

after treatment, respectively ($P < 0.001$). The 400 μM and 1.4 mM SSRI treatments resulted in 86% decreases in TEER 24 h after treatment, and decreased 75% after 48 hr after treatment ($P < 0.001$). The 40 μM , 400 μM , and 1.4 mM MAOI treatments decreased TEER 1.63, 17.8, and 12.2% respectively, 24 h after treatment and 16.2, 37.9, and 41.9% respectively, 48 hr after treatments. Treatment of pBMEC with SSRI + MAOI, resulted in similar decreases in TEER seen with SSRI treatment alone. In conclusion, while both SSRI and MAOI resulted in tight junction disruption in pBMEC. SSRI resulted in larger decreases in TEER relative to the control when compared to MAOI treatments. *Project supported by NRI Grant #2007-35206-17898 from USDA-CSREES.*

Key Words: serotonin, tight junctions, serotonin reuptake transporter

553 Detection of bioluminescent *Staphylococcus aureus* through bovine mammary gland tissue *ex vivo*. J. Curbelo*, K. Moulton, E. Schenck, and S. Willard, *Mississippi State University, Mississippi State.*

Mastitis infections caused by *Staphylococcus aureus* (*S. aureus*) causes serious problems for the dairy industry due to its high degree of pathogenicity, and new models are needed for monitoring mastitis infections. The objectives of this study were 1) to conduct photonic imaging (PE) of a transformed bioluminescent *S. aureus*-lux (Caliper Life Sci.) through bovine mammary gland tissue (BMGT); and 2) to evaluate the

significance of an optical clearing agent (OCA; corn syrup, 75%) in increasing transference of PE through BMGT. To accomplish this, $n=10$ bovine mammary quarters with intact teats were excised in half ($n=20$ sections) and placed over 5 ml of concentrated *S. aureus*-lux inoculums (3.5×10^{14} CFU) inside a black plastic box (opened top) for imaging and PE quantified in relative light units per second (RLU/s). Then, one of the teat sections was treated with OCA for 3 h and the other section was used as control and imaged, as previously described. Due to the high degree of variation of PE between the 20 teat sections, they were categorized by color (black, mixed and white). The mean PE of all teats (non-OCA treated) was 2.30 ± 0.53 RLU/s. However, when analyzed by color, PE through black teats were significantly lower than mixed and white teats ($P=0.01$; 0.68 ± 0.08 vs. 3.77 ± 1.50 and 3.48 ± 0.78 RLU/s, respectively). In addition, when applying OCA to teats, resulted in a greater detection of PE numerically, but not statistically ($P=0.18$); however when the significance of the OCA was evaluated by color, a higher PE was found in mixed colored teats treated with OCA vs non-OCA treated ($P=0.01$; 1.44 ± 0.12 vs 0.53 ± 0.05 RLU/s, respectively). In summary, this study indicates that detection of *S. aureus*-lux through BMGT is questionable due to the low PE detected; however, higher PE were detected through white and mixed colored teats. The OCA used in this study did not increase PE overall. For future studies, the color of the teats may need to be considered due to differences in PE transference, and further studies are needed to enhance penetration of the dense tissue of the mammary gland for photonic pathogen detection.

Key Words: *S. aureus*, biophotonics, mammary gland tissue

Nonruminant Nutrition: Minerals and Vitamins

554 Effects of phytase supplementation on apparent and standardized total tract digestibility of P in corn, soybean meal, and distillers dried grains with solubles (DDGS) fed to growing pigs. F. N. Almeida* and H. H. Stein, *University of Illinois, Urbana.*

An experiment was conducted to measure the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) by growing pigs of P in corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS) without and with supplementation of microbial phytase. Seven diets were prepared. Two diets were based on corn, 2 diets were based on SBM, 2 diets were based on DDGS, and 1 diet was a P-free diet. Corn, SBM, or DDGS were the only sources of P in the diets. One of the diets with each ingredient contained no microbial phytase while the other diet contained 500 units/kg of phytase (Optiphos, Enzyvia LLC, Sheridan, IN). A total of 42 growing barrows (initial BW: 13.5 ± 3.9 kg) were randomly allotted to the 7 dietary treatments with 6 pigs per treatment. All pigs were fed experimental diets for 10 d with the initial 5 d being an adaptation period to the diet, whereas feces were collected quantitatively during the final 5 d of the experiment using the marker to marker procedure. The ATTD of P was calculated for corn, SBM, and DDGS and the effect of microbial phytase was calculated as well. The basal endogenous losses of P (ELP) were measured from pigs fed the P-free diet and values for ATTD of P in the 6 P-containing diets were corrected for the basal ELP to calculate STTD values for P in each of these diets. Results showed that the addition of phytase increased ($P < 0.001$) ATTD of P from 19.9 to 57.8% in corn and from 41.5 to 68.4% in SBM, but the ATTD of P in DDGS without phytase (68.6%) was not different from the ATTD of P in DDGS with phytase (71.0%). The ELP was 199 mg/kg DMI. The addition of phytase also increased ($P < 0.001$) STTD of P in corn and SBM (from 26.4 to 64.4% and from 48.3 to 74.9%, respectively), but the STTD values of

P in DDGS without and with phytase (72.9 and 75.5%, respectively) were not different. In conclusion, the addition of phytase increased the ATTD and STTD of P in corn and SBM, but had no influence on the ATTD and STTD of P in DDGS.

Key Words: digestibility, pigs, phosphorus

555 Determination of the stability of Zn, Mn, Cu and Fe glycinate in aqueous solution by electrospray QqTOF mass spectrometry. S. Oguey*¹, V. Vacchina², R. Lobinski³, and D. Bravo¹, ¹*Pancosma, Geneva, Switzerland*, ²*UT2A, Pau, France*, ³*CNRS, Pau, France*.

The crystalline structure of trace mineral complexes based on glycine (BT) was well known, however setting analysis methods of the products in diluted media (feed, stomach juice, plasma,...) required a previous identification and characterisation of the complexes existing in aqueous solution. The chemical structure of BT with elements of the first transition series (Fe, Cu, Mn and Zn) in aqueous solution was investigated by mass spectroscopy with an electrospray QqTOF mass spectrometer (ESI-QqTOF-MS) either in full scan TOF mode or in tandem mass spectrometry (MS/MS) mode. Each BT was dissolved in a 50% methanol and 10 mM ammonium acetate buffer at pH 7. BT Fe solutions were previously degassed to prevent iron oxidation and iron hydroxide precipitation. Once the chemical structures determined at pH 7, the pH of the BT solutions was varied from 2 to 7 by step of 1 in order to determine the stability of the complexes in solution, and the evolution of the amount of the complex and free glycine ions was observed. BT in aqueous solution at pH 2 were still detected as glycine complexes. The chemical structure was slightly different from the structure found in solid, except for manganese which was identical. Their general formula

was $[M(\text{Gly})(\text{SO}_4)(\text{H}_2\text{O})_x]_n$. For $M=\text{Zn}$ and $M=\text{Fe}$, 2 complexes were detected ($n=1$, $x=1$ and $n=2$, $x=0$), for $M=\text{Cu}$, 3 complexes were found ($n=1$, $x=1$; $n=2$ $x=1$ and $n=3$ $x=0$) and for $M=\text{Mn}$, 4 anhydrous ($x=0$) complexes were observed ($n=1,2,3,4$). All the complexes were detected on the whole pH range. An acid pH induced a partial degradation of the complexes, as free glycine seemed to be more abundant while the acidity of the solution was increased. The small complexes were more degraded than the larger complexes. The identification of glycine complexes in aqueous solution rendered possible the development of analytical traceability methods.

Key Words: chelate, traceability, trace mineral complex

556 Analysis of Zn, Mn, Cu and Fe glycinate by size-exclusion liquid chromatography coupled to an inductively coupled plasma mass spectrometry detection. S. Oguey^{*1}, V. Vacchina², R. Lobinski³, and D. Bravo¹, ¹*Pancosma, Geneva, Switzerland*, ²*UT2A, Pau, France*, ³*CNRS, Pau, France*.

Analyzing trace mineral complexes based on glycine (BT) in diluted media (feed, stomach juice, plasma, etc.) required us to find separation conditions where BT could be detected specifically. The intent of the study was to investigate size exclusion chromatography (SEC) with an inductively coupled plasma mass spectrometer detector (ICP-MS) to determine the stability of BT on a chromatographic column and to separate the glycine complexes from metal sources present in the matrix (like metal sulfates). Ammonium acetate (AAC) was chosen as a buffer and the mobile phase was optimized in terms of ionic strength (10 to 200 mM AAC) and pH (6, 7.4 and 8). A SEC column with an optimal separation range between 0.1 and 7 kDa was chosen. The BT solutions were injected with the selected mobile phase and the ICP-MS data were recorded. The metal remaining on the column was eluted by EDTA, which was removed before the next injection. The recovery was evaluated by comparison of the quantity of metal injected with the quantity collected at the retention time of the glycinate. BT Cu and Mn were eluted with an ionic strength equal or over 10mM AAC. Both were stable at pH 6 and 7.4. At pH 8.5 BT Cu was stable while BT Mn slightly interacted with the column. The stability of BT Zn was maximized with a high ionic strength (200 mM AAC) and a neutral pH. BT Fe required a deoxygenated mobile phase to avoid oxidation and precipitation in the column, an ionic strength of 40 mM AAC or higher, and a slightly acid or neutral pH. At 200 mM AAC and pH 7.4, the recovery was always over 85% for all 4 BT. Metal sulfates also had variable behaviors. Zinc sulfate did not elute from the column while copper, iron and manganese sulfate eluted with a slight time decay. Consequently size-exclusion could be used as a purification method for BT.

Key Words: chelate, traceability, size-exclusion chromatography

557 Femurs are more accurate than fibulas as predictors of whole body bone mineral content in growing pigs. T. D. Crenshaw^{*}, L. E. Hoffman, J. R. Danielson, and D. K. Schneider, *University of Wisconsin, Madison*.

An assessment of skeletal tissue is considered standard procedure for quantifying animal responses to supplemental sources and amounts of Ca and P. If the primary concern involves entire animal responses, then a measure of whole body bone mineral content offers a logical standard. However, individual excised bones are often used to minimize costs and expedite project completion. Justification for selection of a

specific bone is often vague and dependent on ease of collection rather than documented accuracy of the individual bone as a reliable predictor. Variable responses in nutrient bioavailability among experiments may be explained, in part, by the bone chosen for assessment. The pig fibula has been proposed as the preferred bone based on ease of collection and processing. The current objective was to compare accuracy of fibula and femur ash as predictors of whole body bone mineral content in growing pigs. Data were collected from 96 pigs (~25 kg final wt.) that were fed diets with various concentrations and sources of Ca and P over a 27 d trial. Pigs were scanned with a GE Lunar Prodigy DXA instrument (software version 10.10.038, adult standard scan mode) to determine whole body bone mineral content (BMC, g). Pigs were killed and the ash content of the fibula and femur from the right leg were determined. Regression analysis were used to predict whole body BMC from either fibula or femur ash. Femur ash predicted BMC ($R^2 = 0.94$) and showed minimal deviation in residual patterns or slope of responses across BMC values. However, fibula ash had a lower prediction of BMC ($R^2 = 0.78$) and deviated with BMC magnitude. Fibula ash over estimated BMC at low values, but under estimated BMC values at high values. The pattern in fibula ash allowed a reduction in the ability to detect differences between mineral sources and allowed a bias in detection of differences in relative bioavailability compared with estimates based on femur ash or whole animal BMC. Thus, femurs provide a more accurate assessment of mineral status in young growing pigs than fibulas.

Key Words: ash, bioavailability, DXA

558 Effect of supplemented mined humate on growth, loin quality, and pathological status of liver and kidneys in pigs. C. M. Ballou^{*}, Y. Zhao, Y. B. Kim, A. C. Chaytor, and S. W. Kim, *North Carolina State University, Raleigh*.

This study used mined humate from Utah (LiveEarth Prodcuts, Emery, UT) to test its supplemental effect on growth, loin quality, and liver health of pigs. Forty-eight pigs at 24.0 ± 0.8 kg BW were allotted to 2 treatments, 6 replicates per treatment, and 4 pigs per pen. Treatments were CON (control) and HA (0.5% humate). Pigs were fed the assigned experimental diets ad libitum for 6 wks. Body weight and feed intake were measured biweekly. At the end of wk 6, 2 pigs representing the average BW of each pen were selected and bled (8 mL) through jugular vein at 0900. After bleeding, pigs were euthanized to collect loin, liver, and kidney. Loin samples were used for quality measures including loin eye area, loin color, marbling score, and fat content. Serum samples were used to measure indicators of liver function including levels of creatinine, total protein, albumin, total bilirubin, alkaline phosphatase alanine aminotransferase, aspartate aminotransferase, and cholesterol in serum. Liver and kidneys were weighed and tissue samples were used for histological evaluation for tissue damage measurements including vacuolation, necrosis, inflammation, megakaryosis, fibrosis, and bile ductule hyperplasia. Pigs fed HA tended to grow faster ($P=0.054$, 917.2 vs. 868.5 g/d) than pigs fed CON during 6 wk feeding period. Feed intake (2.08 vs. 2.03 kg/d) and gain/feed ratio (0.44 vs. 0.43) did not differ between treatments. Loin eye area, loin color, marbling score, and loin fat content did not differ between treatments. Measurements for liver function test did not differ between treatments. Weight of liver and kidneys did not differ between treatments. Microscopic examinations of the livers and kidneys did not reveal any evidence of toxicity in both CON and HA groups. Collectively, dietary supplementation of mined humate at 0.5% can improve the growth of pigs but did not cause clinical or pathological changes in the liver and kidneys of pigs.

Key Words: growth, humate, pig

559 Effects of EcoCare® Feed on mineral excretion of pigs during the finishing phase. T. Walraven^{*1}, S. Carter¹, M. Lachmann¹, J. Bundy¹, J. Jarrett¹, and B. De Rodas², ¹Oklahoma State University, Stillwater, ²Land O'Lakes Purina Feed, Gray Summit, MO.

We have previously reported that EcoCare® Feed reduced N and P excretion of finishing pigs without affecting growth performance or carcass traits. To determine mineral excretion, eighty crossbred (D x (L x Y)) pigs (30 kg BW) were blocked by BW and sex, and randomly allotted to 1 of 2 dietary treatments. Pigs were housed in an environmentally-controlled building with 4 identical rooms (20 pigs/room, 2 rooms/trt). Each room contained a shallow pit, pull plug system. A fortified corn-soybean meal-based diet served as the control. The test diet (EcoCare®, EC) was similar to the control diet except that CP and available P were reduced (~2.6% units for CP; ~0.11% unit for avail. P) with additions of Lys, Thr, Met, EC Pak (containing phytase) and EC premix. To maintain similar Ca:P ratio, Ca content of the EC diet was reduced. The EC premix provided approximately 20% less Fe, Zn, Cu, and Mn than the mineral premix used in the control diet. All trace minerals were included in an inorganic form with the exception of Se. Pigs and feeders were weighed at each phase change, and pit volume was measured. Feed and pit samples were collected for mineral analysis. On d 0 and 122, 6 and 24 pigs (6/room), respectively, were ground to estimate initial and final body composition. There was no difference (P > 0.10) in initial or final ash or mineral concentration; thus, accretion was similar (P > 0.10) for pigs fed the control or EC diet. The intake of minerals was reduced (P < 0.05) for pigs fed EC. The excretion (g/d) of Ca (7.44 vs. 4.51), P (8.2 vs. 5.7), K (18.4 vs. 14.6), Mg (2.91 vs. 2.55), S (2.12 vs. 1.79), Fe (0.433 vs. 0.343), Zn (0.247 vs. 0.205), Cu (0.026 vs. 0.020), and Mn (0.080 vs. 0.064) was reduced (P < 0.05) for pigs fed EC compared to those fed the control. In general, the excretion of minerals as a % of intake was reduced (P < 0.05) for pigs fed EC. These results suggest that EcoCare® Feed reduced mineral excretion without affecting mineral accretion or growth performance.

Key Words: pigs, mineral, excretion

560 Effects of combining multiple dietary manipulations on growth performance and nutrient excretion of finishing pigs. T. Walraven^{*}, S. Carter, J. Jarrett, M. Bible, and H. J. Kim, Oklahoma State University, Stillwater.

Eighty-eight crossbred (D x (L x Y)) pigs (32 to 114 kg BW) were used to evaluate the effects of reducing dietary CP, Ca, and P with the additions of phytase and organic trace minerals on nutrient excretion during a 94-d finishing period. Pigs were stratified by sex, weight, ancestry and randomly allotted to 1 of 2 dietary treatments. Pigs were housed in an environmentally-controlled building with 4 identical rooms. Each room contained a shallow pit, pull plug system (22 pigs/room, 2 rooms/trt). Dietary treatments were fed in 4 dietary phases and consisted of a fortified corn-soybean meal diet and a Reduced Excretion (REx) diet. The REx diet had a 3% units decrease in CP with Lys, Thr, Met, and Trp added as needed and a reduction in available phosphorus of 0.10% with phytase inclusion. Also, in the REx diet, monocalcium P replaced dicalcium P and CaCl replaced 50% of the CaCO₃ in the control diet. Furthermore, organic sources of Fe, Zn, and Cu replaced inorganic sources of these minerals in the control diet. All diets within phase were formulated on a SID lysine basis (0.92, 0.79, 0.65, and 0.56% for Phases 1 to 4). Feed and slurry samples were collected weekly along with pig weights, feed intake, pit volume, and pH of slurry. Diet did not affect (P < 0.10) ADG (822 vs. 839 g), ADFI (2.27 vs. 2.28 kg), or G:F (0.365 vs. 0.367). Daily DM intakes were similar (P > 0.10), but N (57.8 vs. 47.4 g/d) and P (11.1 vs. 8.6 g/d) intakes were reduced (P < 0.05) for pigs fed REx. Also, slurry pH for pigs fed REx was reduced (7.4 vs. 6.8; P < 0.05). Slurry DM concentration was similar (P > 0.05), but slurry N (15.1 vs. 11.8%) and P (2.84 vs. 1.98%) tended to be reduced (P < 0.10) with REx. The daily excretion of DM, N (33.9 vs. 24.4 g) and P (6.8 vs. 4.5 g) for pigs fed REx was reduced (P < 0.05) compared to those fed the control. These results suggest that combining dietary manipulations is an effective method of reducing N and P excretion. *This project was supported by the National Pork Board.*

Key Words: pigs, diet, excretion

Physiology and Endocrinology: Impact of Gonadal Steroids on Brain Development and Function

561 Feedback and fitness: Consequences of non-classical estrogen receptor α signaling in the brain. J. E. Levine^{*}, Northwestern University, Evanston, IL.

Ovarian estrogens exert critically important actions in hypothalamic neurons to regulate ovulatory cyclicality, reproductive behaviors, and energy homeostasis. Estrogen receptor alpha (ER α) appears to mediate most of these effects, as disruption of ER signaling leads to infertility and metabolic syndrome. ER signaling mechanisms may include "classical genotropic" effects mediated by direct binding of receptor dimers to DNA, "non-classical genotropic" effects involving tethering of ERs to other transcription factors, and "non-classical non-genotropic" actions mediated by cytoplasmic ERs coupled to membrane-initiated signal transduction pathways. Our studies make use of novel ER mutant mouse models to ascertain the cellular mechanisms by which ER mediates E2 effects on these physiological and behavioral processes. We have utilized a novel mutant ER knock-in mouse model, which confers non-classical genotropic and non-genotropic signaling in the absence of classical signaling, to determine that non-classical ER signaling can convey E2 effects integral to homeostatic feedback control of reproductive hormone secretions, as well as E2 actions governing paracopulatory behavior and body weight regulation.

Key Words: estrogen, body weight, reproduction

562 Nongenomic actions of estrogens directly on the ovine pituitary facilitates LH secretion. T. Nett^{*1}, A. Arevalo-Arreguin¹, and T. Davis², ¹Colorado State University, Fort Collins, ²University of Idaho, Moscow.

In addition to the well known genomic actions of steroid hormones, there are nongenomic effects of these hormones that are only now being recognized. Our laboratory has investigated the nongenomic effects of estradiol (E) on secretion of LH in sheep. For these studies, we have employed E conjugated to either BSA (E-BSA) or to a novel 15 amino acid peptide (E-PEP), neither of which appear to induce classic genomic effects attributed to estradiol. Both compounds (as well as E) inhibited (P < 0.01) GnRH-induced secretion of LH from cultured ovine pituitary cells in a dose dependent manner. Each of the estrogens essentially abolished the LH secretion induced by 2 nM GnRH when present in the culture media at 20 nM or greater. Based on these data, ovariectomized (OVX) ewes were administered E or E-BSA and concentrations of LH were monitored for the next 24 hr. Both E and E-BSA resulted in a rapid decrease (<20 min, P < 0.01) in serum concentrations of LH. Interestingly, only E decreased secretion of FSH. Likewise, E, but not E-BSA stimulated an ovulatory-like surge of LH beginning approximately 12 hr after treatment. Although there was no ovulatory-like surge of LH in ewes treated with E-BSA, there was a slight increase in LH at the