

Effect of rearing practices and pre-slaughter handling on the Longissimus thoracis and the Semitendinosus muscle proteomes of young bulls

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Introduction: Cattle are herd animals that co-habit in groups, respecting a social hierarchy. Hierarchy influences behaviour and stress levels within the herd and is dependant upon several factors, including animal horn status (Reiche et al., 2020). Pre-slaughter cattle face several stressful challenges, including; mixing, handling, transportation and lairage; with rearing experiences influencing their reactivity to these challenges (Grandin, 2021; Terlouw et al, 2021). Rearing conditions and pre-slaughter stress affects muscle physiology, thereby impacting meat quality (Picard et al, 2019; Terlouw et al., 2021). However, different muscle types may react differently to rearing practices (Picard et al, 2019). In this study, we compare the separate and interactive effects of “horn status”, “rearing conditions” and “pre-slaughter stress” on the Longissimus thoracis (LT) and Semitendinosus (ST) muscle proteomes to decipher the underlying pathways.

Materials and Methods: Eighty-one young Limousin x Swiss dairy bulls were used, half of which were disbudded using a hot iron at the age of 2 months. The experiment respected the Swiss laws of animal protection in agreement with the Cantonal Veterinary Office of Fribourg, Switzerland (No. 2015_21_FR). The calves were allocated to one of two-rearing conditions: non-mixed (NM) or mixed (M). Six-rearing groups were formed, with 2 repetitions per condition: non-mixed (NM) with horns (n=27), non-mixed disbudded (n=27) and mixed (n=27; with and without horns). The animals were slaughtered at 13 months: half under a supplementary stress protocol and half under a limited stress protocol using a balanced experimental design (Reiche et al. 2019). LT and ST muscles were sampled at 48h post-mortem for shotgun proteomics (Zhu et al. 2021). The proteomics data were analysed considering the three factors (rearing conditions (RC), horn status (HS) and slaughter condition (SC)) and their interactions. Proteins significantly associated with at least one of the three main factors were further analysed by bioinformatics using Metascape® for Gene Ontology (GO) (Gagaoua et al. 2021).

Results: 253 proteins were identified in LT and 529 in ST muscle. The number of proteins identified as differentially abundant at $P < 0.05$ according to main factors for LT and ST respectively, were RC (40 vs 12), HS (16 vs 95) and SC (10 vs 19). RC and HS were the most impacting factors on LT and ST muscle proteomes, respectively, with 40/253 proteins influenced by RC in LT, while 95/529 proteins were influenced by HS in ST. SC had a weak and similar effect on LT (10) and ST (19) muscle proteomes. Nine common differentially abundant proteins were identified in the two muscles (CMBL, ACTN1, PYGM, CHCHD3, SYNPO2, PHKA1, PDLIM7, HPX and MYOZ3), from which CMBL and HPX were significantly influenced by HS in both muscles. The comparative GO enrichment analysis of the proteins influenced by HS in both muscles identified three common GO terms: “GO:0006575: cellular modified amino acid metabolic process”, “GO:0016491: oxidoreductase activity” and “GO:0015980: energy derivation by oxidation of organic compounds”. These enriched terms highlight a mitochondrial activity with activated alternative carbohydrate metabolic processes. Several muscle-specific GO terms were identified. For instance, “GO:0003779: actin binding” and “GO:0006096: glycolytic process” were enriched in ST, whereas “GO:0022900: electron transport chain” was found in LT. For RC and SC, no commonly enriched terms were identified amongst LT and ST. However, several specific enriched ontology terms emerged; likely the “GO:0015980: energy derivation by oxidation of organic compounds” highly enriched in LT within RC and “GO:0009055: electron transfer activity” in ST within SC.

Conclusions: The proteomes of LT and ST muscles were influenced differently by stress-modulating rearing practices. Furthermore, the protein changing patterns differed with muscle, with seven influenced proteins in both muscles, but related to different factors. Contrasting patterns as a consequence of different factors may be related to differences in the contractile and metabolic properties of the two investigated muscles.

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Key words: Post-mortem muscle proteome, Cattle mixing, Horn status, Pre-slaughter stress