**PE1.17 Effect of compensatory growth in pigs on skeletal muscle protease expression 142.00**

*Chanporn Chaosap (1) sbxcc1@nottingham.ac.uk, T Parr( 2), J Wiseman 1,*
*(1)Division of Animal Sciences, School of Biosciences The university of Nottingham Sutton Bonington campus, United Kingsom*
*(2)Division of Nutritional Sciences, School of Biosciences The university of Nottingham Sutton Bonington campus, United Kingdom*

***Abstract*— The purpose of the current study was to examine whether a compensatory feed regime in pigs influenced the gene expression of enzymes involved with muscle proteolysis in Longissimus Dorsi (LD). Pigs at 73 d of age were fed a commercial diet at 0.70 of ad libitum (R) for 40 d followed by a return to ad libitum for a further 42 days whilst the control group was fed ad libitum (AL) throughout. Animals on R and AL feed regimes were slaughtered at the end of the restriction period (SL1), two days after re-feeding (SL2) and after 42 days re-feeding (SL3) (n=8 for each group). The pigs on the R regime at SL1 and SL2 had significantly lower carcass weight (P<0.05) but at SL3 there was no difference, whilst the LD (proportion of carcass weight) at SL1 and SL2 was higher in R (P<0.05 and P=0.069 respectively) but not different at SL3. At SL1 the level of plasma IGF-I was significantly lower (P<0.05) in R but this recovered and was not significantly different at SL2 and SL3. The compensatory regime was associated with changes in gene expression of enzymes involved with muscle proteolysis. Caspase 3 mRNA levels in R were increased (P<0.05) relative to AL at SL1 and SL2 but not at SL3. The levels of the muscle-associated E3-ligases MAFbx and MuRF1 decreased in the R group at SL3 (P=0.091 and P<0.05). The compensatory growth feeding regime had differential effects on the proteolytic systems at different points of the regime. However, there was no effect on meat quality (as measured by shear force), suggesting the changes observed in expression of the proteolytic systems, although they may be related to changes in protein turnover, are not associated with altered meat quality.**

C. Chaosap and J. Wiseman are in the Division of Animal Sciences, School of Biosciences Sutton Bonington Campus The University of Nottingham, Leicestershire LE12 5RD, UK (corresponding author to provide phone: +44 0115 9516128; fax: +44 0115 9516122; e-mail: sbxcc1@nottingham.ac.uk and Julian.wiseman@.nottingham.ac.uk).

T. Parr is in the Division of Nutritional Sciences, School of Biosciences Sutton Bonington Campus The University of Nottingham, Leicestershire LE12 5RD, UK. (e-mail: tim.parr@nottingham.ac.uk).

***Index Terms*—gene expression, calpain, caspase, MuRF1 , MAFbx. Pig meat quality**

## I. INTRODUCTION

Compensatory growth is the process by which animals show a period of enhanced growth when they are re-fed on a higher plane of nutrition after a period of feed restriction. The mechanism by which this process is mediated is believed to be through an overall increased protein turnover in the animals undergoing compensation [1]. The associated increase in the level of protein degradation has been suggested to have a subsequent positive effect on post-mortem proteolysis and could potentially influence meat tenderness [2].

There are a number of enzymes involved in protein degradation in muscle that can be broadly separated into those which are involved in general degradation and those that initiate the process. Examples of the latter are enzymes such as the calpains, caspases and E3-ligases. $Ca2^{+}$-dependent protease calpains, are believed to be involved in the process of protein turnover in muscle [3]. Of the two calpain isofroms, calpain 1, calpain 2, it is the activity of the former that is strongly associated with determining the extent of meat tenderisation [4]. The caspases, the proteinases associated with cell death (apoptosis), have been implicated in proteolysis in muscle, being associated with specific physiological processes involving regulated protein degradation [5]. More recently it has been reported that changes in caspase activity was associated with variations in meat tenderness and that caspases were capable of degrading myofibril proteins [6,7]. A third system has been associated with protein degradation in muscle that is the Ubiqutin (Ub) E3- ligases MAFbx and MuRF1 that, through the generation of Ub-conjugated proteins, identify peptides for degradation by the proteasome [8]. Although closely associated with muscle protein degradation in conditions where there are significant changes in muscle mass [8], the proteasome is thought not to have a significant role in the postmortem tenderisation process [4]. In previous studies compensatory growth regimes have been shown to affect the level of IGF-I with decreased levels during the restriction phase that has been reported to be elevated during the compensatory phase [9]. Both MAFbx and MuRF1 mRNA expression are known to be decreased by elevated by IGF-I levels [10], suggesting that growth regimes that alter IGF-I levels may

| Target transcript | Accession no. | Primer / Probe | Nucleotide Sequences (5'-3') |
|---|---|---|---|
| Calpain 1 | AF263610 | Forward | GACACCCTCCTGCACCGA |
| | | Reverse | TCCACCCACTCCCCAAACT |
| | | Probe | CCACACGGCCAAAGCTTCCAGAATG |
| Calpain 2 | U01181 | Forward | ACATGCACACCATCGGCTTT |
| | | Reverse | CGCTCTGTGCGTCAGGAAG |
| | | Probe | TTCCGGAAGAGTTGACCGGACAGACC |
| Caspase 3 | AB029345 | Forward | TTGAGGCAGACTTCTTGTATGCAT |
| | | Reverse | CGCTGCACAAAGTGACTGGA |
| | | Probe | TTCTACAGCACCTGGTTACTATTCCTGGCG |
| MuRF1 | EW363322 | Forward | GGAGATGTTTACCAAGCCGG |
| | | Reverse | TGGTCCAGTAGGGATTTGCAG |
| | | Probe | CGAAAGTGTGCCAACGACATCTTCCAG |
| MAFbx | EW308124 | Forward | CATTGCCCAAAAGAACTTCATG |
| | | Reverse | GGGTCTGGAGGAGTTCCCTT |
| | | Probe | AAAAAGTGGTACTGAAAGTGCTTGAAGACCAGC |
| Cyclophilin | F14780 | Forward | ACCGTCTTCTTCGACATCGC |
| | | Reverse | GCACGGAAGTTTTCTGCTGCTT |
| | | Probe | CTTGGGCCGCGTCTCCTTCGAG |

influence these enzymes that identify proteins for protein degradation.

The aim of the current study was to examine the effect of a compensatory growth regime in pigs on the plasma level of IGF-I and whether this was associated with changes in the expression of proteolytic enzymes that have been associated with muscle growth and post mortem proteolysis.

## II. MATERIALS AND METHODS

### *a. Animals*

Forty-eight female pigs were allocated to 3 slaughter groups (SL1, SL2, SL3 with slaughter at ages 114d, 116d, and 156d, each group containing 16 animals). All animals were fed a commercial diet (13.9 MJ metabolizable energy and 165 g crude protein/kg) ad libitum from 60 to 73 days at which point, within each slaughter group, n=8 were restricted (0.70 of ad libitum) whilst remaining animals were fed ad libitum. At 114 days all animals were fed ad libitum until 156 days. Thus for SL1, 8 pigs were fed ad libitum and 8 were restricted for 40 days; for SL2 8 animals were fed ad libitum and 8 fed restricted for 40 days then re fed ad libitum for 2 days; for SL3 (control) 8 were fed ad libitum for 82 days and 8 fed restricted for 40 days then re fed ad libitum for 42 days. At slaughter plasma was extracted from blood and longissimus dorsi (LD) samples were taken and snap-frozen in liquid Nitrogen then stored at -70°C until analysed. After 48 hours storage at 4°C postmortem, LD chops were dissected from the the carcass, vacuum packed then conditioned at 4°C for 8 days, then frozen at -70°C until analysed.

*Table 1*. Pig Q-PCR primers and dual labeled probes (5'FAM and 3'TAMRA)

### *b. Shear Force*

The shear force was measured by cooking the 8 day conditioned LD chops in a water bath at 80˚C to an internal temperature of 78˚C. The samples were cooled overnight at 4˚C before shearing cored samples using a Stevens CR Analyzer fitted with Volodkevich-type jaws. The mean values of eight shearings on sub samples of 1 $cm^2$ cross section were recorded.

### *c. IGF-I*

Plasma IGF-I was analysed by the active mouse/rat IGF-I enzyme linked immunosorbant assay(ELISA) kit (Diagnostic systems laboratories, INC).

### *d. Gene Expression*

Total RNA was extracted from 100 mg of crushed LD using Tripure reagent (Roche). Residual genomic DNA in the extracted total RNA was digested with RQ1 RNase-Free DNase (Promega). To determine the relative levels of mRNA transcripts equal quantities of total RNA was used to synthesise first strand cDNA using random hexamers and MMLV Reverse Transcriptase (Promega) which was then subjected to quantitative PCR (Q-PCR) using the primers and dual labelelled fluorescent probe sets (Table 1) using LightCycler® 480 Probes Master reaction mixture on a LightCycler® 480 (Roche) using a protocol as specified by the manufacturer. The Q-PCR reactions were conducted in triplicate using 1 ng of RNA equivalence and cyclophilin mRNA expression was used as internal standard. Relative transcript quantification was performed using standard curve method as described by the manufacturer (Roche).

*e. Statistical analysis*

The statistical model was a feeding regime comparison; data were analyzed by one-way ANOVA separately for each slaughter group (Genstat edition 11th).

## III. RESULTS AND DISCUSSION

As was expected the restricted-fed group had lower carcass weight than the group fed ad libitum both at SL1($P<0.05$) and SL2 ($P=0.052$) but after the compensatory phase (SL3) there was no significant difference in carcass weight, Table 2. However, the LD in the restricted fed animals was significantly larger than the ad libitum-fed animals at SL1 and SL2($P<0.05$)

| Trait | Ad lib | Restriction | P value | SED |
|---|---|---|---|---|
| SL1 | | | | |
| Carcass weight (kg) | 55.2 | 48 | 0.035 | 3.06 |
| LD* | 0.0308 | 0.0326 | 0.066 | 0.00087 |
| IGF-I (ng/ml) | 282 | 132 | 0.010 | 50.2 |
| Shear Force (kg) | 5.9 | 7.4 | 0.110 | 0.89 |
| SL 2 | | | | |
| Carcass weight (kg) | 58 | 51 | 0.052 | 3.2 |
| LD | 0.0311 | 0.0334 | 0.013 | 0.00080 |
| IGF-I (ng/ml) | 244 | 173 | 0.110 | 41.5 |
| Shear force (kg) | 6.5 | 7.2 | 0.320 | 0.69 |
| SL3 | | | | |
| Carcass weight (kg) | 95 | 92 | 0.534 | 4.5 |
| LD | 0.0313 | 0.0310 | 0.776 | 0.00114 |
| IGF-I (ng/ml) | 201 | 227 | 0.31 | 24.6 |
| Shear force (kg) | 5.4 | 5.9 | 0.31 | 0.47 |

*Table 2* Effect of a compensatory growth feeding regime in pigs on plasma IGF-I and carcass characteristics.

* wet weight LD as a proportion of carcass weight

but this difference was not significant once the animals reached SL3 (Table 2).

At SL1 the restricted fed pigs had 53% lower levels of plasma IGF-I than the control ($P<0.05$) but, after re-feeding the restricted group for two days at ad libitum levels, the plasma IGF-I levels had recovered, there being no significant difference between the groups ($P=0.11$), Table 2. Examination of 8 day conditioned LD shear force at all the slaughter dates showed no significant between the feeding groups, Table 2.

*Table 3* Effect of a compensatory growth feeding regime in pigs on the expression levels of proteolytic system components in LD.

| Trait | | Relative expression to cyclophilin mRNA | | | |
|---|---|---|---|---|---|
| | | Ad lib | Restriction | P value | SED |
| SL1 | | | | | |
| | MAFbx | 0.6 | 0.4 | 0.330 | 0.19 |
| | MuRF1 | 0.9 | 1.1 | 0.500 | 0.28 |
| | Calpain 1 | 0.7 | 0.8 | 0.575 | 0.22 |
| | Calpain 2 | 0.9 | 1.0 | 0.605 | 0.31 |
| | Caspase 3 | 0.5 | 1.0 | 0.024 | 0.23 |
| SL2 | | | | | |
| | MAFbx | 0.5 | 0.6 | 0.480 | 0.17 |
| | MuRF1 | 0.9 | 0.9 | 0.760 | 0.12 |
| | Calpain 1 | 0.5 | 0.6 | 0.069 | 0.06 |
| | Calpain 2 | 0.8 | 0.9 | 0.295 | 0.10 |
| | Caspase 3 | 0.58 | 0.75 | 0.020 | 0.07 |
| SL3 | | | | | |
| | MAFbx | 2.1 | 1.2 | 0.091 | 0.51 |
| | MuRF1 | 1.4 | 1.1 | 0.020 | 0.11 |
| | Calpain 1 | 1.0 | 0.8 | 0.562 | 0.23 |
| | Calpain 2 | 0.7 | 0.7 | 0.555 | 0.07 |
| | Caspase 3 | 0.9 | 0.7 | 0.590 | 0.24 |

The expression level of both MAFbx and MuRF1 was not significantly different at SL1 and SL2. However, after the full re-feeding period (SL3), the restricted group showed lower gene expression than the control group for both MAFbx ($P<0.1$) and MuRF1 ($P<0.05$) (Table 3). E3 ligase activity is associated with protein degradation [8], the increase in expression of these at SL3 may be associated with increased protein turnover via the proteasome system. For the calpain system the expression of calpain 2 mRNA was not affected by the changes in feed regime. For calpain 1, re-feeding pigs ad libitum for two days (SL2) led to an increase in its expression ($P=0.069$). Previous studies examining calpain activity in compensatory growth showed the highest activity of calpain in ad libitum fed animals [2], however in our study the level of gene expression was only significant after immediate refeeding (SL2). At the end of the restricted feeding period (SL1) and two days after (SL2), the level of caspase 3 mRNA was significantly increased ($P<0.05$) whilst at SL3 there was no effect ($P>0.05$). Caspase activity in skeletal muscle has been associated with remodeling of muscle proteins during atrophy [5]. In our study, caspase gene expression was elevated in animals where there was restriction of nutrients or immediately after, this may reflect an immediate proteolytic adaptation and perhaps associated remodeling effect in the muscle to altered states of nutrition.

## IV. CONCLUSION

In the current study there was a compensatory recovery in the carcass weights of the animals after a period of restriction but this was not associated with a relative increase in LD weight in relation to the carcass, suggesting the carcass compensation was via some other tissue depot. The results demonstrated that restricted feeding reduced the level of plasma IGF-I but this recovered on ad libitum feeding although it did not increase during the compensatory period. The period where IGF-I levels were significantly different was associated with changes in the levels of caspase 3 and calpain 1 enzymes that have been implicated in initiating remodeling of proteins. In contrast to previous studies where IGF-I was shown to influence MAFbx and MuRF1 [10], in the animals that had different circulating levels of IGF-I there was no difference in their expression. Although there were changes in the expression levels of these proteolytic system components this was not associated with changes in meat quality.


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