Effects of Botanicals on Diarrhea, Systemic Immunity, and Intestinal Health of Weaned Pigs

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ABSTRACT

Botanicals, phytogenic feed additives, and essential oils may have potential benefits in swine production for their health, immunity, and growth promoting effects. Therefore, the objective of this study was to investigate the effects of dietary botanical supplementation on blood profiles and intestinal morphology of weaned piglets experimentally infected with a pathogenic F18 *Escherichia coli* (*E. coli*). Sixty weaned piglets (around 21 days old; 7.15 ± 0.97 kg) were individually housed and randomly allotted to one of five dietary treatments (n = 12): negative control (NC), positive control (PC), high dose of botanicals blend 1 (BB1, 100 mg/kg), and low or high dose of botanicals blend 2 (BB2, 50 mg/kg and 100 mg/kg). Both botanical blends were comprised of capsicum oleoresin but different garlic extract varieties. The experiment lasted 28 days from day -7 to +21 relative to *E. coli* inoculation. All piglets except the NC group were orally inoculated with F18 *E. coli* (10^{10} cfu per dose, 3 doses) for 3 consecutive days. Growth performance and diarrhea scores were recorded throughout the experiment. Blood samples were collected on d 0, 5, 14, and 21 post-inoculation (PI) for complete blood count test. Intestinal segments were collected on d 5 and 21 PI for intestinal morphology analysis. Data were analyzed by ANOVA in PROC MIXED of SAS with a randomized complete block design. *E. coli* infection reduced ($P < 0.05$) pig body weight and growth rate throughout the experiment and reduced ($P < 0.05$) neutrophils and lymphocytes but increased ($P < 0.05$) monocytes on d 4 PI. No differences in weight gain or average daily gain were observed among the botanical blend treatments compared with the control. Supplementation of high dose BB1 or BB2 reduced ($P < 0.05$) frequency and severity of diarrhea of challenged pigs during the experimental period. Pigs supplemented with 100 mg/kg of BB2 had less ($P < 0.05$) lymphocytes than pigs in PC on d 4 PI. Pigs fed with 50 mg/kg of BB2 had

lower ($P < 0.05$) lymphocytes and monocytes than pigs in PC on d 21 PI. Pigs supplemented with 100 mg/kg of BB2 had the greatest ($P < 0.05$) duodenal villi width, jejunum villi height and area, and colon crypt depth than pigs in PC on d 5 PI. In conclusion, dietary supplementation of botanicals reduced diarrhea and tended to affect systemic immunity and enhance intestinal morphology of weaned pigs infected with *E. coli*.

KEY WORDS: botanical blends, blood profile, *Escherichia coli* infection, growth performance, intestinal morphology, post-weaning diarrhea, weaned pigs

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LIST OF ABBREVIATIONS

AB/PAS	Alcian blue periodic acid-Schiff
ADFI	average daily feed intake
ADG	average daily gain
AGP	antibiotic growth promoter
APC	antigen presenting cell
ATP	adenosine triphosphate
BB	botanical blend
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
CBC	complete blood count
COX-2	cyclooxygenase-2
ETEC	enterotoxigenic *Escherichia coli*
FOXP3	forkhead box P3
Ig	immunoglobin
IL	interleukin
IFN-γ	interferon-gamma
IRF	interferon regulatory factors
LDL	low-density lipoproteins
LPS	lipopolysaccharides
LT	heat labile
MAPK	mitogen-activated protein kinases
MCH	mean corpuscular hemoglobin

MCHC	mean corpuscular hemoglobin concentration
MCP-1	monocyte chemoattractant protein-1
MHC	major histocompatibility complex
NC	negative control
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffer saline
PC	positive control
PI	post-inoculation
PRR	pattern recognition receptor
ROS	reactive oxygen species
ST	heat stable
STAT3	signal transducer and activator of transcription 3
TCR	T cell receptor
TNF-α	tumor necrosis factor alpha

CHAPTER 1

LITERATURE REVIEW

1.1 Current Status of Global Swine Production

The massive and expected increase in global population has spurred livestock producers to bolster their production rates to meet the growing global food demand (Raney, 2009). In 2019, the global production of meat estimated nearly 335 million tons, with global trade in meat and meat products estimating 36 million tons (F.A.O., 2019). This growth in meat production will be guided by growing populations, increasing income, and improving economies and diet quality, both in developing and developed countries. Pork and pig-related products have remained an important protein source across developing and developed countries (F.A.O., 2009). The global meat market is projected to increase 18% by 2028, of which 16% increase accounts for pork consumption and pork imports (O.E.C.D. and F.A.O., 2019). The increase in global demand and consumption of pork products will be met by enhancing the efficiency in animal productivity and health and increasing animal agriculture sustainability (O.E.C.D. and F.A.O., 2019).

Improvements toward swine production and management will be achieved by growth and investment in areas such as nutrition, genetics, and sustainable animal agriculture. Sustainability within animal production systems can be met through advancements in animal and human health security, standards for animal welfare, decreasing environmental degradation and resource expenditure, and socio-economic credibility within the livestock and food production sector (Scholten et al., 2019). Some developing countries, for instance, have used alternative non-traditional feed sources, such as palm kernel, to increase profitability due to local palm kernel production (Verhulst, 1992).

Antibiotic growth promoters (AGPs) have been used in global animal agriculture for the past 50 years to improve growth and feed efficiency (Dibner and Richards, 2005). AGPs remain in limited use primarily within intensive pig production, with penicillins and tetracyclines being documented as the most used in developing and developed countries (Cuong et al., 2018; Lekagul et al., 2019). However, stricter regulations regarding the use of AGPs for growth promotion throughout livestock production have been enforced on a global scale as the emergence of antibiotic-resistance pathogens strains have increased. The use of in-feed AGPs for the purpose of livestock growth promotion was fully banned in the E.U. in 2006 (U.S. GAO, 2011), while the U.S. introduced a voluntary requirement for limited use of AGPs for growth promotion and disease resistance in 2017 (F.D.A., 2016). Difficulties in maintaining pig health that have arisen because of these regulations are encouraging enterprise in research for AGP alternatives, further understanding in intestinal immunology and physiology, and improving animal welfare management.

1.2 Weaning Stress and Diarrhea

Weaning is an important and challenging stage for pig health and development in the swine industry. Piglets are abruptly weaned from sows when they are 3-4 weeks of age and are then moved to segregated housing and pens. The commercial weaning practices are markedly different from the gradual 11-12 week weaning period seen in sows and piglets reared in natural environments, thus, has serious negative impacts on the health and development of piglets (Worobec et al., 1998). Numerous stress factors exist within the weaning process, such as sudden maternal separation, transportation and handling, environmental and dietary changes, and reestablishment of the social hierarchy (Lallès et al, 2004; Campbell et al., 2013). Previous literature has associated weaning and post-weaning stress with reduced feed intake, nutrient

absorption, and gut integrity, resulting in an increase in inflammatory responses and susceptibility to diarrhea (Hampson, 1986; Nabuurs et al., 1993; McCracken et al., 1999; Lallès et al, 2004).

1.2.1 Effect of Weaning Stress on Intestinal Physiology

The sudden transition from sow's milk to solid feed is challenging for newly weaned piglets because of the changes in diet form, palatability, and nutrient sources and digestibility. As a result, malnourishment and decreased feed intake and growth rate are often exhibited in weaned piglets (Campbell et al., 2013). Studies have revealed that weaning stress and anorexia has negative impacts on intestinal morphology, primarily causing villous atrophy and crypt hypertrophy (Pluske et al., 1997; McCracken et al., 1999; Boudry et al., 2004). Villi height is correlated with the estimated number of villous enterocytes, the most abundant epithelial cells responsible for nutrient absorption (Barszcz and Skomiał, 2011). Crypt depth indicates the capacity of crypt stem cells maturing into functional enterocytes (Hampson and Kidder, 1986). Villous atrophy is the shortening of intestinal villi and reduction in villous surface area due to decreased rate of epithelial cell renewal from crypt cells (Argenzio et al., 1990; Pluske et al., 1997). Crypt hypertrophy is an increase in crypt cell size and is associated with increases in crypt cell number (crypt hyperplasia) and crypt depth (Ferreira et al., 1990). Reduction in villi height to crypt depth ratio possibly indicates impaired digestive, absorptive, and secretory capabilities by the small intestine.

The activities of brush border enzymes, such as lactase, sucrase, and maltase, reflect the maturation and digestive capacity of the small intestine (Hampson and Kidder, 1986). Dietary change during weaning period is responsible for a reduction in brush border enzyme activities (Bourdry et al., 1986). Electrolyte absorption and secretion is also reduced in newly weaned

piglets, which contributes to the nutrient malabsorption in the small intestine (Nabuurs et al., 1994).

The initial defense mechanism of the gastrointestinal barrier is composed of the epithelial cell layer covered by mucins and glycoproteins that are produced by goblet cells (Barszcz and Skomiał, 2011). Gut permeability, epithelial leakage, and epithelial polarity are regulated by epithelial tight junction proteins, such as transmembrane occludin and claudins and intracellular zona occludens (Edelblum and Turner, 2009; Groschwitz and Hogan, 2009; Turner, 2009; Marchiando et al., 2010; Shea-Donohue, 2018). Studies have shown that weaning stress could reduce goblet cell count, mucin production and tight junction protein expression, resulting in increased gut permeability and disease susceptibility (Peace et al., 2011; Hu et al., 2013a; McLamb et al., 2013). Weaning stress has also been shown to induce stress signaling pathways and to activate intestinal mast cells, which further impair the mucosal barrier development and function (Moeser et al, 2007; Smith et al., 2009).

1.2.2 Effect of Weaning Stress on Immunity

The innate immune system is the immune system's initial response for recognizing foreign pathogens, which involves non-specific defense by many innate immune cells, such as macrophages, neutrophils, mast cells, and dendritic cells (Alberts et al., 2002; Krystal-Whittemore et al., 2016; Germic et al., 2019). Macrophages are long-lived phagocytes that reside in body tissues to sense and respond to pathogens (Watanabe et al., 2019), while neutrophils are short-lived phagocytes that quickly respond to the detection of pathogens by migrating from the blood circulation into tissues (Rosales, 2018). Both macrophages and neutrophils are essential defenders against common pathogens and bacterial infections (Janeway, Jr. et al., 2001a). Pattern recognition receptors (PRRs) present on the innate immune cells distinguish pathogen-associated

molecular patterns (PAMPs) that are located on pathogen surfaces (Alberts et al., 2002). Once a PAMP has been recognized, the PRRs activate several intracellular signaling pathways, such as NF-κB, mitogen-activated protein kinases (MAPKs), and interferon regulatory factors (IRFs), which regulate the expression of genes associated with immune responses (Mogenson, 2009). Cytokines are small protein and glycoprotein signaling molecules that are critical for the early innate immune response and the activation and recruitment of adaptive immune cells (Alberts et al., 2002; Pié et al., 2004; Mogenson, 2009). Cytokines that worsen disease symptoms are considered as pro-inflammatory and cytokines that reduce inflammation and promote healing are considered as anti-inflammatory (Dinarello, 2000). The pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6 are produced by activated macrophages and intestinal epithelial cells (Stadnyk, 1994; Johnson, 1997). They have been shown to affect intestinal mucosal immunity by increasing epithelial tight junction permeability. They additionally affect systemic immunity by preventing apoptosis of epithelial cells and promoting epithelial cell proliferation (Onyiah and Colgen, 2016). The anti-inflammatory cytokines IL-10 and IL-22 help decrease an inflammatory response by preventing activation of pro-inflammatory cytokine IL-6 through modulating the signal transducer and activator of transcription 3 (STAT3) signaling pathway, maintaining epithelial barriers by activating proliferative and anti-apoptotic mechanisms, and promoting tissue expression of mucins and antimicrobial peptides (Sanjabi et al., 2009).

The adaptive immune system is slow to develop on the initial exposure to a new pathogen. However, unlike the innate immune system, the adaptive immune system remembers previous encounters with specific pathogens and acts upon those memories to destroy the pathogen if it returns in the host (Alberts et al., 2002). T and B lymphocytes are adaptive

immune cells produced by the thymus and bone marrow, respectively (Bonilla and Oettgen, 2010). Their development features rearrangement of germ-line gene segments to construct genes for antigen-specific receptors known as T cell receptors (TCRs) and immunoglobulins (B cell receptors). The immense diversity of rearrangements allows for developing receptor specificity to any possible antigens (Chaplin, 2010). Antigen presenting cells (APCs) include macrophages, dendritic cells, and B cells, and are responsible for initiating an adaptive immune response by presenting pathogen-specific surface proteins (antigens) (Gaudino and Kumar, 2019). Once the PRRs present on the APCs bind to their appropriate PAMPs, APCs consume the pathogen via phagocytosis, pinocytosis, or endocytosis. The pathogen is degraded into pathogen-specific surface proteins (antigens), which are then displayed on the APC surface by major histocompatibility complex (MHC) molecules within the APCs. T cells corresponding to the appropriate class of MHC molecules recognize the presented antigens via antigen-specific receptors, prompting T and B cells to memorize and eliminate future incidences of the antigen. (Janeway, Jr. et al., 2001b; Wieczorek et al., 2017; Gaudino and Kumar, 2019). This relationship is an integral mechanism that connects innate immunity with adaptive immunity (Iwasaki and Medzhitov, 2019). Memory T and B cells are produced during the initial primary response to an antigen, which allow for the production of higher affinity TCRs and immunoglobins (Ig) in the event that the same pathogen and antigens are encountered by the immune system (Bonilla and Oettgen, 2010).

The immune system of postnatal piglets is underdeveloped and relies heavily on maternal colostrum and milk for initial immune protection before their adaptive immune system matures (Stokes, 2017). The development of the gut mucosal immune system occurs in a programmed sequence beginning from birth to 5 weeks of age and onward, reaching mature immune levels at

around 7 weeks of age (Stokes et al., 2004). The immunity of newly weaned pigs is relatively immature, as 3-week old weaned piglets produce less IgG1 when compared with 9-week old weaned piglets (Bailey et al., 2004). $CD8^+$ T cells are largely absent in piglets 2-4 weeks old, supporting the immaturity of weaned piglet immunity (Stokes et al., 2004).

Weaning stress has multiple effects on the pig innate and adaptive immune system. Moeser et al. (2007) and Smith et al. (2009) reported that weaned pigs exhibit disrupted epithelial and mucosal barrier function. Other studies have also shown that weaning stress increases intestinal pro-inflammatory cytokine levels, which further negatively impact gut permeability and epithelial function (McKay and Baird, 1999; Pié et al., 2004; McLamb et al., 2013, Hu et al., 2013a).

1.2.3 Post-Weaning Diarrhea

Post-weaning diarrhea is an important cause of economic loss in the global swine industry. It commonly occurs in piglets during the first 1-2 weeks after weaning and is characterized by watery diarrhea that lasts for 1-5 days. Post-weaning diarrhea could result in reduced weight gain and increased morbidity, mortality, and medical costs (Fairbrother et al., 2005; Dubreuil et al., 2016). Susceptibility of post-weaning diarrhea is affected by multiple factors, including weaning stress, weaning age, dietary change, and increased exposure to environmental pathogens (Cox et al., 2012). Post-weaning diarrhea can result in mortalities of weaned pigs ranging from 1.5 to 25%. Swine farms affected by post-weaning diarrhea had 5.9% greater mortality than swine farms unaffected by post-weaning diarrhea (Fairbrother and Nadeau, 2019; Amezcua et al., 2002).

Enterotoxigenic *Escherichia coli* (ETEC) infection is one of the most common causes of post-weaning diarrhea in pigs (Nagy and Fekete, 2005). ETEC infections are transmitted to

animals by oral ingestion from the environment. The bacteria travel to the ileum, where they colonize and secret enterotoxins (Dubreuil et al., 2016). Diarrhea caused by ETEC infections has been observed in multiple animal species, including pigs, calves, sheep, and dogs, with different enterotoxins affecting animal species differently (Foster and Smith, 2009; Fairbrother et al., 2005).

The two main virulence factors of ETEC are adhesins and enterotoxins (Dubreuil et al., 2016). ETEC adhesins are hair-like surface filaments and fimbriae that carry specific receptors for attachment to the epithelial cells of the small intestine (Proft and Baker, 2009). The two common fimbriae types associated with ETEC and diarrhea in pigs are F4 and F18, with their genes present in 92.7% of ETEC in pigs affected by post-weaning diarrhea (Frydendahl, 2002). Pre-weaning diarrhea is more affiliated with F4 fimbriae, while post-weaning diarrhea is affiliated with both F4 and F18 fimbriae (Fairbrother et al., 2005). ETEC enterotoxins are extracellular proteins and peptides that bind to their specific receptors expressed on the surface of epithelial and endothelial cells in the small intestine, affecting intestinal permeability, local and systemic immunity (O'Brien and Holmes, 1996). Enterotoxins produced by ETEC consist primarily of heat stable (ST; STa and STb) toxins, heat labile (LT) toxins, and Shiga-toxins (Kopic and Geibel, 2010). STa and LT toxins increase gut permeability and net intestinal fluid secretions by increasing chloride and water secretion by epithelial crypt cells and inhibiting sodium chloride absorption by intestinal villi (Nataro and Kaper, 1998). STb toxins affect intestinal morphology by reducing villi epithelial cells and inducing villous atrophy (Whipp et al., 1987; Dubreuil, 2008). Shiga-toxins are responsible for an increase in immune cytokines, systemic inflammation, and occurrence of edema disease and fluid secretion (Tarr et al., 2005; Scheutz et al., 2012; Meng et al., 2014). Lipopolysaccharides (LPS) are a glycolytic PAMP

located on the outer membrane of ETEC and other gram-negative bacteria (Beatty et al., 1999). LPS may induce expression of numerous pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-α, which contributes to the excessive inflammation associated with ETEC infection (Schilling et al., 2001; Trent et al., 2006; Liu et al., 2018a).

1.3 Alternatives to Synthetic Antibiotics

Various nutritional strategies have been proposed as potential alternatives to in-feed AGPs to improve the health and production of weaned pigs (Kil and Stein, 2010; Liu et al., 2018b). Zinc and copper are inorganic micro minerals that exhibit antimicrobial properties when fed to pigs in quantities higher than their nutritional requirement (Liu et al., 2018b). Dietary supplementation of zinc and copper may improve growth performance and disease resistance in weaned pigs by upregulating tight junction protein expression, reducing inflammation, and inhibiting pathogen growth in the small intestine (Hu et al, 2013b; Espinosa and Stein, 2021). High dose zinc oxide may improve growth performance of nursery pigs, as well as maintain membrane integrity of enterocytes subjected to an $F4^+$ ETEC infection (Roselli et al., 2003; Hollis et al., 2005).

Spray-dried plasma is another effective alternative to in-feed AGPs during the first two weeks post-weaning (Pérez-Bosque et al., 2016). Spray-dried porcine plasma has been shown to improve growth performance, intestinal morphology, and immune status of weaned pigs (Bosi et al, 2004; Nofrarías et al., 2006; Ruckman et al., 2020). The benefits of spray-dried plasma are attributed to its high concentration of immunoglobins, which provides the weaned pig with immunoglobins normally obtained from sow milk and colostrum (Svendsen and Brown, 1973; Pierce et al., 2005). Despite its benefits, spray-dried plasma is considered not practical for use in

large scale swine production due to its high cost and production desire to minimize use of animal products in feed (Patterson et al., 2010; Gerber et al., 2014).

Organic acids, such as formic acid and benzoic acid, are effective at improving growth rate, feed conversion ratio, and intestinal morphology, and decreasing post-weaning diarrhea caused by ETEC in weaned pigs (Mroz, 2005; Bosi et al., 2007; Halas et al., 2010; Luise et al., 2017). Organic acids aid in nutrient digestion and pathogen monitoring by maintaining the pH of the gastrointestinal tract and demonstrating antimicrobial effects on gram-negative and gram-positive bacteria (Sun and Kim, 2017; Kovanda et al., 2019).

Prebiotics are defined as non-digestible oligosaccharides that improve host health by promoting activity and/or growth of intestinal microbiota (Gibson and Roberfroid, 1995; Lindberg, 2014; Liu et al., 2018b). Oligosaccharide supplementation may benefit weaned pigs by improving growth performance, digestive and absorptive capacities of the intestine, immune function, and disease resistance (Halas and Nochta, 2012; Wu et al., 2017; Zhao et al, 2019; Xing et al., 2020).

Direct-fed microbials/probiotics, which includes *Bacillus*, lactic acid-producing bacteria, and yeast, are live microbes that can be orally supplemented to positively influence the health, gut microbiota, and immune function of the host (Fuller, 1992; Khan et al., 2016). Supplementation of direct-fed microbials may improve growth performance, gut health, and immune function, while decreasing systemic inflammation and diarrhea severity in weaned pigs (Shen et al., 2009; Dowarah et al., 2017; Kim et al., 2019; He et al., 2020). These benefits may be associated with the modification of gut environment to support more favorable microbiota, competing binding site and nutrients with harmful pathogens, and secreting antimicrobial metabolites (Yang et al., 2015; Khan et al., 2016).

1.4 Botanical Extracts

Botanical extracts are natural plant-derived compounds that benefit health of humans and health, feed efficiency, and production of livestock animals (Temiz and Ozturk, 2020). Botanical extracts have been considered as potential replacements for in-feed AGPs because of their antioxidant (Nakatani, 1992; Rice-evans et al, 1995), antimicrobial (Hammer et al., 1999), anti-inflammatory (Liu et al., 2012), and antimicrobial properties (Dorman and Deans, 2000).

1.4.1 Definition, Sources, and Types

Botanical extracts (also called plant extracts, phytobiotics, and/or phytogenic feed additives) are naturally occurring compounds derived from the non-woody constituents of aromatic plants. Botanical extracts include herb extracts, spices, essential oils, and/or oleoresins (Windisch et al., 2008). These compounds are primarily secondary metabolites, which exhibit low molecular weight and provide defense for the plant against physiological and environmental stress (Wenk, 2003). There are various extraction processes for obtaining botanical extracts, including steam distillation, hydrolyzation, solvent extraction, gas chromatography, freeze drying, and rotary evaporation (Herman et al., 2019; Sezen et al., 2019).

Each botanical extract and essential oil consists of various plant secondary metabolites and molecular compounds, such as alcohols, esters, aldehydes, ketones, and phenols (Tabanca et al., 2007; Yap et al., 2014). The three major categories of plant secondary metabolites are terpenes and terpenoids, alkaloids, and phenolic compounds (Croteau et al., 2000). The two most active plant secondary metabolites are terpenes/terpenoids and phenylpropanoids (Simitzis, 2017). Terpenes are groups of molecules structured around the hydrocarbon isoprene, a simple five-carbon molecule that can build upon itself by binding with other isoprene units (Zwenger and Basu, 2008). Terpenoids are terpenes modified by an additional molecule, such as oxygen or

an oxygenated compound (Umay, 2007). Monoterpenes (C10) and sesquiterpenes (C15) are the most commonly found terpenes and terpenoids in essential oils and are essential components for the health benefits of essential oils and botanical extracts (Graßmann, 2005). Phenylpropanoids are organic plant compounds synthesized from phenylalanine and consist of an aromatic six-carbon ring with a three-carbon chain, and can offer beneficial pharmacological properties (Sá et al., 2014).

Each plant secondary metabolite has its own unique chemical properties, which are affected by individual factors, such as plant species and part of the plant used, as well as external factors, such as environment and climate, harvest time, and extraction method (Zeng et al., 2015a). Individual and blends of botanical extracts and essential oils have been reviewed for their beneficial uses in swine, poultry, and ruminant production (Burt, 2004; Windisch et al., 2008; Zeng et al., 2015a; Ratika and Singh, 2018). Examples of terpenes commonly studied for use in human and animal nutrition include thymol (thyme), carvacrol (oregano), and limonene (citrus), while examples of phenylpropanoids include eugenol (clove), cinnamaldehyde (cinnamon), and anethole (anise) (Simitzis, 2017; Sezen et al., 2019).

1.4.2 Biological Activities of Botanical Extracts

1.4.2.1 Antioxidant Activities

Botanical extracts and essential oils have been reviewed and reported to have antioxidative properties (Nakatani, 1992; Rice-evans et al., 1995; Amorati et al., 2013). Oxidative stress and oxidative deterioration are sources responsible for chronic and degenerative disorders and occur when there is an imbalance in free radicals and reactive oxygen species (ROS) present in a biological system (Tungmunnithum et al., 2018). Free radicals and ROS are

independent molecules and compounds containing one or more uncharged electrons and are produced from normal metabolic activity. They can react with polyunsaturated fatty acids to produce secondary volatile aromatic compounds (aldehydes, ketones, oxygenated compounds), which have adverse effects on lipids, proteins, and carbohydrates. Lipid peroxidation can cause oxidative deterioration in phospholipid membranes, which can lead to severe cell damage, loss of cell function, and further development of various cardiovascular diseases and cancer (Simitzis and Deligeorgis, 2011; Pisoschi and Pop, 2015).

The antioxidative properties of botanical extracts and essential oils are attributed to their phenolic compounds, specifically terpenes/terpenoids, phenylpropanoids, and flavonoids (Windisch et al., 2008). Phenolic compounds have high reactivity to ROS and produce phenoxyl radicals upon reaction. Phenoxyl radicals are neutralized into nonradical particles upon reacting quickly with another phenoxyl radical (Amorati et al., 2013). This prevents free radicals and ROS from reacting with lipids, which prevents oxidation from occurring and aiding lipid metabolism (Simitzis, 2017). Flavonoids are large polyphenolic compounds and secondary metabolites of plants commonly found in plant pigments. Flavonoids exhibit beneficial antioxidative properties by preventing oxidation of low-density lipoprotein (LDL) cholesterol, scavenging ROS, and chelating transitional metal ions (Craig, 1999; Simitzis and Deligeorgis, 2011).

Over one hundred spices and medicinal plants and herbs have been analyzed for their antioxidative properties and effectiveness (Nakatani, 1992; Ruberto and Baratta, 2000; Wei and Shibamoto, 2007). Noteworthy phenolic compounds that exhibit strong antioxidative potential are thymol and carvacrol (thyme and oregano) and eugenol (clove and basil) (Amorati et al., 2013). The essential oils of rosemary and sage were found to have strong antioxidative properties

as free radical scavengers and inhibiting lipid peroxidation (Cuvelier et al., 1994; Bozin et al., 2007). Essential oils from clove displayed higher antioxidant activity at lower concentrations when compared to traditional synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Jirovetz et al., 2006). Effective antioxidant properties have further been identified and exhibited in plants belonging to the families Lamiacae (mints, lemon balm), Apiacae (anise, celery, parsley, coriander, cumin, fennel), and Asteraceae (yarrow, wormwood, chamomile), and the genus *Pinus* and *Juniperus* (Mimica-Dukić et al., 2016). Tannins have also been shown to exert antioxidant and free radical scavenging activity in vitro (Smeriglio et al., 2017).

1.4.2.2 Antimicrobial Activities

The antimicrobial and antibacterial properties of botanical extracts and essential oils have been extensively reviewed (Burt, 2004; Simitzis, 2017; Herman et al., 2019; Sezen et al., 2019). The phenolic compounds present in botanical extracts and essential oils are hydrophobic. This property allows phenolic compounds to disrupt bacterial cell membranes, which can cause ion leakage, induce inhibition of adenosine triphosphate (ATP) and protein synthesis, and decrease bacterial growth and proliferation (Faleiro, 2011; Lopez-Romero et al., 2015; Yap et al., 2021). The presence of a hydroxyl group attached to the phenyl ring of phenolic compounds, such as thymol and carvacrol, inactivates microbial enzymes by disrupting ion transport across the bacterial cytoplasmic membrane (Ultee et al., 2002). These two modes of action are more susceptible toward gram positive bacteria, as the phenolic compounds can interact with the bacterial cell membrane directly, unlike gram negative bacteria that possess a hydrophilic external cell wall (Cimanga et al., 2002; Nazzaro et al., 2013). Some phenolic compounds, however, can pass through the porin proteins of the outer membrane and cause membrane

conformational changes and ion leakage in gram-negative bacteria by releasing the lipopolysaccharides within the bacterial membrane (Helander et al., 1998; Yang et al., 2018). The synergisms and antagonisms that combine major and minor phenolic and flavonoid constituents might also possess strong antimicrobial activities (Bassolé and Juliani, 2012; Herman et al., 2019).

Various botanical extracts and essential oils were found to exert antimicrobial, antibacterial, and antifungal effects against both gram-positive and gram-negative bacteria and yeast cells (Hammer et al., 1999). Thyme, tea tree, cassia, lemon grass, and oregano exhibited significant antimicrobial activity against multiple strains of gram-positive and gram-negative bacteria (Abers et al., 2021). Oregano, mint, and turmeric essential oils display the widest range of antimicrobial activity (Sezen et al., 2019). Eugenol, thymol, and carvacrol display antimicrobial activities even higher than sulfanilamide, an antibacterial drug (Guimarães et al., 2019). Carveol, carvone, citronellol, citronellal, α-terpineol, linalool, eucalyptol and α-pinene negatively impact membrane integrity and growth of *Escherichia coli* and *Staphylococcus Aureus* (Zengin and Baysal, 2014; Lopez-Romero et al., 2015). Other botanical extracts that demonstrate high antimicrobial activity include clove (Arora and Kaur, 1999), rosemary and sage (Bozin et al., 2007), cinnamon (Hakim et al., 2020), garlic, and ginger (Karuppiah and Rajaram, 2012). Tannins are a heterogenous group of phytogenic feed additive composed of polyphenolic compounds found in plant and tree constituents, such as proanthocyanidins, that protect the plant from predation by reducing palatability and protecting against pathogens (Girard and Bee, 2020). Tannins may possess antibacterial properties by destabilizing bacterial membranes through hydrogen bonding and hydrophobic interactions and decreasing adhesion between bacterial and intestinal epithelial cells (Smeriglio et al., 2017; Girard and Bee, 2020).

A major concern regarding botanical extracts as antimicrobials is that bacteria may develop a resistance to the phenolic compounds over time with large-scale clinical use. However, the multi-component composition of botanical extracts may reduce the possible development of bacterial resistance because multiple bacterial adaptations would need to occur (Kon and Rai, 2012; Yap et al., 2014). Becerril et al. (2012) found that 48 clinical isolates and 12 reference strains of gram-negative bacteria were all susceptible to oregano and cinnamon essential oils independently of their antibiotic resistance profile, with only 3 strains altering and increasing their antibiotic resistance profile after 50 passages in the presence of subinhibitory levels of oregano essential oil. Additionally, the synergistic effects of phenolic compounds with existing antibiotics may be effective in inhibiting multi-drug resistant bacteria and reducing incidence of bacterial resistance (Hammer et al., 2012; Faleiro et al., 2013; Willing et al., 2018).

1.4.2.3 Anti-inflammatory Activities

The constituents of medicinal plants, botanical extracts, and essential oils have been stated to possess anti-inflammatory and immunomodulating properties (Pérez et al., 2011). Phenolic compounds are able to alter the expression of pro- and anti-inflammatory cytokines, increase leukocyte count, and promote cell-mediated active immune responses (Sá et al., 2014; Peterfalvi et al., 2019). Terpenes can decrease expression of pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) and inflammatory mediators (PGE_2) by inhibiting the NF-κB and cyclooxygenase-2 (COX-2) pathway (Borges et al., 2019). Terpenes promote gene expression of anti-inflammatory cytokines IL-10 and IL-11, which can inhibit inflammatory cytokine production and expression of forkhead box P3 (FOXP3) protein, a regulator of regulatory T cell (Wang et al., 2016; Toden et al., 2017).

Anastasiou and Buchbauer (2017) reviewed the immunomodulatory, immunosuppressive, and anti-inflammatory properties of various botanical extracts, including oregano, mint, thyme, rosemary, and cinnamon varieties. Anethol, capsicum oleoresin, carvacrol, cinnamaldehyde, eugenol, garlicon, and turmeric oleoresin were found to decrease TNF-α and IL-1β secretion in LPS-treated porcine macrophages *in vitro* (Liu et al., 2012). Essential oils from *Citrus* species containing the phenolic compounds limonene and citral were effective at inducing an anti-inflammatory response by reducing cell migration, cytokine production, and protein extravasation in formalin- and carrageenan-induced inflammation in mice (Amorim et al., 2016). Other plants and essential oils that have been used as anti-inflammatory agents include chamomile, lavender, eucalyptus, pine, and clove (Miguel, 2010; Gheisar and Kim, 2018). Proanthocyanidins, a class of polyphenolic tannin, may suppress LPS-induced secretion of IL-6 while promoting secretion of IL-10 (Williams et al., 2017).

1.4.3 Application in Swine Production

Botanical extracts and phytogenic feed additives have been considered as potential antibiotic alternatives for pigs due to their antimicrobial, antioxidative, and immuno-regulatory properties. The beneficial effects of botanical extracts and phytogenic feed additives on swine production and health have been previously reviewed (Windisch et al., 2008; Simitzis, 2017; Liu et al., 2018).

1.4.3.1 Effects on Growth Performance and Nutrient Digestibility

Dietary supplementation of botanical extracts and phytogenic feed additives has been shown to increase growth performance in weaned pigs by enhancing feed intake by up to 19% (Franz et al., 2010; Zeng et al., 2015a). These increases are associated with improvements toward growth rate but not feeding preference by the animals (Zhai et al., 2018). Botanical

extracts supplementation can significantly improve the apparent total tract digestibility of nutrients in piglets, which is attributed to promotion of bile and digestive enzyme secretion and decreases in endogenous nutrient loss (Zeng et al., 2015a; Zhai et al., 2018). Botanical extracts can also improve ileal protein and amino acid digestibility in pigs by increasing mRNA expression of peptide and amino acid transporters and nutrient absorption (Maenner et al., 2011; Reyer et al., 2017).

Supplementation of a botanical blend of thymol and cinnamaldehyde lead to improvements in body weight gain and feed conversion ratio in weaned pigs, with linear improvements observed with increments of 50 mg/kg up to 200 mg/kg (Li et al., 2012, Su et al., 2018). A similar botanical blend containing thymol, cinnamaldehyde, carvacrol, and eugenol increased average daily gain from d 0 to 14 of weaned pigs, as well as improved nitrogen digestibility and production of intestinal enzymes (Huang et al., 2010). An increase in nitrogen digestibility was also observed with dietary supplementation of oregano, anise, orange peel, and chicory (Ahmed et al., 2013), as well as fenugreek, clove, and cinnamon (Cho et al., 2005). Tannins from chestnut wood extract were found to improve feed efficiency when fed to weaned piglets at 1.13, 2.25, and 4.5 g/kg (Biagia et al., 2010). Other phenolic compounds can also improve feed efficiency and nutrient digestibility, such as phytoncides from pine species and resveratrol from grapes and berries (Zhang et al., 2012; Ahmed et al., 2013).

1.4.3.2 Effects on Disease Resistance and Diarrhea

Phenolic compounds, such as carvacrol and thymol, can damage the outer bacterial membrane of gram-negative bacteria *in vitro*, increasing the potency of their antimicrobial properties (Helander et al., 1998; Omonijo et al., 2017). The resulting decrease in intestinal pathogen count can lead to improved intestinal integrity and protection against bacterial diseases

(Zeng et al., 2015a). An important consideration for the use of botanical extracts is their ability to alleviate disease, such as diarrhea caused by F4 and F18 enterotoxigenic *Escherichia coli* in weaned pigs (Sun and Kim, 2017).

Botanical supplementation of thyme, cinnamon, and oregano resulted in decreased intestinal counts of *E. coli* in the rectum and feces of pigs shortly after weaning (Namkung et al., 2004; Li et al., 2012; Zeng et al., 2015b). A decrease in diarrhea frequency and diarrhea score was reported with supplementation of a blend of thymol and cinnamaldehyde (Li et al., 2012) as well as a blend of thymol, cinnamaldehyde, carvacrol, and eugenol (Huang et al., 2010). Botanical extracts were effective at alleviating disease and reducing diarrhea frequency and score in weaned pigs challenged with F4 or F18 *E. coli* (Devi et al., 2015; Liu et al., 2013a). Supplementation of thymol and carvacrol reduced diarrhea incidence and intestinal oxidative stress in weaned pigs infected with *E. coli* (Pu et al., 2018; Tan et al., 2020). The antioxidative properties of thymol and cinnamaldehyde were also reported to help decrease diarrhea prevalence in weaned pigs by preventing endogenous peroxidation and promoting gene expression of antioxidative enzymes (Yuan et al., 2017; Su et al., 2018; Tian and Piao, 2019). Liu et al. (2013b) also found that feeding botanical extracts reduced the disease challenge in weaned pigs infected with porcine reproductive and respiratory syndrome virus. A botanical blend of eucalyptus, lavender, and pine essential oil alleviated adverse symptoms and horizontal transmission of African swine fever virus in piglets (Babikian et al., 2021). The commercially available phytogenic feed additives Patente Herba® and Patente Herba® Plus (Patent Co. DOO, Mišićevo, Serbia), containing thyme, oregano, coriander, and sweet chestnut extracts, showed effective treatment and control of *Brachyspira hyodysenteriae* (swine dysentery) and *Lawsonia intracellularis* (proliferative nteropathy) in weaned pigs (Nikola et al., 2018; Drašković et al.,

2018; Drašković et al., 2020). Supplementation of tannins from chestnut extract were found to decrease fecal scores, diarrhea incidence, and ETEC shedding in weaned pigs experimentally infected with F4 ETEC (Girard et al., 2020).

1.4.3.3 Effects on Intestinal Health

Botanical extracts can enhance intestinal health by several potential mechanisms: 1) modifying the intestinal environment to promote the growth of favorable microflora; 2) enhancing intestinal barrier function; 3) regulating intestinal immunity. Botanical extracts can improve intestinal health by modifying the intestinal environment to promote the growth of favorable microflora, such as *Lactobacillus* and *Bifidobacterium* (Modina et al., 2019; Ruzauskas et al., 2020). These probiotic bacteria can subsequently decrease pathogen populations and reduce the potential risk of bacterial diseases (Franz et al., 2010; Simitzis, 2017). Supplementation of botanical extracts further improves intestinal health and gut mucosal function by increasing gastric retention time and promoting gene expression of tight junction proteins and mucin production (Manzanilla et al., 2004; Liu et al., 2014). An increase in intestinal villi height and crypt depth was also observed with botanical extract supplementation, which benefits intestinal health by increasing intestinal absorptive capacity and decreasing pathogen pressure (Zeng et al., 2015a; Yuan et al., 2017).

Thymol supplementation in the diets of weaned pigs was shown to increase and intestinal health by promoting *Lactobacillus* growth and intestinal barrier function (Van Noten et al., 2020). Zeng et al. (2015b) and Tian and Piao (2019) reported an increase in intestinal villi height and villus height to crypt depth ratio in weaned pigs when fed a diet supplemented with thymol and cinnamaldehyde. Essential oil from oregano extracts improved average daily gain and promoted growth of beneficial intestinal microflora *Lactobacillus* and *Bifidobacterium* in

weaned pigs (Salamon et al., 2019; Ruzauskas et al., 2020). Tannins from Zou et al. (2016) showed that oregano essential oil decreased intestinal *E. coli* populations, improved villus height, and promoted gene expression of tight junction proteins occludin and zona-occludens-1 in pigs (Zou et al., 2016). A botanical blend of carvacrol and thymol beneficially affected intestinal health by promoting growth of *Lactobacillus* and reducing populations of *Enterococcus* and *E. coli*, while not affecting mRNA expression of tight junction proteins (Wei et al., 2017). Supplementation of capsicum oleoresin, garlic botanical, and turmeric oleoresin also promote gut mucosal immunity by upregulating gene expression of tight junction proteins, mucins, and membrane transport proteins (Liu et al., 2014).

1.4.3.4 Effects on Systemic Immunity

Botanical extracts display various immunoregulatory activities that can mitigate immune responses to pathogens and improve pig health (Xiong et al., 2018). The anti-inflammatory activities of botanical extracts and phenylpropanoids come from their ability to prevent the activation of the NF-κB signaling pathway, to modulate production of pro- and anti-inflammatory cytokines, and to decrease gene expression of inflammatory mediators (Sá et al., 2014; Liu et al., 2018; Omonijo et al., 2018). Botanical extract supplementation can also improve the immune status of weaned pigs by increasing lymphocyte proliferation rate, phagocytosis rate, and immunoglobin concentration in serum (Zeng et al., 2015a; Zhai et al., 2018; Tan et al., 2021).

Supplementation of thymol and cinnamaldehyde significantly increased serum levels of immunoglobins IgG, IgA, and IgM and antibodies C3 and C4 in weaned pigs, indicating an improvement in immune status (Li et al., 2012; Zeng et al., 2015b; Su et al., 2018). A similar botanical blend of thymol, cinnamaldehyde, carvacrol, and eugenol stimulated an immune

response in weaned pigs by increasing white blood cell count (Huang et al., 2010). Capsicum oleoresin, turmeric oleoresin, and garlic botanical are all effective at reducing inflammation in pigs shortly after weaning by decreasing serum levels of TNF-α and ileal macrophages and neutrophils (Liu et al., 2013a). A subsequent study showed that the same botanical extracts can exhibit immunomodulatory abilities in weaned pigs by stimulating mRNA expression of immune response genes associated with regulatory proteins, neutrophil recruitment, and antigen processing (Liu et al., 2014). Oregano essential oil supplementation decreased expression of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1), and interferon-gamma (INF-γ) in pigs compared to a control diet, as well as decreased activation of the NF-κB and STAT3 signaling pathway while under stress-inducing stimuli (Zou et al., 2016; Cappelli et al., 2021). Weaned pigs fed a diet supplemented with carvacrol, cinnamaldehyde, and capsicum had increased blood monocytes and lamina propria lymphocyte density (Nofrarías et al., 2006).

SUMMARY

Post-weaning diarrhea caused by ETEC infection is an important cause of weaned pig's mortality and economic loss in the global swine industry. Weaning stress is associated with reduced intestinal health and systemic immunity, which can lead to impaired growth performance, health, and disease resistance in weaned pigs. Numerous feed additives have been researched and reviewed for their potential as replacements for in-feed antibiotic growth promoters. Botanical extracts, phytogenic feed additives and essential oils are secondary metabolites of non-woody aromatic plant constituents and primarily consisting of terpenes and terpenoids, alkaloids, and phenolic compounds. Botanical extracts may provide beneficial effects on newly weaned pigs by promoting intestinal morphology, disease resistance, and systemic

immunity. Dietary supplementation of numerous botanical extracts can also reduce frequency of diarrhea in weaned pigs by modulating intestinal microbiota and inhibiting the growth of pathogens. These improvements toward pig health and immunity through nutritional intervention will be beneficial for the growth and sustainability of the global swine industry.

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CHAPTER 2

EFFECTS OF DIETARY SUPPLEMENTATION OF BOTANICALS ON DIARRHEA, PERFORMANCE, BLOOD METABOLITES, AND INTESTINAL MORPHOLOGY OF WEANED PIGS EXPERIMENTALLY INFECTED WITH A PATHOGENIC *ESCHERICHIA COLI*

Abstract

Botanicals, phytogenic feed additives, and essential oils have been reviewed as potential replacements for in-feed antibiotics in swine production for their health, immunity, and growth promoting effects. Therefore, the objective of this study was to investigate the effects of dietary botanical supplementation on blood metabolites and intestinal morphology of weaned piglets experimentally infected with a pathogenic F18 *Escherichia coli* (*E. coli*). Sixty weaned piglets (around 21 days old; 7.15 ± 0.97 kg) were individually housed and randomly allotted to one of five dietary treatments (n = 12): negative control (NC), positive control (PC), high dose of botanicals blend 1 (BB1, 100 mg/kg), and low or high dose of botanicals blend 2 (BB2, 50 mg/kg and 100 mg/kg). The experiment lasted 28 days from day -7 to +21 relative to *E. coli* inoculation. All piglets except the NC group were orally inoculated with F18 *E. coli* (10^{10} cfu per dose, 3 doses) for 3 consecutive days. Growth performance was recorded throughout the experiment and diarrhea scores were recorded daily. Blood samples were collected on d 0, 5, 14, and 21 post-inoculation (PI) for complete blood count test. Intestinal segments were collected on d 5 and 21 PI for intestinal morphology analysis. Data were analyzed by ANOVA in PROC MIXED of SAS with a randomized complete block design. *E. coli* infection reduced ($P < 0.05$) pig body weight and growth rate throughout the experiment and reduced ($P < 0.05$) neutrophils

and lymphocytes but increased ($P < 0.05$) monocytes on d 4 PI. No differences in weight gain or average daily gain were seen among the botanical blend treatments compared to the control. Supplementation of high dose BB1 or BB2 reduced ($P < 0.05$) frequency and severity of diarrhea of challenged pigs during the experimental period. Pigs supplemented with 100 mg/kg BB2 had less ($P < 0.05$) lymphocytes than pigs in PC on d 4 PI. Pigs fed with 50 mg/kg BB2 had lower ($P < 0.05$) lymphocytes and monocytes than pigs in PC on d 21 PI. Pigs supplemented with 100 mg/kg BB2 had the greatest ($P < 0.05$) duodenal villi width, jejunum villi height and area, and colon crypt depth than pigs in PC on d 5 PI. In conclusion, dietary supplementation of botanicals reduced diarrhea and tended to affect systemic immunity and enhance intestinal morphology of weaned pigs infected with *E. coli*.

KEY WORDS: blood profile, botanical blends, growth performance, intestinal morphology, post-weaning diarrhea, weaned pigs

Introduction

Post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* (*E. coli*) is one of the leading causes of economic losses within the global swine industry. *E. coli* infection results in reduced weight gain, feed intake, and increased morbidity and mortality among post-weaning piglets 1-2 weeks of age (Fairbrother et al., 2005; Cox et al., 2012). The restriction on the use of in-feed antibiotics for growth promotion in domestic animal production (FDA, 2016) has resulted in increased research efforts to explore antibiotic alternatives with regard to improving disease resistance, growth promotion, and overall health of weaned pigs.

Phytogenic feed additives, or botanicals, are plant-derived substances and compounds that promote animal production performance and animal food product quality when supplemented to livestock feed (Simitzis, 2017; Herman et al., 2019). Such botanicals include essential oils, oleoresins, and other constituents of herbs and spices that are classified depending on their method of extraction or processing (Windisch et al., 2008). Essential oils are the products of secondary metabolism of herbs and spices and are composed primarily of phenolic compounds, such as terpenes, flavonoids, and anthocyanins (Zengin and Baysal, 2014). Phenolic terpenes have shown to exhibit antioxidative (Wei and Shibamoto, 2007), antimicrobial and antibacterial (Burt, 2004), and anti-inflammatory properties and activity (Sá et al., 2014). The beneficial effects of phytogenic feed additives, botanicals, and essential oils on health, immunity, and growth promotion have attracted growing research efforts into evaluating their potential as in-feed antibiotic replacements for swine. Studies have shown that dietary supplementation of essential oil mixtures lead to improvements in growth performance, intestinal morphology, and immunity of weaned pigs (Kroismayer et al, 2008; Maenner et al, 2011; Li et al, 2012). Although the beneficial effects of phytogenic feed additives on growth performance, disease resistance, and immunity have been shown in weaned pigs challenged with *E. coli* and *Salmonella* (Liu et al., 2013; Ahmed et al., 2013), there is limited research on the effects of phytogenic feed additives on weaned piglets challenged with F18 *E. coli*, one of the dominant strains of *E. coli* associated with post-weaning diarrhea (Cox et al., 2012). Therefore, the objective of this study was to investigate the influence of dietary supplementation of two blends of botanical extracts comprised of capsicum oleoresin and garlic extract varieties on diarrhea score, growth performance, systemic immunity, and gut integrity of weanling pigs experimentally infected with a pathogenic F18 *E. coli*.

Materials and Methods

Animals, housing, experimental design, and diet

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC #20809) at the University of California, Davis (UC Davis). The study was performed on 60 weanling pigs (crossbred, initial body weight (BW): 7.17 ± 0.97 kg) with an equal number of gilts and barrows. All pigs were selected from the Swine Teaching and Research Center of UC Davis. The sows and piglets used in this experiment did not receive *E. coli* vaccines, antibiotic injections, or antibiotics in creep feed prior to involvement with the study. All piglets used in this study were susceptible to F18 *E. coli*, which was confirmed by genotyping analysis based on the methods of Kreuzer et al. (2013).

After weaning, all pigs were randomly assigned to one of the five treatments in a randomized complete block design with body weight within sex and litter as the blocks and pig as the experimental unit. There were 12 replicates per treatment. The 5 dietary treatments included: 1) negative control: control diet, without *E. coli* challenge; 2) positive control: control diet, with *E. coli* challenge; 3) supplementing with 100 mg/kg of botanicals blend type 1 (BB1), with *E. coli* challenge; 4) supplementing with 50 mg/kg of botanicals blend type 2 (BB2, 50 mg/kg), with *E. coli* challenge; and 5) supplementing with 100 mg/kg of botanicals blend type 2 (BB2, 100 mg/kg), with *E. coli* challenge. BB1 and BB2 have similar proprietary formulation of botanical actives, including approximately 0.3% capsicum oleoresin and 12% garlic extracts, except that different varieties of garlic was used. Pigs were individually housed (pen size: 0.61 × 1.22 m) in several environmentally controlled rooms at the Cole facility at UC Davis. Pigs in the negative control were housed in a separate room. The experiment lasted 28 days, including 7 days' adaptation and another 21 days after the first *E. coli* inoculation (d 0). All pigs had *ad*

libitum access to feed and water throughout the experimental period. The lights were on at 07:00 and off at 19:00 daily.

A 2-phase feeding program was applied in the current study with the first two weeks as phase I and the last two weeks as phase II. Spray-dried plasma, antibiotics, and high levels of zinc oxide exceeding recommendation were not included in the diets. All diets were formulated to meet the nutrient requirements of weaned pigs based on NRC (2012, Table 2-1). The experimental diets were fed to pigs throughout the entire experiment. All diets were provided in mash form.

After 7 days of adaptation, all pigs except pigs in the negative control were orally inoculated with 3 mL of F18 *E. coli* for 3 consecutive days from d 0 post-inoculation (PI). The F18 *E. coli* was isolated from a field disease outbreak by the University of Illinois Veterinary Diagnostic Lab (isolate number: U.IL-VDL # 05-27242). The F18 *E. coli* expresses heat-labile toxin (LT), heat-stable toxin b (STb), and Shiga-like toxins (SLT-2). The inoculums were prepared by the Western Institute for Food Safety and Security at UC Davis and were administered at 10^{10} CFU per 3 mL dose in phosphate buffer saline (PBS).

Clinical observations and sample collections

The procedures for this experiment were adapted from the methods of Liu et al. (2013) and He et al. (2020). Diarrhea scores and alertness scores were recorded twice daily from d 0 PI. The diarrhea score of each pig was assessed visually each day by 2 independent evaluators, with the score ranging from 1 to 5 (1 = normal, solid feces; 2 = moist, solid feces; 3 = mild diarrhea; 4 = severe diarrhea; 5 = watery diarrhea). The alertness score of each pig was assessed visually each day by 2 independent evaluators as well, with the score ranging from 1 to 3 (1 = normal; 2 = slightly depressed or listless; 3 = severely depressed). All pigs had an alertness score of 1

throughout the entire duration of the study (Data were not reported). Pigs were weighed at the beginning of the experiment (d -7), d 0 (first inoculation day), 5, 14, and 21 PI. Feed intake was recorded throughout the study. Average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio was calculated for each interval from d -7 to 0, d 0 to d 5 PI, and d 5 to d 21 PI.

Fecal samples were collected from the rectum of all pigs on d -7 and 0 before *E. coli* inoculation, and d 2, 5, 7, 9, 14, and 21 PI using a cotton swab to test β-hemolytic coliforms (Liu et al., 2013). Blood samples were collected from the jugular vein of all pigs at the beginning of the study (d -7), d 0, 4, 14, and 21 PI. Whole blood samples were used for measuring total and differential blood cell count by complete blood count (CBC) analysis.

Thirty pigs (6 pigs/treatment) were euthanized on d 5 PI near the peak of infection, and the remaining pigs were euthanized at the end of the study on d 21 PI during the recovery period from *E. coli* infection. Pigs were anesthetized with 1 mL mixture of 100 mg telazol, 50 mg ketamine, and 50 mg xylazine (2:1:1) by intramuscular injection. Following anesthesia, pigs were euthanized with an intracardiac injection of 78 mg sodium pentobarbital (Vortech Pharmaceuticals, Ltd., Dearborn, MI). Four 3 cm segments from the duodenum, the middle of jejunum, ileum (5 cm away from the ileocecal junction), and distal colon were collected and fixed in methanol Carnoy's solution (methanol, chloroform, and glacial acetic acid, 6:3:1 *v/v/*v) for Alcian blue periodic acid-Schiff (AB/PAS) staining to measure intestinal morphology.

Detection of β-hemolytic coliforms

The procedures detecting β-hemolytic coliforms in this study were adapted from Kim et al. (2019). Fecal samples were plated onto Columbia Blood agar with 5% sheep blood to identify hemolytic coliforms that lyse red blood cells. Fecal samples were also plated onto MacConkey agar to confirm that they were lactose-fermenting bacteria and flat pink colonies. All plates were

incubated at 37°C for 24 h in an air incubator. Populations of both total coliforms and β-hemolytic coliforms were assessed visually with a score from 0 to 8 (0 = no presence of bacterial growth; 8 = very abundant bacterial growth). The ratio of scores of β-hemolytic coliforms to total coliforms was calculated.

Determination of total and differential blood cell counts

Whole blood samples were analyzed for total and differential blood cell counts by the Comparative Pathology Laboratory at UC Davis. A multiparameter, automated programmed hematology analyzer (Drew/ERBA Scientific 950 FS Hematological Analyzer, Drew Scientific Inc., Miami, FL) was used for CBC analysis. Neutrophil to lymphocyte ratio was calculated.

Intestinal morphology

The fixed intestinal tissue segments were embedded in paraffin, sectioned at 5µm, and stained with high iron diamine and Alcian blue. The slides were scanned using an Olympus BX51 microscope at 10 times magnification. All histological measurements were performed in the image processing and analysis software Image J (NIH). Ten straight and integrated villi and their associated crypts were selected from each sample to evaluate villi height, villi width, villi area, crypt depth, number of goblet cells per villus, and cross-sectional area of sulfo- and sialomucin as described by Deplancke and Gaskins (2001) and Almeida et al. (2013). Only crypt depth and cross-sectional area of sulfomucin was measured in the distal colon because of the lack of villi in this part of the gastrointestinal tract.

Statistical analysis

Normality of data was verified and outliers were identified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified and removed as values that deviated from the treatment mean by more than 3 times the interquartile range. Data were

analyzed by ANOVA using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. The statistical model included diet as fixed effect and blocks as random effects. Treatment means were separated by using the LSMEANS statement and the PDIFF option of PROC MIXED. Statistical significance and tendency was considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Growth performance

E. coli infection reduced ($P < 0.05$) body weights on d 5, 14, and 21 PI, ADG from d 0 to 5 PI, d 14 to 21 PI, d 0 to 14 PI, and d 0 to 21 PI, ADFI from d 0 to 5 PI and d 0 to 14 PI, when pigs in positive control were compared with those in negative control (Table 2-2). In comparison to positive control, supplementation of botanicals blend did not affect BW, ADFI, and gain:feed ratio throughout the experiment, with the exception that pigs fed with 50 mg/kg of BB2 had smaller ($P < 0.05$) gain:feed ratio from d 5 to 14 PI than pigs in positive control. Interestingly, pigs supplemented with 100 mg/kg of BB1 or BB2 had similar body weights on d 14 and 21 PI, and similar ADG and feed efficiency from d 0 to 21 PI, compared with pigs in negative control.

Diarrhea score

Pigs in negative control group had the lowest diarrhea score throughout the study. Pigs in positive control group had the highest ($P < 0.05$) daily diarrhea score from d 1 to 4 PI among all treatment groups, while pigs in 100 mg/kg of BB2 group had the lowest diarrhea score during the same period (Figure 2-1). Pigs in 50 mg/kg of BB2 had higher ($P < 0.05$) diarrhea score on d 6 PI than pigs in the other groups. The average diarrhea scores of pigs in all treatments returned to normal range (diarrhea score < 3) after d 7 PI.

E. coli infection increased ($P < 0.05$) frequency of diarrhea (diarrhea score ≥ 3) to approximately 33.33% from d 0 to 5 PI and to approximately 10.76% during the entire experiment, compared with negative control (Figure 2-2). Supplementation of BB1 or BB2 reduced ($P < 0.05$) frequency of diarrhea from d 0 to 5 PI and the entire experiment compared with negative control, except for 50 mg/kg of BB2.

Fecal culture

No β-hemolytic coliforms were identified in fecal samples of pigs in the negative control. In the four disease challenge groups, no β-hemolytic coliforms were identified in fecal samples from pigs in the three botanicals blend-supplemented groups on d 14 PI. No β-hemolytic coliforms were identified in fecal samples of all pigs on d 21 PI. However, no differences were observed in *E. coli* challenged pigs fed with BB1 or BB2, when compared with positive control.

Total and differential blood cell count

Pigs in positive control had higher ($P < 0.05$) monocytes than pigs in negative control and high dose of BB1 and BB2 at the beginning of the experiment (Table 2-3). Pigs supplemented with BB1 or BB2 had less ($P < 0.05$) lymphocytes than pigs in negative control. *E. coli* infection reduced ($P < 0.05$) neutrophils and lymphocytes and increased ($P < 0.05$) monocytes, eosinophils, and basophils on d 4 PI, when positive control was compared with negative control. Supplementation of BB1 or BB2 did not impact white blood cell profiles, with the exception that pigs supplemented with 100 mg/kg of BB2 had less ($P < 0.05$) lymphocyte counts than pigs in positive control.

Pigs supplemented with 100 mg/kg ofBB1 had the greatest ($P < 0.05$) monocyte counts, while pigs supplemented with 50 mg/kg of BB2 had the least ($P < 0.05$) monocyte counts among all treatments on d 14 PI. Pigs supplemented with 50 mg/kg of BB2 had less ($P < 0.05$)

percentage of monocytes compared with pigs in positive control and the other two botanicals blend groups. At the end of the study (d 21 PI), pigs fed with 100 mg/kg of BB1 still had the greatest ($P < 0.05$) white blood cell count and lymphocyte count, but pigs supplemented with 50 mg/kg of BB2 had the lowest ($P < 0.05$) lymphocytes and monocytes among all 5 treatments.

No differences were observed in red blood cell profile among the five treatments at the beginning of the experiment (Table 2-4). Pigs supplemented with 100 mg/kg of BB1 had the lowest ($P < 0.05$) red blood cell count and packed cell volume on d 0 before *E. coli* inoculation. Pigs supplemented with BB1 or BB2 also had lower ($P < 0.05$) hemoglobin and packed cell volume than pigs in the negative control on d 0. *E. coli* infection reduced ($P < 0.05$) mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) on d 4 PI when positive control was compared with negative control. However, this was not the case on d 14 and 21 PI. Supplementation of BB1 or BB2 did not impact MCH and MCHC on d 4 PI when compared with positive control. However, pigs supplemented with 100 mg/kg of BB1 or 50 mg/kg of BB2 had increased ($P < 0.05$) total blood protein level on d 14 PI than pigs in positive control. At the end of the study (d 21 PI), pigs supplemented with 50 mg/kg of BB2 had lower ($P < 0.05$) hemoglobin and packed cell volume than pigs in positive control.

Intestinal morphology

Compared with negative control, *E. coli* challenge reduced ($P < 0.05$) duodenum villi height and villi area on d 5 PI (Table 5). Supplementation of 100 mg/kg of BB2 increased ($P < 0.05$) duodenal villi width, jejunal villi height and area, and distal colon crypt depth in pigs on d 5 PI, compared with pigs in positive control. Compared with the negative and positive control groups, 50 mg/kg and 100 mg/kg of BB2 increased ($P < 0.05$) duodenum villi width and jejunum villi height on d 5 PI, and both BB1 and BB2 increased ($P < 0.05$) crypt depth on d 5 PI.

At the end of the experiment, pigs supplemented with 100 mg/kg of BB2 had greater ($P < 0.05$) duodenal crypt depth and sialomucin area than pigs in negative control. Compared with negative control, BB1 and BB2 increased ($P < 0.05$) sialomucin area on d 21 PI. Additionally, supplementation of 50 mg/kg of BB2 had increased ($P < 0.05$) jejunal crypt depth than pigs in the positive and negative controls.

Discussion

The results of the current study imply that dietary supplementation of botanical blends significantly reduced frequency of diarrhea and improved systemic immunity and intestinal morphology of weaned pigs experimentally infected with F18 *E. coli*. These findings agree with current knowledge regarding essential oils and phytochemicals as feed additives for weaned pigs, which has shown improved immune function and overall health when fed a basal diet supplemented with different essential oil products (Maenner et al, 2011; Li et al., 2012; Liu et al., 2013). The increases in growth performance were minimal in the current experiment due to the short experimental period and small numbers of animals. However, at the end of this experiment, the infected pigs that were supplemented with botanical blends grew to similar body weights compared with pigs in the negative control. This observation suggests that supplementing botanical blends may accelerate the recovery of weaned pigs from *E. coli* infection.

F18 *E. coli* challenge

F18 enterotoxigenic *E. coli* is an important cause of diarrhea and diarrhea-associated deaths among newly weaned pigs, which is responsible for economic loss and antibiotic use in the global swine industry. Toxins (i.e., LT, STb, and SLT-2 toxins) released by F18 *E. coli* could increase intestinal permeability and induce intestinal damage and inflammation (Dubreuil et al.,

2016; Becker et al., 2020). The presence of β-hemolytic coliforms in feces has been used to signify bacterial presence and pathogenicity of *E. coli* (Bhakdi et al., 1988). The results of the current study confirmed the detrimental impacts of F18 *E. coli* in weaned pigs, as indicated by increased frequency of diarrhea and presence of fecal β-hemolytic coliforms, decreased growth performance, and reduced intestinal villi height and area. These findings agree with previous studies in which a same F18 *E. coli* strain (Liu et al., 2013; Kim et al., 2019; He et al., 2020) or different *E. coli* strains (Nabuurs et al., 1993; Dubreuil et al., 2016; Sun and Kim, 2017) were used. Similar to other bacterial infection, F18 *E. coli* infection also induced systemic inflammation, as indicated by increased overall white blood cell counts, neutrophils, and lymphocytes (Liu et al., 2013). The decreased villi height and area caused by *E. coli* infection observed in the current study agrees with previous research. F18 *E. coli* infection has been shown to affect intestinal morphology by decreasing villi length and increasing crypt depth in weaned pigs (Cox et al., 2012; Liu et al., 2013; Becker et al., 2020). *E. coli* infection leads to decreased nutrient, electrolyte, and water absorption by enterocytes and increased water and electrolyte secretion by crypt cells, promoting the occurrence of post-weaning diarrhea (Nabuurs et al., 1993). The heat-labile, heat-stable, and Shiga- enterotoxins produced by F18 *E. coli* further cause water and electrolyte secretion in the host intestine (Nataro and Kaper, 1998; Dubreuil et al., 2016). Interestingly, the F18 *E. coli* challenge in the current study had remarkable impacts on monocytes, eosinophils, and basophils, instead of the two most dominant immune cells in the blood circulation. Monocytes are responsible for producing monocyte-derived macrophages as well as exhibiting pathogenic activity and other immunological functions (Murray, 2018). Eosinophils and basophils are immune cells involved in innate and adaptive immunity such as expression of immunoglobin receptors, production of cytokines, and expression of antibacterial

functions through extracellular DNA traps (Rigoni et al., 2018; Yousefi et al., 2015). The significant increase in monocytes, eosinophils, and basophils is attributed to the pathogenic *E. coli* infection, which also agrees with previous studies (Liu et al., 2013; Xiong and Pamer, 2015).

Botanicals and phytogenic feed additives

Phytogenic feed additives, botanicals, and essential oils show significant promise as feed supplements to enhance overall health and disease resistance of weaned pigs. Single constituents, blends of multiple botanicals, and combinations of antibiotics and botanicals have been reported to increase growth performance, immune function, and gut health in weaned pigs (Su et al., 2018; Zhang et al., 2020). The results of the current study showed that supplementation of both botanical blends had no statistically significant impacts on BW, ADFI, and feed efficiency compared with pigs in positive control. However, pigs supplemented with 100 mg/kg of botanical blends had similar performance compared with non-challenged pigs. The botanical blends used in the current study were composed of extracts from capsicum and different varieties of garlic. Previous studies have displayed the beneficial effects of these specific phytogenic additives in weaned pigs with or without disease challenge. For instance, individual inclusion of 10 mg/kg of capsicum oleoresin or garlic botanical in diets of unchallenged weaned pigs resulted in increased ADG from d 0-5 post-weaning (Liu et al., 2013). Cho et al. (2005) found that supplementing a commercial botanical blend of fenugreek, clove, and cinnamon (0.03%) enhanced growth performance of weaned pigs compared with a negative control in a 49-day period. Similar results were also found in weaned pigs fed with the same botanical blend (0.05%) when they were inoculated with F4 *E. coli* (Devi et al., 2015). A botanical blend composed of oregano, cinnamon, and capsicum oleoresin was found to increase ADG by 82% during the first week post-weaning and increase ADFI by 39% and 21% during the first- and second-week post-

weaning, respectively (Nofrarías et al., 2006). Pigs supplemented with 100 mg/kg of either botanical blend performed similar in the present study, indicating the similarity of garlic variety.

Post-weaning diarrhea

Post weaning diarrhea typically lasts approximately 5 days and is occasionally hallmarked by severe watery diarrhea (Dubreuil et al., 2016). Post weaning diarrhea caused by F18 *E. coli* is one of primary issues in weaned pigs due to decreased growth performance and increased mortality and antibiotic treatment. Previous studies have shown that dietary inclusion of botanicals such as thymol, cinnamaldehyde, and carvacrol are capable of reducing frequency of diarrhea due to their anti-microbial and anti-inflammatory properties (Huang et al., 2010; Li et al., 2012). In the current study, frequency of diarrhea was reduced in the pigs fed with 100 mg/kg of BB1 and BB2. This observation agrees with the findings in Liu et al. (2013), in which dietary supplementing capsicum oleoresin or garlic botanical decreased diarrhea incidence of *E. coli* challenged pigs. Botanical extracts exhibit antimicrobial activities through their hydrophobic phenolic compounds, which can disrupt bacterial cell membranes and inhibit the growth of gram-negative bacteria like *E. coli* (Faleiro, 2011; Lopez-Romero et al., 2015). Clove and garlic botanical extracts demonstrated significant *in vitro* antimicrobial activity against *E. coli* and other gram-negative bacteria (Arora and Kaur, 1999; Karuppiah and Rajaram, 2012), which is reflective of the reduced frequency of diarrhea observed in the present study.

Blood metabolites and systemic immunity

Supplementation of botanicals resulted in increased WBC, monocytes, and lymphocytes compared with negative control on d 14 and 21 PI. Pigs supplemented with 100 mg/kg of BB1 had the greatest WBC, monocyte, and lymphocyte counts among all botanical treatments, while pigs fed with 50 mg/kg BB2 had the lowest monocyte and lymphocyte counts on d 21 PI. These

findings suggest the immune-modulating effects of botanical blends. Similarly, Liu et al. (2013) reported that supplementation of botanicals (garlic botanicals, capsicum oleoresin, or turmeric oleoresin) resulted in a decrease in total WBC in *E. coli* challenged pigs on d 11 PI, indicating these botanical decreased systemic inflammation caused by *E. coli* infection. Other botanical extract supplementation has also been shown to have beneficial immunomodulatory effects in weaned pigs and other animals (Carrasco et al., 2010; Zeng et al., 2015a). WBC count is used as a measure of pathogenic infection risk and immunological activity (Gordon-Smith, 2009). Monocytes and lymphocytes are major components of innate and adaptive immunity. Monocytes reduce pathogenic infection by recruiting monocyte-derived macrophages (Murray, 2018), while T and B lymphocytes memorize pathogen-specific antigens in the case of future exposures (Gaudino and Kumar, 2019). The increases in WBC, monocytes, and lymphocytes observed in the current study indicate that these two types of botanical blends could modulate the systemic immunity, which may further impact the overall health of weaned pigs. Further analysis on serum cytokines, acute phase proteins, and antibodies is necessary.

Intestinal morphology

Supplementation of both botanical blends increased duodenal villi width and jejunal villi height on d 5 PI, and inclusion of botanical blend 2 increased duodenal and jejunal crypt depth on d 21 PI. Similarly, increases in villi height and villi height to crypt depth ratio have been observed in previous studies when weaned pigs were supplemented with 250 mg/kg of thymol and cinnamaldehyde (Zeng et al., 2015b), 100 mg/kg of thymol and cinnamaldehyde (Tian and Piao, 2019), and 10 mg/kg of capsicum oleoresin or garlic botanicals (Liu et al., 2013). Increases in villi height and width are positively correlated with improved nutrient absorption by villous enterocytes (Barszcz and Skomiał, 2011; Shen et al., 2009; Li et al., 2012), while increases in

crypt depth are associated with improved capacity for crypt stem cells to mature into enterocytes (Hampson and Kidder, 1986). Additionally, supplementation of both botanical blends increased duodenal crypt sialomucin area of weaned pigs at the end of the study, regardless of doses. This observation is most likely due to the increased crypt depths. Intestinal acidomucins are classified as sulfomucin or sialomucin depending on the presence of a terminal acidic sulfate group or neutral sialic acid (Croix et al., 2011). Newly formed goblet cells within crypts switch mucin production from sialomucin to sulfomucin as they mature into enterocytes and migrate up from the crypt to the villus. An increased crypt depth can give immature goblet cells more time to develop from sialomucins to sulfomucins, resulting in effective maturity of mucin types produced by crypt goblet cells (Brown et al., 1998).

Conclusion

In conclusion, dietary supplementation of botanical blends consisting of capsicum oleoresin and garlic extract varieties improved disease resistance, immunity, and intestinal morphology of weaned pigs experimentally infected with F18 *E. coli*. Both BB1 and BB2 were effective at reducing frequency of diarrhea of *E. coli* infected pigs, compared with the positive control. However, no differences in β-hemolytic coliforms were observed in pigs supplemented with botanical blends. Among the botanical blend treatments, 100 mg/kg of BB2 was most effective at enhancing intestinal villi height, villi width, and crypt depth on d 5 PI, as well as regulating systemic immunity of weaned pigs on d 21 PI. These results support the potential of botanicals and phytogenic feed additives on improving growth performance and disease resistance of weaned pigs when antibiotics in-feed is restricted. Further research is needed to confirm the beneficial effects of the botanical blends on performance with a large animal

population, as well as to determine the underlying mechanisms of each individual botanical constituent.

Tables and Figures

Table 2-1. Ingredient compositions of experimental diets[1]

Ingredient, %	Control, phase I	Control, phase II
Corn	51.55	58.44
Dried whey	15.00	10.00
Soybean meal	21.00	24.00
Fish meal	4.00	3.00
Soy protein concentrate	3.00	-
Soybean oil	2.10	1.30
Limestone	0.95	0.95
Dicalcium phosphate	0.55	0.52
L-Lysine·HCl	0.48	0.48
DL-Methionine	0.24	0.21
L-Threonine	0.21	0.20
L-Tryptophan	0.09	0.09
L-Valine	0.13	0.11
Salt	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30
Total	100.00	100.00
Calculated energy and nutrient		
Metabolizable energy, kcal/kg	3390	3333
Net energy, kcal/kg	2545	2501
Crude protein, %	20.49	19.32
Arg,[3] %	1.15	1.09
His,[3] %	0.47	0.45
Ile,[3] %	0.78	0.72
Leu,[3] %	1.55	1.47
Lys,[3] %	1.42	1.32
Met,[3] %	0.55	0.50
Thr,[3] %	0.89	0.83
Trp,[3] %	0.31	0.29
Val,[3] %	0.97	0.89
Met + Cys,[3] %	0.85	0.79
Phe + Tye,[3] %	1.39	1.31
Ca, %	0.83	0.75
Total P, %	0.65	0.59
Digestible P, %	0.42	0.36

[1]In each phase, three additional diets were formulated by adding 100 mg/kg of botanical blend type 1, or 50 or 100mg/kg of botanical blend type 2 to the control diet, respectively.

[2]Provided by United Animal Health (Sheridan, IN). The premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]Amino acids are indicated as standardized ileal digestible amino acids.

Table 2-2. Growth performance of weaned pigs fed diets supplemented with two different botanical blend (BB1 or BB2)

Item[1]	Negative control	Positive control	BB1 100 mg/kg	BB2 50 mg/kg	BB2 100 mg/kg	SEM[3]	*P*-value
BW, kg							
d -7	6.96	7.12	7.23	7.10	7.17	0.30	0.98
d 0 PI	7.34	7.21	7.69	7.36	7.55	0.30	0.82
d 5 PI	9.62^{a}	8.43^{b}	8.84^{ab}	8.70^{ab}	8.87^{ab}	0.40	<0.10
d 5 PI[2]	9.88	8.53	9.62	9.00	8.98	0.40	0.17
d 14 PI[2]	15.85^{a}	13.38^{b}	14.63^{ab}	12.87^{b}	14.17^{ab}	0.62	<0.05
d 21 PI[2]	21.38^{a}	18.00^{b}	19.52^{ab}	17.40^{b}	19.10^{ab}	0.78	<0.05
ADG, g							
d 0 to 5 PI	390	260	271	282	268	40.2	0.15
d 5 to 14 PI[2]	663^{a}	539^{bc}	557^{ab}	430^{c}	576^{ab}	38.8	<0.05
d 14 to 21 PI[2]	790^{a}	660^{b}	680^{ab}	650^{b}	705^{ab}	35.2	<0.10
d 0 to 14 PI[2]	564^{a}	443^{b}	482^{ab}	392^{b}	483^{ab}	35.2	<0.05
d 0 to 21 PI[2]	640^{a}	515^{b}	554^{ab}	477^{b}	557^{ab}	29.5	<0.05
ADFI, g							
d 0 to 5 PI	557^{a}	320^{b}	319^{b}	339^{b}	362^{b}	37.0	<0.01
d 5 to 14 PI[2]	919^{a}	824^{ab}	759^{b}	743^{b}	760^{b}	49.8	<0.10
d 14 to 21 PI[2]	1,180	1,142	1,162	1,089	1,131	66.0	0.78
d 0 to 14 PI[2]	785^{a}	651^{b}	625^{b}	605^{b}	626^{b}	39.3	<0.05
d 0 to 21 PI[2]	919	816	803	764	794	44.5	0.12
Gain:Feed							
d 0 to 5 PI	0.69	0.85	0.95	0.83	0.60	0.14	0.43
d 7 to 14 PI[2]	0.75^{a}	0.69^{a}	0.74^{a}	0.55^{b}	0.77^{a}	0.039	<0.01
d 14 to 21 PI[2]	0.69	0.59	0.62	0.63	0.64	0.034	0.23
d 0 to 14 PI[2]	0.75^{a}	0.71^{ab}	0.78^{a}	0.62^{b}	0.78^{a}	0.031	<0.05
d 0 to 21 PI[2]	0.72^{a}	0.65^{ab}	0.71^{a}	0.62^{b}	0.72^{a}	0.030	<0.05

[1]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; PI = post inoculation. Each least squares mean represents 11-12 observations. [a,b,c]Means without a common superscript are different ($P < 0.05$).

[2]Each least squares mean represents 6 observations.

[3]SEM = standard error of the mean

Table 2-3. Total and differential white blood cells in weaned pigs fed diets supplemented with two different botanical blend (BB1 or BB2)

Item[1]	Negative control	Positive control	BB1 100 mg/kg	BB2 50 mg/kg	BB2 100 mg/kg	SEM[2]	*P*-value
d -7 before inoculation							
WBC, 10^3/μL	9.52	11.00	9.47	9.30	9.44	0.75	0.52
Neu, 10^3/μL	5.55	5.42	4.52	4.40	4.76	0.55	0.43
Lym, 10^3/μL	4.64	5.02	4.28	4.15	3.94	0.35	0.22
Mono, 10^3/μL	0.19^{b}	0.30^{a}	0.20^{b}	0.24^{ab}	0.22^{b}	0.028	<0.10
Eos, 10^3/μL	0.51	0.55	0.42	0.48	0.48	0.15	0.96
Baso, 10^3/μL	0.032	0.027	0.031	0.023	0.027	0.010	0.96
Neu, %	49.36	46.54	47.37	46.32	51.27	2.55	0.56
Lym, %	42.67	45.57	45.85	45.83	40.53	2.78	0.55
Mono, %	1.90	2.82	2.37	2.47	2.22	0.28	0.23
Eos, %	5.78	4.84	4.06	5.16	5.73	1.15	0.81
Baso, %	0.29	0.23	0.35	0.22	0.25	0.097	0.87
Neu:Lym	1.16	1.11	1.07	1.08	1.39	0.14	0.40
d 0 before inoculation							
WBC, 10^3/μL	11.94	10.14	9.48	9.14	10.75	0.72	0.14
Neu, 10^3/μL	5.97^{a}	4.18^{b}	4.39^{ab}	4.04^{b}	5.59^{ab}	0.55	<0.10
Lym, 10^3/μL	5.42^{a}	5.19^{ab}	4.59^{bc}	4.39^{c}	4.23^{c}	0.26	<0.05
Mono, 10^3/μL	0.38	0.39	0.32	0.33	0.31	0.038	0.47
Eos, 10^3/μL	0.67^{a}	0.36^{b}	0.35^{b}	0.35^{b}	0.28^{b}	0.085	<0.05
Baso, 10^3/μL	0.024	0.023	0.020	0.023	0.026	0.005	0.96
Neu, %	48.53	39.74	45.62	43.96	48.95	2.87	0.16
Lym, %	43.44	52.90	47.85	48.43	44.01	2.98	0.18
Mono, %	2.91	3.77	3.30	3.68	2.96	0.32	0.20
Eos, %	4.94	3.37	2.98	3.68	2.94	0.67	0.22
Baso, %	0.175	0.225	0.248	0.251	0.195	0.048	0.74
Neu:Lym	1.15	0.80	1.07	0.94	1.19	0.13	0.21

d 4 post inoculation							
WBC, $10^3/\mu L$	14.78	14.50	12.46	13.40	12.42	0.80	0.15
Neu, $10^3/\mu L$	7.37^{a}	5.45^{b}	5.09^{b}	4.54^{b}	5.34^{b}	0.54	<0.05
Lym, $10^3/\mu L$	6.44^{a}	5.42^{b}	4.95^{bc}	4.90^{bc}	4.30^{c}	0.35	<0.05
Mono, $10^3/\mu L$	0.34^{b}	0.87^{a}	0.97^{a}	1.00^{a}	0.83^{a}	0.091	<0.05
Eos, $10^3/\mu L$	0.53^{b}	2.28^{a}	2.21^{a}	2.28^{a}	2.23^{a}	0.28	<0.05
Baso, $10^3/\mu L$	0.023^{b}	0.103^{a}	0.068^{ab}	0.028^{b}	0.108^{a}	0.019	<0.05
Neu, %	49.75^{a}	37.80^{bc}	39.86^{b}	32.57^{c}	39.77^{b}	2.28	<0.05
Lym, %	44.90^{a}	38.02^{b}	36.03^{b}	40.77^{ab}	35.74^{b}	2.60	<0.05
Mono, %	2.44^{b}	6.08^{a}	7.13^{a}	7.49^{a}	6.65^{a}	0.70	<0.05
Eos, %	3.68^{b}	17.34^{a}	16.44^{a}	17.14^{a}	17.14^{a}	1.85	<0.05
Baso, %	0.153^{b}	0.654^{a}	0.438^{ab}	0.207^{b}	0.700^{a}	0.113	<0.05
Neu:Lym	1.11	1.03	1.18	0.83	1.18	0.11	0.17
d 14 post inoculation							
WBC, $10^3/\mu L$	17.73	18.37	23.09	15.21	16.87	2.12	0.16
Neu, $10^3/\mu L$	6.50	6.65	7.08	6.46	6.34	0.73	0.96
Lym, $10^3/\mu L$	9.57	9.11	10.65	8.15	8.64	1.24	0.77
Mono, $10^3/\mu L$	0.90^{bc}	1.18^{ab}	1.61^{a}	0.58^{c}	1.07^{abc}	0.21	<0.05
Eos, $10^3/\mu L$	1.03	0.85	1.23	0.64	0.88	0.17	0.27
Baso, $10^3/\mu L$	0.080	0.088	0.089	0.107	0.156	0.032	0.42
Neu, %	36.55	38.55	31.08	37.16	37.04	3.81	0.72
Lym, %	52.38	49.06	53.16	54.06	49.90	3.21	0.77
Mono, %	4.94^{ab}	6.02^{a}	6.86^{a}	3.34^{b}	5.89^{a}	0.88	<0.10
Eos, %	5.81	6.12	5.93	4.49	6.38	1.05	0.68
Baso, %	0.40	0.44	0.42	0.39	0.89	0.16	0.18
Neu:Lym	0.71	0.84	0.64	0.76	0.79	0.12	0.80
d 21 post inoculation							
WBC, $10^3/\mu L$	9.25^{b}	11.98^{ab}	13.67^{a}	10.52^{ab}	10.81^{ab}	0.98	<0.10
Neu, $10^3/\mu L$	4.02	4.73	5.62	5.20	4.93	0.61	0.50
Lym, $10^3/\mu L$	4.96^{bc}	6.37^{ab}	7.13^{a}	4.59^{c}	5.80^{abc}	0.53	<0.05
Mono, $10^3/\mu L$	0.37^{ab}	0.38^{ab}	0.48^{a}	0.26^{b}	0.36^{ab}	0.056	<0.10

Eos, $10^3/\mu L$	0.43	0.48	0.38	0.26	0.26	0.12	0.69
Baso, $10^3/\mu L$	0.063	0.025	0.050	0.057	0.075	0.025	0.62
Neu, %	39.66^{ab}	35.39^{b}	41.21^{b}	47.93^{a}	46.59^{a}	2.65	<0.05
Lym, %	50.22^{ab}	56.83^{a}	51.45^{ab}	41.31^{b}	44.29^{b}	3.80	<0.10
Mono, %	3.37	3.25	3.14	2.23	2.87	0.33	0.18
Eos, %	4.29	2.46	3.51	2.47	2.88	1.02	0.74
Baso, %	0.557	0.177	0.590	0.236	0.446	0.190	0.48
Neu:Lym	0.808^{ab}	0.629^{b}	0.817^{ab}	1.108^{a}	1.126^{a}	0.129	<0.10

[1]WBC = white blood cell; Neu = neutrophil; Lym = lymphocyte; Mono = monocyte; Eos = eosinophil; Baso = basophil. Each least squares mean represents 11-12 observations on d -7, d 0, and d 4 post-inoculation. Each least squares mean represents 5-6 observations on d -7, d 0, and d 4 post-inoculation. [a,b,c]Means without a common superscript are different ($P < 0.05$).

[2]SEM = standard error of the mean

Table 2-4. Red blood cell profile in weaned pigs fed diets supplemented with two different botanical blend (BB1 or BB2)

Item[1]	Negative control	Positive control	BB1 100 mg/kg	BB2 50 mg/kg	BB2 100 mg/kg	SEM[3]	*P*-value
d -7 before inoculation							
RBC, 10^6/µL	6.94	7.08	6.65	6.89	6.76	0.14	0.28
HGB, g/dL	10.16	9.73	9.42	9.41	9.38	0.24	0.12
HCT, %	32.65	32.76	30.85	31.33	31.51	0.81	0.20
MCV, fL^2	48.20	44.75	44.67	44.65	45.18	1.12	0.10
MCH, pg	14.98	13.33	13.06	12.96	13.28	0.59	0.11
MCHC, g/dL	31.08	29.75	29.95	29.90	29.77	0.45	0.21
RDW, %	21.02	23.00	22.90	22.32	22.69	0.88	0.43
Platelets, 10^3/µL	503	582	569	523	588	26.09	0.11
MPV, fL^2	8.84	9.26	8.84	8.98	9.00	0.29	0.83
Total protein, g/dL	4.50	4.56	4.60	4.71	4.50	0.11	0.58
d 0 before inoculation							
RBC, 10^6/µL	7.42[a]	7.44[a]	6.77[b]	7.04[ab]	7.14[ab]	0.20	<0.10
HGB, g/dL	11.25[a]	10.73[a]	9.69[b]	9.99[b]	10.03[b]	0.26	<0.05
HCT, %	34.32[a]	32.46[ab]	29.64[c]	30.80[bc]	30.84[bc]	0.73	<0.05
MCV, fL^2	45.67	42.57	42.88	42.79	43.31	1.07	0.28
MCH, pg	14.94	13.98	13.63	13.52	13.67	0.63	0.50
MCHC, g/dL	32.41	32.79	32.73	32.37	32.27	0.49	0.94
RDW, %	21.25	23.30	22.79	21.69	23.01	0.88	0.34
Platelets, 10^3/µL	357	336	324	358	340	24.91	0.85
MPV, fL^2	9.90	9.95	9.50	9.39	9.97	0.41	0.75
Total protein, g/dL	4.86	5.03	4.96	4.89	5.06	0.12	0.72
d 4 post inoculation							
RBC, 10^6/µL	6.59	7.03	6.86	6.62	6.71	0.18	0.49
HGB, g/dL	9.81	9.75	9.44	9.22	9.18	0.29	0.37
HCT, %	30.29	31.12	29.73	28.82	30.03	0.90	0.49

MCV, fL^2	46.10	43.04	42.98	42.53	43.36	1.09	0.12
MCH, pg	15.10[a]	13.52[b]	13.82[ab]	13.03[b]	13.29[b]	0.51	<0.05
MCHC, g/dL	32.78[a]	31.31[b]	31.11[b]	31.15[b]	30.55[b]	0.44	<0.05
RDW, %	21.67	23.81	23.20	22.13	23.91	0.84	0.16
Platelets, $10^3/\mu L$	340	306	348	314	343	27.40	0.75
MPV, fL^2	9.85	9.70	9.75	9.63	10.01	0.42	0.96
Total protein, g/dL	4.70	4.80	5.08	4.76	4.89	0.13	0.28
d 14 post inoculation							
RBC, $10^6/\mu L$	6.40	6.87	6.76	6.59	7.13	0.20	0.20
HGB, g/dL	10.73	10.79	10.34	9.63	10.70	0.40	0.23
HCT, %	34.36	34.00	33.73	30.59	33.63	1.10	0.15
MCV, fL^2	53.37	49.08	48.90	48.71	49.90	1.44	0.18
MCH, pg	17.18[a]	16.06[ab]	15.53[b]	15.28[b]	15.38[b]	0.47	<0.10
MCHC, g/dL	30.84	31.66	30.86	31.40	30.84	0.41	0.51
RDW, %	25.08	26.88	25.26	25.66	27.70	1.31	0.48
Platelets, $10^3/\mu L$	472	354	514	560	385	64.99	0.19
MPV, fL^2	9.65	10.18	9.80	9.95	9.65	0.51	0.95
Total protein, g/dL	4.69[ab]	4.46[b]	4.91[a]	4.81[a]	4.43[b]	0.10	<0.05
d 21 post inoculation							
RBC, $10^6/\mu L$	4.95	5.60	5.31	4.85	5.28	0.18	<0.10
HGB, g/dL	8.47[a]	8.45[a]	8.09[ab]	7.39[b]	7.89[ab]	0.24	<0.05
HCT, %	26.10[a]	26.82[a]	26.22[a]	23.43[b]	25.84[a]	0.72	<0.05
MCV, fL^2	51.94	47.95	48.12	48.73	48.90	1.26	0.28
MCH, pg	16.53	15.34	14.87	15.36	15.24	0.41	0.10
MCHC, g/dL	31.24	31.44	30.71	31.59	31.20	0.37	0.40
RDW, %	22.75	23.78	23.21	25.81	25.00	0.91	0.13
Platelets, $10^3/\mu L$	499	396	433	525	537	54.95	0.35
MPV, fL^2	9.15	9.22	8.32	9.32	9.27	0.43	0.46
Total protein, g/dL	4.93	4.78	5.12	4.95	4.83	0.12	0.28

[1]RBC = red blood cell; HGB = hemoglobin; HCT = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular

hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume.

Each least squares mean represents 11-12 observations on d -7, d 0, and d 4 post-inoculation.

Each least squares mean represents 5-6 observations on d -7, d 0, and d 4 post-inoculation.

[a,b,c]Means without a common superscript are different ($P < 0.05$).

[2]fL= femtolitre (10^{-15} L).

[3]SEM = standard error of the mean

Table 2-5. Intestinal morphology of weaned pigs fed diets supplemented with two different botanical blend (BB1 or BB2)

Item[1]	Negative control	Positive control	BB1 100 mg/kg	BB2 50 mg/kg	BB2 100 mg/kg	SEM[2]	*P*-value
d 5 PI							
Duodenum							
Villi height, µm	340.11[a]	236.31[b]	264.29[ab]	265.68[ab]	275.13[ab]	20.72	<0.05
Villi width, µm	154.6[ab]	129.69[b]	138.78[b]	156.27[ab]	174.49[a]	7.89	<0.05
Crypt depth, µm	549.65	552.44	568.58	573.16	569.81	32.32	0.98
Villi area, µm	50,314[a]	29,099[b]	34,723[b]	39,263[ab]	41,208[ab]	3,572	<0.05
Sulfomucin area, % of total villi area	5.55	4.32	5.31	4.79	5.45	5.48	0.45
Sialomucin area, % of total villi area	1.68	1.31	2.10	2.77	2.26	4.77	0.24
Goblet cell number, per villi	23.25	18.68	18.00	18.16	20.03	1.99	0.34
Jejunum							
Villi height, µm	293.49[ab]	250.82[b]	240.06[b]	296.05[ab]	352.34[a]	25.41	<0.05
Villi width, µm	130.78	119.44	110.11	116.48	120.55	6.77	0.31
Crypt depth, µm	468.18	456.53	371.35	395.53	425.68	29.13	0.13
Villi area, µm	34,228[ab]	25,999[b]	22,593[b]	30,056[ab]	39,676[a]	4,158	0.06
Sulfomucin area, % of total villi area	7.20	3.91	4.73	5.49	3.28	1.83	0.43
Sialomucin area, % of total villi area	2.07	0.75	0.88	1.38	0.87	0.51	0.35
Goblet cell number, per villi	20.93	14.18	18.47	16.26	17.05	2.18	0.28
Ileum							
Villi height, µm	241.28	197.65	217.32	209.94	240.19	16.61	0.31
Villi width, µm	113.80	119.31	115.61	118.66	115.00	6.54	0.97
Crypt depth, µm	342.60	301.74	345.10	316.85	338.15	27.04	0.76
Villi area, µm	23,469	20,189	22,146	20,844	24,174	2,370	0.74

Sulfomucin area, % of total villi area	6.56	9.62	9.19	9.66	9.25	1.07	0.23
Sialomucin area, % of total villi area	2.41	1.98	1.71	1.55	1.78	0.46	0.74
Goblet cell number, per villi	17.72	19.36	17.66	20.30	21.12	1.92	0.63
Distal Colon							
Crypt depth, µm	227.98[b]	235.9[b]	280.37[ab]	269.31[ab]	327.95[a]	17.62	<0.05
Sulfomucin area, % of total villi area	15.74	17.33	17.60	19.44	16.44	1.99	0.74
d 21 PI							
Duodenum							
Villi height, µm	318.98	264.62	346.91	308.87	300.34	31.80	0.47
Villi width, µm	200.30	192.19	181.54	196.80	179.23	9.74	0.48
Crypt depth, µm	557.78[b]	543.74[b]	560.35[b]	589.88[ab]	670.47[a]	33.81	0.09
Villi area, µm	51,569	39,254	50,297	49,671	45,686	5,772	0.54
Sulfomucin area, % of total villi area	5.72	6.57	6.67	7.67	6.72	0.65	0.22
Sialomucin area, % of total villi area	1.113[b]	2.545[ab]	1.755[b]	2.015[ab]	3.118[a]	0.52	<0.05
Goblet cell number, per villi	32.80	31.34	35.88	32.45	27.78	3.62	0.62
Jejunum							
Villi height, µm	491.45	431.83	486.09	418.34	478.83	28.87	0.26
Villi width, µm	131.24	126.08	132.45	143.58	142.03	7.98	0.49
Crypt depth, µm	297.35[b]	277.56[b]	305.79[ab]	348.3[a]	309.07[ab]	17.57	0.08
Villi area, µm	53,796	45,580	53,701	48,589	58,446	3,487	0.14
Sulfomucin area, % of total villi area	3.91	2.84	3.06	4.24	2.26	0.62	0.10
Sialomucin area, % of total villi area	1.33	1.32	0.97	0.95	0.93	0.22	0.47
Goblet cell number, per villi	21.17	15.94	17.05	17.23	15.76	2.26	0.46
Ileum							
Villi height, µm	351.96	321.66	312.28	291.50	304.49	22.83	0.41
Villi width, µm	158.65	158.68	171.41	161.65	158.24	9.43	0.86

Crypt depth, µm	345.49	333.34	331.98	353.81	311.18	20.25	0.60
Villi area, µm	45,350	41,352	43,874	37,216	39,869	3,982	0.59
Sulfomucin area, % of total villi area	6.35	6.45	5.82	5.76	5.89	0.95	0.98
Sialomucin area, % of total villi area	1.29	1.36	1.44	0.94	1.47	0.27	0.64
Goblet cell number, per villi	24.18	19.38	17.12	17.72	16.42	3.34	0.48
Distal Colon							
Crypt depth, µm	425.11	417.93	428.54	390.72	378.89	20.61	0.36
Sulfomucin area, % of total villi area	23.01	21.51	31.22	25.65	26.59	2.66	0.14

[1]Each least squares mean represents 5-6 observations. [a,b]Means without a common superscript are different ($P < 0.05$).

[2]SEM = standard error of the mean

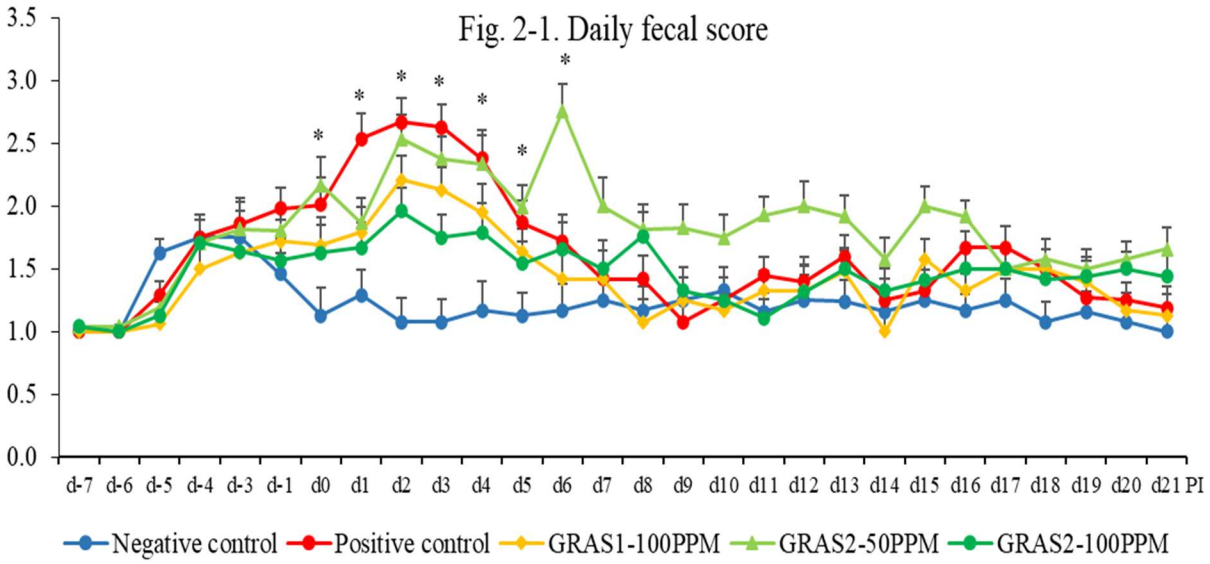

Figure 2-1. Daily fecal score of *Escherichia coli*-infected weaned pigs fed diets supplemented with 100 mg/kg of botanical blend type 1 (BB1), or 50 or 100 mg/kg of botanical blend type 2 (BB2). Diarrhea score = 1, normal feces, 2, moist feces, 3, mild diarrhea, 4, severe diarrhea, 5, watery diarrhea. PI = post inoculation. *$P < 0.05$, indicating diarrhea scores were different among treatments. Each least squares mean represents 12 observations before d 5 PI and each least squares mean represents 6 observations after d 5 PI.

Fig. 2-2. Frequency of diarrhea, d 0 to 21 PI

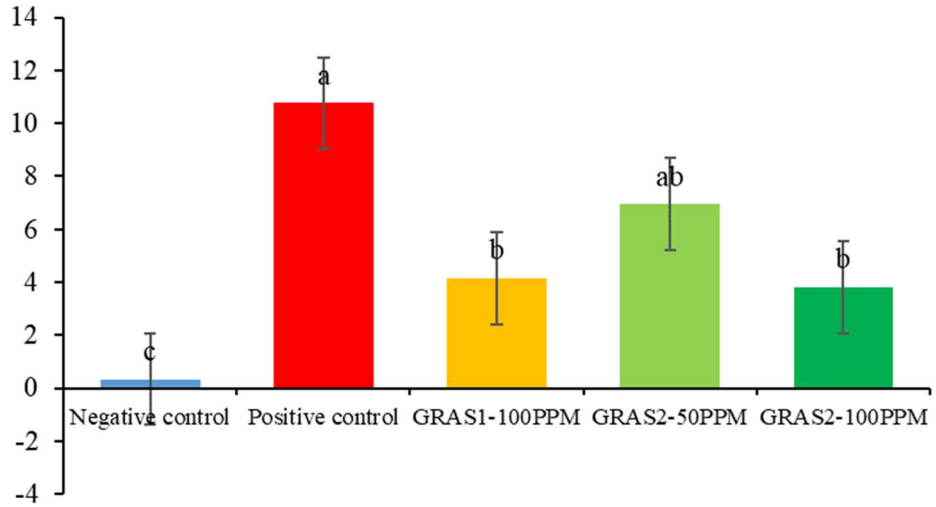

Fig. 2-2. Frequency of diarrhea, d 0 to 5 PI

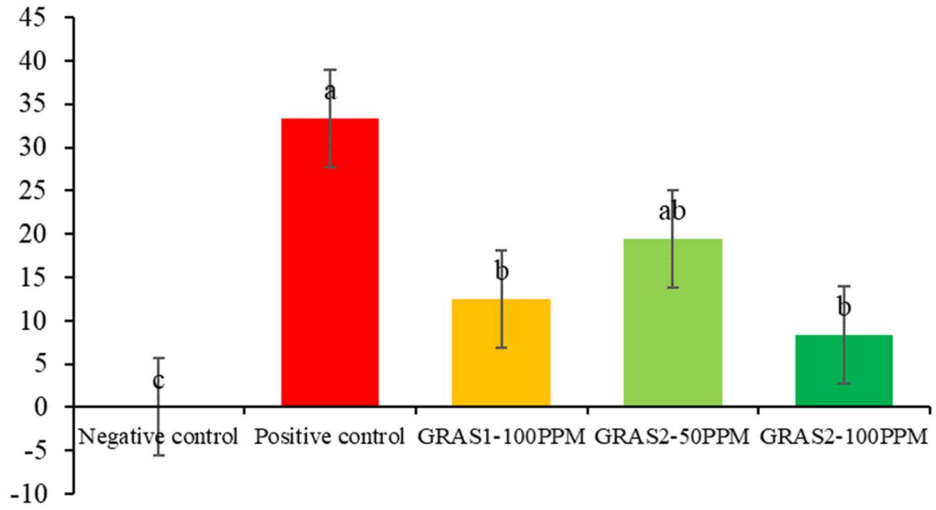

Figure 2-2. Frequency of diarrhea (d 0 to 21 PI and d 0 to 5 PI) of *Escherichia coli*-infected weaned pigs fed diets supplemented with 100 mg/kg of botanical blend type 1 (BB1), or 50 or 100 mg/kg of botanical blend type 2 (BB2). Frequency of diarrhea was calculated as the percentage of pig days with diarrhea score ≥ 3 in the total of pig days. [a,b,c]Means without a common superscript are different ($P < 0.05$).

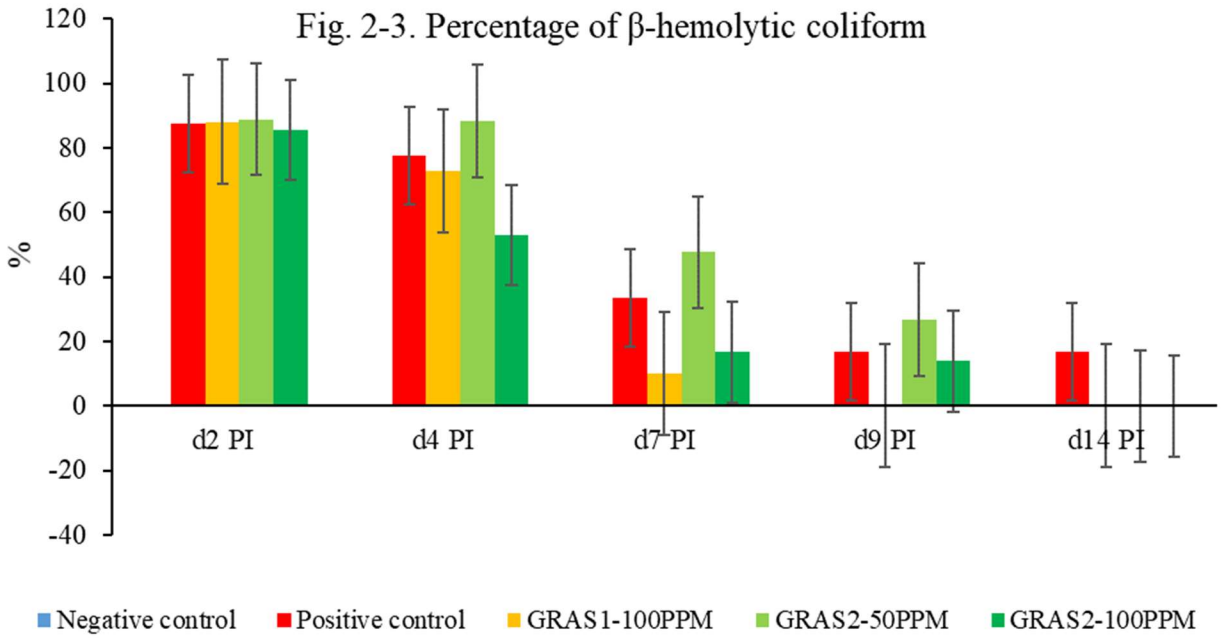

Figure 2-3. The percentage (%) of β-hemolytic coliform in fecal samples of *Escherichia coli* challenged pigs fed diets supplemented with 100 mg/kg of botanical blend type 1 (BB1), or 50 or 100 mg/kg of botanical blend type 2 (BB2). No β-hemolytic coliforms were observed in the fecal samples of pigs in the negative control. No β-hemolytic coliforms were observed in the fecal samples of pigs before *E. coli* challenge and d 14 and 21 post inoculation (PI). Each least squares mean represents 12 observations on d 2 and 4 PI and each least squares mean represents 6 observations after d 7, 9, and 14 PI.

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Chapter 3

GENERAL SUMMARY AND CONCLUSION

Post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* (*E. coli*) is an important cause of economic losses in the global swine industry and can lead to reduced weight gain and increased morbidity and mortality in weaned pigs. The restricted use of in-feed antibiotic growth promoters in swine production has led to increased considerations toward alternative antibiotic replacements for disease resistance and health benefits in pigs. Botanical extracts, phytogenic feed additives and essential oils are secondary metabolites of non-woody aromatic plant constituents and primarily consisting of terpenes and terpenoids, alkaloids, and phenolic compounds. Dietary supplementation of botanical extracts has shown beneficial health effects when fed to weaned pigs by exhibiting antioxidant and antimicrobial properties, promoting immune function, and increasing disease resistance. Therefore, the objective of this study was to investigate the effects of dietary supplementation of botanical blends on growth promotion, diarrhea, blood profiles, and intestinal morphology in weaned pigs experimentally infected with a pathogenic *E. coli*.

In this study, two blends of botanical extracts comprised of capsicum oleoresin and garlic extract varieties (BB1, 100 mg/kg and BB2, 50 or 100 mg/kg) were supplemented into diets to investigate their effects on diarrhea, growth performance, systemic immunity, and gut integrity of weaned pigs experimentally infected with a pathogenic F18 *E. coli*. Although there were no statistical differences in weight gain or average daily gain among experimental treatments, supplementation of all botanical blend treatments significantly reduced incidence of diarrhea.

Supplementation of high dose botanical blend 2 was the most effective one among the botanical blend treatments at improving systemic immunity and intestinal morphology of weaned pigs, as indicated by increased white blood cell, monocyte, and lymphocyte count as well as villi height, villi width, and crypt depth. These results indicate that dietary supplementation of botanical blends may improve disease resistance and systemic immunity of weaned pigs and increase recovery from post-weaning *E. coli* infection.

In conclusion, supplementation of the blends of capsicum oleoresin and garlic extract exhibits very promising benefits on promoting disease resistance of weaned pigs. The potential mechanisms may include but not limited to the enhanced systemic immunity and intestinal integrity. However, further research should be considered to explore the changes of intestinal microbiome and their metabolites, which will help us to understand how botanical extracts affect the intestinal environment and the interactions of diet-microbiome-host. It will be also interesting to investigate which component(s) in botanical blends contribute to their benefits in weaned pigs.