

[1] SOMATOTROPIN MECHANISM IN LACTATING DAIRY COWS: FROM BASIC SCIENCE TO COMMERCIAL APPLICATION. D. E. Bauman, *Department of Animal Science, Cornell University, Ithaca, NY 14853 USA*

Bovine somatotropin, one of the first biotechnology products, results in an increase in milk yield and an unprecedented improvement in efficiency of nutrient use. Beginning with studies in the 1930s with pituitary-derived bST to present day studies with recombinant bST, investigations have covered a wide range of management and environmental conditions. Studies have included animal-related factors such as nutrition, bioenergetics, metabolism, health and well-being, and consumer-related factors such as milk quality, manufacturing characteristics and product safety. Results from these world-wide investigations have been remarkably consistent and led to commercial approval of bST in over 25 countries. Investigations have also allowed delineation of biological concepts in lactation. Overall, bST is a homeorhetic control involved in orchestrating many physiological processes to allow greater synthesis of milk. These coordinated actions are both direct and indirect. Direct effects involve adaptations in a variety of tissues and the metabolism of all nutrient classes - carbohydrates, lipids, protein and minerals. Mechanisms include alterations in key enzymes, intracellular signal transduction systems, and tissue response to homeostatic signals. As a result physiological processes and nutrient partitioning are coordinated in a manner to support an increase synthesis of milk. Indirect effects are thought to be mediated by the IGF system and involve the mammary gland. Specific changes include increased cellular rates of milk synthesis and enhanced maintenance of secretory cells, thereby improving lactation persistency. Indirect effects of bST are modulated by environmental and management factors, especially nutritional status. This modulation of the ST/IGF system is a central component in allowing ST to play a key role in regulating nutrient utilisation across a range of physiological situations from well-managed, high performance cows to cows in an adverse environment. USA commercial use of bST began in 1994 and adoption has been extremely rapid for an agricultural technology. From the consumer perspective, bST was unique and some anti-technology and animal rights groups loudly proclaimed dire health effects. However, introduction of bST had no impact on consumption of milk or milk products, and milk labeled as recombinant bST-free currently occupies a niche market of <1% of fluid milk. From a producer perspective, commercial use verified results from scientific studies and enhanced net farm income. In particular, farmers observed the critical role of quality of management in the milk response to bST. In addition, improved lactation persistency allowed producers to move to longer calving intervals which resulted in reduced culling rates and improvements in animal health and well-being on a herd basis. Overall, ST is a key homeorhetic control regulating nutrient partitioning, and the ST/IGF system plays a key role in animal performance and well-being across a range of physiological situations.

Key words: Somatotropin, IGF, Lactation, Metabolism

[2] GROWTH HORMONE AND MAMMARY GROWTH. K. Sejrsen¹, S. Purup¹, M. Vestergaard¹, M.S. Weber², C.H. Knight³. ¹*Danish Institute of Agricultural Sciences, Foulum, DK-8830 Tjele, Denmark,* ²*Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0315, USA,* ³*Hannah Research Institute, Ayr, KA6 5HL, UK.*

The involvement of growth hormone (GH) in regulation of mammogenesis was established in the 1950's by Lyons and coworkers in classical studies in rodents. Since then it has been shown that GH also is involved in the regulation of mammary development in ruminants. We are unaware of any studies in pigs investigating the effect of GH on mammary growth. In ruminants, exogenous GH enhances mammary growth during puberty and pregnancy. The effect of GH on mammary growth in lactation is less clear. Experiments suggest an increase in mammary gland size after a period of GH treatment. However, administration of GH to goats in early lactation did not influence mammary cell proliferation suggesting an effect on rate of cell death.

In spite of GH-receptor gene expression in mammary tissue, GH does not bind to mammary cells and has no effect on mammary cell proliferation *in vitro*. Most evidence suggests that the effect of exogenous GH is mediated via increased levels of IGF-I in the blood. IGF-I binds to mammary tissue and stimulates mammary cell proliferation *in vitro*. This mechanism of action of GH is supported by data showing increased mitogenic effect of serum from GH-treated animals, an effect that can be blocked by antibodies to IGF-I. Concurrent with increased circulating levels of IGF-I, GH-treatment leads to an increase in the level IGFBP-3, indicating higher half-life of IGF-I.

Endogenous secretions of GH and IGF-I, however, often change in opposite directions making it difficult to understand the mechanism of action behind endogenous variation in GH and mammary growth. One example relates to the observed negative effect of high feeding level on mammary growth in heifers in relation to puberty. In agreement with the involvement of GH in regulation of pubertal mammary growth, circulating level of GH is reduced by high feeding level. However, IGF-I is increased – not decreased - by high feeding level and serum from heifers fed on the high feeding level has higher mitogenic activity than serum from heifers on moderate feeding level. These paradoxical effects cannot be explained by changes in circulating levels of IGF binding proteins, but the sensitivity of mammary tissue to IGF-I is reduced by high feeding level. This reduction in mammary tissue sensitivity to IGF-I suggests that local regulation is involved. We have shown that IGF-I and IGF binding proteins are expressed locally in the mammary glands of heifers, that mammary extracts stimulate mammary cell proliferation and that IGF-antibodies and IGFBP-3 can inhibit the effect of mammary gland extracts *in vitro*.

Key words: Mammary Growth, GH, IGF-I, IGFBP, Mechanism of Action

[3] MANIPULATION OF MILK PRODUCTION AND QUALITY BY USE OF SOMATOTROPIN IN DAIRY RUMINANTS OTHER THAN COW. A. Baldi *Institute of Animal Nutrition, Faculty of Veterinary Medicine, University of Milan, 20133 Milano, Italy*

The ability of exogenous somatotropin to enhance milk production is well established, not only in cows but also in other dairy ruminants, in which the shape of milk yield response is very similar to that observed in dairy cows. Since milk from ewes, goats and buffaloes is mainly used for cheese making, the effect of somatotropin on the composition and manufacturing characteristics of milk is of great interest in these species. In dairy ewes, we found an increase in milk yield (20-30% in Comisana ewes) following treatment with recombinant somatotropin (bST), which did not affect the gross composition of the milk or the coagulating properties (1), except in advanced stage of lactation when the milk protein and fat percentages were reduced at the peak of milk production response and coagulation time was shorter in bST treated ewes than untreated animals. In dairy goats administration of bST is known to increase overall milk yield by 14 to 28% compared to untreated goats. This effect was enhanced by more frequent milking. Our studies (2) and those of others in Italian river buffalo showed that bST treatment increased milk yield by 12% to 17% or even more when the treatment was associated with dietary protected fat supplementation (Ca soaps). The milk fat and protein composition did not change following treatment except in late stages of lactation. Thus the increase in milk fat observed in dairy cows at the onset of treatment does not occur in dairy buffaloes. However the fatty acids composition of milk fat was affected by treatment: long chain and unsaturated fatty acids percentages in milk fat increased evidencing that a mobilization of body fat reserves occurs in bST treated buffalo. In the advanced stages of lactation, we found that, in buffaloes treated with bST, the milk yield response was even higher than that found in earlier stages, however there was a concomitant decrease in milk fat percentages at the peak of milk production response. In general, studies on dairy ruminants show that treatment with bST increases milk production in the short term (immediate post-injection period) and that there is a medium to long term effect on persistency of lactation. There is evidence that lactational involution can be at least partially reversed by bST administration and that this could be related to an increase in mammary parenchymal volume and a decrease in plasmin-plasminogen activities (3) in treated animals. In species with a seasonal breeding pattern (i.e ewes, goats and buffaloes), in which all the animals progress through lactation in a synchronous manner, the possibility to modulate the lactational persistency could be of economic interest for dairy production.

References: Polidori *et al.*, 1997, *J. Dairy Sci.* **80**, 2137; Dell'Orto *et al.*, 1996, *Ann. Zootech.* **45**, 377; Baldi *et al.*, 1997, *Livest. Prod. Sci.* **50**, 43.

Key words: Somatotropin, Milk quality, Ewes, Goats, Buffalo

[4] MODULATION OF THE INFLAMMATORY REACTION AND NEUTROPHIL DEFENCE OF THE BOVINE LACTATING MAMMARY GLAND BY GROWTH HORMONE. C. Burvenich*, M.Paape^o, D.Hoeben*, A.-M.Massart-Leën*.

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Mastitis is economically the most important disease in dairy industry, which is attributable to large milk production (MP) losses. Because it has been shown that recombinant bovine somatotropin (rBST) increases MP in healthy animals we decided to study the effect of this hormone on MP and composition during inflammation of the mammary gland. We further studied neutrophil function in healthy cows treated with rBST. The role of rBST was studied during experimentally induced *Escherichia coli* mastitis in cows immediately after parturition. It was concluded that rBST could have positive effects on the clinical evolution of mastitis in severely diseased cows. Treatment of these cows tended to reverse the severe response into a moderate response. Further, we have shown that rBST enhanced diapedesis of neutrophils into the mammary gland during acute *E. coli* mastitis. In another study cows were experimentally infected with *Streptococcus uberis*. Cows were treated with rBST one week before and after infection, whereas control cows received the excipient. After infection, MP in the infected quarters of rBST treated cows decreased 24%, and in the control cows 40%. In both groups, the decline in production in the uninfected quarters was 6 and 14%, respectively. In the rBST group, MP was completely restored 3 weeks after infection. In the control group, total production and production of the infected quarters remained below pre-infection levels. The results indicate that treatment of cows with rBST significantly reduces MP losses after experimental infection with *E. coli* and *S. uberis*. Different mechanisms could be postulated but the exact action remains unknown. One possibility is that growth hormone could have enhanced the rate of gluconeogenesis and oxidation and thus could have induced a more efficient utilisation of gluconeogenic metabolites. Another possibility is that it acted through its effect on neutrophil function. The influence of 10 daily rBST-injections of cows after peak lactation on the H₂O₂-production capacity of isolated blood neutrophils, was examined. Five to eight days after the onset of rBST administration the PMA-induced superoxide production of neutrophils as well as the H₂O₂-production started to raise to a maximum around the 10th day. The enhanced capacity was then about 50-80 % higher than controls. A few days after stopping the rBST injections, these reactive oxygen species diminished and returned to initial levels. During the period of enhanced H₂O₂-production, the average density of blood neutrophils was significantly lowered (from 1.105 to 1.098). During the period of enhanced H₂O₂-production, there was no effect on zymosan induced oxidative burst. In a study performed on cows immediately after parturition it was found that administration of rBST caused a moderate left shift of neutrophils (immature forms in peripheral blood). Simultaneously, a significant increase in the absolute leukocyte count was observed. The results might be ascribed to an action of rBST on the bone marrow due to a release of stored cells, or an increased recruitment of immature granulocytes from the bone marrow or an activation of the colony-stimulating factor in the bone marrow by rBST. This phenomenon could also explain some beneficial effects of rBST on acute *E. coli* mastitis because in control mastitis studies it was shown that the severity of this disease was inversely related to the number of circulating neutrophils before the start of inflammation in the gland. In protein tyrosin phosphorylation studies in bovine neutrophils, rBST increased phosphorylation of numerous molecule weight moieties with intense staining of a band corresponding to a molecular weight (mw) of L-selectin which is involved in diapedesis. All these findings suggest a physiological role for BST in regulation of immune function.

Key words: Inflammation, Somatotropin, Neutrophil, Bovine, Mammary gland

[L/G1] PIT-1 POLYMORPHISMS AND MILK PRODUCTION PARAMETERS IN ITALIAN-FRIESIAN BULLS. I. Parmentier¹, N. Gengler², F. Mortiaux¹, S. Massart¹, C. Bertozzi¹, D. Portetelle¹, R. Renaville¹. ¹*Department of Applied Biochemistry and Biology,* ²*Department of Agronomy, Economy and Development, University Faculty of Agricultural Sciences, B-5030 Gembloux, Belgium.*

Pit-1 is a pituitary-specific transcription factor responsible for hormone expression in mammals. Mutations in the gene encoding Pit-1 have been found in growth hormone disorder in mice and humans. A mutation situated at the exon 6 level of the gene Pit-1, has been observed in our laboratory by PCR amplification and digestion of the PCR product with Hinfl. Among the three patterns observed (AA, AB and BB), the AB pattern was found significantly superior to the BB pattern for milk and protein yield ($P < 0,05$) and significantly inferior for fat percentage ($P < 0,01$) in 91 Holstein-Friesian bulls (Renaville et al., 1997). Findings for the AA pattern were based on only 2,2% of the animals and were therefore considered as preliminary results.

The aim of this study was to search eventual association between a second mutation, found at the exon 2 level with a SSCP (Single Strand Chain Polymorphism) method, and milk traits of the 91 Holstein-Friesian bulls used in the previous study. We have also determined the eventual correlation between the combinations issue from the association of the two mutations (situated at the exon 2 and 6 level) and the milk traits. A fixed linear model was fitted on the Daughter Yield Deviation (DYDs) for milk, fat and protein yield. Observations were weighted according to the reliability for DYDs and the model contained Pit-1 pattern fixed effect.

The SSCP method shows two patterns called C and D. The frequency of these patterns is 59,5% for C and 40,5% for D. No significant association was obtained between these patterns and the milk traits. On the other hand, the association of the AB pattern with the C pattern is significantly superior for the milk and protein yields than the other possible combinations (AB+D, BB+C, BB+D). We have also observed that the effect of the combination of the AB and C patterns on milk and protein yields is superior to the effect of the AB pattern alone ($381,8 \pm 171,5$ kg milk and $12,7 \pm 5,6$ kg protein against $120,3 \pm 49,7$ kg milk and $4,4 \pm 1,6$ kg protein respectively). In conclusion, this study shows possible evidence of an interesting relationship between Pit-1 the association of the two pit-1 polymorphisms and milk traits in dairy cattle.

Reference: Renaville et al., 1997, *J. Dairy Sci.*, **80**.

Key words: Pit-1, Polymorphisms, Mutations, Milk traits.

[L/G2] INFLUENCE OF GENETIC VARIANTS OF BOVINE GROWTH HORMONE ON MILK PRODUCTIVITY. J.H.J. van der Werf, F.J. Verburg, F.L. Harders, G.J. Garssen, M.F.W. te Pas. *DLO Institute for Animal Science and Health, P.O. Box 65, 8200 AB Lelystad, The Netherlands.*

Bovine growth hormone (bGH) is well known to play a pivotal role in growth, mammary development and lactation in cattle. Two naturally occurring variants of this protein are known, with either a Val or Leu at amino acid position 127. Exogenous bGH-Val¹²⁷ has been shown to have a larger effect on stimulated fat corrected milk production than bGH-Leu¹²⁷ administration. This report describes studies investigating the effect on productivity of genetically different animals. The ultimate goal is to investigate possibilities to use this genetic polymorphism in breeding programs.

Animals were genotyped for their genetic constitution at the GH locus. A PCR fragment of 282 bp containing the polymorphic site was amplified and digested with *AluI*, resulting in either a 150 bp and 132 bp digestion product associated with Val¹²⁷, or a 150 bp, 81 bp, and 51 bp product associated with the Leu¹²⁷ variant.

In a first experiment 352 Holstein heifers were genotyped, originating from two populations with very different genetic merit for milk production capacity. The overall allele frequency for Leu¹²⁷ was 0.95. Full production records were available from 302 heifers. Statistical analysis revealed that the heterozygous Leu/Val genotype showed an increased daily milk yield of $+1.93 \pm 0.93$ kg/day ($p = 0.04$), and an increased protein yield of $+58 \pm 26$ g/day ($p = 0.03$) compared to the Leu/Leu genotype. A single gene effect of this magnitude could increase the annual genetic gain in a breeding program by up to 40% when it would be exploited. No significant effects on milk composition or body weight at first parity were found. These results are in line with those of treatment with exogenous bGH Val¹²⁷. The low frequency of the positive Val¹²⁷ allele is remarkable and may suggest involvement of deleterious effects on other physiologic important processes.

In a second experiment, however, 390 heifers from other herds within the same selection program showed no significant effect of GH genotype: 0.20 ± 0.84 kg/day for first parity milk yield and -0.05 ± 0.08 g/day for protein yield.

In two additional experiments 978 Holstein Friesian (HF) and 43 Jersey sires, respectively, with known estimated breeding values (progeny test) for milk production characteristics were genotyped. No significant genetic differences were observed, although the HF population showed a tendency for increased milk yield for the Val¹²⁷/Val¹²⁷ genotype.

The conclusion from these data is that the initial large effect of the bovine GH polymorphism on milk productivity could not be confirmed in subsequent data sets. Therefore, selection for milk production characteristics based upon GH genotype data seems therefore does not seem to show great promise.

Key Words: GH, RFLP, Lactation

[L/G3] K-CASEIN , β -LACTOGLOBULIN AND GROWTH HORMONE GENES POLYMORPHISMS IN ITALIAN BOVINE BREEDS. M. Messina, E. Vrech, P. Pezzi*, A. Prandi, *Faculty of Agronomy, I-33100 Udine, Italy and *Faculty of Veterinary, I-40064 Bologna, Italy*

The polymorphisms of κ -casein is utilised to improve milk quality as genetic marker in the selection of dairy cattle breed. β -lactoglobulin polymorphisms seems to be related with milk yield and milk protein composition. Growth hormone (GH) affects milk production and plays a key role in nutrient utilisation, mammary control and growth. GH polymorphism could be related to its plasma levels and utilised in a selection program (Lucy *et al.*, 1993; Yao *et al.*, 1996). The aim of this work was to investigate a polymorphism of GH gene, κ -casein gene and of β -lactoglobulin gene to improve the early identification of genetic value of dairy cattle in terms of quantity and quality of milk yield. We have examined 182 Italian Friesian (IF) cows, 118 Brown Mountain (BM) and 157 Italian Simmental (IS) animals (91 cows and 14 bulls). Genomic DNA was extracted from blood of the cows and from the semen of the bulls. Genotyping of animals was determined by AS-PCR for GH gene and by PCR-RFLP for κ -casein and β -lactoglobulin. The site of the mutation investigated for the GH gene corresponding to the base n° 2291 of the sequence of Gordon *et al.* (1983).

K-casein genotypic frequency (%) was: AA=41.2, AB=27.5, BB=4.9, AE=17.6, BE=7.7 and EE=1.1 in IF, AA=15.2, AB=40.7, BB=43.2 and BC=0.8 in BM and AA=42.9, AB=39, BB=12.4, AC=5.7 in IS.

β -lactoglobulin genotypic frequency (%) was: AA=21.4, AB=51.6, BB=26.9 in IF, AA=16.1, AB=55 BB=28.8 in BM and AA=35.2, AB=33.3, BB=31.4 in IS.

GH genotypic frequency (%) was: AA=84.1, AC=12.6, CC=3.3 in IF, AA=96.6, AC=3.4 in BM and AA=82.9, AC=13.3 and CC=3.8 in IS.

These previous results confirm the higher frequency of BB genotype for K-casein in BM instead of IS and IF. The allele C is present only in IS while it is absent the allele E. On the contrary, in the IF is present the allele E and the allele C is missing. In the β -lactoglobulins the distribution of the genotype is similar in the three breeds with a clear prevalence of the genotype AB. In the GH homozygote for C has been found in IF (3.3%) and IS (3.8%), and in the IS the genotype AC is more frequently present than in the IF. The frequency of AC in BM is lower (3.4) instead of IS (13.3) and IF (12.6). The genic frequency of AA and AC in the IF breed is in accordance with what already found by Yao *et al.* in the GH gene. The obtained results will be utilised in the study of the correlations between the genotype and the production quali-quantitative of the milk.

References: Yao *et al.*, 1996, *Genetics*, **144**, 1809-1816; Gordon *et al.*, 1983, *Mol. Cell. Endocrin.*, **33**, 81-95; Lucy *et al.*, 1993, *Domest. Anim. Endocrinol.*, **10**, 325-333

Key word: GH, milk proteins, polymorphism, PCR-RFLP, AS-PCR.

[L/G4] SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP) ANALYSIS OF EXON 4 AND 5 OF GROWTH HORMONE GENE IN SERRANA TRANSMONTANA GOAT BREED. M.C. Varejão, E. Bastos, R. Chaves, A. Cravador*, J. Azevedo**, H. Guedes-Pinto, *Dept. de Genética e Biotecnologia; **Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, P5000 Vila Real, Portugal, *Unidade de Ciências e Tecnologias Agrárias, Universidade do Algarve, Campus de Gambelas, P8000 Faro, Portugal.*

The origin of Serrana goat breed is lost in time; however the wild goat *Capra pyrenaica*, from the Serra da Estrela, is probably its ancestor. The Serrana is the most representative of Portuguese breeds and is considered to have great productive potentialities and considerable expansion possibilities due to its productive and reproductive indices, high degree of ruggedness and high quality products: cheese and meat (1).

The aim of this study was to examine the genetic variability of this portuguese breed at the molecular level for exon 4 and 5 of growth hormone (GH) gene and to try to correlate specific DNA polymorphism with zootechnical parameters of interest with a view to improving future molecular assisted selection.

The single strand conformation polymorphism (SSCP) method depends on the conformation of the single strand DNA fragment and its base sequence, under non-denaturing conditions. One base difference molecule can lead to a different conformation, which can result in different mobilities in polyacrylamide gel (2).

The goat genomic DNA was obtained from blood taken from flocks owned by members of the "Associação Nacional de Caprinicultores da Raça Serrana" (ANCRAS). The DNA extraction was carried out by the high salt method (3). The PCR products of the exon 4 and 5, 214 and 364 bp size respectively, were denaturated and loaded in a polyacrylamide gel. The gel was silver stained. Six conformation patterns for exon 4 of the GH gene were found. However for exon 5, so far two patterns have been detected. The correlation between those results and milk production parameters are also being evaluated, in order to find molecular markers suitable for molecular assisted selection. (Supported by PRAXIS 3/3.2/CA/1991/95 Project.)

References: (1) Almendra, 1994, Edição da Associação Nacional de Caprinicultores da Raça Serrana (ANCRAS) 28p; (2) Orita *et al.*, 1989, *Genomics* **5**, 874; (3) Montgomery *et al.*, 1990, *New Zealand J. of Agricultural Res.* **33**, 437.

Key words: SSCP, Goat, GH, Polymorphism

[75] IDENTIFICATION OF A CONSERVED SEQUENCE IN MANY BOVINE mRNAs INVOLVED IN PHYSIOLOGICAL FUNCTIONS SUSTAINING PRODUCTION. G. Damiani, S. Panelli*, S. Pirovano*, A. Caroli**, P. Bolla**, G. Pagnacco** *IDVGA - CNR, I-20133 Milano, Italy, *University of Pavia, I-27100 Pavia, Italy, ** University of Milan, I-20133 Milano, Italy.*

Stress response, both at cellular and at the organism level, is a co-ordinated series of metabolic events that enable the adaptation to threatening conditions for the maintenance of cell or soma integrity. The activation of a quiescent cell (anabolic phase) into an active cell (catabolic phase) is an example of phase transition phenomena, which may be induced by the stress signals. Using a differential display reverse transcription PCR (DDRT-PCR) we amplified and sequenced several cDNA fragments corresponding to mRNAs transcribed or modified in cultured bovine cells after the activation with different proliferating factors. The differential expression of some of the identified mRNAs fragments were confirmed by quantitative PCR. Our experimental results and a comparison between the differentially transcribed mRNAs and the sequences available in the public database revealed the presence of a 260 bp SINE sequences Bov-ID shared between mRNAs expressed preferentially during the response to environmental stresses (as the interleukin 1-alpha and beta, and the ACTH receptor) or to regulatory factors (as the prolactin receptor, and the lysozyme). The same sequence is present also in many important genes (as the growth hormone, the prolactin receptor, and the casein genes). Many of the shared sequences are located in the 3' untranslated regions of the mRNAs and contain sequences similar to the adenosine and uridine rich elements named AUREs. Several observations suggest that the AURE-containing mRNAs tend to be unstable and a family of AURE binding proteins regulates their decay rates. We speculate that in mammalian cells many mRNAs of the catabolic phase are constitutively expressed but are rapidly degraded in normal conditions. When a stress or a regulatory signal is present, it is able to induce conformational changes in the AURE binding proteins, which stabilise the AURE-containing mRNAs. These observations suggest that common pools of molecules and mechanisms are related to the phenomenon of activation of the catabolic processes of energy transduction for activities which allow the adaptation of cell and organism to stressing conditions. Therefore the genetic variations in these sequences may be related with different productive characteristics.

Key Words: Bovine, mRNA, Production, Genetic variation, Stress response

[L/M1] SPECIFIC INFLUENCE OF ANDROGENS ON MOUSE AND BOVINE MAMMARY CELL LINES. M. Baratta, S. Grolli*, A. Poletti**, C. Tamanini. *Istituto di Fisiologia Veterinaria, Via del Taglio 8, 43100 Parma, Italy; *Istituto di Biochimica Veterinaria, Università di Parma, Italy; **Istituto di Endocrinologia, Università di Milano, Italy.*

Different steroids have been shown to exert a specific influence on cell proliferation and β -casein gene expression in a mouse mammary cell line (Grolli *et al.*, 1997). The aim of this study was to investigate androgens receptor (AR) expression during mammary cell proliferation and differentiation in mouse (HC11) and bovine (BME-UV) cell lines. HC11 were cultured during proliferation in RPMI 1640 with FCS 0.5 %, insulin (5 μ g/ml), EGF (10 ng/ml). BME-UV were cultured in 50% DMEM/F12, 30% RPMI 1640, 20% NCTC 135 with FCS 0.5%. Cells were stimulated with testosterone (T) and dihydrotestosterone (DHT) at the concentrations of 0.01-10 μ M for 24 or 48 h. Cyproterone acetate (Cyp, 3 μ M), a DHT receptor antagonist, was used to block specific androgen effects. Proliferative effects were measured by MTT dye reduction assay. To measure β -casein gene expression, HC11 cells were transfected with p β cCAT, a chimeric rat- β casein gene promoter- CAT gene construct and cultured in RPMI 1640, FCS 3%, dexamethasone (1 μ g/ml), insulin (5 μ g/ml), PRL (from 0.005 to 25 μ g/ml) and the above mentioned androgens. CAT ELISA was used to determine gene expression. RT-PCR was performed from total and/or mRNA in proliferating cells (50-70% of confluence) and in confluent cells. Mouse specific primer for AR in reverse transcription was 5'-tgatctgtggagatgaagct-3'; in PCR reaction, AR primers were 5'-atctcgtggagttgtgaac-3' sense and 3'-agtcacccctgcttcataac-5' antisense; annealing temperature was 42 C° and cycle number was 32. Positive and negative controls were obtained from prostate and heart, respectively. T slightly affected HC11 cell proliferation (4-12% increase vs control) after 24 h while DHT exerted a noteworthy effect (10-42%). After 48 h, T enhanced its effect (up to 28-44%) as well as DHT (up to 37-77%). In BME-UV, T and DHT stimulated cell proliferation (3-23% and 5-25%, respectively). After 48 h T slightly enhanced cell proliferation (0-10%) while DHT maintained a considerable influence (10-35%). Cyp added to treated cells significantly ($p < 0.05$) reduced the proliferative effects. AR gene was expressed in proliferating and differentiated HC11. These observations are consistent with previous reports about a specific effect of androgens in mammary gland and lead to hypothesize an activity of this class of steroids in mammary physiology. In particular, androgens seem to stimulate cell proliferation and β casein gene expression; this influence may be mediated by androgen receptors. Furthermore, HC11 and BME-UV cell lines appear to be useful to investigate steroids influence on mammary cell development and differentiation. This research was supported by C.R.P.A Regione Emilia Romagna, Italy.

Reference: Grolli *et al.*, 1997, Biol. Reprod., 56, Suppl.1, 481.

Key words: Mammary cells, Androgens, Receptor, β -casein expression

[L/M2] INVOLVEMENT OF BAX AND BCL-2 PROTEIN IN REGULATION OF MAMMARY EPITHELIAL CELLS APOPTOSIS. T. Motyl, P. Wareski, T. Ploszaj, S. Janczewska*, A. Orzechowski, Z. Ryniewicz**, J. Skierski***. *Department of Animal Physiology, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland, *Surgery Research and Transplantation Department, Medical Research Center Institute, Polish Academy of Sciences, Warsaw, Poland, **Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland and ***Flow Cytometry Laboratory, Drug Institute, Warsaw, Poland*

The induction or inhibition of mammary epithelial cells apoptosis depends on the endocrine (prolactin, GH, IGF-I) and autocrine/paracrine (EGF, TGF- α , TGF- β , FIL) regulation of Bax:Bcl-2 - death:life rheostat. In the present study the role of Bax and Bcl-2 proteins as a checkpoint of the effector stage of apoptosis was investigated using two experimental models: mammary tissue of dairy goat and prolactin-dependent HC11 mouse mammary epithelial cells. The importance of TGF- β 1 as a local, intramammary gland apoptogenic cytokine and CPP32 (caspase 3) has been also assessed.

Immunohistochemical analysis of goat's mammary tissue explants revealed moderate or high level of Bax, CPP32 and TGF- β 1 within alveolar cells, dependent on the period of lactation and the extent of secretory tissue involution. Drying off was generally associated with increased Bax content, whereas CPP32 and TGF- β 1 levels were similar to that observed in mid lactation. In drying off period, the content of CPP32 was higher in less involuted lobuli than in those exhibiting destructive changes. The development of mammary gland in early lactation was associated with the lowest Bax and CPP32 content in secretory tissue. Single widespread apoptotic cells (determined by TUNEL method) were visible both in lactating and involuting mammary tissue, however their relative number (as percent of total cell number), was higher in the course of drying off, than in early or mid lactation.

Bax transcript evaluated by Bax mRNA/GAPDH mRNA ratio (where GAPDH served as a reference gene) was down regulated by administration of EGF (10 ng/ml) or prolactin (5 μ g/ml) to the culture of HC11 mouse mammary epithelial cells. Conversely, withdrawal of EGF and/or prolactin, which can mimic endocrine pattern at the end of lactation, was associated with increased *bax* expression in HC11 cells. Administration of TGF- β 1 (1 ng/ml) strongly enhanced Bax transcript, decreased Bcl-2 protein level (flow cytometric analysis with FITC-conjugated monoclonal anti-Bcl-2 antibody) and in the consequence induced apoptosis both in cells treated or not treated with EGF or prolactin.

In conclusion: 1) high expression of Bax, CPP32 and TGF- β 1 with simultaneous low level of Bcl-2 protein in mammary epithelial cells in mid and late lactation coincides in time with apoptotic cell number increase, thereby suggesting the involvement of these proteins in the molecular mechanism of mammary epithelial cells apoptosis; 2) apoptogenic stimuli like: TGF- β 1 or EGF deficiency are associated with up-regulation of Bax and down-regulation of Bcl-2, whereas survival signals induced by EGF or prolactin reverse this relation.

Key words: Bax, Bcl-2, Apoptosis, Mammary gland

[L/M3] ARGUMENTS FOR DIRECT ACTIONS OF GROWTH HORMONE ON MAMMARY EPITHELIAL CELLS. H. Jammes, L. Belair, J. Djiane. Unité d'Endocrinologie Moléculaire, INRA, 78352 Jouy en Josas, France

Growth hormone (GH) plays an important role in the mammary gland development and the direct action of GH on epithelial cells is currently under consideration. The presence of mRNA coding for GH receptor has been evidenced in mammary gland (Hauser et al., 1990 ; Jammes et al., 1991). Using a monoclonal anti GHR (Mab263, a gift from Dr. M. Waters, Australia.) we found a GH receptor-like immunoreactivity in human mammary epithelial cells suggesting the ability of GH to act directly on mammary cells via the activation of endogenous GH receptor. The interaction of GH with its receptor has been shown to lead to the tyrosine phosphorylation of JAK2, the receptor itself, and STAT family of transcription factors (Argetsinger *et al.*, 1993; Ihle *et al.*, 1995). After rabbit mammary acini stimulation with 500 ng/ml bGH for 15 min, lysates were prepared, immunoprecipitated with GHR antibody (Mab 263) and subjected to Western Blot analysis using the monoclonal antibody directed against phosphotyrosine (4G10, UBI). Only one immunoreactive band was visualized using ECL system (Amersham) at 120Kd, indicating that GH was able to induce the tyrosine phosphorylation of its own receptors in mammary cells.

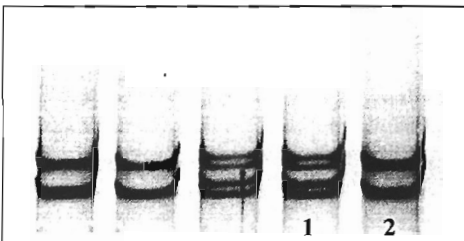
The interactions between nuclear factors induced by GH stimulation and regulatory sequence of Spi 2.1 gene (GHRE) and α casein S1 gene (α S1 Cas) were analysed by standard band shift assays. In presence of labelled oligonucleotide GHRE, a binding activity of nuclear extracts was obtained for epithelial clusters stimulated by GH in dose-dependent manner. In presence of an excess of unlabelled oligonucleotide, the GHRE binding was decreased. In order to determine the nature of nuclear factor involved in the GHRE binding different antibodies anti-STATs were used. For GHRE, nuclear factor involved has been identified as STAT5 (a gift from Dr. Ph. D Groner). In presence of labelled (α S1Cas oligonucleotide, GH was unable to induce a binding activity. This result was consistent with the absence of GH effect on milk protein gene activation.

In order to find a cellular response to GH stimulation, we have used the mouse mammary epithelial cell line HC11 (Ball *et al.*, 1988). A binding of 125 I-human GH (ligand for the both lactogenic and somatogenic receptors), was observed on whole HC11 cells. Increasing concentrations of unlabeled recombinant bovine GH or recombinant human GH were able to decrease the 125 I -human GH binding with a similar affinity. At maximal concentration (10^{-7} M), bovine GH inhibited 50% of binding of 125 I -human GH, suggesting the presence of specific somatogenic receptors on the HC11 cell surface. But the specific binding was too low to determine the number of GH receptor/ cell. The EGF-induced mitogenesis of HC11 cells was analysed by 3 H thymidine incorporation. EGF induced DNA synthesis in a dose-dependent manner with an ED50 of 10^{-10} M and a maximally effective dose of 10^{-9} M. The concomitant presence of GH at maximally effective dose (10^{-8} M), induced a decrease of 3 H-thymidine incorporation obtained with lower EGF concentrations. At higher EGF concentrations, GH lack to be effective. This result was probably due to ratio between EGF and GH receptor number at cell surface.

Key words: GH, Mammary epithelial cells, Transducing signal.

[L/M4] SINGLE STRAND CONFORMATION POLYMORPHISM DETECTION OF EXON 7 OF β -CASEIN GENE IN "CHURRA DA TERRA QUENTE" PORTUGUESE INDIGENOUS OVINE BREED. E. Bastos*, A. Cravador, C. Varejão*, R. Chaves*, H. Guedes-Pinto*. *Universidade de Trás-os-Montes e Alto Douro, Dept. Genética e Biotecnologia, Ap. 202, Vila Real, Portugal. **Universidade do Algarve, UCTA, Campus de Gambelas, Faro, Portugal.**

Milk protein polymorphisms provide a powerful tool for the knowledge of their molecular biology and physicochemical properties. It is becoming increasingly recognised that genetic variation may offer a source of differentiation that can improve milk technology, including cheesemaking. "Churra da Terra Quente" is an important ovine breed of North Portugal used for milk and meat production. The milk is almost entirely processed into cheese, so it is important to define milk characteristics, especially the protein polymorphisms and their influence on cheesemaking properties. In the present study we used a non-radioactive SSCP protocol that allowed the detection of genetic variability at the exon 7 of β -casein gene in "Churra da Terra Quente" animals.



Genomic DNA was isolated from blood samples of animals by a phenol-chloroform-proteinase K protocol. The choice of the direct and reverse primers for the amplification of exon 7 of β -Cn was based on ovine β -Cn gene sequence (1). The SSCP analysis of the 510 bp amplified product was carried out basically by the method described in (2) with some modifications. The electrophoretic run was performed during 16 hours, at 400V and 15°C in a 15% nondenaturing polyacrylamide gel, without glycerol. The DNA bands were visualised through silver staining. Our study showed genetic diversity in the animals studied with this methodology.

We detected two conformation patterns: pattern 1 with four bands (frequency of 37.5%) and pattern 2 with two bands (frequency of 52.5%). In 10% of the animals there was no amplification suggesting a mutation in the primer region. The SSCP analysis is a very reliable and simple method of detecting single-base substitutions and other alterations, but it doesn't allow the identification of the exact mutation. Actually, the fragments with the two distinct patterns are being sequenced in order to inform us what specific mutation occurred. We think that this preliminary approach could be advantageously used to characterise the genetic variability within this breed. Further studies will be developed to analyse the effect of the polymorphisms detected on milk composition and its technological properties

References: (1) Provot *et al.*, 1995, *Gene*. 154: 259-263; (2) Hongyo *et al.*, 1993, *Nucleic Acids Res.* 21: 3637-3642.

Key words: β -casein, Milk, Polymorphism, SSCP

[L/M5] CHARACTERIZATION OF A PARTIAL BOVINE cDNA ENCODING PITUITARY GROWTH HORMONE-RELEASING HORMONE RECEPTOR (GHRH-R) AND MAPPING OF GHRH-R TO CHROMOSOME 4 USING A NOVEL PCR-RFLP. E.E. Connor, M.S. Ashwell*, S.M. Kappes**, G.E. Dahl. *Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742-2311, *Agricultural Research Service, USDA, Beltsville, MD 20705; and **Meat Animal Research Center, USDA, Clay Center, NE 68933.*

Growth hormone-releasing hormone receptor may be an important gene controlling growth performance and carcass characteristics of beef cattle. Previously, we found that differences among beef bulls in their growth hormone response to injection of GHRH are related to differences among animals in growth performance (1). Sequencing of bovine GHRH-R cDNA would allow development of specific probes to study variation in GHRH-R mRNA and GHRH-R expression among animals. The purpose of this study was to sequence and characterize bovine pituitary GHRH-R cDNA and to identify polymorphisms within the gene for linkage mapping. Total RNA was isolated from bovine pituitary tissue and used as template for reverse transcriptase-mediated polymerase chain reaction (PCR). Sequence-specific primers were designed from highly homologous regions of human, porcine and ovine pituitary GHRH-R (GenBank Accession Nos. L09237, L01406 [human], L11869 [porcine] and M.Thorner and B. Gaylinn, personal communication [ovine]). An ~1100-bp fragment was amplified, cloned into a plasmid vector, and sequenced to verify partial sequence of bovine GHRH-R. The fragment was used as a probe to screen a bovine pituitary cDNA library (Stratagene #937723). A partial cDNA encoding 270 amino acids was sequenced and found to have high sequence homology with porcine, human, rat and mouse GHRH-R at 91, 86, 83 and 81%, respectively. Primers were designed from the bovine GHRH-R sequence to amplify an ~450-bp intronic fragment between putative exons 6 and 7. A PCR-restriction fragment length polymorphism (PCR-RFLP) with *Eco57I* was identified within this intronic fragment among the USDA-MARC bovine reference family. The GHRH-R was mapped to bovine chromosome 4 (BTA4; <http://sol.marc.usda.gov/>) between markers INRA072 ($Z=30.5$, $\theta=0.02$) and BMS3013 ($Z=10.2$, $\theta=0.00$). The GHRH-R gene maps to human chromosome 7 (HSA7; 2) and swine chromosome 18 (SSC18; 3), providing additional support to the orthologous relationship among BTA4, HSA7 and SSC18.

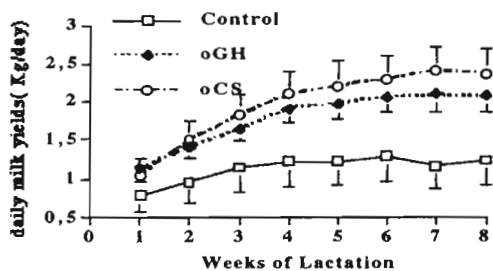
References: Connor *et al.*, 1997, *J. Anim. Sci.* **75**, 170; Gaylinn *et al.*, 1994, *Genomics* **19**, 193; Sun *et al.*, 1997, *Animal Genetics*, **28**, 351

Key Words: GHRH-R, cDNA, PCR-RFLP, Cattle

[L/P1] DEMONSTRATION OF THE MAMMOTROPHIC ROLE OF OVINE CHORIONIC SOMATOTROPIN. G. Kann, A., Gertler*, J. Djiane, *Laboratoire de Biologie Moléculaire et Cellulaire, INRA, 78352 Jouy en Jossas, France and *Dept. of Biochemistry, Food and Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel.*

Ovine Chorionic Somatotropin (oCS) is secreted by the ewe placenta in important amounts in both maternal and fetal compartments. The present study demonstrate that it may have an important role in the mammogenesis of the ewe during pregnancy. Its effect were compared to that already described for ovine Growth Hormone (oGH).

One-year-old nulliparous ewes (n=40) randomly allocated in 3 experimental groups were induced to lactate by the mean of a 7 days E₂ 0.5 mg/kg + P₄ 1.25 mg/kg treatment (D1-D7) followed (or not) on days 10 up to 20 by an hormonal treatment including either 56 µg/kg/day recombinant oGH or 158 µg/kg/day recombinant oCS. All ewes received 25 mg hydrocortisone acetate (HC) twice daily on D18-20. 13 ewes (7 control and 6 experimental) were slaughtered at the end of hormonal treatments (D21). Milk yields of the 27 surviving ewes were daily recorded during eight weeks. Specific RIA were run for plasmas taken twice daily (D1-21), and hourly (on D13 during a 12 h period). of oPRL, oGH, oCS, IGF-I, β-casein was analysed in mammary tissues with a specific RIA. DNA contents of the mammary tissue was measured and oGH receptors estimated in both liver and mammary tissues from slaughtered ewes.



The estro-progestative treatment enhanced the plasma levels of oPRL, oGH, and IGF-I between D0 and D7 1.5, 2.3, and 2.6 times respectively (P=0.002). oGH treatment induced a highly significant enhancement of IGF-I plasma levels from D11 up to D20 when a similar effect appeared for oCS treated ewes only from D17 up to D20 (P<0.01). The mammary epithelium DNA concentration at D21 (mg/g fresh tissue) was higher (P<0.01) for oGH or oCS treated ewes giving evidence for mammogenic effect of both hormones. Milk yield (Fig.) was higher in the both oGH (n=9) and oCS (n=9) groups (P<0.001) than that of control ewes (n=9) from week 3 up to week 8 of lactation. No β-casein was measured in tissue from control ewes, whereas E₂ + P₄ + HC treated ewes had higher casein concentrations regardless of

subsequent hormonal treatment on days 11-20 (P<0.001). β-casein concentrations in mammary parenchyma of oGH treated ewes did not differ from control ewes; however, oCS clearly enhanced expression of β-casein (P<0.001).

These results suggest that mammogenesis and/or lactogenesis is in part controlled by somatotrophic hormones such as ovine GH and oCS.

Key Words: Lactation, Mammogenesis, Somatotrophic hormones, Placenta

[L/P2] THE EFFECTS OF EARLY LACTATION CONCENTRATE LEVEL AND GLUCOGENIC FEED ON PLASMA INSULIN AND GLUCAGON LEVELS IN DAIRY COWS AND HEIFERS. T. Kokkonen, A. Tesfa, M. Tuori, K. Hissa*, L. Syrjälä-Qvist. *Department of Animal Science, PB 28, 00014 University of Helsinki. Finland. *Suomen Rehu Ltd, PB 105, 00241 Helsinki. Finland.*

Insulin and glucagon are considered to be important homeostatic (short term) hormonal regulators of ruminant metabolism. Low insulin concentrations and low tissue responsivity to insulin during early lactation promote mammary gland glucose supply. Glucagon enhances hepatic glycogenolysis and gluconeogenesis (1, 2).

16 multiparous Friesian dairy cows and 16 primiparous Friesian dairy heifers were fed two levels of compounded concentrate and two levels of glucogenic feed (propylene glycol 24.4 %, polyols 25 %, beet molasses 25 %, xylose molasses 25 %, nicotine amide 0.6 %) for twelve weeks starting from calving. The amounts of concentrate for dairy cows were 11 kg/d (fresh weight; LC group) and 15 kg/d (HC) and for heifers 9 kg/d (LC) and 12 kg/d (HC) respectively. The levels of glucogenic feed were 0 l/d (G0) and 1 l/d (G1) for all animals. Wilted grass silage was given *ad lib*. Milk production and feed consumption were measured daily during the whole trial from calving through twelve weeks of lactation. Blood samples were taken before the afternoon feeding one week before the estimated calving date and two, four and six weeks after calving.

There were positive correlations (p<0.10) between milk yield and glucagon, NEFA and β-hydroxybutyrate concentrations in older cows but not in heifers. A higher concentrate level increased milk yield in older cows but not in heifers.

Plasma insulin levels were quite variable. There were no significant effects of concentrate on insulin levels although they seemed to be higher with higher amounts of concentrate. There was a tendency (p<0.10; in weeks 4 and 6) towards higher plasma insulin levels in heifers fed 12 kg/d concentrate. This was in accordance with greater liveweight gain (p<0.10) in these heifers. The effect of glucogenic feed on insulin level was very variable without any clear trend. Plasma glucagon levels were higher (p<0.05) in dairy cows fed a high concentrate diet. There were tendencies towards decreased glucagon levels with glucogenic feed in both parity groups.

The higher concentrate level increased plasma insulin levels and live weight gain in heifers indicating increased nutrient partition to non-mammary tissues. Older cows responded to a higher concentrate level with increased milk yield and increased plasma glucagon concentrations.

References: 1) Bauman & Elliot, 1983, pp. 437 - 468. In: *Biochemistry of Lactation* (ed. T.B. Mepham); 2) Brockman & Laarveld, 1986, *Livest. Prod. Sci.* 14: 313 - 334.

Key words: Insulin, Glucagon, Glucogenic feed, Concentrate level

[L/P3] EFFECTS OF DUODENAL INFUSIONS OF ARGININE OR CASEIN ON PLASMA GROWTH HORMONE, AND PROLACTIN CONCENTRATIONS, MAMMARY BLOOD FLOW, AND MILK PRODUCTION IN LACTATING COWS.

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High doses of Arginine (Arg) given intravenously transiently increased plasma concentrations of growth hormone (GH) (1,3) and prolactin, followed (1) or not (3) by an increase in milk yield in dairy cows. The milk yield effect could be explained by an increase in mammary blood flow (MBF) consecutive to GH increase. That mechanism has also been proposed as an explanation for effect of casein postprandial infusion on milk yield (2). The aim of this study was to analyse the changes in GH and prolactin concentrations, MBF and milk yield following duodenal pulsed infusions of Arg or casein. Three cows were given Arg (infusion time: 2h; L-Arg HCL, 250 g per infusion), or casein as isonitrogenous control (calcium caseinate, 485 g), or water (5 l) as negative control, according to a Latin square design. Infusions were performed twice daily after feed distribution at 8:00 and 20:00 on three consecutive days. The diet, so formulated as to meet recommended energy and protein levels, consisted of corn silage with a 75:25 DM/concentrate ratio. The cows were milked twice daily at 6:00 and 18:00. Blood samples were taken from the carotid artery every hour over the last day of each period. MBF was continuously measured by an ultrasonic flow probe implanted around the left external pudic artery.

Table 1: Effects of infusions on GH, prolactin, MBF and milk yield.

	Control	Casein	Arg	SED
GH ¹ , ng/ml	5.7	6.2	5.9	0.28
Prolactin ¹ , ng/ml	12.6	17.9	15.1	2.21
MBF ^{1,2} , L/min	3.6	3.8	3.5	0.10
Milk yield, kg/d	16.9	17.7	17.6	0.41

¹ measured during the two hours of infusion; ² for an half udder.

Arg and casein infusions had no effect on GH, prolactin concentrations or on MBF measured during infusion and daily milk yield (table 1). These results are consistent with the absence of effect of Arg administered in the abomasum (3). Surprisingly, pulsed casein infusion did not increase milk yield as usually recorded with continuous infusion (2). However, the absence of effect of casein infusion on MBF, GH and prolactin concentrations are in agreement with a previous study (2). To conclude, postprandial supplementation of Arg appeared to be an impractical way of increasing GH secretion and milk yield. This study also suggests that the increase in milk yield induced by casein might be linked to a mechanism that require a lapse of time (more than two hours of infusion) to establish itself.

References: Chew *et al.*, 1984, *J. Dairy Sci.*, **67**, 2507; Guinard *et al.*, 1994, *J. Dairy Sci.*, **77**, 2221; Vicini *et al.*, 1988, *J. Dairy Sci.*, **71**, 658.

Key words: Arginine, Casein, GH, Milk yield

[L/P4] EFFECTS OF EXPOSURE TO ELECTRIC AND MAGNETIC FIELDS ON MELATONIN AND PROLACTIN LEVELS IN DAIRY COWS SUBJECTED TO SHORT PHOTOPERIODS.

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The intensity of electric and magnetic fields is relatively high in areas adjacent to high-tension lines. It has been suggested that the very low frequency electric and magnetic fields (EMF) generated by alternating current (AC) from high voltage lines may have biological effects. One previous study from our laboratory showed increased feed intake (FI) in dairy cows exposed to EMF (1). It has been reported in some species that EMF may suppress pineal release of melatonin (MLT) in a similar fashion as light does (2). Previous studies show that FI of cattle increases under long photoperiods, and this is observed in association with increased circulating prolactin (PRL) levels (3). The hypothesis that EMF may increase FI by altering the response to photoperiod was tested by subjecting dairy cows kept under short day conditions to controlled EMF exposure, to determine if endocrine responses resemble those of cows exposed to lengthening days. Sixteen lactating, pregnant Holstein cows were confined to wooden metabolic cages and exposed to a vertical electric field of 10 kV/m and a horizontal magnetic field of 30 Φ T. The animals were assigned to two replicates according to a cross-over design with treatment switch back. Each group was exposed to EMF for 16 h/d in either of two sequences of three periods each: for the first replicate 28 d off/28 d on/28 d off, and for the second replicate, 28 d on/28 d off/28 d on. All animals were maintained under short day conditions (8 h light/16 h dark) during the trial. Feed intake and milk production were recorded daily. Samples were analyzed weekly for milk composition. Hourly blood samples were taken for 24 h at the end of each exposure period for the determination of MLT and PRL concentrations by RIA. Dry matter intake was increased ($P < 0.01$) under EMF exposure (17.03 vs. 16.04 kg/d, SE=0.4). Serum PRL was also increased (16.6 vs. 12.7 ng/ml, SE=0.8, $P < 0.02$). Plasma MLT showed a small numerical decrease, which was non-significant for the scotophase but significant ($P < 0.05$) for the photophase (LS means for the 8 h light period were 9.9 vs. 12.4 pg/ml, SE=1.3). Production of milk and milk components was not significantly affected by exposure to EMF. In general, these results suggest that the response of dairy cattle to EMF exposure may resemble the effect of a long photoperiod. The latter has previously been associated with decreased MLT and increased PRL levels in the cow.

References: 1) Burchard *et al.*, 1996. *J. Dairy Sci.* **79**:1549; 2) Kato *et al.*, 1994. *Neurosci. Lett.* **166**:59; 3) Peters *et al.*, 1981. *J. Dairy Sci.* **64**:1671

Key Words: Dairy cows, PRL, MLT, EMF

[L/P5] EFFECT OF CULTURE CONDITIONS ON BOVINE MAMMARY CELL PROLIFERATION STIMULATED BY IGF-I AND INSULIN. F. Cheli, F. Fantuz, M. Bojanic*, A. Baldi. *University of Milano, I-20134 Milano, Italy, *Institute of Biotechnology, YU-81000 Podgorica, Yugoslavia.*

Mammary gland functions depend upon the coordinated cellular events of cell growth, differentiation and death. Nutrition which influences animal development, also influences mammogenesis. Studies to better evaluate the role of nutrition and nutrient-hormone interactions require simple *in vitro* systems. The role of insulin and IGF-I in regulating mammary functions is well documented (1).

The aim of this study was to evaluate the role of different culture conditions in modulating the response of bovine mammary epithelial cells to the proliferative factors insulin and IGF-I. The BME-UV1 cell line was used as a model system. BME-UV1 cells respond to exogenous IGF-I (2). The cells were grown in serum-free DME-F12 culture medium. Cellular proliferation at different incubation times was evaluated both by incorporation of BrdU and enumeration using a hemocytometer.

The first experiment examined the effect of insulin and IGF-I on proliferation of BME-UV cells cultured in the presence of excess methionine or lysine (5x) compared to concentrations in the control medium. The proliferative response to hormones was influenced by extracellular methionine and lysine concentrations in the culture medium. Cell proliferation of bovine mammary cells was stimulated by high lysine concentrations. We found that excess lysine enhanced cell proliferation stimulated by insulin and IGF-I by +13% and +25%, respectively, compared to excess methionine.

The second experiment examined the effect of retinoic acid (RA) on cell proliferation. We found that retinoic acid caused a linear growth inhibition of cell proliferation over a concentration range 0.05-0.5 μM . Further increase in the concentration of RA to 5 μM had no further effect on cell growth. RA inhibited cell proliferation stimulated by insulin and IGF-I at 72 h. However RA was less effective in inhibiting BrdU incorporation when cells were stimulated with insulin (-21%) than when cells were stimulated with IGF-I (-35%). There is known to be a strong correlation between the expression of plasminogen activator (PA) and cell proliferation (3). Therefore we measured PA activity in the medium of cells cultured in the presence of 1 μM of RA, in order to examine whether the effects of RA on cell growth may be mediated through changes in PA activity. We found that the inhibition of cell growth by RA was associated with lower PA activity in the culture medium.

In conclusion the present work has provided evidence that nutrient concentration in the culture medium play an important role in modulating cell proliferation stimulated by hormones *in vitro*.

References: Prosser *et al.*, 1988, In "Biotechnology in growth regulation" (Ed RB Heap, CG Prosser and GE Lamming), 141; Zavizion *et al.*, 1996, *In vitro Cell. Dev. Biol. - Animal* 32,149; Politis, 1995, *J Dairy Sci.* 79,1097.

Key words: Mammary cell, IGF, Insulin

[L/P6] LOCALIZATION AND HORMONAL REGULATION OF IGF-I AND IGF-II GENE EXPRESSION DURING OVINE MAMMARY GLAND DEVELOPMENT. De Wei, L. Belair, G. Kann, J. Djiane, H. Jammes. *Laboratoire de Biologie Cellulaire et Molculaire, INRA, 78352 Jouy en Josas, France*

As previously demonstrated, both type I and type II receptors for IGF-I and IGF-II were present in sheep and cow mammary tissue. IGF-I and IGF-II are potent mitogens for mammary epithelial cells. Overexpression of IGF-I in the mammary glands of mice leads to ductal hypertrophy and incomplete involution (Hadsell *et al.*, 1996) while overexpression of IGF-II increases the incidence of mammary adenocarcinoma (Bates *et al.*, 1995). But little is known about the physiological regulation of IGFs synthesized within the mammary gland. In the present study, both the localisation and the evolution of IGF-I and IGF-II mRNA in the mammary glands of ewes during pregnancy and lactation were determined by *in situ* hybridization and Northern blot analysis.

In situ hybridization analysis revealed that at day 15 of pregnancy, the IGF-I mRNA were located in stoma surrounding the terminal ducts. In latter stages of pregnancy, the expression of IGF-I mRNA was progressively more important in the epithelial cells. During lactation (day 48 and day 70), the expression of IGF-I gene was located mainly in epithelial tissue. By Northern Blot, two IGF-I mRNA transcripts were expressed with a low intensity at all pregnancy and lactation stages analysed.

At the beginning of pregnancy, IGF-II mRNAs were located in stroma cells as observed by *in situ* hybridization. Expression was not detectable in epithelial cells. As the mammary gland development is associated with a reduction of stroma components, the IGF-II mRNA amount decreased dramatically. No IGF-II mRNA staining was observed during the first part of lactation. The northern Blot analysis confirms these results. To better understand the hormonal regulation, the IGF-I and IGF-II gene expression was studied in mammary gland from virgin ewes after hormonal induction of lactation. The classical treatment for experimental induction of lactation is : 0.5 mg/kg estradiol and 1.25 mg/kg progesterone twice daily for 7 consecutive days. In order to obtain a better mammary gland development, a treatment with 1 mg/kg hydrocortisone twice daily on experimental days 18 to 20 was also tested. In addition, ewes were injected from day 11 to 20, twice daily, with ovine growth hormone (0.18 mg/kg). On day 20, the animals were killed and samples of mammary gland collected. By Northern blot, IGF-I transcripts were only detectable in mammary gland samples from ewes injected with the association of estradiol - progesterone - followed by hydrocortisone injections, which induced a great development of the epithelial tissue. The epithelial development was amplified with oGH treatment but the IGF-I mRNA amount were not modified. In mammary gland from control and estradiol - progesterone ewes, a specific signal was found for IGF-II mRNA. The addition of hydrocortisone decrease markely the IGF-II gene expression.

In conclusion, these data bring support to the ideas that in mammary gland, IGF-I gene expression occurs in epithelial tissue during the mammary gland development and IGF-II gene expression is limited in stroma cells..

Key words: IGF-I, IGF-II, Mammary gland, Hormonal regulation

[L/P7] EFFECTS OF COMBINED TREATMENT OF rbST AND ENDOTOXIN ON MILK YIELD AND METABOLIC-ENDOCRINE STATUS IN DAIRY COWS. E. Trevisi, R. Lombardelli, G. Folli, G. Berton. *Istituto di Zootecnica, Faculty of Agriculture, U.C.S.C., 291 00 Piacenza, Italy.*

rbST increases milk yield in dairy cows, particularly when administered after the peak of lactation. Nevertheless, in some case, the milk yield response is unsatisfactory, with no apparent reason. Aim of this research was to clarify whether the presence of an inflammatory status, simulated by injecting endotoxin, can contribute to impair the effect of GH. Three dairy cows (667±10 kg of b.w.) in average-late lactation received, in a latin square design, 3 treatments, as daily injections: i) rbST, ii) rbST together with endotoxin, iii) pyrogen free water. rbST (Ely Lilly, 25 mg/d dissolved in carbonate-bicarbonate saline solution, pH 9.4) was injected for 8 consecutive days; while endotoxin (from *E. coli* serotype 055:B5, SIGMA, 0.1 µg/kg b.w.) was injected only in the first 3. The interval without treatments was of 14 days. All the injections were made 1 hour after the morning forage meal. Individual daily feed intake and milk yield at every milking were recorded. Blood samples were taken at -1, 0, 1.5, 3, 6, 9, 12, 24 hours from injections, the day before and 10 days after the end of treatments, as well as the day 1, 2, 3 and 8 of each treatment. At the same time animal behaviour and rectal temperature were recorded. Metabolic profile was determined on all the samples; while IGF-I, insulin, glucagon, cortisol, T3 and rT3 were analysed only on samples taken at -1, 3, 12, 24 h after injection. Two-way ANOVA (time and treatment) was used for statistical valuation.

The contemporary treatment with rbST and *E.coli* endotoxin depressed the milk yield response, compared with rbST alone; nevertheless this reduction (15-20% compared to the previous yield) was observed only for 36-48 h, while an increase, with reference to the zero time yield, did occur after the end of endotoxin treatment. The rectal temperature rise (1.5 °C during the 1st day) was also noticeable (1°C) in the next two days of endotoxin injection. Feed intake was reduced only for approximately 24 h. Moreover, treatments determined quite different patterns of hormones variations, particularly because:

- with GH alone, there was a considerable reduction of glucagon and a small rise of insulin, while IGF-I was strongly raised (nearly 2 fold after 3 days of treatment);
- with GH plus endotoxin, insulin was also raised, but glucagon did not decline while T3 was temporary reduced and IGF-I had a slower raise in the 2 cows that had a higher milk yield loss.

At the metabolic level, the most important differences seemed to be the higher values of NEFA and urea when GH was supplied together with the endotoxin. With GH alone, the NEFA were also raised (although to a lesser extent) and urea was strongly reduced. It can be therefore suggested that the cytokines, released after the endotoxin injection, may have modified the effects of GH and therefore reduced its positive influence on the mammary gland activity. (*Research supported by INVERNIZZI Foundation.*)

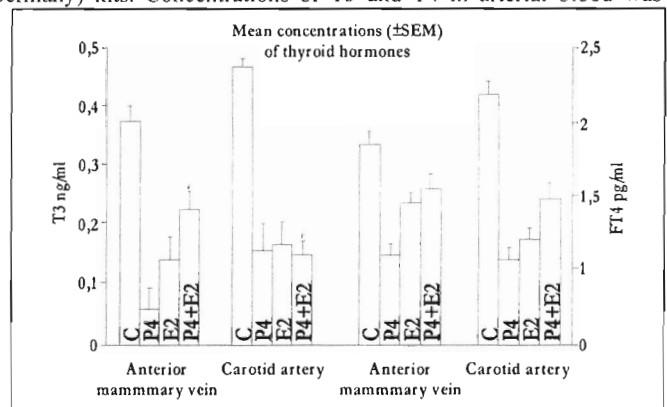
Key words: GH, Endotoxin, Milk yield, Dairy cows

[L/P8] THE LEVEL OF THYROID HORMONES (FT4, T3) IN THE SYSTEMIC ARTERIAL BLOOD AND VENOUS OUTFLOW FROM MAMMARY GLAND AFTER STEROID TREATMENT IN OVARIECTOMIZED PIGS. J. Staszkiwicz, M. Koziowski. *Department of Animal Physiology, University of Agriculture and Technology, 10-718 Olsztyn-Kortowo, Poland*

There is an evidence that thyroid hormones are involved in mammary gland development. The aim of this study was to determine influence of steroid hormones, i.e. estradiol-17β (E2) and progesterone (P4), on concentration of thyroid hormones (fT4, T3) in the systemic arterial blood and venous outflow from mammary gland in ovariectomized prepubertal gilts.

Pigs (n=31) were divided into 4 groups: Group I (control) - i.m. injections of corn oil for 12 days; Group II - i.m. injections of increasing doses of P4 (10-100 mg/day) for 12 days; Group III - i.m. injections of E2 (400 µg/day) for 3 days; Group IV - i.m. injections of E2 (400 µg/day) for 3 days and increasing doses of P4 (10-100 mg/day) for 12 days. On day 13 (Group I and III) or 15 (Group II and IV) plastic catheters were inserted into carotid artery for collecting arterial blood, comparable to that supplying (by analogy) mammary gland, and into anterior mammary vein for collecting venous outflow from mammary gland. Levels of thyroid hormones were determined using Byk Sangtec Diagnostica (Germany) kits. Concentrations of T3 and T4 in arterial blood was compared to concentrations of T3 and T4 in venous (see figure). The concentrations of fT4, a precursor of T3, and T3, biologically active form of thyroid hormones, were lower in both vessels of experimental groups compared to control group. In control group the level of T3 was significantly higher in arterial blood compared to venous outflow. After injections of E2+P4 the level of T3 was significantly higher in venous outflow compared to arterial blood. Results suggest:

- ✓ steroid hormones decrease the level of thyroid hormones in blood plasma
- ✓ an administration of E2 plus P4 increases conversion of fT4 to T3 in area of mammary gland in ovariectomized gilts.



Key words: Thyroid hormones, Mammary gland, Steroid hormones

[L/P9] HOMOLOGOUS RADIOIMMUNOASSAY FOR BOVINE INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-2 (IGFBP-2). D. Portetelle, L. Vleurick, S. Massart, C. Bertozzi, I. Parmentier, F. Mortiaux, R. Renaville. *Faculty of Agronomy, B-5030 Gembloux.*

Insulin-like growth factor-I and -II (IGF-I, -II) circulate in biological fluids bound to six different IGF-binding proteins (IGFBPs). If IGFBP-3 is the major carrier of the IGFs in adult plasma, IGFBP-2 is the most abundant IGFBPs prior to puberty. Numerous studies also demonstrate that IGFBP-2 plasma levels are altered by BST treatment, feeding-restriction or parturition. However, in these investigations, IGFBP-2 levels were evaluated by Western ligand blotting, a more qualitative than quantitative method. Then, after producing antibodies against bovine IGFBP-2, the objectives of this study were 1) to develop a homologous radioimmunoassay, 2) to measure the changes in plasma bovine IGFBP-2 during the nycthemeral period, feeding-restriction period and in response to recombinant BST. Recombinant bovine IGFBP-2 (rbIGFBP-2, GroPep Inc., Australia) conjugated to ovalbumin was used to generate antibodies in rabbit. The antiserum was assessed for cross-reactivity with other IGFBPs by immunoblotting. The same band at 34-35 kDa in plasma was seen with purified recombinant IGFBP-2; suggesting that the antiserum is specific for the protein. In the RIA, the antiserum gave 40% specific binding of ^{125}I -rbIGFBP-2 at a titre of 1:10,000. ^{125}I -rbIGFBP-2 was prepared using the chloramine T method. Samples were diluted at 1:10 with assay buffer. After preincubation of 100 μl sample with 200 μl antibody (diluted in assay buffer) for 2h at room temperature, 200 μl ^{125}I -rbIGFBP-2 in assay buffer (20,000 c.p.m.) were added in each tube. After incubation for 20h at 4°C, the tubes were incubated with 1 ml second antibody solution for 1 h at room temperature before centrifugation. The pellets were counted in a gamma counter. Parallel displacement curves shows strong cross-reactivity with ovine serum but no cross-reactivity with rat, pig or poultry plasma. The addition of IGF-I or -II to control pool of bovine plasma did not significantly alter control IGFBP-2 values in RIA. The intrassay and interassay coefficients of variation are 5% and 9%, respectively. The ED50 for the assay is 7.5 ng rbIGFBP-2 per tube and the minimal detectable dose is 0.15 ng/tube. Six nycthemeral periods realized on 3 young bulls bleeding in two occasions showed that IGFBP-2 plasma levels were stable and 2 or 3 samples were sufficient to characterize in animal. In feeding-restricted bulls, IGFBP-2 levels were significantly increased while refeeding declined protein concentrations. Finally, cows treated with recombinant BST had significantly lower levels of IGFBP-2 as compared with control values. In conclusion, using the RIA method for bovine IGFBP-2 developed in this research, it is now possible to investigate the relationship between IGFBP-2 and the animal status. (This research was supported by Ministry of Agriculture grant (# 5736A))

Key words: IGFBP-2, Bovine, RIA.

[L/P10] EFFECT OF RECOMBINANT BOVINE SOMATOTROPIN (BST) ON MILKING CHARACTERISTICS OF DAIRY COWS. A. Daxenberger, H. Sauerwein*, H. Worstorff, R. M. Bruckmaier. *Institute of Physiology, Research Centre for Milk and Food, D - 85354 Freising-Weihenstephan, Germany, *University of Bonn, D - 53115 Bonn, Germany*

The galactopoietic effect of bST treatment in dairy cows is widely documented. However, no information is available about bST related changes of the milk flow pattern. The aim of this study was to investigate the effect of bST on milking characteristics of dairy cows.

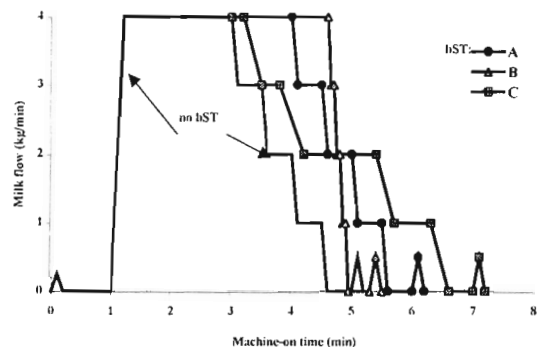
31 Brown-Swiss dairy cows covering the whole lactational period of their first to sixth lactation were treated once with the bST preparation *POSILAC*[®] (Monsanto, St. Louis, USA). Milk yields and continuous milk flow curves were recorded during routine milking (twice daily) using a strain gauge recorder plant. Cows were mechanically prestimulated for one minute (*Stimopuls*, Westfalia, Germany). Evaluation of the data was based on the total milk yield (TMY), peak flow rate (PFR), average flow rate (AFR) and total machine-on time (TMT) during the main effect period (days 7 up 14 after bST treatment) compared to the control period (days -8 to -1). Effects of bST on the various traits were tested by paired t-test (AUC, main effect period vs. control period). Different reaction of the 4 udder quarters is judged by the changes of the milk flow curves (MFC) of each cow.

Average TMY increased by 12.6 % (± 1.35 SEM; $P < 0.001$) from 27.5 to 30.8 kg/day and ranged individually from -1.8 to 34.4 %. It is accompanied by a significantly longer TMT (4.88 % ± 1.08 SEM; $P < 0.001$) spread between -7.7 and 25.9 %, whereas PFR was, with a range from -5.03 to 17.37 %, not altered significantly (0.98 % ± 0.93 SEM; $P = 0.202$) by bST treatment. In contrast to PFR, bST application resulted in a significant AFR increase ($P < 0.001$) of 6.3 % (± 1.19 SEM) from 2.19 to 2.33 min (range from -13.2 to 26.4 %). The variations of MFCs, that correspond to increased in milk yields of at least 6 % (29 cows), could be classified in 3 groups (see figure): the slope of the descending period of the MFC after bST treatment either was greater (B; $n = 5$), equal (A; $n = 12$) or smaller (C; $n = 12$) than in the control phase.

In general, the slope of the MFC descent reflects differences of milk yields of the individual quarters. Our findings suggest, that not only individual animals, but also different quarters of one cow can react in different ways to bST. This might correspond to different quarter-specific galactopoietic effects of bST. Further investigations are necessary.

Key words: bST, Milking characteristics, Udder quarters

Figure: Schematic drawing of typical MFCs, before and after bST treatment



[L/P11] EFFECT OF SELECTION FOR MILK YIELD ON GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR-I (IGF-I) PROFILES IN MULTIPAROUS HOLSTEIN COWS DURING THE PERIPARTURIENT PERIOD. W.J. Weber, L.H. Baumgard, Q. Shi, H. Chester-Jones, L.B. Hansen, B.A. Crooker. *Department of Animal Science, University of Minnesota, St. Paul, MN, USA.*

Effects of selection for milk yield on the somatotrophic axis has not been thoroughly evaluated in ruminants. Objectives were to determine effects of selection for milk yield on the somatotrophic axis. Current production of control line (CL) and selection line (SL) cows from a breeding project established in 1964 is 6,200 and 11,100 kg/305 d. Plasma samples (Study A) were obtained every 15 min over a 7 h interval from 9 CL and 9 SL cows at -13 and 9 d postpartum (PP) and analyzed for GH. Study B used 10 CL and 12 SL cows, half of which received GH (14 mg/d) beginning 15 d PP. Samples for GH analyses were collected as in study A and at 65 d PP. In both studies, serum samples for IGF-I analyses were collected from -14 to 84 d PP. Profile characteristics for GH were determined using CLUSTER. Endocrine results were analyzed as repeated measures using PROC MIXED of SAS and reported as least square means which differed when $P < .05$. Administration of GH in early lactation did not affect milk yield and production data did not differ between studies. Data from studies A and B were combined. Milk yield of CL and SL cows was 29.8 and 37.9 kg/d during 1-84 d PP. Energy balance was greater (1.3, -3.6 Mcal/d) in SL cows prepartum but did not differ between lines after parturition and averaged -12.4 Mcal/d at 9 d PP, reached a nadir (-15.4 Mcal/d) at 7 d PP, and remained negative until 60 d PP. GH profiles differed between -13 and 9 d PP but were consistent for Study A and B. There was an interaction of line and day PP on mean GH as the increase from -13 to 9 d PP was greater in SL (2.7 to 7.4 ng/ml) than in CL cows (2.3 to 4.5 ng/ml). The increased GH in SL cows was due to greater GH baseline (3.3, 4.5 ng/ml) and peak height (5.5, 7.8 ng/ml). GH peak frequency (3.8 peaks/7 h) was not affected by line or day PP. At 65 d PP, the GH induced increase in baseline GH (Study B) was greater in CL (1.9, 7.7 ng/ml) than SL (4.8, 7.4 ng/ml). Similar effects were observed for mean GH. There was a trend ($P = 0.15$) for an interaction of line and day PP on IGF-I (Study A) as IGF-I in CL and SL cows were similar prepartum (128 ng/ml) but was less ($P = 0.10$) in SL cows (67, 92 ng/ml) from 21 to 84 d PP. In a previous study, a similar early lactation reduction in IGF-I in SL cows continued through 140 d PP which is later than the return to positive energy balance.

In summary, selection for milk yield increased mean GH and GH peak height but did not affect GH peak frequency. These effects were independent of energy balance. Although PP energy balance was similar for SL and CL cows, IGF-I tended to be less in SL than CL cows except near the energy balance nadir (1-14 d PP). These results suggest non-nutritional factors are also influencing the effect of selection on circulating GH and IGF-I.

Key words: GH, IGF-I, Selection, Lactation

[L/P12] THE GRAM-NEGATIVE MASTITIS IN EARLY LACTATION MAY INTERFERE WITH IGF-I AND INSULIN LEVELS, THYROID AND ADRENAL FUNCTIONS AND METABOLISM IN DAIRY COWS. Sz. Jánosi, Gy. Huszeniczka, M. Kulcsár, P. Kőrödi, S. J. Dieleman*, P. Ribiczei-Szabó, J.A. Nikolic**, J. Bartyk, P. Rudas. *University of Veterinary Science, H-1400 Budapest, P.O.Box 2, Hungary; *Faculty of Veterinary Medicine, Yalelaan 7, 3584, CL Utrecht, The Netherlands; **Institute for the Application of Nuclear Energy, Banatska 31B, 11080 Zemun, Yugoslavia.*

The interrelation of endotoxin induced (Gram-negative, GN) mastitis diagnosed at the beginning of lactation with thyroid, adrenal, endocrine pancreatic and ovarian functions and metabolism were studied in 3 field trials. When acute mastitis was observed on days 1st to 45th (*Exp. 1* and *2*) or 2nd to 8th (*Exp. 3*) postpartum (pp) the clinical symptom were recorded and scored and milk samples were taken to identify the pathogen(s). In *Exp. 1*, the cows (n=46) with outbreak of clinical symptoms in the morning period were serially sampled six times 6 h apart for assaying the IGF-1, insulin, cortisol, T4 and T3. In *Exp. 2*, the TRH induced T4 and T3 release were followed 12-24 h after the clinical manifestation (n=5). To compare the data of cows with and without mastitis in *Exp. 3*, blood samples were taken from >2 parity cows (n=199) on day 1-3 pp (e.g. before the outbreak of mastitis) and thereafter four times 7 days apart and the IGF-1, insulin, cortisol, T4, T3, acetoacetate (ACAC), POH-butyrate (BHB), non-esterified fatty acid (NEFA) and total cholesterol (TCh) levels were assayed. On day 1-3 and again on day 28-35 also the ACTH-stimulated cortisol and TRH-challenged T4 and T3 responses were estimated. The pp resumption of ovarian cyclicity was followed by progesterone profiles. In *Exp. 1*, the cows with GN mastitis showed decreased IGF-1 and insulin levels as well as a <24 h elevation in cortisol. After a temporary increase the T4 and T3 levels obviously decreased only in the most severe cases. The Gram-positive (GP) mastitis and the most cases of those with no detectable pathogen (NDP) were followed by less severe clinical symptoms and no characteristic endocrine changes. Decreased TRH induced T4 response was observed only in the most severe cases of GN mastitis, but not in any other forms (*Exp. 2*). In *Exp. 3* on days 1-3 pp both the hyperketonaemia (BHB: >1 mmol/l) and the lower than normal (<mean - SD of healthy non-ketoneic cows) ACTH-stimulated cortisol response predisposed the cows for a within some days clinical manifestation of GN mastitis as well as for a more severe (sometimes fatal) course. Although both in cows with GN and NDP mastitis a sudden drop was observed in daily milk production and in the IGF-1, T4, T3, neither the glucose, ACAC and BHB levels nor the time of the first ovulation differed from those in healthy ones: the endocrine alterations were reflected only by a some days elevation in NEFA as well as by a 2-3 weeks depression in TCh. These data confirmed the involvement and clinical importance of certain endocrine and metabolic factors in pathogenesis of endotoxin induced mastitis caused by GN pathogens.

Key words: Endotoxine mastitis, IGF-1, Adrenal gland

[L/A1] EFFECT OF BOVINE SOMATOTROPIN ON MILK PRODUCTION OF INDIGENOUS COWS IN THE SAHELIAN TROPICS. M. Cissé, M. Seck, M. Ba-Diao, I. Sané. *Senegalese Institute of Agricultural Research (ISRA), BP 2057, LNERV-Hann, Dakar, Sénégal.*

The positive effect of bovine somatotropin (bST) on milk production is well documented and bST mechanisms partially elucidated (1, 3). However, experiences using bST in low milk yielding cows are very scarce in Africa. This trial was conducted in the periurban traditional herds in order to evaluate the effect of a prolonged release formulation of bST on milk yield of cows. Forty-two Djakore cows, a crossbred Gobra (*Bos indicus*) x N'Dama (*Bos taurus*), 18 primiparous and 24 multiparous at 8.7±2.9 weeks of lactation, were equally allocated in a control and 2 bST-treated groups, and managed in the sahelian pasture without complementation. BST (250 or 500 mg) was biweekly injected subcutaneously from the last month of the rainy season (September) until the 3rd month of the dry season (January) (6 injections per cow). Cows were milked twice a day after calf sucking during 15 days before the start of injections, and at 3 and 10 days after bST administration, corresponding to the maximal release of the hormone (2). The milk response to each dose of bST was estimated using the following model: $Y_{(ije)} = \mu + A_i + B_j + C_{(xl)} + AB_{ij} + BC_{(jxl)} + e_{(ijle)}$, μ =adjusted mean, A_i =parity effect, B_j =bST effect, $C_{(xl)}$ =covariate, AB_{ij} ="bSTxparity" and $BC_{(jxl)}$ ="bSTxcovariate" interaction, $e_{(ijle)}$ =residual. Through the total period, increase in the milk yield was 18.3% (ns) with the dose of 250 mg and 41.4% ($p<0.01$) with 500 mg of bST. With the dose of 500 mg, the significant increase in collected milk during the rainy season is to be related to the abundance and good quality of pasture. Milk consumed by calves was not recorded and could bias the estimation of the milk response to bST because numerous factors like age of calves, season and change in milk composition due to bST administration (1) can influence this consumption and the milking volume. At the end of the experience, cows receiving 500 mg of BST lost more condition than did the others. These results suggest to take precautions related to feeding for bST use during the dry season because of pasture deficit.

Table 1: Milk yield (kg/cow/day) and bST effect¹.

Dose of bST,mg/14d					bST effect			
		0	250	500	250	rSD	500	rSD
Rainy season	mean±sd	2.2±0.7	2.7±0.8	3.2±0.9	0.5ns	0.8	0.9**	0.8
	extremes	0.7-3.4	1.3-4.9	1.8-6.7				
Dry season	mean±sd	1.4±0.3	1.6±0.4	2.0±0.6	0.2ns	0.4	0.5*	0.5
	extremes	1.0-2.1	1.2-2.4	1.3-3.2				
Total period	mean±sd	1.7±0.3	2.0±0.5	2.4±0.7	0.3ns	0.4	0.7**	0.5

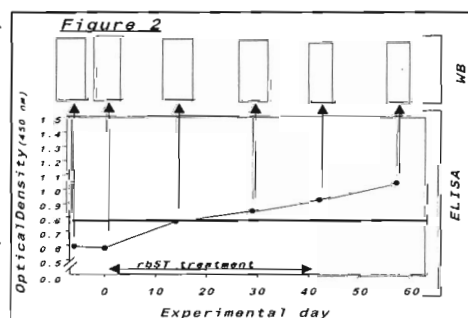
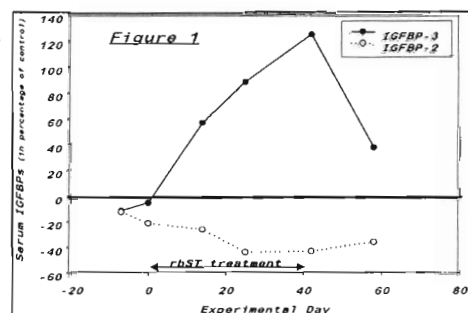
¹:significant at $p<0.01$:**, $p<0.05$:*, $p<0.10$:+, or not significant: ns.

References: Chilliard 1988. *Ann. Zootech.*, 37, 159; Cissé et al., 1991. *J. Dairy Sci.*, 74, 1382; Cissé 1994. *Rev. Sen. Rech. Agr. Hal.*, 4, 5.

Key words: bST, Cow, Milk collected after sucking, Sahelian pasture

[L/A2] ANTIBODY FORMATION AND PLASMA PROFILES OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-BINDING PROTEINS IN GROWING HEIFERS AFTER SUSTAINED-RELEASE EXOGENEOUS BST ADMINISTRATION. C. Bertozzi¹, D. Portetelle¹, S. Massart¹, D. Deaver², L. Vleerick¹, I. Parmentier¹, F. Mortiaux¹ and R. Renaville¹. ¹Department of Applied Biochemistry and Biology, University Faculty of Agricultural Sciences, B-5030 Gembloux, Belgium ; ²Department of Dairy and Animal Sciences PennState University, PA 16802-3502 University Park, USA.

Our objectives were to determine the effects of bovine somatotropin on antibody formation and concentrations of IGF-I and IGFBP-2 and 3 in order to provide a screening test to identify treated animals. Belgian White Blue heifers were injected (n=6) with bST preparation (*Posilac*® Monsanto) or not injected (n=6, control) every two weeks during 40 days. IGF-I and IGFBP-2 were measured by RIA and IGFBP-3 was evaluated using a Western Ligand blotting. Blood bST antibody was estimated by a qualitative method (Western Blotting, WB) as well as by a quantitative method (ELISA). During bST treatment plasma IGF-I increased significantly on d 10 after the first injection and remained significantly higher than in the control group until the end of the trial. Plasma IGFBP-3 was positively and significantly ($P<0.05$) correlated with bST then IGFBP-2 was negatively and significantly ($P<0.05$) correlated with bST treatment (figure 1). Results showed that the calculated IGFBP-3/IGFBP-2 ratio amplifies differences between treated and control animals. bST treated animals developed circulating anti-bST antibodies which were detected by WB after the second injection and during the rest of experiment period (figure 2). WB technique although suited for our objectives, is not adapted for great sampling studies. Therefore we have tried to develop an ELISA method, more easy to use in a practical approach. Mean O.D. in treated group ten days after the first injection was observed over the maximum individual value within control group in whole duration of the experiment (this value defines the positive level) and until 20 days after the last injection. In conclusion, some parameters on the somatotropic axis and especially IGFBP-3/IGFBP-2 ratio could provide a good method to screen rbST treated animals. However, the detection of rbST antibodies must inform efficiently that a rbST treatment exists. (This research was supported by Ministry of Agriculture, DG6, grant5736).



Key Words: bST, IGF, IGFBPs, Antibody