Mixing horned and disbudded bulls during rearing: effects on post-mortem muscle proteome and related biochemical pathways

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Introduction: The slaughter period is often stressful for animals (Terlouw et al., 2021). For example, for cattle, disruption of social groups, handling practices, transportation, loading, unloading, unfamiliar conditions and mixing animals are known stress factors (Picard et al., 2010; Terlouw et al., 2021). Stress before slaughter may have negative effects on meat quality (Diaz et al., 2020; Terlouw et al., 2021). An earlier study on young bulls found that the presence of horns, the mixing of horned and disbudded bulls and pre-slaughter stress levels affected meat quality (Reiche et al., 2019). This study used the same experiment to apply a proteomics approach to help decipher the effect of horn status, rearing conditions and pre-slaughter stress on the muscle proteome and underlying pathways with a focus on mixing horned and disbudded bulls.

Material and Methods: Eighty-one young Limousin x Swiss dairy bulls were used in this trial, half of which were disbudded using a hot iron at the age of 2 months. The experiment respected the Swiss laws of animal protection and authorized by 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; half of each group with horns, half disbudded). 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 as detailed in Reiche et al. (2019). *Longissimus thoracis* muscle samples were taken at 48h post-mortem and used for shotgun proteomics as in Zhu *et al.* (2021). The proteomics data were analyzed by considering the 3 factors (rearing conditions, horn status and slaughter protocol) including the interactions. In this communication, only the proteins influenced by rearing condition were considered for bioinformatics analysis and analysed by the means of Metascape® platform to investigate the main enriched Gene Ontology (GO) terms as described in Gagaoua et al. (2021a,b).

Results: A total of 265 proteins were identified, among which 40 differed significantly ($P < 0.05$) in abundance between the rearing conditions. Thirteen of the 40 proteins were listed by Gagaoua et al. (2021a) as known biomarkers of beef tenderness. The pathway enrichment analysis of the 40 proteins revealed the importance of generation of precursor metabolites and energy (GO: 0006091) as the top enriched GO term for mixed (M) animals followed by others related to the respiratory electron transport and ATP metabolic process. Seventeen of the 40 proteins were down regulated in the M group. These proteins were mainly linked to mitochondrial protein import (R-HSA-1268020), respiratory electron transport and ATP metabolic process. The up-regulated proteins in the M group were related to carbohydrate metabolism (R-HSA-5663084), muscle structure development and glycogen metabolism.

Conclusion: The mixing of horned and disbudded bulls influenced the muscle proteome, relative to non-mixing. Proteins which displayed differences in abundance were related to key biological pathways such as energy and carbohydrate metabolism, including the mitochondrial energy pathways and respiratory chain. Some of these proteins are known to be related to tenderness.

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