form aggregates and to aggregate with the other whey proteins. Studies using various kinds of two-dimensional polyacrylamide gel electrophoresis (PAGE) analysis as the major tool with simple solutions of pure whey proteins confirmed that β-lactoglobulin (β-Lg) was the most important whey protein in these aggregations. A previously unknown group of intermediates, the non-native β-Lg monomers, was of particular interest and some characteristics of these and other early heat-induced intermediates were determined. The changes in the positions of the disulphide bonds in β-Lg as a consequence of heat treatment were identified from mass spectroscopy-based analyses. $\otimes$ Heated mixtures of α-lactalbumin (α-La) and β-Lg were found to contain 1:1 disulfide-bonded dimers as well as non-native monomers, dimers, trimers, etc. of both α-La and β-Lg The findings from this and other model systems were then tested in WPC solutions using one- and two-dimensional PAGE. $\otimes$ In heat-treated milk the whey proteins interact to form disulfide bonds with the casein micelles and κ-casein (κ-Cn) is the most significant casein in this reaction and β-Lg was the most important whey protein in this reaction. In a model system, κ-Cn and β-Lg formed 1:1 aggregates as well as large polymeric aggregates. A heat-induced complex of β-Lg and κ-Cn was isolated from a heated mixture of casein micelles and β-Lg by chromatography. Analysis of this complex identified a number of novel disulfide bonds between β-Lg and κ-Cn. $\otimes$ These results have shown that β-Lg is critical to the heat-induced changes in both milk and WPC, and have lead us to re-evaluate the likely mechanism for the initial changes within β-Lg in response to heat-treatment.

Key Words: β-Lactoglobulin - κ-casein complex, Non-native β-lactoglobulin monomers, Heat-induced β-Lg - α-La complex

200 Functional properties of whey proteins. M. Britten*, *FRDC, Agriculture and Agri-Food Canada, St-Hyacinthe, Qc., Canada.*

In recent years, the use of whey proteins in formulated foods has increased. Health conscious consumers recognize their high nutritional value. Specific biological activities have also been attributed to whey proteins which makes them suitable ingredients for the formulation of functional foods. Along with a healthy image, whey protein provides foods with improved texture and overall quality. A better control of protein polymerization is however required to optimize their use. Heating a whey protein dispersion leads to polymer formation. The pH, calcium and protein concentrations during treatment determine aggregate size, shape and hydration. These characteristics influence their behavior in food systems. Controlled aggregation of whey protein is used to increase the viscosity and improve the mouth feel of liquid products. Added to cheese milk, whey protein aggregates are trapped in the curd and increase the yield, moisture and reduce firmness of cheese. In specific aggregation conditions, whey proteins form opaque dispersions and can be used as clouding agents in beverages. Gel formation is usually induced by heating native whey protein dispersions. However, it can also be obtained from polymerized whey protein dispersions by acidification or by the addition of salts. Use of polymerized whey proteins in yogurt formulations increases firmness and reduces syneresis. Whey proteins are also used in the preparation of emulsions and foams. They adsorb at interfaces and form a membrane which prevents emulsion coalescence or foam collapse. Whey protein membrane has also been shown to provide protection against lipid oxidation. The combination of interfacial adsorption and gel formation properties is used to produce solid-like emulsions and foams. This approach finds applications in baked foods or in the development of nutrient carriers. Whey protein polymerization offers new means to control food texture and stability. It should support the development of formulated food especially designed for health conscious consumers.

Key Words: protein polymers, gel formation, emulsions

201 Technological, functional and biological properties of peptides obtained by enzymatic hydrolysis of whey proteins. S.F. Gauthier* and Y. Pouliot, *Centre de recherche STELA, Universite Laval, Quebec, Canada.*

The study of peptides released by enzymatic hydrolysis of whey proteins has been initially focussing at functional properties in model systems. Our first work showed that sequences 41-60 and 21-40 from β-lactoglobulin (β-LG) were responsible for improved emulsification properties in tryptic hydrolysates of β-LG. Further work showed that adding negatively charged peptides from chymotryptic hydrolysates of whey proteins could prevent phase separation of dairy-based concentrated liquid infant formulas, as a replacement of carrageenin. Hydrolysis of whey proteins using bacterial enzymatic extracts was also successful in improving heat stability of whey proteins in an acidic beverage. Recent work demonstrated the occurrence of interactions between peptides β-LG 102-105, β-LG 142-148 and the native β-LG. These latest results suggest that β-LG could be used as a carrier for bioactive peptides. Finally, the emerging functional foods and nutraceuticals have triggered the development of new knowledge on the biological activity of whey proteins. Whey proteins are recognized to comprise peptide sequences having ACE-inhibiting properties. Our work led to the development of whey protein enzymatic hydrolysate that has demonstrated antihypertensive properties when orally administered to SHR rats at a dosage of 75 mg/kg. Our work has shown that the enzymatic hydrolysis of whey proteins is not only improving their functional and technological properties but it is also providing powerful tools to exploit their full potential by generating bioactive peptides.

202 The quantitative analysis of whey proteins - where we are and where we are going. DE Otter* and EA Foegeding, *North Carolina State University, Raleigh, NC.*

As whey proteins become ingredients in more sophisticated nutriceutically and functionally based foods and dairy products it is imperative that they can be accurately quantified. My presentation will highlight some of the research presently being undertaken to address this issue and to suggest directions for future research.
Previous work has concentrated on quantifying the major whey proteins; α-lactalbumin, β-lactoglobulin, bovine serum albumin and immunoglobulin. There is however an increasing demand to also quantify the minor components, such as glycomacropeptide, lactoferrin and folate binding protein; the bioactivity of the different proteins/peptides; and the amount of native and denatured protein. Researchers are using a number of diverse methods for measuring the individual whey proteins. The idiosyncrasies of each method must be considered when quoting values for the individual proteins.
The cornerstone of any successful quantitative method is the availability of well-defined calibration standards. A wide array of analytical techniques such as nitrogen analysis, UV spectroscopy, PAGE, HPLC and CE has been used to characterise a set of whey protein standards. These techniques all have their individual limitations but when used together they give a good estimate of protein purity. An alternative method based on the unique amino acid 'fingerprint' of each whey protein has also been used to characterise the standards (Tsao et al., The Food Technologist, 28 (3), 94, 1998). Using an in-line nitrogen detector to accurately quantify the nitrogen content of specific HPLC peaks has further enhanced the characterisation. The identity and integrity of the proteins in these peaks has been verified by HPLC-mass spectrometry.
Another important component of method development is method validation and accreditation. This usually involves an inter-laboratory comparison study under the auspices of an international organisation such as AOAC or IDF. The steps involved for an HPLC method for the quantification of bovine IgG that is currently going through this process will be described.

Key Words: Whey proteins, Quantitative analysis, Calibration standards

Extension Education and International Animal Agriculture
The Impact of Governmental Policies on North American Animal Agriculture

203 Mexico: Agricultural policies, trade agreements and challenges for the animal scientist. M Garcìa-Winder*[1], [1] *T.C. Jacoby & Co.*

The importance of the agricultural sector in Mexico is not only determined by the number of people living in rural areas (25% of total population), or because of its contribution to GNP (6.3%), but also because it is a sector with higher poverty levels and in more need of development. The incorporation to GATT, was the beginning of profound transformations. As a consequence agricultural policies have been

radically modified to respond to the challenges of open economies and international trade. During the last decade these policies have been directed towards providing direct support to production, to enhance trade and to promote the development and transfer of technology. Presently, Mexico has signed several free trade agreements with NAFTA being the most notable one. Animal production has shown remarkable annual growth (4.9% for meat, 4.3% for dairy and 6.3% for egg) during the last 10 years. Due to increased demand and trade liberalization, the net trade balance for the sector has been negative, accounted primarily by a deficit in dairy and meat, a condition that is also observed in processed animal products, where dairy cause more than 50% of this deficit. In contrast with the importance of the rural sector, during the last decade public spending has not only been reduced but diversified among programs and institutions with many contradictory goals. Among challenges for the future are the need to increase production, productivity and sustainability of the livestock sector, the development of new products for a more demanding population, improvement of animal welfare and protection and recovery of natural resources. These challenges require animal scientist willing to find new paradigms and to participate in activities traditionally considered outside the scope of animal science.

Key Words: Agricultural policies, Trade agreements, Animal science

Milk Synthesis
Regulation of Mammary Gland Function by Growth Factors and Downstream Signaling Cascades

204 Effect of transforming growth factor-beta-1 on mammary development. K. Plaut*, A. Dean, and T. Patnode, *University of Vermont, Burlington, VT/USA.*

The mammary gland is dependent on hormones and growth factors for development and differentiation. While studies have been conducted to understand the role of growth factors that stimulate mammary growth, few studies have focused on the role of growth inhibitors in mammary development. Transforming growth factor - beta 1 (TGF-β1) is a potent inhibitor of mammary growth and stimulates the extracellular matrix (ECM). The ECM effects mammary development because it provides the scaffolding that supports the epithelial cells of the gland. We studied the effect of TGF-β on mammary development in cell lines and mammary tissue. Incubation of mammary epithelial cells with 2.5 - 5 ng/ml of TGF-β1 + 10% fetal bovine serum (FBS) results in at least a 50% reduction in cell growth compared to FBS controls. Using flow cytometry, we characterized changes in the cell cycle in response to TGF-β. NOG-8 cells were synchronized by serum starvation for 48 hours followed by incubation in media supplemented with 10% FBS and 0 or 2.5 ng/ml TGF-β1. Cells were harvested at 6, 48, and 72 hours post-treatment. There were fewer cells in G0/G1 and a higher proportion of dying cells in the TGF-β treated cells compared to controls at all time points. A cell cycle specific mini-array is being used to determine which genes in the cell cycle are changed in response to TGF-β1. In addition to the effects on cell cycle, TGF-β also effects the production of extracellular matrix proteins, which causes a change in cell shape and function. Cells exposed to 5 ng/ml TGF-β1 changed from a cobblestone morphology to elongated fibroblast-like morphology and expressed high levels of fibronectin as determined by immunocytochemistry. The cells continued to be growth inhibited. Last, studies are underway to determine the in vivo effects of TGF-β on the mammary gland of heifers. Changes in DNA synthesis, cell cycle gene expression and expression of extracellular matrix proteins are being used to measure the response. These studies will lead to a better understanding of how TGF-β effects mammary cell growth.

Key Words: mammary, transforming growth factor beta, growth inhibitor

205 Mammary development, growth and plasma levels of IGF-I and IGF-binding proteins in gilts provided different energy levels from weaning to puberty. MT Sorensen*, M Vestergaard, S Purup, and K Sejrsen, *Danish Institute of Agricultural Sciences, Foulum, Denmark.*

We investigated the effect of feeding level from weaning (d 28) to slaughter at puberty (d 162) on growth rate, mammary development and plasma levels of IGF-I and IGF-binding proteins (IGFBP) in 10 litters of 4 female pigs. From d 28 to 90 (period 1) and from d 90 to 162 (period 2), pigs were fed either ad libitum (A) or restrictively (R; i.e. 30% lower feed intake in period 1 and 25% lower in period 2) in a 2x2 factorial design with treatments named AA, AR, RA and RR. In period 1, ADG of A-gilts was 622 g vs. 522 g for R-gilts ($P<0.001$). At the end of period 1, A- compared with R-gilts had higher plasma levels of IGF-I (303 vs. 220 ng/ml, $P<0.01$) and IGFBP-3 (770 vs. 564, arbitrary units, $P<0.01$), but lower IGFBP-2 (291 vs. 396 a.u., $P<0.02$) and 28 kDa IGFBP ($P<0.06$). In period 2, ADG of RA- and AA-gilts was 1012 g vs. 792 g for RR- and AR-gilts ($P<0.001$). Furthermore, RA-gilts showed compensatory growth compared with AA-gilts (1054 vs. 971 g/d, $P<0.07$) with no difference in feed intake. At the end of period 2, there was a tendency for higher plasma IGF-I ($P<0.15$) in AA- and RA-gilts compared with AR- and RR-gilts whereas IGFBP-2 and 28 kDa IGFBP were reduced ($P<0.01$). The amount of dissected mammary tissue was higher in AA- and RA-gilts compared with AR- and RR-gilts (86 vs. 59 g/gland, $P<0.001$), and although DNA concentration was lower in AA- and RA-gilts compared with AR- and RR-gilts (342 vs. 397 μg/g tissue, $P<0.04$), total amount of mammary DNA was highest in AA- and RA-gilts. The concentration of mammary RNA was not affected by treatment. Feeding level in period 1 did not affect the mammary measures. We conclude that to obtain high mammary growth, a period with ad libitum feeding before puberty is needed, however, this period does not have to commence at weaning. Furthermore, differences in growth rate are associated with differences in IGF-I and IGFBPs, and female pigs fed restrictively from weaning to d 90 and ad libitum until puberty grow as fast as do continuously ad libitum fed pigs.

Key Words: mammary, gilt, IGF

206 Polycation-mediated transfection of the porcine mammary gland. M. Amstutz*[1], S. Reuss[1], R. Neiswander[2], T. Meek[1], S. Courtney[1], and F. Schanbacher[2], [1] *The Ohio State University Agricultural Technical Institute,* [2] *Ohio Agricultural Research and Development Center, Wooster USA.*

Production of recombinant proteins in the milk of livestock has thus far been limited to transgenic and viral-mediated gene transfer methods. Our previous studies have demonstrated the feasibility of polycation-mediated transfection of bovine and murine mammary cells *in vitro* and the guinea pig mammary gland *in vivo*. These experiments were conducted to determine if direct intramammary infusion of polycation-DNA complexes in the porcine mammary gland would result in recombinant human growth hormone (hGH) secretion in milk. A second parity Yorkshire sow (sow 1) was tranquilized on day 112 of gestation. Teat ends were cleaned with alcohol and mammary glands transfected by infusing each teat opening with 50 ml of HBSS containing either; DEAE-dextran (DEAE) 1.25 mg/ml (sham transfection), or DEAE-dextran 1.25 mg/ml and plasmid DNA (50 ug/ml). Following parturition milk samples were collected daily, defatted, and stored at -80°C until assayed for hGH by radioimmunoassay. Milk from sham transfected and uninfused mammary glands contained no hGH. Milk from the DEAE-DNA transfected mammary gland contained hGH on all 14 days of lactation with expression peaking on day 6 at 5.5 ng hGH/ml and declining to 1.5 ng/ml by day 14. A second experiment was conducted as described above utilizing a first litter gilt (sow 2). Milk from two mammary glands transfected with DEAE-DNA again contained hGH throughout the first 12 days of lactation while sham and control samples contained none. Expression profiles for sow 2 were similar to sow 1 with expression peaking at 1.1 (gland 3) and 0.88 (gland 2) ng hGH/ml on days 8 and 9 respectively. Differences in hGH expression levels may be due to variation in time from transfection to parturition (3 days for sow 1 vs. 6 days for sow 2) or parity differences. Although expression levels differ between animals these results demonstrate the feasibility of transfecting the porcine mammary gland via direct intramammary infusion.

Key Words: Transfection, Porcine, Polycation