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Establishing an Ideal Inclusion Rate of Fermented Soybean Meal and Sodium Butyrate on Growth Performance, Complete Blood Cell Count, and Nutrient Utilization in Nursery Pigs

Kristopher A. Bottoms
University of Arkansas, Fayetteville

Establishing an Ideal Inclusion Rate of Fermented Soybean Meal and Sodium Butyrate on Growth Performance, Complete Blood Cell Count, and Nutrient Utilization in Nursery Pigs

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Kristopher A. Bottoms
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_____
Charles Maxwell, PhD
Thesis Director

_____
Jason Apple, PhD
Committee Member

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Craig C. Coon, PhD
Committee Member

Abstract

To evaluate increasing levels of sodium butyrate (SB) in nursery diets on growth performance (Experiment 1 & 2), complete blood cell count (Experiment 2), and the optimal level of fermented soybean meal for maximum performance in weanling pigs (Experiment 3), weaned pigs were blocked with initial body weight (BW) and allotted to dietary treatments. Treatments were: 1) Control (C) moderately complex corn-soybean-meal based supplemented with 0.05% benzoic acid (BA), but devoid of SB; C diet supplemented with 0.05%, 0.10%, or 0.15% SB (Experiment 1). Treatments in experiment 2 consisted of 1) a moderately complex corn-soybean-meal based diet devoid of SB and BA (NC), 2) The NC diet supplemented with 0.5% BA, 3, 4 and 5) NC diet supplemented with 0.5% BA and 0.05%, 0.10% or 0.15% SB, respectively. Treatments in experiment 3 consisted of 1) a fermented soybean protein-poultry by-product diet (C), 2, 3, and 4) C diet was replaced with 5%, 10%, or 15% fermented soybean meal, respectively (FSBM; Experiment 3). Blood was collected at the beginning and end of each phase to determine complete blood cell count (Experiments 2 & 3). Data were analyzed by MIXED procedures of SAS (SAS Inst., Cary, NC) with dietary treatment as a fixed effect, while facility by treatment interactions (Experiment 1 & 2) and initial BW blocks as random effects (all experiments). In exp. 1 & 2, increasing dietary SB increased weight gain ($P < 0.05$), ADFI ($P \leq 0.05$), and final BW ($P < 0.05$). For exp. 2, total white blood cell ($P = 0.07$) and eosinophil cell count increased with increasing SB ($P = 0.08$). Lymphocyte cell count decreased ($P = 0.09$) with increasing SB. In exp. 3, with increasing FSBM in the diet, overall feed efficiency (d 0-40; $P = 0.07$) increased, and ADG ($P = 0.05$) and ADFI ($P = 0.04$) increased during phases 1 & 2 (d 0-29). The heaviest BW was observed in pigs fed 10% FSBM on d 29 ($P = 0.06$), but the difference diminished by the end of the trial. Pigs fed 10% FSBM had the lowest WBC,

neutrophil, and red blood cell count. These experiments suggest that feeding SB and 10% FSBM during the nursery phase improves growth performance and alters blood cell characteristics in weanling pigs.

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Contents

List of Terms

ANFs – anti-nutritional factors

AID – apparent ileal digestibility

ATTD – apparent total tract digestibility

BW – body weight

CBC – complete blood cell count

DSBM – de-hulled soybean meal

DP – degree of polymerization

FSBM – fermented soybean meal

GIT – gastrointestinal tract

MCH – mean corpuscular hemoglobin

MCHC – mean corpuscular hemoglobin concentration

MCV – mean corpuscular volume

MPV – mean platelet volume

NDSBM – non-de-hulled soybean meal

PLT - platelet

PWD – post-weaning diarrhea

RBC – red blood cell

RCBD – randomized complete block design

RCDW – red cell distribution width

SB – sodium butyrate

SBM – soybean meal

SID – standardized ileal digestibility

UA – University of Arkansas at Fayetteville

UIUC – University of Illinois at Urbana-Champaign

WBC – white blood cell

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Introduction

Introduction

In modern-day pig production facilities, piglets are generally weaned from sows between 21-28 days after birth. This weaning period marks the beginning of the nursery phase, which often inflicts environmental, nutritional, psychological, and social stress on newborn piglets. Due to a lack of immunity against disease, along with other added stressors such as a change in diet from liquid to solid, this period is often associated with inflicting marked changes in the piglet's gastrointestinal tract (GIT) physiology, histology, microbiology and immunology (Kelly, D. 1990; Boudery, et. al., 2004; Campbell, et. al., 2013). The GIT during weaning undergoes rapid changes in size, protein turnover rate, microbiota count and composition; resulting in quick, extensive alterations in digestive, absorptive, barrier, and immune functions (Pluske, et al., 1997; Lallés, et al., 2007; Hampson, 1986; Smith, et. al., 1984; Hampson, et. al. 1983).

During the nursery phase following weaning, pigs generally exhibit low feed intake, sub-optimal growth, and often develop post-weaning diarrhea (PWD). PWD is a multi-factorial disease that can be of bacterial origin or other insults that reduce the gut barrier function which leads to increasing microbial challenges (Halas et. al., 2007). Signs are generally characterized by frequent discharge of watery feces from the pigs. This consequently leads to body weight loss and deterioration of feed efficiency; ultimately instigating high rates of morbidity and/or mortality among the nursery pig population within the operation (Pluske, et al., 1997; Jacela, et al., 2009; Hampson, et al., 1994; Halas, et al., 2007; Heo, et al., 2012). However, there are several varieties of processed feed and additives that can diminish the onset of PWD and decrease mortality rates among the nursery population.

In modern day swine production facilities, soy products are generally fed to weaned pigs in the form of soybean meal and its derivatives, due to its cost-effectiveness, high-quality protein

content, and richness in limiting amino acids lysine, threonine, and tryptophan—which are present in low concentrations in commonly fed cereal grains. Soybean meal and other soybean products contain relatively high amounts of magnesium, potassium, and sulfur; therefore negating the need to supplement these minerals in their diet (Stein, et al., 2019). However, soybean meal and its derivatives contain many anti-nutritional factors (ANF's) that limits growth, results in a transient hypersensitivity to soy protein, and increases the incidence of PWD within nursery pigs (Engle 1994; Li et. al., 1990; Li et. al., 1991).

In order to abate negative nutritional effects leading to PWD and high rates of morbidity and mortality in nursery pigs, soybean meal can be processed in various ways to deactivate ANF's, such as fermentation or enzyme treatment. In addition, feed additives are commonly supplemented in the diet during the nursery phase to improve the pig's gastric health, production efficiency and performance. Feed-additive products commonly used in swine diets include natural and synthetic substances. Commonly used feed additives for nursery pig diets include: acidifiers, antibiotics, mold inhibitors, mycotoxin binders, antioxidants, phytase, pre-biotics and pro-biotics. However, the magnitude and consistency of the response may vary, depending on inclusion rate and other dietary factors. Acidifiers are commonly marketed as growth-promoting products and as alternatives for in-feed antibiotics. Butyrate is one of the latest organic acids to be utilized for this purpose, and is proving to be a very effective acidifier, particularly in less complex nursery diets and in combination with other acidifiers.

These chapters aim to review the function, inclusion rate, growth, and immune response of commonly used feed acidifiers (butyrate) and processing techniques (fermentation) in soybean meal fed to nursery pigs.

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Chapter 1: Literature Review

Literature Review

Soybeans

Purpose within agriculture

Soybeans are important crops in the United States due to their widespread use in animal feed, human food, and production of biofuels. Approximately 33% of the world's soybeans are produced in the United States, and are used for a variety of purposes (ASA, 2012). However, nearly all soybeans produced are processed for their oil. By using raw soybeans, soy processors are able to separate the soybean oil from the soybean meal (SBM). Unrefined soybean oil is commonly used in the production of a variety of products, including industrial lubricants, solvents, cleaners, and paints. A smaller percentage of soybeans are further processed to create refined soybean oil. Refined soybean oil is often used in the development of food products for human consumption; and is also used in the production of biodiesel fuel through a three step process known as transesterification (Noureddini et. al., 1997). This process removes the glycerine from the oil, leaving behind pure soy biodiesel. This renewable, non-toxic biodiesel is cleaner burning than petroleum-based diesel oil, can reduce particulate emissions, and is environmentally friendly.

Soybean Composition

Soybeans contain about 8% seed hull, 90 % cotyledons and 2% germ (USDA, 2009). In addition to being rich in oil, soybeans are also rich in protein and carbohydrates. When the hull is removed, soybeans contain 20% oil, 40% protein, 30% carbohydrates, 5-6% water and 4-5% minerals on a DM basis (USDA, 2009). Once the oil is extracted, the remaining SBM contains about 48% protein, 35% carbohydrates, 7-10% water, 5-6% minerals (USDA, 2009). Whole full-

fat soybeans contain approximately 20% fat, compared with de-hulled soybeans, which contains less than 2% fat on an as-fed basis (Figure I, Table I). The digestible energy and metabolizable energy concentrations within full-fat soybeans are greater than in de-hulled soybeans [4,193 kcal/kg and 3,938 kcal/kg vs. 3,619 kcal/kg and 3,294 kcal/kg, respectively (Table II); due to the reduced concentration of fatty oils.

Carbohydrates

Soybeans are made of 30-35% carbohydrates, making them the second most abundant contributor of carbohydrates within the pig's diet, with grains being the most abundant source. These carbohydrates make up 60-70% of the total energy intake in piglets and growing pigs' diet (Bach Knudsen et al., 2013). The dietary carbohydrates found within soybeans are comprised of a diverse group of compounds, consisting of a wide range of chemical, physical, and physiological properties. They range from simple mono- and di-saccharides to complex, organized polysaccharides that make up cell walls (Cummings and Stephen, 2007; Englyst et al., 2007; Bach Knudsen et al., 2013). In swine nutrition, carbohydrates are primarily classified by molecular size (degree of polymerization [DP]), the type of linkage (α or β), and composition of individual monomers. This classification method separates carbohydrates into 3 main groups: sugars (DP 1-2), oligosaccharides (DP3-9), and polysaccharides (DP ≥ 10). Polysaccharides are further divided into starch (α-1:4,1,6-D-glucans) and non-starch polysaccharides (NSP; Bach Knudsen et al., 2016). Oligosaccharides (DP3-9) are considered anti-nutritional factors (ANF's) for nursery pigs, because they reduce pig growth performance and increase the incidence of diarrhea; therefore, nursery pigs require the processing of soybeans before being consumed.

Protein and Amino Acids

Soybeans contain protein that exhibits an excellent balance of amino acids that compares well with the requirements of weanling pigs. Raw soybeans contain approximately 35% crude protein and 19% fat (Stein, 2019). However, once raw soybeans are crushed into SBM, the majority of the fat is removed; resulting in the SBM containing less than 2% fat (Stein, 2019). Prior to crushing, soybeans can be de-hulled. Once de-hulled, the resulting product of de-hulled soybean meal (DSBM) contains approximately 48% crude protein. If the soybeans are not de-hulled prior to crushing, the resulting non-de-hulled soybean meal (NDSBM) produced contains approximately 43% crude protein. Since DSBM contains approximately 48% crude protein on an as fed basis, it is often referred to as high-protein soybean meal. On the other hand, NDSBM contains approximately 44% crude protein, therefore it is commonly referred to as low-protein soybean meal (Stein et al., 2019). Whole full-fat soybeans can be fed after heat treatment, to increase the energy concentration of the pig's diet, but due to the relatively high value of soybean oil, it is not usually economical for the producer.

Amino Acid Digestibility

Stein et. al. (2019) noted that amino acids within soy protein have a greater digestibility by nursery pigs than amino acids in most other cereal grains. The concentration of both lysine and tryptophan, two of the essential amino acids for pigs, is greater in SBM than that of almost all other plant proteins (Stein, 2019; Table III). The concentration of many other essential amino acids such as threonine, isoleucine, and valine is also relatively high in SBM. As a result, the amino acids within SBM complement the concentration of limiting amino acids found within cereal grains—therefore making it possible to formulate diets that are able to meet the requirements of weanling pigs.

The digestibility of amino acids found within soy protein fed to weanling pigs in the form of SBM is characterized by its standardized ileal digestibility (SID). Amino acids found within soybean protein have a greater SID than that of proteins from other plant ingredients. As a result, there is a large proportion of dietary amino acids absorbed when SBM is included in the nursery pig's diet. The digestibility of amino acids found within DSBM is greater than those found within NDSBM; whereas the digestibility of amino acids found within heat treated-full fat soybeans is usually greater than that of de-hulled soybean meal (Baker and Stein, 2010).

Nursery Pig Gastrointestinal Tract Development

For producers, feed costs represent the single largest cost of getting a commercial pig to market, so it is imperative to feed weanling pigs a diet that maximizes profitability, while maintaining an optimal level of performance in the pig at the lowest cost. However, the weaning period is a crucial stage in the growth and development of pigs. This weaning period presents many challenges for the development of healthy pigs – seeing that their digestive system has to adapt to a dry pelleted diet instead of liquid sow's milk. As a result, during this weaning period, the nursery pigs' gastrointestinal tract (GIT), physiology, histology, microbiology, and immunology markedly change (Kelly, D. 1990; Boudery, et. al., 2004; Campbell, et. al., 2013). The GIT undergoes rapid changes in structure (villous height, crypt depth, size, shape, tight junction integrity), protein turnover rate, microbiota mass, and function (loss of surface area, inflammation) – resulting in quick, extensive alterations in digestive, absorptive, barrier and immune functions (Pluske, et al., 1997; Lallés, et al., 2007; Hampson, 1986; Smith, et. al., 1984; Hampson, et. al. 1983). Within the GIT, the small intestine makes the greatest anatomical, physiological and immunological adaptation with changes in the pig's dietary consumption while adjusting to stress (Stokes et al., 1994; Cranwell, 1995; Xu, 1996; Burrin et. al., 2003; Pluske et.

al., 2003; Boudry et al., 2004). These changes within the pigs GIT are linked to local blood flow to the GIT with a reduction in basal vascular resistance, accumulation of colostral proteins in enterocytes, and changes in epithelial cell turnover – specifically, increased mitosis and increased inhibition of apoptosis (Zabielski et. al., 2008).

In addition, nursery pigs develop an unfavorably high pH in their stomach, due to their limited capacity to secrete an adequate amount of hydrochloric acid (HCl; Kidder et. al., 1978).The HCl within the monogastric stomach is a major determinant of stomach pH, and a greater HCl production leads to a lower pH. By having a high pH in the stomach, nursery pigs have a reduced ability to digest proteins within solid diets. Moreover, increased amounts of undigested protein entering the duodenum accelerates pathogenic bacterial growth in the lower GIT (Partanen et. al., 1999) – leading to poor digestion and growth performance.

As a result, these changes are compounded by the loss of immune protection provided by sow's milk prior to weaning; resulting in the onset of a transient hypersensitivity cell-mediated immunological disease to many feed products. As a result, diets fed to weanling pigs usually contain highly digestible ingredients and additives that are designed to aid in the transition from consumption of the sows' milk to solid diets.

Soybeans in Nursery Diets

Since animal proteins and animal by-products are generally more expensive than plant-based proteins, producers commonly feed plant-based proteins like SBM to the weanling pigs due to its cost-effectiveness and high protein content. However, weanling or nursery pigs often experience a transient hypersensitivity to soy protein during weaning, but can begin to develop tolerance after 7 to 10 days (Jones et. al., 2010; Barrat et. al., 1978). Consequently, weanling pigs often exhibit a high incidence of post-weaning diarrhea (PWD), an intestinal disease, leading to

depressed feed intake and growth performance – which can result in high mortality rates among the population.

Additionally, there are many ANF's found within soybeans and SBM that limit its use in the weanling pig's diet. For instance, oligosaccharides found within soybeans are widely considered as an ANF for weanling pigs due to their ability to reduce the pig's growth performance and increase the incidence of PWD. According to recent studies, feeding unprocessed or solvent-extracted SBM to weanling pigs can lead to intestinal, morphological, physiological changes, and a pronounced immune response (Peisker, 2001; Min et. al., 2004; Kim et. al., 2007; Cho et al., 2008). In order to combat the ANF's commonly associated with feeding soy protein to nursery pigs, the soy protein is processed in various ways that aid in the pigs' development. Ingredients such as probiotics, prebiotics, enzymes, minerals, acidifiers, and antibiotics are also commonly added to the diet to aid weanling pigs in the digestion of soy protein (Pettigrew, 2006; Stein, 2006).

Acidifiers & Digestion

Dietary acidifiers (organic and inorganic acids) have recently gained interest within the swine industry due to their cost-effectiveness and ability to abate soy protein's ANF's in the nursey pig's diet and the need to replace antibiotics in swine diets. The addition of dietary acidifiers have been shown to enhance growth performance, stimulate intestinal blood flow, decrease the occurrence of PWD, expedite small intestine development, and improve intestinal morphology, immune system function, feed efficiency, and overall growth in weanling or nursery pigs (Jacela et. al., 2009; Jozefiak et.al., 2004; Galfi and Bokori, 1990; Piva, 2002).

In nursery pigs, efficient soy protein digestion requires the maintenance of a low gastric pH, because a low stomach pH activates proteolytic enzymes, such as pepsin (Kidder et. al., 1978;

Kil et. al., 2011). These acidic conditions play an important role in preventing harmful bacteria from passing into the lower GIT (Maxwell et. al., 1995). An abundance of harmful bacteria within the G.I. tract leads to a high incidence of PWD, resulting in poor protein digestion and growth performance. To overcome these issues correlated with stomach pH in nursery pigs, the supplementation of diet acidifiers is necessary in the pigs' diet. Recent studies have suggested that acidifiers improve nutrient digestion in weanling pigs; ultimately protecting the pig's GIT from pathogenic invasion and proliferation (Ravindran et. al., 1993; Partanen, 1999; Kim et. al., 2005).

Mode of Action

The exact mode of action of acidifiers within the pigs' diet is not fully understood. However, several researchers have proposed that the efficacy of acidifiers on improving growth performance in nursery pigs correlates primarily with a decreased pH in the stomach and lower GIT, modulation of microbial populations, and improvement in nutrient digestion (Ravindran et. al., 1993; Partanen et. al., 1999).

Effects on pH in the GIT

The addition of acidifiers are believed to decrease the diet pH in the nursery pig's stomach in a dose-dependent manner. In addition, the pK_a value of acidifiers, as well as the quantity of other dietary components (mineral supplements high in acid-binding capacity) are believed to affect the overall efficacy of the acidifier (degree of pH reduction within the GIT; Kim et. al., 2005). Several experiments have examined the effects that various acidifiers have on the reduction of the stomach and GIT pH of nursery pigs (Kil, et. al., 2011). Some experiments reported a significant reduction in stomach and GIT pH, while others failed to detect any

significant difference. Within the GIT, Yun (2005) observed a significant pH decrease in the ileum and the cecum by feeding increasing concentrations of acidifiers to nursery pigs, whereas others reported no difference. As a result, the effects of acidifiers on the pH of the GIT appear to vary based on the acidifier being used. Further research needs to be conducted to determine the exact mode of action of acidifiers on stomach and GIT pH, and to determine the effects on pH in the lower GIT.

Effects on Microbial populations

Several studies have determined that low pH in the nursery pig stomach plays an important role in preventing harmful bacteria from invading and proliferating the nursery pig's GIT (Maxwell et. al.,1995; Fuller et. al., 1977). These studies suggest that, by having a low pH in the GIT, preferable conditions are established for the growth of beneficial bacteria that aid in nutrient digestion (Fuller et. al., 1977). Since weanling pigs often have an overgrowth of pathogenic bacteria, and a reduced population of favorable bacteria within the GIT, it is believed that the pig's high stomach pH and increased amount of undigested feed within the lower GIT is the cause for such microbial imbalances (Smith et. al., 1963; Partanen et. al., 1999). As a result, several studies hypothesize that adding various acidifiers to nursery pig diets can ultimately favor the growth of beneficial bacteria, by reducing the amount of harmful bacteria – effectively reducing the pH within the pig's stomach (Partanen et. al., 1999).

In vitro experiments have suggested that the addition of organic acidifiers may have antimicrobial effects, especially on harmful bacteria that is pH-sensitive. These antimicrobial effects were also seen without influencing the growth of beneficial bacteria that is pH-insensitive (Gauthier, 2002). However, other experiments in which dietary acidifiers were used *in vivo* in nursery pigs resulted in few significant benefits for microbial populations within the GIT being

reported. Instead, the addition of various dietary acidifiers resulted in a slight depression of beneficial bacteria in the small intestine and large intestine (Kil et. al., 2010). Limited data makes it extremely difficult to explain the contrasting results related to acidifiers being used *in vitro* and acidifiers being used *in vivo.* Further research needs to be performed to verify the effects that dietary acidifiers have on microbial populations within the nursery pig's GIT.

Effects on Nutrient Digestion

Since dietary acidifiers decrease the stomach pH and increase pepsin activation in the stomach, many researchers have studied whether adding dietary acidifiers to nursery diets improves protein and amino acid digestibility or not. Several studies have additionally studied whether dietary acidifiers delay the passage rate of gastric digesta in to the pig's duodenum, as well as whether it stimulates pancreatic enzyme secretion due to the acidification of the stomach contents. Researchers have suggested that these results are, in fact, true; and that the addition of dietary acidifiers allow further digestion of protein and other nutrients within the pig's GIT (Ravindran et. al., 1993; Partanen et. al., 1999). Several experiments have also used various dietary acidifiers to measure apparent total tract digestibility (ATTD) of protein within nursery pigs. Some studies show results that the inclusion of dietary acidifiers within the diet improved the ATTD of protein by an average of 1% (Kil et. al., 2011). However, the addition of different dietary acidifiers had various ATTD on proteins, suggesting that some acidifiers are more effective than others.

On the other hand, data on the apparent ileal digestibility (AID) of protein and AA's for nursery pigs are limited (Kil et. al., 2011). Results have been inconsistent, with some studies suggesting that the use of dietary acidifiers improve the AID of proteins and AA's (Blank et. al., 1999); whereas, other studies suggest that the use of dietary acidifiers in nursery diets have either

a negative impact, or no impact at all, on the AID of protein and AA's (Gabert et. al., 1995; Gabert et. al., 1995). The reason for the conflicting results is unclear, but it might be related to various differences in sources of protein, dietary levels, and/or other dietary components within the experiments (Blank et. al., 1999).

In addition to protein and AA digestibility, studies have reported that dietary acidifiers have chelating properties towards minerals, and may improve the overall digestibility of several minerals, including Ca and P by 8 to 10% (Ravindran et. al., 1993; Radcliffe et. al., 1998). In growing pigs, the ATTD of Ca and P has been extensively studied, and has been shown to improve when fed various dietary acidifiers (Mroz et. al., 2000; Jongbloed et. al., 2000; Mroz et. al., 2000; Kemme et. al., 1999; Jongbloed et. al., 2000; Sauer et. al., 2009, Buhler et. al. 2010). However, little to no data exists for studying the effects that dietary acidifiers have on the ATTD of Ca and P in nursery pigs.

These findings suggest that more research needs to be done to determine the exact mode of action of dietary acidifiers in the GIT of pigs. In addition, these findings suggest that the mechanism for digestibility may differ between dietary acidifiers. As a result, more research needs to be done to address these issues within nursery pigs.

Effects on Growth Performance

Several studies have elucidated the effects that dietary acidifiers have on nutrient digestibility and microbial population within nursery pigs. As a result, dietary acidifiers have been shown to improve growth performance within these pigs. Acidifiers such as dietary citric acid, fumaric acid, benzoic acid, as well as their salt derivatives, have been shown to significantly improve average daily gain (ADG) and feed efficiency (gain:feed ratio) in weanling pigs (Partanen et. al., 1999; Kil et. al., 2011). However, the most pronounced positive response to dietary acidifiers

have been noted to occur in the immediate post-weaning period, then decrease as the pigs mature (Ravindran et. al., 1993). In previous studies, benzoic acid, was shown to significantly improve ADG by 14.7% and ADFI by 10.8% in the first or second week post-weaning; as well as over the entire experimental period (Halas et. al., 2010; Torrallardona et. al., 2007; Guggenbuhl et. al., 2007). Kluge et. al. (2006) also reported that when benzoic acid is supplemented in the diet, similar improvements are observed, but with a tendency toward dose dependence.

As a result, the concept of including dietary acidifiers in the nursery pig's diet to improve health and growth performance is reasonable since they can lower the pH through diet acidification, can increase nutrient digestibility for proteins and AA's, and can prevent pathogenic bacterial proliferation in the GIT. However, further research needs to be conducted to verify the clear mode(s) of action of acidifiers when relating to growth performance in nursery pigs.

Fermentation of Soybean Meal

SBM is generally fed to nursery pigs due to its overall cost-effectiveness and high protein content. However, SBM contains many ANF's that need to be eliminated in order for nursery pigs to tolerate it well by their GIT. One of the processes that has proven to eliminate ANF's and increase acceptability by nursery pigs is through a process known as fermentation.

Fermented SBM (FSBM) is produced from SBM using various fungal and bacterial strains (predominantly *Aspergillus oryzae* and *Lactobacillus subtilis*, respectively; Mukherjee et. al., 2016). During the fermentation process, large protein, lipid and carbohydrate molecules are broken down to smaller molecules such as peptides, AA, fatty acids and sugars (Kwon et al., 2010). This fermentation process is thought to eliminate residual trypsin inhibitors and some oligosaccharides, (ANF's) within the soybean meal that can decrease pig performance.

Several studies have evaluated and documented the beneficial effects of feeding FSBM to swine and poultry (Feng et. al., 2007; Liu et. al., 2007; Mukherjee et. al., 2015). Studies feeding FSBM to nursery pigs reported that pigs have increased trypsin activity, as well as total protease in the duodenum and jejunum of their GIT. However, no effect was seen in the ileum (Feng et. al., 2007). Several other studies reported that nursery pigs fed increasing amounts of FSBM had improved ADG and G:F by 3.2% and 11.3%, respectively, compared with pigs fed regular SBM (Jones, et. al., 2010; Zhu et. al., 2017). These findings suggest that the fermenting process allows nursery pigs to better absorb and digest nutrients found within the FSBM than regular SBM would allow them to; making FSBM a better option for producers to enhance nursery pig performance, while eliminating ANF's that are commonly associated with regular SBM. However, the ideal inclusion rate of FSBM within the nursery pig diet is not fully understood.

Immune response

Acidifiers and FSBM both have chemical properties that aid to eliminate ANF's found within SBM, while enhancing growth performance and immune responses in nursery pigs. Both additives have the ability to decrease the stomach and GIT pH, enhance digestibility of nutrients, and prevent pathogenic invasions – effectively decreasing the onset of diarrhea within nursery pigs. As a result, pigs fed FSBM exhibited a decreased incidence of diarrhea during weaning and improved blood biochemical parameters and immune function (Zhu et. al., 2017).

Recent studies have examined the effects that dietary acidifiers have on immune function in nursery pigs. One study reported that dietary acidifiers have the ability to alter gut microflora, which is necessary for the development of the immune system (Blum et. al., 2002; Guo et al., 2008). Dietary acidifiers are able to stimulate specific and non-specific immune functions by modulating the composition of intestinal microbiota against harmful organisms (Van der Wielen

et. al., 2000), as well as lower the intestinal pH in order to decrease disease sensitivity. In addition, nursery pig diets containing dietary acidifiers have been found to increase IgG concentration compared to diets that do not contain them (Dibner et. al., 2002) – effectively reducing the incidence of subclinical infections and PWD.

These findings suggest that FSBM and dietary acidifiers are able to alter blood cell characteristics and improve immune function in nursery pigs by means of inhibiting ANF's found within SBM. As a direct result, the incidence of subclinical infections and PWD become drastically reduced. However, the ideal inclusion rate of FSBM within the diet to effectively enhance blood cell characteristics and improve immune function in nursery pigs needs to be further studied.

Scope of Research

Acidifiers, along with fermented soy proteins used in nursery pig diets, have proven to be very beneficial for pig's physiology, histology, immune response, and for maximizing growth performance and efficiency. With acidifiers and fermented soy proteins gaining more notoriety within the swine industry for their positive nutritional effects in pig development, inclusion rates and blood characteristics associated with such processes and additives are discussed in the next chapters. To accomplish this, two experiments were conducted to determine inclusion rate to maximize growth performance (experiment 1 & 2) and analyze complete blood cell characteristics (experiment 2) when nursery pigs were fed sodium butyrate with a moderately complex corn-soybean-meal diet containing benzoic acid. Further examining the inclusion rates, growth performance, and blood cell characteristics in nursery pigs, an additional chapter (experiment 3) has been added, examining the ideal inclusion rate for fermented soybean meal in nursery pig diets.

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Appendix

Figure I: Nutrient composition of soybean and soybean meal (SBM).

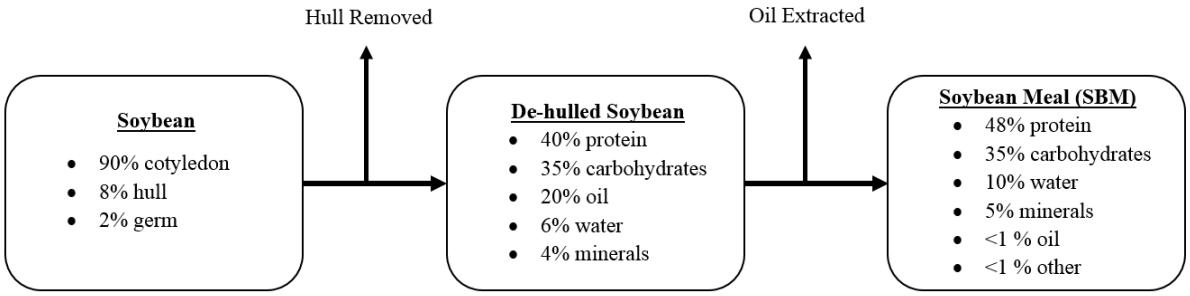

Table I: Nutrient composition (%) of soybeans, soybean meal (SBM), and other soybean products (as-fed basis)

Product	Full-fat soybeans	Dehulled SBM	Non-dehulled SBM	Enzyme Treated SBM	Fermented SBM	Soy Protein Concentrate	Soy protein isolate
Dry Matter	92.36	89.98	88.79	92.7	92.88	92.64	93.71
Crude Protein	37.56	47.73	43.9	55.62	54.07	65.20	84.78
Ether Extract	20.18	1.52	1.24	1.82	2.3	1.05	2.76
Carbohydrates and lignin	29.73	34.46	37.27	28.21	29.53	20.28	2.00
Ash	4.89	6.27	6.38	7.05	6.98	6.11	4.17

[I]Values obtained from NRC (2012).

Table II**:** Concentration of energy (kcal/kg) in soybeans, soybean meal (SBM), and other soybean products (as-fed basis)[I].

Product	Full-fat soybeans	Dehulled SBM	Non-dehulled SBM	Enzyme Treated SBM	Fermented SBM	Soy Protein Concentrate	Soy Protein Isolate
Gross energy	5227	4256	4257	4451	4533	4605	5386
Digestible energy	4193	3619	3681	3914	3975	4260	4150
Metabolizable energy	3938	3294	3382	3536	3607	3817	3573
Net energy	2874	2087	2148	-	-	2376	2187

[I]Values obtained from NRC (2012).

Table III: Crude protein and amino acid concentration and digestibility in soy products (as-fed basis)[I].

	Concentration, %			Standardized ileal digestibility, %		
Product	Full-fat soybeans	44% SBM, non-de-hulled	48% SBM, de-hulled	Full-fat soybeans	44% SBM, non-de-hulled	48% SBM, de-hulled
CP	35.38	43.34	47.26	92.1	84.7	86.9
Indespensable AA						
Arg	2.73	3.26	3.36	94.9	93.5	94.3
His	0.96	1.21	1.21	89.8	89.8	89.8
Ile	1.62	1.98	2.06	87.1	87.1	87.8
Leu	2.71	3.47	3.56	87.9	86.8	89.2
Lys	2.25	2.87	2.98	89.3	88.5	88.9
Met	0.55	0.65	0.68	88.6	89.5	89.0
Phe	1.81	2.26	2.19	89.4	87.4	88.2
Thr	1.41	1.78	1.87	84.7	84.2	84.5
Trp	0.42	0.61	0.65	85.7	85.9	90.4
Val	1.71	2.11	2.12	86.0	84.6	85.8
Dispensable AA						
Ala	1.50	1.99	2.80	91.1	82.5	83.4
Asp	4.00	5.12	5.23	89.7	85.4	85.3
Cys	0.58	0.70	0.68	82.5	82.4	83.0
Glu	6.32	8.07	8.38	90.7	86.1	87.0
Gly	1.52	1.92	1.94	89.2	80.8	81.4
Pro	1.78	2.28	2.27	153.7	112.6	112.8
Ser	1.77	2.27	2.29	88.6	85.8	86.9
Tyr	1.30	1.67	1.70	89.0	88.7	88.8

[I]Values obtained from NRC (2012).

Chapter 2: Effect of Sodium Butyrate (SB) on growth performance and Complete Blood Cell Count in Nursery Pigs

Effect of Sodium Butyrate (SB) on Growth Performance and Complete Blood Cell Count in Nursery Pigs: Two Facility Study

Kristopher A. Bottoms, Tsungcheng Tsai, Casey L. Bradley, Hans H. Stein, Laia Blavi, Hannah Maxwell, Joshua Knapp, Charles V. Maxwell

Abstract:

A total of 344 weaned pigs (21 ± 2 d of age) were used at the University of Arkansas (UA, n = 216) and University of Illinois at Urbana-Champaign (UIUC, n = 128) to evaluate increasing levels of sodium butyrate (SB) on growth performance and complete blood cell count. Pigs at each facility were blocked by initial BW and randomly allotted to 1 of 4 dietary treatments with 9 replications/diet and 6 pigs/pen at UA; and 8 replications/diet and 4 pigs/pen at UIUC. Treatments included a control corn-soybean-meal based diet and 3 diets in which 0.05%, 0.10% or 0.15% SB was added to the control diet. At UA, a negative control diet devoid of BA and SB was also included. Feed was manufactured at each facility. Pigs were fed in 3 phases: 7 d, 14 d, and 14 d at UIUC and 7 d, 14 d, and 19 d at UA for phase 1, 2, and 3, respectively. At UA, blood was collected at the beginning of the experiment and at the end of each phase to determine complete blood cell count. Pen fecal samples were collected at the end of phase 3 at the UA station to determine nutrient digestibility by using titanium dioxide as indigestible marker. Data for growth performance for both facilities were pooled and analyzed as a RCBD; whereby treatment interactions were random effects. Data for nutrient digestibility were analyzed as a RCBD using PROC MIXED procedure of SAS (Cary, NC). Treatment was the fixed effect. Orthogonal contrasts were used to assess linear and quadratic responses to the inclusion of increasing levels of SB in diets. Increasing dietary SB increased weight gain (quadratic, P <

0.05), ADFI (quadratic, $P \leq 0.05$), and final BW (quadratic, $P < 0.05$). Total white blood cell and eosinophil cell count tended to increase with increasing SB (quadratic, $P = 0.07$ and $P = 0.08$, respectively). The lymphocyte cell count tended to decrease (linear, $P = 0.09$) with increasing SB. DM (treatment, $P = 0.01$), NDF (treatment, $P = 0.02$), ADF (treatment, $P = 0.02$), Phosphorus (treatment, $P = 0.01$), Nitrogen (treatment $P = 0.01$), and ash (treatment, $P = 0.01$) were higher in pigs fed SB than other treatments. In addition, absolute butyrate (mM; quadratic, $P = 0.07$) and total VFA (mM; quadratic, $P = 0.08$) increased with increasing levels of SB supplementation. Results indicated that feeding SB during the nursery phase improved growth performance and tended to alter blood cell count.

Keywords: sodium butyrate, growth performance, complete cell count, nursery pig

Introduction

Weaning is a critical period that usually inflicts environmental, nutritional, psychological, and social stress on newborn piglets. Due to a lack of immunity against disease, along with other added stressors such as a change in diet from liquid to solid, this period is often associated with inflicting marked changes in the piglet's gastrointestinal tract (GIT), physiology, histology, microbiology, and immunology (Kelly, D. 1990; Boudry, et. al., 2004; Campbell et. al., 2013). The GIT during weaning undergoes rapid changes in size, protein turnover rate, microbiota count and composition; resulting in quick, extensive alterations in digestive, absorptive, barrier, and immune functions (Pluske, et al., 1997; Lallés, et al., 2007, Hampson, 1986; Smith, et. al., 1984; Hampson, et. al. 1983; Boudry, et. al., 2004). Due to the rapid morphological changes in their GIT, weaned piglets often exhibit a reduction in feed intake—consequently leading to

malnutrition, growth depression, intestinal inflammation, and overall decreased pig performance (Lallés, et. al, 2004; Pié, et. al., 2004).

In order to abate anti-nutritive effects commonly exhibited in early-weaned piglets, acidifiers are commonly added to feed in the early-weaned piglet's diet. Acidifiers improve growth performance in early-weaned piglets, however the magnitude and consistency of the response varies—depending on inclusion rate and other dietary factors (Jacela, et. al, 2009). The exact mode of action of acidifiers has not been fully elucidated; however, acidifiers are commonly marketed as growth-promoting products and as alternatives for in-feed antibiotics. Sodium butyrate (SB) is one of the latest organic acids to be utilized for this purpose. SB has been used as a substrate for metabolism; and has been reported to stimulate intestinal blood flow, expedite small intestine development, improve intestinal morphology, and improve the overall growth of early-weaned pigs (Jozefiak et. al., 2004; Galfi, et. al., 1990; Piva, et. al., 2002). As a result, SB has been proven to serve as an effective acidifier, particularly in less complex nursery diets, and in combination with other acidifiers. These studies were conducted to determine the optimal inclusion rate, and evaluate the effects of increasing levels of sodium butyrate in moderately complex nursery diets on complete blood cell count and growth performance in diets containing benzoic acid as an acidifier.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Arkansas reviewed and approved the protocols for experiment 2 (IACUC #: 18132).

Experiment 1 – University of Illinois - Urbana-Champaign

Animals and Experimental Design

A total of 128 weanling pigs (PIC) were allotted (21 ± 2 d of age) to one of 4 dietary treatments. The pigs were individually weighed and blocked by initial body weight and sex. Pigs remained in the same pens throughout the experiment. Each treatment contained 8 replicate pens per treatments with 4 pigs per pen. A three-phase feeding program was utilized with pigs fed different diets in each of the three phases. Pigs remained on the same dietary treatment throughout the entire study period. Pigs were housed at the University of Illinois, Urbana-Champaign conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment.

Experimental Diets

All feed used for the duration of the study in experiment 1 was manufactured by the UIUC. Each diet was antibiotic-free, contained 0.5% benzoic acid (BA, Vevovitall®, DSM Nutritional Products, Parsippany, NJ), and was formulated without pharmaceutical levels of Cu and Zn. Phase 1 diet was fed for 7 days, phase 2 diet was fed for 14 days, and phase 3 diet was fed for 14 days (Tables 1 & 2). Each diet was formulated to meet the nursery pigs' nutrient requirements (NRC, 2012). During each phase, pigs were fed one of the following dietary treatments: Treatment 1, the control diet (BA), consisted of a moderately complex corn-soybean-meal nursery diet that was devoid of sodium butyrate (SB, Villimax 70 ®, DSM Animal Nutrition). Treaments 2, 3, and 4 were the control diet, but were each supplemented with 0.05% SB, 0.10% SB, and 0.15% SB, respectively.

Sample Collection and Processing

At the start of the study, and at the end of each phase, individual pig weights and pen feed disappearance were measured for each phase to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Experiment 2 – University of Arkansas, Fayetteville

Animals and Experimental Design

A total of 216 (PIC line 26 x 380) weanling pigs were allotted (21 ± 2 d of age) to one of 4 dietary treatments. The pigs were individually weighed and blocked by initial body weight and sex. Pigs remained in the same pens throughout the experiment. Each treatment contained 9 replicate pens per treatments with 6 pigs per pen. A three-phase feeding program was utilized with pigs fed different diets in each of the three phases. Phase 1 (d 0-7) lasted 7 days, phase 2 (d 7-21) lasted 14 days, and phase 3 (d 21-40) lasted 19 days. Pigs remained on the same dietary treatment throughout the entire study period. Pigs were housed in 1.49 x 1.20 M^2 pens at the University of Arkansas conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment. Ambient temperature was set at 85˚F upon pig arrival, and was reduced by two degrees per week until a 75˚F setting for the housing temperature was achieved by the end of the study.

Experimental Diets

All feed used for the duration of the study was manufactured by the UA. Diets were antibiotic-free, and were formulated without pharmaceutical levels of Cu and Zn. The phase 1 diet was fed for 7 days, the phase 2 diet was fed for 14 days, and the phase 3 diet was fed for 19 days (Tables 1 & 2). During phase 1 (Tables 3 & 4), phase 2 (Tables 5 & 6), and phase 3 (Tables

7 & 8), pigs were fed one of the following dietary treatments: Treatment 1 (BA) was a moderately complex corn-soybean-meal nursery diet that was formulated to meet the nursery pigs' nutrient requirements (NRC, 2012). Treatment 1 contained 0.5% benzoic acid (BA, Vevovitall®, DSM Nutritional Products, Parsippany, NJ), an acidifier, and was devoid of sodium butyrate (SB, Villimax®, DSM Nutritional Products, Parsippany, NJ). Treatments 2, 3, and 4 each consisted of the BA diet, which was supplemented with 0.05% SB, 0.10% SB, and 0.15% SB, respectively. Treatment 5, the negative control diet (NC), was the same as treatment one, but was devoid of SB and BA. Titanium dioxide was added in phase 3 diets (d 21-40).

Sample Collection and Processing

At the start of the study, and at the end of each phase, individual pig weights and pen feed disappearance were measured for each phase to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) by phase. Individual pig weights were recorded on d 0, 7, 21, and 40. Feed samples were obtained for each batch of feed mixed. These samples were accumulated for each phase, and were stored in a -20˚C freezer until study completion in order to be subsampled for nutrient analysis. Fecal samples were collected for two consecutive days at the end of the study (d 40), and were stored at -20˚C until study completion to be analyzed for Apparent Total Tract Digestibility (ATTD) of nutrients and volatile fatty acid content.

Samples were analyzed for Fecal Volatile Fatty Acid (VFA) content *via* gas chromatography (Hewlett Packard 5890 Series II Gas Chromatograph, Wilmington, DE) by using 1g of fresh fecal samples. Fecal samples were dried in a drying oven (Shel Lab, Model: SMO28-2, Cornellus, OR) at 55°C; and were then ground through a 2mm screen in a Wiley Mill

Grinder (Arthur H. Thomas, Philadelphia, PA). Ground fecal samples were then dried in an oven (BWR Scientific Gravity oven, Model: 1370 GM, Radnor, PA) at 103°C overnight to determine DM content using AOAC Official Method 930.15 (AOC International, Rockville, MD). Dried, ground fecal and feed samples were ashed in an ashing oven (Thermolyne/Sybron Ashing Oven, Model: FA1938) at 600°C for 8 hrs, and were analyzed for Ash Content (Ash) using the AOAC Official Method 942.05 (AOC International, Rockville, MD). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by batch procedures outlined by the ANKOM Technology Method 13 (ANKOM Technology, Macedon, NY) and the ANKOM Technology Method 12 (ANKOM Technology, Macedon, NY), respectively, using a ANKOM 200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY). Nitrogen (N) content was determined *via* Dumas Combustion Method, and was analyzed with a CHN-analyzer (Na-2000 N-Protein, Fisons Instruments S.p.A., Rodano [MI], Italy). Gross Energy (GE) was analyzed *via* rapid combustion procedure using a calorimeter (Parr 6200 Calorimeter, Moline, Illinois). Mineral content (Calcium & Phosphorus) was determined using methods established by Jones et. al., 1990. Digestion was conducted on an Environmental Express Hot Block (Charleston, SC); and the resulting digestate was analyzed on an Inductively Coupled Plasma Atomic Emission Spectrophotometer (Spectro Arcos 160 SOP, Model: FHS16, Kleve, Germany).

Nutrient Digestibility (University of Arkansas – Fayetteville)

Fecal samples from each pen were collected one day before the end of the study in phase 3 in order to evaluate nutrient digestibility. Fecal samples were stored in a -20°C freezer prior to analysis. Nutrient Digestibility was analyzed by detecting Titanium Dioxide (TiO_2) within feed and fecal samples by using methods established by Short et. al., 1996 and analysis *via* spectrometer (Synergy™ HTX Multi-Mode Microplate Reader, Biotek, Winooski, VT).

Feed samples were obtained for each batch of feed mixed, and was stored in a -20°C freezer until study completion. Concentration of GE, CP, calcium, and phosphorus was determined from both diets and fecal samples to calculate apparent total tract digestibility (ATTD) of nutrients. ATTD for each nutrient was analyzed using the equation established by Miller et. al., 1990:

$$\%\ \text{Digestibility of nutrient} = 100 - 100 * \frac{\%\ \text{Indicator in feed x}\ \%\ \text{nutrient in feces}}{\%\ \text{Indicator in feed x}\ \%\ \text{Nutrient in feed}}$$

Leukocyte Differentiation (University of Arkansas - Fayetteville):

At the University of Arkansas (UA), blood was collected at the beginning of the experiment and at the end of each phase to determine complete blood cell count. On d 0, 7, 21 and 40 of the study, the piglet with the closest-pen-average-BW from each pen was selected, and an attempt was made to select the same gender pig within blocks. Blood samples (n=40) were collected via jugular vena puncture into a 10 mL K2-EDTA vacutainer tube (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) for leukocyte differential analysis. Whole blood samples were removed from ice and allowed to equilibrate to room temperature before leukocyte differential determination, and were centrifuged prior to analysis (Beckman Coulter Centrifuge, Model: ALLEGRA-6R, Indianapolis, IN). Blood samples were analyzed by a blood hematologic system (Hemavet 950 FS, Drew Scientific, Waterbury, CT). All samples were analyzed within 6-12 hours after collection.

Statistical Analysis

Performance data was analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Dietary treatment was the lone fixed effect, blocks based on initial BW

was the random effect, and pen served as the experimental unit for ANOVA. The level of sodium butyrate was used in IML procedure to generate coefficients for orthogonal contrast for BA and treatments 2, 3, and 4. Orthogonal contrasts were also used to determine the linear, quadratic, and cubic effects of various levels of SB on growth performance. Probability values were considered statistically significant at $P < 0.05$, and $0.05 < P < 0.10$ considered a statistical trend.

Results

Experiment 1 (University of Illinois – Urbana-Champaign & University of Arkansas – Fayetteville)

Growth Performance

Data from both the University of Illinois – Urbana Champaign (UIUC, Experiment 1) and the University of Arkansas – Fayetteville (UA, Experiment 2) were pooled and station alone with station by treatment interaction were coded as random effects for statistical analysis. Growth performance in experiment 2 at the UA was not good during phase 1 due to a diarrheal (*Esche*richia coli) outbreak. All pigs at the UA were treated with a water delivery antibiotic (Aureomycin) for one week.

Pigs fed increasing levels of SB tended to increase BW on d 7 (Quad P = 0.07), d 21 (Quad P = 0.04), and d 40 (Quad P = 0.02); with the heaviest BW group appearing in pigs fed 0.05% SB (Table 9, Figure 1). When feeding increasing dosages of SB to pigs, we observed a quadratic improvement on ADG on d 21-40 (phase 3, P = 0.04), d 0-21 (Phase 1 & 2, P = 0.04), and d 0-40 (overall, P = 0.02); with pigs fed 0.05% SB gaining the most weight (Tables 9 & 11, Figure 2).

Similar to ADG, feed intake increased quadratically on d 0-7 (Phase 1, P = 0.05), d 7-21 (Phase 2, P = 0.04), d 21-40 (Phase 3, P = 0.05), d 0-21 (Phase 1 & 2, P = 0.03), and d 0-40 (overall, P = 0.02). Again, pigs fed 0.05% SB resulted in the most feed consumption (Table 10, Figure 3). As for G:F ratio, the impact of adding SB on feed efficiency was not significant across phases. Only in phases 1 & 2 did we observe a linear tendency for increased G:F ratio in pigs fed increasing levels of SB (Table 10, Figure 4).

Experiment 2 (University of Arkansas – Fayetteville)

Growth Performance

This study is being presented separately, since a negative control (NC) devoid of benzoic acid was included. Growth performance was not good in phase 1 & 2 due to a diarrheal (*Escherichia coli)* outbreak. Starting on d 21 post-weaning, all pigs were treated with water delivery antibiotic (Aureomycin) for one week. Pigs fed BA had reduced BW compared to NC fed pigs (Table 11, Figure 5) on d 7 (BA vs NC, P < 0.01) and on d 14 (BA vs NC, P = 0.06). Pigs fed BA also had a lower ADG (BA vs NC, P = 0.01) on d 0-7 (Table 11, Figure 6). This reduction in BW was substantially reduced at the end of the study (BA vs NC, P = 0.95) due to numerically greater ADG in pigs fed BA from d 21-40 than NC fed pigs (0.524 vs. 0.495 kg, BA vs NC, P = 0.16, Table 12).

Pigs fed increasing levels of SB lost less weight than BA fed pigs from d 0-7 (P = 0.01), but tended to increase ADG linearly on d 7-21 (Linear SB effect, P = 0.08, Table 11, Figure 6). The overall effect from d 0-21 was a linear increase in ADG (Linear SB effect, P = 0.04, Table 11, Figure 6). With this change in ADG, BW increased from 0% SB fed pigs to reach a plateau

in pigs fed 0.05% SB on d 7 (Quad. P = 0.09, Table 11, Figure 5), while a linear increase in BW was observed on d 21 (P = 0.02, Table 11).

Pigs fed BA had reduced intake on d 0-7 (BA vs NC, P = 0.01), and d 7-21 (BA vs NC, P = 0.06) when compared to NC fed pigs (Table 12, Figure 7). A quadratic increase in intake was observed from d 0-7 (P = 0.05), d 0-21 (P = 0.09), d 21-40 (P = 0.08) and d 0-40 (P = 0.05) with increasing levels of SB fed to pigs (Table 12, Figure 7). Pigs fed 0.05% SB were consistently shown to have the highest intake when compared to other treatments. As for feed efficiency, pigs fed increasing levels of SB showed a linear increase in G:F ratio on d 0-21 (Table 12, Figure 8).

Complete Blood Cell Count (CBC)

According to CBC results, pigs fed BA had a higher monocyte count (P = 0.07; fraction over WBC, P = 0.09), MCV (P = 0.01), MCH (P < 0.01), and MCHC (P = 0.04) than NC fed pigs (Table 13, Figure 9). A quadratic response was observed on WBC (P = 0.07), neutrophil (P = 0.10), and eosinophil (P = 0.08) concentration when pigs were fed increasing levels of SB (Table 13, Figures 9 & 10); while a linear reduction was observed in lymphocyte (Table 13, Figure 9), MCHC (Table 13, Figure 11) and platelet (Table 13, Figure 12) concentration. MCV was reduced in all treatments from d 0-7, and started to elevate from d 7-40 (Table 14, Figure 13). The increase in magnitude for MCV from d 7-40 was lower in NC fed pigs than all other treatments (Figure 13, treatments by day interaction, P = 0.03).

Digestibility & Volatile Fatty Acid Content

Similar to G:F ratio, pigs fed the SB diet were shown to have higher ATTD of DM, GE, N, ash, NDF, ADF, and P than pigs fed other dietary treatments (Table 15). In the feces

examined, absolute butyrate (mM) and total VFA (mM) increased quadratically with increasing levels of SB supplementation (Table 16).

Discussion

These studies demonstrate that increasing the inclusion rate of SB in the nursery diets that include BA has the potential to improve growth performance (ADG, ADFI, BW) and alter blood cell characteristics. Several recent studies have also suggested that the addition of SB in diets promoted the growth of nursery or weanling pigs (Galfi et. al., 1990; Piva et. al., 2002; Kotunia et. al., 2004; Lu et. al., 2007). Pigs fed increasing levels of SB exhibited an increase in BW and ADFI during the overall study; with the heaviest BW and ADFI group appearing in pigs fed 0.05% SB. Pigs fed increasing levels of SB showed an increase in G:F on d 0-21 over pigs fed the control diet.

However, the addition of BA during the early weaning phase reduced ADG, ADFI, BW, and G:F ratio during early weaning. Continuing the feeding of BA in subsequent phases resulted in improved growth and significantly higher G:F ratio on d 21-40. Other researchers have suggested that feeding nursery pigs diets with 0.5% BA significantly improves ADG and ADFI in the first or second week of weaning (Halas et. al., 2010), suggesting that time of introducing BA in nursery pig diets needs to be further examined. In addition, very few experiments have been conducted to examine the period that nursery pigs should be supplemented with BA to improve growth performance. However, one recent study suggests that pre-weaning SB supplementation is the most efficient period to stimulate body growth and feed intake after weaning (Le Gall et. al., 2009).

Similar to G:F ratio, pigs fed the SB diet had higher ATTD of DM, GE, N, ash, NDF, ADF, and P than pigs fed control treatments, suggesting that SB can improve overall digestibility

in nursery pigs. However, other researchers suggest that dietary acidifiers have no effect or depress growth performance in nursery pigs (Kil et. al., 2011). Digestibility and VFA results suggest that the mechanism on improving growth performance differs among acidifiers. Some studies have suggested that acidifiers' mode of action correlates with improvement in nutrient digestion, whereas other researchers have speculated upon other possible mechanisms (Partanen et. al., 1999; Kil et. al., 2011; Ravindran et. al., 1993). Researchers have proposed the alternative hypothesis that organic acidifiers may stimulate the intermediary metabolism of nutrients within nursery pigs – ultimately leading to improved nutrient utilization and energy; and the hypothesis that organic acidifiers may serve as immediate energy sources for intestinal epithelial cells (Ravindran et. al., 1993; Partanen et. al., 1999). However, little experimental evidence supports these mechanisms within pigs. As a result, further research needs to be done to determine the mechanism of SB acting as an acidifier in nursery pigs.

As for CBC, pigs fed BA had a higher monocyte, MCV, and MCHC value than pigs fed the NC diet. When pigs were fed increasing levels of SB, an increased response was seen on WBC, neutrophil, and eosinophil concentration; whereas a reduction was observed in lymphocyte, MCHC, and platelet concentration. These findings suggest that SB has a positive effect on altering blood characteristics that can modify the immune system and in nursery pigs. A recent study supports this finding by suggesting that SB and other organic acidifiers can modify immune system characteristics by lowering the secretion of immune-response mediators, which can reduce the incidence of subclinical infections in monogastric animals (Dibner et. al., 2002).

Conclusion

Together with CBC results, we conclude that pigs fed a low dosage of sodium butyrate, in combination with benzoic acid, can improve growth performance and alter blood cell characteristics that affect immune function in nursery pigs. However, the period of introducing BA and SB, and the mechanism in which they act upon in nursery pigs needs to be further studied.

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Appendix

Table 1. Experimental diet composition by phase (University of Illinois - Urbana-Champaign & University of Arkansas – Fayetteville).

	University of Illinois – Urbana-Champaign			University of Arkansas - Fayetteville		
Ingredients	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 1**	**Phase 2**	**Phase 3**
Corn[1], %	29.02	31.42	49.53	29.90	32.18	49.88
DDGS, %	5.00	15.00	15.00	5.00	15.00	15.00
Dried Whey, %	8.00	4.00	0.00	8.00	4.00	0.00
Soybean meal, %	22.65	28.05	29.30	22.65	28.05	29.30
Oats, %	15.00	12.50	0.00	15.00	12.50	0.00
Fish meal, %	5.00	3.15	0.00	5.00	3.15	0.00
Lactose, %	0.25	0.00	0.00	0.25	0.00	0.00
Enzymatic SBM, %	9.50	0.00	0.00	9.50	0.00	0.00
Soybean oil, %	2.50	2.50	2.50	2.50	2.50	2.50
Benzoic Acid[2], %	0.50	0.50	0.50	0.50	0.50	0.50
Other[3], %	2.58	2.95	3.24	2.58	2.95	3.24

[1]Sodium butyrate (0.05%, 0.1%, 0.15%) was added to phase 1, 2, 3 respectively.
[2]Benzoic Acid = VevoVitall® (DSM Nutritional Products, Parsippany, NJ)
[3]Other contained: limestone, monocalcium phosphate, trace minerals, vitamins, amino acids, and phytase.
* Diets were antibiotic free, and were formulated without pharmaceutical levels of zinc and copper.

Table 2. Experimental diets calculated analysis by phase (University of Illinois - Urbana-Champaign & University of Arkansas – Fayetteville).

	University of Illinois, Urbana-Champaign			University of Arkansas, Fayetteville		
Calculated Analysis	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 1**	**Phase 2**	**Phase 3**
ME (kcal/kg)	3274	3233	3451	3455	3429	3402
CP (%)	25.62	23.94	22.17	26.50	25.03	22.84
SID Lysine (%)	1.5	1.35	1.23	1.46	1.42	1.28
Available P (%)	0.45	0.4	0.33	0.41	0.30	0.22
Ca (%)	0.85	0.8	0.7	0.76	0.66	0.56
SID M+C:Lys	-	-	-	58.07	58.09	58.00
SID Thr:Lys	-	-	-	60.00	60.09	60.00
SID Trp:Lys	-	-	-	19.20	18.06	17.26

*Sodium butyrate (0.05%, 0.1%, 0.15%) was added to phase 1, 2, 3 respectively. 0.50% Benzoic Acid was added to phases 1, 2, and 3. Diets were antibiotic free, and were formulated without pharmaceutical levels of zinc and copper.

Table 3. Nursery phase 1 diet composition (University of Arkansas – Fayetteville).

Ingredients, %	Treatments				
	Negative Control (NC)	**0.5% Benzoic Acid (BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
Corn, Yellow Dent	30.400	29.900	29.850	29.800	29.750
Soybean meal, 48%	22.650	22.650	22.650	22.650	22.650
Corn DDGS, >6 and <9% Oil	5.000	5.000	5.000	5.000	5.000
Poultry Fat	2.500	2.500	2.500	2.500	2.500
Hamlet 300	9.500	9.500	9.500	9.500	9.500
Monocalcium P	0.300	0.300	0.300	0.300	0.300
Limestone	0.400	0.400	0.400	0.400	0.400
Salt	0.250	0.250	0.250	0.250	0.250
L-Lysine	0.170	0.170	0.170	0.170	0.170
DL-Methionine	0.108	0.108	0.108	0.108	0.108
L-Threonine	0.020	0.020	0.020	0.020	0.020
Whey (NSNG)	8.000	8.000	8.000	8.000	8.000
Lactose(NSNG)	0.250	0.250	0.250	0.250	0.250
Oat groat	15.000	15.000	15.000	15.000	15.000
ZnO	0.013	0.013	0.013	0.013	0.013
Vitamin Premix (NB-6508)[1]	0.250	0.250	0.250	0.250	0.250
Trace Mineral Premix (NB-8534)[2]	0.150	0.150	0.150	0.150	0.150
Phytase[3]	0.015	0.015	0.015	0.015	0.015
Benzoic acid[4]	0.000	0.500	0.500	0.500	0.500
Sodium Butyrate[5]	0.000	0.000	0.050	0.100	0.150
Ethoxiquin (Quinguard)	0.030	0.030	0.030	0.030	0.030
Menhaden Meal	5.000	5.000	5.000	5.000	5.000

[1]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca as $CaCO_3$, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[2]The mineral premix provided the following per kg of complete diet: 84 mg of Ca as $CaCO_3$, 165 mg of Fe as $FeSO_4$, 165 mg of Zn as $ZnSO_4$, 39.6 mg of Mn as $MnSO_4$, 16.5 mg of Cu as $CuSO_4$, 0.3 mg of I as CaI_2, and 0.3 mg of Se as Na_2SeO_3.

[3]Phytase = Ronozyme HiPhos 2700 (GT).

[4]Benzoic Acid = VevoVitall ® (DSM Nutritional Products, Parsippany, NJ).

[5]Sodium Butyrate = Villimax ® (DSM Nutritional Products, Parsippany, NJ).

Table 4. Nursery phase 1 diet calculated analysis (University of Arkansas – Fayetteville).

	Treatments				
Calculated Analysis:	**Negative Control (NC)**	**0.5%Benzoic Acid (BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
ME (kcal/kg)	3454.700	3453.000	3451.300	3449.600	3471.700
CP (%)	26.500	26.500	26.500	26.490	26.550
SID Lysine (%)	1.460	1.460	1.460	1.460	1.460
Total P (%)	0.680	0.680	0.680	0.680	0.680
Available P (%)	0.410	0.410	0.410	0.410	0.410
Aval. P (%) with Phytase	0.520	0.520	0.520	0.520	0.520
Ca (%)	0.761	0.761	0.761	0.761	0.761
Zinc(ppm)	289.600	289.600	289.600	289.600	289.700
Copper(ppm)	25.300	25.300	25.300	25.300	25.320
SID Lysine/Mcal ME	4.230	4.240	4.240	4.240	4.220
SID M+C:Lys	58.070	58.070	58.060	58.050	58.140
SID Thr:Lys	60.000	60.000	60.000	59.990	60.040
SID Trp:Lys	19.200	19.200	19.200	19.200	19.210
SID Ile:Lys	67.840	67.840	67.830	67.830	67.870
SID Val:Lys	74.030	74.020	74.020	74.010	74.090
SID Leu:Lys	128.870	128.850	128.830	128.800	129.070
SID His:Lys	41.050	41.040	41.040	41.030	41.090

Table 5. Nursery phase 2 diet composition (University of Arkansas - Fayetteville).

Ingredients, %	Treatments				
	Negative Control (NC)	**0.5% Benzoic Acid (BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
Corn, Yellow Dent	32.680	32.180	32.130	32.080	32.030
Soybean meal, 48%	28.050	28.050	28.050	28.050	28.050
Corn DDGS, >6 and <9% Oil	15.000	15.000	15.000	15.000	15.000
Poultry Fat	2.500	2.500	2.500	2.500	2.500
Monocalcium P	0.105	0.105	0.105	0.105	0.105
Limestone	0.550	0.550	0.550	0.550	0.550
Salt	0.350	0.350	0.350	0.350	0.350
L-Lysine	0.400	0.400	0.400	0.400	0.400
DL-Methionine	0.144	0.144	0.144	0.144	0.144
L-Threonine	0.098	0.098	0.098	0.098	0.098
L-Tryptophan	0.014	0.014	0.014	0.014	0.014
Whey (NSNG)	4.000	4.000	4.000	4.000	4.000
Oat groat	12.500	12.500	12.500	12.500	12.500
ZnO	0.013	0.013	0.013	0.013	0.013
Vitamin Premix (NB-6508)[1]	0.250	0.250	0.250	0.250	0.250
Trace Mineral Premix (NB-8534)[2]	0.150	0.150	0.150	0.150	0.150
Phytase[3]	0.015	0.015	0.015	0.015	0.015
Benzoic Acid[4]	0.000	0.500	0.500	0.500	0.500
Sodium Butyrate[5]	0.000	0.000	0.050	0.100	0.150
Ethoxiquin (Quinguard)	0.030	0.030	0.030	0.030	0.030
Menhaden Meal	3.150	3.150	3.150	3.150	3.150

[1]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca as $CaCO_3$, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[2]The mineral premix provided the following per kg of complete diet: 84 mg of Ca as $CaCO_3$, 165 mg of Fe as $FeSO_4$, 165 mg of Zn as $ZnSO_4$, 39.6 mg of Mn as $MnSO_4$, 16.5 mg of Cu as $CuSO_4$, 0.3 mg of I as CaI_2, and 0.3 mg of Se as Na_2SeO_3.

[3]Phytase = Ronozyme HiPhos 2700 (GT).

[4]Benzoic Acid = VevoVitall ® (DSM Nutritional Products, Parsippany, NJ).

[5]Sodium Butyrate = Villimax ® (DSM Nutritional Products, Parsippany, NJ).

Table 6. Nursery phase 2 diet calculated analysis (University of Arkansas – Fayetteville).

Calculated Analysis:	Treatments				
	Negative Control (NC)	**0.5% Benzoic Acid (BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
ME (kcal/kg)	3428.500	3426.800	3425.100	3423.400	3445.500
CP (%)	25.030	25.020	25.020	25.010	25.070
SID Lysine (%)	1.420	1.420	1.420	1.420	1.430
Total P (%)	0.570	0.570	0.570	0.570	0.570
Available P (%)	0.300	0.300	0.300	0.300	0.300
Aval. P (%) with Phytase	0.410	0.410	0.410	0.410	0.410
Ca (%)	0.663	0.663	0.663	0.663	0.663
Zinc(ppm)	288.800	288.800	288.800	288.800	288.900
Copper(ppm)	24.150	24.150	24.150	24.150	24.170
SID Lysine/Mcal ME	4.150	4.160	4.160	4.160	4.140
SID M+C:Lys	58.090	58.080	58.070	58.070	58.160
SID Thr:Lys	60.090	60.090	60.080	60.080	60.130
SID Trp:Lys	18.060	18.060	18.060	18.060	18.070
SID Ile:Lys	62.240	62.240	62.230	62.230	62.280
SID Val:Lys	69.220	69.210	69.210	69.200	69.290
SID Leu:Lys	129.790	129.770	129.750	129.730	130.000
SID His:Lys	39.270	39.260	39.260	39.260	39.310

Table 7. Nursery phase 3 diet composition (University of Arkansas - Fayetteville).

	Treatments				
Ingredients, %	**Negative Control (NC)**	**0.5% Benzoic Acid(BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
Corn, Yellow Dent	49.880	49.830	49.780	49.730	50.380
Soybean meal, 48%	29.300	29.300	29.300	29.300	29.300
Corn DDGS, >6 and <9% Oil	15.000	15.000	15.000	15.000	15.000
Poultry Fat	2.500	2.500	2.500	2.500	2.500
Monocalcium P	0.240	0.240	0.240	0.240	0.240
Limestone	0.708	0.708	0.708	0.708	0.708
Salt	0.475	0.475	0.475	0.475	0.475
L-Lysine	0.425	0.425	0.425	0.425	0.425
DL-Methionine	0.130	0.130	0.130	0.130	0.130
L-Threonine	0.094	0.094	0.094	0.094	0.094
Copper Sulfate	0.004	0.004	0.004	0.004	0.004
Vitamin Premix (NB-6508)[1]	0.250	0.250	0.250	0.250	0.250
Trace Mineral Premix (NB-8534)[2]	0.150	0.150	0.150	0.150	0.150
Phytase[3]	0.015	0.015	0.015	0.015	0.015
Benzoic Acid[4]	0.500	0.500	0.500	0.500	0.000
Sodium Butyrate[5]	0.000	0.000	0.500	0.100	0.150
Ethoxiquin (Quinguard)	0.030	0.030	0.030	0.030	0.030
TiO2	0.300	0.300	0.300	0.300	0.300

[1]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca as $CaCO_3$, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[2]The mineral premix provided the following per kg of complete diet: 84 mg of Ca as $CaCO_3$, 165 mg of Fe as $FeSO_4$, 165 mg of Zn as $ZnSO_4$, 39.6 mg of Mn as $MnSO_4$, 16.5 mg of Cu as $CuSO_4$, 0.3 mg of I as CaI_2, and 0.3 mg of Se as Na_2SeO_3.

[3]Phytase = Ronozyme HiPhos 2700 (GT).

[4]Benzoic Acid = VevoVitall ® (DSM Nutritional Products, Parsippany, NJ).

[5]Sodium Butyrate = Villimax ® (DSM Nutritional Products, Parsippany, NJ).

Table 8. Nursery phase 3 diet calculated analysis (University of Arkansas – Fayetteville).

Calculated Analysis:	Treatments				
	Negative Control (NC)	**0.5% Benzoic Acid (BA)**	**BA + 0.5% SB**	**BA + 0.10% SB**	**BA + 0.15% SB**
ME (kcal/kg)	3402.000	3400.300	3398.600	3396.900	3418.900
CP (%)	22.840	22.840	22.840	22.830	22.890
SID Lysine (%)	1.280	1.280	1.280	1.280	1.280
Total P (%)	0.480	0.480	0.480	0.480	0.480
Available P (%)	0.220	0.220	0.220	0.220	0.220
Aval. P (%) with Phytase	0.320	0.320	0.320	0.320	0.320
Ca (%)	0.563	0.563	0.563	0.563	0.564
Zinc(ppm)	195.900	195.900	195.900	195.900	196.000
Copper(ppm)	32.360	32.360	32.360	32.350	32.380
SID Lysine/Mcal ME	3.770	3.770	3.770	3.770	3.750
SID M+C:Lys	58.000	57.990	57.980	57.980	58.070
SID Thr:Lys	60.000	59.990	59.990	59.980	60.040
SID Trp:Lys	17.260	17.260	17.260	17.260	17.270
SID Ile:Lys	61.920	61.910	61.910	61.900	61.960
SID Val:Lys	68.690	68.690	68.680	68.670	68.770
SID Leu:Lys	137.310	137.290	137.260	137.240	137.530
SID His:Lys	40.850	40.850	40.840	40.840	40.900

Table 9. Effect of adding sodium butyrate (SB) on BW and ADG in nursery pigs (LS means; University of Illinois – Urbana - Champaign & University of Arkansas – Fayetteville pooled results).

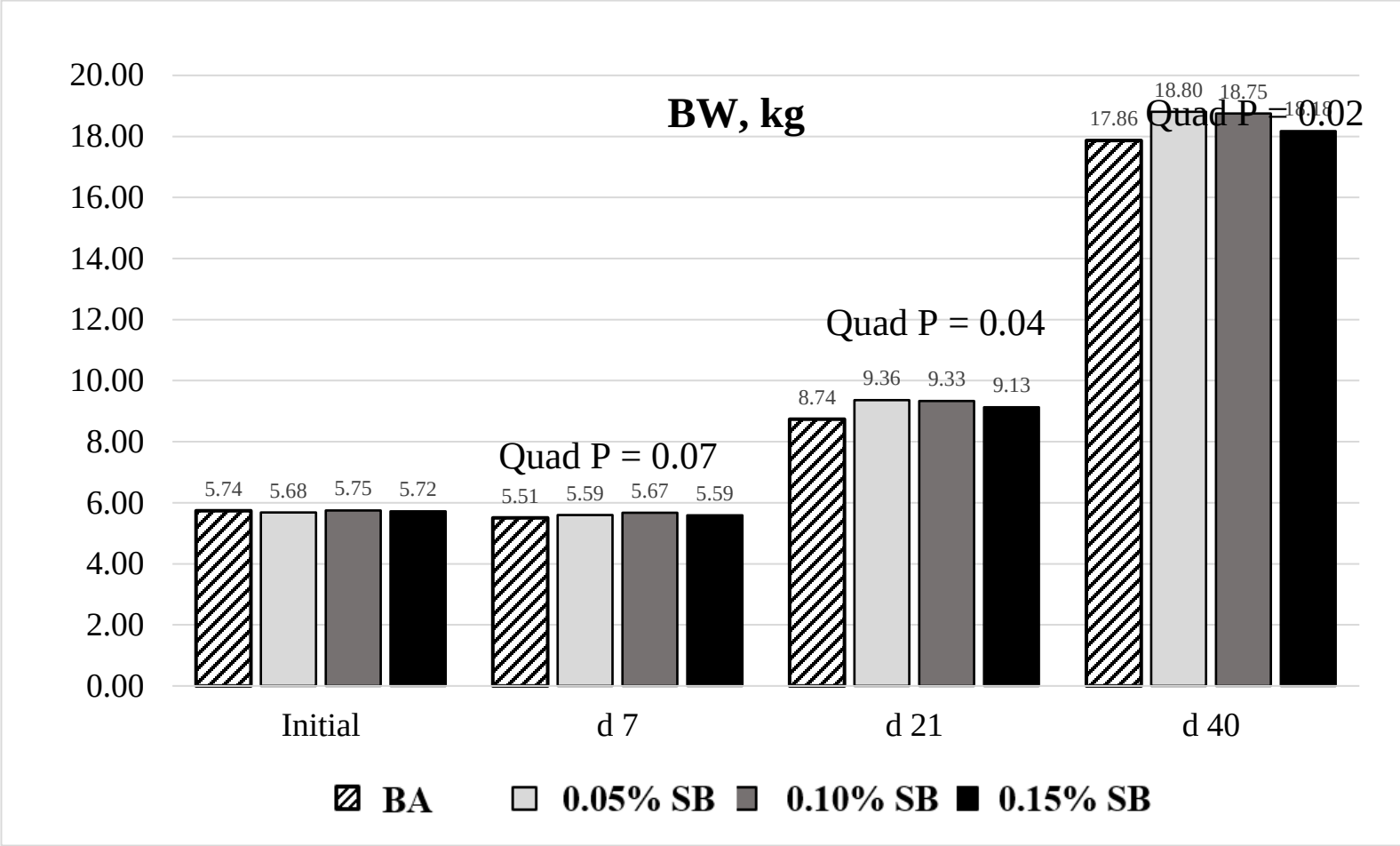
	Treatment							
	BA	**0.05% SB**	**0.1% SB**	**0.15% SB**	**SEM**	**Trt**	**Linear SB**	**Quad SB**
BW, kg								
d 0	5.739	5.678	5.747	5.720	0.315	0.416	0.926	0.595
d 7	5.506	5.595	5.674	5.589	0.352	0.107	0.123	0.070
d 21	8.741	9.360	9.333	9.126	0.418	0.119	0.212	0.044
d 40	17.863	18.804	18.753	18.179	0.437	0.120	0.533	0.022
ADG, kg								
d 0-7	-0.033	-0.016	-0.010	-0.019	0.010	0.195	0.160	0.098
d 7-21	0.239	0.277	0.271	0.259	0.014	0.030	0.370	0.068
d 21-40	0.531	0.553	0.553	0.525	0.014	0.220	0.746	0.040
d 0-21	0.147	0.179	0.175	0.165	0.010	0.123	0.246	0.043
d 0-40	0.322	0.349	0.347	0.331	0.009	0.112	0.551	0.020

Data from University of Arkansas and University of Illinois were combined, and station as well as station x treatments interaction were included as random effect for statistical analysis using MIXED procedure of SAS. IML procedure was requested to generate parameter which later being used in orthogonal contrast for dosage response of sodium butyrate (SB). Due to lack of station by treatment interaction effect, only treatment effects were presented.
Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Table 10. Effect of adding sodium butyrate (SB) on ADFI and G:F ratio in nursery pigs (LS means; University of Illinois – Urbana - Champaign & University of Arkansas – Fayetteville pooled results).

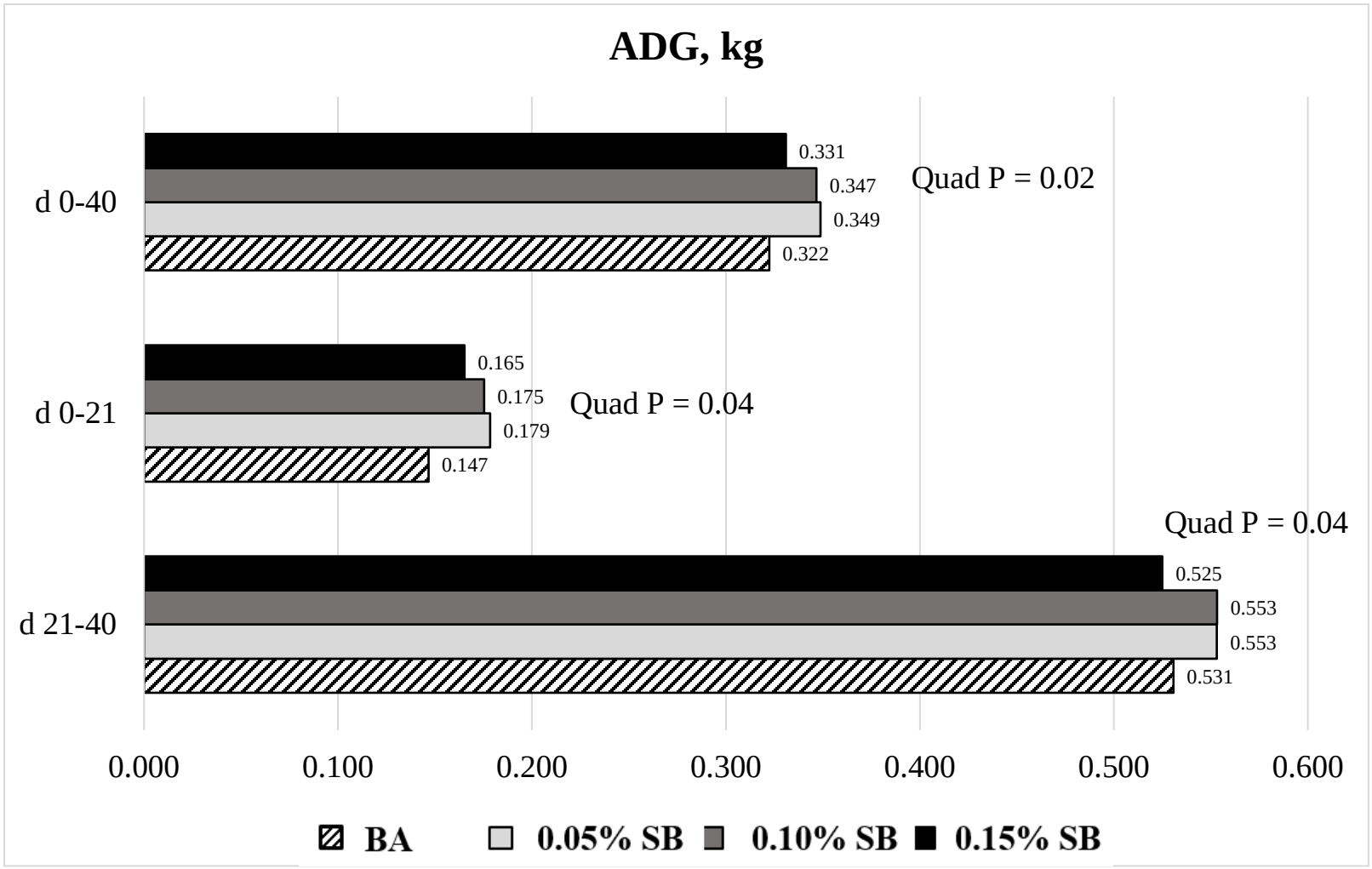
	Treatment							
	BA	**0.05% SB**	**0.1% SB**	**0.15% SB**	**SEM**	**Trt**	**Linear SB**	**Quad SB**
ADFI, kg								
d 0-7	0.073	0.095	0.086	0.083	0.006	0.1021	0.464	0.0501
d 7-21	0.335	0.370	0.371	0.349	0.019	0.1697	0.4655	0.0357
d 21-40	0.802	0.842	0.836	0.801	0.022	0.2596	0.9096	0.0489
d 0-21	0.245	0.276	0.273	0.258	0.013	0.1265	0.4334	0.0277
d 0-40	0.497	0.533	0.528	0.504	0.014	0.136	0.7673	0.0224
G:F								
d 0-7	-0.749	-0.237	-0.210	-0.311	0.187	0.0909	0.0781	0.0721
d 7-21	0.709	0.739	0.738	0.746	0.030	0.754	0.3488	0.6705
d 21-40	0.666	0.655	0.665	0.659	0.016	0.9551	0.8484	0.8798
d 0-21	0.214	0.246	0.244	0.244	0.015	0.2049	0.1071	0.2089
d 0-40	0.436	0.446	0.450	0.447	0.011	0.7934	0.4228	0.5414

Data from University of Arkansas and University of Illinois were combined, and station as well as station x treatments interaction were included as random effect for statistical analysis using MIXED procedure of SAS. IML procedure was requested to generate parameter which later being used in orthogonal contrast for dosage response of sodium butyrate (SB). Due to lack of station by treatment interaction effect, only treatment effects were presented.
Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$



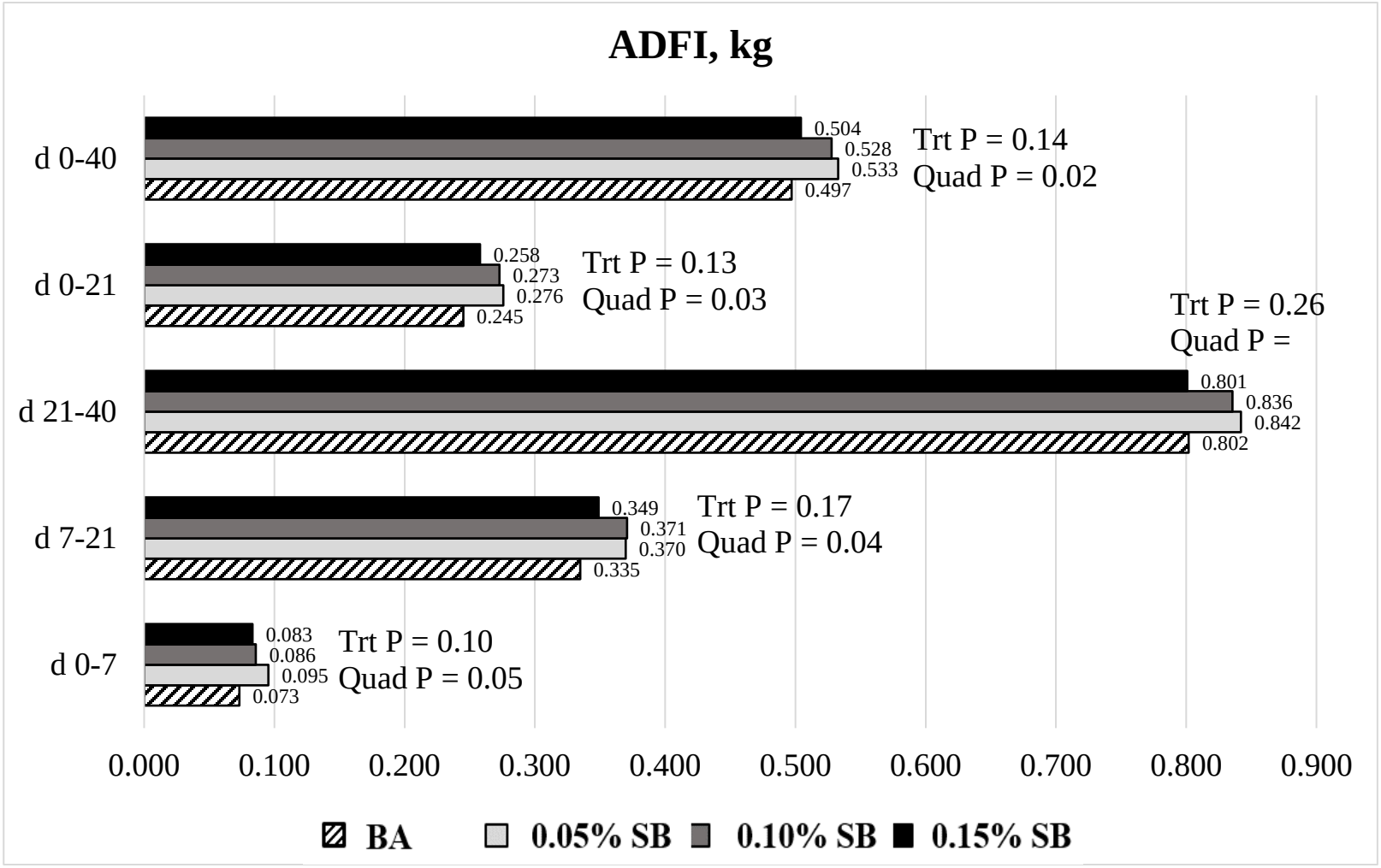
Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$
*Due to no treatments x facility interaction being observed, only contrast results from treatment effect were reported.

Figure 1. Effect of adding sodium butyrate (SB) on BW in nursery pigs (LS means; University of Illinois – Urbana -Champaign & University of Arkansas – Fayetteville pooled results).



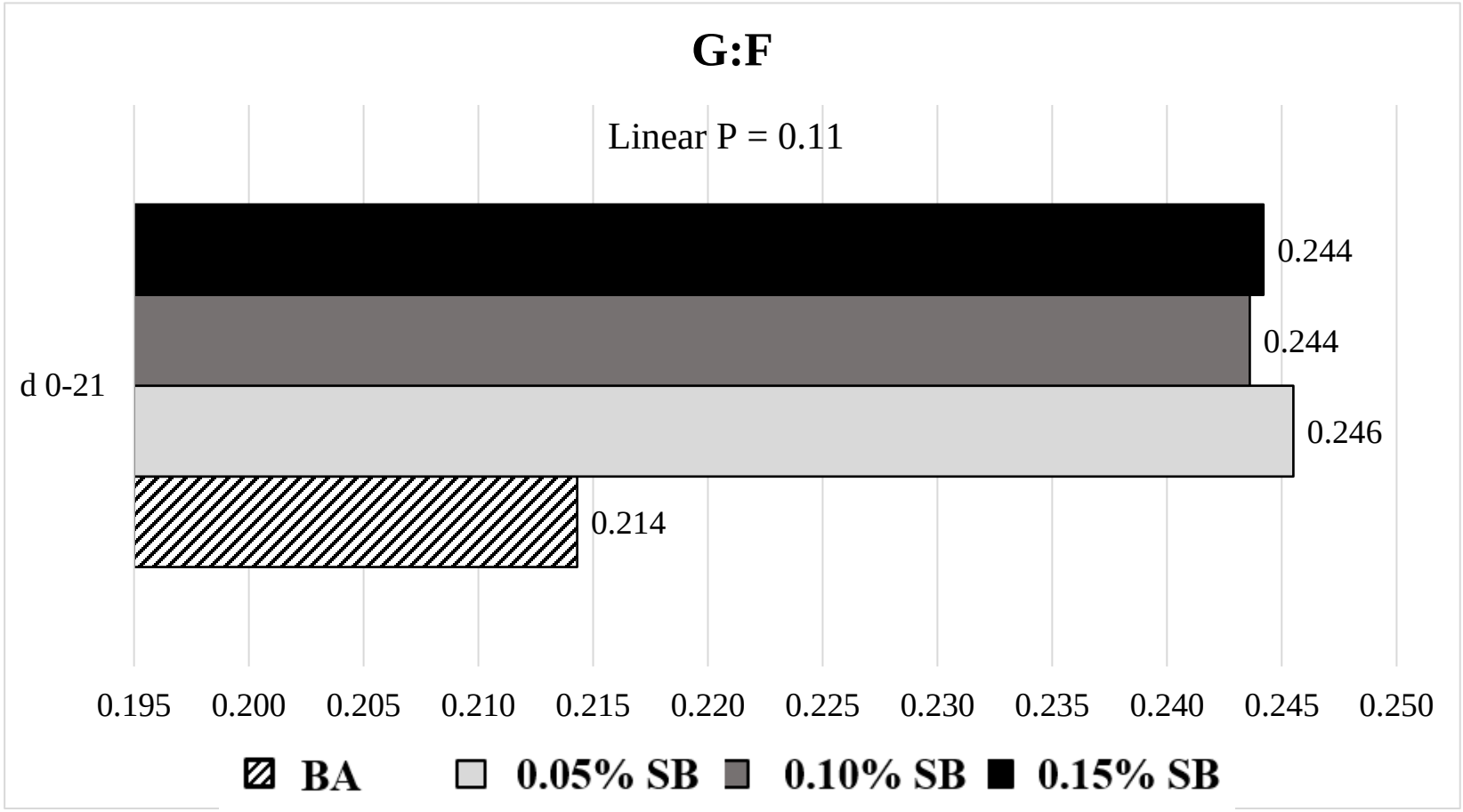
Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 2. Effect of adding sodium butyrate (SB) on ADG in nursery pigs (LS means; University of Illinois – Urbana-Champaign & University of Arkansas – Fayetteville pooled results).



Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 3. Effect of adding sodium butyrate (SB) on ADFI in nursery pigs (LS means; University of Illinois – Urbana-Champaign & University of Arkansas – Fayetteville pooled results).



Significant value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

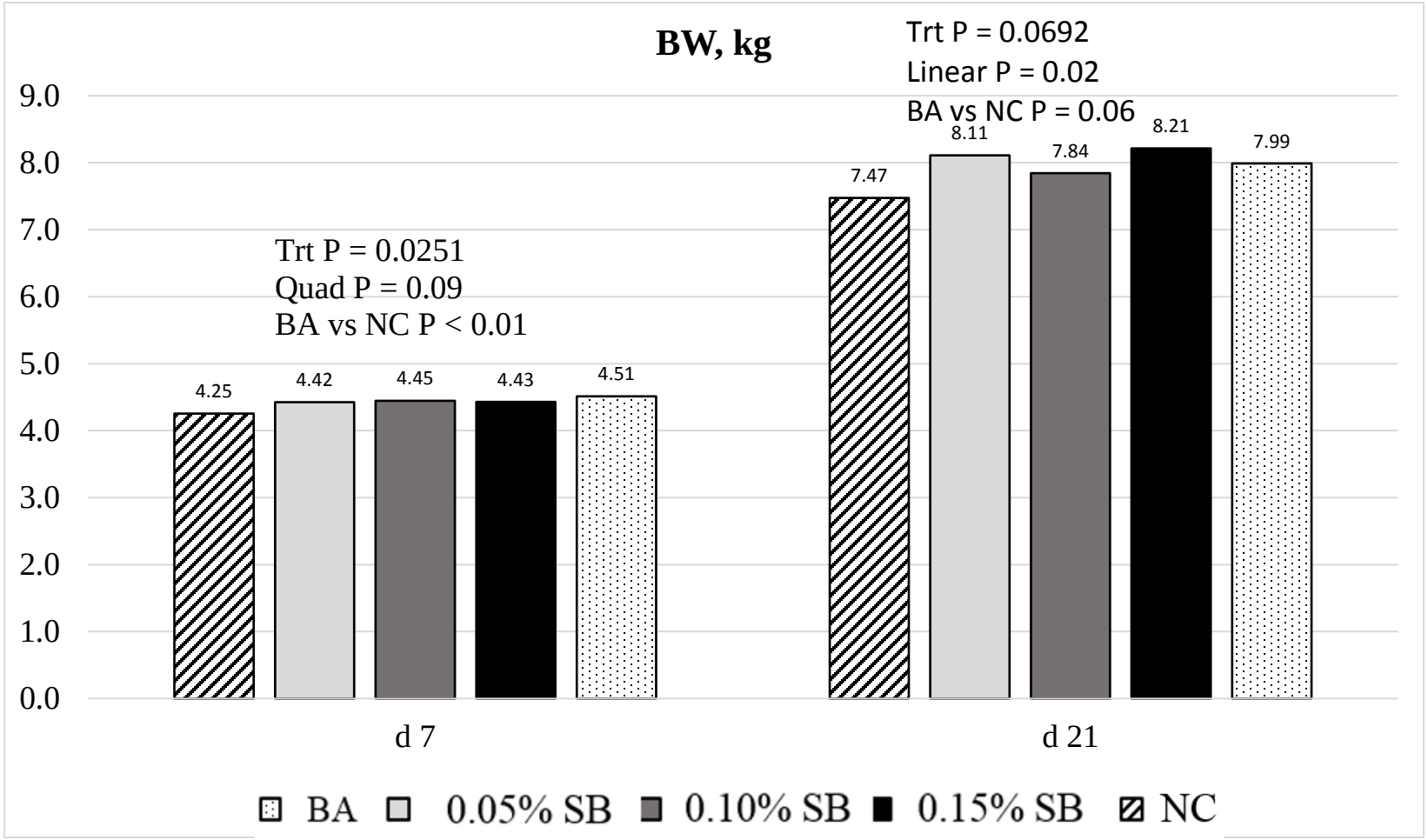
Figure 4. Effect of adding sodium butyrate (SB) on G:F ratio in nursery pigs (LS means; University of Illinois – Urbana-Champaign & University of Arkansas – Fayetteville pooled results).

Table 11. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on BW and ADG in nursery pigs (LS means; University of Arkansas - Fayetteville).

	Treatment									
	NC	**BA**	**BA + 0.05% SB**	**BA + 0.1% SB**	**BA + 0.15% SB**	**SEM**	**Trt**	**Linear SB**	**Quad SB**	**BA vs NC**
BW, kg										
d 0	4.740	4.710	4.600	4.710	4.680	0.210	0.399	0.950	0.403	0.752
d 7	4.510	4.250	4.420	4.450	4.430	0.200	0.025	0.028	0.090	0.002
d 21	7.990	7.470	8.110	7.840	8.210	0.310	0.069	0.025	0.479	0.058
d 40	17.400	17.440	18.210	17.910	17.860	0.550	0.541	0.575	0.292	0.948
ADG, kg										
d 0-7	-0.032	-0.066	-0.032	-0.037	-0.036	0.009	0.054	0.040	0.075	0.012
d 7-21	0.247	0.229	0.260	0.242	0.266	0.013	0.192	0.077	0.774	0.272
d 21-40	0.495	0.524	0.529	0.530	0.508	0.016	0.353	0.448	0.352	0.157
d 0-21	0.155	0.132	0.165	0.150	0.167	0.010	0.072	0.036	0.410	0.091
d 0-40	0.317	0.318	0.339	0.330	0.329	0.011	0.476	0.596	0.267	0.907

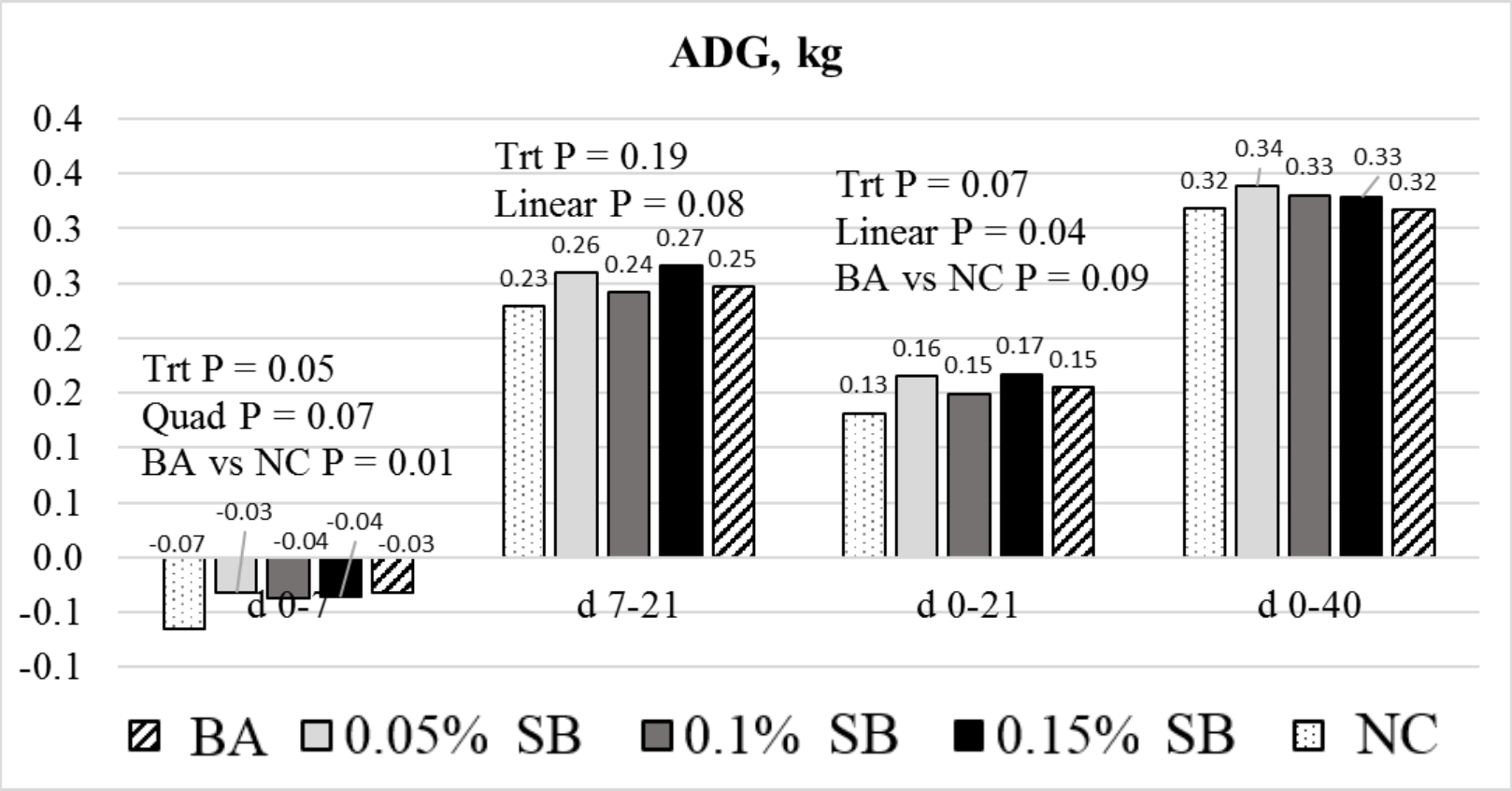
Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$



Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 5. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on BW in nursery pigs (LS means; University of Arkansas – Fayetteville).



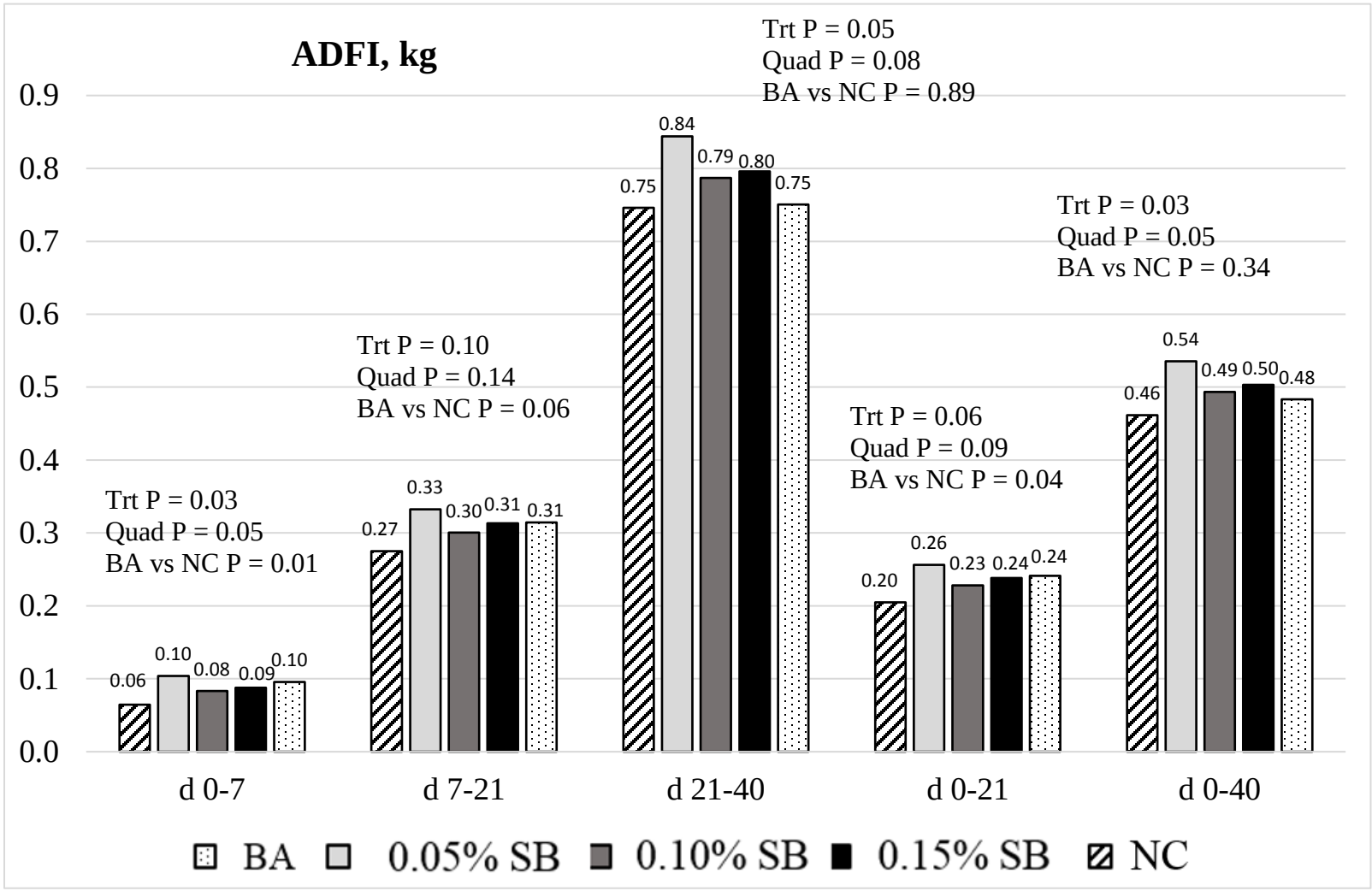
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 6. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on ADG in nursery pigs (LS means; University of Arkansas – Fayetteville).

Table 12. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on ADFI and G:F ratio in nursery pigs (LS means; University of Arkansas – Fayetteville).

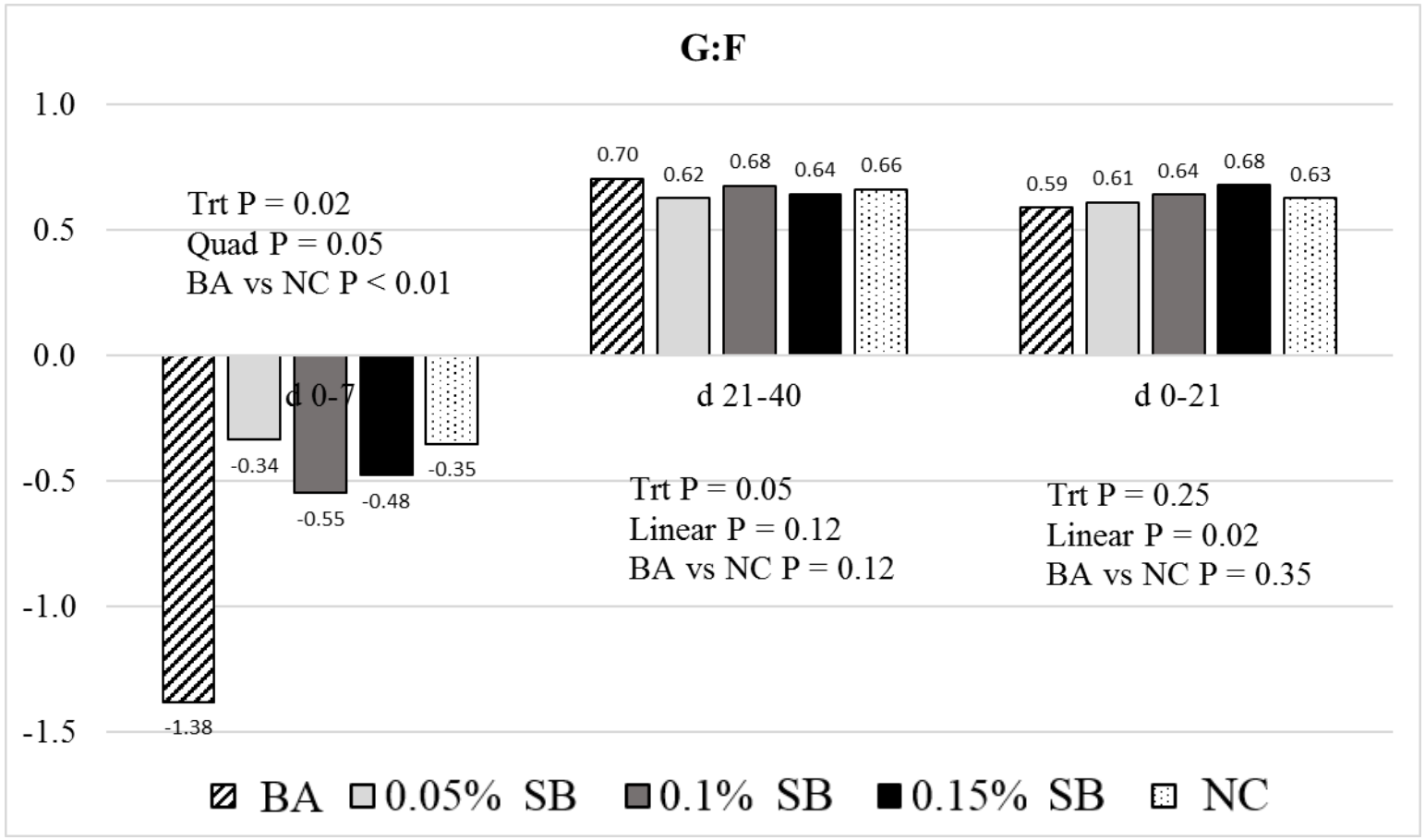
	Treatment									
	NC	**BA**	**BA + 0.05% SB**	**BA + 0.1% SB**	**BA + 0.15% SB**	**SEM**	**Trt**	**Linear SB**	**Quad SB**	**BA vs NC**
ADFI, kg										
d 0-7	0.095	0.064	0.104	0.083	0.088	0.008	0.028	0.200	0.046	0.014
d 7-21	0.314	0.275	0.332	0.300	0.313	0.016	0.099	0.208	0.137	0.064
d 21-40	0.751	0.746	0.844	0.787	0.796	0.026	0.051	0.400	0.076	0.891
d 0-21	0.241	0.205	0.256	0.228	0.238	0.012	0.055	0.182	0.091	0.036
d 0-40	0.483	0.462	0.535	0.494	0.503	0.017	0.034	0.252	0.050	0.341
G:F										
d 0-7	-0.352	-1.380	-0.336	-0.549	-0.477	0.242	0.021	0.025	0.049	0.005
d 7-21	0.776	0.789	0.760	0.793	0.831	0.028	0.451	0.196	0.229	0.749
d 21-40	0.662	0.704	0.625	0.676	0.642	0.019	0.052	0.121	0.232	0.122
d 0-21	0.627	0.590	0.610	0.641	0.676	0.030	0.252	0.025	0.784	0.353
d 0-40	0.652	0.678	0.620	0.667	0.650	0.018	0.256	0.654	0.276	0.326

Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$



Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 7. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on ADFI in nursery pigs (LS means; University of Arkansas – Fayetteville).



Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 8. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on G:F ratio in nursery pigs (LS means; University of Arkansas – Fayetteville).

Table 13. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on CBC in nursery pigs (LS means separated by treatments; University of Arkansas – Fayetteville).

	Treatment										
	NC	**BA**	**BA + 0.5% SB**	**BA + 0.1% SB**	**BA + 0.15% SB**	**SEM**	**Trt**	**Trt*Day**	**Linear SB**	**Quad SB**	**BA vs NC**
Concentration, k/µl											
WBC	14.555	14.212	15.236	14.569	13.114	0.698	0.272	0.469	0.192	0.066	0.714
Neutrophil	7.086	6.592	7.313	6.896	6.154	0.473	0.433	0.553	0.400	0.109	0.437
Lymphocyte	5.947	5.992	6.284	5.755	5.545	0.330	0.294	0.240	0.093	0.310	0.898
Monocyte	0.377	0.480	0.442	0.432	0.409	0.040	0.467	0.386	0.220	0.852	0.074
Eosinophil	1.075	1.091	1.130	1.401	0.906	0.151	0.258	0.394	0.680	0.080	0.940
Basophil	0.052	0.052	0.061	0.066	0.064	0.010	0.740	0.705	0.339	0.532	0.973
Percentage over WBC											
Neutrophil	44.753	44.148	44.172	43.940	44.360	1.700	0.994	0.971	0.945	0.878	0.739
Lymphocyte	45.587	44.977	45.871	44.118	45.815	1.907	0.950	0.860	0.923	0.819	0.805
Monocyte	2.709	3.323	2.929	2.954	3.199	0.254	0.463	0.389	0.761	0.211	0.089
Eosinophil	6.727	7.217	6.700	8.782	6.361	0.678	0.102	0.275	0.874	0.162	0.608
Basophil	0.309	0.350	0.335	0.372	0.389	0.044	0.742	0.833	0.438	0.721	0.512
NLR	1.170	1.099	1.177	1.197	1.104	0.096	0.869	0.862	0.927	0.287	0.529
RBC, M/µl	7.141	6.643	6.911	6.820	6.876	0.236	0.655	0.684	0.560	0.647	0.127
Hemoglobin, g/dL	6.879	6.939	7.132	6.754	6.919	0.289	0.890	0.667	0.709	0.956	0.870
Hematocrit, %	28.957	28.192	29.220	28.682	28.569	1.076	0.950	0.484	0.889	0.545	0.564
MCV	40.321	42.336	42.034	41.723	41.112	0.561	0.091	0.032	0.115	0.783	0.012
MCH, Pg	9.432	10.891	10.134	9.649	9.736	0.358	0.039	0.034	0.015	0.240	0.005
MCHC, g/dL	23.140	25.065	23.826	22.875	23.219	0.645	0.122	0.248	0.026	0.222	0.036
RDW, %	28.117	28.705	28.412	27.891	28.180	0.396	0.650	0.177	0.241	0.465	0.293
PLT, k/µl	382.690	414.870	358.920	365.320	324.120	29.899	0.135	0.675	0.018	0.763	0.359
MPV, fL	7.866	8.282	7.583	7.922	8.012	0.378	0.775	0.631	0.781	0.298	0.438

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Mean corpuscular volume (MCV): average of red cells

Mean corpuscular hemoglobin (MCH): hemoglobin amount per red blood cell

Mean corpuscular hemoglobin concentration (MCHC): hemoglobin amount relative to size of hemoglobin per red blood cell

Red cell distribution width (RDW): calculation of variation in size of red blood cell

Mean platelet volume (MPV): calculation average size of platelets

Table 14. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on CBC in nursery pigs (LS means separated by day; University of Arkansas – Fayetteville).

	Day					
	0	**7**	**21**	**40**	**SEM**	**Day**
Concentration, k/µl						
WBC	7.600	20.270	16.270	13.210	0.600	<0.0001
Neutrophil	2.770	12.190	7.370	4.900	0.390	<0.0001
Lymphocyte	4.580	6.550	6.620	5.860	0.310	<0.0001
Monocyte	0.160	0.360	0.630	0.570	0.030	<0.0001
Eosinophil	0.080	1.020	1.570	1.800	0.120	<0.0001
Basophil	0.000	0.070	0.080	0.080	0.010	<0.0001
Percentage over WBC						
Neutrophil, % of WBC	35.590	59.650	44.660	37.190	1.620	<0.0001
Lymphocyte	61.260	34.060	41.590	44.180	1.530	<0.0001
Monocyte	2.090	1.770	3.880	4.340	0.190	<0.0001
Eosinophil, % of WBC	0.990	4.520	9.400	13.710	0.550	<0.0001
Basophil	0.060	0.310	0.470	0.570	0.040	<0.0001
NLR	0.630	1.920	1.130	0.920	0.090	<0.0001
RBC, M/µl	5.340	7.030	8.170	6.970	0.210	<0.0001
Hemoglobin, g/dL	4.260	5.270	9.410	8.760	0.260	<0.0001
Hematocrit, %	20.790	25.750	35.600	32.760	0.990	<0.0001
MCV	39.290	36.210	43.530	46.990	0.380	<0.0001
MCH, Pg	8.500	7.330	11.500	12.550	0.270	<0.0001
MCHC, g/dL	21.230	20.160	26.440	26.680	0.490	<0.0001
RDW, %	32.060	29.300	28.850	22.830	0.360	<0.0001
PLT, k/µl	540.130	461.520	299.730	175.360	27.530	<0.0001
MPV, fL	8.350	7.700	7.620	8.060	0.250	0.2528

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

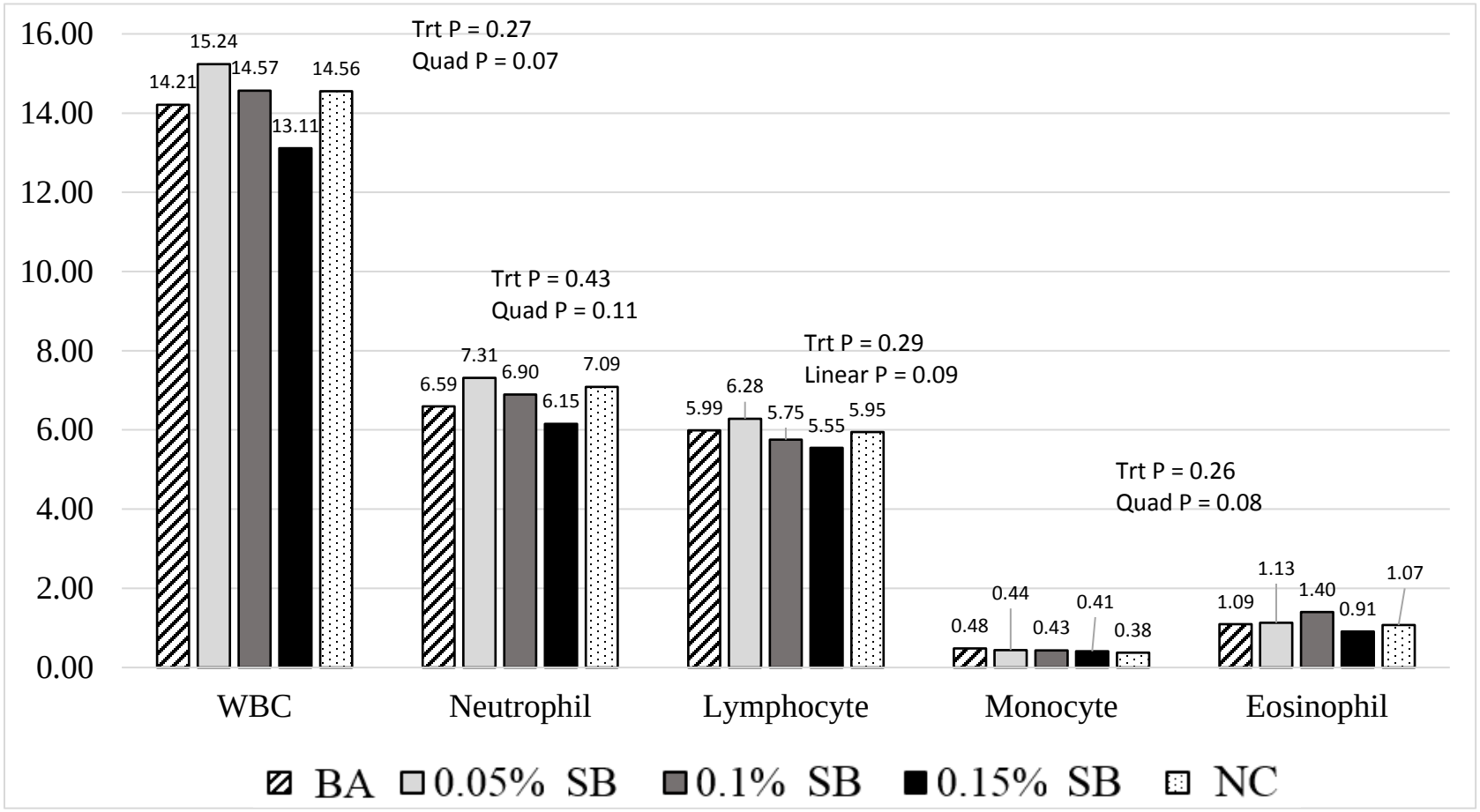
Mean corpuscular volume (MCV): average of red cells

Mean corpuscular hemoglobin (MCH): hemoglobin amount per red blood cell

Mean corpuscular hemoglobin concentration (MCHC): hemoglobin amount relative to size of hemoglobin per red blood cell

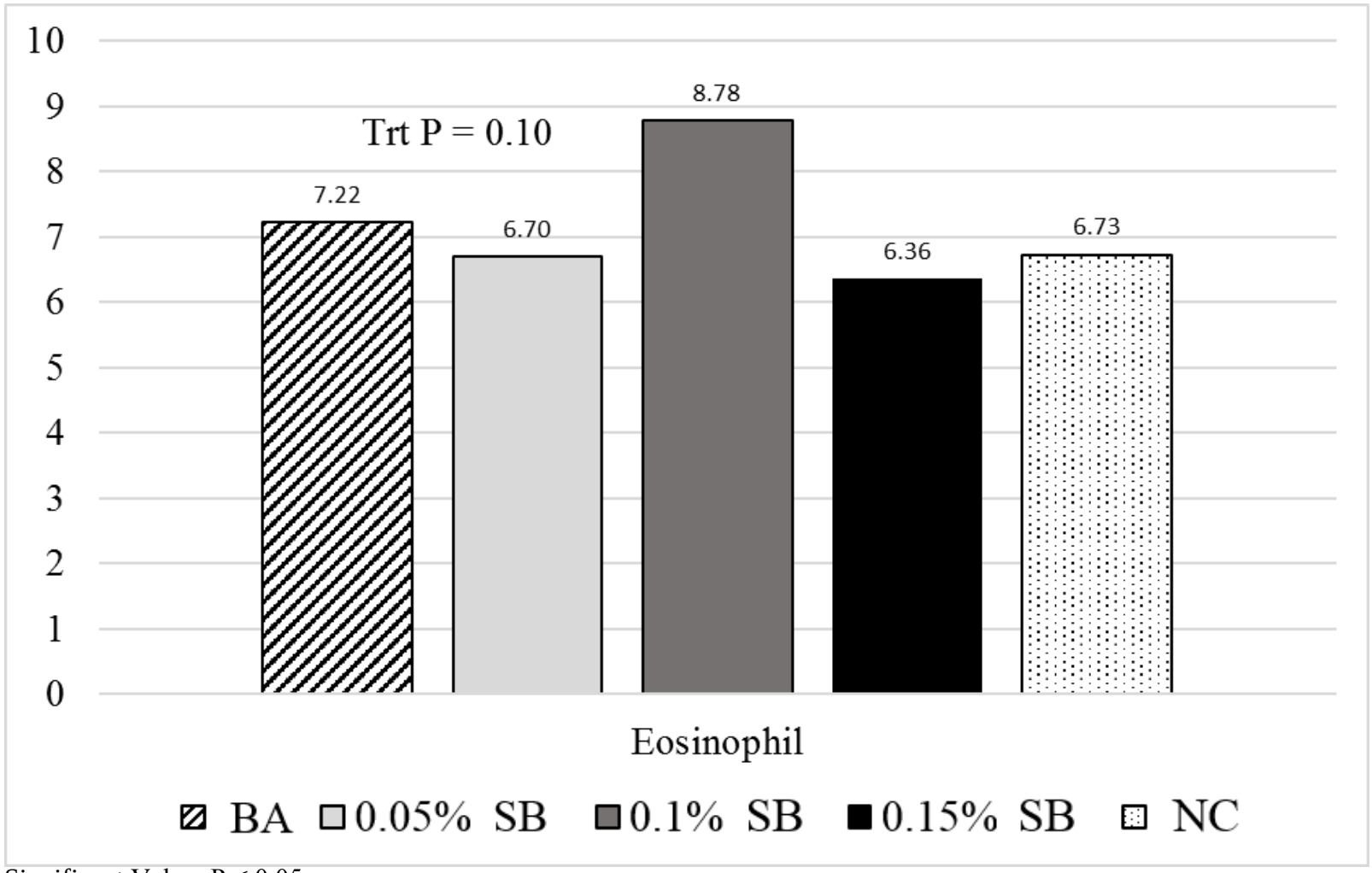
Red cell distribution width (RDW): calculation of variation in size of red blood cell

Mean platelet volume (MPV): calculation average size of platelets



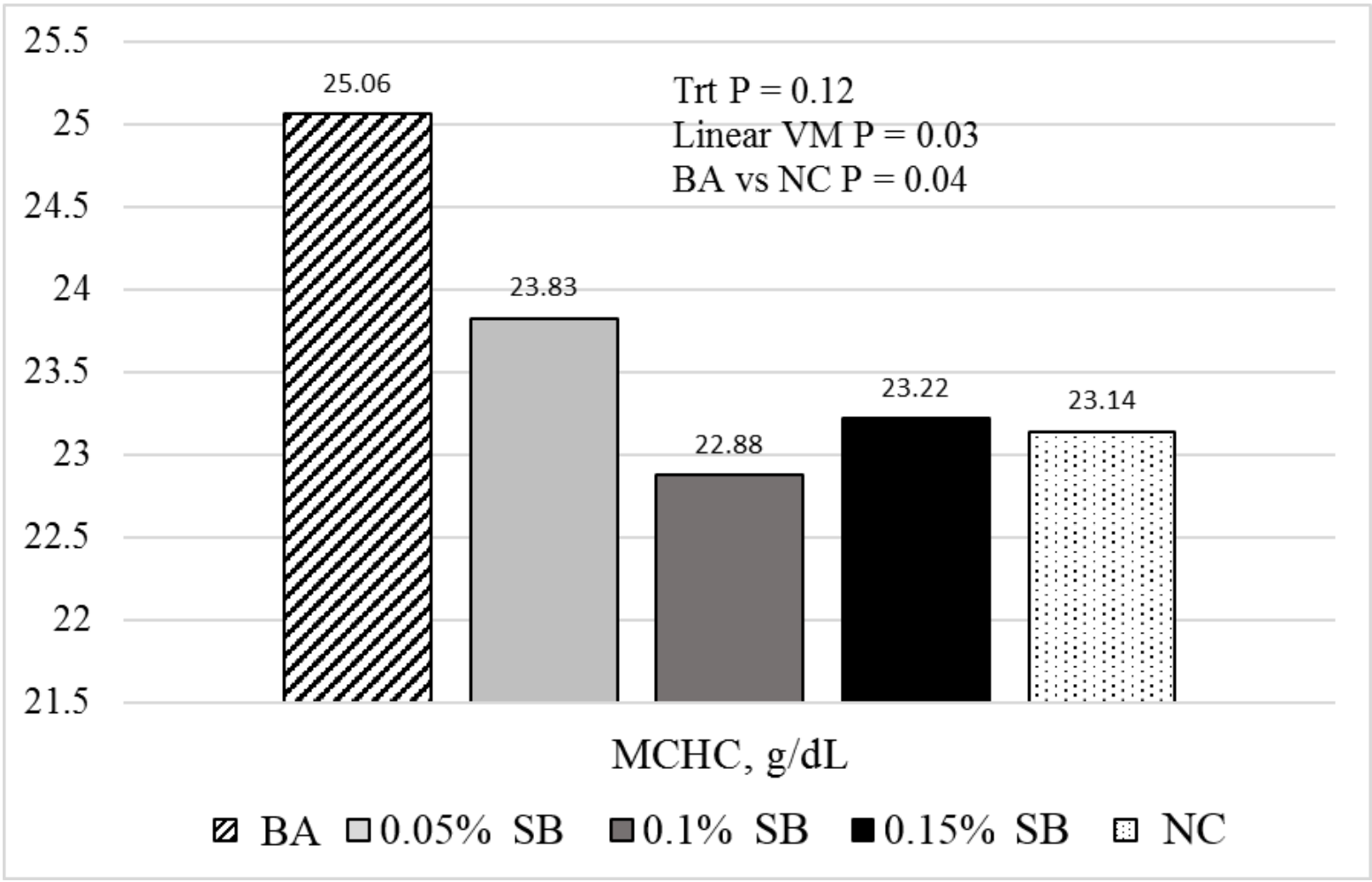
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 9. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on WBC, neutrophil, and lymphocyte counts in nursery pigs (LS means; University of Arkansas – Fayetteville).



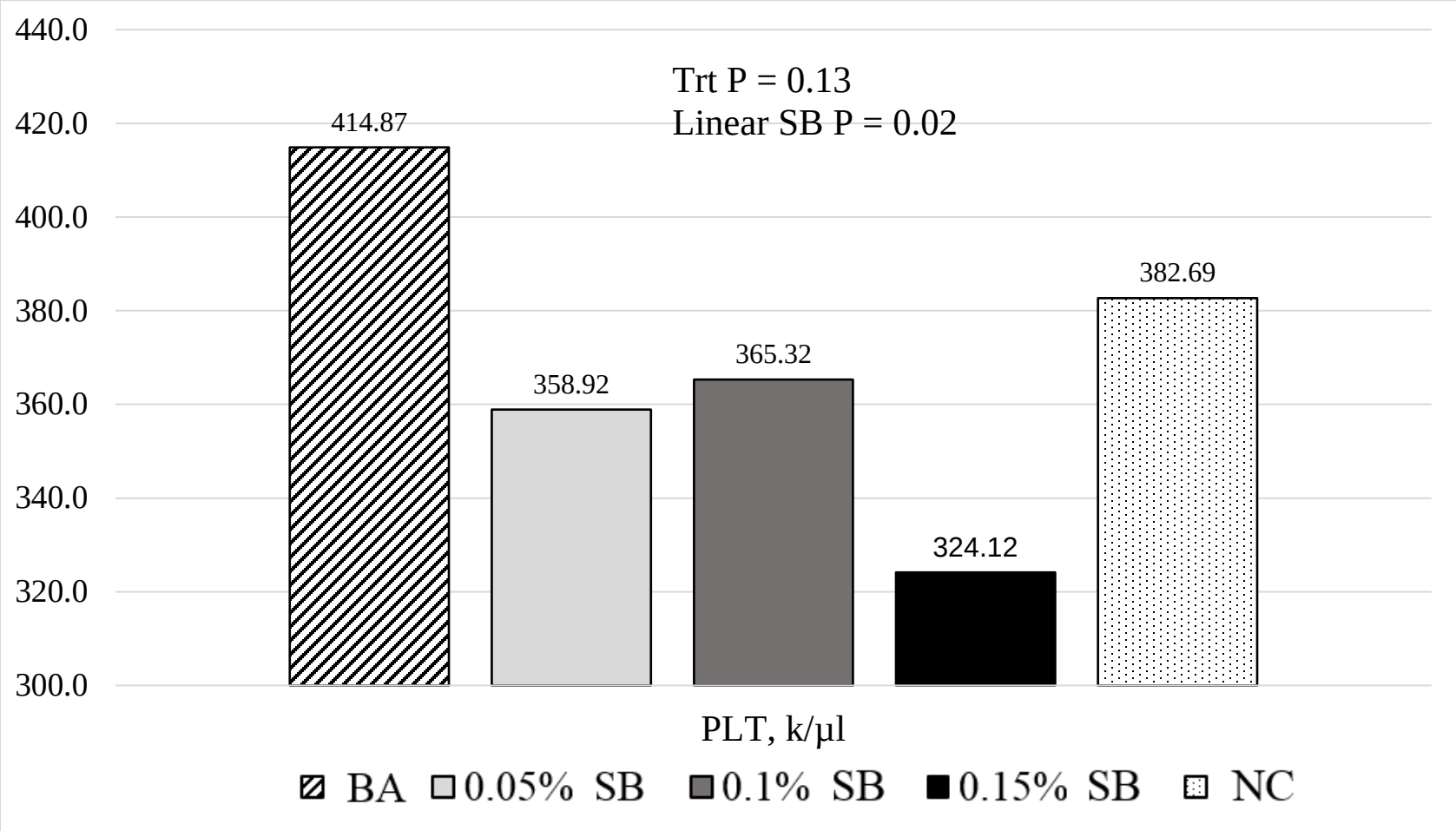
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 10. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on eosinophil counts in nursery pigs (LS means; University of Arkansas – Fayetteville).



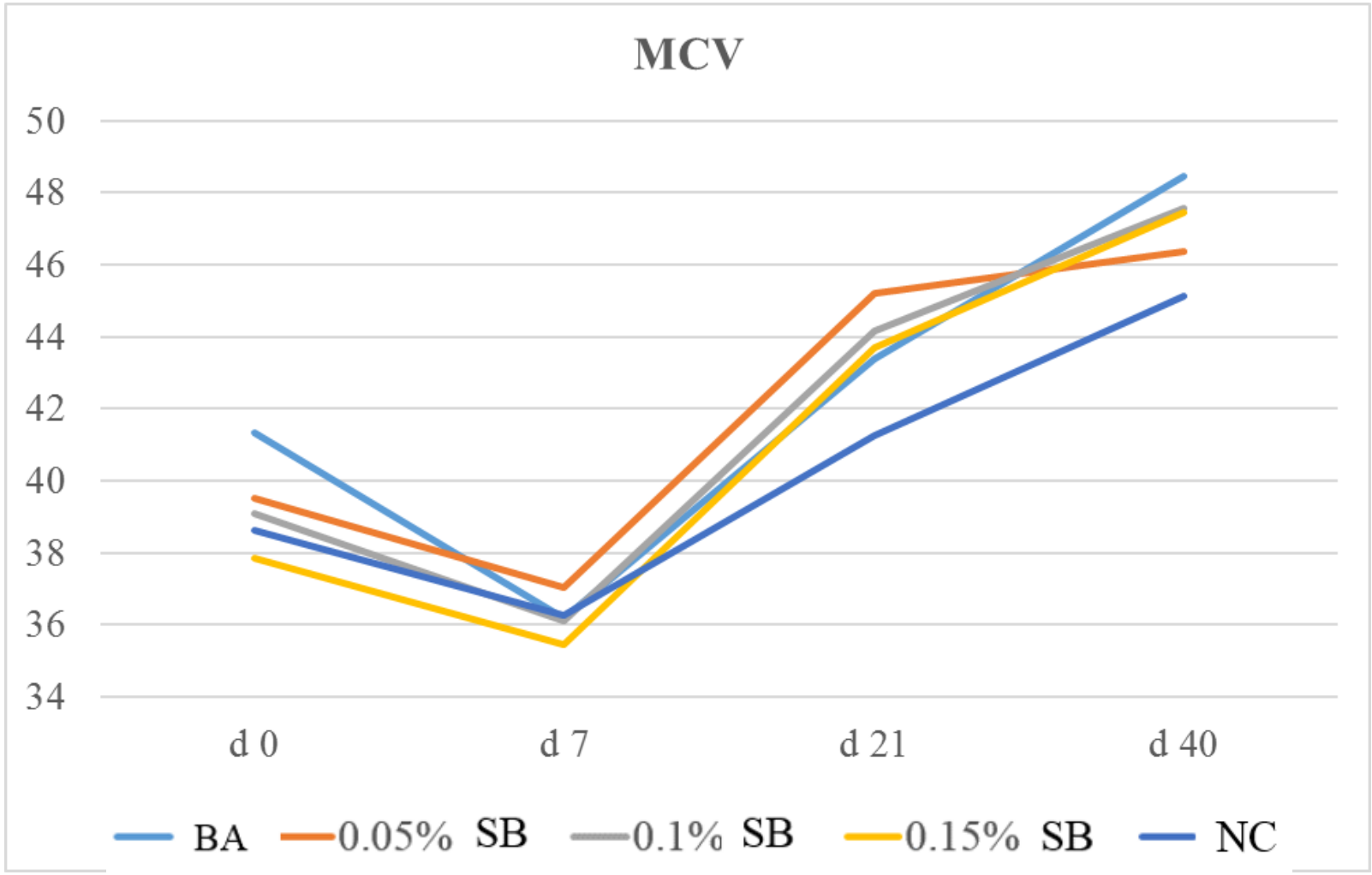
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 11. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on MCHC in nursery pigs (LS means; University of Arkansas - Fayetteville).



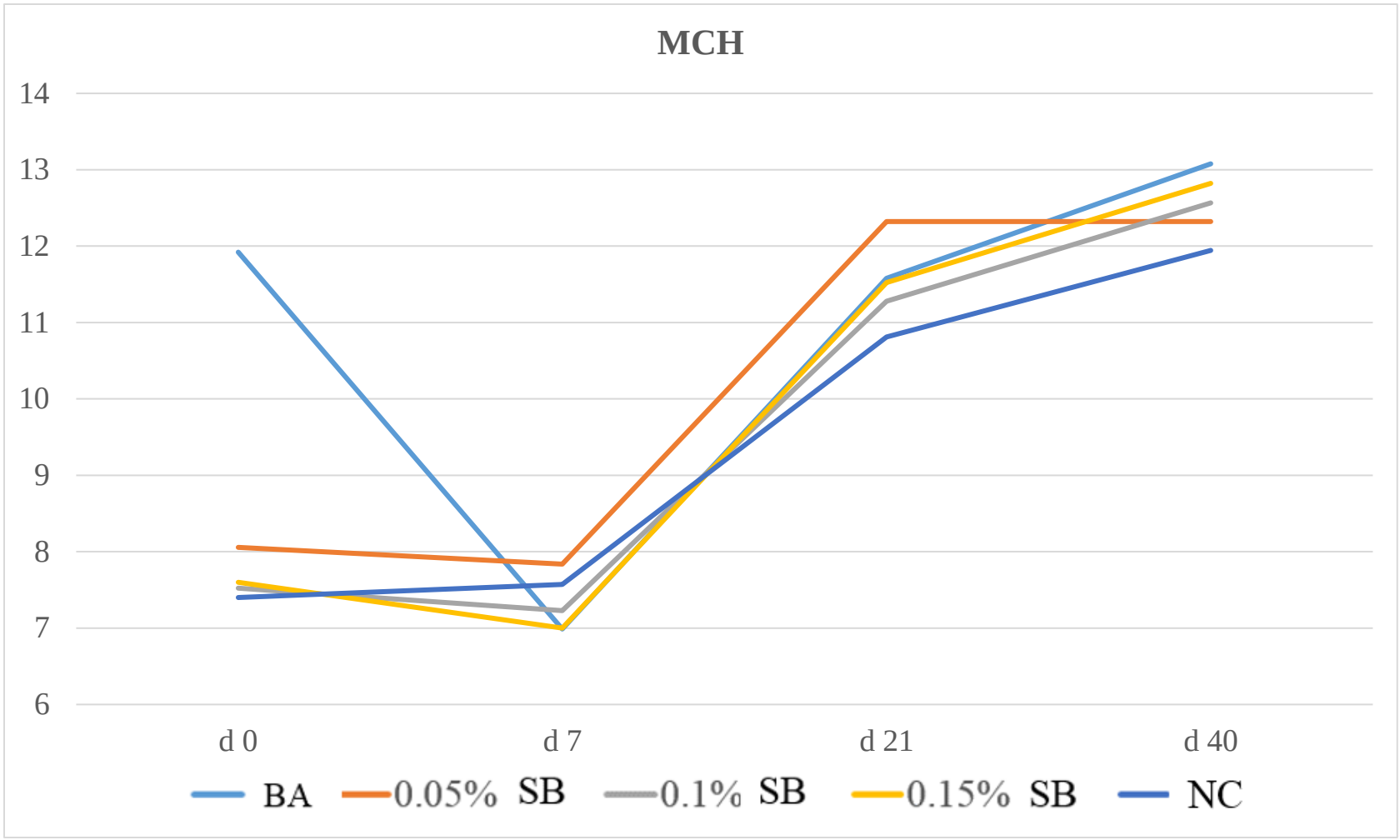
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 12. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on PLT in nursery pigs (LS means; University of Arkansas – Fayetteville).



Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 13. Treatment by day interaction effect of adding benzoic acid (BA) and sodium butyrate (SB) on MCV in nursery pigs (LS means; University of Arkansas – Fayetteville).



Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 14. Treatment by day interaction effect of adding benzoic acid (BA) and sodium butyrate (SB) on MCH in nursery pigs (LS means; University of Arkansas – Fayetteville).

Table 15. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on apparent total tract nutrient digestibility (LS means; University of Arkansas – Fayetteville).

	Treatment												
	NC		BA		BA + 0.05% SB		BA + 0.1% SB		BA + 0.15% SB		SEM	Trt	Linear SB	Quad SB
DM	0.92	a	0.941	b	0.925	a	0.92	a	0.918	a	0.003	<.0001	0.1137	0.7139
Calories	0.781	a	0.837	b	0.794	a	0.777	a	0.776	a	0.01	0.0002	0.1683	0.5048
Fat	0.578	a	0.801	c	0.742	b	0.766	bc	0.745	bc	0.021	<.0001	0.9173	0.3715
Nitrogen	0.953	ab	0.968	c	0.959	b	0.954	ab	0.95	a	0.002	<.0001	0.0084	0.713
Ash	0.524	b	0.651	c	0.552	b	0.458	a	0.5	ab	0.021	<.0001	0.0829	0.0092
NDF	0.633	a	0.73	b	0.648	a	0.618	a	0.615	a	0.018	<.001	0.1511	0.4692
ADF	0.587	b	0.658	c	0.548	ab	0.544	ab	0.516	a	0.024	0.001	0.3187	0.6686
Phosphorus	0.393	a	0.579	c	0.488	b	0.369	a	0.357	a	0.028	<.0001	0.0016	0.1139
Calcium	0.58	ab	0.67	b	0.654	b	0.541	a	0.539	a	0.031	0.0097	0.0128	0.153
Magnesium	0.177	a	0.309	c	0.165	b	-0.043	a	-0.078	a	0.046	<.0001	0.0003	0.1054
Sulfur	0.72	ab	0.811	c	0.754	b	0.721	b	0.686	a	0.013	<.0001	0.0004	0.9264
Sodium	0.808	ab	0.863	b	0.803	ab	0.804	ab	0.746	a	0.023	0.0263	0.0931	0.3015
Iron	-0.107	ab	0.056	bc	0.178	c	-0.047	ab	-0.178	a	0.073	0.0112	0.0013	0.5847
Manganese	-0.53	b	-0.087	c	0.577	d	0.253	cd	-2.435	a	0.147	<.0001	<.0001	<.0001
Zinc	-0.181	b	0.308	c	0.296	c	-0.14	b	-0.538	a	0.074	<.0001	<.0001	0.8274
Copper	-0.366	ab	0.355	c	-0.106	b	-0.367	ab	-0.696	a	0.127	<.0001	0.0022	0.8258
Boron	0.816	a	0.885	c	0.853	b	0.82	a	0.815	a	0.01	<.0001	0.0125	0.2567

[a,b,c] Rows with different superscripts indicate significant differences between the groups.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Table 16. Effect of adding benzoic acid (BA) and sodium butyrate (SB) on fecal VFA concentration (LS means; University of Arkansas – Fayetteville).

	Treatments								
	NC	**BA**	**BA + 0.05% SB**	**BA + 0.1% SB**	**BA + 0.15% SB**	**SEM**	**Trt**	**Linear SB**	**Quad SB**
Absolute Conc., mM									
Acetate	39.529	36.696	37.139	39.364	37.066	1.340	0.399	0.970	0.178
Propionate	17.237	17.429	17.670	19.346	17.527	0.902	0.476	0.911	0.124
Butyrate	12.846	12.567	13.046	14.868	12.599	0.889	0.344	0.724	0.069
Iso-butyrate	1.709	1.825	1.713	1.867	1.582	0.135	0.602	0.496	0.191
Valerate	3.987	3.973	3.915	4.333	3.838	0.314	0.815	0.859	0.231
Iso-valerate	2.452	2.716	2.487	2.660	2.237	0.228	0.603	0.443	0.293
Total VFA	77.761	75.205	75.969	82.438	74.848	3.198	0.450	0.806	0.082
Percentage of total VFA									
Acetate	50.952	49.061	49.223	47.715	49.880	1.118	0.362	0.681	0.189
Propionate	22.162	23.161	23.202	23.462	23.364	0.491	0.336	0.813	0.762
Butyrate	16.419	16.525	16.986	18.080	16.707	0.690	0.450	0.777	0.154
Iso-butyrate	2.193	2.420	2.237	2.263	2.099	0.131	0.532	0.462	0.557
Valerate	5.126	5.218	5.095	5.253	4.997	0.238	0.941	0.768	0.473
Iso-valerate	3.148	3.614	3.257	3.226	2.952	0.251	0.465	0.397	0.695

Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Chapter 3: Establishing Ideal Inclusion Rate of Fermented Soybean Meal in Nursery Rations

Establishing ideal inclusion rate of fermented soybean meal (FSBM) in nursery rations

K. A. Bottoms, T. Tsai, Joshua Knapp, Hannah Maxwell, C.V. Maxwell, A.J. Mercado, B. Bass, T. Weeden

Abstract

This experiment was conducted to determine the optimal level of fermented soybean meal (FSBM, Fermex 200, Purina Animal Nutrition, Arden Hills, MN) in nursery diets. A total of 176 weaned pigs (± 5.96 kg BW) were blocked by initial BW and allotted to 1 of 4 treatments (12 replicates per treatment). Pens were assigned randomly to dietary treatments. Treatments were: 1) Control (C) enzymatic soybean protein-poultry by-product diet, 2) C diet supplemented with 5% FSBM, 3) C diet supplemented with 10% FSBM, and 4) C diet supplemented with 15% FSBM to achieve FSBM1, FSBM2, and FSBM3 treatments, respectively. Pigs remained on the same dietary treatment for phase 1 (d 0-14) and 2 (d 14-29), while a common diet was fed in phase 3 (d 29-40) to evaluate subsequent impact of protein sources from the early nursery period. Individual pig weights and pen feed disappearance were recorded weekly for all pens. Blood was taken via jugular venipuncture, and was analyzed for complete blood cell count on d 0, 14, 29, and 40 from one pig/pen (n = 44) that represented the average BW for each pen. Data were analyzed using MIXED procedure of SAS (Cary, NC) with dietary treatment as the fixed effect, and initial BW block as the random effect. Orthogonal contrasts were performed to test for linear, quadratic and cubic responses to increasing levels of FSBM. A quadratic response to increasing FSBM was observed in ADG (P = 0.06) and ADFI (P = 0.04) during the combined phase 1 and 2 periods (d 0-29). Moreover, the heaviest average BW was observed in pigs fed 10% FSBM on d 29 (quadratic, P = 0.06); however, the difference diminished by the end of the

trial. A tendency for a linear increase with increasing levels of FSBM was observed in overall feed efficiency (d 0-40, P = 0.075). Pigs fed 10% FSBM has the lowest WBC, neutrophil and red blood cell count. Results of this study suggest FSBM fed to pigs improves growth performance and alters blood cell characteristics; and 10% is the optimal level of FSBM to include in early nursery diets.

Keywords: Fermented soybean meal, growth performance, blood characteristics, nursery pig

Introduction

Soybean meal is the premier source of protein used in diets fed to pigs. The balance of amino acids in soy protein complement the amino acids in most cereal grains, resulting in balanced complete diets being formulated (Stein et al., 2008). However, due to several anti-nutritional factors such as antigens, oligosaccharides, and lectins, soybean meal is not well tolerated by weanling pigs – ultimately resulting in a transient depression in growth rate and decreased efficiency of nutrient utilization (Anderson et al., 1979; Cho et al., 2007). Fermentation or enzyme-treated soybean meal eliminates some of the anti-nutritional factors in the meal, which can alternatively enhance growth performance and feed efficiency in nursery pigs (Cervantes-Pahm and Stein, 2010). In addition, recent studies have suggested that fermented soybean meal can be used in nursery pig diets instead of animal proteins without adversely affecting growth (Jones et al., 2010; Kim et al., 2010). However, the optimal inclusion rate of fermented soybean meal in nursery diets to achieve maximum performance has not been defined. As a result, this study was conducted to determine the optimal inclusion rate of a fermented soybean meal (Ferm Ex 200, Purina Animal Nutrition, Sharview, MN) in order to achieve maximum growth performance in nursery pigs.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Arkansas reviewed and approved the protocols for this experiment (IACUC #: 10041).

Animals and Experimental Design

Nursery Phase

A total of 176 PIC C-29 X PIC 380 weanling pigs (± 5.96 kg BW) were blocked by initial BW and allotted to 1 of 4 treatments (12 replicates per treatment). The pigs were individually weighed and blocked by initial body weight and sex. Pigs remained in the same pens throughout the experiment. A three-phase feeding program was utilized: Phase 1 (d 0-14), Phase 2 (d 14-29), Phase 3 (d 29-40); with pigs fed different diets in the first two phases; while a common diet was fed to all pigs during phase 3 to evaluate the subsequent impact of protein sources from the early nursery period. Pigs remained on the same dietary treatment throughout the entire study period. Pigs were housed in 1.49 x 1.20 M^2 pens at the University of Arkansas conventional nursery facility, with *ad libitum* access to feed and water for the duration of the experiment. Ambient temperature was set at 85˚F upon pig arrival, and was reduced by two degrees per week until a 75˚F setting for the housing temperature was achieved by the end of the study.

Experimental Diets

Dietary formulation for Phases 1 and 2 were provided by Purina, whereas formulation for Phase 3 was provided by the University of Arkansas. Diets for nursery phase 1, phase 2, and phase 3 were each fed for 14 days (Table 17 & 18). During phase 1 and phase 2, pigs were fed one of the following dietary treatments: Treatment 1, the control diet (C) was formulated with an

enzymatic soybean protein-poultry by-product, to meet nutrient requirements (NRC, 2012). Treatments 2, 3, and 4 were the control diet supplemented with 5% FSBM, 10% FSBM, 15% FSBM, to achieve FSBM1, FSBM2, and FSBM3 treatments, respectively (Table 17 & 18). A common phase 3 diet was fed to all pigs to evaluate the subsequent impact of protein sources from the early nursery period (Table 19).

Sample Collection and Processing

At the start of the study, and at the end of each phase, individual pig weights and pen feed disappearance were measured for each phase to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). Individual pig weights were recorded on D 0, 7, 14, 21, 28, and 42. Feed samples were obtained for each batch of feed mixed. These samples were accumulated for each phase, and were stored in a -20˚C freezer until study completion in order to be subsampled for nutrient analysis.

Growth performance

Body weight (BW) from individual pigs was monitored at d 0, and individual pig BW was recorded at the beginning of the study (d 0), weekly (d 7, 14, 21, 28, 35) and at study completion (d 40). Pen feed disappearance was also recorded weekly (d 7, 14, 21, 28, 35) and at study completion (d 40) in order to calculate ADG, ADFI, and G:F ratio.

Leukocyte Differentiation

On d 0, 14, 29, and 40, one piglet from each pen that represented the average BW for each pen was selected for blood collection, and an attempt was made to select the same gender pig within blocks. Blood samples (n=44) were collected via jugular vena puncture into a 10 mL K2-EDTA vacutainer tube (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes,

NJ) for leukocyte differential analysis. Whole blood samples were removed from ice and allowed to equilibrate to room temperature before leukocyte differential determination, and samples were analyzed within 6-12 hours after collection. Samples were analyzed by a blood hematologic system (Hemavet 950 FS, Drew Scientific, Waterbury, CT).

Statistical Analysis

Performance data was analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Dietary treatment was the lone fixed effect, blocks based on initial BW were the random effect, and pen served as the experimental unit for ANOVA. Orthogonal contrasts were used to determine the linear, quadratic, and cubic effects of various levels of FSBM on growth performance. Probability values were considered statistically significant at $P < 0.05$, and $0.05 < P < 0.10$ considered a statistical trend.

Results

Growth Performance

Main effects of increasing levels of FSBM on growth performance results are presented in Tables 20 and 21. Overall health status was good, with only three pigs having been removed from the trial (one from treatments 1, 2, and 4). Pigs fed increasing levels of FSBM showed an increase in BW (Table 20, Figure 15) at d 7 (linear effect, $P = 0.08$), d 21 and d 29 (quadratic effect, $P = 0.05$ and $P = 0.06$, respectively). Results of growth performance suggested that fermented soybean meal improved ADG. ADG (Table 20, Figure 16) was shown to quadratically increase when increasing levels of FSBM was added in the diets on d 14-21 ($P < 0.04$), d 21-29 ($P = 0.03$), d 14-29 ($P = 0.02$; overall phase 2), and d 0-29 ($P = 0.06$; phase 1 & 2). However,

when compared to pigs fed other levels of FSBM, the pigs fed 10% FSBM exhibited a far superior growth rate (Table 20, Figure 16).

ADFI was shown to quadratically increase in pigs fed increasing levels of FSBM (Table 21, Figures 17 & 18) on d 0-7 ($P < 0.04$), d 14-21 ($P = 0.06$), d 14-29 ($P = 0.03$; overall phase 2), and d 0-29 (Phase 1 & 2). A linear increase in feed intake was observed in pigs on d 14-21 ($P < 0.02$, Table 21, Figure 17). However, once pigs were fed a common phase 3 diet, and treatments were withdrawn, a linear reduction on feed intake was observed in pigs previously fed increasing levels of FSBM ($P < 0.04$; Table 21, Figure 17). As a result, a linear reduction in ADFI was observed for the overall period (Table 21, Figure 18; $P < 0.01$). When compared to other dietary levels of FSBM, pigs fed 15% FSBM had the lowest FI (Table 21, Figures 17 & 18). In addition, a tendency for a linear increase in feed efficiency was observed when pigs were fed increasing levels of FSBM (Table 21, Figure 19; $P = 0.075$).

Complete Blood Cell Count (CBC)

Nursery pigs that were fed increasing levels of FSBM were shown to have altered blood cell characteristics (WBC's, neutrophils, and RBC's; Tables 22 & 23). With increasing levels of FSBM, pigs showed increased absolute value and percentage of eosinophils (Table 22, Figure 20; Cubic $P = 0.02$), and neutrophils (Table 22, Figure 20; Cubic effect, $P = 0.02$); with the highest level appearing in pigs fed 15% FSBM. Regarding RBC count, pigs fed increasing amounts of FSBM exhibited a decrease in RBC count (Table 22, Figure 22). However, pigs that consumed diets supplemented with 15% FSBM presented a RBC count similar to pigs fed the 0% FSBM control diet (Table 22, Figure 22; Quadratic effect, $P = 0.09$). Both mean corpuscular volume (Cubic effect, $P < 0.01$) and mean platelet volume (Linear effect, $P < 0.01$) decreased

with an increasing amount of FSBM above 5% (Table 22, Figure 23). However, percentage of red blood cell distribution width increased in pigs fed 10% and 15% of FSBM (Table 22, Figure 23; Linear effect, $P < 0.01$). Moreover, platelet level linearly decreased with an increasing level of FSBM (Table 22, Figure 24; Linear effect, $P < 0.01$).

With a lower monocyte count at weaning in FSBM groups, adding FSBM in the diets increased the pigs' monocyte count to the level of the C on day 14 (Table 23, Figure 25). Despite no difference being observed on d 29 in the pigs' monocyte count, the monocyte count increased more than two fold on d 40 (Table 23, Figures 25 & 26). Results suggest that pigs fed 5% FSBM had the highest monocyte count, while pigs fed 10% FSBM had the lowest monocyte count (Table 22). Pigs fed the C diet and the 15% FSBM diet exhibited a monocyte count that was intermediate to the 5% FSBM and 10% FSBM diets (Table 22, Figures 25 & 26; Treatment by day interaction, $P < 0.02$).

Hematocrit and hemoglobin shared similar results. From d 0 to d 29, a lack of a response was observed (Figures 27 & 28). However, on d 40 at study completion, pigs fed more than 5% FSBM exhibited reduced hematocrit and hemoglobin values.

After pigs were fed FSBM diets for 14 days, a linear increase in basophil concentration and its percentage over WBC was observed (Table 23, Figures 29 & 30). The same response was observed at the study completion However, on d 29, only pigs fed 15% FSBM had increased basophil levels (Figure 30). Both MCH (Table 22, Figure 31; $P = 0.04$) and MCHC (Table 22, Figure 32; $P = 0.02$) were lower with increasing levels of FSBM before treatments were administered. This response was shown to persist on d 29 and d 40 (Figures 31 & 32).

Nevertheless, results suggest that pigs fed the 5% FSBM diet had the highest MCH and MCHC among all other treatments.

Discussion

This study demonstrated that increasing the inclusion rate of FSBM in the diet has the potential to improve growth performance (ADG, BW, G:F) and alter blood cell characteristics. During phases 1 & 2 (d 0-29), increasing levels of FSBM resulted in numerically superior ADG and ADFI. Moreover, the heaviest BW was observed in pigs fed 10% FSBM on d 29; however, the difference diminished during the final phase, once all pigs were fed the same diet. Pigs fed 10% FSBM exhibited a tendency to linearly increase overall feed efficiency with increasing level of FSBM. Pigs fed 15% FSBM had the lowest FI, suggesting that 15% inclusion rate for FSBM is too high, and can cause negative effects on growth performance in nursery pigs. However, results suggested that a 10% inclusion rate of FSBM is optimal for improving growth performance and feed efficiency in nursery pigs. Similar effects of FSBM inclusion on growth performance and feed efficiency were reported in various other studies, demonstrating that FSBM may be able to enhance growth performance in nursery or weanling pigs (Jones et. al., 2010; Min et. al., 2004; Kim et. al., 2007; Cho et. al., 2008). This improved performance could be associated with overall pig health status and the fermentation process of SBM, which is thought to eliminate residual trypsin inhibitors and some oligosaccharides in soybean meal that can decrease pig performance (Jones et. al., 2010; Hong et. al, 2004; Feng et. al., 2007).

During phase 3, when pigs were fed a common phase 3 diet, there was a decrease in feed intake with increasing levels of FSBM. There is considerable data indicating that first exposure to SBM in nursery pigs may lead to increased diarrhea and reduced growth performance (Kiers

et. al., 2003; Friesen et. al., 1993). Although increasing the level of FSBM during phase 1 and 2 delays the anti-nutritive effects associated with the inclusion of dietary SBM in the nursery pigs' diet, reduced pig performance in phase 3 suggests that the anti-nutritive effects associated with feeding SBM is still present at the end of phase two; although the effect may be diminished. Several other studies suggest a similar response occurs when feeding high levels of plasma protein in phase 1 nursery diets (Weaver et. al., 2014; Crenshaw et. al., 2016).

Nursery pigs fed 10% FSBM has the lowest WBC, neutrophil, and red blood cell count. Similar effects of the inclusion of FSBM on altering blood cell characteristics (WBC's, neutrophils, and RBC) were reported in various other studies, demonstrating that FSBM has the potential to improve blood biochemical parameters in nursery pigs (Zhu et. al., 2017; Xin et. al., 2007). The current studies suggest that a 10% inclusion rate FSBM is most effective at improving growth performance and altering blood characteristics in nursery pigs.

Conclusion

The inclusion FSBM in the diet enhanced growth performance and altered blood characteristics (WBC's, neutrophils, and RBC's). ADG, BW, G:F, and blood cell characteristics were all increased when FSBM was included in the diet. However, pigs fed 15% FSBM had the lowest FI; suggesting that there is a maximum inclusion percentage in the diet that will effectively enhance growth performance in nursery pigs. Pigs fed 10% inclusion rate of FSBM is the optimal level of FSBM to include in early nursery diets.

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Appendix

Table 17. Diet composition (Phases 1 & 2).

	Phase 1				Phase 2			
Ingredients	**Control (C)**	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C + 15% (FSBM3)**	**Control (C)**	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C+ 15% (FSBM3)**
Corn, %	31.532	30.813	30.021	27.710	49.567	48.634	47.001	43.384
Oat Mill Byproduct, %	7.500	7.500	7.500	7.500	-	-	-	-
Soybean Meal,%	18.000	18.000	18.000	18.000	26.500	26.500	26.500	26.500
HP 300, %	6.663	2.450	0.000	0.000	4.308	0.244	0.000	0.000
AP 920 Bovine Plasma, %	4.000	4.000	4.000	4.000	-	-	-	-
Poultry Byproduct Meal, 65% CP, %	5.000	5.000	2.892	0.000	5.000	5.000	1.340	0.000
Calcium Carbonate, %	0.535	0.531	0.651	0.824	0.427	0.455	0.586	0.731
Mono-Dical Phos, %	0.448	0.444	0.573	0.703	0.725	0.717	0.921	0.919
Salt, %	0.077	0.028	0.101	0.130	0.535	0.538	0.575	0.588
Choice White Grease, %	2.525	2.525	2.525	2.525	2.020	2.020	2.219	2.278
Intellibond C (CuCl), %	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
Choline Chlor-70, %	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Lysine 98.5%, %	0.297	0.302	0.324	0.285	0.350	0.350	0.350	0.252
DL Methionine, %	0.169	0.162	0.165	0.153	0.168	0.158	0.159	0.125
L-Threonine, %	0.118	0.115	0.119	0.093	0.155	0.149	0.142	0.088
L-Tryptophan 98%, %	0.028	0.026	0.022	0.006	0.045	0.042	0.031	0.007
L-Valine, %	0.039	0.035	0.036	0.000	0.074	0.066	0.050	0.000
Zinc Oxide 72, %	0.399	0.399	0.399	0.399	0.261	0.261	0.261	0.261
Dairylac 80, %	21.951	21.951	21.951	21.951	9.146	9.146	9.146	9.146
Fermented Soybean Meal[1], %	0.000	5.000	10.000	15.000	0.000	5.000	10.000	15.000
KemGest, %	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
KSU VitPmx w/O Phy (NB-6508), %	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
U Of A Swine Trace Mineral (NB-8534), %	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Phytase[2], %	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040

[1]Fermented Soybean Meal = (Ferm Ex 200 ®, Purina Animal Nutrition, Sharview, MN).

[2]Phytase = Ronozyme® Hiphospate GT 500 (DSM Nutritional Products, Parsippany, NJ).

Table 18: Calculated Analysis of Diets (Phases 1 & 2).

	Phase 1				Phase 2			
Calculated Analysis	**Control (C)**	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C + 15% (FSBM3)**	**Control (C)**	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C+ 15% (FSBM3)**
Protein, %	22.62	22.58	22.21	22.47	22.05	22.17	21.93	22.99
Calcium, %	0.75	0.75	0.75	0.75	0.7	0.7	0.68	0.7
Phosphorus, %	0.7	0.7	0.7	0.7	0.65	0.65	0.65	0.65
Lactose, %	18	18	18	18	7.5	7.5	7.5	7.5
Ca/P Ratio	1.07	1.07	1.07	1.07	1.08	1.08	1.06	1.08
Copper Ad	175	175	175	175	175	175	175	175
Zinc Ad	2900	2900	2900	2900	1900	1900	1900	1900
SID Lys	1.4	1.4	1.4	1.4	1.35	1.35	1.35	1.35
SID M+C	0.812	0.812	0.812	0.812	0.78	0.78	0.78	0.78
SID Thr	0.882	0.882	0.882	0.882	0.85	0.85	0.85	0.85
SID Trp	0.266	0.266	0.266	0.266	0.25	0.25	0.25	0.25
SID Val	0.966	0.966	0.966	0.968	0.93	0.93	0.93	0.93
ME Swine	1568	1564	1561	1562	1517	1513	1520	1522

Table 19. Diet composition and calculated analysis (Phase 3).

	Phase 3
Ingredients	**Control (C)**
Corn, %	55.450
SBM 48%, %	28.900
DDGS,%	10.000
Poultry Fat, %	2.500
Monocalcium P, %	0.440
Limestone, %	1.075
Salt, %	0.500
DL-Methionine, %	0.083
Copper Sulfate, %	0.100
Vitamin Premix (NB-6508), %	0.250
Trace Mineral Premix (NB-8534), %	0.150
Phytase[1], %	0.019
L-Lysine, %	0.429
L-Threonine, %	0.102
L-Tryptophan, %	0.008
Calculated Analysis	
Protein, %	21.621
Calcium, %	0.651
Phosphorus, %	0.516
Ca/P Ratio	1.260
Copper Ad	281.613
Zinc Ad	199.857
SID Lys	1.282
SID M+C	0.744
SID Thr	0.770
SID Trp	0.219
SID Val	1.003
ME Swine	1551

[1]Phytase = Ronozyme® Hiphospate GT 500 (DSM Nutritional Products, Parsippany, NJ).

Table 20. Effect of feeding increasing levels of fermented soybean meal (FSBM) on BW and ADG in nursery pigs (LS means).

	FSBM					P - Value			
	Control (C)	C + 5% (FSBM 1)	C + 10% (FSBM2)	C + 15% (FSBM3)	SEM	Trt	Linear	Quad.	Cub.
BW, kg									
d 0	5.93	5.98	5.96	5.99	0.34	0.5174	0.2242	0.6991	0.4333
d 7	6.39	6.46	6.7	6.53	0.32	0.0775	0.0843	0.1772	0.1288
d 14	8.58	8.56	8.98	8.64	0.43	0.2165	0.4006	0.3251	0.0941
d 21	12.24	12.54	12.55	11.89	0.55	0.1774	0.3261	0.0513	0.7087
d 29	16.66	16.77	16.95	15.92	0.62	0.0853	0.1252	0.0604	0.3315
d 40	22.26	22.56	22.33	21.61	0.79	0.5971	0.3418	0.3133	0.9834
ADG, kg									
d 0-7	0.066	0.068	0.105	0.078	0.013	0.0956	0.177	0.2153	0.0736
d 7-14	0.313	0.3	0.326	0.301	0.02	0.5078	0.8753	0.667	0.1518
d 14-21	0.457	0.484	0.446	0.406	0.019	0.0103	0.0088	0.0382	0.3541
d 21-29	0.597	0.605	0.629	0.549	0.021	0.0464	0.1777	0.0304	0.1772
d 29-40	0.508	0.526	0.489	0.506	0.035	0.7955	0.7162	0.9945	0.3562
d 0-14	0.19	0.184	0.216	0.19	0.013	0.2162	0.5358	0.3619	0.0722
d 14-29	0.531	0.541	0.531	0.479	0.016	0.0058	0.0058	0.0183	0.6781
d 0-29	0.37	0.372	0.379	0.343	0.012	0.0623	0.0908	0.0568	0.2888
d 0-40	0.408	0.415	0.409	0.391	0.014	0.5637	0.3017	0.3138	0.9921

Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

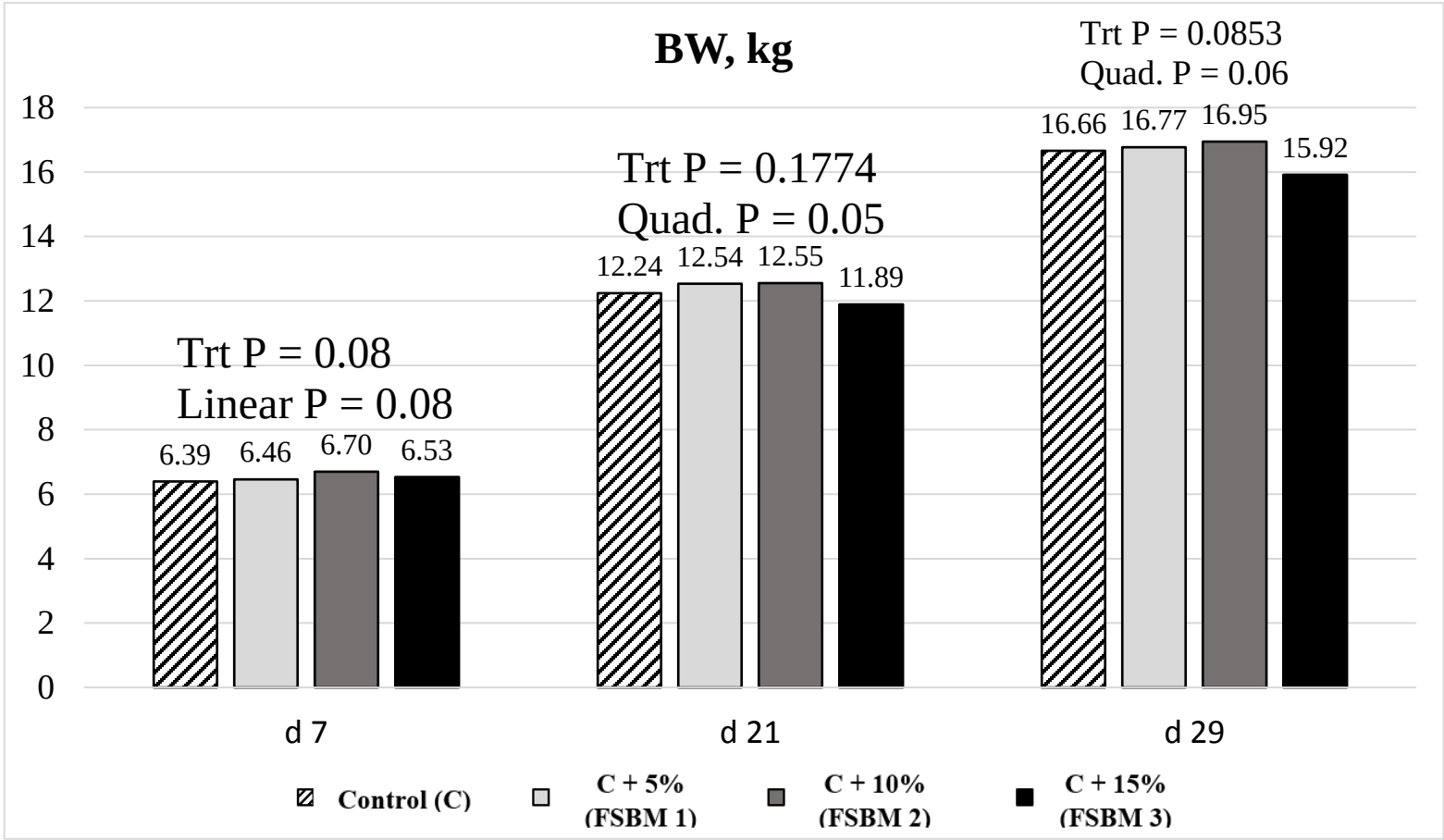
Table 21. Effect of feeding increasing levels of fermented soybean meal (FSBM) on ADFI and feed efficiency in nursery pigs (LS means).

	FSBM					P - Value			
	Control (C)	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C + 15% (FSBM3)**	**SEM**	**Trt**	**Linear**	**Quad.**	**Cub.**
ADFI, kg									
d 0-7	0.115	0.131	0.150	0.128	0.008	0.057	0.154	0.034	0.242
d 7-14	0.356	0.327	0.351	0.306	0.022	0.244	0.152	0.682	0.162
d 14-21	0.574	0.574	0.548	0.500	0.027	0.086	0.019	0.290	0.967
d 21-29	0.789	0.808	0.817	0.737	0.028	0.128	0.195	0.057	0.492
d 29-40	1.040	1.029	0.987	0.930	0.044	0.184	0.035	0.542	0.929
d 0-14	0.240	0.229	0.250	0.217	0.013	0.152	0.312	0.316	0.070
d 14-29	0.674	0.683	0.673	0.611	0.022	0.012	0.009	0.033	0.640
d 0-29	0.463	0.464	0.469	0.420	0.015	0.017	0.026	0.037	0.261
d 0-40	0.626	0.619	0.612	0.565	0.020	0.025	0.007	0.176	0.551
G:F									
d 0-7	0.531	0.451	0.702	0.598	0.098	0.243	0.269	0.891	0.091
d 7-14	0.904	0.916	0.929	1.061	0.082	0.463	0.175	0.445	0.741
d 14-21	0.801	0.833	0.830	0.816	0.026	0.809	0.728	0.376	0.843
d 21-29	0.759	0.751	0.773	0.747	0.020	0.809	0.877	0.651	0.398
d 29-40	0.492	0.515	0.487	0.545	0.019	0.112	0.114	0.334	0.087
d 0-14	0.807	0.796	0.860	0.886	0.043	0.380	0.121	0.665	0.549
d 14-29	0.778	0.789	0.793	0.776	0.015	0.843	0.967	0.383	0.840
d 0-29	0.783	0.791	0.809	0.800	0.014	0.587	0.285	0.534	0.535
d 0-40	0.650	0.665	0.669	0.684	0.013	0.334	0.075	0.981	0.698

Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

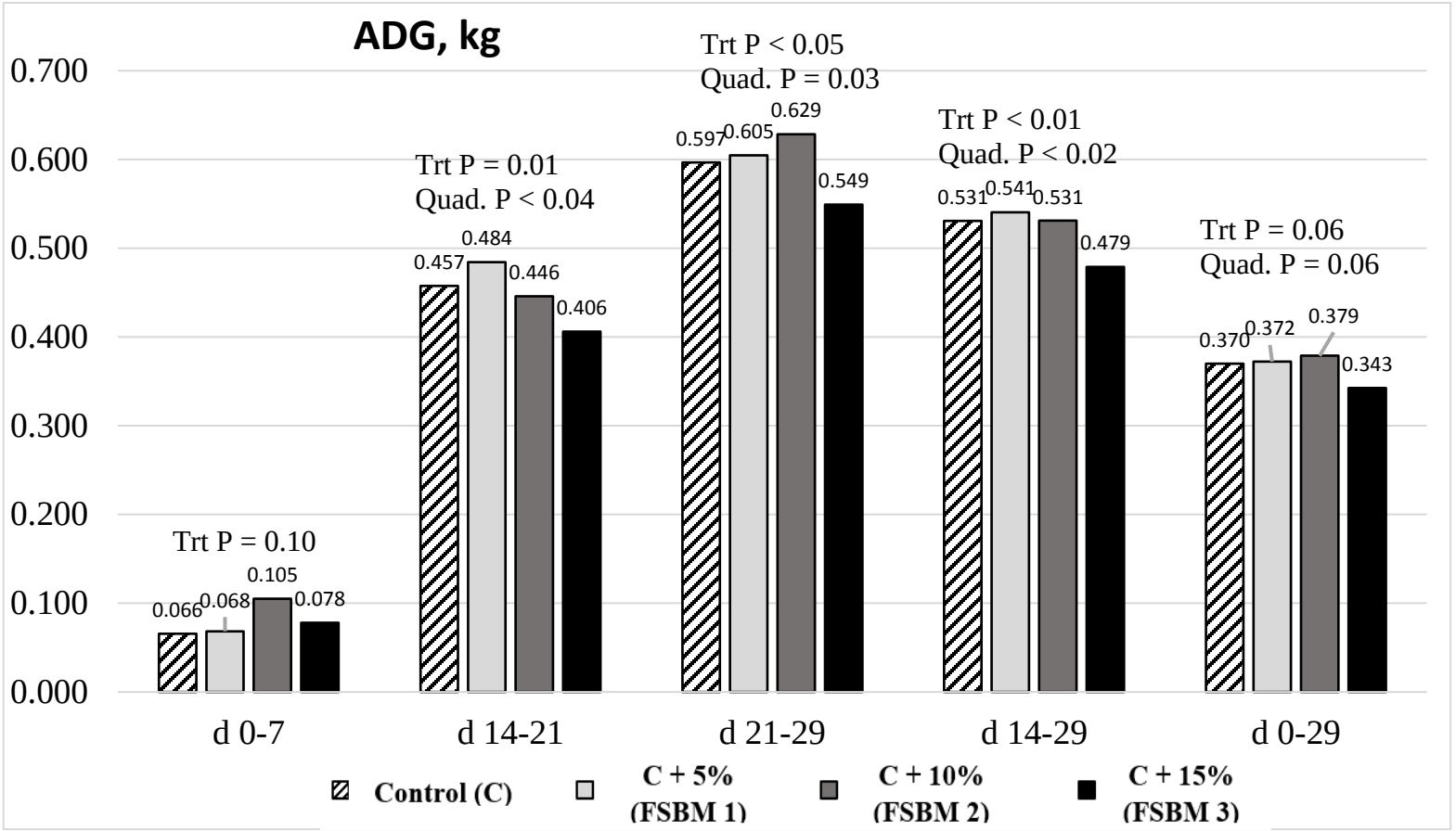


Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 15. Effect of increasing levels of fermented soybean meal (FSBM) on BW in nursery pigs (LS means).

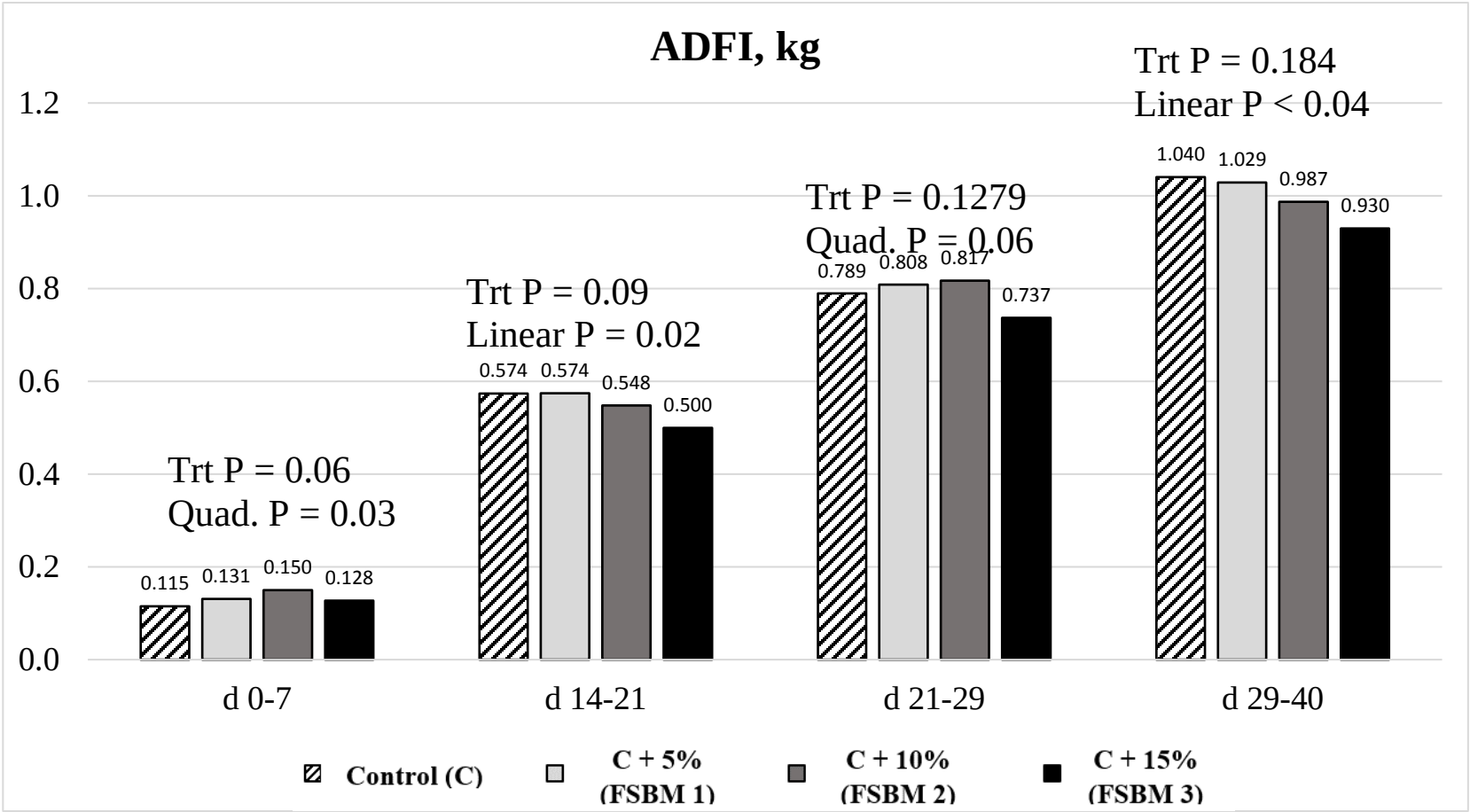


Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 16. Effect of increasing levels of fermented soybean meal (FSBM) on ADG in nursery pigs (LS means).

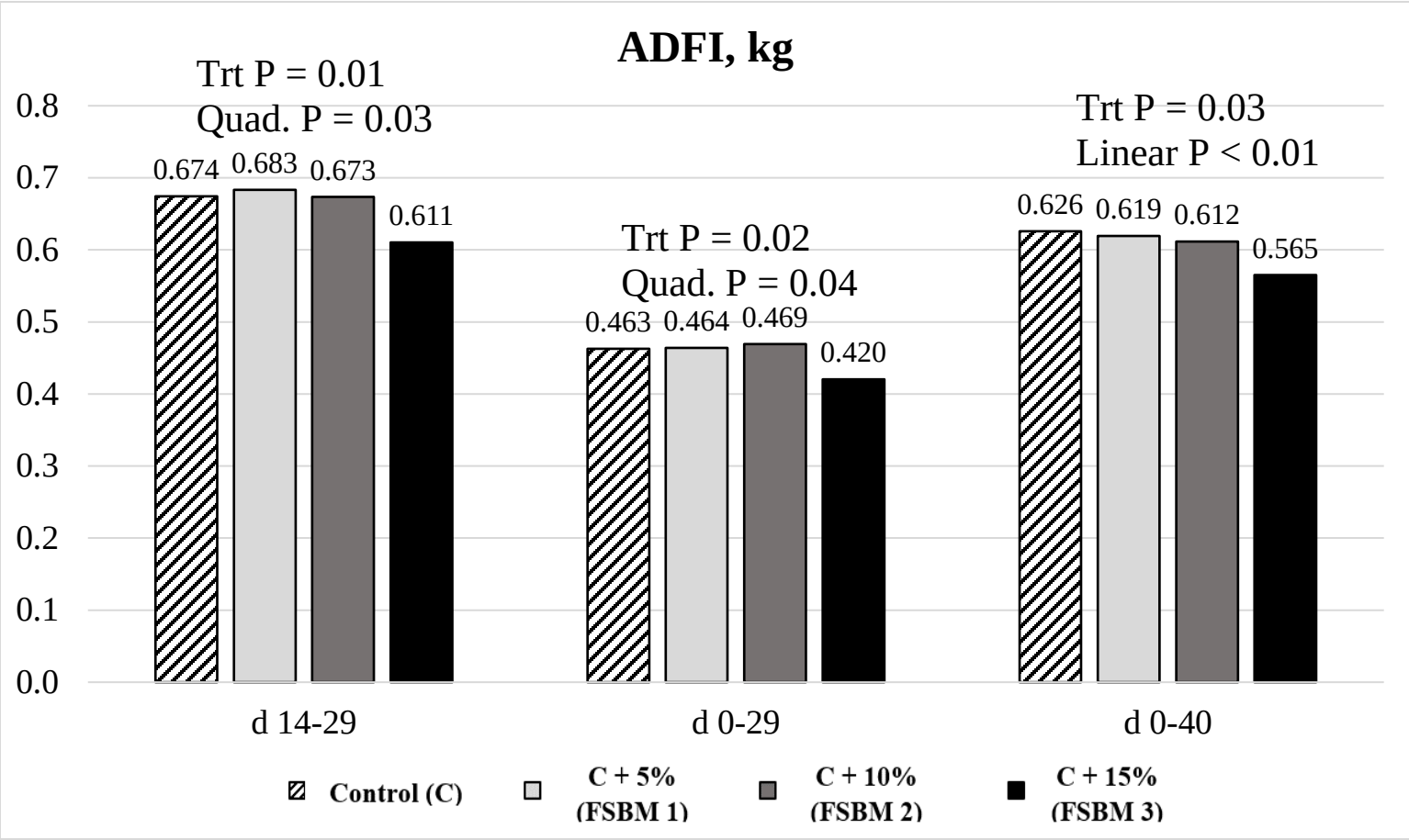


Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 17. Effect of increasing levels of fermented soybean meal (FSBM) on ADFI in nursery pigs (LS means).

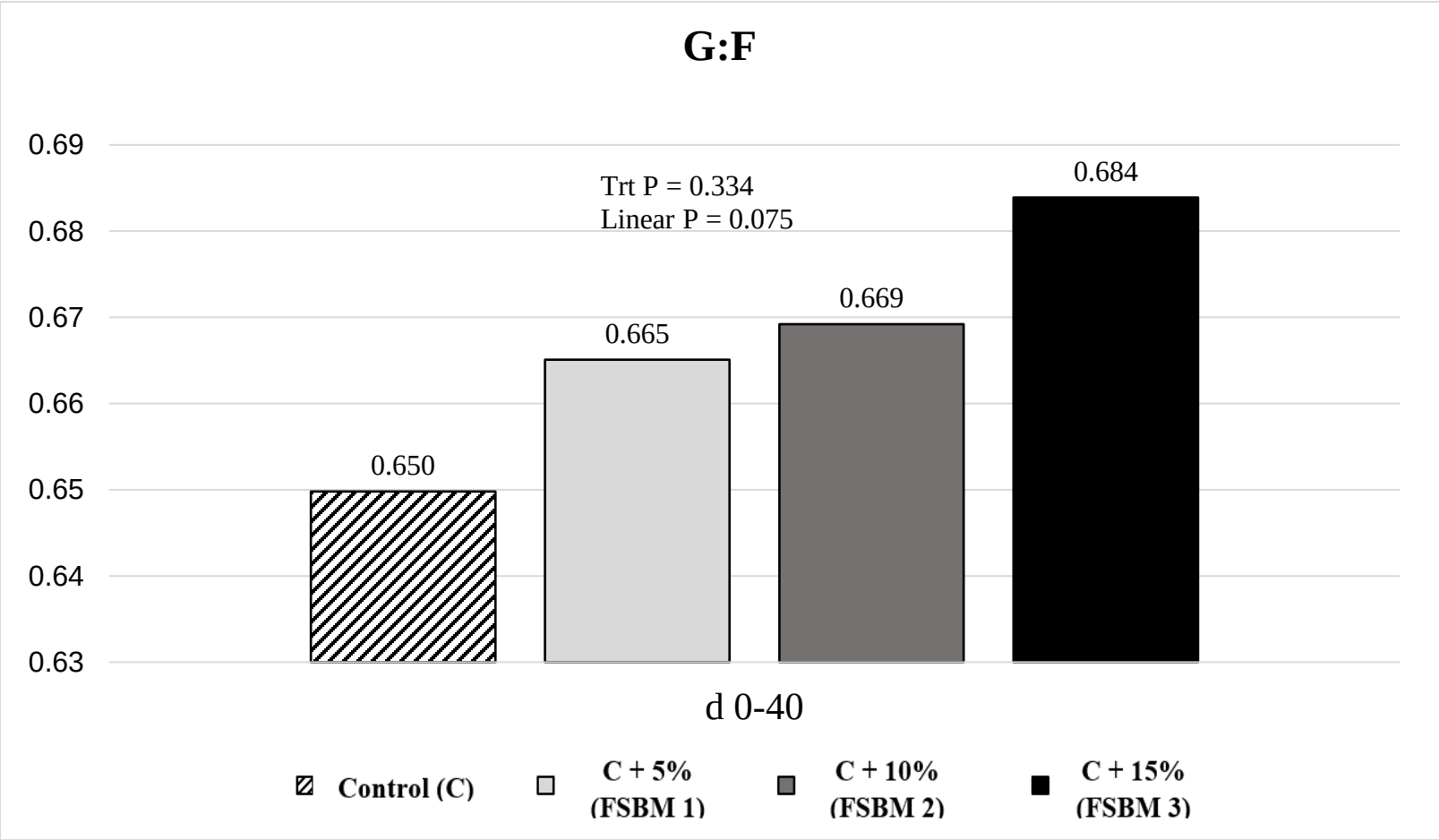


Weaned pigs were blocked by initial BW and allotted to pens. Pens then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 18. Effect of increasing levels of fermented soybean meal (FSBM) on ADFI in nursery pigs (LS means).



Weaned pigs were blocked by initial BW and allotted to pens. Pens were then randomly allotted to dietary treatments. Pigs were fed three feeding regimes: Phase 1 (d 0-14); Phase 2 (d 14-29); Phase 3 (d 29 to 40) for this trial. Data were analyzed using MIXED procedure of SAS with treatment as main effect and pen was the experimental unit. IML procedure was used to generate coefficient for orthogonal contrast.
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 19. Effect of increasing levels of fermented soybean meal (FSBM) on overall feed efficiency in nursery pigs (LS means).

Table 22. Effect of feeding incremental levels of fermented soybean meal (FSBM) on CBC in nursery pigs (LS means separated by treatments).

	FSBM					P-Value				
	Control (C)	**C + 5% (FSBM1)**	**C + 10% (FSBM2)**	**C + 15% (FSBM3)**	**SEM**	**Trt**	**Trt*day**	**Linear**	**Quad**	**Cubic**
Concentration, k/µl										
WBC	15.010	16.361	14.674	16.952	0.599	0.021	0.435	0.122	0.435	0.009
Neutrophil	6.621	7.350	6.559	7.470	0.355	0.110	0.657	0.243	0.785	0.031
Lymphocyte	6.675	6.833	6.516	7.318	0.309	0.294	0.401	0.248	0.300	0.248
Monocyte	0.711	0.756	0.529	0.607	0.054	0.013	0.014	0.025	0.756	0.016
Eosinophil	0.890	1.239	1.041	1.373	0.108	0.004	0.330	0.006	0.931	0.016
Basophil	0.083	0.115	0.139	0.184	0.013	<0.0001	0.000	<0.0001	0.525	0.544
Percentage over WBC										
Neutrophil	0.530	0.588	0.525	0.598	0.028	0.110	0.657	0.234	0.785	0.031
Lymphocyte	0.534	0.547	0.521	0.585	0.025	0.294	0.401	0.248	0.300	0.248
Monocyte	0.057	0.060	0.042	0.049	0.004	0.013	0.014	0.025	0.756	0.016
Eosinophil	0.071	0.099	0.083	0.110	0.009	0.004	0.330	0.006	0.931	0.016
Basophil	0.007	0.009	0.011	0.015	0.001	<0.0001	0.000	<0.0001	0.525	0.544
NLR	105.500	115.400	102.800	107.100	6.600	0.501	0.599	0.784	0.644	0.152
RBC, M/µl	7.280	7.180	6.900	7.280	0.140	0.188	0.179	0.661	0.090	0.191
Hemoglobin, g/dL	9.340	9.070	8.050	8.310	0.190	0.000	0.042	<0.0001	0.165	0.019
Hematocrit, %	34.480	33.780	30.980	32.240	0.650	0.001	0.049	0.001	0.133	0.034
MCV	47.200	47.210	44.700	44.260	0.500	<0.0001	0.240	<0.0001	0.608	0.018
MCH, Pg	12.850	12.610	11.580	11.370	0.190	<0.0001	0.036	<0.0001	0.960	0.035
MCHC, g/dL	27.170	26.800	25.850	25.660	0.220	<0.0001	0.023	<0.0001	0.661	0.151
RDW, %	27.050	27.290	28.780	28.510	0.430	0.006	0.262	0.002	0.545	0.107
PLT, k/µl	395.600	372.450	289.800	258.340	22.570	<0.0001	0.381	<0.0001	0.853	0.268
MPV, fL	9.720	9.080	8.060	7.610	0.260	<0.0001	0.705	<0.0001	0.657	0.347

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

Mean corpuscular volume (MCV): average of red cells

Mean corpuscular hemoglobin (MCH): hemoglobin amount per red blood cell

Mean corpuscular hemoglobin concentration (MCHC): hemoglobin amount relative to size of hemoglobin per red blood cell

Red cell distribution width (RDW): calculation of variation in size of red blood cell

Mean platelet volume (MPV): calculation average size of platelets

Table 23. Effect of feeding increasing levels of fermented soybean meal (FSBM) on CBC in nursery pigs (LS means separated by day).

	Day					P – Value
	0	14	29	40	SEM	day
Concentration, k/µl						
WBC	9.060	20.200	13.550	20.180	0.600	<0.0001
Neutrophil	4.260	11.150	5.310	7.280	0.360	<0.0001
Lymphocyte	4.410	7.290	6.110	9.530	0.310	<0.0001
Monocyte	0.260	0.480	0.440	1.410	0.050	<0.0001
Eosinophil	0.200	1.000	1.630	1.720	0.110	<0.0001
Basophil	0.040	0.220	0.050	0.210	0.010	<0.0001
Percentage over WBC						
Neutrophil	0.340	0.890	0.420	0.580	0.030	<0.0001
Lymphocyte	0.350	0.580	0.490	0.760	0.020	<0.0001
Monocyte	0.020	0.040	0.040	0.110	0.004	<0.0001
Eosinophil	0.020	0.080	0.130	0.140	0.010	<0.0001
Basophil	0.000	0.020	0.000	0.020	0.000	<0.0001
NLR	101.600	157.500	90.100	81.600	6.600	<0.0001
RBC, M/µl	6.040	6.980	7.280	8.340	0.140	<0.0001
Hemoglobin, g/dL	6.910	8.150	8.790	10.920	0.190	<0.0001
Hematocrit, %	27.200	30.300	33.200	40.800	0.600	<0.0001
MCV	45.300	43.500	45.700	48.900	0.500	<0.0001
MCH, Pg	11.400	11.700	12.100	13.200	0.200	<0.0001
MCHC, g/dL	25.200	26.900	26.500	26.900	0.200	0.010
RDW, %	33.100	30.100	25.700	22.700	0.400	<0.0001
PLT, k/µl	468.900	265.800	417.700	163.800	22.600	<0.0001
MPV, fL	8.557	8.575	8.959	8.372	0.264	0.309

Significant Value: $P \leq 0.05$

Values tended to be significant at $0.05 \leq P \leq 0.10$

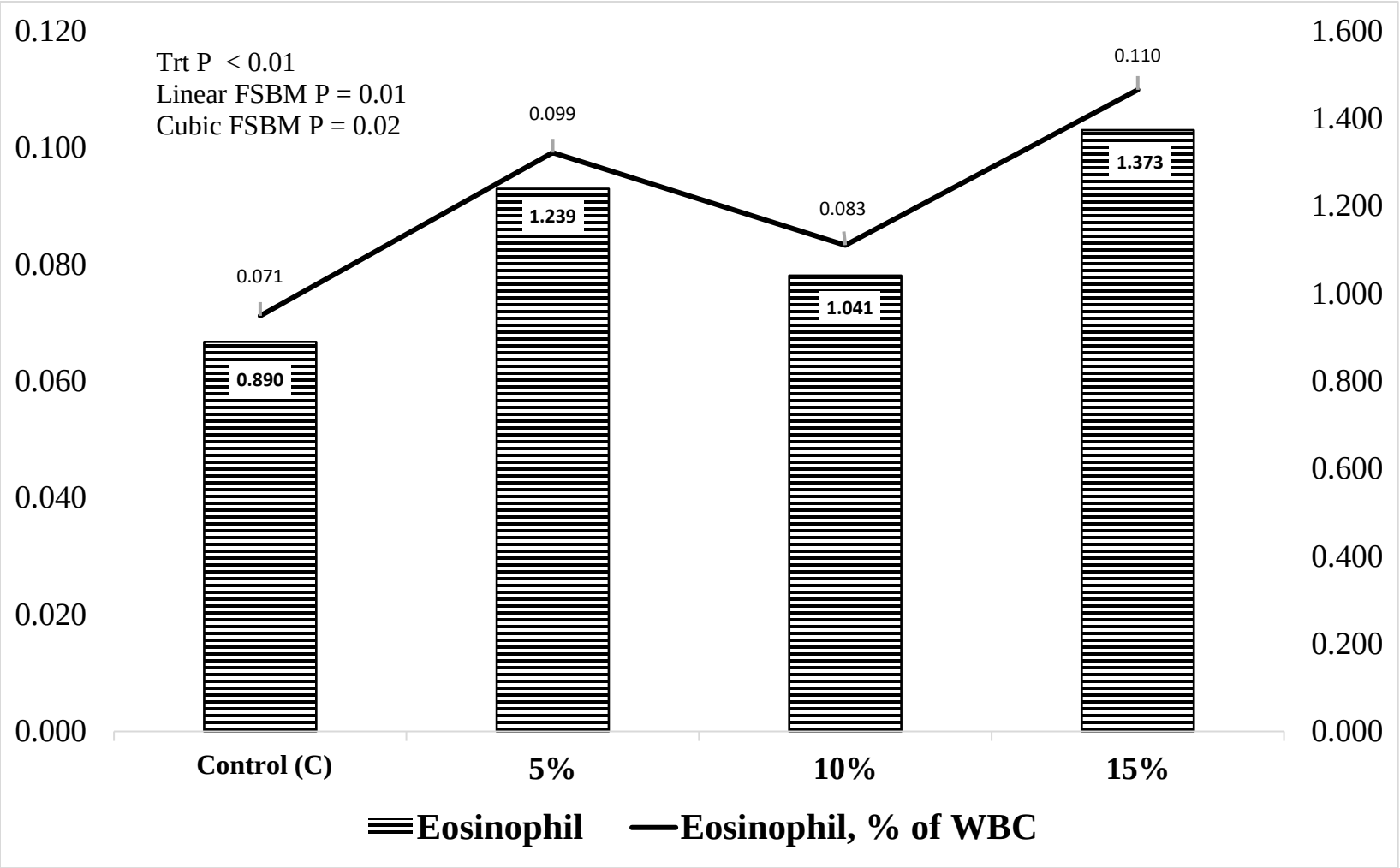
Mean corpuscular volume (MCV): average of red cells

Mean corpuscular hemoglobin (MCH): hemoglobin amount per red blood cell

Mean corpuscular hemoglobin concentration (MCHC): hemoglobin amount relative to size of hemoglobin per red blood cell

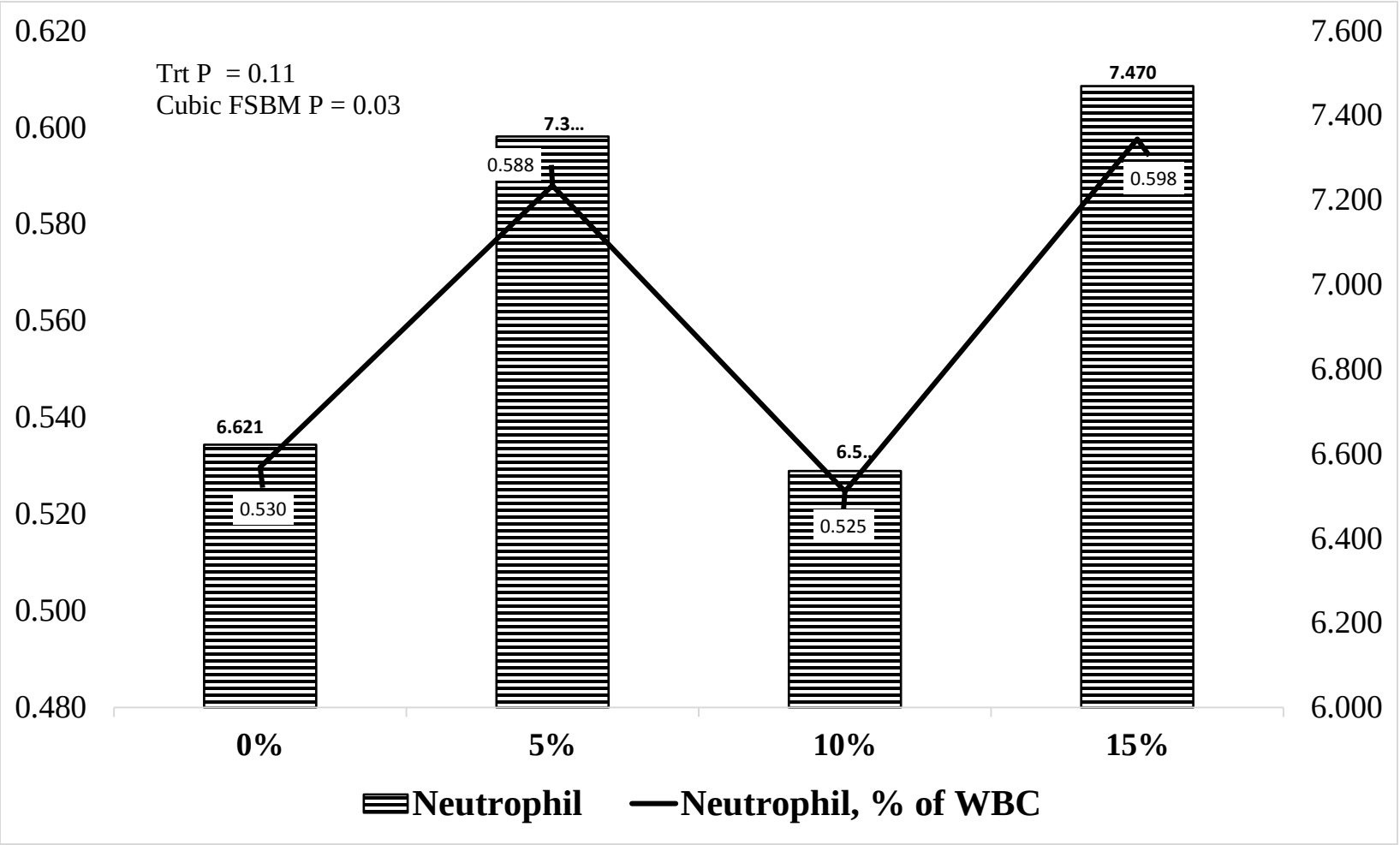
Red cell distribution width (RDW): calculation of variation in size of red blood cell

Mean platelet volume (MPV): calculation average size of platelets



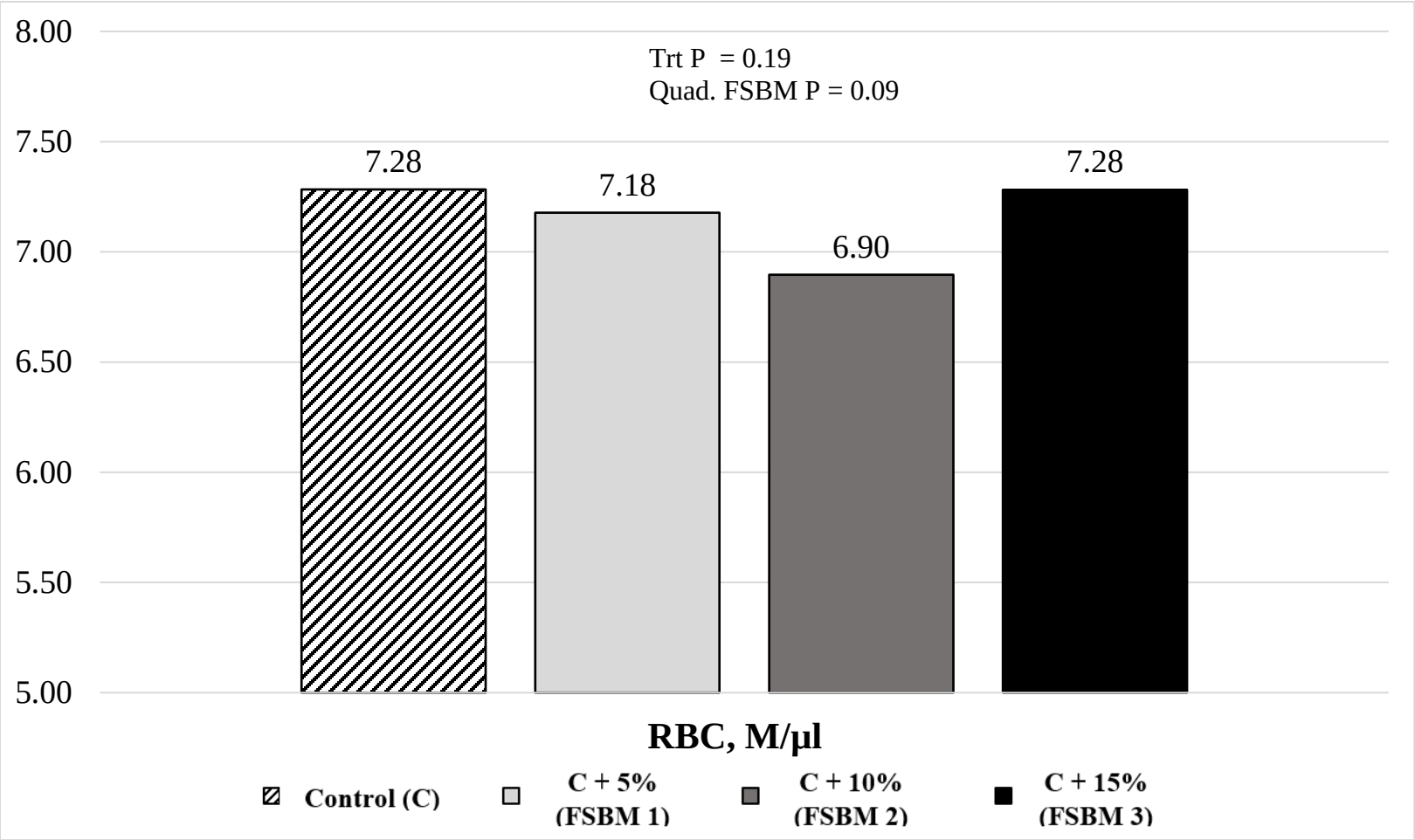
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 20. Effect of increasing levels of fermented soybean meal (FSBM) on eosinophil concentration, and eosinophil percentage over WBC.



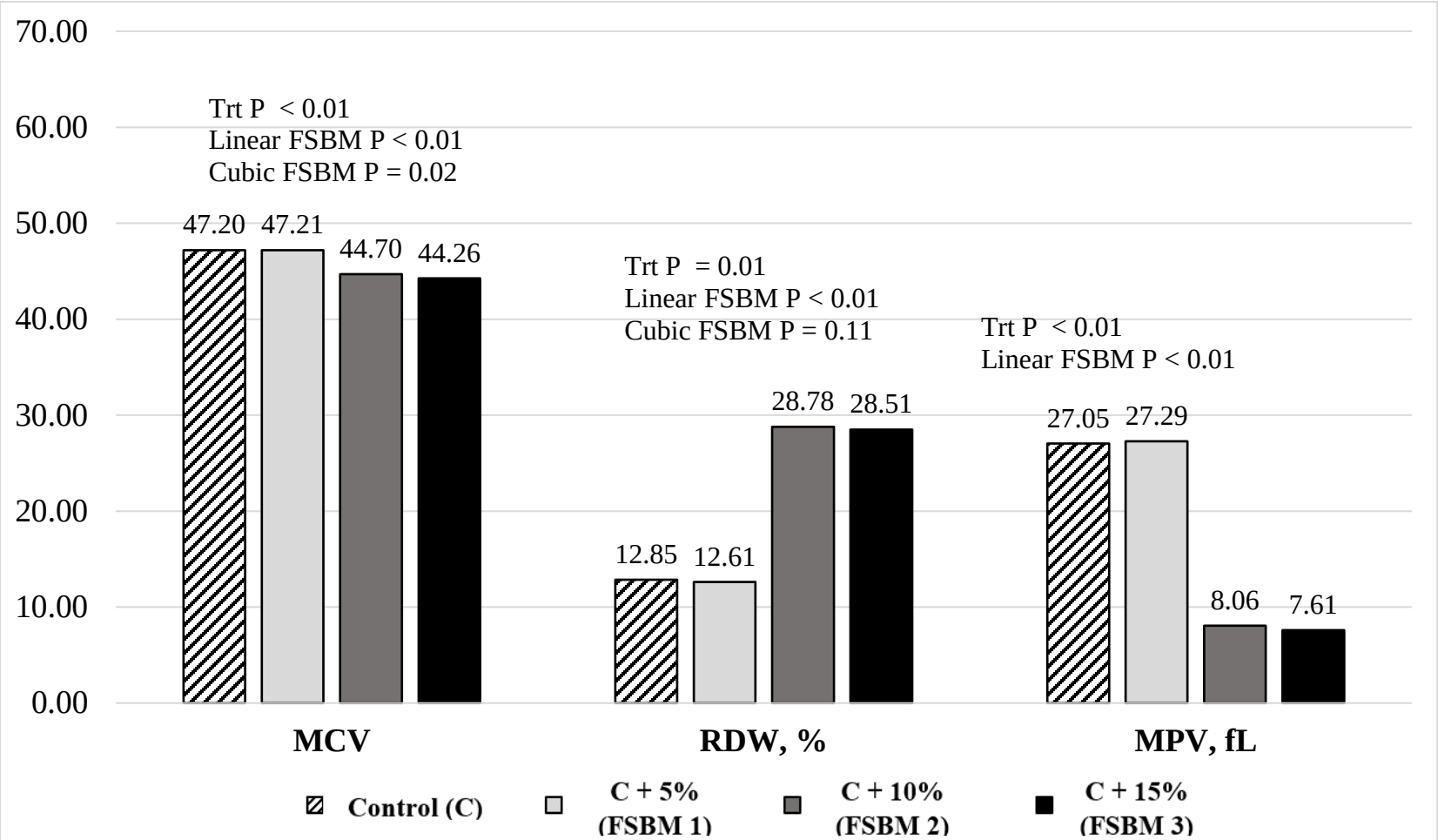
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 21: Effect of increasing levels of fermented soybean meal (FSBM) on neutrophil concentration and neutrophil percentage over WBC.



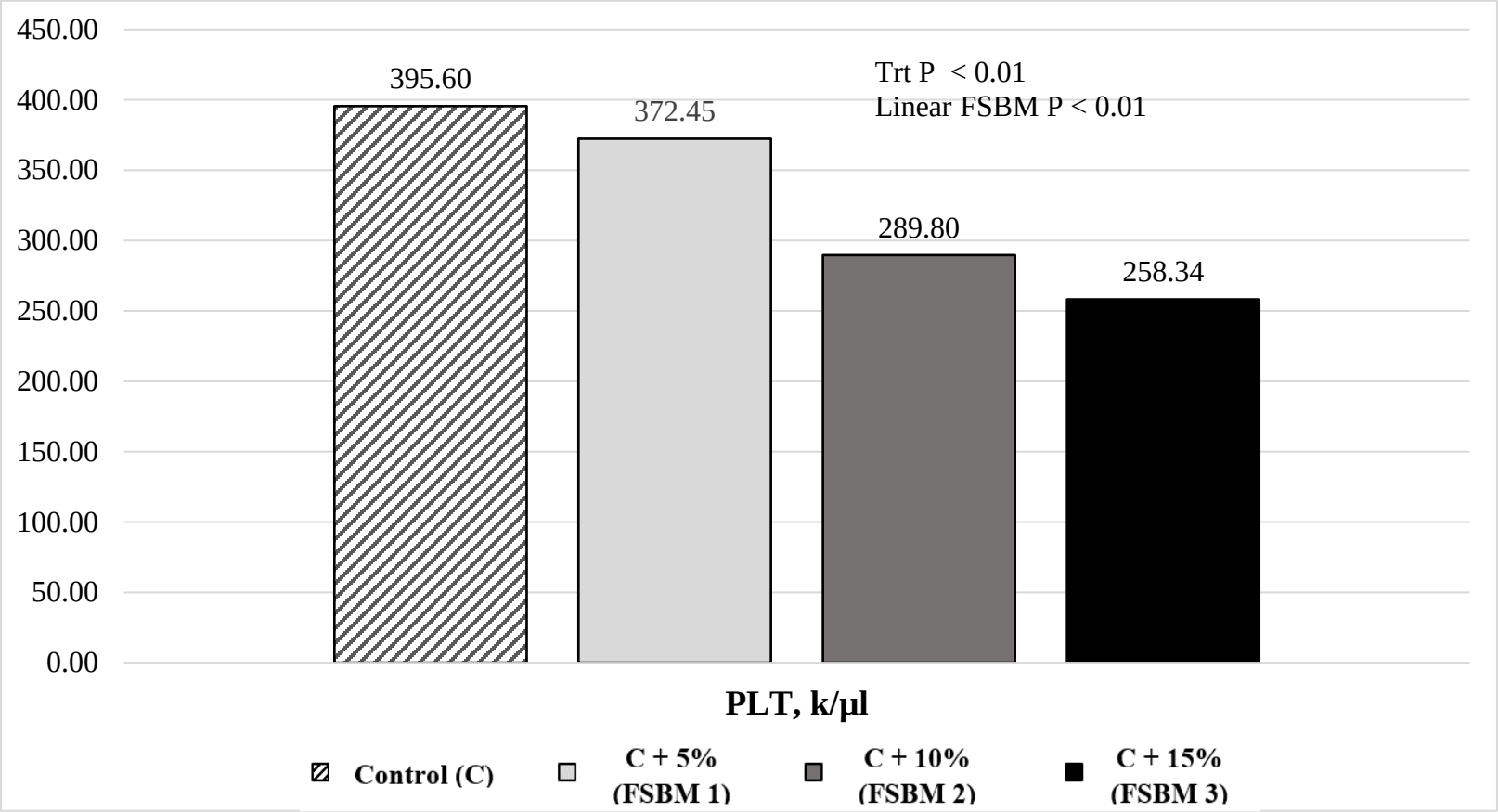
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 22. Effect of increasing levels of fermented soybean meal (FMSB) on RBC count.



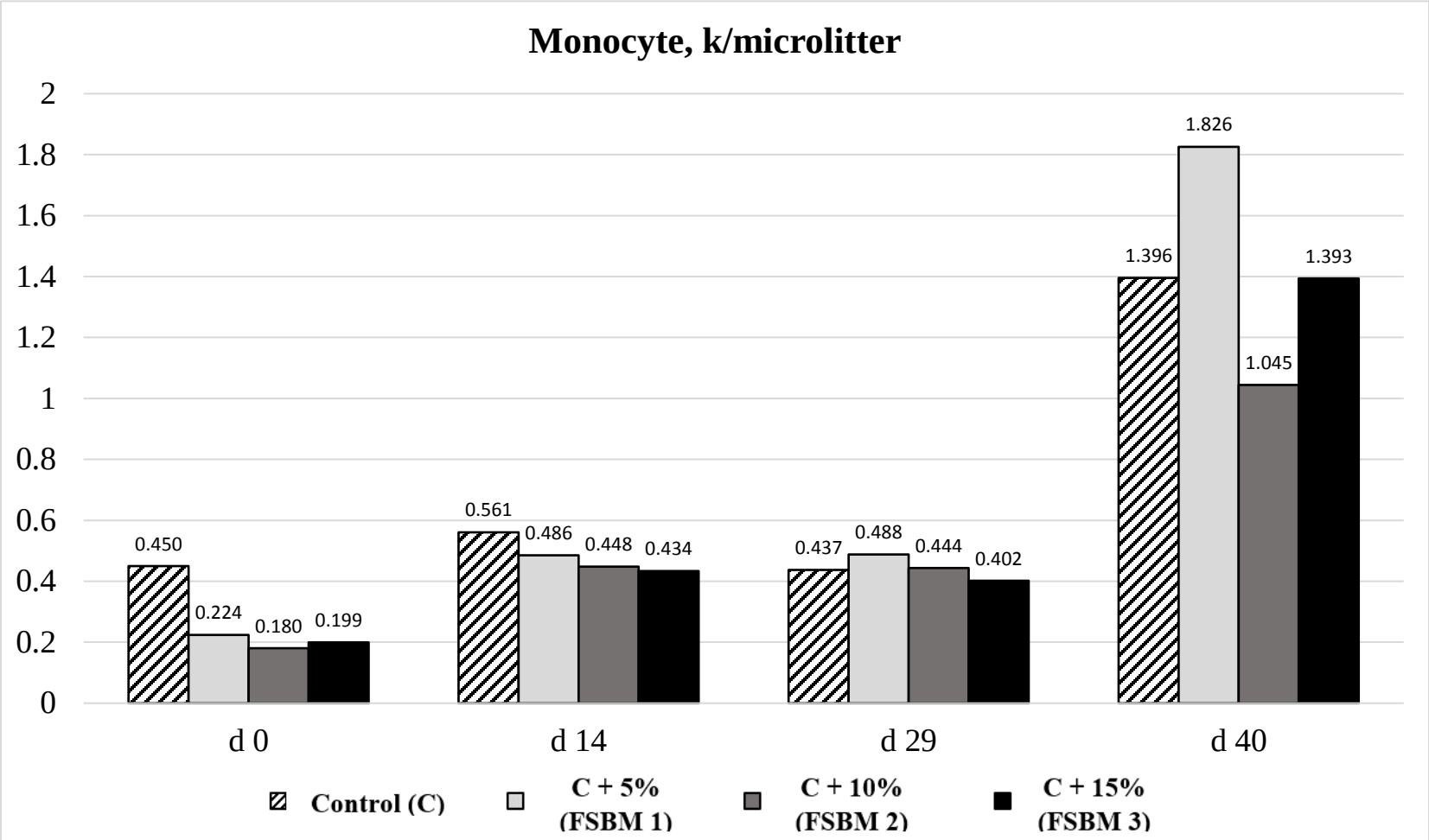
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 23. Effect of increasing levels of fermented soybean meal (FSBM) on MCV, RDW and MPV.



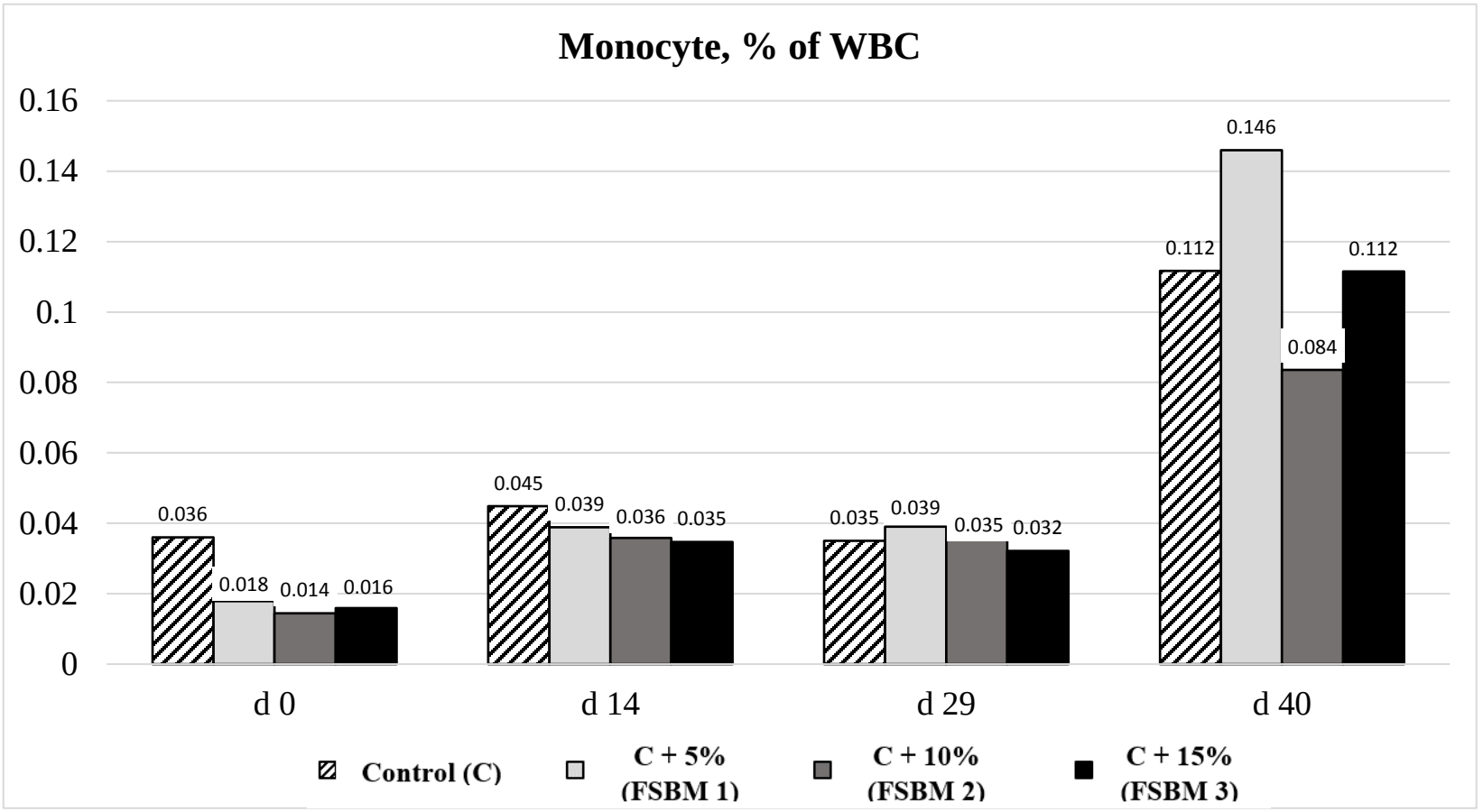
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 24. Effect of increasing levels of fermented soybean meal (FSBM) on PLT.



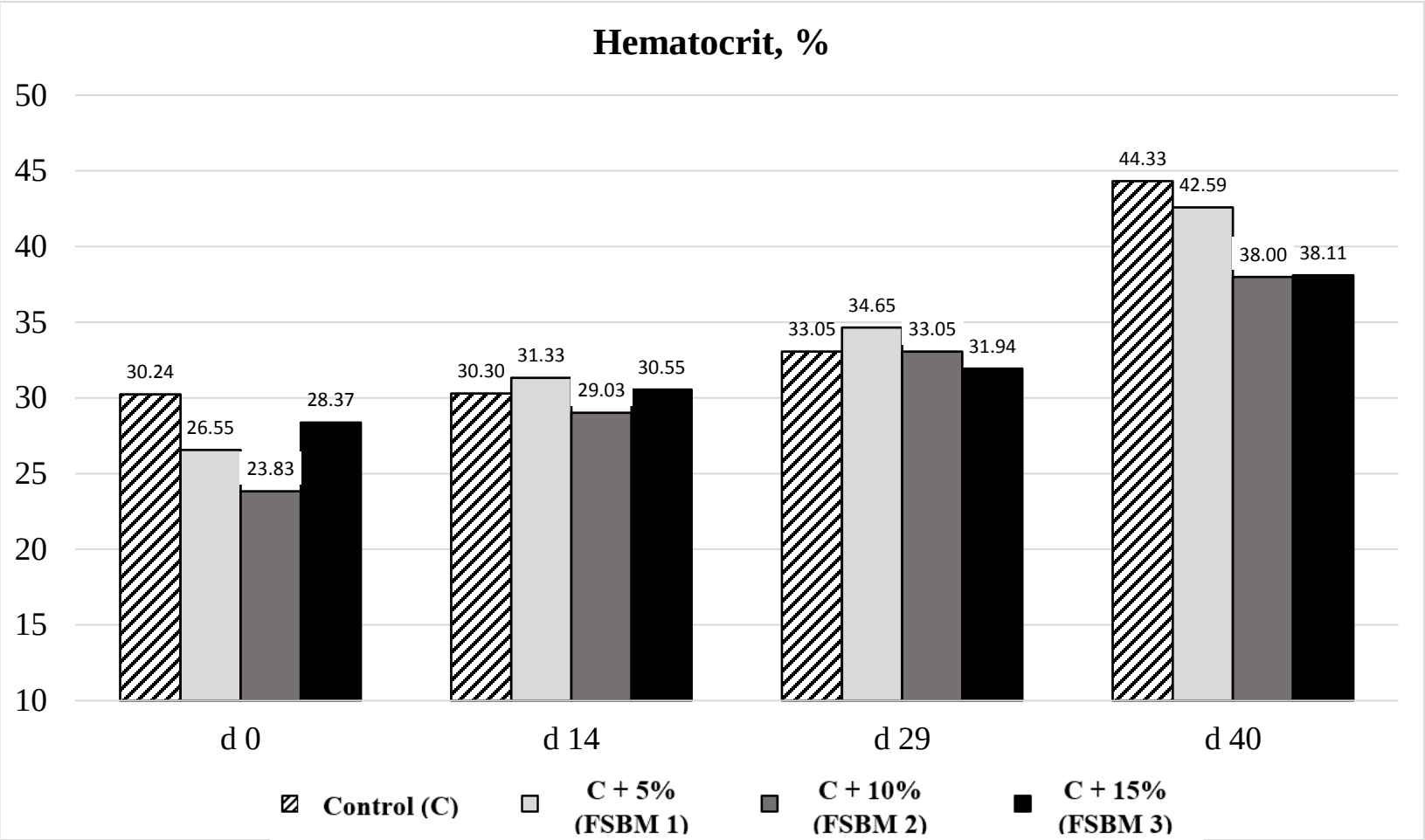
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 25. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on monocyte concentration.



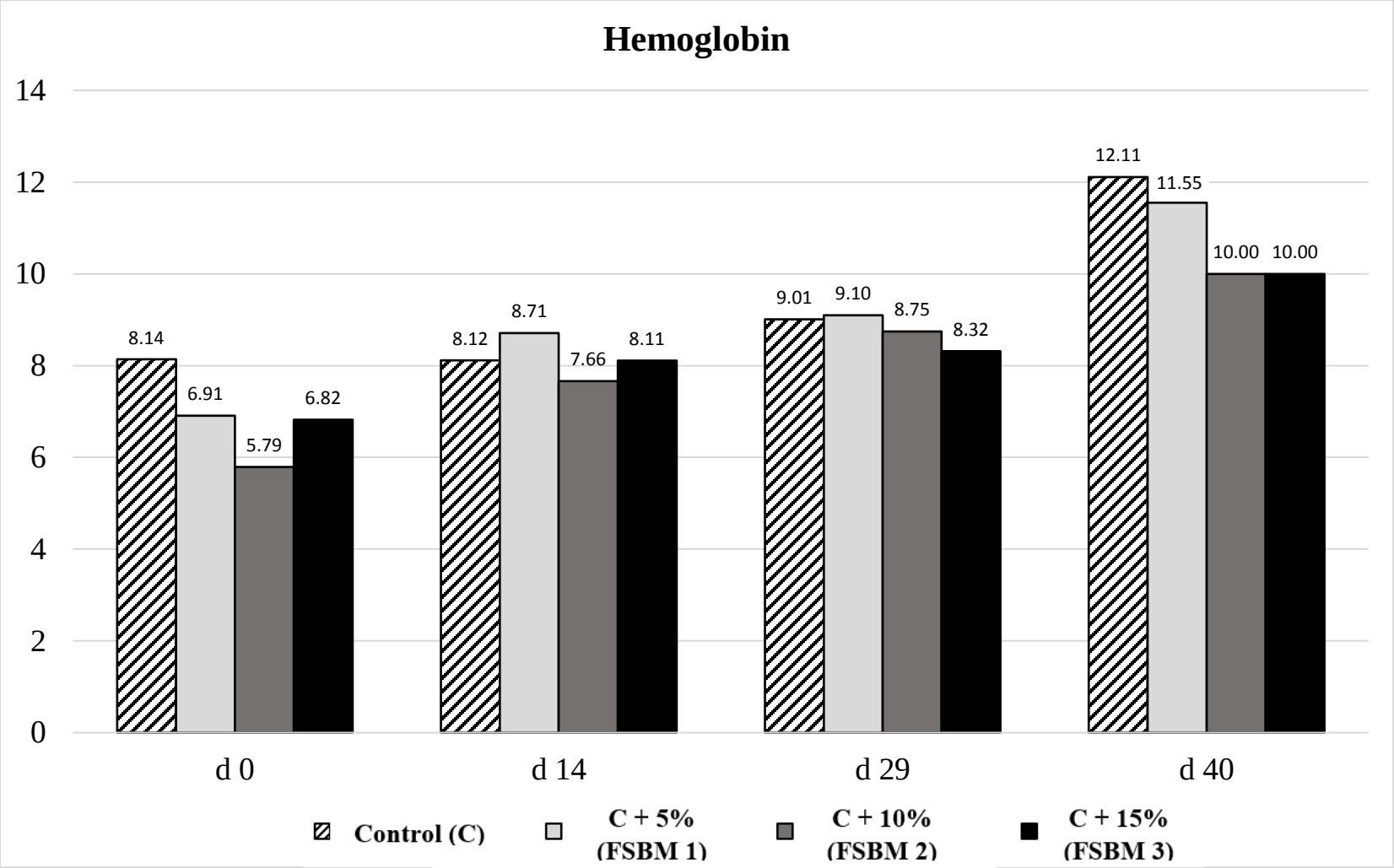
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 26. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on monocyte percentage of WBC.



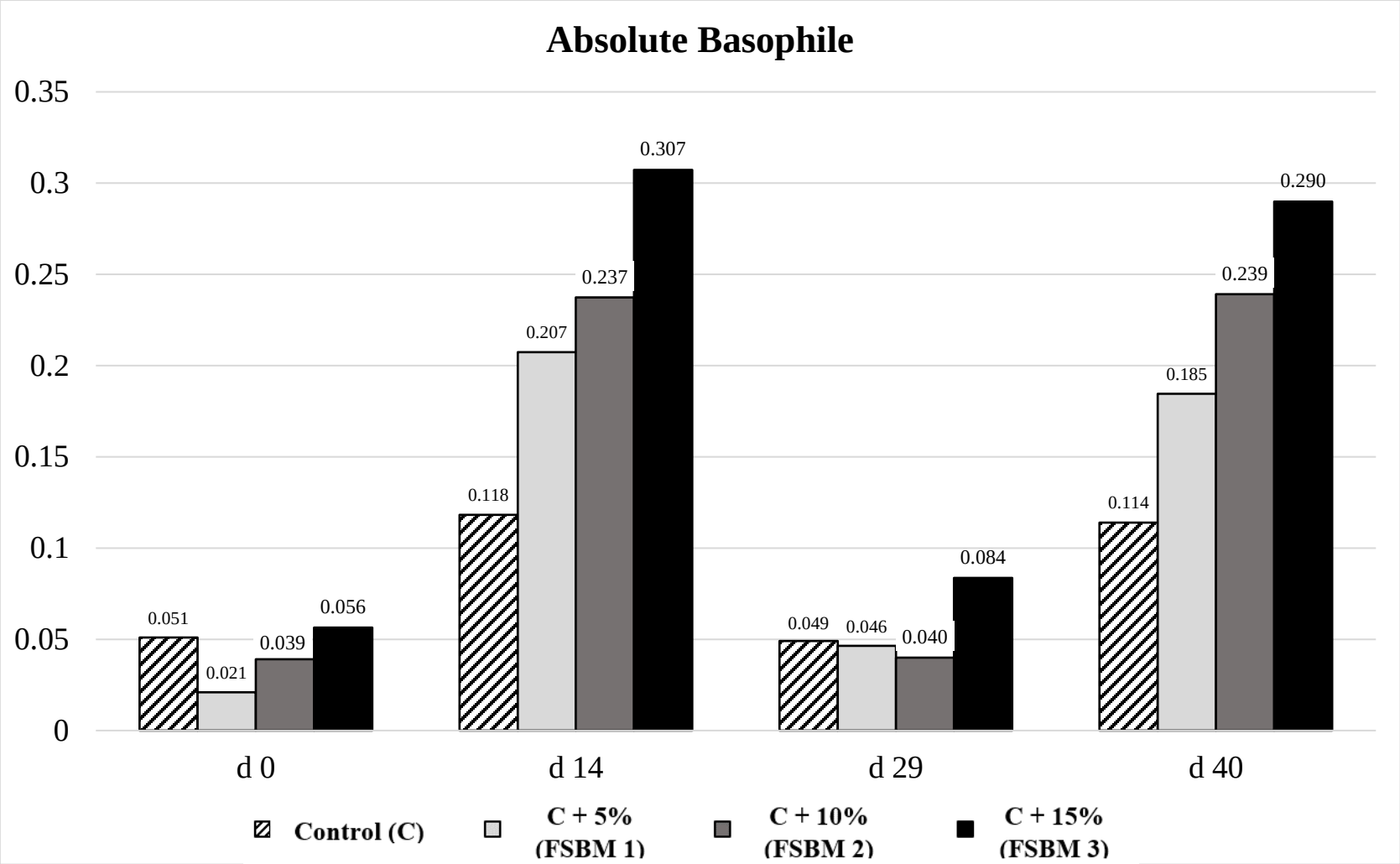
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 27. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on hematocrit percentage.



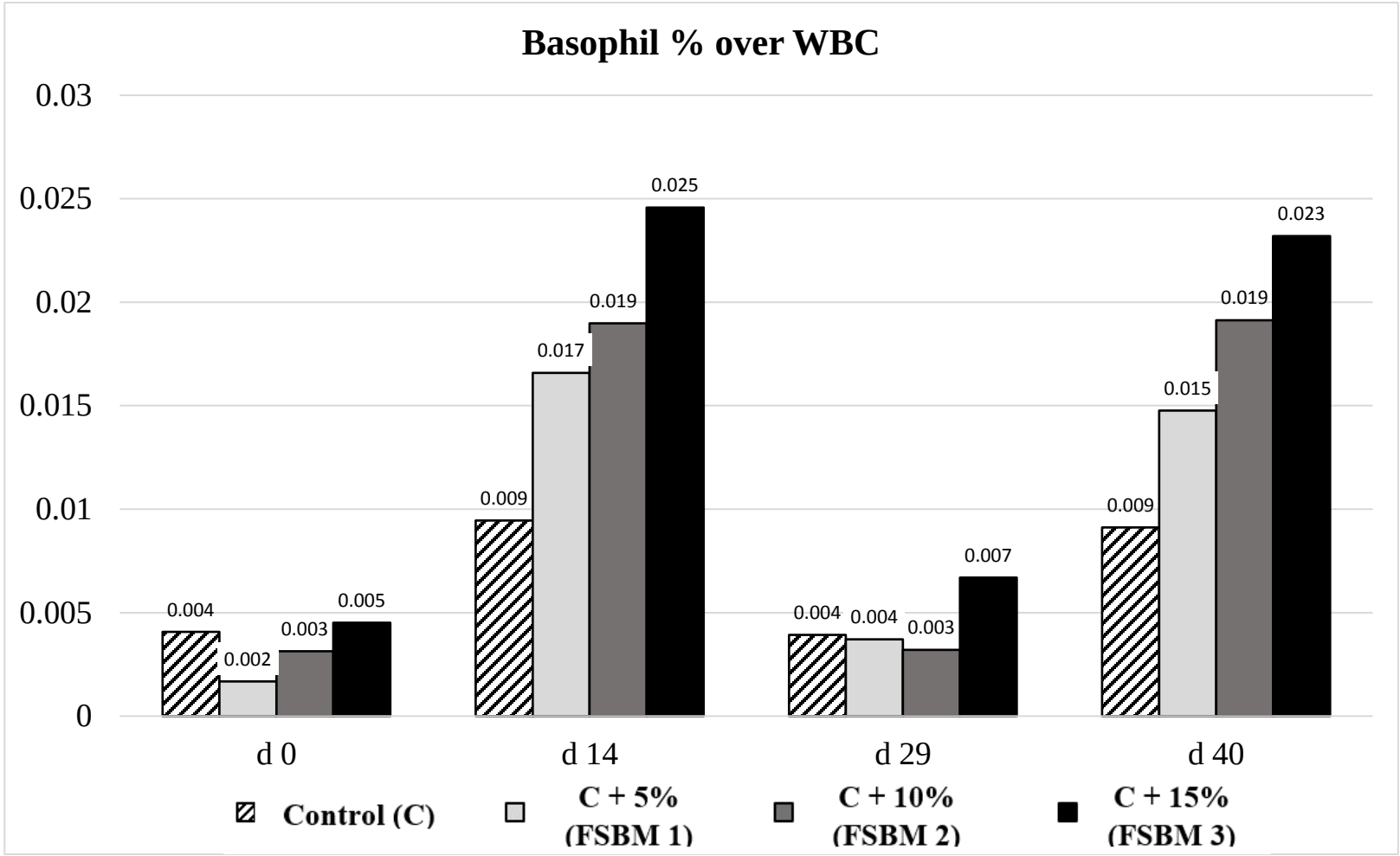
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 28. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on hemoglobin.



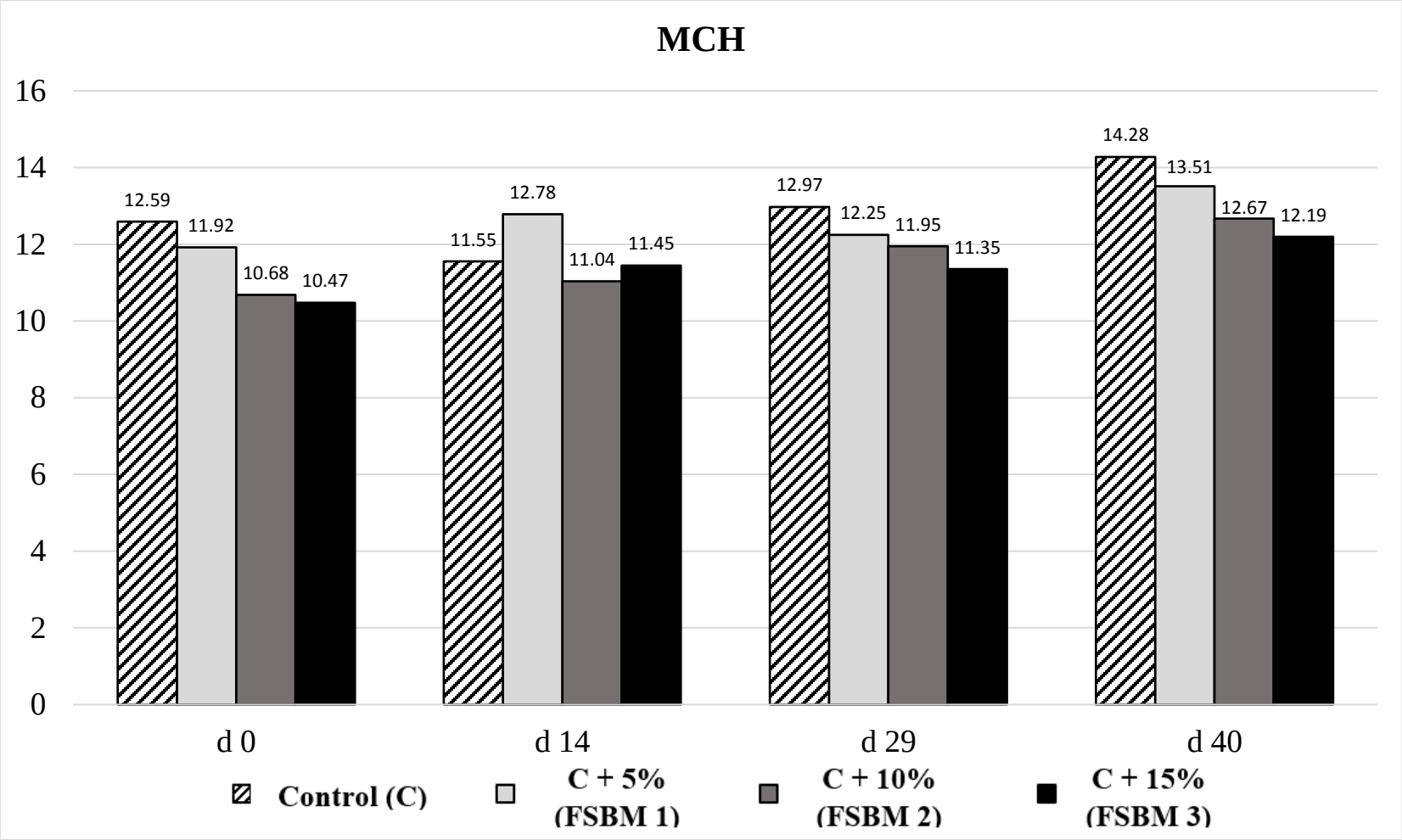
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
Significant Value: $P \leq 0.05$
Values tended to be significant at $0.05 \leq P \leq 0.10$

Figure 29. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on basophil concentration.



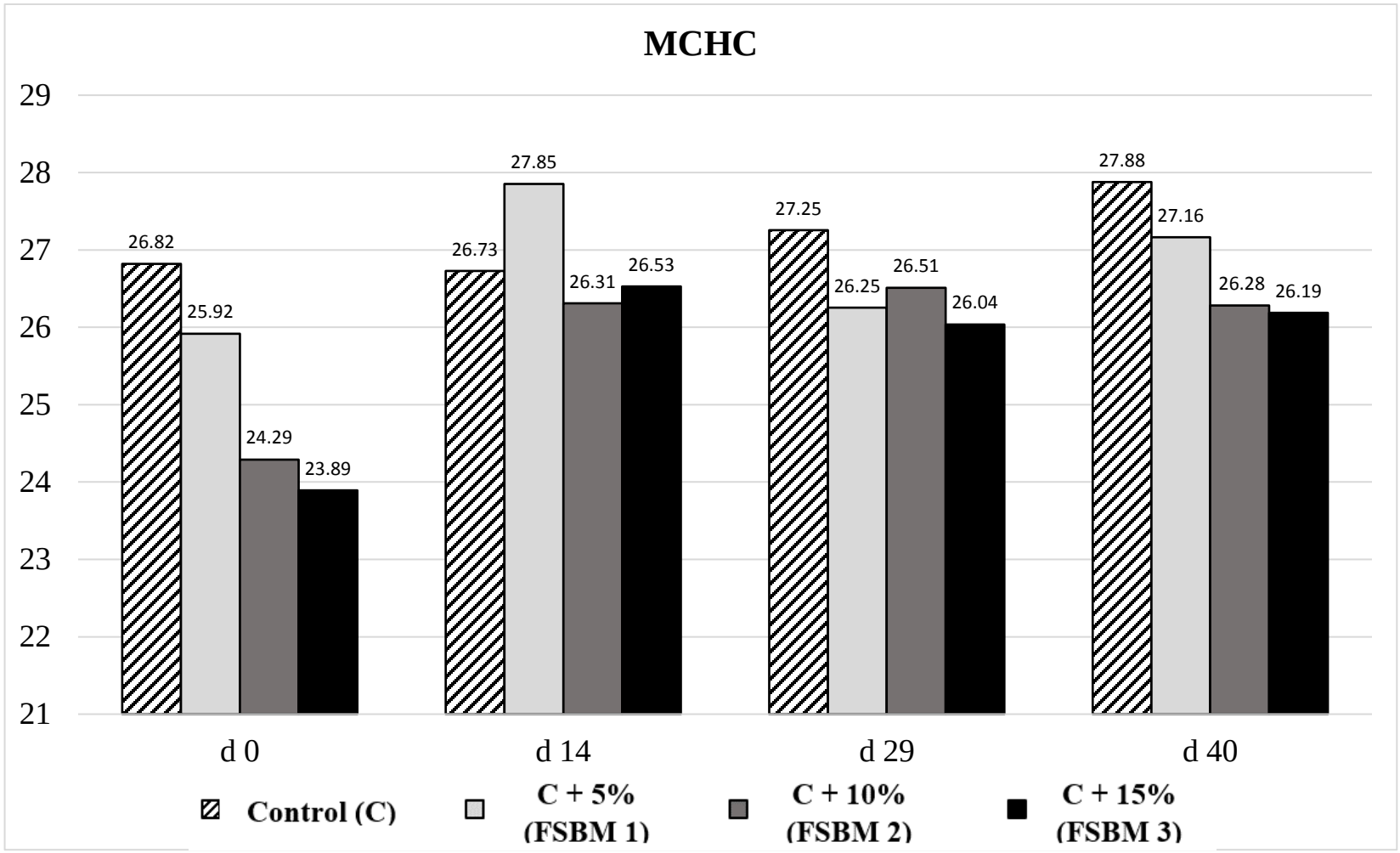
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc). *Significant Value: $P \leq 0.05$

Figure 30. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on basophil percentage over WBC.



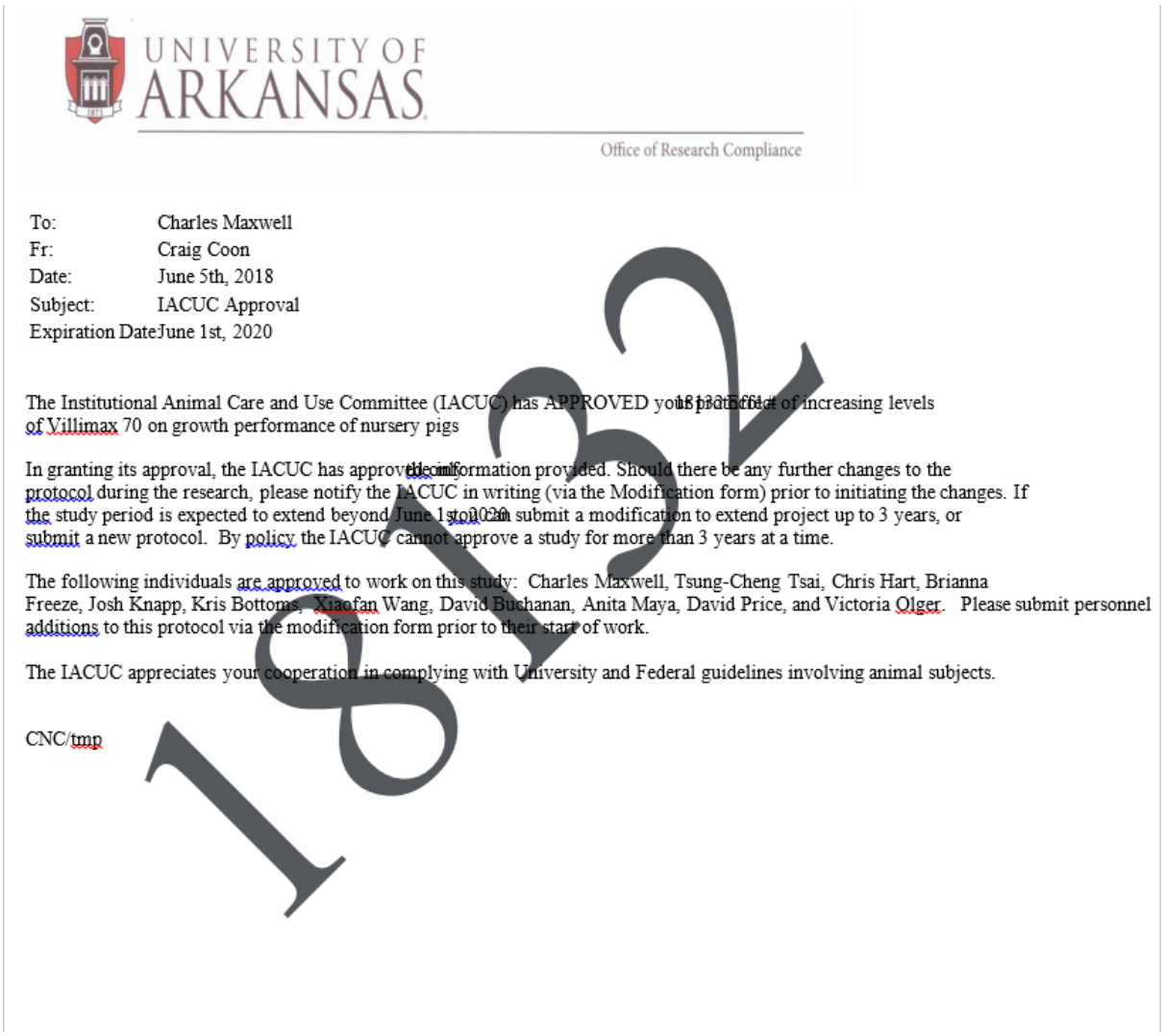
Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc). *Significant Value: $P \leq 0.05$

Figure 31. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on MCH.



Samples were collected at d 0, 14, 29 and 40. Whole blood was assayed by Hemavet instrument (Drew Scientific Inc).
*Significant Value: $P \leq 0.05$

Figure 32. Treatment by day interaction effect of increasing levels of fermented soybean meal (FSBM) on MCHC.



UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Charles Maxwell
Fr: Craig Coon
Date: June 5th, 2018
Subject: IACUC Approval
Expiration Date June 1st, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol titled "Effect of increasing levels of Villimax 70 on growth performance of nursery pigs

In granting its approval, the IACUC has approved the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond June 1st, 2020 submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Charles Maxwell, Tsung-Cheng Tsai, Chris Hart, Brianna Freeze, Josh Knapp, Kris Bottoms, Xiaofan Wang, David Buchanan, Anita Maya, David Price, and Victoria Olger. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

18132

Figure 33: IACUC Approval Document (SB Study).

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Charles Maxwell
Fr: Craig Coon
Date: April 19th, 2018
Subject: IACUC Approval
Expiration Date: April 6th, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18110:** *Establishing ideal inclusion rate of FermEx 200 in nursery rations.*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond April 6th, 2020 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Charles Maxwell, Tsung-Cheng Tsai, Chris Hart, Brianna Freeze, Josh Knapp, Xiaofan Wang, David Buchanan, Anita Maya, David Price, Jordan Volkmann, Kriss Bottoms, and Michael Price. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

18110

Figure 34: IACUC Approval Document (FSBM Study).