Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs

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Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs[1]

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ABSTRACT: Lysozyme is a 1,4-β-*N*-acetylmuramidase that has antimicrobial properties. The objective of this experiment was to determine the effect of a purified granulated lysozyme, compared with antibiotics, on growth performance, small intestinal morphology, and *Campylobacter* shedding in 10-d-old pigs. Forty-eight pigs (n = 16 per treatment), with an initial BW of 4.0 ± 0.1 kg ($P > 0.40$), were weaned at 10 d of age, blocked by litter and sex, and assigned to pens (8 pigs/pen). Each block was randomly assigned to consume 1 of 3 liquid dietary treatments for 14 d: a control diet, the control diet + lysozyme (100 mg/kg of diet), or the control diet + antibiotics (neomycin and oxytetracycline, 16 mg/kg of diet). Pigs were weighed and blood was sampled on d 0, 7, and 14. Blood was analyzed for plasma urea N and IgA. After 14 d of treatment, pigs were killed and samples of the jejunum and ileum were collected and fixed to measure villus height and crypt depth. Rectal swabs were taken on d 0, 7, and 14 of treatment, and samples of ileal and cecal contents were taken at d 14 of treatment to determine the presence of *Campylobacter*. Pigs consuming lysozyme and antibiotics gained BW at a faster rate than did control pigs over the course of the study (402 ± 12 and 422 ± 14 g/d, respectively, vs. 364 ± 14 g/d; $P < 0.02$), resulting in heavier ending BW (9.9 ± 0.3, 9.9 ± 0.3, and 9.0 ± 0.2 kg for pigs in the lysozyme, antibiotic, and control groups, respectively; $P < 0.03$). Immunoglobulin A decreased and plasma urea N increased over the course of the study ($P < 0.1$), regardless of dietary treatment ($P > 0.6$). Crypt depth was increased in pigs fed lysozyme- and antibiotic-treated diets, compared with pigs fed the control diet, in both the jejunum (60.0 ± 2.8 and 62.2 ± 3.0 μm, respectively, vs. 50.7 ± 3.1 μm; $P < 0.03$) and ileum (76.0 ± 7.5 and 72.2 ± 5.0 μm, respectively, vs. 52.4 ± 3.5 μm; $P < 0.02$). Villus height did not differ in the jejunum ($P > 0.2$) but was increased in the ileum of pigs consuming the lysozyme- and antibiotic-treated diets, compared with pigs fed the control diet (312 ± 20 and 314 ± 10 μm, respectively, vs. 263 ± 15 μm; $P < 0.4$). Small intestinal total mucosa and mucosal protein concentrations, as well as disaccharidase-specific activities, were not altered by lysozyme or antibiotics ($P > 0.05$). *Campylobacter* was detected in 27% of control samples but in only 5% of samples from pigs fed antibiotics and 8% of samples from pigs fed lysozyme ($P < 0.01$). Thus, granulated lysozyme is a suitable alternative to antibiotics for 10-d-old pigs consuming manufactured liquid diets.

Key words: antimicrobial, *Campylobacter*, immunoglobulin A, lysozyme, swine

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INTRODUCTION

Recently, swine producers have been pressured to reduce or remove dietary antibiotics. Antibiotics have been fed at subtherapeutic amounts as growth promoters for more than 50 yr, and the majority of swine produced in the United States receive antibiotics in their feed at some point during the production process. The addition of antibiotics to swine diets benefits producers by improving feed efficiency and decreasing susceptibility to bacterial infections (Verstegen and Williams, 2002). In previous studies, *Salmonella* was found to be prevalent in 62% of swine before the growing phase of production (Wells et al., 2005). After 8 wk on diets including chlortetracycline, <15% of the pigs were *Salmonella* positive. In addition, increased *Campylobacter* shedding is associated with reduced performance in growing pigs (Wells et al., 2010).

[1]Mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

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Lysozyme is a 1,4-β-*N*-acetylmuramidase that enzymatically cleaves the glycosidic linkage in the peptidoglycan component of bacterial cell walls, leading to cell death (Ellison and Giehl, 1991). Hydrolysis products produced from the loss of cellular membrane integrity enhance IgA secretion, macrophage activation, and rapid clearance of bacterial pathogens (Kawano et al., 1981). Previous studies using lysozyme delivered the enzyme via transgenic goat milk or transgenic rice. In these studies, beneficial changes were observed in intestinal morphology (Brundige et al., 2008), intestinal microflora (Maga et al., 2006), and metabolite profiles (Brundige et al., 2010). These findings indicate that inclusion of lysozyme may prove to be a viable alternative to antibiotics in swine feed. However, the large-scale use of transgenic sources of lysozyme may prove problematic. Therefore, the objective of this study was to determine if purified, granulated lysozyme in manufactured liquid diets would reduce the presence of *Campylobacter* and improve the small intestinal morphology and growth performance of 10-d-old pigs.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee of the US Meat Animal Research Center.

Animal Care and Dietary Treatment

Forty-eight pigs (n = 16 per treatment) were weaned from the sow at 10 d of age and used in a randomized complete block design. Pigs were blocked by litter and sex and assigned to 1 of 6 pens (8 pigs per pen). Pigs were housed in specialized pens consisting of an enclosed portion maintained at approximately 32°C and an area open to ambient temperature. Ambient temperature was maintained at approximately 24°C. Each block was randomly assigned to 1 of 3 experimental diets that met or exceeded NRC (1998) recommendations for required nutrients: 1) a control diet (nonmedicated milk replacer, Milk Specialties Inc., Carpentersville, IL), 2) the control diet + antibiotics (neomycin and oxytetracycline, 16 mg/L; Pennfield Animal Health, Omaha, NE), and 3) the control diet + lysozyme (100 mg/L, Entegard, Bioseutica USA Inc., Rhinebeck, NY). The manufactured liquid diet was delivered via a gravity-flow feeding system (Oliver and Miles, 2010), with 30-L Nalgene carboys (Fisher Scientific, Pittsburgh, PA) used to accommodate each pen of 8 pigs. New supplies of the manufactured liquid diet were added twice daily (0800 and 2000 h) to ensure freshness and allow pigs to consume diets ad libitum. The liquid diet was prepared on a daily basis and stored at 4°C. Feed disappearance was measured gravimetrically on a daily basis, and pig BW were measured on d 0, 7, and 14. All components of the feeding system were cleaned thoroughly before the first feeding (0800 h).

Sample Collection and Analytical Procedure

On d 0, 7, and 14 of treatment, 5 mL of blood was collected into heparinized syringes (20 IU of Li-heparin/mL of blood (Sarstedt, Newton, NC) via jugular venipuncture and placed immediately on ice. After collection, blood samples were centrifuged at 800 × *g* for 10 min at 4°C, with plasma collected and frozen at −20°C until further analyses. Plasma was analyzed for IgA and plasma urea N (**PUN**) concentrations. The IgA concentrations were determined by commercial ELISA (Bethyl Laboratories Inc., Montgomery, TX). Plasma was analyzed in duplicate for urea N (Marsh et al., 1965) and measured using a Technicon Autoanalyzer System (Technicon Autoanalyzer Systems, Tarrytown, NY). The sample mean for PUN pools was 8.0 ± 0.3 m*M* and the intraassay CV was 3.8%. The sample mean for IgA pools was 10.2 ± 0.8 mg/mL and the intraassay CV was 4.9%.

Pigs were killed via CO_2 stunning followed by exsanguination on d 14 of treatment, and 3- and 10-cm segments of jejunum and ileum were collected. The 3-cm segments were processed, embedded, and stained according to procedures described previously (Luna, 1968). Briefly, freshly cut intestinal sections were rinsed in cold PBS, and then fixed in freshly prepared chilled fixative solution (10 mL of formalin, 70 mL of 95% ethanol, 15 mL of distilled water, 5 mL of acetic acid). Intestinal segments were dehydrated over a 2-d period by using increasing concentrations of ethanol and chloroform. Sections were embedded in paraffin, and cross sections were cut with a microtome (American Optical Co., Buffalo, NY) approximately 10 μm thick. Sections were stained with hematoxylin and eosin, and morphometric measurements were performed by 1 person using light microscopy with a computer-assisted morphometric system (Bioquant Image Analysis Corp., Nashville, TN). The height and crypt depth of 8 well-oriented villi per sample were measured.

The 10-cm segments of jejunum and ileum were opened longitudinally, and the mucosa was collected, weighed, and homogenized in 4 vol (vol/wt) of 0.9% NaCl solution. All samples were frozen at −20°C until further analysis. The homogenates were then assayed for intestinal lactase and maltase activities according to the methods described by Dahlqvist (1964). Protein content of the mucosa homogenate was determined using a bicinchoninic acid reagent (Smith et al., 1985). Glucose liberation was measured using an immobilized enzyme system (model 2700 YSI, Yellow Springs Instruments, Yellow Springs, OH).

Rectal swabs were collected for assessment of *Campylobacter* shedding on d 0, 7, and 14. On d 14, samples of contents found in the ileum and cecum were collected. Rectal swabs and digesta samples were determined by the procedures described by Wells et al. (2005, 2010). Briefly, swabs for *Campylobacter* were enriched with 13 mL of Bolton broth with supplement (Oxoid, Basingstoke, UK) and lysed horse blood cells (Lampire Bio-

logical Labs, Pipersville, PA). Tubes were gently mixed, capped tightly, and incubated for 4 h at 37°C, followed by 44 h at 42°C. A 10-μL aliquot was plated onto Campy-Cephex agar and grown using MicroAero Packs in an AnaeroPack System (Mitsubishi Gas Chemical, New York, NY) for 48 h at 42°C. Colonies formed were confirmed as *Campylobacter* positive by PCR for the presence of the *lpxA* gene (Klena et al., 2004). Rectal swabs and samples of digesta were also measured for *Salmonella* as described previously (Wells et al., 2005).

Statistical Analyses

Data were subjected to ANOVA using the GLM procedure (Minitab Inc., State College, PA). Data were evaluated for the effects of treatment (control, antibiotic-treated, and lysozyme-treated diets), day, sex, and all appropriate interactions. No statistically significant sex differences were observed. Thus, sex data were combined. Day and treatment responses were contrasted by using a protected LSD test (Steel et al., 1997). For ADFI and feed efficiency, the experimental unit was the pen of pigs. For BW, ADG, and all blood and tissue measurements, the experimental unit for all statistical procedures was the individual pig. The significance level for all tests was set at $P < 0.05$. The presence of *Campylobacter* was analyzed using Fisher exact methods as described previously (Wells et al., 2005, 2010).

RESULTS

Pigs gained 334 ± 12 g/d from d 0 to 7 of treatment, regardless of dietary treatment (Table 1; $P > 0.19$). However, from d 7 to 14, pigs fed the lysozyme- and antibiotic-treated diets gained BW at a greater rate compared with pigs fed the control diet ($P < 0.05$), which resulted in an overall faster ADG from d 0 to 14 (402 ± 12 and 422 ± 14 g/d vs. 364 ± 14 g/d, respectively; $P < 0.02$). As a result of the increase in ADG, pigs consuming the antibiotic- and lysozyme-treated liquid diets were heavier after 14 d of treatment compared with control pigs (9.9 ± 0.3, 9.9 ± 0.3, and 9.0 ± 0.2 kg, respectively; $P < 0.03$). Regardless of dietary treatment, pigs consumed 2,066 ± 52 g of DM/pen per day from d 0 to 7. From d 7 to 14, pigs fed the antibiotic-treated liquid diets consumed more feed than pigs fed the control diet (3,783 ± 204 vs. 3,359 ± 207 g of DM/pen per day; $P < 0.4$). Overall, from d 0 to 14, pigs fed the antibiotic- and lysozyme-treated diets consumed more feed than pigs fed the control diet (2,967 ± 118 and 2,803 ± 19 g of DM/pen per day, respectively, vs. 2,655 ± 284 g of DM/pen per day; $P < 0.05$). Feed efficiency (G:F) was not altered by dietary treatment ($P > 0.37$).

Dietary treatment did not affect circulating IgA or PUN concentrations (Figure 1; $P > 0.6$). Circulating IgA decreased over the course of the experiment ($P < 0.01$), with pigs fed the lysozyme-treated diet showing the greatest decrease (treatment × day; $P < 0.03$). Regardless of dietary treatment, PUN concentration increased over the course of the experiment ($P < 0.01$).

The amounts of jejunal and ileal mucosa and mucosa protein did not differ, regardless of dietary treatment (Table 2; $P > 0.42$). In addition, the specific activities of lactase and maltase were not affected by dietary treatment ($P > 0.37$).

Regardless of dietary treatment, villus height did not differ in the jejunum (Figure 2; $P > 0.21$) However,

Table 1. Performance by 10-d-old pigs fed control, control + antibiotics, or control + lysozyme manufactured liquid diets from 10 to 24 d of age[1]

	Diet		
Variable	Control	Control + antibiotics	Control + lysozyme
BW, g			
d 0	3,922 ± 97	3,944 ± 138	4,119 ± 135
d 7	6,079 ± 173	6,288 ± 189	6,346 ± 227
d 14	9,040 ± 207[a]	9,898 ± 255[b]	9,862 ± 252[b]
ADG, g/d			
d 0 to 7	320 ± 15	345 ± 13	337 ± 15
d 7 to 14	414 ± 15[a]	490 ± 18[b]	459 ± 15[b]
d 0 to 14	364 ± 14[a]	422 ± 14[b]	402 ± 12[b]
ADFI, g of DM/pen per day			
d 0 to 7	1,951 ± 78	2,148 ± 48	2,098 ± 97
d 7 to 14	3,359 ± 207[a]	3,783 ± 204[b]	3,509 ± 10[ab]
d 0 to 14	2,655 ± 284[a]	2,967 ± 118[b]	2,803 ± 19[b]
G:F, g/g of DM			
d 0 to 7	1.18 ± 0.02	1.20 ± 0.05	1.17 ± 0.03
d 7 to 14	1.01 ± 0.01	1.01 ± 0.01	1.01 ± 0.01
d 0 to 14	1.06 ± 0.02	1.12 ± 0.01	1.08 ± 0.01

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Control = diet of nonmedicated milk replacer (Milk Specialties Inc., Carpentersville, IL); control + antibiotics = control diet + neomycin and oxytetracycline (16 mg/L; Pennfield Animal Health, Omaha, NE); control + lysozyme = control diet + Entegard (100 mg/L, Bioseutica USA Inc., Rhinebeck, NY). Values are least squares means ± SEM; for BW and ADG, n = 14 to 16; for ADFI and G:F, n = 2.

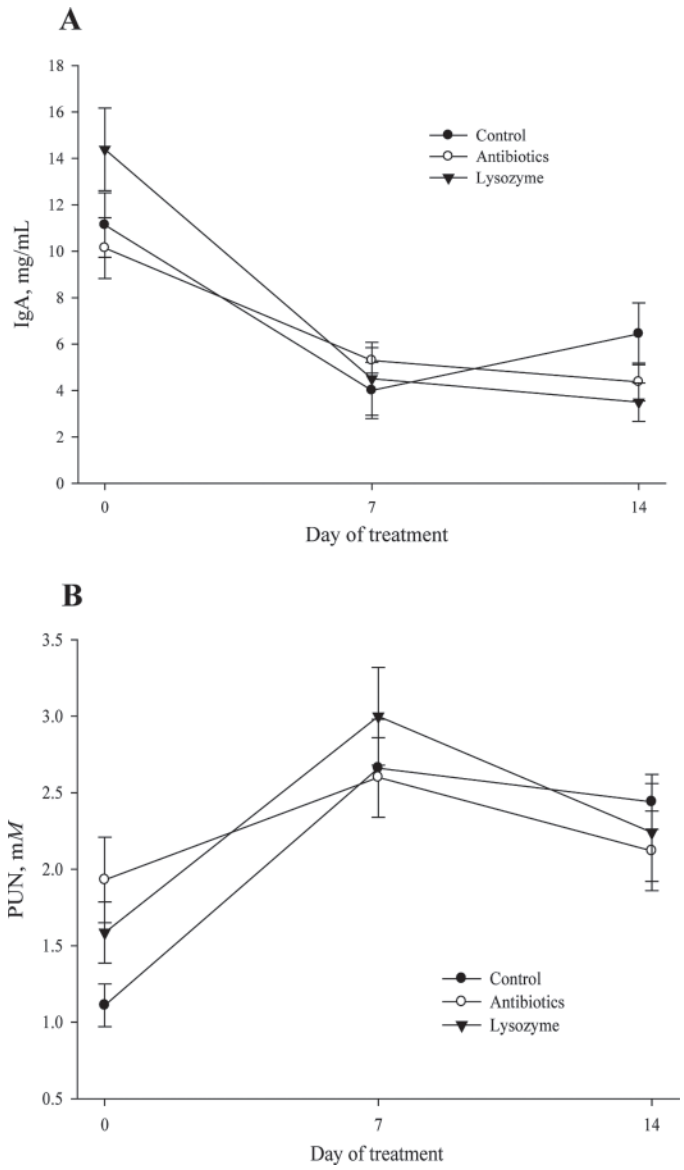

Figure 1. Effect of antibiotics or lysozyme in manufactured liquid diets on circulating A) IgA and B) plasma urea N (PUN) in pigs weaned from the sow at 10 d of age. Control = diet of nonmedicated milk replacer (Milk Specialties Inc., Carpentersville, IL); antibiotics = control diet + neomycin and oxytetracycline (16 mg/L; Pennfield Animal Health, Omaha, NE); lysozyme = control diet + Entegard (100 mg/L, Bioseutica USA Inc., Rhinebeck, NY). Values shown are means ± SEM; n = 14 to 16. No treatment differences were observed ($P > 0.05$). The IgA decreased and PUN increased from d 0 to 7 ($P < 0.05$). There was a treatment × day interaction for IgA ($P < 0.03$).

villus height was greater in the ileum of pigs consuming antibiotics or lysozyme, compared with control pigs (314 ± 20 and 312 ± 10 μm, respectively, vs. 263 ± 15 μm; $P < 0.04$). In addition, crypt depths in pigs fed the antibiotic- and lysozyme-treated diets, compared with pigs consuming the control diet, were greater in both the jejunum (60.0 ± 2.8 and 62.2 ± 3.0 μm, respectively, vs. 50.7 ± 3.1 μm; $P < 0.03$) and ileum (76.0 ± 7.5 and 72.2 ± 5.0 μm, respectively, vs. 52.4 ± 3.5 μm; $P < 0.02$).

Campylobacter shedding in samples from pigs consuming the antibiotic- (5%) or lysozyme-treated (8%) diets did not differ ($P > 0.05$), but both were less ($P < 0.01$) compared with shedding in pigs consuming the control diet (27%; Figure 3.). *Salmonella* shedding was not detectable in any sample measured (data not shown).

DISCUSSION

The use of antibiotics in livestock feed is well established and can improve growth rates in several species, including swine (Schwarz et al., 2001; Cromwell, 2002; Thymann et al., 2007). In the current study, we showed that pigs weaned from the sow at 10 d of age and consuming either antibiotics or lysozyme in liquid diets demonstrated an increase in ADG. Over the 2-wk trial, pigs consuming lysozyme gained BW 10% faster than did control pigs, which is comparable with pigs consuming antibiotics. In contrast, in studies using human lysozyme (**hLZ**) from transgenic goat milk, pigs consuming the hLZ did not show an improvement in growth (Maga et al., 2006; Brundige et al., 2008). Two factors may explain the lack of growth rate improvement in these studies compared with the current experiment. Growth improvement attributable to the hLZ may have been masked because of the antibiotics in both the control and experimental diets in the study by Brundige et al. (2008). It is unclear whether the diets of Maga et al. (2006) included antibiotics. In addition, both Brundige et al. (2008) and Maga et al. (2006) fed dry, pelleted nursery diets in addition to the hLZ goat milk. It is unclear how much hLZ was consumed by pigs in relation to the dry diets in these studies. Presumably, the pigs consumed a significant amount because of the changes in intestinal morphology and microflora, but this may not have been a sufficient amount to affect growth rate. Similar to previous studies of pigs, hLZ produced in transgenic rice did not improve the growth rate of chicks fed a diet containing 152 mg of hLZ/kg of feed (Humphrey et al., 2002). However, chicks had significantly improved feed efficiency over those reared on a diet containing neither the transgenic protein nor antibiotics. In the current study, no changes in feed efficiency were observed in pigs consuming diets with antibiotics or lysozyme. However, because of the study design, our statistical power to detect differences in feeding variables was greatly limited.

Skeletal muscle accretion is most rapid in young, growing pigs. Therefore, in the young pig, PUN concentration is a reliable indirect measurement to show the oxidation of dietary AA. Plasma urea N concentrations were not different between treatments in the current study and were comparable with those in our earlier work with similarly aged pigs (Oliver and Miles, 2010). This indicates that pigs consuming lysozyme were utilizing dietary AA for protein synthesis similarly to pigs consuming the control and antibiotic-treated diets. Plasma urea N increased during the study for all treatment groups. Presumably, this was due to a slight excess in AA while consuming manufactured liquid di-

Table 2. Ileal and jejunal mucosal mass, protein mass, lactase activity, and maltase activity of 24-d-old pigs fed control, control + antibiotics, or control + lysozyme manufactured liquid diets from 10 to 24 d of age[1]

	Diet		
Variable	Control	Control + antibiotics	Control + lysozyme
Jejunum			
Mucosa,[2] g/10 cm	3.9 ± 0.2	4.4 ± 0.2	4.0 ± 0.2
Protein,[3] g/10 cm	163 ± 9	136 ± 6	158 ± 8
Lactase activity, μmol/(min·g of protein)	72.7 ± 9.4	67.2 ± 7.8	70.3 ± 8.1
Maltase activity, μmol/(min·g of protein)	65.3 ± 5.3	64.1 ± 6.2	67.9 ± 3.4
Ileum			
Mucosa, g/10 cm	2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.2
Protein, g/10 cm	130 ± 11	120 ± 7	128 ± 9
Lactase activity, μmol/(min·g of protein)	37.4 ± 4.6	30.9 ± 3.6	31.5 ± 3.2
Maltase activity, μmol/(min·g of protein)	63.8 ± 9.1	67.4 ± 9.3	64.4 ± 8.9

[1]Control = diet of nonmedicated milk replacer (Milk Specialties Inc., Carpentersville, IL); control + antibiotics = control diet + neomycin and oxytetracycline (16 mg/L; Pennfield Animal Health, Omaha, NE); control + lysozyme = control diet + Entegard (100 mg/L, Bioseutica USA Inc., Rhinebeck, NY). Values are least squares means ± SEM; n = 14 to 16.

[2]Wet weight of mucosa from a 10-cm section of small intestine.

[3]Protein content of mucosa from a 10-cm section of small intestine.

ets ad libitum because the sow likely limited intake by 10 d of age (Boyd et al., 1995; Azain et al., 1996).

Small intestinal morphology has typically been used as an estimate of intestinal health in pigs (Argenzio and Liacos, 1990; Li et al., 1990; Zijlstra et al., 1996; Oliver et al., 2002). In the current study, pigs consuming lysozyme had villus heights and crypt depths that were similar to those of pigs consuming antibiotics, both of which were improved compared with the villus heights and crypt depths the control group. In contrast, pigs consuming hLZ from goat milk did not show changes in villus height or crypt depth in the ileum or jejunum (Brundige et al., 2008; Cooper et al., 2011). Similar to growth performance measures, this may have been due to decreased lysozyme consumption or the presence of antibiotics in the diets. Humphrey et al. (2002) also

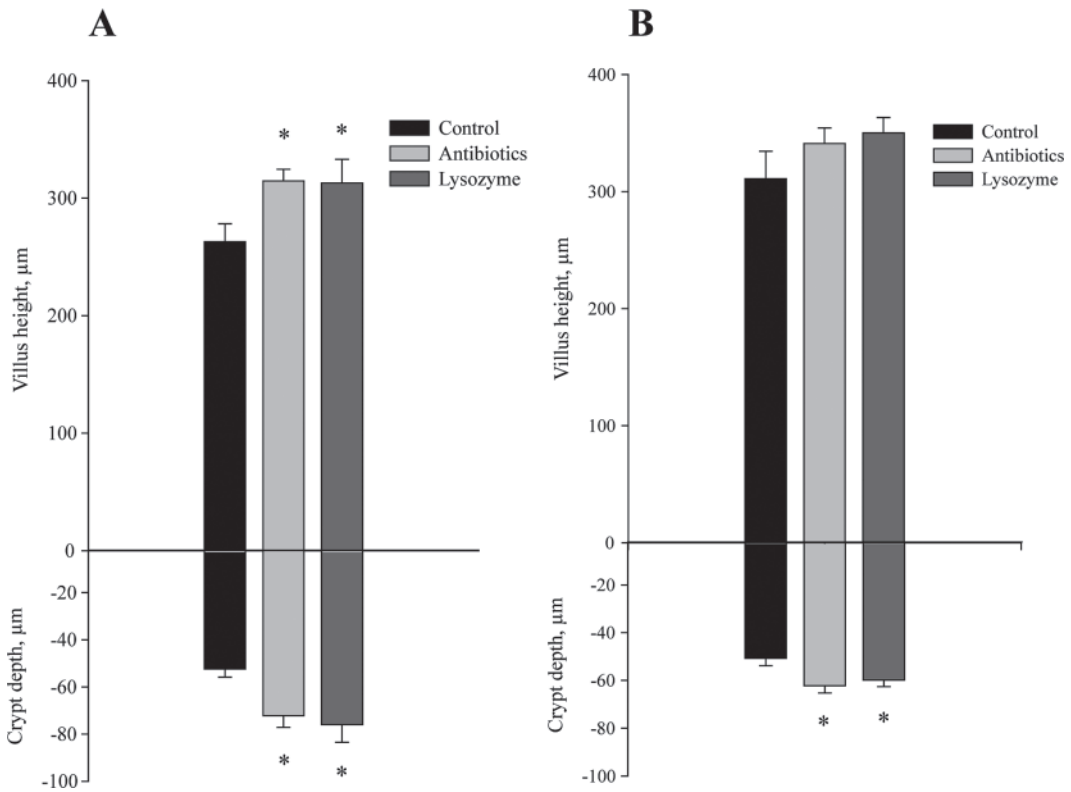

Figure 2. Effect of 14 d of antibiotics or lysozyme in manufactured liquid diets on mean villus heights and crypt depths in the A) ileum and B) jejunum of pigs weaned from the sow at 10 d of age. Control = diet of nonmedicated milk replacer (Milk Specialties Inc., Carpentersville, IL); antibiotics = control diet + neomycin and oxytetracycline (16 mg/L); lysozyme = control diet + Entegard (100 mg/L, Bioseutica USA Inc., Rhinebeck, NY). Values shown are means ± SEM; n = 14 to 16. An asterisk (*) indicates the mean differs from the control ($P < 0.05$). All other comparisons, no difference ($P > 0.05$).

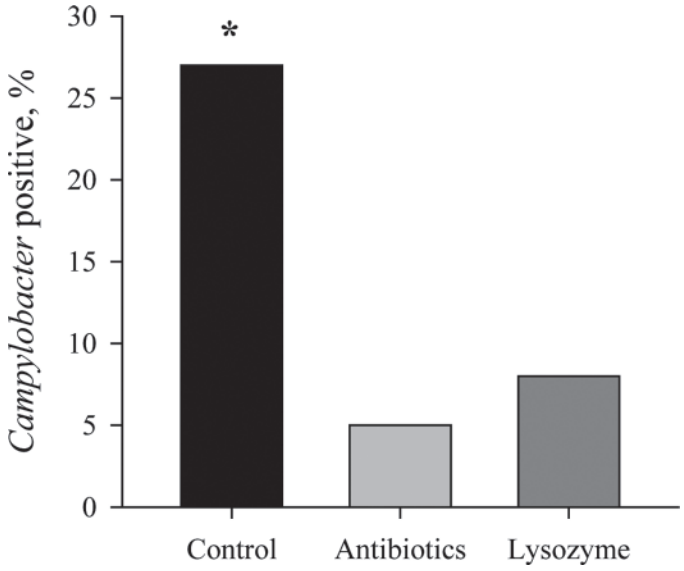

Figure 3. Effect of 14 d of antibiotics or lysozyme in manufactured liquid diets on the percentage of rectal swab and digesta samples positive for *Campylobacter* in pigs weaned from the sow at 10 d of age (n = 64). Control = diet of nonmedicated milk replacer (Milk Specialties Inc., Carpentersville, IL); antibiotics = control diet + neomycin and oxytetracycline (16 mg/L); lysozyme = control diet + Entegard (100 mg/L, Bioseutica USA Inc., Rhinebeck, NY). An asterisk (*) indicates the mean differs from pigs fed antibiotics and lysozyme ($P < 0.01$).

did not observe changes in villus height in the ileum of chicks fed rice expressing hLZ. However, duodenal villus heights were increased in chicks consuming lysozyme. Similar to the current experiment, Nyachoti et al. (2011) observed increased villus heights in the ileum.

Contrary to the current experiment, Thymann et al. (2007) did not observe morphological changes attributable to antibiotics in 24-d-old weaned pigs. Similarly, Shen et al. (2009) did not observe changes in villus heights or crypt depths in 28-d-old weaned pigs resulting from antibiotic treatment. However, an improvement in the villus height-to-crypt depth ratio was observed. Similar to the current experiment, Piva et al. (2008) observed increased villus heights in the proximal and mid jejunum. Clearly, the responses of villus and crypt structures were variable in response to antibiotics. It is likely that antibiotics, and lysozyme in the current study, have a real effect on small intestinal morphology. In the current study, young pigs consuming milk diets had very uniform villus heights and crypt depths, which facilitated the detection of changes attributable to antibiotics and lysozyme. Presumably, the increase in villus height leads to a greater absorptive capacity in the small intestine, which indicates a possible mechanism by which lysozyme and antibiotics can improve growth rates.

Intestinal disaccharidase-specific activities are another measure of intestinal health (Correa-Matos et al., 2003) and maturity in response to dietary factors (Oliver et al., 2002). The disaccharidase response to antibiotics has not been studied, but disaccharidase-specific activities are decreased in the small intestine of piglets after infection with *Salmonella* Typhimurium (Correa-Matos et al., 2003). In the current study, no differences in lactase or maltase activities were observed in either the jejunum or the ileum resulting from the consumption of antibiotic- or lysozyme-treated diets. Therefore, any beneficial effect of antibiotics or lysozyme on the small intestinal microflora was not sufficient to alter the normal activities of lactase and maltase.

Immunogobulin A is produced in response to bacterial interactions with the host animal in the gastrointestinal tract (Suzuki and Fagarasan, 2008). Little information is available on the effects of subtherapeutic antibiotic use on IgA in pigs. Dietary treatment did not have an effect on circulating IgA concentrations in the current study. However, a day of treatment effect was observed. From d 0 to 7, IgA concentrations decreased for all treatment groups, and this concentration was maintained throughout the remainder of the experiment. This was expected because of the normal decrease in maternal IgA provided from sow colostrum. Spray-dried plasma proteins have antimicrobial-like properties when consumed by pigs and have been shown to reduce the concentration of circulating IgA in nursery pigs (Van den Broeck et al., 1999; Bosi et al., 2004). However, these experiments used enterotoxigenic *Escherichia coli* K88 challenges and reported K88-specific IgA concentrations.

Pigs consuming lysozyme in the liquid diet for 2 wk had less *Campylobacter* shedding compared with pigs consuming the control diet. *Campylobacter* shedding was observed in 27% of samples collected from pigs consuming the control diet, whereas *Campylobacter* shedding was observed in only 5 and 8% of samples from pigs consuming antibiotics and lysozyme, respectively. Similarly, Maga et al. (2006) reported that the intestinal microflora of pigs consuming pasteurized milk from hLZ transgenic animals was markedly different from the intestinal microflora of those receiving milk from nontransgenic control animals. Young weaned pigs fed milk from hLZ transgenic animals had significantly fewer numbers of coliforms and total *E. coli* in the duodenum than those fed nontransgenic control feed (Maga et al., 2006). However, only the number of coliforms present in the lower small intestine of the hLZ-fed group was different from that of the group fed the control diet. Similarly, Nyachoti et al. (2011) observed decreased coliforms in mucosal scrapings from pigs challenged with *E. coli* K88 and consuming a water-soluble lysozyme.

Coliforms represent a variety of gram-negative bacteria common to the gastrointestinal tract, and *E. coli* are a specific member exclusive to the mammalian intestines (Katouli and Wallgren, 2005). Lysozyme is known to be more active against gram-positive bacteria (Costerton et al., 1974). Therefore, it is likely that the hLZ consumed was active against the predominant gram-positive species in the gut, thereby reducing competition and allowing more gram-negative coliforms to grow (Maga et al., 2006).

Campylobacter shedding was measured in the current study because of its potential effect on human health. In addition, increased *Campylobacter* spp. shedding is associated with reduced performance in growing pigs (Wells et al., 2010). *Campylobacter* is gram negative, and it is not intuitive why shedding should be reduced by lysozyme. *Campylobacter* is resistant to lysozyme (Hughey and Johnson 1987; Carneiro de Melo et al. 1998); therefore, it is likely that the reduction in *Campylobacter* was due, in part, to changes in the gastrointestinal health and microflora by lysozyme that indirectly reduced *Campylobacter* colonization and shedding.

Feeding antibiotics remains efficacious at improving animal productivity after more than 50 yr of steady use. However, public perception regarding the transfer of antibiotic-resistant genes from commensal bacteria of poultry, pigs, and cattle to human pathogens has driven a search for alternate strategies. The antibacterial properties of lysozyme show no indication that bacteria have become resistant to the protein in nature; however, the development of bacterial resistance after prolonged feeding has yet to be examined. To our knowledge, this study is the first to demonstrate improved growth performance in response to lysozyme consumption in pigs. In addition, pigs consuming the lysozyme-treated liquid diet had improved small intestinal morphology and decreased *Campylobacter* prevalence in the gastrointestinal tract. Thus, we conclude that granulated lysozyme is a suitable alternative to antibiotics in 10-d-old pigs consuming liquid diets.

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