Milk Protein and Enzymes Symposium: Milk Proteins and Peptides: Bioactivity and Digestion

379 Structural bases for the nutritional and biological properties of the caseins. H. M. Farrell Jr.*[1], E. L. Malin[1], E. M. Brown[2], and A. Mora-Gutierrez[3], *[1]USDA, ERRC, Dairy and Functional Foods RU, Wyndmoor, PA, [2]USDA, ERRC, Biobased and Other Animal Coproducts RU, Wyndmoor, PA, [3]Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX.*

Our understanding of the nutritional and biological properties of the caseins has paralleled the description of the chemistry of these proteins. For example, following the first amino acid analyses of the caseins, it became apparent that these proteins are an excellent source of the essential amino acids needed for human nutrition, and casein became the standard reference protein for nutritional studies. Upon elucidation of the primary sequences of the caseins, it was discovered that inherent in these sequences were several peptides, the so-called casomorphins, which if liberated during digestion would have physiological (endorphin-like) activity. The enumeration of other potentially biologically active peptides followed. In addition, anti-bacterial activities have been ascribed to several other casein peptides. Although many of these activities have been demonstrated in vitro, few have been demonstrated in vivo in clinical or feeding studies. Notable exceptions to this rule have been antihypertensive peptides, an anti-stress peptide from α_{S1}-casein, and the role of phospho-peptides in calcium uptake. The advent of 3D molecular modeling has now allowed us to portray these peptides at the molecular level and will allow the comparisons of the structures of the various biologically active peptides, leading to a better understanding of the molecular mode of action of the casein peptides.

Key words: casein structure, casein bioactive

380 Digestibility of whey protein aggregates and fibrils under simulated gastro-intestinal environments. H. Singh*, M. Peram, S. Loveday, B. Libby, and Y. Aiqain, *Riddet Institute, Massey University, Palmerston North, New Zealand.*

The digestion and metabolism of proteins continues to generate considerable scientific interest, driven mainly by the need to understand the release of bioactive peptides, and factors affecting protein allergenicity and satiety. It is well known that β-lactoglobulin (β-Lg), which represents about 60% of whey proteins in bovine milk, is resistant to hydrolysis by pepsin under gastric conditions because of its stable, globular tertiary structure at low pH (<pH 3). However, denatured β-Lg (caused by heating or high pressure) can be readily digested by pepsin under gastric conditions. Under denaturing conditions, β-Lg is able to form different kinds of aggregates, including spherical aggregates and elongated fibrils, depending on the protein concentration, pH, temperature and heating time. Although many studies on the mechanism of β-Lg aggregate formation and structures have been carried out, little work has been carried out on the digestion of different forms and structures of these aggregates under gastro-intestinal conditions. Therefore the objective of our study was to understand the relationship between heat-induced structural changes in β-Lg and in vitro gastric digestibility. Heating of β-Lg at 90°C and neutral pH resulted in the formation of non-native aggregates of different sizes (dimmers, trimers, oligomers), linked through disulfide and non-covalent bonds. The digestibility of non-native β-Lg aggregates varied significantly depending on the type of aggregates. Some dimers were resistant to digestion, while high molecular weight aggregates were digested fast. β-Lg fibrils formed by heating β-Lg solutions at 80°C and pH 2.0 for 20 h consisted of peptides, generated through acid/heat-induced hydrolysis. Using matrix-assisted laser desorption/ionization mass spectometry (MALDI-MS), we found that the peptides in the fibrils were more susceptible for pepsin attack than the other peptides, possible because of their hydrophobic nature.

Key words: whey proteins, digestibility, protein aggregation

381 Peptides derived from whey protein: Endothelium and vascular bioactive function. E. D. Bastian* and L. W. Ward, *Glanbia Nutritionals Inc., Twin Falls, ID.*

Novel, high-leucine peptides derived from whey protein were tested for vascular, endothelial response in 20 young individuals. A randomized, placebo-controlled, crossover design was used which consisted of 2 wk supplementation, 2 wk washout, and 2 wk crossover supplementation. Peptide and placebo dose was 5 g/day. Subjects participated in 2 vascular testing days, each preceded by 2 wk of supplementation. After consuming the dose, vascular function in the forearm was measured serially for 120 min. Macrovascular and microvascular function was assessed using brachial-artery, flow-mediated dilation (FMD) and venous-occlusion, strain-gauge plethysmography. Six blood draws were taken across the 120 min measurement period and analyzed for total nitrates/nitrites. Placebo had no impact on FMD at 30, 60 and 90 min post ingestion. When peptides were ingested, FMD increased at the 3 time points by 14.0, 26.9, and 15.4% compared with placebo and baseline measurements. Plasma total nitrates/nitrites significantly decreased for the 120 min post-ingestion period and were lower at 120 min in placebo (−25%) compared with peptide (−18%). These data indicate that supplementation with high-leucine, whey-derived peptides improves vascular function in young, healthy individuals.

Key words: bioactive, peptide, vascular

382 The structure of dairy products modifies the kinetics of protein hydrolysis and the release of bioactive peptides in the gut during digestion. D. Dupont*[1,2], K. Bouzerzour[1,2], F. Barbe[1,2], Y. Le Gouar[1,2], and O. Menard[1,2], *[1]National Institute for Agricultural research, Rennes, France, [2]Agrocampus Ouest, Rennes, France.*

Digestion provides nutrients and energy essential to the survival and growth of the organisms. But little is known about the influence of food structure on its digestibility and nutritional properties. The objectives of our group are therefore to understand how dairy products are disintegrated during digestion and how the structure of the matrix will modify the protein digestibility. To reach this goal, static and dynamic in vitro models simulating what happens in the GI tract have been developed and in vivo experiments have been performed using the pig as a model. Digested samples collected in the different parts of the gut were characterized by SDS-PAGE, ELISA and/or mass spectrometry in order to quantify and identify milk proteins and peptides throughout the GI tract. Two applications will be presented. In a first experiment, digestion of an infant formula by 28 days-old piglets showed that caseins were rapidly hydrolysed in the stomach whereas whey proteins were partially resistant. Large milk protein fragments were shown to resist digestion and were detected in the ileum. Peptides

known to carry biological (anti-hypertensive, immunomodulating) properties were identified in the small intestine of piglets. In a second experiment, six dairy matrices (liquid milks, acid and rennet gels with or without heat-treatment) of similar composition but with different microstructure were manufactured from the same raw skim milk in a pilot plant. Each sample was given to six multi-catheterized adult mini-pigs. Effluents were then taken at the very end of stomach during 7 h after the meal and milk proteins and peptides quantified. Compares to liquid milks, acid gels showed a delayed gastric emptying and a slower release of caseins and β-lactoglobulin in the duodenum. When gel was stirred, an intermediate behaviour was observed. Results also showed that heat treatment of milk induced a slower release of caseins in the small intestine. All these results emphasize the role played by the structure of the food matrix on the digestion of dietary proteins.

Key words: digestion, dairy products, milk proteins

383 Effects of dietary milk fat globule membrane in the gut and on systemic lipid metabolism. R. Ward*[1], R. Jimenez-Flores[2], A. Zhou[1], and K. Hintze[1], *[1]Utah State University, Logan, [2]California Polytechnic State University, San Luis Obispo.*

In milk, fat droplets are coated by an epithelial membrane that results from a novel secretion process within the mammary gland. The milk fat globule membrane (MFGM) is composed of primarily polar lipids and membrane proteins and is the most diverse fraction of milk. While MFGM is present in all dairy products to some extent, it is present in several co products of dairy processes such as churn buttermilk and whey, and may be recovered for use a bioactive ingredient. Despite the fact that MFGM is a unique ingredient in the food supply and it is rich in potentially bioactive constituents, few studies have been conducted to measure its effect on physiology and nutrition. In the last several years our labs have begun to investigate the potential of this material as a food ingredient through studies with cells in culture, with rodents, and currently with humans. Our results to date indicate that MFGM has myriad effects within the gut, and also appears to affect systemic lipid partitioning. For example, MFGM reduces the number of aberrant crypt foci in a rat model of colon cancer and that it prevents gut leakiness and systemic inflammation induced by lipopolysaccharide in mice. Furthermore, supplementation of rat diets high in sucrose with MFGM appears to reduce the development of fatty liver via effects on gene expression and lipid transport. Lastly, dietary MFGM has significant effects on the fecal microbiome profile and presence of fecal small molecules. In sum, these results indicate that inclusion of MFGM into food products may have demonstrable and valuable bioactive effects.

Key words: MFGM, gut, metabolism