782 Dietary whey and casein produce differential effects on energy balance, gut hormones, glucose metabolism, and taste preference in diet-induced obese rats. Adel Pezeshki*, Andrew Fahim, and Prasanth Chelikani, *University of Calgary, Calgary, AB, Canada.*

Milk proteins decrease food intake, body weight, and improve glycemic control; however, little is known about the underlying mechanisms of improvements in energy balance and glucose metabolism. The objectives of this study were to determine the effects of dietary whey, casein, and a combination of the 2, on food intake, energy expenditure, body composition, gut hormones, glucose tolerance, metabolic markers, and taste preference in diet-induced obese rats. In experiment 1, obese rats were randomly assigned to isocaloric high-fat diets (n = 12/group; 33% calories from fat) containing egg white (control; 14% protein calories), whey (WH; 40% protein calories), casein (CA; 40% protein calories) or whey + casein (WHCA; 40% protein calories). Measurements included various behavioral and metabolic parameters. In experiment 2, following an 8-d training period, preference for WH, CA, WHCA or control diets was assessed on consecutive days. Data were analyzed by MIXED procedure. WH, CA and WHCA decreased food intake, body weight and fat content with WH and CA producing pronounced effects. The hypophagic effects of WH were likely due to reduced dietary preference. WH and CA but not WHCA improved glucose tolerance, with WH being more effective. WH reduced energy expenditure at early stages, whereas CA and WHCA increased energy expenditure at later stages of the study. WH, CA and WHCA decreased plasma leptin, glucose-dependent insulinotropic polypeptide and interleukin-6, whereas WH increased glucagon-like peptide-1 concentrations. The CA and WHCA diets increased plasma membrane glucose transporter-4 (GLUT4) to total GLUT4 ratio in skeletal muscle; protein abundance of other markers of glucose and energy metabolism (total Akt, AMPKα, COX-IV, HADH, SIRT3) in the adipose and cardiac tissues did not differ. Overall, our data demonstrate that dietary whey, casein, and their combination, improve energy balance through divergent effects on food intake, energy expenditure, glucose tolerance and gut hormone secretion, with whey being more efficacious. These findings have potential significance for developing whey-based functional foods and nutraceuticals for weight and diabetic control.

Key Words: whey, casein, energy balance

783 Characterization of the bovine milk proteome produced by Holstein and Jersey breeds of dairy cows. Rink Tacoma*, Lam Ying Wai, Julia Ganister Fields, and Sabrina Greenwood, *University of Vermont, Burlington, VT.*

Low-abundance milk proteins are of interest because of their diverse bioactivity. The objective of this study was to characterize the low-abundance protein profile within the whey fraction of milk produced by 2 dairy cattle breeds. A 7-d trial was conducted with 6 Jersey (80 ± 49 DIM) and 6 Holstein (75 ± 21 DIM) cows paired by DIM and parity, housed in the same tie-stall barn. Cows were all maintained on the same TMR diet and were fed individually to determine daily DMI. Milk samples were collected at a.m. and p.m. milking during the experiment. Milk composition (protein, fat, milk urea nitrogen (MUN), SCC) was determined in all samples collected and subsamples were collected and stored at −80C for low-abundance protein analysis. Milk samples for low-abundance protein analysis were thawed, pooled within animal and a mammalian protease inhibitor was added to each sample before centrifugation to isolate the whey fraction. Samples were depleted of casein and separated by SDS-PAGE. Samples were excised and subjected to a tryptic digest followed by LC-MS/MS analysis on a linear ion trap (LTQ)-Orbitrap Mass Spectrometer (MS). Product ion spectra were searched using SEQUEST on Proteome Discoverer 1.4 against a curated Bovine database. A linear mixed model was used to perform a repeated measures analysis on milk parameters and DMI in SAS (9.4). DMI ($P = 0.0108$), milk protein % ($P = 0.0021$), protein yield (kg/d; $P = 0.0068$) and fat % ($P = < 0.001$) were different between breeds whereas MUN ($P = 0.86$) and SCC ($P = 0.59$) were not different between breeds. MS analysis identified 947 proteins including over 45 proteins present at significantly different peptide counts between breeds ($P < 0.05$) with fold differences in peptide counts ranging from 0.2 to 6.5. Some known bioactive proteins were present at significantly different levels, including lactotransferrin ($P = 0.0026$) and complement C2 ($P = 0.0001$), whereas other known bioactive proteins including osteopontin ($P = 0.17$) and lactoperoxidase ($P = 0.29$) were present at similar levels in both breeds. This work provides insight into the low-abundance protein composition of milk produced by 2 dairy breeds.

Key Words: bioactive, whey

784 Lactoferrin and lactalbumin are more effective than whey protein in improving energy balance and glucose tolerance in diet-induced obese rats. Rizaldy Zapata*[1], Adel Pezeshki[1], Arashdeep Singh[1], Mary Chou[2], and Prasanth Chelikani[1], *[1]University of Calgary, Calgary, AB, Canada, [2]Advanced Orthomolecular Research Inc., Calgary, AB, Canada.*

Whey protein is often reported to promote satiety, aid in weight loss and stimulate energy expenditure. However, there is limited information on the relative efficacies of the individual components of whey – lactalbumin and lactoferrin – on energy balance and diabetic control. Our objectives were to compare the effects of whey with lactalbumin and lactoferrin on weight, body composition, food intake, energy expenditure, glucose tolerance and meal-induced hormone responses in diet-induced obese (DIO) rats. The DIO rats were randomized to receive one of 3 isocaloric and isonitrogenous high-fat diets (n = 8/group; 40% calories from fat, 30% protein calories): (1) whey (WH), (2) lactalbumin (LA), or (3) lactoferrin (LF) and were followed for 65 d (d). Food intake, energy expenditure, body composition, glucose tolerance and plasma satiety hormone concentrations were measured. Data were analyzed by repeated measures ANOVA or ANCOVA. Compared with WH, LF decreased food intake, and decreased energy expenditure during the dark period for the first 14 d of treatment. LA did not alter food intake, but increased energy expenditure during the first 3 h of the dark period after ~34 d of treatment interventions. LF reduced body weight by reducing adipose mass after 7 d whereas LA did not alter body weight and composition. When food intake was used as a covariate, LF reduced blood glucose and plasma leptin, but neither LA nor LF altered plasma concentrations of satiety hormones (GLP-1, GIP, PYY, insulin, amylin). Though glucose tolerance did not differ between WH, LA and LF by 30 d, LA and LF improved glucose tolerance by 60 d. In summary, lactoferrin is more effective than whey in inducing hypophagia, promoting fat loss, improving glucose tolerance and in decreasing plasma leptin, whereas lactalbumin is effective in increasing energy expenditure and improving

glucose tolerance. These components seem to be more beneficial than just whey itself in improving energy balance and glucose tolerance.

Key Words: whey, lactoferrin, lactalbumin

785 Partial hydrolysis of whey protein using immobilized enzymes and conjugation of these hydrolyzates with the aim of lowering whey protein allergenicity. Yuansheng Gong*[1], Lei Xu[1], and John A. Lucey[1,2], *[1]Department of Food Science, University of Wisconsin-Madison, Madison, WI, [2]Center for Dairy Research, University of Wisconsin-Madison, Madison, WI.*

Our previous research indicated that conjugation of whey protein isolates (WPI) with dextran (DX) via the Maillard reaction could provide an alternative approach to decrease the immunogenicity of whey protein. It has been well established that partial enzymatic hydrolysis of proteins also reduces their antigenicity. We want to explore a combination of partial protein hydrolysis and conjugation to decrease whey protein immunogenicity. In this study, we partially hydrolyzed whey protein by immobilized trypsin and chymotrypsin. All the hydrolyzed proteins were then conjugated with dextran (molecular weight 40kDa). Trypsin and chymotrypsin from bovine were immobilized onto (aldehyde) agarose (6% gels). WPI was partially hydrolyzed by these enzymes at 40, 45 and 50°C. Samples of hydrolyzed whey protein at 30, 60, 120, 180 and 240 min of hydrolysis were analyzed by SDS-PAGE and HPLC. The molecular weight of hydrolyzates was measured by Size-exclusion chromatography coupled with multi-angle laser light scattering detector. The degree of hydrolysis (DH) was 5 and 12% after 30 and 180 min respectively of hydrolysis at 50°C. Molecular weights of most hydrolyzates were between 1 to 5 kDa after 180 min hydrolysis at 50°C. Two hydrolyzates with DH values of 5 and 12% were conjugated with 40 kDa molecular weight dextran via our novel aqueous Maillard reaction method. Conjugates were separated and purified by chromatography. The IgE binding capacity was determined by ImmunoCap method using blood serum from cow's milk protein patient. The IgE binding capacity of conjugated whey protein hydrolyzates is currently being investigated and will be reported. Partial hydrolysis of whey protein plus conjugation with dextran may provide another option to reduce whey protein allergenicity.

Key Words: hydrolysis, conjugation, allergenic

786 Effect of Maillard modification on reducing immunogenicity of whey protein isolate. Lei Xu*[1], Yuansheng Gong[1], and John A. Lucey[1,2], *[1]University of Wisconsin-Madison, Department of Food Science, Madison, WI, [2]Wisconsin Center for Dairy Research, Madison, WI.*

A growing concern around the world is the number of people that are suffering from food protein allergies, especially among infants and young children. One potential approach is to block IgE binding epitopes of the protein allergen via the Maillard reaction with polysaccharides to decrease the allergy potential. Dairy infant formula is often formulated to a high proportion of whey proteins, and if infants are sensitive to whey proteins then hydrolysis of whey proteins is often used. The goal of this research was to reduce the immunogenicity of whey protein isolate (WPI) by conjugating WPI with dextran (DX). During this study, the effect of the molecular weight (M_W) of DX, ranging from 1 to 2000 kDa, on the immunogenicity of WPI-DX were explored. Our data indicated that the WPI to DX molar ratios in the conjugates made from DX with M_W values of 1 (G1), 3.5 (G3.5), 10 (G10), 150 (G150), 500 (G500), and 2000 kDa (G2000) were 1:4, 1:3, 1:2, 1:1.5, 1:1, and 1:1, respectively. With the increase in the M_W of DX, the M_W values of the corresponding conjugates were also increased, as determined by size exclusion chromatography with multiangle laser light scattering. The immunogenicity of conjugates were evaluated by IgE binding capacity of conjugates incubated with serum from blood samples obtained from patients with cow's milk protein allergy. Our results showed that WPI-DX conjugates have a lower WPI-specific IgE binding capacity than native WPI, with the lowest IgE binding capacity obtained in G10 conjugate, demonstrating that glycation via Maillard reaction did significantly reduce the immunogenicity of WPI. Furthermore, atomic force microscopy images suggested that conjugation of WPI with small M_W dextran resulted in greater surface coverage on the protein compared with large dextran conjugates, hence significantly reducing protein immunogenicity by creating steric hindrance that limited IgE binding.

Key Words: whey protein isolate, dextran, immunogenicity