Determining the effects of branched chain amino acids, manganese, and xylanase on growing-finishing pig growth performance and carcass characteristics

by

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Abstract

Experiment 1 used a total of 1,200 pigs to determine the effects of added Val, Ile, and Trp in high Leu on growing-finishing pig growth performance and carcass characteristics in order to validate a prediction model. Experiments 2 and 3 used a total of 3,888 pigs to determine the effects of manganese source and level on growing-finishing pigs growth performance and carcass characteristics. Experiment 4 u sed a total of 1,944 pigs to determine the effects of increasing added xylanase in nutrient adequate diets on growing-finishing pigs growth performance and carcass characteristics. Experiment 1 determined that increasing Val or Ile in high Lys-HCl-DDGS-based diets improved growth performance and final BW compared with pigs fed diets containing high levels of Lys-HCl without added Val and Ile. The addition of Trp alone could not overcome the negative effects of growth performance of pigs fed high Leu diets. These results demonstrate that negative effects of high Leu concentrations in corn-DDGS-based diets can be reversed by increasing the ratios of Val and Ile to Lys. In Exp. 2 and 3, growth performance was improved when 8 and 32 mg/kg of Mn is supplemented compared to 16 mg/kg and when pharmacological levels of Cu are supplemented; pigs fed Mn hydroxychloride had improved growth performance. Also, as Mn concentration in the diet increased, regardless of source, total Mn concentration in the liver increased but increased less for pigs fed Mn hydroxychloride. In Exp. 4, when xylanase was added to nutrient adequate diets, there was improved carcass yield when intermediate levels were fed, however, there was no impact on growth performance or mortality.

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Chapter 1 - Improving performance of finishing pigs with added Valine, Isoleucine, and Tryptophan: Validating a meta-analysis model

Abstract

Based on results of a recent meta-analysis, we hypothesized that increased dietary Val, Ile, or Trp could correct possible amino acid interactions as a result of excess dietary Leu in diets containing high levels of corn protein, namely dried distiller's grains with solubles (DDGS). A total of 1,200 pigs (PIC TR4 × (Fast LW × PIC L02); initially 33.6 ± 0.6 kg) were used in a 103-d study. The 6 dietary treatments were corn-soybean meal-DDGS-based diets as follow: 1) high soybean meal and low level of Lys-HCl (HSBM), 2) high Lys-HCl and moderate Ile, Val, Trp (AA above NRC 2012 estimates; NC), 3) moderate Lys-HCl and high Ile, Val, and Trp (PC), and PC with either increased 4) L-Val (PC+Val), 5) L-Ile (PC+Ile), 6) or L-Trp (PC+Trp). Pigs fed the NC diet were predicted to have the poorest ADG, the PC diet to be intermediate, and pigs fed the HSBM, PC+Val, PC+Ile, and PC+Trp have the same and highest predicted ADG. Diets contained 30% DDGS until pigs reached approximately 98.5 kg and then contained 20% DDGS for the remainder of the study. In the grower period (34 to 90 kg), average daily gain (ADG) was greater ($P < 0.05$) for the pigs fed HSBM and PC+Val diets than the NC with pigs fed other diets intermediate. Pigs fed HSBM were more ($P < 0.05$) efficient (G:F) than the NC and PC pigs fed other diets intermediate. In the finisher period (90 to 136 kg, ADG was greater ($P < 0.05$) for pigs fed PC+Ile than that of the NC with pigs fed other diets intermediate. Pigs fed PC+Val had greater ($P < 0.05$) average daily feed intake (ADFI) than the NC with pigs fed other diets intermediate. However, PC+Ile pigs were more ($P < 0.05$) efficient than PC+Val pigs fed other

diets intermediate. Overall, final body weight, ADG, and hot carcass weight were greater ($P <$ 0.05) for pigs fed HSBM, PC+Val, and PC+Ile diets than the NC pigs fed other diets intermediate. Pigs fed the PC+Val diet had greater ($P < 0.05$) ADFI than the NC with pigs fed other diets intermediate. No differences were detected between treatments for overall G:F or other carcass characteristics. In conclusion, increasing Val or Ile in high Lys-HCl-DDGS-based diets improved growth performance and final BW compared with pigs fed diets containing high levels of Lys-HCl without added Val and Ile. These results demonstrate that negative effects of high Leu concentrations in corn-DDGS-based diets can be reversed by increasing the ratios of Val and Ile relative to Lys.

Key words: Branch chain amino acids, finishing pig, isoleucine, leucine, tryptophan, valine

Introduction

Branched-chain amino acids (BCAA) are a collective group of structurally similar amino acids and are comprised of isoleucine (Ile), leucine (Leu), and valine (Val); all of which also share the same first steps in catabolism to form keto-acids (Harris et al., 2005). Excess of any one of the BCAA leads to an increase in catabolism of all the BCAA, with Leu being the most potent stimulator of the muscle-containing enzyme, branch-chain amino acid transferase (BCAT), which is responsible for BCAA catabolism (Harper et al., 1984). This becomes increasingly important in diets containing high amounts of corn protein, as Leu is disproportionally higher than Val and Ile in corn and corn by-products (NRC, 2012). Large neutral amino acids (LNAA), such as tryptophan (Trp), also share the same brain transporters as BCAA (Pardridge, 1977; Fernstrom, 2013). Tryptophan is a precursor for the neurotransmitter serotonin, which is involved in feed intake regulation (Henry et al., 1992; Fernstrom, 2013). In

turn, an excess of BCAA may affect the transport of Trp into the brain and lead to a decrease in serotonin activity and, thus, feed intake.

Although not consistent, diets containing high levels of Leu have been shown to have negative effects on pig growth performance (Kwon et al., 2019a; Millet et al., 2015; Wiltafsky et al., 2010). The decrease in growth performance has been hypothesized as a result of an imbalance in BCAA. Based on an extensive literature review, Cemin et al. (2019) developed a growth prediction model suggesting that the inclusion of different combinations of Ile, Val, and/or Trp can reverse the decrease in growth performance as a result of excess Leu. If this model is accurate, it will create a platform for further advancements in diet formulation, which will allow nutritionists to create more nutritionally balanced diets. Therefore, our hypothesis was that dietary additions of Val, Ile, or Trp can ameliorate the poor performance of pigs fed diets containing high concentrations of Leu; which in turn, would also validate the model developed by Cemin et al. (2019).

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research facility owned and operated by New Fashion Pork (Jackson, MN). The two barns were tunnel-ventilated with completely slatted concrete flooring and deep pits for manure storage. Each pen (2.4 × 5.8 m) was equipped with adjustable gates and a 3-hole, dry feeder (Thorp Equipment, Inc., Thorp, WI) and a pan waterer. Feed and water were offered ad libitum and feed additions were delivered and recorded using a robotic feeding (FeedPro; Feedlogic Corp., Willmar, MN). The study was conducted from July 17, 2019 to November 28, 2019.

Approximately 1,200 finishing pigs (PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN, USA; Fast Genetics, Saskatoon, SK, Canada; initial BW 33.6 ± 0.63 kg) in two barns were used in a 103-d growth trial. Pigs were housed in mixed gender (10 barrows and 10 gilts) pens with 20 pigs per pen and 10 replicates per treatment. Pens were assigned to 1 of 6 dietary treatments in a complete randomized block design with initial BW and pen location within barn as blocking factors.

Prior to diet formulation, a composite sample of corn, SBM, and DDGS was collected and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed for a complete amino acid profile (Table 1; Method 982.30, AOAC Int., 2006). These total AA values for corn, SBM, and DDGS were multiplied by NRC (2012) standardized ileal digestibility coefficients and these values were used in diet formulation. Experimental diets were fed in 4 phases (Tables 2 and 3) from d 0 to 16, 16 to 40, 40 to 64, and 64 to 103, which correspond to body weights of approximately 34 to 51, 51 to 75, 75 to 99, and 99 kg to market, respectively. Experimental diets were corn-SBM-DDGS-based with 30% DDGS fed in phases 1 to 3 and 20% DDGS fed in phase 4.

Experimental treatments consisted of: 1) high SBM and low feed grade AA (HSBM) with Val:Lys, Ile:Lys and Trp:Lys ranging from 85 to 90, 76 to 78, and 19.3 to 19.9%, respectively, across the 4 dietary phases; 2) negative control (NC) with low SBM and high levels of feed grade AA with Val:Lys, Ile:Lys and Trp:Lys ranging from 64 to 68, 51 to 53, and 17.0 to 17.5%, respectively, across the 4 dietary phases 3) positive control (PC) with a medium feed grade AA inclusion with Val:Lys and Trp:Lys held constant at 70 and 19.0%, respectively, and with Ile:Lys ranging from 58 to 61% across the 4 dietary phases; 4) PC with high Val:Lys (PC+Val) ranging from 76 to 80% across the 4 dietary phases; 5) PC with a high Ile:Lys (PC+Ile) ranging

from 66 to 68% across the 4 dietary phases, and 6) PC with a high Trp:Lys (PC+Trp) ranging from 21.1 to 23.1% across the 4 dietary phases. Pigs fed the NC diet were predicted to have the poorest ADG, the PC diet to be intermediate, and pigs fed the HSBM, highest predicted ADG. The PC+Val and PC+Trp treatments were developed by increasing the Val:Lys and Trp:Lys, respectively, until the model of Cemin et al. (2019) predicted the same ADG of the HSBM treatment. Because the model predicts that the response to Ile is quadratic, the PC+Ile treatment was developed by increasing the Ile:Lys to come as close as possible to the predicted ADG of the HSBM diet. Each pig was tagged with an RFID tag at the beginning of the trial in order to be individually identified. Pigs were individually weighed on d 0 and 76 in order to evaluate if the response to dietary treatment was influenced by pig sex. Pigs were weighed approximately every 14 days to determine ADG, ADFI, and G:F. On d 83, four to six of the heaviest pigs in each pen were selected and marketed to achieve a consistent inventory of 14 pigs remaining in each pen. The pigs marketed on d 83 were included in the growth data, but not in the final pen carcass data. On the last day of the trial (d 103), final pen weights were obtained, and the remaining pigs were transported to a U.S. Department of Agriculture-inspected packing plant (Triumph Foods, St. Joseph, MO) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Loin depth and back fat depth were determined ultrasonically using a Fat-O-Meater (SFK; Herlev, Denmark) inserted approximately 7 cm off the mid-line of the pig and between the 10^{th} and 11^{th} rib. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Samples of complete diets were obtained from 5 feeders of each treatment in each barn approximately 4 d after the beginning and 4 d prior to the end of each phase. Feed samples were

delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of diets were combined within dietary treatment, and a composite sample from each phase for each treatment was analyzed (University of Missouri-Columbia, MO, Table 2 and 3). Samples were analyzed for crude protein (Method 990.03; AOAC Int., 2006), P (Method 966.01; AOAC Int., 2006), and Ca (Method 985.01; AOAC Int., 1990).

Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1; 2018-07-02, R Foundation for Statistical Computing, Vienna, Austria) with pen considered as the experimental unit, body weight and pen location within barn as blocking factors, and treatment as a fixed effect. Preplanned pairwise comparisons using the Tukey-Kramer adjustment were used to evaluate differences in treatment means. Results were considered significant at $P \leq 0.05$.

At the conclusion of the study, in order to validate and compare the predicted ADG model to the actual ADG observed, the equation's intercept term was adjusted until the predicted ADG matched the actual ADG for the pigs fed the HSBM diets. The equation with the adjusted intercept term was then used to predict the ADG of the remaining five treatments. The relationship between the actual and predicted ADG was calculated (actual ADG/predicted ADG) to illustrate the accuracy of the prediction model.

Results

Chemical analysis

Results of amino acid analysis of the corn, soybean meal, and DDGS used in diet formulation indicated a lower Lys content in corn but greater in soybean meal than listed by NRC (2012; Table 1). There were no notable differences revealed between the chemical and

calculated analysis of the complete diets (Tables 2 and 3). The analyzed level of CP, Ca, and P followed the targeted expected dietary levels.

Growth performance and carcass characteristics

In the grower period from 34 to 90 kg (d 0 to 54), pigs fed the HSBM and PC+Val diets had greater ($P < 0.05$) ADG than those fed the NC, with pigs fed PC, PC+Ile, and PC+Trp intermediate (Table 4). Pigs fed HSBM were also more ($P < 0.05$) efficient than the NC and PC pigs with those fed PC+Val, PC+Ile, and PC+Trp intermediate. There was no difference ($P > 0.05$) in ADFI among pigs fed any of the treatments during the grower period.

During the finishing period from 90 to 136 kg (d 54 to 103), ADG was greater ($P < 0.05$) for pigs fed PC+Ile than that of pigs fed the NC with those fed HSBM, PC, PC+Val, and PC+Trp intermediate. Pigs fed PC+Val had greater ($P < 0.05$) ADFI than pigs fed NC with those fed HSBM, PC, PC+Ile, and PC+Trp intermediate. Pigs fed the PC+Ile treatment were more ($P < 0.05$) efficient than pigs fed PC+Val with those fed HSBM, NC, PC, and PC+Trp intermediate.

Overall, pigs fed the HSBM, PC+Val, and PC+Ile diets had greater ($P < 0.05$) ADG and final BW than the pigs fed the NC diet with those fed PC and PC+Trp intermediate. Pigs fed the PC+Val diets had greater ($P < 0.05$) ADFI than pigs fed NC with those fed HSBM, PC, PC+Ile, and PC+Trp intermediate. There were no differences ($P > 0.05$) between treatments for G:F. Similar to overall ADG and final BW, pigs fed the HSBM, PC, PC+Val, and PC+Ile diets had heavier ($P < 0.05$) HCW than the pigs fed the NC diet with those fed PC+Trp intermediate. There was no evidence for treatment differences ($P > 0.05$) observed for any other carcass characteristic or percentage carcass yield.

There was no ($P > 0.10$) treatment × sex interaction on growth performance (data not shown). Day 76 BW standard deviation was also not influenced by sex within treatment. The d 76 BW standard deviation for barrows for barrows averaged 25.0 and averaged 25.6 for gilts.

Model validation

When comparing the model predicted ADG proposed by Cemin et al. (2019) to actual ADG in the grower period (Table 5), the model accurately predicted ADG of pigs fed the NC and PC+Val diets, but over-predicted ADG for the pigs fed the PC (2.2%), PC+Ile (2.5%) and PC+Trp (3%) diets. In the finisher period, the model accurately predicted ADG of the PC+Val treatment and over-predicted the ADG for the PC+Trp treatment by 1.6%, but under-predicted the ADG for pigs fed the NC, PC and PC+Ile diets by 2.8, 2.6, and 2.9%, respectively. For the overall experimental period, the model was quite accurate for most treatments with ADG of the pigs fed the NC, PC, PC+Val and PC+Ile predicted within 0.4% of actual. However, the model over-predicted ADG of pigs fed the PC+Trp diet by 2.2%.

Discussion

An imbalance in BCAA can occur in swine diets comprised of corn and corn by-products that are also supplemented with high amounts of feed-grade AA. The imbalance is a result of the high Leu content relative to Val and Ile in corn protein (NRC, 2012). Unlike most amino acids, BCAA are first transported to skeletal muscle to be degraded and through the action of BCAT are reversibly converted to α-keto acids. The α-keto acids are then transported to the liver where they are decarboxylated by branched-chain α-keto acid dehydrogenase complex (BCKD). Leucine is the most potent stimulator of BCAT and BCKD. Thus, high dietary concentrations of Leu would lead to catabolism not only of itself, but also Ile and Val (Harper et al., 1984). If ratios of Ile and Val are close to requirement estimates, as in the case in diets supplemented with

L-Lys, DL-Met, L-Thr, and L-Trp, increased degradation may potentially reduce pig growth performance.

Tryptophan is a LNAA that is a precursor for serotonin, which plays a role in appetite regulation (Henry et al., 1992). Large neutral amino acids share the same brain transporters as BCAA and an excess in BCAA, specifically Leu, has been negatively correlated with Trp up-take and serotonin levels in the brain, ultimately leading to a decrease in ADFI and growth performance (Wessels et al., 2016a, 2016b).

A prediction model for ADG based on a meta-analysis by Cemin et al. (2019) suggests that increased concentrations of Val, Ile, or Trp might reverse the decreased performance of pigs fed diets containing high Leu. The current study focused on validating this model by adding Val, Ile, or Trp to diets containing high concentrations of Leu. The HSBM dietary treatment with addition of low feed grade amino acids contains a high Leu level, but also has high Val, Ile, and Trp ratios relative to Lys and therefore should have had the best ADG. In order to validate the model's prediction for negative ADG from high Leu along with an imbalance in BCAA, the NC diet was formulated to contain the most L-Lys HCL and the lowest amount of SBM and by doing so it resulted in a predicted decrease in ADG because of an imbalance in BCAA. The PC diet was the base for the remaining treatments and contained high levels of L-Lys HCL; however, less than that of the NC, and had slightly greater L-Val, L-Ile, and L-Trp than the NC. The PC was formulated to have intermediate predicted ADG compared to the HSBM and the NC, while the PC+ Val, PC+Ile, and PC+Trp where formulated to match the ADG of the HSBM treatment.

The HSBM diets in our experiment had the highest dietary Leu concentration; however, the increased levels of SBM and reduced L-Lys HCl also resulted in elevated dietary levels of Val, Ile, and Trp. These greater levels of other BCAA and Trp negate or lessen the negative

effects of high Leu. Meanwhile, despite the reduction of Leu in the NC diet compared to the HSBM diets, the NC diets also had the lowest dietary levels of Val, Ile, and Trp with levels being above, but near NRC, 2012 requirement estimates. Thus the NC diet contains Val, Ile, and Trp that meet the pig's requirements relative to lysine in diets without excess Leu, but may not meet the needs when diet contain excess Leu. Using the model by Cemin et al. (2019), it accurately predicted the actual 4.4% reduction in ADG for pigs fed the NC treatment when compared to those fed the HSBM diets. The reduction in ADG observed in our study is in agreement with observations by Kwon et al. (2019a). They also observed decreased ADG when an imbalance in BCAA arise, namely an increase in Leu concentrations with no change in Val or Ile.

The equation for ADG by Cemin et al. (2019) predicts that adding Val or Ile to the diet can reverse the negative effects of high Leu concentrations. However, the model predicts that Ile has a lesser ability to reverse the negative effects of Leu than Val. In our study, pigs fed the diets with increased Val were able achieve ADG that was almost identical to performance of pigs fed the HSBM diet as predicted by the model. The model accurately predicted the ADG in both the grower and finisher phases for the pigs fed PC+Val. The increased ADG of pigs fed increased Val in high Leu diets is in agreement with the results from Gloaguen et al. (2011) and Millet et al. (2015); where increased Val was observed to ameliorate the decrease in ADG of pigs fed excess Leu.

The improvement in overall ADG and lower G:F for pigs fed the PC+Val diets in our study was primarily driven by increased feed intake. High levels of Leu have also been shown to decrease ADFI (Gloaguen et al., 2011; Millet et al., 2015; Kwon et al., 2019a) possibly a result of over-stimulating mammalian target rapamycin (mTOR) receptors. Mammalian target

rapamycin receptors is a signaling pathway that stimulates protein synthesis for cell growth (Schmelze and Hall, 2000) but over-stimulation can lead to inhibition of feed intake (Cota et al., 2006). Valine, however, has been shown to decrease or inhibit the transport of Leu through the blood brain barrier (Hargreaves and Pardridge, 1988; Hjelle et al., 1978), which could lead to a reduction in mTOR stimulation. Although mTOR stimulation was not measured, the resulting increase in feed intake in the finishing period that occurred for the PC+Val treatment may have been a result to the reduction of Leu crossing the blood-brain barrier and preventing mTOR over stimulation.

According to the model of Cemin et al. (2019), Ile alone cannot reverse the negative effects of high Leu concentrations and may need to be used in combination with Val or Trp. This would be in agreement with Harper et al. (1954) where increased Ile was only able to partially recover growth in rats fed high dietary Leu. In the present study, the model of Cemin et al. (2019) accurately predicted ADG for the PC+Ile treatment, but when broken down into two different time periods, the PC+Ile dietary treatment under performed in ADG in the grower period and then over performed in the finisher period when compared to the predicted model. These results may indicate that Ile deficiency relative to Leu in the NC diet may have been more detrimental in the finisher period than during the growing period allowing for a greater response to dietary addition of Leu. Although the Ile:Lys ratio was similar in the grower and finisher phases, the Leu:Ile ratio was greater in the finisher phase as the Leu content of the diets increased with greater inclusion of corn.

Van Milgen et al. (2012) demonstrated using a meta-analysis that the requirement for Ile increased when pigs were fed diets containing blood meal or blood cells and believed this to be the result of the high concentration of Leu in these products creating an imbalance in BCAA.

Like Val, but to a lesser extent, Ile has been shown to decrease up-take of Leu by the brain (Hargreaves and Pardridge, 1988). Parr et al. (2004) observed a linear reduction in plasma urea nitrogen (PUN) as Ile increased in the diet for finishing pigs. Although Leu levels were not stated in the publication, the resulting decrease in PUN may have been due to a decrease in catabolism stimulated from a BCAA imbalance caused by excess Leu. A potential decrease in catabolism might be the reason for the improvement in G:F that was observed for the pigs fed PC+Ile in the finishing period. Over the course of multiple experiments, Dean et al. (2005) observed mixed results when evaluating the Ile requirement in finishing pigs and this may have been a result of different Leu levels across the 6 experiments. Retrospectively, the experiments where increasing dietary Ile improved growth performance may have been a result of correcting an imbalance in BCAA caused by true digestible Leu:Lys being at or greater than1.32 in the diets and when no response observed; true digestible Leu:Lys levels were at or below 0.99 which may not have been high enough to create a BCAA imbalance, thus making incremental inclusion of Ile unnecessary.

Our results show that the model of Cemin et al. (2019) over-predicted the response to dietary addition of Trp during the grower, finisher, and overall periods. This result was unexpected and may have been due to not having a high enough added Trp in the PC+Trp diet. Kwon et al. (2019b) were partially able to overcome the negative effects on ADG and ADFI from excess dietary Leu with 23 and 28% Trp:Lys ratios, which were greater than the Trp:Lys ratios used in our experiment (approximately 21 to 23% of Lys). Another possible reason for the model's over-prediction may be because Val or Ile might be more deficient relative to Leu in the PC diets in this experiment and that correcting the BCAA imbalance was more important than correcting a LNAA imbalance. If we used greater levels of Val or Ile in the PC+Trp diet, we

might have observed increased ADG for those fed the PC+Trp diets. Early research conducted by Rogers et al. (1967) observed that Trp needed to be supplemented in combination with Val and Ile in order to fully alleviate the decreased growth from excess dietary Leu in rats. However additional research is needed to verify this response in pigs.

Tryptophan also plays a key role in feed intake as it is a precursor for serotonin. Because LNAA and BCAA compete for the same brain receptors, an imbalance in BCAA can lead to a decrease in Trp uptake in the brain and in turn reduce serotonin synthesis. Henry et al. (1992) observed ADFI and serotonin concentrations in the hypothalamus were more reduced in gilts than barrows when Trp was fed at deficient levels. More importantly, Wessels et al. (2016a, 2016b) observed a negative correlation between increasing amounts of Leu on Trp and serotonin levels in the brain. Our results show that the PC+Trp treatment did not improve ADFI in the presence of high Leu, again possibly suggesting that Trp alone may not be able to overcome an imbalance in BCAA caused by excess Leu. Additionally, these data show that increased inclusion of Val, Ile, or Trp did not influence ADG of barrows and gilts differently.

Dietary treatment had no influence on any measured carcass characteristics except for HCW. The HCW response was directly correlated to the improvements in overall ADG. High Leu diets have not been shown to effect carcass yield, backfat, or loin depth (Hyun et al., 2003; 2007). Our results would suggest that the additions of Val, Ile, or Trp in diets with high Leu also do not affect carcass characteristics.

In conclusion, the ADG model proposed by Cemin et al. (2019) accurately predicted the overall growth for pigs fed the NC, PC, PC+Val, and PC+Ile diets; however, the model over-predicted ADG for pigs fed the PC+Trp diet. Pigs fed the high Val diets were able to reverse the negative effects of excess Leu starting in the grower period, whereas pigs fed PC+Ile diets

overcame the negative effects of high dietary Leu in the finishing period. The over-prediction for ADG of the PC+Trp treatment may have been result of also needing additional Val and/or Ile above the levels used herein to reduce the negative effects of Leu or the model may underestimate the amount of added Trp needed in the diet. Further research is needed to validate the ADG prediction model when combinations of Val, Ile, and Trp are used in high Leu diets.

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Table 1.1 Amino acid analysis of corn, soybean meal, and distillers dried grains with solubles (DDGS); as-fed basis[1]

Amino acid, %	Corn	Soybean meal	DDGS
Alanine	0.43	1.95	1.54
Arginine	0.26	3.34	1.18
Aspartic Acid	0.44	5.15	1.62
Cysteine	0.15	0.66	0.55
Glutamic acid	1.02	7.92	2.95
Histidine	0.18	1.18	0.75
Isoleucine	0.21	2.04	1.02
Leucine	0.66	3.41	2.68
Lysine	0.21	3.41	0.97
Methionine	0.12	0.61	0.46
Phenylalanine	0.29	2.33	1.06
Proline	0.53	2.42	1.85
Serine	0.30	2.24	1.07
Threonine	0.22	1.78	1.01
Tryptophan	0.05	0.62	0.19
Tyrosine	0.08	1.14	0.83
Valine	0.28	2.07	1.31

[1]A representative sample of each ingredient was collected, homogenized, and submitted to Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed in duplicate. These values, multiplied by standardized ileal digestibility coefficients derived from NRC (2012) were used in diet formulation.

Table 1.2 Phase 1 and 2 diet composition (as-fed basis)[1,2]

	Phase 1						Phase 2					
Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Ingredients, %												
Corn	41.92	55.53	50.94	50.87	50.89	50.92	47.00	60.35	56.03	55.91	55.92	56.01
Soybean meal (46.5% CP)	24.27	10.31	15.07	15.08	15.08	15.07	19.34	5.71	10.14	10.15	10.15	10.14
DDGS, > 6 and < 9% Oil[3]	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Choice white grease	1.50	1.00	1.15	1.15	1.15	1.15	1.55	1.05	1.20	1.25	1.25	1.20
Calcium carbonate	1.25	1.20	1.22	1.22	1.22	1.22	1.19	1.14	1.15	1.15	1.15	1.15
Monocalcium P, 21% P	0.29	0.44	0.40	0.40	0.40	0.40	0.14	0.30	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.15	0.58	0.43	0.43	0.43	0.43	0.16	0.58	0.44	0.44	0.44	0.44
DL-Methionine	-	0.08	0.05	0.05	0.05	0.05	-	0.05	0.04	0.04	0.04	0.04
L-Threonine	-	0.14	0.08	0.08	0.08	0.08	-	0.14	0.08	0.08	0.08	0.08
L-Tryptophan	-	0.05	0.04	0.04	0.04	0.06	-	0.05	0.05	0.05	0.05	0.07
L-Valine	-	0.06	-	0.07	-	-	-	0.03	-	0.07	-	-
L-Isoleucine	-	-	-	-	0.05	-	-	-	-	-	0.06	-
Vitamin-mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[5]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis												
Standardized ileal digestible (SID) amino acids, %												
Lysine	0.98	0.98	0.98	0.98	0.98	0.98	0.87	0.87	0.87	0.87	0.87	0.87
Isoleucine:lysine	76	53	61	61	66	61	77	51	59	59	66	59
Leucine:lysine	168	133	145	145	145	145	175	137	150	150	150	150
Methionine:lysine	29	30	30	30	30	30	30	29	30	30	30	30
Methionine + cysteine:lysine	60	55	57	57	57	57	63	55	58	58	58	58
Threonine:lysine	67	62	62	62	62	62	68	62	62	62	62	62
Tryptophan:lysine	19.9	17.5	19.0	19.0	19.0	21.1	19.5	17.0	19.0	19.0	19.0	21.8
Valine:lysine	85	68	70	76	70	70	86	64	70	77	70	70
Lysine:net energy, g/Mcal	3.92	3.92	3.92	3.92	3.92	3.92	3.45	3.45	3.45	3.45	3.45	3.45

Net energy kcal/kg	2,499	2,499	2,499	2,499	2,499	2,499	2,524	2,524	2,524	2,524	2,524	2,524
STTD P, %[6]	0.39	0.39	0.39	0.39	0.39	0.39	0.35	0.35	0.35	0.35	0.35	0.35
Chemical analysis[7]												
Crude protein, %	21.44	16.52	18.00	18.38	17.78	18.42	20.34	15.48	17.38	17.94	17.08	16.83
Total Ca, %	0.67	0.76	0.86	0.76	0.60	0.86	0.69	0.82	0.83	0.70	0.78	0.95
Total P, %	0.60	0.61	0.60	0.57	0.56	0.57	0.59	0.56	0.55	0.57	0.58	0.65

[1]Phase 1 diets were fed from d 0 to 16 (33.6 to 50.8 kg) and phase 2 diets were fed from d 16 to 40 (50.8 to 74.9 kg).

[2]HSBM = high soybean meal, NC = negative control, PC = positive control, PC+Val = positive control + valine, PC+Ile = positive control + isoleucine, PC+Trp = positive control + tryptophan.

[3]DDGS = dried distillers grains with solubles

[4]Vitamin and mineral premix provided per kg of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2,507 IU vitamin A, 318 IU vitamin D,12 IU vitamin E, 0.01 mg vitamin B12, 11.6 mg niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin

[5]Smizyme TS G5 2,500 (Origination Inc., St. Paul, MN) provided 626 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.

[6]Standardized total tract digestible P.

[7]A composite sample of each dietary treatment for each phase was collected, homogenized, and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed.

Table 1.3 Phase 3 and 4 diet composition (as-fed basis)[1,2]

	Phase 3						Phase 4					
Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Ingredients, %												
Corn	51.34	62.55	60.58	60.46	60.45	60.55	62.44	71.87	70.00	69.87	69.88	69.92
Soybean meal (46.5% CP)	15.07	3.58	5.55	5.56	5.56	5.55	13.96	4.27	6.24	6.25	6.25	6.25
DDGS, > 6 and < 9% Oil[3]	30.00	30.00	30.00	30.00	30.00	30.00	20.00	20.00	20.00	20.00	20.00	20.00
Choice white grease	1.50	1.10	1.20	1.25	1.25	1.20	1.45	1.10	1.15	1.20	1.20	1.20
Calcium carbonate	1.16	1.13	1.13	1.13	1.13	1.13	1.10	1.07	1.07	1.07	1.07	1.07
Monocalcium P, 21% P	0.15	0.33	0.30	0.30	0.30	0.30	0.30	0.44	0.40	0.40	0.40	0.40
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.15	0.50	0.44	0.44	0.44	0.44	0.13	0.42	0.36	0.36	0.36	0.36
DL-Methionine	-	0.03	0.03	0.03	0.03	0.03	-	0.05	0.04	0.04	0.04	0.04
L-Threonine	-	0.11	0.10	0.10	0.10	0.10	-	0.11	0.09	0.09	0.09	0.09
L-Tryptophan	-	0.04	0.05	0.05	0.05	0.07	-	0.04	0.04	0.04	0.04	0.06
L-Valine	-	0.02	-	0.06	-	-	-	0.02	-	0.07	-	-
L-Isoleucine	-	-	-	-	0.08	-	-	-	-	-	0.07	-
Vitamin-mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis												
Standardized ileal digestible (SID) amino acids, %												
Lysine	0.76	0.76	0.76	0.76	0.76	0.76	0.67	0.67	0.67	0.67	0.67	0.67
Isoleucine:lysine	78	53	58	58	67	58	77	53	58	58	68	58
Leucine:lysine	187	150	157	157	157	157	183	148	155	155	155	155
Methionine:lysine	32	29	30	30	30	30	32	32	32	32	32	32
Methionine + cysteine:lysine	67	58	59	59	59	59	67	61	62	61	61	62
Threonine:lysine	70	64	66	66	66	66	69	65	66	66	66	66
Tryptophan:lysine	19.3	17.0	19.0	19.0	19.0	22.2	19.7	17.5	19.0	19.0	19.0	23.1
Valine:lysine	90	68	70	78	70	70	88	68	70	80	70	70
Lysine:net energy, g/Mcal	3.00	3.00	3.00	3.00	3.00	3.00	2.61	2.61	2.61	2.61	2.61	2.61
Net energy, kcal/kg	2,537	2,537	2,537	2,537	2,537	2,537	2,568	2,568	2,568	2,568	2,568	2,568

STTD P, %[6]	0.34	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.34	0.34
Chemical analysis[7]												
Crude protein, %	18.64	14.34	14.62	14.06	14.80	14.97	14.98	13.55	13.23	12.91	13.61	13.10
Total Ca, %	0.63	0.66	0.63	0.71	0.75	0.53	0.79	0.64	0.54	0.58	0.77	0.73
Total P, %	0.54	0.52	0.58	0.55	0.54	0.51	0.46	0.50	0.42	0.43	0.50	0.47

[1]Phase 3 diets were fed from d 40 to 64 (74.9 to 98.5 kg) and phase 4 diets were fed from d 64 to 103 (98.5 to market, respectively)

[2]HSBM = high soybean meal, NC = negative control, PC = positive control, PC+Val = positive control + valine, PC+Ile = positive control + isoleucine, PC+Trp = positive control + tryptophan.

[3]DDGS = dried distillers grains with solubles.

[4]Vitamin and mineral premix provided per kg of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2507 IU vitamin A, 318 IU vitamin D,12 IU vitamin E, 0.01 mg vitamin B12, 11.6 mg niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin

[5]Smizyme TS G5 2,500 (Origination Inc., St. Paul, MN) provided 626 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.

[6]Standardized total tract digestible P.

[7]A composite sample of each dietary treatment for each phase was collected, homogenized, and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed.

Table 1.4 Effects of supplemental Val, Ile, Trp on growth performance of growing-finishing pigs[1,2]

Item[3]	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	SEM	Probability, *P* =
Initial BW, kg	33.5	33.5	33.6	33.6	33.6	33.5	0.63	0.994
d 54 BW, kg	91.5^{a}	88.1^{c}	89.2^{bc}	91.2^{ab}	89.8^{abc}	89.9^{abc}	0.61	< 0.001
Final BW, kg	136.3^{a}	130.6^{b}	134.3^{ab}	136.0^{a}	135.4^{a}	133.9^{ab}	0.96	< 0.001
Grower (d 0 to 54)								
ADG, kg	1.078^{a}	1.019^{b}	1.040^{ab}	1.074^{a}	1.049^{ab}	1.046^{ab}	0.0107	< 0.001
ADFI, kg	2.313	2.265	2.291	2.343	2.301	2.303	0.0202	0.175
G:F	0.466^{a}	0.450^{b}	0.454^{b}	0.459^{ab}	0.456^{ab}	0.454^{ab}	0.0038	0.007
Finisher (d 54 to 103)								
ADG, kg	0.983^{ab}	0.956^{b}	0.985^{ab}	0.978^{ab}	1.006^{a}	0.967^{ab}	0.0121	0.080
ADFI, kg	3.022^{ab}	2.936^{b}	3.011^{ab}	3.047^{a}	3.036^{ab}	2.976^{ab}	0.0296	0.042
G:F	0.325^{ab}	0.326^{ab}	0.327^{ab}	0.321^{b}	0.331^{a}	0.325^{ab}	0.0023	0.049
Overall (d 0 to 103)								
ADG, kg	1.035^{a}	0.990^{b}	1.015^{ab}	1.031^{a}	1.029^{a}	1.010^{ab}	0.0084	< 0.001
ADFI, kg	2.629^{ab}	2.563^{b}	2.611^{ab}	2.656^{a}	2.629^{ab}	2.602^{ab}	0.0207	0.027
G:F	0.394	0.387	0.389	0.388	0.392	0.388	0.0021	0.060
Carcass characteristics								
HCW, kg	99.8^{a}	95.9^{b}	98.7^{a}	100.0^{a}	99.3^{a}	98.7^{ab}	0.78	0.005
Carcass yield, %	73.2	73.4	73.4	73.4	73.3	73.7	0.298	0.931
Backfat depth, mm[4]	15.1	15.6	15.2	15.8	15.3	15.4	0.28	0.335
Loin depth, mm[4]	65.5	64.2	65.0	64.5	65.2	65.1	0.41	0.136
Lean, %[4]	54.9	54.5	54.8	54.5	54.7	54.7	0.14	0.190

[1]A total of 1,200 pigs in two groups were used in a 103-d study with 20 pigs per pen and 10 replicates per treatment.

[2]HSBM = high soybean meal, NC = negative control, PC = positive control, PC+Val = positive control + valine, PC+Ile = positive control + isoleucine, PC+Trp = positive control + tryptophan.

[3] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[4]Adjusted using HCW as covariate.

[a,b,c]Means with different superscripts are significantly different ($P \leq 0.05$).

Table 1.5 Comparison of predicted ADG based on the model versus the actual ADG[1,2]

Item[3]	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Grower (d 0 to 54)						
Actual ADG, kg	1.078	1.019	1.040	1.074	1.049	1.046
Predicted ADG, kg	1.078	1.025	1.063	1.078	1.076	1.078
Actual vs predicted, %[4]	100	99.4	97.8	99.6	97.5	97.0
Finisher (d 54 to 103)						
Actual ADG, kg	0.983	0.956	0.985	0.978	1.006	0.967
Predicted ADG, kg	0.983	0.930	0.960	0.983	0.978	0.983
Actual vs predicted, %[4]	100	102.8	102.6	99.5	102.9	98.4
Overall						
Actual ADG, kg	1.035	0.990	1.015	1.031	1.029	1.010
Predicted ADG, kg	1.035	0.990	1.015	1.035	1.033	1.035
Actual vs predicted, %[4]	100.0	100.0	100.0	99.6	99.6	97.8

[1]Prediction equation used was derived by Cemin et al. (2019)[5], the intercept term was adjusted until the predicted ADG matched the actual ADG of HSBM treatment. The adjusted intercept term equation was then used to predict the ADG of the remaining treatments.

[2]HSBM = high soybean meal, NC = negative control, PC = positive control, PC+Val = positive control + valine, PC+Ile = positive control + isoleucine, PC+Trp = positive control + tryptophan.

[3]ADG = average daily gain

[4]Actual vs predicted = actual ADG/predicted ADG

Chapter 2 - Determining the effects of manganese source and level on growth performance and carcass characteristics of growing-finishing pigs

Abstract

Two experiments were conducted to determine the effect of manganese (Mn) source and level on finishing pig growth performance and carcass characteristics. Dietary treatments were arranged in a 2 × 3 factorial with main effects of Mn source ($MnSO_4$; Eurochem, Veracruz, Mexico, or Mn hydroxychloride (IBM); Micronutrients, Indianapolis, IN), and increasing added Mn concentration (8, 16, and 32 mg/kg). The trace mineral premix was formulated without added Mn. Copper was added to all diets at 10 and 150 mg/kg in Exp. 1 and 2, respectively. In both experiments 1,994 pigs (PIC; 337 × 1050; initially 34.5 ± 0.50 and 40.0 ± 0.77 kg) were used with 27 pigs per pen and 12 replicates per treatment. Diets were corn-soybean meal-distillers dried grains with solubles-based and were fed in 4 phases. In Exp. 1, there was a marginal Mn source × level interaction (quadratic, $P = 0.057$) for overall G:F, with a decrease then increase in pigs fed IBM, but G:F increased with increasing Mn from $MnSO_4$. There was no evidence for Mn source differences for ADG or ADFI, but pigs fed 16 mg/kg of Mn tended to have decreased (quadratic, $P < 0.05$) ADG and final BW compared to other levels. For carcass yield, there was a tendency for Mn source × level interaction (quadratic, $P = 0.075$) where carcass yield did not change by increasing $MnSO_4$ but was greatest for 16 mg/kg Mn from IBM. Loin depth increased (source × level, $P = 0.041$) for pigs fed increasing Mn from $MnSO_4$ but decreased when Mn was increased from IBM. Pigs fed the intermediate level of Mn tended to

have the lightest HCW (quadratic, $P = 0.071$) and decreased loin depth (quadratic, $P = 0.044$). Liver Mn concentration increased (linear, $P = 0.015$) as added Mn increased and tended to be greater ($P = 0.075$) when Mn was supplied by $MnSO_4$ compared to IBM. In Exp. 2, there was no ($P > 0.10$) Mn source × level interaction observed for ADG, ADFI, and G:F. Pigs fed IBM had increased ($P < 0.05$) final BW, ADG, and ADFI compared to pigs fed $MnSO_4$. Pigs fed 16 mg/kg of Mn tended ($P = 0.088$) to have reduced ADFI when compared pigs fed 8 and 32 mg/kg of Mn. In conclusion, there appears to be little benefit in growth performance by feeding more than 8 mg/kg of added Mn. When pharmacological levels of Cu were fed in Exp. 2, pigs fed IBM had improved growth performance compared with those fed $MnSO_4$. Further research is needed to understand the potential benefits of Mn hydroxychloride fed in conjunction with pharmacological levels of Cu on pig growth performance.

Key words: Copper, finishing pig, growth, manganese, manganese hydroxychloride

Introduction

Manganese is an essential trace mineral that is a key component in carbohydrate, lipid and protein metabolism, as well as playing a role in mitochondrial superoxide dismutase (MnSOD) activity and bone development (Suttle, 2010). According to the NRC (2012), the quantitative requirement for Mn for nursery and finishing diets ranges from 2 to 4 mg/kg. Assuming bioavailability is not a concern, many swine diets today meet the NRC (2012) estimated requirement for Mn from the major dietary ingredients before a trace mineral premix is added to the diet. However, due to the unknown bioavailability of the innate Mn in ingredients, swine diets typically contain added Mn through a trace mineral premix. In a survey conducted by Flohr et al. (2016), swine diet Mn levels were found to be supplemented at as low as 3.3 mg/kg

and as high as 40 mg/kg throughout the entire finishing period. Therefore, there is a wide discrepancy of Mn supplementation in commercial swine diets.

Manganese is typically supplemented in swine diets as manganese sulfate; however, different mineral sources can also be utilized within swine diets, such as hydroxychloride trace minerals. To our knowledge little research has been complete to observe the effects of Mn hydroxychloride in finishing diets or how other dietary levels of trace minerals such as Cu may affect the response. Therefore, the objective of our study was to determine the effects of increasing dietary levels of Mn and source of Mn on growth performance and carcass characteristics of growing-finishing pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in this experiment.

Animals and diets

Two studies were conducted with a total of four barns (two barns per study) at a commercial research-finishing site in southwest Minnesota (New Horizon Farms, Pipestone, MN). Each barn was naturally ventilated and double-curtain-sided with a slatted concrete floor and deep manure storage. Each pen (3.05 × 5.49 m^2) was equipped with a 5-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a bowl waterer for *ad libitum* access to feed and water. The first experiment was conducted from October 3, 2018 to February 27, 2019 and the second experiment was conducted from December 5, 2019 to April 22, 2020.

In each experiment, two groups of 972 pigs (1,944 total pigs, PIC 337 × 1050; initial body weight (BW; 34.5 ± 0.50 kg and 40.0 ± 0.77 kg) were used in 107- or 100-d growth trials. Pigs were housed in mixed gender pens with 27 pigs per pen and 12 pens per treatment. Daily

feed additions to each pen were achieved by using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record feed amounts for individual pens. The treatments were structured as a randomized complete block design and arranged in a 2 × 3 factorial with main effects of Mn source ($MnSO_4$, Eurochem, Veracruz, Mexico; or Mn hydroxychloride, IBM; IntelliBond M, Micronutrients USA, LLC, Indianapolis, IN, US) and increasing Mn (8, 16, or 32 mg/kg). All treatment diets were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN and were formulated to meet or exceed NRC (2012) requirement estimates for growing-finishing pigs for their respective weight ranges (Table 1). The same basal diets were fed in both experiments. Diets were fed in meal form and in 4 dietary phases within each experiment.

In Exp. 1, dietary phases were fed from 34.5 to 56.7 kg, 56.7 to 72.6 kg, 72.6 to 99.8 kg, and 99.8 kg to market. For Exp. 1, the grower period was from 34.5 to 72.6 kg and the finisher period was 72.6 kg to market, respectively. Experimental diets were corn-soybean meal-DDGS-based and were formulated with a Mn, Cu, and Zn free premix. Manganese, Cu, and Zn were added to the diet by a hand-made premixes, which were added in place of corn in the diet. Each hand addition contributed the desired source of Mn, MnSO4 or IBM, and Mn level, 8, 16, or 32 mg/kg to the appropriate treatment, along with hand additions to provide 10 mg of Cu from Intellibond C (Micronutrients, Indianapolis, IN, US) and 80 mg of Zn from Intellibond Z (Micronutrients, Indianapolis, IN, US) per kg of the diet.

In Exp 2., dietary phases were fed from 40.0 to 49.0 kg, 49.0 to 77.0 kg, 77.0 to 104.3 kg, and 104.3 kg to market. For Exp. 2, the grower period was from 40.0 to 77.0 kg and the finisher period was 77.0 kg to market, respectively. Additions of dietary levels of Mn were the same as

in Exp. 1. All diets contained 80 mg/kg of Zn, similar to Exp. 1., however, the premix used in Exp. 2 provided 150 mg of Cu from Intellibond C per kg of the diet.

In both experiments, pigs were weighed approximately every 14 days to determine ADG, ADFI, and G:F. On d 86 for Exp. 1 and d 84 for Exp. 2, the 3 heaviest pigs in each pen were selected and marketed. These pigs were included in the calculation of pen growth performance, but not the carcass characteristics. On the last day of Exp. 1, final weights were obtained, and pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Loin depth and backfat were measured by optical probe (SFK; Herlev, Denmark) at the 10th rib. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm. For Exp. 2, because of the ongoing outbreak of COVID-19; only final weights were obtained and no carcass data was collected.

Chemical analysis

Representative diet samples were obtained from all feeders of each treatment and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of the diets were combined within dietary treatment, and two composite samples from each treatment were analyzed in duplicate (Cumberland Valley Analytical Services, Hagerstown, MD). Samples were analyzed for Mn, Cu, and Zn content (Method 985.01; AOAC Int., 2000).

Mineral content of the liver was also determined in Exp. 1. Liver samples were collected from 3 random pigs per pen from pigs marketed at the end of the study in the second group. Each liver sample was collected from the same location from the liver lob that is attached to the

gallbladder on each individual pig. The liver samples were dried at 105°C for 24 h. Liver tissues were then acid digested using trace metal grade nitric acid in preparation for heavy metal determination. Liver samples were analyzed for Mn at the Kansas State University Veterinary Medicine Diagnostic Laboratory using an inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, NexION 350X, Waltham, MA). Bismuth, germanium and rhodium served as internal standards. All runs included bovine liver Trace Elements and Methylmercury in freeze-dried muscle tissue (National Institute of Standards and Technology, Gaithersburg, MD) standards as appropriate for verification of instrument accuracy.

Statistical analysis

Data from both experiments were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria) with pen considered the experimental unit, BW as blocking factor, and treatment as fixed effect. Predetermined orthogonal contrasts were used to evaluate the interactive effects of Mn source and level. Interactions ($P \leq 0.10$) were evaluated linearly or quadratically within source. All results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results

Chemical analysis

The analyzed dietary Mn, Cu, and Zn were consistent with calculated values used in diet formulation for Exp. 1 and 2 and followed the intended Mn titration additions (Table 2).

Experiment 1

There was no evidence of difference ($P > 0.10$) for a Mn source × level interaction or main effect of source and level on any growth performance response in the grower and finisher

phases. Overall, there was a marginal Mn source × level interaction (quadratic, $P = 0.057$) for G:F, with G:F improving as Mn increased when supplied from $MnSO_4$, but decreasing and then increasing when Mn was supplemented from IBM. There was no evidence for Mn source differences for ADG or ADFI, but pigs fed 16 mg/kg of Mn had the poorest (quadratic, $P < 0.05$) ADG and lightest final BW compared to 8 or 32 mg/kg.

There was a tendency for Mn source × level interaction (quadratic, $P = 0.075$) for carcass yield, where yield did not change by increasing $MnSO_4$, but was greatest for pigs fed 16 mg/kg Mn from IBM. Loin depth increased (linear, source × level, $P = 0.035$) for increasing Mn from $MnSO_4$ but decreased when Mn was increased from IBM. The intermediate level of Mn had the lightest HCW (quadratic, $P = 0.071$) and decreased loin depth (quadratic, $P = 0.044$). No evidence of difference ($P > 0.10$) was observed for concentration of Cu and Zn in the liver. Manganese concentration increased (linear, $P = 0.015$) as Mn supplementation increased and tended to be greater ($P = 0.075$) when Mn was supplied by $MnSO_4$ compared to IBM.

Experiment 2

For Exp. 2 growth-promoting levels of Cu were included in all diets and in the grower period, there was a Mn source × level interaction (linear, $P = 0.029$) observed for G:F, with G:F improving as Mn increased from IBM but decreasing with increased Mn from $MnSO_4$. There was no evidence ($P > 0.10$) for Mn source or Mn level effect on ADG, but ADFI was greater ($P = 0.034$) when Mn was provided by IBM. Pigs fed 16 mg/kg Mn tended ($P = 0.052$) to have decreased ADFI when compared to those fed 8 or 32 mg/kg, regardless of source.

In the finisher period, there was a Mn source × level interaction (linear, $P = 0.039$) for ADG, with ADG improving as supplemental Mn was increased for $MnSO_4$ but decreasing when Mn was increased for pigs fed IBM. There was no evidence ($P > 0.10$) of difference for Mn level

to influence ADFI, but ADFI was greater ($P = 0.049$) for pigs fed Mn from IBM compared to pigs fed Mn from $MnSO_4$. There was no evidence of difference ($P > 0.10$) for a Mn source or level effect on G:F in the finisher period.

Overall, there was no evidence ($P > 0.10$) for a Mn source × level interaction for final body weight or any observed growth responses. Pigs fed Mn provided by IBM had greater ($P < 0.05$) ADG and ADFI and heavier ($P < 0.05$) final BW than pigs fed Mn from $MnSO_4$. Regardless of source, pigs fed 16 mg/kg of Mn tended (quadratic, $P = 0.088$) to have lower overall ADFI than pigs fed 8 or 32 mg/kg of Mn. There was no evidence of difference ($P > 0.10$) for Mn source or level effect on G:F.

Discussion

According to the NRC (2012), the Mn requirement estimate for growing-finishing pigs is 2 to 4 mg/kg of the diet. However, the majority of the research for determining the Mn requirement was conducted more than 50 years ago. Due to the unknown bioavailability of Mn from ingredients commonly used in diets, Mn is usually added to swine diets through a trace mineral premix, frequently as $MnSO_4$. Manganese hydroxychloride is another source of Mn that can be added to swine diets trace mineral premixes. Hydroxychloride-based minerals are manufactured through the reaction of hydrochloric acid, high purity forms of metal, and water. The product of this reaction is hydroxychloride crystals that contain the desired metal covalently bonded to chloride and hydroxyl groups. The covalent bonds processed by hydroxychloride minerals reduce the ability for the minerals to react with other components of the diet and potentially improve bioavailability (Cao et al., 2000).

To our knowledge, this is the first study that evaluated the effects of Mn hydroxychloride on pig growth performance and carcass characteristics. In the Exp. 1, there was no main effect of

Mn source on any influence growth performance and carcass characteristics, although there was a source × level interaction on G:F and loin depth. However, in Exp. 2, pigs fed supplemental Mn from IBM had increased ADG and ADFI and heavier final BW when compared to pigs fed $MnSO_4$. The reason for these inconsistent effects of IBM on growth performance is not clear, but could have been a result of the higher levels of dietary Cu in Exp. 2. Pharmacological levels of Cu have been shown to improve growth performance in growing-finishing pigs (Coble et al., 2017). Copper is excreted from the body through bile, however, when dietary Mn was increased to 200 mg/kg in rats, Mercandante et al. (2016) observed a reduction in Cu levels in the bile, signifying a hepatobiliary metabolism relationship between Mn and Cu. With the potential of improved bioavailability of the Mn hydroxychloride, less Cu may have been excreted allowing for potentially greater utilization of Cu in pigs being fed Mn hydroxychloride. However, this theory warrants further investigation.

Grummer et al. (1950) reported improvement in ADG and G:F when 40 mg/kg of Mn was added to a basal diet containing 12 mg/kg of Mn but saw no further improvement in growth performance at 80 or 160 mg/kg of Mn. Grummer et al. (1950) did not test levels of Mn supplemented below 40 mg/kg but based on our results, there is no additional benefit to add more than 8 mg/kg of supplemental Mn to the diet on growth performance or carcass characteristics. Apple et al. (2004) also observed no additional benefit when Mn supplementation was greater than 20 mg/kg on ADG, ADFI, or on carcass characteristics, but they did not evaluate levels less than 20 mg/kg of supplemental Mn. In additional research, Apple et al. (2004) did observe an improvement in G:F when Mn was supplemented at 320 mg/kg. In both of our experiments, regardless of source, 16 mg/kg of Mn reduced growth performance. In Exp. 1, final BW and ADG were reduced and ADFI was reduced in Exp. 2. The reduction in growth and

intake for the intermediate Mn level is not fully understood but may be a result of a potential unknown metabolic interaction occurring when 16 mg/kg of Mn is supplemented. It is also not fully understood as to why pigs fed 32 mg/kg of Mn had similar growth performance as pigs fed 8 mg/kg when performance was reduced when 16 mg/kg of Mn was supplemented. Further research may be needed to understand the negative response that occurred in both experiments when 16 mg/kg of Mn was supplemented.

Our results from Exp. 1 suggest there is no evidence of difference in HCW, yield, and carcass characteristics between pigs fed the different Mn sources when growth promoting levels of copper are not feed in the diet; however, 16 mg/kg of supplemental Mn did reduce loin depth and tended to produce lighter HCW. The reduction in HCW and loin depth appears to be directly correlated with the lighter final BW and reduced ADG that occurred for the 16 mg/kg in Exp. 1. Plumlee et al. (1956) visually observed an increase in fat deposition when pigs were fed Mn deficient diets of 0.5 mg/kg, which indicates that Mn can affect fat deposition; however, source nor level of Mn influenced percentage lean or backfat depth in our studies. Similarly, Apple et al. (2004) and Sawyer et al. (2007) also did not observe changes in carcass characteristics from added dietary Mn; however, they did observe improvements in fresh pork color and cooked pork tenderness when 320 to 350 mg/kg of Mn was fed.

Manganese absorption occurs in the small intestine and is transported into the body by the divalent metal transporter 1 (DMT1; Au et al., 2008). Copper and Zn can also be transported by DMT1 (Zheng et al. 2012), suggesting dietary Mn intake may affect the absorption of Cu and Zn. When 200 mg/kg of dietary Mn was fed to rats, Mercandante et al. (2016) observed an increase in Mn and Cu concentration in the liver on d 7, but Cu levels returned to control levels by d 61. Although lower levels of dietary Mn were fed in our experiments; our results indicate

that the dietary Mn levels fed in our study, regardless of source, did not affect Cu or Zn levels in the liver. Manganese concentration in the liver increased as dietary Mn increased, which agrees with observations of Grummer et al. (1950) and Mercandante et al. (2016). Manganese liver concentrations, however, were less when IBM was the dietary Mn source. The lower level of liver Mn concentration when supplemental Mn was fed from IBM could potentially be the result of increased Mn utilization or increased Mn excretion for the hydroxychloride form of Mn, however, neither of these reasons were measure in this study.

In conclusion, our results suggest that supplementing growing-finishing diets with greater than 8 mg/kg of Mn did not lead to any improvements in growth performance and carcass characteristics. More research is needed to further understand the potential benefits of Mn hydroxychloride fed in conjunction with pharmacological levels of Cu on pig growth performance and to understand the reason a depression in growth performance was observed at the intermediate supplementation level.

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Table 2.1 Composition of basal diets, Exp. 1 and 2 (as-fed basis)[1]

Items	Phase 1	Phase 2	Phase 3	Phase 4
Ingredients, %				
Corn	58.80	66.88	72.51	80.66
Soybean meal (46.5% CP)	26.60	18.77	13.29	15.35
DDGS[2]	10.00	10.00	10.00	---
Beef tallow	1.50	1.50	1.50	1.50
Limestone, ground	1.08	1.00	0.95	0.73
Monocalcium phosphate (21% P)	0.90	0.75	0.65	0.75
Salt	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.37	0.39	0.39	0.30
DL-Methionine	0.06	0.03	0.01	0.02
L-Threonine	0.09	0.09	0.10	0.10
L-Tryptophan	0.02	0.03	0.03	0.03
Phytase[3]	0.04	0.04	0.04	0.04
Vitamin-trace mineral premix[4,5]	0.15	0.15	0.15	0.15
Mn Source[6]	+/-	+/-	+/-	+/-
Calculated analysis				
Standardized ileal digestible amino acids, %				
Lysine	1.15	0.97	0.84	0.79
Isoleucine:lysine	63	61	59	60
Leucine:lysine	140	147	155	147
Methionine:lysine	31	30	29	29
Methionine and cysteine:lysine	55	55	56	56
Threonine:lysine	62	62	64	65
Tryptophan:lysine	19	19	19	20
Valine:lysine	70	70	70	70
Lysine:net energy, g/Mcal	4.62	3.82	3.26	3.05
Net energy, kcal/kg	2,486	2,539	2,574	2,594
Crude protein, %	20.8	17.8	15.6	14.4
Calcium, %	0.73	0.63	0.57	0.52
Standardized total tract digestible phosphorus, %	0.52	0.47	0.41	0.39

[1]In Exp.1 phases 1, 2, 3, and 4 were fed from 34.5 to 56.7 kg, 56.7 to 72.6 kg, 72.6 to 99.8 kg, and 99.8 kg to market, respectively. In Exp. 2 phases 1, 2, 3, and 4 were fed from 40.0 to 49.0 kg, 49.0 to 77.0 kg, 77.0 to 104.3 kg, and 104.3 kg to market, respectively.

[2]DDGS = dried distillers grains with solubles.

[3]Optiphos 2000 (Huvepharma Inc. Peachtree City, GA) provided 858.7 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.

[4]Provided per kg of diet: 80 mg Zn, 110 mg Fe, 0.33 mg I, 0.30 mg Se, 5,290 IU vitamin A, 1,322 IU vitamin D, 26 IU vitamin E, 1.2 mg vitamin K, 22.5 mg niacin, 7.5 mg pantothenic acid, 2.25 mg riboflavin, and 10.5 μg vitamin B12.

[5] Tribasic copper chloride (IntelliBond C, Micronutrients, Indianapolis, IN) provided 10 and 150 mg/kg of Cu for Exp. 1 and Exp. 2, respectively

[6]Mn hydroxychloride (IntelliBond M, Micronutrients, Indianapolis, IN); or Mn sulfate ($MnSO_4$, Eurochem, Veracruz, Mexico).

Table 2.2 Chemical analysis of Exp. 1 diets (as-fed basis)[1]

	$MnSO_4$, mg/kg			IBM, mg/kg		
Mineral, mg/kg	8	16	32	8	16	32
Experiment 1						
Cu	40.1	31.3	33.4	32.6	33.0	39.8
Mn	29.9	35.9	50.8	29.8	38.4	50.5
Zn	120.8	117.4	125.4	121.9	116.1	121.3
Experiment 2						
Cu	217.1	207.0	193.7	197.4	198.9	206.4
Mn	33.8	35.2	49.5	34.5	41.6	51.5
Zn	126.1	131.9	129.9	126.1	131.3	124.6

[1]Values represent means from 16 composite samples (4 per phase). For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD). IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

Table 2.3 Interactive effects of Mn source and level on grow-finish pig growth performance, Exp. 1[1]

	$MnSO_4$, mg/kg			IBM, mg/kg				Source × level, *P* =	
Item[2,3]	8	16	32	8	16	32	SEM	Linear	Quadratic
BW, kg									
Initial	34.5	34.4	34.5	34.5	34.5	34.5	0.50	0.846	0.646
Grower	71.8	71.5	71.7	72.5	72.0	72.1	1.14	0.725	0.889
Final	133.6	132.3	134.5	134.8	132.5	134.3	1.19	0.483	0.681
Grower									
ADG, kg	0.92	0.91	0.92	0.93	0.93	0.93	0.013	0.638	0.884
ADFI, kg	1.92	1.92	1.91	1.94	1.93	1.94	0.034	0.896	0.823
G:F	0.477	0.476	0.481	0.482	0.479	0.478	0.0051	0.366	0.954
Finisher									
ADG, kg	0.96	0.95	0.97	0.96	0.94	0.96	0.013	0.876	0.772
ADFI, kg	2.89	2.82	2.86	2.86	2.86	2.84	0.029	0.914	0.170
G:F	0.334	0.338	0.340	0.355	0.328	0.339	0.0038	0.952	0.117
Overall									
ADG, kg	0.95	0.94	0.96	0.96	0.94	0.95	0.008	0.351	0.593
ADFI, kg	2.51	2.46	2.49	2.50	2.49	2.48	0.025	0.904	0.289
G:F	0.377	0.381	0.385	0.384	0.377	0.384	0.0030	0.348	0.057
Carcass Characteristics									
HCW, kg	98.4	96.9	98.4	98.9	98.3	98.6	0.77	0.600	0.329
Carcass yield, %	73.5	73.3	73.2	73.4	74.2	73.3	0.20	0.970	0.075
Backfat depth, mm	17.0	16.6	16.9	17.0	17.1	17.0	0.31	0.981	0.108
Loin depth, mm	67.7	68.0	69.2	69.2	68.3	68.9	0.45	0.035	0.633
Lean, %[4]	56.6	56.7	56.8	56.8	56.6	56.8	0.20	0.564	0.115

[1]A total of 1,944 pigs (initial BW of 34.5 kg) were used in two groups with 27 pigs per pen and 6 replicates per treatment. Mn sources were Mn sulfate ($MnSO_4$, Erachem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio.

[3]The grower period was from d 0 to d 42 in group 1 and from d 0 to 39 in group 2. The finisher period was from d 42 to 106 in group 1 and from d 39 to 107 in group 2.

[4]Adjusted using HCW as covariate.

Table 2.4 Main effects of Mn source and level on growth performance, Exp 1.[1]

	Source				Level, mg/kg				Probability, *P* =	
Item[2]	$MnSO_4$	IBM	SEM	Probability, *P* =	8	16	32	SEM	Linear	Quadratic
BW, kg										
Initial	34.5	34.5	0.50	0.711	34.5	34.5	34.5	0.50	0.753	0.663
Grower	71.7	72.2	1.06	0.144	72.1	71.8	71.9	1.08	0.725	0.889
Final	133.5	133.9	0.90	0.588	134.3	132.4	134.6	0.96	0.483	0.016
Grower										
ADG, kg	0.92	0.93	0.010	0.201	0.93	0.92	0.92	0.011	0.827	0.566
ADFI, kg	1.92	1.94	0.029	0.271	1.93	1.93	1.93	0.030	0.719	0.868
G:F	0.478	0.480	0.0042	0.639	0.479	0.478	0.480	0.0043	0.880	0.649
Finisher										
ADG, kg	0.96	0.95	0.006	0.315	0.96	0.96	0.97	0.009	0.383	0.102
ADFI, kg	2.86	2.85	0.019	0.823	2.89	2.82	2.86	0.022	0.506	0.203
G:F	0.337	0.334	0.0022	0.261	0.334	0.333	0.340	0.0029	0.096	0.362
Overall										
ADG, kg	0.95	0.95	0.005	0.625	0.95	0.94	0.95	0.005	0.366	0.009
ADFI, kg	2.48	2.49	0.018	0.788	2.50	2.47	2.48	0.020	0.449	0.225
G:F	0.381	0.381	0.0020	0.826	0.380	0.379	0.384	0.0020	0.057	0.163
Carcass characteristics										
HCW, kg	97.9	98.6	0.56	0.167	98.7	97.7	98.5	0.61	0.899	0.071
Carcass yield, %	73.3	73.7	0.20	0.118	73.5	73.8	73.3	0.20	0.394	0.217
Backfat depth, mm[4]	16.9	17.1	0.17	0.522	17.0	16.9	17.0	0.21	0.932	0.258
Loin depth, mm[4]	68.3	68.8	0.25	0.127	68.4	68.1	69.0	0.30	0.109	0.044
Lean, %[4]	56.8	56.7	0.11	0.126	56.7	56.7	56.8	0.13	0.623	0.544

[1]A total of 1,944 pigs (initial BW of 34.5 kg) were used in two groups with 27 pigs per pen and 12 replicates per treatment. Mn sources were Mn sulfate ($MnSO_4$, Eurochem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio.

[3]The grower period was from d 0 to d 42 in group 1 and from d 0 to 39 in group 2. The finisher period was from d 42 to 106 in group 1 and from d 39 to 107 in group 2.

[4]Adjusted using HCW as covariate.

Table 2.5 Interactive effects of Mn source and level on grow-finish pig growth performance, Exp. 2[1]

	$MnSO_4$, mg/kg			IBM, mg/kg				Source × level, *P* =	
Item[2,3]	8	16	32	8	16	32	SEM	Linear	Quadratic
BW, kg									
Initial	40.0	40.0	40.0	40.0	40.0	39.9	0.77	0.821	0.650
Grower	75.9	75.1	74.8	76.3	75.4	75.8	1.20	0.468	0.699
Final	129.9	129.8	130.0	132.6	130.4	131.2	1.32	0.391	0.207
Grower									
ADG, kg	0.89	0.88	0.87	0.90	0.88	0.89	0.013	0.330	0.615
ADFI, kg	1.93	1.89	1.92	1.98	1.93	1.95	0.034	0.578	0.879
G:F	0.463	0.464	0.452	0.454	0.458	0.459	0.0051	0.029	0.652
Finisher									
ADG, kg	0.95	0.96	0.97	0.99	0.97	0.97	0.011	0.039	0.258
ADFI, kg	2.81	2.79	2.83	2.88	2.83	2.85	0.030	0.272	0.782
G:F	0.340	0.344	0.343	0.346	0.343	0.340	0.0041	0.267	0.402
Overall									
ADG, kg	0.93	0.93	0.93	0.95	0.93	0.94	0.007	0.284	0.213
ADFI, kg	2.43	2.41	2.45	2.50	2.45	2.46	0.029	0.236	0.856
G:F	0.381	0.384	0.379	0.382	0.380	0.380	0.0038	0.801	0.332

[1]A total of 1,944 pigs (initial BW of 40.0 kg) were used in two groups with 27 pigs per pen and 12 replicates per treatment. Mn sources were Mn sulfate ($MnSO_4$, Erachem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[3]The grower period was from d 0 to d 42 in group 1 and from d 0 to 38 in group 2. The finisher period was from d 42 to 97 in group 1 and from d 38 to 100 in group 2.

Table 2.6 Main effects of Mn source and level on growth performance, Exp. 2[1]

	Source				Level, mg/kg				Probability, *P* =	
Item[2]	$MnSO_4$	IBM	SEM	Probability, *P* =	8	16	32	SEM	Linear	Quadratic
BW, kg										
Initial	40.0	40.0	0.76	0.683	40.0	40.0	40.0	0.76	0.977	0.920
Grower	75.3	75.8	1.12	0.202	76.1	75.2	75.3	1.14	0.153	0.182
Final	129.9	131.4	1.18	0.011	131.2	130.1	130.5	1.21	0.391	0.201
Grower										
ADG, kg	0.879	0.891	0.0095	0.169	0.895	0.879	0.881	0.0104	0.273	0.221
ADFI, kg	1.914	1.952	0.0287	0.034	1.956	1.911	1.932	0.0301	0.478	0.052
G:F	0.460	0.457	0.0041	0.407	0.459	0.461	0.456	0.0043	0.340	0.319
Finisher										
ADG, kg	0.961	0.976	0.0069	0.049	0.974	0.965	0.968	0.0079	0.650	0.352
ADFI, kg	2.810	2.852	0.0213	0.049	2.844	2.809	2.839	0.0240	0.930	0.140
G:F	0.342	0.343	0.0032	0.846	0.343	0.344	0.341	0.0035	0.612	0.671
Overall										
ADG, kg	0.926	0.940	0.0042	0.009	0.940	0.928	0.932	0.0048	0.309	0.103
ADFI, kg	2.432	2.457	0.0238	0.023	2.466	2.432	2.457	0.0254	0.912	0.088
G:F	0.381	0.381	0.0032	0.865	0.382	0.382	0.379	0.0034	0.809	0.332

[1]A total of 1,944 pigs (initial BW of 40.0 kg) were used in two groups with 27 pigs per pen and 12 replicates per treatment. Mn sources were Mn sulfate ($MnSO_4$, Eurochem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).
[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW= hot carcass weight.
[3]The grower period was from d 0 to d 42 in group 1 and from d 0 to 38 in group 2. The finisher period was from d 42 to 97 in group 1 and from d 38 to 100 in group 2.

Table 2.7 Interactive effects of Mn source and level on grow-finish pig micromineral liver concentrations, Exp. 1[1,2]

	$MnSO_4$, mg/kg			IBM, mg/kg				Source × level, *P* =	
Item	8	16	32	8	16	32	SEM	Linear	Quadratic
Micromineral, mg/kg									
Cu	38.9	38.1	40.0	38.3	39.4	38.0	4.27	0.815	0.752
Mn	8.63	8.88	9.87	8.07	8.51	8.88	0.44	0.560	0.663
Zn	242.1	243.6	244.4	203.7	238.7	232.0	17.5	0.521	0.380

[1]A total of 36 pens were used in the second marketed group with 3 pigs per pen and 6 replicates per treatment. Mn sources were Mn sulfate ($MnSO_4$, Erachem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

[2]Liver micromineral analysis done by ICP-MS.

Table 2.8 Main effects of manganese source and level on grow-finish pig micromineral liver concentrations, Exp. 1[1,2]

	Source			Probability,	Level, mg/kg				Probability, *P* =	
Item	$MnSO_4$	IBM	SEM	*P* =	8	16	32	SEM	Linear	Quadratic
Micromineral, mg/kg										
Cu	39.0	38.6	2.43	0.902	38.6	38.7	39.0	2.92	0.925	0.994
Mn	9.1	8.5	0.25	0.075	8.5	8.7	9.4	0.30	0.015	0.989
Zn	243.3	224.9	11.7	0.166	222.9	241.1	238.2	13.3	0.427	0.346

[1]A total of 36 pens were used in the second marketed group with 3 pigs per pen and 18 replicates per source and 12 replicates per level. Mn sources were Mn sulfate ($MnSO_4$, Erachem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

[2]Liver micromineral analysis done by ICP-MS.

Chapter 3 - Determining the effects of added xylanase in nutrient adequate diets on growth performance and carcass characteristics of growing-finishing pigs

Abstract

A total of 1,944 mixed sex growing-finishing pigs (PIC 337 × 1050; initially 22.5 ± 0.53 kg) were used in a 131-d growth trial to determine the effects of increasing added xylanase in nutrient adequate diets on grow-finish pig growth performance and carcass characteristics. Xylanase is a naturally occurring enzyme derived from bacteria and fungi that can degrade the linear polysaccharide beta-1, 4-xylan; thus, breaking down the non-starch polysaccharides and potentially improving nutrient utilization. Pens of pigs were assigned to 1 of 6 treatments in a randomized complete block design with initial weight as a blocking factor. There were 27 pigs per pen and 12 pens per treatment. Experimental diets were fed in 5 phases and were corn-soybean meal-dried distillers grains with solubles-based. Dietary phases were fed from 22.5 to 36.3, 36.3 to 63.5, 63.5 to 88.5, 88.5 to 108.9, and 108.9 kg to market. The 6 dietary treatments were formulated to contain increasing added xylanase (Belfeed B 1100 MP; Jefo Nutrition, Inc., Saint-Hyacinthe, Quebec), derived from *Bacillus subtilis*, to provide 0, 5, 10, 20, 40, or 75 IU/kg of enzymatic activity. Data were analyzed using the lmer function in the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria) with pen as the experimental unit, body weight as a blocking factor, and treatment as a fixed effect. From d 0 to 70, there was a tendency (quadratic, $P = 0.064$) for average daily gain (ADG) to decrease and then return to control values with increasing added xylanase, but there was no evidence ($P > 0.10$) of differences for average daily feed intake (ADFI) or feed efficiency (G:F). From d 70 to

131 and overall, there was no evidence of difference ($P > 0.10$) observed for ADG, ADFI, and G:F. There was no evidence for difference ($P > 0.10$) for the number of pigs receiving injectable treatments or mortalities among treatments. For carcass traits, increasing xylanase increased then decreased (quadratic, $P = 0.010$) percentage carcass yield. Increasing added xylanase marginally increased (linear, $P = 0.066$) backfat depth and decreased (linear, $P = 0.038$) percentage lean. In conclusion, when xylanase was added to nutrient adequate diets, there was improved carcass yield when intermediate levels were fed, however, there was no impact on growth performance or mortality.

Key words: Carcass, enzyme, finishing pig, growth performance, xylanase

Introduction

It is a common practice in today's commercial swine production to add distillers dried grains with solubles (DDGS) and other by-product ingredients to diets in order to reduce feed cost. These ingredients can increase dietary non-starch polysaccharides (NSP) such as arabinoxylan, cellulose, and hemi-cellulose. As example, the NSP content of corn DDGS can be up to 25.0% of the dry matter (Jaworski et al., 2015). Non-starch polysaccharides are poorly utilized by the pig, can increase intestinal digesta viscosity, and can reduce digestibility of other ingredients (Laerke et al., 2015). Although results have not been consistent, various enzymes added to swine diets have been shown to potentially lessen the negative effects of NSP and improve digestibility of nutrients (Barrera et al., 2004, Nortey et al., 2007).

Xylanase is a naturally occurring enzyme derived from bacteria and fungi that catalyzes the hydrolysis of xylan components of NSP (Polizeli et al., 2005). The xylan bonds that are

hydrolyzed by xylanase is dependent on the nature of the substrate molecule, the chain length, and the degree of branching (Polizeli et al., 2005). Xylanases classified as endo-1,4-β-xylanase hydrolyze the glyosidic bonds of linear polysaccharide β-1, 4-xylans (Polizeli et al., 2005). The xylan bonds that are hydrolyzed are typically those from hemicellulose, more specifically arabinoxylan. The degradation of the linear polysaccharide beta-1, 4-xylan could result in an increased nutrient availability in the diet which could improve pig growth performance (Nortey et al., 2008). The addition of xylanase also has been shown to reduce intestinal viscosity caused by NSP and help improve digestibility (Brufau et al., 2006). The xylanase source, Belfeed B 1100 MP, is derived from the bacteria *Bacillus subtilis* and is classified as an endo-1,4-β-xylanase. Bacterial derived xylanases have been shown to have alkali tolerance and better thermostability than xylanases derived from fungi (Mandal et al., 2015). The ability to be more stable at lower pH allows *Bacillus subtilis* xylanase strains to pass through the stomach and activate in the more neutral pH of the small intestine.

Another potential benefit of xylanase is cleaving arabinoxylan creating arabinoxylan-oligosaccharides (AXOS), which have been linked to potential prebiotic effects (Grootaert et al., 2007). The release of AXOS lead to the bacterial fermentation of volatile fatty acids, which can improve gut integrity. Interestingly, Zier-Rush et al. (2016) observed a reduction in mortality of finishing pigs fed added dietary xylanase, which could have potentially been driven by the release of AXOS when xylanase was added to the diet.

Further investigation under commercial conditions is needed to understand the effects of xylanase, deriving from *Bacillus subtilis,* in diets for growing-finishing pigs. Therefore, the objective of this study was to determine the effects of xylanase in nutrient adequate diets on

growth performance, carcass characteristics, and mortality of growing-finishing pigs raised in a commercial environment.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted in two barns at a commercial research-finishing site in southwest Minnesota (New Horizon Farms, Pipestone, MN). Each barn was naturally ventilated and double-curtain-sided with a slatted concrete floor and deep manure storage. Each pen (3.05 × 5.49 m) was equipped with a 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a bowl waterer for *ad libitum* access to feed and water. The study was conducted from November 30, 2018 to May 1, 2019.

Two groups of approximately 972 pigs (1,944 total pigs, PIC 337 × 1050; initial body weight (BW); 22.5 ± 0.53 kg) were used in a 131-d growth trial. Pigs were housed in mixed gender pens with 27 pigs per pen and 12 pens per treatment (6 replications per group). Daily feed additions to each pen were achieved by using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record feed amounts for individual pens. Pens of pigs were assigned to 1 of 6 dietary treatments in a randomized complete block design with BW as a blocking factor.

All treatment diets (Table 1) were manufactured at the New Horizon Farms Feed Mill in Pipestone, MN and were formulated to meet or exceed NRC (2012) requirement estimates for growing-finishing pigs for their respective weight ranges. All diets consisted of the same basal formulation and were fed in meal form. Experimental diets were fed in 5 phases, with dietary phases fed from: 22.5 to 36.3, 36.3 to 63.5, 63.5 to 88.5, 88.5 to 108.9, and 108.9 kg to market. Experimental diets were corn-soybean meal-DDGS-based with dietary treatments formulated to include enzymatic xylanase (Belfeed B 1100 MP; Jefo Nutrition, Inc., Saint-Hyacinthe, Quebec)

activity at levels of: 0, 5, 10, 20, 40, or 75 IU/kg. Xylanase was included in the diet in place of corn.

Pigs were weighed approximately every 14 d to determine ADG, ADFI, and G:F. On d 102, the 2 heaviest pigs in each pen were selected and marketed. These pigs were included in the calculation of pen growth performance, but not the carcass characteristics. On the last day of the trial, final weights were obtained, and pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Loin depth and backfat were measured by optical probe (SFK; Herlev, Denamrk) at the 10^{th} rib. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Samples of complete diets were obtained from 6 feeders of each treatment approximately 4 d after the beginning and 4 d prior to the end of each phase from each group. Feed samples were delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of diets from both groups were combined within dietary treatment, and a composite sample from each phase for each treatment was analyzed in duplicate (Ward Laboratories, Inc., Kearney, NE, Table 1.). Samples were analyzed for dry matter (Method 930.15; AOAC Int., 2007), crude protein (Method 990.03; AOAC Int., 2007) and neutral detergent fiber (Method 991.43; AOAC Int., 2007). Composite samples were also analyzed for xylanase enzymatic activity (Puratos, Brussels, Belgium, Table 2). Dietary treatments with no added xylanase were analyzed and used as a baseline to identify the relative xylanase activity in other diets.

Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria) with pen considered as the experimental unit, BW as blocking factor, barn as a random effect, and treatment as a fixed effect. Initial BW was used as a covariate for all growth performance characteristics and carcass characteristics except: backfat, percentage lean, and loin depth. For the analysis of backfat, percentage lean, and loin depth HCW was used as a covariate. Predetermined orthogonal contrasts were used to evaluate the effects of added dietary xylanase. All results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Diet Analysis

Analysis of the complete diets for dry matter, crude protein and neutral detergent fiber (NDF) were consistent to the formulated values (Table 1). Diets were considered nutrient adequate as they were formulated to meet or exceed the NRC (2012) requirement estimates for growing-finishing pigs and are considered to have moderate NDF content. Analyzed xylanase activity followed the expected increasing dose curve (Table 2). Some variation in analytical results was expected with low xylanase levels in final diets being difficult to analyze, especially when less than 10 IU/kg was included in the diet.

Growth Performance and Carcass Characteristics

From d 0 to 70, there was marginal response (quadratic, $P = 0.064$) for ADG, with growth decreasing and then increasing back to the control value with increasing xylanase. However, there was no evidence ($P > 0.10$) of difference for ADFI and G:F. On day 70, there was a significant increase (linear, $P = 0.041$) in BW as xylanase increased. From d 70 to 131 and

overall, there was no evidence of differences ($P > 0.10$) for ADG, ADFI, G:F, and final BW among treatments.

Pigs fed diets containing high contents of non-starch polysaccharides (NSP) have been shown to have decreased growth performance and increase in intestinal viscosity which could be a result of the low digestibility of NSP (Laerke et al., 2015; Nortey et al., 2007). Xylanase can help hydrolyze the hemicellulose of NSP components, specifically arabinoxylans, in order to improve nutrient digestibility and reduce intestinal viscosity (Laerke et al., 2015). Although the authors found that intestinal viscosity was reduced when xylanase was added to the diet, it was thought that other NSP components may be causing the reduction in nutrient digestibility. Arabinoxylan, a component of fiber, can act as an antinutritional factor that can reduce the digestibility of other nutrients. Nortey et al. (2007, 2008) observed an improvement in energy, AA, and P digestibility when xylanase was added to wheat based diets and suggested improvements in digestibility may be due to solubilization of arabinoxylans and releasing of bound nutrients. Weilman et al. (2016) and Wightman et al. (2019) observed that improvements in energy digestibility from xylanase did not occur immediately, but rather after pigs were fed xylanase for a period of time, suggesting xylanase needs to be fed for a longer duration of time prior to observing a response. A reason for this response may also be due to the increased ability for fermentation as the pig matures, which allows for better utilization of the AXOS that is created from using xylanase.

Improvements in nutrient digestibility with added xylanase have not been consistently reflected in growth performance. The ability to observe improvements in feed efficiency with xylanase addition could be related to diet composition. Nortey et al. (2007) and Barrera et al. (2004) observed an improvement in feed efficiency when xylanase was added to swine diets

primarily composed of wheat and wheat by-products, while Moran et al. (2016) and Wightman et al. (2019) showed no improvement in any growth response criteria with diets comprised of corn and corn DDGS. This could be due to wheat having greater arabinoxylan and total NSP content (63.0 and 9.5%, respectively) compared to that of corn (48.6 and 8.1% respectively; Jaworski et al., 2015). Endo-1,4-β-xylanase, such as that used in the current study, is most effective at degrading the linear polysaccharide β-1, 4-xylans of arabinoxylans. Thus, it may be more beneficial to use xylanase in diets containing ingredients high in arabinoxylan content, such as wheat and wheat by-products. This may explain the lack of improvement in overall growth performance in the current study, whereas Barrera et al. (2004) and Nortey et al. (2007) observed improvements in feed efficiency.

When evaluating carcass characteristics, there was a quadratic response ($P = 0.010$) for carcass yield as added xylanase increased. Carcass yield increased and then decreased with increasing added xylanase. Zier-Rush et al. (2016) observed that increasing xylanase numerically improved carcass yield. The reason for xylanase to effect yield is not fully understood. These data show, as xylanase increased, backfat tended (linear, $P = 0.066$) to increase while percentage lean decreased (linear, $P = 0.038$). The evidence of xylanase changing backfat and percentage lean is not consistent with other trials. Barnes et al. (2011) and Jang et al. (2017) found no influence of xylanase on any carcass characteristics. Added xylanase in the current study had no evidence of impact ($P = 0.10$) on HCW and loin depth.

Zier-Rush et al. (2016) observed that increasing added dietary xylanase in growing-finishing diets linearly increased pig viability and numerically decreased mortality. This improvement in survivability is thought to come from xylanase breaking down arabinoxylans into AXOS. The AXOS is then fermented and converted to short chain fatty acids, such as

butyrate (Sanchez et al. 2008). Butyrate can ameliorate mucosal inflammation and helps strengthen the epithelial defense barrier (Canani et al., 2011). The benefits from the increased butyrate production in the intestinal tract is thought to be the main reason for the decrease in mortality. However, there was no evidence for differences ($P > 0.10$) found among treatments for the percentage of pigs receiving injectable medical interventions and overall mortality in the present study. More replication may be needed to observe small changes in mortality that can result from xylanase inclusion. Further research is needed to understand the effects of xylanase on mortality in growing-finishing pigs.

In conclusion, this study provided no evidence that feeding xylanase in nutrient adequate corn-SBM-DDGS based diets had an impact on growth performance, mortality, or the percentage of pigs that required health-related treatments, but did increase carcass yield when intermediate levels were fed.

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Table 3.1 Diet composition (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Ingredients, %					
Corn	50.82	58.29	64.10	64.10	84.33
Soybean meal, 46.5% CP	20.54	13.27	7.46	7.64	12.44
DDGS[2]	25.00	25.00	25.00	25.00	---
Beef tallow	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	1.10	1.10	1.10	1.10	0.85
Monocalcium P, 21% P	0.15	---	---	---	0.25
Salt	0.50	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.53	0.54	0.53	0.41	0.30
DL-Methionine	0.04	---	---	---	0.04
L-Threonine	0.11	0.09	0.09	0.04	0.09
L-Tryptophan	0.03	0.04	0.04	0.03	0.02
Phytase[3]	0.04	0.04	0.04	0.04	0.04
Mineral-vitamin premix[4]	0.15	0.15	0.15	0.15	0.15
Xylanase[5]	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lysine	1.17	1.00	0.85	0.76	0.72
Isoleucine:lysine	60	59	58	65	60
Leucine:lysine	150	159	171	192	153
Methionine:lysine	30	28	30	34	33
Methionine + cysteine:lysine	55	54	58	65	61
Threonine:lysine	61	60	61	63	65
Tryptophan:lysine	18.0	18.0	18.0	19.0	19.0
Valine:lysine	70	70	71	80	70
SID lysine:net energy, g/Mcal	4.73	3.96	3.33	2.98	2.77
Net energy, kcal/kg	2,475	2,521	2,554	2,552	2,596
Ca, %	0.54	0.50	0.48	0.48	0.44
STTD P[6], %	0.40	0.35	0.34	0.34	0.28
Chemical analysis, %					
DM, %	88.89	88.68	87.87	87.86	86.46
CP, %	21.45	20.78	16.28	16.30	13.57
NDF, %	11.53	11.85	12.42	12.33	8.67

[1]Phases 1, 2, 3, 4, and 5 were fed from 22.7 to 36.4 kg, 36.4 to 63.6 kg, 63.6 to 88.6 kg, 88.6 to 109.1 kg, and 109.1 kg to market, respectively.

[2]DDGS = dried distillers grains with solubles.

[3]Optiphos 2000 (Huvepharma Inc. Peachtree City, GA) provided 858.3 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.

[4]Provided per kg of diet: 111 mg Zn, 111 mg Fe, 33 mg Mn, 17 mg Cu, 0.33 mg I, 0.30 mg Se, 2400 IU vitamin A, 600 IU vitamin D, 12 IU vitamin E, 1.2 mg vitamin K, 22.5 mg niacin, 7.5 mg pantothenic acid, 2.25 mg riboflavin, and 10.5 μg vitamin B12[5]Belfeed B 1100 MP (Jefo Nutrition, Inc., Saint-Hyacinthe, Quebec) replaced corn in the diet.

[6] Standardized total tract digestible P.

Table 3.2 Relative analyzed xylanase activity by phase[1,2]

	Dietary phase				
Xylanase, IU/kg	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
5	-1.4	-1.7	2.3	3.1	1.6
10	0.5	8.5	5.0	11.0	7.5
20	16.3	16.9	16.2	24.7	13.8
40	32.4	49.4	28.4	35.9	31.6
75	62.3	68.8	58.6	66.1	73.7

[1]Phases 1, 2, 3, 4, and 5 were fed from 22.7 to 36.4, 36.4 to 63.6, 63.6 to 88.6, 88.6 to 109.1, and 109.1 kg to market, respectively.

[2]Diets with less than 10 IU/kg activity are difficult to analyze due to low activity.

Table 3.3 Effects of added xylanase on growth performance and carcass characteristics of growing-finishing pigs[1,2]

	Xylanase, IU/kg							Probability, *P* =	
Item[3]	0	5	10	20	40	75	SEM	Linear	Quadratic
BW, kg									
d 70	79.0	80.1	78.6	79.4	79.3	80.5	0.79	0.041	0.245
d 131	134.3	134.4	132.7	133.6	133.0	134.6	1.18	0.919	0.154
d 0 to 70									
ADG, kg	0.79	0.80	0.77	0.77	0.78	0.79	0.013	0.773	0.064
ADFI, kg	1.62	1.58	1.55	1.56	1.59	1.60	0.022	0.556	0.130
G:F	0.490	0.507	0.498	0.498	0.493	0.497	0.0051	0.634	0.830
d 70 to 131									
ADG, kg	0.92	0.90	0.90	0.92	0.90	0.90	0.009	0.645	0.930
ADFI, kg	2.67	2.60	2.69	2.70	2.65	2.67	0.030	0.741	0.671
G:F	0.345	0.345	0.335	0.343	0.341	0.339	0.0032	0.330	0.616
Overall (d 0 to 131)									
ADG, kg	0.86	0.85	0.84	0.86	0.85	0.85	0.007	0.696	0.242
ADFI, kg	2.20	2.14	2.18	2.19	2.17	2.18	0.027	0.700	0.965
G:F	0.393	0.398	0.387	0.393	0.390	0.390	0.0026	0.314	0.205
Treatments, %[4]	2.02	3.09	4.71	2.16	3.70	3.70	1.230	0.361	0.742
Mortality, %	3.70	3.70	3.36	3.08	3.40	5.56	1.272	0.188	0.277
Carcass traits									
HCW, kg	98.1	96.9	97.2	98.1	96.8	96.6	0.903	0.264	0.915
Carcass yield, %	72.78	72.18	73.48	73.31	73.35	72.02	0.434	0.220	0.010
Backfat, mm[5]	16.4	16.3	15.8	16.9	16.7	16.7	0.34	0.066	0.703
Loin depth, mm[5]	69.6	69.2	69.7	70.0	69.2	69.2	0.93	0.683	0.828
Lean, %[5]	57.2	57.2	57.6	57.0	57.0	56.8	0.210	0.038	0.782

[1]A total of 1,944 pigs in two groups were used in a 131-d study with 27 pigs per pen and 12 replicates per treatment.

[2]Initial BW was used as a covariate for all criteria except backfat, loin depth, and lean %. Initial BW were 22.3, 22.6, 22.5, 22.3, 22.6, and 22.7 kg for diets containing 0, 5, 10, 20, 40, and 75 IU/kg of xylanase, respectively.

[3]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[4]Treatments = total injectable medication treatments divided by pigs per pen.

[5]Adjusted using HCW as covariate.

Appendix A - A multi-trial analysis evaluating the effects of pharmacological levels of Intellibond Copper on growing-finishing pig growth performance and carcass characteristics

Summary

A multi-trial analysis was conducted to evaluate the effects of pharmacological levels of added Intellibond Copper (IBC) on growing-finishing pig growth performance and carcass characteristics compared to pigs fed control diets containing typical additions of copper from a trace mineral premix. Data from 8 trials and 331 observations were included in the final database. Inclusion rates for IBC were either 75 (1 trial), 150 (7 trials), or 200 (1 trial) ppm. Data were analyzed using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria). Pigs fed IBC throughout the entire grow-finish period had greater ($P < 0.05$) overall ADG, ADFI, and final BW and tended ($P = 0.085$) to be more efficient than pigs fed control diets. There was no evidence for differences ($P > 0.10$) between dietary treatments for the percentage of pigs marketed. Pigs fed IBC also had ($P < 0.05$) heavier HCW and greater carcass ADG compared to pigs fed a control diet. There were no differences ($P > 0.10$) between pigs fed the control or IBC diets for carcass characteristics, carcass yield, and carcass feed efficiency. In conclusion, the addition of IBC at growth promotional levels throughout the entire growing-finishing period increased final BW, HCW and ADG on a live and carcass basis.

Key words: Finishing pig, growth performance, tribasic copper chloride

Introduction

Copper is an essential trace mineral that plays an important role in hemoglobin synthesis and is needed for the activation of enzymes necessary for normal metabolism. According to the NRC (2012)[1], growing-finishing pigs require 5 to 6 ppm of copper. However, it has been shown that feeding pharmacological levels of copper (75 to 200 ppm) during the growing-finishing period can improve growth performance. Multiple trials have been conducted to evaluate the effects of the copper source, IntelliBond C (IBC), when supplemented at pharmacological levels, on growth performance and carcass characteristics; however, an analysis summarizing results of all the studies has not been conducted. Therefore, the objective of this analysis was to summarize the results of 8 trials that evaluated the effects of pharmacological levels of IBC on growing-finishing pig growth performance and carcass characteristics.

Database

All data was derived exclusively from experiments that were verified to be comparing a control diet containing copper levels derived only from the trace mineral premix versus diets containing IBC supplemented at pharmacological levels throughout the entire growing-finishing period. A total of 6,790 pigs from 8 trials with 331 observations (pens) were recorded in the final analysis (Table 1). Inclusion rates of growth promotional IBC consisted of 75 (1 trial), 150 (7 trials), or 200 (1 trial) ppm. Feeding duration of supplemental IBC in four trials was from 80 to 99 d and four trials fed IBC from 100 to 120 d in duration. For initial pig inventory, two trials had 9 pigs per pen, 1 trial had 20 pigs per pen, 2 trials had 26 pigs per pen, and 3 trials had 27

[1] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.

pigs per pen. Two trials had less 500 pigs on test, 3 trials had 501 to 1,000 pigs on test, 3 trials had greater than 1,000 pigs on test. In order to be included in the final growth performance database, observations needed to have a recorded final BW and the same basal diet composition needed to be used for both the control and IBC treatments throughout the entire study. In order to be included in the final carcass characteristics database, observations needed to have a recorded HCW. If an observation had any other measured carcass characteristics missing, that observation was omitted only from that specific measurement. Categorical variables such as barn, diet composition, gender, growing season, pen space, and ractopamine usage were also included in the final database. Some trials were conducted as factorials measuring the influence of IBC supplementation dependent on basal diet formulation and in these cases the basal diet formulations were treated as separate observations. Therefore, treatment diet compositions were consisted of: corn-soybean meal (3 trials), corn-soybean meal-wheat midds (1 trial), corn-soybean meal-dried distillers grains with solubles (2 trials), and corn-soybean meal-DDGS-bakery meal (4 trials). Three trials used mixed sex pens of pigs while the remaining 5 trials sorted pens by gender. Season was determined by the month when the majority of the pigs were marketed for the trial, with 5 trials marketed in the fall, 2 trials in the winter, and 1 trial in the spring. Pen space across all trials ranged from 6.50 to 6.85 square feet per pig. Ractopamine was fed in 5 of the 8 trials.

Data from each trial was recorded in a spreadsheet that included growth performance and carcass characteristics. Growth performance data was categorized into grower (75.1 ± 3.92 lb to 150.2 ± 3.57 lb), finisher (150.2 ± 3.57 lb to 279.8 ± 2.05 lb), and overall (75.1 ± 3.92 lb to 279.8 ± 2.05 lb) periods. Measured growth performance data included initial, grower, and final BW along with ADG, ADFI, and F/G for each respective period. Percentage of pigs marketed was

also measured by taking the number of pigs marketed divided by the number of pigs placed. Measured carcass data included: HCW, carcass yield, carcass ADG, carcass F/G, backfat, percentage lean, and loin depth.

Statistical analysis

Data were analyzed using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria). The Weights statement in R was used to account for heterogeneity in the variance of the residuals. Response variables were weighted based on the number of observations for each study using the inverse of the squared SEM. To determine the SEM for each treatment within each trial, the lm function from the lme4 package in R was used. Study, barn, season, experimental diet composition, and gender were included as a random effect. The statistical inclusion of terms as covariates in the model was determined at $P < 0.05$. Initial BW was used as a covariate for all growth performance characteristics and carcass characteristics except backfat, percentage lean, and loin depth, where HCW was used as a covariate. No categorical variable was used as a covariate because all categorical variable by treatment interactions were nonsignificant ($P > 0.05$).

From the results, an economic comparison scenario was devised. Prices used were: IBC ($3.85/lb), total feed cost without added IBC ($50.00/pig), and HCW ($0.65/lb). For this scenario, IBC was included at 0.56 lb/ton to achieve 150 ppm of additional Cu supplementation in the diet. Revenue was calculated as HCW multiplied by $0.65. Income over feed cost (IOFC) was calculated as revenue minus total feed cost.

Results and Discussion

There was no evidence ($P > 0.05$) for categorical variable by treatment interactions, which is likely a result of not having sufficient data for the categorical variables of diet composition, gender, growing season, pen space, or ractopamine usage, to truly assess if these variables influence the growth response to feeding pharmacological levels of IBC.

In the grower period, pigs fed pharmacological levels of IBC had greater ($P < 0.05$) ADG, ADFI and heavier BW at the end of the grower period when compared with pigs fed control diets (Table 2). In the finisher period, pigs fed pharmacological levels of IBC had greater ($P < 0.05$) ADG and tended ($P = 0.075$) to have increased ADFI compared with pigs fed control diets. There were no differences ($P > 0.10$) in feed efficiency between dietary treatments in both the grower and finisher period.

Overall, pigs fed pharmacological levels of IBC had greater ($P < 0.05$) ADG, ADFI and final BW and tended to have improved ($P = 0.085$) feed efficiency compared with pigs fed control diets. There were no differences ($P > 0.10$) between dietary treatments for the percentage of pigs marketed. Pigs fed IBC throughout the growing-finishing period also had greater ($P < 0.05$) HCW and ADG on a carcass weight basis when compared to pigs fed the control diets. There was no evidence for differences ($P > 0.10$) between pigs fed the control or IBC diets for backfat, loin depth, percentage lean, carcass yield, or carcass feed efficiency. In conclusion, the addition of IBC at growth promotional levels throughout the entire growing-finishing period increased final BW, HCW and ADG on a live and carcass basis.

In order to determine the potential economic implications of the multi-trial analysis results, the following example is provided: Prior to the addition of pharmacological levels of IBC, growing-finishing pigs in this fixed time scenario of 100 d have an ADFI of 5.91 lb/day. When 150 ppm of supplemental IBC is added to the diet, an increase in $1.32 of additional feed

cost will accrue from the inclusion of IBC and from the increase in ADFI, which is now 5.99 lb/day. The usage of pharmacological levels of IBC, however, will result in a 2.9 lb increase in HCW and would increase revenue per pig by $1.89, if HCW is valued at $0.65. Overall, the improvement in IOFC is $0.56 per pig for this economic scenario.

Table A.1 Summary of experiments used in the multi-trial analysis to determine growth performance and carcass characteristics from pharmacological levels of IBC[1]

Experiment[2]	Avg. length, d	Barns, n	Total pens, n	Total pigs, n	Avg. Initial BW, lb	Avg. Final BW, lb	IBC level, ppm	Diet composition	Season	Pen gender	Ractopamine	Initial pen space, ft^2
Trial 1	111	1	21	570	55.3	281.2	75, 150	CSDB	Winter	Mix	Yes	6.85
Trial 2	120	1	48	1,248	63.8	276.6	150	CSDB	Fall	Mix	Yes	6.85
Trial 3	120	1	24	632	58.3	289.0	150	CSDB	Spring	Mix	Yes	6.85
Trial 4	118	1	42	1,133	60.8	277.8	150	CS, CSDB	Winter	Mix	Yes	6.85
Trial 5	81	3	101	2,005	103.7	280.1	150	CSD	Fall	Same	Yes	6.50
Trial 6	103	2	19	518	83.6	284.8	200	CSD	Fall	Mix	No	6.85
Trial 7	87	2	38	342	62.2	276.5	150	CS	Fall	Same	No	N/A
Trial 8	82	2	38	342	70.2	268.5	150	CS	Fall	Same	No	N/A

[1]A total of 6,790 pigs were used in eight experiments to evaluate the effects of diets containing no added copper or diets with 75 to 200 ppm of additional copper from IntelliBond C (IBC, Micronutrients, Indianapolis, IN) on growth performance. A total of 6,737 pigs were used in eight experiments to evaluate the effects of diets containing no supplemental copper or diets with 75 to 200 ppm of additional copper from IBC on carcass characteristics.

[2]BW= body weight. CS = corn-soy. CSD = corn-soy-dried distillers grains with solubles. CSDB = corn-soy-dried distillers grains with solubles-bakery meal

Table A.2 Effects of added Intellibond C on grow-finish pig growth performance and carcass characteristics[1,2]

Item[3]	Control	IBC	SEM	Probability, *P* =
BW				
Grower, lb[4]	147.8	150.2	3.57	< 0.001
Final, lb[5]	276.0	279.8	2.07	< 0.001
Grower[4]				
ADG, lb	2.05	2.12	0.041	< 0.001
ADFI, lb	4.55	4.68	0.093	< 0.001
F/G	2.25	2.25	0.033	0.563
Finisher[5]				
ADG, lb	2.18	2.21	0.065	0.015
ADFI, lb	6.72	6.77	0.202	0.075
F/G	3.07	3.08	0.037	0.198
Overall				
ADG, lb	2.11	2.15	0.065	< 0.001
ADFI, lb	5.91	5.99	0.164	0.008
F/G	2.82	2.80	0.047	0.085
Percent marketed, %[6]	91.7	91.0	1.80	0.247
Carcass characteristics				
HCW, lb	205.3	208.2	1.56	< 0.001
Carcass yield, %	74.5	74.6	0.03	0.486
Carcass ADG, lb	1.57	1.60	0.047	< 0.001
Carcass F/G	3.79	3.76	0.078	0.105
Backfat, in[7]	0.76	0.75	0.041	0.463
Lean, %[7]	55.4	55.2	1.42	0.914
Loin depth, in[7]	2.61	2.61	0.078	0.984

[1]A total of 6,790 pigs were used in eight experiments to evaluate the effects of diets containing no added copper or diets with 75 to 200 ppm of additional copper from IntelliBond C (IBC, Micronutrients, Indianapolis, IN) on growth performance. A total of 6,737 pigs were used in eight experiments to evaluate the effects of diets containing no supplemental copper or diets with 75 to 200 ppm of additional copper from IBC on carcass characteristics.

[2]Initial BW was used as a covariate. Initial BW were 73.8 and 75.1 lb for control and IBC, respectively

[3]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. F/G = feed-to-gain ratio. HCW = hot carcass weight.

[4]Grower period is from 70 to 150 lb.

[5]Finisher period is from 150 lb to marketing.

[6]Percent marketed = pigs marketed/pigs placed.

[7]HCW was used as a covariate.