreas weight between the animals fed the baseline diet and groups BD60 and BD90.

3.3 Loin tissue dissection, composition and fatty acid content

No diet effect was observed for the proportions of muscle, fat, bone or connective tissues relative to total loin weight (Table 3). Accordingly, the proximate composition of the loin was similar between the treatment groups (Table 4). There was a significant decrease in the unsaturated fatty acid content in groups BD60 and BD90 compared with group BD, and PUFA/SFA, UFA/SFA ratios were also decreased quadratically.

3.4 Meat protein profile

The electrophoretic profile of the *longissimus lumborum* muscle revealed 37 bands in common among all tested animals. Identification of the bands excised from the gel by ESI-Q-ToF revealed 41 different proteins (Table 5).

The proteins were grouped according to gene ontology and their role in the qualitative traits of meat. Most proteins participate in biological processes associated with 32% metabolic processes, 40% muscle contraction, 40% regulation and 28%

Table 1. Means and standard errors of dry matter intakes, weight gains, peripheral metabolites and glutathione peroxidase in adult goats fed during 28 days with distinct nutritional levels.

Parameters	Group				*p* Value				
	BD	BD30	BD60	BD90	Diet	Time	D vs. T	Linear	Quadratic
Goats exposed, n	11	10	10	10					
In vivo performance									
DM intake, g.kg / MW	52.0 ± 0.4a	62.3 ± 0.4b	65.9 ± 0.5c	71.3 ± 0.6d	0.01	0.01	0.01	0.01	0.01
DM intake, %BW	2.2 ± 0.02a	2.6 ± 0.02b	2.8 ± 0.02c	3.0 ± 0.03d	0.01	0.01	0.01	0.01	0.01
Daily weight gain, g.day	1.9 ± 19.7a	51.1 ± 16.6b	66.7 ± 14.6b	104.3 ± 20.9b	0.01	0.01	0.08	0.49	0.74
Total weight gain, kg	0.0 ± 0.4a	1.3 ± 0.2b	1.7 ± 0.4bc	2.7 ± 0.4c	0.01	-	-	0.33	0.61
Metabolites									
Glucose, mg.dl	55.3 ± 1.9	58.5 ± 2.4	55.4 ± 1.2	53.3 ± 3.0	0.44	0.08	0.40	0.26	0.22
Protein, mg.dl	7.3 ± 0.3	7.3 ± 0.1	6.9 ± 0.3	7.1 ± 0.1	0.39	0.05	0.98	0.32	0.28
Albumin, mg.dl	3.5 ± 0.1a	3.1 ± 0.1b	2.8 ± 0.1b	2.4 ± 0.1c	0.01	0.85	0.05	0.53	0.97
Cholesterol, mg.dl	75.4 ± 3.5a	61.8 ± 3.5b	61.0 ± 3.0b	60.7 ± 2.1b	0.01	0.66	0.48	0.01	0.02
Glutathione peroxidase*, $U.L^{-1}$	105.6 ± 21.2	118.8 ± 28.7	129.5 ± 32.3	107.4 ± 23.9	0.91	-	-	0.73	0.73

*performed at 28th day of feeding before pre-slaughter starving period. Diets = effect of diets; Time = effect of diet supplementation time length; D vs. T = interaction effect. Contrast: Linear = linear effect of increasing level of diets; Quadratic = quadratic effect of increasing levels of diet.

tissue development, most of them have a 40% molecular binding function and 36% catalytic activity, and as a cellular component, 44% participate in the cytoskeleton and 28% do cytosol. As regards the quality of the meat product, the most recorded functions were associated with physicochemical parameters such as 36% tenderness, 28% drip loss and 24% fat deposition.

The Figure 1 shows the SDS-PAGE 1D gels, which shows the electrophoretic profile of LL of goats muscle proteins. In Figure 2 was given the results of the comparison between diets for band intensity. Except for bands 3 (MYH1 and MYH4 proteins) and 35 (TNNC1 and PPIA proteins), all 35 protein bands were affected by the nutritional treatment (Figure 2). Apparently, the intensity of the bands decreased as the feeding level was increased. For 22 bands, a lower expression was detected in groups BD60 and BD90 than in groups BD and BD30, whereas for 35 bands lesser expression was seen in group BD90 compared with groups BD and BD30.

3.5 Multivariate analysis

Figure 3 shows the score plot for the results of multivariate analysis performed in the protein bands. The diets groups were colored green (BD), black (BD30), blue (BD60) and red (BD90). The first, second and third principal components of the multivariate model retained 71%, 10% and 5% of the total variance, respectively, accounting for 86% of the cumulative variance together. The prediction model generated with PLS-DA showed accuracy measures of the area under the curve (AUC) of 96.3%, 96.3%, 100% and 98.1% for treatments BD, BD30, BD60 and BD90 ($P < 0.01$), respectively.

The 14 identified protein bands with variable importance (VIP) scores > 1.0 (Gosselin et al., 2010) in the multivariate model were grouped in Figure 4, corresponding to 37.84% of the total bands. Seven of these VIP group are part of structural contractile muscle complex: Myosin light chain 1/3 skeletal muscle (MYL1, bands 36, 32, and 31), Myosin light regulatory chain 2, (MYLPF, band 34), Myosin heavy chain (MYH, bands 1, 2, 3, 6), Desmin (DES, band 14), Alpha-actinin-3 (ACTN3, band 2), Fast skeletal troponin I (TNNI2, band 31), Tropomyosin (TPM, bands 37, 24 and 19), one is involved with oxygen transport, Hemoglobin subunit beta-A (HBB, band 37), another protein belonging to the lysosome involved in lipid degradation, the Peroxiredoxin-6 (PRDX6, band 27) and seven are relationship with energetic metabolism, glycolytic pathway and citric acid cycle: ATP synthase subunit beta and Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex (ATP5F1B and DLST, band 15), Fructose-bisphosphate aldolase A and Glyceraldehyde-3-phosphate dehydrogenase (ALDOA and GAPDH, band 19), Pyruvate kinase (PMK, band 14), Phosphoglycerate mutase 2 (PGAM2, band 27) and Glycogen phosphorylase, muscle form (PYGM, band 6). In the VIP bands, 16 proteins were not repeated between the bands, about 21.92% of the total proteins reported in Table 5.

Table 2. Means and standard errors of anatomical records performed at slaughter in adult goats fed during 28 days with distinct nutritional levels.

Parameters	Group				*p* Value		
	BD	BD30	BD60	BD90	Diet	Linear	Quadratic
FW*, kg	29.3 ± 1.2	29.5 ± 1.0	28.8 ± 0.8	31.3 ± 1.0	0.35	0.40	0.35
Heart, g	129.1 ± 7.3	133.0 ± 8.2	127.0 ± 8.9	145.0 ± 4.8	0.33	0.92	0.80
Lungs, g	260.9 ± 13.4	272.0 ± 17.5	231.0 ± 16.0	235.0 ± 11.1	0.16	0.92	0.80
Liver, g	434.5 ± 26.6a	471.0 ± 24.9ab	482.0 ± 27.5ab	544.0 ± 14.7b	0.02	0.81	0.60
Spleen, g	53.6 ± 3.9	58.0 ± 7.8	51.0 ± 2.8	60.0 ± 2.6	0.53	0.67	0.63
Pancreas, g	36.4 ± 4.9a	49.0 ± 5.7ab	56.0 ± 4.3b	61.0 ± 4.6b	0.01	0.26	0.43
Kidneys, g	73.6 ± 4.3a	77.0 ± 3.3a	78.0 ± 3.3a	91.0 ± 3.5b	0.01	0.32	0.19
OAT**, g	503.6 ± 82.1a	531.0 ± 74.9a	623.0 ± 74.9a	898.0 ± 107.6b	0.01	0.26	0.15
Empty Stomachs, kg	5.7 ± 0.3	6.1 ± 0.3	5.5 ± 0.4	5.3 ± 0.3	0.33	0.42	0.35
Empty Intestines, kg	2.1 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	0.68	0.36	0.38
Weights as % of FW							
Heart, %	0.44 ± 0.01	0.45 ± 0.02	0.44 ± 0.03	0.46 ± 0.01	0.81	0.78	0.73
Lungs, %	0.90 ± 0.04	0.92 ± 0.05	0.80 ± 0.05	0.75 ± 0.02	0.05	0.50	0.35
Liver, %	1.48 ± 0.05a	1.60 ± 0.07ab	1.67 ± 0.08ab	1.74 ± 0.04b	0.03	0.50	0.69
Spleen, %	0.18 ± 0.01	0.20 ± 0.03	0.18 ± 0.01	0.19 ± 0.01	0.78	0.86	0.87
Pancreas, %	0.12 ± 0.01a	0.17 ± 0.02ab	0.19 ± 0.01b	0.19 ± 0.01b	0.01	0.12	0.20
Kidneys, %	0.25 ± 0.01a	0.26 ± 0.01ab	0.27 ± 0.01ab	0.29 ± 0.01b	0.05	0.87	0.68
OAT, %	1.65 ± 0.2a	1.78 ± 0.2a	2.1 ± 0.2a	2.8 ± 0.3b	0.01	0.39	0.23
Empty Stomachs, %	19.6 ± 0.7ab	20.8 ± 0.8a	18.9 ±1.2ab	16.8 ± 0.8b	0.01	0.09	0.05
Empty Intestines, %	7.06 ± 0.37	7.9 ± 0.45	7.6 ± 0.40	6.9 ± 0.3	0.26	0.05	0.06

*Fasting weight: weight performed at pre-slaughter starving period. **Omentum adipose tissue. Diets = effect of diets. Contrast: Linear = linear effect of increasing level of diets; Quadratic = quadratic effect of increasing levels of diet.

Table 3. Means and standard errors of loin tissue dissection of adult goats fed during 28 days with distinct nutritional levels.

Parameters	Group				*p* Value		
	BD	BD30	BD60	BD90	Diet	Linear	Quadratic
Loin,g	148.2 ± 8.8a	126.5 ± 7.7ab	111.1 ± 7.9b	130.7 ± 7.4ab	0.02	0.05	0.06
Dissection, g							
Muscle	70.1 ± 5.4a	56.4 ± 2.9ab	50.2 ± 5.5b	66.5 ± 4.8ab	0.02	0.06	0.06
Adipose	8.3 ± 2.1	7.2 ± 1.3	4.3 ± 0.7	4.4 ± 0.8	0.08	0.50	0.66
Bone	32.0 ± 1.7	29.3 ± 2.6	27.4 ± 1.9	26.7 ± 1.7	0.27	0.51	0.63
Connective	22.6 ± 2.0	20.5 ± 2.2	17.5 ± 1.5	18.3 ± 1.6	0.22	0.34	0.44
Waste	14.3 ± 1.7	12.9 ± 2.0	11.6 ± 1.0	15.5 ± 1.8	0.38	0.12	0.11
*Weights as % LW**							
Muscle, %	47.1 ± 1.9	45.0 ± 1.7	44.4 ± 2.3	50.8 ± 1.8	0.10	0.05	0.06
Adipose, %	5.5 ± 1.4	6.9 ± 1.3	3.8 ± 0.6	3.2 ± 0.5	0.21	0.73	0.60
Bone, %	21.9 ± 1.2	23.0 ± 1.1	25.4 ± 2.0	20.5 ± 1.0	0.10	0.05	0.06
Connective, %	15.2 ± 0.9	15.9 ± 1.0	15.8 ± 1.0	14.0 ± 0.9	0.40	0.21	0.18
Waste, %	9.7 ± 1.2	10.0 ± 1.0	10.6 ± 0.8	12.0 ± 1.3	0.44	0.79	0.50

*Loin weight. Diets = effect of diets. Contrast: Linear = linear effect of increasing level of diets; Quadratic = quadratic effect of increasing levels of diet.

For the analysis of the protein network, some of these were evaluated considering their expression pattern between groups as well as their relationship to the dietary treatments, energy metabolism and muscle function, in addition to their contribution to discrimination by the VIP multivariate model. According to this analysis, the MYL1 (band 36, Figure 5A), MYH1 (band 1, Figure 5B) and TPM2 (band 37, Figure 5C) proteins showed interactions with a group of other proteins related to structural function and muscle contraction (such as troponin and actin-myosin complex proteins) and GAPDH (band 19, Figure 5D) shows interaction with a protein set present in the energy generation and cellular respiration pathways.

Table 4. Means and standard errors of proximate composition and fatty acid concentration of the loin from adult goats fed during 28 days with distinct nutritional levels.

Parameters	Group				*p* Value		
	BD	BD30	BD60	BD90	Diet	Linear	Quadratic
Composition, %							
Moisture	75.2 ± 0.2	75.6 ± 0.5	75.8 ± 0.3	75.2 ± 0.3	0.54	0.17	0.17
Protein	13.0 ± 1.0	15.2 ± 0.4	13.1 ± 0.9	13.9 ± 1.1	0.41	0.35	0.36
Ash	1.1 ± 0.05	1.1 ± 0.01	1.1 ± 0.06	1.3 ± 0.09	0.16	0.43	0.33
Total lipids	7.7 ± 0.7	10.0 ± 1.8	7.2 ± 1.0	7.8 ± 1.1	0.40	0.64	0.64
Fatty acids, %							
SFA	40.4 ± 3.5a	47.4 ± 2.2ab	61.1 ± 5.2b	53.0 ± 5.4ab	0.01	0.05	0.09
UFA	59.7 ± 3.5a	52.6 ± 2.2ab	38.3 ± 5.1b	44.8 ± 5.6b	0.01	0.07	0.13
MUFA	3.8 ± 1.2	2.8 ± 1.2	3.6 ± 0.3	4.0 ± 1.3	0.11	0.05	0.05
PUFA	55.9 ± 3.3a	49.7 ± 2.8ab	37.7 ± 4.9b	40.7 ± 5.2b	0.01	0.17	0.28
UFA/SFA	1.6 ± 0.2 a	1.1 ± 0.1ab	0.7 ± 0.1b	1.0 ± 0.2ab	0.01	0.02	0.03
PUFA/SFA	1.5 ± 0.2a	1.1. ± 0.1ab	0.7 ± 0.1b	0.9 ± 0.2b	0.01	0.03	0.03

SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Diets = effect of diets. Contrast: Linear = linear effect of increasing level of diets; Quadratic = quadratic effect of increasing levels of diet.

Table 5. Proteins of the *longissimus lumborum* from adult goats fed during 28 days with distinct nutritional levels. Proteins were separated by one-dimensional SDS-PAGE and identified by tandem mass spectrometry (ESI-Q-ToF).

Band	Protein name	Gene	NCBI or SwissProt accession nunber	MS/MS Protein score	Sequence coverage (%)	Number of peptides
1	Myosin-1 isoform X1	MYH1	XP_017920146.1[a]	938	9	15
2	Myosin-1 isoform X1	MYH1	XP_017920146.1[a]	1082	11	15
	Alpha-actinin-3 isoform X1	ACTN3	XP_017898846.1 [a]	1131	20	16
3	Myosin-1 isoform X1	MYH1	XP_017920146.1 [a]	2261	22	33
	Myosin-4	MYH4	XP_017920145.1 [a]	1637	15	23
4	Myomesin – 2	MYOM2	XP_017897599.1 [a]	447	5	6
	Myosin-binding protein C, fast-type	MYBPC2	XP_017918332.1 [a]	221	3	3
5	Myosin-binding protein. slow-type isoform X5	MYBPC1	XP_005680574.1 [a]	291	4	4
6	Myosin-2	MYH2	XP_017920148.1 [a]	978	9	17
	Glycogen phosphorylase, muscle form	PYGM	PYGM_BOVIN [b]	139	2	2
7	Myosin-2	MYH2	XP_017920148.1 [a]	1104	10	17
8	Alpha-actinin isoform X2	ACTN1	XP_013831501.2 [a]	1075	19	15
9	Alpha-actinin-3 isoform X2	ACTN3	XP_013831501.2 [a]	394	6	5
	Myosin 1 isoform X1	MYH1	XP_017920146.1 [a]	153	1	2
	Glycogen phosphorylase, muscle form	PYGM	PYGM_BOVIN [b]	473	9	6
10	Albumin precursor. Partial	ALB	ACF10391.1[b]	565	12	9
	Serum albumin	ALB	XP_005681801.1 [a]	565	12	9
	Heat shock protein 70.1	HSP70.1	AEM24982.1[b]	441	14	6
	Glycerol-3-phosphate dehydrogenase, mitochondrial	GPD2	GPDM_BOVIN[b]	74	2	1
11	No indetificate protein					
12	Pyruvate kinase PKM isoform X2	PKM	XP_005685234.1 [a]	1000	34	17
13	Pyruvate kinase PKM isoform X2	PKM	XP_005685234.1 [a]	1000	22	9
	Glucose-6-phosphate isomerase	GPI	XP_017917783.1 [a]	298	10	4
14	Pyruvate kinase PKM isoform X2	PMK	XP_005685234.1 [a]	217	10	3
	Desmin	DES	DESM_BOVIN [b]	796	31	10
15	ATP synthase subunit beta. Mitochondrial	ATP5F1B	XP_005680388.1 [a]	641	22	8
	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	DLST	ODO2_BOVIN [b]	67	2	1
16	Beta-enolase isoform X1	ENO3	XP_005693511.1 [a]	701	31	13

[a]NCBIprot database (v. 20180429 with 152462470 sequences; 55858910152 residues) and [b]SwissProt database (v. 2019_01 with 559077 sequences; 200828568 residues)

Table 5. Continued...

Band	Protein name	Gene	NCBI or SwissProt accession nunber	MS/MS Protein score	Sequence coverage (%)	Number of peptides
	Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial	PDHA1	ODPA_BOVIN [b]	59	3	1
17	Beta actin, partial	ACTB	AAS68014.1[a]	48	80	2
18	Fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_005697728.1 [a]	304	16	4
	Creatine kinase M-type	CMK	XP_005692693.1 [a]	732	32	35
19	Fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_005697728.1 [a]	770	38	21
	Tropomyosin alpha-1 chain	TPM1	TPM1_BOVIN [b]	153	8	2
	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	G3P_BOVIN [b]	63	4	1
20	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	G3P_BOVIN [b]	403	25	5
	Fast skeletal muscle troponin T	TNNT3	AEP14536.1 [b]	347	22	5
21	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	G3P_BOVIN [b]	562	36	30
	Tropomyosin beta chain	TPM2	TPM2_BOVIN [b]	336	30	10
22	Fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_005697728.1 [a]	534	30	9
	Tropomyosin beta chain	TPM2	TPM2_BOVIN[b]	393	24	8
	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	G3P_BOVIN [b]	231	34	5
23	Tropomyosin alpha-1 chain	TPM1	TPM1_BOVIN [b]	863	24	10
	Malate dehydrogenase, mitochondrial	MDH2	MDHM_BOVIN [b]	261	14	4
	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	G3P_BOVIN [b]	151	8	1
24	Tropomyosin alpha-1 chain	TPM2	TPM2_BOVIN [b]	876	23	11
25	Lactate dehydrogenase	LDHC	NP_001272647.1 [a]	286	13	4
	Malate dehydrogenase, mitochondrial	MDH2	MDHM_BOVIN [b]	203	16	4
	LIM domain-binding protein 3 isoform X2	LDB3	XP_017897677.1 [a]	391	9	5
	Myozenin-1	MYOZ1	MYOZ1_BOVIN [b]	181	8	2
	Beta-enolase	ENO3	ENOB_BOVIN [b]	85	3	1
26	Keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_017920532.1 [a]	156	5	2
	NADH dehydrogenase [ubiquinone]	NDUFS3	NDUS3_BOVIN [b]	93	9	2
27	Phosphoglycerate mutase 2	PGAM2	XP_005695544.1 [a]	474	40	8
	Peroxiredoxin-6	PRDX6	PRDX6_BOVIN [b]	103	13	3
28	Triosephosphate isomerase	TPI1	XP_017904037.1 [a]	740	36	10
	Phospholgycerate mutase 2	PGAM2	PGAM2_BOVIN [b]	167	24	4
	Glutathione S-transferase Mu 1	GSTM1	GSTM1_BOVIN [b]	89	5	1
29	Myosin light chain 1/3 skeletal muscle isoform X1	MYL1	XP_005676539.1 [a]	534	48	9
	Myosin light chain 6B	MYL6B	XP_005680423.1 [a]	275	31	4
30	Myosin light chain 1/3 skeletal muscle isoform X1	MYL1	XP_005676539.1 [a]	841	64	19
31	Myosin light chain 1/3 skeletal muscle isoform X1	MYL1	XP_005676539.1 [a]	458	40	4
	Fast skeletal troponin I isoform. partial	TNNI2	AAK56403.1 [b]	244	31	4
32	Myosin lignt chain 2	MYL2	AJP08204.1[a]	365	34	7
33	Myosin regulatory lignt chain 2, skeletal muscle	MYLPF	NP_001272683.1 [a]	599	62	4
	Tropomyosin alpha-1	TPM1	TPM1_BOVIN [b]	58	3	1
34	Myosin light regulatory chain 2, skeletal muscle	MYLPF	NP_001272683.1[a]	864	79	16
	Myosin light chain 1/3 skeletal muscle	MYL1	XP_005676539.1[a]	85	5	1
	Tropomyosin alpha-1	TPM1	TPM1_BOVIN [b]	141	3	2
35	Troponin C, slow skeletal and cardiac muscles	TNNC1	TNNC1_BOVIN [b]	90	15	3
	Peptidyl-prolyl cis-trans isomerase A	PPIA	PPIA_BOVIN [b]	71	10	1
36	Myosin light chain 1/3 skeletal muscle isoform X1	MYL1	XP_005676539.1 [a]	539	37	10
37	Hemoglobin subunit beta-A	HBB	HBBA_CAPHI [b]	69	7	1
	Tropomyosin beta chain	TPM2	TPM2_BOVIN [b]	91	8	2

[a]NCBIprot database (v. 20180429 with 152462470 sequences; 55858910152 residues) and [b]SwissProt database (v. 2019_01 with 559077 sequences; 200828568 residues)

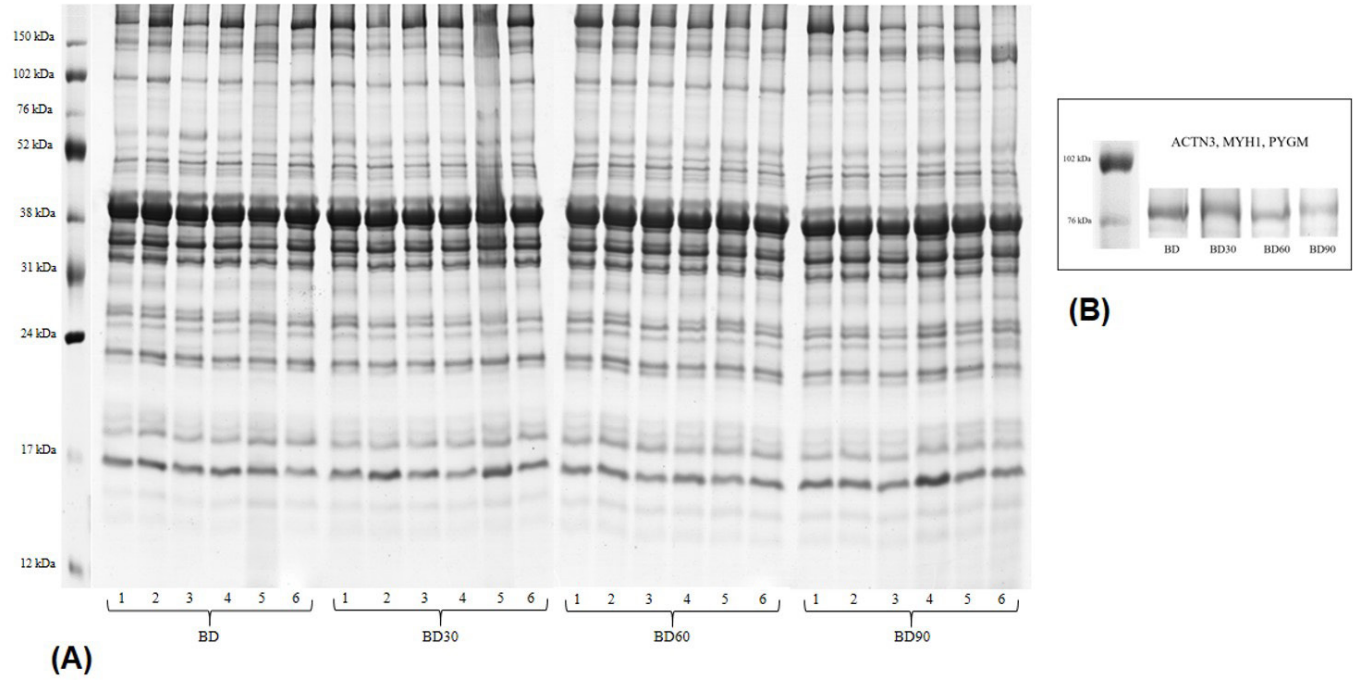

Figure 1. Figure 1A shows the electrophoresis gels bands intensity of SDS-PAGE 1D from nutritional groups (BD, BD30, BD60, BD90). The Figure 1B shows the visual differences between groups in band 9, where the proteins Alpha-actin 3, Myosin 1 and Glycogen phosphorylase are present. The left lane corresponds to the molecular weight scale (225-12 kDa molecular weight standard mix).

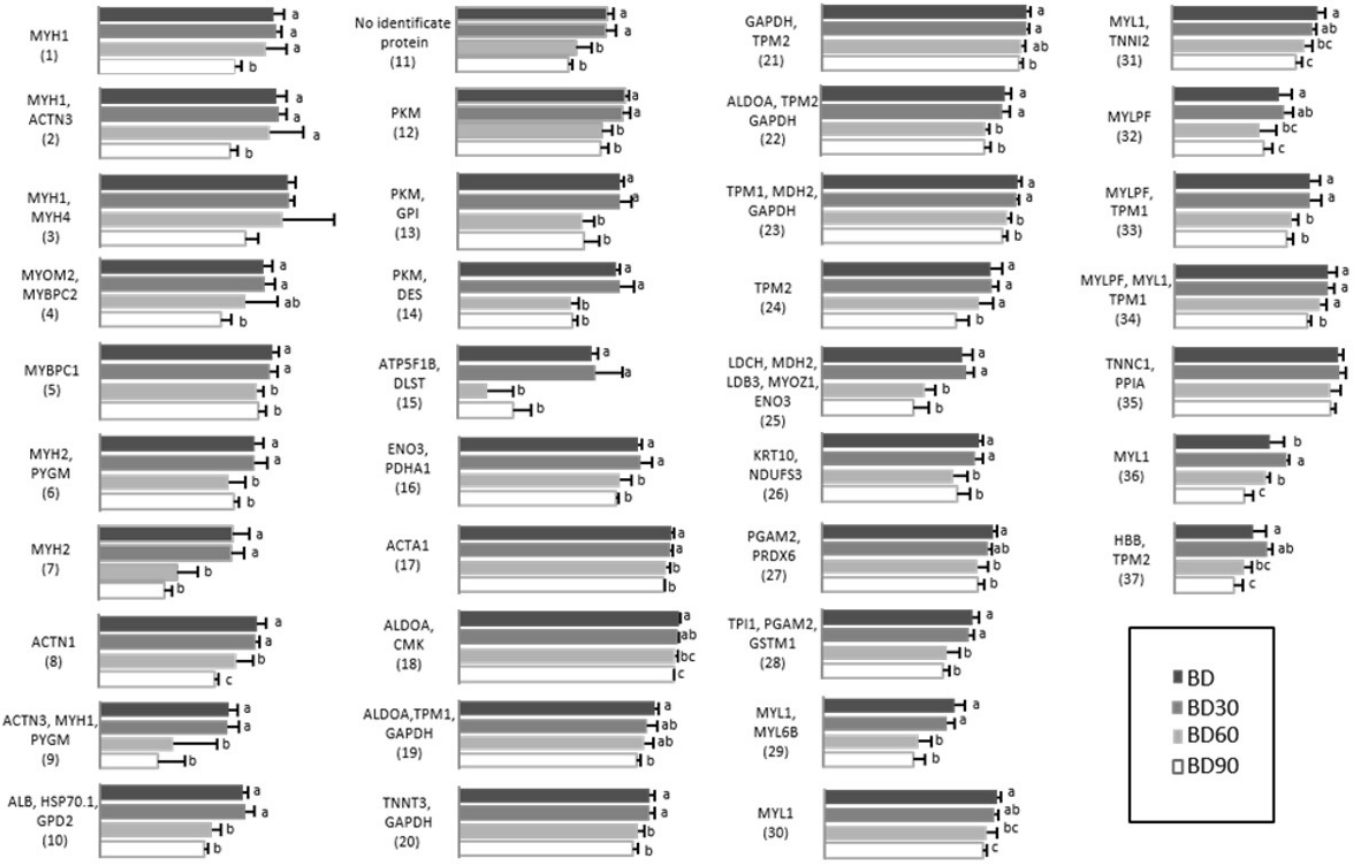

Figure 2. Comparison between proteins identified in *longissimus lumborum* from adult goats fed during 28 days with distinct nutritional levels (BD, BD30, BD60, BD90). In parentheses, the number of the band obtained in the 1D SDS-PAGE is indicated. Values are expressed as means ± SEM. Means with different letters differ ($p < 0.05$).

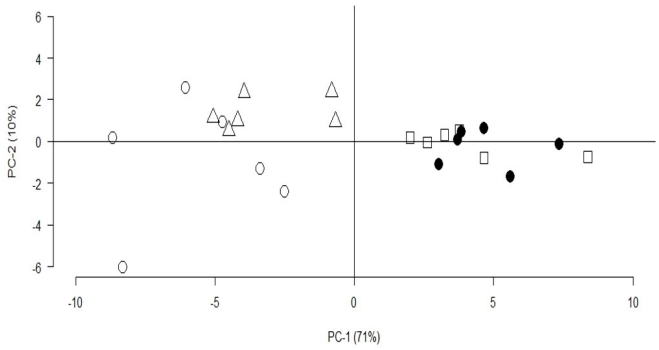

Figure 3. Score plot colored of the first (PC-1) and second (PC-2) principal components of multivariate analysis and value of each variance retained (PC-Var.) according to the proteins identified from *longissimus lumborum* of adult goats nutritional groups. The nutritional groups in the figure were: BD (Solid circle), BD30 (Empty square), BD60 (Empty circle) and BD90 (Empty triangle). Each symbol in the figure represents an individual goat analyzed.

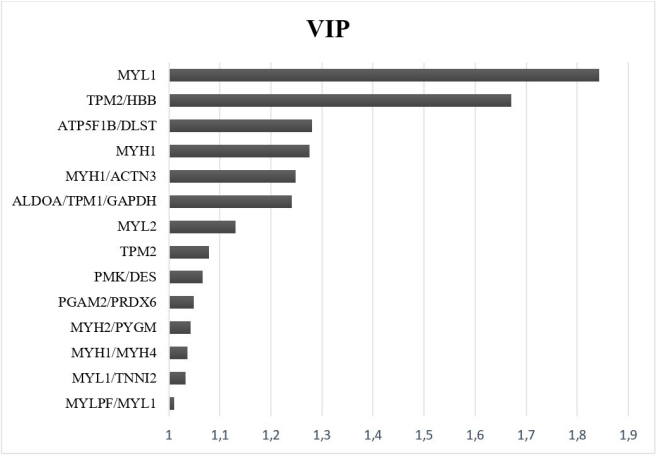

Figure 4. Protein bands with Variable of importance (VIP) value > 1.0 in the PLS-DA multivariate model.

4 Discussion

In adult ruminants destined for slaughter, nutritional strategies for the finishing phase are usually aimed at gains in weight and conformation, mainly through adipose tissue deposition in the carcass. One of the consequences of increasing the energy density of diets is the marked hepatic accumulation of glycogen, the main substrate for lipogenesis. In the present study, the diets exceeding the nutritional requirements for maintenance stimulated an increase in feed intake, body weight and the weight of organs such as liver and pancreas, proven to be active in animal metabolism (Meyer et al., 2015). In goats, Mushi et al. (2009) also reported an increase in liver weight and glycogen concentration in goats receiving high levels of dietary supplements. Regarding this last phenomenon, some authors (Goetsch et al., 2011) suggest that a compensatory growth of this organ may occur to meet the new metabolic demands of the animal.

According to the maturity stage of the experimental animals, the diets did not induce changes in carcass lean mass or loin tissue and chemical composition. As expected, weight gain was mainly directed towards increased visceral adipose mass. In fact, in goats, as in other ruminant species, the growth rate reaches a limiting point in maturity at which the deposition of adipose tissue exceeds that of other tissues (Irshad et al., 2013). This usually occurs due to adipocyte hypertrophy with triglycerides, which are generated from the degradation of dietary carbohydrates and drained into the tissues by the portal hepatic system (Cianzio et al., 1985). In goats, adipose tissue deposition is late and directed towards accumulation of omental fat in the abdominal cavity, in contrast to discrete intramuscular deposition (Webb, 2014). For this reason, goat carcasses are characterized as lean meat, with more favorable nutritional aspects.

Increasing the nutritional level of the diets also significantly reduced the PUFA content of the loin intramuscular adipose tissue. This phenomenon was offset by the corresponding increase in SFA content (more evidently in groups BD60 and BD90), which in turn altered the PUFA/SFA ratio. The change in intramuscular fatty acid profile is related to alterations in the activity of the lipid metabolism enzymes fatty acid synthase (FAS), acetyl-CoA-carboxylase α (ACCa), involved in the synthesis of SFA, and stearoyl-CoA desaturase (SCD), which have desaturase action on SFA to convert them into MUFA (Smith et al., 2009).

The changes induced by supplementing the feed with high levels of non-fibrous carbohydrates alter the rumen dynamics by reducing the pH, which can compromise the lipolytic processes in the rumen, interfering with the synthesis of fatty acids performed by microorganisms (Liu et al., 2019). As a result, the SFA content of the *longissimus dorsi* muscle of sheep increases, as described by Liu et al. (2019). Studies suggest that the activity of these enzymes is sensitive to changes in dietary availability, with a negative regulation of FAS, ACCa and SCD occurring under feed deprivation, in sheep (El-Sayed et al., 2019); and to increasing intramuscular fat levels, in cattle. Researchers have reported a positive regulation of FAS and ACCa that resulted in increased SFA contents (Ward et al., 2010).

A general analysis of the obtained results reveals a segmentation of the applied dietary treatments, with a clear approximation between the responses of groups BD and BD30, as opposed BD60 and BD90. This behavior was also observed by Safari et al. (2009) in goats supplemented with increasing levels of concentrated feed. In their study, the chemical and physical parameters of the carcass showed similarity between treatments with 0% and 33% as well as the groups that received over 60% of concentrate supplementation.

Indeed, the distinct responses between the diet groups were evident in the results pertaining to the muscular protein profile of *longissimus lumborum* muscle, which showed a generalized reduction in protein expression in the BD60 and BD90 groups. This suggests that increased nutritional intake by adult animals may change the muscle protein level, possibly as a consequence of changes in intracellular gene modulation (Baik et al., 2017).

In adult ruminants, the increase in lean mass provided by increased feed availability is not effective, since the flow of excess

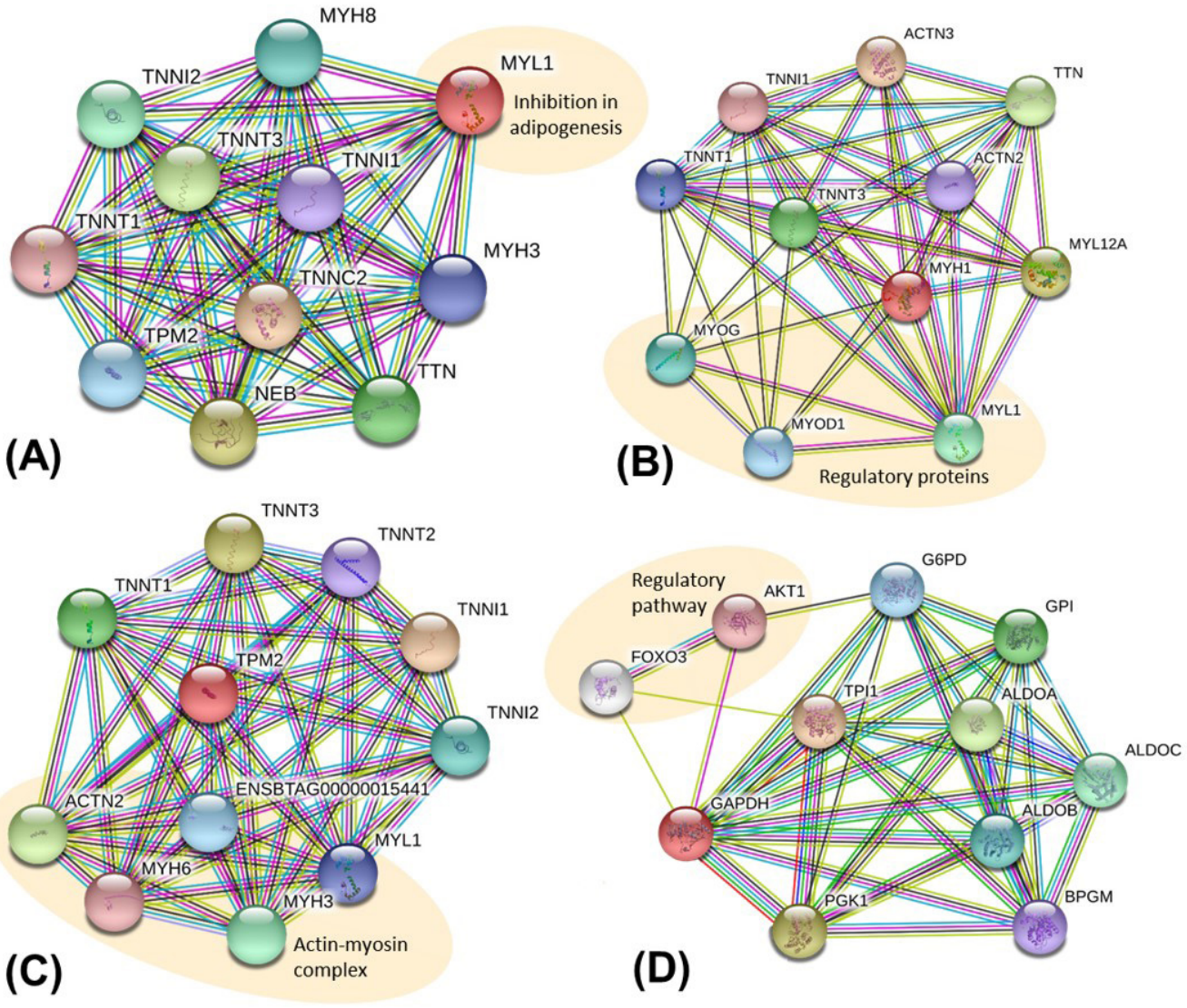

Figure 5. In-silico interaction analysis of proteins set with VIP > 1 obtained from multivariate model. Proteins were analyzed with the web-based STRING software. Interaction search was set at "medium" confidence. Analyzed proteins were as follows: MYL1 (A), MYH1 (B), TPM2 (C), GAPDH (D), Green: textming; black: coexpression; blue: databases; and pink: experiments.

nutrients is directed towards lipid conversion. Adipose tissue accumulation in adult animals can act as a signal for a decrease in protein deposition in muscle tissue (Brameld et al., 2003).

The existing regulatory mechanism in postnatal cell growth is mainly through the action of myostatin in adult muscle tissue, which prompts a negative regulation in the gene expression of the main structural proteins that make up the muscle, namely, the heavy chain of myosin, troponin I and desmin (Durieux et al., 2007). On the other hand, intracellular lipid accumulation is known to be detected by the endoplasmic reticulum (ER), which leads to accumulation of unfolded proteins in the lumen of the organelle, causing a phenomenon called lipotoxicity (Mandl et al., 2009), characterized by an excess of SFA. As a result, lipid storage in non-adipose cells is increased, leading to changes in the expression pattern of genes related to ER stress as well as pro and anti-apoptotic pathways (Bax, BCL-2) and protein stability, including heat-shock proteins (HSP). In mice, Deldicque et al. (2010) demonstrated in myogenic cells that the negative regulation of protein synthesis in skeletal muscle tissue occurs due to ER stress, which is in turn induced by increased energy levels in the diet. Thus, changes in HSP70 expression have been associated with increased aggregation of unfolded enzymes (Sugimoto et al., 2003), with HSP70 being a chaperone related to the effectors of the pathway in the inactivated state (Deldicque et al., 2012).

Changes in the expression of the muscle protein profile may interfere with quality parameters, making it necessary to analyze reference regions of the skeletal muscle structure of the carcass

such as the *longissimus thoracis et lumborum* muscle (Silva et al., 2007). Thus, the study of the protein profile provides information about the molecular events of transformation of muscle into meat, which function as quality biomarkers (Paredi et al., 2012) that can be used as predictive indices of sensory quality (Zuo et al., 2016).

At the molecular level, the relationship between fat deposition, mRNA expression and proteomic study in skeletal muscle shows that ruminants with high fat deposition rates have a significant decrease in the expression of MYL3 (Zhang et al., 2010). In our study, mass spectrometry identified the MYL1 protein in band 36, this protein band was what most contributed to the VIP values of the multivariate model for the separation between dietary treatments. It is known that this protein may have regulatory function in the myosin chains (Heissler & Sellers, 2014), and the inhibition of its expression during adipogenesis results in negative regulation of the other myosins, as demonstrated in previous studies, suggesting that myofibrillar proteins are inhibited during adipogenesis (Singh et al., 2007). In its network of in silico interactions, MYL1 exhibits co-expression interactions with all proteins, including TPM2, present in the band placed in the VIP score.

Such myofibrillar proteins are composed of the myosin family, comprised of two heavy chains and four light chains, as well as actins, troponins and tropomyosins, forming the contractile structure of the muscle (Bouley et al., 2004). Based on information from the analysis of interactions *in silico*, we can confirm that these proteins have strong functional associations with each other, suggesting that changes in expression may cause generalized alterations in this protein group.

Using spectrometry, we identified the most abundant proteins that make up the goat muscle (Wang et al., 2016), e.g. those with glycolytic (PMK and ALDOA), transport (HBB) and stress (HSP70) functions, and almost 50% of contractile proteins, including ACT, TNNT, TNNI, TPM, DES, MYL, MYHC and, notably, myosin isoforms that characterize the adult muscle (Murphy et al., 2016).

It is noteworthy that protein expression does not occur in isolation, thus the molecular regulation between the glucose metabolism and skeletal muscle development is associated with events that occurred in the animal muscle (Hocquette et al., 1998). Therefore, a correlation between energy metabolism proteins found in the VIP score may be achieved through changes at expression and co-expression levels. In our findings, the band 15 which was third in VIP value, identifying DLST and ATP5F1B proteins in this band, which participate directly in the Krebs cycle inside the mitochondria, with DLST being responsible for converting 2-oxoglutarate into succinyl-CoA, which then forms succinate, ATP and CoA (Schmid et al., 2004). A reduction in the pattern of protein sub-expression in response to the increment of dietetic levels was verified in the referred band, being likely to consider that there was modification in mitochondrial regulation and glucose gathering in high energy diets. Previous studies sustain that high energy diets may promote damage to mitochondrial function, showing reduced levels of intracellular ATP, which triggers a significant reduction of the myosin heavy chains (MYHC) expression, in addition to exhibit activation of stress factors in the ER and increment of skeletal muscle apoptosis (Yuzefovych et al., 2013).

Furthermore in cattle, increased fat deposition in the muscle induces an underexpression of mRNA of the d-subunit of ATP (Zhang et al., 2010), which in turn may be associated with a gradual decrease in the rate of energy consumption in the muscle (Wang et al., 2009). Related to the energy-metabolism, the presence of GAPDH was identified in bands 19 to 23, associated with several proteins linked to the processes of intracellular energy production and muscle contraction, such in band 19 where ALDOA proteins were identified, involved in the synthesis of D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose and TPM1 involved in calcium-dependent regulation of striated muscle contraction (Kim & Dang, 2005). In addition, the protein MDH2, identified in band 23 along with the protein GAPDH, is part of the tricarboxylic acid cycle in the mitochondrial matrix and; for all bands where these proteins involved in the energy metabolism were detected there was a reduction of protein expression in the animal groups which received higher levels of energy (Seidler, 2013).

In the network of interactions *in silico*, we observed that GAPDH associates with protein AKT1 kinase, which acts in regulatory process of myostatin. The regulation of myostatin may also respond to the events of energy metabolism, through the inhibition of AKT1, which possess the function of regulating glucose uptake (Egerman & Glass, 2014), which may result in the induction of muscular atrophy by blocking of the protein synthesis pathaway mediated by negative regulation of AKT levels (Sharma et al., 2015).

Among the molecular aspects of meat quality, tenderness is related to the degree of *post mortem* proteolysis of the myofibrillar structure (actin-myosin-troponin interactions), whose integrity interferes with the *post mortem* meat tenderization process (Kim et al., 2008). The process of conversion of muscle to meat begins with apoptosis, since it involves energy expenditure and the action of HSP proteins (Ouali et al., 2006), in two main pathways: the intrinsic mitochondrial pathway and the ER stress pathway (Chen et al., 2020), which are responsible for the oxidative stress that promotes meat tenderness and the response of HSPB1 and HSPB6, respectively (D'Alessandro et al., 2012).

Differential expression of myofibrillar proteins such as MYH (bands 1-3, 6, 7 and 9), ACTN (bands 3, 8 and 9), TPM (bands 19, 21-24, 33, 34 and 37), DES (band 14), as well as glycolytic proteins, for example LDH (band 25), ENO3 (bands 16 and 25), TPI (band 28) and GAPDH (bands 19-23) have been described as biomarkers of quality prediction meat. According to Lana & Zolla (2016) the abundance of proteins and/or factors are correlated to the stimulation of increase in resistance in meat, as high levels of actin; increased phosphorylation of the actin fragments (characterized as a marker of apoptotic resistance); high levels of tropomyosin 1; and high desmin content of meat with a low pH value. Some authors showed protein biomarkers related to meat tenderness, through the association between lower abundance of myosin heavy chain (MYHC), lactate dehydrogenase chain B (LDHB) and higher abundance of ENO3 associated with high tenderness scores in bovine´s *longissimus thoracis* muscle (Picard et al., 2014).

It is known that ENO3 converts glucose to pyruvate, GADPH synthesizes pyruvate from D-glyceraldehyde 3-phosphate and TPI controls the glycerol content through the reversible conversion of glyceraldehydes-3-phosphate. The expression of these energy-metabolism proteins in the identified bands, can be considered a useful tool in the prediction of meat quality, as research shows that the expression of TPI and ENO3 in the muscle decreases as the amount of fat increases in cattle (Kim et al., 2009).

Glycolytic proteins are strongly linked to meat quality, as they regulate its pH. The muscle-to-meat transformation process is usually accompanied by a decline in pH, which in turn is associated with softness and color (Page et al., 2001). The lactate dehydrogenase protein identified is responsible for catalyzing the conversion of L-lactate and NAD+ into pyruvate and NADH in the final stage of anaerobic glycolysis, and the increase in LDHA after slaughter correlates with the decrease in pH, revealing an inversely proportional relationship between protein expression and pH (Polati et al., 2012). pH values above 5.8 induce protein denaturation, causing the myofibrils to shrink (Cassar-Malek et al., 2007). Several authors have demonstrated how the pH decrease in meat can be associated with high values of ENO 3, LDH-B, myosin binding protein H-like (MyBP-H) and Hsp70 (Gagaoua et al., 2015). Glycolytic enzymes have their expression increased by a faster energy metabolism, resulting in a faster pH decline in the meat product (Cassar-Malek & Picard, 2016).

5 Conclusions

In summary, the increase of nutritional balance in finish diets was able to stimulate the alimentary intake but have been shown to promote a body gain mainly by deposit of visceral fat in adult goats.

There were no evidences that finish diets support a quality enhance of tissue structure and chemical composition of loin, but high diet levels show to have a negative effect on fatty acid profile of the loin by increasing of saturated portion of intramuscular fat.

At the same time, we demonstrated that nutritional supply induced a sub-expression of proteins of muscle. Some of these proteins are glycolytic and structural function and participate of important metabolic events in the transformation of muscle into meat, which significantly effort on meat quality traits.

Finally we can conclude that in the our experimental conditions the increase of level in diets for finishing in adult goats did not produce positive effects on animal conformation but it profoundly modifies the expression of fundamental proteins for attributing the qualitative aspects of the meat product. For this reason, a careful definition of the best feeding plan for goats destined for finishing should be carefully considered and studied in the future.

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