PROTEIN EXPRESSION PROFILES IN MUSCLE ARE AFFECTED BY BETA-AGONIST TREATMENT IN CATTLE

Phillip Strydom[1*], Vibeke Høst[2], Eva Veiseth-Kent[2] and Ennet Moholisa[1]

[1] Animal Production Institute, Agricultural Research Council of South Africa, Irene, 0062, South Africa;

[2]Nofima AS, Norway

*Corresponding author email: pstrydom@arc.agric.za

I. INTRODUCTION

Beta agonists, such as zilpaterol, are used to increase feed efficiency and lean tissue deposition in feedlot cattle. Their mechanisms include an increase in lipolysis and protein synthesis and a decrease in lipogenesis and protein degradation [1]. However, beta-agonists also induce muscle toughness by increasing calpastatin activity and reducing calpain activity [1, 2], with less evidence of effects on background tenderness, i.e. muscle fibre type and connective tissue characteristics. All these processes will involve changes in protein expression related to changes in growth and meat quality characteristics [3]. Our study investigates meat tenderness related characteristics and muscle protein expression profiles in beef longissimus muscle as affected by the beta-agonist, zilpaterol.

II. MATERIALS AND METHODS

Eight Bonsmara steers were grain fed under commercial feedlot conditions for approximately 110 days. Four steers were supplemented with the beta–agonist, zilpaterol (AZ) for the final 30 days on feed and the remaining four received no zilpaterol (AC). The longissimus muscle (LL) aged for 3 and 14 days was used to measure Warner-Bratzler shear force resistance (WBSF) on oven-broiled samples (70°C) and myofibrillar fragments length (MFL) 4] by video image analyses (400X magnification). Collagen properties were measured on unaged samples [5]. For proteome studies, muscle samples were snap-frozen immediately after slaughter. Proteins were extracted by homogenizing muscle tissue in a SDS-DTT-Tris buffer, and peptide generation was performed with the FASP method [6]. Samples were analysed by a QExactive LC-MSMS system, with three injections per sample in randomized order. Raw data was analysed using the LFQ-procedure in MaxQuant, and Partial Least Squares Regression (PLSR) in Unscrambler was used to reveal significant changes in protein expression profiles.

III. RESULTS AND DISCUSSION

The results discussed here are from a sub-sample of a larger trial and the results and conclusions should therefore be considered with caution. However, it can be confirmed that the differences in meat quality parameters between treatments reported in Tble 1 are similar to the differences found in the larger studyAZ steaks were tougher at 3 days after slaughter than AC steaks and the disadvantage of zilpaterol was not negated by aging after 14 days. MFL values mirrored WBSF values, although the differences at 14 days were smaller than at 3 days. Shorter MFLs are generally associated with a higher degree of proteolysis and therefore tenderisation [1, 4]. The lower collagen content of AZ samples ($P = 0.024$) could be linked to muscle hypertrophy, in agreement with the study of Strydom et al. [1]. For the proteomics analysis, all proteins quantified in at least one sample per treatment group were included in the PLSR analysis (727 proteins). The results showed a systematic variation in protein expression between treatments and the first two principal components (PC) explained 86 and 13 % of the variation in the data set, respectively. There was a clear separation of the two treatment groups along PC1 (86% of variation), and the expression profile of 80 proteins had a significant contribution to this grouping. The changes in protein expression profiles indicates a shift in energy metabolism from an oxidative energy production in the AC animals, towards a more glycolytic energy production in the AZ animals. In addition, AZ animals had elevated levels of proteins related to RNA translation, protein folding, as well as myofibrillar- and cytoskeleton-related proteins compared to the AC animals, further supporting the muscle hypertrophy effect of zilpaterol. In support, Strydom et al. [1] reported

higher ratios and larger fibre areas for fast glycolytic (white) muscle fibres and a general trend towards larger fibre areas for all muscle fibre types of zilpaterol treated animals.
Because the loin muscle is a low connective tissue muscle, the positive effect of lower collagen content in AZ samples was probably masked by the negative effect of zilpaterol on other characteristics. Hypertrophy of fast glycolytic fibres are generally associated with tougher meat [7], but the same review reports that fast glycolytic fibres have a faster rate of maturation than slow oxidative fibres.

Table 1 Meat quality characteristics showing the effect of beta-agonist treatment

Item	AC	AZ	SEM	P value
Total collagen[1]	2.0	1.4	0.156	0.024
% Soluble collagen	9.7	10.5	0.897	0.544
Shear force 3 days p.m. (kg)	4.1	5.6	0.280	0.007
Shear force 14 days p.m.(kg)	3.1	4.2	0.232	0.011
MFL 3 days (μm	28.1	42.2	4.49	0.068
MFL 14 days (μm)	19.3	29.3	1.92	0.010

[1]Hydroxy-proline N/Total N x 10^3; p.m. – post mortem; MFL - Myofibril fragment lengths

IV. CONCLUSION

Protein expression in this study indicates that zilpaterol treatment of cattle induces muscle hypertrophy that also coincides with a shift towards a more glycolytic energy production. However, when considering the differences in initial tenderness (3 days) and more specific further tenderization (14 days) between control and zilpaterol treated animals, muscle hypertrophy explains some but not all the mechanisms involved in tenderness differences. Further bioinformatics investigations of the proteomics data may reveal more information regarding the effects of zilpaterol both in the living animals as well as during post mortem storage.

ACKNOWLEDGEMENTS

This work was supported by Red Meat Research and Development South Africa.

REFERENCES

1. Strydom, P.E., Frylinck, L., Montgomery, J.L., & Smith, M.F. (2009). The comparison of three beta-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Science 81:557–564.
2. Wheeler, T. L., & Koohmaraie, M. (1997). Effects of the adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinases activities, and meat tenderness in steers. Journal of Animal Science 70: 3035−3043.
3. Keller, W., Hayes, C., Shabb, J., Muhonen, W., Maddock-Carlin, K. (2016). Effect of implant strategy and supplementation of zilpaterol hydrochloride on the skeletal muscle proteome from beef steaks aged up to 14 days. Beef Research. Accessed 15 April 2018: www.beefresearch.org/productqualityresearch.aspx?newsid=5948.
4. Culler, R. D., Parrish, F. C., Smith, G. C., & Cross, H. R. (1978). Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. Journal of Food Science 43: 1177–1180.
5. Weber, R. (1973). The determination of hydroxyproline and chloride in meat and meat products: Simultaneous operation with nitrogen and phosphorus determinations. Technicon International Division SA, Technical Report 7.
6. Wisniewski, J. R., Zougman, A., Nagaraj, N. & Mann, M. (2009). Universal sample preparation method for proteome analysis. Nature methods 6: 359-362.
7. Lefaucheur, L. (2010). A second look into fibre typing--relation to meat quality. Meat Science 84:257-70.