

ABSTRACT

BLOOMER, SARAH ALISSA. Combinational Use of Na-Butyrate and Phytobiotics on Growth Performance and Intestinal Health of Nursery Pigs. (Under the direction of Dr. Sung Woo Kim.)

The period after weaning is accompanied by significant stressors that compromise pig gastrointestinal and immune health. Sodium butyrate (SB) and phytobiotics (PH) have generated interest as potential antibiotic alternatives to alleviate post-weaning stress. Research findings involving the use of these additives have a wide variation in results, however, and a limited number of studies have reported on the effects of using these additives in combination.

The objective of this study was to determine the effects of encapsulated SB (Ultramix C, Nutriad, Elgen, IL), PH (Apex Swine, Nutriad), and a sequential use of SB and PH on growth performance, fecal score, gut morphology, immune status, and oxidative stress status in nursery pigs. Fifty-six weaned pigs (21 d of age; 6.9 ± 0.6 kg BW) were individually housed and allotted to 5 dietary treatments through a randomized complete block design. Pigs were fed for 33 d in 3 phases (phase 1: 0 to 7 d; phase 2: 7 to 19 d; and phase 3: 19 to 33 d). Treatments were: 1) a basal diet with no additives (n = 12); 2) basal diet + SB in phases 1 (0.2%) and 2 (0.1%) (n = 12); 3) basal diet + 0.033% PH in phase 3 (n = 12); 4) basal diet + SB in phases 1 (0.2%) and 2 (0.1%) + 0.033% PH in phase 3 (n = 12); and 5) basal diet + SB in phase 1 (0.2%) and phases 2 and 3 (0.1%) (n = 8). Diets were formulated to meet or exceed the nutrient requirements estimated by NRC (2012) and pigs had *ad libitum* access to feed and water. Feed intake and BW were recorded at the beginning and end of each phase to calculate ADG, ADFI, and G:F, and fecal scores were assessed every odd day from d 3 to 19 and on d 26 and 33. Blood samples were collected at the end of phases 2 and 3 to measure

tumor necrosis factor alpha (TNF- α), IL-6, and immunoglobulin G (IgG). Four pigs from each of the first 4 treatments (16 total) were euthanized at the end of phase 2 and the remaining pigs from all treatments (40 total) were euthanized at the end of phase 3 for sample collection. Jejunal tissue, jejunal mucosa, and ileal digesta samples were obtained to measure villus height (VH), villus width (VW), crypt depth (CD), rate of enterocyte proliferation, TNF- α , IL-6, myeloperoxidase, malondialdehyde (MDA), and protein carbonyl. Data were analyzed using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC). Initial BW and sex were blocking factors and pen was the experimental unit. Fixed effects were either SB and PH or treatment. Block was considered a random effect. Sodium butyrate decreased ($P < 0.05$) phase 2 and phase 1 to 2 ADFI but had no effect on ADG and G:F. Sodium butyrate tended ($P = 0.063$) to decrease jejunal villus height to crypt depth ratio (VH:CD) at the end of phase 2 and increased ($P < 0.05$) the rate of enterocyte proliferation at the end of phase 3. Phytobiotics increased ($P < 0.05$) phase 3 and overall G:F without affecting ADG and ADFI. Neither SB nor PH affected fecal score, jejunal VH, VW, and CD, or gut health measurements. There were tendencies for interaction between SB and PH for VH:CD ($P = 0.074$), MDA ($P = 0.087$), and serum IL-6 ($P = 0.052$). In conclusion, SB and the sequential use of SB and PH had minimal effects on growth performance and intestinal health when added to the diets of nursery pigs. However, PH supplementation 19 to 33 d post-weaning improved feed efficiency.

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Combinational Use of Na-Butyrate and Phytobiotics on Growth Performance and Intestinal Health of Nursery Pigs

by
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DEDICATION

This work is dedicated to my parents,

Joe and Holly Bloomer,

for being with me every step of the way.

BIOGRAPHY

Sarah A. Bloomer was born on June 3rd, 1994 in Dallas, Texas and was raised in Wake Forest, North Carolina. Growing up with dogs and riding horses, she had an interest in animal health and production from a young age. She received Bachelor of Science degrees in Animal Science and Agricultural Business Management in 2016 from North Carolina State University. Later that year she started her Master of Science degree with Dr. Sung Woo Kim at N.C. State, focusing in swine nutrition. Her thesis studies the effects of supplementing encapsulated sodium butyrate and phytobiotics on the growth and gut health of nursery pigs.

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TABLE OF CONTENTS

LIST OF TABLES	viii
LIST OF FIGURES	x
LIST OF ABBREVIATIONS.....	xi
CHAPTER I. LITERATURE REVIEW.....	1
Introduction.....	2
Butyric Acid.....	3
Methods of delivery	4
Modes of action.....	6
Effects on growth performance.....	7
Effects on gastrointestinal content and digestibility	14
Effects on diarrhea incidence.....	15
Effects on pH	16
Effects on intestinal morphology and gut integrity.....	17
Effects on intestinal microbiota	19
Effects on immune status and oxidative stress	20
Phytobiotics.....	21
Modes of action.....	22
Effects on growth performance.....	23
Effects on digestibility and noxious gas emissions.....	29
Effects on diarrhea incidence.....	31
Effects on intestinal morphology and enterocyte proliferation.....	32
Effects on intestinal microbiota	34
Effects on immune status and oxidative stress	36
Butyric Acid and Phytobiotics in Combination	39
Scope of the Present Thesis	41
Literature Cited.....	43
CHAPTER II. COMBINATIONAL USE OF NA-BUTYRATE AND PHYTOBIOTICS ON GROWTH PERFORMANCE AND INTESTINAL HEALTH OF NURSERY PIGS ..	57
Abstract.....	58
Introduction.....	60

Materials and Methods.....	61
Animals and experimental design.....	61
Experimental diets	63
Growth performance and fecal score	63
Sample collection.....	63
Intestinal morphology	64
Immune and oxidative stress measurements.....	65
Statistical analysis.....	66
Results.....	67
Growth performance and fecal score	67
Intestinal morphology	67
Immune and oxidative stress status.....	68
Discussion.....	69
Conclusion	77
Literature Cited	79

LIST OF TABLES

Table 1.	Composition of experimental diets, as-fed basis.....	89
Table 2.	Growth performance of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning	91
Table 3.	Growth performance of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.....	92
Table 4.	Growth performance of pigs fed experimental diets from 19 to 33 d post-weaning	93
Table 5.	Fecal scores of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning	94
Table 6.	Fecal scores of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning	95
Table 7.	Fecal scores of pigs fed experimental diets from 19 to 33 d post-weaning	96
Table 8.	Histological measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning	97
Table 9.	Histological measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning	98
Table 10.	Histological measurements of pigs fed experimental diets from 19 to 33 d post-weaning	99
Table 11.	Immune status measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning	100
Table 12.	Immune status measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning	101
Table 13.	Immune status measurements of pigs fed experimental diets from 19 to 33 d post-weaning	102
Table 14.	Oxidative stress indicators of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning	103
Table 15.	Oxidative stress indicators of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning	104

Table 16.	Oxidative stress indicators of pigs fed experimental diets from 19 to 33 d post-weaning	105
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LIST OF FIGURES

Figure 1. Jejunal villus and crypt histological measurements 106

LIST OF ABBREVIATIONS

ADG: average daily gain

ADFI: average daily feed intake

BW: body weight

CD: crypt depth

CP: crude protein

d: day

DFM: direct fed microbial

DM: dry matter

FCR: feed conversion ratio

FDA: Food and Drug Administration

F:G: feed to gain ratio

g: gram

G:F: gain to feed ratio

IFN- γ : interferon gamma

IgA: immunoglobulin A

IgG: immunoglobulin G

IgM: immunoglobulin M

IL: interleukin

kg: kilogram

MCT: monocarboxylate transporter

MDA: malondialdehyde

mg: milligram

MPO: myeloperoxidase

NF- κ B: nuclear factor kappa B

PH: phytobiotics

SB: sodium butyrate

SCFA: short chain fatty acid

SEM: standard error of the mean

SID: standardized ileal digestibility

SMCT: Na⁺ coupled monocarboxylate transporter

STTD: standardized total tract digestible

TGF- β : transforming growth factor beta

TNF- α : tumor necrosis factor alpha

VH: villus height

VH:CD: villus height to crypt depth ratio

VFA: volatile fatty acid

VFD: Veterinary Feed Directive

VW: villus width

wk: week

CHAPTER I
LITERATURE REVIEW

Introduction

In the period following weaning, young pigs are susceptible to a wide variety of stressors, including diarrhea, oxidative stress, and bacterial pathogens. These stressors impair piglet growth and overall gut health. To combat this, nursery pig diets are often supplemented with additives such as antibiotics, which have been proven to increase growth rate and feed utilization while decreasing symptoms of post-weaning stress (Vondruskova et al., 2010). However, it has become apparent in the past decades that the widespread use of antibiotics, particularly as growth promoters, in food animal production may lead to the pervasiveness of antibiotic-resistant bacteria in both animals and humans (Durso and Cook, 2014). This issue, in conjunction with increased global focus on food production safety, helped push for the creation of the Veterinary Feed Directive (VFD) by the U.S. Food and Drug Administration (FDA). The VFD reduces the number and uses of antibiotics commercially available to producers, which therefore limits the number of supplements that can remedy the adverse effects of weaning. As a result, the swine industry has an increased demand for the development of new non-antibiotic alternatives for nursery pig production.

Alternative additives examined by researchers include acidifiers, feed enzymes, trace minerals, and microbiota modifiers (Thacker, 2013). Acidifiers, such as citric, fumaric, and formic acids, are proposed to decrease the pH of the pig gastrointestinal tract, thus improving nutrient digestion and safeguarding against pathogens (Kil et al., 2011). Phytase and carbohydrase enzymes potentially play a role in improving digestion and decreasing phosphorus excretion, and dietary inclusion of trace minerals such as copper and zinc has been shown to improve piglet growth performance (Partridge, 2001; Jacela et al., 2010a; Lei et al., 2013). Microbiota modifiers include prebiotic and direct fed microbial (DFM)

additives. Prebiotics promote the growth of favorable microorganisms in the intestine, and DFM deliver live bacterial cultures, such as beneficial *Bacillus* and *Bifidobacterium* species, directly to the gut (Jacela et al., 2010b). Both types of additives have been reported to enhance disease resistance and promote growth to the same degree as antibiotics in nursery pig production (Kritas and Morrison, 2005; Halas and Nochta, 2012).

Two additional alternatives that have increasingly gathered interest in the research community are butyric acid and phytobiotics. Both are derived from natural sources and have shown promise in improving broiler growth and gut efficiency in poultry production due to their antimicrobial and antioxidative properties (Peng et al., 2016; Liu et al., 2017). Although extensive research has been conducted on their effects in swine, the results for both are highly variable. Differences in inclusion levels, type of butyrate encapsulation, the specific mix of phytobiotics, and the targeted stage of production contribute to this variability. This review will evaluate the previous uses of butyric acid and phytobiotics in pig production and their resulting impacts on growth and gut health.

Butyric Acid

Short chain fatty acids (SCFA) are a group of organic acids that consist of 1 to 7 carbon atoms in either straight- or branched-chain forms. They are produced in the intestine via bacterial fermentation of plant materials such as cellulose and fibers (Guilloteau et al., 2010). The predominant SCFA are acetic, propionic, and butyric acids. Of these, butyric acid is the most readily oxidized to carbon dioxide and is the predominant energy source for epithelial cells of the ileum and colon (Fleming and Gill, 1997; Chen et al., 2015). Butyric acid has also been shown to improve growth performance and inhibit harmful intestinal

bacteria at lower concentrations than other SCFA and organic acids (Gálfi and Bokori, 1990; Lu et al., 2008). However, several contradictory results exist, particularly relating to butyric acid's effects on growth performance (Weber and Kerr, 2008; Fang et al., 2014; Jang et al., 2017). This variability necessitates a need for further analysis of butyric acid's efficacy in promoting growth and intestinal health in newly weaned pigs.

Methods of delivery

Butyric acid is an oily liquid and has an observable rancid odor. As a result, feeding salts is preferred over free acids because they are relatively odorless and are easier to handle in the feed manufacturing process (Lallès et al., 2009; Guilloteau et al., 2010). Sodium butyrate (SB) appears to be the most frequently used among the butyrate salts as a feed additive in pigs. Specifically, SB has been found to improve the growth performance of weaned pigs, improve gastrointestinal barrier function, and inhibit the growth of harmful intestinal bacteria (Piva et al., 2002; Le Gall et al., 2009).

Butyrate is often encapsulated to deter prompt absorption and metabolism in the upper gastrointestinal tract of the stomach and duodenum. Many encapsulation materials exist; studies in nursery pig diets have used inulin (Lacorn et al., 2010), polyacrylic acid resin (Fang et al., 2014), and vegetable fats (Mallo et al., 2012). Manzanilla and his colleagues (2006) found that unprotected butyrate in the diet of newly weaned pigs is detectable in the stomach but not in the cranial jejunum. Additionally, in a trial with 528 newly weaned pigs, Mallo et al. (2012) showed that pigs supplemented with vegetable fat-coated SB had higher final body weights and improved ADG after 39 d compared to piglets that were fed an unprotected monoglyceride of butyric acid (2 butyric acid per molecule of glycerol). These

studies indicate that encapsulation is necessary to prevent the immediate absorption of oral butyric acid until it reaches the mid and lower gastrointestinal tract.

Even with encapsulation, however, there can be complications with delivering butyrate to defined intestinal parts. Lacorn et al. (2010) attempted to coat butyric acid with inulin, which has a low pre-cecal digestibility, and deliver it to the colon of finishing pigs. The authors evaluated intestinal chyme for inulin content and detected highest values in the jejunum, decreased values in the ileum, and values below the detection limit (<2 mg/g) in the colon. In their aforementioned study, Mallo et al. (2012) also determined that SB should be coated in a fat matrix that would be primarily digested by pancreatic lipase in the small intestine, but noted that even if protected, a significant amount is still absorbed in the upper gut. For these reasons, attention to the type and degree of encapsulation is necessary when desiring for butyrate to be delivered to the lower gut.

Another source of study variability concerns the composition of the encapsulated matrix of the final product. Most authors do not publish the source of their encapsulation (e.g., type of vegetable fat), bead diameter, and butyric acid inclusion percentages in the matrix, and these characteristics can vary from one feed supplier to another (Moquet et al., 2016). In swine trials where the matrix composition was stated, butyric acid inclusion ranged from 30% (Fang et al., 2014) to 85% (Biagi et al., 2007) of the final product. This can make comparing the findings of one study to another more difficult, especially considering that the specific composition of a butyrate matrix can affect its location of release in the gastrointestinal tract.

Modes of action

There are several suggested mechanisms by which butyric acid affects gut efficiency and health in swine. In the intestine, butyrate has been shown to affect colonocyte energy production, increase enterocyte proliferation and apoptosis of malignant cells, control pathogens and cytokine production, and synthesize ketone bodies (Guilloteau et al., 2010). Short chain fatty acids can pass through the apical membrane of epithelial intestinal cells by diffusion as uncharged acids or by active transport via H⁺ coupled (MCT) or Na⁺ coupled (SMCT) monocarboxylate transporters (Halestrap and Price, 1999). Li et al. (2003) demonstrated that when bound to SMCT-2, butyrate increases apoptosis of malignant tumor cells. At the same time, butyrate can decrease apoptosis of normal enterocytes by influencing gene expression and protein synthesis (Sengupta et al., 2006).

Research indicates that a direct effect by butyrate via blood circulation is unlikely because it is almost entirely metabolized in the gut or liver (Guilloteau et al., 2010). However, butyric acid can act indirectly when coupled with receptor proteins located on cells proximal to the gut. Butyrate induces the production of host defense peptides, which increase cell proliferation by stimulating the development and repair of epithelial cells (Bartholome et al., 2004; Guilloteau et al., 2009). Butyrate can also bind to specific G-protein coupled receptors involved in the regulation of lipid and glucose metabolism in the gut (den Besten et al., 2013). These receptors stimulate the production of peptides that have been shown to improve the development and function of the immune system and enhance gut motility (Cox et al., 2009; Soret et al., 2010). Findings have also indicated that while butyrate receptors exist in a variety of cell types, they are most commonly found in immune cells (Guilloteau et al., 2010). Other studies, including one by Weber and Kerr (2008), indicate that butyrate

increases cortisol response and IL-6 mRNA expression, which affects the bodily response to inflammatory stimuli.

Effects on growth performance

Butyric acid has been shown to have a variety of effects on growth performance (ADG, ADFI, and G:F) in all phases of swine production. Despite the several published studies on the topic, results are highly inconsistent even within a single stage of production. In most studies, butyric acid was found to improve either all growth parameters or none at all; however, a few studies achieved mixed results within ADG, ADFI, and G:F (Piva et al., 2002; Manzanilla et al., 2006; Le Gall et al., 2009; Huang et al., 2015). This variability can most likely be attributed to the varying levels of butyric acid inclusion in the experimental diets, as well as to differences in the environmental conditions of the studied pigs.

A few studies have evaluated the effects of SB on sow growth performance and the resulting performance of their piglets. Jang et al. (2017) fed diets containing either 0, 500, or 1,000 ppm of encapsulated SB to sows in mid to late gestation and found no differences between treatments in ADFI, G:F, and BW at farrowing. From the sows supplemented with either 0 or 1,000 ppm of SB, 72 of their offspring were supplemented with 0, 500, or 1,000 ppm of SB from weaning to 35 d post-weaning. Pigs born from sows treated with 1,000 ppm of SB tended to have lower F:G (feed to gain) for the first 2 wk post-weaning compared to pigs from the 0 ppm sow treatment (Jang et al., 2017). However, there were linear decreases in d 35 BW and overall period ADG and ADFI as SB inclusion in the nursery diets increased (Jang et al., 2017). The authors reported no difference in nursery treatment F:G nor any interaction between the sow and nursery treatments. These findings indicate that even though

high levels of SB supplementation in sows can improve offspring feed efficiency, there is no effect on the growth performance of the sows themselves. Additionally, SB supplementation in nursery pigs may reduce growth and feed intake.

Two other studies evaluated the effects of butyric acid on sow and offspring growth performance. From d 77 of gestation to 21 d post-farrowing, Lu and colleagues (2012) supplemented primiparous sows with either 0 or 0.3% butyrate. Their piglets were weaned at 21 d and consumed no SB through 12 wk of age. Pigs from sows supplemented with butyrate had significantly higher BW at 12 wk than pigs from sows that did not receive butyrate (Lu et al., 2012). Average daily gain, ADFI, and G:F were not measured. In another study, sows supplemented with 6 g/kg of SB in lactation did not experience changes in ADFI compared to sows fed 0 g/kg (Wang et al., 2014). Their piglets, which were fed a creep feed containing 6 g/kg SB from 7 to 28 d of age, did not have differences in weaning weight (d 21), final BW (d 28), ADG, ADFI, and feed conversion ratio (FCR) compared to piglets that received 0 g/kg and were from sows that received no SB inclusion (Wang et al., 2014). These conflicting results may be a consequence of the doubled inclusion rate of butyrate between the studies. Sodium butyrate may also have a disagreeable smell at high inclusion levels, which would influence feed intake and therefore growth. However, this does not explain why Jang et al. (2017) still found negative impacts of SB in their sow and nursery study at a relatively low inclusion level.

Relatively little research has been collected on the efficacy of neonatal butyrate supplementation on improving growth. Kotunia et al. (2004) found that piglets given a milk formula with 3 g/kg of SB had greater BW at 10 d of age and higher ADG from 3 to 10 d of age than pigs given 0 g/kg of SB. However, these results should not be over-interpreted

because the initial BW of the pigs receiving SB was also higher than the control group. Neonatal pigs administered with 0.1 g/(kg · BW) of oral SB solution from 0 to 21 d of age had no difference in final BW or overall ADG in comparison to pigs given a saline solution (Yu et al., 2017).

Although supplementing neonatal piglets with butyric acid may have questionable effects on growth performance before weaning, two studies have shown that the addition of butyric acid to the pre-weaning diet may have positive effects on post-weaning growth (Le Gall et al., 2009; Lu et al., 2012). Le Gall et al. (2009) investigated the effects of both pre-weaning (0 or 60 g/L SB solution, 5 to 28 d of age) and post-weaning (0 or 3 g/kg SB, 28 to 40 d of age) butyric acid inclusion on pre- and post-weaning growth. While there were no differences in pre-weaning growth between treatments, pigs given 60 g/L SB before weaning had greater BW at 40 d of age and increased ADG and ADFI post-weaning in comparison to pigs given 0 g/L (Le Gall et al., 2009). Likewise, pigs given 500 mg/d of butyrate from d 4 of age to weaning (d 21) had higher BW at 12 wk of age and improved ADG for the post-weaning period compared to pigs given 0 mg/d of butyrate (Lu et al., 2012). These studies suggest that butyrate inclusion in the pre-weaning diet of pigs may have long-lasting effects on growth performance. Therefore adding butyric acid to the diets of neonatal piglets could be beneficial in preparing their gastrointestinal system for the post-weaning phase.

In the post-weaning nursery phase, numerous studies have shown that butyrate can improve BW, ADG, ADFI, and G:F (Gálfi and Bokori, 1990; Lu et al., 2008; Wen et al., 2012). Gálfi and Bokori (1990) supplemented the diets of 164 nursery pigs weaned at 31 d of age with 0 or 15 mmol/kg of SB for 79 d. Although there were no differences in ADFI, the SB treatment group performed better than the control group with improved final BW, ADG,

and F:G (Gálfi and Bokori, 1990). In similar studies conducted by Lu et al. (2008) and Wen et al. (2012), nursery pigs were supplemented with 0, 500, or 1,000 mg/kg of SB. Both studies had 96 pigs weaned at 21 d, however the former trial lasted 30 d and the latter 21 d. Pigs given 1,000 mg/kg of SB in both studies had increased BW at the end of the trial period and improved ADG, ADFI, and G:F compared to both the 0 and 500 mg/kg SB treatments. Both studies also reported that the 500 mg/kg SB treatment group had no difference in BW, ADG, ADFI, and G:F compared to the control group, which indicates that SB may have a minimum level of inclusion at which it can still improve growth characteristics.

Manzanilla et al. (2006) and Huang et al. (2015) evaluated the effects of SB inclusion in comparison to traditional antibiotics in nursery pigs. In the first study, 24 pigs weaned between 18 and 22 d received either a basal diet with no additives (CON), a diet with 0.04% avilamycin (AB), or a diet with 0.3% SB (AC). Compared to CON, AB and AC treated groups had improved G:F 7 to 14 d after weaning and for the overall period of 0 to 14 d (Manzanilla et al., 2006). There were no differences between AB and AC for ADG, ADFI, or G:F for the overall trial (Manzanilla et al., 2006). In the second study, 90 pigs weaned at 28 d were given either a basal diet with no additives (CON), with antibiotics (100 mg/kg of kitasamycin, 40 mg/kg of colistin sulfate; PC), or with reduced antibiotics and SB (50 mg/kg of kitasamycin, 20 mg/kg of colistin sulfate, 1,000 mg/kg of SB; ASB). PC and ASB treatments improved ADG and G:F compared to CON for the 28 d period (Huang et al., 2015). In addition, the authors found no growth performance differences between PC and ASB. These studies suggest that SB may be able to partly or fully replace the use of antibiotic growth promoters in nursery pig diets without reducing growth performance.

The remaining nursery studies evaluated for this review concluded that the addition of sodium butyrate to the diet of nursery pigs either has no effect, a negative effect, or mixed effects on growth performance. Research by Biagi et al. (2007), Fang et al. (2014), and Grilli et al. (2016) found no effects of SB on final BW, ADG, ADFI, and G:F for weaned pigs. They evaluated 0, 1,000, 2,000, or 3,000 ppm; 0 or 1,000 mg/kg; and 0 or 960 mg/kg of SB, respectively, and all 204 pigs were weaned at 28 d of age. Hanczakowska et al. (2016) found no effects in BW and ADG for pigs given diets with 3 g/kg SB versus 0 g/kg. In a pair of trials conducted by Mallo et al. (2012), there were no differences in growth performance in pigs fed 0, 2, or 2.5 kg/t of SB.

Weber and Kerr (2008) and Jang et al. (2017) found that increasing levels of coated SB linearly reduced growth performance in nursery pigs. As mentioned previously, increasing levels of 0, 500, and 1,000 ppm of SB linearly decreased final BW and overall ADG and ADFI in weaned pigs over a 35 d period (Jang et al., 2017). Weber and Kerr (2008) supplemented pigs with 0, 0.05, 0.1, 0.2, or 0.4% SB over a 4 wk period. Both ADG and ADFI tended to linearly decrease as the level of SB increased in the diet 14-28 d post-weaning and for the overall period (Weber and Kerr, 2008). In the aforementioned study by Le Gall et al. (2009), weaned pigs supplemented with 3 g/kg SB post-weaning had a tendency for increased overall ADG and ADFI compared to pigs that received no SB in their diets, but not greater final BW or improved G:F. Lastly, in a trial with 40 pigs that were given either 0 or 0.8 g/kg of SB for 56 d, Piva et al. (2002) observed that even though final BW, ADG, and ADFI increased, overall G:F decreased.

Butyric acid supplementation has also been studied in growing and finishing pigs. Gálfi and Bokori (1990) added 0 or 15 mmol/kg of SB to the diets of 164 pigs for 139 d until

they reached an average BW of 100 kg. The SB treatment improved final BW and overall ADG, ADFI, and G:F compared to the control treatment (Gálfi and Bokori, 1990).

Øverland et al. (2008), on the other hand, found no influence of butyric acid on final BW, ADG, ADFI, and FCR for 20 growing pigs supplemented with either 1.2% Ca-butyrate or 1.5% inulin-coated butyric acid for 78 d. These results are supported by a study conducted by Lacorn et al. (2010), where 18 finishing pigs were given either 0 or 150 g/d of inulin-coated butyrate for 6 d, and butyrate was found to have no influence on final BW. Similarly, Walia et al. (2016) fed 177 finishing pigs either 0 or 3 kg/t of SB for 24 d and found no differences in growth performance. It is important to note the acute differences in both sample size and trial duration between these studies. The short length of study combined with the initially higher average BW (97 kg) in Lacorn et al. (2010) most likely contributed to the difference in results observed by Gálfi and Bokori (1990). In addition, both Øverland et al. (2008) and Lacorn et al. (2010) did not use the Na salt of butyrate, whereas Gálfi and Bokori (1990) and Walia et al. (2016) did, which may have led to differing rates of absorption of butyric acid and therefore different effects within the upper and lower gut.

In immune challenging conditions, the effects of butyric acid on growth performance continue to vary in different stages of production. In a study by Jang et al. (2017), 72 pigs from sows supplemented with 0 or 1,000 ppm SB mid to late gestation were injected intramuscularly at weaning and 14 d post-weaning with 4 mg of ovalbumin. The pigs were also allotted to diets containing SB at 0, 500, and 1,000 ppm, creating a split-plot design. Pigs from the 1,000 ppm SB sow treatment had greater BW than 0 ppm sow treatment pigs on d 14 and 35 post-weaning, and had greater overall ADG, ADFI, and F:G (Jang et al., 2017). This indicates that some aspect of butyric acid inclusion in sow gestation diets may

influence piglet immune status and growth later in challenged conditions. Furthermore, unlike the linear decreases in growth performance found by Jang et al. (2017) due to increasing levels of SB inclusion in non-challenged weaned pigs, the authors found the opposite to be true for the challenged pigs. Increasing levels of SB supplementation linearly increased BW 35 d post-weaning, overall ADG, and overall ADFI (Jang et al., 2017). There was also a quadratic response for d 14 BW, d 0 to 14 ADG, and overall G:F (Jang et al., 2017). Therefore, SB addition to sow and nursery pig diets may have the strongest effect immediately after weaning for pigs that are immunosuppressed.

In contrast, other studies involving challenged conditions in pigs showed no effects of butyric acid inclusion on growth performance (Weber and Kerr, 2008; Walia et al., 2016). Weber and Kerr (2008) injected 25 ug/(kg · BW) of *Escherichia coli* lipopolysaccharide (LPS) into 108 weaned pigs that were assigned to 1 of 3 treatments: a basal diet with no additives (CON), CON + 0.2% SB (SB), and CON + 55 mg/kg carbadox (AB). They found that in the 2 wk period following injection, there were no differences in growth performance between CON and SB, and SB decreased ADG and tended to decrease G:F in comparison to the AB treatment. In addition, in a study by Walia et al. (2016) involving 177 growing pigs infected with monophasic *Salmonella typhimurium*, there were no differences in growth performance for pigs fed 0 or 3 kg/t SB.

Collectively, these studies demonstrate that there are a myriad of responses in growth performance to butyric acid supplementation in all phases and environments of swine production. Results tend to indicate that SB is more effective at promoting growth in nursery pigs if fed earlier after weaning rather than later. Supplementing diets with butyric acid during gestation, lactation, and/or before weaning could also promote nursery pig

performance. In grower and finisher pigs, the role of butyric acid in promoting growth performance is still unclear, as variations in trial length, initial BW, type of butyric acid, and level of inclusion differed greatly in the literature. Pigs supplemented with 1,000 mg/kg of SB experienced both positive and negative effects on growth, which indicates that butyric acid may be affecting the gastrointestinal system of the pigs in different ways. Therefore, exploring the effects of butyric acid on digestibility, gut health, oxidative stress, immune status, and intestinal morphology is crucial to understanding the full extent of butyrate's impact in the gut of pigs.

Effects on gastrointestinal content and digestibility

Butyric acid and other organic acids are thought to delay gastric emptying, which improves the digestibility of feedstuffs (Partanen and Mroz, 1999). Higher intestinal content and percentage of DM in the gut may reflect a slower emptying rate (Manzanilla et al., 2004). Several studies have shown that SB has, at least to some extent, effects on gastrointestinal content and digestibility, particularly related to volatile fatty acid (VFA) and SCFA content. In the small intestine, SB addition to the diet was shown to decrease SCFA content in jejunal chyme and increase acetic acid, n-butyric acid, and VFA concentrations in the ileum in pigs post-weaning (Gálfi and Bokori, 1990; Hanczakowska et al., 2016). In the colon, butyric acid had no effect on total VFA concentration but did increase butyric and lactic acid, and tended to increase acetic acid (Gálfi and Bokori, 1990; Mallo et al., 2012). No effects on propionic acid in pigs were observed in the upper or lower gut (Gálfi and Bokori, 1990; Mallo et al., 2012; Hanczakowska et al., 2016). Short chain fatty acids are primarily produced by microbial fermentation in the intestine, so the change in SCFA content

could be related to microbial modulation by butyric acid (Gancarcikova et al., 2009). Sodium butyrate was reported to have no effect on the DM content of the stomach, ileum, cecum, colon, or rectum (Manzanilla et al., 2006). This is slightly contrasted by Le Gall et al. (2009), who noted higher DM content in the stomach of pigs supplemented with SB compared to pigs fed no supplement.

Butyric acid's effects on digestibility in swine are not widely reported in the literature. When provided during the neonatal phase, SB had no apparent effects on ileal digestibility of DM or N, but increased fecal digestibility of DM, N, and organic matter (Le Gall et al., 2009). Le Gall and colleagues (2009) also found that providing SB in post-weaning diets tended to decrease ileal DM digestibility, significantly decreased ileal N digestibility, and decreased fecal apparent digestibility of DM and organic matter. Although data for swine is limited, in broiler production butyric acid inclusion has been found to improve digestibility of total tract crude fat, apparent ME, and several amino acids in the ileum (Kaczmarek et al., 2016). Accordingly, further investigation is needed to determine the effects of butyric acid on energy and nutrient digestibility in pigs.

Effects on diarrhea incidence

Two nursery pig studies examined the effects of SB on diarrhea incidence. In a study comparing 0 and 1,000 mg/kg of SB addition, Fang et al. (2014) found that pigs given SB had significantly reduced diarrhea incidence in the period immediately following weaning. Huang et al. (2015) supported this by reporting that pigs supplemented with a diet containing reduced levels of antibiotics and 1,000 mg/kg of SB had lower diarrhea incidence than pigs with no additives. This impact on diarrhea was not significantly different than the decrease in

diarrhea incidence caused by another treatment with a full antibiotic load (Huang et al., 2015). Poor fecal consistency may be the consequence of an immature or unhealthy digestive tract and is a common symptom of post-weaning stress. Sakata (1987) suggested that SCFA, including butyric acid, can stimulate the development and growth of the gastrointestinal tract. This would explain the improvement in fecal consistency suggested by the above studies.

Effects on pH

The pH balance in the gut is an important component of a healthy microenvironment and digestive system. Several trials in neonatal, nursery, and growing pigs have shown that SB inclusion in the diet does not affect the pH of gastric and intestinal contents (Gálfi and Bokori, 1990; Manzanilla et al., 2006; Biagi et al., 2007; Mazzoni et al., 2008; Hanczakowska et al., 2016). In pigs given 3 g/kg of SB from 7 to 60 d of age, SB did not change the level of acidity of chyme over the entire length of the gastrointestinal tract (Hanczakowska et al., 2016). Similarly, the addition of 60 g/L of SB pre-weaning and/or 3 g/kg post-weaning did not show any changes in gastric pH in both the pre- and post-weaning periods (Mazzoni et al., 2008). Manzanilla et al. (2006) found no effects in stomach, ileal, cecal, or colonic pH at 0.3% SB, whereas Biagi et al. (2007) reported that cecal pH increased linearly as SB inclusion increased from 0.1 to 0.3%. Lastly, in a study involving nursery and growing pigs, SB inclusion in the diet at 15 mmol/kg did not decrease the pH of the gastrointestinal contents at 79 or 139 d of age. Collectively, these studies indicate that including butyric acid in the diet does not affect the overall pH of the swine digestive tract.

Effects on intestinal morphology and gut integrity

Weaning in young pigs is associated with a multitude of changes in gut morphology, including a reduction in villus height and a deepening of crypts (Piva et al., 2002). These changes decrease the absorptive capacity of the intestine, causing a reduction in feed intake and efficiency (Campbell et al., 2013). In addition, the epithelial layer of the intestine serves as a crucial barrier between the internal and external environments. Decreased gut integrity allows toxins, bacteria, and other antigens to cross this barrier and cause inflammation, diarrhea, and reduced growth (Campbell et al., 2013). In this regard, the effects of butyric acid on both intestinal morphology and gut integrity is important in understanding its role in overall gut health.

In studies examining neonatal histology, butyric acid was generally concluded to have no effect on duodenal and jejunal villus heights, crypt depths, and villus height to crypt depth ratio (VH:CD) (Kotunia et al., 2004; Hanczakowska et al., 2016). However, Kotunia et al. (2004) also reported mixed results with decreased duodenal villus height and increased ileal villus height and crypt depth in neonatal piglets given 0.3% versus 0% SB. In growing and finishing pigs, Claus et al. (2007) and Lacorn et al. (2010) found no effect of butyrate addition to diets on jejunal villus height, yet Gálfi and Bokori (1990) reported increased villus height in the ileum.

Histological findings in nursery pigs are highly variable. Duodenal, jejunal, and ileal villus heights and VH:CD were increased by 1,000 mg/kg of SB addition in research by Lu et al. (2008) and Wen et al. (2012). In addition, duodenal, jejunal, and ileal crypt depths were decreased when supplementing at a minimum of 1,000 mg/kg SB (Lu et al., 2008; Wen et al., 2012; Huang et al., 2015). In some cases, butyric acid inclusion showed negative results.

Jejunal villus height (Huang et al., 2015), jejunal crypt depth (Manzanilla et al., 2006), and cecal crypt depth (Biagi et al., 2007) were all negatively influenced by SB addition to the diet. Overall, a great deal of studies found no effect of butyric acid on histological measurements for nursery pigs, including duodenal, jejunal, ileal, cecal, and colonic villus heights, crypt depths, and VH:CD (Manzanilla et al., 2006; Biagi et al., 2007; Lu et al., 2008; Le Gall et al., 2009; Huang et al., 2015). For all phases of production, the type of butyric acid product used and the dosage level appear to be insignificant between the results of the studies. Therefore, it cannot be confirmed if butyric acid has positive effects on the intestinal microvilli.

Butyric acid addition to swine diets may influence the rates of enterocyte proliferation, mitosis, and apoptosis. In nursery trials, 0.3% SB did not affect mitotic rates in the jejunum, ileum, and colon (Manzanilla et al., 2006), but did increase intestinal epithelial proliferation (Le Gall et al., 2009). Apoptotic rates in nursery pigs were not affected (Le Gall et al., 2009). In growing and finishing pigs, the mitotic rates of the jejunum and ileum were not affected by SB inclusion (Claus et al., 2007; Lacorn et al., 2010). Lacorn and colleagues (2010) also found increased proliferation rates in the colon. Both Claus et al. (2007) and Lacorn et al. (2010) did not find differences in apoptosis in the gastrointestinal tract, but noted that butyrate-fed pigs had numerically reduced apoptotic rates.

Gut barrier integrity can be evaluated by the expression of tight junction proteins and brush border enzyme activity. Occludin and claudin-1 are transmembrane proteins that function as anchors for pairs of epithelial cells, sealing the epithelial layer and regulating permeability (Turner, 2009). In a study with newly weaned pigs, Grilli et al. (2016) found that 960 mg/kg of SB supplementation in the diet increased occludin expression in the

duodenum and tended to increase expression in the jejunum. Claudin-1 expression, however, was reduced in the duodenum, jejunum, and ileum, and tended to be reduced in the colon (Grilli et al., 2016). Another nursery trial also reported increased jejunal and colonic occludin expression (Huang et al., 2015). When SB was provided postnatally, no effects were observed in the specific activities of brush border enzymes, but the total activities of jejunal maltase, sucrase, aminopeptidase A, aminopeptidase N, and dipeptidyl peptidase IV were reduced (Le Gall et al., 2009). Kotunia and colleagues (2004) supported this finding by reporting no effects of 0.3% SB inclusion on the same enzymes in the jejunum.

Effects on intestinal microbiota

The redistribution of gut microbiota by certain feed additives can influence several aspects of overall gut health, including fermentation and digestibility (Le Gall et al., 2009; Huang et al., 2015). Adding 1,000 mg/kg of SB to the diet of nursery pigs decreased the abundance of bacteria from families *Lactobacillaceae*, *Pasteurellaceae*, and *Enterobacteriaceae* in the ileal lumen, increased levels of *Ruminococcaceae* and *Lachnospiraceae* in the colon (Huang et al., 2015), and reduced viable counts of *Escherichia coli* in the small intestine and proximal colon (Lu et al., 2008; Wen et al., 2012). However, while Huang et al. (2015) reported increased *Clostridium* levels in the ileum and colon, Lu et al. (2008) and Wen et al. (2012) reported decreased levels in the entire length of the small intestine and colon. In grower and finisher pigs, the addition of 1.5% butyrate to the diet reduced the total level of coliforms in the jejunum (Øverland et al., 2008).

Studies involving an immune challenge with *Salmonella typhimurium* found that butyric acid supplementation reduced fecal shedding (Boyen et al., 2008; Walia et al., 2016).

Two grams/kilogram of butyrate added to nursery diets had a tendency to decrease *Salmonella* shedding and also tended to reduce colonization of the intestine and associated lymph nodes (Boyen et al., 2008). In grower pigs, the addition of 3 kg/t of SB to the diet reduced *Salmonella* shedding by 45% 12 to 28 d post-inoculation compared to pigs fed a diet with no additives (Walia et al., 2016). Together, these studies indicate that butyric acid inclusion in the diet may be a useful remedy to immune stress caused by *Salmonella* strains.

Effects on immune status and oxidative stress

Butyric acid is thought to have several anti-inflammatory and antioxidative properties that may reduce immune and oxidative stress symptoms in pigs (Guilloteau et al., 2010). Several studies have evaluated cytokine, immunoglobulin, and other immune status marker levels in neonatal piglets, nursery pigs, and sows. Butyric acid inclusion in the diet was reported to reduce levels of tumor necrosis factor alpha (TNF- α) in serum (Lu et al., 2008; Wen et al., 2012) and mucosal tissue (Huang et al., 2015) of the small intestine of nursery pigs. In contrast, Grilli et al. (2016) found increased levels of TNF- α in duodenal mucosa in pigs supplemented with SB. Other studies reported no changes in levels of TNF- α in serum (Weber and Kerr, 2008; Huang et al., 2015) and jejunal mucosa (Le Gall et al., 2009). Although Lu et al. (2008) and Wen et al. (2012) reported downregulation of IL-6 by butyric acid supplementation in serum, Weber and Kerr (2008) found no effects. In addition, Huang et al. (2015) observed no difference in serum IL-1 and IL-2 levels in pigs fed SB or no additives. Lastly, butyric acid inclusion in the diet was reported to increase levels of interferon gamma (IFN- γ) in serum and duodenal mucosa, yet decrease levels in the colon (Huang et al., 2015; Grilli et al., 2016).

Fang et al. (2014) and Jang et al. (2017) reported no change in serum immunoglobulin levels of IgA and IgM with the addition of SB to the diet in nursery pigs. However, while Fang and colleagues (2014) found that butyric acid increased levels of serum IgG, Jang et al. (2017) found no difference between SB and control diets. Other immune status parameters that butyric acid significantly affected were increasing serum cortisol response in the presence of LPS (Weber and Kerr, 2008) and decreasing the levels of nuclear factor kappa B (NF- κ B) in the ileal mucosa (Wen et al., 2012). Lastly, Manzanilla et al. (2006) found no effect of SB addition on lymphocyte levels or the ratio of lymphocytes to nuclei in the small intestine and colon.

Limited data are available on the influence of butyric acid on oxidative stress in nursery pigs. Huang et al. (2015) supplemented newly weaned pigs with either 0 or 1,000 mg/kg of SB and a reduced level of antibiotics. The authors found that pigs fed SB had increased levels of both superoxide dismutase and glutathione peroxidase as compared to control pigs. There were also decreased levels of malondialdehyde (MDA) in the serum and mucosa of pigs fed butyric acid (Huang et al., 2015). Together, the up and downregulation of these markers indicate that butyric acid may have a role in reducing the oxidative stress of nursery pigs, although more research should be completed to confirm this.

Phytobiotics

Phytobiotics (PH) are plant-derived compounds which may have positive effects on animal growth and health due to their antibacterial, anti-inflammatory, and antioxidative properties (Yan et al., 2010; Zeng et al., 2015a). In addition, their aromatic and oily characteristics have been shown to increase the palatability of feed with no flavor or aroma

difference in the finished meat product (Holden and McKean, 2001). Phytobiotic mixtures are often referred to as botanicals or phytogenics and can be comprised of herbs, spices, essential oils, and oleoresins. Herbs, such as garlic, oregano, and thyme, are classified as non-woody flowering plants. Spices are herbs with a prolific smell or taste, such as cinnamon and ginger (Jacela et al., 2010b). Essential oils are volatile lipophilic compounds derived by cold expression or by alcohol and steam distillation of herbs and other plants, and oleoresins are extracts derived from non-aqueous solvents (Windisch et al., 2008).

Commercially available PH supplements can vary considerably in composition. A group of 35 studies published between 1991 and 2015 that studied the effects of PH in swine diets were evaluated for PH ingredient composition. Oregano, thyme, and cinnamon derivatives were used in more than a fifth of the studies, followed closely by ingredients derived from garlic, pepper, and citrus fruit. However, several additional plant sources were also used, including clove, rosemary, anise, fenugreek, nettle, yam, and/or chicory powder. Due to their great diversity in chemical properties, low inclusion levels in diets, and relatively unknown stability through feed processing, studies involving PH supplementation in swine are widely varied in results (Zeng et al., 2015a). The second part of this review will focus on summarizing the reported effects of PH addition to diets on pig growth and intestinal health.

Modes of action

Herbs and spices, due to their aromatic attributes, are thought to improve the palatability of feed at low inclusion levels, which can increase feed intake and therefore weight gain (Holden and McKean, 2001). They are also proposed to enhance gut function by

increasing digestive secretions and intestinal enzyme activity (Windisch et al., 2008). Essential oils, which are hydrophobic, are able to disperse and disrupt bacterial cell and mitochondrial membranes (Li et al., 2012). This may contribute to antibacterial properties. However, it is generally accepted that the largest common antibacterial mode of action is related to the phenolic compounds found in a large variety of PH compounds (Lambert et al., 2001). This commonly includes, but is not limited to, plants from the *Labiatae* family (e.g. mint, oregano, thyme), from the *Umbelliferae* family (e.g. anise, coriander), and plants with flavonoids (e.g. garlic, onion). Phenolic compounds have been found to have a variety of beneficial properties, including acting as antioxidants, free radical scavengers, and metal chelators (Mandal et al., 2017). Other non-phenolic PH are speculated to act in a variety of ways dependent on their specific structures and characteristics (Zeng et al., 2015a). Burt (2004) surmises that several of these individual modes of action are still centered around the ability of PH components to modify cell membrane structures, transmembrane proteins, and motive forces reliant on the membrane. In addition to the diversity of species and processing techniques, specific PH characteristics are also dependent on factors such as harvest time, climate conditions, and the part of the plant used (Màthé, 2009). The specific nature of each individual PH is important to consider when evaluating their efficacy in improving swine health.

Effects on growth performance

Several different varieties of PH have been reported to have positive influences on growth performance in sows, nursery, grower, and finisher pigs (Grela et al., 1998; Hong et al., 2004; Allan and Bilkei, 2005). However, certain mixes have been shown to have negative

effects on growth, and considerably more studies have shown no influence at all (Muhl and Liebert, 2007; Liu et al., 2013). This can be partly attributed to the differences in inclusion levels and specific mixture of PH, however a closer investigation is needed to evaluate if PH are able to improve overall growth performance in swine.

The inclusion of PH into the diet of sows during gestation and lactation generally creates improvements in sow and neonatal growth performance (Allan and Bilkei, 2005; Miller et al., 2009). Sows supplemented with 1,000 ppm of oregano leaves, flowers, and herbs during gestation and lactation had improved ADFI compared to sows that received no oregano (Allan and Bilkei, 2005). This result is supported by Miller et al. (2009), who reported that sows fed 2 kg/t of a PH mixture of oregano, anise, citrus, and fructooligosaccharides from 10 d prior to farrowing through weaning had higher ADFI than sows fed a basal diet with no additives. In addition, the 21 d average litter weight of sows fed the PH mixture was higher (Miller et al., 2009). Another study conducted by Maass et al. (2005) had different outcomes. Sows fed a gestation diet with 1.2 or 3.6% purple coneflower herb inclusion had decreased ADG and no differences in final BW, ADFI, and G:F compared to sows fed a diet with no herbs (Maass et al., 2005). In lactation diets with 0, 0.5, or 1.5% purple coneflower addition, there were no differences in BW, ADG, ADFI, and G:F, and there were no recorded differences in the growth performance of the neonatal piglets from all sow treatments (Maass et al., 2005). These studies suggest that oregano and other similar herbs may be able to improve sow and neonatal growth performance more than the addition of purple coneflower herb.

Many of the PH mixtures in nursery pig trials that produced positive results in growth performance contained active components from cinnamon, clove, thyme, and/or anise

from 0.01 to 1% of the diet. The following PH mixtures and inclusion levels produced increased ADG in newly weaned pigs compared to control diets with no additives: 0.03% fenugreek, clove, and cinnamon essential oils (Cho et al., 2006); 0.1 and 0.3% ginseng, Chinese yam, sunflower, Chinese licorice, and balloon flower (Huang et al., 2012); 0.2% citrus fruit and chestnut tree extract (Hong et al., 2004); 0.01% thymol and cinnamaldehyde (Li et al., 2012a); 50, 100, and 150 g/t of thymol and cinnamaldehyde (Li et al., 2012b); 0.25% Chinese rhubarb root (Straub et al., 2005); and 0.025% cinnamon and thyme (Zeng et al., 2015b). In the study conducted by Cho et al. (2006), the 0.03% PH mixture also increased ADFI throughout the entire observed 6 wk period after weaning, and in the study by Li et al. (2012b), the 100 and 150 g/kg mixtures increased ADFI and BW from 2 to 5 wk post-weaning. Liu et al. (2013) also found improved ADFI in the 4 wk period following weaning in pigs fed nursery diets containing either 10 mg/kg of capsicum or 10 mg/kg of turmeric oleoresins. Improvements in feed efficiency were found in trials involving 0.1 and 0.3% ginseng, Chinese yam, sunflower, Chinese licorice, and balloon flower (Huang et al., 2012); 150 g/t of thymol and cinnamaldehyde (Li et al., 2012b); 300 mg/kg of anise, clove, and peppermint (Maenner et al., 2011); 0.25% Chinese rhubarb root (Straub et al., 2005); and 0.025% thymol and cinnamaldehyde (Zeng et al., 2015b). For the study by Huang et al. (2012), the improvements in ADG and F:G only occurred in the first 14 d after weaning, and not in the total observed 4 wk period. The Straub et al. (2005) study also included diets with 0.5% and 1% Chinese rhubarb, however these inclusion levels did not create any significant differences in growth performance compared to the control diet.

Contrasting with the above studies, a few studies in nursery pigs produced negative responses in growth performance when PH were added to the diet. An essential oil mixture of

anise, citrus, and oregano that was added to diets at a 0.1% inclusion level decreased G:F 3 to 4 wk post-weaning compared to pigs that were fed a diet with no additives (Kommera et al., 2006). The same effect was observed in a 6 wk study with 1.8% inclusion of purple coneflower (Maass et al., 2005). Liu et al. (2013) found that 10 mg/kg of garlic and 10 mg/kg of turmeric oleoresins tended to decrease ADFI in the first 2 wk following weaning compared to pigs fed no additives, which is surprising given the very low inclusion level of the PH ingredients. ADG and ADFI were decreased in the third wk post-weaning in a study by Namkung et al. (2004) for pigs fed a diet containing 0.75% cinnamon, thyme, and oregano herbs. Lastly, Straub and colleagues (2005) observed that a 1% inclusion rate of Chinese rhubarb root decreased final BW, ADG, and ADFI compared to pigs that were fed a 0.25% inclusion level. It is difficult to reason why these studies in particular observed negative influences of PH because both the inclusion levels and mixtures were not extraordinarily different than those seen in studies with positive results.

It is important to note the considerable number of nursery pig studies that reported no differences in at least 3 of the 4 growth parameters (final BW, ADG, ADFI, or feed efficiency) between pigs fed diets supplemented with PH and diets with no additives. Maass et al. (2005) found no differences in BW, ADG, and ADFI for pigs fed 0 or 1.8% purple coneflower, and Jugl-Chizzola et al. (2005) found no differences in BW, ADG, and G:F for pigs fed 0 or 10 g/kg of thyme. No differences between the control diet and PH treatments for ADG, ADFI, and G:F were found in studies using 1,000 or 10,000 mg/kg of garlic (Horton et al., 1991), 0.1% cinnamon, oregano, thyme, rosemary, and cloves (Huang et al., 2010), and 0.03% carvacrol, cinnamaldehyde, and capsicum (Manzanilla et al., 2006; Nofrarias et al., 2006). Finally, with the following PH mixtures and inclusion levels researchers reported no

effect of PH in all 4 growth parameters: 0.1, 0.5, or 1% thyme herb and essential oil (Hagmüller et al., 2006); 40 mg/kg of oregano, anise, citrus, and chicory powder essential oils (Kroismayr et al., 2008a); 150 or 300 mg/kg of carvacrol, cinnamon, and capsicum (Manzanilla et al., 2004); 0.5, 0.1, or 0.15% carvacrol, thymol, or chestnut meal tannins (Muhl and Liebert, 2007); 700, 1,400, or 2,100 ppm of thyme, clove, oregano, eugenol, and carvacrol (Oetting et al., 2006); 0.1, 0.25, and 0.5% garlic powder (Holden and McKean, 2001); and 100 mg/kg of fennel oil or 100 mg/kg of caraway oil (Schöne et al., 2006). Collectively, these studies covered a wide range of inclusion levels and ingredients, which makes it difficult to confirm any direct effects of PH supplementation on growth performance in newly weaned pigs.

One nursery pig study in particular had interesting results in growth performance. Liu and colleagues (2013) conducted a split-plot design where pigs were fed either a basal diet with no additives (CON), 10 mg/kg of capsicum oleoresin (CAP), 10 mg/kg of garlic (GAR), or 10 mg/kg of turmeric oleoresin (TUR). After 2 wk, half of the pigs were then inoculated with porcine reproductive and respiratory syndrome (PRRS), and growth parameters were evaluated for both before and after inoculation. At the end of the trial period, pigs that were not immunosuppressed and were fed either CAP or TUR diets had increased, and GAR decreased, ADFI compared to CON (Liu et al., 2013). The complete opposite was true for pigs that suffered from PRRS; the CAP and TUR diets decreased ADFI, and GAR increased ADFI compared to CON (Liu et al., 2013). In addition, Liu et al. (2013) reported that only PRRS challenged pigs fed CAP, TUR, or GAR experienced increased G:F in the second 2 wk of the study; no effect was observed in the non-challenged group. The authors attribute these differences to the specific characteristics of capsicum, garlic, and turmeric and their

modes of action along the gut. The results also indicate that PH supplementation may be beneficial to negating the growth impairments of pigs infected with PRRS.

A wide assortment of herbs, spices, and oils have been found to improve the growth performance of grower and finisher pigs (Grela et al., 1998; Maass et al., 2005; Janz et al., 2007; Yan et al., 2010; Devi et al., 2015). In particular, garlic and oregano seem to be popular additives for pigs past the nursery phase. A study by Janz et al. (2007) investigated the preference of finisher pigs for diets supplemented with 0.05% of either rosemary essential oil, garlic essential oil, oregano oleoresin, or ginger oleoresin. The authors found that there was a tendency for pigs to consume the garlic treatment the most and oregano and ginger diets the least, with no differences between the treatments on final BW, ADG, or G:F. Grela et al. (1998) reported that an addition of 50 g/kg of a nettle, garlic, and wheat grass mixture to grower and finisher pigs improved ADG and decreased the FCR for pigs fed the mixture versus pigs fed no additives, with no effect on final BW. In a 6 wk grower study, the addition of 0.05, 0.1, or 0.15% of a mixture containing ginger and balloon root spices found that the 2 smaller inclusion levels (0.05 and 0.1%) increased ADG (Devi et al., 2015). This suggests that the inclusion of a plant extract at 0.15% negates positive effects in the growing and finishing phases, which is supported by a study by Maass et al. (2005) where 0.15% purple coneflower cob addition to the diet of grower pigs had no influence on final BW, ADG, or ADFI. On the other hand, very small PH inclusion has been shown to still exert positive effects. Yan et al. (2010) supplemented grower and finisher pigs with 0.01% of a rosemary, thyme, and oregano mixture and reported improved ADG in the first 6 wk of the grower phase and overall. In addition, overall ADFI and G:F were improved compared to the pigs that received no additive (Yan et al., 2010).

Altogether, studies in swine evaluating the effects of PH supplementation have reported various results in growth performance. As with butyric acid addition, the efficacy of PH in improving feed intake and efficiency may be subject to a number of different factors that vary from study to study. Accordingly, a closer analysis of the actions of PH on the internal gastrointestinal tract and microbial environment is necessary to understand how plant extracts can influence growth performance and gut health overall.

Effects on digestibility and noxious gas emissions

Phytobiotics may affect the digestibility of feed in a variety of ways. Phytogetic compounds can modulate the gut microbiota community, especially in newly weaned pigs, or stimulate the secretion of gastric and pancreatic juices (Wenk, 2003; Huang et al., 2010). In addition, the improvement of digestibility in pigs may lead to decreased production of fecal noxious gases, which are the primary components of pig waste that contribute to air pollution (Yan et al., 2010). Therefore, investigating the effect of PH on both digestibility and noxious gas production is necessary to determine its degree of influence on the gastrointestinal tract and gut microbiota.

Mixtures of plant extracts have been shown to increase the digestibility of DM, N, energy, CP, and several amino acids in nursery and growing pigs (Oetting et al., 2006; Yan et al., 2010; Maenner et al., 2011; Li et al., 2012a; Zeng et al., 2015b). Studies using 0.1 and 0.3% ginseng, Chinese yam, sunflower, Chinese licorice, and balloon flower (Huang et al., 2012) and 0.025% cinnamaldehyde and thymol (Zeng et al., 2015b) reported increases in DM, energy, and CP digestibility in nursery pig diets. An addition of 0.01% thymol and cinnamaldehyde to nursery pig diets increased DM and CP digestibility and tended to

increase the digestibility of P (Li et al., 2012a). In grower and finisher pigs, 0.15% ginger and balloon root spices increased DM digestibility and 0.1% of the same mixture increased the digestibility of N (Devi et al., 2015). Increases in DM digestibility in newly weaned pigs was also reported with PH mixtures containing 0.03% fenugreek, clove, and cinnamon (Cho et al., 2006) and 700, 1,400, or 2,100 ppm of thyme, clove, oregano, eugenol, and carvacrol (Oetting et al., 2006). Lastly, a study by Maenner et al. (2011) in nursery pigs demonstrated that 300 mg/kg of either menthol-based or cinnamaldehyde-based extracts improved the amino acid digestibility of every amino acid except Met, and that menthol-based extracts also improved CP digestibility. However, both Yan et al. (2010), with a 0.1% mixture of thyme, rosemary, and oregano for grower pigs, and Huang et al. (2010), using a 0.1% supplement of cinnamon, oregano, thyme, rosemary, and cloves for nursery pigs, reported increases in DM, N, and energy digestibility only in the initial phases (6 and 4 wk, respectively) of their studies. This indicates that the positive effects on digestibility by PH addition to the diet may not be long lasting.

A few studies reported that addition of PH did not affect the digestibility of some ingredients. In both Maenner et al. (2011) and Zeng et al. (2015b), no changes in digestibility were reported in Ca or P, and Maenner and colleagues (2011) also reported no changes in crude fat, crude ash, starch, or Na digestibility. Manzanilla et al. (2006) reported no differences in total tract starch digestibility in pigs fed 0 or 0.03% carvacrol, cinnamaldehyde, and capsicum. Nevertheless, the overall results indicate that PH mixtures, at the very least, can improve the apparent digestibility of DM, N, and energy in both nursery and growing pigs.

This increase in digestibility may help reduce fecal noxious gas emissions. Decreases in emissions of ammonia by the addition of PH to the diets of nursery and growing pigs were reported in studies by Huang et al. (2010), Yan et al. (2010), and Devi et al. (2015). In addition, Devi et al. (2015) found decreased levels of acetic acid and methyl mercaptan in pigs given 0.05, 0.1, or 0.15% of a spice mixture. Cho et al. (2006) found no effects of 0.03% fenugreek, clove, and cinnamon essential oils on emissions of hydrogen sulfide, ammonia, or VFA in nursery pigs, which is supported by both Devi et al. (2015) and Yan et al. (2010), who also found no differences in hydrogen sulfide emission in pigs fed a basal diet or a basal diet with PH addition. Overall, it appears that PH supplementation may both increase digestibility and reduce some fecal noxious gas output, although the extensiveness to either is still being investigated.

Effects on diarrhea incidence

Several authors have published work on the effect of PH supplementation on diarrhea incidence and fecal consistency in newly weaned pigs. Fecal score was improved in pigs fed 0.01% or 0.025% inclusion levels of thymol and cinnamaldehyde compared to pigs that were fed no additive (Li et al., 2012a; Zeng et al., 2015b). A reduction in diarrhea incidence was reported in studies involving the following PH mixtures and inclusion levels: 0.1 or 0.2% citrus fruit and chestnut tree extract (Hong et al., 2004); 0.3% ginseng, Chinese yam, sunflower, Chinese licorice, and balloon flower (Huang et al., 2012); and 50, 100, or 150 g/t of thymol and cinnamaldehyde (Li et al., 2012b). Interestingly, both Hong et al. (2004) and Huang et al. (2012) reported a significant reduction in diarrhea incidence only in the first few d or first wk post-weaning, but this could be due to the fact that as weaned pigs age their

overall gut health improves and diarrhea incidence decreases. Additive mixtures containing 0.05 to 1.8% of ingredients such as fenugreek, clove, cinnamon, oregano, thyme, rosemary, anise, citrus, capsicum, chestnut meal, and purple coneflower were involved in studies that did not find any effect of PH supplementation on reducing diarrhea incidence or improving fecal consistency (Manzanilla et al., 2004; Maass et al., 2005; Cho et al., 2006; Kommera et al., 2006; Muhl and Liebert, 2007; Huang et al., 2010). The varying results in the effects of PH mixtures on diarrhea incidence may be related to the individual active components' ability to modulate the gut microbiota and digestibility.

Effects on intestinal morphology and enterocyte proliferation

Phytobiotics supplementation potentially influences the gut morphology of newly weaned pigs. Improvement in villus height and reduction in crypt depths can increase the ability of a pig to absorb nutrients and reduce energy losses (Oetting et al., 2006). In addition, villus atrophy is primarily caused by an increased rate of apoptosis and a decreased rate of enterocyte proliferation (Zeng et al., 2015a). Therefore, the action of PH on the morphology and proliferation of intestinal cells is an important factor to consider when evaluating the overall effect of PH on swine intestinal health.

The majority of the reported positive effects by PH supplementation on gut morphology are found in the jejunum. A 0.025% mixture of cinnamaldehyde and thymol (Zeng et al., 2015b) and either 0.1 or 0.3% mixtures of ginseng, Chinese yam, sunflower, Chinese licorice, and balloon flower (Huang et al., 2012) were found to increase the villus height in the jejunum of nursery pigs compared to pigs that received no PH. Huang et al. (2012) also reported a decrease in jejunal crypt depth, an increase in jejunal VH:CD, and an

increase in duodenal and ileal villus heights, although the latter two results were only significant for the 0.3% PH mixture. A combination of thymol and cinnamaldehyde was also reported to increase jejunal VH:CD in another study (Li et al., 2012a).

Largely, however, reported results from nursery pig trials found no influence of PH addition to the diet on histological measurements of the intestine. No effect on jejunal villus height and crypt depth were reported in several works (Manzanilla et al., 2004; Namkung et al., 2004; Manzanilla et al., 2006; Nofrarias et al., 2006; Oetting et al., 2006; Kroismayr et al., 2008b; Li et al., 2012a). Nofarias et al. (2006) also reported no effects of 0.03% carvacrol, thyme, and oregano on jejunal or ileal crypt depth and jejunal VH:CD, which was supported by results from Manzanilla et al. (2006) and Oetting et al. (2006). Li et al. (2012a), Oetting et al. (2006), and Zeng et al. (2015b) found no effects of PH supplementation on duodenal villus height, crypt depth, or VH:CD. Addition of PH to the diets of nursery pigs also had no effect on ileal villus height, crypt depth, or VH:CD, as reported in studies by Manzanilla et al. (2006), Nofarias et al. (2006), Li et al. (2012a), and Zeng et al. (2015b). Finally, in the cecum and colon, several authors reported no effects of plant extracts on crypt depth with PH mixtures of 0.004 to 0.03% oregano, anise, citrus, chicory, cinnamon, or capsicum (Manzanilla et al., 2006; Nofrarias et al., 2006; Kroismayr et al., 2008b).

The scope of findings relating to enterocyte proliferation is limited. Nofrarias et al. (2006) found no difference in the mitotic rate of jejunal, ileal, or colonic crypts in pigs fed a 0.03% addition of carvacrol, cinnamaldehyde, and capsicum compared to pigs fed a control, no-additive diet. Furthermore, Kroismayr et al. (2008b) did not find any impact of 40 mg/kg addition of oregano, anise, citrus peels, and chicory powder essential oils on the level of Caspase 3 in any section of the gastrointestinal tract, including the liver, spleen, and

associated lymph nodes. Caspase 3 is part of a family of enzymes responsible for executing cell apoptosis, and so a reduction in activity would most likely indicate a reduction in the rate of cell apoptosis. In summary, the addition of plant extracts to pig diets does not seem to influence gut morphology or improve enterocyte proliferation, although more data are needed to reach a conclusion on the latter.

Effects on intestinal microbiota

Perhaps the most influential action of supplemental plant extracts in the gastrointestinal tract of pigs is related to their modulation of gut microbiota. Intestinal bacterial colonies can influence digestibility, post-weaning diarrhea incidence, and pig growth performance (Huang et al., 2010; Huang et al., 2012; Li et al., 2012b). The ability to lyse bacterial cell walls is a potent antibacterial factor associated with many active components in plant extracts (Burt, 2004). Therefore, the reported modulation of aerobes, anaerobes, lactic acid bacteria, and *Enterobacteria* was reviewed.

Studies which reported the effect of PH supplementation on the total count of aerobic and anaerobic bacteria in the gut found either a decrease in bacteria or no difference between pigs supplemented with PH and pigs that received no additives (Muhl and Liebert, 2007; Kroismayr et al., 2008a; Li et al., 2012a; Zeng et al., 2015b). An inclusion of 40 mg/kg of oregano, anise, citrus peels, and chicory powder essential oils tended to decrease total aerobe and anaerobe counts in the ileum (Kroismayr et al., 2008a) and a blend of 0.01% thymol and cinnamaldehyde decreased levels of aerobic bacteria in the rectum (Li et al., 2012a). Furthermore, Zeng et al. (2015b) reported decreased levels of anaerobic bacteria in the colon and rectum of pigs fed 0.025% cinnamaldehyde and thymol compared to pigs that received

no additive. Numerous studies reported no differences in either aerobic or anaerobic bacterial levels between pigs supplemented with PH and pigs given a control diet. This includes aerobes in the ileum, cecum, colon, rectum, and feces, and anaerobes in the cecum, colon, and rectum (Muhl and Liebert, 2007; Kroismayr et al., 2008a; Li et al., 2012a; Zeng et al., 2015b).

Bacteria from the order Lactobacillales, including the commonly known *Lactobacilli*, are generally accepted to be beneficial in maintaining a healthy intestinal environment due to their ability to control pathogenic bacteria (Manzanilla et al., 2004). Multiple studies with a variety of PH mixtures (thymol, cinnamaldehyde, carvacrol, capsicum, ginseng, Chinese yam, Chinese licorice, sunflower, balloon flower) and inclusion levels (0.015 to 0.3%) reported increased levels of *Lactobacilli* in the jejunum, ileum, colon, rectum, and feces (Manzanilla et al., 2004; Maenner et al., 2011; Huang et al., 2012; Li et al., 2012b; Zeng et al., 2015b). Several other authors, however, with similar PH combinations and inclusion levels, reported no change in *Lactobacilli* concentration in the stomach, ileum, cecum, colon, rectum, and feces (Namkung et al., 2004; Muhl and Liebert, 2007; Kroismayr et al., 2008a; Maenner et al., 2011; Li et al., 2012a; Zeng et al., 2015b). Maenner et al. (2011) also evaluated the modulation of 300 mg/kg of menthol-based plant extracts on *Enterococci* counts in the stomach, ileum, and colon, and found that in the ileum the levels significantly decreased compared to pigs fed 0 mg/kg of the extracts.

Enterobacteriaceae is a large family of gram-negative bacteria that includes familiar pathogens such as *Salmonella* and *Escherichia coli* (*E. coli*). Manzanilla et al. (2004) reported no differences in cumulative levels of *Enterobacteria* in the jejunum of newly weaned pigs fed 0, 150, or 300 mg/kg of carvacrol, cinnamaldehyde, and capsicum.

However, 0.1, 0.5 and 1% thyme herb and essential oil (Hagmüller et al., 2006) and 50, 100, and 150 g/t of thymol and cinnamaldehyde (Li et al., 2012b) were found to decrease the amount of *E. coli* fecal shedding in weaned pigs compared to pigs that received 0% thyme or 0 g/kg of thymol and cinnamaldehyde. In addition, the supplementation of PH mixtures containing low inclusion levels (0.01 to 0.025%) of thymol, cinnamaldehyde, or both resulted in decreased prevalence of *E. coli* in the cecum, colon, and rectum (Jugl-Chizzola et al., 2005; Li et al., 2012a; Zeng et al., 2015b). Only a few authors reported no differences in *E. coli* counts in the stomach, ileum, cecum, or colon (Namkung et al., 2004; Maenner et al., 2011; Zeng et al., 2015b).

In all of the above microbial reports, there does not appear to be a tendency for one specific type of PH or inclusion level to have a more significant result than another, although this can be difficult to determine due to the vast variety of mixtures of ingredients. Collectively, these studies indicate that the addition of PH to the diets of pigs generally increases *Lactobacilli* counts and can decrease potentially pathogenic bacteria such as *E. coli*. This can help improve the health of the intestinal tract and, overall, improve the growth performance of pigs.

Effects on immune status and oxidative stress

In addition to modulating gut microbiota, the active components of PH are thought to possibly affect the immune system and antioxidant capability of pigs, especially when stressed. Although the microbial population in the intestine can have a general effect on immune and antioxidant function, PH may also have more direct effects (Manzanilla et al., 2004). For example, capsicum and cinnamaldehyde, two common ingredients in PH

mixtures, have been shown to exert anti-inflammatory properties by inhibiting the activation and proliferation of T cells (Koh et al., 1998). A review of the literature found mixed results concerning the effect of PH supplementation on both immune status and oxidative stress markers in the intestinal tracts of pigs.

In general, the quantity of lymphocytes, white blood cells, red blood cells, and neutrophils in serum, plasma, jejunal, and ileal samples were unchanged in nursery and grower pigs that received some form of PH supplementation (Horton et al., 1991; Hong et al., 2004; Namkung et al., 2004; Maass et al., 2005; Cho et al., 2006; Manzanilla et al., 2006; Kroismayr et al., 2008a,b; Huang et al., 2010; Devi et al., 2015). In the first wk after weaning, Huang et al. (2012) reported decreased levels of serum IgG in pigs fed 0.03% ginseng, Chinese yam and licorice, sunflower, and balloon flower extracts compared to pigs fed no additives. This is in contrast to both Li et al. (2012b) and Zeng et al. (2015b), who found increased levels of serum IgG in pigs fed combinations of thymol and cinnamaldehyde post-weaning. Several authors published results stating that IgG levels in serum (Cho et al., 2006; Huang et al., 2010; Yan et al., 2010) and plasma (Namkung et al., 2004; Li et al., 2012a) were not different between pigs fed PH or no PH. While Li et al. (2012a) found no differences in plasma IgA levels in pigs fed PH, Huang et al. (2012), Li et al. (2012b), and Zeng et al. (2015b) found increases in serum IgA levels. There were no reported effects of PH supplementation on serum or plasma IgM (Li et al., 2012a; Li et al., 2012b; Zeng et al., 2015b).

Levels of serum TNF- α and IL-1 β were unchanged in nursery pigs supplemented with 10 mg/kg of capsicum, garlic, or turmeric oleoresins, but decreased when the same pigs were inoculated with PRRS (Liu et al., 2013). This indicates that PH may provide an anti-

inflammatory response in an immunosuppressed state. Namkung et al. (2004) also reported no changes in pigs fed 0 or 0.75% cinnamon, thyme, and oregano on the levels of plasma TNF- α and IL-1 β . Interestingly, a study using 0.01% thymol and cinnamaldehyde reported an increase in plasma TNF- α post-weaning (Li et al., 2012a). The same study reported a decrease in plasma levels of IL-6 by pigs fed the PH mixture, but pigs that were not challenged with PRRS in the study by Liu et al. (2013) showed no effects of PH addition to diets on serum levels of IL-6. Lastly, Kroismayr et al. (2008b) found that pigs supplemented with 40 mg/kg of oregano, anise, citrus peels, and chicory powder essential oils had decreased NF- κ B in the ileum and a tendency for decreased NF- κ B in the colon compared to pigs fed 0 mg/kg of PH.

Only a few studies have evaluated the potential antioxidative properties associated with PH supplementation in swine. Total antioxidant capacity was reported to be increased by additions of 0.01 to 0.025% thymol and cinnamaldehyde in the diets of newly weaned pigs (Li et al., 2012a; Zeng et al., 2015b). In the study by Zeng et al. (2015b), activities of superoxide dismutase and glutathione peroxidase were reportedly increased by PH addition to the diets, whereas in the study by Li et al. (2012a), there were no changes. The findings by Li et al. (2012a) are supported by Frankič et al. (2010), who found no effect of adding 271.2 mg/kg of carvacrol, cinnamaldehyde, and capsaicin to the diets of nursery pigs on glutathione peroxidase levels. The authors also found that PH supplementation decreased activity of 8-Oxo-2'-deoxyguanosine (8-OHdG). Lastly, no effect on the level of MDA in serum, plasma, and urine was observed in pigs supplemented with PH such as rosemary oil, garlic oil, oregano oleoresin, ginger spice, and capsicum (Janz et al., 2007; Frankič et al., 2010; Zeng et al., 2015b). On the whole, PH do not appear to have a large effect on enhancing the immune

status or antioxidative properties of pigs, but further research on the individual modes of action of PH ingredients should be completed to reach further conclusions.

Butyric Acid and Phytobiotics in Combination

Limited knowledge exists on the interactive effects of butyric acid and PH when used in combination or consecutively as feed additives for nursery pigs. Due to their different modes of action, combining organic acids and PH might be beneficial because they could potentially have synergistic effects and act on different parts of the digestive tract (Pathak, 2014). Some studies in poultry production have supported this theory. Pathak (2014) gave broilers a combination of cinnamaldehyde and calcium formate and reported an increase in BW, but no changes in FCR, in birds given the combination compared to birds that received no additive. In addition, birds given both the organic acid and the essential oil had increased intestinal villi height and reduced counts of *Salmonella* and *Clostridium* (Pathak, 2014). Combinations of oils from oregano, laurel, sage, myrtle, fennel seed, and citrus peel and formic, lactic, citric, propionic, and ammonium formate acids reportedly improved broiler BW and ADG compared to a basal diet with no additives (Bozkurt et al., 2012). However, it was noted that the positive effects on BW and ADG only occurred at low dose combinations of the additives (0.9 g/kg of organic acids and 12 mg/kg of essential oils) rather than high dose combinations (2.7 g/kg and 36 mg/kg). Lastly, a study by Cerisuelo et al. (2014) studied the effects of adding 0, 50, or 100 mg/kg of cinnamaldehyde and thymol and 0 or 1 g/kg of SB to the diets of broiler chicks inoculated with *Salmonella enterica*. The authors found no differences in growth performance between the dosage levels, but did report that a

combination of 50 mg/kg of PH and 1 g/kg of SB decreased fecal shedding of *Salmonella* by 6 wk compared to broilers fed just PH.

In swine production, the number of studies that have evaluated organic acids and PH in combination are fewer, and no studies have evaluated the effect of the additives when used sequentially. Cho and Kim (2015) reported that weanling pigs fed a mixture of citric and sorbic acids with thymol and vanillin had improved growth performance and increased nutrient digestibility than pigs fed a diet with no additives. Grilli et al. (2015) also evaluated the effect of citric and sorbic acids in combination with thymol and vanillin on the intestinal barrier function and inflammatory response in newly weaned pigs. A combinational use of the organic acids and PH lowered ileal mRNA expression of transforming growth factor- β (TGF- β) and IL-12 and tended to reduce expression of IL-6 and IFN- γ (Grilli et al., 2015). In ileal mucosa, the organic acids and PH treatment tended to reduce TGF- β protein content but had no apparent effect on IL-6, IL-10, IL-12, IFN- γ , or TNF- α protein content (Grilli et al., 2015). In addition, serum samples of pigs fed the combinational diet had higher TNF- α and tended to have higher IL-12 and IFN- γ compared to pigs fed no additives.

Collectively, these studies indicate that a synergistic effect between butyric acid and PH in nursery pigs is possible. Butyrate is thought to predominately act in the distal small intestine by inducing the production of host defense peptides and by binding to G-protein coupled receptors which stimulate peptides that improve gut function and motility (Bartholome et al., 2004; den Besten et al., 2013). It is also an important energy source for enterocytes and has been reported to increase enterocyte proliferation and malignant cell apoptosis (Guilloteau et al., 2010). Together these actions contribute to improvements in growth performance, reductions in diarrhea incidence, and improved immune response. In

addition, certain botanical mixtures have been shown to directly exert antibacterial actions throughout the gastrointestinal tract by interacting directly with bacterial cell and mitochondrial membranes (Li et al., 2012). Phytogetic compounds are also thought to increase gut motility, enhance digestive enzyme activity, and promote antioxidative functions, which can improve overall growth and gut health (Windisch et al., 2008; Mandal et al., 2017). However, the prevalence of synergistic effects may vary widely depending on the dosage of both butyric acid and PH, the specific mixture of PH used, and the environmental conditions of the animals being studied. In addition, these synergistic effects may not be apparent when the additives are fed sequentially rather than in combination. Therefore more research is required to determine the overall effect of combinational uses of both additives on growth and gut health in nursery pigs.

Scope of the Present Thesis

The post-weaning period is accompanied by significant stressors that compromise pig gastrointestinal and immune health. Although antibiotics were traditionally used to improve piglet growth performance and feed efficiency, increasing consumer demand for antibiotic-free meat and growing FDA oversight of the use of antibiotics in production has caused the swine industry to shift its focus from antibiotics to other feed additives that can still improve the growth performance and intestinal health of newly weaned pigs.

Sodium butyrate and phytobiotics are two such feed additives that have been studied extensively for their effects on gut morphology, microbiota, immune status, and growth performance. In the initial period after weaning, SB provides an energy source for enterocytes and may contribute to intestinal villi rebuilding (Guilloteau et al., 2010). In

addition, although the intense aromatic properties of PH may decrease feed intake initially after weaning, recent findings have suggested that PH can have positive effects on growth and intestinal health when supplemented later in the nursery phase (Park et al., 2016).

Therefore, supplementing SB in the initial period after weaning followed by PH may support increased resistance to post-weaning stressors and improved growth. However, past results for both additives in all aspects of swine performance and gut health are extremely varied, and only a few studies have focused on the combinational effects of the additives for pigs in the post-weaning period. Further analysis should be completed to accurately evaluate if consecutively providing SB followed by PH benefits nursery pig production. Accordingly, the objective of this thesis is to examine the effects of standalone SB, PH, or a sequential use of SB and PH on the overall growth performance and intestinal health of newly weaned pigs.

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CHAPTER II
COMBINATIONAL USE OF NA-BUTYRATE AND PHYTOBIOTICS ON GROWTH
PERFORMANCE AND INTESTINAL HEALTH OF NURSERY PIGS

Abstract

The objective of this research was to determine the effects of encapsulated sodium butyrate (SB; Ultramix C, Nutriad, Elgen, IL), phytobiotics (PH; Apex Swine, Nutriad), and a sequential use of SB and PH on growth performance and intestinal health of nursery pigs. Fifty-six weaned pigs (21 d of age; 6.9 ± 0.6 kg BW) were individually housed and allotted to 5 dietary treatments through a randomized complete block design. Pigs were fed for 33 d (phase 1: 0 to 7 d; phase 2: 7 to 19 d; phase 3: 19 to 33 d). Treatments were: 1) a basal diet with no additives (n = 12); 2) basal diet + SB in phases 1 (0.2%) and 2 (0.1%) (n = 12); 3) basal diet + 0.033% PH in phase 3 (n = 12); 4) basal diet + SB in phases 1 (0.2%) and 2 (0.1%) + 0.033% PH in phase 3 (n = 12); and 5) basal diet + SB in phase 1 (0.2%) and phases 2 and 3 (0.1%) (n = 8). Diets were formulated to meet or exceed the nutrient requirements estimated by NRC (2012) and pigs had *ad libitum* access to feed and water. Feed intake and BW were recorded at the beginning and end of each phase to calculate ADG, ADFI, and G:F, and fecal scores were assessed every odd day from d 3 to 19 and on d 26 and 33. Blood samples were drawn at the end of phases 2 and 3 to measure tumor necrosis factor alpha (TNF- α), IL-6, and immunoglobulin G (IgG). Four pigs from each of the first 4 treatments (16 total) were euthanized at the end of phase 2 and the remaining pigs from all treatments (40 total) were euthanized at the end of phase 3 for sample collection. Jejunal tissue, mucosa, and ileal digesta samples were obtained to measure gut histology, TNF- α , IL-6, myeloperoxidase (MPO), malondialdehyde (MDA), and protein carbonyl. Data were analyzed using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC). Initial BW and sex were blocking factors and pen was the experimental unit. Fixed effects, depending on the analysis, were either treatment or SB and PH. Block was a random effect. Sodium butyrate

decreased ($P < 0.05$) phase 2 and phase 1 to 2 ADFI but had no effect on ADG and G:F. Sodium butyrate tended ($P = 0.063$) to decrease villus height to crypt depth ratio (VH:CD) at the end of phase 2 but increased ($P < 0.05$) the rate of enterocyte proliferation at the end of phase 3. Phytobiotics increased ($P < 0.05$) phase 3 and overall G:F without affecting ADG and ADFI. Neither SB nor PH affected concentrations of TNF- α , IL-6, IgG, MPO, MDA, or protein carbonyl and had no effect on villus height, villus width, crypt depth, or fecal scores. There were tendencies for interaction between SB and PH for VH:CD ($P = 0.074$), MDA ($P = 0.087$), and serum IL-6 ($P = 0.052$). In conclusion, SB and the sequential use of SB and PH had minimal effects on growth performance and gut health when added to the diets of nursery pigs. However, PH supplementation 19 to 33 d post-weaning improved feed efficiency.

Introduction

Post-weaning stress in young pigs is a predominant concern in the swine industry due to potential economic losses associated with reduced growth and increased piglet mortality. Antibiotics, particularly in the form of growth promoters, have formerly been used to reduce the symptoms of post-weaning stress and improve growth rate, feed utilization, and intestinal health (Vondruskova et al., 2010). With increased governmental regulation of the use of antibiotics as growth promoters and growing consumer demand for antibiotic-free production, the availability of additives that can reduce the symptoms of post-weaning stress have dwindled. An increasing number of studies have suggested that two antibiotic alternatives, sodium butyrate and phytobiotics, may be useful in nursery pig production by enhancing growth performance and intestinal health (Vidanarachchi et al., 2005; Guilloteau et al., 2010).

Butyric acid is a SCFA that is regularly fed as an encapsulated salt to reduce unpleasant odors, increase ease of handling, and target delivery to the lower gut (Lallès et al., 2009; Mallo et al., 2012). Several studies have shown that SB addition to nursery pig diets can promote growth performance, improve fecal consistency, increase intestinal villi height, and decrease the abundance inflammatory cytokines (Gálfi and Bokori, 1990; Lu et al., 2008; Wen et al., 2012; Huang et al., 2015). Phytobiotics are commonly comprised of herbs, spices, essential oils, and oleoresins and have several potential modes of action. Phytobiotic mixtures can enhance feed palatability and intake, reduce diarrhea incidence, and support beneficial gut microbiota (Manzanilla et al., 2004; Zeng et al., 2015b). Results from studies involving SB or PH have wide variation, however, and more research is needed to reach any definitive conclusions on their effectiveness as feed additives in nursery pig production.

The scope of knowledge on the combinational use of these additives in nursery pig diets is also limited. Supplementing SB in the initial period after weaning can provide an energy source for enterocytes and allow intestinal villi to rebuild after the stress of weaning (Guilloteau et al., 2010). After the initial recovery period, PH addition to the diet can further enhance intestinal health and improve growth (Park et al., 2016). In addition, PH appear to act directly on the intestinal environment and SB evidently operates more indirectly (Lambert et al., 2001; Guilloteau et al., 2010). Therefore, it is possible that when used consecutively in the nursery phase of production their modes of action complement each other and they can both exhibit positive reactions in the gut and peripheral tissues. The purpose of this study was to further evaluate the effects of standalone SB and PH on growth performance and intestinal health of pigs in the period following weaning and to also assess the response in pigs fed a combination of both additives.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University (Raleigh, NC).

Animals and experimental design

Fifty-six newly weaned barrows and gilts (PIC 337 x Camborough 22; 7.0 ± 0.6 kg BW) at 21 d of age were used for the experiment. Pigs were blocked by initial BW (high and low) and sex, and 5 dietary treatments were randomly assigned within each block. The feeding programs were: 1) a basal diet with no additives; 2) basal diet + SB in phases 1 (0.2%) and 2 (0.1%); 3) basal diet + 0.033% PH in phase 3; 4) basal diet + SB in phases 1

(0.2%) and 2 (0.1%) + 0.033% PH in phase 3; and 5) basal diet + SB in phase 1 (0.2%) and phases 2 and 3 (0.1%). The experimental phases were determined by mean BW targets and lasted 0 to 7 d (phase 1; initial to 8 kg BW), 7 to 19 d (phase 2; 8 to 12 kg BW), and 19 to 33 d (phase 3; 12 kg to final BW). All pigs were housed indoors at the North Carolina State University Metabolism Education Unit (Raleigh, NC) in individual pens (1.50 x 0.74 m) with slatted floors. The pigs had *ad libitum* access to feed and water via feeders and nipple waterers in each pen. The average temperature and humidity in the pen area for the duration of the trial was 29.9°C and 69.6%, respectively.

The first 4 experimental treatments had 12 replicates per treatment in phases 1 and 2. There were 3 replicates per treatment in each block. At the end of phase 2, 1 pig per treatment in each block (4 pigs per treatment, 16 total) closest to the mean BW for their treatment and block were euthanized via captive bolt gun and exsanguination for sample collection. The remaining pigs (8 replicates per treatment) were euthanized at the end of phase 3. The fifth experimental treatment had 8 replicates for the entire length of the trial. At the end of phase 3, all 8 pigs were sampled.

Three analyses were conducted for this experiment. The first analysis studied the effects of supplementing SB in phases 1 and 2 only. For growth performance and fecal score data, this involved the comparison of 24 pigs that were fed the control diet and 24 pigs that were fed SB in phases 1 and 2. Blood- and tissue-based measurements were analyzed from the 8 pigs fed the control diet and 8 pigs fed SB that were euthanized at the end of phase 2. The second analysis evaluated the effects of supplementing SB in phases 1 and 2 only or all 3 phases. The treatments used in this analysis were the control diet, the pigs fed SB in phases 1 and 2 only, and the pigs fed SB in all 3 phases. The third analysis used a 2 x 2 factorial

arrangement of treatment. The first factor was SB (0 or 0.2/0.1% SB in phases 1/2) and the second factor was PH (0 or 0.033% PH in phase 3). In the second and third analyses there were 8 pigs per treatment for all measurements.

Experimental diets

The composition of the experimental diets is described in Table 1. All diets were formulated to meet or exceed the nutrient requirements estimated by NRC (2012) and were manufactured as mashed feed at the North Carolina State University Feed Mill (Raleigh, NC). The source of vegetable fat encapsulated SB was Ultramix C (Nutriad, Elgen, IL). The PH additive was a blend of garlic, thyme, cinnamon, anise, and rosemary plant extracts and essential oils (Apex Swine; Nutriad).

Growth performance and fecal score

Pigs were weighed initially and on d 7, 14, 19, 26, and 33 to evaluate average BW and ADG. Feed intake was determined by weighing feeders and recording the amount of feed given. Feed efficiency was calculated as G:F. Fecal score was visually assessed every odd day from d 3 to 19 and on d 26 and 33. Scoring was scaled as: 1) completely solid and defined in shape; 2) mostly solid; 3) semisolid, loose shape; 4) mostly liquid; and 5) completely liquid with no shape.

Sample collection

Blood samples were collected from the jugular vein on d 18 for pigs euthanized on d 19 and on d 31 for pigs euthanized on d 33. Blood was collected in sterile BD Vacutainer

tubes (Becton, Dickinson and Company; Franklin Lakes, NJ) that were then centrifuged at 3,000 x g for 15 min at 4°C to obtain a serum supernatant. Serum samples were aliquoted to 1.5 mL polypropylene tubes (Thermo Fisher Scientific; Waltham, MA) and stored at -80°C until further analysis of total protein, IgG, TNF- α , and IL-6 concentrations.

After euthanasia, the gastrointestinal tracts were immediately removed and sampled. Digesta samples from the ileum (20 cm before the ileocecal junction) were collected in 50 mL polypropylene Falcon tubes (Corning Inc.; Corning, NY) and placed on ice. They were then stored at -20°C prior to analysis of myeloperoxidase (MPO) activity. Jejunal mucosa scrapings (75 cm before the ileocecal junction) were collected and snap frozen in liquid nitrogen before being stored at -80°C until further analysis of total protein, MDA, protein carbonyl, TNF- α , and IL-6 concentrations. Lastly, 5 cm sections of intact jejunal tissue were rinsed in saline solution and placed in 50 mL polypropylene Falcon tubes containing 45 mL of 10% buffered formaldehyde solution for subsequent histological evaluation.

Intestinal morphology

The jejunal tissues fixed in 10% formaldehyde solution remained submerged for 3 wk at room temperature before being cut into 0.75 cm thick cross sections. The cross sections (2 per pig) were then placed into cassettes and submerged in ethanol before being sent to the North Carolina State University College of Veterinary Medicine Histopathology Lab (Raleigh, NC) for hematoxylin and eosin (H & E) staining and histology slide preparation. The microscope slides were then evaluated and photographed using an Olympus CX31 microscope (Tokyo, Japan) and Infinity Analyze and Capture software (Lumenera Corporation; Ottawa, Canada). Twenty clearly defined villi and crypts were measured per pig

to obtain measurements in villus height, villus width, and crypt depth. Width was measured at the base, middle, and tip of each villus. Figure 1 illustrates a model of the histological measurements.

Cell proliferation was measured in 20 jejunal crypts using a Ki67 antibody stain (Histopathology Lab). The intact crypt was cropped using an online photo editor (LunaPic) and enterocyte proliferation was calculated using a browser-based ImageJS application (Almeida et al., 2012), which measured the percentage of Ki67-positive nuclei to the percentage of total nuclei. The same person performed all morphological analyses.

Immune and oxidative stress measurements

Digesta samples were allowed to thaw and then centrifuged twice at 1,150 x g for 10 minutes at 4°C. For each sample, 750 uL of the resulting supernatant was transferred to 2 mL polypropylene microcentrifuge tubes (Thermo Fisher Scientific) and centrifuged again at 7,000 x g for 10 minutes at room temperature. The samples were then assayed undiluted for determination of MPO activity using a fluorometric assay kit (ab111749, Abcam; Cambridge, UK). Myeloperoxidase activity was reported in $\mu\text{U}/\text{mL}$, where 1 unit of MPO activity equals the amount of MPO that oxidizes an aminophenyl fluorescein substrate to generate 1.0 μmol of fluorescein per minute at 25°C.

The jejunal mucosa samples were prepared for analysis by homogenizing (Tissuemiser; Thermo Fisher Scientific) 0.495 to 0.505 g of frozen mucosa with 1 mL of phosphate-buffered saline solution while on ice. The resulting homogenates were then centrifuged for 20 min at 7,000 x g, after which the supernatant was aliquoted into 1.5 mL polypropylene tubes and stored at -80°C.

Total protein concentrations of serum and mucosa samples were determined using a Pierce BCA Protein Assay Kit (#23225, Thermo Fisher Scientific). Immune status indicators (TNF- α and IL-6) were measured from serum and mucosa using Porcine TNF- α (PTA00) and IL-6 (P6000B) Quantikine ELISA Kits (R&D Systems, Minneapolis, MN). Serum samples were diluted 15,000x and evaluated for IgG concentration using a Pig IgG ELISA Quantitation Set (E100-104, Bethyl Laboratories; Montgomery, TX). Oxidative stress status was determined by measuring MDA and protein carbonyl levels. Malondialdehyde was measured by an OxiSelect TBARS MDA Quantitation Assay Kit (STA-330, Cell Biolabs, Inc.; San Diego, CA) and protein carbonyl was measured using an OxiSelect Protein Carbonyl ELISA Kit (STA-310, Cell Biolabs, Inc.). All assays were completed using duplicate samples and followed the manufacturers' instructions. Control samples supplied by the manufacturers were used to verify that the assays were completed correctly, and intra-assay coefficient variables were calculated for each assay to monitor variation between sample duplicates. The 96-well plates were read using a BioTek Syngery HT plate reader (BioTek; Winooski, VT).

Statistical analysis

The experiment was organized as a randomized complete block design with initial BW and sex as blocking factors and pen as the experimental unit. Depending on the analysis, fixed effects were either treatment or SB and PH. Block was considered a random effect. Data were checked for normality using the Shapiro-Wilk test via the Univariate procedure in SAS 9.3 (SAS Institute; Cary, NC). All normally distributed data were analyzed with ANOVA using the Mixed procedure in SAS. Fecal score data, which are nonparametric, and

data determined to not be normally distributed were analyzed using either the Wilcoxon-Mann-Whitney test or the Kruskal-Wallis test via the SAS Npar1way procedure. Outliers were defined as values above or below 1.5 times the interquartile range and were removed from the data. Significance was defined as $P < 0.05$ and tendencies were defined as $0.05 \leq P < 0.10$.

Results

Growth performance and fecal score

Sodium butyrate did not affect average BW, ADG, or G:F compared to the control diet when supplemented in phases 1 and 2 only or all 3 phases (Tables 2 to 4). However, SB decreased ADFI ($P < 0.05$) in phase 2 (509 g/d) and the overall phase 1 to 2 period (382 g/d) compared to pigs fed the control diet (578 and 427 g/d, respectively) (Table 2). There were no differences in ADFI in phases 1 and 3.

Supplementing PH in the diet increased phase 3 ($P < 0.05$) and overall ($P < 0.01$) G:F (Table 4). However, ADG and ADFI were not affected by PH. There were no interactive effects between SB and PH. Fecal scores were not affected by SB and/or PH in all phases (Tables 5 to 7).

Intestinal morphology

Sodium butyrate supplementation in phases 1 and 2 tended to decrease ($P = 0.063$) VH:CD (2.09) compared to control (2.12) but had no effects on villus height, villus width, or crypt depth (Table 8). When supplemented in all 3 phases, SB had no effect on villus height, villus width, crypt depth, and VH:CD (Table 9). By the end of phase 3, SB supplementation

increased ($P < 0.05$) enterocyte proliferation when supplemented in phases 1 and 2 only (23.9%) and all 3 phases (23.8%) compared to pigs that received no SB (20.8%).

In the factorial analysis, SB and PH did not affect villus height, villus width, crypt depth, or VH:CD (Table 10). There was a tendency for interaction ($P = 0.074$) between SB and PH for VH:CD. Pigs that were fed SB only (1.79) or PH only (1.72) tended to have greater VH:CD than pigs fed the control diet (1.62) or both additives sequentially (1.58). Although SB supplementation in phases 1 and 2 increased ($P < 0.05$) enterocyte proliferation by the end of phase 3, PH had no effect, and there was no interaction effect between SB and PH.

Immune and oxidative stress status

Including SB and/or PH in the diets did not affect immune status measurements. Sodium butyrate had no effect on jejunal mucosa and serum TNF- α and IL-6, serum IgG, and ileal digesta MPO when supplemented in phases 1 and 2 only or all 3 phases (Tables 11 to 13). Adding PH to the diet in phase 3 did not affect immune status parameters (Table 13). However, the effect of PH on serum IL-6 tended to be contingent on ($P = 0.052$) whether or not SB was added to the diet.

Sodium butyrate and/or PH supplementation did not affect oxidative stress status measurements (Tables 14 to 16). There were no differences between the SB and control treatments for jejunal mucosa MDA and protein carbonyl at the end of both phases 2 and 3 (Tables 14 to 16). Phytobiotic addition to the diet did not affect MDA or protein carbonyl concentrations at the end of both phases 2 and 3. However, there was a tendency for interaction ($P = 0.087$) between SB and PH for MDA (Table 16). Pigs that were fed

standalone SB or PH tended to have lower MDA concentrations (5.78 and 5.83 μM , respectively) than pigs fed the control diet (7.63 μM) or both additives consecutively (8.03 μM).

Discussion

The decrease in phase 2 and overall phase 1 to 2 ADFI for pigs fed SB compared to pigs fed the control diet is similar to results from previous studies (Weber and Kerr, 2008; Jang et al., 2017). Both authors reported that increasing levels of coated SB from 0, 500, and 1,000 ppm or 0, 0.05, 0.1, 0.2, and 0.4% linearly decreased ADFI in the first 4 to 5 wk post-weaning (Weber and Kerr, 2008; Jang et al., 2017). However, these studies also reported decreases in final BW and ADG, which were not observed in the present study. A few nursery studies have reported an increase in ADFI (Lu et al., 2008; Wen et al., 2012) and comparably more no effect on ADFI (Galfi and Bokori, 1990; Manzanilla et al., 2006; Biagi et al., 2007; Fang et al., 2014; Grilli et al., 2016) in pigs fed 960 to 3,000 mg/kg of SB compared to those fed diets with no additives.

The lack of influence of SB on BW, ADG, and G:F throughout the entire study is also reported in several studies that included SB from 960 to 3,000 mg/kg in the post-weaning period (Biagi et al., 2007; Mallo et al., 2012; Fang et al., 2014; Grilli et al., 2016; Hanczakowska et al., 2016). Only a few studies reported an increase in one or more of the growth parameters using the same inclusion range of SB (Galfi and Bokori, 1990; Lu et al., 2008; Wen et al., 2012). The decrease in ADFI in this study could be the result of an unpleasant aroma or flavor from the SB addition, although this is unlikely due to the encapsulation of the butyrate salt and the low inclusion level in the diet. Moreover, the pigs

consuming SB did not have changes in ADG or G:F. Thus, adding SB to the diet may decrease feed intake but not overall growth. Feeding SB in all 3 phases did not alter growth performance compared to pigs fed SB in just phases 1 and 2. Therefore, it is possible that SB has the strongest influence on growth performance in the first 3 wk post-weaning, which is supported by Piva et al. (2002).

The PH mixture used in this study contained extracts and essential oils from garlic, thyme, cinnamon, anise, and rosemary. Average daily gain and ADFI were not affected ($P = 0.762$ and 0.529 , respectively) by supplementing PH in phase 3, whereas G:F was improved ($P < 0.05$). This could be attributable to numerical increases in ADG, numerical decreases in ADFI, and a smaller standard error for the calculated G:F values. In addition, the improvement in G:F could be due to changes in factors not measured in this study, such as gut motility and digestibility. These results are consistent with studies that used 0.017 to 0.025% thyme and cinnamon (Li et al., 2012a; Zeng et al., 2015b) and 0.005 to 0.03% anise (Maenner et al., 2011; Charal et al., 2016).

In the present study, PH addition did not affect BW, ADG, and ADFI, which is concurrent with several nursery pig studies involving the active components of the PH product used for this study. Including 10 g/kg of thyme was reported to not affect BW or ADG (Jugl-Chizzola et al., 2005), and 0.1, 0.5, or 1% thyme herb and essential oils did not affect BW, ADG, or ADFI (Hagmüller et al., 2006). Pigs given 40 mg/kg of a PH blend containing anise (Kroismayr et al., 2008a) or diets containing 0.1 to 1.0% garlic (Horton et al., 1991; Holden and McKean, 2001) also had no differences in growth performance. Huang et al. (2010) reported no changes in ADG and ADFI for pigs given a 0.1% blend of cinnamon, thyme, rosemary, oregano, and cloves. One study in grower and finisher pigs

reported that pigs fed a diet containing 0.05% garlic had higher feed intake than pigs fed diets with 0.05% rosemary, oregano, or ginger (Janz et al., 2007). This reported preference for garlic could be negated in this study due to the inclusion of other PH ingredients in the mixture, in addition to the low inclusion level in the diet.

Sodium butyrate and/or PH addition to the diet did not influence fecal score, which is inconsistent with findings on SB by Fang et al. (2014) and Huang et al. (2015) and with findings on PH mixtures that included 0.005 to 0.025% thyme and cinnamon (Li et al., 2012a; Li et al., 2012b; Zeng et al., 2015b). Both Fang et al. (2014) and Huang et al. (2015) reported that pigs fed 1,000 mg/kg of SB had decreased diarrhea incidence in the period following weaning compared to pigs fed 0 mg/kg of SB. The difference in results between the current study and the two SB studies could be due to a difference in scoring systems and because Fang et al. (2014) and Huang et al. (2015) directly studied diarrhea incidence and not overall fecal consistency. A large number of studies involving PH mixtures reported no change in fecal score or diarrhea incidence (Manzanilla et al., 2004; Maass et al., 2005; Cho et al., 2006; Kommera et al., 2006; Muhl and Liebert et al., 2007; Huang et al., 2010). In this study, the lack of improvement in fecal score for pigs fed a PH diet could be due to the fact that PH was not supplemented until 19 d post-weaning, at which point the pigs had already recovered from any weaning-induced diarrhea.

During the post-weaning phase, the intestinal tract of the weanling pig undergoes several changes in gut morphology, including a reduction in villus height and an increase in crypt depth (Piva et al., 2002). These changes may decrease feed efficiency because they reduce the absorptive capacity of the intestine (Campbell et al., 2013). Therefore, improvements in villus height and reductions in crypt depth can increase nutrient absorption

and reduce energy loss (Oetting et al., 2006). In this study, jejunal villus height, villus width, and crypt depth measurements were not affected by supplementing SB or PH, which is consistent with findings by Manzanilla et al. (2004), Namkung et al. (2004), Manzanilla et al. (2006), Nofrarias et al. (2006), Oetting et al. (2006), Biagi et al. (2007), Kroismayr et al. (2008b), Le Gall et al. (2009), and Li et al. (2012b).

The pigs that were fed SB in phases 1 and 2 only and were sampled at the end of phase 2 had a tendency for reduced VH:CD compared to pigs fed a control diet. However, this tendency was not observed in pigs that were fed SB in phases 1 and 2 only and sampled at the end of phase 3. In addition, both villus height and crypt depth measurements were not different between the two treatments. This indicates that the effect of SB on VH:CD may be only apparent in the initial phase after weaning.

The tendency for negative interaction on VH:CD between the SB and PH feeding programs is not reported in literature. Pigs fed exclusively SB or exclusively PH had a tendency for higher VH:CD compared to pigs fed neither of the supplements or both consecutively. Lu et al. (2008) and Wen et al. (2012) reported that 1,000 mg/kg of SB addition increased VH:CD, and several studies involving PH mixtures in nursery pigs reported similar findings (Huang et al., 2012; Li et al., 2012b; Zeng et al., 2015b). It is possible that there is an inhibitory effect between SB and PH related to the intestinal epithelium, although this is doubtful due to the fact that the additives were not supplemented at the same time. A deeper analysis of the specific modes of action between SB and the active ingredients in the PH mixture used in this study would need to be conducted to be able to fully explain this interactive effect.

Post-weaning stress can inhibit the development and repair of the epithelial layer of the small and large intestines (Montagne et al., 2007). Therefore, the rate of enterocyte proliferation is an important indicator of overall intestinal health in newly weaned pigs. Decreased rates of intestinal epithelial cell proliferation can lead to villus atrophy, which reduces absorptive capability (Zeng et al., 2015a). Butyric acid is thought to induce the production of host defense peptides, which in turn stimulate the development of epithelial cells (Bartholome et al., 2004; Guilloteau et al., 2009). The results of the current study confirm this: SB supplementation in phases 1 and 2 only and in all 3 phases increased the rate of enterocyte proliferation in the jejunum compared to pigs fed a control diet. These results are also supported by previous findings with nursery and grower pigs by Le Gall et al. (2009) and Lacorn et al. (2010).

Phytobiotic supplementation in phase 3 did not affect the rate of enterocyte proliferation in the jejunum. There are limited reports of PH mixtures modulating the structure of the epithelial lining. Nofrarias et al. (2006) reported that nursery pigs supplemented with carvacrol, cinnamaldehyde, and capsicum had no changes in jejunal crypt mitotic rates compared to pigs fed a control diet. The primary modes of action related garlic, thyme, cinnamon, anise, and rosemary active compounds do not appear to directly affect the rate of enterocyte proliferation in newly weaned pigs.

Young pigs are susceptible to pathogenic infection after weaning due to stress-induced greater intestinal permeability and mucosal inflammation (Moeser et al., 2007). The immune system response is chiefly mediated and executed by proteins such as cytokines and immunoglobulins. Cytokines are cell signaling proteins commonly secreted by immune cells to modulate cellular immune response (Ibelgaufts, 2016). Two of these cytokines, TNF- α

and IL-6, are primarily involved in activities regarding activation of NF- κ B, inflammatory regulation, and cellular apoptosis (Wajant et al., 2003; Hoene and Weigert, 2008).

Immunoglobulins are large proteins that bind to antigens and pathogens to neutralize them. One isotype, IgG, is the major antibody found in serum and controls infection in tissues (Meulenbroek, 2008). Lastly, MPO is a peroxidase enzyme produced by primary granules of neutrophils that catalyzes the production of bacteria-killing hypochlorous acid and tyrosyl radicals (Klebanoff, 2005). Collectively, TNF- α , IL-6, IgG, and MPO are useful markers for analyzing the overall immune status of the pig.

Butyrate is thought to increase cortisol levels in response to post-weaning stress and upregulate the expression of anti-inflammatory proteins (Weber and Kerr, 2008). Some agents in the PH mixture used in this study are also speculated to exert immune response properties. Garlic contains several organosulfur compounds that exhibit antimicrobial effects against gram-negative and gram-positive bacteria (Mikaili et al., 2013), and anise contains trans-anethole and estragole compounds, both of which have antibacterial characteristics (Shojaii and Fard, 2012). However, neither SB nor PH inclusion in the diets affected the concentrations of TNF- α , IL-6, IgG, and MPO in blood serum, jejunal mucosa, and/or ileal digesta. This could potentially be due to the fact that the pigs in this study were not inoculated with a direct immune challenge. These results have also been reported in nursery pig studies by Cho et al. (2006), Weber and Kerr (2008), Le Gall et al. (2009), Huang et al. (2010), Liu et al. (2013), and Jang et al. (2017). However, contradictory findings exist. Pigs supplemented with SB have been reported to experience reduced levels of mucosal TNF- α (Huang et al., 2015), reduced levels of IL-6 (Lu et al., 2008), and increased levels of serum IgG (Fang et al., 2014). Likewise, PH mixtures have been suggested to decrease serum IgG

(Huang et al., 2012), increase serum IgG (Li et al., 2012a; Zeng et al., 2015b), and decrease IL-6 concentrations (Li et al., 2012b) in nursery pigs. These reports, as well as the results from this study, are most likely varied due to the differing inclusion rates of the additives and the different active ingredients present in the PH mixtures.

Oxidative stress can occur in the post-weaning period due to the accumulation of reactive oxidative species in conjunction with an impaired antioxidant defense system in the pig (Deng et al., 2010). Reactive oxidative species can rapidly oxidize nutrient molecules such as lipids and carbohydrates and cause damage to DNA and tissues (Zhao and Shen, 2004). Huang et al. (2015) suggested that SB could play a role in reducing oxidative stress in nursery pigs by increasing concentrations of glutathione peroxidase. Phenolic compounds are commonly found in plants from the *Labiatae* (e.g. rosemary, thyme) and *Umbelliferae* (e.g. anise, coriander) families and are widely known to possess antioxidant characteristics due to their reduction-oxidation properties and chemical structures (Balasundram et al., 2006; Brenes and Roura, 2010). In addition, dietary garlic was reported to increase antioxidant activity and improve blood lipid metabolism in rats (Gorinstein et al., 2006), and cinnamon was demonstrated by Kamel (1999) to have antioxidant activity. For these reasons both MDA and protein carbonyl concentrations were measured to observe if a consecutive use of SB and PH could reduce oxidative stress. Malondialdehyde is a highly reactive compound produced by the degradation of lipids by reactive oxygen species (Ma et al., 2011). Protein carbonylation is a result of amino acid oxidation with metals and hydrogen peroxide or through hydroxyl radical-mediated lipid oxidation (Grimsrud et al., 2008). Thus, both MDA and protein carbonyl are useful markers for evaluating oxidative stress status.

Despite the suggested antioxidant properties of SB and PH, neither additive affected the MDA or protein carbonyl concentrations in the jejunal mucosa of the pigs studied. Although there are no reported studies with the same results concerning SB in swine mucosa, Ciric et al. (2017) reported that pigs fed 0.3 and 0.5% SB did not have significantly decreased levels of MDA in kidney and liver tissue compared to pigs fed a control diet. In addition, several authors using a variety of PH compounds reported no changes in MDA concentrations in pig serum, plasma, and urine (Janz et al., 2007; Frankič et al., 2010; Zeng et al., 2015b). The findings in the current study are contradicted by Huang and colleagues (2015), who found that pigs given 1,000 mg/kg of butyric acid had decreased levels of MDA in both serum and mucosa compared to pigs given no butyric acid. Other authors, particularly those studying the effects of PH, reported positive antioxidant results when evaluating the concentration of glutathione peroxidase and superoxide dismutase (Zeng et al., 2015b) and total antioxidant capacity (Li et al., 2012b; Zeng et al., 2015b). Therefore, while it does not appear that SB and PH directly affected the concentration of MDA and protein carbonyl in nursery pigs, other antioxidative effects could still exist.

Interleukin 6 and MDA concentrations tended to have an interactive effect between SB and PH. In both cases, pigs fed either SB or PH only tended to have decreased levels of serum IL-6 and mucosal MDA compared to pigs fed the control diet or SB and PH consecutively. As with the VH:CD interaction, these tendencies indicate that the effectiveness of the PH treatment may rely on whether or not SB was fed in the initial phase after weaning. Interleukin 6 is secreted by T cells and macrophages and signals through a type 1 cytokine receptor complex to create a signaling cascade (Schuster et al., 2003; Hoene and Weigert, 2008), and MDA is synthesized from an eicosanoid called thromboxane A₂

(Ma et al., 2011). Sodium butyrate could potentially generate changes at the secretory and/or signaling level for IL-6 and MDA, although it is unknown if and how these modifications would inhibit the active compounds of the PH mixture when fed later in the nursery phase. Further research would need to be conducted to also understand why there was an interaction tendency for IL-6 in jejunal mucosa but not in serum.

Conclusion

In summary, PH addition to the diets of nursery pigs improved feed efficiency, but SB and the consecutive use of SB and PH showed minimal effects on overall growth performance and gut health. Sodium butyrate decreased ADFI 0 to 19 d post-weaning and increased the rate of enterocyte proliferation by 33 d post-weaning but had no effects on improving ADG, G:F, BW, fecal score, villus height, and immune or oxidative stress responses. Likewise, although pigs fed a diet containing PH experienced improved G:F 19 to 33 d post-weaning, there were no enhancements on indicators of intestinal health. This study proposed some evidence that when used consecutively, the additives have the potential to negatively affect gut morphology and immune response. Conclusively, the results from this study suggest that 0.2/0.1% inclusion of SB and a sequential use of SB and 0.033% PH do not provide a meaningful advantage to overcoming the symptoms of post-weaning stress compared to pigs fed diets with no additives. However, adding PH to the diets of nursery pigs 19 to 33 d post-weaning can improve feed efficiency. Further research is needed to identify the specific modes of action between SB and PH to determine their degree of interaction. Finally, due to the wide variation of results in the literature concerning SB and PH supplementation, additional data are needed to determine the optimal inclusion levels of SB

and PH in the diet and the best combination of plant-derived compounds for a PH supplement.

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Table 1. Composition of experimental diets, as-fed basis.

Item	Pig BW	Phase 1 5 to 7 kg	Phase 2 7 to 11 kg	Phase 3 11 to 25 kg
Ingredient, %				
Corn, yellow dent		40.40	50.05	55.14
Soybean meal, dehulled		20.00	24.00	30.00
Corn DDGS, > 6 and < 9% oil		0.00	0.00	9.00
Whey permeate, 80% lactose		20.00	10.00	0.00
Fish meal		5.00	3.00	0.00
Poultry meal		5.00	5.00	0.00
Poultry fat		2.00	2.00	2.00
Blood plasma		4.75	3.00	0.00
L-Lys HCl		0.39	0.29	0.37
DL-Met		0.19	0.12	0.09
L-Thr		0.12	0.06	0.07
Limestone, ground		0.65	0.79	0.98
Dicalcium phosphate		0.10	0.29	0.95
Salt		0.22	0.22	0.22
Trace mineral premix ¹		0.15	0.15	0.15
Vitamin premix ²		0.03	0.03	0.03
Supplement ³		1.00	1.00	1.00
Total		100.00	100.00	100.00
Calculated composition ⁴				
ME, kcal/kg		3,408	3,402	3,354
SID ⁵ Lys, %		1.50	1.35	1.23
SID ⁵ Met + Cys, %		0.82	0.74	0.68
SID ⁵ Thr, %		0.88	0.79	0.73
SID ⁵ Trp, %		0.25	0.24	0.22
Ca, %		0.85	0.80	0.70
Total P, %		0.69	0.64	0.59
STTD ⁶ P, %		0.47	0.40	0.33
Analyzed composition				
DM, %		92.84	92.73	91.36
CP, %		24.20	24.08	22.64
Crude fat, %		5.24	4.79	4.54
Crude ash, %		6.27	5.59	4.70
NDF, %		7.70	7.90	8.97
ADF, %		3.03	3.06	4.07
Non-fiber carbohydrate, %		49.43	50.38	50.51

Table 1. Continued

Aflatoxin, µg/kg	3.00	0.00	1.83
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^{1,2}Premix is added to provide the following nutrients per kg of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B12; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin; 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.

³Supplement: corn (1%) for basal diet with no additives; corn (0.8%) + sodium butyrate (0.2%) for phase 1 inclusion; corn (0.9%) + sodium butyrate (0.1%) for phases 2 and 3 inclusion; corn (0.967%) + phytobiotics (0.033%) for phase 3 inclusion.

⁴Calculated based on NRC (2012)

⁵Standardized ileal digestible

⁶Standardized total tract digestible

Table 2. Growth performance of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning¹.

Item	Sodium butyrate ^{2,3}		SEM	<i>P</i> value
	No	Yes		
BW, kg				
Initial	7.0	7.0	0.3	0.865
d 7	8.0	8.0	0.3	0.989
d 14	10.4	10.2	0.4	0.665
d 19	13.1	12.7	0.5	0.394
ADG, g/d				
Phase 1	147	150	17	0.876
Phase 2	424	393	18	0.209
Overall	322	303	16	0.342
ADFI, g/d				
Phase 1	166	163	12	0.822
Phase 2	578	509	24	0.024
Overall	427	382	19	0.048
G:F				
Phase 1	0.84	0.89	0.06	0.515
Phase 2	0.74	0.78	0.02	0.164
Overall	0.76	0.80	0.02	0.146

¹Phase 1 represents 0 to 7 d, phase 2 represents 7 to 19 d, and overall represents 0 to 19 d post-weaning.

²Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion in phase 1 and 0.1% in phase 2.

³Each value represents the mean of 24 and 23 individually housed pigs, respectively. One gilt from the sodium butyrate group was removed as an outlier due to poor health.

Table 3. Growth performance of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.¹

Item	Sodium butyrate ^{2,3}			SEM	<i>P</i> value
	None	Phase 1 and 2	Phase 1 to 3		
BW, kg					
Initial	7.0	6.9	7.0	0.2	0.822
d 7	8.0	8.2	8.1	0.2	0.862
d 14	10.3	10.5	10.2	0.5	0.909
d 19	13.0	13.0	13.0	0.6	1.000
d 26	16.9	16.9	16.5	0.7	0.912
d 33	21.1	21.0	21.0	1.0	0.996
ADG, g/d					
Phase 1	135	174	152	32	0.672
Phase 2	417	395	407	36	0.918
Phase 3	580	580	575	41	0.991
Overall	426	427	424	27	0.996
ADFI, g/d					
Phase 1	163	187	159	22	0.514
Phase 2	570	551	547	38	0.899
Phase 3	790	831	828	53	0.690
Overall	577	594	584	32	0.910
G:F					
Phase 1	0.79	0.94	0.83	0.15	0.788
Phase 2	0.73	0.72	0.74	0.04	0.960
Phase 3	0.73	0.70	0.69	0.02	0.175
Overall	0.74	0.72	0.72	0.02	0.843

¹Phase 1 represents 0 to 7 d; phase 2, 7 to 19 d; phase 3, 19 to 33 d; and overall, 0 to 33 d, post-weaning.

²Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion in phase 1 and 0.1% in phases 2 and 3.

³Each value represents the mean of 8 individually housed pigs, except for the Phase 1 and 2 group, which had one gilt removed as an outlier due to poor health.

Table 4. Growth performance of pigs fed experimental diets from 19 to 33 d post-weaning.^{1,2,3}

Item	SB ⁴	No		Yes		SEM	P value		
	PH ⁵	No	Yes	No	Yes		SB	PH	SB x PH
BW, kg									
d 26		16.9	16.8	17.0	16.9	0.2	0.633	0.618	0.944
d 33		21.1	21.4	21.2	21.2	0.4	0.949	0.762	0.716
ADG, g/d									
Phase 3		580	599	589	587	29	0.949	0.762	0.716
Overall		427	436	431	430	13	0.942	0.746	0.677
ADFI, g/d									
Phase 3		791	812	841	774	40	0.875	0.529	0.237
Overall		578	571	600	544	19	0.891	0.115	0.214
G:F									
Phase 3		0.73	0.74	0.70	0.76	0.02	0.586	0.045	0.175
Overall		0.74	0.76	0.73	0.79	0.02	0.774	0.007	0.224

¹Phase 3 represents 19 to 33 d, and overall 0 to 33 d, post-weaning.

²Phase 3 initial BW was used as a covariate to control for treatment variation from phases 1 and 2.

³Each value represents the mean of 8 individually housed pigs, except for the SB only group, which had one gilt removed as an outlier due to poor health.

⁴Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.1% inclusion in phase 3.

⁵Phytobiotics (Apex Swine; Nutriad) were added to diets at 0.033% inclusion in phase 3.

Table 5. Fecal scores of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning.¹

Item	Sodium butyrate ^{2,3}		SEM	<i>P</i> value
	No	Yes		
Fecal score ⁴				
d 3	3.47	3.65	0.29	0.693
d 5	2.98	3.27	0.17	0.355
d 7	2.82	2.83	0.15	0.896
d 9	2.68	2.33	0.19	0.157
d 11	2.79	2.46	0.22	0.233
d 13	2.39	2.42	0.16	0.991
d 15	2.50	2.18	0.19	0.189
d 17	2.43	2.43	0.21	0.952
d 19	2.39	2.47	0.17	0.980
Phase 1	3.10	3.14	0.14	0.818
Phase 2	2.53	2.39	0.13	0.265
Overall	2.71	2.63	0.12	0.570

¹Phase 1 represents 0 to 7 d, phase 2 represents 7 to 19 d, and overall 0 to 19 d post-weaning.

²Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion in phase 1 and 0.1% in phase 2.

³Each value represents the mean of 24 individually housed pigs.

⁴Fecal score was evaluated on a scale of 1 to 5, with completely solid feces receiving a score of 1 and completely liquid feces receiving a score of 5.

Table 6. Fecal scores of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.¹

Item	Sodium butyrate ^{2,3}			SEM	P value
	None	Phase 1 and 2	Phase 1 to 3		
Fecal score ⁴					
d 3	3.50	3.80	4.00	0.49	0.819
d 5	3.00	3.00	3.29	0.32	0.699
d 7	2.57	3.00	3.00	0.30	0.455
d 9	2.38	2.38	3.00	0.34	0.440
d 11	2.38	2.63	2.88	0.37	0.657
d 13	2.38	2.25	2.75	0.32	0.576
d 15	2.50	2.29	2.75	0.35	0.568
d 17	2.43	2.71	2.43	0.37	0.948
d 19	2.43	2.29	2.75	0.32	0.573
d 26	2.38	2.71	2.50	0.27	0.491
d 33	2.25	2.00	2.25	0.30	0.805
Phase 1	3.01	3.21	3.19	0.28	0.884
Phase 2	2.45	2.41	2.78	0.25	0.686
Phase 3	2.31	2.38	2.38	0.23	0.959
Overall	2.56	2.60	2.79	0.22	0.952

¹Phase 1 represents 0 to 7 d; phase 2, 7 to 19 d; phase 3, 19 to 33 d; and overall, 0 to 33 d, post-weaning.

²Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion in phase 1 and 0.1% in phases 2 and 3.

³Each value represents the mean of 8 individually housed pigs.

⁴Fecal score was evaluated on a scale of 1 to 5, with completely solid feces receiving a score of 1 and completely liquid feces receiving a score of 5.

Table 7. Fecal scores of pigs fed experimental diets from 19 to 33 d post-weaning.^{1,2}

Item	SB ³	No		Yes		SEM	<i>P</i> value		
		No	Yes	No	Yes		SB	PH	SB x PH
Fecal score ⁵									
d 26		2.38	2.12	2.69	2.38	0.29	0.201	0.317	0.916
d 33		2.25	2.13	2.00	2.50	0.30	0.823	0.569	0.308
Phase 3		2.31	2.19	2.38	2.44	0.23	0.448	0.754	0.686
Overall		2.56	2.60	2.60	2.64	0.18	0.792	0.940	1.000

¹Phase 3 represents 19 to 33 d, and overall 0 to 33 d, post-weaning.

²Each value represents the mean of 8 individually housed pigs.

³Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.1% inclusion in phase 3.

⁴Phytobiotics (Apex Swine; Nutriad) were added to diets at 0.033% inclusion in phase 3.

⁵Fecal score was evaluated on a scale of 1 to 5, with completely solid feces receiving a score of 1 and completely liquid feces receiving a score of 5.

Table 8. Histological measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning.

Item	Sodium butyrate ^{1,2}		SEM	P value
	No	Yes		
Jejunal histology				
Villus height, μm	443	421	15	0.329
Villus base, μm	120	125	4	0.357
Villus width, μm	112	118	5	0.362
Villus tip, μm	76	82	4	0.256
Crypt depth, μm	270	269	14	0.898
VH:CD ³	2.12	2.09	0.02	0.063
Enterocyte proliferation ⁴ , %	12.43	18.09	2.27	0.105

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 19 d post-weaning.

²Each value represents the mean of 8 individually housed pigs.

³Villus height to crypt depth ratio

⁴Proliferation was evaluated by staining cells with a Ki67 antibody. Ki67 is a protein expressed in the nuclei of proliferating cells.

Table 9. Histological measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.

Item	Sodium butyrate ^{1,2}			SEM	<i>P</i> value
	None	0 to 19 d	0 to 33 d		
Jejunal histology					
Villus height, μm	410	430	397	19	0.431
Villus base, μm	135	143	137	5	0.615
Villus width, μm	136	146	141	5	0.396
Villus tip, μm	97	97	100	5	0.893
Crypt depth, μm	270	263	259	13	0.838
VH:CD ³	1.62	1.79	1.64	0.11	0.470
Enterocyte proliferation ⁴ , %	20.80 ^a	23.94 ^b	23.84 ^b	0.61	0.003

^{ab}Values without a common superscript within a row were significantly different ($P < 0.05$).

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 33 d post-weaning.

²Each value represents the mean of 8 individually housed pigs.

³Villus height to crypt depth ratio

⁴Proliferation was evaluated by staining cells with a Ki67 antibody. Ki67 is a protein expressed in the nuclei of proliferating cells.

Table 10. Histological measurements of pigs fed experimental diets from 19 to 33 d post-weaning.¹

Item	SB ²	No		Yes		SEM	<i>P</i> value		
	PH ³	No	Yes	No	Yes		SB	PH	SB x PH
Jejunal histology									
Villus height, μm		410	428	430	393	18	0.693	0.596	0.125
Villus base, μm		135	138	143	139	6	0.462	0.929	0.554
Villus width, μm		136	138	146	136	6	0.401	0.362	0.246
Villus tip, μm		97	95	97	94	4	0.898	0.551	0.842
Crypt depth, μm		270	268	263	267	12	0.726	0.937	0.765
VH:CD ⁴		1.62	1.72	1.79	1.58	0.11	0.850	0.526	0.074
Enterocyte proliferation ⁵ , %		20.80	21.36	23.94	22.80	0.68	0.001	0.654	0.190

¹Each value represents the mean of 8 individually housed pigs.

²Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.1% inclusion 19 to 33 d post-weaning.

³Phytobiotics (Apex Swine; Nutriad) were added to diets at 0.033% inclusion 19 to 33 d post-weaning.

⁴Villus height to crypt depth ratio

⁵Proliferation was evaluated by staining cells with a Ki67 antibody. Ki67 is a protein expressed in the nuclei of proliferating cells.

Table 11. Immune status measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning.

Item	Sodium butyrate ¹		SEM	<i>P</i> value
	No	Yes		
Serum ²				
TNF- α , pg/mL	106.9	87.2	9.9	0.138
IL-6, pg/mL	0.80	0.71	0.26	0.837
IgG, mg/mL	2.97	2.92	0.22	0.877
Jejunal mucosa ²				
TNF- α , pg/mg	1.05	0.90	0.19	0.867
IL-6, pg/mg	1.31	1.17	0.31	0.760

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 19 d post-weaning.

²TNF- α : tumor necrosis factor alpha; IL-6: interleukin 6; IgG: immunoglobulin G

Table 12. Immune status measurements of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.

Item	Sodium butyrate ¹			SEM	<i>P</i> value
	None	0 to 19 d	0 to 33 d		
Serum ²					
TNF- α , pg/mL	150.8	112.2	171.6	33.6	0.289
IL-6, pg/mL	5.27	4.52	4.13	0.73	0.857
IgG, mg/mL	2.94	3.42	3.38	0.27	0.242
Jejunal mucosa ²					
TNF- α , pg/mg	0.39	0.29	0.31	0.07	0.415
IL-6, pg/mg	0.56	0.64	0.57	0.11	0.879
Ileal digesta ²					
MPO, μ U/mL ³	16.86	17.29	17.71	1.00	0.838

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 33 d post-weaning.

²TNF- α : tumor necrosis factor alpha; IL-6: interleukin 6; IgG: immunoglobulin G; MPO: myeloperoxidase

³One unit of MPO activity equals the amount of MPO that oxidizes aminophenyl fluorescein substrate to generate 1.0 μ mol of fluorescein per minute at 25°C.

Table 13. Immune status measurements of pigs fed experimental diets from 19 to 33 d post-weaning.

Item	SB ¹	No		Yes		SEM	<i>P</i> value		
	PH ²	No	Yes	No	Yes		SB	PH	SB x PH
Serum ³									
TNF- α , pg/mL		159.8	133.7	114.2	147.7	22.0	0.472	0.865	0.182
IL-6, pg/mL		5.27	4.11	4.52	6.43	0.77	0.306	0.622	0.052
IgG, mg/mL		2.94	2.86	3.42	2.93	0.21	0.202	0.192	0.353
Jejunal mucosa ³									
TNF- α , pg/mg		0.39	0.34	0.29	0.32	0.07	0.358	0.901	0.506
IL-6, pg/mg		0.56	0.45	0.64	0.77	0.14	0.182	0.922	0.395
Ileal digesta ³									
MPO, μ U/mL ⁴		16.86	19.72	17.29	17.61	1.20	0.495	0.199	0.302

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.1% inclusion 19 to 33 d post-weaning.

²Phytobiotics (Apex Swine; Nutriad) were added to diets at 0.033% inclusion 19 to 33 d post-weaning.

³TNF- α : tumor necrosis factor alpha; IL-6: interleukin 6; IgG: immunoglobulin G; MPO: myeloperoxidase

⁴One unit of MPO activity equals the amount of MPO that oxidizes aminophenyl fluorescein substrate to generate 1.0 μ mol of fluorescein per minute at 25°C.

Table 14. Oxidative stress indicators of pigs fed diets supplemented with sodium butyrate from 0 to 19 d post-weaning.

Item	Sodium butyrate ¹		SEM	<i>P</i> value
	No	Yes		
Jejunal mucosa				
Malondialdehyde, μM	11.23	9.74	2.09	0.580
Protein carbonyl, nmol/mg	0.31	0.38	0.17	0.955

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 19 d post-weaning.

Table 15. Oxidative stress indicators of pigs fed diets supplemented with sodium butyrate from 0 to 19 d only or 0 to 33 d post-weaning.

Item	Sodium butyrate ¹			SEM	<i>P</i> value
	None	0 to 19 d	0 to 33 d		
Jejunal mucosa					
Malondialdehyde, μ M	7.63	5.78	9.24	1.23	0.179
Protein carbonyl, nmol/mg	0.79	0.65	0.56	0.13	0.455

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.2% inclusion 0 to 7 d and 0.1% inclusion 7 to 33 d post-weaning.

Table 16. Oxidative stress indicators of pigs fed experimental diets from 19 to 33 d post-weaning.

Item	SB ¹	No		Yes		SEM	<i>P</i> value		
	PH ²	No	Yes	No	Yes		SB	PH	SB x PH
Jejunal mucosa									
Malondialdehyde, μ M		7.63	5.83	5.78	8.03	1.14	0.877	0.845	0.087
Protein carbonyl, nmol/mg		0.79	0.59	0.65	0.69	0.16	0.882	0.581	0.379

¹Encapsulated sodium butyrate (Ultramix C; Nutriad, Elgen, IL) was added to diets at 0.1% inclusion 19 to 33 d post-weaning.

²Phytobiotics (Apex Swine; Nutriad) were added to diets at 0.033% inclusion 19 to 33 d post-weaning.

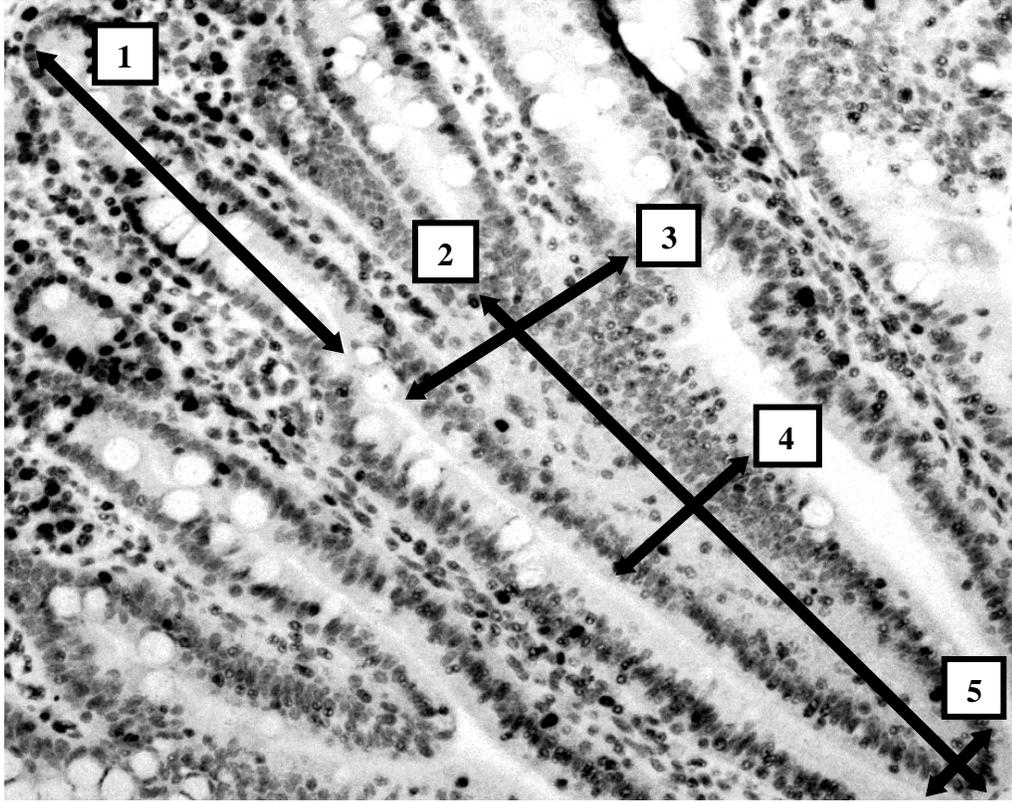


Figure 1. Jejunal villus and crypt histological measurements.
1) Crypt depth; 2) villus height; 3) villus width at the base; 4) villus width at the middle; 5) villus width at the tip.