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Midwest Swine Nutrition Conference

Schedule of Presentations

8:15 a.m	Registration
9:00	Welcome and Introductions *Dennis Liptrap, Ralco Animal Nutrition*
9:05	The State of the U.S. Animal Protein Sector *Pablo Sherwell, Head of Rabo Research Food and Agribusiness NA, Rabobank*
9:50	Potential Nutritional Considerations to Prevent Sow Mortality *Casey Bradley, DSM Nutritional Products and Monique Pairis-Garcia, The Ohio State University*
10:25	Break
11:00	Impact of Dietary Amino Acid Balance on Nitrogen and Energy Efficiency in Sows: From Theory to Practice *Nathalie Trottier, Michigan State University*
11:30	Copper Levels and Sources for Sows *Merlin Lindemann, University of Kentucky*
12:00	Lunch
1:00	Development and Nutritional Value of Advanced Soybean Products Used in Diets for Young Pigs *Hans H. Stein, University of Illinois*
1:35	Nutritional Regulation of Piglet Gut Health *Sheila Jacobi, The Ohio State University*
2:10	Break
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3:15	Strategic Therapeutic Antibiotic Use Compared to the Challenge of Not Using Antibiotics for Growing Pigs *Dean Boyd, The Hanor Company*

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The State of the U.S. Animal Protein Sector

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The U.S. animal protein sector is going through an inflection point. After a few years of positive margins, the sector is moving to an economic downturn. Hogs, in particular, are shifting to the negative side, and this scenario is likely to deteriorate faster and deeper than previously anticipated.

This inflection point in the industry comes after several subsectors began investing and expanding around the same time in 2015/16. This expansion is the natural result of stable domestic meat consumption combined with strong exports, positive margins, and limited production capacity

However, the economic scenario for the sector is changing as there are many factors that came into play concurrently.

First, the expansion is tangible in almost every protein (except for turkey), and it is creating an oversupply and, consequently, more competition among meats. This year, beef and pork production will grow over 4 percent respectively, poultry only around 1.5 percent, but all in all meat production will increase around 3.6 percent. In addition, plans to keep expanding in 2019 are (at least at this point) on their way.

Second, new packing plants are facing tremendous challenges securing adequate labor. The boost in the economy and the increase in labor demand in other industries, such as energy, construction, restaurants, etc., is leaving the meat industry with few options. Changes in demographics and immigration laws have only made the situation worse.

Third, a trade war with two of the most important U.S. meat customers, Mexico and China, is complicating the allocation of additional supplies. Moreover, since there is not a timeline on how long this is going to last, planning uncertainty is rising. As long as these tariffs are in place, other competitors such as Brazil, Europe, and Canada will gain market share in traditional U.S. export markets.

As a result of the above, we anticipate animal protein margins to keep declining until a more balanced market is reached. However, a correction in the market will take time as investments in the industry have only recently been made. In fact, we expect that margins will deteriorate further before they recover. The situation for packers could be more controllable as long as they are able to manage margins.

The presentation will conclude with our mid-term outlook for the sector and discuss some challenges and opportunities as we move forward.

Potential Nutritional Considerations to Prevent Sow Mortality

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Summary

Sow mortality has always been a challenge for the swine industry, but there has been a spike in sow mortality over the last few years, with limited understanding of the root causes. Furthermore, the incidences of sow uterine and rectal prolapses have also substantially increased within this time frame. It is well documented that lameness is also a leading cause of mortality, and most likely the predominant or secondary reason for culling in sow herds.

Within human medicine, Pelvic Floor Disorder and/or vaginal prolapses are also anticipated to steadily increase in next few decades; thus, there is substantial research dedicated to understanding this similar issue we are observing in sows with prolapses. The main characteristics identified in human subjects are impaired collagen and elastin metabolism in the supporting ligaments and muscle architecture. Furthermore, metabolic stressors such as mycotoxin contaminated feeds and/or oxidative stress can alter nutrient requirements of animals and potential hormonal responses within the sow. For example, zearalenone is known to have estrogen like responses that could cause laxity issues within ligaments like that found in human medicine, resulting in injury and thus lameness or uterine ligament support.

Vitamin C is an essential component in collagen and elastin metabolism, but a dietary requirement has not been established for swine because they are generally considered capable of synthesizing enough on their own. Additionally, there is a considerable amount of data indicating that the greater bioavailability of certain trace minerals, especially organic vs. inorganic sources, may reduce lameness and mortality in sow herds. In humans and swine, there is mounting evidence that glycine, a "nonessential" amino acid, is likely deficient for optimizing certain metabolic pathways, including collagen formation, especially during pregnancy. Thus, the objective of this paper is to present novel perspectives from human medicine, and discuss the potential for nutrients that are often overlooked in today's nutritional programs for sows.

Introduction

Sow mortality has always been a concern in sow herds, and any percentage of sow mortality presents challenges for all management levels. It is well known that replacement gilts do not start becoming profitable until their third or fourth parity within a system. Thus, increasing discussions around rising sow mortality rates in the USA over the last years has involved nearly all segments of the industry. Nevertheless, the real question remains largely unanswered: What are the primary underlying causes for the apparent increase in sow mortality and, ultimately, what can we focus on with interventions?

Hindering our ability to define strategies to combat mortality, there is limited information on the precise reasons that sows are dying. This is largely due to imprecise record keeping and the lack of expertise and time to conduct necropsies on farms. In 2007, Sanz et al. assessed sow mortality in a 5,200-sow unit over a 20-wk period in which all deaths were necropsied. The authors reported that 47% of sows were euthanized, 28% were expected deaths, and 25% died unexpectedly. Locomotion issues were the main reason for sow mortality in all stages of production, with arthritis and osteochondrosis-dyschondroplasia as the main lesions found. Other reasons for mortality included: prolapses, farrowing difficulty, gastric ulcers, and circulatory or respiratory problems.

Most recently, Dr. Jeremy Pittman of Smithfield Foods discussed the rise in mortality in sows (as reported by Day, 2017) and the dramatic increase in prolapses. He also reported a long list of potential factors: mycotoxins, hypocalcemia, vitamin deficiency, use of phytase, litter sizes, constipation, genetics, change in estrogen and/or other hormone levels, oxidative stress, meal vs. pelleted feed, high and rapid feed intake, bloating, coughing, piling, housing design, diarrhea, colitis, and excess lysine. Without any clear solutions to the current mortality problems, the objective of this paper is to discuss potential similarities with human medicine and the possibilities for nutritional interventions.

Uterine Prolapses—Human Medicine Perspective

Pelvic Floor Disorder and vaginal and/or uterus prolapses have also been a problem in human medicine. Multiple-pregnancies are known as one of the main correlating factors for these issues in women; however, with modern advances in science, these problems have been studied in greater detail. Even in humans, the incidence of these cases continues to increase, and are estimated to increase by 45% or double in frequency over the next 30 years, which may be related to the aging population (Luber et al, 2001).

Swine as a Biological Model for Humans

There are two main supportive tissues for the reproductive tract, supportive ligaments and muscles. The pelvic support ligaments, cardinal and uterosacral ligaments (Figure 1), suspend the pelvic organs to the pelvic side walls over the levator plate, while the pelvic floor muscles close the urogenital hiatus, allowing the organs (uterus, bladder, rectum) to rest on top of them. Work conducted at Virginia Tech University, Tan (2015) dissected the tissues of sows to better understand the mechanical properties of the supportive ligaments and how they would compare to humans for use as a potential biological model. He reported finding loose connective tissue in all specimens, with a larger amount of ground substance in the left cardinal ligament (**LCL**), while the right cardinal ligament (**RCL**) contained a larger number of smooth muscle fibers and adipose cells. Within the uterosacral ligament (**USL**) there was dense connective tissue, characterized by considerable amounts of collage and elastin fibers, but this was not identified in the other ligaments. Additionally, elastin content was significantly higher in the USL than either CL. Tensile strength was found to vary considerably with the location of the ligaments, but was relatively similar within the same ligament.

As noted with Tan's work, collagen and elastin are the two main structural constituents of supportive ligaments. During pregnancy, elastin within the female reproductive organs (which provides extension and recoil to tissues) will dramatically increase and then decrease back to pre-gravid levels after parturition. Within the literature on humans, there are several publications indicating that improper collagen and elastin metabolism is correlated to prolapses or pelvic floor disorders in women. For instance, Zong et al. (2010) reported that tropoelastin

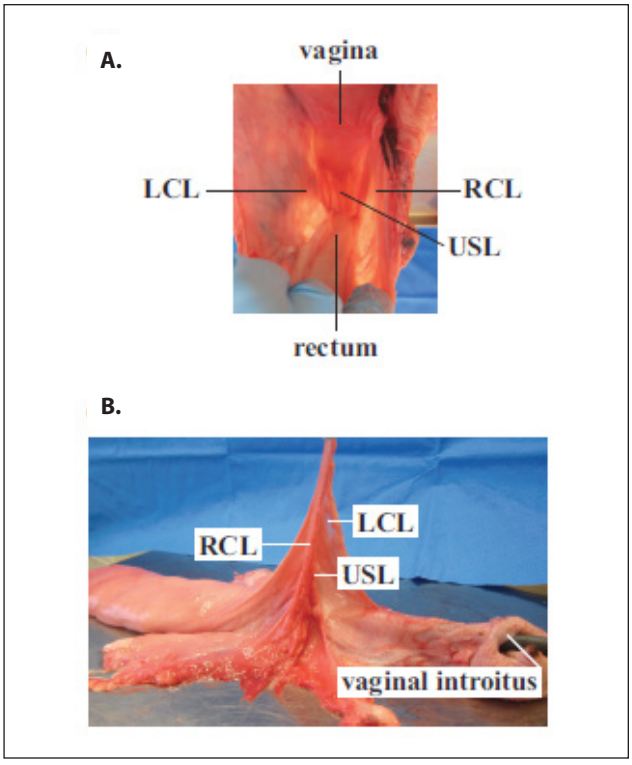

Figure 1. Adapted from Tan et al., 2015. **A.** Sow reproductive support ligaments: Left cardinal ligament (LCL), uterosacral ligament (USL), and right cardinal ligament (RCL) and their location relative to the rectum or vagina. **B.** Ligament (LCL, USL, and RCL) attachment to the cervix.

(432%), mature elastin (55%), proMMP-9 (90%), and active MMP-9 (106%) were increased in women with prolapses, while active MMP-2 (41%) was decreased when compared to the control. Furthermore, Megadhana and Suwiyoga (2016), found high expression of estrogen receptor alpha and collagen III with low expression of elastin in sacrouterine ligaments as risk factors in stage III-IV uterine prolapses. Because of the change in collagen or elastin metabolism, the tissue that is formed is weak, but may not be less, as Hahn et al. (2014) reported an increased diameter of the collagen fibril that lacked proper formation and strength for women with a pelvic organ prolapse when compared to the control.

Hormonal Influences

In a review, Leblanc et al. (2017) discussed the importance of estrogens as a regulating factor of metabolism for connective tissues, like bone, muscle, and cartilage. Primarily they influence the development, maturation, and function of the female reproductive organs, but are also involved in developmental processes of the bone formation. A great example of how estrogen may impact mobility or lameness can be shown in humans, in which injury rate of the cruciate ligament is approximately 2 to 8 times higher than in men. Furthermore, pre-menopausal women are at greater risk, and there is some evidence that suggests tendon-injuries in female athletes might differ in different phases of the menstrual cycle, with the laxity in the knee of women peaking between ovulation and post-ovulation. Additionally, the authors discussed the change in "stiffness" of a tendon; whereas, relaxin, for example, decreases the stiffness of some ligaments during pregnancy and leaves the female open to specific injuries. Furthermore, Vitamin D is a steriod prohormone that is essentially involved in regulating calcium homeostasis, but may also be important in regulating reproductive processes by influencing estrogen synthesis (Reese and Casey, 2015).

Collagen and Osteochondrosis

It has been noted that many lame and culled sows have osteochondrosis (**OC**) lesions (Figure 2) in their joints at slaughter, and that this is also a leading reason for lameness in sows. It has been suggested that OC lesions, or the development of lesions, can potentially arise *in utero*. Early work has defined OC as focal failure of endochondral ossification with subsequent persistence of the growth cartilage in the epiphyseal growth plates. These lesions can heal over time, but scar tissue can still be seen in older animals. Genetics, conformation, injury or trauma, and nutritional deficiencies have all been linked to the prevalence of OC. However, recently Laverty and Girad (2016) indicated that there are now several studies demonstrating a consistent increase in type II collagen synthesis in young animals of multiple species. This increase in type II collagen synthesis may be indicative of repair, but there is a lack of coordinating synthesis of the cartilage matrix proteoglycan molecule. This appears to demonstrate altered type II collagen metabolism may be involved in early stages of OC; thus, indicating an importance of collagen metabolism for both bone and ligaments in swine.

Other Potential Metabolic Stressors

Mycotoxins

Zearalenone is the primary mycotoxin that comes into question when we are having reproductive issues in sows. However, many times feed samples are obtained and no mycotoxins are present, or low levels are found and not considered problematic. Nevertheless, there is a growing body of evidence that zearalenone concentrations as low as 75 ppb can impact the reproductive tract in as little as 6 days post contamination. Research conducted with 10.8 kg gilts indicated that both length and weight of the reproductive tract was increased with zearalenone contaminated feed (Oguey and Durox, 2016). This is alarming because, traditionally, gilts of this size are fed a standard nursery program and not

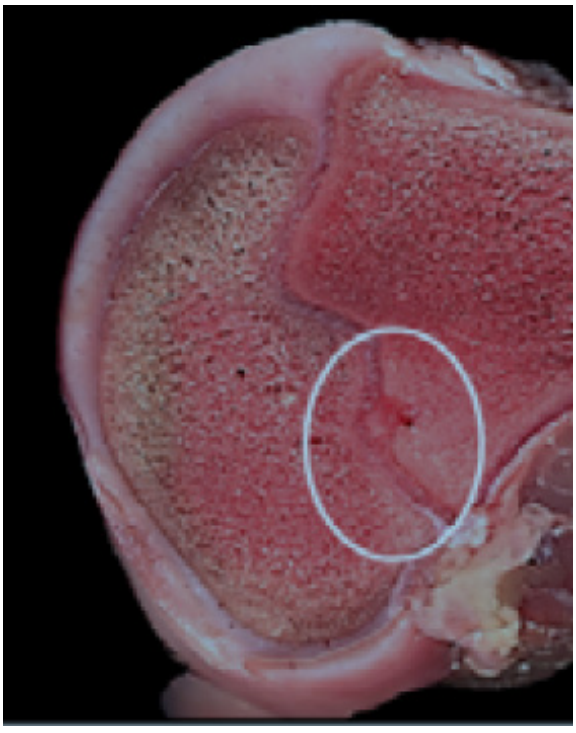

Figure 2. Osetochondrosis lesion in a femur of a growing pig (photo provided by T. Crenshaw, University of Wisconsin, Madison).

a specialized gilt development program. There is, however, limited research evaluating whether pulses of contaminated feed throughout a gilt or sow's lifetime may impact reproductive health and longevity. Additionally, mycotoxin mitigation could potentially interfere with the absorption of certain essential vitamins and other nutrients, and that may or not be considered during diet formulation.

Lameness and Oxidative Stress

Within dairy, it has been demonstrated that oxidative stress is involved in lameness. For instance, Zhoa et al. (2015) reported that lame cows had a 17.7% increase in malondialdehyde (**MDA**), a 7.7% decrease in superoxide dismutase (**SOD**), 18% decrease in metallothionein (**MT**), and 16% increase in the oxidized glutathione to reduced glutathione (**GSSG/GSH**) ratio. Furthermore, trace mineral metabolism was altered as indicated by the reduction of trace mineral levels (Zn, Cu, Mn) in serum, hair, and hoof keratin, where these minerals are known to play a role in protection from oxidative stress. Furthermore, CTX-II and COMP was elevated in the serum lame cows, indicative of cartilage breakdown.

Micronutrient Considerations

Vitamin C

It has generally been considered that swine can endogenously produce their own Vitamin C to meet their requirements, and that they may only require supplementation during stressful conditions. However, a line of pigs has been identified to have a mutated gene so that they lack the ability to synthesize Vitamin C. This genetic line has been kept for research purposes. Within this line, Vitamin C deficiency presented classical signs of "Scurvy" in sows and the skeletal system of their offspring in as little as 25-30 d after removal of ascorbic acid from the diet (Wegger and Palludan, 1994). A review by Mahan et al. (2004) indicated there may be key time points during the reproductive cycle that supplementation C would be beneficial, but the relative body of work indicates no additional benefits of supplementing vitamin C to sows that are typical.

Because vitamin C has not been recognized to be important for supplementation in typical swine, very little swine research has occurred for this vitamin. Has vitamin C metabolism unknowingly been altered in modern, swine genetic improvement programs? Or, are some prolific sows unable to produce adequate amounts for their optimum health and the health of their progeny? As previously discussed, zearalenone contamination can add additional weight and length to the reproductive tract, but it also causes oxidative stress in the liver. Shi et al. (2017) demonstrated that supplementing gilts with 150 mg/kg of Vitamin C was able to alleviate the oxidative stress damage of zearalenone contaminated feed. Thus, their research confirms a potential benefit with supplemental Vitamin C in swine diets, particularly when presented with a mycotoxin contamination.

Vitamin C is also a key regulator in collagen formation. The lack of vitamin C can lead to impaired collagen formation or, at a minimum, reduced strength of collagen formed structures, such as ligaments. As described before, improper collagen and elastin metabolism have been found to be related to pelvic floor disorders and prolapses (**POP**) in women. Findik et al. (2016) demonstrated that Vitamin C supplementation during pregnancy in rats may have aided in the prevention of POP by increasing the production of type I and type III collagen in the cardinal and uterosacral ligaments.

Vitamin D

Although a requirement for dietary vitamin D supplementation is recognized for domestic swine, much of the previous research used to establish published requirement estimates has been based on a rather limited number of studies. Only recently have there been studies evaluating the effects of differing levels and sources of vitamin D supplementation on reproductive and progeny performance, immune function, the incidence of OC, or other variables related to greater health, longevity, or reproductive efficiency. Lauridsen et al. (2010) reported that the number of stillborn pigs per litter was reduced when maternal diets were supplemented with 1,400 or 2,000 IU vitamin D/kg rather than 200 or 800 IU/kg. When an increased level of vitamin D supplementation was provided to gilts throughout gestation and lactation using the addition of 2,000 IU of 25(OH) D_3/kg to a diet already containing 2,000 IU vitamin D_3/kg, Zhou et al. (2016) reported that the higher level of supplementation (using the combination of vitamin D forms) improved the reproductive performance, milk quality and bone status of sows, as well as the bone characteristics of the newborn piglets. Using 2 dietary vitamin D treatments similar to that of Zhou et al. (2016) (i.e., basal level of vitamin D_3 vs. basal diet + 2,000 IU 25(OH)D_3/kg), Sugiyama et al. (2013) reported that the growth performance and bone density was not different from 6 to 110 kg BW, but serum levels of 25(OH) D_3 were increased, and the incidence of osteochondrotic lesions of the humerus and femur at 110 kg BW was greatly reduced, for the pigs fed added vitamin D in the form of 25(OH)D_3. Higher levels of vitamin D than those recommended for preventing classical deficiencies are needed to optimize health and performance,

but the 25(OH)D_3 form of the vitamin is very effective for increasing the level of vitamin D supplementation.

Trace Minerals - Organic vs Inorganic Sources

It has been well established that there is a wide range of trace mineral sources with varying bioavailability for animals. Chelated (organic) minerals have been intensively studied in both cows and sows for their capability to reduce lameness and claw lesions. The potential causes of claw lesions associated with lameness are genetics, flooring, nutrition, injury and other factors. As a sow's BW increases, claw sole area increases quadratically while claw volume increases linearly. Also, the rear inner claws generally remain smaller in sole area and volume than the other claws (Figure 3, Bradley, 2010). As reported by Bradley (2010), organic trace mineral supplementation did not impact overall quantity of lesions presence, but did appear to reduce the severity of claw hemorrhages found, which were most likely due to injury. Further work has demonstrated organic trace mineral supplementation to lower the frequency and/or severity of lesions in sows (Lisgara et al., 2016).

Boron

Boron is the fifth element on the periodic table and it belongs to group 13, that also contains aluminum. Most ICP analysis can detect aluminum, but are not programmed to analyze boron. Similarly, there is no approved boron food additive for feed. It was accepted that boron was essential for plant nutrition as early as 1926 (Sommer and Lipman), which was considered unique until the 1980's because to date it did not appear essential for livestock. In a chick study, Hunt and Nielsen (1981) suggested that it stimulated growth and partially prevented leg abnormalities in Vitamin D deficient chicks. More recently using swine, the supplementation of boron at 25 ppm in a grower diet reduced the incidence of lameness (Johnson et al., 2005).

Macronutrient Considerations—Collagen Formation

Protein and/or amino acids and energy requirements, in general, have been studied in much greater depth than micronutrients for sows. However, NRC Swine (2012) cited only 4 trials to determine the SID lysine values for gestating sows, and 3 of those studies were published 40+ years ago. There were 10 studies for lactating sows, but the most recent publication was from 2001. Swine diets are normally only formulated to the fourth or fifth limiting amino acid (lysine, tryptophan, threonine, valine, and isoleucine), while nonessential amino acids are rarely considered. For instance, glycine is not considered a limiting amino acid, but is important for collagen formation. Glycine comprises one-third of the amino acid residues of collagen. In metabolism, glycine is utilized in two ways: 1) building block for porphyrins, purine bases, creatine, glutathione, bile salts, and hippuric acid; and 2) for amino acid synthesis of

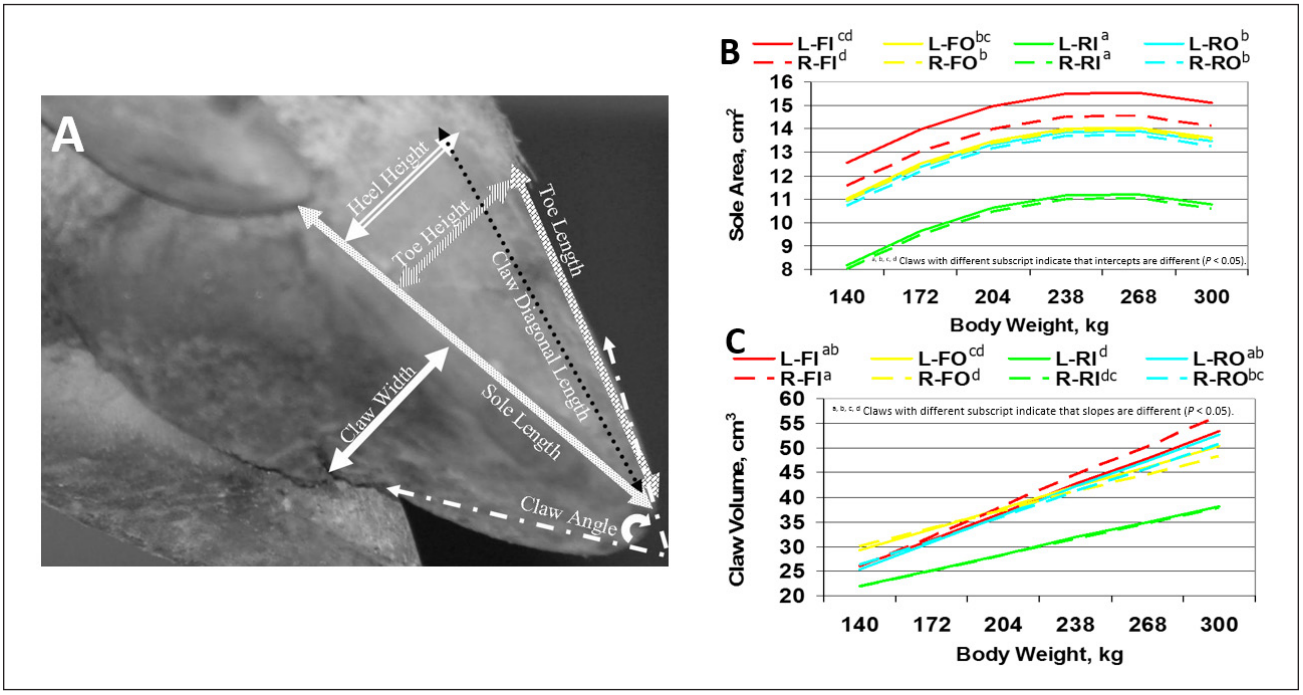

Figure 3. A: Diagram of different claw measurements. **B:** Regression plot of how a sow's sole area (SA) changes with BW (SA = Claw Width x Sole Length. **C:** Regression plot of how a sow's claw volume (CV) changes with BW (CV = SA x heel height). L = left foot, R = right foot, FI = Front Inner Claw, FO = front outer claw, RI = rear inner claw, RO = rear outer claw. (Bradley, 2010).

proteins, especially collagen. Within human nutrition, Meléndez-Hevia et al. (2009) reported that glycine is a semi-essential amino acid and should be supplemented at higher levels to promote healthy metabolism. The authors detailed the inability for glycine to be adequately present for metabolic needs, but especially for proper collagen synthesis, which may be further compromised during pregnancy and in aging populations. Thus, as we consider lower crude protein diets in growing animals and sows with and the use of synthetic essential amino acids, the non-essential amino acids should be evaluated beyond traditional growth performance and reproductive parameters.

Conclusions

Within this review of pelvic floor disorders and prolapses in humans, there are numerous possibilities to influence the condition of supportive ligaments via nutrition. Furthermore, stressors within a sow's lifetime may have a negative effect on her longevity. A "perfect storm" could be occurring within today's sow herds to create the increases in sow mortality and uterine prolapses. Knowledge and research to solve the current issues of concern are mostly lacking, and the traditional methods of measuring sow reproductive traits for greater profitability are insufficient to solve the existing and future problems. This review has provided some unique insights into opportunities for improving our understanding of nutritional requirements that may be helpful in reducing the risks for sow mortality.

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Impact of Dietary Amino Acid Balance on Nitrogen and Energy Efficiency in Sows: from Theory to Practice

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Summary

Aggressive reduction in dietary crude protein and crystalline amino acid supplementation to improve dietary amino acid balance minimizes urea-nitrogen synthesis and ammonia emission without compromising lactation performance. Improving dietary amino acid balance also increases milk yield and true milk protein yield in peak lactation, and amino acid and global nitrogen utilization efficiency for milk protein production. Individual amino acid efficiencies are dynamic, and correct use of these efficiency values is critical for accurate prediction of amino acid requirements. Future dietary formulations using reduced crude protein diets to minimize nitrogen excretion and ammonia emission will require amino acid requirements based on near maximum biological efficiency estimates.

Introduction

The use of crystalline amino acids (**CAA**) in conjunction with reduction in dietary crude protein (**CP**) allows for improvement of dietary amino acid (**AA**) balance and increases whole body nitrogen (**N**) and energy utilization efficiency for milk production in sows (Huber et al., 2015; Zhang et al., 2018). Improvement in dietary AA balance reduces N excretion and ammonia emission (Hubert et al., 2015; Chamberlin et al., 2015b). The global improvement in N utilization is due to incremental change in utilization efficiency of individual dietary AA (Huber et al., 2015). Except for that of lysine (**Lys**), utilization efficiencies of the nine remaining essential AA have not been systematically estimated (NRC, 2012). Prediction of dietary AA requirement through the use of models (NRC, 2012) are dependent on valid AA efficiency estimates. Amino acid efficiencies however are dynamic, (White et al., 2016), and dependent on their own dietary concentrations and that of total N. This plasticity complicates the application of AA efficiency for prediction of requirements. The objectives of this presentation are to provide a summary of the data we have thus far collected from studies with sows fed diets with improved AA balance. We will discuss the impact of such diets on sow performance, urea excretion and ammonia emissions, AA efficiencies and future direction on implementation strategies of AA efficiency estimates for prediction of AA requirements.

Sow Performance and Milk Protein

Across studies, results show that feeding reduced CP diets containing as low as 12.56% CP with CAA to meet the SID requirement of the limiting AA does not impact piglet ADG, litter growth rate, and sow feed intake (Table 1). In the studies by Chamberlin et al. (2015b), Huber et al. (2015) and Zhang et al. (2018), sows lost more weight and retained less N in their body when fed the lowest level of CP. The greater loss in sow body weight (**BW**) appears to be associated with greater body protein loss rather than body fat. It is possible that other AA may have been limiting, leading to body protein mobilization, and reflecting an underestimation of dietary requirements in NRC (2012) for total N or an essential AA for which the dietary concentrations were

Table 1. Performance of lactating sows fed diets reduced in crude protein (CP) concentration with supplemental crystalline amino acids.[1]

Study	CP, %	SID Lys, %	SID Thr, %	SID M+C, %	SID Trp, %	Litter gain, kg	Piglet ADG, g/d	Sow BW loss, g/d	Sow fat change Δ	Sow protein Δ
Manjarin et al., 2012	17.52	1.11	0.69	0.55	0.21	1.71	214	228	-	-
	13.53	0.85	0.53	0.42	0.16	2.26	282	232	-	-
Huber et al., 2015	17.62	0.74	0.59	0.50	0.18	1.86	186	414	-	-
	14.63	0.74	0.59	0.50	0.18	2.18	221	433	-	-
Huber et al., 2015	16.03	0.74	0.59	0.50	0.18	2.32	238	143	−0.1	+0.2
	15.70	0.74	0.59	0.50	0.18	2.53	256	176	−0.2	−0.8
	14.29	0.74	0.59	0.50	0.18	2.41	243	190	−0.1	−1.2
	13.22	0.74	0.59	0.50	0.18	2.60	260	285	−0.2	−2.7
Chamberlin et al., 2015a	17.16	0.78	0.53	0.48	0.18	2.53	262	270	-	-
	14.79	0.78	0.49	0.42	0.15	2.64	278	413	-	-
	12.56	0.78	0.49	0.41	0.15	2.56	258	358	-	-
Chamberlin et al., 2015b	17.16[2]	0.78	0.53	0.48	0.18	2.60	265	500	−0.06	-
	12.56[2]	0.78	0.49	0.41	0.15	2.80	279	300	−0.13	-
	17.16[3]	0.78	0.53	0.48	0.18	2.40	244	700	−0.15	-
	12.56[3]	0.78	0.49	0.41	0.15	2.30	238	800	−0.10	-
Zhang et al., 2018	18.74	0.90	0.61	0.54	0.21	2.43	251	62	−0.05[4]	+5.4[6]
	13.78	0.90	0.58	0.49	0.17	2.56	255	395	−0.17[5]	−11.2[6]

1 NE=2,580 to 2,600 kcal/kg.
2 Sows were housed under thermal neutral environmental temperature.
3 Sows were housed under thermal heat stress environmental temperature.
4 Corresponds to 77 g/d body fat loss.
5 Corresponds to 312 g/d body fat loss.
6 Body protein loss, g/d.

lowest in the lowest CP diets (e.g., arginine (**Arg**), histidine (**His**), leucine (**Leu**), phenylalanine (**Phe**), and Phe + tyrosine (**Tyr**).

Depending on the stage of lactation, milk casein concentration and casein to true protein (**TP**) ratio are either unaffected by AA balance, or greater. Both Huber et al. (2015) and Chamberlin et al. (2015b) reported 25% increases in casein concentration and yield (data not shown), and casein:TP in peak lactation (Table 2).

Overall Efficiency of Nitrogen Utilization

Utilization of dietary N for milk production increases in response to decreasing dietary CP concentration and increasing CAA supplementation, provided that the supply of potentially limiting AA meet their estimated requirements. In both studies by Huber et al. (2015) and Chamberlin et al. (2015a and 2015b) feeding reduced CP diets lowered milk urea-N over 2-fold in early lactation and by more than 5-fold in peak lactation (Figures 1 and 2). In both studies, milk urea-N concentration from early to peak lactation did not change in sows fed the reduced CP diet, but nearly doubled in sows fed a non-reduced CP diet. Similarly, in early lactation, plasma urea-N of sows fed a low CP diet was nearly half that of control, and in peak lactation, plasma urea-N of sows fed a low CP diet was reduced, indicating greater utilization of N compared to the low-CP fed sows. These changes are equally reflected in the urinary N excretion response (Figure 2, right panel) and a remarkable decrease in ammonia emissions under either thermal neutral or thermal heat stress environments (Figure 3).

The urea cycle is an energy expensive process, whereby two molecules of ammonia, and one molecule of carbon dioxide are used for the synthesis of one molecule of urea ($(NH_2)_2CO$). The reduction in MUN coupled with the reduction in plasma urea-N without a decrease in true protein or casein yield suggests sows fed near optimum AA balance are metabolically more efficient and that energy cost associated with excess AA catabolism and urea synthesis can be minimized. The recent study by Zhang et al. (2018) showed that feeding sows with a CAA-supplemented diet containing 13.78% CP compared to a 18.74% CP diet increased milk N production per unit of ME and NE in early (20% increase) and peak (over 30% increase) lactation periods, with a drastic increase in efficiency of dietary N into milk N (Table 3).

Efficiency of Amino Acid Utilization

Earlier, Manjarín et al. (2012) reported that decreasing dietary CP by 4% (from 17.5% to 13.5%) and meeting the limiting AA requirement via CAA supplementation increased the efficiency of Lys and Arg utilization by the mammary gland. As discussed previously, aggressive reduction in dietary CP and CAA inclusion rates can minimize serum and milk urea-N and ammonia emis-

sion without compromising lactation performance, indicating that the dietary AA balance is improved. Within this range of dietary CP concentrations, improving dietary AA balance also led to increased estimated milk yield and estimated true milk protein yield during peak lactation, and improved N utilization efficiency for milk protein production. The global improvement in N utilization is due to incremental change in utilization efficiency of individual dietary AA (Huber et al., 2015). Amino acid efficiencies are dynamic (White et al., 2016) as depicted in Table 4. Amino acid efficiency estimates across dietary CP concentrations and CAA inclusion rates generally increase, and quite considerably for some AA (Arg, His, Ile, Leu) with dietary improvement in AA balance. Note that efficiency estimates for Val reported by Huber et al. (2015) decrease with reduction in CP while those of Zhang et al. (2018) increase. This discrepancy stems from the fact that the highest CP diet in Zhang et al. (2018) did not contain crystalline valine (Val) while that of Huber et al. (2015) contained crystalline Val and was likely over Val requirement. The NRC (2012) efficiency estimates were not systemically determined, except for that of Lys, and therefore it is not

Table 2. Milk casein concentration and milk casein to true protein ratio from lactating sows fed diets reduced in crude protein concentration with supplemental crystalline amino acids.

		Early lactation, d 3-7		Peak lactation, d 14-18	
Study	**CP, %**	**Casein, %**	**Casein:TP[1]**	**Casein, %**	**Casein:TP**
Hubert et al., 2015	16.03	3.63	0.70	3.29	0.79
	15.70	3.30	0.65	3.21	0.77
	14.29	3.64	0.68	3.38	0.84
	13.22	3.50	0.67	3.41	0.77
Chamberlin et al., 2015a	17.16	3.70	0.78	2.85	0.67
	14.79	3.25	0.66	3.15	0.80
	12.56	3.76	0.79	3.81	0.90
Chamberlin et al., 2015b	17.161	2.77	0.69	2.65	0.76
	12.561	2.81	0.74	2.45	0.73
	17.162	2.65	0.76	2.57	0.73
	12.562	2.45	0.73	2.64	0.75
Zhang et al., 2018	18.74	4.52[2]	-	4.46[2]	-
	13.78	4.23[2]	-	4.52[2]	-

[1] True protein.
[2] True protein concentration, %.

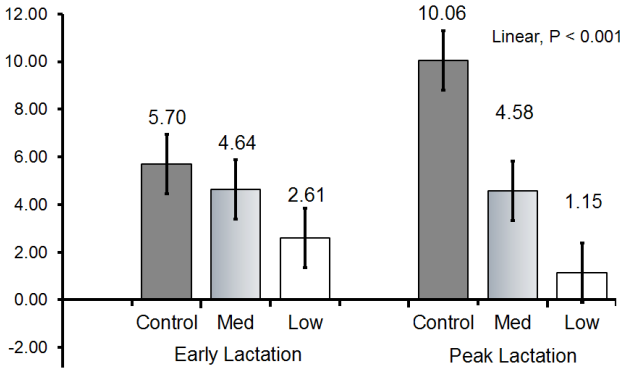

Figure 1. Milk urea concentration (mg/dL) in sows fed diets containing 17.55, 15.25 and 12.98 % CP (Control, Med and Low, respectively), in early (d 3-7) and peak (d 14-17) lactation. From Chamberlin et al. (2015a).

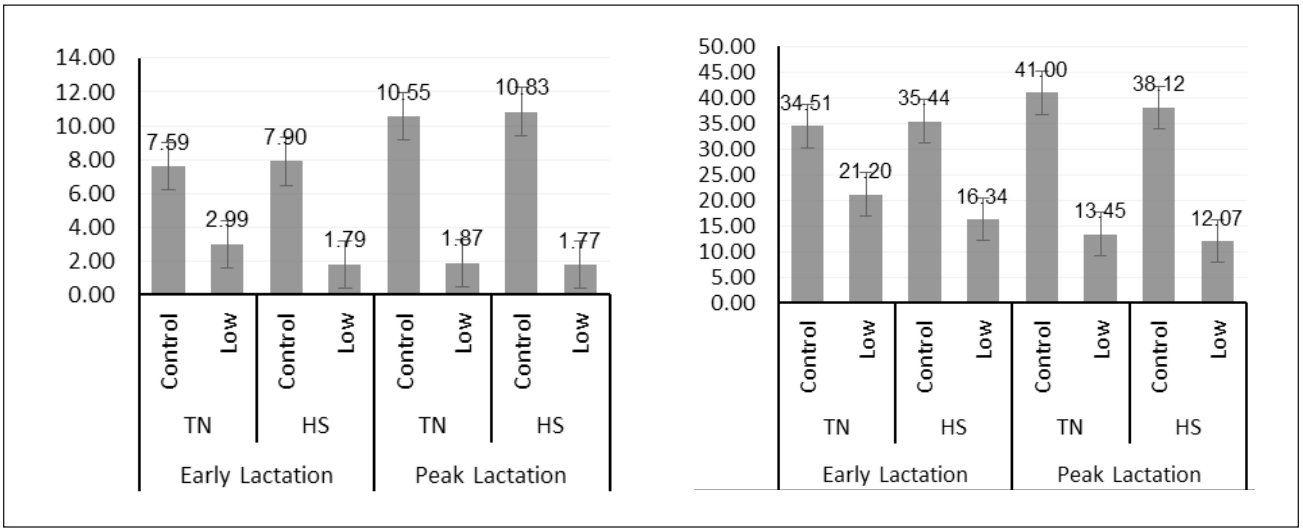

Figure 2. Milk (left panel) and urinary (right panel) urea nitrogen (mg/dL and g/d, respectively) from sows exposed to heat stress (HS) and thermo-neutral temperature (TN) and fed a Low protein diet or a Control diet during lactation. From Chamberlin et al. (2015b).

Table 3. Energetic utilization of milk nitrogen deposition.

Days in lactation	CP	Milk N/ME intake, mg/kcal[1]	Milk N/NE intake, mg/kcal[2]	N output/ N intake, %[3]
4 to 7	18.74	3.62	4.83	40.4
	13.78	4.45	5.86	67.5
14 to 17	18.74	3.74	3.73	41.7
	13.78	4.98	4.89	56.5

Zhang et al. (2018).
[1] SEM = 0.25
[2] SEM = 0.35
[3] SEM = 3.8

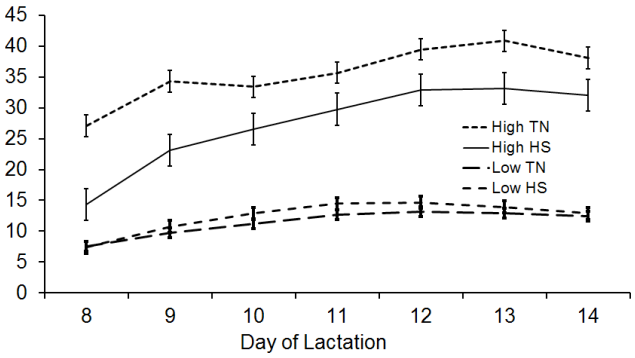

Figure 3. Air ammonia production in individual lactating sows and their litters. Sows were fed diets containing 17.55 and 12.98% CP, and housed under either thermal neutral (TN) or thermal heat stress (HS) environments. From Chamberlin et al. (2015b).

surprising that many AA differ quite substantially from those reported by Huber et al. (2015) and Zhang et al. (2018). In addition, AA efficiency values from Zhang et al. (2018) are notably higher than those of Huber et al. (2015) for peak lactation, and this may be related to the use of sow BW loss over a 21-d period rather than the specific peak lactation period of d 14 to 17. Consequently, Trp for instance is over 100%. Hence, for practical purpose in estimating requirement using a modeling approach, efficiency estimates generated over a 21-d period would be more relevant and biologically correct. Maximum biological efficiency estimates are needed to predict AA requirement. Efficiency estimates below the maximum biological efficiency leads to over estimation of AA requirement.

We have conducted a meta-analysis using the current available studies on AA requirements for lactating sows and generated AA efficiency estimates. Our estimated values were too low to use in practical formulation, except for that of Lys, which was in line with the estimated value of Huber et al. (2015), Zhang et al. (2018) and NRC (2012). This makes sense because the great majority of studies are designed to determine the minimum Lys requirement. This exemplifies the need to conduct systematic experiments with formulations targeted to generate maximum biological efficiency values for Thr, methionine+cystien (Met + Cys), Trp, Ile, Phe, Val, and Leu. Accurate efficiency values for Arg and His remain debatable because of de novo synthesis and de novo availability, respectively.

Table 4. Efficiencies of SID amino acid utilization for milk production in sows fed diets with reduced crude protein concentrations and supplemental crystalline amino acids, %.

	Crude protein concentration, %						
Item	**19.24[1]**	**16.58[2]**	**15.35[2]**	**14.15[2]**	**13.78[1]**	**12.89[2]**	**NRC[3]**
Early lactation (d 3-7)							
Arg	30.1	35.4	36.8	40.2	50.6	39.5	
His	52.2	58.4	65.7	68.6	69.8	73.6	
Ile	43.5	48.9	53.0	51.3	62.5	50.6	
Leu	43.2	46.4	53.9	53.7	64.2	59.6	
Lys	60.1	67.7	71.0	64.3	61.1	62.6	
Met	64.9	55.7	60.1	48.4	57.6	44.2	
Met + Cys	53.1	59.2	66.0	58.9	60.5	58.4	
Phe	37.4	42.0	48.2	49.6	48.7	53.1	
Phe + Tyr	44.3	55.3	60.4	61.2	61.6	60.4	
Thr	55.7	58.6	64.0	56.7	60.0	54.1	
Trp	62.0	49.9	54.3	48.7	87.8	51.8	
Val	51.3	44.5	46.6	42.6	50.7	39.4	
N	44.4	55.3	60.4	61.2	62.7	60.4	
Essential AA	52.2	-	-	-	62.5	-	
Peak lactation (d 14-18)							
Arg	33.5	31.7	41.0	43.8	69.9	53.6	81.6
His	58.1	51.8	64.8	67.8	93.8	81.5	72.2
Ile	48.4	43.1	50.6	51.0	83.6	54.3	69.8
Leu	48.2	41.0	49.9	50.8	85.6	59.5	72.3
Lys	67.0	59.9	68.4	65.4	82.1	67.6	67.0
Met	71.8	50.0	58.9	48.8	77.1	47.0	67.5
Met + Cys	59.0	52.6	61.6	57.1	80.7	57.9	66.2
Phe	41.7	37.0	45.8	48.5	65.2	56.9	73.3
Phe + Tyr	49.3	48.9	59.6	60.3	82.2	68.2	70.5
Thr	61.9	52.6	60.0	57.4	80.2	59.3	76.4
Trp4	68.4	44.2	48.8	46.6	114.0	52.3	67.4
Val	57.1	39.3	45.0	44.5	68.0	45.0	58.3
N	49.8	48.9	59.6	60.3	84.7	68.2	75.9
Essential AA	58.1	-	-	-	83.3	-	-

[1] Zhang et al., 2018. Unpublished.
[2] Huber et al., 2015.
[3] NRC, 2012. Values are over a 21-d lactation period and are not restricted to the peak lactation period.

Conclusion

Aggressive reduction in dietary CP and CAA supplementation to improve dietary AA balance minimizes urea-N synthesis and ammonia emission without compromising lactation performance. Improving dietary AA balance also increases milk yield and true milk protein yield in peak lactation, and AA and global N utilization efficiency for milk protein production. Individual AA efficiencies are dynamic, and correct use of these efficiency values is critical for accurate prediction of AA requirements. Future dietary formulations using reduced CP diets to minimize N excretion and ammonia emission will require AA requirements based on near maximum biological efficiency estimates.

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Copper Levels and Sources for Sows

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Summary

In the present study, 31 crossbred gilts were fed experimental diets to determine the effects of dietary Cu sources [copper sulfate (CuSO4) or tribasic copper chloride (TBCC)] and levels (20, 120, or 220 mg/kg) on performance and health of sows and their progeny. Sows fed TBCC diets had greater adjusted weaning weight and adjusted lactation weight gain for the litter and/or the individual piglet ($P < 0.10$), respectively, when compared to sows that received $CuSO_4$ diets. Increasing dietary Cu level linearly increased ($P = 0.06$) live born piglet weight. Sows fed TBCC diets had greater apparent total tract digestibility of dry matter and nitrogen during lactation ($P < 0.05$). Increasing Cu levels increased levels of milk fat and Cu (linear, $P < 0.05$); but linearly decreased lactose and Zn levels ($P < 0.05$). Lactating sows fed TBCC diets had a greater activity of Cu/Zn superoxide dismutase (SOD) and ceruloplasmin in serum than those fed $CuSO_4$ diets ($P < 0.05$). Increasing dietary Cu levels increased total and Cu/Zn SOD activity for lactating sows (linear, $P < 0.05$). Sows fed TBCC diets had lower concentrations of Cu ($P = 0.04$), but greater concentrations of iron and manganese ($P < 0.05$) in the liver, when compared to those fed $CuSO_4$ diets. In addition, liver Cu concentration increased with increasing dietary Cu levels (linear and quadratic, $P < 0.05$). Increasing sow dietary Cu levels did not influence tissue trace mineral concentration in neonatal piglets, but resulted in the elevation of Cu concentrations in the liver of weanling piglets (linear, $P < 0.0001$). Results of this study suggest that the source of Cu affects reproductive performance, and that higher dietary Cu levels in sow diets than the NRC (2012) requirement estimates are beneficial to sows and piglets without showing any apparent adverse effects.

Introduction

Sow productivity is a key determinant of swine enterprise profitability. Sow productivity in the major pork producing countries, including China, the European Union, and the U.S., has increased by 11 to 28% from 2001 to 2013, regarding weaned or finished pigs produced per sow per year (Lu, 2018). However, greater sow productivity inevitably leads to increased mobilization of minerals from body tissues, and reproductive capacity can be compromised if the mineral needs for reproductive demands exceed body stores and dietary intake (Mahan, 1990). It has been demonstrated that body mineral contents, including calcium, phosphorus, magnesium, potassium, sodium, aluminum, zinc, and copper declined in sows that had completed three parities as compared to those of similarly aged, nongravid gilts (Mahan and Newton, 1995).

Copper is required by pigs to serve many biological roles in the body, such as supporting iron metabolism, protecting tissues from oxidative damage, and maintaining immunity (Hill and Spears, 2001). The latest edition of the NRC estimates that the Cu requirement of growing pigs is 3 to 6 mg/kg and for gestating and lactating sows is 10 and 20 mg/kg, respectively (NRC, 2012). Moreover, pharmacological concentrations of Cu (125 to 250 mg/kg) have been extensively studied and demonstrated to enhance growth performance of weanling and growing pigs (Cromwell, 2001). Regarding breeding herds, dietary supplementation of pharmacological levels of Cu (250 mg/kg) in gestation and lactation diets from Parity 1 to 6 has been demonstrated to improve piglet weight at birth and weaning, as well as increase Cu concentrations in sow liver and kidney (Cromwell et al., 1993). However, no study has been reported to assess the effects of high dietary Cu from different sources on reproducing sows. Therefore, the objective of the present study was to determine the long-term effects of both dietary Cu source and level on performance and health of sows and their progeny.

Experimental Procedures

Animals and experimental design

A total of 31 eight-month-old gilts [Yorkshire; (Yorkshire × Landrace) × Duroc] were provided with a common gestation diet containing 8 mg/kg Cu as copper sulfate (**$CuSO_4$**) from breeding until their pregnancies were confirmed at 55 ± 2 d post-breeding. Upon confirmation of pregnancy, gilts were allotted to a 2 × 3 factorial arrangement in a completely randomized design with 2 Cu sources [$CuSO_4$ or tribasic copper chloride (**TBCC**)] and 3 Cu levels (20, 120, and 220 mg/kg). All experimental animals received their respective diets until they were removed from the study or until they weaned their 4th litter.

Experimental Diets

Corn-soybean meal diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestating and lactating sows. Diets 1 to 3 were designed to contain 20, 120, and 220 mg/kg of Cu as TBCC, respectively; Diets 4 to 6 were designed to contain 20, 120, and 220 mg/kg of Cu as $CuSO_4$, respectively. Titanium dioxide was added at a level of 0.30% by replacing an equal amount of corn as an indigestible marker.

Data and Sample Collection

The number and weight of total piglets born, born alive, and stillborn were recorded within 12 h after farrowing; and the number and weight of piglets at weaning was also recorded. The weight of the piglet and litter at weaning were adjusted to a common 21-d lactation as described by Jang et al. (2017). Diet samples were collected at the feed mill for every mixing batch. Fecal samples were collected for lactating sows during the 2nd and 3rd parity from d 15 to 17 post-farrowing by grab sampling. Blood samples were collected via vena cava puncture from sows during the 2nd to 4th parity at d 15 post-farrowing. During the 3rd and 4th parity, milk samples were collected at d 15 post-farrowing from each sow after intravenous injection of 1 mL oxytocin. At the beginning of the study, 6 open gilts from the same breeding group were slaughtered for tissue collection (liver, heart, right kidney, and both ovaries). After completing at least 3 parities, sows were slaughtered for the same tissue collection. One piglet was sacrificed at birth, and another one at weaning for tissue collection (liver, kidneys, and heart) for the 3rd and 4th litter of each sow.

Sample Analysis

Feed and fecal samples were analyzed for dry matter (**DM**), gross energy (**GE**), ether extract (**EE**), and nitrogen. Malondialdehyde (**MDA**) concentration, superoxide dismutase (**SOD**) activity, and ceruloplasmin (**Cp**) activity were analyzed in serum samples. Milk composition analysis was conducted using Foss Milkoscan™ FT 120 device.

Statistical Analysis

All data were subjected to ANOVA using the GLM procedure in SAS (Statistical Analysis System, Cary, NC, USA) for a completely randomized design. The individual litter served as the experimental unit and results are reported as least squares means. The statistical model included the Cu source, Cu level, and sow parity, as well as their interactions. With the purpose of achieving appropriate statistical power, the α level used for determination of statistical significance was set at 0.10 for sow and litter performance data (α level at 0.15 for tendency), and set at 0.05 for all other data (α level at 0.10 for tendency).

Results

Reproductive Performance

A total of 107 litters were farrowed in the present study (Table 1). Chi-square analysis showed that the litter distribution did not differ across dietary treatments. One sow from Diet 1 was culled because of reproductive failure (no litter farrowed), and another sow from Diet 3 was removed because of death from gastric torsion (after completion of 3 parities).

Sows fed the TBCC diets had more stillborn piglets (P = 0.03), greater adjusted weaning weight of piglets (P = 0.07), and adjusted litter (P = 0.10) and piglet (P = 0.05) weight gain than $CuSO_4$ fed sows (Table 2). In addition, tendencies for greater litter weight of total born piglets (P = 0.11), piglet weight at weaning (P = 0.13), piglet weight

Table 1. Distribution of number of litters[1, 2]

Copper source:	**Tribasic copper chloride**			**Copper sulfate**		
Copper level, mg/kg:	**20**	**120**	**220**	**20**	**120**	**220**
Item *Diet No.:*	**1**	**2**	**3**	**4**	**5**	**6**
No. of gilts allotted	5	6	5	5	5	5
No. of litters						
1st-parity litters	4	6	5	5	5	5
2nd-parity litters	4	6	5	5	5	5
3rd-parity litters	3	6	5	4	4	4
4th-parity litters	3	4	4	4	2	4
Total	14	22	19	18	16	18
Average no. of litters/sow	3.5	3.7	3.8	3.6	3.2	3.6

[1] No significant difference across treatments.
[2] One gilt from Diet 1 did not farrow throughout the experiment, and one sow from Diet 3 died after completion of the 3rd parity.

Table 2. Effects of dietary copper source and level on litter performance[1]

Copper source:	**Tribasic copper chloride**			**Copper sulfate**					
Copper level, mg/kg:	**20**	**120**	**220**	**20**	**120**	**220**		***P* values**	
Item *Diet No.:*	**1**	**2**	**3**	**4**	**5**	**6**	**SEM**	**Source**	**Level**
No. of litters	8	15	12	13	11	8			
Lactation length, d	21.1	20.3	19.8	20.7	20.6	20.0	0.5	0.96	0.20
Litter size									
Total born	10.67	10.64	11.51	10.93	10.02	9.83	0.83	0.33	0.83
Live born	10.00	9.77	10.36	10.47	9.77	9.61	0.83	0.89	0.85
Stillborn	0.56	0.81	1.16	0.47	0.33	0.22	0.26	0.03	0.81
Weaning	9.11	8.58	9.09	9.17	8.45	8.28	0.70	0.61	0.66
Litter weight, kg									
Total born	17.12	15.96	18.52	14.86	15.57	16.34	1.20	0.11	0.35
Live born	16.26	14.84	16.77	14.32	15.25	15.54	1.25	0.37	0.66
Weaning	58.38	55.30	58.68	57.02	53.02	47.66	4.52	0.19	0.59
Litter gain[6]	43.94	42.01	44.10	44.20	39.45	34.25	3.54	0.17	0.40
Piglet weight, kg									
Total born[5]	1.53	1.54	1.67	1.41	1.56	1.59	0.10	0.46	0.30
Live born[2]	1.54	1.56	1.74	1.42	1.57	1.61	0.10	0.31	0.17
Weaning	6.40	6.48	7.10	6.35	6.41	5.88	0.35	0.13	0.95
Piglet gain[4, 6]	4.82	4.92	5.33	4.91	4.80	4.24	0.29	0.12	0.95
Adjusted weight, kg									
Litter weight at weaning	57.80	56.59	61.14	57.52	53.68	49.01	4.17	0.14	0.79
Piglet weight at weaning[3]	6.34	6.65	7.41	6.44	6.49	6.06	0.31	0.07	0.57
Litter weight gain[6]	43.36	43.31	46.56	44.70	40.11	35.60	3.14	0.10	0.63
Piglet weight gain[4, 6]	4.76	5.08	5.64	5.00	4.88	4.41	0.24	0.05	0.84

1 Data were collected from the 2nd to 4th parity litters.
2 Linear response to copper level ($P = 0.06$).
3 Copper source × copper level interaction ($P = 0.06$). A linear response to increasing copper level ($P = 0.02$) within tribasic copper chloride diets whereas no response within copper sulfate diets ($P > 0.28$).
4 Copper source × copper level interaction ($P = 0.02$). An increasing linear response to increasing copper level ($P = 0.02$) within tribasic copper chloride diets whereas a decreasing linear response within copper sulfate diets ($P = 0.05$).
5 Tendency of linear response to copper level ($P = 0.12$).
6 Because 1 piglet was sacrificed at birth for the 3rd and 4th litters, adjustment of excluding the sacrificed piglets was applied on live born litter size and litter weight that was used in the calculations of survival rate, litter gain, and piglet gain.

gain during lactation ($P = 0.12$), and adjusted litter weight at weaning ($P = 0.14$) were observed for TBCC fed sows compared to $CuSO_4$ fed sows. Litter performance (litter size, litter weight, and piglet weight) was not affected by Cu level except for the weight of total piglets born (linear. $P = 0.12$) or born alive (linear, $P = 0.06$). An interaction of Cu source and level was detected on adjusted piglet weight at weaning ($P = 0.06$), which showed a linear increase with increasing Cu levels in TBCC diets ($P = 0.02$) but no response to Cu level within $CuSO_4$ diets ($P > 0.28$). Moreover, the interaction was also observed on adjusted piglet weight gain ($P = 0.02$), which showed an increasing linear response to increasing copper level ($P = 0.02$) within TBCC diets but a decreasing linear response within $CuSO_4$ diets ($P = 0.05$).

Apparent Total Tract Digestibility

Sows fed TBCC diets had greater apparent total tract digestibility (**ATTD**) of DM, nitrogen ($P < 0.05$), and GE ($P < 0.10$) when compared to the ones fed $CuSO_4$ diets (Table 3). The increasing dietary Cu levels elevated the ATTD of DM (linear, $P < 0.05$) and GE (linear, $P < 0.06$).

Hematology and Antioxidant Status

Lactating sows fed TBCC diets had greater activity of total SOD ($P = 0.08$), Cu/Zn SOD ($P = 0.04$), and Cp ($P = 0.01$) compared to sows fed the $CuSO_4$ diets (Table 4). The activity of total and Cu/Zn SOD increased linearly ($P < 0.03$) with increasing dietary Cu levels. In addition, the activity of Mn SOD and Cp quadratically changed with increasing dietary Cu levels ($P < 0.05$). However, hematocrit, hemoglobin, or MDA were not affected by dietary Cu source or level ($P > 0.23$).

Milk Composition

Milk from sows that were fed TBCC diets had greater levels of protein than that from sows that were fed $CuSO_4$ diets ($P = 0.02$, Table 5). Furthermore, the TBCC fed sows tended to have greater levels of fat, GE, and total solids, but a lower level of lactose in milk than the $CuSO_4$ fed sows ($P < 0.10$). With increasing dietary Cu levels, concentrations of fat and Cu ($P < 0.05$), as well as gross energy and total solids ($P < 0.10$) linearly increased in milk. However, linearly decreased levels of lactose (P

Table 3. Effects of dietary copper source and level on apparent total tract digestibility (%) during lactation[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item *Diet No.:*	1	2	3	4	5	6	SEM	Source	Level
No. of observations	6	12	10	9	7	6			
Dry matter[2]	87.17	88.15	87.51	86.41	86.79	87.65	0.36	0.03	0.09
Nitrogen[3]	87.90	89.25	88.16	87.22	87.36	87.96	0.54	0.04	0.38
Gross energy[4]	87.71	88.33	87.82	86.70	87.04	88.16	0.41	0.06	0.19
Ether extract	76.61	78.51	73.78	67.23	76.04	79.70	3.57	0.51	0.28

[1] Fecal samples were collected from the 2nd and 3rd parity sows during d 15 to 17 of lactation.
[2] Linear response to copper level ($P = 0.02$).
[3] Sow parity effect ($P < 0.05$).
[4] Tendency of linear response to copper level ($P < 0.06$).

= 0.03) and Zn ($P = 0.09$) were observed in milk as dietary Cu level increased. In addition, non-fat solids in milk decreased from sows fed the 20 to 120 mg/kg Cu diets and then increased from the 120 to 220 mg/kg Cu diets (quadratic, $P < 0.05$).

Tissue Trace Mineral Levels

The experimental sows had a greater concentration of Cu (liver and heart) and Zn (liver) when compared to baseline gilts ($P < 0.05$, Table 6). Moreover, the experimental sows tended to have greater concentration of Cu in the kidney than the baseline gilts ($P < 0.10$). Sows fed the TBCC diets had lower concentrations of Cu ($P = 0.04$), but greater concentrations of Fe and Mn ($P < 0.05$) in liver than sows fed the $CuSO_4$ diets. In addition, liver Cu concentration increased with increasing dietary Cu levels (linear and quadratic, $P < 0.05$). The concentration of Cu in kidney, heart, and ovary was not affected by either Cu source or level ($P > 0.37$).

Trace mineral concentrations were not affected by sow dietary Cu source or level in various organs of neonatal piglets ($P > 0.20$, Table 7). Whereas for weanling piglets, increasing dietary sow Cu levels resulted in an increase of Cu concentration in liver (linear, $P < 0.01$, Table 8). However, Zn concentrations in liver tended to decrease as maternal dietary Cu levels increased (linear, $P < 0.10$).

Discussion

Sow and Litter Performance

Pigs are Cu tolerant among domestic animal species (Hill and Spears, 2001) and high concentrations of dietary Cu (100 to 250 mg/kg) are commonly used in nursery and growing-finishing diets as a growth promoter. Sows have a much longer lifespan than growing pigs. Thus, one of the concerns of applying pharmacological levels of Cu in sow diets might be the Cu toxicity due to over-accumulation of Cu in tissues. In the present study, sows fed high Cu diets (120 and 220 mg/kg) for 2.5 yr produced a numerically greater number of litters than sows fed 20 mg/kg Cu (38 and 37 vs. 32 litters; Table 1). This demonstrates that feeding high Cu to sows long-term may not induce any negative effect

Table 4. Effects of dietary copper source and level on Htc, Hb, and antioxidant status of lactating sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item *Diet No.:*	1	2	3	4	5	6	SEM	Source	Level
Lactation									
No. of observations	8	15	13	13	10	12			
Htc, %[3]	35.58	35.40	34.92	33.29	34.95	34.54	1.04	0.23	0.78
Hb, g/dL	11.62	11.92	11.80	11.28	11.53	11.64	0.34	0.30	0.67
Total SOD, U/mL[3, 4]	39.57	43.09	45.77	37.16	39.30	41.74	2.36	0.08	0.09
Cu/Zn SOD, U/mL[3, 4]	24.90	25.58	30.92	21.29	22.73	26.82	2.05	0.04	0.02
Mn SOD, U/mL[3, 5]	14.67	17.51	14.86	15.87	16.57	14.92	1.14	0.91	0.14
Cp, U/mL[5, 6]	0.153	0.164	0.130	0.128	0.139	0.117	0.010	0.01	0.02
MDA, μM	6.69	6.94	6.54	6.11	7.01	6.12	0.48	0.45	0.32

[1] Htc, hematocrit; Hb, hemoglobin; SOD, superoxide dismutase; Cp, ceruloplasmin; MDA, malondialdehyde.
[2] Blood samples were collected on d 15 of lactation from the 2nd to 4th parity sows.
[3] Sow parity effect ($P < 0.05$).
[4] Linear response to copper level ($P < 0.05$).
[5] Quadratic response to copper level ($P < 0.05$).
[6] Tendency of linear response to copper level ($P < 0.10$).

Table 5. Effects of dietary copper source and level on nutrient concentrations in milk (as-is basis)[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item *Diet No.:*	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	9	9	8	6	8			
Fat, %[2]	5.72	5.78	6.19	4.98	5.06	6.03	0.38	0.10	0.07
Protein, %	4.60	4.62	4.75	4.44	4.19	4.57	0.12	0.02	0.11
Lactose, %[2]	5.95	5.95	5.93	6.16	6.00	5.93	0.06	0.10	0.10
Gross energy, Mcal/kg[3]	1.03	1.03	1.08	0.96	0.94	1.05	0.04	0.07	0.06
Total solids, %[3]	17.26	17.31	17.93	16.50	16.22	17.57	0.45	0.06	0.05
Non-fat solids, %[4]	10.97	10.90	11.08	10.95	10.61	10.90	0.12	0.11	0.13
Minerals, µg/mL[5]									
Copper[2]	1.12	1.23	1.34	1.01	1.12	1.36	0.05	0.13	< 0.0001
Iron	2.05	1.66	1.51	1.38	1.63	1.48	0.23	0.22	0.62
Zinc[2]	4.41	3.83	3.74	4.20	3.33	3.49	0.39	0.32	0.17

[1] Milk samples were collected from the 3rd and 4th parity sows.
[2] Linear response to copper level ($P < 0.05$).
[3] Tendency of linear response to copper level ($P < 0.10$).
[4] Quadratic response to copper level ($P < 0.05$).
[5] Manganese was below the detectable level with atomic absorption spectrophotometer.

on fertility of sows. It is in agreement with the results of Cromwell et al. (1993), who reported that there was no difference in the number of litters produced by sows fed diets containing 0 or 250 mg/kg of supplemental Cu (above the basal NRC level in the diets) as $CuSO_4$ for 6 parities.

In the present study, increasing dietary Cu levels did not affect litter size of total born, live born, and weaning piglets ($P > 0.66$; Table 2). Cromwell et al. (1993) previously reported a significant improvement of litter size of total born for sows fed 250 mg/kg supplemented diets compared to sows fed diets without Cu supplementation ($P < 0.10$). The increasing dietary Cu levels linearly increased piglet weight of total born ($P = 0.12$) and live born ($P = 0.06$) piglets; meanwhile, maternal liver Cu concentrations also exhibited a linear increase with increasing dietary Cu levels ($P < 0.0001$; Table 6). These results might indicate an association between fetal development and maternal Cu status. This speculation is in accordance with a human study that demonstrated Cu concentrations in the placenta from 20 to 40 yr old mothers at 37 to 41 wk of gestation were positively correlated with neonate weight (Özdemir et al., 2009). Significant interactions between dietary Cu source and level were observed on adjusted piglet weight at weaning ($P = 0.06$) and adjusted piglet weight gain ($P = 0.02$), which showed increasing linear response within TBCC diets with no response or decreasing linear response within $CuSO_4$ diets, to the increasing dietary Cu levels. Tribasic copper chloride has been demonstrated to be a less prooxidative form of Cu than $CuSO_4$ in the intestinal lumen of animals and feed. Studies showed

Table 6. Effects of dietary copper source and level (mg/kg) on tissue trace mineral concentration (DM basis, mg/kg) of sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate						
Copper level, mg/kg:	20	120	220	20	120	220	Baseline		*P* values	
Item *Diet No.:*	1	2	3	4	5	6	gilts	SEM	Source	Level
No. of observations	3	6	4	4	4	4	6			
Liver										
Copper[3, 4, 5]	123.9	232.5	923.0	298.0	459.8	1372.5	72.1	129.8	0.04	< 0.0001
Iron	1363.9	1416.6	979.6	934.0	993.9	852.1	1156.1	159.5	0.04	0.41
Manganese[3]	8.29	8.07	7.03	6.82	6.84	6.73	6.12	0.58	0.04	0.14
Zinc[3]	260.1	234.1	226.4	255.0	236.3	282.9	165.0	27.1	0.46	0.58
Copper										
Kidney[6]	46.62	38.89	40.73	39.51	36.91	52.71	23.14	11.36	0.97	0.98
Heart[3]	11.51	11.47	11.91	11.58	12.10	14.05	9.82	1.06	0.37	0.43
Ovary	9.88	7.62	10.15	7.69	7.27	8.56	5.34	2.18	0.47	0.99

[1] A single degree of freedom contrast was conducted to compare experimental sows vs. baseline gilts.
[2] Tissue samples were collected from sows that completed the 3rd and 4th parity.
[3] Difference for the contrast of experimental sows vs. baseline gilts ($P < 0.05$).
[4] Linear response to copper level ($P < 0.0001$).
[5] Quadratic response to copper level ($P = 0.04$).
[6] Tendency of difference between experimental sows vs. baseline gilts ($P < 0.10$).

Table 7. Effects of dietary copper source and level on tissue trace mineral concentration (DM basis, mg/kg) of piglets at birth[1]

Copper source:	**Tribasic copper chloride**			**Copper sulfate**					
Copper level, mg/kg:	**20**	**120**	**220**	**20**	**120**	**220**		***P* values**	
Item *Diet No.:*	**1**	**2**	**3**	**4**	**5**	**6**	**SEM**	**Source**	**Level**
No. of observations	5	10	9	8	6	8			
Liver									
Copper	156.8	174.4	182.2	154.2	147.4	188.7	20.5	0.65	0.29
Iron	496.3	793.7	698.7	675.7	571.4	778.1	144.7	0.92	0.58
Manganese	5.62	5.75	6.05	5.14	6.12	6.07	0.43	0.93	0.27
Zinc	357.4	412.7	431.8	406.8	390.4	409.9	61.9	0.97	0.82
Copper									
Kidney[2]	11.85	10.69	11.30	12.36	10.15	13.57	1.18	0.45	0.20
Heart	10.17	9.92	9.93	10.04	9.96	10.14	0.19	0.80	0.71

1 Tissue samples were collected from piglets in the 3rd and 4th parity litters.
2 Tendency of quadratic response to copper level ($P = 0.08$).

that high dietary levels of $CuSO_4$ may result in elevated MDA concentration in duodenum of nursery pigs and decreased liver vitamin E level in broilers as compared to the same level of Cu in the form of TBCC (Fry et al., 2012; Huang et al., 2015; Luo et al., 2005). In addition, the prooxidant activity exerted by $CuSO_4$ had greater detrimental effects on dietary vitamin activity during storage when compared to TBCC (Lu et al., 2010; Miles et al., 1998).

Apparent Total Tract Digestibility

Lactating sows fed TBCC diets had greater ATTD of DM, nitrogen, and GE than those fed $CuSO_4$ diets. It might suggest that TBCC fed sows absorbed more nutrients and consequently provide more nutrients to their progenies through milk. This is consistent with the results of the present study that piglets from TBCC fed sows had greater weaning weight and weight gain during lactation than those from $CuSO_4$ fed sows.

Antioxidant Status

The activity of total and Cu/Zn SOD in sow serum increased linearly with increasing dietary Cu levels regardless of Cu sources ($P < 0.05$). Copper is a critical constituent of Cu/Zn SOD; it is reversibly oxidized and reduced by successive encounters with to yield O_2 and H_2O_2 during SOD catalysis (Tainer et al., 1983). It has been reported that 50 and 250 mg/kg of supplemental Cu as $CuSO_4$ increased serum and erythrocyte SOD activity of growing pigs, respectively, when compared with pigs fed diets without supplemental Cu (Feng et al., 2007; Gonzales-Eguia et al., 2009). Moreover, it also has been reported that greater dietary Cu may lead to elevated SOD activity in other species including beef cattle (40 vs. 0 mg/kg supplemental Cu) and broiler chickens (50 vs. 0 mg/kg supplemental Cu) (Song et al., 2011; Correa et al., 2014).

Table 8. Effects of dietary copper source and level on tissue trace mineral concentration (DM basis, mg/kg) of piglets at weaning[1]

Copper source:	**Tribasic copper chloride**			**Copper sulfate**					
Copper level, mg/kg:	**20**	**120**	**220**	**20**	**120**	**220**		***P* values**	
Item *Diet No.:*	**1**	**2**	**3**	**4**	**5**	**6**	**SEM**	**Source**	**Level**
No. of observations	5	9	10	8	5	8			
Liver									
Copper[2]	358.1	399.3	537.1	379.7	444.9	541.6	27.0	0.29	< 0.0001
Iron	152.3	343.3	224.1	299.0	207.6	417.5	80.4	0.32	0.46
Manganese	9.69	9.18	10.41	10.55	9.45	9.83	0.67	0.74	0.41
Zinc[3]	391.8	280.3	322.3	384.4	356.3	292.1	42.7	0.72	0.15
Copper									
Kidney	29.86	22.06	29.00	32.47	28.45	32.77	4.57	0.27	0.38
Heart[4]	8.68	9.06	9.04	8.78	9.17	8.98	0.22	0.79	0.25

1 Tissue samples were collected from piglets in the 3rd and 4th parity litters.
2 Linear response to copper level ($P < 0.0001$).
3 Tendency of linear response to copper level ($P = 0.06$).
4 Sow parity effects ($P < 0.05$).

Milk Composition

In the current study, the improved milk composition of sows fed TBCC diets compared to those fed $CuSO_4$ diets may explain the greater adjusted piglet weight at weaning, as well as the greater adjusted litter and piglet weight gain of litters from TBCC fed sows. In addition, the greater nutrient concentrations in milk might also indicate an improved mammary gland development. Mammary gland development occurs throughout many stages of growth and reproduction in swine, and various hormones are involved in the control of mammary development (Farmer and Hurley, 2015). Growth hormone (**GH**), which is secreted by the pituitary gland, regulates the development of mammary gland mainly through insulin-like growth factor-1 (IGF-1) (Ruan and Kleinberg, 1999; Gallego et al., 2001). It has been demonstrated by many studies that high dietary Cu levels increased expression of GH and growth hormone-releasing hormone mRNA, as well as serum IGF-1 levels in growing pigs (Wang et al., 2016; Yang et al., 2011; Zhou et al., 1994). Therefore, it is speculated that the greater nutrient levels in milk produced by TBCC fed sows may be associated with the Cu-induced changes of hormone secretion.

Tissue Trace Mineral Levels

Trace mineral concentrations of neonatal piglets did not differ across treatments. This indicates that transfer of trace minerals from the maternal tissues to fetuses was not affected by dietary Cu source and level. However, liver Cu concentrations of weanling piglets linearly increased as sow dietary Cu level increased ($P < 0.05$). This is associated with Cu concentrations in milk, which exhibited a significant linear increase with increasing sow dietary Cu levels. Moreover, since Cu level was concentrated in sow feces (Diet 1 to 6: 296, 964, 1607, 407, 1127, and 1782 mg/kg during gestation; 185, 876, 1559, 252, 961, and 1583 mg/kg during lactation); and the suckling piglets were able to access sow feces during lactation, the greater liver Cu accumulation of weanling piglets might also be partially attributed to coprophagy.

Conclusions

In the current study, sows fed with TBCC diets had improved nutrient digestibility, lower Cu but greater Fe and Mn concentrations in liver, higher nutrient levels in milk, greater serum antioxidant enzyme activity during lactation, and greater litter performance, when compared to sows fed with $CuSO_4$ diets. Additionally, increasing dietary Cu levels linearly increased digestibility of DM and GE during lactation, the antioxidant enzyme activity of sows, nutrient levels in milk, total born and live born piglet weight, and liver Cu concentrations in sows and weanling piglets. Results of the present study indicate that TBCC might be a superior Cu source compared to $CuSO_4$ for sows; the greater dietary Cu levels is beneficial to improve piglet birth weight, serum SOD activity, and Cu concentration in tissue of sows and piglets without showing any apparent negative effects on sows and progenies.

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Development and Nutritional Value of Advanced Soybean Products Used in Diets for Young Pigs

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Summary

Soybean meal is an excellent source of protein for pigs because of its excellent amino acid profile and its high amino acid digestibility. However, the presence of anti-nutritional factors such as trypsin inhibitors, lectins, oligosaccharides, and antigenic proteins reduces the inclusion level in weanling diets. The oligosaccharides can be reduced or eliminated by processing the soybean meal via aqueous ethanol extraction, fermentation, or enzymatic treatment. Reducing the concentration of oligosaccharides increases the concentration of other nutrients such as crude protein. Soy protein concentrate and soy protein isolate have low levels of stachyose and raffinose and antigenic proteins, and greater digestibility of amino acids and energy than soybean meal. Enzyme-treated soybean meal also has low concentration of oligosaccharides, and fermented soybean meal contains no stachyose or raffinose. However, fermented soybean meal sometimes has a reduced digestibility of lysine because of Mailard reactions caused by excess heating during drying after fermentation, but this is usually not observed in enzyme-treated soybean meal where the amino acid digestibility is similar to soybean meal.

Introduction

Soybeans is one of the most important crops in the U.S. and the co-product soybean meal (**SBM**) is the primary source of protein in swine diets because of its favorable concentration and balance of digestible amino acids (**AA**). Domestic livestock and poultry consumed 28 million metric tons of SBM in 2010, using nearly 80% of all the soybean meal processed in the U.S. However, raw soybeans contain anti-nutritional factors: trypsin inhibitors, lectins, phytic acid, oligosaccharides (raffinose, stachyose, and verbascose), and antigenic protein (glycinin and β-conglycinin; Baker, 2000; NRC, 2012; He et al., 2015). To reduce the concentration of some of the anti-nutritional factors such as trypsin inhibitors, soybean products need to be heated before being fed to swine because trypsin inhibitors are heat-labile.

Soybeans contain approximately 37% crude protein and 20% fat (Table 1). However, after crushing, most of the fat is removed via solvent extraction, and the resulting SBM contains less than 2% fat. The concentration of total carbohydrates in intact soybeans is 35 to 40% with approximately 15% being non-structural carbohydrates, such as sucrose, uronic acid, oligosaccharides, and free sugars. The concentration of oligosaccharides (raffinose, stachyose, and verbascose) in soybeans is between 4 and 7% (NRC, 2012). However, in SBM the concentration of oligosaccharides may be between 7 and 11%, where the concentration of stachyose is between 5.1 and 7.3% and the concentration of raffinose is between 1.1 and 3.8% (Cervantes-Pahm and Stein, 2010; NRC, 2012).

The main oligosaccharides in soybeans are raffinose, stachyose, and verbascose, and they are commonly called α-galactosides because of the presence of galac-

Table 1. Nutritional composition of U.S. soybeans and U.S. soybean meal (NRC, 2012).

	Full-fat soybeans	48% U.S. SBM, dehulled
Dry matter, %	92.36	89.98
Crude protein, %	37.56	47.73
Ash, %	4.89	6.27
Ether extract, %	20.18	1.52
Carbohydrates, %		
Sucrose	6.42	4.30
Raffinose	0.77	3.78
Stachyose	3.89	7.33
Verbascose	0.03	0.00
Starch	1.89	1.89
NDF	10.00	8.21

tose in the structure of these three oligosaccharides. Raffinose consists of one unit of glucose, one unit of fructose, and one unit of galactose. Stachyose and verbascose have a structure that is similar to raffinose with the exception that they contain two or three units of galactose, respectively. The glucose and fructose units in the oligosaccharides are connected by an α-(1-2) glycosidic linkage, whereas an α-(1-6) linkage connects glucose to galactose and also connects the galactose units in stachyose and verbascose. Therefore, the glycosidic linkages in the α-galactosides may be hydrolyzed by the enzyme α-galactosidase. However, pigs do not secrete the digestive enzymes necessary to cleave α-1,6 linkages and raffinose, stachyose, and verbascose are, therefore, considered indigestible by pigs, and are fermented in the digestive tract (Canibe and Bach Knudsen, 1997). However, it has been demonstrated that the ileal digestibility of α-galactosides is between 50 and 80% (Bengala-Freire et al., 1991; Canibe and Bach Knudsen, 1997; Smiricky et al., 2002), which indicates that there is considerable fermentation taking place in the small intestine of pigs. This fermentation allows pigs above approximately 20 kg to utilize the energy from the oligosaccharides in the form of short chain fatty acids. However, younger pigs fed large quantities of SBM do not handle the fermentation of these oligosaccharides very efficiently, and negative side effects such as diarrhea, gastrointestinal discomfort, and a reduction of weight gain are observed (Liying et al., 2003). Inclusion of SBM in diets fed to weanling pigs is, therefore, usually restricted to less than 20%. The total tract digestibility is considered to be 100% because any α-galactosides that are not fermented in the small intestine are rapidly fermented in the large intestine.

Removal of the Oligosaccharides

Removing the oligosaccharides from conventional SBM may be achieved by removing the non-protein constituents from dehulled and deffated soybeans, by fermentation or by enzyme treatment. Therefore, different products can be obtained: soy protein concentrate (**SPC**), soy protein isolate (**SPI**), fermented soybean meal (**FSBM**), or enzyme-treated soybean meal (**ESBM**).

Soy Protein Concentrate

Soy protein concentrate by definition contains a minimum of 65% crude protein (**CP**) on a dry matter (**DM**) basis (Endres, 2001), and it is produced by acid leaching, extraction with aqueous alcohol, or by denaturing the protein with moist heat before extraction with water (Endres, 2001). Thus, SPC contains fewer trypsin inhibitors, sucrose, raffinose, and stachyose than SBM (Oliveira and Stein, 2016), and the concentration of CP and AA are greater than in SBM (Table 2). The AA digestibility in SPC is similar to SBM, except for some AA where the standardized ileal digestibility (**SID**) is greater in SPC than in SBM (Oliveira and Stein, 2016; Pedersen et al., 2016). However, SPC contains more DE and ME than SBM (Oliveira and Stein, 2016).

Reduction in particle size of SBM improves the digestibility of most indispensable AA (Fastinger and Mahan, 2003) and the values of DE and ME (Rojas and Stein, 2015). Therefore, when SPC is ground to 70 or 180 μm the SID of arginine, isoleucine, phenylalanine, and tryptophan is greater than in SBM. However, there are no differences among conventional SBM and SPC ground to 70, 180, or 700 μm in DE and ME (Casas et al., 2017).

Addition of SPC to weanling pig diets at the expense of animal proteins does not affect the growth performance during the initial 4 weeks post-weaning (Guzmán et al., 2016; Casas and Stein, 2017), but SPC reduces the incidence of post-weaning diarrhea (Guzmán et al., 2016). Therefore, SPC may be used in diets fed to weanling pigs as a replacement for animal proteins.

Table 2. Nutrient composition of soybean meal, soy protein concentrate, and soy protein isolate (NRC, 2012).

	Soybean Meal	Soy Protein Concentrate	Soy Protein Isolate	Fermented SBM	Enzyme-treated SBM
Dry matter, %	89.9	92.6	93.7	92.7	92.9
Crude protein, %	47.7	65.2	84.8	55.6	54.1
Ether extract, %	1.5	1.1	2.8	1.8	2.3
Ash, %	6.3	6.11	4.2	7.1	7.0
Carbohydrates, %					
Sucrose	4.30	0.67	0.13	-	-
Raffinose	3.78	0.46	-	-	-
Stachyose	7.33	0.91	-	-	-
Verbascose	0.00	-	-	-	-
Amino Acids, %					
Arginine	3.45	4.75	6.14	3.95	3.70
Histidine	1.28	1.70	2.19	1.41	1.37
Isoleucine	2.14	2.99	3.83	2.48	2.55
Leucine	3.62	5.16	6.76	4.09	4.25
Lysine	2.96	4.09	5.19	3.20	3.14
Methionine	0.66	0.87	1.11	0.71	0.75
Phenylalanine	2.40	3.38	4.40	2.78	2.87
Threonine	1.86	2.52	3.09	2.13	2.09
Tryptophan	0.66	0.81	1.13	0.72	0.69
Valine	2.23	3.14	4.02	2.57	2.67

Soy Protein Isolate

Soy protein isolate contains at least 80% CP (Middelbos and Fahey, 2008). It is produced by solubilizing the protein at neutral and slightly alkaline pH, and the extract is then precipitated by acidification to obtain the protein isolate (Berk, 1992). Therefore, most of the non-protein constituents from soybeans are removed, and SPC therefore contains very few trypsin inhibitors, limited fiber, and practically none of the oligosaccharides (Table 2). The allergenic proteins glycinin and β-conglycinin are deactivated in soy protein isolate, and also in SPC because they are produced by extraction at temperatures greater than 50°C (Sissons et al., 1982). The AA digestibility of SPI is similar to that in casein (Cervantes-Pahm and Stein, 2010) and similar to SBM, but for some AA, SPI has greater SID values than SBM (Pedersen et al., 2016). Soy protein isolate is well tolerated by weanling pigs (Li et al., 1991) but its high cost of production makes it uncommon in commercial pig feed production.

Fermented Soybean Meal

Fermented soybean meal is produced by inoculating conventional soybean meal with the bacterium *Aspergillus oryzae* or other microbes (Hong et al., 2004). Raw soybeans are soaked in distilled water for 3 hours and placed in an autoclave at 100–120°C for 20 minutes. After that, autoclaved soybeans are cooled to room temperature for 3 hours. The soybeans are then inoculated with *A. oryzae* and placed in an incubator for 48 hours at 30°C with 90% moisture. After fermentation, soybeans are dried at 50–60°C and ground in a hammer mill (Hong et al., 2004).

Fermented soybean meal contains more DM, CP, and ash than conventional SBM (Table 2). The absence of sucrose, stachyose, and raffinose in FSBM is attributed to the production of α-galactosidase by *Aspergillus oryzae* during the fermentation process (Cervantes-Pahm and Stein, 2010). The disappearance of these saccharides is the main reason for the analyzed increase in the concentration of other nutrients in FSBM as compared with SBM.

Yoon (2012) analyzed 4 different samples of FBSM and observed that the concentration of CP is between 53 and 58%, which is greater than in conventional SBM. However, the SID of lysine is lower in FSBM than in SBM (Cervantes-Pahm and Stein, 2010) and lower lysine-to-CP ratio compared with SBM, SPC, SPI, and ESBM (Pedersen et al., 2016). This is likely a result of the heat that is used during drying of FSBM, which may result in heat damage. Heat damage may result in Maillard reactions that can destroy some of the lysine in the FSBM (Stein et al., 2009), and Maillard reactions can also result in reduced SID of lysine (Stein et al., 2009).

Peptide size distribution does not differ between SBM and FSBM and there is no evidence for hydrolysis of the peptides in FSBM (Cervantes-Pahm and Stein, 2010). The concentration of glycinin and β-conglycinin in FSBM is similar to SBM, which is in contrast with SPI that has low concentration of the allergenic proteins (Cervantes-Pahm and Stein, 2010). Antigenic proteins glycin and β-conglycinin reduce ADG and G:F in young pigs (Zhao et al., 1998) and may reduce villus height in the small intestine and decrease nitrogen digestibility in pigs (Li et al., 1991).

Enzyme-treated Soybean Meal

Enzyme-treated SBM is produced by treating dehulled solvent-extracted SBM for several hours with a proprietary blend of enzymes (Goebel and Stein, 2011). Enzyme treatment removes sucrose and reduces the concentrations of oligosaccharides and allergenic proteins (Cervantes-Pahm and Stein, 2010). Therefore, the concentration of CP and other nutrients is greater in ESBM than in SBM (Table 2). In addition, there are no differences in SID of AA between SBM and ESBM (Cervantes-Pahm and Stein, 2010; Pedersen et al., 2016). Several studies have demonstrated that ESBM is well accepted by young pigs, and ESBM may, therefore, replace animal proteins in starter diets for pigs.

Conclusion

The reduction of oligosaccharides and antigenic proteins in SPC, SPI, FSBM and ESBM increases the nutritional value to young pigs and the AA digestibility is usually similar to that in SBM or greater. As a result, these special soybean products may be used in diets fed to weanling pigs as a replacement for fishmeal or others animal proteins.

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Nutritional Regulation of Piglet Gut Health

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Summary

Gastrointestinal health issues rank among the highest causes of neonatal morbidity and mortality across most mammalian species, including pigs (USDA, 2012). Gut health is an area of research with significant impact to swine nutrition because without a healthy intestinal tract there are severe impacts from poor nutrient digestion and absorption and slow growth to increased intestinal pathogens which can result in death. However, the definition of gut health is vague without a precise etiology. Nevertheless, it is known that nutrition has a great impact on gut development. The stresses of production, growth, and changing nutrition over the growth period often present a challenge to optimum intestinal health, and therefore, efficient growth for optimal least cost swine production. For years, animal livestock has utilized antimicrobial growth promotants to mitigate some of the challenges of gut health and to optimize efficient growth. However, with pressure to reduce the feeding of antimicrobial growth promotants in livestock production, it has forced the industry to look at alternative ways to optimize growth performance while minimizing use of antimicrobials. The development of new/different management and feeding strategies to stimulate gut development and a healthy microbiome in the neonate is necessary, and understanding how nutritional bioactive compounds promote gut and microbiome development in early life is necessary for optimizing gut health and immune function throughout production.

Introduction

The definition of gut health is more than a homeostatic gastrointestinal tract. Bischoff (2011) defined gut health by five major criteria: 1. the effective digestion and absorption of food, 2. absence of GI illness, 3. a normal and stable microbiome, 4. effective immune status, and 5. status of well-being. This definition of gut health does not only associate health with pathogenic disease disruption of intestinal health, but allows for inclusion of the concepts of how prevention and avoidance of gastrointestinal disease are part of the gut health equation. For the swine industry we know many stressors involved in production can disturb pig performance, and it is important to understand how developing nutritional and management programs may enhance gut health through prevention and/or treatment to optimize pig performance.

One of the five criteria that Bischoff (2011) defines as a part of gut health is a normal and stable microbiome. In recent years, the focus on how microbiome affects the overall health of humans and animals has become a focus of the nutritional community because diet significantly impacts the gut microbiome (Isaacson and Kim, 2012). Intestinal microbiota are acquired early in life and play many roles in host's health. They affect maturation of the gut, program/mature the developing immune system, competitively exclude pathogenic organisms that cause disease, and the microbes are critical in nutrient digestion such as fiber and synthesis of vitamins. Additionally, imbalance in a homeostatic microbial population in the gut is known to cause disruption of gut barrier function (Pluske et al., 2018), and gut barrier function is critical in maintenance of the five criteria of gut health. Therefore, optimal nutritional support for the developing intestinal tract, microbiome, and immune system of young pigs is important for efficient growth and health, and likely critical for life-long health of the intestinal track given the impact on mucosal immune development (Frese et al., 2015). Bioactive components are particularly important in prevention and possibly management of gut health. With new feed supplements being developed to moderate neonatal in-

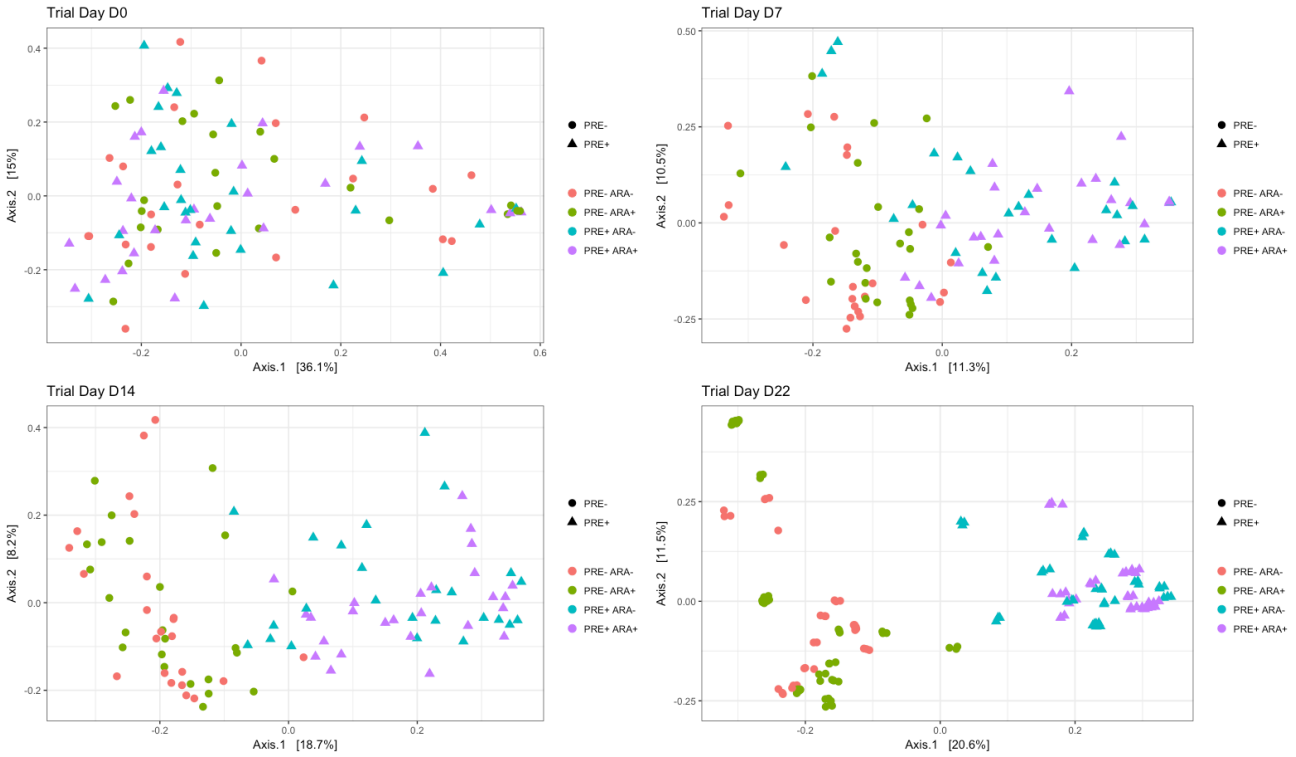

Figure 1. Principle Coordinate Analysis of Changing Colonic Microbial Taxa in Suckling Piglets. Each plot represents a either trial day 0, 7, 14 or 21. Dietary treatments are PRE = prebiotics; ARA = arachidonic acid; or a combination of PRE+ARA; Supplementation is denoted by + or – in the figure legends on the principle coordinate plots.

testinal issues that improve survivability and improve production performance in swine research on understanding how nutrients could be used in preventive health are important. Additionally, there are increasing pressures to mitigate intestinal challenges and improve piglet performance without the use of antimicrobial compounds and high mineral feeding that are known to enhance performance, but also have negative environmental impacts (Pluske, 2013). Therefore understanding how other bioactive compounds may alter microbial populations in the developing piglet may lead to a better understanding of how we use nutrition to optimize gut health and immune status in pigs.

Studies of gut microbiota show that early postnatal environmental exposures play a critical role in determining the overall composition of the adult animal and programing of the adult immune system (Kau et al., 2011). Early dietary exposure to mother's microbiota and nutrients of maternal milk which stimulate microbial composition are some of the first exposures of the neonatal GI tract to colonization (Bode, 2012). Of the dietary components known to modulate neonatal gut microbial populations, the oligosaccharide component composition are the most influential. Oligosaccharides, like prebiotics, are undigested by the neonate and have several functions in the development of young piglets gastrointestinal tract including pathogen binding, immune system development, and serving as a growth factor to beneficial bacteria (Tao et al., 2010; Salcedo et al., 2016). In addition to prebiotics developing the microbiome, they also are known to increase short chain fatty acid (**SCFA**) production by the microbiome. SCFA are produced by gut microbiome members (Nicholson et al., 2012) (e.g., *Eubacterium*, *Roseburia*, *Faecalibacterium*, and *Coprococcus*) directly from sugar to produce a mixture of fatty acids - acetate, propionate, butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate, and hexanoate (Nicholson et al., 2012). These SCFA's are associated with decreased colonic pH, inhibition of pathogens; increased water and sodium absorption; and an energy source to colonic epithelial cells. Acetate accumulates in circulation making it a very good candidate for pre- or probiotic supplementation[13]. Acetate producing bacteria include *Akkermansia muciniphila, Bacteroides, Bifidobacterium, Prevotella, Ruminococcus* (Koh et al., 2016). Treatments that increase these organisms will result in health benefits for an animal and decrease pathogens.

A second beneficial SCFA is butyrate. Acetate can be converted to butyrate by the microbiome, which is a primary energy source for colonocytes, is anti-inflammatory, promotes cell function and differentiation (Koh

et al., 2016) - which also occurs with bacterial assocation, and significantly changes response to inflammation (Kol et al., 2014). Butyrate also regulates immune cells responses and tight junctions involved in gut barrier function (Koh et al., 2016; Yan and Ajuwon, 2017).

The effects of dietary long-chain polyunsaturated fatty acids (**LCP-UFA**) on intestinal microbiota have not been as well researched. Neilsen et al. (2007) showed that daily fish oil supplementation for one month influenced the composition of the fecal bacteria in human babies. Similarly, piglets fed formula containing LCP-UFA significantly influenced overall bacterial composition and the size of the Bacteroides community (Anderson et al., 2011). These and others report positive effects of LCPUFA on the numbers of different lactic acid bacteria which have benefit to the developing intestine (Anderson et al., 2011). Therefore, we are interested in how diet not only provides essential nutrients for growth and development, but how individual nutrients have critical bioactive roles in modulating gut physiology. Understanding the interaction of these nutrients are a way for nutritionist to modify dietary programs for optimizing piglet growth and survivability.

Table 1. Dietary Effects on Intestinal pH

	CONT	PRE	ARA	PRE + ARA	SE	*P*-Value
Ileum	7.36	7.29	7.36	7.31	0.05	0.647
Cecum	6.22[a]	5.74[b]	6.24[a]	5.84[b]	0.06	0.001
Proximal Colon	6.38[a]	5.71[b]	6.34[a]	5.78[b]	0.07	0.001
Distal Colon	6.50[a]	6.05[b]	6.60[a]	6.15[b]	0.07	0.001

[a,b]Means within a row with different superscripts differ ($P<0.05$)
Dietary treatments are CONT = control, PRE = prebiotics, ARA = arachidonic acid, or a combination of PRE+ARA.

Table 2. Dietary Effects on VFA Concentrations in Distal Colon

	CONT	PRE	ARA	PRE + ARA	SE	*P*-Value
Acetate	41.5[a]	51.4[b]	44.7[a]	48.6[b]	2.2	0.001
Propionate	12.3[a]	18.0[b]	13.7[a]	16.2[b]	1.5	0.001
Butyrate	7.2[a]	8.4[b]	7.9[a]	8.4[b]	.3	0.001
Total	71.0[a]	87.4[b]	76.8[a]	83.1[b]	3.3	0.001

[a,b]Means within a row with different superscripts differ ($P<0.05$).
Dietary treatments are CONT = control, PRE = prebiotics, ARA = arachidonic acid, or a combination of PRE+ARA.

Methods

Full term crossbred pigs were vaginally delivered and allowed to suckle colostrum for 24 hours and then individually housed and fed milk replacer in an environmentally controlled barn. Pigs were allocated, according to body weight and litter origin, to one of four dietary treatments differing in prebiotic and fatty acid composition: 1) formula containing no prebiotic and baseline LCPUFA (CONT), 2) formula enriched with 4g/L GOS + 4g/L PDX (PRE), 3) formula enriched with 2.5% ARA (LCPUFA), or 4) formula enriched with both PRE & LCPUFA (PRE+LCPUFA). They were fed to ~60% ad libitum with fresh diets offered three times a day for 21 days. Fecal swabs were collected weekly and microbiome analysis was performed via 16S rDNA Illumina sequencing. On day 21, pigs were euthanized, intestinal pH and colonic mucosal scrapings and digesta were immediately collected and snap frozen for further analysis. Fatty acid composition of colonic mucosa was analyzed via GC-MS and volatile fatty acids composition of the colonic digesta was analayzed via GC-FID. Data were analyzed according to a randomized block design using general linear models procedures of SAS (SAS, Cary, NC). Microbiome were analyzed by using R multivariate analysis.

Results

Diet did not affect growth ($P > 0.1$). Microbial taxa significantly changed from d 0 to d 21 in a diet-dependent manner with diets containing prebiotics clustering together by d 7 post-feeding and becoming more closely associated with longer feeding times ($P < 0.05$). Specifically, an increase in bacterial taxa belonging to the phyla Bacteroidetes was observed ($P < 0.05$). Fatty acid inclusion in the diets significantly changed the fatty acid composition of the colonic mucosa. Colonic mucosal arachidonic acid (**ARA**) percent (ARA)

Table 3. Dietary Effects on Fatty Acid Composition (%wt) of Colonic Mucosa

	CONT	ARA	SE	*P*-Value	-PRE	+PRE	SE	*P*-Value
C18:0	21.36	21.32	0.94	0.98	23.65[a]	19.03[b]	1.02	0.002
C18:1c	23.49	22.54	0.72	0.35	22.37	23.66	0.78	0.21
C18:2c n6	14.10[a]	12.81[b]	0.43	0.04	11.26[b]	15.67[a]	0.46	0.001
C18:3 n3	0.40	0.40	0.04	0.99	0.31[b]	0.48[a]	0.03	0.001
C20:4 n6	3.83[a]	6.67[b]	0.33	0.001	3.74[b]	6.67[a]	0.37	0.001

[a,b]Means within a row with different superscripts differ ($P<0.05$).
CONT = control, ARA = arachidonic acid, PRE = prebiotics.

(w/w) increased from 3.83 in controls to 6.67 in pigs fed diets enriched in ARA ($P < 0.001$), with reciprocal reductions in linoleate concentration. Dietary prebiotics (PRE) also increased ARA and linoleate concentration but reduced stearate concentration in colonic mucosa ($P < 0.002$). Additionally, dietary PRE reduced colonic pH associated with increased concentration of VFAs ($P < 0.05$). In conclusion, addition of PRE to milk replacer progressively alters microbial taxa over the first three weeks of life and increases colonic fermentation. Dietary ARA has minimal impact on microbial taxa but enriches colonic mucosa.

Conclusions

The aim of the study was to understand the impact of dietary fatty acids and prebiotics on changes in intestinal microbial populations that may have beneficial outcomes on piglet gut health. To investigate this goal we fed one-hundred and two crossbred day old piglet on milk replace supplemented with prebiotics or LCPUFA for 21 days. Additionally, the interaction of bioactive nutrients was also investigated by including a treatment with a combination of prebiotic plus LCPUFA. Diets containing dietary prebiotics had the most significant effects on gut microbial population of the developing neonate. Microbial populations of piglets being fed prebiotics had a progressive shift in microbial taxa over the first three weeks of life. The early instability of the microbial population in the gut lends itself to a time when diet could have significant impact on microbial communities and could impact long-term intestinal microbe populations and modify intestinal health. Piglets fed prebiotics also had increased total volatile fatty acids in the colonic digesta due to an increase in the individual VFAs, actetate, propionate, and butyrate at the end of the study. The decrease in colonic digesta pH caused by changes in microbial populations, and the production of VFA are associated with competitive exclusion of pathogenic microorganism (Nicholson et al., 2012; Koh et al., 2016).

Arachidonic acid did not significantly change microbial composition of the piglet intestine, but did modify the fatty acid composition of cell membranes. Further analysis will need to be completed to understand what impact this could have on gut health. However, previously we have shown metabolites of long-chain polyunsaturated fatty acids have significant impact on gut barrier function following an intestinal insult (Jacobi et al., 2011). Modifying the gut environment during development using nutritional interventions may have significant impact on gut health during challenges later in swine production. Although long term studies would need to be completed in swine to truly understand the impact of early neonatal nutrition in programming gut microbial population that affect overall intestinal health in later production stages, it seems reasonable there could be a significant impact to improve swine gut health.

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Glutamine and Transport Stress

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Summary

In modern swine production, newly weaned pigs are often subjected to multiple stressors including weaning stress, transport stress, and thermal stress, and these have the potential to increase the incidence of animal disease, morbidity, and mortality, especially when they occur concomitantly. In order to promote stress recovery, prevent the onset of disease, and improve animal welfare, producers often administer dietary antibiotics for 14 to 42 days after pigs enter nurseries or wean-to-finish facilities. However, due to increased consumer concern regarding the use of antibiotics in animal production and regulatory changes for antibiotic use, it has become increasingly important to develop antibiotic alternatives that can help pigs recover from stressful events as effectively as dietary antibiotics. It was determined in this study that replacing dietary antibiotics with 0.20% L-glutamine improved the productivity of weaned pigs at a similar level as dietary antibiotics throughout the nursery phase. However, the positive effects of dietary antibiotics and L-glutamine during the initial 14 days post-weaning on pig productivity were diminished as pigs entered the grow-finish phase, and no carcass characteristics were altered by nursery dietary treatments. Comparing seasons, pigs weaned in the spring had greater growth performance compared to pigs weaned in the summer resulting in heavier carcass weights and greater loin depth for spring weaned pigs. In conclusion, 0.20% L-glutamine supplementation improved pig health and productivity after weaning and transport similarly to antibiotics; however, the positive growth effects of dietary antibiotics and L-glutamine provided the first 14 days post-weaning were diminished during the grow-finish phase.

Introduction

Weaning is a complex stressor associated with social, environmental, and metabolic stress in pigs (Lallés et al., 2004). In newly weaned pigs, stress is induced by separation from the sow, relocation and mixing piglet groups, and a radical change in diet that often reduces or eliminates feed intake in the first 48 hours post-weaning (Brooks et al., 2001). As a result, piglets undergo a variety of physiological and metabolic changes that can negatively impact welfare. Changes may result from elevated blood cortisol levels (Moeser et al., 2007), compromised feed intake (Maenz et al., 1994), altered intestinal morphology (Lallés et al., 2004), and dehydration due to the switch from an all liquid (milk) to a solid diet. Unfortunately, in commercial production systems, weaning stress may be compounded by transport stress, which can induce significant weight loss with only 4 hours (**h**) of travel time (Hicks et al., 1998), and ambient temperature likely plays a critical role in determining total stress load incurred by weaned piglets (Lambooy, 1988).

Historically, swine producers used dietary antibiotics to help newly weaned pigs overcome the stress of weaning and associated stressors (Chiba, 2010). However, due to several consumer and regulatory factors, it has become increasingly important to develop antibiotic alternatives that can help pigs recover from stressful events as effectively as dietary antibiotics. Previous research determined that inclusion of 0.20% L-glutamine in the diets of newly weaned and transported pigs could improve growth rate and well-being more effectively than dietary antibiotics (chlortetracycline + tiamulin; Johnson and Lay, 2017). However, this study was conducted under controlled conditions utilizing simulated transport and individual housing. Therefore, this study's objectives were to evaluate the impact of replacing dietary antibiotics with 0.20% L-glutamine on swine

growth performance, health status, welfare, and carcass characteristics of pigs in a production environment following weaning and transport during different seasons (summer or spring). We hypothesized that withholding dietary antibiotics would negatively impact the overall well-being of piglets, and that diet supplementation with 0.20% L-glutamine would have a similar effect on piglet health and productivity as dietary antibiotics in a production environment.

Materials and Methods

All procedures involving animal use were approved by the Purdue University Animal Care and Use Committee and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). Mixed sex crossbred pigs [n = 480; 5.62 ± 0.06 kg initial body weight (**BW**); Duroc x (Yorkshire x Landrace)] were weaned and transported at 18.4 ± 0.2 d of age in central Indiana during the summer of 2016 and the spring of 2017. One day prior to weaning and transport, all pigs were individually weighed, blocked by BW, and randomly allotted to pens, and pens of pigs were allotted to three dietary treatments with 10 pens per dietary treatment per season of weaning and transport. Initially, each pen contained eight pigs. Dietary treatments were antibiotics [A; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or 0.20% L-glutamine (**GLN**; Ajinomoto North America, Inc., Raleigh, NC). The treatments were arranged in a 2 × 3 factorial with main effects of season (summer or spring) and diet (**A, GLN, NA**).

On the day of weaning and transport, pigs were removed from sows and herded up a ramp into a gooseneck livestock trailer (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA) providing 0.07 m^2 per pig. The loading ramp to the trailer was 2.13 m in length providing an 11.0^0 incline. Two data loggers (Hobo®; data logger temperature/RH; Onset®; Bourne, MA) were evenly spaced within the trailer to measure ambient temperature ($\mathbf{T_A}$) and relative humidity (**RH**) in 5 minute (**m**) intervals. During transport, the average trailer T_A during the summer season was 29.4 ± 0.2°C and during the spring was 11.0 ± 0.2°C with trailer RH being 64.3 ± 0.8% and 63.1 ± 0.9%, respectively. Trailers were bedded with wood shavings and ventilation openings were adjusted based on the T_A.

Piglets were transported as a group in the trailer for approximately 12 h and 819 km without feed or water. Total transport time was determined by adding loading time, time spent in the trailer, unloading time, and the time it took to be sorted into their respective pens in the nursery facility. The average time to wean and load the trailer was 55 m. The drivers were the same and followed the identical route for the summer and spring season. The transport route was approximately 50% two-lane roads and 50% four-lane roads. The route was approximately 273 km in length and was completed three times during the transport phase for each season. The route took, on average 3 h 16 m to complete. The driver was changed and the truck was refueled after each time the 273 km route was completed. At the conclusion of the 12 h transport, piglets were unloaded from the trailer, individually weighed, and placed into pens. The average time to unload the trailer, weigh the pigs, and place into pens was 1 h 10 m.

Nursery Phase

Following transport, pigs were placed in their assigned pens and provided their respective dietary treatments for 14 d in two weekly phases (d 0 to 14 of the nursery phase). Following the dietary treatment period, all pigs were fed common antibiotic free diets from d 14 to the end of the nursery phase (d 34; Table 1). Diets were corn-soybean meal-based and formulated to meet or exceed nutrient requirements (NRC, 2012) in meal form in four phases during the nursery period (Table 1). Pig BW and feed consumption were recorded every 7 d during the nursery period to calculate ADG, ADFI, and G:F. On d 13 and 33, one pig per pen was harvested to collect plasma and gut tissue samples for biomarker assays and intestinal histology.

Therapeutic antibiotic administration was recorded for the duration of the trial (weaning to market). Pigs were treated when exhibiting clinical signs of illness. Treatment dose, product given, date given, pig and pen identification, and reason administered were recorded. Reason for therapeutic administration was then categorized for post-hoc analysis. Categories were: enteric challenge (scours or loose watery stool), respiratory challenge (coughing, thumping, or labored breathing), lameness (carrying a limb or difficulty walking or swollen joints), unthriftiness (BW loss, poor gain, loss of body condition, or rough hair coat), and all other treatments (Streptococcus suis, skin infection, and abscess).

The nursery facility where the initial 34 d of the trial was conducted contained pens (1.22 m × 1.37 m) that provided initially approximately 0.21 m^2 per pig. All pens contain one 5-hole dry self-feeder and a cup waterer to allow for ad libitum access to feed and water. The nursery barn has a shallow pit for manure storage and completely slatted plastic floors. The nursery room operated on mechanical ventilation using a 4-stage digital controller (Airstream TC5-2V25A, Automated Pro-

duction Systems, Assumption, IL, USA). During d 0 to 14 post-weaning, the nursery room average daily T_A during the summer season was 31.5 ± 1.82°C and during the spring was 30.6 ± 0.68°C. From d 14 to 34 the nursery T_A was 28.7 ± 1.14°C and 26.0 ± 0.84°C for the summer and spring seasons, respectively.

Grow-Finish Phase

On day 34, all pigs were moved to the grow-finish facility for the remainder of the trial and pen integrity was maintained. Common antibiotic free diets were corn-soybean meal-DDGS-based diets provided in meal form to meet or exceed nutrient requirements (NRC, 2012) in six phases during the grow-finish period (Table 2). Pigs and feeders were weighed every 21 d during the grow-finish period to determine ADG, ADFI, and G:F.

The grow-finish facility contained pens (1.68 m × 4.27 m) that provided approximately 1.19 m^2 per pig. All pens contained one 2-hole dry self-feeder and a nipple waterer to allow for ad libitum access to feed and water. The grow-finish barn had a shallow pit for manure storage and completely slatted concrete floors. The barn was mechanically ventilated. During d 0 to 62 of the grow-finish phase, the

Table 1. Composition of Nursery Diets, as fed.

	Phase 1[1]			Phase 2[2]				
Item	**A[5]**	**GLN[6]**	**NA[7]**	**A**	**GLN**	**NA**	**Phase 3[3]**	**Phase 4[4]**
Ingredient, %								
Corn	30.81	31.18	31.38	37.52	37.89	38.09	51.63	57.38
SBM, 48% CP	13.95	13.95	13.95	18.00	18.00	18.00	25.65	30.70
DDGS, 7% oil	---	---	---	---	---	---	---	5.00
Soybean Oil	5.00	5.00	5.00	5.00	5.00	5.00	3.00	---
Choice White Grease	---	---	---	---	---	---	---	3.00
Limestone	0.79	0.79	0.79	0.74	0.74	0.74	0.86	1.33
Monocal. Phos.	0.40	0.40	0.40	0.49	0.49	0.49	0.49	0.74
Vitamin Premix[8]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral Premix[9]	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Selenium Premix[10]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.30	0.35
Plasma Protein	6.50	6.50	6.50	2.50	2.50	2.50	---	---
Spray Dried Blood Meal	1.50	1.50	1.50	1.50	1.50	1.50	---	---
Soy Concentrate	4.00	4.00	4.00	3.00	3.00	3.00	2.50	---
Select Menhaden Fish Meal	5.00	5.00	5.00	4.00	4.00	4.00	4.00	---
Dried Whey	25.00	25.00	25.00	25.00	25.00	25.00	10.00	---
Lactose	5.00	5.00	5.00	---	---	---	---	---
L-Lysine-HCL	0.07	0.07	0.07	0.2	0.2	0.2	0.28	0.40
DL-Methionine	0.22	0.22	0.22	0.23	0.23	0.23	0.18	0.17
L-Threonine	0.04	0.04	0.04	0.09	0.09	0.09	0.12	0.14
L-Tryptophan	---	---	---	0.01	0.01	0.01	0.01	---
Zinc Oxide	0.375	0.375	0.375	0.375	0.375	0.375	0.375	---
Copper Sulfate	---	---	---	---	---	---	---	0.10
Aureomycin 50[12]	0.40	---	---	0.40	---	---	---	---
Denagard 10[13]	0.18	---	---	0.18	---	---	---	---
L-Glutamine[14]	---	0.20	---	---	0.20	---	---	---
Banminth 48[15]	---	---	---	---	---	---	---	0.10
Clarifly, 0.67%[16]	---	---	---	---	---	---	0.08	0.07
Calculated chemical composition								
ME, kcal/kg	3536	3536	3536	3510	3510	3510	3418	3396
Fat, %	7.27	7.27	7.27	7.36	7.36	7.36	5.73	5.86
CP, %	24.6	24.6	24.6	22.9	22.9	22.9	22.3	21.3
SID Lysine, %	1.55	1.55	1.55	1.45	1.45	1.45	1.35	1.25
Ca, %	0.90	0.90	0.90	0.85	0.85	0.85	0.80	0.75
Total P, %	0.75	0.75	0.75	0.71	0.71	0.71	0.64	0.57
Avail. P, %	0.60	0.60	0.60	0.55	0.55	0.55	0.45	0.36

1 Fed d 0 to 7 post-weaning and transport.
2 Fed d 7 to 14 post-weaning and transport.
3 Fed d 14 to 21 post-weaning and transport.
4 Fed d 21 to 34 post-weaning and transport.
5 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)].
6 Pigs provided 0.20% L-glutamine
7 Pigs provided no dietary antibiotics
8 Provided per kilogram of the diet: vitamin A, 6,614 IU; vitamin D_3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B_{12}, 0.039 mg.
9 Provided per kilogram of the diet available: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg.
10 Provided 0.3 ppm selenium
11 Provided 600 FTU per kg of the diet
12 Aureomycin (Zoetis, Parsippany, NJ) provided 441 ppm chlortetracycline in the diet.
13 Denagard (Elanco Animal Health, Greenfield, IN) provided 38.6 ppm tiamulin in the diet.
14 Ajinomoto North America, Inc., Raleigh, NC
15 Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet.
16 Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm (Phase 3) and 4.7 ppm (Phase 4) diflubenzuron in the diet.

room average daily T_A for the summer weaned pigs was 22.4 ± 1.14°C and for the spring weaned pigs was 25.5 ± 2.64°C. From d 62 to 125 the T_A was 19.9 ± 0.83°C and 25.7 ± 2.48°C for the pigs weaned in the summer and spring seasons, respectively.

Marketing

At the end of the 159 d experiment, pigs from each pen were tattooed with pen number and shipped approximately 48 km to Indiana Packers Corporation (Delphi, IN). Pigs were slaughtered under commercial conditions with carbon dioxide stunning. Standard carcass criteria of loin and backfat depth, hot carcass weight (**HCW**), fat-free lean index, and yield were collected. Fat depth and loin depth were measured with an optical probe (Fat-O-Meater, SFK Technology A/S, Herlev, Denmark) inserted between the third and fourth rib from the last rib (counting from the posterior of the carcass) and 7 cm from the dorsal midline of the hot carcass. Lean percentage was calculated according to the Indiana Packers Corporation (2015) formula and the fat-free lean percentage was calculated according to Schinckel et al. (2010) procedures.

Table 2. Composition of grow-finish diets, as fed.

Item	Phase 1[1]	Phase 2[2]	Phase 3[3]	Phase 4[4]	Phase 5[5]	Phase 6[6]
Ingredient, %						
Corn	61.47	64.65	66.40	71.10	82.38	68.67
SBM, 48% CP	23.20	16.15	9.75	5.25	4.25	15.10
DDGS, 7% Fat	10.00	15.00	20.00	20.00	10.00	10.00
Choice White Grease	2.00	1.00	1.00	1.00	1.00	3.00
Limestone	1.37	1.35	1.39	1.32	1.16	1.26
Monocal. Phos.	0.47	0.32	0.05	0.00	0.10	0.27
Vitamin Premix	0.150[7]	0.150[7]	0.125[8]	0.120[9]	0.100[10]	0.150[7]
Trace Mineral Premix	0.10[11]	0.09[12]	0.08[13]	0.07[14]	0.05[15]	0.10[11]
Selenium Premix	0.05[16]	0.05[16]	0.05[16]	0.05[16]	0.025[17]	0.05[16]
Phytase[18]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.30	0.30	0.25	0.30
L-Lysine-HCL	0.42	0.46	0.48	0.46	0.37	0.42
DL-Methionine	0.11	0.08	0.05	0.01	0.00	0.10
L-Threonine	0.130	0.130	0.120	0.105	0.095	0.160
L-Tryptophan	0.010	0.030	0.035	0.040	0.030	0.030
Paylean 2.25[19]	---	---	---	---	---	0.15
Availa Zn 120[20]	---	---	---	---	---	0.042
Clarifly, 0.67%[21]	0.07	0.09	0.07	0.08	0.09	0.10
Calculated chemical composition						
ME, kcal/kg	3373	3337	3351	3359	3371	3438
Fat, %	5.29	4.69	5.06	5.15	4.73	6.40
CP, %	19.34	17.59	15.99	14.18	11.90	16.01
SID Lysine, %	1.10	0.98	0.85	0.73	0.60	0.90
Ca, %	0.70	0.65	0.60	0.55	0.50	0.60
Total P, %	0.50	0.47	0.41	0.38	0.35	0.42
Avail. P, %	0.32	0.30	0.26	0.24	0.20	0.26

1 Fed d 0 to 21 of the grow-finish phase.
2 Fed d 21 to 42 of the grow-finish phase.
3 Fed d 42 to 62 of the grow-finish phase.
4 Fed d 62 to 83 of the grow-finish phase.
5 Fed d 83 to 104 of the grow-finish phase.
6 Fed d 104 to 125 of the grow-finish phase.
7 Provided per kilogram of the diet: vitamin A, 3,968 IU; vitamin D_3, 397 IU; vitamin E, 26.5 IU; vitamin K, 1.3 mg; riboflavin, 5.3 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B_{12}, 0.023 mg.
8 Provided per kilogram of the diet: vitamin A, 3,307 IU; vitamin D3, 331 IU; vitamin E, 22.1 IU; vitamin K, 1.1 mg; riboflavin, 4.4 mg; pantothenic acid, 11 mg; niacin, 16.5 mg; vitamin B12, 0.020 mg.
9 Provided per kilogram of the diet: vitamin A, 3,174 IU; vitamin D_3, 317 IU; vitamin E, 21.2 IU; vitamin K, 1.0 mg; riboflavin, 4.2 mg; pantothenic acid, 10.6 mg; niacin, 15.8 mg; vitamin B_{12}, 0.019 mg.
10 Provided per kilogram of the diet: vitamin A, 2,647 IU; vitamin D_3, 265 IU; vitamin E, 17.7 IU; vitamin K, 0.9 mg; riboflavin, 3.5 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg; vitamin B_{12}, 0.016 mg.
11 Available mineral provided per kilogram of the diet: iron, 97 mg; zinc, 97 mg; manganese, 12 mg; copper, 9 mg; iodine, 0.37 mg.
12 Available mineral provided per kilogram of the diet: iron, 87 mg; zinc, 87 mg; manganese, 10.8 mg; copper, 8.1 mg; iodine, 0.33 mg.
13 Available mineral provided per kilogram of the diet: iron, 77.6 mg; zinc, 77.6 mg; manganese, 9.6 mg; copper, 7.2 mg; iodine, 0.29 mg.
14 Available mineral provided per kilogram of the diet: iron, 68 mg; zinc, 68 mg; manganese, 8.4 mg; copper, 6.3 mg; iodine, 0.26 mg.
15 Available mineral provided per kilogram of the diet: iron, 48.5 mg; zinc, 48.5 mg; manganese, 6 mg; copper, 4.5 mg; iodine, 0.18 mg.
16 Provided 0.3 ppm selenium
17 Provided 0.15 ppm selenium
18 Provided 600 FTU per kg of the diet
19 Paylean (Elanco Animal Health, Greenfield, IN) provided 7.5 ppm ractopamine HCl in the diet
20 Zinpro Corporation, Eden Prairie, MN
21 Clarifly (Central Life Sciences, Schaumburg, IL) provided 4.7, 6.0, 5.4, 6.7 ppm diflubenzuron in the diet, respectively.

Statistics

Data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC), with pen as the experimental unit. Main effects of season and diet and their interactions were tested. The assumptions of normality of error, homogeneity of variance, and linearity were confirmed post-hoc. All therapeutic treatment rate data were log-transformed to meet assumptions of normal-

ity; however, all log-transformed data are presented as arithmetic means for ease of interpretation. All non-transformed data are presented as LS means. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

Results

Nursery Phase

When comparing the main effect of dietary treatment, ADG was greater overall ($P = 0.01$; 14.9%) from d 0 to 14 of the nursery period in A and GLN fed pigs compared to NA pigs, but no ADG differences were detected between A and GLN pigs (Table 3). An increase in ADFI ($P = 0.04$) was detected from d 0 to 14 of the nursery phase for A compared to NA pigs, but no differences were observed between GLN versus A and NA pigs. Feed efficiency (G:F) was greater ($P = 0.01$; 7.7%) from d 0 to 14 of the nursery phase for A compared to NA and GLN pigs, but no differences were observed between NA and GLN pigs. Day 14 BW was greater overall ($P = 0.01$) for A (8.65 kg) and GLN (8.50 kg) pigs compared to NA (8.19 kg) pigs; however, no differences were detected between A and GLN pigs (Table 3).

Overall, from d 0 to 34 of the nursery period, ADG ($P = 0.01$; 7.9%), ADFI ($P = 0.09$), G:F ($P = 0.01$; 4.3%), and final BW ($P = 0.04$) were greater for pigs fed A compared to NA fed pigs, with GLN fed pigs being intermediate and not different from A and NA pigs (Table 3). No other dietary treatment growth performance differences ($P > 0.05$) were detected during the nursery phase.

During the spring season, pigs tended to have reduced ADFI ($P = 0.08$; 5.1%) compared to the summer from d 0 to 14 of the nursery phase (Table 3). However, from d 14 to 34 of the nursery phase, ADG tended to be increased ($P = 0.09$) and G:F was increased ($P = 0.01$) during the spring compared to the summer (3.7 and 7.4%, respectively; Table 3). Overall, from d 0 to 34 of the nursery period, G:F was reduced ($P = 0.04$; 4.1%) during the summer compared to the spring (Table 3). No other seasonal effects were observed during the nursery period ($P > 0.05$).

A diet x season interaction was detected ($P = 0.04$) from d 14 to 34 of the nursery phase where G:F was greater in the spring in NA (0.69 ± 0.01) and GLN (0.68 ± 0.01) pigs compared to NA pigs (0.61 ± 0.01) during the summer. However, no differences were observed between A pigs (0.66 ± 0.01) during the spring and A (0.64 ± 0.01) and GLN (0.63 ± 0.01) pigs during the summer (data not presented). No other diet x season interactions were detected ($P < 0.05$).

On d13, plasma TNF-α was reduced (P=0.02) in A (36.7±6.9 pg/mL) and GLN pigs (40.9±6.9 pg/mL) versus NA pigs (63.2±6.9 pg/mL). On d33, villus height:crypt depth tended (P=0.09) to be greater in A (2.71±0.09) and GLN (2.72±0.09) compared to NA (2.54±0.09) fed pigs.

Grow-Finish Phase

There were no carry-over effects of nursery dietary treatments observed ($P > 0.17$) during the grow-finish period (Table 4). From d 0 to 62 of the grow-finish phase, G:F was reduced ($P = 0.01$; 4.3%) for the pigs weaned during the summer compared to the spring (Table 4). Average daily gain, ADFI, and G:F were reduced ($P = 0.01$; 14.6, 4.4, and 12.1%, respectively) for the summer weaned pigs from d 62 to 125 of the grow-finish phase compared to the spring pigs (Table 4). Overall, from d 0 to 125 of the grow-finish period, ADG, G:F, and final BW were reduced ($P = 0.01$; 9.2%, 5.1%, and 9.8 kg, respectively) in the summer weaned pigs compared to the spring (Table 4). No other growth performance differences were observed ($P > 0.05$) during the grow-finish period with any comparison (Table 4).

Treatment rates

From d 0 to 14, there was a tendency ($P = 0.07$) for a diet by season interaction for enteric treatments with pigs fed A having the lowest and similar percentage of pigs treated in the summer (3.13%) and spring (3.13%), however pigs fed GLN had a similar low percentage of pigs treated during the summer (3.75%) but the greatest percentage in the spring (8.19%) while the pigs fed NA diets had the greatest (6.88%) enteric therapeutic treatments during the summer and a reduced intermediate level of therapies (4.55%) in the spring (Table 5). Pigs treated for other reasons were greater ($P \leq 0.02$) from d 0 to 14 during the spring (1.08%) compared to the summer (0.00%), regardless of dietary treatment (Table 5).

A diet x season effect was detected ($P = 0.04$) where pigs treated for lameness from d 14 to 34 was greater in the spring for GLN pigs (2.12%) compared to all other treatments. However, no differences were observed between A (0.56%) and NA (0.00%) pigs during the spring, and A (0.48%), GLN (0.00%), and NA (0.00%) pigs during the summer (Table 5).

During d 62 to 125 of the grow-finish phase, enteric disease treatments tended ($P < 0.08$) to be reduced by A (0.00%) treatment and highest for the GLN (1.17%) treatment with NA (0.34%) pigs being intermediate (Table 6). From d 62 to 125, treatment for unthriftiness was reduced ($P = 0.01$) in GLN (0.00%) and NA pigs (0.00%) compared to A pigs (1.11%).

Table 3. Effect of dietary treatment and season on nursery pig growth performance.[1]

	Main Effects								
	Season		Diet				*P*		
Parameter	**Summer[2]**	**Spring[3]**	**A[4]**	**GLN[5]**	**NA[6]**	**SE**	**D[7]**	**S[8]**	**D x S**
Day 0 to 14									
Initial BW, kg	5.64	5.51	5.58	5.59	5.57	0.29	0.99	0.70	0.99
ADG, g	210.1	206.1	224.2^{a}	210.8^{a}	189.2^{b}	10.19	0.01	0.56	0.82
ADFI, g	274.6	260.6	277.1^{a}	272.1^{ab}	253.6^{b}	13.21	0.04	0.08	0.92
G:F	0.80	0.80	0.84^{a}	0.79^{b}	0.77^{b}	0.01	0.01	0.91	0.17
Day 14 BW, kg	8.44	8.46	8.65^{a}	8.50^{a}	8.19^{b}	0.52	0.01	0.83	0.97
Day 14 to 34									
ADG, g	439.0	455.7	458.0	447.4	436.7	12.05	0.21	0.09	0.43
ADFI, g	693.5	674.7	702.3	680.4	669.6	22.81	0.16	0.19	0.63
G:F	0.63	0.68	0.65	0.66	0.65	0.01	0.78	0.01	0.04
Day 0 to 34									
ADG, g	347.4	355.9	364.5^{a}	352.7^{ab}	337.7^{b}	10.18	0.01	0.23	0.58
ADFI, g	525.9	509.0	532.2^{x}	517.1^{xy}	503.2^{y}	17.43	0.09	0.12	0.77
G:F	0.70	0.73	0.73^{a}	0.71^{ab}	0.70^{b}	0.01	0.03	0.01	0.07
Final BW, kg	17.20	17.62	17.78^{a}	17.49^{ab}	16.96^{b}	0.74	0.04	0.11	0.69

1 A total of 10 pens were used per dietary treatment per season.
2 Pigs weaned and transported for 12 h during July 2016.
3 Pigs weaned and transported for 12 h during April 2017.
4 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
5 Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
6 Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
7 Dietary treatment
8 Season
a,b Letters indicate significant differences ($P \leq 0.05$) within a row and main effect.
x,y Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and main effect.

Table 4. Effect of dietary treatment and season on grow-finish pig growth performance.[1]

	Main Effects								
	Season		Diet				*P*		
Parameter	**Summer[2]**	**Spring[3]**	**A[4]**	**GLN[5]**	**NA[6]**	**SE**	**D[7]**	**S[8]**	**D x S**
Day 0 to 62									
ADG, kg	0.76	0.77	0.78	0.76	0.76	0.01	0.32	0.37	0.62
ADFI, kg	1.79	1.75	1.80	1.76	1.75	0.03	0.40	0.14	0.88
G:F	0.44	0.46	0.45	0.46	0.45	0.01	0.80	0.01	0.36
Day 62 BW, kg	64.72	65.50	65.99	65.02	64.31	0.96	0.22	0.32	0.76
Day 62 to 125									
ADG, kg	0.82	0.96	0.88	0.89	0.90	0.02	0.41	0.01	0.36
ADFI, kg	2.83	2.96	2.87	2.91	2.90	0.05	0.72	0.01	0.42
G:F	0.29	0.33	0.30	0.31	0.31	0.01	0.17	0.01	0.62
Day 0 to 125									
ADG, kg	0.79	0.87	0.83	0.83	0.83	0.01	0.95	0.01	0.58
ADFI, kg	2.31	2.35	2.33	2.33	2.32	0.03	0.97	0.21	0.60
G:F	0.37	0.39	0.38	0.38	0.38	0.01	0.54	0.01	0.56
Final BW, kg	117.37	127.19	122.77	121.73	122.34	1.23	0.83	0.01	0.64

1 A total of 10 pens were used per dietary treatment per season.
2 Pigs weaned and transported for 12 h during July 2016.
3 Pigs weaned and transported for 12 h during April 2017.
4 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
5 Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
6 Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
7 Dietary treatment
8 Season

Table 5. Effect of dietary treatment and season on therapeutic antibiotic treatment rate during the nursery period.[1]

	Main Effects									
	Summer[2]			Spring[3]				*P*		
Parameter	**A[4]**	**GLN[5]**	**NA[6]**	**A**	**GLN**	**NA**	**SE**	**D[7]**	**S[8]**	**D x S**
Day 0 to 14										
Enteric[9]	3.13^{y}	3.75^{y}	6.88^{xy}	3.13^{y}	8.19^{x}	4.55^{xy}	2.31	0.31	0.38	0.07
Lame[10]	1.88	1.88	1.25	0.63	1.39	0.63	1.02	0.73	0.27	0.89
Unthrifty[11]	1.88	1.25	1.25	0.00	0.69	1.25	1.02	0.92	0.22	0.48
Respiratory[12]	---	---	---	---	---	---	---	---	---	---
Other[13]	0.00	0.00	0.00	1.25	0.69	1.25	0.86	0.86	0.02	0.86
Day 14 to 34										
Enteric	0.00	0.95	0.48	0.00	0.00	0.56	0.66	0.36	0.33	0.37
Lame	0.48^{b}	0.00^{b}	0.00^{b}	0.56^{b}	2.12^{a}	0.00^{b}	1.00	0.08	0.06	0.04
Unthrifty	0.00	0.00	1.03	1.03	1.15	1.03	0.80	0.64	0.14	0.57
Respiratory	0.00	0.00	0.48	0.00	0.53	0.00	0.53	0.58	0.94	0.20
Other	0.00	0.00	0.00	0.00	0.53	0.00	0.53	0.31	0.27	0.31

1 A total of 10 pens were used per dietary treatment per season.
2 Pigs weaned and transported for 12 h during July 2016.
3 Pigs weaned and transported for 12 h during April 2017.
4 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
5 Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
6 Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
7 Dietary treatment
8 Season
9 Percent of pigs within pen treated with therapeutic antibiotics for enteric challenge.
10 Percent of pigs within pen treated with therapeutic antibiotics for lameness.
11 Percent of pigs within pen treated with therapeutic antibiotics for un-thriftiness.
12 Percent of pigs within pen treated with therapeutic antibiotics for respiratory challenge.
13 Percent of pigs within pen treated with therapeutic antibiotics for all other conditions.
a,b,c Letters indicate significant differences ($P \leq 0.05$) within a row.
x,y Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row.

Table 6. Effect of season and dietary treatment on therapeutic antibiotic treatment rate for enteric challenges during the grow-finish period.[1]

	Summer[2]			Spring[3]				*P*		
Parameter	**A[4]**	**GLN[5]**	**NA[6]**	**A**	**GLN**	**NA**	**SE**	**D[7]**	**S[8]**	**D x S**
Day 0 to 62										
Enteric[9]	0.56	0.00	0.00	0.56	0.67	0.67	0.67	0.81	0.31	0.77
Lame[10]	0.56	0.56	1.89	0.00	0.00	0.00	1.06	0.41	0.02	0.41
Unthrifty[11]	1.78	0.00	0.67	0.00	0.00	0.56	0.99	0.24	0.17	0.16
Respiratory[12]	11.11	10.78	8.00	0.56	1.11	2.22	2.92	0.60	<0.01	0.77
Other[13]	1.11	2.33	1.33	0.00	0.00	0.56	1.11	0.69	0.02	0.45
Day 62 to 125										
Enteric	0.00	0.67	0.00	0.00	1.67	0.67	0.93	0.08	0.19	0.58
Lame	2.22	0.67	0.67	0.00	0.00	0.00	1.05	0.21	0.01	0.21
Unthrifty	0.56	0.00	0.00	1.67	0.00	0.00	0.93	0.01	0.28	0.30
Respiratory	10.33	8.22	12.17	7.33	7.39	6.78	4.20	0.81	0.49	0.86
Other	0.56	0.00	0.00	0.00	0.00	0.00	0.56	0.37	0.32	0.37

1 A total of 10 pens were used per dietary treatment per season.
2 Pigs weaned and transported for 12 h during July 2016.
3 Pigs weaned and transported for 12 h during April 2017.
4 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
5 Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
6 Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
7 Dietary treatment
8 Season
9 Percent of pigs within pen treated with therapeutic antibiotics for enteric challenge.
10 Percent of pigs within pen treated with therapeutic antibiotics for lameness.
11 Percent of pigs within pen treated with therapeutic antibiotics for un-thriftiness.
12 Percent of pigs within pen treated with therapeutic antibiotics for respiratory challenge.
13 Percent of pigs within pen treated with therapeutic antibiotics for all other conditions.

Table 7. Effect of season and dietary treatment on carcass characteristics.[1]

	Main Effects								
	Season		Diet				*P*		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	S[8]	D x S
No HCW[9] covariate									
HCW, kg	92.42	97.44	95.32	95.54	93.93	1.32	0.60	<0.01	0.70
Loin depth, mm	64.0	67.4	65.8	65.9	65.5	0.72	0.93	<0.01	0.60
Backfat, mm	21.4	22.1	21.7	21.6	21.7	0.59	0.99	0.31	0.40
Yield, %	77.2	75. 7	76.6	76.4	76.4	0.19	0.68	<0.01	0.46
Lean, %[10]	54.42	54.61	54.51	54.55	54.47	0.25	0.97	0.53	0.54
Fat-free lean, %[11]	48.69	48.79	48.74	48.79	48.69	0.30	0.97	0.76	0.50
HCW covariate									
Loin depth, mm	64.4	67.00	65.7	65.7	65.7	0.69	0.99	0.01	0.66
Backfat, mm	22.0	21.4	21.6	21.5	22.0	0.49	0.75	0.33	0.57
Yield, %	77.3	75.5	76.5	76.3	76.4	0.17	0.69	0.01	0.63
Lean, %	54.20	54.82	54.54	54.60	54.38	0.23	0.78	0.04	0.71
Fat-free lean, %	48.41	49.06	48.77	48.85	48.58	0.27	0.77	0.07	0.68

1 A total of 10 pens were used per dietary treatment per season.
2 Pigs weaned and transported for 12 h during July 2016.
3 Pigs weaned and transported for 12 h during April 2017.
4 Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
5 Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
6 Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
7 Dietary treatment
8 Season
9 Hot carcass weight
10 Equation used: 54.672154 - (0.412525 × backfat, mm) - (0.002982 × hot carcass weight, kg × 2.20462) + (0.1433242 × loin depth, mm) (Indiana Packers Corporation, 2015)
11 Equation used: 51.2 - (0.510 × backfat, mm) + (0.131 × loin depth, mm) (Schinckel et al., 2010)

Pigs treated for lameness were greater ($P < 0.02$) from d 0 to 62 and d 62 to 125 during the summer (1.00%, 1.19%, respectively) compared to the spring (0.00%, 0.00%, respectively), regardless of dietary treatment (Table 6). Treatment for respiratory challenges were greater ($P < 0.01$) from d 0 to 62 during the summer (9.96%) compared to the spring (1.30%). Pigs treated for other challenges were also greater ($P < 0.02$) during the summer compared to the spring from d 0 to 62.

Carcass Characteristics

No dietary treatment effects were observed ($P > 0.60$) on carcass characteristics (Table 7). Hot carcass weight and loin depth were increased ($P < 0.01$; 5.4% and 5.5%, respectively) and carcass yield was reduced ($P < 0.01$; 2.0%) for pigs weaned during the spring compared to the summer (Table 7). When HCW was used as a covariate, loin depth and lean percentage were increased ($P = 0.01$; 4.0% and 1.1%, respectively) and carcass yield was reduced ($P = 0.01$; 2.3%) for the spring weaned pigs compared to the summer (Table 7). Fat-free lean percentage for the spring weaned pigs tended to be greater ($P = 0.07$; 1.3%) compared to the summer when HCW was included as a covariate (Table 7).

Discussion

The need to wean and transport pigs is necessary to reduce the risk of infectious disease through multi-site production (Harris, 2000). However, the resultant stress response can reduce growth performance and welfare in newly weaned pigs (Campbell et al., 2013), especially in the absence of dietary antibiotics (Heo et al., 2013). Despite this, the use of in-feed antibiotics has been reduced in swine production due to consumer preference, legislative action, and concerns about antibiotic resistance, putting the welfare and productivity of newly weaned and transported pigs at risk and necessitating the development of effective alternatives. Recent work has described improved welfare and productivity in piglets provided GLN compared to A and NA following weaning and simulated transport (Johnson and Lay, 2017). In accordance with the aforementioned study, piglets provided GLN after weaning and transport in the present study had improved growth performance compared to NA pigs during the 14 d dietary treatment period, regardless of season of transport. Additionally, no growth performance differences were detected between GLN and A pigs in our current study. Although reasons for this discrepancy are currently unknown, it may be due to differences in study design since the

transport procedure was simulated and piglets were individually housed in the previous study (Johnson and Lay, 2017). While the mechanism(s) of action for improved growth performance has yet to be fully discerned, GLN can serve as energy source for enterocytes thus reducing jejunal atrophy and intestinal epithelial damage (Wu et al., 1996; Yi et al., 2005). Therefore, it is possible that piglets provided supplemental GLN had improved intestinal barrier function leading to greater pathogen resistance, reduced translocation of bacteria (Peng, 2004) and subsequently an improvement in growth performance (Jiang et al., 2009; Johnson and Lay, 2017). This is partially supported by the greater villus height:crypt depth ratios of pigs fed GLN and A in our study. Additionally, we observed at the end of the dietary feeding period (d13), plasma TNF-α was reduced 38.6% for pigs fed GLN and A, further supporting these dietary effects on improving intestinal health and function by feeding GLN and A. These advantages observed in early nursery growth performance may suggest that GLN supplementation could serve as an alternative to dietary antibiotics in production systems.

Although growth performance was improved in GLN and A pigs during the dietary treatment period and the advantage was maintained for the overall nursery period, no differences were detected when compared to NA pigs from d 34 to market when all pigs were fed common antibiotic free diets during the grow-finish period. However, these results were somewhat expected as previous research has described a loss of growth performance differences once dietary antibiotic treatments ceased (Skinner et al., 2014). This may be due to variability differences that diminished the growth rate advantages as the studies progressed or the performance advantages of feeding dietary treatments are limited only to the period when fed. Therefore, it could be suggested that feeding GLN to pigs for a longer duration could have extended the growth benefits; however, further work would be needed to confirm this hypothesis and any increase in growth performance would need to be balanced with the cost of including GLN in diets.

Therapeutic injectable antibiotics are one of many options currently available to aid in the control of pathogens and disease in addition to good biosecurity practices, vaccinations, and dietary antibiotics. In the present study, pigs receiving A had fewer therapeutic antibiotic treatments for enteric challenges compared to GLN pigs during the spring from d 0 to 14 post-weaning, but no differences were detected during the summer. While this may indicate that dietary antibiotic treatments were more effective at reducing pathogen impact compared to GLN, the lack of overall dietary treatment differences may suggest that the season of weaning and transport influences the impact of GLN on therapeutic treatments. Regardless, the increase in therapeutic treatments did not appear to coincide with a reduction in growth performance and this may be due to differences in the mode of action between A and GLN treatments whereby dietary antibiotics reduce pathogen colonization (Pluske et al., 2002) and GLN fed pigs may improve gut barrier function (Wang et al., 2015).

In the present study, no dietary treatment carcass trait differences were detected, confirming previous reports that providing dietary antibiotics for a limited period in the nursery phase would have no impact on meat quality (Skinner et al., 2014). While the effects of providing GLN on carcass characteristics in pigs are unknown, previous reports in broilers reported that GLN supplementation during heat stress improved meat yield (Dai et al., 2011). However, because broilers were provided GLN until harvest in the aforementioned study and pigs in the present study were only provided GLN for 14 d, it is likely that the lack of carcass trait differences are related to the duration of dietary inclusion.

Despite the lack of dietary treatment differences on carcass characteristics, pigs weaned in the spring had greater HCW and loin depth and increased lean percentage and fat-free lean percentage when HCW was used as a covariate compared to summer weaned pigs. While the mechanism(s) for the improvement in carcass characteristics are unknown, we speculate that health status may have impacted the carcass differences observed in the current study due to the differences in therapeutic antibiotic treatment rate between seasons. This response appears to be consistent with previous work by Holck et al., (1998) and Williams et al., (1997) who reported improved carcass characteristics when pigs were reared under higher health status. This suggests that poorer health status may have decreased growth rate and subsequently reduced lean tissue accretion rate. This potential advantage in health status during the spring may have allowed the pigs to grow and deposit lean tissue at a rate closer to their genetic potential because previous studies determined that when pigs were exposed to chronic immune system activation in a health compromised environment, cytokine concentration was elevated (Williams et al., 1997) thereby suppressing lean growth. Less environmental pathogens as indicated by reduced therapeutic antibiotic use could have decreased immune system and cytokine activation thus allowing the potential for increased muscle accretion rate.

Conclusion

Weaning and transport is stressful to pigs and antibiotics have been routinely used to help young pigs overcome these challenges. Despite the advantages in growth performance and productivity found from the use of dietary antibiotics, alternatives to antibiotics are needed. We determined that L-glutamine supplemented at 0.20% improved pig health and productivity after weaning and transport similarly to antibiotics during the nursery phase; however, the positive effects of early nursery dietary antibiotics and L-glutamine were diminished during the grow-finish phase. However, pigs not provided dietary antibiotics had decreased growth rate during the nursery phase. Future work should address if the benefits of feeding dietary antibiotics and L-glutamine are additive and to better understand the mechanism involved for L-glutamine supplementation to improve pig growth performance following weaning and transport stressors.

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Strategic Therapeutic Antibiotic Use Compared to the Challenge of Not Using Antibiotics for Growing Pigs

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Summary

This paper provides clarity on the challenges of not using antibiotics for therapeutic purposes, compared to using them strategically. A decision to not utilize medically important antibiotics in pigs, from birth (or after weaning) to harvest, should not be made without delivering on management imperatives. One must relate the increase in production cost to the 'market opportunity.' Several groups decided to have presence in the no antibiotic 'niche' market, notwithstanding the possible erosion of profit. Triumph Foods conducted an experiment to serve as template for modeling the value proposition for a no therapeutic antibiotic program. In this instance, piglets did not receive antibiotics from birth to market (NAE) as compared to no antibiotics after weaning (NA-W). We also defined critical factors that must be met (e.g. PRRSv free sow herd, robust sire and sow lines, 23-25 d wean age) in order to make the NA approaches most successful. In a NA program, marginal profit is a problem for pork (and beef), since only selected parts of the carcass are sought. Poultry have a large advantage to pork, since they can be vaccinated in the egg phase, and their life-cycle is short; however, mortality does increase and some flocks have to be removed from the NAE program. Suitable tools do not exist for total replacement of therapeutic antibiotics, to treat respiratory disease. However, there are examples of antibiotic 'alternatives' that are auxiliary helpmates; in other words, they have not proven to replace antibiotics, but using them in combination further improves the outcome. Alternatives to enteric disease antibiotics can be more helpful, but they are not entirely satisfactory either. Zinc oxide has proven almost indispensable for weaned pigs; its use helping to reduce therapeutic treatment. Vaccine development and strict farm biosecurity also combine to reduce therapeutic medical treatment. Complete antibiotic removal undercuts humane animal care, so they must be used for pigs that become ill. Precision rearing is more possible with pigs than with poultry, since segregation of sick pigs to medical treatment pens is logistically possible and part of normal practice. Finally, alternatives for antibiotic growth promoters are satisfactory; AGP not being particularly effective with modern methods.

Introduction

This paper presents a comparison of strategic therapeutic antibiotic use to no antibiotics ever, from birth to harvest (NAE), from the perspective of performance and financial implications. Antibiotic alternatives have not proven completely satisfactory in total replacement, but some appear to play a meaningful auxiliary role; the outcome sometimes being to improve the ability to thrive, compared to antibiotics alone. Helping food animals to survive is a very different matter from the discussion of alternatives to antibiotic growth promoters. In the latter case, there are some effective choices.

The attraction to provide meat from animals that have never received antibiotics, follows on EU legislation to remove medically important antibiotics from food animals. Some North American food corporates have made the decision to provide meat products from NAE-sourced animals (e.g. Chick-fil-A, Panera Bread, Chipotle). This has given rise to groups desiring to satisfy this niche with the hope of achieving a market advantage. Marketing decisions were made by corporates in the Poultry sector (e.g. Perdue) to convert 'all' (e.g. 80-85%) of their production to that specification. Foster Farms was one of the few, in the poultry sector, that elected not to follow suit on the basis of humane animal care and responsible therapeutic antibiotic stewardship.

In the pork sector, Smithfield Foods made an early decision to have presence in this market (therapeutic and AGP). This was followed by Tyson and the Clemens Food group (NAE), and some smaller producers. At present, and for the foreseeable future (4-6 years), this pork market niche is small and driven by a small segment of consumers. Food companies that have made this commitment occupy a minute share of the pork market. Product needs are easily met with present commitments; commoditization has been rapid.

Before going directly into NAE (or NA-W) niche meat production, it is well to understand (1) what Europeans mean by 'no antibiotics'; NAE or after weaning (NA-W); (2) how financial evaluations compare for limited, therapeutic antibiotic use versus NAE and (3) what production imperatives need to be delivered on, prior to 'eliminating' therapeutic antibiotics for growing pigs; These 3 matters form the basis for our paper.

Understand 'Antibiotic-free' Program Specifications

In review of many programs associated with restricted or limited antibiotics, we have discovered that some are mistakenly referred to as antibiotic-free, but they are not. One such instance was in conjunction with a farm and processing plant visit in Europe, where there were antibiotics given to every pre-weaned pig, during piglet processing. The pork product from this farm was part of an antibiotic-free market and that dose of antibiotics was approved and widely used by the farmers in the program. We will show, from our studies, that providing a precautionary antibiotic treatment at processing has value prior to and after weaning. Understanding the differences in specifications of the marketing program is important when evaluating the cost and performance of one program compared to another. Proof of delivery on the requirements for a NAE program is significantly more onerous that for NA-W.

Business Reality of NAE in Pig Production

There are a number of road blocks to an NAE program in North America. First, the public segment is small (national, global), and market saturation was easily achieved; thus evolving to a commodity product with challenged marginal return. Second, marginal cost per pig marketed, is not normally known, but it increases even on pigs derived from sow farms of high health status. The primary driver is elevated mortality, pre- and post-weaning to market. When this cost reality is juxtaposed against the demand for only selected cuts, the financial outcome is not encouraging. The third element is the most significant road block to viability – the respiratory pathogen PRRSv. We consider this pathogen to be the first limit to viable NAE (or NA-W) production. When the vaccine is combined with key management steps, PRRSv can be controlled, but not 100% of the time.

The PRRS virus causes immune suppression with deadly, secondary pathogens (e.g. *strep suis, salmonella, E. coli*) arising thereafter. A key to NAE success is to control sow farm PRRSv and lateral transmission once pigs are placed in the field. Pipestone systems has had relative good success, more recently (2017 to current), with their sow farm filtration method, combined with innovative biosecurity enhancement (*Dr. Barry Kerkart, pers. communication*). This places them, and others like them, in a good position for NAE since, as stated, antibiotic alternatives for the respiratory pathogens, *PRRSv, Mycoplasma pneumonia* and *HPS*, are non-existent.

Extending on point number three, the number of diseases (endemic, epidemic) have increased, rather than decreased. New epidemic disease is not expected to subside with PEDv introduction into North America. Key pig growing states have also concentrated in density. These are business realities that need to be considered when discussing NAE. However, this point is clear – that decisions to enter the NAE niche market are usually made without understanding how production cost will change.

Value Proposition for Therapeutic Antibiotics vs NAE

Triumph Foods conducted an integrated study to inform owner leaders, as to the cost of a NAE program, from birth to harvest (Zier-Rush et al., 2017). This data formed the template from which to model opportunity prospects, under various market conditions. The study involved comparison between conventional therapeutic medication for disease control and NAE, to harvest at approximately 290 lbs. The study was robust in that 3 robust sire lines were compared to a high performance reference line (Line D). Pigs were derived from a high health sow farm; characteristic of the status required for a NAE program. Pigs in the NAE group received $2.71 in vaccine per pig, compared to $2.21 for those in the Conventional group. The cost of therapeutic antibiotic treatment, to treat or prevent disease was $2.78 per pig. The cost of non-antibiotic treatment (e.g. aspirin, grazix) for the NAE group was $0.85 per pig. Thus, vaccine and supportive treatment was provided to facilitate NAE viability.

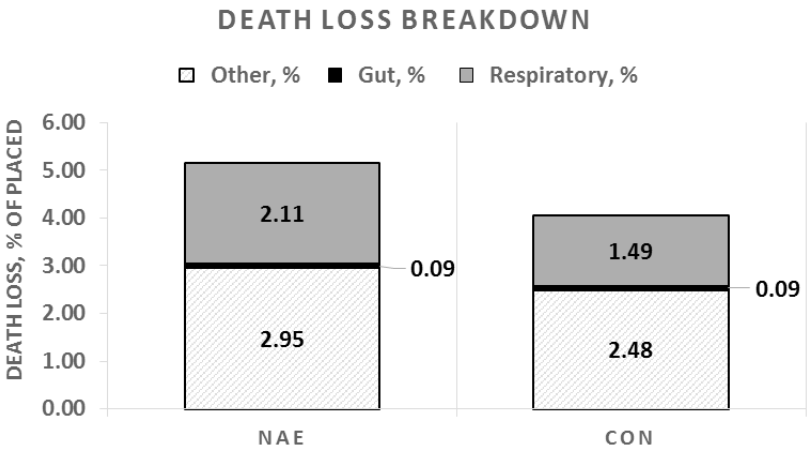

Figure 1. Proportion of mortality judged to be either respiratory, enteric (Gut) or other (known, unknown) causes. Gut and Other, P>0.33, Respiratory P=0.117.

The test was conducted on a 4800 head, commercial W-F site in Iowa, which was retrofitted for research. There was a decline in full-value pigs (FVP) marketed, when antibiotic treatment was removed (feed, water, injectable). Humane care required that pigs, from either treatment, deemed to be in need of medical treatment was removed to a medical treatment pen. Their remaining contribution to the data involved viability.

The first outcome of NAE was noted for viability to weaning at about 21 d of age. There was a 1.78% increase in prewean mortality (data not shown), when a single injection of Excede was not given, at processing, to NAE pigs (included pigs < 6 lbs at weaning). Pigs are not born into a sterile environment, so this proved that the ability to thrive benefit of precautionary antibiotic treatment at processing.

The advantage of antibiotic treatment, to prevent or treat disease, is shown in Table 1. Antibiotic treatment increased FVP, from placement to plant (94.8 vs 92.4%). This was a composite of the advantage in viability and pigs not culled to a lower value market (<230 lbs). The preponderance of mortality was due to respiratory as compared to enteric (Gut) cause, and the ability to medically treat pigs for respiratory challenge made a difference (Figure 1). The marginal advantage to NAE strategy would increase further if immune stress was greater than imposed here. This may require an NAE (or NA-W program to remove a house or site from the program (Steve Pollmann, p.c.). The percent of pigs completing the NAE strategy (not removed for medical treatment) ranged from 82.3 to 77.8% for sire lines tested (Figure 2). Each of the robust lines had a higher percent of pigs that survived (data not shown), and a higher percent that required no antibiotics compared to the benchmark sire.

Based on our sire line comparisons, differences among sires increase as immune stress increases (data not shown). Imposing additional stress, such as pelleted diets, during elevated respiratory immune stress makes the situation even worse.

Table 1. Summary of pig viability and ability to thrive for pigs receiving no antibiotics from birth (NAE) or treated with therapeutic antibiotics (CON) to prevent or treat disease[1]

Variable	NAE	CON	SEM	P-Value
No. pigs tagged at birth (EU)	2969	2953	-	-
Pre-wean death + rejection at wean (< 6.0 lbs), %	12.03	10.25	0.84	0.131
No. pigs placed into Wean - Finish test site	2367	2234	-	-
Full value pigs to harvest, % placed (> 230 lbs)	92.42	94.80	0.51	0.001
Mortality, %	5.16	4.07	0.44	0.079
Cull for low weight (< 230 lbs), %	2.49	1.13	0.28	0.001
Total antibiotic injections, % placed	17.61	34.95	1.50	<0.001
NAE pigs marketed receiving no antibiotics, % placed	80.7	NA	-	-

[1] Pigs were pooled across sire line (Sire line x antibiotic strategy, NS) for the main effects of NAE or Conventional treatments.

The primary driver of financial value was viability (Table 1), as the advantage conferred by antibiotics was negligible for growth rate (+0.02 *P*=0.003), feed efficiency (-0.02, NS), carcass yield (NS) and proportion of saleable meat per unit of carcass (NS). When all aspects of growth, viability, full-value (and cull value) price and value of saleable meat was placed against production cost, the value proposition for NAE emerged (Table 2). The relative advantage among sire lines is also shown.

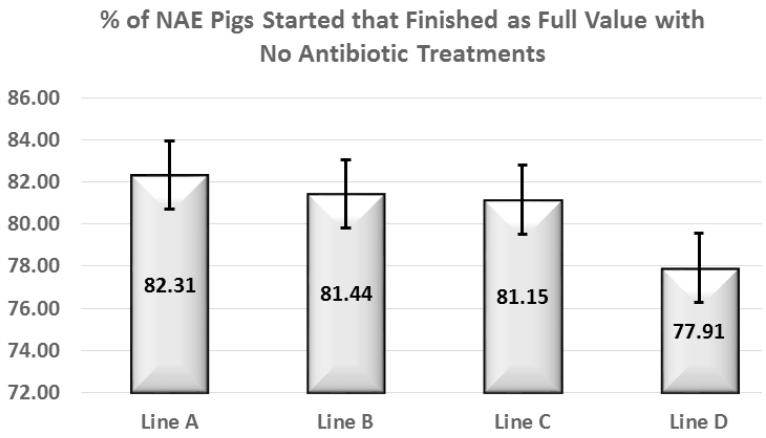

Figure 2. Comparison among sire lines for ability of their progeny to achieve market weight without antibiotic treatment (feed, water, injection). All pigs were from the NAE treatment. *P*-value sire line = 0.233, SEM = 1.63, Line A vs Line D, *P*-value = 0.052.

On the fixed-time basis that we operate under (most of the time), final weight plays a significant role in feed cost and revenue received. Under almost optimum health conditions, the comparative value of NAE was -$4.63 per pig placed. This estimate could be used to compute what breakeven price you would need if only 25% of the carcass cuts were saleable to an NAE market (>$16 per carcass).

Production Imperatives for Disease Resilience

In order to deliver on an NAE program, every effort should be taken to deliver a healthy and disease resilient weaned pig. Negotiation to deliver on a program that commits to no antibiotics after weaning, is most desirable. Our blue-print of management imperatives for an 'NAE' program, is presented in Table 3. These represent the sum of our experience, based on internal research and practice; in most cases, the scientific basis is building.

The first-limiting step in an antibiotic-free option is to control sow farm PRRSv (Table 3) and lateral transmission after pigs are transferred to the field. The sow farm should either be PRRSv naïve, or strain presence should be the vaccine strain. A sow farm that is PRRSv stable is not acceptable, for our perspective, since this tends to be transient. PRRS virus is immunosuppressive, which opens the door to secondary bacterial pathogens (e.g. *Strep suiis*, *Hemophilus parasuis*). Resident pathogens, such as mycoplasma pneumonia, exhibit greater pathogenicity with a PRRSv background.

A second imperative is to utilize robust sire and sow lines. There are clear differences in sire robustness, as illustrated in Figure 2. Viability differences are also known among sow lines (internal research), but this is not as well known. We learned that segregating progeny of first-litter sows, and confining NAE to mature sow progeny is beneficial (T. Donovan, B. Meuer, p.c. 2004); the mature sow provides better protection in her colostrum and milk than first litter females. Colostrum management is likewise vital and pigs must receive a minimum volume (>200 g/pig; Vallet et al., et al., 2015). The best source is from the mother (Bandrick et al., 2008). Colostrum contains cellular immunity (e.g. natural killer cells, CD-4, CD-8) and these are specific to mom and her progeny. Cellular immune cells do not translocate to progeny blood, unless they are derived from the mother (Tuboly et al., 1988; Tuboly and Bernath, 2002). For this reason, pigs must remain with their mother for 24 h and split-sucking is advised to ensure that all pigs receive adequate colostrum. Antibody absorption is not mother specific, however.

Table 2. Summary of marginal financial differences under fixed time conditions of 164 d for NAE vs Conventional therapeutic antibiotics and for the four sire lines ($/pig placed)[1].

	Comparison for Therapeutic Antibiotics				Sires Pooled	
Financial Sector	**Line A**	**Line B**	**Line C**	**Line D**	**NAE**	**Conventional**
Final weight at fixed time, lbs[2]	296.0	291.5	290.9	289.9	295.3	296.0
Full-value pigs (>230 lbs), %	95.02	96.03	95.14	93.02	92.42	94.80
Total Production Cost, $/pig placed	105.63	105.68	104.87	102.92	106.27	106.06
Total Revenue including Cull Pigs, $	154.35	151.18	150.72	147.97	149.80	154.21
Net Margin, $	48.72	45.50	45.85	45.05	43.53	48.15
Marginal difference vs Control, $	3.67	0.45	0.80	-	-4.63	-

[1] Values extracted from the complete financial evaluation in Research Memo 2016-07.
[2] Average weight of full-value and culled pigs, combined. In the NAE vs Conventional comparison – for pigs that were removed for medication (as with Conventional pigs) their weight and financial value was not included in this analysis.

Another non-negotiable for increasing disease resilience is elevating wean age. Average wean age must be sufficient to keep the number of litters weaned at <21 d to less than 5%. Low wean age has proven detrimental to viability, growth (Main et al., 2004) and even lean content (Cabrera et al. 2010). The basis for this was revealed with the elegant studies of the laboratories of Moeser (Moeser, 2015; Moeser et al., 2017; Pohl et al., 2017) and Blikslager (Ziegler and Blikslager, 2017). They showed how multiple weaning stressors, esp. early weaning (e.g 14-15 d), compromise gastrointestinal barrier development, and this has life-long consequences for gut health. This breach in the gut barrier has long-term consequences that include reduced viability. Thus, the mother influences her progeny, profoundly, and well beyond the colostrum phase (Odle et al., 2017).

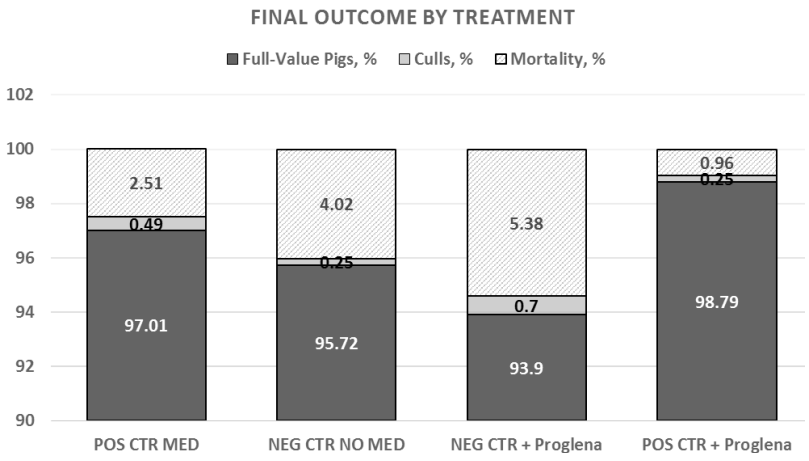

Figure 3. Comparison of therapeutic antibiotics (MED) to no antibiotics after weaning (NO MED) and for an algae source of 1-3 β-glucan (Proglena), alone or in combination with therapeutic antibiotics

We emphasize two other facets. Geographical region for pig placement and biosecurity processes must be expertly managed. Care to place pigs in a 'pig-free' region, however, can be foiled by intermingling of pigs, from two or more farms. Single sow farm pig flow is essential because death loss is lower and medical needs are less than intermingled flows (Wade et al, 2009). The biological basis for this is beginning to emerge with the study of microbial communities (enteric, tonsil, systemic); which are remarkably diverse and environmentally specific. Sow farms appear to have a unique microflora (beneficial and pathogen).

Auxiliary Help to Antibiotics with Respiratory Challenge

Antibiotic alternatives are more likely to be successful in preventing enteric pathogens, than for respiratory infection. In order for antibiotics to be effective against respiratory pathogens, they must be absorbed and go to the lung to be effective. In other words, getting the molecule to the pathogen is what has to happen. What non-antibiotic product is known to be absorbed, and then to become lethal to the pathogen (bacteria, virus), to reduce proliferation or enhance the immune response in the respiratory tract? A prospective alternative, whose residence remains enteric, is not expected to be effective. An exception was observed by feeding spray-dried plasma to animals challenged nasally with PRRSv or Influenza virus. Plasma somehow caused gut active lymphoid tissue to orchestrate changes in the lung, ahead of respiratory pathogen challenge (Campbell et al., 2016).

Table 3. Management imperatives that are not negotiable for No Antibiotic pig production.

- Sow farm free of the disease pathogen, PRRSv
- Robust sire line
- Robust sow line(s)
- Immature first-litter female progeny are immune inferior to mature progeny (cattle, sows)
- Minimum Colostrum intake from maternal sow
- Minimum 23-25 day wean age (<5% under 21 days age)
- Strategic antibiotic injection at processing (not born into sterile environment)
- Pigs placed in single sow farm flows (not intermingled)
- Place weaned pigs in low pig density area (pathogen plumes, cause lateral infections)
- All-in All-out site management to reduce pathogen concentration
- Female pig flows thrive better than males
- Vaccines field validated for effectiveness
- Transition Diets stress minimum (composition, budget delivered on)
- Pigs requiring antibiotic medication removed to medical pens (advantage to poultry)

In our research, and from what has been reported, we are not aware of an alternative to therapeutic antibiotics for respiratory pathogens. We have studies that show an auxiliary or additive role that an immune stimulant can have, when combined with therapeutic antibiotics. Weaned pigs are the most vulnerable target, since transition from mother to dry diets and with change in residence is their most challenging 'moment' beyond birth. The lack of immune development is an additional risk. Strategic medication and diet format (composition, budget) can minimize the challenge during this immunologically vulnerable time.

Pathogenic disease-stressors cause the pig to utilize the innate immune system, to the extent that it has developed. Medications are a means to reduce pathogen exposure and this partially compensates for an under-

developed immune response. Our latest study (Dion and Boyd, 2018) illustrates this facet and the format we use to test proposed antibiotic alternative challengers; in conditions of low and high respiratory immune stress (e.g. PRRSv stable, unstable sow farm). This study shows that a specific algae source of 1-3 β-glucan (>90% of β-glucan is in the 1-3 configuration) added to the benefit observed with therapeutic antibiotics alone, in the face of a respiratory challenge. In the absence of antibiotics, it provided no measurable improvement, thereby showing the value of antibiotics in combatting respiratory assault, and the additive nature of improving the response by an immature immune system. Earlier, we observed immune enabling with a yeast source of 1-3 β-glucan (Cenzone Yeasture).

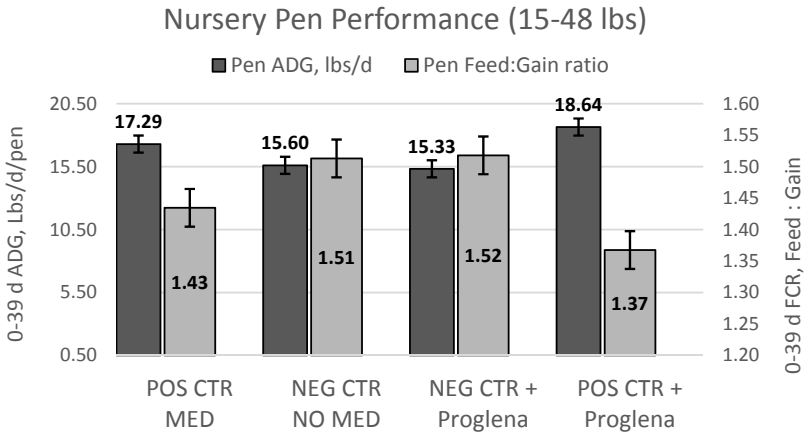

Figure 4. Comparison of therapeutic antibiotics (MED) to no antibiotics after weaning (NO MED) and an algae source of 1 -3 β-glucan (Proglena), alone or in combination with therapeutic antibiotics using Pen ADG and FCR. This is the multiple of viability (retention) and individual pig ADG and FCR.

The objective of the study was to compare our therapeutic antibiotic program (Pulmotil, CTC/Denagaard), for treating weaned pigs derived from a sow farm with active PRRSv virus (unstable), to a non-antibiotic challenger (algae source 1-3 β-glucan, proglena), for growth, viability and ability to thrive (indicated by medical treatment, low weight culls). A negative control (no antibiotics) group was compared to the antibiotic control. The modality of proglena was to facilitate earlier development of the adaptive immune system (Dalmo and Bogwald, 2008). This source of the 1-3 β-glucan (*Euglena gracilis*) was of particular interest because the algae provided survival protection against influenza virus infection in mice (Nakashima et al., 2017).

The study involved 2,156 weaned pigs (22.8 d avg age; range, 19 to 27 d) that were PRRSv (1-7-4) positive at weaning. Pigs were removed to medical pens, as required, but remained on respective diet treatments. The nursery remained positive for PEDv and porcine delta-

Table 4. Growth and viability comparison of therapeutic antibiotics (MED) to no antibiotics after weaning (NO MED) and for an algae source of 1-3 β-glucan (Proglena), alone or in combination with therapeutic antibiotics[1]

Item	POS CTR MED	NEG CTR NO MED	NEG CTR + Proglena	POS CTR + Proglena	SEM	TRT	1v2
No. Pens	19	20	20	19	-	-	-
No. Pigs	418	440	440	418	-	-	-
Initial Weight, lbs	15.7	15.6	15.7	15.6	0.65	0.641	0.336
Period 1, 0-20 d[2]							
End Weight, lbs	29.5	27.6	27.6	29.8	0.90	<0.001	<0.001
Gain, lbs	13.7	12.0	11.9	14.2	0.38	<0.001	<0.001
ADG, lbs/d	0.69	0.60	0.60	0.71	0.02	<0.001	<0.001
ADFI, lbs/d	0.80	0.75	0.75	0.81	0.02	0.007	0.023
FCR, feed/gain ratio	1.16	1.25	1.25	1.15	0.02	<0.001	<0.001
Nursery Period, 0-39 d[2]							
End Weight, lbs	49.5	45.5	46.0	50.4	1.37	<0.001	<0.001
Gain, lbs	33.8	30.4	30.4	34.8	0.85	<0.001	<0.001
ADG, lbs/d	0.87	0.78	0.78	0.89	0.02	<0.001	<0.001
ADFI, lbs/d	1.17	1.08	1.07	1.18	0.03	<0.001	<0.001
FCR, feed/gain ratio	1.35	1.40	1.38	1.34	0.01	<0.001	<0.001
Full-Value Pigs, %	97.0	95.7	93.9	98.8	1.24	0.002	0.427
Average injections per pen	1.3	10.4	9.9	2.6	0.83	<0.001	<0.001

[1] Initial age = 22.8 days, ranged from 19 to 27 days.
[2] Normal performance traits did not account for dead pig weights, and included: ADG = (avg. pig weight end – avg. pig weight initial)/ (days on the period); ADFI= (feed delivered- feed weighed back at the end of the period)/ (pig days on the period); FCR= ADFI/ADG. FVP is defined as the percentage of pigs that weighed > 24.2 lbs at the end of the trial (<4 standard deviations from the mean, which is possible where diet stress exists resulting in a skewed left tail of the distribution).

corona virus throughout the study. Pigs were considered as under moderate immune stress.

Performance is summarized in Table 4. The primary driver of the value proposition is improved FVP, and this was compromised by not using antibiotics (97.0 vs 95.7% for not medicated; treatments, $P<0.002$). Proglena alone did not reduce mortality (Figure 3), but when combined with antibiotics, mortality tended to decline compared to antibiotics alone (0.97 vs 2.51%); further, FVP was numerically improved (98.8 vs 97.0%, $P=0.29$); the latter would require about 100 EU pens/treatment to prove statistically (SD=4.8) at the 0.05 level of significance. The number of Baytril injections (doses per pen) was 8-fold higher for No MED as compared to MED pigs ($P<0.05$). The impact of MED and MED plus immune facilitation, on the ability to thrive, as compared to No MED was reflected in improved ADG and FCR (Table 4). When death rate is different among treatments, the multiple of retained pigs and growth rate is a better illustration of the treatment effect – pen ADG and pen FCR (Figure 4).

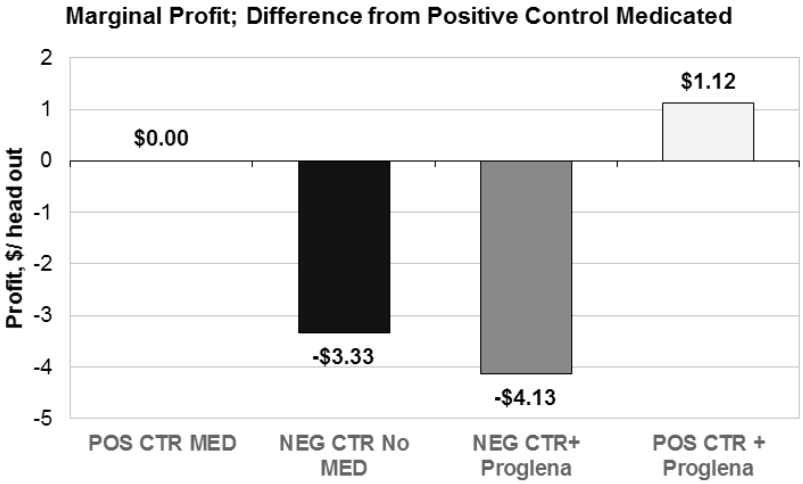

Figure 5. Financial evaluation for marginal return over cost for therapeutic antibiotics (MED) to no antibiotics after weaning (NO MED) and an algae source of 1-3 β-glucan (Proglena), alone or in combination with therapeutic antibiotics.

The financial outcome presented in Figure 5 considered gain value, but it also accounted for the value of dead weaned pigs, which are not free ($30-36/weaned pig cost). Treatments that elevate death loss during the nursery phase include (a) no medication and (b) cheap diet composition (or low budget). This might also be applied to reducing the dose of a life-saving vaccine to reduce cost, if that decision results in greater death. Failure to use MED resulted in a negative return over investment (ROI) of -$3.33/pig, but the combination of MED and Proglena β-glucan increased the marginal ROI to $1.12 per pig out. Thus, MED is the most effective means to minimize death from respiratory disease, that we have tested in this critical period; but facilitation of the adaptive immune system, is a comparatively cheap auxiliary tool to further improve viability and FCR. This would be more difficult to prove under conditions of high health.

Disease Resilience, Health Promotion and AGP Alternatives

Global respiratory disease threat requires the use of medically important antibiotics to survive. Diseases include PRRSv, influenza, mycoplasma pneumonia, hemophillus parasuis and their mutation variants. However, the global pathogen library is even more ominous than this. Antibiotics are a powerful pathogen controlling or ameliorating technology. Failure to control respiratory disease leads to secondary, bacterial challenges (respiratory, enteric), increased mortality and reduced sustainability. On the other hand, growth promoting antibiotics (AGP) are not essential to pig production. More effective alternatives are emerging and these tend to improve gut barrier resilience and reduce enteric pathogen load through various mechanisms.

Given the disease threats that are before the American Pig Industry, and with a mind toward reducing dependence on therapeutic antibiotics, or elimination in the case of a No Antibiotics program, Table 5 provides example tactics (classes) that are deemed sufficiently effective to be noted. The framework presents examples of alternative antibiotic growth promoters (AGP) as well as those that significantly reduce pathogens (lethality, proliferation, attachment); pathogens that would otherwise increase mortality. We specifically avoided listing the 'kitchen sink' of alternative AGP and refer readers to reviews by Liu et al. (2018), Pluske (2013) and Heo et al. (2012) and to footnote 1 of Table 5.

Modest Look Forward – Medically Important Antibiotics in Food Animals

We do not expect antibiotics, which are medically important to food animals, to be eliminated in our working life-time by American legislative mandate. We believe that there will be more pressure placed on documentation to trace outcomes relative to stated purpose for use. This is what Europe has evolved to; did it work or not work ? Greater regulation around the use of therapeutic antibiotics, specifically critically important for human use, is expected.

Table 5. Example tactics deemed sufficiently effective in promoting immunity or growth[1].

Category	Class or Aspect	Explanation
Production technique	Sow farm is foundation	Air filtration, Biosecurity, Location, Farm size
	Genetic lines	Viability under disease stress is very different
	Colostrum, mother ➔progeny	Immune cells, antibodies; minimum 200 g/pig
	Sow Milk	Bioactive molecules
	Wean age, >23 d	Early weaning (14-16 d), immune compromise
Immune enabling	Vaccines	Strong therapeutic antibiotic compliment
	Wean pig diets 1-3	'Cheap' composition, Inadequate budget causes Pathogen proliferation, Barrier disruption
	1-3 β-glucan (algae, yeast)	Facilitates Adaptive immune element
	Specific Nutrients	E, D (esp. 25-OH D_3), C, Zn, Cu, Arginine, 18:2
Immune tempering – (Inflammation hyper-response)	Aspirin, salicylates, dexamet.	Tempers excessive lethal inflammation
	Animal plasma	Reduce inflammation
	Soybeans	Reduce inflammation (e.g. Isoflavones)
	Fish meal	Reduce inflammation
Respiratory auxiliary help[2]	Animal plasma	Oral consumption, systemic immune response
	1-3 β-glucan (algae, yeast)	Algae beneficial to Influenza challenged mice
Resp. antibiotic alternative[3]	Unaware of proven alternative	See Table 4, Figures 3-4
Enteric health, growth promotion *(See Pluske 2013)*	Zinc Oxide	Proven *E. Coli* control
	Copper salts	Pathogen control, growth promotion
	Egg Immunoglobulins	Targeted Pathogen control
	Enzyme Xylanase	Improve viability via favorable microbiota shift
	Botanicals	Pathogen control (Grazix, Carvacrol, Thymol)
	Medium-chain fatty acids	Pathogen control (6, 8, 10 carbons)
	Organic acids	Pathogen control, growth promotion
Emerging tactics – pathogen Control (respiratory, enteric)	Gene editing	Crispr technology, PRRSv attachment inability
	Epidermal growth factor	Oral EGF ameliorates diarrhea, epithelia repair[4]
	Bacteriocins	Colicin, Anti-microbial peptides
	Bacteriophage viruses	Replicate in host to destroy pathogen
OTHER: Metabolic Modifiers[5] and Digestion end-products[5]	Ractopamine	β-agonist stimulation of protein synthesis
	Immuno-Castration	Anti-GNRF injection allows intact benefit for time
	Ionophore Narasin	Binds gram (+) bacteria for hypertonic death ➔ Propionate : Acetate + Butyrate ratio increases

1 List of enteric alternatives for antibiotic growth promotion (proactive pathogen disruption) is long. Classes include probiotics, prebiotic fermentable substrates, specifically stressed yeast to generate internal stress-resistant biomolecules, dietary carbohydrate modification (less soluble NDF, less total NDF) and a longer list of botanical products (than named above) including capucins (antioxidant reduction). Interferons are likewise noted as these naturally occurring molecules interfere with virus reproduction.

2 Auxiliary helps to antibiotics when respiratory system becomes infected.

3 Antibiotic alternatives to antibiotic treatment of respiratory infection (e.g. PRRSv, Influenza, Hemophilus parasuis).

4 Basis for this prospective technology is presented by Zijltra et al. 1994 (repair epithelia, villi after rotavirus infection of piglets) and Yonghua et al. 2016 (Soya-sourced r-EGF found to be as bio-efficacious as human EGF in mechanism involved in preventing necrotizing enterocolitis). SOYA presented as a prospective biopharma generator of EGF, which promotes epithelial growth, proliferation, cytokine response tempering, maximizing digestive enzyme secretions, decreases cell death and IgA generation to ameliorate gut allergens.

5 This class is intended to bring attention to example components whose mechanism is not associated with Pathogen control, per se; rather to change Metabolism or Digestion end products. These are examples of a growing list.

The AVMA established 'Core Principles of Antimicrobial Stewardship' that we ascribe to (https://www.avma.org/KB/Policies/Pages/Antimicrobial-Stewardship-Definition-and-Core-Principles.aspx). Central to the 5 principles, is using an evidence-based approach in making decisions to use antimicrobial drugs and then continually evaluating outcomes of the therapy.

Technologies such as the PRRSv resistant pig and better vaccine technologies will be key prevention mechanisms for disease. A recent decision by the EU court, to categorize gene deletion as creating a GMO (PRRSv crispr technology) is a set-back, but this decision may not be widely accepted in key global markets. There are also other mechanisms of selecting for reduced disease responsiveness in the pig (molecular typing and mapping) and on disease agents (e.g. modified live pathogen vaccines selected against virulence).

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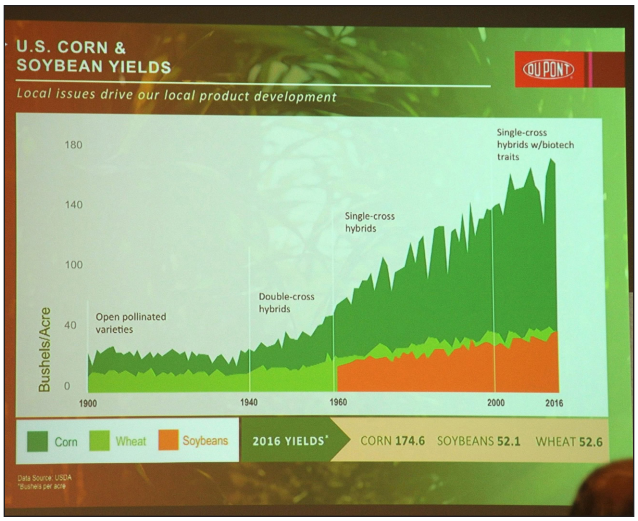
Charles Zila and Sara Larsson, DuPont Pioneer, Windfall, IN, Speakers



Pioneer's graph showing yield of corn, wheat, and soybeans from 1900 to 2016.

Gretchen Hill, Michigan State University, Speaker

Dennis Liptrap, Ralco Nutrition, Moderator

Scott Radcliffe, Purdue University, Videographer

Discussing Brian Richert's presentation.

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