The effects of prebiotics on growth performance and in vitro immune biomarkers in weaned pigs[1]

Joice V. San Andres,[†,‡] Gabriel A. Mastromano,[†] Yanshuo Li,[†] Huyen Tran,[†] Justin W. Bundy,[†] Phillip S. Miller,[†] and Thomas E. Burkey[†,2]

[†]Department of Animal Science, University of Nebraska, Lincoln, NE 68583; and [‡]Department of Animal Science, Central Luzon State University, 3120, Philippines

ABSTRACT: The objective of the experiment was to investigate the effects of prebiotics in nursery pigs on growth performance and immune biomarkers. Sixty-four weaned pigs (31 ± 1 d; BW 8 ± 0.1 kg) of mixed gender were housed (4 pigs/pen) in an environmentally controlled nursery with ad libitum access to feed and water over a 35-d study. Pigs were randomly assigned to one of four treatments: control (53% corn, 32% SBM, 7% fishmeal, 8% others), control + 2.5% GroBiotic-S (GS), control + 0.05% chicory (CL), or control + 0.5% chicory (CH). Feeders and pigs were weighed weekly. On day 21, blood samples were obtained from three pigs/treatment for collection of peripheral blood mononuclear cells (PBMC). Isolated PBMC were cultured and subsequently challenged with lipopolysaccharide (LPS; 20 ng/mL). Cell culture supernatants were collected for quantification of the pro- and anti-inflammatory cytokines, interleukin (IL)-8 and IL-10, respectively. Dietary treatment had no effect on BW. At days 28 to 35, pigs fed GS (790 ± 15 g), CL (704 ± 15 g), or CH (692 ± 15 g) had greater ($P < 0.05$) ADG compared with control (643 ± 15 g) pigs. In addition, overall (days 0–35), pigs fed GS (823 ± 18 g), CL (783 ± 18 g), or CH (782 ± 18 g) had greater ($P < 0.05$) ADFI compared with control, and ADFI for GS-fed pigs was greater ($P < 0.05$) than either CL or CH. There was no difference in G:F among treatments. In vitro LPS challenge increased ($P < 0.05$) IL-8 secretion from PBMC isolated from CL (23,731 ± 3,221 pg/mL) pigs compared with control (10,061 ± 3,221 pg/mL) and CH (12,411 ± 3,221 pg/mL) pigs. Secretion of IL-10 from PBMC isolated from CL (63 ± 9 pg/mL) pigs was greater ($P < 0.05$) compared with control (22 ± 9 pg/mL) pigs and tended ($P < 0.1$) to be greater compared with CH (34 ± 9 pg/mL) pigs. Results indicate that inclusion of prebiotics in nursery pig diets has positive effects on growth performance and may have immunomodulatory effects (in vitro) on cells isolated from prebiotic-fed pigs.

Key words: growth performance, immune biomarker, prebiotic, weaned pigs

Transl. Anim. Sci. 2019.3:1315–1325
doi: 10.1093/tas/txz129

[1]A contribution of the University of Nebraska Agricultural Resource Division supported in part by funds provided through the Hatch Act. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA or the University of Nebraska and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

[2]Corresponding author: tburkey2@unl.edu

Received April 26, 2019.
Accepted July 25, 2019.

INTRODUCTION

Prebiotics are nondigestible fibers that can be fermented and favorably change the composition and activity of gut microorganisms (Slavin, 2013). This, consequently, may increase production of beneficial bacterial metabolites such as short-chain fatty acids (Le Bourgot, 2017; Mao, 2017; Shang et al., 2017; Taciak et al., 2017) and promote growth (Chen, 2017; Keerqin et al., 2017; Lee et al., 2017). In addition, prebiotic oligosaccharides can reduce intestinal inflammation by modulating intestinal microbiota or influencing cytokine expression by itself (Zenhom et al., 2011). Immune modulation is important in nursery pigs because of their susceptibility to paracellular transport of endotoxin and pathogens (Mani et al., 2012) as a result of impaired intestinal barrier function (Smith et al., 2010) and reduction in brush-border alkaline phosphatase activities which play a role in detoxification of endotoxin (Lalles, 2010). Inflammation and endotoxin can cause suppressed appetite and channeling of nutrients away from growth toward sustaining immune system requirements (Mani et al., 2012).

Chicory (*Cichorium intybus* L.) has considerable amounts of prebiotic compounds such as insulin and oligofructose (Lepczyński et al., 2017). These fiber components have been shown to stimulate the growth of beneficial bacteria such as increased populations of lactic acid bacteria in the ileum and butyrate-producing bacteria in the colon (Liu et al., 2012). It is also proposed that inulin-type fructans induce production of pro-and anti-inflammatory cytokines through detection of dendritic cells, via receptor ligation of pathogen recognition receptors (PRRs) such as toll-like receptors (TLRs) and galectins (Vogt et al., 2015). However, work in the areas of mechanisms, dose–response associations, and structure–function relationships are needed to fully elucidate immunomodulatory effects of chicory.

GroBiotic-S (for swine) or GroBiotic-A (for aquaculture) (International Ingredient Corporation, St. Louis, MS) is prebiotic mixture of brewer's yeast, dairy ingredient components, and dried fermentation products that has been reported to have beneficial effects such as enhanced growth performance and disease resistance on fish (Li and Gatlin, 2005; Sealey et al., 2007; Buentello et al., 2010; Zheng et al., 2011; Adel et al., 2017). GroBiotic-A was observed to alter microbial communities and increase butyrate concentrations in the intestinal tract (Burr et al., 2010). These positive effects of GroBiotic supplementation may be worthwhile as feed additives for animals, especially animals in stressful situations (e.g., at weaning) and animals exposed to greater pathogenic loads.

Although there have been studies on the influence of chicory on growth performance and immune regulation in pigs, there is little information on the basis by which such effects are achieved. GroBiotic-A supplementation, on the other hand, has been demonstrated to have benefits in aquaculture but its effects in pigs have not been extensively evaluated. We hypothesized that positive influence of chicory pulp and GroBiotic-S may be through modulation of immune response in pigs. The present study is aimed at determining the effects of supplementing low and high concentrations of chicory and GroBiotic-S on growth performance of nursery piglets and on the in vitro immune response of peripheral blood mononuclear cells to secondary challenge with lipopolysaccharide.

MATERIALS AND METHODS

The protocol for animal use in the study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Nebraska-Lincoln (UNL) with IACUC number EXP13401.

Animals, Housing, and Experimental Diets

Sixty-four piglets (Nebraska female x Danbred sire) weaned at 31 ± 1 d of equal numbers of males and females and initial body weight of 8 ± 0.1 kg were obtained from Eastern Nebraska Research and Extension Center. The pigs were housed in a climate-controlled nursery equipped with stainless-steel feeders and nipple drinkers. Feed and water were offered ad libitum throughout the 35-d experiment. There were four pigs (two males and two females) per pen with four pens per treatment.

Pens were assigned to four dietary treatments: basal diet (control), control + 2.5% GroBiotic-S (GS), control + 0.05% chicory (CL), or control + 0.5% chicory (CH) following completely randomized design. Diets were formulated to meet or exceed NRC (2012) recommendation with no antibiotic inclusion. Chicory and GS were incorporated in the diet by partially replacing cornstarch in the formulation. The ingredient composition and calculated analysis of the experimental diets are presented in Table 1.

GS is a free-flowing powdered prebiotic feed additive derived from dairy fractions, partially autolyzed brewer's yeast, and dried fermentation

Table 1. Ingredient and chemical composition of experimental diets (%, as-fed basis)

	Diets[1]			
Item	Control	CH	CL	GS
Ingredient, %				
Corn	52.98	52.98	52.98	52.98
Soybean meal, 46.5% CP	32.00	32.00	32.00	32.00
Fishmeal	7.00	7.00	7.00	7.00
Corn starch	3.00	2.50	2.95	0.50
GroBiotic-S[2]	–	–	–	2.50
Dicalcium phosphate, 18.5% P	1.00	1.00	1.00	1.00
Limestone	0.20	0.20	0.20	0.20
Vitamin and Mineral Premix[3]	0.40	0.40	0.40	0.40
Corn oil	3.00	3.00	3.00	3.00
Chicory pulp[4]	–	0.50	0.05	–
Salt	0.30	0.30	0.30	0.30
L-Lysine-HCL	0.12	0.12	0.12	0.12
Calculated nutrient content, %				
ME, MJ/kg	14.07	14.07	14.07	14.07
CP, %	20.55	20.55	20.55	20.55
Lys, %	1.31	1.31	1.31	1.31
Met, %	0.37	0.37	0.37	0.37
Thr, %	0.77	0.77	0.77	0.77
Trp, %	0.25	0.25	0.25	0.25
Arg, %	1.44	1.44	1.44	1.44
Ile,%	0.88	0.88	0.88	0.88
Val,%	0.96	0.96	0.96	0.96
His,%	0.56	0.56	0.56	0.56
Leu, %	1.73	1.73	1.73	1.73
Ca, %	0.74	0.74	0.74	0.74
P, %	0.48	0.48	0.48	0.48
SID[5] Lys/ME	3.89	3.89	3.89	3.89

[1]Diets: Control, basal diet; control + 2.5% GroBiotic-S, GS; CL, control + 0.05% chicory; CH, control + 0.5% chicory.

[2]GroBiotic-S is a yeast-based prebiotic provided by International Ingredient Co., St. Louis, MO.

[3]Supplied per kg of diet: vitamin A (as retinyl acetate), 5,500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as α-tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; and vitamin B_{12} (as cyanocobalamin), 33.0 mg, copper (as $CuSO_4 \cdot 5H_2O$), 10 mg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg; iron ($FeSO_4 \cdot 2H_2O$), 125 mg; manganese (MnO), 15 mg; selenium (Na_2SeO_3), 0.3 mg; and zinc ($ZnSO_4 \cdot H_2O$), 125 mg.

[4]Chicory is a prebiotic derived from chicory pulp.

[5]SID = Standardized ileal digestible.

products supplied by International Ingredient Corporation (St. Louis, MS). On as-fed basis GS typically contains about 32% crude protein, 2% crude fat, 2% crude fiber and 53% carbohydrates (simple and complex including oligosaccharides), 6% Ash, 5% moisture, and 14.98 MJ/kg of metabolizable energy. The chicory pulp is a ground (<600 μm) prebiotic obtained from Bill Bar Company (Overland Park, KS). Chicory pulp on an as-fed basis has 87% dry matter, 8% total sugars, 7.5% inulin, 18.5% total cellulose, 8% hemicellulose, and 27% pectin.

Pigs were weighed at the start of experiment and weekly thereafter (days 0, 7, 14, 21, 28, and 35) to measure average daily gain (ADG). Average daily feed intake (ADFI) was measured in a similar manner as ADG. Gain to feed ratio (G:F) was calculated accordingly based on ADG and ADFI and expressed as g gain/g feed intake.

PBMC Cell Culture, LPS Challenge, and Cytokine Quantification

Blood collection. An in vitro experiment using cells derived from the animals used in the experiment was done to evaluate the effects of dietary treatments on immunity. On day 21 of the study, blood samples were collected from three pigs (two males and one female) per treatment for isolation of peripheral blood mononuclear cells (PBMC). Blood samples were obtained (6–10 mL)

via jugular venipuncture using heparinized glass blood tubes.

Isolation of PBMC. Isolation of PBMC was done using the procedure adapted from Goff et al. (1996). Briefly, blood samples were centrifuged at room temperature (18–25°C) within 2 h after collection and mononuclear cells and platelets were obtained after aspirating supernatants. Centrifugations were done for 20 min at 1,500 to 1,800 × *g*, 15 min at 300 × *g*, and 10 min for 300 × *g*. Cells were mixed in between centrifugation and washed with phosphate-buffered saline (PBS, Invitrogen). Trypan blue dye exclusion (Sigma–Aldrich Co., St Louis, MO) was used to determine cell viability and cell density was evaluated using a hemocytometer (Fisher Scientific Inc., Pittsburgh, PA).

In vitro LPS challenge. Isolated PBMC were suspended in RPMI 1640 (Roswell Park Memorial Institute; Hyclone, South Logan, UT) medium with 10% fetal bovine serum (Sigma–Aldrich, St. Louis, MO) and 1% antibiotics (Invitrogen) for cell culture. Cells were seeded (1×10^6 cells/well) onto 24-well plates (BD Falcon, Corning Inc., Corning, NY) and incubated overnight (37°C, 5% CO_2) to allow for PBMC to adhere. The next day, nonadherent cells were discarded, and residual adherent cells were incubated for a further 2 h in fresh medium without antibiotics. Subsequently, cells were challenged with lipopolysaccharide (LPS; 20 ng/mL) and incubated for 48 h. Three 24-well plates were used with three replicates of PBMC isolated from each pig (three pigs for each of the four treatments) for non-LPS-challenged (LPS-) and LPS-challenged (LPS+) cells.

Quantification of IL-8 and IL-10 in cell culture supernatants. Cell culture media samples were harvested for analysis of immune biomarkers secreted from PBMC. Quantification of cytokines CXCL8, also known as interleukin-8 (IL-8), and interleukin-10 (IL-10) from PBMC was done using commercial porcine-specific ELISA kits for IL-8 (catalog number 45-IL8-E02; R&D System, Minneapolis, MN) and IL-10 (catalog number 45-I10PO-E01; R&D System, Minneapolis, MN). The range of detection for IL-8 was 16.75 to 38111.89 pg/mL and 5.42 to 94.48 pg/mL for IL-10. The intra-assay CV for IL-8 and IL-10 was 19.71% and 7.96%, respectively. The inter-assay CV for for IL-8 and IL-10 was 27.11% and 17.55%, respectively. The optical density (OD) of samples was read by spectrophotometer at 450 nm and corrected at 570 nm (Fluorstar Optima; BMG Labtech, Durham, NC) for both cytokines.

Statistical Analyses

All data were analyzed as completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Means are presented as least-squares means. Pen was considered as experimental unit and random effect for growth performance. Each well was considered as experimental unit and random effect for analysis of cytokine concentrations. Dietary treatment was considered as a fixed effect in the model for growth performance. The model for cytokine concentrations included diet, LPS challenge, and their interaction with plate as a random effect. Variability in the data is expressed as standard error (SE) and the threshold for significance was set at $P < 0.05$. Comparison among means for parameters with significant difference was done using Tukey's honest significant difference test using SAS.

RESULTS

Growth Performance

Growth performance data are presented in Table 2. Dietary treatments did not affect the body weight of pigs throughout the duration of the study. However, ADG of pigs given GS (790.18 ± 15.03 g) was greater than those fed Control (643.43 ± 15.03 g) and comparable to CH (704.11 ± 15.03 g) and CL (692.14 ± 15.03 g) fed pigs at days 28 to 35 of the experiment ($P < 0.004$). Overall ($P < 0.018$) and days 28 to 35 ($P < 0.015$) ADFI of pigs fed GS (822.53 ± 10.08 and 1239.02 ± 18.04 g, respectively) were greater than those fed Control (739.29 ± 10.08 and 1029.43 ± 18.04 g, respectively) but similar to those given CH (782.18 ± 10.08 and 1159.19 ± 18.04 g, respectively) and CL (782.71 ± 10.08 and 1172.50 ± 18.04 g, respectively). There were no observed differences among treatments in terms of G:F throughout the experiment.

Cytokine Concentrations of LPS-Challenged Isolated PBMC

Mean concentrations of cytokines IL-8 and IL-10 of isolated PBMC from pigs given dietary treatments with or without LPS challenge in vitro are presented in Figures 1 and 2. LPS increased secretion of IL-8 ($P < 0.0001$) and IL-10 ($P < 0.0001$) from isolated PBMC. Dietary treatments did not influence cytokine concentrations but an interaction between treatments given to pigs and LPS challenge was observed for IL-8 ($P < 0.0008$) and IL-10 ($P < 0.001$) secretion from isolated PBMC. Concentrations of IL-8 and IL-10 in

Table 2. Performance of nursery pigs fed basal diet (Control), control + 0.05% chicory (CL), control + 0.5% chicory (CH), and control + 2.5% GroBiotic-S (GS)

	Treatment					
Item	Control	CH	CL	GS	SEM	*P*
BW, kg						
d 0	7.94	8.02	7.96	7.98	0.05	0.964
d 7	9.60	9.90	9.88	9.73	0.09	0.592
d 14	13.01	13.56	13.33	13.35	0.14	0.624
d 21	16.25	16.47	16.45	16.87	0.19	0.711
d 28	21.68	22.07	21.87	22.61	0.26	0.620
d 35	26.19	27.00	26.71	28.15	0.31	0.147
ADG, g/d						
d 0 to 7	236.38	268.16	277.69	249.64	8.99	0.389
d 7 to 14	487.43	499.46	495.65	518.21	11.91	0.860
d 14 to 21	462.10	415.71	445.71	502.86	14.44	0.186
d 21 to 28	776.76	800.18	774.29	820.00	16.31	0.735
d 28 to 35	643.43[b]	704.11[ab]	692.14[ab]	790.18[a]	15.03	0.004
Overall (d 0 to 35)	521.22	542.25	535.79	576.18	8.36	0.120
ADFI, g/d						
d 0 to 7	319.22	339.29	366.97	340.18	9.05	0.343
d 7 to 14	599.36	666.97	621.78	665.53	13.79	0.227
d 14 to 21	691.93	701.61	748.39	773.93	18.84	0.396
d 21 to 28	990.50	1,043.75	1,003.93	1,094.72	19.01	0.218
d 28 to 35	1,092.43[b]	1,159.29[ab]	1,172.5[ab]	1,239.02[a]	18.04	0.018
Overall (d 0 to 35)	739.29[b]	782.18[ab]	782.71[ab]	822.53[a]	10.08	0.015
G:F, g/kg						
d 0 to 7	730.44	790.16	741.94	733.45	15.03	0.502
d 7 to 14	808.17	784.44	795.39	782.95	11.72	0.888
d 14 to 21	666.04	591.23	595.43	644.17	16.26	0.297
d 21 to 28	785.91	767.28	771.50	750.56	8.97	0.624
d 28 to 35	579.89	606.58	590.27	637.62	10.63	0.250
Overall (d 0 to 35)	700.95	693.05	684.82	700.65	4.23	0.530

[a,b]Means within a row that do not have a common superscript differ, $P < 0.05$.

[1]Sixty-four weaned pigs (Nebraska female x Danbred sire; 31 ± 1 d of age) were randomly assigned to 16 pens (four pigs/pen and four pens/treatment).

LPS challenged PBMC from pigs given CL (23,731 ± 3221.54 g and 63.03 ± 9.83 g pg/mL, respectively) was greater compared with PBMC from pigs fed Control (10,061 ± 3221.54 g and 21.77 ± 9.83 g pg/mL, respectively), but was comparable to those given CH (12,411 ± 3221.54 g and 33.93 ± 9.83 g pg/mL, respectively) and GS (16,941 ± 3221.54 g and 42.39 ± 9.83 g pg/mL, respectively) diets.

DISCUSSION

Growth Performance

Feeding of GS in nursery pigs enhanced ADG (22.81%) of pigs compared with Control at days 28 to 35. Increase in ADFI in GS fed pigs was observed during days 28 to 35 (13.48%) and days 0 to 35 (11.26%). These improvements in growth and feed intake were similarly reported in studies in fish (Sealey et al., 2007; Zheng et al., 2011; Adel et al., 2016; Wang et al., 2016; Adel et al., 2017) which demonstrated that the inclusion of GS at 1 to 2% in diets of juvenile Nile tilapia (*Oreochromis niloticus*), starry flounder (*Platichthys stellatus*), and sturgeon (*Huso huso* Linnaeus, 1754) increased weight gain during an 8-wk experiment by as much as 32.80%, 17.37%, and 41.45%, respectively. Effects of GS on growth, feed intake, and feed efficiency have been attributed by researchers to proliferation of beneficial microbes and their metabolites (Sealey et al., 2015; Adel et al., 2017). Beneficial microbes such as lactic acid bacteria can produce bacteriocins, lactic acid, and other compounds that inhibit growth of certain pathogens and improve intestinal environment and reduce inflammation, which may result to improve growth (Ringø et al., 2005).

Prebiotic treatments show increase in beneficial short-chain fatty acids (SCFA) production that can

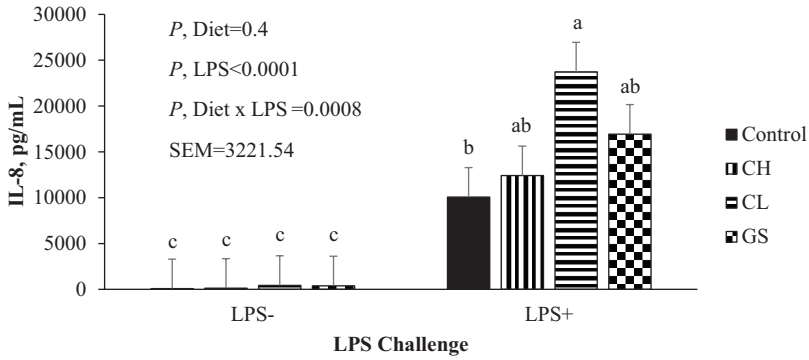

Figure 1. Interleukin 8 (IL-8) secretion by porcine peripheral blood mononuclear cells (PBMC) isolated from nursery pigs fed dietary treatments (Control, basal diet; CH, control + 0.5% chicory; CL, control + 0.05% chicory; GS, control + 2.5% GroBiotic-S). Blood was collected from pigs (n = 3/dietary treatment) at day 21 for isolation of PBMC. Isolated PBMC from each pig were assigned to one of two ex vivo treatments including lipopolysaccharide (LPS) challenge (20 ng/mL) or without LPS challenge and was replicated three times. Feeding of CL increased the secretion of IL-8 in PBMC in response to in vitro LPS challenge ($P_{Diet\ x\ LPS}$ = 0.0008). Bars with different superscripts are different (P < 0.05).

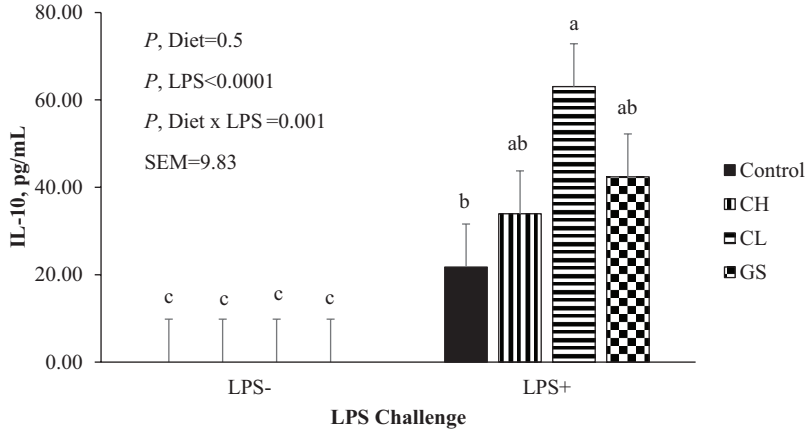

Figure 2. Interleukin 10 (IL-10) secretion by porcine peripheral blood mononuclear cells (PBMC) isolated from nursery pigs fed dietary treatments (Control, basal diet; CH, control + 0.5% chicory; CL, control + 0.05% chicory; GS, control + 2.5% GroBiotic-S). Blood was collected from pigs (n = 3/dietary treatment) at day 21 for isolation of PBMC. Isolated PBMC from each pig were assigned to one of two ex vivo treatments including lipopolysaccharide (LPS) challenge (20 ng/mL) or without LPS challenge and was replicated three times. Feeding of CL increased the secretion of IL-10 in PBMC in response to in vitro LPS challenge ($P_{Diet\ x\ LPS}$ = 0.0008). Bars with different superscripts are statistically different (P < 0.05).

help maintain intestinal health (Keerqin et al., 2017; Mao, 2017; Shang et al., 2017). Increase in butyrate production was also documented in GS supplementation in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Butyrate is an important source of energy for colonocytes (Wong et al., 2006) and has been reported to enhance pancreatic secretion and digestibility in guinea pigs and calves (Katoh and Tsuda, 1987; Harmon, 1992; Guilloteau et al., 2010). Moreover, butyrate increased activity of elastase II which is an enzyme with broad specificity for substrate containing medium and large hydrophobic amino acids (Guilloteau et al., 2009). Higher digestibility of crude protein, organic matter, and energy has been reported in hybrid striped bass fed GS (Burr et al., 2008). In addition, GS contains dried yeast that contains mannan oligosaccharides (MOS), which had been reported to improve growth in early weaned piglets (Tang et al., 2005) and growing pigs (Davis et al., 2002) by increasing plasma serum growth hormone and insulin-like growth factor-I concentrations. MOS can also reduce inflammation, which likewise may result in improved performance (Che et al., 2011; Sun et al., 2015).

Growth performance of pigs given CL and CH was comparable to GS fed pigs and was numerically greater than those given Control. In lambs, chicory feeding increased average daily liveweight gain (Tzamaloukas et al., 2006). Effects of dietary fiber inclusion in the diet on growth performance are often related to its prebiotic effects. Fructans derived from chicory such as inulin and its hydrolysate oligofructose have demonstrated prebiotic effects such as increased relative abundance of lactic acid bacteria, *Bifidobacteria* and butyrate producing bacteria in pigs (Liu et al., 2012). In addition, chicory root also contains other bioactive compounds such as phenolic compounds, sesquiterpene lactones, monomeric flavonoids, alkaloids,

and pectin (Nwafor et al., 2017). These compounds reduce inflammation, modulate lipid metabolism, inhibit growth of certain pathogens, and improve redox balance which increase immunocompetence that may result to improve growth performance (Kocsis, 2003; Karioti, 2008; Schumacher et al., 2011; Ferioli and D'Antuono, 2012).

Abundance in species of lactic acid bacteria and *Bifidobacteria* has been associated with improved growth in weanling pigs (Abe et al., 1995; Modesto et al., 2009). These bacteria and their fermentation products, such as SCFA and polyamines, improve energy supply to the colonic epithelial cells (Donohoe et al., 2011) and aid in the absorption of minerals such as calcium and magnesium (Ohta et al., 1995; Sako et al., 1999). It was also observed that chicory root feeding stimulates proliferation of microbes such as *Megasphaera elsdinii* that produces growth-promoting metabolites in colon of pigs (Mølbak et al., 2007). *Megasphaera elsdinii* can ferment lactate produced by *Bifidobacteria* into SCFA and lactate as a major carbon source, it produces growth-associated product propionate (Soto-Cruz et al., 2002). Propionate plasma concentration was positively correlated with live weight gain in piglets (Hansen et al., 2012).

Biomarkers of Immunity

The diversion of nutrients away from growth to support immune-related processes is a hindrance to obtain optimum growth and it is recognized that many metabolic processes respond directly or indirectly to cytokines (Spurlock, 1997). In the present study, response to LPS challenge of isolated PBMC was affected by chicory supplementation. Isolated PBMC with LPS challenge from pigs fed CL had greater secretion of IL-8 and IL-10 than Control. Advantage of chicory supplementation in disease-challenged animals has been demonstrated in some studies. In lambs, feeding of chicory increased immunity against trickle infection which was attributed to indirect immunological response indicated by greater amounts of cells (mucosal mast cells and globule leucocytes) associated with immune response against parasite in the abomasum and reduced number of worms recovered (Tzamaloukas et al., 2006). Chicory root inclusion (13.8%) in the diet of growing pigs increased proportion of *Bifidobacterium thermacidophilum* and *Megasphaera elsdinii* and can be a course for inhibition of dysentery-causing bacteria *Brach. hyodysenteriae* in colon of pigs through cross-feeding (Mølbak et al., 2007).

Prebiotics such as inulin and oligofructose, which are among the main bioactive compounds in chicory, have been observed to modulate functions of the immune system and increase IL-10 secretion in rats (Roller et al., 2004; Watzl et al., 2005). Inulin was observed to boost LPS-induced secretion of IL-10 in mice (Capitán-Cañadas et al., 2014). Anti-inflammatory effects of phenolic compounds from chicory have also been observed in humans (Schumacher et al., 2011). In the clinical study, humans given caffeine-free chicory coffee (20 g chicory/300 mL) had reduced phenylpyruvate tautomerase enzymatic activity and serum concentration of proinflammatory cytokine macrophage migration inhibitory factor (MIF). Migration inhibitory factor is an integral component of the host antimicrobial alarm system and stress response that promotes the pro-inflammatory functions of immune cells (Calandra and Roger, 2003).

The IL-8 and IL-10 secretions from PBMC isolated from GS fed animals were comparable to those fed CL and was numerically greater than the animals given the Control diet. Effects of GS supplementation on immunity had been reported in studies with juvenile fish such as red drum, Nile tilapia, starry flounder, and sturgeon (Buentello dro et al., 2010; Zheng et al., 2011; Adel et al., 2016; Wang et al., 2016; Adel et al., 2017). In these studies, nonspecific markers of immunity such as blood lysozyme activities, serum total protein, total Ig concentrations, plasma catalase, neutrophil oxidative production, and superoxide dismutase activity were increased by inclusion of GS (1–2%) in the diet. Moreover, increased survivability in fish challenged with bacteria such as *Streptococcus iniae, Yersinia ruckeri, Listeria monocytogenes, Escherichia coli* (Adel et al., 2016), and *Aeromonas hydrophila* (Zheng et al., 2011) or parasite infection such as *Amyloodinium ocellatum* (Buentello et al., 2010) was also observed with GS feeding.

GS is a feed additive based on dried brewer's yeast, which contains polysaccharides such as mannanoligosaccharide (MOS) and ß-glucans in its cell wall (Kogan and Kocher, 2007) which can have immunomodulatory effects. MOS (0.2%) feeding has been observed to improve immune responses of nursery pigs challenged with porcine reproductive and respiratory syndrome virus (PRRSV) indicated by reduced tumor necrosis factor (TNF)-α and increased serum IL-10 (Che et al., 2011). Supplementation of β-glucans (25–200 ppm) extracted from the cell wall of brewer's yeast had also been demonstrated to enhance plasma TNF-α and

IL-10 secretion in LPS- (25 µg/kg) challenged pigs (Che et al., 2012).

In the present study, results indicate that chicory supplementation may affect immune response to endotoxin challenge by altering immune biomarkers associated with inflammation. IL-8 and IL-10 increase in the LPS-challenged isolated PBMC from CL fed pigs compared with those given the Control diet implies that response to infectious disease by the animal can be influenced by bioactive compounds present in chicory such as inulin, oligofructose, and polyphenols. IL-8 is a cytokine involved in the activation of neutrophils from the blood to the site of infection and considered as one of the principal mediators of inflammation (Baggiolini et al., 1995; Bosi et al., 2004). It is expressed in enterocytes and macrophages to recruit other inflammatory cells and is highly selective for neutrophil movement to inflammatory sites. Neutrophils are a relevant class of inflammatory cells in infectious disease (Bosi et al., 2004). In contrast, production of IL-10 principally by macrophages and Th2 T-cells inhibits a cell-mediated immune response and macrophage function (Moore et al., 1993). Cytokines (e.g., IL-10) can hinder macrophage function including the oxidative burst, nitric oxide production, phagocytosis, proinflammatory cytokine production, and accessory functions of macrophages in T-cell activation (Aste-Amezaga et al., 1998).

IL-8, which is largely produced by the epithelial cells themselves, appears to be a major mediator of the recruitment of polymorphonuclear leukocytes (PMNs) to the subepithelial area and transmigration of these cells through the epithelial lining (Sansonetti et al., 1999). By mediating eradication of bacteria at their epithelial entry site, although at the cost of severe epithelial destruction, IL-8 therefore appears to be a key chemokine in the control of bacterial translocation. Low concentrations or absence of IL-8 in the serum of infected patients is associated with more severe diseases such as fatal Lassa fever (Mahanty et al., 2001).

IL-10 is a potent anti-inflammatory cytokine produced by a variety of cells but mainly by macrophages, B cells, and T cells (Moore et al., 1993). Increase in IL-10 is associated with infectious diseases such as those caused by *Mycoplasma hyopneumoniae* and PRRSV (Thanawongnuwech and Thacker, 2003). It plays a critical role in shaping immune responses by serving as a key anti-inflammatory cytokine secreted by activated macrophages as a feedback control mechanism to prevent excessive inflammatory responses (Sellon et al., 1998; Stanley et al., 2012). Severe inflammation was observed in IL-10 deficient germ-free mice challenged with *Helicobacter hepaticus* which highlights the importance of IL-10 in regulating immunopathology (Sellon et al., 1998).

Weak pro-and anti-inflammatory response from infection is associated with severe disease outcome (Mahanty et al., 2001). Hence, it is important to have a check and balance of these cytokines to ameliorate disease consequences such as those elucidated in LPS challenge. Based on the results of the study, chicory supplementation and to some extent, GS, may help modulate secretion of some cytokines in response to endotoxin challenge such as LPS and can be beneficial in animals that are prone to conditions that can cause inflammation.

CONCLUSION

Based on the results of the present study, GS feeding may improve growth performance of nursery pigs particularly during days 28 to 35 after weaning. In addition, in vitro LPS challenge using peripheral blood mononuclear cells obtained from pigs fed chicory at low concentrations may increase secretion of cytokines (IL-8 and IL-10). In conclusion, GS and chicory may be used in nursery pig diets to improve feed intake, growth rate, and modulate in vitro secretion of selected cytokines.

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