RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Replacing dietary antibiotics with L-glutamine and synbiotics following weaning and transport in pigs – **NPB #18-020**

Investigator: Dr. Jay S. Johnson

Institution: USDA-ARS Livestock Behavior Research Unit

Co-Investigators: Drs. Brian Richert and Susan Eicher

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Industry Summary:

Immediately following weaning, pigs must adapt to multiple stressors such as transportation, handling, litter mixing, crowding, and delayed access to feed and water. Antibiotics have traditionally been used to help combat the negative impacts of weaning and transport in pigs. However, their use has been limited in the United States swine industry due to consumer pressures and concerns regarding antibiotic resistance. Therefore, nutraceutical supplements such as L-glutamine and synbiotics may be beneficial alternatives to improve piglet health and welfare following weaning and transport stress. The results from this study suggest that the provision of dietary antibiotics and 0.20% L-glutamine may improve some biological markers of immune function (e.g., haptoglobin). In addition, adding synbiotics (probiotic and prebiotic mix) may be of benefit to intestinal health through an increase in goblet cells that produce mucin to protect the intestine from pathogenic bacteria. Moreover, withholding dietary antibiotics resulted in an increase in gene expression for pro-inflammatory cytokines in the intestine. Despite the improvements in some metrics of intestinal health for pigs provided either nutraceuticals or antibiotics, an increase in growth performance was not detected, but this may be due to the lack of stress in the research setting relative to previous experiments. Taken together, results from this experiment demonstrate that both nutraceuticals and antibiotics can improve biomarkers of immune function and intestinal health, but growth performance improvements may not be apparent when pigs are transported and housed under non-stressful conditions.

Keywords: antibiotics, L-glutamine, pigs, synbiotics, transport, weaning

Scientific Abstract:

Dietary antibiotic use has been limited in United States swine production due to concerns regarding antibiotic resistance. However, this may negatively impact the health, productivity and welfare of pigs. Therefore, the study objective was to determine if combining dietary synbiotics and 0.20% L-glutamine would improve pig growth performance and intestinal health following weaning and transport when compared to traditionally used dietary antibiotics. Because previous research indicates that L-glutamine improves swine growth performance and synbiotics reduce enterogenic bacteria, it was hypothesized that supplementing diets with 0.20% L-glutamine (**GLN**) and synbiotics [**SYN;** 3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day)] would have an additive effect and improve pig performance and intestinal health over that of dietary antibiotics. Mixed sex pigs (N = 226; 5.86 ± 0.11 kg BW) were weaned (19.4 ± 0.2 d of age) and transported for 12 h in central Indiana. Pigs were blocked by BW and allotted to 1 of 5 dietary treatments (5 to 6 pigs/pen); antibiotics (**A**; chlortetracycline [441 ppm] + tiamulin [38.5 ppm]), no antibiotics (**NA**), GLN, SYN, or the NA diet with both the GLN and SYN additives (**GLN+SYN**) fed for 14 d. From d 14 post-weaning to the end of the grow-finish period, all pigs were provided common antibiotic-free diets. Data were analyzed using PROC MIXED in SAS 9.4. Overall, haptoglobin was greater ($P = 0.03$; 216%) in NA pigs compared to A pigs. A diet x day effect was detected where on d 13, GLN and A pigs tended to have reduced ($P = 0.07$; 75.2 and 67.3%, respectively) haptoglobin compared to NA pigs. On d 34, the jejunal goblet cell count per villi and per mm tended to be greater ($P < 0.08$; 71.4 and 62.9%, respectively) in SYN pigs compared to all other dietary treatments. Overall, jejunal mucosa TNFα gene expression tended to be greater ($P = 0.09$; 40.0%) in NA pigs compared to A pigs on d 34. In addition, jejunal mucosa TNFα gene expression tended to be greater ($P = 0.09$; 33.3, 41.2, and 60.0%, respectively) in GLN pigs compared to SYN, GLN+SYN, and A pigs on d 34. Although it was determined that some metrics of pig health were improved by the addition of GLN and SYN (i.e., haptoglobin, goblet cell count), overall there were very few differences detected between dietary treatments and this may be related to the stress-load incurred by the pigs.

Introduction

In United States swine production systems, weaning pigs is associated with social, environmental, and metabolic stress (Lallés et al., 2004) that is often exacerbated by transport to wean-to-finish facilities. This process increases cortisol levels (Moeser et al., 2007), decreases food intake (Brooks et al., 2001), and increases disease susceptibility (Deprez et al., 1986). As a result, weaning and transport stress decreases intestinal integrity and can cause pathological disorders (Spreeuwenberg et al., 2001) leading to reduced growth performance and increased morbidity and mortality (Jayaraman and Nyachoti, 2017). Therefore, developing mitigation strategies and improving upon current management practices is key to increasing pig health and growth performance post-weaning and transport.

Antibiotics have traditionally been used to help combat the negative impacts of weaning and transport in pigs. However, their use has been limited in the United States swine industry due to consumer pressures and concerns regarding antibiotic resistance (Smith et al., 2010). Therefore, nutraceutical supplements may be a beneficial alternative to improve pig health and welfare following weaning and transport stress. Specifically, L-glutamine, a conditionally essential amino acid, has been shown to improve pig health and growth performance similarly (Duttlinger et al., 2019) or better (Johnson and Lay, 2017) than dietary antibiotics when fed at

0.20% of the diet for 14 d following weaning and transport stress. Although supplementary L-glutamine may be an effective replacement for dietary antibiotics (Johnson and Lay, 2017; Duttlinger et al., 2019), it is currently unknown whether combining this nutraceutical with other supplements such as probiotics or prebiotics may have an additive effect to improve the efficacy compared to dietary antibiotics. The combination of probiotics and prebiotics (i.e., synbiotics) have been used to promote gastrointestinal balance and health in livestock species and have been considered potential alternatives to growth promoting antibiotics. Reports in pigs indicate that synbiotics can enhance immune function, improve performance (i.e., ADFI and ADG), and reduce diarrhea incidence (as reviewed by Cheng et al., 2014), and this is likely due to their reductive effects on pathogenic bacteria that promotes infection. However, it is currently unknown whether combining synbiotics with 0.20% L-glutamine in the diets of newly weaned and transported pigs will have an additive effect to improve health and growth performance. Therefore, the study objective was to determine if combining dietary synbiotics [**SYN;** 3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN] and 0.20% L-glutamine (**GLN**; Ajinomoto North America Inc., Raleigh, NC) would improve pig intestinal health and growth performance following weaning and transport when compared to traditionally used dietary antibiotics [**A**; chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)]. We hypothesized that supplementing diets with GLN and SYN immediately following weaning and transport would have an additive effect and improve pig performance and intestinal health over that of A.

Objective:

To determine if combining dietary synbiotics (Lactobacillus + FOS + β-glucan) and 0.20$ L-glutamine will results in a greater improvement in swine health and performance following weaning and transport in a production environment when compared to dietary antibiotics (CTC + tiamulin).

Materials & Methods:

General

All live animal procedures were approved by the Purdue University Animal Care and Use Committee (protocol #1801001678A002) and animal husbandry practices were in accordance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). Mixed-sex crossbred pigs [$N = 226$, 5.86 ± 0.11 kg initial BW; Duroc × (Landrace × Yorkshire)] were individually weighed 1 d prior to weaning and then transported on the day of weaning (19.4 ± 0.2 d of age) in central Indiana for 12 h in May 2018. Following transport, pigs were placed in their assigned pens and allotted to 1 of 5 dietary treatments with 8 pens per treatment, balanced for sex and litter. Each pen initially contained 5 to 6 pigs and provided 0.72 m^2 per pig, which was above the range of 0.16 to 0.37 m^2 per pig required for group-housed nursery pigs (3 to 27 kg BW; Federation of Animal Science Societies, 2010). Dietary treatments were 1) a diet containing no dietary antibiotics (**NA**), 2) the NA diet with GLN, 3) the NA diet with SYN, 4) the NA diet with the both the GLN and SYN additives (**GLN+SYN**), or 5) a diet containing A.

Transportation

Transportation procedures were performed as previously described (Duttlinger et al., 2019). Briefly, pigs were weaned and herded up a ramp (2.13 m long, 11° incline) into a gooseneck livestock trailer (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA) bedded with wood shavings, and transported for 12 h (approximately 819 km). Pig density in the trailer was 0.07 m^2 per pig, which was within the recommended range of 0.060 to 0.084 m^2 for 4.54 to 9.07 kg pigs, respectively (Federation of Animal Science Societies, 2010). Two data loggers (Hobo; data logger temperature/RH; accuracy ±0.20°C; Onset; Bourne, MA) were spaced evenly within the transport trailer to measure relative humidity (**RH**; 77.86 ± 0.44%) and ambient temperature ($\mathbf{T_A}$; 23.72 ± 0.10 °C) in 1-min intervals. Ventilation openings were adjusted based on the T_A (National Pork Board, 2015). Pigs were not provided feed or water during transport, and total transport time included loading the trailer, transport, unloading the trailer, and sorting pigs into their respective pens. The transport route consisted of approximately 50% two-lane roads and 50% four-lane roads and was 273 km in length. This route was completed 3 times with stops to refuel the truck each time that lasted approximately 10 min. After arrival at the wean-to-finish facility, pigs were individually weighed, sorted into their respective pens in a mechanically-ventilated wean-to-finish facility, and given access to dietary treatments.

Sentinel pigs

Six sentinel pigs (n = 3 gilts and 3 barrows) were transported to obtain descriptive data during the transport procedure. Blood samples were collected (BD vacutainers; Franklin Lakes, NJ) from all sentinel pigs via jugular venipuncture prior to transport and 24 h post-transport. Immediately following the 24 h blood collection, all sentinel pigs were euthanized for collection of jejunum and ileum tissue samples.

Nursery phase

Following weaning and transport, all pigs were placed in their respective pens. All pens contained a single-hole gravity feeder and nipple waterer to allow for *ad libitum* feed and water access. The nursery barn had slatted concrete floors and a shallow pit for manure storage. The room operated on mechanical ventilation using a 4-stage digital controller (Airstream TC5-2V25A, Automated Production Systems, Assumption, IL). During the nursery phase, the room average T_A and RH was measured using mounted data loggers (Hobo; data logger temperature/RH; accuracy ±0.20°C; Onset; Bourne, MA) and was 27.93 ± 0.04°C and 48.41 ± 0.59%, respectively.

Upon placement in their pens, pigs were assigned to dietary treatments for 14 d in 2 phases (Tables 1 and 2). Following the dietary treatment phase, all pigs were fed a common antibiotic-free diet from d 14 to 34 (end of the nursery phase; Table 1). All nursery diets were corn-soybean meal based, fed in 4 phases, and were formulated to meet or exceed the nutrient requirements of the pigs at a given phase (NRC, 2012; Table 1). Feeders and pigs were individually weighed in 7 d intervals throughout the nursery phase to determine ADG, ADFI, and G:F on a per pen basis.

Therapeutic antibiotic injections given to pigs throughout the nursery phase were recorded and classified according to the illness symptoms displayed as previously described (Duttlinger et al., 2019). Categories included: enteric challenge (e.g. scours or loose watery stool), respiratory challenge (e.g. coughing or labored breathing), lameness (e.g. carrying a limb,

difficulty walking, or swollen joints), unthriftiness (e.g. BW loss, poor gain, loss of body condition, or rough hair coat), and all other symptoms (e.g. side paddling associated with *Streptococcus suis* infection, skin infection, and abscess). The research farm staff were trained to identify and treat individual pigs and were blinded to dietary treatments. Treatment dose, product given, pig and pen identification, and reason for antibiotic was recorded and used for *post hoc* analysis.

Blood was obtained via jugular venipuncture from one pig per pen immediately post-transport, and at d 2, 13, and 32 post-weaning. The same pig was used for all blood drawings through d 13. For the d 32 post-weaning blood collection, a different pig in each pen was used. Sex of the selected pig was balanced across treatments within day.

On d 14 and 34 of the nursery phase, one pig per pen balanced by sex within each day was euthanized for tissue collection. A 15 cm section of the jejunum was collected either 1.2 m (d 14) or 1.8 m (d 34) proximal from the pyloric sphincter. In addition, a 15 cm ileal section was collected immediately prior to the ileo-cecal junction. Intestinal sections were rinsed with phosphate-buffered saline to remove digesta. Digesta was collected from the jejunum and ileum into 15 mL conical tubes and snap frozen in liquid nitrogen for later analysis of *Lactobacillus* counts. Seven cm sections of jejunum and ileum were placed into 10% neutral-buffered formalin for later histology. The remaining 7 cm sections were opened longitudinally and mucosa was collected by gently scraping the luminal surface with a glass slide, snap frozen in liquid nitrogen, and stored at -80 °C until further processing.

Grow-finish phase

On d 34 post-weaning and transport, pigs remained in their original wean-to-finish pens and were fed a common antibiotic free and corn-soybean meal-DDGS-based diet (Table 3) in 6 phases. During the grow-finish phase, the room average T_A and RH was measured using mounted data loggers (Hobo; data logger temperature/RH; accuracy ±0.20°C; Onset; Bourne, MA) and was 25.73 ± 0.07°C and 70.00 ± 0.32%, respectively. Pigs and feeders were individually weighed in 21 d intervals to obtain ADG, ADFI, and G:F. In addition, therapeutic antibiotic injection rate was recorded for the entire grow-finish phase using the same criteria as defined in the nursery phase.

Blood parameters

For sentinel pigs, blood was collected into lithium heparin (6 ml, BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ), EDTA (5 ml, BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ), and serum separation tubes (5 ml, BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ). For all other pigs, blood was collected into EDTA (5 ml, BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ) and serum separation tubes (5 ml, BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ). Sentinel pig blood in the lithium heparin tube was analyzed on site via a Vet Scan i-Stat1 machine with a CG8+ cartridge (model 300V; Abaxis Inc., Union City, CA) to quantify blood glucose and hematocrit levels. Serum separation and EDTA tubes were centrifuged at 1500 × g at 4 °C, and then serum and plasma were aliquoted and stored at -80 °C until further analysis.

Cortisol concentrations were analyzed via a commercial radioimmunoassay kit (ImmuChem Cortisol CT, MP Biomedicals LLC, Solon, OH) according to manufacturer's instructions. The intra-assay coefficient of variation (**CV**) was 5.64%, and the inter-assay CV was 7.90%. Haptoglobin levels were determined with a commercial kit (Porcine Haptoglobin

ELISA Assay, ALPCO, Salem, NH) using a 1:10,000 dilution as per the manufacturer's instructions. The intra-plate CV was 1.92% and the inter-plate CV was 19.25%.

To analyze leukocyte counts, blood was smeared on slides, stained (Hema 3 Stat Pack, Fisher Scientific, Pittsburgh, PA), and cover slipped. Blood smears were analyzed by one trained individual who was blinded to dietary treatments. Leukocytes were counted under 100X magnification and percent neutrophils and percent lymphocytes were identified according to nuclear and cytoplasmic staining and morphology. One hundred leukocytes were counted for each slide, and abundance of neutrophils and lymphocytes were expressed as a percentage of total cells counted. The neutrophil to lymphocyte ratio was then calculated as a biomarker of the physiological stress response of pigs (McGlone at al., 1993) by dividing the number of neutrophils by the number of lymphocytes on each slide.

Intestinal morphology

Histological analyses were performed as previously described (Johnson et al., 2016). Briefly, jejunum and ileum tissue samples were placed in a 10% formalin solution for 24 h and then referred to the Purdue University Histology and Phenotyping Laboratory for sectioning (10-µm thickness) and staining with toluidine blue with fast green counter stain. For histological measures, correct identification of goblet cells and the location of the crypt/villi interface were verified by a pathologist prior to making histological measurements. To avoid selection bias (e.g. selection of large prominent villi only), a random location on the slide was selected at 4 or 10X magnification depending on villi height, and the first 6 villi meeting the selection criteria were imaged (MoticamBTW, Motic, Hong Kong, China) for later analysis. ImageJ 1.47v software (National Institutes of Health; Bethesda, MD) was used to measure mean villus height (µm) and mean crypt depth (µm) within each image. The number of goblet cells present on each measured villi were also counted. Goblet cell count for each villi was divided by the corresponding villi height to obtain goblet cell count per mm of villi (Horn et al., 2009). All histology measures were averaged per pig prior to data analysis.

Gene expression

Using a commercial kit, mRNA was extracted (RNeasy Mini Kit, QIAGEN, Germantown, MD) and reverse transcription was performed (Invitrogen TaqMan Reverse Transcription Reagents, ThermoFisher Scientific, Carlsbad, CA) from intestinal mucosal scrapings for gene expression according to the manufacturers' instructions. Real-time PCR was performed using a master mix (Invitrogen TaqMan Universal PCR Master Mix, ThermoFisher Scientific, Carlsbad, CA) and primer and probe sets (Invitrogen TaqMan Gene Expression Assays combined primer and probe sets, ThermoFisher Scientific, Carlsbad, CA) for the following genes (primer and probe sets identified with assay ID): tumor necrosis factor-α (**TNFα**; assay ID Ss03391318_g1), interleukin-8 (**IL-8**; assay ID Ss03392437_m1, zonula occludens-1 (**ZO-1**; assay ID Ss03373514_m1), glucagon-like peptide 2 receptor (**GLP2R**; assay ID Ss04322851_m1), claudin-1 (**CL-1**; assay ID Ss04246284_s1) and 18S ribosomal RNA (**18S**; assay ID Hs03003631_g1). Results were quantified by the standard curve method, and data are expressed as the relative abundance of the genes of interest to the reference gene (18S).

Bacteriological analysis

Total *lactobacillus* was calculated by spread-plating (100 uL) on a Ragosa agar (Becton, Dickinson and Company, Sparks, MD) and the plates were incubated at 37°C for 24 h. Based on

previously published studies (Wall et al., 2010; Saez et al., 2011; Rostagno et al., 2011; Walsh et al., 2012), the challenge model used a marked strain (a nalidixic acid–resistant Salmonella Typhimurium) to allow for a convenient and reliable recovery method.

Statistics

Data were analyzed as a randomized complete block design with initial BW as the blocking factor. Pen was considered the experimental unit. Data were primarily analyzed via Proc GIMMIX of SAS 9.4 (SAS Institute Inc., Carry, NC), with Proc MIXED used to analyze for influential outliers via Cook's D test (Cook, 1977). Dietary treatment was considered a fixed effect while block was considered a random effect. Data collected over time were analyzed with repeated measures with the time unit as a fixed effect, the subject as pen(treatment*block), and using the optimal covariance structure for each response variable as determined by the BIC goodness of fit criteria (Littell et al., 1998). A Kenward-Rogers degrees of freedom correction was applied to all repeated measures analysis via the ddfm=kr option of the model statement (Kenward and Roger, 1997). Means of significant main effects or interactions were separated with a Tukey's adjustment. Neutrophil: lymphocyte and therapeutic injection rate data were log transformed prior to analysis to meet the assumptions of normality and homogeneity of variances but results are presented as arithmetic means for ease of interpretation. Post-transport blood samples were used as a covariate to analyze haptoglobin data. *Lactobacillus* data were log transformed to meet normality assumptions, and back-transformed means are reported. Results are presented as least square means ± 1 SEM unless otherwise stated. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

Results:

Sentinel data

All sentinel data are for descriptive purposes only. Neutrophil to lymphocyte ratio, blood cortisol, blood glucose, and blood hematocrit concentrations during pre-transport and post-transport are presented in Table 4.

Growth performance

Nursery phase

No growth performance differences were observed with any comparison ($P > 0.05$) during the nursery phase (Table 5).

Grow-finish phase

No growth performance differences were observed with any comparison ($P > 0.05$) during the grow-finish phase (Table 5).

Therapeutic injections

Nursery phase

From d 15 to 34, lameness treatments tended to be greater ($P = 0.08$; 5.0%) in NA pigs compared to all other dietary treatments (Table 6). No other therapeutic injection differences were observed with any comparison ($P > 0.05$) during the nursery phase (Table 6).

Grow-finish phase

No therapeutic injection differences were observed with any comparison ($P > 0.05$) during the grow-finish phase (Table 6).

Blood parameters

Haptoglobin tended to be the greatest ($P = 0.07$; 754.52 ± 128.51) in NA pigs on d 6 and the least (89.18 ± 119.94) in A pigs on d 6 (Fig. 2). On d 13, GLN and A pigs tended to have reduced ($P = 0.07$; 75.2 and 67.3%, respectively) haptoglobin compared to NA pigs (Fig. 2). Overall, haptoglobin was greater ($P = 0.03$; 216%) in NA pigs compared to A pigs (Fig. 2). No other blood parameter differences were observed ($P > 0.05$) with any comparison (Fig. 1, 2, 3).

Intestinal morphology

On d 34, the jejunal goblet cell count per villi and per mm tended to be greater ($P < 0.08$; 71.4 and 62.9%, respectively) in SYN pigs compared to all other dietary treatments (Table 7). No other jejunal morphological differences were observed ($P > 0.05$) with any comparison (Table 7).

No ileum morphological differences were observed ($P > 0.05$) with any comparison (Table 7).

Gene expression

Overall, jejunal mucosa TNFα gene expression tended to be greater ($P = 0.09$; 40.0%) in NA pigs compared to A pigs on d 34 (Table 8). In addition, jejunal mucosa TNFα gene expression tended to be greater ($P = 0.09$; 33.3, 41.2, and 60.0%, respectively) in GLN pigs compared to SYN, GLN+SYN, and A pigs on d 34 (Table 8). No other jejunal mucosa gene expression differences were observed ($P > 0.05$) with any comparison (Table 8).

No ileal mucosa gene expression differences were observed ($P > 0.05$) with any comparison (Table 8).

Discussion:

Pigs are exposed to multiple stressors throughout their lifespan (i.e., weaning and transport), which threaten their health, performance, and welfare (as reviewed by Sutherland et al., 2014) and reduce intestinal barrier function (Hu et al.. 2013; Wang et al., 2015). Because the intestine is the first line of defense between the pig and their environment (Pitman et al., 2000), maintaining intestinal health and function is paramount to positive swine production and welfare outcomes. Traditionally, A have been used in swine production systems to improve intestinal health and reduce disease susceptibility (Pluske et al., 2013). However, concerns about antibiotic resistance have limited their use, thereby necessitating the development and evaluation of effective alternatives such as SYN to improve immune function and growth rate (Krause et al., 2010; Roselli et al., 2017) and GLN to improve post-weaning and transport growth performance (Johnson and Lay, 2017; Duttlinger et al., 2019) and increase biomarkers of improved intestinal barrier function (e.g., tight junction protein abundance; Wang et al. 2015). However, in the present study, no differences related to intestinal barrier function were detected for GLN, SYN, or A-fed pigs relative to NA-fed pigs. While the lack of intestinal barrier function differences for SYN pigs were expected because SYN functions through modulating the composition of the gastrointestinal tract, specifically the microflora, by altering levels of bacteria (Roberfroid,

1998), it was surprising that improvements were not detected for GLN pigs considering the aforementioned increases in tight junction protein abundance for GLN when compared to NA pigs (Wang et al., 2015). However, this discrepancy may be due to either study design differences or tissue collection time points. Previous research indicates that the optimal inclusion level of GLN for improving markers of intestinal barrier function is 1.00% as-fed (Wang et al., 2015), whereas in the present study, GLN was included at only 0.20% as-fed. As a result, it is possible that had greater levels of GLN been included in the diet, an increase in tight junction protein gene expression would have been detected. Alternatively, the lack of differences may be explained by the tissue collection timing because in the previous study, intestinal measures were taken on d 7 post-weaning (Wang et al., 2015), and in the present study they were taken on d 14. Therefore, had measures been taken closer to the initial insult (e.g., weaning and transport), the diet-induced improvements in intestinal barrier function could have been more apparent.

Previous studies have demonstrated improvements in villus height, crypt depth, and/or villus height:crypt depth in weaned pigs supplemented with either GLN (Wu et al., 1996; Lee et al., 2003; Hsu et al., 2010; Wang et al., 2015; Johnson and Lay, 2017), A (Johnson and Lay, 2017), or SYN (Marinho et al., 2007; Wang et al., 2018). This response is generally attributed to GLN enhancing the maturation of intestinal crypt cells (Wu et al., 1996), SYN producing short-chain fatty acids to improve digestion and absorption (Wang et al., 2018), and A reducing pathogen colonization (Pluske et al., 2002). However, despite these data, no dietary treatment differences related to intestinal morphology were detected in the present study. Although specific reasons for this discrepancy are currently unclear, it may be due to either study design differences, level of GLN inclusion, type of SYN used, or tissue collection timing. For example, the majority of studies that detect GLN-induced improvements in morphological markers of intestinal health used either 1.00% GLN as-fed (Wang et al., 2015; Lee et al., 2003; Wu et al., 1996) or 2.00% GLN as-fed (Hsu et al., 2010). As for the one study to our knowledge that detected intestinal morphology improvements using 0.20% GLN (Johnson and Lay, 2017), a simulated transport under heat stress conditions was used and piglets were housed individually, which may have altered the response to GLN supplementation relative to the present study. During stress, the requirements for GLN exceeds the ability of the body to produce sufficient amounts by itself (Lacey et al., 1990), and GLN supplementation may be necessary to meet the animal's requirements. Because the stress load in the present study was not significant (i.e., comfortable transport temperature, greater nursery pen space per pig than commercial conditions, lack of a neutrophil:lymphocyte response over time), it is possible that the GLN requirements by the pigs were being met by endogenous production and that supplementing GLN was not necessary. Alternatively, several studies using GLN collected intestinal tissue at d 7 post-weaning (Wang et al., 2015), thus it is possible that if intestinal tissue had been collected sooner than 14 d in the present study, differences would have been detected. In addition, the effects of SYN supplementation are not always consistent and intestinal morphology outcomes may depend on the type and quantity of pre- and probiotics used as some studies have not observed any intestinal morphology differences for pigs supplemented with SYN following weaning (Shim et al., 2005; Mair et al., 2010). Furthermore, the effects of dietary A supplementation on intestinal morphology can vary depending on chemical composition, bacterial spectrum of the intestine, absorption patterns, and the pathogen load of the environment (as reviewed by Cromwell, 2002). Therefore, either the reduced stress-load or the type and quantity of SYN used in the present study may have contributed to the lack of a dietary treatment response on intestinal morphology.

Although no differences related to villus height or crypt depth were detected, on d 34 goblet cells tended to be increased in SYN pigs relative to all other dietary treatments. Although no studies to our knowledge have associated SYN supplementation with increased goblet cell count in pigs, previous research demonstrates increased mucin production in humans and rats provided probiotics (Mack et al., 1999; Caballero-Franco et al., 2007). Because goblet cells produce mucin, which protects against pathogens and prevents intestinal disease (Kim and Ho, 2010), it is possible that SYN caused an increase in goblet cell production as a mechanism to increase mucin production by the intestine. However, this hypothesis would have to be proven in subsequent experiments.

The process of weaning and transport of pigs causes immune system activation and the production of pro-inflammatory cytokines (Pié et al., 2004; Hu et al., 2013). However, this response may be mitigated by the addition of GLN because it is an immunomodulator that inhibits the production of pro-inflammatory cytokines in pigs (Yi et al., 2005; Jiang et al., 2009), and previous research demonstrates a reduction in circulating TNFα for pigs fed GLN and A diets after weaning and transport compared to NA-fed pigs (Duttlinger et al., 2019). Despite this however, in the present study, no dietary treatment-related cytokine gene expression differences were detected in the jejunum or ileum during the diet treatment period. Although this response confirms previous reports in pigs fed SYN (Mair et al., 2010), it is surprising that no GLN or A diet differences were observed considering the fact that several studies report decreased cytokine production for GLN-fed (Yi et al., 2005; Jiang et al., 2009; Duttlinger et al., 2019) or A-fed (Song et al., 2013; Oliver et al., 2014) pigs. While reasons for this discrepancy are currently unclear, the lack of differences for GLN treated pigs may be related to the stress-load incurred as GLN treated pigs in previous reports were subjected to lipopolysaccharide (Jiang et al., 2009), Escherichia coli K88+ (Yi et al., 2005), or heat stress (Duttlinger et al., 2019) challenges during the weaning process and the use of GLN by immune cells is significantly elevated during times of infection or high catabolism (Curi et al., 1986; Newsholme et al., 1987). Therefore, because pigs in the present study were weaned and transported under relatively benign conditions, and likely were not suffering from a high stress-load (as indicated by a lack of neutrophil:lymphocyte differences), it is likely that the stress-load was not harsh enough to elicit a dietary treatment response from GLN, and previous studies have observed no cytokine differences in pigs that were simply weaned and then fed GLN (Johnson et al., 2006). Furthermore, the lack of cytokine differences related to the A treatment may be due to the fact that no therapeutic antibiotics treatment differences between dietary treatments were detected indicating an overall lack of illness, thereby reducing the pigs' requirements for A to alleviate immune-challenges as previously described (as reviewed by Cromwell, 2002).

Although no diet treatment-related cytokine differences were detected on d 14 during the diet treatment period, jejunal TNFα gene expression tended to be greater for GLN compared to SYN, GLN+SYN, and A pigs on d 34 during the common diet period. The greater cytokine gene expression for GLN pigs during the common diet period was unexpected as others have reported no differences in circulating TNFα levels during the common diet period for GLN pigs compared to A or NA pigs (Duttlinger et al., 2019). However, because only a tendency was detected, and all pigs had been off their dietary treatments for 20 d when intestinal tissue was obtained for these measures, it is unlikely that this tendency was related to the dietary treatment. Furthermore, since there were no therapeutic injection differences between dietary treatments, the relationship between GLN pigs and TNFα during the common diet period is unknown and should be further investigated.

Haptoglobin is an acute phase protein that is released in response to cytokine activation (González-Ramón et al., 2000), and may be used as an indicator of health status (Harding et al., 1997) and as an immune biomarker (i.e., biomarker of non-specific infection or inflammation) to assess the efficacy of feed additives in pigs (Saco et al., 2010). In the present study, serum haptoglobin levels were reduced overall for A compared to NA pigs and tended to be reduced for both A and GLN pigs compared to NA pigs at the end of the diet treatment period. These data may indicate that A and GLN treatments effectively improved immune status of the pigs as previously described (Fraile et al., 2009). The reductive effects of GLN on haptoglobin was likely in response to the effects of GLN as an immunomodulator that reduces the production of pro-inflammatory cytokines in pigs (Yi et al., 2005; Jiang et al., 2009). As for the A treatment, previous research demonstrates that A administration reduces cytokine levels (Song et al., 2013; Oliver et al., 2014) and this may be a mechanism by which haptoglobin levels were reduced in the present study. Although GLN and A treatments were effective in reducing haptoglobin levels compared to the NA treatment, no differences were detected when GLN was combined with SYN. Although this is somewhat surprising, it should be noted that although no significance or tendencies were detected, haptoglobin was reduced numerically by 37.1% relative to the NA-fed pigs. Therefore, it is possible that if more blood samples were taken or a greater number of experimental units had been used, then differences may have been detected. Regardless, the reduction in haptoglobin levels for GLN and A pigs may indicate that even during times of low stress, these dietary additives can be effective in improving the immune status of newly weaned and transported pigs. However, more research should be conducted to confirm this hypothesis.

In pigs, growth performance is closely linked to gastrointestinal function (Pluske, 2013). In general, a decrease in intestinal health (i.e., increased permeability and inflammation) is associated with reduced growth rate (Shen et al., 2009; Li et al., 2012). Therefore, because no dietary treatment-related differences in gastrointestinal function were detected in the present study, it is unsurprising that no performance differences were observed. In previous studies, improvements in growth performance have been detected in pigs provided A (Wan et al., 2016; Waititu et al., 2016; Johnson and Lay, 2017; Duttlinger et al., 2019), GLN (Johnson and Lay, 2017; Duttlinger et al., 2019; Wu et al., 1996), or SYN (Piva et al., 2005; Wang et al., 2018). However, this response has generally been accompanied by improvements in intestinal health and function including morphological markers of intestinal health (Wu et al., 1996; Wan et al., 2016; Wang et al., 2018; Johnson and Lay, 2017), improved intestinal barrier function (Wang et al., 2015), and a reduction in inflammatory biomarkers (Song et al., 2013; Oliver et al., 2014; Duttlinger et al., 2019). In addition to the effects of intestinal function, the reduced stress-load in the present study may have played a role in the lack of dietary treatment growth performance differences. In the case of GLN, it is well-established that under normal homeostatic conditions, pigs can synthesize enough GLN to meet its needs (Wu et al., 1995). During times of stress (i.e., early weaning, diarrhea challenges) however, endogenous GLN levels may be compromised and pigs may require supplementation to meet their requirements (Lobley et al., 2001). For example, in previous studies where growth performance differences were detected for newly weaned and transported pigs supplemented with 0.20% GLN compared to NA, pigs were subjected to heat stress (Johnson and Lay, 2017; Duttlinger et al., 2019) whereas pigs in the present study were weaned and transported under mild environmental conditions. Furthermore, because pen crowding compromises growth performance (Smith et al., 2004; Oh et al., 2010) and increases stress (Sutherland et al., 2010) in newly weaned pigs, this may explain the lack of growth performance differences since pigs in the present study, pigs had 60% more pen space during the

nursery phase compared to the Johnson and Lay (2017) study, and 243% more pen space during the nursery phase compared to the Duttlinger et al. (2019) study. The increased pen space allocation in the present study may also explain the lack of growth performance improvements for A compared to NA pigs since previous reports indicate that the need for A is reduced by improving pen space (NCR-89 Committee on Confinement Management of Swine, 1986). While the lack of growth performance differences for GLN and A pigs may be explained by the total stress-load incurred, reasons why no improvements were detected for SYN pigs are unclear. Some reports indicate that SYN supplementation can improve G:F (Piva et al., 2005), while others observed an increase in BW gain for SYN supplemented pigs (Shim et al., 2005). However, the discrepancies between the current study and the previous reports (Piva et al., 2005; Shim et al., 2005) may be due to differences in the inclusion levels or types of SYN compared to the current study and how this correlates with growth performance outcomes should be investigated in future research.

Therapeutic antibiotic injections are used to treat diseases in pigs (Rosengren et al., 2008) and the therapeutic antibiotic treatment rate may be used as an indicator of illness (Duttlinger et al., 2019). In the present study, no dietary treatment-related therapeutic treatment differences were detected for diseases related to intestinal health (i.e., enteric challenges). Although a tendency was detected where NA pigs were treated for lameness more often compared to all other dietary treatments during the common diet period, this increase may not have been directly related to the dietary treatment as feed additives would likely have no impact on motor function. Interestingly, the primary reason for therapeutic treatments during the first 14 d of the nursery phase in the present study was for enteric health challenges, with roughly 41% of all pigs being given therapeutic treatments compared to an average of 5% as seen in a previous study (Duttlinger et al., 2019). Therefore, is possible that the high percentage of therapeutic treatments in the present study may have masked any dietary treatment differences that could have been present. Reasons for the increased overall treatment rate in the present study may be related to the fact that the caretaker to pig ratio is generally increased in research settings, which can improve individualized pig care. Therefore, had this study been performed in a commercial facility, it is possible that results would have been more consistent with previous studies where pigs were provided A, GLN, or SYN.

These data suggest that weaning and transport stress can have negative effects on the health of pigs. Differences between studies measuring intestinal health and growth performance in pigs may be due to weaning, pen space, and transport conditions. Other potential explanations for the inconsistency in results using antibiotic alternatives may include differences in management practices, pig microbiome, and pig immune status (Heo et al., 2013; Pluske et al., 2018). In future studies, other measures of pig welfare, such as skin lesions, body temperature, and mortality rates may be useful when developing antibiotic alternatives in swine production. Moreover, in developing antibiotic alternatives, it may be necessary to understand how early life stressors, such as weaning and transport, effect long-term immune function to promote prolonged intestinal health in pigs.

Conclusion:

Weaning and transport are one of the most stressful phases of a pig's life and A have traditionally been used to improve pig health, welfare, and performance during this process. Currently, antibiotic use is being limited in United States swine production; therefore, the evaluation and development of effective alternatives is paramount for ensuring positive animal

welfare outcomes. Previous research has determined that the provision of supplementary GLN or SYN may be effective in improving growth performance and intestinal health of newly weaned pigs. However, it was unknown whether a combination of GLN and SYN would provide additional benefits and it was hypothesized that combining GLN and SYN would improve pig growth performance and health to a greater extent than A following weaning and transport. Although it was determined that some metrics of pig health were improved by the provision of GLN and SYN (i.e., haptoglobin, goblet cells), overall there were very few differences detected between dietary treatments. Although reasons for the lack of differences are currently unclear, it may be due to either the stress-load incurred by the pigs in the present study or the provision of therapeutic antibiotic treatments in a research setting that mitigated any dietary impact that would have been present. While the results from this experiment do not prove our hypothesis, they provide valuable information about factors to consider in the design of future experiments related to evaluating antibiotic alternatives and potentially the role of the stress-load on the efficacy of nutritional supplements in pigs.

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Table 1. Composition of nursery diets

Item	Phase 1[1]	Phase 2[2]	Phase 3[3]	Phase 4[4]
Ingredient, % as fed				
Corn	31.09	37.85	49.22	55.30
SBM, 48% CP	13.90	17.95	20.80	22.81
DDGS	-	-	7.50	15.00
Soybean oil	5.00	5.00	3.00	-
Choice white grease	-	-	-	3.00
Limestone	0.80	0.74	1.08	1.48
Monocalcium phosphate	0.38	0.49	0.07	0.51
Vitamin premix[5]	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.13	0.13	0.13	0.13
Selenium premix[7]	0.05	0.05	0.05	0.05
Phytase[8]	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.30	0.35
Plasma protein	6.50	2.50	-	-
Spray dried blood meal	1.50	1.50	-	-
Soy concentrate	4.00	3.00	2.50	-
Select menhaden fish meal	5.00	4.00	4.00	-
Dried whey	25.00	25.00	10.00	-
Lactose	5.00	-	-	-
Lysine-HCl	0.07	0.20	0.34	0.48
DL-Methionine	0.22	0.23	0.16	0.13
L-Threonine	0.04	0.09	0.12	0.14

L-Tryptophan	0.01	0.02	0.03	0.03
Zinc oxide	-	-	0.28	-
Copper sulfate	-	-	-	0.08
Banminth 48[9]	-	-	-	0.10
Clarifly, 0.67%[10]	0.14	0.09	0.08	0.07
Calculated chemical composition				
ME, kcal/kg	3545.00	3520.00	3429.00	3407.00
CP, %	24.62	22.88	21.81	20.05
SID Lys, %	1.55	1.45	1.30	1.15
Ca, %	0.90	0.85	0.80	0.75
Avail. P, %	0.60	0.55	0.40	0.35

[1]Fed d 0 to 7 post-weaning and transport.
[2]Fed d 7 to 14 post-weaning and transport.
[3]Fed d 14 to 21 post-weaning and transport.
[4]Fed d 21 to 35 post-weaning and transport.
[5]Provided per kilogram of the diet: vitamin A, 6,614 IU; vitamin D_3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg.
[6]Provided available minerals per kilogram of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg.
[7]Provided 0.3 ppm Se.
[8]Provided 600 FTU per kg of the diet.
[9]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet.
[10]Clarifly (Central Life Sciences, Schaumburg, IL) provided 9.5 ppm (Phase 1), 6.1 ppm (Phase 2), 5.4 ppm (Phase 3), and 4.7 ppm (Phase 4) diflubenzuron in the diet.

Table 2. Analyzed chemical composition of treatment phase nursery diets on an as-fed basis

	Phase 1[1]					Phase 2[2]				
Item	NA[3]	GLN[4]	SYN[5]	GLN+SYN[6]	A[7]	NA	GLN	SYN	GLN+SYN	A
DM, %[8]	90.80	91.10	90.60	90.70	91.30	89.90	90.10	90.40	89.50	89.50
CP, %[8]	23.50	24.40	23.50	24.20	24.10	22.10	22.20	22.30	22.50	22.20
Chlortetracycline, ppm[9]	-	-	-	-	346.20	-	-	-	-	373.00
Tiamulin, ppm[10]	-	-	-	-	34.70	-	-	-	-	33.10
Total Glu, %[8]	3.60	3.80	3.70	3.80	3.70	3.50	3.80	3.80	3.80	3.70
Total Lys, %[8]	1.60	1.60	1.60	1.58	1.63	1.46	1.42	1.51	1.43	1.49
Total Met, %[8]	0.50	0.50	0.50	0.52	0.52	0.48	0.47	0.48	0.47	0.48
Total Met + Cys, %[8]	0.90	0.94	0.90	0.91	0.93	0.8	0.81	0.82	0.82	0.83
Total Thr, %[8]	1.09	1.12	1.10	1.13	1.15	1.01	1.02	1.03	0.98	0.99
Total Trp, %[8]	0.32	0.32	0.31	0.32	0.33	0.28	0.28	0.30	0.30	0.28
Total Val, %[8]	1.17	1.17	1.19	1.19	1.22	1.04	1.06	1.09	1.05	1.08
Total Ile, %[8]	0.88	0.89	0.90	0.89	0.92	0.85	0.85	0.88	0.84	0.86
Total Arg, %[8]	1.25	1.26	1.23	1.25	1.31	1.19	1.23	1.28	1.22	1.24

[1]Phase 1 was fed from 0 to 7 d post-weaning and transport.
[2]Phase 2 was fed from 7 to 14 d post-weaning and transport.
[3]Pigs provided a diet with no dietary antibiotics.
[4]Pigs provided a diet with 0.20% L-glutamine (Ajinomoto North America Inc., Raleigh, NC).
[5]Pigs provided a diet with synbiotics [3 strains of *Lactobacillus* (1.2 x 10^9 cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN].
[6]Pigs provided a diet with 0.20% L-glutamine and synbiotics.
[7]Pigs provided a diet with dietary antibiotics [chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)].

[8]Samples submitted to Ajinomoto Heartland Inc., Chicago, IL.
[9]Samples submitted to Zoetis, Parsippany, NJ.
[10]Samples submitted to Elanco Animal Health, Greenfield, IN.

Table 3. Composition of grow-finish diets

Item	Phase 1[1]	Phase 2[2]	Phase 3[3]	Phase 4[4]	Phase 5[5]	Phase 6[6]
Ingredient, % as fed						
Corn	59.20	60.89	61.51	64.64	73.64	67.91
SBM, 48% CP	15.50	10.00	4.65	1.76	0.55	11.45
DDGS	20.00	25.00	30.00	30.00	22.50	15.00
Choice white grease	2.00	1.00	1.00	1.00	1.00	2.50
Limestone	1.54	1.52	1.45	1.34	1.23	1.36
Monocalcium phosphate	0.20	0.05	-	-	-	0.10
Vitamin premix[7]	0.15	0.14	0.13	0.12	0.10	0.13
Trace mineral premix[8]	0.10	0.09	0.08	0.07	0.05	0.08
Selenium premix[9]	0.05	0.05	0.05	0.05	0.03	0.05
Phytase[10]	0.10	0.10	0.05	-	-	0.10
Salt	0.35	0.35	0.30	0.30	0.25	0.30
Lysine-HCl	0.50	0.52	0.54	0.50	0.44	0.45
DL-Methionine	0.08	0.05	0.02	-	-	0.10
L-Threonine	0.13	0.12	0.12	0.10	0.09	0.16
L-Tryptophan	0.03	0.04	0.05	0.05	0.04	0.03
Paylean 2.25[11]	-	-	-	-	-	0.15
Availa Zn 120[12]	-	-	-	-	-	0.04
Clarifly[13]	0.07	0.09	0.07	0.08	0.09	0.10
Calculated chemical composition						
ME, kcal/kg	3363.00	3329.00	3342.00	3347.00	3362.00	3405.00
CP, %	18.60	16.80	15.70	14.50	12.60	15.40
SID Lys, %	1.00	0.90	0.80	0.70	0.60	0.85

Ca, %	0.70	0.65	0.60	0.55	0.50	0.60
Avail. P, %	0.30	0.29	0.27	0.23	0.18	0.25

[1]Fed from the end of the 5-week nursery phase (d 0) to d 26 of the grow-finish phase.
[2]Fed d 26 to 47 of the grow-finish phase.
[3]Fed d 47 to 68 of the grow-finish phase.
[4]Fed d 68 to 89 of the grow-finish phase.
[5]Fed d 89 to 110 of the grow-finish phase.
[6]Fed d 110 to 124 of the grow-finish phase.
[7]For each 0.01% premix inclusion in the diet the following vitamins were provided per kg: vitamin A, 265 IU; vitamin D_3, 26.5 IU; vitamin E, 1.7 IU; vitamin K, 0.09 mg; riboflavin, 0.33 mg; pantothenic acid, 0.87 mg; niacin, 1.33 mg.
[8]For each 0.01% premix inclusion in the diet the following minerals were provided per kilogram of the diet: iron, 9.4 mg; zinc, 9.4 mg; manganese, 1.3 mg; copper, 0.009 mg; iodine, 0.023 mg.
[9]Provided 0.3 ppm Se at 0.05% inclusion and 0.15 ppm Se at 0.025% inclusion.
[10]Provided 600 FTU per kg of the diet.
[11]Paylean (Elanco Animal Health, Greenfield, IN) provided 7.5 ppm ractopamine HCl in the diet.
[12]Zinpro Corporation, Eden Prairie, MN.
[13]Clarifly (Central Life Sciences, Schaumburg, IL) provided 0.67 ppm diflubenzuron in the diet for every 0.01% inclusion in the diet.

Table 4. Descriptive effects of transport on blood parameter concentrations from newly weaned sentinel pigs[1]

Parameter[2]	Pre-Transport[3]	Post-Transport[4]	SEM
Neutrophil: lymphocyte	0.70	1.00	0.30
Cortisol, ng/mL	14.10	113.30	12.20
Glucose, mg/dL	122.30	126.70	4.90
Hematocrit, %	26.40	26.30	1.50

[1]Pigs weaned and transported for 12 h during May 2018.
[2]Six sentinel pigs (n = 3 gilts and 3 barrows) were selected for blood parameter descriptive data.
[3]Blood samples were collected immediately prior to transport.
[4]Blood samples were collected immediately post-transport.

Table 5. Effects of dietary treatment on nursery and grow-finish performance in pigs.

	Diet treatment						*P*-value
Parameter	NA[1]	GLN[2]	SYN[3]	GLN+SYN[4]	A[5]	SEM	Diet[6]
Nursery phase[7]							
Days 0 to 14							
Initial BW, kg	5.92	5.90	5.94	5.91	5.94	0.65	0.61
ADG, kg	0.11	0.12	0.12	0.11	0.14	0.02	0.83
ADFI, kg	0.17	0.17	0.17	0.17	0.18	0.02	0.99
G:F	0.66	0.75	0.64	0.70	0.73	0.05	0.52
Day 14 BW, kg	7.51	7.62	7.55	7.51	7.90	0.70	0.37
Days 14 to 35							
ADG, kg	0.38	0.36	0.37	0.39	0.42	0.03	0.34
ADFI, kg	0.59	0.60	0.58	0.62	0.66	0.04	0.62
G:F	0.65	0.61	0.64	0.64	0.65	0.03	0.63
Days 0 to 35							
ADG, kg	0.27	0.27	0.27	0.28	0.31	0.03	0.76
ADFI, kg	0.42	0.43	0.42	0.44	0.47	0.04	0.92
G:F	0.65	0.66	0.64	0.66	0.68	0.03	0.77
Day 35 BW, kg	15.32	15.11	15.22	15.60	16.69	1.17	0.14
Grow-finish phase[8]							
Days 0 to 68							
ADG, kg	0.87	0.85	0.84	0.87	0.83	0.03	0.85
ADFI, kg	1.97	1.91	1.93	1.97	1.93	0.11	0.99
G:F	0.46	0.46	0.45	0.46	0.45	0.01	0.93
Day 68 BW, kg	73.35	71.92	72.27	73.47	72.65	2.55	0.90
Days 68 to 124							
ADG, kg	0.97	1.02	1.02	0.96	0.97	0.04	0.62
ADFI, kg	2.96	2.97	3.05	2.95	2.91	0.09	0.62
G:F	0.32	0.34	0.33	0.32	0.33	0.01	0.74

Days 0 to 124							
ADG, kg	0.92	0.93	0.93	0.91	0.90	0.03	0.87
ADFI, kg	2.45	2.44	2.49	2.46	2.42	0.10	0.99
G:F	0.39	0.40	0.39	0.39	0.39	0.01	0.97
Final BW, kg	124.83	127.17	127.94	127.00	126.84	4.17	0.92

[1]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[2]Pigs provided 0.20% L-glutamine (Ajinomoto North America Inc., Raleigh, NC) for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[3]Pigs provided synbiotics [3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[4]Pigs provided 0.20% L-glutamine and synbiotcs for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[5]Pigs provided dietary antibiotics [chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[6]Dietary treatment.
[7]Nursery phase fed from d 0 to 34 post weaning and transport.
[8]Grow-finish phase fed from the end of the 5-week nursery phase (d 0) to d 124 of the grow-finish phase.

Table 6. Effects of dietary treatment on therapeutic antibiotic treatment rate in pigs.

	Diet treatment						*P*-value
Parameter	NA[1]	GLN[2]	SYN[3]	GLN+SYN[4]	A[5]	SEM	Diet[6]
Nursery phase[7]							
Days 0 to 14							
Enteric[8]	38.30	43.30	43.30	43.80	35.80	6.50	0.64
Lame[9]	2.10	2.10	2.10	0.00	6.70	1.90	0.31
Unthrifty[10]	0.00	0.00	0.00	2.10	0.00	0.40	0.42
Respiratory[11]	-	-	-	-	-	-	-
Other[12]	0.00	2.10	2.10	0.00	0.00	0.80	0.58
Days 15 to 34							
Enteric	0.00	3.10	2.50	2.50	0.00	1.60	0.76
Lame	5.00^{x}	0.00^{y}	0.00^{y}	0.00^{y}	0.00^{y}	0.70	0.08
Unthrifty	2.50	0.00	0.00	0.00	0.00	0.50	0.42
Respiratory	2.50	3.10	0.00	3.10	0.00	1.80	0.76
Other	-	-	-	-	-	-	-
Grow-finish phase[13]							
Days 0 to 124							
Enteric	-	-	-	-	-	-	-
Lame	-	-	-	-	-	-	-
Unthrifty	3.10	0.00	4.20	3.10	0.00	2.00	0.76
Respiratory	0.00	6.30	0.00	0.00	10.40	2.60	0.27
Other	3.10	0.00	0.00	0.00	6.30	1.90	0.58

[1]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.

[2]Pigs provided 0.20% L-glutamine (Ajinomoto North America Inc., Raleigh, NC) for 14 d post-weaning and transport and then fed common antibiotic-free diets.

[3]Pigs provided synbiotics [3 strains of *Lactobacillus* (1.2 x 10^{9} cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN] for 14 d post-weaning and transport and then fed common antibiotic-free diets.

[4]Pigs provided 0.20% L-glutamine and synbiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.

[5]Pigs provided dietary antibiotics [chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[6]Dietary treatment.
[7]Nursery phase fed from d 0 to 34 post weaning and transport.
[8]Percent of pigs within pen treated with therapeutic antibiotics for enteric challenge.
[9]Percent of pigs within pen treated with therapeutic antibiotics for lameness.
[10]Percent of pigs within pen treated with therapeutic antibiotics for unthriftiness.
[11]Percent of pigs within pen treated with therapeutic antibiotics for respiratory challenge.
[12]Percent of pigs within pen treated with therapeutic antibiotics for all other conditions.
[13]Grow-finish phase fed from the end of the 5-week nursery phase (d 0) to d 124 of the grow-finish phase.
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row.

Table 7. Effect of dietary treatment on morphological measures of intestinal health in pigs.

	Diet Treatment						*P*-value
Parameter	NA[1]	GLN[2]	SYN[3]	GLN + SYN[4]	A[5]	SEM	Diet[6]
Day 14[7]							
Jejunum							
Villi height, µm	340.20	294.20	294.40	375.10	276.50	33.00	0.29
Crypt depth, µm	325.90	341.90	379.10	343.40	353.80	17.90	0.30
VH:CD ratio[8]	1.05	0.90	0.78	1.09	0.79	0.10	0.13
Goblet cell count/villi	3.20	1.90	2.10	2.20	1.70	0.60	0.40
Goblet cell count/mm	9.20	6.60	7.00	7.10	5.60	1.80	0.65
Ileum							
Villi height, µm	279.90	260.30	307.00	281.70	327.40	27.60	0.54
Crypt depth, µm	253.40	266.80	258.30	222.80	237.40	13.40	0.16
VH:CD ratio	1.12	1.03	1.20	1.32	1.39	0.14	0.46
Goblet cell count/villi	5.30	5.80	5.90	5.70	7.20	0.90	0.59
Goblet cell count/mm	18.70	21.40	19.60	19.30	22.30	2.20	0.68
Mast cell count/mm^2	426.00	410.00	485.00	363.00	500.00	54.00	0.23
Day 34[9]							
Jejunum							
Villi height, µm	400.20	431.80	456.80	418.50	482.40	26.40	0.20
Crypt depth, µm	385.10	398.60	356.50	376.30	373.60	16.30	0.48
VH:CD ratio	1.05	1.14	1.31	1.13	1.31	0.10	0.27
Goblet cell count/villi	2.60^y	3.30^y	5.10^x	3.10^y	2.90^y	0.70	0.06
Goblet cell count/mm	6.80^y	7.50^y	11.40^x	7.40^y	6.30^y	1.50	0.07
Ileum							
Villi height, µm	381.70	413.10	397.70	393.50	415.20	20.20	0.77
Crypt depth, µm	304.10	314.30	295.60	303.00	312.00	9.30	0.64
VH:CD ratio	1.26	1.33	1.35	1.32	1.33	0.08	0.96
Goblet cell count/villi	7.90	8.90	9.00	9.60	8.80	1.10	0.87
Goblet cell count/mm	20.80	21.90	22.20	25.10	21.70	2.80	0.84

[1]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.

[2]Pigs provided 0.20% L-glutamine (Ajinomoto North America Inc., Raleigh, NC) for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[3]Pigs provided synbiotics [3 strains of *Lactobacillus* (1.2 x 10^9 cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[4]Pigs provided 0.20% L-glutamine and synbiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[5]Pigs provided dietary antibiotics [chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[6]Dietary treatment
[7]Day 14 post transport and weaning.
[8]VH:CD ratio, villi height:crypt depth ratio.
[9]Day 34 post transport and weaning.
[a,b]Letters indicate significant differences ($P < 0.05$) within row and dietary treatment.
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and dietary treatment.

Table 8. Effect of dietary treatment on gene expression of intestinal tissues in pigs.[1]

	Diet treatment						*P*-value
Parameter	NA[2]	GLN[3]	SYN[4]	GLN + SYN[5]	A[6]	SEM	Diet[7]
Day 14[8]							
Jejunum							
TNF-α[9]	0.33	0.26	0.27	0.26	0.30	0.04	0.74
IL-8[10]	1.27	1.16	1.47	1.18	1.47	0.29	0.90
GLP2R[11]	0.27	0.26	0.30	0.28	0.28	0.04	0.95
ZO-1[12]	0.85	0.93	1.06	1.06	0.99	0.17	0.76
CL-1[13]	0.87	0.67	0.84	0.81	0.68	0.17	0.89
Ileum							
TNF-α	1.17	1.07	0.82	1.20	0.76	0.20	0.44
IL-8	0.60	0.55	0.51	0.46	0.54	0.09	0.87
GLP2R	0.58	0.58	0.67	0.47	0.59	0.09	0.73
ZO-1	1.79	1.49	1.14	1.38	1.48	0.16	0.11
CL-1	1.97	1.30	1.51	1.44	1.28	0.34	0.68
Day 34[14]							
Jejunum							
TNF-α	0.21[xy]	0.24[x]	0.18[yz]	0.17[yz]	0.15[z]	0.02	0.09
IL-8	0.84	0.83	0.77	0.67	0.62	0.16	0.81
GLP2R	0.43	0.49	0.30	0.38	0.35	0.07	0.31
ZO-1	1.07	0.97	0.86	0.86	0.79	0.12	0.45
CL-1	1.39	0.81	1.31	1.06	0.96	0.20	0.11
Ileum							
TNF-α	0.40	0.45	0.31	0.39	0.37	0.11	0.93
IL-8	0.40	0.41	0.40	0.37	0.37	0.08	0.99
GLP2R	0.27	0.29	0.28	0.30	0.35	0.04	0.67
ZO-1	1.17	1.37	1.19	1.37	1.14	0.14	0.71
CL-1	1.36	1.66	1.70	2.27	1.51	0.43	0.61

[1]Results were quantified by the standard curve method, and data are expressed as the relative abundance of the genes of interest to the reference gene (18s).

[2]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[3]Pigs provided 0.20% L-glutamine (Ajinomoto North America Inc., Raleigh, NC) for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[4]Pigs provided synbiotics [3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[5]Pigs provided 0.20% L-glutamine and synbiotics for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[6]Pigs provided dietary antibiotics [chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] for 14 d post-weaning and transport and then fed common antibiotic-free diets.
[7]Dietary treatment.
[8]Day 14 post transport and weaning.
[9]TNF-α, tumor necrosis factor alpha.
[10]IL-8, interleukin 8.
[11]GLP2R, glucagon like peptide 2 receptor.
[12]ZO-1, zonula occludens 1.
[13]CL-1, claudin 1.
[14]Day 34 post transport and weaning.
[x-z]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and dietary treatment.

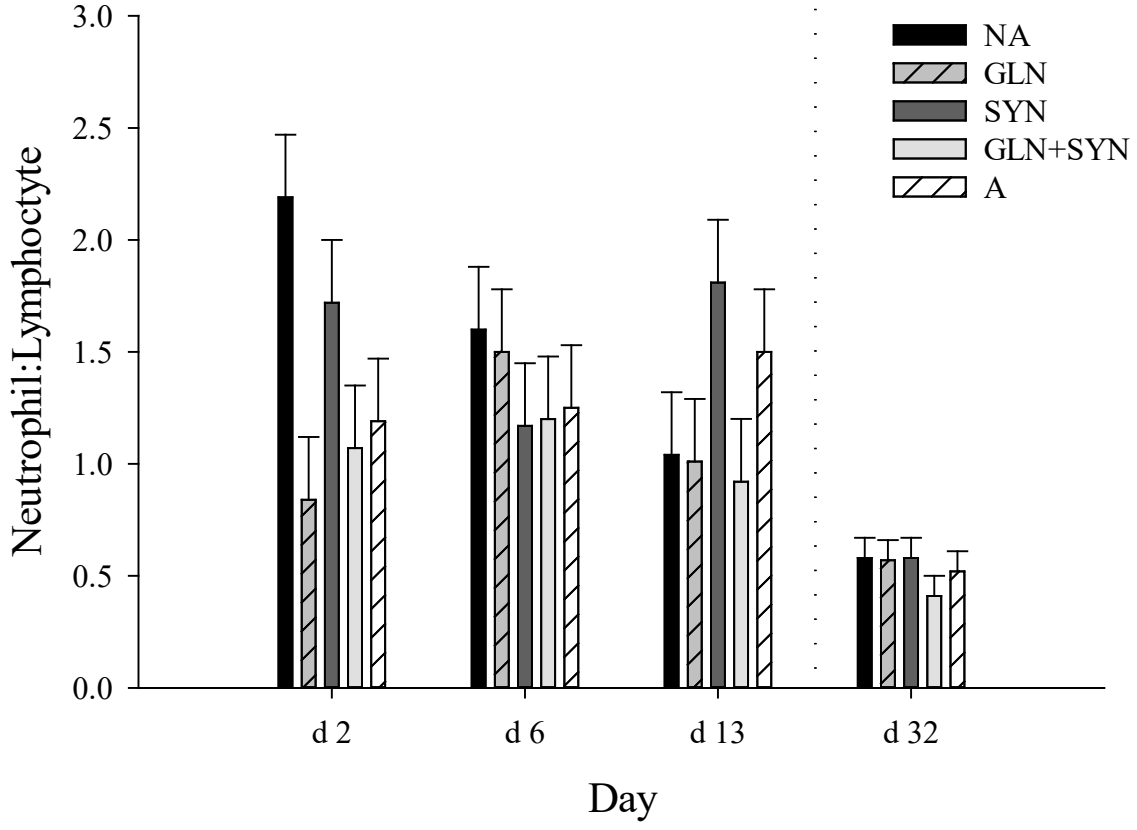

Figure 1. Effects of supplementing newly weaned and transported pigs with no dietary antibiotics (**NA**), 0.20% L-glutamine (**GLN**; Ajinomoto North America Inc., Raleigh, NC), synbiotics [**SYN;** 3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN], GLN+SYN, and dietary antibiotics [**A**; chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] on neutrophil:lymphocyte on d 2, 6, and 13 post-weaning and transport during the diet treatment period, and on d 32 post-weaning and transport during the common diet period. Error bars indicate ± 1 SE. No significant differences ($P \leq 0.05$) or tendencies ($0.05 < P \leq 0.10$) were detected for neutrophil:lymphocyte with any comparison.

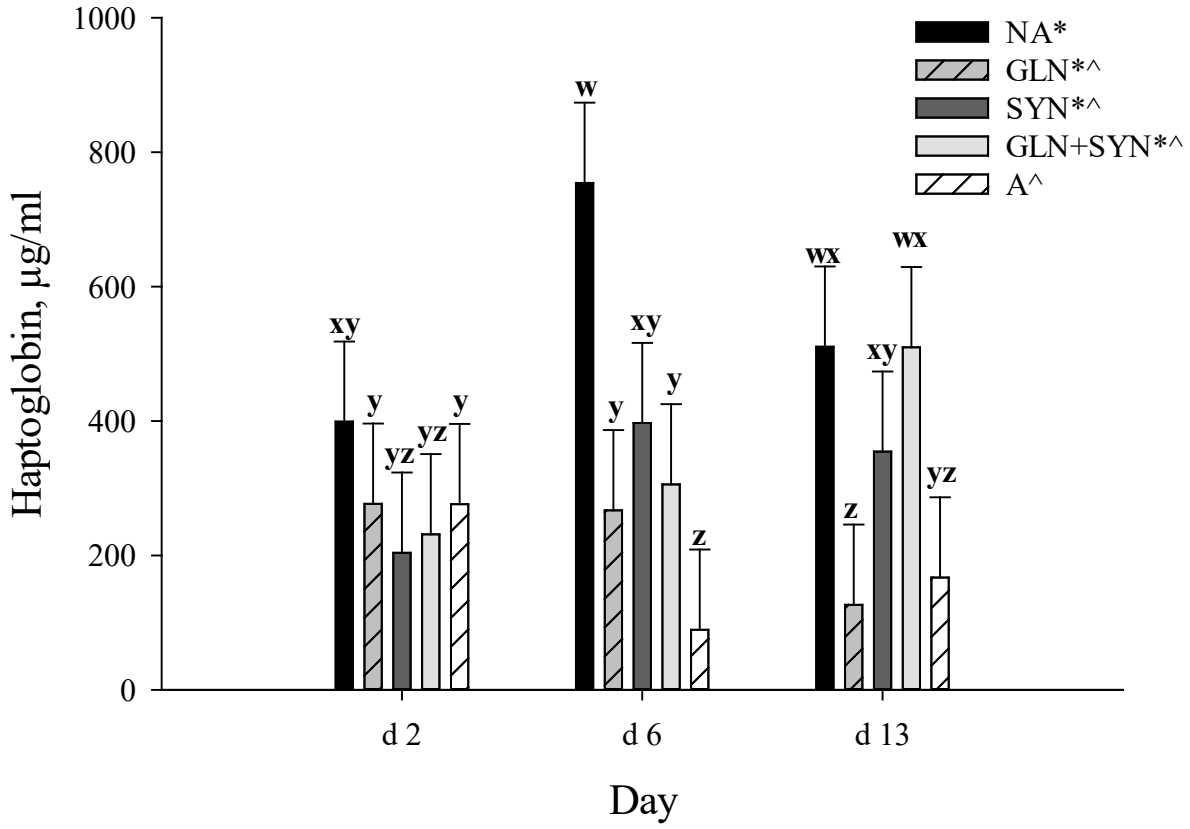

Figure 2. Effects of supplementing newly weaned and transported pigs with no dietary antibiotics (**NA**), 0.20% L-glutamine (**GLN**; Ajinomoto North America Inc., Raleigh, NC), synbiotics [**SYN;** 3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN], GLN+SYN, and dietary antibiotics [**A**; chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] on circulating haptoglobin measured on d 2, 6, and 13 post-weaning and transport during the diet treatment period. Error bars indicate ± 1 SE. Letters$^{w\text{-}z}$ indicate diet treatment by day tendencies ($0.05 < P \leq 0.10$). Symbols$^{*,\wedge}$ on the legend indicate overall dietary treatment differences ($P < 0.05$).

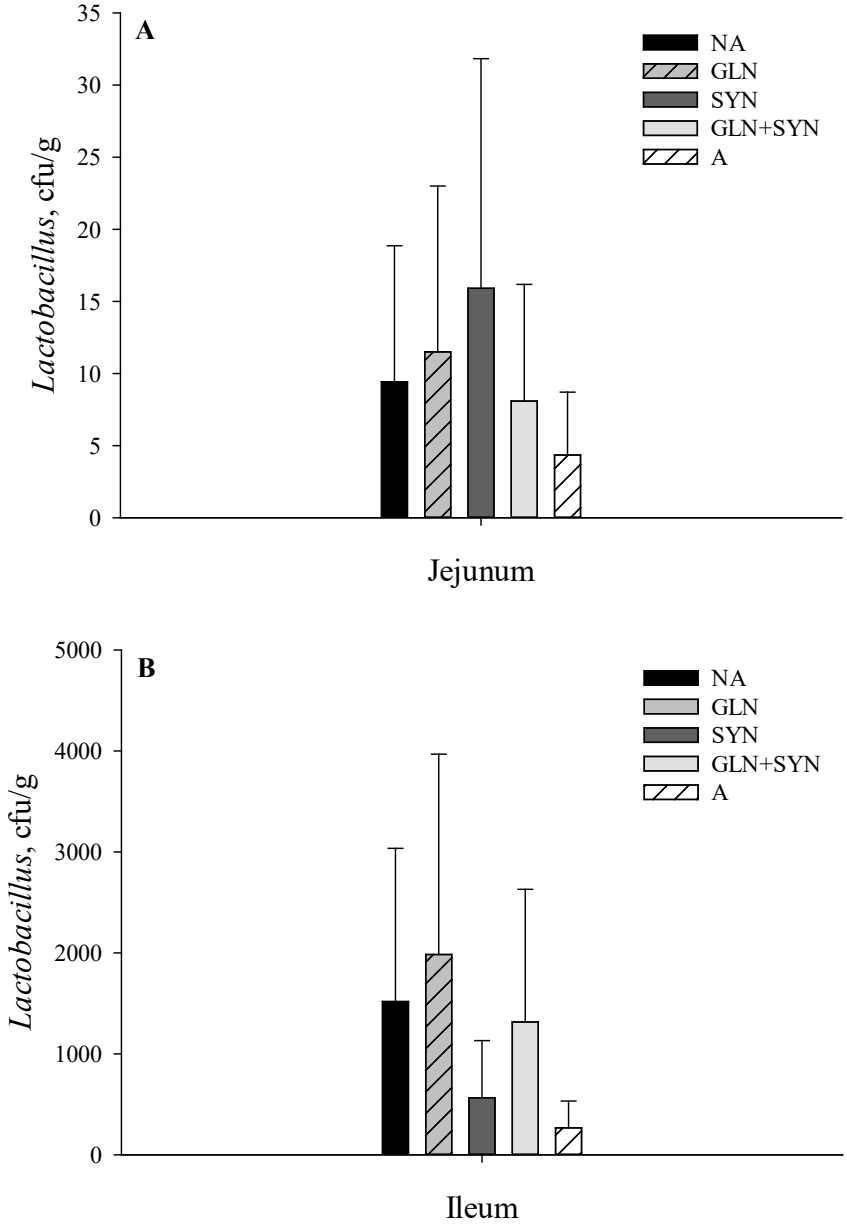

Figure 3. Effects of supplementing newly weaned and transported pigs with no dietary antibiotics (**NA**), 0.20% L-glutamine (**GLN**; Ajinomoto North America Inc., Raleigh, NC), synbiotics [**SYN;** 3 strains of *Lactobacillus* (1.2 x $10^{\wedge 9}$ cfu/g of strain/pig/day) + β-glucan (0.01 g/pig/day) + fructooligosaccharide (0.01 g/pig/day); BioMatrix International, Princeton, MN], GLN+SYN, and dietary antibiotics [**A**; chlortetracycline (441 ppm; Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (38.5 ppm; Denagard, Elanco Animal Health, Greenfield, IN)] on *Lactobacillus* counts in (A) jejunum and (B) ileum determined on d 13 post-weaning and transport during the diet treatment period. Error bars indicate ± 1 SE. No significant differences ($P \leq 0.05$) or tendencies ($0.05 < P \leq 0.10$) were detected for *Lactobacillus* counts with any comparison.