SESSION 4:

PROTEIN METABOLISM

Communication no. 27

3-Hydroxy-3-methylbutyrate affects muscle cathepsin D and calpain activities in rats during the post-dexamethasone recovery period. M. Jank[a], S. Rosochacki[b], J. Wilczak[b], B. Balasinska[b], P. Ostaszewski[b] ([a] Department of Animal Physiology, Veterinary Faculty, Warsaw Agricultural University, Nowoursynowska 166, 02-787 Warsaw, Poland; [b] Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-551 Mroków, Poland)

Stress of any type (surgery, trauma, sepsis) is associated with metabolic changes resulting in an increase in proteolysis. During stress the level of glucocorticoids is always high. These hormones inhibit protein synthesis but their action on proteolysis is less clear. Our first objective was to study the roles of calpains and cathepsines in mediating the action of glucocorticoids in skeletal muscle. Accordingly, we investigated the action of synthetic glucocorticoid (Dexamethasone – DEX) on two enzymes of muscle protein degradation: calpain II and cathepsin D. Our main objective was to assess the modulatory effect of 3-hydroxy-3-methylbutyrate (HMB) on the post-dexamethasone recovery period since some observations in humans indicate that HMB decreases muscle protein breakdown in situations of elevated muscle proteolysis (Nissen et al., J. Appl. Physiol. 81 (1996) 2095–2104).

The experiment was performed on Sprague-Dawley rats divided into four groups: C – control pair fed, D – 5 days DEX treatment, Drec – 5 days DEX followed by 5 days recovery, DHMB – 5 days DEX followed by 5 days HMB treatment. DEX and HMB were administrated by an intragastric tube. After an overnight fast, general anesthesia with diethyl ether was given and a sample of quadriceps femoral muscle was excised and stored in liquid nitrogen. Blood samples were also collected. Calpain activity, principally calpain II, was determined after the isoelectric precipitation separation of enzymes and inhibitors. Cathepsin D and acid activity with or without specific inhibitors, leupeptin and pepstatin were also determined.

Five day DEX administration resulted in an increase in calpain activity ($P < 0.05$) in group D. After a 5-day recovery, calpain activity significantly decreased ($P < 0.01$) in the group fed HMB (DHMB) and this value dropped to around 50 % of the control value. DEX treatment did not significantly change cathepsin D activity (group D). During post-DEX recovery, cathepsin activity increased in the group not treated with HMB (DRec; $P < 0.01$), whereas HMB administration activity decreased. In conclusion, lysosomal- and Ca-dependent proteinases involved in intracellular protein degradation differ in their activity following DEX treatment. HMB modulates the post-dexamethasone recovery period.

Communication no. 28

Growth hormone and milk protein gene polymorphism in relation to the levels of some hormones, enzymes and metabolites in growing dairy cattle. J. Oprzadek, L. Zwierzchowski, M. Lukaszewicz, E. Dymnicki, Z. Reklewski, R. Grochowska (Institute of Genetics and Animal Breeding, Polish Academy of Science, Jastrzebiec, 05-551 Mroków, Poland)

Two-hundred-and-sixty-two progenies of both sexes, sired by 31 AI Polish Friesian bulls were genotyped for growth hormone (GH), κ-casein (CASK) and β-lactoglobulin (BLG) gene variants. The levels of thyroxine (T_4), triiodothyronine (T_3), insulin (IRI),

glucose (GLU), urea (UR), creatinine (CR) and cholesterol (CHOL) and the activity of alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were recorded four times during controlled growth and feeding, between the ages of 6 and 8 months. The relationships between particular genotypes of GH, CASK, and BLG and sampled physiological indicators were studied assuming a two-locus epistasis. The genotype substitution of LL to VV, AA to BB and AA to BB effects for GH, CASK and BLG, respectively, as well as the dominance effects of LV, AB and AB were estimated as dependent on the accompanying genotypes at the other two loci. In addition, the model of analysis included the fixed effects of sex, yearseason of birth, number of sampling and age at sampling nested within the sampling number. It was also assumed that the sires were indifferent with regard to their 'random' genotype, and were not included in the model to avoid confusion between sire and genotype effects. Out of the three hormones studied only the IRI level was independent of the chosen loci. The T_3 level was significantly influenced by the interaction between GH and BLG loci, and T_4 by CASK and BLG epistasis. All three loci were involved in moulding the ALT level, GH*BLG in AST and GH*CASK in AP. UR was not affected by the accompanying genotypes, while CHOL and CR levels depended on both CASK and BLG, and their interaction, and GLU depended on BLG owing to the AB dominance effect. All three loci are of economic importance in dairy cattle. At the same time they appear to be marker loci for some active protein and metabolite levels which themselves may be indicative of the animal's producing potential. We should also consider a fixed aggregate marker genotype in predicting productivity of animals, rather than just a single locus.

Communication no. 29

Diagnostics and occurrence of the D128G (BLAD) mutation responsible for immune disorders in cattle and its significance in Polish dairy cattle breeding.
K. Lubieniecki, G. Grzybowski (Institute of Genetics and Animal Breeding, Polish Academy of Science, Jastrzebiec, 05-551 Mroków, Poland)

The purpose of this investigation was: 1) to determine the frequency of the BLAD mutation in the Polish black-and-white cattle population, 2) to compare the effectiveness of genotype identification in the CD18 locus using PCR-RFLP analysis and solid phase sequencing of the PCR products, and 3) to determine the effects of the BL allele on cattle productivity.

Tests were carried out on samples of blood, semen and hair roots from bulls from different Polish AI Stations, as well as on samples of milk from cows. The effect of the BL allele on productivity was estimated from the statistical analysis of milk productivity of 140 cow – half sisters (71 wild type and 69 carriers of the BL allele). All were offspring of a bull imported from France – Feodal 3590063415 (license number 70101-4-9).

It was shown that about 5 % of the AI bulls are carriers of the BLAD mutation. The most useful method for large scale diagnostic tests is the PCR-RFLP method modified at the Institute of Genetics and Animal Breeding of the Polish Academy of Science, which includes an internal control of the PCR product digestion. Solid-phase sequencing can be used for monitoring a larger region in the CD18 gene, allowing a quick detection of any new abnormalities in the DNA sequence of this region.

The presence of the allele in a cow's genome is linked to increased productivity of milk and protein. Comparison of lifetime production showed that BL/TL cows produced 734.56 kg of milk and 23.79 kg of