The protein profile in the *longissimus thoracis* muscle from Nellore bulls harvested with different weights reveals proteins that may be related to variations in beef color

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I. INTRODUCTION

The beef color is the meat quality property that most influences consumers' purchase decisions [1]. Diet and production systems can considerably influence final carcass weight and, consequently, affect final pH and meat color. Among the beef color parameters, redness is considered the most important for acceptability criteria [2]. Meat obtained from carcasses with an unfavorable color may suffer economic penalties and, therefore, represent a financial loss for producers and the beef industry. The goal of this study was to evaluate the beef color of cattle harvested at two slaughter weights and similar age by differential proteomics in the *longissimus thoracis* muscle, using the strategy Label-Free Proteomics.

II. MATERIALS AND METHODS

Sixteen Nellore bulls with the same physiological maturity separated into groups according to final carcass weight: Light (127.32±1.92, N=8) and heavy (152.68±1.11kg, N=8) were used. *Longissimus thoracis* samples were collected after slaughter and stored in liquid nitrogen to perform differential proteomics analysis. After a 24-hour chilling period, the pHu was measured (pH meter Testo 205, Lenzkirch, Germany) and 2.54 cm thick steaks were obtained from the *Longissimus* muscle between the 12^{th}-13^{th} ribs for meat color analysis. The meat color measurement was obtained using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA). The experimental design was a completely randomized with eight repetitions per treatment, and each animal was considered an experimental unit. The SAS software (9.4; SAS Institute Inc., Cary, NC, USA) was used to analyze the data. To evaluate the effects of weight (Light and Heavy) on color parameters, the PROC GLM procedure was applied. The significance was considered when $P \leq 0.05$. The raw data collected by the mass spectrometer was converted into mzXML (extensible mark-up language) files using CompassXport software, version 3.0 (Bruker Daltonics, Germany). In this conversion, the mass/charge ratio (m/z) values were encoded using 64-bit precision. The PEAKS software, version 8.5 (Bioinformatics Solutions Inc., Canada) was used to process the mzXML files, using the PEAKS DB procedure [3] to identify the proteins present in the samples.

III. RESULTS AND DISCUSSION

There was a difference in the a* color component ($P = 0.006$), indicating a redder color for cattle in the heavy group. Similarly, chroma, the color saturation index, was also higher for the heavy group ($P = 0.019$; Table 1). Proteins identified in the meat of Nellore bulls with different carcass weights have been enriched for the following pathways: Metabolic pathways, Oxidative Phosphorylation, Electron Transport Chain, Glycolysis/Gluconeogenesis, Amino Acids Biosynthesis, and Muscle Contraction (FDR ≤ 0.05; Table 1). A total of 22 proteins were identified only in the muscle of the “Light carcass”

group, including Phosphopyruvate hydratase (ENO2) and Stress-70 protein (HSPA9), which are reported as putative protein biomarkers correlated with beef color traits [4].

Table 1 – Meat pH and color parameters of Nellore bulls with different carcass weights and distribution of proteins identified only in meat from the "light carcass" group according to their shared function in different biological processes enrichments in the Gene Ontology network (GO).

	LIGHT			HEAVY			*P-value*
	Mean ± SEM	Min	Max	Mean ± SEM	Min	Max	
CW, kg	125.17 ± 1.83	116.10	133.10	150.41 ± 1.06	145.5	155.5	<0.001
pHu	5.66 ± 0.05	5.61	5.77	5.64 ± 0.05	5.54	5.70	0.482
L*	35.47 ± 1.05	29.47	40.97	36.18 ± 1.00	32.26	40.77	0.632
a*	13.93 ± 0.46	11.52	15.82	15.85 ± 0.39	14.00	17.69	0.006
b*	12.47 ± 0.44	9.14	13.48	13.52 ± 0.58	10.39	15.85	0.167
Chroma	18.74 ± 0.52	16.04	20.42	20.87 ± 0.63	17.45	23.23	0.019

Biological Process[1]	**Gene**	**FDR[2]**
Mitochondrial electron transport, NADH to ubiquinone	NDUFS1; NDUFA8; NDUFA7	0.009
Pyruvate metabolic process	DLAT; ENO2; GAPDHS	0.018
Oxidative Phosphorylation	NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.009
Aerobic respiration	SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.001
Generation of precursor metabolites and energy	GAPDHS; ENO2; SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.000
Cellular metabolic process	TUFM; HSPA9; ALDH7A1; AKR1B1; GAPDHS; ENO2; SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7; DLAT	0.001

[1]Biological Process (Gene Ontology). Software String 12.0. [2]False Discovery Rate – *P-value* corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).
CW = carcass weight; SEM = standard error of the mean; Min = minimum; Max = maximum.

IV. CONCLUSION

This study indicates that heavier cattle of the same age compared to lighter ones tend to yield beef with greater a* and chroma attributes while having no difference in final pH. Standardizing harvesting weight can mitigate color variations in meat from animals with normal pH levels. Furthermore, proteins associated with the oxidative pathway may influence the coloration of beef from cattle of different weights.

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