of AbiK$^+$ cells, expression of the early genes was normal but no late transcript was detected at any time. Together with previous results on phage DNA replication and late protein production, these results confirm that AbiK rapidly blocks macromolecule synthesis during ul36 infection.

Key Words: Bacteriophage, Gene expression, *Lactococcus lactis*

143 Monitoring endospores and endospore-forming bacteria populations in commercial skim milk powder production plants. C. Murillo* and Rafael Jimenez-Flores, *California Polytechnic State University, San Luis Obispo, CA.*

The microflora of milk powder consists of a wide array of microorganisms of which special attention is given to *Bacillus* endospores. *Bacillus* endospores survive pasteurization and spray drying and inhabit the final powder product in the dormant state indefinitely. Once the powder is reconstituted, endospores may germinate, and through their enzymatic activity become detrimental to quality. The objectives of this study are to 1) enumerate mesophilic and thermophilic endospore populations during commercial, low-heat skim milk powder production, and 2) characterize the microbial ecology of this process using Terminal Restriction Fragment Patterns (TRFPs) in conjunction with the Ribosomal Data Base, and 3) compare the changes in bacteria populations during processing of low-heat, skim milk powder. Our approach is to observe these changes in commercial operations and to use the DPTC pilot plant as a model system. Fluid and powder skim milk samples were collected from two commercial milk powder facilities. Sampling points included the raw milk silo, separator, evaporator, and spray dryer. Microbial evaluation was normalized based on total solids. Every sample was evaluated for total aerobic plate count and mesophilic and thermophilic endospore counts. For TRFPs community DNA was extracted, amplified by PCR using 16s rDNA probes, and digested with *Hae*III and *Dpn*II. Endospore formers are predominant in condensed and powdered milk, and tent to increase in the powder with increasing processing time. In raw milk mesophilic and thermophilic endospores ranged from <25CFU/g to 70CFU/g and <25CFU/g to 10^2 CFU/g, respectively. In powder they ranged from <25CFU/g to 10^3 CFU/g and <25CFU/g to 10^5 CFU/g, respectively. Both endospore counts from skim milk showed an increasing trend with run time and rendered the powder out of the 10^3 CFU/g limit. In commercial samples TRF patterns successfully described microbial populations and a drastic change was observed between raw and powder milk for most runs.

Key Words: Endospore, Milk Powder, Production

144 Influence of lactococcal cell envelope proteinases on accelerated Cheddar cheese ripening. S. I. Myaka*, L. E. Metzger, and L. L. McKay, *MN-SD Dairy Food Research Center, University of Minnesota, St. Paul, MN.*

The proteolytic enzymes produced by lactococcal starters have an essential role in cheese ripening. Secondary proteolysis is initiated by the cell envelope proteinase (CEP) and cheese quality has been linked to particular CEPs. Additionally, Cheddar cheese ripening can be accelerated through the use of a "quick lysis" strain of *L. lactis*. Previously, thermolytic, isogenic strains of *L. lactis* subsp. lactis were constructed to possess different lactococcal CEPs. The objective of this research was to investigate the influence of lactococcal CEPs type a, c, d, e, and g from five isogenic, thermolytic strains on Cheddar cheese ripening. Three replicates of stirred curd Cheddar cheese were produced using these strains. Since the cultures undergo lysis during cheese manufacture, the cheeses were acidified with Glucono-delta-lactone after the cooking step. Additionally a control cheese was produced at each time with a commercial direct vat set starter culture using a conventional stirred-curd Cheddar cheese making procedure. There were no significant differences ($p \geq .05$) in cheese composition among the treatments and the mean moisture, fat on a dry basis, salt to moisture ratio, and pH ranged from 36.6 to 38.1%, 50.5 to 51.6%, 4.9 to 5.1%, and 5.2 to 5.3 respectively. Proteolysis was determined at 3 weeks, 2 months, and 4 months of ripening whereas descriptive sensory analysis (15 judges) was performed at 2 and 4 months of ripening. As expected the level of pH 4.6 soluble nitrogen, 70% ethanol soluble nitrogen, and free amino acids increased in all treatments during ripening and at 4 months the level of free amino acids was 1.68, 1.30, 1.89, 1.56, 1.20, and .97 mg Leu/g cheese respectively for type a, c, d, e, g, and control. There were no significant ($p \geq .05$) differences in overall flavor among the cheeses. However, bitterness was significantly higher ($p \leq .05$) in the control cheese. These results indicate that the level of free amino acids produced during ripening can be increased using a thermolytic culture, but the different CEPs investigated had no significant ($p \geq .05$) effect on overall cheese flavor after 4 months of ripening.

Key Words: Accelerated ripening, Cell envelope proteinase

145 Exopolysaccharides from lactic acid bacteria: microbial physiology and fermentation kinetics. L DE VUYST*[1] and F VANINGELGEM[1], [1]*Vrije Universiteit Brussel.*

Exopolysaccharides (EPS) from lactic acid bacteria (LAB) can be subdivided into two groups, namely homopolysaccharides (HoPS) and heteropolysaccharides (HePS). Recently, HePS receive renewed interest, since they play an important role in the rheology, texture, and mouthfeel of fermented milk drinks. For instance, the creamy, smooth texture is one of the aspects of the quality of yoghurt that seems to be improved by the ability of the yoghurt bacteria to produce HePS, even though only small amounts of HePS are being synthesized in milk. Many different types of HePS are secreted by LAB strains with respect to sugar composition and molecular size. HoPS are polymerised extracellularly from sucrose as donor molecule and supplier of energy. HePS are made by the polymerization of repeating unit precursors formed in the cytoplasm. The repeating units are assembled at the membrane by specific glycosyltransferases (GTF) through the sequential addition of activated sugars (sugar nucleotides), followed by export and polymerisation into a final HePS. Several enzymes and/or proteins are involved in the biosynthesis and secretion of HePS; some of them are unique to HePS formation. Glucose-1-phosphate and fructose-6-phospate are the precursor molecules for HePS biosynthesis. A major difference between cells and strains grown on different carbohydrate sources is the capacity to synthesize sugar nucleotides. Instability of HePS production and variability of polymer yields are well-documented problems in the dairy industry. Therefore, a well-understood optimal carbon flux and supply of sugar nucleotides in stable, EPS-producing, industrial strains is a key issue for their economical exploitation. Whereas mesophilic LAB strains seem to produce maximum amounts of HePS under conditions not optimal for growth, for instance low temperatures, HePS production from thermophilic LAB strains appears to be growth-associated,*i.e.* maximum production during growth and under conditions optimal for growth. HePS degradation often takes place upon prolonged incubation of HePS-producing LAB strains due to glycohydrolase activity.

Key Words: Eps, Lactic acid bacteria, Physiology

Nonruminant Nutrition
Nutritional Values of Phytase and Other Enzymes

146 Impacts of site-directed mutations and expression systems on efficacy of *Escherichia coli* phytases in diets for weanling pigs. C. H. Stahl*, J. M. Gentile, T. W. Kim, K. R. Roneker, and X. G. Lei, *Cornell University.*

Our laboratory has previously expressed an *E. coli* phytase (ECAP) and its two variants (Mutant U and AppA2) in *Pichia pastoris* using an inducible promoter. To reduce the fermentation cost, we expressed AppA2 in a constitutive expression system and characterized the biochemical properties of the produced enzyme. The objective of this study was to compare the efficacy of these three enzymes produced from the two systems in a corn-soybean meal diet for young pigs. Thirty pigs (9.45 ± .95 kg BW) were fed the diet plus ECAP, Mutant U, and AppA2 produced by the inducible system, AppA2 produced by the constitutive system, or .16% inorganic P. All phytases were included at 500 U/kg diet. Growth performance, and plasma inorganic P concentrations were assessed weekly. At wk 1, pigs fed inorganic P had higher ($P < .05$) plasma inorganic P levels than all other groups, with the exception of the group receiving mutant U phytase. This difference disappeared by wk 2. There were no significant differences in overall ADG, ADFI, or gain:feed

among the five treatment groups. In conclusion, all of the phytases examined were comparatively efficacious in improving the bioavailability of phytate-P from a corn-soybean meal diet to young pigs. Mutant U may be slightly more efficacious than the wild type (ECAP) or AppA2 under the conditions of this study.

Key Words: Phytase expression, Pigs, Phosphorus

147 Relative effectiveness of an experimental consensus phytase to inorganic phosphorus and an *Escherichia coli* phytase in diets for weanling pigs. J. M. Gentile*, K. R. Roneker, S. E. Crowe, W. G. Pond, and X. G. Lei, *Cornell University*.

Consensus phytase is a stable biosynthetic enzyme derived from the sequences of multiple homologous phytases. Two experiments were conducted to compare its effectiveness with inorganic P (P_i) and an *E. coli* phytase Mutant U in improving phytate-P availability to pigs. In Experiment 1, 36 pigs (3 wk old) were fed a corn-soybean meal basal diet (BD) plus consensus phytase at 0, 250, 500, 750, 1000, or 1250 U/kg for 5 wk. In Experiment 2, 36 pigs (4 wk old) were fed BD supplemented with .1% P_i, .2% P_i, 750 U consensus, 450 U consensus, 450 U Mutant U, or 225 U consensus + 225 U Mutant U/kg. In Experiment 1, plasma inorganic P concentration, plasma alkaline phosphatase activity, bone strength, and growth performance of pigs were improved ($P < .05$) by supplemental consensus phytase. Pigs fed 750 U/kg displayed the best overall responses of all measures. In Experiment 2, pigs fed 450 U consensus/kg had similar plasma phosphorus concentrations and bone strengths to those fed .1% P_i. Pigs fed 450 U Mutant U and 750 U consensus/kg were not significantly different from those fed .2% P_i in plasma inorganic P concentration or bone strength. In conclusion, experimental consensus phytase is effective in releasing phytate-P from the corn-soy diet for weanling pigs, and 750 U/kg seems to be an appropriate dosage. In addition, Mutant U may be more effective than consensus phytase at 450 U/kg, but no synergistic effect was seen by combining these two enzymes.

Key Words: Consensus phytase, *E. coli* phytase, Phosphorus

148 True phosphorus digestibility is improved with little change in the endogenous phosphorus outputs associated with soybean meal in the transgenic phytase weanling enviropig^{TM}. A. Ajakaiye*, M. Z. Fan, C. W. Forsberg, J. P. Phillips, R. G. Meidinger, M. Z. Weiderkehr, T. Archbold, S. P. Golovan, R. R. Hacker, and D. Barney, *University of Guelph, Guelph, Ontario, Canada*.

The objectives of this study were to compare true phosphorus (P) digestibility and the endogenous P outputs between the transgenic phytase weanling enviropig^{TM} and the non-transgenic weanling pig by regression analysis technique using soybean meal (SBM) as a "model" test ingredient. Four transgenic G_1 phytase pigs, with an average initial BW of 6.1 kg, were fitted with a simple T-cannula at the distal ileum and fed four diets according to a 4 x 4 Latin square design. The diets were cornstarch-based and contained four levels of P (0.99, 1.95, 2.94 and 3.96 g/kg DMI) from SBM. Chromic oxide (0.35%) was used as a digestibility marker. Each experimental period consisted of 8 d with 4-d adaptation and 4-d collection of salivary juice, ileal digesta, and fecal samples. There were no effects ($P > 0.05$) of diets, animals and periods as well as circadian rhythm (am vs pm) on salivary phytase activity (144.2 ± 42.8 - 152.4 ± 57.22 μmol/mg protein/min). Compared with the results of the non-transgenic weanling pig reported in our previous studies (Fan *et al.,* 2001, J. Nutr. 131:2388-2396), there were differences ($P < 0.05$) in the true ileal (93.3 ± 5.4 vs 50.7 ± 7.1%) and fecal 88.2 ± 3.3 vs 48.5 ± 5.4%) P digestibility values in SBM. However, there were no differences ($P > 0.05$) in the ileal (0.54 ± 0.13 vs 0.86 ± 0.09g/kg DMI) and the fecal (0.30 ± 0.09 vs 0.31 ± 0.06 g/kg DMI) endogenous P outputs associated with SBM between the transgenic and the non-transgenic weanling pigs. In conclusion, the transgenic phytase weanling enviropig^{TM} is effecient in utilizing P associated with plant feed ingredients with little change in the metabolism of gastrointestinal endogenous P.

Key Words: Phosphorus, True digestibility, Weanling transgenic phytase pig

149 Effect of microbial phytase on energy availability as assessed by protein and fat deposition in pigs. J. L. Shelton*[1], L. L. Southern[1], T. D. Bidner[1], M. Persica[1], J. Braun[2], B. Cousins[3], and F. McKnight[3], [1]*LSU Agricultural Center,* [2]*BASF AG, Ludwigshafen, Germany,* [3]*BASF Corporation, Mount Olive, NJ.*

A comparative slaughter experiment (EXP) was conducted to determine the effect of phytase (Natuphos®) on energy availability as assessed by protein and fat deposition in pigs. Crossbred barrows (initial and final BW of 26 and 52 kg) were allotted to four treatments: 1) corn-soybean meal (C-SBM) diet fed at 2.9 x maintenance (M), 2) C-SBM fed at 3.2 x M, 3) Diet 1 + 500 FTU phytase, or 4) Diet 2 + 500 FTU phytase. The diets contained 1.15% total Lys and all other AA met or exceeded 115% of the requirement. Calcium and available P were fed at 115% of the requirement in diets with no added phytase and decreased by 0.10% in diets with added phytase. Pigs were penned individually and fed at 0600 and 1700 hr daily, and water was available constantly. Eight pigs were slaughtered and ground at the beginning of the EXP to determine initial energy, protein, fat, and ash content using chemical analyses. At the end of the EXP, all 48 pigs were killed for determination of carcass traits and protein and fat content by TOBEC analysis. Six pigs per treatment were ground for chemical determination of energy, protein, fat, and ash content. Pigs fed at 3.2 x M had an increase ($P<0.10$) in ADG, fat deposition, retained energy (RE) in the carcass and in the carcass + viscera, and kilograms of protein in the carcass. Phytase increased ($P<0.05$) tenth-rib backfat thickness at both energy levels. No other response variables were significantly ($P>0.10$) affected by phytase as determined by univariate statistical analysis, but phytase in the diet of pigs fed at 2.9 x M increased every response variable measured. Multivariate analysis (MANOVA) that included the response variables ADG, gain:feed, loin muscle area, tenth-rib backfat thickness, protein deposition, fat deposition, and RE, showed that phytase increased ($P<0.07$) energy availability in pigs fed at 2.9 x M. The results of this EXP show that phytase increases energy availability in diets for pigs.

Key Words: Energy, Phytase, Pigs

150 The effect of citric acid alone or in combination with microbial phytase on gastric pH, and P and DM ileal and fecal digestibilities. J.P. Rice*[1], J.S. Radcliffe[1], and R.S. Pleasant[2], [1]*Purdue University, West Lafayette, IN,* [2]*Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA*.

Eight crossbred barrows fitted with steered ileo-cecal valve cannulas and percutaneous endoscopic gastrostomy (PEG) tubes were used in a 4X4 Latin square design to test the effects of citric acid alone or in combination with microbial phytase on gastric pH and P and DM apparent ileal (AID) and apparent fecal (ATTD) digestibility. Pigs were individually housed, allowed ad libitum access to water, and fed at 9% of metabolic BW ($BW^{.75}$). Water intake was measured daily. Diets were corn-soybean meal based and contained Cr_2O_3 as an indigestible marker. Dietary treatments were: 1) negative control, 0.53% Ca, 0.39% aP; 2) Diet 1 + 3.0% citric acid; 3) Diet 1 + 750 U/kg phytase, and 4) Diet 2 + 750 U/kg phytase. Each period consisted of a 7d adjustment, a 3d total collection, a 12h ileal collection, and a 3d adjustment followed by a second 12h ileal collection. Samples from the second collection were pooled by pig, freeze-dried and, along with fecal samples, analyzed for Cr, P, and DM content. Gastric pH was monitored continuously via an endogastric pH probe that was introduced into the gastric lumen through the PEG tube. Data were analyzed using the GLM procedures of SAS. Water intake was unaffected by diet ($P<0.10$). Citric acid addition to the diet decreased stomach pH by 11.4% ($P<0.001$) while phytase addition increased stomach pH ($P<0.001$) by 12.5%. A phytase x acid interaction was observed for gastric pH ($P<0.001$) with the combination of citric acid and phytase causing a greater decrease (14.3%) in stomach pH than acid alone. Phytase increased P AID by 15.5% ($P<0.001$) and ATTD by 7.2% ($P<0.001$), but had no effect ($P>0.10$) on DM digestibility. The addition of citric acid did not effect ($P>0.10$) the AID or ATTD of P, but did improve DM ATTD ($P<0.001$) and AID ($P<0.001$). Phytase x acid interactions were observed for P and DM ATTD ($P<0.001$) and AID ($P<0.001$). For all interactions, the combination of phytase and acid caused a greater increase in digestibility than an additive effect of phytase and acid. Feeding pigs citric acid and phytase in combination has the potential to increase nutrient digestibilities to a greater extent than citric acid or phytase alone.

Key Words: Pig, Citric acid, Phytase