RESEARCH REPORT
ANIMAL SCIENCE

Title: Effect of lysozyme or antibiotics in ameliorating the effects of an indirect diseases challenge – **NPB #12-148** **revised**

Investigator: William T. Oliver

Institution: USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933-0166

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INDUSTRY SUMMARY

Subtherapeutic levels of antibiotics are used in swine feed as growth promotants, to improve feed efficiency, and to reduce the susceptibility to bacterial infections. As a result, the use of antibiotics improves the profitability of production for swine producers. However, swine producers are currently under pressure to eliminate subtherapeutic antibiotic use throughout the production cycle. Finding safe and effective alternatives to traditional antibiotics will give swine producers viable options in the event that the removal of traditional antibiotics is needed. Research conducted at the U.S. Meat Animal Research Center determined that feeding an antimicrobial enzyme, lysozyme, to nursery pigs was as effective as traditional antibiotics in increasing growth performance, including growth, nutrient accretion and feed efficiency, and decreasing pathogen shedding. In addition, lysozyme was effective in pigs under a chronic immune stimulation. Thus, lysozyme is a suitable alternative to antibiotics in swine nursery diets, and lysozyme ameliorates the effects of a chronic immune challenge.

Key Findings:

- Lysozyme improves growth performance and feed efficiency in pigs similarly to antibiotics.
- An immune response decreases the performance of pigs in the nursery; both lysozyme and antibiotics improve growth performance in nursery pigs under a chronic immune challenge.
- Pigs consuming lysozyme have decreased shedding of *Campylobacter* compared to antibiotic and control fed pigs.

KEY WORDS: Antibiotics, Lysozyme, Swine, Nursery, Immune

SCIENTIFIC ABSTRACT

Lysozyme is a 1,4-β-N-acetylmuramidase that has antimicrobial properties. The objective of this study was to determine the effect of lysozyme and antibiotics on growth performance and immune response during an indirect disease challenge. Two replicates of 600 pigs each were weaned from the sow at 26 d of age, blocked by litter and gender, and then randomly assigned to one of 24 pens in either a nursery room that had been fully disinfected or a nursery room left unclean since the previous group of pigs. Within a room, pigs were randomly assigned to either control diets (C; 2 phase nursery regime), control diets + antibiotics (C + A; chlortetracycline/Denegard), or control diets + lysozyme (C + Lyso; 100 mg/kg diet). Pig weights and feed disappearance were measured and blood was collected on d 0, 14, and 28 of treatment. A group of 20 pigs were killed at 24 d of age for initial body composition analysis and 10 pigs of median weight were killed per diet room combination for body composition analysis after 28 d of treatment. Control + A and C + Lyso fed pigs grew at a faster rate for the 28 d study (318±14, 320±15, vs. 288±15 g/d, respectively; $P<0.05$), regardless of immune status ($P>0.05$). The indirect immune challenge did not alter growth performance from d 0 to 14 of treatment, but decreased ADG from d 14 to 28 of the study (415±15 vs. 445±13**;** $P<0.05$). Feed intake was not altered by the immune challenge *(*$P>0.61$) or dietary treatments ($P>0.10$), but feed efficiency was worsened by the indirect immune challenge ($P<0.05$) and improved by both C + A and C + Lyso diets ($P<0.01$). The immune challenge did not alter nutrient accretion ($P>0.25$), but both C + A and C + Lyso the accretion of whole-body lipid ($P<0.01$) and protein ($P<0.09$). Blood levels of tumor necrosis factor-α (TNF-α, $P<0.01$),

haptoglobin (P<0.09), and C-reactive protein (CRP, P<0.01) were higher due to the indirect immune challenge, compared to pigs reared in the clean nursery (P<0.05). In addition, pigs consuming antibiotics or lysozyme had lower TNF-α, haptoglobin, and CRP compared to control pigs, regardless of nursery environment (P<0.04). Thus, lysozyme or antibiotics improve pig performance during an indirect immune challenge. Thus, lysozyme is a suitable alternative to antibiotics in swine nursery diets, and lysozyme ameliorates the effects of a chronic immune challenge.

INTRODUCTION

Antibiotics have been fed at subtherapeutic levels to swine as growth promoters for more than 50 years, and the majority of swine produced in the U.S. receive antibiotics in their feed at some point in their production cycle. These compounds benefit the producers by minimizing production losses by increasing feed efficiency and decreasing susceptibility to bacterial infection and disease (Verstegen and Williams, 2002). Lysozyme is a 1,4-β-N-acetylmuramidase that enzymatically cleaves a glycosidic linkage in the peptidoglycan component of bacterial cell walls, which results in the loss of cellular membrane integrity and cell death (Ellison, III and Giehl, 1991). We have identified lysozyme (May et al., 2012; Oliver and Wells, 2013) as a suitable alternative to antibiotics.

It is well established that immune system activation, including pro-inflammatory cytokine and acute phase protein production, prevents animals from reaching their genetic growth potential (see review by Cook, M.E, 2011). Poultry and swine reared in germ-free environments grow at a faster rate than animals reared in conventional production environments (Drew et al., 2003; Loynachan et al., 2005). In addition, utilizing a clean vs. dirty environment model elicits an immune response that decrease animal performance (Roura et al., 1992; Bassaganya-Riera et al., 2001; Renaudeau, 2009). In pigs, an immune response does not generally result in decreased feed conversion (Williams et al., 1997a, 1997b; Renaudeau, 2009). However, both lysozyme (Oliver and Wells, 2013) and antibiotics (Verstegen and Williams, 2002) improve feed efficiency in nursery swine. In addition, Nyachoti et al. (2012) reported that lysozyme alleviated the piglet response to an oral challenge of

Escherichia coli K88, similarly to traditional antibiotics. Thus, we propose to determine the efficacy of lysozyme in ameliorating the effect of an immune response in pigs weaned from the sow at 24 d of age.

OBJECTIVES

a. Determine if lysozyme and/or antibiotics improves growth performance, including composition of gain, of nursery pigs during an indirect disease challenge.

b. Determine if lysozyme and/or antibiotics alters the immune response of nursery pigs during an indirect disease challenge.

c. Determine if lysozyme and/or antibiotics decreases the shedding of bacteria potentially harmful to humans, including *Campylobacter*, *Salmonella*, and shigatoxinic *Escherichia coli* (stx genes) during an indirect disease challenge.

d. Determine if lysozyme and/or antibiotics reduces the subsequent days to market following an immune response in the nursery.

MATERIALS AND METHODS

Animal Care and Dietary Treatment

The experimental protocol was approved by the Animal Care and Use Committee of the U.S. Meat Animal Research Center (USMARC). Two replicates of 600 pigs were weaned from the sow at 26 days of age. Pigs were blocked by litter and gender, and then randomly assigned to either a nursery room that had been fully cleaned and disinfected or a nursery room left unclean since the previous group of pigs (Bassaganya-Riera et al., 2001; Renaudeau, 2009). Within a room, pigs were randomly assigned to either control diets (2 phase nursery regime), control diets + antibiotics (cholorotetracycline/Denegard), or control diets + lysozyme (100 mg/kg diet; Entegard, Neova Technologies, Abbotsford, BC, Canada) and allowed to consume diets *ad libitum* for four weeks. All diets met or exceeded NRC recommendations for required nutrients (Table 1: NRC, 1998). Pig weights and feed disappearance were measured on d 0, 14, and 28 of treatment. After four weeks in the nursery, pigs were moved to finishing facilities where weights were recorded every two weeks until 120 kg body weight.

Sample Collection and Analytical Procedures

On d 0, 14, and 28 of treatment, 5 mL of blood was collected from the same 14 pigs per diet room combination (n= 28 per treatment for the entire experiment) into syringes via jugular venipuncture. After collection and blood coagulation, blood samples were centrifuged at 800 × *g* for 20 min at 4°C, with serum collected and frozen at -20°C until further analyses. Serum was analyzed for urea N (BUN), NEFA, cytokines (tumer necrosis factor-α (TNF- α) and IL-6), and acute phase proteins (C-reactive protein (CRP), haptoglobin, and pig major acute phase protein (Pig-MAP)). Blood urea N was measured (Marsh et al., 1965) using a Technicon Autoanalyzer System (Technicon Autoanalyzer Systems, Tarrytown, NY). The sample mean for BUN pools was 8.2 ± 0.3 mM and the intraassay CV was 4.1%. Serum NEFA concentrations were determined by an enzymatic colorimetric method (Zen-Bio, Inc., Research Triangle Park, NC). The sample mean for NEFA pools was 24.1 ± 1.2 µM and the intraassay CV was 4.8%. Acute phase (CRP and haptoglobin, Life Diagnostics, Inc., West Chester, PA; Pig-MAP, Pigchamp Pro Europa, S.L., Segovia, Spain) and cytokines (TNF- α and IL-6, R&D Systems, Inc., Minneapolis, MN) were measured by commercially available ELISA. The sample mean and intraassay CV for pools were: CRP, 112.4 ± 3.9 ng/mL and 5.9%; haptoglobin, 46.2 ± 1.4 ng/mL and 3.8%; Pig-MAP, 0.76 ± 0.16 µg/mL and 9.8%; TNF-α, 186.9 ± 4.6 pg/mL and 5.2%; and IL-6, 45.6 ± 0.9 pg/mL and 4.8%.

An initial group of pigs (24d of age; n=10 per replicate) were killed (Beuthanasia-D, Shering-Plough Animal Health Corp., Union, NJ) for initial body composition analysis (proximate analysis, AOAC, 1997). After four weeks of treatment, 10 pigs of median weight (n = 20 per treatment for the entire experiment) were killed per diet room combination for body composition analysis. The contents of the gastrointestinal tract, urinary bladder, and gall bladder were removed before the carcasses were stored at -20° C until further analysis.

Statistical Analyses

This experiment was designed as a split-plot with pig within block being the experimental unit for dietary treatment (subplot) and pen within room being the experimental unit for the room treatment (whole plot). Data was analyzed as a 2 x 3 factorial arrangement of treatments within the split-plot design using the

GLM procedure (Minitab Inc., State College, PA). The model included replicate, gender, day, treatment, and all appropriate interactions. Where no statistically significant sex differences were observed, sex data were combined. The significance level for all tests was set at $P < 0.05$ tendencies set at $P < 0.10$.

RESULTS

Pigs were weaned at 26.3 ± 0.1 d of age and weighed 8.6 ± 0.1 kg, regardless of nursery or dietary treatment ($P > 0.22$). From d 0 to 14, pigs consuming antibiotics or lysozyme grew at a faster rate compared to pigs consuming control diets (Table 1; $P <0.05$), regardless of nursery ($P > 0.80$). Similarly, pigs fed antibiotic or lysozyme grew at a faster rate from d 14 to 28 of the study ($P < 0.001$). In addition, pigs reared in the dirty nursery grew at a slower rate that pigs reared in the clean nursery ($P < 0.05$). This resulted in an ADG for pigs consuming antibiotics or lysozyme that was 10 % greater than pigs consuming control diets ($P < 0.001$). However, overall ADG was not affected by nursery ($P > 0.11$). Due to the changes in ADG, pigs consuming antibiotics or lysozyme were heavier at the completion of the 28 d study, compared to control pigs ($P < 0.001$). The improved growth response observed in the nursery carried into the remainder of production, in that days to market (120kg) were reduced by 5 days in antibiotic and lysozyme pigs compared to control pigs ($P < 0.04$). Pigs reared in the dirty nursery tended to have a increased days to market ($P < 0.09$). In addition, male pigs reached market weight 6 days earlier than female pigs (data not shown; $P < 0.01$), regardless of dietary treatment or nursery environment ($P > 0.43$). No differences in ADFI were observed during the course of the study, regardless of dietary treatment or nursery environment ($P > 0.11$). Pigs reared in the dirty nursery had decreased feed efficiency (G:F) from day 14 to 28 ($P < 0.04$), but not d 0 to 14 ($P > 0.15$), which led to an overall 5 % worsening of G:F ($P < 0.05$). Pigs consuming antibiotics or lysozyme had improved feed efficiency compared to control fed pigs from d 0 to 14 of treatment ($P < 0.003$), but not from d 14 to 28 ($P > 0.16$). For the entire 28-d experiment, antibiotics and lysozyme improved feed efficiency by approximately 6.5 % ($P < 0.006$).

Regardless of dietary treatment or nursery environment, pigs had lower lipid and ash, and higher water, as a proportion, after 28 d of treatment compared to the initial reference group (Table 3; $P < 0.05$). Protein

percentage of all pigs were similar to the reference group ($P > 0.10$). Nursery environment did not alter body composition ($P > 0.68$), but both antibiotic and lysozyme fed pigs had less whole-body lipid ($P < 0.01$), as a proportion, compared to the control group. No differences were observed in water or ash accretion rates during the 28-d study, regardless of dietary treatment or nursery environment (Table 5; $P > 0.25$). No differences due to nursery environment on lipid or protein accretion rates were observed during the study ($P > 0.65$). However, lipid accretion was decreased in pigs consuming antibiotics or lysozyme, compared to control pigs ($P < 0.02$). In addition, pigs consuming antibiotics or lysozyme tended to accrue more protein ($P < 0.09$).

Dietary treatment ($P > 0.90$) and nursery environment ($P > 0.31$) had no affect on circulating NEFA concentrations. In addition, there were no sex differences on NEFA ($P > 0.74$). Circulating NEFA decreased from d 0 to 14 ($P < 0.001$), and NEFA concentrations at d 14 were similar to concentrations at d 28 ($P > 0.85$). Sex had no affect on circulating BUN ($P > 0.12$). Overall, BUN was greater in control pigs than both antibiotic and lysozyme fed pigs ($P < 0.01$). In addition, antibiotic fed pigs tended to have a larger BUN compared to pigs consuming lysozyme ($P < 0.10$). Blood urea nitrogen increased during the course of the study ($P < 0.001$), but a dietary treatment x day interaction was observed ($P > 0.001$). Control fed pigs had a higher BUN on d 14 of treatment compared to antibiotic or lysozyme fed pigs, while there were no differences between dietary treatment on d 28 ($P > 0.14$). In addition, pigs in the dirty nursery had higher BUN compared to pigs in the clean nursery, regardless of dietary treatment ($P < 0.05$).

No IL-6 or TNF-α differences dues to sex were observed ($P > 0.75$). In addition, circulating IL-6 decreased over the course of the experiment (Figure 2A; $P < 0.001$) to undetectable levels at d 28, regardless of dietary treatment ($P > 0.13$) or nursery environment ($P > 0.50$). Similarly, TNF-α decreased from day 0 to 14 of treatment (Figure 2B; $P < 0.001$), but levels were similar between d 14 and d 28 ($P > 0.68$). TNF-α was lower in pigs consuming antibiotics or lysozyme compared to control pigs ($P < 0.04$), and this was more evident in pigs reared in the dirty nursery environment (dietary treatment x nursery environment, $P = 0.003$).

Sex had no effect on acute phase proteins ($P > 0.82$). Circulating PigMAP increased from d 0 to14 (Figure 3A; $P < 0.001$), but was unchanged from d 14 to d 28 ($P > 0.24$). PigMAP was not affected by dietary

treatment ($P > 0.20$) or nursery environment ($P > 0.97$). Haptoglobin (Figure 3B; $P < 0.001$) and CRP (Figure 3C; $P < 0.001$) both increased from d 0 to d14 of dietary treatment and were unchanged ($P > 0.62$) from d 14 to 28. Haptoglobin concentration was lower in antibiotic and lysozyme fed pigs, compared to pigs consuming the control diet ($P < 0.02$). Pigs reared in the dirty nursery environment tended to have higher haptoglobin concentrations ($P < 0.09$). Similarly, CRP concentrations were lower for pigs consuming antibiotic or lysozyme vs. control diets ($P < 0.01$) and higher for pigs reared in the dirty nursery environment ($P < 0.001$).

Salmonella prevalence was very low and did not differ between sexes, dietary treatments or nursery environments (data not shown, $P > 0.10$). *Campylobacter coli* prevalence was not affected by sex or nursery environment (Figure 4; $P > 0.10$). *Campylobacter coli* prevalence in feces was similar between dietary treatments at d 0, but higher in both control and antibiotic fed pigs compared to pigs consuming lysozyme at d 28 ($P < 0.01$).

DISCUSSION

Antibiotics are used in feed as a growth promotants for several species, including swine (Cromwell, 2002; Schwarz et al., 2001; Thymann et al., 2007). In addition, antibiotics improve feed efficiency, and to reduce the susceptibility to bacterial infections in swine (Verstegen and Williams, 2002). As a result, the use of antibiotics improves the profitability of production for swine producers. However, the use of antibiotics is banned in some countries and there is pressure to eliminate or reduce their use. Finding safe and effective alternatives to traditional antibiotics will allow swine producers to keep the competitive advantage of antibiotics without the stigma associated with their use. In the current study we showed that pigs consuming diets with either CTC/Denegard or lysozyme had improved growth rates and feed efficiency. This is similar to our previous work in young, milk-fed pigs (May et al., 1012) and nursery pigs (Oliver and Wells, 2013). In addition, the current experiment an indirect disease challenge model was used to assess the effect of antibiotics or lysozyme of performance during a subclinical immune response. In the current study, a chronic immune stimulation did not decrease growth rate or daily feed intake in pigs over the 28 d study. This contradicts previous work that showed modest decreases in ADG and ADFI in pigs utilizing a similar model (Bassaganya-

Riera et al., 2001; Renaudeau, 2009; Williams et al., 1997). However, ADG was decreased due to chronic immune stimulation during the second two-week period of current study. Due to this difference, feed efficiency was worsened in chronically stimulated pigs, similarly to previous work (Williams et al., 1997). Due to these changes, as well as changes in cytokines and acute phase proteins, we are confident that the clean vs. dirty challenge model produced pigs with a subclinical immune response. In the current experiment, both antibiotics and lysozyme improved growth rate and feed efficiency in pigs with a chronic immune stimulation. It is well established that antibiotics improves growth rate during bacterial infections in swine (Verstegen and Williams, 2002), but this is the first evidence of lysozyme improving growth rate during an immune challenge. These data differ from Nyachoti et al. (2012), who did not observe a change in growth rate in pigs consuming antibiotics or lysozyme. However, those pigs were given a more acute challenge of *Escherichia coli* K88 for only 7d, while the current study used a chronic challenge for four wk.

Previous work has shown that antibiotic supplementation in feeds may influence body composition in pigs, depending on the experiment. Van Lunen (2003) observed greater P2 lean, but no difference in P2 fat depths, in pigs consuming tylosin phosphate. However, Song et al. (2001) did not observe differences in LM area or backfat depth in pigs fed bacitracin methylene disalicylate. In the current study, accretion rates of both fat and protein as measured by the slaughter balance technique were altered in pigs consuming either antibiotics or lysozyme. Lipid accretion was decreased and protein accretion tended to increase in these pigs. The data for antibiotic pigs may differ from previous work due to the different antibiotics use (CTC/Denegard vs. tyloin or bacitracin). To our knowledge, this is the first report of lysozyme altering the partitioning of nutrients in pigs. In the current study, a chronic immune stimulation did not alter accretion rates. This differs from Bassanganya-Riera et al. (2001) who observed lower fat, as a proportion, in pigs reared in a dirty environment. However, Williams et al. (1997b) determined that, in spite of differences in N retention differences, chronic immune stimulation does not alter the efficiency of lysine utilization. Nitrogen retention differences would indicate differences in the partitioning of nutrients for protein and fat, but Williams et al. (1997b) determined this was due to the low immune stimulation pigs greater biological capacity for accretion. Although protein and lipid

accretion was lower due to a high immune status (Williams et al., 1997b), this is likely due to the greater difference in growth rates observed in that study. No accretion differences between nursery groups in the current study is likely due to a lower, more chronic, immune system activation in the current study. Nonetheless, due to performance, cytokine, and acute phase protein differences in the dirty nursery, we conclude that lysozyme decreases fat and increases protein deposition in both clean nursery environments and pigs under a chronic immune stimulation.

Circulating urea N is a reliable indirect measurement to show the oxidation of dietary AA in young pigs (Oliver and Miles, 2010). Blood urea N was increased in control pigs compared to pigs consuming lysozyme or antibiotics. This contradicts earlier work out of our lab (Oliver and Wells, 2013). However, the BUN is expected due to increased protein accretion observed in lysozyme or antibiotic fed pigs since feed intake was similar between treatment groups. Those pigs consuming lysozyme or antibiotics utilized more of their dietary amino acids for protein deposition than control pigs. It is likely that our earlier work had too few animals to detects a response in BUN. In addition, the growth response observe due to antibiotics and lysozyme was slightly larger in the current study. Similarly to other work (Bassaganya-Riera et al., 2001), chronically immune stimulated pigs had lower BUN than pigs in the clean nursery. Circulating NEFA is an indirect measure of lipolysis, fatty acids available for uptake into tissues, or both and lipolysis rates should be relatively low in nursery pigs. In spite of differences in lipid accretion, no differences in NEFA due to diet or immune activation were observed. Unlike previous work in 10 to 20-d-old pigs accruing more protein and less lipid (Oliver and Miles, 2010), the rates of lipolysis in 26 to 54 d old pigs were likely fast enough to preclude the detection of differences due to lipid use in the current study.

Cytokines and acute phase proteins were measured to both confirm the chronic immune stimulation and to determine the effect of antibiotics and lysozyme on the immune response. Interleukin-6 and PigMAP were unaffected by immune status. However, the variability of PigMAP concentrations likely prohibited the ability to detect any differences between nurseries or dietary treatments. In contrast, circulating levels of the cytokine TNF-α and the acute phase proteins haptoglobin and CRP were higher in chronically immune stimulated pigs

compared to pigs reared in the clean nursery. These changes in cytokines and acute phase proteins, as well as the performance changes observed, indicate that an acceptable level of immune response was generated in pigs reared in the dirty nursery to make inferences into the affect of antibiotics and lysozyme on chronically immune stimulated pigs. In the current study, pigs consuming antibiotics or lysozyme had lower TNF-α, haptoglobin, and CRP, compared to control pigs. This was true of pigs chronically immune challenged or reared in the clean nursery. These data agree with others in that both antibiotics and lysozyme can ameliorate an immune response. Lee et al. (2012) observed lower haptoglobin levels in antibiotic fed pigs compared to nonmedicated controls. In addition, Nyachoti showed lower circulating TNF-α post challenge in pigs consuming lysozyme. Bothe of these studies used acute *Escherichia coli* challenges, but demonstrate that both antibiotics and lysozyme fed pigs have a reduced immune response when exposed to pathogens.

Similarly to our early work examining lysozyme in liquid diets for young pigs (May et al., 2012), pigs consuming lysozyme had less *Campylobacter* shedding compared to pigs consuming the control diet at the end of the study, regardless of immune status. Similarly, Maga et al. (2006) reported intestinal microflora of pigs that consumed pasteurized milk from hLZ transgenic animals to be markedly different from those that received milk from non-transgenic 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 non-transgenic 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 the control-fed group. Similarly, Nyachoti et al. (2011) observed decreased coliforms in mucosal scraping 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 due to 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* is, in part, due to changes in the gastrointestinal health (May et al., 2012; Oliver and Wells, 2013) and microflora by lysozyme that indirectly reduce *Campylobacter* colonization and shedding.

The use of subtherapeutic levels antibiotics in diets increase the performance of swine. However, the industry is under increased pressure to reduce or remove antibiotics from swine diets due the perceived danger of their use. This study confirms that lysozyme improves growth rates and feed efficiency in nursery diets. In addition, lysozyme, as well as antibiotics increased protein deposition at the expense of lipid accretion in the current study. An immune response in pigs redirects nutrients away from growth and toward the immune system, which decreases the efficiency of nutrient use. In the current study, both lysozyme and antibiotics decreased the severity of the immune response in pigs under a chronic immune challenge. In addition, pigs consuming lysozyme had a decreased prevalence of *Campylobacter* in the rectum after 28 days of treatment. Thus, we conclude that lysozyme is a suitable alternative to antibiotics in swine nursery diets, and that lysozyme ameliorated the effects of a chronic immune challenge.

Table 1. Composition and calculated analysis of the dietary treatments[1]

	Phase 1 (d 0 to 14)			Phase 2 (d 14 to 28)		
Item	Control	C + A	C + Lyso	Control	C + A	C + Lyso
Ingredients, %						
Corn	50.8	49.6	50.5	63.3	62.1	63.0
Soybean meal, 465 g/kg	24.3	24.4	24.3	26.5	26.6	26.6
Fish Meal	5.0	5.0	5.0	2.5	2.5	2.5
Blood meal	1.3	1.3	1.3	1.3	1.3	1.3
Whey	12.5	12.5	12.5	0.0	0.0	0.0
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0
Monocalcium Phosphate	0.8	0.8	0.8	1.2	1.2	1.2
Limestone	0.8	0.8	0.8	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.4	0.4	0.4
Zinc oxide	0.3	0.3	0.3	0.0	0.0	0.0
Vitamin premix[2]	0.3	0.3	0.3	0.3	0.3	0.3
Mineral premix[3]	0.2	0.2	0.2	0.2	0.2	0.2
Lysine HCl, 980 g/kg	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine, 985 g/kg	0.2	0.2	0.2	0.1	0.1	0.1
L-Threonine, 985 g/kg	0.2	0.2	0.2	0.1	0.1	0.1
CTC/Denagard[4]	0.0	1.0	0.0	0.0	1.0	0.0
Copper sulfate	0.0	0.1	0.0	0.0	0.1	0.0
Lysozyme[5]	0.0	0.0	0.3	0.0	0.0	0.3

Calculated analysis[5]						
ME, MJ/kg	14.4	14.3	14.4	14.4	14.3	14.4
CP, g/kg	210	210	210	209	208	209
TID lysine, g/kg	13.5	13.5	13.5	13	13	13
Ca, g/kg	8.7	8.7	8.7	8.1	8.1	8.1
Available P, g/kg	4.4	4.4	4.4	3.8	3.8	3.8

[1]Expressed on as-fed basis; A, antibiotics; Lyso, lysozyme; TID, true ileal digestible.

[2]Provided the following per kilogram of diet: vitamin A, 9000 IU; vitamin D_3, 200 IU; vitamin E, 19 IU; vitamin K, 2.2 mg; thiamine, 2.2 mg; riboflavin, 4.4 mg; niacin, 33 mg; pantothenic acid, 22 mg; vitamin B_{12}, 0.028 mg; vitamine B_6, 2.2 mg; folic acid, 1.35 mg; and biotin, 0.11 mg.

[3]Provided the following per kilogram of diet: Fe, 78 mg; Cu, 7 mg; Co, 0.80 mg; Zn, 168 mg; Mn, 60 mg; I, 0.79 mg; and Se, 0.13 mg.

[4]Chlortetracycline/Denagard, Provides a final concentration of 55 mg of CTC and 1.65 g Denagard per kg diet.

[5]Entegaurd, Neova Technologies, Inc.; provides a final concentration of 100 mg lysozyme/kg diet.

Table 2. Effect of an indirect immune challenge on pigs weaned at 26 d of age and fed control (C), C + antibiotics (C+A), or C + lysozyme (C+Lyso) diets for 28 d[1]

Variable	Clean Nursery			Dirty Nursery				*P*-values		
	Control	C + A	C + Lyso	Control	C + A	C + Lyso	SEM	Diet	Nursery	Diet x Nursery
Live weight, kg										
d 0	8.46	8.65	8.53	8.46	8.65	8.60	0.12	0.226	0.799	0.947
d 14	10.83	11.08	11.10	11.18	11.09	11.19	0.14	0.922	0.394	0.718
d 28	16.67	17.53	17.45	16.49	17.25	17.36	0.15	<0.001	0.715	0.752
ADG, kg/d										
d 0 to 14	0.138	0.172	0.170	0.163	0.174	0.179	0.018	0.048	0.980	0.768
d 14 to 28	0.415	0.462	0.471	0.401	0.444	0.439	0.014	<0.001	0.046	0.199
d 0 to 28	0.292	0.317	0.322	0.286	0.315	0.314	0.015	<0.001	0.109	0.426
ADFI, $kg \cdot pen^{-1} \cdot d^{-1}$										
d 0 to 14	3.57	3.78	3.42	3.52	3.57	3.26	0.19	0.486	0.530	0.962
d 14 to 28	8.70	7.24	7.29	8.88	7.95	7.22	0.32	0.477	0.612	0.827

d 0 to 28	6.25	5.55	5.36	6.15	5.76	5.24	0.38	0.112	0.866	0.870
G:F										
d 0 to 14	0.587	0.641	0.663	0.605	0.671	0.694	0.019	0.002	0.159	0.955
d 14 to 28	0.712	0.724	0.688	0.668	0.694	0.657	0.012	0.161	0.032	0.922
d 0 to 28	0.665	0.690	0.674	0.625	0.683	0.662	0.013	0.005	0.049	0.389
Days to Market[2]	96.4	92.1	92.8	98.1	93.6	93.4	0.3	0.041	0.086	0.682

[1]Values are least squares means; n = 16 per treatment.

[2]Days to reach 120 kg BW.

1

Table 3. Effect of an indirect immune challenge on the composition of the empty body and tissue accretion rates of pigs weaned at 26 d of age and fed control (C), C + antibiotics (C+A), or C + lysozyme (C+Lyso) diets for 28 d [1]

	Body composition, %			
Treatment	Protein	Lipid	Ash	Water
Initial				
d 0	14.9 ± 0.2	13.3 ± 0.2	4.5 ± 0.1	68.0 ± 0.2
Clean Nursery				
C, d 28	15.1 ± 0.2	9.9 ± 0.2‡	4.2 ± 0.1‡	71.1 ± 0.2‡
C + A, d 28	15.2 ± 0.2	8.9 ± 0.2‡†	4.1 ± 0.1‡	71.8 ± 0.2‡
C + Lyso, d 28	15.1 ± 0.2	9.2 ± 0.2‡†	4.1 ± 0.1‡	71.5 ± 0.2‡
Dirty Nursery				
C, d 28	15.0 ± 0.2	9.9 ± 0.2‡	4.2 ± 0.1‡	71.1 ± 0.2‡
C + A, d 28	15.2 ± 0.2	9.2 ± 0.2‡†	4.1 ± 0.1‡	71.6 ± 0.2‡
C + Lyso, d 28	15.2 ± 0.2	9.1 ± 0.2‡†	4.1 ± 0.1‡	71.6 ± 0.2‡

	Accretion rates, g/d			
Clean Nursery				
C, d 0 to28	45.3 ± 1.2	19.4 ± 1.9	11.9 ± 0.7	229.2 ± 6.7
C + A, d 0 to 28	51.0 ± 1.2†	16.9 ± 1.8‡	12.1 ± 0.4	239.8 ± 7.9
C + Lyso, d 0 to 28	47.7 ± 1.0†	16.2 ± 1.4‡	10.9 ± 0.6	235.5 ± 4.4
Dirty Nursery				
C, d 0 to 28	44.9 ± 1.3	18.9 ± 2.0	11.6 ± 0.6	224.5 ± 6.2
C + A, d 0 to 28	50.6 ± 1.1†	15.4 ± 1.8‡	11.8 ± 0.6	236.6 ± 5.5
C + Lyso, d 0 to 28	48.1 ± 1.5†	15.0 ± 1.6‡	10.8 ± 0.8	232.5 ± 6.0

[1]Values are least squares means ± SEM; n = 18 to 20 per treatment. For body composition: ‡Mean differs from d 0 ($P < 0.05$); and †Antibiotic and lysozyme differs from control, within nursery, ($P < 0.05$). No differences in composition due to nursery were observed. For accretion rates: ‡Mean differs from control, within nursery ($P < 0.05$); and †Mean tends to differ from control, within nursery ($P < 0.10$). No differences in composition due to nursery were observed.

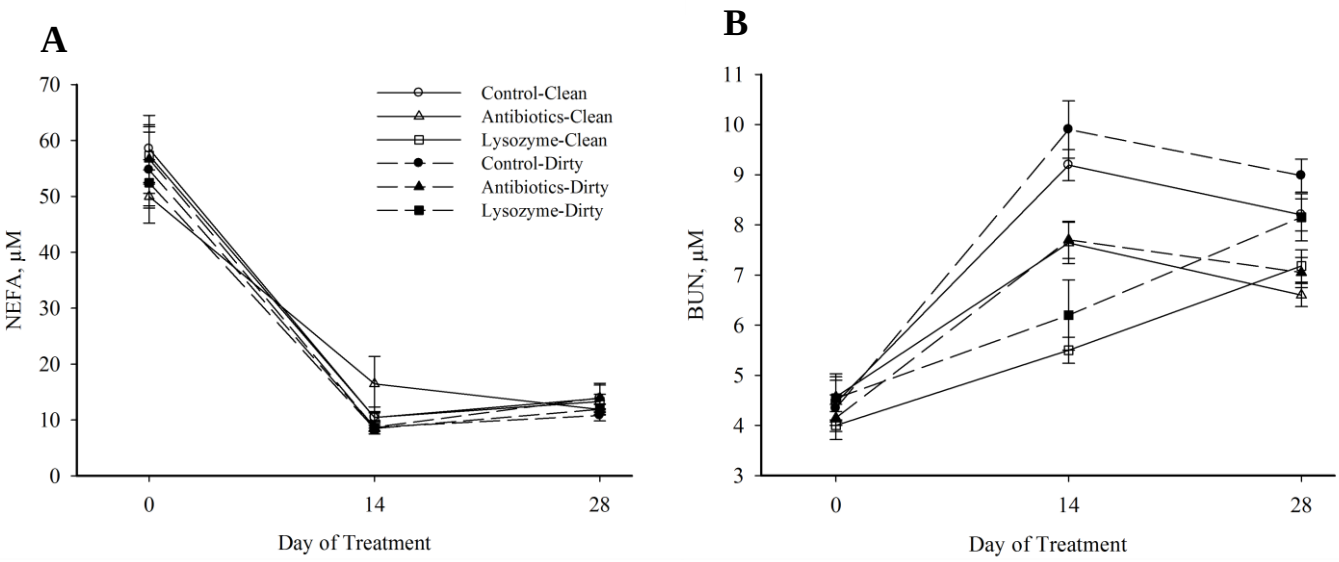


Figure 1. Effect of an indirect immune challenge and antibiotics or lysozyme in nursery pig diets on A) NEFA and B) blood urea N (BUN) in pigs weaned from the sow at 26 d of age. Values shown are means ± SEM; n = 31 or 32. Dietary treatment ($P > 0.90$) and nursery environment ($P > 0.31$) had no affect on circulating NEFA concentrations. NEFA decreased from d 0 to 14 ($P < 0.001$), but was similar between d 14 and d 28 ($P > 0.85$). Overall, BUN was greater in control pigs than both antibiotic and lysozyme fed pigs ($P < 0.01$). Antibiotic fed pigs had larger BUN than lysozyme fed pigs ($P < 0.10$). BUN increased with day ($P < 0.001$), but a dietary treatment x day interaction was observed ($P > 0.001$). Control fed pigs had a higher BUN on d 14, but there were no differences between dietary treatment on d 28 ($P > 0.14$). Pigs in the dirty nursery had higher BUN compared to pigs in the clean nursery($P < 0.05$).

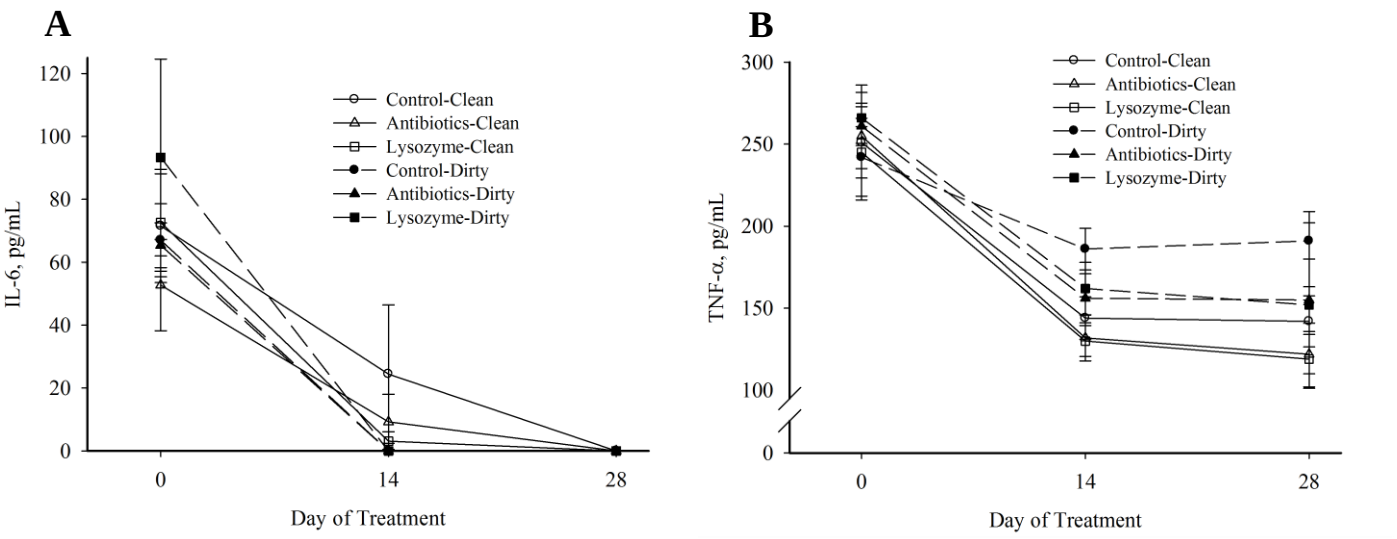
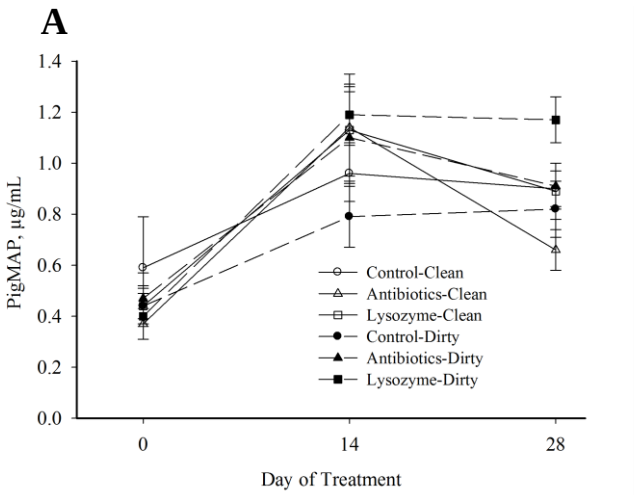
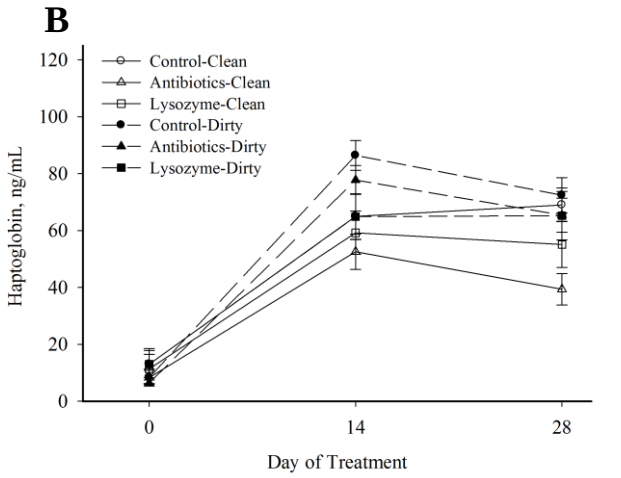


Figure 2. Effect of an indirect immune challenge and antibiotics or lysozyme in nursery pig diets on A) Interleukin-6 (IL-6) and B) Tumor necrosis factor-α (TNF-α) in pigs weaned from the sow at 26 d of age. Values shown are means ± SEM; n = 30 to 32. Dietary treatment ($P > 0.13$) and nursery environment ($P > 0.50$) had no affect on circulating NEFA concentrations and IL-6 decreased over the course of the experiment ($P < 0.001$) to undetectable levels at d 28. TNF-α decreased from day 0 to 14 of treatment ($P < 0.001$), but levels were similar between d 14 and d 28 ($P > 0.68$). TNF-α was lower in pigs consuming antibiotics or lysozyme compared to control pigs ($P < 0.04$), which was more evident in pigs reared in the dirty nursery environment (dietary treatment x nursery environment, $P = 0.003$).


A
PigMAP, µg/mL
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
14
28
Day of Treatment
Control-Clean
Antibiotics-Clean
Lysozyme-Clean
Control-Dirty
Antibiotics-Dirty
Lysozyme-Dirty


B
Haptoglobin, ng/mL
120
100
80
60
40
20
0
0
14
28
Day of Treatment
Control-Clean
Antibiotics-Clean
Lysozyme-Clean
Control-Dirty
Antibiotics-Dirty
Lysozyme-Dirty

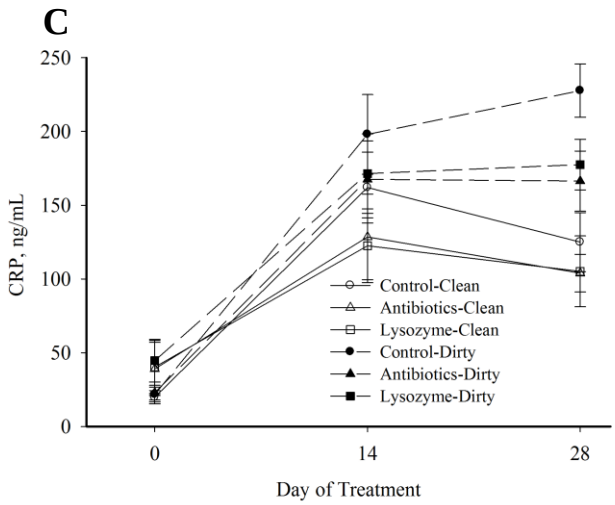

C
CRP, ng/mL
250
200
150
100
50
0
0
14
28
Day of Treatment
Control-Clean
Antibiotics-Clean
Lysozyme-Clean
Control-Dirty
Antibiotics-Dirty
Lysozyme-Dirty

Figure 3. Effect of an indirect immune challenge and antibiotics or lysozyme in nursery pig diets on A) pig major acute phase protein (PigMAP), B) haptoglobin, and C) C-reactive protein (CRP) in pigs weaned from the sow at 26 d of age. Values shown are means ± SEM; n = 30 to 32. PigMAP increased from d 0 to14 ($P < 0.001$), but was unchanged from d 14 to d 28 ($P > 0.24$). PigMAP was not affected by dietary treatment ($P > 0.20$) or nursery environment ($P > 0.97$). Haptoglobin ($P < 0.001$) and CRP ($P < 0.001$) both increased from d 0 to d14 of dietary treatment and were unchanged ($P > 0.62$) from d 14 to 28. Haptoglobin and CRP was lower in antibiotic and lysozyme fed pigs, compared to control pigs ($P < 0.02$). Pigs reared in the dirty nursery environment had higher haptoglobin ($P < 0.09$) and CRP ($P < 0.001$) concentrations.

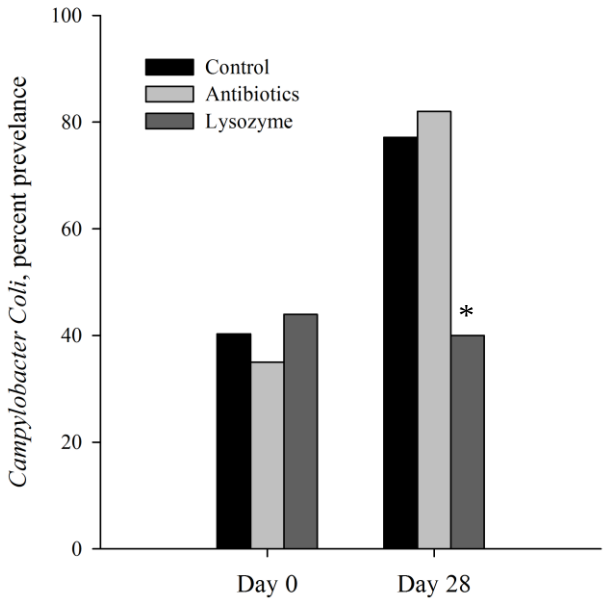


Figure 4. Effect of 28 d of antibiotics or lysozyme nursery pig diets on the percentage of rectal swab samples positive for Campylobacter in pigs weaned from the sow at 10 d of age (n = 200). Mean differs from antibiotic- and lysozyme-fed pigs, within day ($P < 0.01$).