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Appreciation is expressed to the Indiana Farm Bureau and their staff for hosting the 2013 Midwest Swine Nutrition Conference and providing the facilities for this function.

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Contents

Enriching People's Lives: A 2013 Report on the Importance of Animal Source Foods

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"Almost certainly, the first essential component of social justice is adequate food for all mankind."
—Norman Borlaug, 1970 Nobel Peace Prize winner

Overview

Food security has far-reaching ramifications for health, productivity and quality of life at the individual, community and global levels. Historically, as societies emerge from poverty, they begin to consume animal source foods. Meat, milk and eggs provide nutrients critical for brain and muscle development and disease prevention. In developed countries, where obesity is an issue, animal source foods offer a healthy alternative to less nutrient-dense choices. In both settings, animal source foods help improve health and productivity, ultimately enriching people's lives.

Need for Balanced Diets

Lack of calories and essential nutrients hinders mental and physical development, weakens the immune system and increases mortality, especially among women and children.[1] When people are unhealthy, societies fall short of their full potential, which hinders national development for generations.

Food Security Defined

The World Health Organization (WHO) declares food security is built on three pillars:

- **Food availability:** sufficient quantities of food available on a consistent basis.
- **Food access:** having sufficient resources to obtain appropriate foods for a nutritious diet.
- **Food use:** appropriate use-based on knowledge of basic nutrition and care, sufficient water and sanitation (see Figure 1).

Food security isn't just about those that don't obtain enough calories each day. There's a spectrum of food security with animal source foods delivering important benefits at all levels.

- **People in developing countries,** living on less than \$2 a day and malnourished. *Animal source foods improve brain, muscle and skeletal development.*
- **People in emerging markets,** living on \$3-\$10 a day and moving into the middle class. *Animal source foods help diversity their diets.*
- **People in developed countries,** ranging from the low-income, experiencing hidden hunger, to the affluent, who need to maintain proper weight. *Animal source foods lessen chronic disease risk and obesity* (see Figure 2).

The Importance of Protein

According to the 2010 Dietary Guidelines Advisory Committee report, animal products provide a greater quantity and quality of protein than plant products.[2] Meat, milk and eggs also provide bioavailable micronutrients such as iron, zinc, calcium, and vitamins A, D and B_{12}. These nutrients are often found to be deficient in malnourished people.[3]

Consider these benefits of animal source foods:

- Babies whose mothers receive enough iron during pregnancy are born with iron stores that help mitigate the risks of poorer cognitive, motor, social-emotional and neurophysiologic development in the short- and long-term. WHO estimates that eradicating iron deficiency could improve national productivity levels by as much as 20 percent.[4]
- In a landmark observational study performed in Kenya, adding animal source foods to the diet—and, in turn, increasing intake of iron, zinc, vitamin B_{12} and riboflavin—was associated with better growth, cognitive performance, motor development and activity in 1st and 2nd grade children.[5] (See Figure 3.)

- High-quality dietary protein contributes to weight loss and maintains a "balanced diet".
    - Protein may be the single most important nutrient influencing metabolic rate.[6]
    - Dietary protein positively impacts several critical body-weight influencers,[7,8] including satiety,[9] and body composition (see Figure 3).

Ultimately, the often-overlooked impact of animal source foods provides the high-quality protein and key micronutrients essential for physical and cognitive growth and well-being. Meat, milk and eggs not only help alleviate hunger, but meet nutritional needs so both people and society can develop and thrive.

Achieving Food Security

Improving nutrition increases productivity, stimulates economic growth and strengthens society as a whole. To reap these benefits, sufficient nutrient-rich foods must be available to meet the needs of the more than 7 billion people worldwide and an estimated 9 billion by the year 2050.[10]

Innovations in animal production have made it possible to produce more animal source foods from fewer animals, which means we can provide protein nutrients to more people without increasing the use of land, feed, and other resources. Advancements in animal nutrition and health help animals use energy needed to produce meat, milk and eggs at their peak level. As a result, the nutritional needs of more people can be met with the same resources.

Achieving food security demands a global vision and long-term strategy. People are hungry for access to the quality, affordable nutrients available in foods such as meat, milk and eggs. But this access will not come without political, economic and social change. This change requires:

- Broadening understanding of nutritional needs and the role of animal source foods.
- Empowering collaboration that ensures choice, which increases access to and affordability of animal source foods.
- Advancing innovation that supports sustainable production of animal source foods.

Get involved in the food security conversation. Help tell the story of the value poultry, eggs and other animal source foods provide to improve individuals, communities and society as a whole.

For more information about the important role of animal source foods in enriching people's lives, visit Elanco.com, follow @Elanco on Twitter or contact Colleen Dekker at 317-276-4076 for a copy of a full white paper on this topic.

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Figure 1

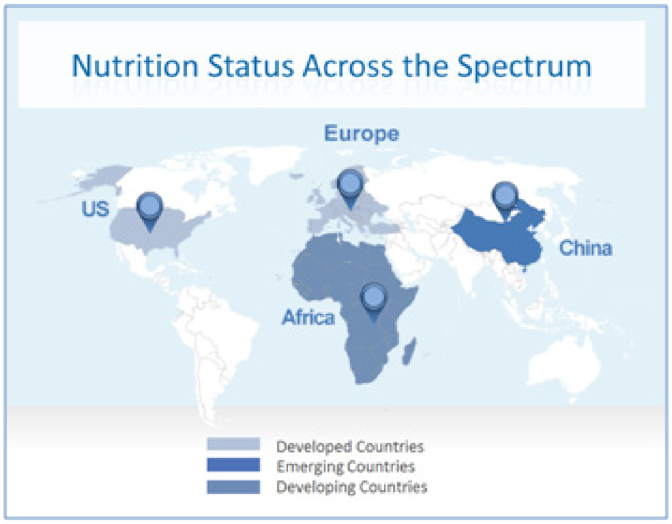
Food Security is Built on Three Pillars

Availability

Access

Utilization

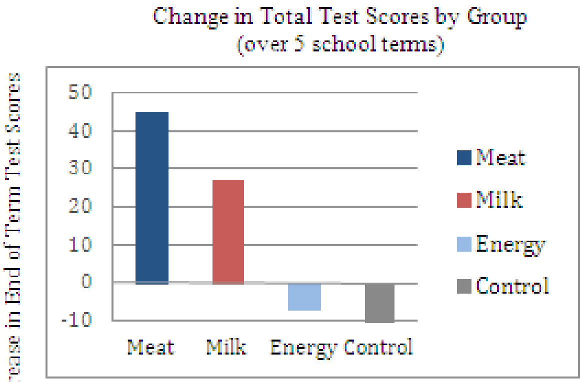
Figure 2



Nutrition Status Across the Spectrum

▨ Developed Countries
▨ Emerging Countries
▨ Developing Countries

Figure 3



Change in Total Test Scores by Group
(over 5 school terms)

Neumann C, Bwibo N, Gewa C. Animal Source Foods Promote Cognitive Function, School Performance, Increased Muscle Growth and Activity in Kenyan Children: From Observational to Evidence-Based Findings. Global Livestock CRSP. June 10, 2009.

Intrauterine Growth Restriction and Arginine Supplementation of Piglets

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Summary

Large profit losses in the swine industry can be attributed to morbidity and mortality of piglets before weaning, especially in the low birth weight (LBW) piglet. Recent evidence suggests sow's milk contains insufficient concentrations of arginine to support optimal growth and health of piglets. Thus, our objective was to assess global metabolomic profiles and the potential for arginine supplementation to promote growth of LBW (≤ 0.9 kg body weight) and average birth weight (ABW, 1.3-1.5 kg body weight) piglets. Piglets were selected in littermate pairs at processing to receive either L-arginine (Arg) or an isonitrogenous control (L-alanine, Ala), weighed daily to assess growth rate, and blood was collected at approximately 16 days of age. In terms of growth, LBW and ABW piglets supplemented with Arg weighed 22.3% and 12.7% less, respectively, at day 16 compared with Ala-supplemented piglets of the same birth weight group. Overall, differences ($P < 0.05$) were observed among treatments for metabolic pathways involving energy (i.e., TCA cycle intermediates), amino acids, nucleotides, and fatty acids. Increased nucleotide turnover, indicating an increase in DNA damage and cell death, was particularly noted in the LBW piglet. However, Arg supplementation reduced these effects to levels comparable to those observed in ABW piglets. Moreover, changes in glucose metabolism suggested the ability to extract energy from dietary sources may have been compromised in the LBW piglet, but these effects were partially recovered by Arg supplementation. We conclude that a reduction in the growth potential of LBW piglets may be associated with alterations in multiple metabolic pathways, and further reduction due to Arg supplementation may have resulted from perturbations in multiple metabolic pathways.

Introduction

Intrauterine growth restriction is often used to describe impaired growth and development of the mammalian fetus or its organs during pregnancy (Wu et al., 2006). In practical terms, a piglet that has experienced prenatal growth restriction is born with a low birth weight (**LBW**) relative to its gestational age. This condition, often referred to as the 'runt' piglet, may affect 15-20% of all pigs born each year in US swine industry (Wu et al., 2006). Herein, we use the term LBW to describe a healthy piglet that is born with a small stature relative to its littermates, as based on objective birth weight criteria. Importantly, LBW piglets still remain at risk for above-normal rates of morbidity and mortality during the pre-weaning period due mainly to underdeveloped function of the gastrointestinal tract (Wang et al., 2008; Wang et al., 2005). Overall, large profit losses in the US swine industry have been attributed to LBW piglets, as even those pigs that survive the pre-weaning period exhibit an 8% reduction in growth rate and require an additional 12 days to reach 102 kg of live weight as compared with heavier pigs (Gondret et al., 2005). Thus, characterizing metabolic changes in the LBW piglet is warranted, especially in combination with postnatal interventions that may improve the LBW condition.

Arginine (**Arg**) is not only a building block for proteins, serving as an essential amino acid for neonatal animals, but also a precursor for the synthesis of important metabolites (e.g., creatine, polyamines, and nitric oxide) related to growth, vasodilation, and immune responses (Wu and Morris, 1998). Whereas piglets have a high physiological requirement for Arg (Flynn et al., 2002; Visek, 1986), sow's milk has been reported to contain inadequate concentrations of this amino acid for optimal growth and metabolic function of the piglet (Wu and Knabe, 1994; Wu et al., 2004). The importance of Arg supplementation for piglets, during both the prenatal and postnatal periods, has been extensively studied. Thus, our aims were to: (1) define objective birth weight criteria for the low birth weight and average birth weight piglet based on recent farrowing records, and (2) determine whether daily oral dosing of Arg alters growth and global metabolite profiles of pre-weaning piglets in a commercially-applicable setting.

Experimental Procedures

Characterization of Piglet Birth Weights

Farrowing records from the University of Illinois Imported Swine Research Laboratory farm were used to create a database of birth weights from 8,207 piglets farrowed between July 8, 2008 and October 21, 2011. Three separate data sets were created based on ultimate disposition of piglets, as follows:

1. All piglets born
2. Piglets denoted as low-viability plus piglets ultimately weaned
3. Piglets ultimately weaned

Litter data were excluded if meeting any of several criteria, including: 1) litter size smaller than 5 piglets, 2) dam of an under-represented breed, or 3) less than 30% of pigs in a litter surviving through weaning (approximately 21 days of age). Any stillborn or pigs that were euthanized for reasons other than low birth weight were also excluded from the final data set.

Experimental Treatment of Piglets

Thirty-two piglets were selected in littermate pairs within 24 h of birth based on body weight; 15 separate litters were used. The LBW piglet was defined as weighing less than 0.9 kg, whereas the average birth weight (**ABW**) piglet was defined as weighing 1.3-1.5 kg, as determined from the population characterization described above. A litter was selected only if it contained both a LBW and an ABW piglet of the same sex. Both the LBW and ABW groups contained equal numbers of gilts and barrows. Within each birth weight group, piglets were assigned to an amino acid solution of either L-arginine (treatment, 145.0 mg/kg body weight) or an isonitrogenous concentration of L-alanine (control, 245.5 mg/kg body weight). In total, there were 4 treatment groups (n = 8 piglets per treatment), including: ABW-Ala, ABW-Arg, LBW-Ala, and LBW-Arg.

Amino acid solutions were prepared fresh once per week by dissolving L-arginine•HCl or L-alanine in sterile saline and buffering to a common pH to prevent gastrointestinal distress; solutions were always stored at 4°C. For dosing, individual piglets were removed from the sow and weighed each morning to calculate the total daily volume of amino acid solution to be dispensed. Every 12 hours, piglets received half of the total daily volume of assigned amino acid solution by oral gavage and were immediately returned to the sow. Gavage of LBW and ABW piglets began at processing (approximately 1 day of age) and continued through the end of the study (16-17 days of age). At study termination, blood was collected from individual piglets into evacuated tubes containing EDTA as an anticoagulant and stored at -80°C pending analysis.

Biochemical Profiling

The metabolome is defined as the complete set of small-molecule metabolites present in any biological sample. Thus, the simultaneous analysis and interpretation of metabolic effects due to treatment is referred to as metabolomics. In our study, piglet blood samples were submitted to a commercial laboratory (Metabolon, Inc.) for global metabolite profiling. At the time of analysis, EDTA-treated blood samples were prepared using a proprietary series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small biomolecules. The resulting extract was divided into two fractions for analysis on either the gas chromatography-mass spectrometry (GC/MS) or liquid chromatography-mass spectrometry (LC/MS) platforms. Thorough quality control steps were taken to ensure integrity of final data, including normalization of data when sample analyses occurred over multiple days. Semi-quantitative methods were used to assess treatment effects on individual metabolites, and data were expressed as fold-changes relative to the control calibrator group (ABW-Ala).

Statistical Analyses

Various statistical models were tested, and the best model (i.e., that with the smallest AIC fit statistic) for characterizing piglet birth weights included the fixed effects of sire breed, dam breed, dam parity (with all 2- and 3-way interactions) and piglet sex, along with the random effects of record year and litter. Studentized residuals were generated to detect outliers, and any observation with an absolute studentized residual greater than 3 was removed from the data set. The process of detecting and removing outliers was repeated until the data sets stabilized, and ultimately, descriptive statistics were generated.

Piglet body weight gain data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute). The statistical model included the fixed effects of birth weight (LBW vs. ABW), amino acid solution (Arg vs. Ala), as well as their interaction. Longitudinal piglet body weight data were analyzed by a repeated measures ANOVA using the same statistical model described above, with day serving as the repeated measure. Piglet sex was excluded from both models when no main or interactive effects of this variable were found. Statistical differences were considered significant at $P < 0.05$.

Following log transformation, all metabolite data were subjected to a 2-way ANOVA using the open-source statistical program 'R.' The statistical model included the fixed effects of birth weight (LBW vs. ABW) and amino acid solution (Arg vs. Ala) as well as their interaction. Statistical differences were considered significant at $P < 0.05$.

Results and Discussion

Descriptive statistics and a histogram of piglet birth weights are shown in Figure 1. Based on the lower 10th percentile of the dataset, the LBW piglet was defined as weighing less than 0.9 kg, while the ABW piglet was defined as weighing 1.3-1.5 kg at birth; these criteria are in good agreement with published values (Hegarty and Allen, 1978). For the dosing study, final body weight of ABW piglets was greater ($P < 0.01$) than LBW pigs (Table 1). Similarly, ADG was greater ($P < 0.01$) for ABW piglets (204 g/d) than for LBW piglets (129 g/d). Rates of body weight gain for the LBW and ABW piglet were similar to previously reported values recorded over a 21-d period (Ramsay et al., 2010).

In general, Arg supplementation reduced ($P < 0.01$) final body weight compared with Ala supplementation, and Arg-supplemented pigs had lower ($P < 0.01$) ADG than Ala-supplemented piglets. Growth depression due to Arg supplementation was unexpected as this amino acid has been shown to induce protein synthesis in *in vitro* studies using porcine cells (Bauchart-Thevret et al., 2010). Moreover, others have reported increased piglet growth when milk replacer formulas were supplemented with Arg (Kim and Wu, 2004). Importantly, the daily Ala and Arg dosages supplied to piglets in our study were based on Arg concentrations found in sow's milk as suggested by Wu et al. (2004). Arginine concentration in sow's milk, however, may vary from 7.7 to 12.4 g/kg (Ball et al., 2007). Unfortunately, Arg concentration in sow's milk used in the present study was not measured, which raises the possibility that depression in growth performance observed herein may have resulted of an imbalance in the dietary amino acid profile that pigs were receiving relative to specific amino acid requirements within each birth weight group. Nevertheless, this indicates that the potential benefits of Arg supplementation to newborn piglets may depend not only on nutritive composition of sow's milk, but also on the milk intake by the piglet. Interestingly, the negative effects of daily Arg supplementation became evident within birth weight group starting around day 11 of the study (Figure 2).

Global metabolic effects of birth weight group and amino acid supplementation were also noted in this study. Per the experimental design, we observed a main effect of Arg supplementation for changes in blood Arg, with LBW piglets exhibiting a greater ($P < 0.05$) fold-change in blood Arg compared with ABW piglets (Figure 3). Fold-changes in other important metabolites are shown in Table 2. Interactive effects ($P < 0.05$) between birth weight group and oral amino acid solution were observed for metabolite categories involving amino acids, energy, lipids, and nucleotides. In ABW piglets, blood glutamate was increased ($P < 0.05$) due to Arg-supplementation, which makes sense considering Arg is primarily synthesized from glutamine and glutamate in the piglet (Ball et al., 2007). Thus, Arg supplementation in ABW piglets may have effectively spared glutamate, thereby requiring greater nitrogen handling in these larger piglets. For LBW piglets, however, Arg-supplementation caused a differential response, with these lighter piglets exhibiting a decrease ($P < 0.05$) in blood glutamate compared with the Ala-supplemented LBW piglets. Furthermore, the fold-change of blood urea was greater in LBW piglets than in ABW piglets (Figure 4), suggesting overall nitrogen handling was different in the LBW piglet. We speculate that amino acid requirements of the LBW piglet are lower than for the ABW piglet, likely due to less lean tissue in the body and lower rates of muscle protein synthesis, which ultimately results in reduced muscle growth and lower body weight gain (Foxcroft et al., 2006). In this scenario, amino acids supplied in excess of physiological requirements would be deaminated to ultimately form urea, and this theory is corroborated by the greater fold-change in blood urea of LBW piglets that were supplemented with Arg.

Changes in metabolites associated with nitrogen handling may also be related to increases in circulating concentrations of nucleotides, the building blocks of RNA and DNA. This observation is in agreement with Yamauchi et al. (2002) who observed that supplementation with Arg decreased the flux of glutamine to Arg in immortalized human intestinal cells *in vitro*, ultimately resulting in enhanced nucleotide synthesis. Our results support this observation as Arg-supplemented ABW piglets also exhibited a significant increase in blood nucleotides (i.e., cytidine, guanosine, and uracil). Fold-changes in blood nucleotides were higher ($P < 0.05$) in ABW piglets supplemented with Arg, but lower in Arg-supplemented LBW piglets compared with Ala-supplemented piglets of the same birth weight group. One possible reason for this observation is that nitric oxide has potential to induce cell apoptosis, thus increasing nucleotide turnover and circulating nucleotide concentrations as observed for Arg-supplemented ABW piglets.

The fact that we did not observe a similar pattern in the LBW piglets is not clearly understood and requires further investigation.

In the piglet dosing study, we also observed an overall decrease ($P < 0.05$) in fold-changes of glucose, malate, and α-ketoglutarate when both piglet birth weight groups were supplemented with Arg, although this reduction was greater in LBW piglets compared with ABW piglets. Arginine is used to synthesize nitric oxide, which plays an important role in energy metabolism, and this gaseous molecule is known to stimulate expression of GLUT-4, a key glucose transporter, thus increasing glucose uptake by cells (Jobgen et al., 2006). Consequently, glucose oxidation is expected to increase due to higher rates of glycolysis and flux through the Krebs cycle. As a result, blood metabolites involved in the Krebs cycle, such as malate and α-ketoglutarate, would be expected to decrease. Again, changes due to the combined effects of birth weight group and amino acid supplementation suggest the Arg-supplemented LBW piglet exhibited generalized metabolic disturbances.

In summary, our results indicate that Arg supplementation to LBW piglets did not improve growth performance as previously thought. In fact, Arg supplementation reduced growth of piglets in both birth weight groups, which may reflect a dietary imbalance of amino acid intake. From the metabolomics analyses, we concluded that oral dosing of Arg altered the global blood metabolite profile of piglets, mainly involving metabolites associated with amino acid, energy, lipid, and nucleotide pathways. Whereas Arg supplementation was able to correct some of the metabolite abnormalities observed in LBW piglets, these effects appeared to be independent of growth mechanisms as piglets of both birth weight groups were negatively impacted by daily dosing of this amino acid. Considering piglets in this study received amino acid supplements while remaining on the sow in a commercially-applicable setting, these results provide industry-relevant information to better understand the implications of Arg supplementation in preweanling piglets of differing birth weight categories.

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Table 1. Body weight and growth performance of piglets[1].

Parameter	Low Birth Weight		Average Birth Weight		SE	P-values		
	Alanine	Arginine	Alanine	Arginine		Birth Weight	Amino Acid	Interaction
Initial body weight, kg	0.80	0.80	1.43	1.38	0.042	0.001	0.401	0.378
Final body weight, kg	3.22	2.50	4.98	4.35	0.204	0.001	0.003	0.830
Average daily gain, g	151.3	106.3	221.6	185.5	12.22	0.001	0.003	0.718

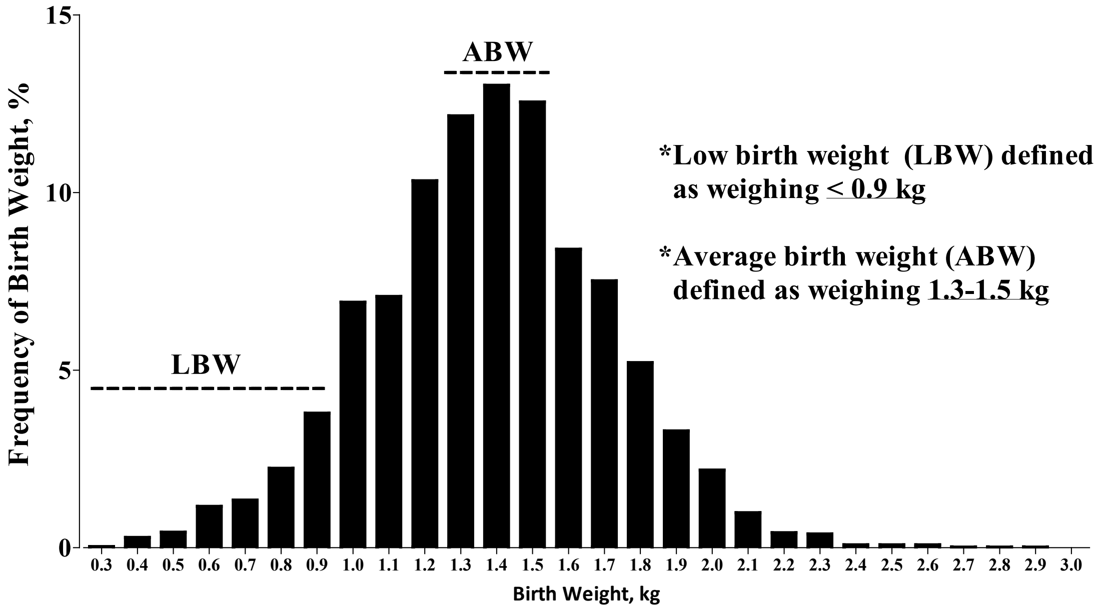
[1] n = 8 piglets per treatment; piglets representing low- and average-birth weight categories received daily oral doses of 245.5 mg L-arginine/kg body weight (treatment) or 145.0 mg L-alanine/kg body weight (control; isonitrogenous to L-arginine dose) throughout the study.

Table 2. Fold-change in blood metabolites from piglets[1].

Metabolic Pathway	Metabolite	ABW		LBW		SD	P-values		
		Ala	Arg	Ala	Arg		Birth Weight	Amino Acid	Interaction
Amino acids	Dimethylarginine	1.00	0.92	1.18	0.86	0.19	0.34	< 0.01	0.04
Amino acids	Glutamate	1.00	1.65	1.68	1.34	0.80	0.29	0.65	0.04
Amino acids	Glutathione	1.00	2.61	1.30	0.97	1.74	0.69	0.79	0.03
Carbohydrate	Glucose	1.00	1.02	1.16	0.95	0.14	0.48	0.05	0.02
Energy	Malate	1.00	1.25	2.61	1.27	1.05	0.03	0.15	0.02
Energy	α-ketoglutarate	1.00	0.69	1.13	0.85	0.34	0.23	< 0.01	0.04
Lipids	Carnitine	1.00	1.14	1.25	0.82	0.31	0.23	< 0.01	0.73
Lipids	Glycerol-3-phosphate	1.00	0.79	1.17	0.53	0.41	0.69	0.07	0.01
Lipids	Octanoylcarnitine	1.00	0.40	1.13	0.55	0.45	0.25	< 0.01	0.57
Lipids	Stearate	1.00	0.78	1.12	1.20	0.38	0.03	0.67	0.26
Nucleotide	Cytidine	1.00	1.23	1.50	0.98	0.38	0.35	0.25	< 0.01
Nucleotide	Guanosine	1.00	2.29	2.95	1.28	1.34	0.15	0.5	< 0.01
Nucleotide	Uracil	1.00	1.75	2.47	0.98	1.02	0.38	0.31	< 0.01

[1] n = 8 piglets per treatment; piglets representing low- and average-birth weight groups received daily oral doses of 245.5 mg L-arginine/kg body weight (treatment) or 145.0 mg L-alanine/kg body weight (control; isonitrogenous to L-arginine dose) throughout the study. Values represent fold-changes in scaled intensity estimates relative to the calibrator group (average birth weight piglets receiving daily oral dose of L-alanine).

Figure 1. Descriptive statistics of piglet birth weights.



*Low birth weight (LBW) defined as weighing < 0.9 kg

*Average birth weight (ABW) defined as weighing 1.3-1.5 kg

Data Set	n	Mean, kg	SD	5th Percentile	10th Percentile	Median, kg
All piglets born	7263	1.37	0.360	0.79	0.93	1.36
Low-viability plus weaned	6178	1.38	0.332	0.86	0.98	1.38
Weaned only	5660	1.43	0.328	0.95	1.04	1.40

[1] Weaned piglets were considered healthy and productive animals, while those categorized as low -viability were euthanized before the typical weaning age of 21 d.

Figure 2. Longitudinal body weights of piglets.

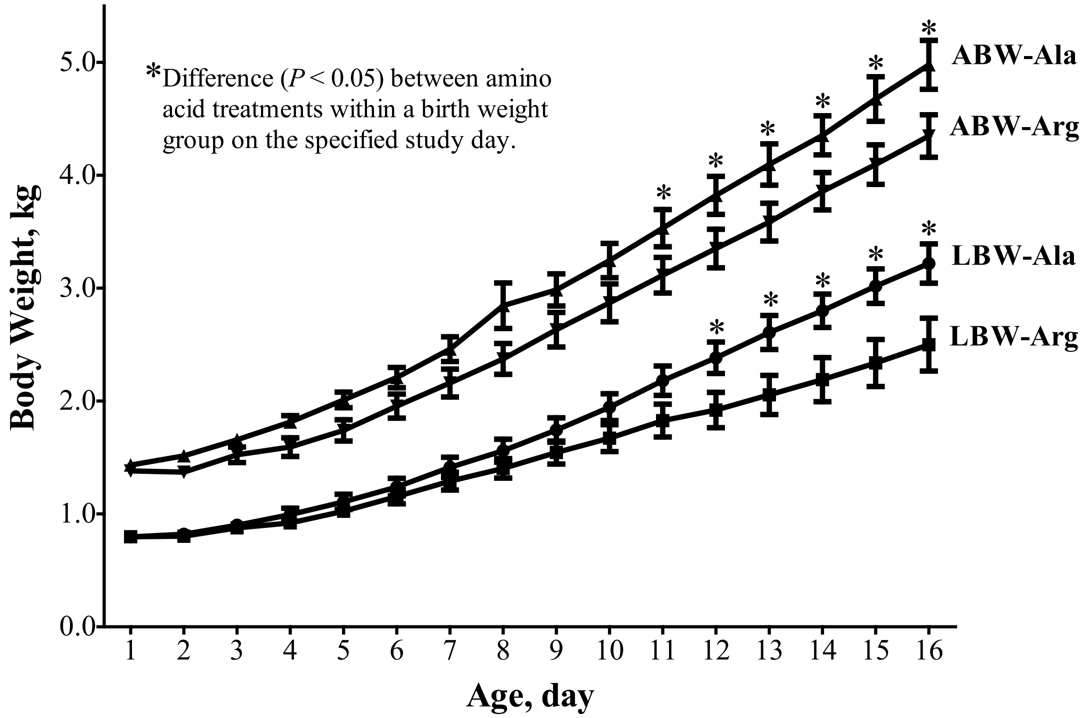


Figure 3. Fold-change in blood arginine from piglets.

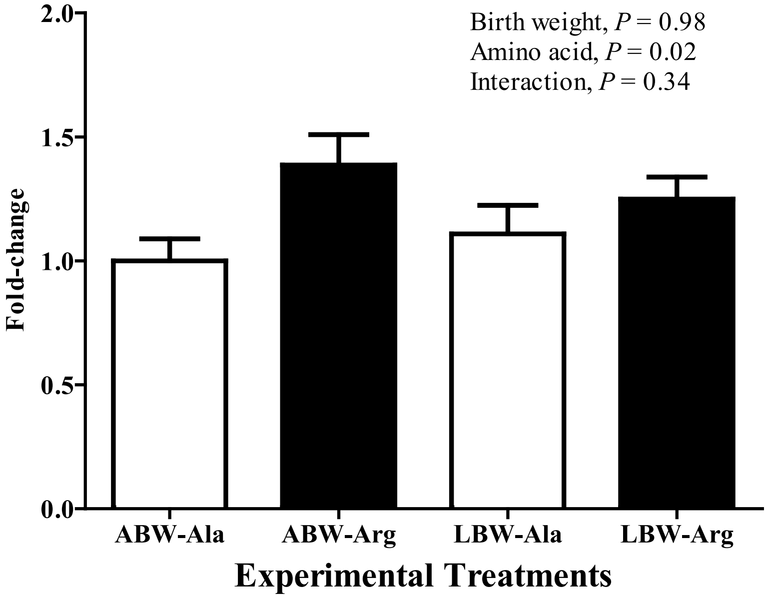
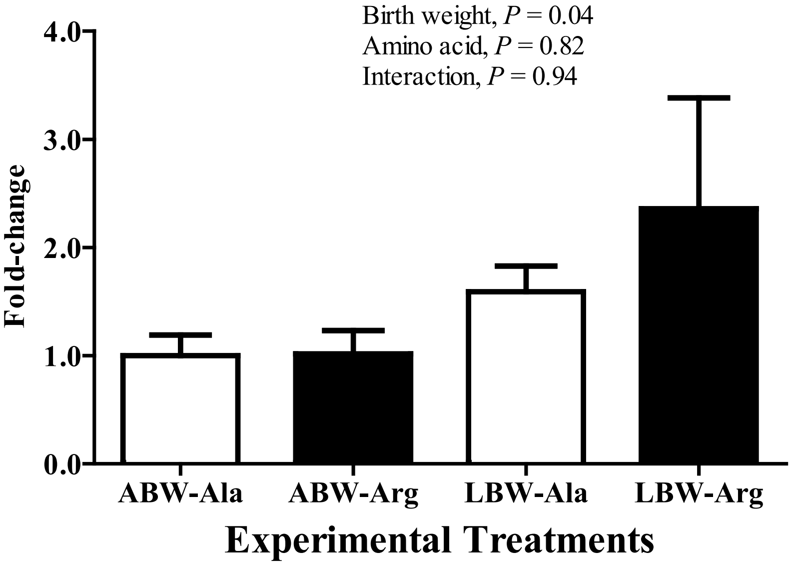


Figure 4. Fold-change in blood urea from piglets.



Stage of Gestation and Sow Age have Dramatic Effects on Amino Acid Requirements

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Summary

We have conducted a number of experiments in which we directly measured the requirements of sows during gestation for energy, using indirect calorimetry, and for amino acids, using indicator amino acid oxidation. When we began this research there were no good models to predict pregnant sow amino acid and energy requirements. There are now several models that are based on the growth of body components, i.e. maternal body and products of conception during gestation. These models add up the amino acid and energy deposition in these body components, together with estimates of maintenance, and then apply estimates of the efficiency of amino acid and energy utilization to calculate requirements. A key output of these models is that the requirements for amino acids and energy are dramatically greater in late than early pregnancy, which is in agreement with our empirical studies specifically designed to determine the effects of stage of pregnancy and age of sows on amino acid and energy requirements. However, there is disagreement regarding the magnitude of requirement changes during late gestation and between gilts and sows, indicating that some of the assumptions used in the models may need reconsideration and that additional research is necessary. For practical application, parity segregated phase feeding is ideal to feed all sows to their individual needs, and thus achieve optimal performance and economic return from both sows and offspring.

Introduction

The recommendations for feeding pregnant sows have recently been revised (NRC 2012). The new recommendations are for greater protein (amino acid) and energy intake during late gestation (LG) compared to early gestation (EG). These revised recommendations have been based on growth models, e.g. GfE (2008), Kim et al. (2009) and NRC (2012). In addition, our empirical results (Moehn et al., 2011) support the general conclusions drawn in the growth models. However, there are significant differences both among the models and when compared to our observed data. This paper will discuss the major concepts of sow feeding during gestation, describe our recent results, and compare agreements and differences between the NRC (2012) model and our empirical data. Finally, the application and implications for commercial sow feeding will be considered.

Concepts in Gestation Feeding

Nutrient and energy requirements during gestation were previously considered to be constant (NRC 1998). However, practical experience with feeding prolific sows showed that it was necessary to increase feed and nutrient intake during late gestation to maintain performance (Shelton et al, 2009, Soto et al 2011).

Feeding a constant level of amino acid and energy during gestation has always been metabolically incorrect because it assumes an equal distribution of nutrient demand throughout gestation, which is untrue. The simplistic approach of single diets for sows was universally applied because it was easy to manage and the innate adaptability of the sow often enabled her to overcome our deficient feeding paradigm. However, sow productivity began to increase dramatically about 12 years ago and with this increased productivity the sow has become less able to overcome the deficiencies of a constant feeding program during gestation.

The metabolic focus of the sow changes during gestation, from the recovery of maternal body tissue following weaning, to the synthesis of fetal tissue in late gestation. Fetal weight, fetal protein content and mammary protein content increase 5-, 18- and 27-fold, respectively, in the last 45 d of gestation (McPherson et al., 2004; Ji et al., 2006). These dramatic increases in fetal weight and protein gain indicate that the requirement for amino acids must be greater in late gestation compared to early gestation. Similarly, the sows' maternal growth rate decreases with age, becoming virtually zero in adult animals, so that mature sows have lower requirements than gilts that are still growing vigorously. Ignoring these dynamics by applying a single phase feeding program will lead to overfeeding during early gestation and underfeeding during late gestation. Overfeeding in early gestation results in a waste of feed and money, while underfeeding in late gestation leads to sows entering lactation in a severe catabolic state.

Experimentally Determined Requirements of Pregnant Sows

Very few amino acid requirement studies of pregnant sows have been performed in the last 10 years. Srichana (2006) used the nitrogen balance technique to determine the lysine requirement of gilts in early, mid and late pregnancy. Zhong et al. (2009) reported the true ileal digestible lysine requirement in gilts up to day 84 of pregnancy as 0.69% in a diet containing 12.5 MJ ME. In contrast, Dourmad and Etienne (2002) did not report differences in lysine and threonine requirements in four consecutive nitrogen balance periods in pregnant sows.

The experimental data from our research group include the requirements in early and late gestation for lysine (Samuel et al., 2012), threonine (Levesque et al., 2011), isoleucine (Franco et al., 2013) and tryptophan (Moehn et al., 2012). In these experiments, each sow received each of six test diets in both early and late gestation. Feed allowance was kept constant throughout gestation. Amino acid requirements were determined using the indicator amino acid oxidation technique simultaneously with indirect calorimetry to measure energy expenditure. The performance of the sows used in these experiments (Table 1) show that these were highly productive sows with weight gains, litter size and litter weights comparable to good commercial production.

A summary of recent research on sow amino acid requirements (Table 2) shows that requirements are dramatically different during late compared to early gestation and that requirements decline substantially as parity increases. Srichana (2006) found no difference in the lysine requirement of 15.0 g/d between early and mid gestation, but reported an increased requirement of 20.0 g/d in late gestation. Samuel et al. (2010) showed that the total lysine requirement of 2nd parity sows was 13.1 g/d and 18.7 g/d in early and late gestation, respectively. For 3rd parity sows, the dietary total lysine requirement was 8.2 g/d and 13.0 g/d for early and late gestation, respectively (Samuel et al., 2010). Levesque et al. (2011) found that 2nd parity sows required 7.2 g/d total threonine in early gestation (day 35 to 53) and 13.6 g/d threonine in late gestation (day 92 to 110), based on indicator amino acid oxidation. In multiparous sows (Levesque et al., 2011), the total threonine requirement was more than doubled from 5.0 g/d in early gestation to 12.3 g/d in the last third of gestation. The tryptophan requirement of 2nd parity sows increased from 1.7 g/d to 2.6 g/d from early to late gestation. The isoleucine requirement of 4th parity sows increased from 3.6 g/d to 9.6 g/d from early to late gestation.

Comparison of Determined Requirement Values to Model Estimates

We compared our experimentally determined requirements to the estimates produced by 2 mathematical models (Table 3). Although there is agreement among the recommendations that requirements are greater in late than early pregnancy and that requirements decline as parity increases, there are some significant and important differences. In early pregnancy, our measured requirement values are lower ($P = 0.04$) than those proposed by NRC (2012), while those modelled by GfE (2008) were intermediate (Table 3). In late pregnancy, our empirical values were similar to those modelled by GfE (2008), and both these sets of recommendations were greater ($P < 0.1$) than the proposed values by NRC (2012). This is despite the fact that NRC (2012) and GfE (2008) based their amino acid requirements on increased energy intake in late pregnancy, while our experiments were conducted with a constant feed allowance.

Our measured requirements and the requirements modelled using the NRC (2012) program agree reasonably well for lysine and tryptophan, but less so for threonine and isoleucine. In addition, the modelled (NRC, 2012) and determined requirement values for lysine and tryptophan were similar to the recommendations by GfE (2008) in both early and late gestation (Table 3). For threonine, the late gestation values were similar for all three recommendations, while in early gestation, our empirical values and the recommendations of GfE (2008) were lower ($P < 0.1$) than the recommendations by NRC (2012). Large differences are evident in the isoleucine requirement of adult sows, ranging from 1.8 to 5.0 g/d in early and from 4.4 to 9.7 g/d in late gestation.

The NRC (2012) model was reviewed at this conference last year (DeLange, 2012). Although this model is a vast improvement over the previous versions (NRC 1998) we believe that it still has some shortcomings.

To estimate amino acid requirements, the NRC (2012) model identified six discreet sow body pools, for which weight and protein content are calculated on a day-by day basis. Thus, daily growth of each pool can be calculated for any user-defined period in pregnancy. For each of the pools, as well as for maintenance, its own specific amino acid pattern is applied so that retention of amino acids can be estimated as the sum of all pools. The protein pools include (see NRC, 2012, Figure 8.5): conceptus (i.e. uterus, placenta and fluids), mammary and foetal protein, and maternal protein pools. The foetal and mammary protein pools increase exponentially with a sharp increase in the third trimester of pregnancy, and contribute most to the increase in amino acid and energy requirements in late gestation.

Maternal protein growth was separated into energy-dependent plus time-dependent protein deposition. The time-dependent protein gain was defined as 'whole-body nitrogen retention that could not be associated with energy intake or reproductive tissues' (NRC, 2012), and thus is the source of our greatest concern. This means that the sum of energy-dependent protein deposition and conceptus protein deposition was less than the whole-body protein deposition observed in a range of experiments, and thus a correction factor had to be introduced. This approach has several problems. First, the need to include a correction factor to account for the unexplained changes in protein deposition indicates that there are important changes in protein metabolism of the sow that we still do not understand; these must be investigated if we are to improve the accuracy and application of the NRC (2012) model. Second, N-balance, which was widely used as experimental basis for the model of Dourmad et al. (2008), upon which much of the NRC (2012) model was based, overestimates maternal protein deposition (Clowes et al., 2003). In addition, because conceptus protein deposition was modeled based on data obtained in serial slaughter experiments, this leaves an unexplained amount of maternal protein deposition that may be an artifact of the scaling of protein deposition based on N-balance experiments. Finally, although the time-dependent protein deposition was derived mainly from experiments in gilts, it was applied to sows of all ages. It is possible that the time-dependent protein deposition in older sows may represent the re-gaining of body protein lost in the preceding lactation. However, body protein loss during lactation differs widely among sows due to genetics, number of piglets, feeding program, environment, etc., therefore a blanket correction is not appropriate. It would be much more preferable if the NRC (2012) model contained a separate term for protein deposition to regain lactation body tissue loss that could be scaled by the user to the actual loss observed.

An additional shortcoming, in our opinion, is that energy intake was treated as a user-defined input. We believe that the NRC (2012) program would be improved by an option of calculating the energy intake needed to achieve a certain performance level or body composition.

Although there is general agreement between modelled and empirically determined energy and amino acid requirements of sows, there are important questions that need further investigation. Despite this, the current recommendations represent requirement values that reflect the changing physiology of pregnant sows. To meet these requirements, parity-segregated phase feeding (PSPF) is the ideal nutritional regimen for pregnant sows.

Feeding Recommendations for Sows

The preferred strategy is to formulate just 2 diets—one corresponding to the highest, the other to the lowest amino acid needs—and then mix these in appropriate ratios to meet the entire range of requirements, from late gestating gilt, which have the highest requirements, to early gestating adult sows, which have the lowest requirements. This approach minimizes nutrient deficiency or excess, and allows sows to perform to their potential while eliminating expensive oversupply of nutrients. Therefore, this approach maximizes the benefits of a PSPF system. Although from a nutrition and feed cost point of view, this option with 'high' and 'low' diets is the best; its implementation incurs some costs to upgrade existing feeding equipment. The increasing use of group sow housing with electronic feeding makes this approach very feasible.

Sows given a constant feed allowance in gestation can achieve good body condition at farrowing. However, the excess nutrients allow sows to deposit body protein and fat in early and mid gestation that is mobilized to offset the inadequate nutrient intake in late gestation. This is metabolically and energetically wasteful because both fat deposition and fat mobilization are less than 100% energy efficient. Therefore, the total feed eaten by a sow during gestation can be reduced slightly by introducing phase feeding and still provide optimal nutrition for sow and conceptus.

In phase feeding, less feed is offered in early and mid gestation (days 0 to 85) and a higher intake in late gestation (days 86 to 114); this will result in less total feed eaten by a sow during pregnancy compared to feeding a single diet. We recommend an increased feed allowance for 1st, 2nd and 3rd parity and older of 0.6 kg/d, 0.5 kg/d and 0.4 kg/d, respectively, during the last 4 weeks of gestation. An increase in late gestation feed allowance does not impair lactation feed intake and may improve litter weight (Miller et al. 2000). These considerations lead to the feed amounts shown in Table 4 for corn-soybean meal diets. These feed amounts apply to sows of average body weight and condition; they should be modified for sows that are too lean or too fat, heavier or lighter than average, and more or less efficient in their nutrient utilization.

The feed allowances in Table 4 were chosen to cover the energy requirement of sows; Table 5 shows the necessary amino acid contents. In addition, an example is given for the amino acid contents that can be found in a single diet for pregnant sows. It becomes clear that a single diet is not adequate to provide sufficient amino acids in late pregnancy for young sows. Conversely, a single diet provides excess amino acids throughout pregnancy for older sows. The consequence is that the single diet may impair performance of young sows and will waste money because it is over-formulated for older sows.

The immediate benefits of PSPF are reduced feed usage for younger sows and lower formulated diets for older sows, thus reducing feed costs throughout a sows' live. Further benefits of phase feeding include an increased sow productive life and improved piglet quality.

Financial Benefits of PSPF

The obvious benefit of PSPF is decreased feed cost, which we estimate to be about $5/sow per gestation cycle. Reducing dietary AA contents in the first ¾ of pregnancy leads to lower diet cost of an additional $5.00 to 10.00 per tonne. Although the diet for late gestation must be better than current single diets for sows and will be more expensive, the diet for early and mid gestation, of which more is used, can be made less expensive. This, however, is the smallest benefit, and pales beside the production benefits. A greater benefit will be in more robust piglets and more even litters which will result in better postweaning pig performance. PSPF is predicted to result in lower culling rates for sows, because of improved rebreeding after the 1st litter (Shelton et al 2009) and because sows will not be entering lactation in a severely catabolic state due to underfeeding in late gestation, especially after the 1st to 3rd litters. An increase of the average litters per sow from 3.5 to 4.0 litters would increase the lifetime performance per sow by half a litter and reduce the replacement rate by 10%. Sasaki et al. (2012) calculated that each additional litter after the 3rd parity increased the net income per sow by approximately US$300, so that an increase of the lifetime performance of 0.5 litters carries a value of $150 per sow place. In addition, if sows had 4.0 instead of 3.5 litters per sow, savings of approximately $30.00 in replacement costs per sow can be realized. The financial potential is too great to be ignored. Although there will be an investment cost in equipment to implementing PSPF the payback should be significant.

Conclusions

Completely different methods—mathematical modeling and experimental animal research—agree that amino acid and energy requirements of sows increase dramatically in late pregnancy. To supply nutrients and energy at the right amounts and at the right time for sows of all ages, parity-segregated phase feeding is the most appropriate approach. Such a feeding regimen will minimize feed costs for pregnant sows and may result in improved sow and piglet performance. Amino acid requirements during the 30 days after breeding, requirements for first litter gilts, and the relationship between response to energy intake and amino acid requirements in late pregnancy need further study.

Acknowledgements

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Table 1. Sow performance during amino acid requirement studies in early (EG) and late gestation (LG) over 3 parities.

Parity	AA studied		BW, kg	Maternal gain, kg	ME intake, MJ/d	Protein retention, g/d	Energy retention, MJ/d	Litter size	Litter weight, kg
2	Lys, Thr, Trp	EG	177	44	34.2	32	3.0	13.8	19.5
		LG	215		34.5	126	-0.7		
3	Lys, Thr	EG	205	40	36.1	38	1.2	13.6	20.1
		LG	244		36.0	119	-0.9		
4	Thr, Ile	EG	240	25	35.6	4	1.5	15.8	22.1
		LG	266		35.5	64	-1.3		

Table 2. Total lysine[1], threonine[2], tryptophan[3] and isoleucine[4] requirements of gestating sows.

		1st parity	2nd parity	3rd, 4th parity
Lysine	Early gestation	15.0	13.1	8.1
	Late gestation	20.0	18.4	13.0
Threonine	Early gestation	n/a[5]	7.0	5.0
	Late gestation	n/a	13.6	12.3
Tryptophan	Early gestation	n/a	1.7	n/a
	Late gestation	n/a	2.6	n/a
Isoleucine	Early gestation	n/a	n/a	3.6
	Late gestation	n/a	n/a	9.7

[1] Srichana (2006) for 1st parity, Samuel et al. (2010) for 2nd and 3rd parity.
[2] Levesque et al. (2011).
[3] Moehn et al. (2012).
[4] Franco et al. (2013).
[5] Not available.

Table 3. Comparison of modelled and determined standardized ileal digestible amino acid requirements of gestating sows in early and late pregnancy in several parities.

Amino Acid	Parity	Stage of pregnancy	Source of requirements		
			NRC (2012)[1]	GfE (2008)[2]	Ball[3]
Lysine	2	Early	11.3	9.4	11.8
		Late	14.0	14.6	17.1
	3	Early	9.9	8.2	6.7
		Late	11.7	13.4	11.6
Threonine	2	Early	8.0	6.6	6.6
		Late	9.5	9.6	9.7
	3	Early	7.6	6.2	4.0
		Late	9.6	9.1	12.0
	4	Early	6.7	3.9	7.0
		Late	8.6	6.8	11.8
Tryptophan	2	Early	2.0	1.8	1.6
		Late	2.7	2.8	2.3
Isoleucine	4	Early	5.0	1.8	3.4
		Late	6.0	4.4	9.1

[1] Calculated using sow and litter performance, feed intake and days in pregnancy as in each experiment.
[2] Mean values given by GfE for parity and stage of pregnancy.
[3] Standardized ileal digestibility calculated assuming NRC (2012) digestibility for amino acids in corn and 100% digestibility for free amino acids.

Table 4. Daily feed allowance (kg/d) of a corn-soybean meal based diet for average sows in good condition in early and late gestation.

	1st parity	2nd parity	3rd parity and older
Early gestation (day 1 to 84)	1.8	2.2	2.4
Late gestation (day 85 to 112)	2.4	2.7	2.8
Average daily feed:			
Phase feeding	1.95	2.32	2.50
Constant allowance	2.00	2.40	2.50

Table 5. Total dietary amino acid contents (g/kg) in parity-segregated phase feeding compared to feeding a single diet for all sows.

		Phase feeding		
		2nd parity	3rd parity	Single diet
Early gestation	Lysine	0.60	0.34	0.60 ?
	Threonine	0.32	0.21	0.45 ?
Late gestation	Lysine	0.68	0.46	0.60 ?
	Threonine	0.50	0.44	0.45 ?

Management and Feeding of Group-Housed Gestating Sows

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Summary

Moving pregnant sows from individual stalls to group housing will require many changes in management and feeding practices to maintain optimal biological performance and sow welfare. Dietary energy requirements of sows may change compared with stall housing but requirements for other nutrients are not likely to change significantly. Producers may need to consider feeding requirements to support optimal sow welfare just like they have historically considered feeding requirements for optimal pig production and sow longevity. Even though sows are housed in groups, caretakers must employ an "animal-directed" approach to satisfy the needs of individual sows. The move from individual stalls to group housing will require a honing and fine-tuning of the caretakers' pig knowledge and observational skills to ensure all sows receive proper care which leads to optimal performance.

Introduction

Market and societal concerns are pushing pork producers in the U.S. to adopt group housing systems for gestating sows. This shift raises many questions concerning the management and feeding of sows in groups. To maintain optimal performance, sows are fed restrictively during gestation which causes sows to experience hunger throughout pregnancy. When sows are housed individually, hungry sows express increased stereotypic behaviors as a way of coping with hunger (Johnston and Holt, 2005). Individually-housed sows have almost no other methods of dealing with reduced supply of feed. However, in group housing systems where sows have greater freedom of movement and greater interaction with contemporaries, sows become aggressive with penmates as they attempt to access more feed to satisfy their hunger. This aggression creates challenges for the proper supply of nutrients to each individual sow, may compromise the welfare of individual sows especially sows at the bottom of the social rank, and may decrease the productive life of sows.

Group housing of sows forces pork producers and their advisors to consider a wide array of factors that interact to provide the proper nutrition of the sow. Some of these factors relate to changes in the biological needs of the sow elicited by the housing system. Other factors relate to the method of feed delivery and specifics of the group housing systems employed. The ultimate goal is to maintain productive sows that experience optimal welfare.

Biological Changes in Nutrient Requirements

Moving sows from an individual housing system to a group housing system is unlikely to change the sow's requirements for amino acids, minerals, or vitamins. However, energy requirements of sows could change as a result of moving to group housing. The changes in energy needs are difficult to predict accurately because not all group housing systems provide similar environmental conditions for the sow. Body weight of sows, expected litter size, thermal environment, and activity level all interact to determine an individual sow's energy requirement. Sow body weight and predicted litter size are not expected to change substantially when sows move from individual to group housing (Table 1; Johnston et al., 2013). In contrast, thermal environment experienced by sows and measured as effective environmental temperature could change dramatically. The effective environmental temperature is a combination of ambient air temperature, humidity, presence of drafts, flooring type (bedded or not), ability of sows to huddle with penmates, and whether sows can stay dry. All these factors converge to determine the temperature sows experience and ultimately influences the sow's daily energy requirement.

The National Research Council (NRC, 2012) recently published a mathematical model to predict energy requirements of gestating sows. This model relies on scientific estimates of the effects of environment and housing conditions on energy requirements of sows.

The NRC model can be used to demonstrate how the environmental conditions mentioned above can influence energy needs of pregnant sows (NRC, 2012; DeRouchey and Tokach, 2013). The scenarios displayed in Table 2 depict a given sow (Parity 2 sow weighing 165 kg at mating and 225 kg at farrowing) under a variety of environmental and housing conditions. The scenarios assume feed offered contains 3,300 kcal ME/kg, 9% fermentable fiber and that there will be 5% feed wastage.

Effects of Temperature and Housing

The lower critical temperature for sows housed individually is 20°C. Once environmental temperatures fall below 20°C, the sow must consume increased energy to maintain body weight gain. Decreasing ambient temperature from 20 to 10°C increases daily energy requirements by 2,163 kcal ME daily (Scenario 1 vs. 2) or 655 g of feed daily. In other words, for every degree below 20°C (defined as a degree of coldness) energy intake must increase 216 kcal (65 g of feed) daily. In contrast, the same temperature drop (from 20 to 10°C) in group housing only requires daily ME intake to increase 721 kcal (Scenario 4 vs. 5) which is equal to 218 g of feed. Because group-housed sows can and do huddle together, less feed is required to adjust to colder temperatures for two reasons. First, the lower critical temperature for group-housed sows is 16°C instead of 20°C for individually-housed sows. So, group-housed sows do not begin to feel the cold and require additional feed until the temperature gets to 16°C. Second, group-housed sows can conserve heat while huddled together so each degree of coldness only requires 120 kcal of ME (36 g of feed) instead of 216 kcal required by individually housed sows. If room temperature and activity level are equal, group-housed sows require about 15% less energy and feed than similar sows housed individually (Scenario 3 vs. 5).

Theoretically, energy requirements of sows will increase once environmental temperatures exceed the sow's upper critical temperature. However, since most pregnant sows are limit-fed, environmental temperatures above the sow's upper critical temperature are not considered important under practical conditions (NRC, 2012).

Effects of Sow Activity

Activity utilizes energy so increases in activity require additional dietary energy. Activity is often defined as standing, walking, and exploratory behaviors in swine. Activity level of sows can increase in any housing system for various reasons. But, one would expect activity of group-housed sows to increase compared with individually-housed sows because sows have greater freedom of movement (Salak-Johnson et al., 2012). Salak-Johnson et al. (2012) reported that activity of group-housed sows with varying floor space allowances ranged from 135 to 240 minutes daily. Increasing activity from 120 to 240 minutes daily increases energy requirements about 313 kcal ME which can be satisfied with an increase of 94 g of feed (Scenarios 1 vs. 4 and 2 vs. 3).

Effects of Bedding

Bedding, usually straw, is almost never used for individually-housed sows but can be used in group-housing systems. Bedding has value as an insulator in cold temperatures and can reduce the energy requirement of group-housed sows by 479 kcal ME daily (Scenario 5 vs. 6). The presence of bedding reduces the lower critical temperature of group-housed sows to 12°C. Bedding's value as an insulator against heat loss is greatly diminished if the bedding becomes wet. Fortunately, sows in group housing systems often have the ability to select the most desirable locations for lying and may be able to avoid uncomfortably wet bedding in cold environments.

Practical Implications

As mentioned previously, it is nearly impossible to provide a broadly applicable, accurate prediction of energy requirements for group-housed sows because conditions are not standard across group housing systems. Moving sows into groups will make them better able to resist cold temperatures due to their huddling behavior; however, they will likely increase activity which will require more energy than individually-housed sows. So, producers can use the guidelines listed here to provide a starting point for adjusting the feeding programs to account for group housing. Of course, careful monitoring of sow body weight gain, sow body condition and sow performance will provide the most accurate assessment of needed changes in nutrient intake levels for pregnant sows moved to group housing.

Diet and Feeding Management to Improve Welfare of Group-Housed Sows

The market-driven shift from individual stalls to group housing of pregnant sows is based on an expected improvement in sow welfare. Improvements in sow welfare resulting from the increased freedom of movement can be erased quickly by excessive and un-controlled aggression among sows in group-housing systems. Aggression at the time sows are mixed in the group and daily aggression, particularly at feeding time, can cause injury, lameness, low feed intake, and poor performance of sows (Kemp and Soede, 2012). Young sows are usually subordinate to older sows in groups and become the recipients of greater aggression than older, dominate sows. The negative effects of this aggression against subordinate sows can be dramatic. Li et al. (2012) reported that farrowing rate increased from 67% to 94% when first-parity sows were mixed and housed with gilts compared with sows. In pens with sows, the first-parity sows were the subordinate females and the recipients of increased aggression and injuries. However, in gilt pens, first-parity sows were not the focus of aggression from older sows. Interestingly, performance of gilts housed with first-parity sows was not reduced. Reduced feed intake during pregnancy, even drastic reductions for as little as 36 hours, can reduce pregnancy rate of sows and litter size. Kongsted (2006) studied 14 commercial herds to understand the relationship among feed intake, stress, and reproductive performance. Backfat gain in the first three weeks after mating was used as a proxy for feed intake of sows mixed in groups at about 8 days postmating. She reported over a 9 percentage point greater pregnancy rate in sows that gained 1 mm of backfat depth weekly compared with sows that lost 1 mm of backfat depth weekly (Fig. 1). So, pork producers need to consider feeding and management approaches to mitigate the negative effects of aggression among sows in group-housing systems so that individual sow performance and welfare can be optimized.

Nutrition and feeding management can play a role in controlling aggression among sows. Historically, producers and swine nutritionists have focused on the nutrient needs of sows to optimize reproductive performance and more recently, sow longevity. However, as the industry moves sows to group-housing systems, we might have to consider nutritional requirements for optimal sow welfare in addition to those needed to satisfy performance and longevity goals. There might be one level of nutrient/feed intake necessary to support optimal reproductive performance and a higher level required to support optimal sow welfare (Johnston and Holt, 2005).

Reducing Aggression at Mixing

One of the periods of most intense aggression among sows is at the time of mixing. Controlling aggression at this time would be very useful in helping mitigate the negative effects of aggression on sow welfare and performance. A common recommendation is to increase feeding levels right before and for a couple days after mixing to reduce aggression (DeRouchey and Tokach, 2013). The central idea is that sows are more satiated by elevated feeding levels and are more inclined to lie down and less apt to seek a fight with penmates. Full-feeding for 24 or 48 hours after mixing can markedly reduce the number of aggressive interactions among sows while feed is available. In many commercial settings, full-feeding is not practical but significant increases (100%) in feeding levels likely will help reduce aggression. One must be on the lookout for increased feed wastage with such high feeding levels.

The amino acid, tryptophan, has been investigated as a supplement that might reduce aggression among sows at mixing (Li et al., 2011). Tryptophan is a precursor of serotonin. Serotonin has sedative effects by suppressing aggression, excitement, anxiety, and pain. Supplementing crystalline tryptophan to the diet at 2.3 times the requirement for three days before and three days after mixing in pens had no significant effects on the incidence of aggression among sows. The authors theorized that the limited feed intake of sows (2.3 kg/d) during the period after mixing overwhelmed the ability of dietary tryptophan to exert any sedative effects on recently weaned and mixed sows.

Managing Individual Nutrient Intake to Control Competition in Group Housing

Throughout gestation, sows housed in groups are subjected to competition among penmates for resources (feed, water, lying space) which may lead to aggression from penmates. Much of this aggression stems from the fact that sows are limit-fed during pregnancy. Limiting feed intake increases frustration in sows and contributes to increased incidence of stereotypic behaviors. Stereotypic behaviors such as sham chewing, bar biting, and nosing or licking the floor when feed is not present are used as an indicator that sows are not satiated. Simply increasing the amount of feed offered will reduce the incidence of stereotypic behaviors because sows are more satiated (Bergeron et al., 2000). Sows that are fuller with feed are less likely to engage in aggressive acts with penmates. Unfortunately, increasing feed intake supports excessive maternal gain in body weight which suppresses feed intake during the subsequent lactation and compromises sow longevity. So, large increases in feed intake to control aggression are not practical in commercial production settings.

An obvious solution is to dilute the energy and nutrient density of the gestation diet so that increased feed intake is possible without the associated excessive increases in sow body weight. Typically, this approach employs high levels of dietary fiber. Stewart et al. (2010) fed sows a wheat-barley-soybean meal control diet (5% crude fiber) or a high fiber diet (15% crude fiber; 30% soy hulls + 14 % sugar beet pulp) to sows housed in dynamic groups with an electronic sow feeder and observed the activity of newly-introduced sows. The high fiber diet significantly increased lying time of sows and decreased standing, sitting, and exploratory behaviors (Table 3). These behavioral changes suggest that the sows were more satiated and content. The high fiber diet had no effect on the occurrences of aggressive behaviors after mixing but did reduce the incidences of head thrusts and biting during the 12 observation periods beginning at mixing and lasting for 3 weeks after mixing. The overall incidence of aggression in this study was very low and may not be characteristic of other dynamic group housing systems. This study suggests that high fiber diets can influence behavior of sows but the evidence supporting a reduction in sow aggression is weak. The apparent increase in sow contentment displayed by increased lying behavior may be elicited by the slow digestion of high-fiber diets and the prolonged postprandial peak in blood glucose concentration. Diets high is fermentable fiber (45% sugar beet pulp) fed to sows elicit a higher and more stable concentration of glucose between meals compared with a conventional grain-based diet. The elevated glucose between meals suggests that sows feel more satiated and are more content.

The beneficial effects of dietary fiber on sow satiety and behavior are not always consistent. Jensen et al. (2012) fed sows a restricted quantity (2.6 to 3.0 kg/d) of high-fiber diets that contained 20% pectin residue, 45% potato pulp, or 33% sugar beet pulp and measured the sows' motivation to consume feed. Compared with a barley-wheat control diet, sows fed the high fiber diets did not express a reduced motivation to consume feed as expected. Only when a high fiber diet containing a mixture of pectin residue, potato pulp, and sugar beet pulp was offered in a semi-ad libitum setting did sows show a reduced motivation to feed. This study suggests that potential benefits of high fiber diets on sow satiation and potentially behavior may be mitigated by offering restricted amounts of feed. Holt et al. (2006) included 40% soybean hulls in a corn-soybean meal based diet hoping to reduce stereotypic behaviors of limit-fed sows housed in individual stalls. Increasing crude fiber from 2.7 to 14.8% had no influence on expression of stereotypic behaviors by sows. The lack of effect may have been do to the highly insoluble character of the fiber used and(or) the limited amount of feed (2.19 kg) offered daily to sows. Presumably, if the high-fiber diet did not influence expression of stereotypic behaviors, it is unlikely the high-fiber diet would influence aggression in group-housed sows, but this was not tested directly. More recently, Li et al. (2013) reported that gestating sows fed a diet containing 40% dried distillers grains with solubles (7.16% ADF) in dynamic groups with access to an electronic sow feeder displayed increased aggression toward penmates compared with sows fed a corn-soybean meal diet (3.10% ADF). In apparent contrast, if sows were housed individually in gestation stalls, the DDGS-containing diet increased lying time and decreased stereotypic behaviors suggesting that sows were more content than contemporary sows fed the corn-soybean meal control diet. The differential effect of dietary DDGS on sow behavior across housing systems is puzzling.

Considered in total, there seems to be little chance for using increased dietary fiber to mitigate aggression among group-housed sows when sows are fed restrictively. If any fiber source could reduce aggression, it seems that a highly fermentable fiber has the best chance of success. Nonetheless, it seems that any potential benefits of feeding high-fiber diets on sow behavior and aggression are overwhelmed by the negative effects of restricting feed intake needed to prevent sows from becoming too fat during gestation. The best approach to controlling aggression from competition for feed may be to employ a non-competitive feeding system (e.g. electronic sow feeder, or free access stalls) but capital and installation costs, and available space in existing or new buildings may not be available for such feeding systems.

Stockmanship

The move from individual stalls to group housing for gestating sows requires important changes in how animal caretakers approach their duties. In a stalled system, a given sow is always in the same place each day. If a given sow is sick and not eating, this is easily detected because her allotment of feed remains well after all other sows have consumed their allotment. If a sow requires special attention for ill-health or other conditions, the caretaker simply records the location of the sick sow and can return to that location multiple times to find the sow. However, in a group system, sows are mobile so a sick sow cannot necessarily be identified by remaining feed or her location. So, caretakers must be more cognizant of sow behavior to identify sick sows, injured sows, and submissive sows that are not consuming sufficient feed. Producers that successfully implement a group housing system must adopt an "animal-directed" approach that focuses on individual sows (Kemp and Soede, 2012).

An animal-directed approach means that caretakers consider and evaluate each individual sow even though they are housed in groups. The first goal is to determine if all sows are consuming their allotted quantity of feed.

As discussed earlier, suboptimal feed intake compromises sow welfare and performance. The submissive sows in a pen are most at risk for low feed intake. Submissive sows may have the following characteristics: 1. they sleep alone rather than with the other sows in the pen; 2. they routinely are lying in the most undesirable locations in the pen; 3. when approached by a human, they are quicker than more dominant sows to flee and not return to the human; 4. they suffer a high number of scratches and injuries on the rear portions of their bodies that were received when retreating from fights; and 5. they seem to lose most of the fights with penmates at mixing time. In systems with electronic sow feeders, the submissive sows tend to be the last sows to access the feeder during a feeding cycle. If submissive sows are not accommodated in some way, they often will display poorer body condition than more dominant penmates. Feed wastage can be a problem in some group housing systems so feed allotments may need to be increased 5 to 10% to account for this wastage if it cannot be controlled (Johnston, 2010). The second goal of an animal-directed approach is to ensure all sows are healthy. Caretakers must be keenly aware of sow behavior to discern which sows are experiencing ill-health. Sows that seem depressed, are slow to rise and move, display obvious injuries or lameness, have bloody discharges or blood-stained hams and rear legs, and are isolated from and not interacting with penmates. Some feeding systems, such as an electronic sow feeder, may help identify sick sows. The feeding order of sows in a pen is rather consistent with an electronic sow feeder (Kruse et al., 2011). Sows that do not eat their daily allotment of feed at their usual spot in the order may be sick and should be given a special inspection.

The National Pork Board has recently commissioned a group of scientists and extension specialists to develop a "How To" management guide for gestation housing systems. The guide was developed for barn managers and barn workers in one of six gestation housing systems. The gestation housing systems include: individual stalls, group pens with electronic sow feeders, group pens with feeding stalls, group pens with free access (self-locking) stalls, group pens with trickle feeding, and group pens with floor feeding. Each guide includes management recommendations for feeding, mixing, health care, building and equipment maintenance, heat detection, worker safety, and many other nuances specific to each housing system. Each guide also includes a suggested routine for daily monitoring of the sows and the barn. These management guides are in final review and should be available sometime late in 2013.

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Table 1. Effects of individual stalls or pens during gestation on sow weight change and subsequent litter size[1].

Item	Stalls	Large Pens[2]	Small Pens[2]	Pooled SE	P value
No. of sows	326	335	154	--	--
No. of stalls or pens	326	13	26	--	--
Sow wt. gain in gestation[3], kg	41.5[a]	33.4[b]	39.5[a]	1.64	0.01
Pigs born live/litter	12.3	12.5	12.2	0.21	0.66
Pigs weaned/litter	10.3	10.2	10.1	0.20	0.89

[1] Johnston et al. (2013)

[2] Large pens = 26 sows/pen; Small pens = 6 sows/pen

[3] Body weight change from day 35 to 109 of gestation

[ab] Means in the same row with different superscripts differ ($P < 0.05$).

Table 2. Energy and feed requirements of gestating sows[1,2].

	Scenario					
	1	2	3	4	5	6
Housing system	**Individual**			**Group**		
Sow standing time[3], min/day	120	120	240	240	240	240
Temperature,°C	20	10	10	20	10	10
Floor type	Slat	Slat	Slat	Slat	Slat	Straw
Feed intake (d 0-90)[4], kg/d	2.11	2.80	2.90	2.21	2.44	2.29
Feed intake (d 0—114)[4], kg/day	2.20	2.89	2.99	2.30	2.53	2.38
Energy required, kcal ME/d	6,890	9,053	9,367	7,203	7,924	7,445

[1] Adapted from NRC (2012).

[2] Assumed parity 2 sow with breeding weight of 165 kg and target final body weight of 225 kg. Diet was assumed to contain 3,300 kcal ME/kg and 9% fermentable fiber.

[3] Estimated from Salak-Johnson et al. (2012).

[4] Estimated feed intake includes required feed and 5% wastage. Feed intake was increased 400 g/d from day 90—114 of pregnancy.

Table 3. Effects of a high-fiber diet on behavior of gestating sows[1]

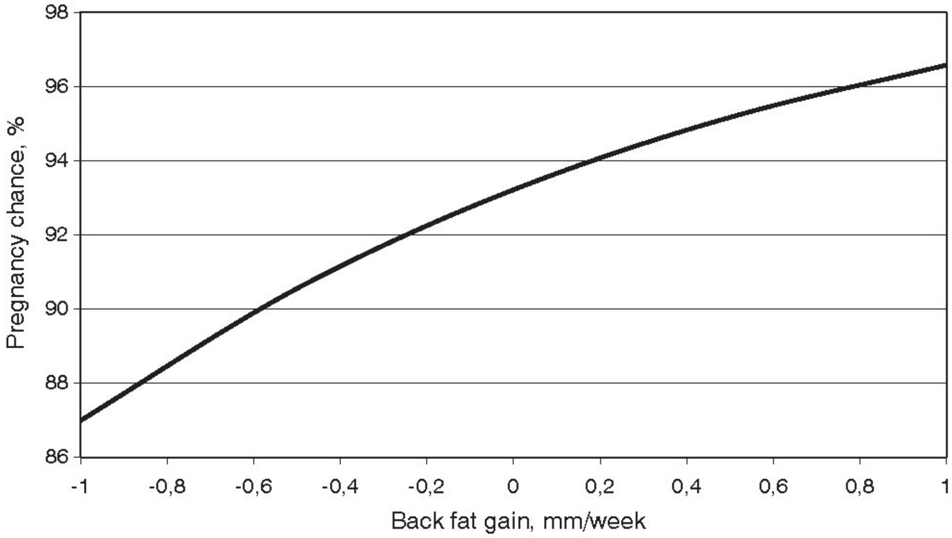
	Diet		
Behaviors	**Control**	**High Fiber**	**P value**
Lying[2]	0.689	0.765	< 0.05
Standing[2]	0.293	0.226	0.05
Sitting[2]	0.018	0.009	< 0.05
Exploration[2]	0.296	0.217	< 0.05
Aggressive encounters[3]	0.05	0.05	NS
Head thrusts[3]	0.02	0.00	< 0.01
Biting[3]	0.02	0.01	< 0.05

[1] Stewart et al. (2010).

[2] Percent of observation time recorded during 12 periods over 3 weeks after mixing.

[3] Occurrences/min of observation time.

Figure 1. Relation between back fat gain in the 3 weeks after mating and pregnancy rate of sows housed in groups (Kongsted, 2006).



Historical and Projected Climatic Changes in the Midwestern USA and Potential Impacts on Livestock Production Systems

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Summary

Weather and its longer term variant climate remain among the most important uncontrollable variables in agricultural production systems. Knowledge of long term trends or changes in climate over time is an essential component in the design of successful livestock production systems and in risk management. In this paper, historical and projected future climatic trends in the Midwestern USA are examined along with a discussion of possible impacts. In general, the climate in the region has become warmer and wetter in recent decades. Future projections all suggest additional warming in the future, with mean annual temperatures increasing from 3-6°C by the end of the century. Annual precipitation is projected to increase in some sections of the region and decrease in others. The frequency of days with heat stress is also projected to increase regionwide. While it is possible to plan for the potential impacts resulting from slow, long term shifts in climate such as the selection of more heat-tolerant breeds, changes in climate variability over time may be more difficult to plan for or to manage.

Historical Temperature Trends

Reliable temperature and precipitation records are available across the Midwest region for approximately the past 125-150 years. Although there is tremendous inter-annual variability in regional temperatures, and there are multiple points in time when temperature shifts occurred, mean temperatures in the region have increased overall since 1900 (Figure 1). Based on the CRUTEM3 gridded data set, annual mean temperatures over the Midwest increased by approximately 0.059°C per decade during 1900-2010 period, increased 0.12°C per decade for the period 1950-2010, and 0.26°C per decade for the period 1979-2010. The trends and temporal patterns are somewhat similar to overall global trends which include an increase in mean temperature of about 0.8°C since 1850 (IPCC, 2007).

The increases in temperature during the past several decades were not consistent across season or time of day, especially since 1980. A relatively greater proportion of the regional warming during the past 30 years has been be associated with warmer nighttime temperatures (i.e. minimum temperatures) (Andresen et al., 2012). Changes in maximum temperature during the same time frame have been relatively modest and in some sections of the region (e.g. IL, IN, MI) have actually decreased, possibly due to landscape cover type changes associated with intensified agriculture over time. Just as importantly, much of the warming occurred during the winter and spring seasons, with relatively little change in temperature found during the summer and fall seasons. Mean average minimum temperatures during the winter season have warmed as much as 3.0-5.0°C over northern sections of the region. In related trends, milder temperatures of the cold season are resulting in both less winter ice cover and thickness of ice cover on the Great Lakes and other lakes in the region and an earlier seasonal warm-up in the spring season with overwintering vegetation breaking dormancy an average of 7-10 days earlier than was the case just 30 years ago. There have also been increases in the length of the frost-free growing season. Averaged across the region, the frost free growing season length averaged about 155-160 days prior to the 1930s, then increased to around 160 days during the 1930s into the 1980s. Since the 1980s, it has continued to increase and now averages about 165-170 days. The increase in length across the region is the result of both earlier last spring freezes and later first fall freezes.

Overall across the region, annual precipitation across the Midwest generally decreased from the late 1800's through the dust bowl years of the mid 1930's, followed by a general increasing trend beginning during the late 1930s that continues to the present (Andresen, 2012), with an overall increase in precipitation during the past century. In general, the 1930's were the driest decade on record regionally, while the recent 2-3 decades were the wettest. There are some seasonal differences in the regional precipitation trends, with the majority of the increase in precipitation since the 1930's occurring during spring, summer, and winter seasons. The increase in precipitation since the 1930's has occurred both as a result of an increase in the number of heavy precipitation events as well as overall increases in the number of wet days and multiple wet day events. Trends in seasonal snowfall during the past 50 years are mixed. Relatively little change or even slight decreases in snowfall have been observed in those parts of the region away from the Great Lakes. In contrast, significant increases in snowfall (in some cases almost a doubling) have been recorded during the same time frame in areas close to, and downwind of the Great Lakes. These increases are associated with increases in lake effect snowfall, which is in part dependent on Great Lakes water temperature and the length of time the Great Lakes are ice free. Given increasing winter temperatures, there is also evidence that the number of days with snow cover on the ground has decreased during the past 1-2 decades.

Finally, while observational datasets are somewhat more limited, there have been other discernible climatic trends in recent decades. Given trends toward more annual precipitation and days with precipitation, there have also been corresponding increases in cloudiness, at least a portion of which was attributed to the recent increase in cloudiness to increases in high level cirriform clouds associated with jet aircraft contrails. Humidity levels across the region have increased in recent decades, with increases of relative humidity of 0.5-2.0% per decade from 1976 to 2004 and a steady increase of the frequency of high dewpoint days during the period 1960-2000 (Schoof, 2013). The increase in precipitation has also been associated with a decreasing frequency of drought over most of the region, including both reduced numbers of extreme and exceptional droughts and lesser areal extent of drought in recent decades. The trend towards a wetter climate and decreasing drought frequency has also had a major impact on the region's agriculture industry in recent decades, with relative increases in crop yields due to less moisture stress and overall more favorable growing conditions (Andresen et al., 2001).

Projected Future Trends

Warming temperatures are projected by global climate model output across all of North America during this century, with the magnitude projected to increase almost linearly with time (Figure 2). The warming in the USA is projected to exceed 2°C by nearly all the models, and to exceed 4°C by more than 5 GCMs out of 21 (Christensen et al., 2007). Relative increases in mean annual temperatures across the Midwest are projected to range from 1-3°C during the middle of the century (2040-2059) and from 3-5°C during the 2080-2099 period. The model projections also suggest distinct seasonality across the region, with relatively greater warming during the winter season.

Seasonal changes in annual mean precipitation by late this century relative to the recent past are given in Figure 3 (from Karl et al., 2009). Overall, annual increases are likely across northern sections of the region while decreases are likely in the south. Annual increases in northern (southern) sections are strongly linked with seasonal increases (decreases) in the winter and spring seasons. In contrast to projections of regional temperature, projections of future precipitation change remain somewhat uncertain for the Midwest. The majority of climate projections are in agreement regarding the sign of the projected change for only the winter season. Regardless, one key result of the model projections is the expectation of relatively drier summers across most all of the Midwest during the summer season. This is critical given the timing of the growing season and coincides with the period of greatest moisture needs by vegetation. As a result, mean annual soil moisture content across the region is projected to decrease even though annual precipitation amounts are expected to increase. The projection of drier summers across much of the central and eastern USA suggests that concurrent increases in evapotranspiration will largely come during the transition and cool seasons (ie spring, fall, and winter).

One important issue in the projections is the expected occurrence and frequency of weather and climate extremes. The majority of the climate projections suggest an increase in the frequency, length, and severity of heat waves, a significant decrease in the number of cold wave episodes, decreases in the number of frost/freeze days, and a comparable increase in growing season length. The projections also suggest an increase in precipitation intensity (with a relatively greater portion of the total annual precipitation falling as extreme events) and for precipitation to become more erratic, which would likely lead to an increasing likelihood of droughts (Karl et al., 2009).

The occurrence of heat waves and accompanying heat stress conditions are of particular importance to the livestock industry in the USA. In the Midwestern region of United States, high summer temperatures are often accompanied by elevated near-surface humidity, which enhances animal heat stress through the reduction of evaporative cooling. The combined effect of temperature and humidity on heat stress is usually quantified as the apparent temperature (also commonly referred to as the heat index). This variable is strongly correlated with the commonly-used Temperature Humidity Index, THI.

Schoof (2013) considered several different global climate models and emissions scenarios to estimate possible future changes in apparent temperature in the Midwest. While the trends varied by individual climate model and emission scenario, he found increases in both summertime air temperature and humidity and resulting increases in apparent temperature from 2.6 – 4.1°C during the 2050-2059 decade and 3.5 – 6.9°C during the 2090-2099 decade. Collectively, the results suggest that heat stress-related weather events will increase significantly in the future, both as a result of increasing air temperatures and increasing humidity levels.

It is interesting to note that with the exception of the frequency of drought, recent historical trends toward a warmer and wetter climate are in general agreement with future projections. This is a critical for agriculture in general, as the industry is much better equipped to adapt and cope with long term, steady changes (e.g. changes in animal breed with a gradual increase in temperature) than with changes in climate variability such as in increase in the frequency and severity of droughts. Due to a potentially changing climate, it is possible that livestock producers may have to rely on much shorter time horizons for long term planning than were utilized in past years (e.g. 5-10 years vs. previous time frames of 30- or 40-years).

Direct Impacts

Most significant potential direct impacts from climate change to livestock production systems in the Midwest region include reductions in productivity and increases in the frequency of potentially life-threatening heat stress. The combination of high air temperatures and relatively high humidity, heat stress results in declines in animal physical activity, feed intake, growth rates and weight gains, and conception rates (Mader and Davis, 2004). Grazing patterns could be altered as well. In a study of the grazing site preferences of bison and cattle, Allred et al. (2013) found that cattle begin to seek thermal refugia (areas of woody vegetation and/or riparian zones) more quickly and at lower threshold temperatures than bison, resulting in significantly different grazing pressure on the landscape. One critical, relatively unknown factor is the impact of future changes of climate variability on animal productivity, which could be much larger than impacts associated with changes in average conditions should variability increase. Collectively, the vast majority of previous research suggests declines in livestock production in a warmer world, resulting in increasing production costs as well as the additional need for modification of production facilities to reduce heat stress (Hatfield et al., 2008).

In an attempt to quantify the potential impacts of increasing temperatures on livestock production systems across the USA, Frank et al. (2001) applied production and response models for confined swine and beef cattle, and milk-producing dairy cattle using climate projections of the future. The simulations were run for one historical and two future climate scenarios: doubled CO_2 by the year 2040 and a tripling of CO_2 concentrations by the year 2090. Example results from this study for swine production during the 2040 time frame are given in Figure 5. In general, changes in production associated with the warmer climate for cattle and dairy production were negative across the country for the 2040 scenario, with decreases ranging from 0.7-2.0% to 2.1-2.2% respectively. For swine production in 2040, significant declines in productivity across southern regions of the country were offset by stable or increasing productivity across northern and western sections (Figure 5). Overall, swine and beef production were affected most in the south-central and southeastern United States while dairy production was affected the most in the U.S. Midwest and Northeast regions. In the Midwest region, there were also seasonal differences, with relative gains during the winter season offset by relative losses during the summer.

It is also important to consider the relative level of animal performance as it may influence the overall risk of the production systems associated with climate change (Hahn et al., 2001). At relatively high performance levels (e.g. large weight gains), the vulnerability of the animal increases, and when coupled with an adverse environment, the animal is at greater risk. Combining an adverse environment with high performance pushes the level of vulnerability and consequent risk to even higher levels. Domestic livestock, in general, can cope with or adapt to gradual changes in environmental conditions; however, rapid changes in environmental conditions or extended periods of exposure to extreme conditions drastically reduce productivity and are potentially life threatening. In an effort to maintain optimum levels of production, climate change will likely result in livestock producers selecting breeds and breed types that have genetically adapted to conditions that are similar to those associated with the climate change.

Indirect Impacts

Overall, the warmer and wetter conditions during the past couple decades have resulted in a number of agronomic impacts related to animal production systems. On one hand, relatively warmer and wetter growing seasons have led to increases in plant available moisture and potential yields of many regional crops (Andresen et al., 2001). Considering potential future changes in feed production, longer growing seasons in the region may allow production of longer season crop types and varieties, many of which have potentially greater yields. This trend has already resulted in profound changes in cropping systems and mixtures across the region, especially across northern sections with increasing production of corn and soybeans. While shifting climate played a major role in these changes, changes in agronomic technology such as improved cultivars and increasing capacity of agricultural implements were also found to be important (Kucharik, 2006). In a study of potential future climate impacts on corn and wheat production in the Great Lakes Basin, Andresen et al. (2013) found the direction and magnitude of simulated impacts depended greatly on the crop and CO_2 emissions scenario. In general, the warmer climate suggested by the climate scenarios led to decreases in corn yields and increases in wheat yields in the non-CO_2 enriched scenarios. For the increasing CO_2 scenarios, corn yields in general decreased across the region (a range of -27.3% to +23.0%) while wheat yields generally increased (ranging from -9.3% to 72.7%). The decreases in corn yield were associated with decreases in growing season precipitation and increases in growing season temperatures while the wheat yield increases appeared to be linked to steady or increasing fall and winter precipitation. The impact of CO_2 enrichment was substantial for the C-3 wheat crop, with increases in yield noted for most scenarios. Largest percentage increases in yield were found at the northernmost study locations, which suggest at least the potential for increasing agronomic productivity with time in northern sections of the region that have not been historically productive.

Changes in climate and atmospheric CO_2 concentrations may also alter forage quality, including fiber content and concentrations of crude protein, non-structural carbohydrates, minerals, and secondary toxic compounds. Recent research suggests both potential positive and negative changes in quality are possible (Hatfield et al., 2008). For example, non-structural carbohydrates can increase under elevated CO_2, while plant N and crude protein concentrations often decline, especially when plant production is enhanced by CO_2 (Hatfield et al., 2008). Reductions in forage quality could possibly have pronounced negative effects on animal growth, reproduction, and mortality and could render livestock production unsustainable unless animal diets are supplemented with N (Milchunas et al. 2005).

The frequency, distribution, and risk of many parasites and disease pathogens may also be impacted by a changing climate. For example, higher humidity and increasing precipitation may favor the increased incidence of fungal pathogens. Warmer winters may allow for greater survival of parasites and disease pathogens while a warmer overall climate may allow for the introduction of exotic species that were previously unable to survive. At a minimum, the addition of new species could result in additional subsequent population changes as the system dynamically responds to the new species composition. Carbon dioxide enrichment could also play an important role as many weeds respond more positively to increasing CO_2 than do crops (Hatfield et al., 2008). Unfortunately, previous research suggests that glyphosate, the most widely used herbicide in the United States, loses its efficacy on weeds grown at CO_2 levels likely to occur in the coming decades (Ziska et al. 1999).

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Figure 1. Annual temperature anomalies for the Midwest relative to 1961-1990. The data are taken from the CRUTEM3 data set and have a spatial resolution of 5 x 5°across the domain 35°N to 50°N and 95°W to 80°W. Data were downloaded from http://www.cru/uea.ac.uk/cru/data/temperature/#datdow. Also shown is a 5 year running mean and linear fits to the annual data for 1900-2010, 1950-2010 and 1979-2010. The shading represents the 95% confidence intervals on the fits. The slopes of the region-wide trend estimates are expressed in °C per decade and are shown for 3 time periods; 1900-2010, 1950-2010, and 1979-2010. (Pryor and Barthelmie, 2011)

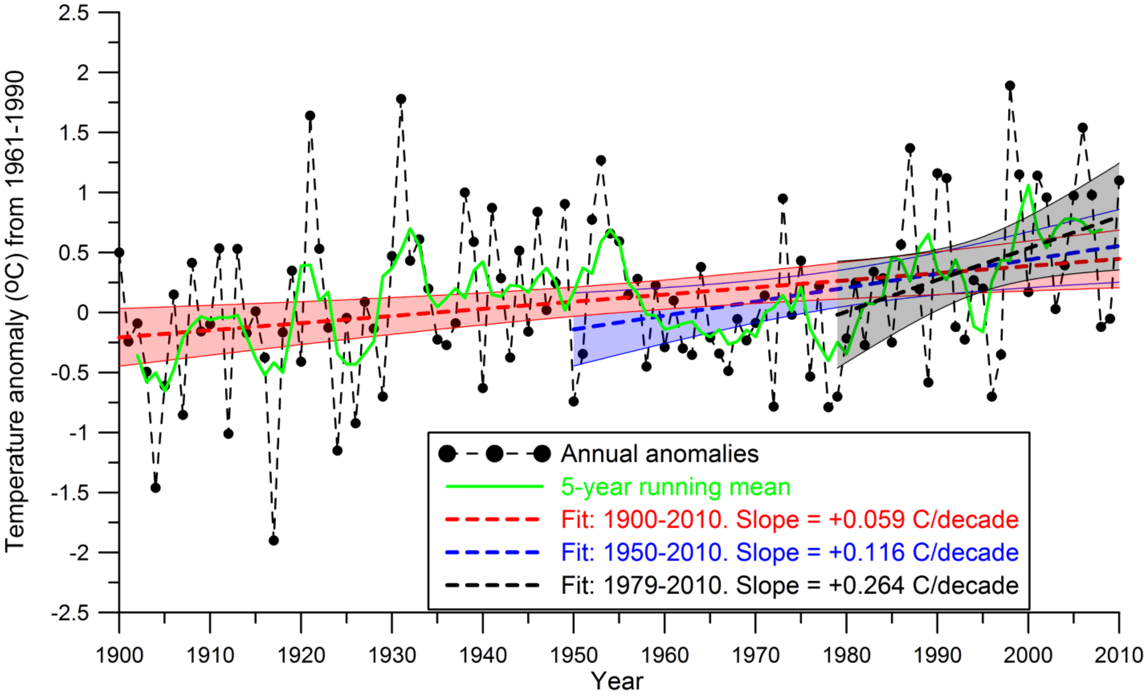


Figure 2. Temperature anomalies with respect to 1901 to 1950 for five North American land regions for 1906 to 2005 (black line) and as simulated (red envelope) by IPCC Fourth Assessment GCM models incorporating known forcings; and as projected for 2001 to 2100 by the same models for the A1B emissions scenario (orange envelope). The majority of the Midwestern USA is within the Central North America region, 'CNA' (middle of the bottom row). The bars at the end of the orange envelope represent the range of projected changes for 2091 to 2100 for the B1 scenario (blue), the A1B scenario (orange) and the A2 scenario (red). The black line is dashed where observations are present for less than 50% of the area in the decade concerned. *(From Christensen et al.,*2007)

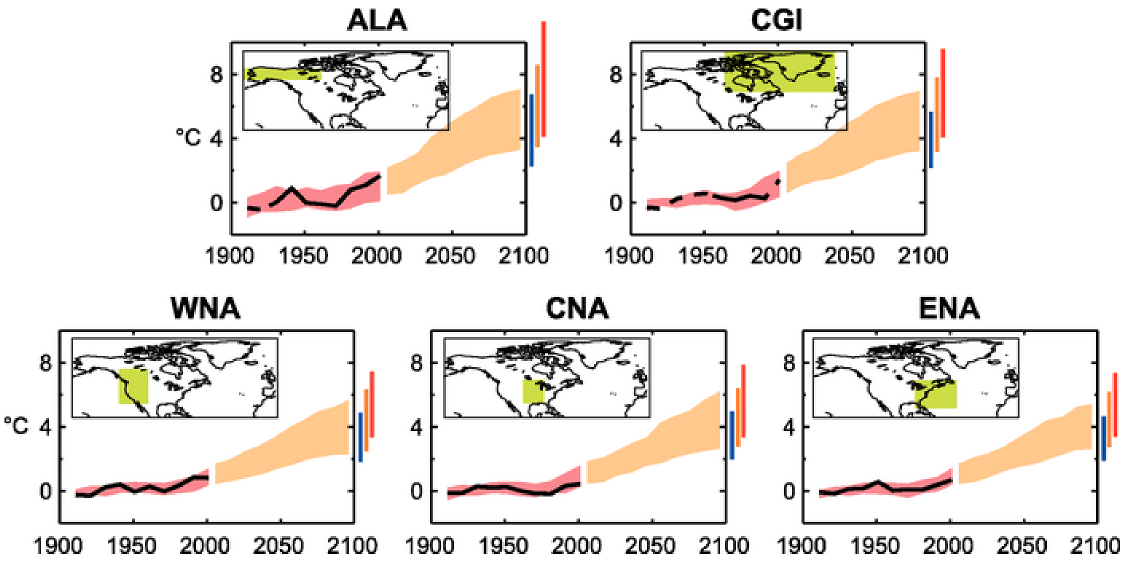


Figure 3. Model-projected future changes in precipitation relative to the recent past as simulated by 15 IPCC Fourth Assessment GCMs. The projections are for the 2080-2099 period versus the 1981-2000 period for a high SRES emissions scenario. Confidence in the projected changes is highest in the hatched areas indicating areas of relatively greater confidence with at least 10 of the 15 models in agreement on the direction of the change. (From Karl et al., 2009)

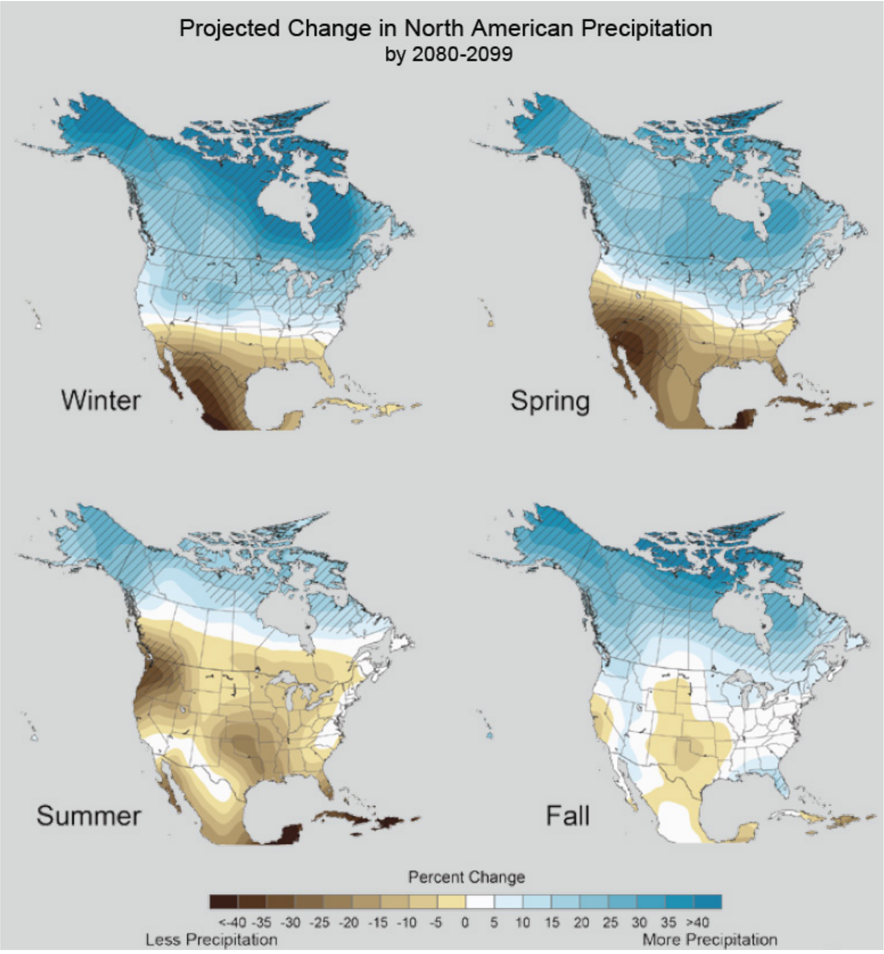


Figure 4. Regionally averaged downscaled climate model projections of changes of maximum temperature T_{max} (top), dew point temperature Td_{max} (middle), and apparaent temperature Ta_{max} (bottom). The line and solid symbol indicate the decadal average of the projection for each greenhouse gas scenario. The open symbols indicate the maximum and minimum values for AOGCM simulations included in the ensemble. (From Schoof, 2013)

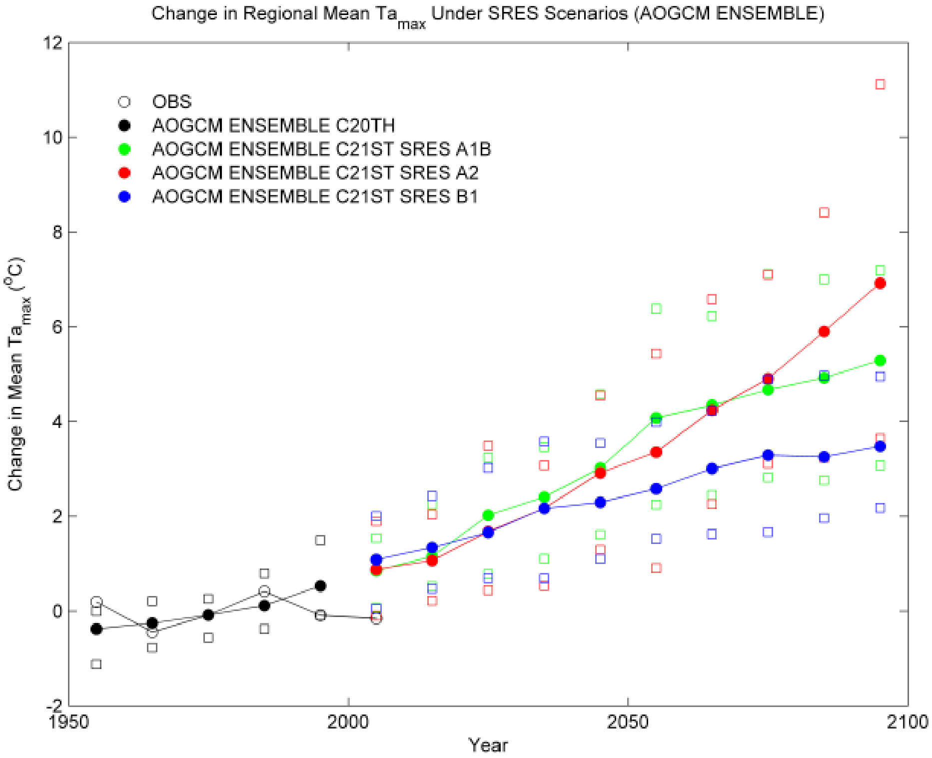
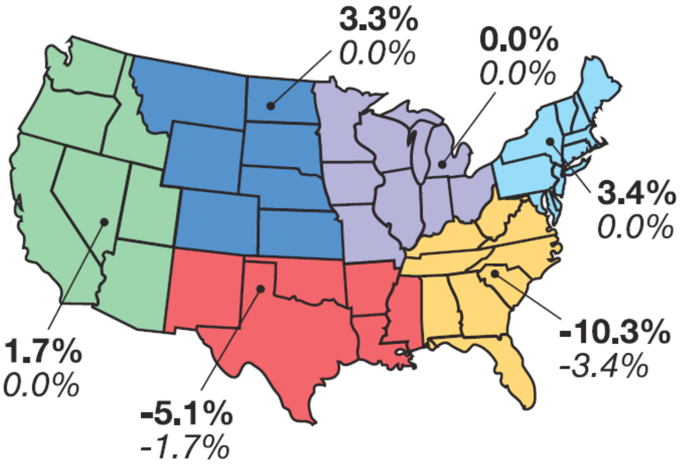


Figure 5. Percent change from historical baseline to year 2040 of days for swine to grow from 50 to 110 kg, beginning June 1 under CGC climate model (bold text) and Hadley climate model (italicized text). From Frank et al. (2001) and Hatfield et al. (2008).



Assessing the Energy Value of Corn Co-products

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Summary

Corn dried distillers grains with solubles (DDGS) have been used extensively in commercial swine diets as an economical energy source. Variability in energy and nutrient content among DDGS sources continues to be a significant challenge when assessing relative feeding value among sources and determining accurate nutrient loading values for use in feed formulation. Implementation of partial oil extraction technology by the majority of U.S. ethanol plants has further increased energy and nutrient content variability. To manage this variability, nutritionists need accurate, inexpensive, and rapid methods to assess energy value among DDGS sources. Prediction equations using chemical and physical measurements that are most predictive of metabolizable or net energy content offer a reasonable approach to estimate DDGS energy content, but understanding their limitations is essential. Fiber content and composition in DDGS plays a significant role in estimation of energy content. Minimizing the impact of dietary fiber on fatty acid digestibility, as well as increasing digestibility of dietary fiber, represent two of the most significant opportunities for improving energy utilization in DDGS. Dietary fiber may reduce energy utilization of DDGS through several mechanisms. Using the most appropriate measure to characterize dietary fiber and its physiologic and nutritional impact in swine diets is essential. A better understanding fiber digestion and fermentation is needed in order to improve the efficacy of exogenous enzymes as a means to enhance energy utilization in DDGS.

Introduction

Energy is the most expensive component of swine diets. Historically, corn grain has served as the primary energy source, but with the extensive growth of the ethanol industry, dried distillers grains with solubles (DDGS) has become an economical and commonly used partial substitute for corn, soybean meal, and inorganic phosphate. In 2012, about 122 million metric tonnes of corn were used to produce over 50 billion liters of ethanol and 33.3 million metric tonnes of distillers grains with solubles for use in livestock feeds (RFA, 2013). During the past two years, annual production of distillers grains with solubles has exceeded that of soybean meal. Of the total distillers grains with solubles produced in 2012, swine consumed about 12%, or nearly 4 million metric tonnes (RFA, 2013). As a result, DDGS has evolved from being considered an alternative ingredient to one that is commonly added to the majority of grower-finisher swine diets. This widespread use of DDGS in swine diets has been primarily due to it being historically priced at 75 to 80% of the price of corn, while containing similar energy and higher protein (lysine) and digestible phosphorus than corn. Due to a shortage of corn and other feedstuffs resulting from the drought in 2012, DDGS price has been near 100% of the price of corn making it less economically attractive, but still a valuable feed ingredient for use in swine diets.

However, the nutrient composition of DDGS is changing. During the past two years, an increasing number of ethanol plants have been partially extracting corn oil from thin stillage prior to manufacturing DDGS. Oil extraction is accomplished relatively inexpensively through the use of centrifugation equipment in ethanol plants. Ethanol industry experts predict that 90% of the ethanol plants in the U.S. will be partially extracting oil by the end of 2013. Partial oil extraction during the DDGS production process has resulted in a reduction in the total amount of DDGS produced, and has raised questions about the economic and feeding value of reduced-oil DDGS in swine diets. As long as extracted distillers corn oil continues to be a profitable revenue source for fuel ethanol plants, it is expected that additional corn oil removal technology will be implemented by many ethanol plants in the near future that will further reduce the oil content of DDGS.

As corn co-products produced by the U.S. fuel ethanol industry continue to evolve in composition and feeding value, nutritionists need to develop "nutritional tools" to: 1) assess metabolizable and net energy content of this increasingly variable co-product, and 2) develop strategies to convert more of the remaining gross energy (provided by fiber, residual starch, unextracted oil, and protein) into energy that can be used by pigs.

Therefore, the objectives of this paper are to discuss approaches and limitations to assess the ME and NE content of DDGS sources of variable oil and nutrient content, as well as propose some research perspectives to better understand the impact of digestibility of dietary fiber and lipids on ME and NE among sources of DDGS.

Estimating ME and NE Content of Corn Co-products with Prediction Equations

The energy and nutrient content in DDGS is more variable (Spiehs et al., 2002) than in corn and soybean meal (Cromwell et al., 1999). Anderson et al. (2012) showed that the metabolizable energy (ME) content among traditional high-oil DDGS sources can range from 3,414 to 4,141 kcal/kg DM. Surprisingly, the range in ME content among 15 reduced-oil DDGS sources was not as wide (3,266 to 3,696 kcal/kg DM) in a recent study conducted by Kerr et al. (2013). Regardless, variability in energy and nutrient content among DDGS sources has created significant challenges for determining relative economic value, as well as establishing accurate nutrient loading values when formulating swine diets. Overestimation of energy and digestible nutrient content can lead to reduced growth performance, while underestimation results in an inability to capture full ingredient value (Min et al., 2011).

In order to manage the variability in energy content of DDGS, nutritionists need rapid, inexpensive, and accurate methods. Although NRC (2012) provides gross energy (GE) and digestible energy (DE) values with standard deviations, as well as metabolizable energy (ME) and net energy (NE) values, for various classifications of corn DDGS co-products, these estimates can quickly become outdated due to evolving production technologies being implemented in the U.S. ethanol industry. *In vivo* determinations of DE, ME, and NE of various DDGS sources are the most accurate, but are impractical because they are expensive, tedious, and time consuming. However, because there are no standardized methodologies (e.g. marker to marker vs. total collection, length of adaptation period, composition of basal diet, etc.) for conducting energy balance studies, *in vivo* DE and ME estimates can vary for samples of feed ingredients among experiments. Near infrared spectroscopy (Rathore et al., 2005) offers the advantages of being fast and inexpensive, but a database in excess of 200 estimates of *in vivo* DE or ME estimates is required in order to develop good calibrations. As a result, we believe that the development and use of accurate prediction equations that include the most significant chemical and physical components, appears to be the most promising approach to achieve the goal of providing rapid, inexpensive, and accurate "tools" for estimating the concentration of GE, DE, ME, and NE among sources of reduced-oil DDGS.

Energy prediction equations have been developed for barley (Fairbairn et al., 1999), meat and bone meal (Adedokun and Adeola, 2005; Olukosi and Adeola, 2009), wheat DDGS (Cozannet et al., 2010), and complete diets (Just et al., 1984; Noblet and Perez, 1993). However, these empirical prediction equations are likely to only be accurate for the nutrient matrices from which they were derived, and not for corn DDGS. Pederson et al. (2007) developed prediction equations specifically for corn DDGS, and equations from Anderson et al. (2012) were developed using a wide variety of corn co-products, including DDGS, but these have not been validated. More recently, Kerr et al. (2013) developed DE and ME prediction equations for reduced-oil DDGS, and these equations are currently being validated at both the university and industry locations.

Most of the DE and ME prediction equations for DDGS from Pederson et al. (2007), Anderson et al. (2012), and Kerr et al. (2013) require a GE estimate as one of the primary predictive factors. Gross energy determinations of feedstuffs are relatively inexpensive, accurate, and the methodology is well accepted, but this measurement is not commonly conducted by commercial laboratories. Ewan (1989) developed a GE prediction equation based on chemical composition of a feed ingredient or diet, but it has not been evaluated for accuracy in predicting GE content of DDGS. Kerr et al. (2013) developed GE prediction equations for reduced-oil DDGS based on chemical composition and physical characteristics, which include ether extract and particle size as the most predictive factors (GE, kcal/kg DM = 4,583 + (50.61 × ether extract, %) – (0.12 × particle size, μm).

The other major component used in the majority of these energy prediction equations is a measure of fiber (i.e. ADF, NDF, or total dietary fiber-TDF). In general, TDF provides better predictability (higher R^2 and lower SE) than NDF or ADF, but its measurement is also not commonly performed in commercial laboratories, and it is more expensive and tedious to measure than NDF and ADF. Although NDF is used extensively for evaluating fiber in ruminant feeds and ingredients, our experience is that there is considerable lab to lab variation in its measurement which can have a significant impact on DE or ME estimates (Table 1). For example, ME estimates using an Anderson et al. (2012) equation can vary by 378 kcal/kg DM for the same DDGS sample, depending on which chemical composition values are used in the equation.

Theoretically, we know that starch, fat, protein, and fiber have a positive influence on energy of a feed ingredient, while ash has a negative effect. Although fiber is often considered to have a negative effect on GE, DE, ME and NE, this is only true if the equations are derived from a standard reference diet. However, ingredients like DDGS have low amounts of starch, and limited data have been reported on whether the remaining starch is resistant or soluble starch. Urriola et al. (2010) reported that total starch in DDGS ranges from 3.8 to 11.4%, but the range in insoluble starch was from 2.0 to 7.6%, suggesting that a high proportion of residual starch is indigestible in pigs. There are several methods that can be used to measure fiber including crude fiber, ADF, NDF, NSP (non-starch polysaccharides), and TDF. Urriola et al. (2010) reported that apparent total tract digestibility of TDF averaged 43.7%, but ranged from 23.4 to 55.0% in DDGS. However, each of these methods has limitations for characterizing the fermentable vs. the non-fermentable complex carbohydrate fractions of feed ingredients used in swine diets. It is likely that the methods currently used to characterize fiber, lab to lab variation in measuring chemical components of feedstuffs, along with our relatively poor understanding of fiber utilization in pigs, are significant factors affecting the accuracy of ME prediction equations.

We have conducted an analysis to compare GE, DE, and ME prediction equations from various published studies for their accuracy in predicting experimentally determined GE and *in vivo* DE and ME estimates using data sets from other studies, in an across study validation. Samples of DDGS evaluated by Kerr et al. (2013) had less concentration of GE (4,996 kcal/kg DM) than samples collected by Pedersen et al. (5,434 kcal/kg DM; 2007), Stein et al. (5,539 kcal/kg DM) 2009) and Anderson et al. (5,420 kcal/kg DM; 2012). The reason for this lower concentration of GE in the Kerr et al. (2013) study is likely due to the selection of sources of DDGS with lower oil content compared to the high-oil DDGS sources evaluated in previous studies. This observation suggests that the reduction of oil content in reduced-oil DDGS impacts GE content, and subsequently DE and ME content, but variation in nutrient digestibility among sources of DDGS is greater, and therefore results in a poor relationship between oil content of DDGS and ME content.

We also observed significant variability in the concentration of chemical components among data sets as a result of using different methods to analyze these constituents. This effect is most noticeable for analytical values of NDF. The concentration of NDF in DDGS samples from Pedersen et al. (2007) was less compared with those from other references (Spiehs, et al., 2002; Stein et al., 2006; Stein et al., 2009; Anderson et al., 2012; Kerr et al., 2013). These differences are consistent with the lab to lab variation in nutrient analysis reported by Cromwell et al. (1999, 2000) when the same samples of soybean meal, corn, and wheat middlings were analyzed in different laboratories.

Challenges of using energy prediction equations

Although energy prediction equations offer the advantage of predicting ME from chemical analysis components, there are several questions that need to be considered when using these equations.

- **Have these equations been validated?** Often times, prediction equations can be fairly accurate for predicting ME within the data set from samples from which they were derived, but may not be as accurate when applied to unknown samples outside of the data set.
- **How robust or diverse were the samples and data from which these equations were derived?** Diversity of samples is usually preferred because equations can be applied across a broader range of sample nutrient content and predict energy content relatively accurately. However, depending on the size of the data set, the R^2 of these equations is usually lower than for equations derived from samples with less variable nutrient composition.
- **Are we using the most predictive components of equations?** Some of the chemical analysis measures required for accurate ME prediction (e.g. GE and TDF) are not routinely measured in commercial labs, or are expensive and time consuming. Because of this, some nutritionists may choose less accurate (lower R^2 and higher SE) equations that contain variables that can be more conveniently measured, if equations have been developed that contain the variables they want to use.
- **What laboratory and laboratory methods are used to determine the chemical and physical components needed in prediction equations?** As previously discussed, lab-to-lab variation exists for routine proximate analysis of feed ingredients, and as a result, can give different results when using these data in energy prediction equations. There are many AOAC or AOCS approved procedures for measuring specific nutritional components, which sometimes are not described in publications when prediction equations are published. The chemical method chosen, along with normal lab-to-lab variation when following the same analysis protocol, can greatly influence the inputs of prediction equations,

and ultimately the accuracy of energy prediction. For example, some measures, such as NDF can be highly variable among labs, and because it is found in high concentrations in DDGS, can dramatically impact ME estimates.

- **Does the magnitude of the coefficients and their direction (positive or negative) make sense in the equation?** Some adjustments for fiber and ether extract in some equations seem counterintuitive.
- **What methods were used to determine DE and ME values of the test sources?** Length of adaptation time to experimental diets, composition of basal diet, use of indigestible markers vs. total collection, age of pig, etc. all influence the accuracy of the original *in vivo* DE and ME estimates from which equations are derived.
- **How was the regression analysis conducted to develop the equations?** Assumptions regarding the intercept, level of significance from which variable are included or excluded from the model, use of backward or forward regressions influence the accuracy of prediction equations.
- **How does particle size of DDGS affect DE and ME content?** Recent studies by Liu et al. (2012) indicate that for each 100 micron reduction in average DDGS particle size, DE increases by 52 kcal/kg DM. Most studies that derived energy prediction equations did not measure physical characteristics of the DDGS samples from which equations were derived.

How Do We Obtain More ME and NE from GE in Corn Co-products?

The concentration of GE in DDGS (4,849 kcal/kg) is greater than in corn (3,933 kcal/kg), but unfortunately, the concentration of ME is similar (3,394 vs. 3,382 kcal/kg). The lower ME:GE in DDGS (70%) relative to corn (86%) is due to the compositional differences between the two ingredients. Corn has a greater concentration of starch, while DDGS has a greater concentration of TDF (31 vs. 14%). Furthermore, despite the fact that DDGS has a greater concentration of oil than corn, it has lower true ileal digestibility of oil than corn (62 vs. 86%; Kim et al., 2013). Now that over 75% of the U.S. ethanol plants are extracting corn oil prior to manufacturing reduced-oil DDGS, the challenge of finding ways to increase energy value of DDGS has become even greater. However, we first must understand the mechanisms that are limiting their consistent positive effects.

Results from a recent study by Kerr et al. (2013) showed that ether extract content of DDGS is a poor predictor of DE and ME content for swine (Table 2). These results were confirmed in a recent follow-up study that showed a poor relationship between DDGS oil content and DE, ME, and NE content of reduced-oil DDGS. We speculate that these responses may be due to a greater than anticipated contribution of energy from dietary fiber. The energy that pigs obtain from dietary fiber is derived from volatile fatty acids produced by microbial fermentation, but the energetic efficiency of dietary fiber is lower than that obtained from lipids and starch (Boisen et al., 2000; Birkett and de Lange, 2001). Urriola et al. (2010) showed that apparent total tract digestibility of dietary fiber in corn DDGS ranges from 23 to 55%. Therefore, it is possible that the variability in energy digestibility is due to interactive effects of fiber and lipid. Furthermore, Kim et al. (2013) showed that the true total tract digestibility of intact corn oil present in DDGS is substantially less (52%) than extracted corn oil (94%). This suggests that much of the lipid present in the fiber matrix of DDGS may be inaccessible to endogenous enzymes, rendering it less digestible and thus, provides a lower contribution toward ME content of DDGS than theoretically possible. Dietary fiber reduces the digestibility of lipids by 1.3 to 1.5% for each 1% increase in dietary fiber level (Just et al., 1983; Degen et al., 2009). Although this reduction in lipid digestibility may seem small on a percentage basis, lipids have 2.25 times greater ME than starch, and as a result, the magnitude of this reduction becomes significant on a caloric basis.

Minimizing the impact of dietary fiber on lipid digestibility, and increasing apparent total tract digestibility of dietary fiber represent two of the most significant opportunities for improving energy utilization in low-energy feedstuffs (Kerr and Shurson, 2013). We have conducted an extensive literature review and have summarized our current knowledge regarding the relationship between dietary fiber and lipid digestion and absorption using a simplified model (Figure 1). The first factor that reduces digestibility of fatty acids is a tight contact between lipids and dietary fiber, where lipids are contained in a fiber matrix that prevents lipase from degrading triglycerides into free fatty acids (Schneeman and Gallaher, 2001). Evidence for this effect is suggested by the lower digestibility of intact oil in corn germ compared with extracted oil (Kil et al., 2010, Kim et al., 2013). Secondly, fiber may also cause a reduction in fatty acid digestibility by increasing the passage rate of digesta, reducing meal retention time, and increasing excretion of undigested fatty acids (Ehle et al., 1982). Third, digesta viscosity is another physicochemical property of some types of fiber such as pectins and guar gum, which may reduce digestibility of cholesterol and fatty acids (Chen et al., 1979) by reducing their transfer through the unstirred water layer (Johnson and Gee, 1981). However, this mechanism has not been studied in pigs. Finally, dietary fiber has been shown to increase endogenous fatty acid losses in rats (Isaksson et al., 1983). Although endogenous losses of fat have been measured in growing pigs using fat-free diets (Bach-Knudsen and Hansen, 1991; Kil et al., 2010), no experiments have been conducted to measure the effect of fiber degrading exogenous enzymes on the endogenous losses of fatty acids from pigs fed DDGS diets.

Fiber is Poorly Understood in Swine Nutrition

The first challenge for improving the utilization dietary fiber is defining the most appropriate measure to characterize dietary fiber and its physiologic and nutritional impact in swine diets (Lee and Prosky, 1995). We believe that measuring TDF provides a more accurate measurement of all indigestible dietary carbohydrates than crude fiber, NDF, and ADF (Bach-Knudsen, 2001). Some swine nutritionists have suggested measuring individual monosaccharides that are part of non-starch polysaccharides (NSP) as a more appropriate fiber measure than TDF. However, measuring individual monosaccharides is expensive, time consuming, and difficult to use when assessing the energy contribution from these individual components due to their interactive effects with other carbohydrates and dietary constituents.

Appropriate exogenous enzymes could provide a significant improvement in obtaining more ME or NE from GE in reduced-oil DDGS. However, various factors contribute to the effectiveness of exogenous carbohydrase enzymes, when added to diets containing a feed ingredient (i.e. substrate) with a high concentration of TDF, such as DDGS. The effectiveness of exogenous enzymes is affected by three factors: 1) ability to improve nutrient digestion and absorption in the small intestine (i.e. apparent ileal digestibility of protein, fat, and starch), 2) ability to improve the extent of fermentation of TDF in the large intestine, and 3) mitigation of the negative impacts of factors such as changes in gut microbes, microbial fermentation products, and feed intake. As a result of the interrelationships of these factors, there can be several scenarios with different energy and nutrient uplift outcomes. These factors are affected by properties of the enzymes as well as properties of the substrate (i.e. reduced-oil DDGS).

Nutrient digestion and absorption in the small intestine of pigs fed high fiber diets is low because nutrients are "trapped" within a matrix of fiber that reduces the interaction between endogenous gastric and pancreatic enzymes and nutrients (Inborr et al., 1993; Le et al., 2013). In corn and corn DDGS, nutrients "trapped" within this fiber matrix consist mainly of insoluble arabinoxylans and cellulose linked to lignins (Jaworski, 2012). This effect has been demonstrated to reduce digestibility of acid hydrolyzed ether extract of intact corn oil (Kim et al., 2013).

It appears that the effect of nutrient entrapment in the fiber matrix varies among sources of DDGS. Apparent ileal digestibility (AID) of TDF is not homogenous among DDGS sources and ranges between 11.4 to 38.2% (Urriola et al., 2010a, b). Sources of DDGS with low AID of TDF also have less AID of crude protein (r = 0.56). This observation suggests that dietary fiber within a DDGS source with low (< 20%) AID of TDF is in a tight structural fiber matrix with low degradation and strong bonds between its components (Benamrouche et al., 2002). As a result, variability of AID of TDF among DDGS sources may be one of the factors that determine the effectiveness of exogenous carbohydrase enzymes in DDGS diets. It is possible that the compositional differences in dietary fiber among DDGS sources influence the effectiveness of carbohydrase enzymes for energy uplift, and may be greater among sources of DDGS with low AID of TDF (< 20%).

Apparent total tract digestibility of TDF among DDGS sources varies from 23 to 57% (Urriola et al., 2010b). This observation suggests that dietary fiber in DDGS sources with < 30% ATTD of TDF is structurally resistant to fermentation in the large intestine, and as a result, has a minimal contribution to digestible energy. Therefore, energy uplift of exogenous carbohydrase enzymes may be greater among DDGS sources with < 30% ATTD of TDF. Similarly, the contribution of energy uplift obtained from the addition of exogenous enzymes to DDGS will be proportionally greater if applied to a source of DDGS with ATTD of TDF < 30%.

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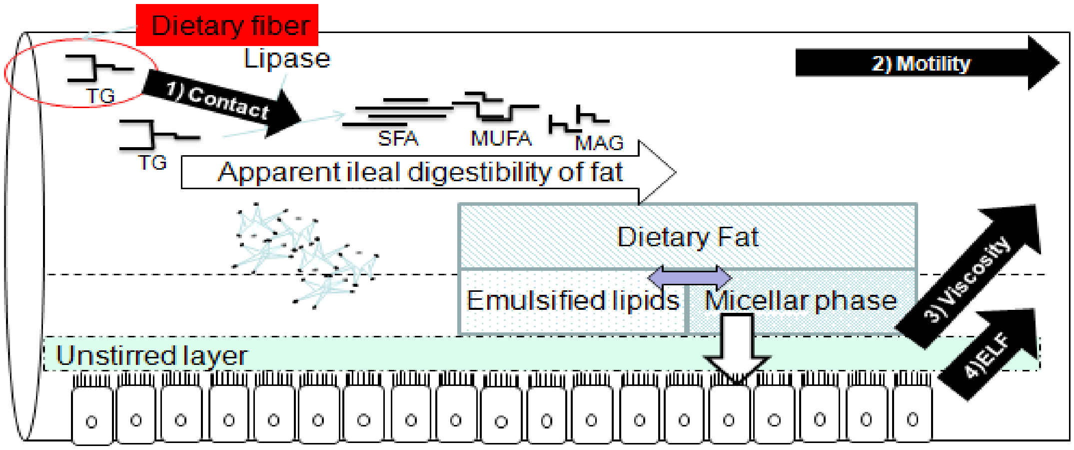
Table 1. Chemical composition (DM basis) of a single DDGS sample varies among laboratories (B.J. Kerr, personal communication).

	Lab 1	**Lab 2**	**Lab 3**	**Lab 4**
Dry matter, %	96.2	95.1	92.4	95.1
Crude protein, %	29.6	30.3	30.2	29.3
Crude fat, %	9.4	13.0	11.1	11.9
NDF, %	32.2	26.8	40.5	27.8
Ash, %	4.2	5.0	4.4	4.3

Table 2. Metabolizable energy ranking of reduced-oil DDGS sources and associated chemical components (Kerr et al., 2013).

DDGS Source	**ME, kcal/kg**	**Crude fat, %**	**NDF, %**	**Crude protein, %**	**Starch, %**	**Ash, %**
15	3,696	10.9	31.6	29.0	3.3	5.4
13	3,604	5.6	31.6	30.6	3.3	6.1
8	3,603	13.2	34.0	30.6	1.3	5.3
11	3,553	11.8	38.9	32.1	1.1	4.9
9	3,550	9.7	28.8	29.8	2.8	5.0
6	3,513	9.6	33.0	30.1	3.4	4.9
7	3,423	10.1	38.2	30.3	2.2	5.0
2	3,400	11.1	36.5	29.7	3.9	4.3
4	3,362	8.6	35.7	32.9	0.8	5.1
3	3,360	10.8	38.6	29.7	1.6	4.6
10	3,327	10.0	35.9	32.7	1.0	5.3
1	3,302	11.2	44.0	27.7	1.8	4.4
12	3,286	4.9	30.5	31.2	3.3	5.8
5	3,277	11.1	39.7	31.6	0.9	5.0
14	3,266	7.5	33.9	30.8	2.5	5.7

Figure 1. A simplified model of the effect of dietary fiber on digestibility of triglycerides (TG), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), monoacylglycerol (MAG), gut motility, viscosity of ileal digesta, and endogenous loses of fat (ELF).



TG: triglyceride; SFA: saturated fatty acids; MUFA= monosaturated fatty acids; MAG: monoacylglycerol; and ELF: endogenous losses of fat

Potential for Mannanase and Glucanase Enzymes in Swine Feeding Programs

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Summary

The mannan content in soybean meal and glucan content in distillers dried grains with solubles (DDGS) may have negative effects on nursery and grow-finish pig performance due to a feed induced immune response in the GI tract of the pig. In a 33-day nursery pig study, a heat tolerant mannanase enzyme treatment exhibited a marked improvement in body weight gain at the beginning of the trial that carried through to the end of the trial. Average final weight for the enzyme treatment was approximately 1 kg greater than the positive control and 1.6 kg greater than the same negative control diet without the enzyme. The diet containing mannanase with glucanase did not improve performance except when the DDGS content was increased to 20% of the diet. In a grower pig study, increasing levels of a β-1,3 glucanase linearly improved feed efficiency (4.8%) during the grower period and numerically improved ADG and final body weight in pigs fed corn-soybean meal diets containing up to 30% DDGS. These data demonstrate the performance response to a β-1,3 glucanase in combination with a native β-1,4 mannanase in the presence of high DDGS inclusion in swine diets. The glucanase response, however, seemed to be dose dependent on optimizing either ADG (lower doses) or G:F (higher doses). Based on these 2 studies, there are clear potential benefits to adding a heat tolerant β-1,4 mannanase and β-1,3 glucanase to swine diets, but swine diets may need to contain a minimum of approximately 15% soybean meal or 20% DDGS to elicit a response to the mannanase and glucanase enzymes, respectively.

Introduction

The swine feed industry has undergone some dramatic changes in the last few years brought about by increased biofuel production and the recent drought. Diets have higher inclusions of biofuel by-products as well as many other non-traditional feedstuffs. Successful use of by-products as an alternative to more expensive feed options is dependent upon a pig's ability to utilize the nutrients available in the by-products to maintain a similar growth rate. By-products often have similar energy content as traditional feedstuffs, but generally contain a higher fibrous fraction. Therefore, supplemental enzymes could potentially be used in a by-product based diet to improve nutrient digestibility and growth performance of pigs. Perhaps what is less understood is the difference in the negative impact of increased fiber between or among fiber fractions or sources and the impact of an enzyme that effectively breaks down the fiber fraction.

Soybean meal and some non-legume feed ingredients contain the soluble non-starch polysaccharide ß-galactomannan (ß-mannan; Hsiao et al. 2006) while dried distillers grains with solubles (DDGS) contains the non-starch polysaccharide β-glucan. The 1,4-β-D mannans and 1,3-β-D glucans of various types are recognized by the innate immune system by several Pattern Recognition Receptors including the serum protein mannose binding lectin, cell surface receptors, and others (DC-SIGN) on key immune system cells (Stahl and Ezekowitz,1998; Huang et al., 2009). The stimulation of the immune system to a pathogen response consists of several proliferation and formation factors of clonal, lymphocytes, lymphoid tissue maturation of Ig, and the synthesis of effector molecules (e.g., complement) and communication molecules (e.g., cytokines). The recognition to non-pathogens is commonly known as a PAMP (Pathogen Associated Molecular Pattern) or MAMP (Microbe Associated Molecular Pattern) and is influenced by the innate immune systems capability to recognize these compounds as non-self due to their molecular pattern. Most are accompanied by a systemic inflammatory response, including metabolic and cellular responses that can markedly impact nutrient partitioning among tissues (Klasing 2007; Gabler and Spurlock, 2008).

Methods

The objective of the studies was to evaluate the efficacy of a heat tolerant variant of β-D-Mannanase - commercially recognized as Hemicell® HT1.5x (Elanco Animal Health, Greenfield, IN) - to potentially mitigate the PAMP response initiated by the soluble substrate ß-galactomannan found in soybean meal and soybean hulls. Hemicell® HT1.5x β-D-Mannanase is an endohydrolyase enzyme that randomly cleaves within the 1,4-β-D mannan main chain of galactomannan, galactoglucomannan and mannan. A second objective was to evaluate a glucanase (Zymannase®; Elanco Animal Health, Greenfield, IN) that is an endohydrolyase enzyme which randomly cleaves within the 1,3-β-D glucan main chain of D-glucose polysaccharides found in DDGS and the 1,4-β-D mannan chain of galactomannan, galactoglucomannan, and mannan found in soybean meal. The differences between the mannanase sources in these two enzymes are in strain development, heat tolerance, pepsin resistance, proteolysis resistance, and operating pH.

Experiment 1: Nursery Pig Trial

One hundred forty-four weanling pigs (avg 17.9 days, 5.44 kg) were used in a 5-week growth trial to determine the effects of enzyme supplementation on feed intake, weight gain, and feed efficiency. A total of 24 pens were utilized for the trial, with 6 pigs per pen. Pigs were divided into pens based upon weight, sex, and litter origin. Pigs were blocked by initial body weight, sex and litter origin and randomly allotted to pens within block. Diets were randomly assigned to pens within a body weight block. Dietary treatments (Tables 1 and 2) were as follows:

1. Positive Control (PC); 3519, 3455, 3374, 3400 kcals/kg ME; by phase, respectively.
2. Negative Control (NC); T1 reduced by 100 kcals/kg primarily through a 2% reduction in added soy oil for phase 1 & 2 and choice white grease in phase 3 & 4.
3. NC + Hemicell-HT (HT) (Heat Stable Mannanase)
4. NC + Zymannase (ZYM) (Native Mannanase + Glucanase)

All diets contained added soybean hulls to increase the mannan content of the diets to levels similar to a 44% CP soybean meal. As the dietary phases advanced, the amount of DDGS increased in the diet from 5% to 20%. Pigs had ad libitum access to feed and water by means of a 5-hole self-feeder and a single nipple waterer in each pen. Pigs were weighed and feed intake recorded on days 0, 5, 12, 19, 26, and 33. Pigs were fed 4 dietary phases over 5 weeks. Phase 1 was days 0 to 5, Phase 2 was days 5 to12, Phase 3 was days 12 to 19, and Phase 4 was days 19 to 33.

Experiment 2: Grower Pig Trial

There were 252 pigs with an initial body weight of 26.0 ± 0.45 kg in the study. Six pigs were housed in each pen (0.70 m2/pig) with 7 pens/diet. All pens were mixed gender with pigs stratified by initial body weight and litter of origin in a randomized complete block design. Individual body weights and pen feed disappearance were recorded weekly. Pigs were fed in 2 dietary phases, each 3 weeks in duration. The amount of DDGS increased from 25 to 30% between the 2 phases. Dietary treatments (Table 3) were as follows:

1. Negative control (NC)
2. NC+ β-mannanase included at a rate of 0.16 MU/kg
3. T2 + β-glucanase included at a rate of 0.044 MU/kg
4. T2 + β-glucanase included at a rate of 0.088 MU/kg
5. T2 + β-glucanase included at a rate of 0.132 MU/kg
6. T2 + β-glucanase included at a rate of 0.176 MU/kg

Results

In experiment 1, the nursery study, overall ADG (days 0 to 33) was greater for enzyme T3 Hemicell® HT1.5x compared to all other treatments (P = 0.002; 363, 345, 396, 374 g/d, by treatment, respectively). This was a 15% and 8% improvement over the negative control for the heat tolerant mannanase and combination of native mannanase with glucanase (T3 and T4, respectively). Pigs fed enzyme dietary treatments (T3 and T4) had increased overall ADFI compared to the Positive and Negative Control treatments ($P = 0.056$) by 12% and 7%, respectively. Overall G:F tended to be different ($P < 0.10$) between the positive and negative control treatments with enzyme treatments being intermediate (0.736, 0.698, 0.717, 0.708, respectively). Final body weight for pigs fed T3 (Hemicell® HT1.5x) were 1.60 kg heavier ($P = 0.032$) than Negative Control fed pigs and tended to be greater than all other treatments ($P < 0.10$). These data demonstrate a growth performance response to Hemicell® HT1.5x and Zymannase® in the presence of up to 2.6% added soybean hulls and 20% DDGS inclusion in nursery diets.

In experiment 2, the grower pig study, overall ADG was greater ($P < 0.05$) for T3, T5, and T6, (β-1,3 glucanase), compared to T1 (negative control) with T2 and T4 being intermediate. Pigs fed enzyme dietary treatments T3 to T6 had linear reductions in overall ADFI (linear, $P < 0.04$) with overall G:F linearly ($P < 0.01$) improved with increasing levels of β-1,3 glucanase (0.400, 0.397, 0.407, 0.405, 0.416, 0.414, respectively). Final body weight for pigs fed T3 (enzyme activity ratio of 0.044/0.16 β-1,3 glucanase:β-1,4 mannanase; respectively) was 1.70 kg greater ($P < 0.05$) than T1 (NC) and final body weight for T5 & T6 tended to be greater than T1 ($P < 0.10$). If performance improvements in body weight gain could be maintained throughout the grow-finish period this would extrapolate to market weights being 3.6 kg heavier per pig. It is clear that increasing levels of β-1,3 glucanase linearly improved feed efficiency (4.8%) during the grower period and numerically improved ADG and final body weight with corn-soybean meal diets containing up to 30% DDGS.

These data demonstrate the potential performance response to a β-1,3 glucanase in combination with a β-1,4 mannanase in the presence of high DDGS inclusion swine diets. The response to glucanase however seems to be dose dependent on optimizing either ADG (lower doses) or G:F (higher doses) in grower pigs.

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Table 1. Phase 1 and Phase 2 Nursery Diets for Experiment 1.

	Phase 1 (d 0-5)		Phase 2 (d 5-12)	
Ingredient, %	**PC**	**NC**	**PC**	**NC**
Corn	31.145	33.265	32.545	34.705
DDGS	0.000	0.000	5.000	5.000
Soybean meal, 48% CP	15.000	15.000	18.000	18.000
Soybean hulls	1.650	1.650	1.980	1.980
Soybean oil	5.000	3.000	4.000	2.000
Limestone, ground.	0.680	0.690	0.620	0.630
Monocalcium phosphate	0.380	0.360	0.390	0.370
Vitamin premix[1]	0.250	0.250	0.250	0.250
Trace mineral premix[2]	0.175	0.175	0.175	0.175
Phytase[3]	0.100	0.100	0.100	0.100
Salt	0.250	0.250	0.250	0.250
Plasma protein	6.500	6.500	2.500	2.500
Soy protein concentrate	3.400	3.300	3.500	3.350
Fish meal	4.000	4.000	4.000	4.000
Dried whey	30.000	30.000	25.000	25.000
Lysine-HCl	0.110	0.110	0.250	0.250
DL-Methionine	0.180	0.170	0.180	0.180
L-Threonine	0.030	0.030	0.090	0.090
L-Tryptophan	0.000	0.000	0.020	0.020
Rabon[4]	0.025	0.025	0.025	0.025
Zinc oxide	0.375	0.375	0.375	0.375
Carbadox-10[5]	0.250	0.250	0.250	0.250
Corn treatment premix[6]	0.500	0.500	0.500	0.500
Total	100.00	100.00	100.00	100.00
Calculated Composition				
ME, kcal/kg	3519.3	3419.7	3455.1	3355.7
Crude protein, %	23.57	23.67	22.99	23.08
Total lysine, %	1.682	1.684	1.620	1.620
TID lysine, %	1.500	1.500	1.452	1.450
Calcium, %	0.850	0.851	0.801	0.801
Available phosphorus, %	0.602	0.600	0.552	0.549

[1] Vitamin premix provided per kg of diet: Vitamin A, 6,615 IU; Vitamin D3, 661.5 IU; Vitamin E, 44.1 IU; Menadione (Vitamin K), 2.205 mg; Vitamin B_{12}, 0.039 mg; Riboflavin, 8.82 mg; d-Pantothenic Acid, 22.05 mg; Niacin, 33.075 mg.

[2] Trace mineral premix provided per kg of diet: Iron, 121.25 g; Zinc, 121.25 g; Manganese, 15.0 g; Copper, 11.3 g; Iodine, 0.46 g; Selenium, 0.30 mg.

[3] Phytase provided per kg of diet: 600 PU

[4] Rabon provided per kg of diet: 0.019 g of tetrachlorvinphos as a larvicide.

[5] Carbadox-10 provided per kg of diet: 55 ppm Carbadox

[6] Corn treatment premixes contained finely ground corn and the enzyme treatments as follows: PC and NC had only finely ground corn; HT had 36 MU/ton (0.04 MU/kg) of heat stable mannanase; Zym had 64 MU/ton of native mannanase and 45 MU/ton (0.05 MU/kg) glucanase.

Table 2. Phase 3 and Phase 4 Nursery Diets for Experiment 1.

	Phase 3 (d 12-19)		Phase 4 (d 19-33)	
Ingredient, %	**PC**	**NC**	**PC**	**NC**
Corn	42.075	44.475	49.050	51.475
DDGS	10.000	10.000	20.000	20.000
Soybean meal, 48% CP	23.500	23.300	20.980	20.780
Soybean hulls	2.590	2.590	2.290	2.290
Swine grease	3.200	1.000	3.210	1.000
Limestone, ground	1.190	1.200	1.470	1.470
Monocalcium phosphate	0.840	0.830	0.460	0.450
Vitamin premix[1]	0.250	0.250	0.250	0.250
Trace mineral premix[2]	0.175	0.175	0.175	0.175
Phytase[3]	0.100	0.100	0.100	0.100
Salt	0.300	0.300	0.350	0.350
Soy protein concentrate	4.000	4.000	0.000	0.000
Dried whey	10.000	10.000	0.000	0.000
Lysine-HCl	0.350	0.350	0.460	0.460
DL-Methionine	0.140	0.140	0.085	0.080
L-Threonine	0.120	0.120	0.135	0.135
L-Tryptophan	0.020	0.020	0.035	0.035
Rabon[4]	0.025	0.025	0.025	0.025
Zinc oxide	0.375	0.375	0.000	0.000
Carbadox-10[5]	0.250	0.250	0.250	0.250
Copper sulfate	0.000	0.000	0.075	0.075
Banmith dewormer 48[6]	0.000	0.000	0.100	0.100
Corn treatment premix[7]	0.500	0.500	0.500	0.500
Total	100.00	100.00	100.00	100.00
Calculated Composition				
ME, kcal/kg	3374.1	3274.4	3400.1	3300.2
Crude protein, %	21.92	22.02	20.22	20.32
Total lysine, %	1.463	1.463	1.323	1.324
TID lysine, %	1.301	1.301	1.150	1.150
Calcium, %	0.801	0.803	0.753	0.752
Available phosphorus, %	0.452	0.452	0.351	0.351

[1] Vitamin premix provided per kg of diet: Vitamin A, 6615 IU; Vitamin D3 661.5 IU; Vitamin E, 44.1 IU; Menadione (Vitamin K), 2.205 mg; Vitamin B_{12}, 0.039 mg; Riboflavin, 8.82 mg; d-Pantothenic Acid, 22.05 mg; Niacin, 33.075 mg.

[2] Trace mineral premix provided per kg of diet: Iron, 121.25 g; Zinc, 121.25 g; Manganese, 15.0 g; Copper, 11.3 g; Iodine, 0.46 g; Selenium, 0.30 mg.

[3] Phytase provided per kg of diet: 600 PU

[4] Rabon provided per kg of diet: 0.019 g of tetrachlorvinphos as a larvicide.

[5] Carbadox-10 provided per kg of diet: 55 ppm Carbadox

[6] Banmith provided per kg of diet: 0.882 g of pyrantel tartrate as a dewormer.

[7] Corn treatment premixes contained finely ground corn and the enzyme treatments as follows: PC and NC had only finely ground corn; HT had 36 MU/ton (0.04 MU/kg) of heat stable mannanase; Zym had 64 MU/ton (0.071 MU/kg) of native mannanase and 45 MU/ton (0.05 MU/kg) glucanase.

Table 3. Basal diet formulations for the grower pig glucanase titration study for Experiment 2.

	Phase 1 (- Enz) (d 0-21)	Phase 2 (-Enz) (d 21-42)
Corn, %	54.63	54.53
Soybean meal, %	16.72	12.35
DDGS, %	25	30
Choice white grease, %	0.50	0.50
Lysine HCl, %	0.23	0.22
Premix, %	0.25	0.25
Remaining ingredients, %	2.67	2.15
Calculated Composition		
ME, kcal/kg	3,323	3,329
SID lys	0.89	0.79
SID met+cys:lys	0.66	0.74
SID trp:lys	0.17	0.17
SID thr:lys	0.62	0.68
SID ile:lys	0.72	0.77
SID val:lys	0.86	0.94

Table 4. Effect of mannanase and glucanase on overall (d 0-33) nursery pig growth performance for Experiment 1.

	T1 (Pos)	T2 (Neg)	T3 Hemicell HT	T4 Zymannase	SE	Diet P <
Enzyme activity	**--**	**--**	**0.04 MU/kg**	**0.08/0.10 MU/kg**		
ADG, g/d	363^{b}	345^{b}	396^{a}	374^{b}	7.5	0.002
ADFI, g/d	495^{by}	495^{by}	554^{ax}	529^{aby}	16.2	0.056
G:F	0.736	0.698	0.717	0.708	0.0162	0.332
F/G	1.361	1.435	1.397	1.416	0.0308	0.302
Final BW, kg	17.45^{aby}	16.92^{by}	18.51^{ax}	17.44^{aby}	0.343	0.032

Meyers et al., 2013.

Table 5. Effect on growth rate with increasing glucanase relative to a constant level of mannanase in grower pig diets from day 0-42 for Experiment 2.

	T1 (NC)	T2	T3	T4	T5	T6	SE	Lin.Glu *P* <	Contrast, T1 to T2
Activity ratios	**--**	**--/0.16**	**0.044/0.16**	**0.088/0.16**	**0.132/0.16**	**0.176/0.16**			
ADG, g	789	802	825	810	818	819	9.08	0.35	0.32
ADFI, kg	1.97	2.02	2.04	2.00	1.97	1.98	0.025	0.04	0.16
G:F	.400	.397	.407	.405	.416	0.414	0.006	0.01	0.64
F/G	2.50	2.52	2.46	2.48	2.41	2.41	0.034	0.01	0.67
Final BW, kg	59.51	60.24	61.24	60.56	60.74	60.78	0.41	0.61	0.23

Rambo et al., 2012.

Copper Source and Concentration Effects on Intestinal Gene Expression and Mineral Balance in the Pig

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Summary

Previous data from our laboratory suggests that Cu from Cu-proteinate can be absorbed through the peptide transporter PepT1 in addition to classically suggested routes of Cu absorption via Ctr1 or DMT1. A reduction in intestinal Cu was observed in subsequent experiments when Cu-triglycinate was added in conjunction with a known competitive inhibitor of PepT1. Therefore, we hypothesized that if Cu from Cu-proteinate could be absorbed while still attached to a di- or tri-peptide that it would also be processed and/or handled differently once absorbed, resulting in alterations in gene expression and body mineral pools. Therefore, this experiment was designed to investigate the effects of Cu source (Cu-sulfate vs. Cu-proteinate) and concentration (0, 4, 25 and 125) on gene expression and mineral concentrations in the intestine, liver, kidney, serum and bile. Using a porcine affymetrix gene array we observed 71 genes that were differentially regulated by Cu-proteinate and Cu-sulfate. Copper source x concentration interactions were also observed for several mineral concentrations in the liver, serum, and bile. This data clearly demonstrates that the source of copper fed and the concentration of copper fed can impact the status of other minerals in a variety of tissues and can have significant impacts on gene expression of a multitude of genes involved in signal transduction, cell adhesion and cell cycle regulation.

Introduction

Copper is an essential trace element added to swine diets. The Cu requirement is roughly 5-6 ppm (NRC, 1998), but the swine industry typically feeds ~ 25 ppm Cu, or at pharmacological concentrations (125-250 ppm Cu) to provide an antibiotic-like response which stimulates growth. This can result in increased copper excretion. Historically, copper sulfate has been the mineral source for adding dietary Cu. However, numerous other Cu sources have entered the market in recent years. Minerals can be added to the diet in a variety of forms, including as inorganic salts or chelated/complexed with organic ligands, which are often referred to as "organic minerals". These organic mineral sources can be chelates (ring forming around mineral) or complexes (no ring around mineral, but covalently attached). Sources include mineral AA complexes, mineral AA chelates, metal AA chelates, metal polysaccharide complexes and metal proteinates (~10 amino acids in length). The inherent chemical variations between organic and inorganic mineral sources may result in different bioavailabilities and possibly different routes of absorption (Ashmead, 1993; Aldridge et al., 2007).

In order to evaluate intestinal absorption of organic and inorganic sources of Cu, several pilot studies were conducted in modified Ussing chambers to explore differences in ion flux (Aldridge et al., 2007). Short circuit current (indicative of active transport)was 252% higher in poultry when challenged with Cu proteinate versus Cu sulfate, and 300% in swine jejunum (unpublished data). An increased change in short circuit current occurred when Cu proteinate was added, above $CuSO_4$, which was likely indicative of increased active Cu absorption, or as a result of AA/peptide movement or both. Additionally, 52% more Cu was located in intestinal tissue 20 min post-challenge in tissue challenged with Cu proteinate compared to $CuSO_4$. These results led to the conclusion that mineral-peptides may be transported through the di- and tri- peptide transporter, PepT1. As a result we hypothesized that Cu from different sources maybe handled differently once absorbed resulting in changes in gene expression and changes in mineral concentrations in various tissues/pools in the body. To test this hypothesis, a 2-wk feeding trial was conducted to investigate the effects of Cu source and dietary Cu concentration on gene expression and mineral concentrations.

Materials and Methods

All experimental protocols were approved by the Purdue University Animal Care and Use Committee.

Animals and experimental design

Seventy crossbred barrows were weaned at 20 ± 1 d of age and used to investigate the effects of Cu source (Copper sulfate vs. Cu proteinate) and concentration (0, 4, 25, and 125ppm) on gene expression and body mineral pools.

Feeding and tissue sampling

Before the start of experimental diets, pigs were fed a commercial, pelleted starter diet for 3 d and then a basal diet containing no Cu supplement for 4 d. On d 7 post-weaning, pigs were moved to individual pens equipped with nipple waterers and trough feeders. Pigs were fed the same diet (no Cu supplement), at 9% of metabolic BW ($BW^{.75}$ per d) for 4 d in 2 daily feedings. They were then weighed (average BW 8.07 kg) and randomly assigned to 1 of 7 dietary treatments (Table 1):

0 ppm supplemental Cu
4 ppm Cu from Cu proteinate
4 ppm Cu from Cu sulfate
25 ppm Cu from Cu proteinate
25 ppm Cu from Cu sulfate
125 ppm Cu from Cu proteinate
125 ppm Cu from Cu sulfate

Diets were supplied in 2 daily feedings at 9% of metabolic BW. Pigs remained on dietary treatments for 14 d. After 7 d, they were reweighed and feed allowances were adjusted accordingly. On d 14 of the experiment, pigs were euthanized by asphyxiation with CO_2 followed by exsanguination. Proximal jejunal tissue was collected for gene expression analyses (Microarray and real-time RT-PCR) and tissue from the proximal jejunum, kidney, liver and bile were collected for ionomic analysis by ICP-MS.

Results

There were no interactions between copper source and dietary Cu concentration on growth performance. Overall, ADG and Gain:Feed were improved (Table 2) numerically as Cu concentration in the diet was increased, but these improvements only approached a trend ($P < 0.12$). Similar to performance data, there were no interactions between Cu source and concentration on tissue Cu concentrations. Intestine, liver, kidney and serum Cu concentrations were similar for 0-25 ppm supplemental Cu, but increased ($P < 0.001$) when 125 ppm Cu were fed, indicating that 125 ppm supplemental Cu is in excess of what the body can homeostaticly regulate (Figure 1). Copper concentration in the bile linearly increased ($P < 0.001$) with increasing Cu concentration in the diet.

No interactions between Cu source and concentration were observed for mineral concentrations in the proximal jejunum and kidney. Increasing dietary supplementation of Cu resulted in linear ($P < 0.05$) increases in Fe and Cd concentrations in both the intestine and kidney. In the intestine Mg and Mo concentrations were also increased ($P < 0.05$) with increasing dietary Cu supplementation, and Zn tended ($P < 0.10$) to increase. Whereas in the kidney, linear ($P < 0.05$) increases in Se, Mn, and Co concentration were observed as dietary Cu increased in addition to Fe and Cd.

Cu source x Cu concentration interactions were observed for some minerals in the liver, serum and bile. However, no consistent pattern emerged. Calcium was the only mineral where an interaction or a trend for an interaction was observed in the liver, serum and bile. However, the interaction seemed to behave differently between locations and no clear-cut conclusions could be made.

Of the ~23,000 possible transcripts present on the porcine genome array, 13,577 were expressed in the proximal jejunum. Compared with the control, a total of 554 genes were differentially expressed with the $CuSO_4$ supplement and 396 with Cu proteinate. Of these transcripts, 71were mutually expressed by both Cu sources ($P < 0.05$, FC > 1.2). Of these 71 genes, 29 were upregulated and 42 were downregulated. Absolute fold changes (FC) between supplemented diets and the unsupplemented diet ranged from 1.2 to 2.1. These fold changes may seem modest, but slight changes in gene expression have been shown to shift cellular functions (Wu et al., 2012). Real-time PCR validated ($P < 0.05$) mRNA levels obtained by microarray analysis for AKAP3, AQP11, PECR, and SERPINA1, using the housekeeping gene RNF5 as a reference (Table 3).

Feeding Cu from Cu proteinate versus $CuSO_4$ for 14 d resulted in 214 genes, which were differentially expressed ($P < 0.05$ and FC > 1.3). Of these transcripts, 142 genes were down regulated and 72 were up-regulated, for Cu proteinate compared to $CuSO_4$ fed pigs. Absolute fold changes ranged from to 1.3 to 3.59. The 214 genes differentially regulated by Cu proteinate and $CuSO_4$ were characterized as 2 ligands, 4 phosphatases, 7 proteases, 9 receptors, 13 kinases, 22 transcription factors, 31 enzymes, and 126 'other' genes not classified in these categories.

Discussion

There has been conflicting data regarding the bioavailability of organic and inorganic sources of Cu in swine diets. In the rat, organic Cu sources have been reported to be absorbed faster than inorganic forms (Kirchgessner and Grassman, 1970; Grassman and Kirchgessner, 1974), and tissue mineral concentrations from several species have been reported to be greater when organic minerals were supplemented to the diet (Ashmead, 1985; Du et al., 1996; Glover and Wood, 2008). Previous data for our lab indicates that PepT1 provides an additional route of enterocyte absorption for Cu(Gly)3, but not for CuSO4 or Cu(Gly)4. This makes sense as both CuSO4 and (Gly)4 are not PepT1 substrates, but the tri-peptide (Gly)3 is.

In the current experiment, growth performance was not different between Cu sources, althought the effect of increasing Cu concentrations in the diet on ADG and Gain:Feed approached a positive trend. The lack of a significant performance response is not surprising given the short duration trial coupled with the fact that animals were limit fed. Hernadez et al. (2008) reported only improvements in feed conversion when increasing concentrations of Cu proteinate (10, 30, 50 mg/kg) were fed over a period of 49 d, but not over 21 d. In a review of the human literature, Harvey et al. (2009) suggested that a 4-wk duration of Cu supplementation was needed to determine Cu-biomarker changes, such as serum Cu, which may prove to be especially true when smaller sample sizes are used in the pig.

Antagonistic and protagonistic Mineral-Mineral interactions can occur at various points in the digestive, absorptive and metabolic processes in the body. Minerals can bind to one another in the gastrointestinal tract forming insoluble salts, and thereby decreasing the bioavailability of each other. They can compete for absorption through the same transport system, they can compete for binding sites on chaperon proteins once absorbed, they can alter gene expression of know binding proteins, etc. While it is known that these interactions can occur and have significant impacts, in general we still tend to study 1 mineral at a time. In this experiment we attempted to determine the impact of Cu source and dietary concentration on mineral concentrations in the intestine, liver, kidney, serum and bile. Interestingly, we did not observe a negative impact of Cu on any mineral concentrations measured. Instead, when effects were observed, mineral concentrations remained the same or increased with increasing dietary Cu. Some Cu source x dietary Cu concentrations effects on mineral concentrations were observed in the liver, serum and bile. These were most pronounced in the bile where concentrations of several minerals from pigs fed 25 ppm Cu from Cu proteinate were higher than those fed 25 ppm Cu from Cu sulfate, but the opposite was observed at 125 ppm Cu. This indicates differential regulation of mineral excretion via the bile between sources and concentrations. It is important to remember that the mineral concentrations in each pool represent the concentration of each mineral at a set point in time and do not necessarily reflect the bioavailability of that mineral and/or changes over time. Certainly effects would likely be different if for instance the trial had lasted 4 wk instead of 2.

With advances in biotechnology, there are increasing tools available to the swine industry to help understand mechanisms at a more basic level. The key of course is for this more basic information to help lead to applied changes. In an attempt to better understand applied differences between Cu sources we investigated gene expression in the proximal jejunum using an affymetrix porcine gene chip, and compared 0 ppm supplemental Cu with 25 ppm supplemental Cu from Cu sulfate or Cu proteinate. We focused our attention on genes that were regulated by added copper regardless of source and those that were differentially regulated between the 2 sources. There were a total of 71 genes that were up (n = 29) or down (n = 42) regulated by the addition of 25 ppm copper from either source. When comparing sources (copper sulfate vs. copper proteinate), we observed 214 genes that were differentially expressed. These genes will hopefully provide better insight and/or mechanisms into observed differences between copper sources. Interestingly, proposed copper transporters CTR1 and DMT1 were not differentially regulated between sources. Using pathway analysis bioinformatics tools it appears that differences may exist between Cu sources in their ability to regulate signal transduction, cell adhesion and cell cycle regulation via regulation of key genes. However, definitive conclusions are premature at this point as this experiment represents one small snap-shop at one point in time.

Based on our data, Cu from copper proteinate can be absorbed while still attached to a di- or tri-peptide via the peptide transporter PepT-1. While this is a possible route of absorption, our data does not allow us to determine what proportion of Cu is absorbed via this route. However, we did observe differences between Cu sources in their ability to alter mineral pools within the body and in their ability to regulate gene expression. These differences could simply be due to differences in the bioavailability of Cu between sources or they could be the result of how the Cu is handled, processed and trafficked once absorbed.

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Table 1. Composition of Cu basal diet.

Ingredients	**% Diet**
Ground Corn	52.55
Whey	5.63
Soybean Meal, 47.5% CP	5.64
Lactose	18.07
Spray dried plasma	5.00
Casein, dried	7.41
Soybean Oil	4.01
Limestone	0.28
Monocalcium phosphate	0.24
Lysine HCL	0.13
DL-Methionine	0.07
L-Threonine	0.03
L-Tryptophan	0.05
Se 600	0.05
Cellulose[1]	0.13
Salt	0.35
Vitamin premix[2]	0.25
Trace mineral premix[3]	0.13
Calculated composition of diet	
ME, kcal/kg	3334
CP, %	16.76
Lys, %	1.35
Met, %	0.44
Met. + Cys., %	0.76
Trp, %	0.24
Thr., %	0.86
Ca, %	0.80
Avail. P%	0.40
Cu, mg/kg	3.73
Zn, mg/kg	15.34
Mn, mg/kg	6.17
Fe, mg/kg	36.27

[1] The basal diet contained cellulose, with additional diets replacing cellulose with 4, 25 or 125 ppm Cu from Cu proteinate or $CuSO_4$.

[2] Supplied per kilogram of premix: 2,640,000 IU Vitamin A; 264,000 IU Vit. D_3; 17,600 IU Vit.E; 880 mg Menadione; 14.5 mg Vit. B_{12}; 13,200 mg Niacin; 8,800 mg Pantothenic Acid; 3,520 mg Riboflavin.

[3] Supplied per kilogram of diet: 6 mg Mn; 150.0 mg Zn; 150.0 mg Fe; and 0.21 mg I.

Table 2. Effect of Cu concentration on ADG and Gain:Feed[1].

	Cu Concentration, mg/kg				SEM	P Value	
	0	**4**	**25**	**125**		**Lin[2]**	**Quad[3]**
ADG, g/d							
d 1-14	203.6	218.5	220.9	225.9	7.0	0.12	0.35
Gain:Feed							
d 1-14	0.55	0.58	0.59	0.60	0.02	0.11	0.19

[1] Each treatment represents 10 pigs.
[2] Linear contrast based on analyzed dietary Cu values.
[3] Quadratic contrast based on analyzed dietary Cu values.

Table 3. RT-PCR validation utilizing Ring Finger 5 (RNF5) as housekeeping gene. A simple t-test was used from SigmaPlot to determine P values. For both Cu sources, the fold change was compared against the control diet containing no supplemental Cu (n = 10).

	Microarray Results Fold Change			RT-PCR Results Fold Change		
Gene	**Cu Proteinate**	**$CuSO_4$**	**P-value**	**Cu Proteinate**	**$CuSO_4$**	**P-value**
AQP11: Aquaporin	1.33	1.3	<0.03	1.76	1.64	0.02
SERPINA1: Serpin peptidase inhibitor	-1.74	-2.08	<0.01	-1.34	-1.74	0.05
PECR: peroxisomal trans-2-enoyl-CoA reductase	-1.67	-1.76	<0.0001	-1.64	-1.94	<0.005
AKAP3:A-kinase anchor protein 3	1.57	1.99	<0.01	1.47	1.73	0.05

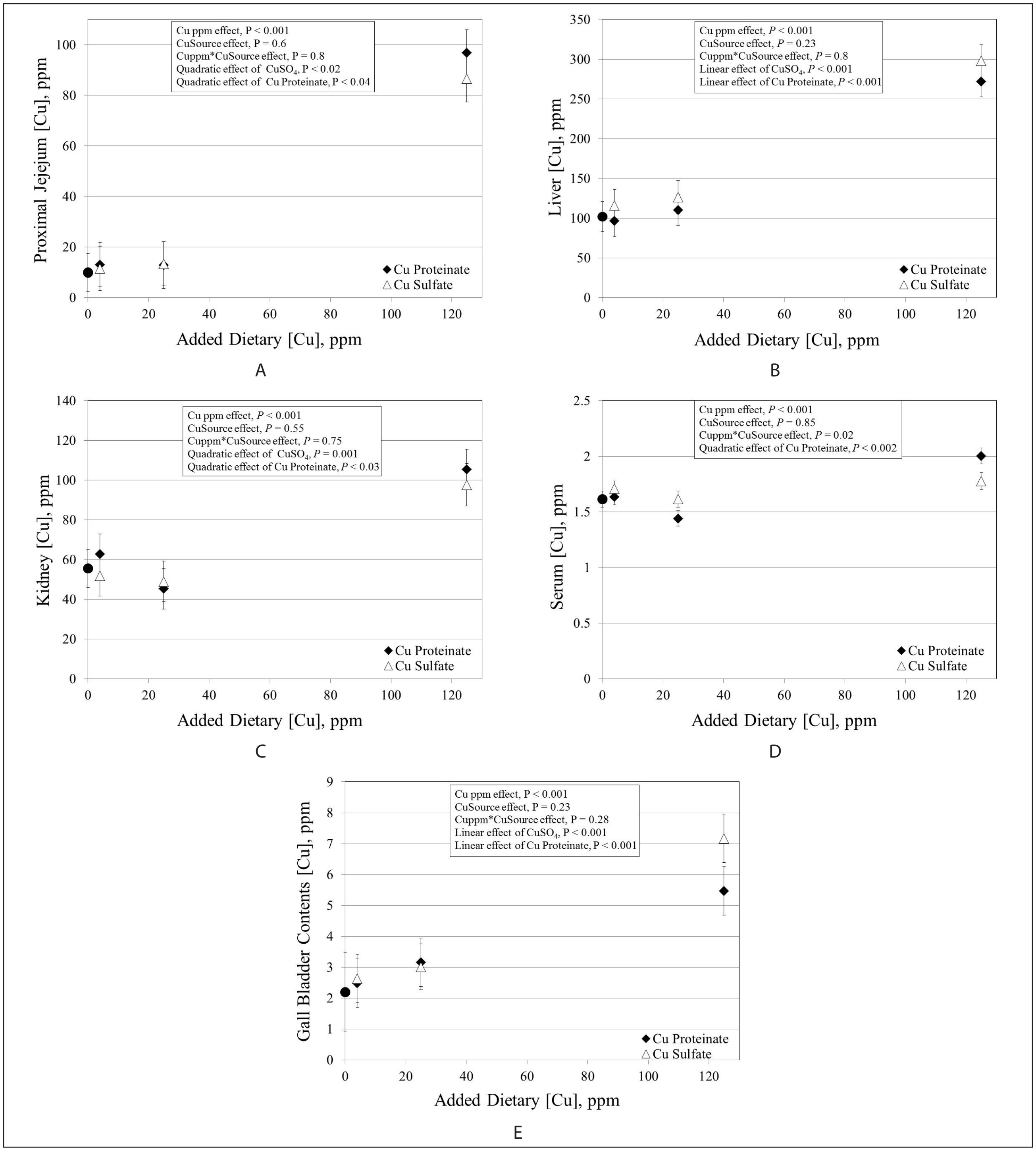

Figure 1. Effect of supplementing 0, 4, 25 or 125 ppm Cu from either Cu proteinate or $CuSO_4$ for two weeks on Cu concentration in the proximal jejunum (A), liver (B), kidney (C), serum (D) and gall bladder contents (dry basis) in young pigs. Values reported represent Least Square means.

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