Meat Science and Muscle Biology: Muscle Growth and Fresh Meat Quality

388 Myostatin regulates MyHC isoform expression during myoblast differentiation in cattle. S. Hayashi*, K. Watanabe, Y. Miura, S. Hayashi, M. Miyake, H. Aso, S. Ohwada, and T. Yamaguchi, *Tohoku University, Sendai, Japan.*

Myostatin (MSTN) belongs to the transforming growth factor beta superfamily and has been shown to function as an inhibitor of skeletal muscle proliferation and differentiation. Spontaneous mutations of the myostatin gene in some cattle breeds are characterized by increased muscle mass (double-muscling: DM) resulting from both muscle hypertrophy and myofiber hyperplasia. To study the effects of MSTN during early differentiation of myoblasts, we prepared myoblast cultures from normal (NM) and DM Japanese shorthorn cattle by enzyme dissociation and examined mRNA expression of the myosin heavy chain (MyHC) isoforms (embryonic, fetal, fast 2a, fast 2x and slow) by semi-quantitative RT-PCR. Further, we established five myostatin deficient myoblast (MDMB) clones from DM myoblast primary culture by limiting dilution method and added 1 µg/ml recombinant MSTN (rMSTN) into the cloned myoblast cultures to investigate it's role in myoblast differentiation. Here, we found that the increasing in number of myoblasts and myotubes was detected in DM cultures as compared with that in NM ones. We also found that mRNA expression of fast 2a and 2x MvHC isoforms were greater in DM cultures than that in NM ones in fusion medium. In MDMB cultures, rMSTN inhibited myotube formation and the expression of fetal and fast 2x MyHC isoforms. Interestingly, there was no effect of rMSTN on the expression of embryonic, fast 2a and slow MyHC isoforms. Our findings suggest that MSTN plays a critical role in regulating the myosin isoform expression of myoblasts and myotubes. These results indicate that MSTN may associate with changes of skeletal myofiber types during bovine muscle development.

Key Words: Bovine, Myostatin, Myoblast

389 Influence of the IGF-II genotype on the calpastatin activity in three muscle in relation to age and development. K. Van den Maagdenberg*¹, A. Stinckens², E. Claeys¹, N. Buys², and S. De Smet¹, ¹Laboratory of Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Ghent, Belgium, ²Centre for Animal Genetics and Selection, Department of Animal Production, K.U.Leuven, Leuven, Belgium.

Recently a new QTN, located in the regulatory sequence of the paternally imprinted IGF-II gene was discovered in the pig. Effects of the IGF-II polymorphism on muscle growth and fat deposition have been reported. The aim of this study was to investigate the effect of the IGF-II paternal allele (Qpat/qpat) on early post mortem calpastatin activity in the pig in relation to age and development. Calpastatin was measured in three muscles (*Longissimus, Semimembranosus* and *Triceps brachii*) from respectively, 6/9, 7/3, 6/6 and 15/14 numbers of Qpat/qpat boars of 4, 8, 16 and 26 weeks of age from two lines (Rattlerow Seghers). Data were analysed with an univariate general linear model with IGF-II genotype (G), age group (A), muscle (M) and line number (L) as fixed factors and the two way interactions GxL and MxA.

Significant effects were found for IGF-II genotype, age group, muscle and the two way interactions GxL and MxA. There was no significant main effect for line number. Calpastatin activity was significantly higher in Qpat animals compared to qpat animals. Calpastatin activity was highest in the oxidative *Triceps brachii* and also the largest difference between Qpat and qpat animals was found in this muscle. Calpastatin activity decreased with age from the group of 4 weeks till the group of 16 weeks. Thereafter, calpastatin activity increased again in the group of 26 weeks. For the *Triceps brachii*, the calpastatin activity increased even to higher values compared to values of the group of 4 weeks.

The results show that the calpastatin activity changes with age and development. The higher calpastatin activity in pigs with the IGF-II genotype (Qpat) suggests a reduced proteolytic enzyme activity.

Key Words: IGF-II, Calpastatin, Muscle

390 Cardiac and skeletal muscle protein synthesis and activation of translation initiation factors are stimulated by leucine, but not isoleucine or valine, in neonatal pigs. J. Escobar*, J. Frank, A. Suryawan, H. Nguyen, and T. Davis, USDA/ARS, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX.

Protein synthesis in skeletal muscle of neonatal pigs increases in response to a physiological increase in plasma leucine. However, the effect of a physiological increase in plasma isoleucine and valine on skeletal muscle protein synthesis has not been investigated in neonates. In the pig, the left ventricular wall grows about 3 times faster than the right ventricular wall during the first 10 d of postnatal life due to increased hemodynamic workload of the left ventricle. Therefore, the effects of individual branched chain amino acids on protein synthesis in the left and right ventricular walls, as well as individual skeletal muscles, were determined. Fasted pigs (5 d of age) were infused intra-arterially with saline or 400 µmol·kg⁻¹·h⁻¹ of leucine, isoleucine or valine and protein synthesis was measured after 60 min. Infusion of leucine, but not isoleucine or valine, increased (P < 0.05) phosphorylation of eukaryotic initiation factor (eIF) 4E binding protein-1, and increased the amount and phosphorylation of eIF4G associated with eIF4E in skeletal muscles composed primarily of white (longissimus dorsi) and red (masseter) muscle fibers, as well as in the left and right ventricular walls. Leucine, but not isoleucine or valine, increased the phosphorylation of ribosomal protein (rp) S6 kinase and rpS6 in longissimus dorsi and masseter but not in the left or right ventricular walls. Phosphorylation of elongation factor 2 was unaffected by treatment. The stimulation (P < 0.05) of protein synthesis by leucine was similar in longissimus dorsi, masseter, and left and right ventricular walls. Isoleucine and valine did not increase protein synthesis in cardiac or skeletal muscles. Thus, leucine, but not isoleucine or valine, stimulates protein synthesis in cardiac and skeletal muscles of neonates by increasing eIF4E availability for eIF4F assembly without affecting elongation factor activation. (NIH AR 44474 and USDA 58-6250-6-001)

Key Words: Protein Synthesis, Branched-Chain Amino Acids, Translation Initiation Factors

391 Histochemical properties and meat quality traits of porcine muscles during growth: Effect of feed restriction in pigs slaughtered at the same age and varying weight. G. Bee*, M. Calderini, C. Biolley, G. Guex, and W. Herzog, Agroscope Liebefeld-Posieux, Swiss Federal Research Station for Animal Production and Dairy Products (ALP), Posieux, Fribourg, Switzerland.

At birth porcine muscle fibers are oxidative and conversion towards white fibers occurs rapidly up to 4 months of age; thereafter, the proportion of white fibers keeps increasing at a slower rate. The aim of the study was to determine through feed restriction the effect of age (< or > 4 month) and weight on histochemical properties of myofibers and meat quality of the LM and light portion of the semitendinosus (STL). Swiss Large White barrows (n = 24) from six litters were given either ad libitum or restrictive access to a grower diet from 21 to 60 kg BW. At d 113 of age six pigs of the ad libitum (BW = 62 kg) and six siblings of the restricted group (BW = 51 kg) were slaughtered. The remaining 12 barrows were fed a finisher diet until slaughter at 154 d of age; the BW of the ad libitum and restricted group was 100 and 87 kg, respectively. Muscle fibers were stained and classified based on the stain reaction as slow-oxidative (SO), fast oxidative-glycolytic (FOG), and fast glycolytic (FG), and fiber area and distribution was determined. In addition percentages of cooking loss and shear force of teh LM and STL were assessed. Regardless of the age at slaughter, pigs of the restricted group had smaller SO (LM: 2245 vs. 2713 µm²; STL: 2300 vs. 3294 μ m²), FG (LM: 3100 vs. 3573 μ m²; STL: 3953 vs. 4481 μ m²), FOG (STL: 3219 vs. 3856 µm²) fibers, and the STL had more FOG (32 vs. 26%) and fewer FG (60 vs. 70%) fibers than pigs of the ad libitum group ($P \le$ 0.04 for each). The muscles of restricted pigs were less tender (shear force for LM: 4.7 vs. 3.5 kg; STL: 4.1 vs. 3.7 kg) and percentages of cooking loss were higher (LM: 18 vs. 14%; STL: 24 vs. 21%) than of pigs in the ad libitum group $(P \le 0.08 \text{ for each})$. Muscle fiber size of FOG (r = -0.32) and FG (r = -0.36) was

negatively ($P \le 0.03$) correlated with shear force values. In conclusion, for pigs at the same age, when slaughter weight was lower (restriction) the selected porcine muscles were partly more oxidative and exhibited smaller myofibers which negatively affected meat tenderness.

Key Words: Age at Slaughter, Muscle Fibers, Meat Quality

392 Role of β -adrenoceptor signaling and AMP-activated protein kinase in the glycolysis of postmortem skeletal muscle. Q. W. Shen*, M. Du, and M. J. Zhu, *University of Wyoming*, *Laramie*.

Postmortem glycolysis is directly linked to the incidences of PSE (Pale, soft and exudative) and DFD (Dark, firm and dry) meats which cause significant loss to meat industry. However, mechanisms controlling postmortem glycolysis are largely unclear. The objective of this study is to show the role of βadrenoceptor signaling and AMP-activated protein kinase (AMPK) in the postmortem glycolysis. Eighteen two-month old C57BL/6J female mice were randomly separated into three groups. Group I received an injection of saline solution only and served as control; Group II received a saline injection and then was forced to swim for 1 min; Group III received an injection of propranolol (1 mg/kg) in saline solution. In addition, 6 C57BL/6J female AMPK knockout mice were assigned to Group IV, which received a saline injection and was forced to swim for 1 min. The longissimus dorsi muscle was sampled at 0, 1 and 24 hr postmortem for pH and enzyme activity measurements. Results showed that AMPK activity had a major role in determining the ultimate muscle pH, while β-adrenoceptor signaling is essential for initial rapid glycolysis. Activation of β-adrenoceptor signaling due to pre-slaughter stress activates glycogen phosphorylase, resulting in a rapid glycogenolysis and glycolysis shortly following slaughter. On the other hand, the activation of AMPK is important for maintaining the activity of glycogen phosphorylase and pyruvate kinase in postmortem muscle, leading to a sustained glycolysis and a low ultimate pH.

Key Words: Postmortem Muscle, Glycolysis, AMP-Activated Protein Kinase

393 The fatty acid composition of Longissimus muscle from grazing cattle supplemented with sunflower oil and fishoil. E. Ermias^{1,2}, F. J. Monahan², and A. P. Moloney*¹, ¹Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland, ²University College Dublin, Belfield, Dublin, Ireland.

Effects of sunflower oil (S) and fish oil (F) supplementation of grazing cattle on the fatty acid profile of muscle, in particular the conjugated linoleic acid (CLA) and vaccenic acid (VA) concentrations were examined. Grazing Charolais crossbred heifers (initial bodyweight = 407 kg, s.d. 31.3) were offered (n = 12/treatment): grazing only(G), or an individual daily supplement of 2.5 kg concentrates that supplied 290 g S(S1), 415g S (S2), 290g S + 85g F (FS1) or 415 g S + 85 g F (FS2). Animals were slaughtered after 150 days and lipids from the Longissimus muscle were separated into neutral (N) and polar (P) fractions prior to methylation and separation by gas chromatography. Data were subjected to analysis of variance and "a priori" contrasts were used to test for effects of S level and F inclusion. Daily bodyweight gain and carcass weight averaged 834 g and 294 kg, respectively and did not differ (P <0.05) between treatments. The N fraction of muscle from G, S1, S2, FS1 and FS2-fed cattle had 1.29, 1.80, 2.18, 2.32 and 2.44 (sem 0.132) g c9,t11CLA /100g fatty acids, respectively. The corresponding values were 2.78, 4.40, 4.96, 5.51 and 5.33 (sem 0.318) for VA, 1.29, 1.66, 1.49, 1.39 and 1.54 (sem 0.083) for linoleic acid and 0.66, 0.44, 0.42, 0.42 and 0.47 (sem 0.030) for linolenic acid. The P fraction of muscle from G, S1, S2, FS1 and FS2-fed cattle had 0.59, 0.88, 1.12, 1.35 and 1.20 (sem 0.101) g c9,t11CLA /100g fatty acids, respectively. The corresponding values were 1.10, 3.19, 3.77, 5.64 and 4.46 (sem 0.529) for VA, 12.0, 17.5, 17.5, 10.9 and 14.4 (sem 1.66) for linoleic acid, 4.61, 2.21, 2.35, 2.19 and 2.53 (sem 0.258) for linolenic acid. 3.76, 1.98, 1.75, 2.37 and 2.31 (sem 0.250) for eicosapentaenoic acid and 0.15, 0.06, 0.02, 0.35 and 0.61 (sem 0.053) for docosahexaenoic acid. It is concluded that (1) supplementing grazing cattle with S-enriched concentrates increased muscle CLA concentration in the N fraction in a dose-dependant pattern, and (2) F consumption increased

the concentration of long-chain n-3 fatty acids in the P fraction but increased CLA concentration only when added to the lower S concentrate.

Key Words: Cattle, CLA, Fatty Acids

394 Effects of corn oil supplementation on carcass quality, rib composition, and tenderness of implanted Angus, Brangus, and Hereford Heifers. J. Long*1, S. Duckett¹, G. Hill², and H. Crowe¹, ¹University of Georgia, Athens, ²University of Georgia, Tifton.

Angus (A; n = 14), Brangus (B; n = 14) and Hereford (H; n = 14) heifers were used to determine the effects of breed and corn oil supplementation on carcass quality, rib composition and tenderness. Heifers within the three breeds were randomly assigned to one of two treatments, control or oil supplementation. All heifers were implanted on d 0 with Revalor-H and received same diets until day 57, when oil treatment allotted heifers were supplemented with 4.7% corn oil DM basis. Cattle were slaughtered and carcass characteristics were recorded. Ribs (IMPS 107) from A and B carcasses (n = 28) were shipped to UGA for color, rib composition, and tenderness determination. Data was analyzed using the GLM procedure with breed, treatment, time (when appropriate), and all interactions in the model. A and H heifers were similar in average live weight (ALW) and were heavier (P < 0.05) than those of B heifers. Hot carcass weights of A were heavier than B and dressing percentages for both A and B carcasses were greater (P < 0.05) than H. Carcasses from A had higher marbling scores, and quality grade than B and H (P < 0.05). B carcasses had lower yield grades (YG) than A and H (P < 0.05). Oil treated carcasses tended (P < 0.10) to have smaller ribeye areas and higher YG. A carcasses had lower pH values (P < 0.05), increased LM L* values and higher s.c. b* values (P<0.05). A carcasses had heavier whole rib, 9-10-11 rib, and LM weights (P < 0.05). Seam and s.c. fat weights were lower (P < 0.05) in B ribs. Oil supplementation increased (P < 0.05) the amount of s.c. fat in 9-10-11 rib section. Warner-Braztler shear values decreased with prolonged aging treatments (P < 0.05) but did not differ between breeds or oil treatments. This study showed that breed had the greatest impact on carcass quality and rib composition but these differences did not translate to decreased tenderness.

Key Words: Beef, Quality, Tenderness

395 Effect of castration of females on productive performance and carcass quality of Iberian pigs. M. P. Serrano¹, D. G. Valencia¹, R. Lázaro¹, M. Nieto², and G. G. Mateos*¹, ¹Universidad Politécnica de Madrid, Spain, ²Copese, Segovia, Spain.

Cured products from Iberian pigs, the ancestral dark hairy pig original from Spain, are characterized for its high quality but the productivity of sows (less than 14 piglets weaned/year) and fattening pigs (average feed to gain ratio from 25 to 150 kg of 4.5) is low. A total of sixty crossbred (Duroc sire × Iberian dam) pigs was used to study the influence of castration on productive performance and carcass quality of females. Each treatment (castrated females; CF, and entire females; EF) was replicated five times (six pigs). The trial lasted 186 d and the pigs were sacrificed with an average live weight of 144 kg. At the end of the trial CF ate more feed (2740 vs 2629 g/d; P ≤ 0.05), had higher carcass yield (81.3 vs 80.0%; $P \le 0.01$), and were fatter (64.4 vs 57.0 mm at P_2 and 58.9 vs 47.6 mm at m. Gluteus medius; $P \le 0.05$) than EF. The m. semimembranosus from CF presented higher pH at 2 and 24 h post mortem (P \leq 0.05) and higher temperature at 2 h post mortem ($P \le 0.001$) than the same muscle from EF. Also EF had heavier shoulders at 24 h post mortem (16.9 vs 16.1 kg; $P \le 0.05$) and after trimming (13.7 vs 12.9 kg; $P \le 0.05$) than CF. Trimmed ham yield (18.9 vs 17.9% $P \le 0.10$), shoulder yield (11.9 vs 11.1%; $P \le 0.05$), and primal cuts yield $(P \le 0.05)$ were higher for EF than for CF. Also, depot fat from the coccyx was more saturated in CF than in EF (39.09 vs 38.22%; $P \le 0.05$ for total saturated fatty acids; 8.80 vs 9.30%; P ≤ 0.10 for total polyunsaturated fatty acids; 8.19 vs 8.61 %; $P \le 0.10$ for linoleic acid content). We conclude that EF had less carcass yield but better productive performance and yield of primal cuts than CF. Therefore, when productive performance, cost of castration, and carcass

quality traits are considered, EF are a good alternative to CF for production of heavy pigs destined to the dry-cured industry.

Key Words: Iberian Pigs, Gilt Castration, Carcass Quality

396 Effects of pump rate and cooked temperature on pork loin instrumental, sensory descriptive and consumer-rated characteristics. R. T. Baublits*, J.-F. Meullenet, J. T. Sawyer, J. M. Mehaffey, and A. Saha, *University of Arkansas, Fayetteville*.

Fresh pork loins (n = 15; muscle sections, n = 30; arranged in an incomplete block design) were utilized to evaluate the effects of untreated muscles (0% pump rate), or muscles enhanced with a solution comprising 0.4% sodium tripolyphosphate and 1.0 % sodium chloride at either a 6% or 12% pump rate, and cooked to a 71°C or 82°C end-point internal temperature on meat quality, instrumental texture characteristics, sensory profiles, and consumer acceptance. Loins enhanced at a 12% pump rate had a higher (P < 0.05) pH than untreated loins. While there were no differences in Warner-Bratzler shear force due to cooked temperature, chops enhanced at a 12% pump rate had lower (P < 0.05)

shear force values than untreated chops. Additionally, chops enhanced at 6% or 12% pump rates had lower (P < 0.05) razor shear force values than untreated chops. Descriptive sensory analyses revealed chops cooked to 71°C had a more intense (P < 0.05) blood serum flavor than chops cooked to 82°C. Untreated chops had less intense (P < 0.05) pork fat flavor, and more intense (P < 0.05) blood serum, livery, and cardboard or oxidized flavor characteristics than chops enhanced at 6% or 12% pump rates. Additionally, there were no differences (P > 0.05) in metallic intensity between enhanced and untreated chops. Sensory panelists reported chops enhanced at 6% or 12% pump rates to generally be more tender than untreated chops. Consumers reported a higher (P < 0.05) overall acceptability for chops enhanced at 6% or 12% pump rates. Overall acceptance scores were in average 2 points higher for the enhanced chops (mean =7.03) than for the untreated chops (mean =4.94) on the 9-point hedonic scale. Furthermore, both sensory panelists and consumers reported chops enhanced at 6% or 12% pump rates to be similar (P > 0.05) in juiciness characteristics, regardless of end-point temperature. However, untreated chops cooked to 82°C were less juicy (P < 0.05) than untreated chops cooked to 71°C, suggesting retained palatability when enhanced chops are cooked to more abusive temperatures.

Key Words: Pork, Enhancement, Cooked Temperature

Milk Protein and Enzymes: Milk Protein Interactions

397 Casein micelles and whey proteins: Physical interactions and functional properties. S. G. Anema*, Fonterra Research Centre, Palmerston North, New Zealand.

The functional properties of milk products are inextricably linked to the denaturation of the whey proteins and their interactions with other milk protein components. As the level of whey protein denaturation is easily measured, milk products are often classified by their level of whey protein denaturation. We examined the denaturation of the major whey proteins in milk, the factors affecting this denaturation, and the relationship between the level of denaturation and the functional performance of the milk in a simple acid gel system. From these studies, models to predict the degree of denaturation of the whey proteins at a range of temperature, heating time, and composition combinations were generated. However, the relationship between the level of denaturation and the functional performance of the milk in acid gels was rather poor.

An understanding of the denaturation reactions of the whey proteins provides information on only the initial steps of a complex series of aggregation reactions that occur when milk products are heated. Analysis and interpretation of these aggregation reactions are often difficult. In our further studies, we examined one specific aggregation reaction considered to be of importance to functionality: the interaction between the denatured whey proteins and the casein micelles. The effects of numerous factors (pH, concentration, composition, heating regime, etc.) on these interactions, and the relationship between these interactions and the functional performance of the milk in our simple acid gel system, were studied. We found that it was possible to substantially manipulate the degree of interaction between the denatured whey proteins and the casein micelles by altering the conditions of the milk at heating. These changes in interaction behavior had a marked effect on the physical properties of the milk and the functional performance in our acid gel system, allowing significant modification of the textural properties. These results exemplify the importance of the aggregation reactions of the denatured whey proteins in determining the properties of heated milk systems.

398 Process-induced intermolecular bonds in milk protein gels and their impact on rheological properties. J. Hinrichs*, *University of Hohenheim, Germany.*

The various texture properties in milk products are mainly determined by the protein content, the interacting protein fractions, the milieu conditions and the

process technology applied. Furthermore, the kind of technology applied may force or enhance the formation of intermolecular interactions stabilizing the nano- and microstructure which on their part may correlate with the functional properties or texture of milk protein gels. An extraction test applying different buffering agents was established in order to quantify the stabilizing covalent and non-covalent bonds in milk gels. The texture properties were characterized by dynamic rheological measurements and penetration tests simultaneously. This enables us to study the process–structure–function relationship in pressure-induced, heat-induced, rennet-induced and acid-induced milk gels.

The combined characterization of the gel structure demonstrated that heat-induced and pressure-induced whey protein gels - predominantly stabilized by covalent disulphide bonds - appeared elastic, which is expressed by the low loss angle. In high pressure-induced whey protein gels, the loss angle declined when the amount of stabilizing disulphide bonds was increased by technological means. Contrary to that, the loss angle remained constant while the storage modulus increased when the amount of calcium bridges respectively the amount of hydrophobic interactions increased in rennet-induced respectively acid-induced gels via technological means. The gels appeared firmer but the viscoelastic properties remained unchanged.

In summary, the kind and quantity of the intermolecular interactions stabilizing milk protein gels can be determined by applying various buffer systems. It seems likely that analysis of process-induced covalent and non-covalent bonds will become an additional tool for texture analysis in scientific issues and for solving technological problems.

399 The 500 Myr story of the evolution of phosphoproteins that made milk possible. C. Holt* and R. A. Clegg, *Hannah Research Institute*, *Ayr*, *UK*.

Because of recent genome sequencing projects, casein genes are now recognised to have evolved as members of a paralogous group of secretory calcium (phosphate) binding phosphoproteins (SCPPs). The group's origins lie in ancient homologues of a protein called SPARC which gave rise to a SPARC-like protein (SPARCL1) and an osteopontin-like protein (OPN) at the end of the Cambrian period, about 500 Myr ago [1,2]. The earliest mineralised tissues (denticles) employing calcium phosphate emerged at this time, to be followed by external bony plates and scales and later endoskeletons. In extant mammals, members of the SCPP family are found in the biological fluids saliva and milk and in the mineralised tissues of bone, dentine and enamel. OPN, however has