EFFECTS ON PORK QUALITY OF GILTS TREATED WITH RACTOPAMINE AND GROWTH HORMONE FOR 7 AND 27 DAYS

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Abstract –Effects of beta-agonist (Ractopamine™) and growth hormone (GH - Reporcin™) were evaluated on gilts' carcass characteristics, and *longissimus* muscle meat quality and proteolytic system activities (caspase 3/7 and proteasome). In two experiments consisting of 7 or 27 day treatment periods, gilts (n=15 each group) were fed a standard commercial diet *ad-libitum (control)* or with the same feed supplemented with beta-agonist at 10mg/kg (7days) or 20mg/kg (27days), or administered growth hormone (10mg every 2 days) in both experiments. Carcass characteristics were assessed at slaughter and *longissimus* muscle samples were assessed for caspase 3/7 and proteasome (chymotrypsin-like, trypsin-like and caspase-like) activities. Growth hormone increased liver weights (P <0.01) but had no effect on muscle weights. Beta-agonist increased muscle weights over the 27 day (P<0.05) and 7 day (P=0.062) treatments and tended to increase shear force values (P=0.09 and 0.107, 7day and 27day respectively). Beta-agonist increased the chymotrypsin-like proteasome activity (P=0.062 and 0.041, 7 days and 27 days, respectively), whereas Growth hormone decreased caspase-like proteasome activity (P=0.071 and 0.025, 7day and 27day, respectively). This study indicates that proteasome activities are not associated with the potentially negative effects on meat quality induced by beta-agonist treatment in pigs.

Key Words – Caspase 3/7, Proteasome, Tenderness.

- INTRODUCTION

Growth promoters are mainly used as supplements to improve rate of gain, improve feed efficiency and to increase carcass meat yield efficiency [1,2]. Feed conversion efficiency (FCE) and carcass composition of the animals are major variable factors which impact on the productivity and profitability of the pig industry. The inconsistent quality of pork meat is a major concern for the industry which is in part caused by variable tenderness. Meat tenderness is partially dependent upon the degree of alteration of the structural components of muscle and associated proteins post-mortem. Reduced activity of the calpain proteinases is associated with tough meat. The levels of calpastatin, a specific inhibitor protein of the calpain proteinases, are strongly related to meat toughness [3]. However it is known that, in addition to the calpain proteinases, both caspase and proteasome proteolytic enzyme systems are involved in skeletal muscle development and remodeling. Caspases are activated early in some pathological events, such as apoptosis [3,4], while the proteasome is a ubiquitin-dependent protein degradation system that cleaves regulatory, mis-folded and damaged proteins into small peptides [5]. Their proteolytic attributes make these enzyme systems possible contributors to improved meat tenderness. The objective of this study was to evaluate the effects of a beta-agonist (BA - Ractopamine™) and porcine growth hormone (GH - Reporcin™) on the caspase3/7 and proteasome variants' (chymotrypsin-like, trypsin-like and caspase-like) activities, as well as tenderness of the *longissimus* muscle from adult gilts treated for 7 or 27 days.

- MATERIALS AND METHODS

Two separate studies were performed, the main difference being the length of treatment, with pigs treated for either 7days (study 1) or 27days (study 2). In each study, 45 White Duroc x

(Landrance x Large White) gilts were sourced from PIC (Alpha Building, Nantwich, Cheshire), acclimated to the feed and environment for 5days, before being allocated to one of three treatment groups (Control, BA or GH, n=15 per treatment). The Control group were fed a standard commercial diet *ad-libitum*, while the BA group were also fed *ad-libitum* the standard commercial diet containing Ractopamine™ at either 10mg/kg (for 7days) or 20mg/kg (for 27days) and the GH group were fed the commercial diet *ad-libitum* and administered Reporcin™ (10mg) by intramuscular injection every other day until the day before slaughter (day 6 or 26). Feed intake was measured daily. Within 15 minutes of slaughter, skeletal muscles (*Semitendinosus* (ST), *Vastus Lateralis* (VLat)) were dissected from the right-side half of the carcass and their weights recorded together with that of the liver and the whole carcass. Samples of *Longissimus dorsi* (LD) muscle were collected at 0hr post-mortem and snap frozen in liquid nitrogen. LD samples were then assayed for caspase 3/7 activity [6] and proteasome activity was assessed as three separate proteolytic activity constituents, chymotrypsin-like, trypsin-like and caspase-like activities [5], all using commercially available kits (Promega, UK). After 48 hrs of conditioning at 4°C, chops were also collected from the LD muscle, vacuum packed and aged for 5 or 8 days at 4°C, then stored at -20°C until analysis of Warner-Bratzler shear force. Data were analysed by ANOVA (using the Genstat statistical package), followed by a *Post Hoc* Dunnett's test. $P<0.05$ was taken as being significant.

- RESULTS AND DISCUSSION

As has been previously reported [7], GH significantly increased liver weight ($p<0.001$) at both time points (Tables 1 & 2). In pigs treated for 7days there were no significant differences in carcass or ST muscle weights or ST % weight (as a proportion of body weight), but BA tended to increase VLat muscle weight and % weight (P=0.062 and 0.055 respectively, Table 1). In pigs treated for 27 days (Table 2), BA significantly increased carcass, VLat and VLat % weights. These studies indicate that a low dose of BA (10mg/kg) combined with a short time period of administration (7 days) was not sufficient to elicit a significant response in lean tissue growth, but a higher dose (20mg/kg) for longer (27 days) did elicit a significant increase in lean tissue growth. There were no significant differences in shear force after 5days of aging in both studies (Tables 1 and 2), but BA treatment tended to increase shear force values after 8 days of ageing (P=0.09 and p=0.107 for the 7 and 27 day studies respectively). Negative effects of BA on meat quality have been reported before and in this study it appeared that these negative effects took time to develop during the conditioning period. No significant differences in LD Caspase 3/7 activities were observed

Table 1 Effects of treating gilts with BA or GH for a 7 day period

Measurement	Control	BA	GH	SED	P-value
Feed conversion efficiency (kg gain/kg feed)	0.327	0.336	0.315	0.041	0.879
Carcass weight (kg)	74.96	75.62	73.59	1.93	0.567
Liver wt (kg)	1.573	1.551	1.859	0.067	<0.001
% Liver wt (g/100g BWt)	1.68	1.65	2.02	0.05	<0.001
Whole muscle weights:					
ST wt (g)	434.21	454.58	417.46	21.23	0.231
% ST wt (g/100g BWt)	0.465	0.484	0.454	0.018	0.267
VLat wt (g)	340.62	376.87	342.51	16.56	0.062
% VLat wt (g/100g BWt)	0.364	0.401	0.373	0.015	0.055

Shear Force (KgF)					
5 days of Ageing	5.24	5.79	5.32	0.187	0.439
8 days of Ageing	5.28	5.55	5.08	0.229	0.09
LD Proteolytic system					
Caspase 3/7 (fluorescence/µg protein)	12.16	12.01	12.15	0.891	0.983
Proteasome:					
Caspase-like (luminescence/µg protein)	28386	32570	27468	2258.8	0.071
Chymotrypsin-like (luminescence/µg protein)	5830	6181	5263	375.3	0.062
Trypsin-like (luminescence/µg protein)	43177	42909	41470	2578.6	0.778

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Table 2 Effects of treating gilts with BA or GH for a 27 day period

Measurement	Control	BA	GH	SED	P-value
Feed conversion efficiency (kg gain/kg feed)	0.843	0.834	0.787	0.260	0.974
Carcass weight (kg)	83.26	87.74	85.21	1.067	0.002
Liver wt (kg)	1.635	1.539	1.915	0.064	<.001
% Liver wt (g/100g BWt)	1.59	1.45	1.82	0.071	<.001
Whole muscle weights:					
ST wt (g)	483.3	534.3	503.9	22.47	0.075
% ST wt (g/100g BWt)	0.471	0.505	0.479	0.025	0.118
VLat wt (g)	384.4	406.1	358.3	15.56	<.001
% VLat wt (g/100g BWt)	0.377	0.383	0.341	0.018	<.001
Warner-Bratzler Shear Force (Kg)					
5 days of Ageing	5.53	5.92	5.58	0.405	0.581
8 days of Ageing	4.93	5.48	5.21	0.257	0.107
LD Proteolytic system					
Caspase 3/7 (fluorescence/µg protein)	13.721	14.33	12.52	1.26	0.363
Proteasome:					
Caspase-like (luminescence/µg protein)	22171	19734	18144	1414	0.025
Chymotrypsin-like (luminescence/µg protein)	18931	20978	17467	1327	0.041
Trypsin-like (luminescence/µg protein)	35865	34809	32716	2220	0.345

between treatments in the two studies (Tables 1 and 2). There were also no effects of treatment on LD trypsin–like proteasome activity in either study. However there were effects on both chymotrypsin-like and caspase-like proteasome activities, with BA tending to increase both activities after the 7day treatment (P=0.062 and P=0.071 respectively, Table 1). In the 27 day treatments (Table 2), gilts treated with GH had lower caspase-like activities (P=0.025), whereas those treated with BA had higher chymotrypsin-like proteasome activities (P=0.041). Of the three proteolytic activities within the proteasome complex, the chymotrypsin-like variant is reported to be the most active [5], which was higher in BA treated gilts in both studies.

IV. CONCLUSION

Of the two commercially available growth promoters used in this study, BA was the most potent but had negative effects on meat quality. Increased calpain mediated proteolysis is recognized as being positively associated with meat tenderness [8]. Although there were effects of BA and GH on some components of the proteasome system, these were in the opposite

direction to that of the observed effects on meat tenderness. Therefore this study indicates that caspase and proteasome activities are unlikely to be associated with the negative effects on meat quality induced by BA treatment of pigs.

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REFERENCES

- Dikeman, M. E. (2007). Effects of metabolic modifiers on carcass traits and meat quality. Meat Science 77: 121 – 135.
- Hope-Jones, M., Strydom, P. E., Frylinck, L. & Webb, E. C. (2010). The efficiency of electrical stimulation to counteract the negative effects of β-agonists on meat tenderness of feedlot cattle. Meat Science 86: 699 – 705.
- Kemp, C. M., Sensky, P. L., Bardsley, R.G. Buttery, P. J. & Parr, T. (2010). Tenderness – an enzymatic view. Meat Science 84: 248-256.
- Herrera-Mendez, C H, Becila, S., Boudjellal, A. & Ouali, A. (2006). Meat ageing: Reconsideration of the current concept. Trends in Food Science & Technology 17: 394-405.
- Strucksberg, K., Tangavelou, K., SchrÖder, R. & Clemen, C. (2010). Proteasomal activity in skeletal muscle: A matter of assay design, muscle type, and age. Analytical Biochemistry 399: 225 – 229.
- Wagner, K. D., Wagner, N., Wellmann, S., Schley, G., Bondke, A., Theres, H. & Scholz, H. (2003). Oxygen-regulated expression of the Wilms' tumor suppressor Wt1 involves hypoxia-inducible factor-1 (HIF-1). FASEB J. 17: 1364 – 1366.
- Brameld, J. M., Atkinson, J. L., Saunders, J. C., Pell, J. M., Buttery, P. J. & Gilmour, R. S. (1996). Effects of growth hormone administration and dietary protein intake on insulin-like growth factor I and growth hormone receptor mRNA expression in porcine liver, skeletal muscle, and adipose tissue. Journal of Animal Science 74: 1832-1841.
- Koohmaraie, M. & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science 74: 34-43.