

# MEAT QUALITY AND HEPATIC AND SKELETAL MUSCLE METABOLOMES AS AFFECTED BY DIETARY PROTEIN RESTRICTION

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**Abstract.** The restriction of nutrients in the diet of animals, might affects the rate of protein turnover in muscle and liver, and could impact on tenderness of the meat. Therefore, a study was conducted, using as a feeding strategy, an abrupt reduction in the level of protein in the diet, during the final phase of finishing. There were used 14 crossbred ewes (Dorper x Santa Ines) in finishing phase, distributed in two groups; the first group received a feeding regimen that attended all nutritional requirements (Control, CT = 13.3% crude protein, n = 7) during all the experimental period and the second group was fed a reduced protein diet for 14 days before slaughter (Low protein, LP = 4.33% of crude protein, n=7). Within the meat quality parameters evaluated, there was difference for shear force (P = 0.0055) and collagen content (P = 0.02). Metabolomic analysis was performed on muscle and liver, observing some differences in amino acids abundance such as valine (P = 0.02) and asparagine (P = 0.01) in muscle and alanine (P = 0.02), glutamate (P = 0.01) and aspartate (P= 0.02) in the liver.

**Key Words – Amino acids, protein turnover, tenderness**

## I. INTRODUCTION

The manipulation of the calpains, through feeding strategies, has been proposed, to alter the balance of synthesis/degradation of the muscle in vivo and thus to improve the tenderness of the meat (Therkildsen & Oksbjerg, 2009). Therefore, as an objective of our study, we evaluated the effect of abrupt reduction of dietary protein during the final phase of finishing of ewes on organoleptic characteristics of the meat and muscular and hepatic metabolomic profile.

## II. MATERIALS AND METHODS

This study was conducted in the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Brazil. All procedures were approved by the Committee of Animal Care and Use (protocol number 65/2016-CEUAP/UFV). Fourteen crossbred ewes (initial body weight = 39.3 + 9.4 kg) were used, which were grouped into two treatments, consisting of two levels of crude protein in the diet. Both groups were fed the same crude protein content in the diet (CP = 13.3%). One group was fed a low crude protein diet (CP = 4.33%) for 14 days before slaughter, while the other group was fed same diet until slaughter. After slaughtering, a sample of M. Longissimus lumborum (LL) and liver were collected and immediately pulverized in liquid nitrogen, for further analysis of GC-MS metabolomics. Other samples were analyzed for shear force (WBSF), sarcomere length, myofibrillar fragmentation index (MFI) and collagen content. The data were subjected to analysis of variance, comparing the means through the Tukey test, when they had a significant effect. For all statistical procedures, was adopted  $\alpha = 0.05$ . All statistical procedures were performed with PROC GLM of SAS 9.0 statistical software (Statistical Analysis System Institute, Inc.). The metabolomic data were analyzed using the web-based tool MetaboAnalyst 3.0 (<http://www.metaboanalyst.ca/>; (Xia & Wishart, 2016)). The metabolite concentration table was uploaded to MetaboAnalyst and the data was log-transformed and Pareto-scaled before analysis. The T test was used to find the metabolites that differed significantly in muscle and liver concentrations between the protein restriction and control treatments (P<0.05).

## III. RESULTS AND DISCUSSION

Water losses, sarcomere length and soluble collagen were not affected by the dietary protein restriction, nonetheless shear force and collagen content were higher in the restricted diet (Table 1). For muscle and liver, 191 and 199 metabolites, respectively, were identified of which the amino acids were detailed. The muscle amino acid profile of the animals with restriction had a few differences, except for the amino acids valine and asparagine, which were less abundant ( $P < 0.05$ ). This result coincides with a study carried out by Palma et al. (2016), with nutritional restriction in different breeds of sheep, who found lower levels of the valine and related it to lower synthesis of muscle protein. On the other hand, Li et al. (2016) found lower levels of the valine and higher concentration of asparagine in the muscle of pigs with protein restriction in the diet for 10 and 25 days. Aspartate, alanine and glutamate in liver were less abundant in animals with protein restriction ( $P < 0.05$ ). The reduced levels of these two amino acids are possibly a consequence of lower degradation.

Table 1 – Means, standard error (SEM) and P value of qualitative characteristics of meat of crossbred ewes fed protein restricted diets.

Items	Diet		SEM	P- value
	Control	Low Protein		
Thawing losses, %	2.35	2.23	0.437	0.743
Cooking losses, %	20.82	21.5	1.193	0.481
Total losses, %	22.7	23.3	1.256	0.576
WBSF, N	20.7 <sup>a</sup>	25.4 <sup>b</sup>	0.155	0.0055
Sarcomere length, $\mu\text{m}$	1.72	1.70	0.047	0.7352
MFI	86.2	80.4	5.108	0.1661
Total collagen, mg/g	2.05 <sup>a</sup>	2.35 <sup>b</sup>	0.152	0.0221
Soluble collagen, %	22.0	22.8	1.298	0.4838

WBSF = Warner-Blatzer Shear Force; MFI= Myofibrillar fragmentation index

#### IV. CONCLUSION

The amino acids valine, asparagine, glutamate, aspartate and alanine, related to energy production, were less abundant in protein restricted diets. Fourteen days of nutritional restriction was not sufficient to impact muscular myofibril degradation, indeed, the dietary protein restriction increased shear force resistance of the meat although, other meat quality parameters assessed as myofibrillar fragmentation index, water losses and sarcomere length, were not affected by the nutritional protein restriction in crossbred ewes.

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