Effects of antibiotic administration or ZnO replacement strategies on nursery pig performance and a commercial organic acid, essential oil blend on performance of wean-to-finish pigs

by

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Abstract

Three experiments were conducted to determine the influence of dietary strategies on nursery pig performance. In Exp. 1, a total of 2,592 pigs were used to determine the effects of two antibiotics (chlortetracycline; CTC vs. tiamulin) and their route of administration (in-feed vs. in-water) on nursery pig growth performance. Although antibiotics did not improve feed efficiency when compared to non-medicated fed pigs, providing CTC in feed with or without tiamulin or tiamulin provided in the water improved nursery pig growth performance. In Exp. 2, 360 weaned pigs were used to evaluate potential replacements for pharmacological levels of Zn (provided by Zn oxide), such as diet acidification (sodium diformate), and dietary crude protein (CP: 21 vs. 18%) on nursery pig performance and fecal dry matter. Although none of the diets had a major influence on fecal dry matter, the addition of pharmacological levels of Zn or sodium diformate independently improved nursery pig performance. In Exp. 3, 1,215 pigs were used to determine the effect of AviPlus, a combination of micro-encapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals, (Vetagro, Inc. Chicago, IL) on growth performance of pigs from weaning to market. When AviPlus was provided during the nursery phase, there was an improvement in G:F in the early and overall nursery phases, but there was no effect on overall wean-to-finish performance.

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Chapter 1 - Evaluating the effect of route of antibiotic administration on nursery pig growth performance

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Abstract

A total of 2,592 pigs (L337 × 1050, PIC Hendersonville, TN; initially 6.6 kg) were used in a 28-d trial to determine the effects of two antibiotics (chlortetracycline; **CTC** vs. Tiamulin**)** and their route of administration (in-feed vs. in-water) on nursery pig growth performance. Pigs were weaned at 21 d of age and placed in a commercial research facility with 27 pigs per pen. After a 7-d pre-trial period with no antibiotic in the feed, pens of pigs were assigned to weight blocks in a randomized complete block design. There were 12 replications with pen as experimental unit for in-feed medication treatments and a pair of pens sharing the same water line as the experimental unit for water medication treatments. The six treatments included a control diet (no medication), CTC provided via feed or water to achieve a target dosage of 22 mg/kg body weight (**BW**), tiamulin provided in feed (5 mg/kg BW) or water (23 mg/kg BW), or a combination of CTC and tiamulin in feed (22 mg/kg and 5 mg/kg), respectively. Experimental diets were provided for 14-d followed by a 14-d period without feed or water-based medication. Treatment differences were tested as a 2 × 2 factorial with main effects of antibiotic (CTC or tiamulin) and route of administration (in-feed or in-water). Pairwise comparisons were also made between the control and the other individual treatments. From d 0 to 14, and 0 to 28 there was an antibiotic × route of administration interaction ($P < 0.05$) observed for average daily gain (**ADG**). The interactions were a result of pigs fed CTC having increased ADG compared to CTC in-water, whereas pigs provided tiamulin in-water had increased ADG compared with tiamulin in feed. An antibiotic × route of administration interaction ($P < 0.05$) was also observed for gain:feed (**G:F**) from d 0 to 14 and 0 to 28. Pigs provided tiamulin in the feed had the poorest G:F, whereas G:F was not different among the other treatments. From d 0 to 14 and 0 to 28, providing CTC in the feed or water or tiamulin in the water improved ($P < 0.05$ or $P < 0.10$) ADG compared to pigs fed the control diet. In summary, our study shows that providing CTC or tiamulin in the water improves growth in nursery pigs. Providing CTC in feed with or without tiamulin or tiamulin provided in the water demonstrated to improve nursery pig growth performance. Administering antibiotics via water may prove to be a more practical strategy due to flexibility of its usage.

Keywords: Administration, antibiotics, chlortetracycline, Nursery pigs, Tiamulin

Introduction

Antibiotics fend off or slow down the growth of bacteria and the diseases they produce and have been shown to improve growth performance and feed efficiency (Cromwell et al., 2002). Chlortetracycline (CTC) and tiamulin have been widely used either alone or in combination in the swine industry (Puls et al., 2019). Chlortetracycline is used to control and for treatment of bacterial enteritis (scours) caused by *Escherichia coli* and *Salmonella* spp. (Jacela et al., 2009). Chlortetracycline belongs to the tetracycline class of antibiotics and is one of the most widely used antibiotics in the swine industry (Helm et al., 2019). Chlortetracycline in nursery diets improves average daily gain (ADG) and gain:feed ratio (G:F) in nursery pigs (Feldpausch et al., 2018; Williams et al., 2018; Capps et al., 2020).

Tiamulin is used for treatment of swine dysentery associated with *Brachyspira hyodysenteriae* and has also been demonstrated to improve growth rate and feed efficiency (Cromwell et al., 1985; Steidinger et al., 2009). Tiamulin belongs to the pleuromutilin class of antibiotics commonly used in pigs and poultry. Many antibiotics that are used in the swine industry have varying formulations which allow them to be administered through different routes. Providing antibiotics in the water provides more flexibility and is more convenient for producers to quickly administer an antibiotic in response to a disease outbreak (Wu et al., 2019). Antibiotics provided in feed will likely be administered later than desired after a diseases diagnosis and might be fed either shorter or longer than targeted compared to a water route. To our knowledge, there is limited data directly comparing CTC and/or Tiamulin administration in the feed or water and to determine if route of administration effects growth performance. Thus, the objective of this study was to identify the effects of administering CTC and/or tiamulin via feed or water on nursery pig growth performance.

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 New Horizon Farms Commercial Research Nursery facility located in Pipestone, MN. Each pen (3.65 × 2.44 m; allowed approximately 0.33 m^2/pig) had plastic slatted floors and was equipped with a 6-hole stainless steel dry feeder and a pan waterer to provide ad libitum access to feed and water. Feed additions to each pen were delivered and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

Pigs were weaned at approximately 21 d of age and placed in pens of 27 pigs based on initial body weight (BW). A total of 2,592 pigs (L337 × 1050 PIC, Hendersonville, TN) were used. After a 7-d pre-trial period, pens of pigs were assigned to weight blocks in a randomized complete block design and allotted to 1 of 6 dietary treatments. The 6 treatments included a control diet (no medication), chlortetracycline (CTC) provided via feed (440 mg/kg) or water to achieve a dosage of 22 mg/kg BW, tiamulin in feed (38.5 mg/kg) to provide 5 mg/kg BW or water (23 mg/kg BW), or a combination of CTC and tiamulin in feed. There were 12 replications per treatment with pen as experimental unit for in-feed medication treatments and pairs of pens as the experimental unit for water medication treatments. Experimental treatments were provided for 14-d followed by a 14-d period without any medication. The diet fed prior to starting the experiment was a pelleted diet that did not contain an antibiotic. Experimental diets were fed in meal form (Table 1.1). Pens of pigs were weighed and feed disappearance was measured weekly to determine ADG, ADFI, and G:F.

Statistical Analysis

Data were analyzed using R Studio (Version 3.5.2, R Core Team, Vienna, Austria) with pen for feed or a pair of adjacent pens for water treatments serving as the experimental unit. The study was a randomized complete block design with weight block included in the model as a random effect. Pre-planned contrast statements were used to evaluate the treatment effects on ADG, ADFI, BW, and G:F. The statistical analysis was conducted as a 2 × 2 factorial with main effect of antibiotic type (CTC or tiamulin) and route of administration (in-feed or in-water and their interactions). Pairwise comparisons were also made between pigs fed the control diet and all other antibiotic-containing treatments. Statistical models were fit using NLME package in R. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 > P \leq 0.10$.

Results

During the pre-test period immediately after weaning (d -7 to 0), there was no evidence for difference ($P > 0.386$) for ADG, ADFI, G:F or BW. Averaged daily gain was 322 g/d, ADFI 450 g/d and G:F was 718 g/kg.

From d 0 to 14, there was an antibiotic × route of administration interaction ($P = 0.002$) observed for ADG (Table 1.2). Pigs fed diets containing CTC had increased ($P < 0.05$) ADG compared with pigs provided CTC in the water whereas pigs provided tiamulin in the water had greater ADG than those fed tiamulin in feed. Pigs provided CTC in the feed or water, tiamulin in the water and the combination of CTC and Tiamulin in feed had increased ADG ($P < 0.05$) when compared to pigs fed the control diet. Pigs provided tiamulin in the feed showed no evidence of difference in ADG compared to the control pigs.

Pigs provided CTC or tiamulin in feed had increased ADFI compared to those fed the control diet with pigs provided CTC or tiamulin in the water having marginally greater ($P <$

0.10) ADFI than control pigs. Pigs provided the combination of CTC and tiamulin in feed were not different than those provided CTC in feed for ADFI.

There was also an antibiotic × route of administration interaction (*P* =0.001) observed for G:F where pigs provided tiamulin in feed had decreased G:F compared to pigs provided CTC in feed or water or tiamulin supplied in water (*P* < 0.05) which were not different from each other. Pigs provided tiamulin in feed also had decreased (*P* < 0.05) G:F compared with pigs fed the control diet.

There was an antibiotic × route of administration interaction (*P* < 0.05) observed for d 14 BW. Pigs provided CTC in-feed had increased BW compared to pigs provided CTC in-water, whereas pigs provided tiamulin in the water had greater BW than those pigs that were provided tiamulin in the feed. Pigs provided CTC in the feed had increased (*P* < 0.05) BW compared to pigs fed the control diet but no evidence of difference was observed among the other treatments.

For the subsequent non-medicated period (d 14 to 28), there was a marginal antibiotic × route of administration interaction (*P* = 0.071) observed for ADG. Pigs previously provided CTC in the feed had greater ADG than pigs provided CTC in the water whereas pigs previously provided tiamulin in the water had greater ADG than pigs previously provided tiamulin in the feed. Pigs previously provided tiamulin in the water had increased ADG (*P* < 0.05) when compared to the control and pigs previously provided CTC in the feed tended to have greater (*P* < 0.10) ADG than the control-fed pigs. For ADFI from d 14 to 28, there were no main effects of antibiotic or route of administration; however, pigs previously provided the combination of CTC and tiamulin in the feed had increased ADFI (*P* < 0.05) and pigs previously provided CTC in the feed or tiamulin in the water tended to have increased (*P* < 0.10) ADFI compared to pigs provided the control treatment. For G:F, there was no evidence of difference between treatments ($P > 0.05$).

Overall (d 0 to 28), there was an antibiotic type × route of administration interaction ($P < 0.05$) observed for ADG. Pigs provided CTC in the feed during the treatment period had increased ADG compared to pigs provided CTC in the water whereas pigs provided tiamulin in water had increased ADG compared to pigs provided tiamulin in the feed. Compared with pigs fed the control diet, pigs provided CTC in feed, tiamulin in the water, or CTC and tiamulin in feed during the treatment period had increased ADG ($P < 0.05$) with pigs provided CTC in the water having marginally greater ($P < 0.10$) ADG than the control. There were no interactions or main effects of antibiotic type or route of administration observed ($P > 0.05$) for overall ADFI. However, pigs provided CTC in the feed, tiamulin in the water, and the combination of CTC and tiamulin in feed had increased ($P<0.05$) ADFI when compared to the control. Pigs provided CTC in the water and tiamulin in the feed also had a tendency ($P < 0.10$) for increased ADFI compared to the control. For G:F from d 0 to 28, there was an antibiotic type × route of administration interaction ($P < 0.05$) observed. Pigs provided tiamulin in the feed had poorer G:F compared to pigs provided other treatments. Finally, for BW on d 28, there was an antibiotic type × route of administration interaction ($P < 0.05$) where pigs provided CTC in-feed from d 0 to 14 had increased BW compared to pigs provided CTC in water, whereas pigs provided tiamulin in water had increased BW compared to pigs provided tiamulin in the feed. Pigs provided CTC in the feed and tiamulin in the water also had increased ($P < 0.05$) BW on d 28 when compared to the control

Discussion

Chlortetracycline is considered medically important because of its use in human medicine. Chlortetracycline in nursery diets improves ADG and G:F in nursery pigs (Feldpausch et al., 2018; Williams et al., 2018; Capps et al., 2020). In two studies, Feldpausch et al. (2018) observed that pigs fed sub-therapeutic or therapeutic dosages of CTC had increased ADG, ADFI, and BW during the treatment period. Helm et al. (2019) also observed an increase in ADG and ADFI when feeding subtherapeutic levels of CTC. In two other studies, Williams et al. (2018) and Capps et al. (2020) observed pigs fed CTC had improved ADG, ADFI, and G:F, which represents much of the other literature on CTC. Williams et al. (2018) and Feldpausch et al. (2018) observed minimal carryover effects on subsequent nursery pig growth performance. In our current study, pigs provided CTC in the feed during the treatment period tended to have improved growth performance in the post treatment period. Overall pigs provided CTC had improved ADG and ADFI compared to pigs fed diets without CTC. Our study agrees with other studies showing that providing CTC in the feed or water improves growth performance compared to other antibiotic treatments and pigs that were not provided antibiotics.

Tiamulin is not considered to be a medically important antibiotic for human medicine. Tiamulin is commonly used for the control and treatment of swine dysentery and ileitis. Tiamulin, like CTC, has been demonstrated to improve growth rate and feed efficiency in swine (Cromwell et al., 1985; Steidinger et al., 2009). Cromwell et al. (1985) evaluated the efficacy of tiamulin as a growth promoter and observed a 14.1% improvement in gain and 5.7% improvement in feed efficiency. In another study, Sotak et al. (2009) compared tiamulin, tiamulin/CTC, and tilmicosin phosphate on nursery pig growth performance. No differences were observed between pigs fed a combination of tiamulin and CTC or tiamulin alone but both antibiotics improved performance relative to pigs fed control diets (Sotak et al., 2009). Therefore, when using tiamulin it could be expected to see improvements in growth when included in nursery swine diets. In the current study, pigs provided tiamulin in the feed had poorer feed efficiency and similar ADG compared with control pigs during the experimental period. This differs from historical literature demonstrating that antibiotics improve ADG, ADFI, and feed efficiency (NCR -89, 1984; Cromwell, 2002; Sotak et al., 2009). We also observed in the current study that pigs provided tiamulin in the water (provided to achieve 23 mg/kg BW) had improved growth performance compared to pigs provided tiamulin in the feed (provided to achieve 5 mg/kg BW). This improvement was observed during the treatment period and the overall period for the experiment. The differences between in feed and in water tiamulin could be attributed to dosage level. Tiamulin dosages in-feed and or in-water were administered as per the FDA guidelines for either treatment or prevention of disease (U.S. Food and Drug Administration, 2020). Tiamulin provided via water to achieve 23 mg/kg BW is more bioavailable in the swine gut compared to tiamulin in the feed provided to achieve 5 mg/kg BW (Riond et al., 1993; Peeters et al., 2016). The lack of response to tiamulin in the feed in our study was different than initially anticipated. In other studies, tiamulin has improved growth performance when provided at low concentrations (ranging from 2.75 – 22 mg/kg) in the feed (Cromwell et al., 1985); however, others (Sotak et al. 2009; Steidinger et al., 2009) have fed higher concentrations (35 g/ton of feed) of tiamulin than used in our study and observed improved ADG and G:F.

Tiamulin is commonly used in combination with CTC in the swine industry (Puls et al., 2019). Steidinger et al. (2009) observed an increase in ADG, ADFI, and G:F when tiamulin and

CTC were fed in combination. Several researchers have recently observed an increase in ADG, ADFI, and improved feed efficiency when providing the combination of CTC and tiamulin (Oliver et al., 2014; Kiarie et al., 2018; Puls et al., 2019). In the current study, we observed that providing CTC in the feed with or without tiamulin and tiamulin in the water improved nursery pig growth performance compared with control pigs. Providing CTC and tiamulin in combination had little effect on subsequent performance but overall pigs provided the combination of CTC and tiamulin had increased ADG, ADFI, and a trend for increased BW on d 28 compared to control pigs. Likewise, Puls et al. (2019) observed pigs fed the combination of CTC and tiamulin resulted in increased overall growth performance compared to pigs fed no antibiotics. Our study agrees that the combination of CTC and tiamulin improves growth performance when compared to pigs that are not offered antibiotics. Most previous experiments compared the combination of CTC and tiamulin to a control, but not to diets containing only tiamulin.

After weaning, pigs can experience many instances of stress in their transition from the farrowing house to a nursery including transportation and environmental stress leading to negative overall affects for growth performance (Mishra et al., 2014; Liu et al., 2018; de Souza et al., 2019). By targeting sensitive populations of bacteria and relieving weaning-associated stress, antibiotics have the potential to improve growth performance in weaned pigs (Verstegen and Williams, 2002). When antibiotics are not included in the diet, pigs can become subject to intestinal stress from pathogenic bacteria (*Escherichia coli*, *Salmonella* spp., and *Brachyspira hyodysenteriae*) (Gaskins et al., 2002). The effect of these antimicrobials on growth performance may be attributed to their bioavailability within the swine gut. Chlortetracycline forms insoluble compounds with aluminum, calcium, iron, and magnesium and binds with lipids and proteins in the gut reducing its absorption (Neuvonen, 1976; Agwuh and MacGowan, 2006). Research has shown that the bioavailability of orally administered CTC in pigs was only 6% (Nielsen and Gryd-Hansen, 1996; Peeters et al., 2016). The low bioavailability indicates that most of the antibiotic remains in the gastrointestinal tract. In contrast, tiamulin bioavailability is up to 90% when administered orally (Riond et al., 1993). Because oral bioavailability has an inverse relationship with intestinal concentration of the antibiotic (Peeters et al., 2016), tiamulin was expected to be available at minimal concentration (<10%) in the gut. Similar to CTC, increasing the amount of tiamulin increases the ultimate concentration in the gut contents (Riond et al., 1993). The tiamulin concentration was greater for the water delivery route than in feed, which should have increased tiamulin in gut contents and after absorption. Thus, a higher inclusion rate of tiamulin through the feed may have provided more benefit in growth performance.

In the swine industry, antibiotics have been widely studied for over 50 years with their growth promotional or subtherapeutic use and effectiveness summarized by Hays (1978), Zimmerman (1986), and Dritz et al. (2002). With improvements in biosecurity, increase in swine herd health, and multi-site production farms, the impact of antibiotics as health management practices and for growth promotion may be lower than in earlier studies (Dritz et al., 2002; Jacela et al., 2009). Antibiotics provided in the water in our study had similar or better responses in some cases with respect to growth performance as in-feed antibiotic treatments. Antibiotics administered through the water could be an effective and convenient practice when administering antibiotics due to their easy initiation and then removal after treatment, similar impact for growth promotion, and decreased chance of AMR (Wu et al., 2019). It is widely accepted that water supply and consumption can significantly affect performance (Brooks and Carpenter, 1990). Immediately after weaning, feed intake is low, but water intake is high (McLeese et al., 1992;

Maenz et al., 1993). Rather than using in-feed antibiotics, water application of antibiotics may prove to be more beneficial for nursery pigs in early stages of growth when pigs are observed to have to low feed intake (Gaskins et al., 2002), but still consume water. Water application also allows more timely administration of antibiotics because they can be added or removed more easily without waiting for a diet change.

In conclusion, our study illustrates the value of orally administered antimicrobials on nursery pig growth performance with minimal effect on subsequent performance from the dietary treatments. The therapeutic levels of either CTC alone or in combination with tiamulin can be used to improve growth performance. This agrees with other literature where CTC in nursery diets improves average daily gain and gain:feed ratio in nursery pigs (Feldpausch et al., 2018; Williams et al., 2018; Capps et al., 2020). Our study also shows that CTC or tiamulin in the water can be as effective as traditional in-feed antibiotics and can also improve growth performance in nursery pigs. The higher dosage of tiamulin achieved through the water provided more growth response than the lower dosage achieved through the feed in this experiment. The water administration of these antimicrobials may be a useful and a more practical strategy due to their ease of use, effectiveness, and flexibility.

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Table 1.1. Composition of experimental diets (as-fed basis)[1]

Ingredient, %	Control (d 0 to 14)[2]	Common diet (d 14 to 28)[3]
Corn	50.60	47.90
Soybean meal	20.95	28.85
Distillers dried grains with solubles	10.00	20.00
Fish meal	5.00	---
Dried whey	10.00	---
Monocalcium P	0.45	0.37
Limestone	1.00	1.4
Sodium chloride	0.50	0.50
L-Lysine-HCl	0.48	0.45
DL-Methionine	0.19	0.12
L-Threonine	0.20	0.14
L-Tryptophan	0.08	0.04
L-Valine	0.13	0.01
Phytase[4]	0.05	0.05
Zinc oxide	0.25	---
Vitamin and mineral premix[5]	0.15	0.15
Chlortetracycline	±	---
Tiamulin	±	---
Total	100	100
Calculated analysis		
Lysine	1.35	1.30
Isoleucine:lysine	57	63
Leucine:lysine	123	143
Methionine:lysine	39	35
Methionine & cystine:lysine	60	60
Threonine:lysine	65	65
Tryptophan:lysine	21	21
Valine:lysine	72	72
Total Lysine, %	1.52	1.50
ME, kcal/kg	3,310	3,279
NE, kcal/kg	2,452	2,381
SID Lys:NE, g/Mcal	5.51	5.46
CP, %	22.2	24.0
Ca, %	0.81	0.70
P, %	0.67	0.56
Available P, %	0.55	0.40
Na, %	0.36	0.28
Cl, %	0.59	0.42

[1] Experimental diets were fed from d 0 to 14 after a 7-day pretrial period.

[2] Antibiotics replaced corn in the control diet to provide chlortetracycline (CTC) at 22 mg/kg body weight and Tiamulin at 5 mg/kg BW or the combination of CTC and tiamulin. Pigs that received antibiotics in the water were fed the control diet.

[3] Common diet fed from day 14 to 28 after treatment

[4] Optiphos 2000, (Huvepharma Inc., Peachtree City, GA) provided 103 phytase units (FTU)/kg of diet, for an estimated release of 0.14% available P.

[5] Each kg of premix contained 66,700 mg Fe from ferrous sulfate, 73,300 mg Zn from zinc oxide, 26,700 mg Mn from manganous oxide, 10,000 mg Cu from copper sulfate, 500 mg I from calcium iodate, 200 mg Se, 5,344,484 IU vitamin A, 100,210 IU vitamin E, 21 mg vitamin B12, 4,007 mg riboflavin, 15,366 mg pantothenic acid, 29,061 mg niacin, 668 mg folic acid, 1,201 mg vitamin B6, 67 mg biotin, 1,336,122 IU vitamin D3, and 1,671 mg vitamin K.

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Table 1.2. Evaluating the route of antibiotic administration and its effect on nursery pig growth performance[1]

		Chlortetracycline[3]		Tiamulin[4]						
Item[2]	Control	In-feed	In-water	In-feed	In-water	Chlortetracycline and Tiamulin	SEM	Antibiotic × route	Antibiotic	Route
d 0 to 14 (treatment period)										
ADG, g	457	500[x]	476[x]	463	479[x]	494[x]	9.1	**0.002**	**0.010**	0.529
ADFI, g	620	675[x]	643[y]	650[x]	641[y]	663[x]	12.6	0.162	0.117	**0.014**
G:F	737	741	741	713[x]	747	745	9.1	**0.001**	**0.025**	**0.001**
d 14 to 28 (post treatment period)										
ADG, g	583	605[y]	594	591	608[x]	602	11.1	**0.071**	0.980	0.669
ADFI, g	908	947[y]	935	931	942[y]	960[x]	22.9	0.375	0.706	0.986
G:F	643	640	637	636	647	630	10.9	0.210	0.657	0.478
Overall (d 0 to 28)										
ADG, g	521	544[x]	536[y]	529	545[x]	549[x]	8.9	**0.004**	0.149	0.899
ADFI, g	766	814[x]	792[y]	794[y]	794[x]	815[x]	17.0	0.231	0.344	0.263
G:F	680	681	678	667[x]	687	675	8.6	**0.004**	0.423	**0.035**
BW, kg										
d -7 (Pre-trial)	6.60	6.60	6.52	6.57	6.57	6.59	0.158	0.642	0.861	0.614
d 0	8.86	8.89	8.80	8.88	8.89	8.81	0.203	0.611	0.646	0.686
d 14	15.35	15.92[x]	15.53	15.43	15.69	15.74	0.292	**0.047**	0.297	0.687
d 28	23.95	24.81[x]	24.27	24.11	24.65[x]	24.60[y]	0.458	**0.020**	0.476	0.991

[1] A total of 2,592 pigs (initially 6.60 kg, BW) were used in a 28-d growth trial with 27 pigs/pen and 12 replicates/treatment. Treatment diets were fed from d 0 to 14. After the experimental period pigs were fed a common diet from d 14 to 28.

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

[3] Chlortetracycline (CTC) provided via feed or water to achieve 22 mg/kg body weight.

[4] Tiamulin in feed (5mg/kg BW) or water (23 mg/kg BW).

[x]Indicates this treatment had performance different from the control in a pairwise comparison ($P < 0.05$).

[y]Indicates this treatment had performance different from the control in a pairwise comparison ($P < 0.10$).

Chapter 2 - The effects of pharmacological levels of zinc, diet acidification and dietary crude protein on growth performance on nursery pigs

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Abstract

This experiment was conducted to evaluate potential replacements for pharmacological levels of Zn (provided by Zn oxide), such as diet acidification (sodium diformate), and low dietary crude protein (CP: 21 vs 18%) on nursery pig performance and fecal dry matter. A total of 360 weaned pigs (Line 200 × 400, DNA, Columbus, NE; initially 5.90 kg) were used in a 42-d growth study. Pigs were weaned at approximately 21-d of age and randomly assigned to pens (5 pigs per pen). Pens were then allotted to 1 of 8 dietary treatments with 9 pens per treatment. Experimental diets were fed in two phases: Phase 1 from weaning to d 7 and phase 2 from d 7 to

21; with all pigs fed the same common diet from d 21 to 42. The eight treatment diets were arranged as a 2 × 2 × 2 factorial with main effects of Zn (110 mg/kg from d 0 to 21 or 3,000 mg/kg from d 0 to 7, and 2,000 mg/kg from d 7 to 21), diet acidification, (without or with 1.2% sodium diformate), and dietary CP (21 or 18%, 1.40 and 1.35% in Phase 1 and 2 vs 1.20% standardized ileal digestible Lys, respectively). Fecal samples were collected weekly to determine dry matter (**DM**) content. No 2- or 3-way interactions ($P > 0.05$) were observed throughout the 42-d study for growth performance; however, there was a Zn × acidifier × CP interaction ($P < 0.05$) for fecal DM on d 7 and for the mean of the 6 collections. Reducing CP without acidification or pharmacological levels of Zn increased fecal DM, but CP had little effect when ZnO was present in the diet. From d 0 to 21, significant ($P < 0.05$) main effects were observed where average daily gain (**ADG**) and gain:feed (**G:F**) increased for pigs fed pharmacological levels of Zn, sodium diformate, or 21% CP ($P < 0.065$). In the subsequent period (d 21 to 42) after the experimental diets were fed, there was no evidence of difference in growth performance among treatments. Overall (d 0 to 42), main effect differences were observed ($P < 0.066$) for pigs fed added Zn or sodium diformate from d 0 to 21 whereas pigs fed 21% CP had greater G:F than those fed 18% CP. Pig weight on d 42 was increased by adding Zn (P < 0.05) or acidifier (P < 0.06) but not CP. In summary, none of the feed additives had a major influence on fecal DM, but dietary addition of pharmacological levels of Zn or sodium diformate independently improved nursery pig performance.

Keywords: Crude protein, diet acidification, nursery pigs, Zn

Abbreviations:

ADFI, average daily feed intake

ADG, average daily gain

CP, crude protein

DM, dry matter

G:F, gain to feed ratio

SID, standardized ileal digestible

Introduction

Weanling pigs are subjected to abrupt changes in diet, environment, and social re-organization stressors. During this time, the GIT goes through changes in physiology, microbiology, and immunology (Heo et al., 2012). As a result, the post-weaning period has a high incidence of diarrhea associated with the proliferation of *Escherichia coli* (Pluske et al., 1997; Heo et al., 2012).

Zinc is an essential trace mineral and when fed at pharmacological doses from ZnO (2,000 to 3,000 mg/kg), improves growth performance and reduces post-weaning diarrhea (Molist et al., 2011; Ren et al., 2020; Wei et al., 2020). However, there is growing public concern that high levels of Zn may pose potential environmental problems when Zn is excreted as waste and is then spread on farm ground (Poulsen, 1998). As a result, the European Union has banned or restricted the use of pharmacological levels Zn in diets for weanling pigs (European Food Safety Authority (EFSA) panel on Additives Products or Substances used in Animal Feed (FEEDAP), 2012; Postma et al., 2015). Because of Zn's positive effects on growth performance and decreased incidence of post-weaning diarrhea, alternative nutritional strategies offering similar benefits as Zn are of high interest to the swine industry.

Among alternative nutritional strategies, acidifiers (organic and inorganic acids) have gained great interest in the swine industry (Kil et al., 2011; Suiryanrayna and Ramana, 2015).

Dietary acidifiers have the potential to reduce the pH of the GIT which improves nutrient digestion in weanling pigs and protects against the invasion and proliferation of pathogenic bacteria (Kil et al., 2011; Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020). A low gastric pH promotes enzyme activity for the digestion and absorption of CP and other nutrients (Canibe et al., 2001; Poeikhampha et al., 2011; Lampromsuk et al., 2012). Htoo and Morales (2012) observed that adding potassium diformate or potassium formate in weanling pig diets increased ADG and G:F. However, results with dietary acidification have been varied, which has been attributed to the type of acid, inclusion rate, and diet formulation (Mroz et al., 2002).

Another option to reduce post weaning diarrhea is lowering CP content of the diet (Heo et al., 2012; Wang et al., 2018). Low CP diets reduce the amount of excess nitrogen in the GIT limiting bacterial fermentation which increases fecal DM (Heo et al., 2012). Low CP, amino acid fortified diets are widely used in Europe as a means of decreasing post weaning diarrhea and reduce nitrogen excretion into the environment. Diet acidification and reduced CP diets may prove to be effective formulation options for nursery pigs when pharmacological levels of Zn cannot be used in the diet. Our hypothesis was that these alternatives to pharmacological levels of Zn work in combination, or independently of each other, offering similar benefits in health and growth performance to that of add Zn. Therefore, the objective of this study was to determine the interactive effects of added ZnO, diet acidification, and CP level on growth performance and fecal dry matter in weanling pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the Kansas State University Segregated Early Weaning Facility (SEW) located in Manhattan, KS. The SEW facility features two identical barns that are completely enclosed, mechanically ventilated, and environmentally regulated. Each pen (1.22 × 1.22 m) has metal tri-bar floors and allowed approximately 0.30 m^2/pig. Each pen contains a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Initial room temperature is set at 30 degrees C and reduced 1 C each week after weaning.

Animals

A total of 360 weanling pigs (Line 200 × 400, DNA, Columbus, NE; initially 5.90 kg), averaging 21 days of age were used in a 42-d growth study. Following the arrival to the research facility, pigs were weighed and assigned to pens. Pens of pigs were then randomly allotted to 1 of 8 treatments in a completely randomized design with 9 replicate pens per treatment, and 5 pigs per pen. The eight treatment diets were arranged as a 2 × 2 × 2 factorial with main effects of added Zn provided by ZnO (3,000 mg/kg from d 0 to 7 and 2,000 mg/kg from d 7 to 21 or 110 mg/kg from d 0 to 21), diet acidification (without or with 1.2% sodium diformate, [Formi-NDF, Addcon, Bitterfeld-Wolfen, Germany]), and dietary crude protein (21 or 18% CP corresponding to 1.40 or 1.20% SID Lys for phase 1 and 1.35 or 1.20% SID Lys in phase 2, respectively). The SID Lys was reduced in the 18% CP diets to maintain a maximum Lys:CP ratio of 7.3. Diets also contained 4% wheat bran in phases 1 and 2 and were balanced to similar Na concentrations by adjusting the addition of salt when sodium diformate was added to the diet. The diet fed from d 0 to 7 was pelleted and the following diets were fed as meal. Experimental diets were fed from d 0 to 21 with a common diet (19.9% CP with no added Zn or acidifier) fed from d 21 to 42 (Tables 2.1., 2.2., and 2.3.).

Diet Preparation

Nursery diets were made at the O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Samples of each diet were collected at the time of manufacturing. Composite samples were kept refrigerated at the KSU Swine Lab until analysis. A representative sample of each diet was collected from the feeders of each treatment, homogenized, ground, and complete diet samples were sent for crude protein (method 990.03; AOAC International, 2019) and Zn analyses (Campbell and Plank, 1991) to the University of Missouri-Columbia Agricultural Experimental Station Chemical Laboratories (Table 2.4.). For the pelleted phase 1 diet, average conditioning temperature target was 51°C and the average hot pellet temperature was 72°C. Retention time was 30 s using a 0.48 × 3.18 cm die (L/D = 6.0) with a 707.60 kg/h production rate and approximately 23°C ambient temperature.

Data Collection

Pigs were weighed and feed disappearance recorded weekly for the 42-d study to determine ADG, ADFI, and G:F. Fecal samples were collected from the same three pigs from each pen on d 7, 14, 21, 28, 35 and 42 of the trial. The three fecal samples from the same pen at each collection time were combined after collection. To determine fecal DM percentage, samples were weighed, then dried in an oven at 41ºC for 48 hours, then weighed again to determine fecal DM.

Statistical Analysis

Experimental data were analyzed using R Studio (Version 3.5.2, R Core Team. Vienna, Austria) with pen serving as the experimental unit. Main effects and all 2- and 3-way interactions of Zn, sodium diformate, and CP were analyzed. Fecal dry matter was analyzed over time as repeated measures. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

The chemical analyses of the experimental diets were similar to those calculated from diet formulation in respect of Zn concentration and CP (Table 2.4.). There were no 2- or 3-way interactions ($P > 0.05$) observed throughout the 42-d study for ADG, ADFI, and G:F (Table 2.5.).

From d 0 to 7 there was no evidence of differences ($P > 0.05$) for ADG, ADFI, G:F, or d 7 BW by the addition of 3,000 mg/kg added Zn. However, from d 7 to 21 and 0 to 21, ADG, ADFI, G:F, and BW were increased ($P < 0.05$) for pigs fed pharmacological levels of Zn compared to those fed 110 mg/kg. For subsequent performance (d 21 to 42), there was no evidence of difference of previous Zn treatment on ADG, ADFI, or G:F. For the overall period, there was a tendency ($P = 0.061$) for improved overall ADG for pigs fed added ZnO from d 0 to 21, but no effect on ADFI or G:F. On d 42, pigs fed pharmacological levels of added Zn from d 0 to 21 had increased ($P < 0.05$) BW compared with those fed 110 mg/kg.

From d 0 to 7, pigs fed added sodium diformate had increased ($P < 0.05$) ADG and d 7 BW with no evidence of difference observed for ADFI or G:F. Pigs fed sodium diformate had improved ADG, and G:F from d 7 to 21 and d 0 to 21 and increased BW on d 21 ($P < 0.05$). From d 21 to 42 during the post-treatment period, there was no evidence of difference that previous sodium diformate treatment impacted ADG, ADFI, and G:F. For the overall period (d 0 to 42), a tendency ($P < 0.10$) was observed for pigs fed sodium diformate from d 0 to 21 to have increased ADG and d 42 BW with no evidence of difference observed for ADFI or G:F.

For the main effect of CP, from d 0 to 7 there was no evidence of difference ($P < 0.10$) in ADG, ADFI, or G:F among pigs fed either 21 or 18% CP. From d 7 to 21, there was no evidence of difference ($P > 0.10$) between pigs fed either 21 or 18% CP for ADG or ADFI; however, pigs fed 21% CP had increased ($P = 0.002$) G:F, and a trend ($P \leq 0.10$) for increased BW on d 21. From d 0 to 21, there was a trend observed where pigs fed 21% CP diets had increased ADG ($P = 0.065$) compared with pigs fed 18% CP diets. There was no evidence of difference between pigs fed 21 or 18% CP in ADFI, but pigs provided 21% CP had increased G:F ($P < 0.001$) compared with those fed 18% CP diets. For the subsequent performance (d 21 to 42), there was no evidence of difference ($P > 0.10$) in ADG, ADFI, G:F, or BW due to CP level previously fed from d 0 to 21. Overall, there was no evidence of difference in ADG, ADFI, or d 42 BW; however, pigs fed 21% CP from d 0 to 21 had improved ($P = 0.001$) G:F compared with those fed 18% CP.

For fecal DM, there was a ZnO × sodium diformate × CP interaction ($P < 0.05$) observed on d 7 and the overall average of the weekly samples. When pharmacological levels of Zn were in the diet, adding sodium diformate or reducing CP had little effect on fecal DM. However, in diets without added Zn, lowering CP without acidification increased fecal DM, but with acidification, low CP decreased fecal DM. The only other change in fecal DM was on d 42, where DM was lower for pigs previously fed low CP diets compared to pigs previously fed high CP.

Discussion

Post-weaning diarrhea (PWD) is a serious issue that affects the global swine industry. Most commonly, PWD is characterized by death loss, diarrhea, dehydration, and has negative effects on a young pig's growth performance that may inhibit growth throughout the pig's life

(Pluske, 2016; Rhouma et al., 2017). Furthermore, stress factors including removal from the sow, changes in diet, environment, and social organization stressors may negatively affect the immune system's response thus leading to further gut dysfunction (Heo et al., 2012; Pluske, 2016; Rhouma et al., 2017). However, feeding strategies can be put into place to mitigate or reduce the instances of PWD and the condition's negative effects on growth.

It is well accepted that pharmacological levels of Zn (usually 2,000 to 3,000 mg/kg of Zn) mitigate PWD, promote growth, and reduce the potential negative effects from weaning (Smith et al., 1997; Carlson et al., 1999; Molist et al., 2011). In a recent study from Wei et al. (2020), pigs were observed to have improved growth performance when supplemented diets with high Zn. In agreement, our study observed that ADG, ADFI, G:F, and BW were improved during the treatment feeding period. While there was no evidence of difference in subsequent performance after high Zn feeding stopped, high Zn had a tendency to improve overall ADG and increased BW on d 42 compared with pigs fed 110 mg/kg of Zn throughout. Likewise, in a study from Ren et al. (2020), Zn supplementation was only effective in the first 2 weeks postweaning.

Although much work has been done with pharmacological Zn to determine the mode(s) of action, the exact mechanisms by which Zn exerts its positive benefits are still unclear. The modes of action of Zn can be grouped into four categories for better understanding: a direct impact on feed intake; gut protection; antibacterial properties; and a high Zn requirement in weaned pigs. Yin et al. (2009) observed that dietary supplementation of Zn resulted in increased secretion of ghrelin by the stomach, which can directly stimulate feed intake and subsequently muscle growth. High levels of Zn have also shown to prevent pathogenic bacteria from attaching to the intestine (Wei et al., 2020). Feeding high Zn also reduces intestinal permeability due to an improvement in tight junctions (Peng et al., 2020). The improved tight junctions prevent translocation of pathogenic bacteria which could be responsible for reduced PWD when feeding high Zn (Huang et al., 1999). Providing pharmacological levels Zn has also shown evidence to reduce intestinal inflammation, improving growth performance due to a potential increase in nutrient digestibility by reducing microbiota populations in the GIT (Katouli et al., 1999; Højberg et al., 2005; Ou et al., 2007). Zinc is considered to be an essential trace mineral with the NRC (2012) having a recommended level for 5 to 11 kg pigs being 100 mg/kg of Zn. However, weaned pigs may have a higher Zn requirement than previously thought. Evidence has shown that weaned pigs have a drop in plasma Zn concentrations which can be counteracted by feeding high doses of dietary ZnO to achieve Zn homeostasis, restoring plasma Zn levels to that of unweaned pigs (Davin et al., 2013). The exact mechanism by which Zn improves growth and reduces PWD is still unknown, but it is likely the result of a combination of multiple modes of action that Zn has in the pig.

While it is widely accepted that providing high Zn improves growth, alternative feeding strategies are needed due to restrictions placed on feeding high Zn diets in the European Union and growing public concern for the environment (Poulsen, 1998; European Food Safety Authority (EFSA) panel on Additives Products or Substances used in Animal Feed (FEEDAP), 2012; Postma et al., 2015). As more regulatory restrictions are likely to come in other countries as well, alternative dietary strategies to minimize PWD and weaning stress are needed. Among the alterative feeding strategies, there has been promising research into diet acidification and lowering crude protein as alternatives to high Zn to diminish PWD effects and stress after weaning.

Acidifiers have gained particular interest to replace ZnO due to their potential to reduce the pH of the gastrointestinal tract (Kil et al., 2011; Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020). Lowering the pH of the stomach promotes pepsinogen conversion to pepsin which improves nutrient digestion in weanling pigs (Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020). At weaning, pigs have low acid secretion and an elevated stomach pH of 5.0 or above can be observed (Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020). Lowering the pH of the stomach also inhibits pathogenic bacteria (Suiryanrayna and Ramana, 2015). Organic acids are believed to reduce production of harmful bacteria, lowering the risk of infections and reducing microbial competition with the pig for nutrients (Suiryanrayna and Ramana, 2015).

Acidification of diets has elicited a wide variation in responses thought to be due to various factors including acid type, dose, and supplementation duration (Tugnoli et al., 2020). Øverland et al. (2008) supplemented 1.0% formic acid in the diet and observed an increase in ADG and improved G:F. In another study, the supplementation of sodium formate improved G:F during the nursery period (Graham et al., 2018). Likewise, in our study the supplementation of an acidifier, sodium diformate, increased ADG and G:F from d 0 to 21. Research and peer reviewed articles with sodium diformate are lacking, but a similar formic acid, potassium diformate, has been observed to improve growth performance in nursery (Paulicks et al., 2008; Poeikhampha et al., 2011; Htoo and Morales, 2012) and finishing (Overland et al., 2000) pigs. However, these growth responses are not always observed. Although a small study (36 nursery pigs), Canibe et al. (2001) supplemented 1.8% potassium diformate in the diet for nursery pigs and observed no effect on growth performance. The authors did observe potassium diformate to have an antimicrobial effect reducing counts of anaerobic bacteria, lactic acid bacteria, coliforms, and yeast when supplemented in the diet without reducing the pH along the GIT (Canibe et al., 2001). The authors attributed the antimicrobial effects of potassium diformate to the undissociated acid being able to penetrate the bacterial cell wall rather than lowing GIT pH.

Likewise, in a study from Mroz et al (2002), potassium diformate, when provided up to 1% in the diet for growing-finishing pigs, showed no evidence of difference in growth performance. However, our study agrees that acidifiers have the potential to improve growth performance similar to other studies in nursery pigs (Paulicks et al., 2008; Poeikhampha et al., 2011; Htoo and Morales, 2012). The lack of consistency in growth responses implies that more data should be gathered on acidifiers and their modes of action to fully understand their role in swine diets.

Due to low feed intake after weaning, pigs are offered high CP diets to maximize the potential for protein deposition (Gloaguen et al., 2014). As long as the pig's requirement for essential amino acids are met and enough nitrogen is present to support nonessential amino acid production, it has been shown that lower dietary CP can be provided as a means to reduce the proliferation of *E. coli* strains associated with PWD (Opapeju et al., 2009; Kim et al., 2011). When excess CP is fed, pigs can also suffer from reduced nitrogen utilization where an excess of amino acids are deaminated and excreted through the urine as urea (Jeaurond et al., 2008; Wang et al., 2018). The undigested protein enters the hind gut leading to diarrhea. By controlling the substrate availability to gastrointestinal microflora, the instances of PWD can be reduced (Wellock et al., 2006) without observing reductions in growth performance when decreasing CP (Hansen et al., 1993; Le Bellego and Noblet, 2002).

Reducing the dietary CP content by reducing amino acid concentrations can result in compromised growth performance (Nyachoti et al., 2006). In the current study, pigs were either provided 21 or 18% CP which corresponded to 1.40 or 1.20% SID Lys for phase 1 and 1.35 or 1.20% SID Lys in phase 2, respectively. Our results show that feeding a low CP diet with lower SID Lys can result in a reduction in growth performance compared to pigs fed high CP diets. Pigs fed high CP had increased BW on d 14 and tended to have increased BW on d 21 compared to pigs fed low CP diets. These results are in agreement with Wellock et al. (2006) and Jansman et al. (2016) which observed reduced growth when SID Lys was found to be below the requirement for optimal growth of pigs during the nursery phase. Nyachoti et al. (2006) fed pigs low CP diets (17% CP) and observed reduced overall ADG, ADFI and final BW. Nyachoti et al. (2006) concluded in their study that low CP diets could limit other amino acids, which could be a driving factor in reduced performance. In the present study, the resulting reduction in performance could be attributed to deficiencies in SID Lys which likely limited growth performance by limiting protein deposition. In order to keep the Lys:CP ratio the same across all treatments, Lys was decreased in low CP diets in the current study. Research conducted by Millet et al. (2018), suggest using a maximum Lys to digestible CP ratio when using low CP diets to maintain adequate amounts of nitrogen to enable sufficient synthesis of non-essential amino acids and to optimize the amino acid profile present in the diet. When low CP diets are provided, non-essential amino acids may be the limiting factor in growth performance (Gloaguen et al., 2014). Evidence suggest that low CP diets have an insufficient supply of non-essential amino acids that can regulate metabolic pathways and when in insufficient supply, can limit growth (Wu, 2014). Thus, when providing low CP diets perhaps one or more dispensable AA should be added to the diet for nursery pigs to maintain growth performance such as Wu (2014). Therefore, simply increasing SID Lys cannot overcome the growth insufficiencies from low CP diets without supplementation of non-essential amino acids (Wang et al., 2018).

Pigs provided low CP may have exhibited reductions in growth performance during the experimental period; however, the low CP diets did result in increased fecal DM on d 7 and the overall average DM of weekly samples. Feeding 18% CP diets increased fecal DM on d 7 and overall when pharmacological Zn and sodium diformate were not in the diet resulting in a Zn ×

acidifier × CP interaction. Protein fermentation in the large intestine can potentially produce toxic compounds such as ammonia and amines which can increase the occurrence of PWD (Heo et al., 2012). Improved fecal DM by feeding a low CP diet can be a result of decreased protein fermentation in the large intestine (Nyachoti et al., 2006; Wellock et al., 2006; Heo et al., 2012).

In conclusion, no interactions of added Zn, the addition of acidifier, or high or low CP were observed for growth measurements, but high Zn, the addition of an acidifier, and high crude protein all independently improved growth performance. Our objective was to test alternative feeding strategies offering similar benefits to pharmacological levels of Zn. Pigs fed diets containing sodium diformate or pharmacological levels of Zn had improved growth performance similar to that observed in other studies. However, reducing crude protein and subsequently reducing SID Lys reduced growth during the experimental period but improved fecal dry matter. The current study shows that the addition of an acidifier may prove to be a useful practice to improve nursery pig growth performance and warrants more research in to understanding acidifiers role in swine diets.

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Table 2.1. Phase 1 diet composition (as-fed basis)[1]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet			
	No acidifier		Added acidifier[2]		No acidifier		Added acidifier[2]	
Item	21% CP	18% CP	21% CP	18%CP	21% CP	18% CP	21% CP	18% CP
Ingredient %								
Corn	41.19	48.63	40.14	47.58	40.79	48.23	39.74	47.18
Soybean meal	17.75	10.25	17.75	10.25	17.75	10.25	17.75	10.25
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Fish meal	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP 300[3]	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Calcium carbonate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Monocalcium phosphate	0.30	0.40	0.30	0.40	0.30	0.40	0.30	0.40
Sodium chloride	0.30	0.30	0.15	0.15	0.30	0.30	0.15	0.15
L-Lysine-HCL	0.43	0.41	0.43	0.41	0.43	0.41	0.43	0.41
DL-Methionine	0.21	0.17	0.21	0.17	0.21	0.17	0.21	0.17
L-Threonine	0.20	0.19	0.20	0.19	0.20	0.19	0.20	0.19
L-Tryptophan	0.06	0.07	0.06	0.07	0.06	0.07	0.06	0.07
L-Valine	0.10	0.13	0.10	0.13	0.10	0.13	0.10	0.13
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Zinc oxide	---	---	---	---	0.40	0.40	0.40	0.40
Na diformate	---	---	1.20	1.20	---	---	1.20	1.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

continued

Table 2.1. Phase 1 diet composition (as-fed basis)[1]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet			
	No acidifier		Added acidifier[2]		No acidifier		Added acidifier[2]	
Item	21% CP	18% CP	21% CP	18%CP	21% CP	18% CP	21% CP	18% CP
Calculated analysis								
Standardized ileal digestible (SID) amino acids								
Lysine	1.40	1.20	1.40	1.20	1.40	1.20	1.40	1.20
Isoleucine:lysine	56	55	56	55	56	55	56	55
Leucine:lysine	108	112	108	111	108	112	108	111
Methionine:lysine	37	37	37	36	37	37	37	36
Methionine & cystine:lysine	58	58	58	58	58	58	58	58
Threonine:lysine	64	65	64	65	64	65	64	65
Tryptophan:lysine	20.1	21.2	20.1	21.2	20.1	21.2	20.1	21.2
Valine:lysine	67	70	67	70	67	70	67	70
Total Lys, %	1.54	1.32	1.54	1.32	1.54	1.32	1.53	1.32
ME[5], kcal/kg	3,398	3,402	3,363	3,367	3,385	3,389	3,349	3,351
NE, kcal/kg	2,553	2,595	2,527	2,567	2,542	2,584	2,516	2,555
SID Lys:NE, g/Mcal	5.48	4.62	5.54	4.67	5.5	4.64	5.56	4.69
Crude protein, %	21.1	18.1	21.0	18.0	21.1	18.1	21.0	18.0
Ca, %	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
P, %	0.67	0.66	0.67	0.66	0.67	0.66	0.67	0.66
STTD P, %	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Na, %	0.40	0.39	0.59	0.59	0.40	0.39	0.59	0.59
Cl, %	0.67	0.66	0.58	0.57	0.67	0.66	0.58	0.57
Zn, ppm	110	110	110	110	2,990	2,990	2,990	2,990

[1] Phase 1 diets were fed from d 0 to d 7

[2] Sodium diformate (Formi-NDF, Addcon, Bitterfeld-Wolfen, Germany) was included in the diet at 1.2% from d 0 to d 21.

[3] HP 300 (Hamlet Protein, Findlay, OH).

[4] HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided an estimated release of 0.12% STTD P.
[5] ME = metabolizable energy. NE = net energy.

Table 2.2. Phase 2 diet composition (as-fed basis)[1]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet			
	No acidifier		Added acidifier[2]		No acidifier		Added acidifier[2]	
Item	21% CP	18% CP	21% CP	18%CP	21% CP	18% CP	21% CP	18% CP
Ingredient, %								
Corn	53.41	60.54	52.40	59.54	53.16	60.30	52.16	59.29
Soybean meal	28.75	21.35	28.75	21.35	28.75	21.35	28.75	21.35
Milk, whey powder	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Calcium carbonate	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Calcium phosphate	0.80	0.90	0.80	0.90	0.80	0.90	0.80	0.90
Sodium chloride	0.55	0.55	0.35	0.35	0.55	0.55	0.35	0.35
L-Lysine-HCL	0.50	0.54	0.50	0.54	0.50	0.54	0.50	0.54
DL-Methionine	0.20	0.23	0.20	0.23	0.20	0.23	0.20	0.23
L-Threonine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
L-Tryptophan	0.04	0.07	0.04	0.07	0.04	0.07	0.04	0.07
L-Valine	0.10	0.17	0.10	0.17	0.10	0.17	0.10	0.17
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[3]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Zinc oxide	---	---	---	---	0.25	0.25	0.25	0.25
Na diformate	---	---	1.20	1.20	---	---	1.20	1.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

continued

Table 2.2. Phase 2 diet composition (as-fed basis)[1]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet			
	No acidifier		Added acidifier[2]		No acidifier		Added acidifier[2]	
Item	21% CP	18% CP	21% CP	18%CP	21% CP	18% CP	21% CP	18% CP
Calculated analysis								
Standardized ileal digestible (SID) amino acids								
Lysine	1.35	1.20	1.35	1.20	1.35	1.20	1.35	1.20
Isoleucine:lysine	55	52	55	52	55	52	55	52
Leucine:lysine	111	110	110	110	110	110	110	110
Methionine:lysine	35	39	35	39	35	39	35	39
Methionine & cystine:lysine	57	61	57	61	57	61	57	61
Thronine:lysine	65	65	65	65	65	65	65	65
Tryptophan:lysine	19.1	21	19.1	21	19.1	21	19.1	21
Valine:lysine	67	70	66	70	67	70	66	70
Total Lysine, %	1.49	1.32	1.48	1.32	1.49	1.32	1.48	1.31
ME[4], kcal/kg	3,243	3,252	3,210	3,217	3,235	3,243	3,201	3,208
NE, kcal/kg	2,401	2,445	2,375	2,419	2,395	2,429	2,368	2,412
SID Lys:NE, g/Mcal	5.63	4.91	5.68	4.96	5.64	4.92	5.7	4.97
Crude Protein, %	20.7	17.9	20.7	17.9	20.7	17.9	20.6	17.9
Ca, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
P, %	0.62	0.61	0.62	0.61	0.62	0.61	0.62	0.61
STTD P, %	0.51	0.51	0.51	0.51	0.51	0.51	0.50	0.50
Na, %	0.35	0.34	0.52	0.52	0.35	0.34	0.52	0.52
Cl, %	0.60	0.61	0.49	0.49	0.60	0.61	0.49	0.49
Zn, ppm	110	110	110	110	1,910	1,910	1,910	1,910

[1] Phase 1 diets were fed from d 7 to d 21

[2] Sodium diformate (Formi-NDF, Addcon, Bitterfeld-Wolfen, Germany) was included in the diet at 1.2% from d 0 to d 21.

[3] HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided an estimated release of 0.13% STTD P.
[4] ME = metabolizable energy. NE = net energy.

Table 2.3. Phase 3 common diet composition (as-fed basis)[1]

Item	Common diet
Ingredients, %	
Corn	65.47
Soybean Meal	28.30
Fat	2.00
Calcium carbonate	0.75
Monocalcium phosphate	1.10
Sodium chloride	0.60
L-Lysine-HCl	0.55
DL-Methionine	0.25
L-Threonine	0.23
L-Tryptophan	0.05
L-Valine	0.16
Vitamin premix	0.25
Trace mineral premix	0.15
Alltech All-Bind HD	0.15
Total	100.00
SID amino acids, %	
Lysine	1.30
Isoleucine:lysine	53
Leucine:lysine	111
Methionine:lysine	39
Metionine and cysteine:lysine	60
Threonine:lysine	63
Tryptophan:lysine	19.3
Valine:lysine	70
Histidine:lysine	35
ME[3], kcal/kg	3,318
NE, kcal/kg	2,534
Crude protein, %	19.9
Ca, %	0.65
STTD P[2], %	0.48

[1] Phase 3 common diets were fed from d 21 to 42.
[2] STTD P = standardized total tract digestible phosphorus.
[3] ME = metabolizable energy. NE = net energy.

Table 2.4. Analyzed diet composition (as-fed basis)[1,2]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet			
	No acidifier		Added acidifier[3]		No acidifier		Added acidifier[3]	
Analyzed composition %[4]	21% CP	18% CP	21% CP	18% CP	21% CP	18% CP	21% CP	18% CP
Phase 1								
Crude protein	20.52	17.48	20.52	17.81	20.92	18.20	20.49	17.76
Zn	181	154	191	101	2,450	2,820	2,650	2,620
Phase 2								
Crude protein	20.06	17.01	20.57	17.76	20.04	17.40	20.03	17.26
Zn	77.6	231	148	156	2,020	1,750	1,440	1,490

[1] Diets were fed in 3 phases from d 0 to 7, 7 to 21, and 21 to 42 for phases 1, 2, and 3, respectively.

[2] Zinc oxide was included in the diet at 3,000 ppm of Zn from d 0 to 7; 2,000 ppm of Zn from d 7 to 21; and no additional Zn other than that from the TM premix from d 21 to 42.

[3] Sodium diformate (Formi-NDF, Addcon, St. Peters, MN) was included in the diet at 1.2% from d 0 to d 21.

[4] Complete diet samples were taken at manufacture. Samples were stored at -20°C until they were homogenized, subsampled, and submitted to University of Missouri-Columbia Agricultural Experimental Station Chemical Laboratories for crude protein and Zn analyses.

Table 2.5. Evaluating the effects of pharmacological levels of Zn, diet acidification, and crude protein on growth performance of nursery pigs[1]

	110 mg/kg added Zn in the diet				3,000/2,000 mg/kg added Zn in the diet							
	No acidifier		Added acidifier[2]		No acidifier		Added acidifier[2]			Probability *P* <		
	21% CP[3]	18% CP	21% CP	18% CP	21% CP	18% CP	21% CP	18% CP				
Item[4]									SEM	Acidifier	Zn	Crude protein
Body weight, kg												
d 0	5.86	5.85	5.85	5.85	5.86	5.84	5.84	5.86	0.014	0.754	0.794	1.000
d 7	6.26	6.19	6.47	6.35	6.29	6.31	6.43	6.32	0.067	0.007	0.717	0.160
d 21	10.58	10.08	10.94	10.89	11.26	11.09	11.71	11.32	0.230	0.006	<0.001	0.094
d 42	23.23	22.55	23.96	24.11	24.23	23.94	24.80	23.84	0.519	0.066	0.048	0.228
d 0 to 7												
ADG, g	57	48	89	72	61	67	84	65	9.8	0.007	0.682	0.172
ADFI, g	85	83	93	96	79	94	92	83	6.8	0.257	0.629	0.771
G:F, g/kg	625	591	964	769	745	605	903	788	93.6	0.184	0.574	0.699
d 7 to 21												
ADG, g	308	270	319	324	356	342	377	357	14.8	0.017	<0.001	0.110
ADFI, g	454	444	467	492	512	529	508	513	21.7	0.506	0.002	0.542
G:F, g/kg	676	610	685	660	701	655	739	696	18.9	0.010	0.005	0.002
d 0 to 21, treatment period												
ADG, g	225	195	243	240	257	249	279	260	11.3	0.004	<0.001	0.065
ADFI, g	331	323	342	360	368	382	369	370	15.8	0.409	0.004	0.582
G:F, g/kg	676	607	709	669	706	652	754	702	17.4	<0.001	0.003	<0.001
d 21 to 42, common period												
ADG, g	603	594	620	629	618	612	617	596	11.3	0.495	0.949	0.621
ADFI, g	874	866	897	919	885	901	923	884	15.8	0.176	0.598	0.891
G:F, g/kg	690	686	691	685	697	680	667	675	17.4	0.108	0.124	0.365
d 0 to 42												
ADG, g	414	393	431	435	438	429	446	428	12.7	0.069	0.061	0.231
ADFI, g	603	592	620	639	627	640	642	627	18.2	0.197	0.113	0.897
G:F, g/kg	687	664	696	680	699	671	692	683	7.5	0.166	0.387	0.001

[1] A total of 360 weanling pigs were used in a 42-d growth study with 5 pigs per pen and 9 pens per treatment. No 2- or 3-way interactions ($P > 0.05$) were observed.

[2] Sodium diformate (Formi-NDF, Addcon, Bitterfeld-Wolfen, Germany) was included in the diet at 1.2% from d 0 to d 21.

[3] Dietary CP, 21 or 18% corresponded to 1.40 or 1.20% SID Lys from d 0 to 7 and 1.35 or 1.20% SID Lys from d 7 to 21.

[4] CP = Crude protein, BW = Body weight, ADG = Average daily gain, ADFI = Average daily feed intake, G:F = Gain-to-feed ratio

Table 2.6. Effects of pharmacological levels of zinc oxide, diet acidification and crude protein in nursery diets on fecal dry matter[1,2]

	110 mg/kg added Zn in the diet				3000/2000 mg/kg added Zn in the diet								
	No acidifier		Added acidifier[3]		No acidifier		Added acidifier[3]						
	21% CP[4]	18% CP	21% CP	18% CP	21% CP	18% CP	21% CP	18% CP		Probability $P <$			
Item[5]									SEM	Interaction[6]	ZnO[7]	Acid[8]	Crude Protein[9]
d 7	24.4	28.1	26.3	25.2	25.7	23.8	24.8	25.6	0.01	**0.011**	0.185	0.939	0.603
d 14	23.1	24.9	24.7	22.8	22.6	24.8	23.5	25.5	0.01	0.236	0.766	0.717	0.161
d 21	23.9	25.6	24.3	24.1	23.1	23.4	23.6	23.7	0.01	0.578	0.154	0.948	0.539
d 28	25.8	25.1	25.8	22.7	24.6	24.6	24.5	25.4	0.01	0.255	0.892	0.559	0.321
d 35	26.0	27.2	26.8	25.6	26.1	25.4	25.9	24.6	0.01	0.523	0.234	0.546	0.504
d 42	28.7	26.5	29.2	25.8	27.5	24.5	26.7	27.1	0.01	0.105	0.130	0.549	**0.006**
Overall[10]	25.3	26.2	26.2	24.4	24.9	24.4	24.8	25.3	0.006	**0.023**	0.113	0.912	0.556

[1] A total of 360 pigs were used in a 42-d growth study with 5 pigs per pen and 9 pens per treatment. To determine fecal DM percentage, samples were weighed, then dried in an oven at 41ºC for 48 hours, then weighed again to determine fecal DM.

[2] Zinc oxide was included in the diet to provide 3,000 ppm of Zn from d 0 to 7; 2,000 ppm of Zn from d 7 to 21; and no additional Zn other than that from the TM premix from d 21 to 42.

[3] Sodium diformate (Formi-NDF, Addcon, Bitterfeld-Wolfen, Germany) was included in the diet at 1.2% from d 0 to d 21.

[4] Dietary CP 21 or 18% CP corresponding to 1.40 or 1.20% SID Lys for phase one and 1.35 or 1.20% SID Lys in phase two respectively.

[5] d = day of collection

[6] Interaction ZnO × Acidifier × Crude Protein. All 2-way interactions were found to be nonsignificant ($P \geq 0.111$).

[7] Main effect of adding ZnO

[8] Main effect of adding an acidifier

[9] Main effect of high or low crude protein

[10] Represents the mean fecal dry matter across every week.

[ab] Means within row with different superscripts differ ($P < 0.05$)

Chapter 3 - Evaluation of a microencapsulated complex of organic acids and essential oils on growth performance of nursery and growing-finishing pigs

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Abstract

A total of 1,215 pigs (L337 × 1050, PIC, Hendersonville, TN) were used to determine the effect of a microencapsulated complex of organic acids and essential oils (MOE; AviPlus; Vetagro, Inc. Chicago, IL), on growth performance from weaning to market. Pigs were weaned at approximately 21 d of age and placed in pens based on initial body weight (BW) with 27 pigs per pen in a randomized complete block design. During the 42-day nursery period, pigs were allotted to 1 of 2 treatments in an unbalanced treatment structure with 15 pens (replications) fed the control diet and 30 pens (replications) fed diets containing 0.30% MOE from d 0 to 21 and 0.10% from d 21 to 42. On d 42, pigs were transported as intact pens from the nursery to the finishing facility. During the finishing period, 3 treatments were applied which included: 1) pigs on the control diet in nursery remained on control diets; 2) 50% of pigs provided MOE in nursery were then fed 0.05% MOE throughout finishing, and 3) 50% of pigs provided MOE in nursery were then fed the control diet throughout finishing. All pens of pigs on treatments 2 and 3 were allotted based on ending nursery BW to the finishing treatment. There were 15 replications per treatment in the finishing period. From d 0 to 21, pigs fed diets with MOE had a tendency for increased ($P < 0.058$) gain:feed (G:F) when compared to pigs fed the control diet; however, there was no evidence of difference ($P > 0.05$) for average daily gain (ADG), average daily feed intake (ADFI), or d 21 BW. From d 21 to 42, there was no evidence of difference ($P > 0.05$) for ADG, ADFI, or G:F. For the overall nursery period (d 0 to 42), pigs fed diets with MOE had increased ($P < 0.05$) G:F (660 vs 670 g/kg) when compared to pigs fed the control diet, but there was no evidence of difference ($P > 0.05$) for d 42 BW, ADG, or ADFI between treatments. From d 42 to 106, there was no evidence of difference ($P > 0.05$) for ADG, ADFI, and G:F. For the overall finishing period (d 42 to 156) and overall experimental period (d 0 to 156), there was no evidence of difference ($P > 0.05$) for BW, ADG, ADFI, or G:F. For mortality and removals, there was no evidence of difference ($P > 0.05$) observed during the nursery, finishing, or overall. In summary, providing MOE during the nursery phase increased G:F in the early and overall nursery phase, but there was no effect on overall wean-to-finish performance.

Keywords: Acidifier, finishing pigs, microencapsulation, nursery pigs,

Abbreviations:

ADFI, average daily feed intake

ADG, average daily gain,

BW, body weight

EO, essential oils

G:F, gain to feed ratio

GIT, gastrointestinal tract

Introduction

Acidifiers (organic and inorganic acids) have gained interest in the swine industry as options to improve pig growth performance (Kil et al., 2011; Suiryanrayna and Ramana, 2015). Acidifiers provided in swine diets have the potential to reduce the pH of the gastrointestinal tract (GIT) protecting the pig from pathogenic bacteria and improve nutrient digestion (Wang et al., 2009; Kil et al., 2011; Suiryanrayna and Ramana, 2015). Similar to acidifiers, essential oils (EO) have gained interest due to their broad spectrum of antimicrobial activity (Falcone et al., 2005). A combination product of microencapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals (AviPlus; Vetagro, Inc. Chicago, IL) is commercially available to swine producers. Essential oils extracted from edible plants such as thymol and vanillin have the potential to affect growth performance and nutrient retention by enhancing enzymatic activity and nutrient absorption (Burt, 2004; Windisch et al., 2008; Oh et al., 2018). The combination of acidifiers together with EOs via microencapsulation may offer synergistic effects on improving swine growth and feed efficiency (Canibe et al., 2005; Grilli et al., 2010; Cho et al., 2014). Grilli et al. (2010) observed that feeding a microencapsulated blend of organic acids and essential oils to nursery pigs improved ADG and ADFI which resulted in a higher BW at the end of their study. Likewise, Cho et al. (2014) observed improved ADG compared to control pigs and higher

BW throughout their experiment when diets for finishing pigs were supplemented with a microencapsulated blend of organic acids and essential oils. Therefore, the objective of our current study was to evaluate a microencapsulated complex of organic acids and essential oils (MOE) during the wean-to-finish period on pig growth performance.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This experiment was conducted at New Horizon Farms research nursery and finishing facilities located in Pipestone, Minnesota. In the nursery, each pen (3.65 × 2.44 m) had plastic slatted floors and was equipped with a six-hole stainless steel dry feeder and a pan waterer allowing ad libitum access to feed and water. Phase 1 diets were manufactured at Hubbard Feeds, Mankato MN, and all other diets were manufactured at the New Horizon Farms feed mill in Pipestone, MN. In the grow-finish phase of the study, each pen (5.49 × 2.74 m) was equipped with a 4-hole stainless steel dry self-feeder and a waterer cup for ad libitum access to feed and water. Feed additions for the nursery and finishing phases to each pen were delivered and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN). Pens of pigs were weighed, and feed delivery and disappearance were determined weekly during the nursery phase and approximately every 2 weeks during the finisher phase. Weights and feed measurements were used to determine growth performance (ADG, ADFI, and G:F). Under the circumstance where a pig died or needed to be removed from the study due to disease or injury, the weight of the pig was recorded, and the pig was classified as a mortality or removal.

A total of 1,215 pigs (L337 × 1050, PIC, Hendersonville, TN) were weaned at approximately 21 d of age and placed in pens based on initial BW. There were approximately 27 pig per pen during the 42-day nursery period. Pens of pigs were allotted to 1 of 2 dietary treatments in an unbalanced treatment structure with 15 pens (replications) fed the control diets and 30 pens fed diets containing 0.30% microencapsulated complex of organic acids and essential oils (MOE) (AviPlus; Vetagro, Inc. Chicago, IL) from d 0 to 21 and 0.10% from d 21 to 42 . Nursery diets were fed in 3 phases, with pharmacological levels of Zn in phase 1 and 2 (3,000 mg/kg added from ZnO in phase 1 and 2,000 mg/kg in phase 2). Diets were formulated to meet or exceed requirement estimates (NRC, 2012).

On d 42, pigs were transported as intact pens from the nursery to a finishing facility. During the finishing period, 3 treatments were applied which included: 1) pigs fed the control diets in nursery remained on control diets; 2) 50% of pigs in nursery fed MOE were then fed 0.05% MOE throughout finishing, and 3) 50% of pigs in nursery fed MOE were then fed control diets throughout finishing. All pens of pigs on finishing treatments 2 and 3 were allotted based on ending nursery BW to ensure nursery ADG had no influence on the finishing period. There were 15 replications per treatment in the finishing period.

Data were analyzed using R Studio (Version 4.0, R Core Team. Vienna, Austria) with pen serving as the experimental unit. The study was a randomized complete block design with weight block included in the model as a random effect. Pre-planned contrast statements were used to evaluate the treatment effects on ADG, ADFI, BW, and G:F. A binomial distribution was used to determine treatment effect on removals and mortality. Nursery data was analyzed as a 1-way treatment structure with 2 treatments. Grow-finish data was also analyzed as a 1-way treatment structure with 3 treatments. Statistical models were fitted using NLME package in R. Results were considered significant at $P < 0.05$ and marginally significant at $0.05 > P < 0.10$.

Results and Discussion

In the current study we evaluated the influence of a combination product of micro-encapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals on wean-to-finish growth performance, mortality and removals. From d 0 to 21, there was no evidence of difference for ADG, ADFI, or d 21 BW. However, a trend for increased ($P < 0.058$) G:F was observed for pigs fed diets with MOE compared with pigs fed the control diets. From d 21 to 42, there was no evidence of difference for ADG, ADFI, G:F, or d 42 BW. For the overall nursery period (d 0 to 42) pigs fed diets with MOE had increased ($P < 0.05$) G:F compared with pigs fed the control diet, but there was no evidence of difference for ADG or ADFI.

For the finishing period, from d 42 to 156, and the overall wean-to-finish period, there was no evidence of difference between treatments for ADG, ADFI, G:F or final BW. For mortality and removals, there was no evidence of difference observed for nursery mortality, removals or total nursery mortality and removals. Similarly, in finishing and overall, no evidence of difference was observed for mortality, removals, or total mortality and removals.

Our data contrasts with other studies using a microencapsulated blend of organic acids and essential oils in growing swine. Grilli et al. (2010) observed a trend for increased ADG and improved BW on d 41 for pigs fed the same microencapsulated blend of organic acids and essential oils. Our study was initiated by the responses to MOE observed by Oh (2018). In that study, two levels of added MOE 0.1% and 0.2% of the diet were evaluated for 22 weeks from weaning to market weight. Oh et al. (2018) observed increased BW, ADFI, ADG, and G:F in nursery pigs when supplementing the diet with 0.2% MOE. The authors also observed that the addition of MOE had a positive influence on increasing fecal Lactobacillus counts. Oh et al.

(2018) attributed this response to an acidifier's potential to decrease the pH of the GIT thus promoting increased nutrient digestion and retention.

Organic acids, including sorbic and citric acid, all have demonstrated evidence as potential growth promoters in nursery pigs. Nursery pigs undergoing stress at weaning have low acid secretion and elevated stomach pH levels of 5.0 and over can be observed (Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020). By lowering the pH of the stomach, acidifiers improve nutrient digestion by promoting pepsinogen conversion to pepsin which occurs rapidly at a pH of 2.0 (Suiryanrayna and Ramana, 2015). Acidifiers also have the potential to have an antimicrobial effect where the undissociated acid penetrates the bacterial cell wall reducing competition for nutrients in the swine GIT (Canibe et al., 2001). In our study, we observed a 1.5% improvement in G:F in pigs fed MOE, but this response was not carried through to the finishing phase or overall.

Pure botanicals, which are also commonly referred to as volatile or ethereal oils, acquired from plants have the potential to improve nutrient and energy utilization (Wenk, 2003; Simonson 2004). Growth performance and nutrient retention can both be improved by botanicals by enhancing enzyme activity related to nutrient absorption (Burt, 2004; Windisch et al., 2008). Commonly, essential oils like thymol and vanillin are used due to their broad spectrum of antimicrobial activity (Falcone et al., 2005; Huang et al., 2010; Gallage et al., 2014). Huang et al. (2010) observed that the inclusion of blended essential oils improved early nursery pig ADG, but the authors observed no other improvements in growth performance. Likewise, in an earlier study from Hong et al. (2004), the authors concluded that plant extracts could be included in the diet of weaned pigs without negatively affecting growth performance. However, in a study from Feldpausch et al. (2018), the authors observed that supplementing Origanum EO failed to affect daily gain, feed intake and resulted in poorer G:F. Organic acids and EOs can be provided together using microencapsulated technology to allow delayed absorption of the acidifiers and EOs without affecting the bioavailability of the protected compound (Piva et al., 1997; Piva et al., 2007). In a study by Cho et al. (2014), they observed that the microencapsulated blend of organic acids and EOs improved growth performance and nutrient digestibility. However, Gerritsen et al. (2010) observed no growth performance differences from supplementing a blend of organic acids.

In conclusion, the addition of a MOE in our study improved G:F in the nursery period but there was no overall effect on wean-to-finish growth performance or mortality and removals.

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Table 3.1. Composition of nursery and grow-finish diets (as-fed basis)[1]

	Nursery diet phases[2]			Finishing diet phases[3]			
Item	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %							
Corn	42.20	47.05	48.35	52.70	59.95	66.15	69.50
Soybean meal	15.05	24.95	27.30	23.95	16.60	10.50	7.20
Whey permeate	17.50	9.00	---	---	---	---	---
Enzymatically treated soybean meal[4]	6.65	---	---	---	---	---	---
Distillers dried grains with solubles	5.00	10.00	20.00	20.00	20.00	20.00	20.00
Fish meal	5.00	5.00	---	---	---	---	---
Beef tallow	---	1.00	1.00	1.00	1.00	1.00	1.00
Corn oil	3.00	---	---	---	---	---	---
Spray-dried blood plasma	2.50	---	---	---	---	---	---
Limestone	0.73	0.80	1.25	1.20	1.30	1.25	1.25
Monocalcium P	0.45	0.30	0.50	---	---	---	---
Sodium chloride	0.30	0.50	0.55	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.40	0.48	0.50	0.45	0.45	0.45	0.40
DL-Methionine	0.20	0.19	0.10	0.04	0.03	---	---
L-Threonine	0.20	0.19	0.13	0.09	0.08	0.08	0.08
L-Tryptophan	0.02	0.03	0.02	0.02	0.03	0.04	0.03
L-Valine	0.10	0.10	0.05	---	---	---	---
Zinc oxide	0.42	0.27	---	---	---	---	---
Phytase[5,6]	0.02	0.05	0.05	0.04	0.04	0.04	0.04
Vitamin premix[7]	0.05	---	---	---	---	---	---
Trace mineral premix[8]	0.13	---	---	---	---	---	---
Selenium premix	0.05	---	---	---	---	---	---
Nursery vitamin and trace mineral premix[9]	---	0.15	0.15	---	---	---	---
Grow-Finish vitamin and trace mineral premix[10]	---	---	---	0.15	0.15	0.15	0.15
Tri-basic copper chloride	---	---	0.02	---	---	---	---

MOE[11]	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
Total	100	100	100	100	100	100	100
Calculated analysis							
Standardized ileal digestible (SID) amino acids							
Lysine	1.40	1.38	1.30	1.18	1.00	0.85	0.73
Isoleucine:lysine	55	57	61	62	61	60	63
Leucine:lysine	115	121	140	148	157	168	185
Methionine:lysine	37	38	33	30	31	30	33
Methionine & cystine:lysine	58	58	57	55	57	58	64
Threonine:lysine	64	63	62	61	61	62	66
Tryptophan:lysine	18.2	18.3	18.3	18.6	18.9	18.8	18.7
Valine:lysine	71	70	72	71	72	73	77
Total lysine, %	1.57	1.56	1.48	1.35	1.15	0.99	0.86
Metabolizable energy, kcal/kg	3,532	3,358	3,287	3,314	3,318	3,327	3,329
Net energy, kcal/kg	2,665	2,500	2,436	2,476	2,516	2,553	2,571
SID Lys:net energy, g/Mcal	5.25	5.52	5.34	4.77	3.98	3.33	2.84
CP, %	22.2	22.8	23.3	21.90	19.00	16.60	15.20
Ca, %	0.70	0.70	0.68	0.54	0.55	0.51	0.50
P, %	0.65	0.63	0.60	0.48	0.45	0.42	0.41
STTD P, %	0.56	0.51	0.47	0.36	0.34	0.33	0.32
Na, %	0.42	0.36	0.29	0.21	0.21	0.20	0.20
Cl, %	0.74	0.67	0.50	0.37	0.37	0.37	0.36

[1] Nursery diets were provided in 3 phases from d 0 to 42. Finishing diets were provided in 4 phases from d 42 to the end of the trial.

[2] Phase 1 nursery diets were provided as a 1.81 kg/pig feed budget. Phase 2 nursery diets were provided after phase 1 till d 21. Phase 3 diets were provided from d 21 to 42.

[3] Diets were provided in 4 phases: Phase 1 diet was fed from 23 to 39 kg, Phase 2 diet was fed from 39 to 64 kg, Phase 3 diet was fed from 64 to 91 kg, and Phase 4 diet was fed from 91 kg to the end of the trial.

[4] HP 300 (Hamlet Protein, Findlay, OH).

[5] Quantum Blue 5G (AB Vista, Plantation, FL) provided 1,959 FTU/kg was used in phase 1 diets.

[6] Optiphos 2000, (Huvepharma Inc., Peachtree City, GA) provided 227 phytase units (FTU)/lb of diet, for an estimated release of 0.14% available P.

[7] Provided per kg of premix: 11,100,196 IU vitamin A; 2,000,360 IU vitamin D; 59,996 IU vitamin E; 6000 mg vitamin K; 50 mg vitamin B12; 8,001 mg riboflavin; 41,000 mg pantothenic acid; 45,002 mg niacin; 1,200 mg folic acid; and 200 mg biotin.

[8] Provided per kg of premix: 160,090 mg Zn from zinc sulfate; 134,000 mg Fe from ferrous sulfate; 40,000 mg Mn from manganese oxide; 13,340 mg Cu from copper sulfate; and 666 mg I from calcium iodate.

[9] Each kg of premix contained 66,700 mg Fe from ferrous sulfate, 73,300 mg Zn from zinc oxide, 26,700 mg Mn from manganous oxide, 10,000 mg Cu from copper sulfate, 500 mg I from calcium iodate, 200 mg Se, 5,344,484 IU vitamin A, 100,210 IU vitamin E, 21 mg vitamin B12, 4,007 mg riboflavin, 15,366 mg pantothenic acid, 29,061 mg niacin, 668 mg folic acid, 1,201 mg vitamin B6, 67 mg biotin, 1,336,122 IU vitamin D3, and 1,671 mg vitamin K

[10] Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; 0.20 g Se from sodium selenite; and 500,000 FTU phytase from OptiPhos® 2000 (Huvepharma Inc., Peachtree City, GA).

[11] A microencapsulated complex of organic acids and essential oils (AviPlus; Vetagro, Inc. Chicago, IL) was provided at 0.30% of the diet from d 0 to 21 and 0.10% of the diet from d 21 to 42. For the finishing period, AviPlus was included at 0.05% of the diet from d 42 to the end of the experiment.

[12] Standard total tract digestible phosphorous

Table 3.2. Evaluation of a microencapsulated complex of organic acids and essential oils (MOE) on growth performance of nursery and growing-finishing pigs[1]

	Nursery Performance		SEM	*P*-value
Item[2]	Control	Added MOE[3]		
d 0 to 42				
ADG, g	383	385	5.37	0.867
ADFI, g	580	573	8.37	0.544
GF, g/kg	660	670	3.00	0.023

	Grow-finish performance				
		Added MOE[3]			
Item[2]	Control	Nursery and finisher[4]	Only in nursery[5]	SEM	*P* -value
d 42 to 156					
d 42 BW, kg	23.0	23.0	23.1	0.329	0.958
ADG, g	919	908	922	5.40	0.187
ADFI, g	2,344	2,341	2,353	22.00	0.917
GF, g/kg	392	388	392	17.00	0.463
d 0 to 156					
d 156 BW, kg	127.6	126.3	128.1	0.735	0.236
ADG, g	756	747	757	4.05	0.125
ADFI, g	1,809	1,797	1,807	17.26	0.858
GF, g/kg	418	416	419	2.00	0.593
Mortality, %					
Nursery	0.5	1.0	0.7	0.50	0.710
Finishing	0.7	0.2	0.7	0.40	0.515

Total nursery and finishing	1.1	1.1	1.3	0.60	0.939
Removals, %[6]					
Nursery	10.0	8.6	8.8	1.60	0.739
Finishing	5.4	7.9	6.7	1.30	0.369
Total nursery and finishing	15.6	16.5	15.6	1.80	0.907
Mortality and removals, %					
Nursery	10.6	9.6	9.6	1.30	0.865
Finishing	6.2	8.1	7.4	1.40	0.544
Total nursery and finishing	16.8	17.7	17.0	1.90	0.928

[1] A total of 1,215 pigs (initial BW of 5 kg) were used in a 156-d growth study with 27 pigs per pen. In the nursery, there were 15 pens (replications) fed the control diet and 30 pens (replications) fed diets containing a microencapsulated complex of organic acids and essential oils (MOE). In the finishing period of the experiment, 3 treatments were applied which included: 1) pigs on the control diet in nursery remaining on control diets; 2) ½ of pigs in nursery fed the MOE were fed the MOE throughout finishing, and 3) ½ of pigs in nursery fed the MOE were then fed the control diets throughout finishing

[2] BW = Body Weight, ADG = Average Daily Gain, ADFI = Average Daily Feed Intake, GF = Feed efficiency.

[3] A combination of micro-encapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals; Vetagro, Inc. Chicago, IL

[4] The MOE inclusion rate of 0.30% of the diet in phase 1 (1.81 kg/pig feed budget) and phase 2 (ended at d 21) and 0.90 kg/ton 0.10% of the diet from d 21 to 42 of the nursery period. Pigs were then placed on diets that provided 0.05% MOE in the diet of throughout the grow-finish period.

[5] Pigs fed diets with 0.30% MOE in phase 1 (1.81 kg/pig feed budget) and phase 2 (ended at d 21) and 0.10% of the diet from d 21 to 42 during the nursery period and then switched to the control diet from d 42 to 156.

[6] Pigs that have an inability to overcome sickness or injury during the trial were removed and marked as a removal.