SPRAY DRIED PLASMA INCREASES NUTRIENT DIGESTIBILITY, IMPROVES GROWTH PERFORMANCE, AND MODULATES THE INFLAMMATORY RESPONSE OF WEANED PIGS AND SOWS

BY

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DISSERTATION

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Abstract

Eight experiments were conducted to investigate effects of spray dried plasma (**SDP**) on nutrient and energy digestibility, growth performance, post-weaning diarrhea, and immune response of weanling pigs and lactating sows. The first 2 experiments were conducted to determine the effect of including SDP in a phase 1 diet fed to newly weaned pigs on energy and nutrient digestibility of a subsequent diet. At weaning, 16 gilts and 24 barrows were fed a phase 1 diet without or with 6% SDP until a common phase 2 diet without SDP was fed beginning on d 18 post-weaning when gilts were cannulated [body weight (**BW**): 6.92 ± 0.42 kg] or on d 21 post-weaning when barrows (BW: 9.37 ± 1.40 kg) were moved into individual metabolism crates. Results indicated that apparent ileal digestibility (**AID**) of acid hydrolyzed ether extract, starch, crude protein (**CP**), and amino acids (**AA**), and apparent total tract digestibility (**ATTD**) of gross energy (**GE**), total dietary fiber (**TDF**), Ca, and P in the phase 2 diet were not affected by inclusion of SDP in the phase 1 diet. Experiments 3 and 4 were conducted to test the hypothesis that dietary SDP increases the digestibility of energy and nutrients originating from other ingredients in the diet. Four phase 2 diets were prepared without SDP and contained cereal grains used in the U.S.A. (corn), Canada (wheat and barley), the European Union (corn, wheat, and barley), and Asia (corn and rice). Four additional diets were prepared by mixing 94% of the previous 4 diets with 6% SDP. Diets were fed to ileal cannulated barrows (BW: 9.30 ± 0.63 kg) or barrows (BW: 9.30 ± 0.97 kg) housed in individual metabolism crates. Differences between measured and predicted values for standardized ileal digestibility (**SID**) and ATTD of energy and nutrients and standardized total tract digestibility (**STTD**) of P in the mixed diets with SDP were calculated. The measured SID of CP and most AA was greater ($P < 0.05$) than predicted in the Canada diet, no differences were observed between measured and predicted values for the European Union

and the Asia diets, and few differences were observed in the U.S.A. diet. The measured ATTD of TDF was greater ($P < 0.05$) than the predicted for the U.S.A. and European Union diets, and the measured ATTD of GE, N, Ca, and P and the STTD of P was greater ($P < 0.05$) than the predicted for the Asia diet compared with the other diets. It was, therefore, concluded that addition of 6% SDP to diets including wheat and barley may increase the SID of CP and AA, and for diets based on rice, SDP may increase the ATTD of energy and nutrients and the STTD of P in the diet. Experiments 5 and 6 tested the hypotheses that 6% SDP complements low CP in a phase 1 diet by improving growth performance, decreasing diarrhea incidence, and reducing inflammation of weanling pigs, and that these parameters will continue to improve for pigs if 2.5% SDP is included in the phase 2 diet. In experiment 5, 160 newly weaned pigs (BW: 5.89 ± 0.39 kg) were fed one of 4 phase 1 diets for 14 d post-weaning: 2 diets had 23.0% CP without or with 6% SDP and 2 diets had 18.5% CP without or with 6% SDP. Results indicated that pigs fed 23% dietary CP had greater ($P < 0.05$) average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) than pigs fed 18.5% dietary CP, and pigs fed 6% SDP had greater ($P < 0.05$) ADG, ADFI, and G:F than pigs fed no dietary SDP. There was a reduction ($P < 0.05$) in diarrhea scores for pigs fed 18.5% dietary CP compared with pigs fed the diet with 23% dietary CP, but diarrhea scores were not influenced by dietary SDP. Circulating cytokine interleukin- (**IL-**) 2 decreased (interaction, $P < 0.05$) and interferon gamma, IL-6, and IL-18 tended to decrease (interaction, $P < 0.10$) on d 7 and 14 if 6% SDP was included in the 18.5% CP diet, but this was not the case if 6% SDP was included in the 23% CP diet. Decreased circulating pro-inflammatory cytokines may indicate a reduction in inflammatory response of pigs fed the diet with 6% SDP and low CP, but based on the observation that this combination did not improve growth performance or diarrhea of pigs during the initial 14 d post-weaning,

experiment 6 was conducted. Three-hundred pigs (BW: 6.36 ± 0.78 kg) were fed a low CP diet without or with 6% SDP from d 1 to 14 post-weaning and fed one of 4 phase 2 diets with 2 levels of SDP (0 or 2.5%) and 2 levels of CP (normal or low) from d 15 to 28 post-weaning. Results indicated that pigs fed the phase 1 diet with 6% SDP did not continue to have improved ADG, ADFI, or final BW in phase 2 if 2.5% SDP was included in the low CP diet. However, diarrhea incidence during phase 2 was less ($P < 0.05$) if 2.5% SDP was included in the phase 2 diet compared with the diet without SDP or if pigs were fed the low CP phase 2 diet compared with the normal CP diet. Ileal mucosa IL-1α and IL-1β decreased ($P < 0.05$) and IL-6 and IL-12 tended to decrease ($P < 0.10$) on d 28 if pigs were fed the phase 1 diet with 6% SDP than the phase 1 diet without SDP, and addition of 2.5% SDP to the phase 2 diet decreased ($P < 0.05$) IL-1β in the ileal mucosa on d 28 compared with the phase 2 diet without SDP. Based on the observation that dietary SDP in phase 1 or phase 2 decreased pro-inflammatory cytokines in the intestine of pigs, experiment 7 was conducted to test the hypothesis that greater inclusion of SDP in diets reduces inflammation in weaned pigs undergoing a sanitation challenge. For 14 d post-weaning, 400 pigs (BW: 6.05 ± 0.80 kg) were fed a phase 1 diet containing 0, 2, 4, 6, or 8% SDP. Results indicated that mucosal IL-2 in the jejunum on d 14 tended to be least (quadratic, $P < 0.10$) at 8% inclusion of SDP and IL-8 tended to increase (linear, $P < 0.10$) as SDP inclusion increased in the diet. Ileal mucosa IL-10 tended to be least at 4 or 6% dietary SDP, but increased with 8% dietary SDP (quadratic, $P < 0.10$). In the blood, the ratio of activated to regulatory-T cells from d 7 to 14 tended to be greatest at 4% dietary SDP, but then decreased as dietary SDP increased (quadratic, $P < 0.10$), whereas lymphocytes linearly decreased ($P < 0.05$) as SDP increased in the diet. It was, therefore, concluded that 8% dietary SDP may increase mucosal cytokine synthesis, but the adaptive immune response was not over-stimulated by 8% SDP.

Experiment 8 tested the hypotheses that during heat stress (26.6 ± 4.8°C), sows in late gestation and throughout lactation fed a diet with 0.5% SDP have improved litter performance and reduced systemic inflammation, and that pigs weaned from sows fed 0.5% SDP have improved growth performance during the initial 14 d post-weaning. Results indicated that there was no effect of SDP on the number of total born pigs, but the percent of low vitality pigs during lactation was less ($P < 0.05$) for sows fed 0.5% dietary SDP compared with sows fed the diet without SDP. Serum cytokines increased ($P < 0.05$) throughout lactation if 0.5% SDP was included in the diet, but the weight loss of sows during lactation tended to be less ($P < 0.10$) if 0.5% SDP was included in the diet. Results indicated that feeding lactating sows a diet with 0.5% SDP did not affect the performance of weaned pigs during the initial 14 d post-weaning. In conclusion, dietary SDP increases the ileal and total tract digestibility of energy and nutrients for ingredients with low AA digestibility or low fiber concentration. Dietary SDP improves ADG, ADFI, G:F, and BW of pigs if fed for 14 or 28 d post-weaning and reduces weight loss of sows if fed throughout lactation. Dietary SDP also increases circulating cytokines in lactating sows, but if SDP is fed to weanling pigs in combination with low CP for 14 or 28 d post-weaning, intestinal and circulating pro-inflammatory cytokines are decreased.

Key words: cytokines, digestibility, growth performance, lactating sows, spray dried plasma, weaned pigs

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CHAPTER 1: Introduction

Farrowing and weaning are stressful periods for pigs that can be exasperated by genetic stressors, challenging environmental conditions, or nutritional stressors. As a consequence of increased stress on the sow, feed intake decreases and the sow enters a negative energy balance in gestation, leading to an increase in inflammatory response and in the number of stillbirths at farrowing (Lucy and Safranski, 2017). The offspring of sows under increased stress may also have reduced survivability and decreased weight at weaning (Crenshaw et al., 2008; Crenshaw et al., 2010; Carter et al., 2018). As a consequence of increased stress associated with weaning, there is a reduction in feed intake, the function of the intestinal mucosa is compromised, and increased intestinal inflammation is observed (Torrallardona, 2010; Peace et al., 2011), which can result in increased diarrhea and decreased growth of the pig (Remus et al., 2013). Therefore, ingredients that are highly digestible and promote feed intake and that contain biologically active proteins to maintain the integrity of the intestinal tract and decrease the inflammatory response of the pig, are imperative in diets to optimize pig health and growth (Torrallardona, 2010).

Spray dried plasma (**SDP**), a by-product of the slaughter industry, is obtained from porcine, bovine, or poultry designated fit for human consumption (Gerber et al., 2014). The blood from these animals is collected in containers with an anticoagulant, and then chilled, transported to a manufacturing plant, inspected, centrifuged to separate the plasma from the erythrocytes, and spray-dried under high temperatures and pressure for a short period of time, resulting in a concentrated, powdered plasma ingredient with approximately 92% dry matter and 78% crude protein (NRC, 2012; Pérez-Bosque et al., 2016; Blázquez et al., 2020). The amino acid (**AA**) composition of SDP is similar to that of sow's milk (van Dijk et al., 2001), and the AA in SDP are highly digestible with a standardized ileal digestibility ranging from 92 to 100%

(Gottlob et al., 2006; Almeida et al., 2013). The high concentration of Glu in SDP may explain the increased palatability of diets with SDP, resulting in increased feed intake of newly weaned pigs (Ermer et al., 1994; van Dijk et al., 2001). However, the beneficial effects of SDP on feed intake and growth performance of weanling pigs may not only be related to increased palatability of the diet or greater digestibility of AA in SDP. There are biologically active proteins, i.e., immunoglobulins (**Ig**), in high concentrations in SDP (Torrallardona, 2010). These Ig, specifically IgG, aid in the protection of mucosal surfaces to prevent pathogen adhesion and subsequent colonization on the enterocytes (Hedegaard et al., 2016). As a consequence, intestinal inflammation of pigs fed a diet including SDP can be reduced with an observed decrease in intestinal mucosa concentrations of tumor necrosis factor-α and interleukin-8 (Bosi et al., 2004; Zhang et al., 2016). However, the mechanisms by which SDP modulates intestinal inflammation and its association with improved growth performance and litter performance of weanling pigs and sows have not been completely elucidated. Therefore, it was the objective of this dissertation to test 5 hypotheses: 1) inclusion of SDP increases the ileal and total tract digestibility of energy and nutrients originating from other ingredients in mixed diets; 2) growth performance is improved and diarrhea incidence of weanling pigs is reduced by inclusion of SDP in diets formulated below AA requirements; 3) intestinal and systemic inflammation is reduced for pigs fed a diet with SDP at or below AA requirements or when weaned to a challenging environment; 4) heat stressed sows fed diets containing SDP during the last third of gestation have less oxidative stress and inflammation and greater litter weaning weights compared with sows fed diets without SDP; and 5) the offspring of sows fed a diet with SDP have greater growth performance and reduced diarrhea compared with the offspring of sows fed a diet without SDP.

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CHAPTER 2: Spray dried plasma for pigs: Literature review

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
CP	crude protein
Ig	immunoglobulin
SDP	spray dried plasma
SID	standardized ileal digestibility
STTD	standardized total tract digestibility
TID	true ileal digestibility
TTTD	true total tract digestibility

Introduction

Production and safety

Blood meal is considered a by-product of the animal industry, because it is a secondary product that is further processed for use after being obtained during the manufacturing of its principal commodity, i.e., meat (Meeker, 2009). Blood makes up approximately 4% of the live weight of an animal, but for pigs, only 2 to 3 L of blood can be collected at the time of slaughter because almost 50% of blood is retained in the capillary system of the animal (Wanasundara et al., 2003;

Bah et al., 2013). Blood that is used for production of blood meal or other co-products is generally from bovine, porcine, or poultry origin and there are a variety of ways that blood can be processed into feed ingredients that are used in the swine, cattle, poultry, aquaculture, and companion animal feed industries. Blood products are primarily used as high-quality protein ingredients to support carnivorous fish or increase growth rate and feed intake of livestock (Bah et al., 2013). Blood products can also be used to increase palatability and improve texture and cohesion of companion animal diets (Ofori and Hsieh, 2014). In the swine industry, spray dried plasma (**SDP**) of porcine or bovine origin is an important ingredient that is often included in diets fed to newly weaned pigs (Nelssen et al., 1999).

In livestock or poultry abattoirs, whole blood is collected and pooled from numerous animals, and prior to slaughter, these animals are inspected by government employed veterinarians and only slaughtered if they are deemed healthy and designated fit for human consumption (Gerber et al., 2014). The blood is collected in containers with an anticoagulant followed by chilling, transportation to a manufacturing plant, blood inspection, and centrifugation to separate the plasma from the red blood cells (Blázquez et al., 2020). The plasma fraction contains approximately 90% water, and is concentrated by membrane filtration or vacuum evaporation prior to drying (Kowalski et al., 2017; Blázquez et al., 2020). Plasma is then pumped into a spray-dryer that rapidly forces the concentrated, liquid plasma through a stream of hot air under high pressure, which results in the formation of droplets. The majority of the moisture in the droplets is then evaporated as it passes through the hot chamber forming small particles that are separated from the air stream as they exit the chamber (Blázquez et al., 2020). This process takes 20 to 90 s with an inlet temperature range of 170 to 310^{o}C and an outlet temperature of greater than 80^{o}C (Pérez-Bosque et al., 2016). Temperatures during this process

are closely monitored because the European Animal Protein Association and the North American Spray Dried Blood and Plasma Producers require a minimum temperature of 80^{o}C to be achieved throughout the plasma during spray-drying (Pérez-Bosque et al., 2016; Blázquez et al., 2020). Temperatures of 80^{o}C or greater may initiate denaturation of proteins, but the outlet air temperature has the greatest impact on pathogen inactivation, and therefore, 80^{o}C as outlet air temperature is necessary for the safety of SDP (Pérez-Bosque et al., 2016; Blázquez et al., 2020).

The duration, temperature, and pressure used during spray-drying is effective in inactivating or eliminating pathogens in the final product (Polo et al., 2005; Pujols et al., 2007; Pujols et al., 2008; Gerber et al., 2014; Pujols and Segalés, 2014; Blázquez et al., 2018a). The World Health Organization recognizes that 99.99% of a virus must be inactivated to assure viral safety of human plasma used for transfusions (WHO, 2004), which is equivalent to a reduction of 4 logarithms of virus (WHO, 2004). Liquid plasma used in animal feed was inoculated with *Porcine reproductive and respiratory syndrome virus*, *African swine fever virus*, *Porcine epidemic diarrhea virus*, or *Swine vesicular disease virus*, and after spray-drying, logarithm reductions of approximately > 4.0, 4.1, > 5.2, or 6.0, respectively, were observed (Polo et al., 2005; Pujols et al., 2007; Pujols and Segalés, 2014; Blázquez et al., 2018a). *Porcine circovirus 2*, one of the most heat resistant viruses that infect swine, was not transmitted to weanling pigs fed diets with SDP containing the virus genome (Pujols et al., 2008). Bacteria of concern, such as *E.coli K88*, *E.coli K99*, *Salmonella typhimurium*, or *Salmonella choleraesuis*, were also inoculated in the liquid plasma and after spray-drying these bacteria had an approximate 7.3, 7.7, 5.4, or 5.3 logarithms reduction, respectively (Blázquez et al., 2018b; Blázquez et al., 2018c). These data demonstrate that the spray-drying process used in the production of SDP is effective in eliminating or inactivating pathogens and preventing pathogen transmission to pigs within the

swine industry (Blázquez et al., 2020). However, the high heat used for these techniques may affect the amino acid (**AA**) concentration and digestibility, and therefore, the quality of blood products (Meeker, 2009).

Nutrient Composition

Blood is composed of 2 fractions, blood cells (erythrocytes, leukocytes, and platelets) and plasma, which make up 20 to 40% and 60 to 80% of the total blood volume, respectively (Tarté, 2011). Whole, unprocessed blood is approximately 22% dry matter, and the blood cells and plasma fractions are approximately 40 and 10% dry matter, respectively (Tarté, 2011). Crude protein (**CP**) concentration of whole, unprocessed blood is approximately 18% with approximately 38 and 8% CP for blood cells and plasma, respectively (Tarté, 2011). However, when whole blood is concentrated by drying and further processing, the CP concentration increases, making it a high-protein ingredient for livestock diets.

Crude protein

Compared with plant ingredients, blood products have a more balanced AA pattern and greater AA digestibility, which is imperative during the nursery phase in the swine industry (Torrallardona, 2010). Although plant ingredients are less expensive, blood products, such as SDP, have a CP and AA composition that is close to that of sow's milk (van Dijk et al., 2001a). A variety of ingredients may be produced from whole blood, such as blood meal or spray dried animal blood, blood cells, and plasma, and the concentration of CP in these products ranges from 77 to 95% with SDP having a CP concentration of approximately 78% (van Dijk et al., 2001a; Almeida et al., 2013). Dried skim milk, casein, and fish meal, which may also be fed after weaning, have CP concentrations of approximately 37, 87, and 64%, respectively (Cervantes-

Pahm and Stein, 2010; NRC, 2012). Plant ingredients commonly fed after weaning, such as fermented soybean meal and soy protein concentrate, have CP concentrations of approximately 54 and 65%, respectively (Cervantes-Pahm and Stein, 2010; NRC, 2012). The CP concentration of SDP is similar to casein, but compared to most other protein ingredients fed to nursery pigs, SDP has a greater concentration of CP. However, due to exposure to temperatures of 80°C or greater during spray-drying, protein denaturation and destruction of AA may occur (Abdul-Hamid et al., 2002), and thus, the concentration of AA may vary in SDP.

Amino acids

The AA composition of SDP is well balanced in relation to the AA requirements of young pigs, except for Met and Ile, which are the 2 limiting AA in SDP (Torrallardona, 2010; NRC, 2012). The concentration of Met and Ile in SDP (0.76 and 2.44% as-fed basis, respectively) is less than the concentration of Met and Ile in soy protein concentrate (0.93 and 3.15% as-fed basis respectively) and casein (2.54 and 4.61% as-fed basis, respectively), similar to the concentration of Met and Ile in fermented soybean meal (0.76 and 2.48% as-fed basis, respectively) and Ile in fish meal (2.33% as-fed basis), and less than the concentration of Met in fish meal (1.59% as-fed basis; Lenehan et al., 2007; Cervantes-Pahm and Stein, 2010; Almeida et al., 2013). Lysine is the most heat labile AA, and is typically the first limiting AA in swine diets. In contrast to Met and Ile, Lys concentration of SDP is 6.95% (as-fed basis), which is greater than the Lys concentration in soy protein concentrate (4.41%) and fish meal (4.50%), but similar to casein (7.02%; Lenehan et al., 2007; Cervantes-Pahm and Stein, 2010; Almeida et al., 2013).

Spray-drying temperatures of 150°C/76°C or 180°C/90°C inlet/outlet air, which are similar to temperatures used during SDP processing, were evaluated in the production of spray-dried protein hydrolysate from Black Tilapia, and it was reported that the greater spray-drying

temperature resulted in a decreased concentration of AA (Abdul-Hamid et al., 2002). The digestibility of CP was also decreased as spray-drying temperature increased; however, CP digestibility was greater than 88% (Abdul-Hamid et al., 2002). Therefore, the high temperatures reached during spray-drying may not affect the high digestibility of high protein ingredients. Additionally, other protein fractions in SDP, specifically immunoglobulins, keep their biological activity after spray-drying (Torrallardona, 2010).

Immunoglobulins

The major fractions of protein in SDP are albumins and globulins, which make up approximately 95% of the protein in SDP, with the other 5% comprised of fibrinogens (Torrallardona, 2010; Biga et al., 2019). Albumin proteins, synthesized by the liver, function as binding proteins and maintain plasma osmotic pressure of the blood to prevent fluids and nutrients from leaking across blood vessel walls (Torrallardona, 2010). Blood globulins are a group of proteins that are synthesized by the liver and the immune system; they function in transporting nutrients, contributing to osmotic pressure, and are involved in immunity (Torrallardona, 2010; Biga et al., 2019). Gamma globulins, or immunoglobulins (**Ig**), are produced by specialized leukocytes (i.e., B lymphocytes) when instructed by the immune system and are often the proteinaceous fraction that have been related to the beneficial effects and mode of action of SDP (Torrallardona, 2010). The Ig in SDP are antibodies that identify and neutralize pathogenic bacteria, viruses, parasites, or cellular antigens on mucosal surfaces and in circulation, aiding in the immune response of young pigs until they are capable of synthesizing their own Ig for protection (Pierce et al., 2005; Hedegaard et al., 2016).

There are 5 types of Ig in SDP: IgD, IgE, IgM, IgA, and IgG (Torrallardona, 2010). The Ig are biologically active and have a binding capacity to cellular receptors and exhibit one or

more immune functions, including activation of the complement system upon initial pathogen infection, opsonization of pathogens for phagocytosis, prevention of pathogenic colonization on mucosal surfaces, stimulation of an allergic response, or neutralization of infectious pathogens (Justiz Vaillant et al., 2020). Immunoglobulin G is the most abundant Ig in SDP, ranging from approximately 16 to 22%, and has the greatest immuno-stimulating impact in young pigs (Pierce et al., 2005; Torrallardona, 2010). Immunoglobulin G is also the only Ig that can cross the placenta, but pigs can also attain circulating IgG through colostrum from the sow by an intestinal transport mechanism that is only available for the initial 24 h after parturition (Torrallardona, 2010; Hedegaard and Heegaard, 2016). The IgG from sow's milk provides passive immunity at the mucosal surfaces and in circulation until pigs are weaned or until the half-life of IgG is reached, approximately 2 to 3 wk after birth (Hedegaard and Heegaard, 2016; Justiz Vaillant et al., 2020). After weaning, IgG can be provided to pigs by including SDP in the diet. The IgG in SDP contains many antigen-binding regions because SDP is produced from plasma that is pooled from thousands of animals (Fernández-Cruz et al., 2009). Species origin of IgG is less important as the antibody response generally works across species (Hedegaard and Heegaard, 2016), and SDP of bovine or poultry origin is, therefore, as effective in protecting weaned pigs against antigens as SDP of porcine origin (Zhang et al., 2016; Balan et al., 2021).

The main site of protection of SDP is within the lumen of the intestine where IgG binds to pathogens, specifically pathogenic bacteria such as *E.coli* and *Salmonella enterica*, preventing their adhesion and subsequent colonization on the enterocytes protecting the villi from damage and the pig from post-weaning diarrhea (Pierce et al., 2005; Torrallardona, 2010; Hedegaard et al., 2016). The presence of IgG at the mucosal level also improves intestinal morphology and increases enzyme activity at the brush border by increasing villus surface area, villus length, and

villus height to crypt depth ratio, and by increasing maltase and lactase activities, respectively (Pierce et al., 2005), thereby enabling a more functional intestinal wall that results in improved digestion and absorption of nutrients.

Minerals

Plasma also contains minerals and the ash content is approximately 2.0 to 7.5%. Products with lower ash concentrations may be produced by ultrafiltration to reduce the concentration of Na (Torrallardona, 2010). In SDP, concentrations of Na, P, and Cl are approximately 2.76, 1.28, and 1.19%, respectively (NRC, 2012; Munoz et al., 2018). Other macro minerals, such as Ca, K, and Mg, are present in lesser concentrations of approximately 0.13, 0.02, 0.03%, respectively (NRC, 2012; Munoz et al., 2018). Because SDP is a blood product it does not contain phytate; therefore, minerals in SDP, including P, have high digestibility by pigs and poultry (Almeida and Stein, 2011; Munoz et al., 2018). As a consequence, inclusion of feed phosphates and phytase may be reduced, resulting in a decrease in overall cost of the diet and in mineral excretion into the environment (Almeida and Stein, 2011; Munoz et al., 2018).

Digestibility of Nutrients and Energy

Among the initial published studies with SDP were experiments conducted to evaluate the effect of replacing milk ingredients with SDP on growth performance of early weaned pigs (Sohn et al., 1991; Hansen et al., 1993; Ermer et al., 1994). Sohn et al. (1991) and Hansen et al. (1993) observed that when SDP replaced dried skim milk or dried whey in diets fed during the initial 2 wk post-weaning, pigs had increased average daily gain (**ADG**) and average daily feed intake (**ADFI**), and an increase in ADG and ADFI was also observed the subsequent 3 wk when a common diet was fed (Sohn et al., 1991). The beneficial effect of SDP on growth performance of

weanling pigs was suggested to be related to increased preference for diets that contained SDP due to greater palatability (Ermer et al., 1994). Others have suggested that the greater performance of pigs fed diets containing SDP can be related to its IgG concentration, because IgG protects intestinal mucosal surfaces from pathogen colonization and increases digestive enzyme activities (Pierce et al., 2005). However, it was not until later that values for apparent ileal digestibility (**AID**) of CP, AA, and energy in SDP were reported (NRC, 1998; Chae et al., 1999). Values for true ileal digestibility (**TID**) of CP and AA, and standardized ileal digestibility (**SID**) of AA and energy in SDP were also reported (Kim et al., 2000; Gottlob et al., 2006; Mateo and Stein, 2007).

Ileal digestibility

There are 3 defined ways that ileal digestibility of CP or AA may be expressed (i.e., AID, SID, and TID), and the distinguishing factor among these digestibility values is how ileal endogenous AA losses are reflected (Stein et al., 2007). Ileal endogenous AA losses are AA in proteins that are endogenously synthesized by the pig and secreted into the intestinal lumen, but not digested or reabsorbed prior to the distal ileum, such as mucin proteins, sloughed cells, digestive enzymes, and serum albumin (Stein et al., 2007). Endogenous AA losses are either 1) basal losses: nonspecific, diet-independent, and represent the minimum loss of AA by the pig, or 2) specific losses: diet-dependent, influenced by fiber or antinutritional factors, and represent extra losses of AA due to ingredient characteristics (Stein et al., 2007). The ileal digestibility value most affected by dietary AA content is AID, because AID is measured using the total outflow of ileal AA at the distal ileum; therefore, nondigested dietary AA and basal and specific endogenous AA losses are included in the calculation (Stein et al., 2007). When AID values are corrected for basal endogenous AA losses, SID values are determined. The effect of dietary AA

levels on ileal digestibility are eliminated in the calculation of SID, and therefore, SID values determined for ingredients are additive in mixed diets (Stein et al., 2005; Stein et al., 2007). However, basal endogenous AA losses may be impacted by the age of the animal (Adeola et al., 2016). Furthermore, both basal and specific endogenous AA losses are subtracted from the total outflow of AA at the distal ileum in the calculation of TID values (Stein et al., 2007). There is no direct way of measuring specific endogenous AA losses, whereas the methodology to measure basal endogenous AA losses has been established (Stein et al., 2007); therefore, values for SID are more commonly determined for feed ingredients and used in diet formulation (Adeola et al., 2016).

Chae et al. (1999) reported AID values for SDP using early- or conventionally-weaned pigs at 10 d old and 3.1 kg or 21 d old and 6.3 kg, respectively, and the AID of Ile and Leu was reduced for SDP when fed to early-weaned pigs compared with conventionally-weaned pigs, but no differences were observed in the AID among other indispensable AA. Across weaning age, the average AID of indispensable AA for SDP was 73.8% with Thr as the least digestible AA (60.3%) and His as the most digestible AA (81.3%), and the AID of CP was 73.2% (Chae et al., 1999), which is similar to the AID of CP (72.1%) reported by Bosi et al. (2001) using pigs weaned at 13 d and sacrificed after 14 d for ileal digesta collection. Kim et al. (2000) and Yun et al. (2005) determined AID and TID of AA in SDP utilizing 18 or 21 d old pigs that were 5.8 or 5.2 kg, respectively, and observed similar mean AID of indispensable AA at 75.6 and 74.6% and similar mean TID of indispensable AA at 84.8 and 85.9%, respectively. The AA with the greatest TID was Ile at 87.7 or 87.8% for pigs weighing 5.8 or 5.2 kg, respectively (Kim et al., 2000; Yun et al., 2005). Mateo and Stein (2007) determined values for SID of AA for SDP that ranged from 82.6 (Thr) to 92.4% (Arg) in pigs with an initial body weight of 5.0 kg, but ileal

digesta were not collected until pigs were 28 d old. These SID values were similar to values from Wu et al. (2018) where 20.6 kg pigs were used and Arg was observed to have the greatest SID of 89.2% and Thr having the least SID of 81.5%. Gottlob et al. (2006) reported SID values for SDP of pigs with an initial body weight of 29.5 kg and observed greater SID values for AA compared with Wu et al. (2018). The SID values for SDP ranged from 92.2 (Thr) to 101% (Trp) or the second greatest AA, Arg, with an SID of 96.8% (Gottlob et al., 2006). Based on these studies, it is concluded that ileal digestibility of AA in SDP can vary due to the age and body weight of the experimental animal; similar observations have been made with soy proteins where results indicated that the SID of AA were less in pigs of less than 20 kg compared with pigs greater than 20 kg (Pedersen et al., 2016). Therefore, it is imperative to determine the ileal digestibility of nutrients in the animal to which the feed ingredient is meant to be fed in practice, and as a consequence, AID and SID of AA in SDP should be determined in newly weaned pigs.

The AA in SDP are relatively well digested by pigs, but the AID of most AA, except His, Lys, and Arg, are less in SDP than in dried skim milk (Chae et al., 1999). However, there were no differences in the AID of Thr, Val, Ile, Leu, Phe, His, and Lys between soy protein isolate and SDP (Chae et al., 1999). The AID of Leu and Val and the TID of Lys was greater for SDP compared with soy protein concentrate, but did not differ for the other AA, with the exception that the TID of Met was greater in soy protein concentrate than in SDP (Yun et al., 2005). Compared with fish meal, the SID of His, Ile, Leu, Met, and Phe was greater in SDP, and the SID of Arg, Lys, Thr, Trp, and Val was greater in fish meal (Mateo and Stein, 2007; Cervantes-Pahm and Stein, 2010), and compared with soybean meal and fermented soybean meal, the SID of most AA, except Arg and Thr, is greater in SDP (Mateo and Stein, 2007; Cervantes-Pahm and Stein, 2010).

The significance of determining SID of AA in feed ingredients is that they are independent of dietary AA content; therefore, values are additive in mixed diets (Stein et al., 2005). It is also assumed that the amount of digestible nutrients in a diet is equal to the sum of that nutrient from the different ingredients in the diet (Xue et al., 2014). However, SDP may increase secretion of certain digestive enzymes as well as improve intestinal morphology of the small intestine (Pierce et al., 2005). Therefore, inclusion of SDP in diets may increase the digestibility of other ingredients in the diet, resulting in a lack of additivity of predetermined SID values among ingredients.

Total tract digestibility

Total tract digestibility experiments are conducted to determine fiber, energy, and mineral (e.g., Ca and P) digestibility values in ingredients, with P digestibility receiving more attention because it is an expensive nutrient to add to swine diets and high concentrations of excreted P in manure is a major pollutant concern for swine producers (Stein, 2017). There is no difference between ileal and total tract digestibility values for Ca or P because there is no net absorption of these minerals from the hindgut (Stein, 2017). Consequently, total tract digestibility of Ca and P are preferred due to the decreased cost and time required to determine these values compared with ileal digestibility values. Values for total tract digestibility are expressed similar to ileal digestibility, where apparent total tract digestibility (**ATTD**) encompasses total fecal output of a nutrient, standardized total tract digestibility (**STTD**) is ATTD corrected for basal endogenous losses of the nutrient, and true total tract digestibility (**TTTD**) is ATTD corrected for total endogenous losses of the nutrient (Stein et al., 2007). These values can be influenced by body weight or physiological state of the animal with improvement of total tract digestibility values observed as body weight increases (Le Goff and Noblet, 2001). Therefore, it is imperative to

determine total tract digestibility of nutrients using the appropriately sized pig that will be consuming these ingredients in practice.

Values for STTD of Ca and P in ingredients are preferred because there are considerable losses of endogenous Ca and P from the intestinal tract of pigs originating from saliva, intestinal epithelial cells, and pancreatic or bile secretions (González-Vega et al., 2013; She et al., 2017; Stein, 2017). However, reported values for ATTD or STTD of P and Ca in SDP are not widely available. Almeida et al. (2011) reported ATTD and STTD of P in SDP to be 91 and 103%, respectively, for 18.8 kg pigs, which indicates that the P in SDP was completely available to pigs. The STTD of P in SDP is greater than in monocalcium phosphate (94.9%) or dicalcium phosphate (88.4%; She et al., 2017), and SDP had a greater STTD of P compared with whey powder (91.2%) and whey permeate (93.1%; She et al., 2017), but all these animal ingredients have high P digestibility. The completely digestible P in SDP means that SDP can be used as an alternative protein source to gelatin in P-free diets used to determine basal endogenous P losses, because it is assumed that all P excreted in the manure of pigs fed a SDP "P-free" diet is of endogenous origin (Stein, 2017). This is important because feeding diets with no P for an extended period of time can lead to symptoms of diarrhea and muscular spasms (She et al., 2017). Further evaluation of the estimated basal endogenous P losses from gelatin- or SDP-based "P-free" diets are needed.

The digestibility of Ca in ingredients has not been evaluated as extensively as the digestibility of P. However, the concentration of digestible Ca in diets fed to pigs may impact P digestibility (Stein et al., 2011; Stein, 2017), and, therefore, it is important to determine ATTD or STTD of Ca in ingredients fed to pigs. The ATTD or STTD of Ca in SDP has not been reported for pigs as SDP is not a significant source of Ca. When SDP is included in a corn-soybean meal

diet, the ATTD of Ca in the diet is increased (Zhang et al., 2015), suggesting that SDP may increase the digestibility of other ingredients in the diet.

The concentration of gross energy in SDP is approximately 4,627 kcal per kg on an as-fed basis (Gottlob et al., 2006). Concentrations of digestible energy and metabolizable energy in SDP are calculated by subtracting the gross energy in the feces and the gross energy in the feces and urine, respectively, from the gross energy in the diet (NRC, 2012). The concentration of digestible energy and metabolizable energy in SDP fed to pigs is approximately 4,546 and 3,979 kcal per kg on an as-fed basis (Gottlob et al., 2006), which is similar to NRC (2012) digestible energy (4,546 kcal per kg) and metabolizable energy (4,017 kcal per kg) values, but slightly less than the digestible energy (4,931 kcal per kg) and metabolizable energy (4,613 kcal per kg) reported on a dry matter-basis by Wu et al. (2018). The concentration of net energy in SDP fed to pigs is approximately 3,020 kcal per kg on an as-fed basis (Gottlob et al., 2006), which is greater than the net energy of SDP (2,506 kcal per kg) reported by NRC (2012). The concentration of digestible energy, metabolizable energy, and net energy in SDP is greater than in fish meal, soy protein concentrate, and whey powder (NRC, 2012).

Growth Performance

Weaning is an extremely stressful event for pigs; it is a time where they are transitioned from highly digestible, liquid sow milk to a less digestible, mainly plant-based, solid diet. This change in diet and associated stress decreases feed intake, and further leads to increased prevalence of diarrhea and decreased growth (Remus et al., 2013). Addition of SDP to newly weaned pig diets instead of plant ingredients provides greater amounts of highly-digestible protein that is more similar in composition to that of sow's milk (van Dijk et al., 2001a). Spray dried plasma is a

relatively expensive protein ingredient, and therefore, experiments have been carried out to determine the optimum inclusion level and feeding duration of SDP that elicits maximum beneficial effects on the growth of newly weaned pigs (van Dijk et al., 2001a; Torrallardona, 2010; Balan et al., 2021).

Spray dried plasma is generally included in diets fed to pigs the initial 2 wk post-weaning. During this time, ADG and ADFI were reported to increase by approximately 27 and 25%, respectively, when SDP was included in the diet, and the feed conversion ratio decreased by 3% (van Dijk et al., 2001a). Similar improvements in growth responses were reported by Balan et al. (2021), and greater improvements in ADG (39% increase), ADFI (32% increase), and feed conversion ratio (5.4% reduction) were reported by Ferreira et al. (2009). However, when the initial 2 wk were analyzed individually, it was observed that SDP had a greater effect on growth performance parameters from d 1 to 7, ADG and ADFI increased by 36 and 17%, respectively, compared with d 8 to 14 where ADG and ADFI increased by 2 and 3%, respectively (Balan et al., 2021). Generally, SDP is not included in diets fed to pigs past 2 wk post-weaning, and when a common diet was fed to all pigs, after being fed a diet with SDP, there were no lasting positive carryover effects of SDP on growth performance (van Dijk et al., 2001a).

The positive growth response to SDP in the diet may be dependent on the protein ingredient it replaced in the control diet. Improvements in growth were observed when SDP replaced plant (e.g., soy protein concentrate, isolated soy protein, soybean meal, and wheat gluten) and animal (e.g., dried skim milk, casein, fish meal, and spray dried egg) ingredients in the control diet (van Dijk et al., 2001a; Torrallardona, 2010; Balan et al., 2021). The positive growth response to SDP may also vary depending on inclusion rate in the diet, and ADG linearly

increases with up to 10% SDP in the diet (Kats et al., 1994). Prediction equations suggest this linear increase as well (Balan et al., 2021); however, crystalline Met must be supplemented due to the low concentration of Met in SDP (Torrallardona, 2010). Improvements in ADG, ADFI, and feed conversion ratio were consistent up to 6% SDP inclusion in the diet, whereas growth responses had greater variability at SDP inclusion rates above 6% (van Dijk et al., 2001a). Therefore, inclusion of 6% SDP to diets for weanling pigs has been considered optimum to elicit improvements in growth performance (van Dijk et al., 2001a; Torrallardona, 2010). However, inclusion level of SDP to optimize the immunocompetence of weanling pigs has not yet been evaluated, and this is imperative as the positive effects of SDP on growth performance may be related to an improvement of pig intestinal health, especially observed in pigs experiencing a greater pathogen load (Balan et al., 2021).

Intestinal Health and Immuno-protective Mechanisms of Spray Dried Plasma

Immediately after weaning, pigs are often observed to have decreased feed intake, or total starvation, and are exposed to greater pathogen loads (Torrallardona, 2010). Pigs are vulnerable to pathogens because their own immune system is not fully developed until 6 to 8 wk after birth, and as a consequence, the functionality and integrity of the intestinal mucosa can become compromised (Torrallardona, 2010). Additionally, other changes in the intestine are observed during the post-weaning period, such as villus atrophy followed by hyper-regeneration of cells, decreased brush border enzyme activity affecting digestion and absorption of nutrients, and colonization of enteric pathogens increasing inflammation and susceptibility to infection, which leads to post-weaning diarrhea (van Dijk et al., 2001b; Lallès et al., 2004; Torrallardona, 2010).

Antibodies (i.e., Ig) that may protect and help maintain the function of the intestinal mucosa can be provided to newly weaned pigs by including SDP in the diet (Torrallardona, 2010).

Intestinal morphology

At birth, the weight of the gastrointestinal tract of pigs is approximately 2% of body weight, and this increases to more than 6% of body weight 2 wk post-weaning, indicating rapid changes in the development of the intestinal tract during this time (Lallès et al., 2004). However, due to increased stress associated with weaning, the development of the intestine may be hindered during the post-weaning period. During the initial 2 d post-weaning (pigs weaned at 21 d), small intestinal tissue and mucosa weight were observed to decrease by 18 and 30%, respectively, and then increase after 8 d post-weaning by 14 and 5%, respectively, compared with pre-weaning tissue and mucosa weight (Lallès et al., 2004). Additionally, duodenum villus height and crypt depth were observed to decrease 2 d post-weaning by 40 and 2%, respectively, and then 8 d post-weaning, crypt depth increased by 41% but villus height continued to be decreased by 37% compared with pre-weaning values (Lallès et al., 2004). Similar trends were observed in the proximal jejunum with villus height significantly decreased on d 2 and 8 post-weaning compared with pre-weaning, and crypt depth was greater on d 8 post-weaning compared with pre-weaning. However, crypt depth in the jejunum and ileum did not differ between pre-weaning and d 2 post-weaning (Montagne et al., 2007). The villus height in the distal ileum slightly decreased on d 2 post-weaning compared with pre-weaning, but on d 8 post-weaning villus height was not different from pre-weaning (Montagne et al., 2007).

Pigs fed diets with 5% SDP for 14 d post-weaning had increased villus height in the duodenum and increased villus height to crypt depth ratio in the duodenum and ileum compared with pigs fed diets without SDP (Tran et al., 2014). In contrast, small intestinal villus height was

reduced in weaned pigs compared with unweaned pigs, but did not differ for weaned pigs fed a diet with 8 or 10% SDP compared with weaned pigs fed a diet with 8 or 10% casein (van Dijk et al., 2001b; van Dijk et al., 2002). Less diarrhea was observed for pigs fed a diet with 5% SDP compared with pigs fed a diet with no or 2.5% SDP for 14 d post-weaning (van Dijk et al., 2001a; Peace et al., 2011). The rate of cell proliferation in the enterocytes was decreased in weanling pigs fed a diet with SDP compared with pigs fed a diet with casein, and as a consequence, less immature enterocytes were present in the small intestine (van Dijk et al., 2001b). Similar results were reported by Jiang et al. (2000); however, van Dijk et al. (2002) observed no differences in enterocyte proliferation between pigs fed a diet with SDP compared with pigs fed a diet that contained less casein than the diet used by van Dijk et al. (2001b). The effect of SDP on villus atrophy in the small intestine is variable, and therefore, further research is required to determine if the beneficial effects of SDP on feed intake, diarrhea prevalence, and pathogen susceptibility may be more closely related to the immune response signaling at the intestinal level.

Cytokines and immunoglobulin A

Cytokines are primarily involved in host responses to disease or infection and are produced when stimulated by noxious or "stressful" events (Dinarello, 2000). One way to categorize cytokines is by their role in infection or inflammation; pro-inflammatory cytokines are released in response to recognition of threats to homeostasis and promote inflammation, whereas anti-inflammatory cytokines suppress genes for pro-inflammatory cytokines (Dinarello, 2000). There is a natural balance between pro- and anti-inflammatory cytokines, and an imbalance can determine the outcome of disease (Dinarello, 2000). For example, pro-inflammatory cytokines can lead to an increase in muscle protein degradation due to greater AA demands and can damage intestinal

barrier function, which results in a reduction of feed intake and growth of newly weaned pigs (Tran et al., 2014; Balan et al., 2021). Tumor necrosis factor-α is a pro-inflammatory cytokine that is produced by macrophages, dendritic cells, and T cells that are regulated by Toll-like receptor 4 in response to microbial infection (Tran et al., 2014). Both Tumor necrosis factor-α and interleukin-1 can initiate the cascade of inflammation by triggering the production of other pro-inflammatory cytokines, such as interleukin-8, that target the endothelium (Dinarello, 2000; Tran et al., 2014). Therefore, the increase in feed intake of pigs fed a diet with SDP may be a result of reduced production of pro-inflammatory cytokines (Torrallardona, 2010).

Cytokines, such as interleukins-4, 6, and 10, can stimulate the production of secretory IgA by epithelial cells in the intestinal mucosa (Mantis et al., 2011). Secretory IgA is the most abundant antibody in the intestinal tract (Mantis et al., 2011), and functions in protecting the mucosal membrane lining the intestine by binding to and neutralizing pathogens (Hansen et al., 2019). Blocking pathogens from attaching to the mucosal membrane prevents infection (Hansen et al., 2019), thereby modulating the immune response to prevent inducing severe inflammation (Corthésy, 2013). Secretory IgA can prevent inflammation by also reducing the production of key pro-inflammatory cytokines tumor necrosis factor-α and interleukin-1β (Hansen et al., 2019).

Pigs fed a diet with 5% SDP have decreased mucosal concentrations of tumor necrosis factor-α in the jejunum and ileum and decreased secretory IgA in the ileum compared with pigs fed a diet without SDP (Zhang et al., 2016), which is in agreement with data reported by Peace et al. (2011) who also reported tumor necrosis factor-α decreased in the colon of pigs fed a diet with 5% SDP. Feeding a diet with 6% SDP to pigs also reduced expression of interleukin-8 in the jejunum (Bosi et al., 2004). The anti-inflammatory cytokine, interleukin-10, was reduced in jejunum and ileum mucosa of pigs fed a diet with 5% SDP compared with pigs fed a diet with no

SDP (Zhang et al., 2016). Additional data on the effect of SDP on regulating the expression of other pro- and anti-inflammatory cytokines at the intestinal level are lacking. The mechanism responsible for the reduction in pro- and anti-inflammatory cytokines may be a result of the protective role SDP has in preventing over-activation of the immune system of pigs after weaning (Zhang et al., 2016). However, further research is needed to evaluate the effect SDP has on regulating Toll-like receptor 4 and other T-cells responsible for the production of cytokines and how that affects the immune response of pigs during high stress events.

Sow Productivity

The number of pigs born per sow has steadily increased over the past 3 decades, and studies have reported a positive correlation between greater litter size and increased stillbirths (Feyera et al., 2018). Farrowing a greater number of pigs is a high energy demanding process for the sow that can lead to increased parturition rate and increased stress, and as a consequence, increased number of stillbirths (Feyera et al., 2018; Crenshaw et al., 2021). Additionally, sows located in regions with warmer summer months may experience heat stress, and heat-stressed sows can have reduced feed intake, increased number of stillbirths, reduced litter size birth weight, and increased inflammatory response at farrowing (Lucy and Safranski, 2017). Pigs under high stress (e.g., weaning, parturition, heat stress) fed diets containing SDP have a more efficient immune response, and therefore, improved recovery time and overall health (Crenshaw et al., 2021).

Heat-stress compromises the integrity of the intestinal tract, because as the sow over-heats, blood flow is diverted to the periphery resulting in vasoconstriction in the intestinal tract, which decreases blood flow and, therefore, supplies of oxygen and nutrients to the intestinal epithelial cells are reduced (Pearce et al., 2013; Ross et al., 2017). As a consequence, tight

junctions are compromised, which may increase endotoxin permeability into circulation (Pearce et al., 2013; Ross et al., 2017). Oxidative stress in sows also may increase during late gestation and lactation, and this can be further increased under a high thermal environment (Zhao and Kim, 2020). Malondialdehyde, a final product of polyunsaturated fatty acid peroxidation in cells and a common marker of oxidative stress (Gaweł et al., 2004), was greater in late gestation compared with late lactation for sows during summer months (Zhao and Kim, 2020). In contrast, glutathione peroxidase is an enzyme that functions in eliminating free radicals produced during oxidative stress in the pig, and by increasing its activity, destruction of intestinal barrier integrity may be mitigated (Liu et al., 2016). However, there is a lack of data on the effect SDP in sow diets has on oxidative stress or immune response markers, such as cytokines, during times of high stress (i.e., heat stress or parturition). Data on sow productivity parameters, such as feed intake, body weight, and litter performance have, however, been reported, which is likely because these data are easier to collect.

Gestating sows fed diets with 0.50 to 2.50% SDP for 4 d prior to parturition have a reduced number of stillborn pigs (Crenshaw et al., 2021). Sows fed diets with 0.50% SDP from d 112 of gestation until d 1 post-parturition had increased total pigs weaned, increased number of pigs weaned at greater than 3.6 kg, and increased litter gain during lactation (Crenshaw et al., 2010). Lactating sows fed diets with 0.50% SDP had increased feed intake, pig pre-weaning survival, and pig weaning weight (Crenshaw et al., 2008; Carter et al., 2018). Lactating sows fed diets with 0.25 or 0.50% SDP during summer months had greater feed intake throughout lactation and pigs weaned from these sows had greater body weight at weaning compared with lactating sows fed diets with 0.25 to 0.50% SDP during winter months (Crenshaw et al., 2007). These observations are consistent with increased feed intake of weanling pigs with greater

pathogen exposure compared with pigs weaned to a clean environment (van Dijk et al., 2001). However, improvements in sow performance is observed with as little as 0.25% SDP in the diet compared with optimal weanling pig performance observed at 6% SDP inclusion to the diet (van Dijk et al., 2001; Crenshaw et al., 2007).

Conclusions

Improving feed intake and reducing post-weaning diarrhea of weanling pigs is imperative to maximize health and production efficiency in swine operations. Additionally, improving sow farrowing performance can lead to improved litter performance and overall health of the offspring when weaned. Inclusion of SDP to diets fed to sows and weanling pigs increases diet palatability and provides highly digestible sources of nutrients. As a consequence, feed intake and daily gain are increased. However, SDP may have a greater effect on improving the immunocompetence of pigs at the intestinal level. More research is needed to determine the immune response elicited by SDP, specifically on cytokine production, and how that subsequently affects the digestibility of other ingredients in a diet, growth performance of weanling pigs, prevalence of post-weaning diarrhea, and sow productivity. Additionally, it is imperative to determine if there are any carryover effects of feeding SDP to sows on weanling pig performance and health.

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CHAPTER 3: There is no carryover effect of inclusion of spray dried plasma (SDP) to a phase 1 diet on the ileal and total tract digestibility of energy and nutrients of a phase 2 diet without SDP fed to weanling pigs

Abstract

Two experiments were conducted to test the null hypothesis that inclusion of spray dried plasma (SDP) in a phase 1 diet fed to weanling pigs will not affect energy or nutrient digestibility of a phase 2 diet without SDP. In Exp. 1, 16 gilts with an initial body weight (BW) of 4.47 ± 0.35 kg were randomly allotted to a phase 1 diet without SDP or a diet with 6% SDP and fed on an ad libitum basis for 14 d. All pigs (BW: 6.92 ± 0.42 kg) were then equipped with a T-cannula in the distal ileum, moved to individual pens, and fed the common phase 2 diet for 10 d with ileal digesta collection on d 9 and 10. In Exp. 2, 24 barrows (initial BW: 6.60 ± 0.22 kg) were randomly allotted to phase 1 diets without or with 6% SDP and given ad libitum access to feed for 20 d. All pigs (BW: 9.37 ± 1.40 kg) were then moved to individual metabolism crates and fed the common phase 2 diet for 14 d with the initial 5 d being the adaptation period to the diet followed by 7 d of collection of feces and urine according to the marker-to-marker procedure. The apparent ileal digestibility (AID) of acid hydrolyzed ether extract (AEE), starch, crude protein (CP), and amino acids (AA) was determined in Exp. 1, and the apparent total tract digestibility (ATTD) of gross energy (GE), insoluble-, soluble-, and total-dietary fiber, Ca, and P, and the retention and biological value of N were determined in Exp. 2. Results of Exp. 1 indicated that the AID of AEE, starch, CP, and AA in phase 2 were not affected by phase 1

treatment. Results of Exp. 2 indicated that the ATTD of GE, insoluble-, soluble-, and total-dietary fiber, Ca, and P and N retention and biological value in phase 2 were also not influenced by phase 1 treatment. In conclusion, feeding weanling pigs a diet with 6% SDP in phase 1 does not affect the AID and ATTD of energy or nutrients in a phase 2 diet without SDP.

Key words: apparent ileal digestibility, apparent total tract digestibility, spray dried plasma, weaned pigs

Abbreviations

AA	amino acid
AEE	acid hydrolyzed ether extract
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
BW	body weight
CP	crude protein
DE	digestible energy
DM	dry matter
GE	gross energy
IDF	insoluble dietary fiber
ME	metabolizable energy
SDF	soluble dietary fiber
SDP	spray dried plasma
TDF	total dietary fiber

Introduction

Weaning is a stressful period for pigs because they are removed from the sow, mixed with pigs from other litters, moved to a new environment, and transitioned from sows' milk to solid feed (Torrallardona, 2010). Weaning often results in a decrease in the total weight of the mucosal layer and tissue in the small intestine (Lallès et al., 2004). Villus height and crypt depth in the duodenum also decrease after weaning leading to reduced digestion and absorption of nutrients (Lallés et al., 2004; Zhang et al., 2015). Highly palatable and digestible ingredients are often used in formulation of diets for newly weaned pigs to promote feed intake and to supply a concentrated source of nutrients (Torrallardona, 2010).

Spray dried plasma (**SDP**) is an animal protein with highly digestible amino acids (**AA**) and P (Mateo and Stein, 2007; Almeida et al., 2011). The AA provided by SDP are 92 to 100% digestible by pigs (Almeida et al., 2013), and when included in diets for weanling pigs, protein synthesis, and also growth performance, were improved (van Dijk et al., 2001). Phosphorus is required for the development and maintenance of skeletal tissue (Veum, 2010), and the P in SDP has a relative bioavailability of 92% and is nearly 100% digestible by pigs (NRC, 1998; Almeida et al., 2011). The beneficial effects of including SDP in diets for weanling pigs on growth performance is more pronounced in the first wk post-weaning compared with the second wk, and continued improvements in growth performance have not always been observed after pigs are fed a diet without SDP (van Dijk et al., 2001). Additionally, SDP contains biologically active peptides, i.e. immunoglobulins, which act in the intestinal tract to prevent pathogen colonization on the mucosal membrane, leading to an improvement in intestinal barrier function and a reduction in intestinal inflammation (Peace et al., 2011). These improvements in intestinal health may result in increased nutrient digestibility; however, data are limited on the effect of including

SDP in a phase 1 diet on the subsequent nutrient digestibility of a phase 2 diet. Therefore, the objective of these experiments was to test the null hypothesis that there is no carryover effect of feeding a diet with SDP to pigs in phase 1 on the ileal and total tract digestibility of energy and nutrients of a diet without SDP fed in phase 2.

Materials and Methods

Two experiments were conducted, and the protocols for both experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before animal work was initiated. In both experiments pigs that were the offspring of Line 359 boars mated to Camborough females were used (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and feeding

In both experiments, SDP (i.e., Appetein B; APC Inc., Ankeny, IA, USA) was used. Three diets were prepared (Tables 3.1, 3.2, and 3.3); 2 phase 1 diets were formulated without or with 6% SDP and a common phase 2 diet without SDP was also used. In the ileal cannulation experiment (Exp. 1), the phase 2 diet contained 0.40% chromic oxide to enable calculation of apparent ileal digestibility (**AID**). Vitamins and minerals were included in all diets to meet or exceed current nutritional requirements for 5 to 7 kg or 7 to 11 kg pigs in phase 1 and phase 2, respectively (NRC, 2012). All diets were provided in meal form and a sample of the main ingredients and all diets were collected at the time of diet mixing and used for chemical analysis.

In Exp. 1, 16 gilts that had an initial body weight (**BW**) of 4.47 ± 0.35 kg at weaning (20 ± 2 d of age) were randomly allotted to a phase 1 diet without SDP or a diet with 6% SDP. Pigs were fed their assigned diet on an ad libitum basis for 2 wk. Pigs were housed in group pens (1.8

× 1.2 m) with 8 pigs per pen and separated by diet in an environmentally controlled room. Each pen had a fully slatted floor, a feeder, and a nipple drinker. After 2 wk, all pigs (BW: 6.92 ± 0.42 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998). Following surgery, pigs were moved to individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors, and a feeder and a nipple drinker installed in each pen. Pens also were equipped with a heat lamp or a floor mat. All pigs were fed the common phase 2 diet without SDP following surgery. Feed was restricted while the pigs recovered from surgery and beginning on d 18 post-weaning feed was provided on an ad libitum basis. All pigs had free access to water.

In Exp. 2, 24 barrows were weaned at 20 ± 2 d (initial BW: 6.60 ± 0.22 kg) and randomly allotted to a phase 1 diet without SDP or a phase 1 diet with 6% SDP. Pigs were housed in group pens (1.8 × 1.2 m) with 6 pigs per pen and separated by diet in an environmentally controlled room and fed their assigned diet for 20 d. All pigs (BW: 9.37 ± 1.40 kg) were then moved to individual metabolism crates that were equipped with a feeder, a nipple drinker, a fully slatted floor, a screen floor, and a urine tray, which allowed for the total, but separate, collection of feces and urine. All pigs were fed the common phase 2 diet without SDP for 14 d. Feed was provided in a daily amount of 3.3 times the maintenance energy requirement (i.e., 197 kcal/kg $BW^{0.60}$; NRC, 2012) and provided in 2 equal meals at 0700 and 1600 h. Water was available at all times throughout the experiment.

Sample collection

In Exp. 1, pig weights were recorded at the beginning of the experiment, before cannulation, and at the conclusion of the experiment. Pigs were fed the phase 2 diet for 10 d after cannulation with ileal digesta collection on d 9 and 10 for 9 h (from 0700 to 1600 h) following standard

procedures (Stein et al., 1998). In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 min, and immediately frozen at -20°C to prevent bacterial degradation of the AA in the digesta. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and finely ground prior to chemical analysis.

In Exp. 2, pigs were fed the phase 2 diet for 14 d with the initial 5 d being the adaptation period to the diet followed by 7 d of collection of feces and urine according to the marker-to-marker procedure (Adeola, 2001). Fecal collection began when the first marker (i.e., indigo carmine), fed in the morning meal on d 6, appeared in the feces, and ceased when the second marker (i.e., ferric oxide), fed in the morning meal on d 13, appeared in the feces. All pigs were weighed at the beginning of the experiment, prior to moving into metabolism crates, and at the end of both the adaptation and collection periods. In addition, orts were collected and weighed daily to determine feed intake. During the collection period, feces were collected twice daily and stored at -20^{o}C immediately after collection, and urine was collected over a preservative of 50 mL of 6 *N* HCl in buckets placed under the metabolism crates. The urine buckets were emptied daily, the weight of the collected urine was recorded, and 20% was stored at -20^{o}C. At the conclusion of the experiment, fecal samples were dried at 65^{o}C in a forced air oven (Metalab Equipment Corp., Hicksville, NY, USA) and finely ground using a 500G stainless steel mill grinder (RRH, Zhejiang, China) prior to chemical analysis, and urine samples were thawed and mixed within animal and diet and 2 subsamples were collected. One urine subsample was lyophilized and the other subsample was stored at -20^{o}C until analysis for N.

Chemical analysis

In both experiments, all diet and ingredient samples were analyzed for dry matter (**DM**; method 930.15, AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019), and gross energy (**GE**) was analyzed using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). The concentration of N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of crude protein (**CP**) as N × 6.25. All diets and ingredients were also sent to the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA) and analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int., 2019] and for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019).

In Exp. 1, diet, ingredient, and ileal digesta samples were analyzed for acid hydrolyzed ether extract (**AEE**) using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). Ileal digesta samples were also analyzed for DM, N, AA, and starch as described for diets and ingredients. The phase 2 diet and all ileal digesta samples were analyzed for chromium (method 990.08; AOAC Int., 2019) at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri.

In Exp. 2, all diet, ingredient, and fecal samples were analyzed for Ca and P by inductively coupled plasma spectroscopy (method 985.01 A, B, and C; AOAC Int., 2019) after wet ash sample preparation (method 975.03 B(b); AOAC Int., 2019) at the Agricultural

Experiment Station Chemical Laboratories at the University of Missouri. The lyophilized urine samples and dried fecal samples were analyzed for GE as described, and fecal samples and urine samples that were not lyophilized were analyzed for N using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a Kjeltec^{TM} 8400 (FOSS Inc., Eden Prairie, MN, USA) with subsequent calculation of CP by a conversion factor of 6.25. Fecal samples were also analyzed for DM and ash, and insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were analyzed according to method 991.43 (AOAC Int., 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber (**TDF**) was calculated as the sum of IDF and SDF.

Calculations

In Exp. 1, AID of CP, AA, starch, and AEE for the phase 2 diet was calculated using the following equation **[3.1]** (Stein et al., 2007):

$$AID\ (\%) = [1 - (AA_d / AA_f) \times (Cr_f / Cr_d)] \times 100 \qquad \textbf{[3.1]}$$

where AID is the apparent ileal digestibility of an AA (%), AA_d is the concentration of that AA in the ileal digesta DM, AA_f is the AA concentration of that AA in the feed DM, Cr_f is the chromium concentration in the feed DM, and Cr_d is the chromium concentration in the ileal digesta DM. The AID for CP, starch, and AEE was also calculated using this equation.

In Exp. 2, apparent total tract digestibility (**ATTD**) of GE was calculated using the following equation **[3.2]** (Almeida and Stein, 2010; NRC, 2012):

$$ATTD\ of\ GE,\ \% = [(GE_i - GE_f) / GE_i] \times 100 \qquad \textbf{[3.2]}$$

where ATTD of GE is the apparent total tract digestibility of GE (%), GE_i is the GE intake (g) from d 6 to 13; and GE_f is the GE output (g) in the feces originating from the feed that was fed from d 6 to 13. The digestible energy (**DE**) and metabolizable energy (**ME**) in the diet

was calculated by subtracting the GE in feces and the GE in feces and urine, respectively, from GE in the diet (NRC, 2012). The ATTD of fiber, N, Ca, and P were also calculated using this equation.

The retention of N for each pig was calculated using the following equation **[3.3]** (Pedersen et al., 2007):

$$Nr = \{[N_i - (N_f + N_u)] / N_i\} \times 100 \quad \textbf{[3.3]}$$

where Nr is the retention of N (%), N_i is the N intake (g) from d 6 to 13, N_f and N_u are N output (g) in feces and urine originating from the feed that was fed from d 6 to 13, respectively. The biological value of N in the diet was calculated by expressing N retention as a percentage of the difference between N intake and N output in feces (Rojas and Stein, 2013).

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA) for both experiments. Outliers were removed if the value deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). Data were analyzed using PROC MIXED of SAS (SAS Inst. Inc. Cary, NC, USA) with the pig as the experimental unit for all analyses. For both experiments, data were analyzed in a randomized complete block design with BW used as the blocking factor. The statistical model to determine differences in AID of CP, AA, starch, and AEE (Exp. 1) or in ATTD of GE, DE, ME, IDF, SDF, TDF, Ca, and P (Exp. 2) between pigs fed a phase 1 diet without or with 6% SDP included phase 1 diet as the fixed effect and pig as the random effect. Treatment means were calculated using the LSMEANS statement in SAS, and if significant, means were separated using the PDIFF option in the PROC MIXED procedure. For all analyses, an alpha value of 0.05 was used to assess significance among means.

Results and Discussion

One pig was removed from the Exp. 1, all other pigs remained healthy throughout both experiments and readily consumed their daily feed allowance.

Experiment 1: Ileal digestibility

The AID of AEE, starch, CP, and all AA in phase 2 were not different between pigs fed the phase 1 diet without SDP and pigs fed the phase 1 diet with 6% SDP (Table 3.4). These results are in agreement with Pendergraft et al. (1993) who reported that the apparent fecal digestibility of DM and N did not differ between pigs fed a phase 1 diet with SDP and pigs fed a diet without SDP after 6 d on a phase 2 diet without SDP. In an experiment with broiler chickens where SDP was fed at 3 inclusion levels (0, 10, or 20 g/kg) from hatch to 10 d of age and then a commercial diet without SDP until 24 d of age (Beski et al., 2016), the ileal digestibility of DM, GE, CP, and AA did not differ among treatments and it was concluded that there were no long-term effects of feeding SDP on the ileal digestibility of nutrients in broiler chickens (Beski et al., 2016). It therefore appears that inclusion of SDP in phase 1 diets for both pigs and broiler chickens does not impact ileal digestibility of nutrients in the subsequent diet. It is, therefore, likely that possible intestinal health benefits provided by SDP in phase 1 do not impact digestibility of nutrients.

Experiment 2: Total tract digestibility

The initial and final BW of pigs in phase 2 were not affected by phase 1 diet (Table 3.5). Feed intake during phase 2 was also not influenced by phase 1 diet. Therefore, there were no differences between treatments for ADFI or ADG during phase 2 of the experiment. This observation is in agreement with van Dijk et al. (2002) who reported that weaned pigs fed a diet

containing 3% SDP during the initial 21 d post-weaning did not have greater growth performance during the subsequent phase compared with control pigs fed no SDP.

Feed intake and the intake of GE, IDF, SDF, TDF, Ca, and P did not differ between pigs fed phase 1 diets without or with SDP, and ATTD of GE, IDF, SDF, TDF, Ca, and P was not influenced by the phase 1 diet (Table 3.6). These data are in agreement with Pendergraft et al. (1993) who observed no differences in ATTD of DM after pigs previously fed a diet without or with SDP were fed a non-SDP diet for 6 d. In the current experiment, the ME in the phase 2 diet fed to pigs previously fed the phase 1 diet with SDP was less ($P < 0.05$) than the ME in the phase 2 diet fed to pigs previously fed the phase 1 diet without SDP, which was the result of a numerical increase in the urine output and urinary GE output of pigs previously fed the phase 1 diet with SDP compared with pigs fed the phase 1 diet without SDP. Thomson et al. (1995) observed greater metabolic energy losses of mice fed a diet with SDP for 21 d compared with the control mice fed a diet without SDP, and liver weights increased for mice fed a diet with SDP compared with control mice, indicating that dietary SDP may result in hyperplasia or hypertrophy of the liver (Thomson et al., 1994). The increased liver weights may have resulted in an increase in the basal metabolic rate resulting in increased metabolic losses (Thomson et al., 1995). Therefore, pigs previously fed a phase 1 diet with SDP may have had increased liver weight resulting in greater maintenance energy requirements, which may have resulted in increased urine loss of energy. However, because liver weights were not determined in the present experiment, we are not able to confirm this hypothesis.

During the 7-d collection period, there were no differences in feed intake, N intake, or N output in feces or urine between pigs previously fed the phase 1 diet without SDP and pigs fed the diet with SDP (Table 3.7). There were also no differences in the ATTD of N, N retention, or

the biological value of N between pigs previously fed a phase 1 diet without SDP or with 6% SDP. These data are in agreement with Pendergraft et al. (1993) who observed no impact of feeding a phase 1 diet with SDP on ATTD of N in phase 2. It therefore appears that provision of SDP in phase 1 does not result in changes in the intestinal tract that will influence digestibility in phase 2. The reason for this may be that the intestinal epithelium exhibits rapid turnover, undergoing complete renewal every 2 to 3 days (Verdile et al., 2019), and therefore, responds to diet can quickly change. As a consequence, even if a phase 1 diet results in increased intestinal health, this change may not be maintained in phase 2.

Conclusions

Spray dried plasma is an excellent source of highly digestible AA and P in diets for weanling pigs. However, data from the current experiments indicate that pigs fed a diet with 6% SDP during the initial 14 to 20 d post-weaning and then switched to a diet without SDP do not have increased AID of AEE, starch, CP, or AA, nor did the ATTD of energy, fiber, Ca, and P, or N retention increase. Therefore, it is concluded that there is no positive carryover effect of SDP in phase 1 diets on energy and nutrient digestibility in phase 2.

Tables

Table 3.1. Ingredient composition of experimental diets, Exp. 1 and Exp. 2

Item, %	Phase 1		Phase 2	
	Exp. 1 and 2		Exp. 1	Exp. 2
Spray dried plasma	–	6.00	–	–
Corn, ground	40.88	43.16	44.87	48.65
Soybean meal, 46% crude protein	25.00	25.00	30.00	25.00
Whey powder, dried	20.00	20.00	15.00	15.00
Soy protein concentrate	8.00	–	–	5.00
Fish meal	–	–	5.00	–
Soybean oil	3.14	3.10	3.00	3.50
Limestone, ground	0.95	1.20	0.86	0.99
Dicalcium phosphate	1.10	0.80	0.30	1.00
Sodium chloride	0.10	0.10	0.10	0.10
L-Lys HCl	0.38	0.29	0.20	0.36
DL-Met	0.20	0.15	0.10	0.16

Table 3.1 (cont.)

L-Thr	0.10	0.05	0.02	0.09
Chromic oxide	–	–	0.40	–
Vitamin mineral premix[1]	0.15	0.15	0.15	0.15

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 3.2. Analyzed nutrient composition of ingredients and diets (as-fed basis), Exp. 1[1]

Item	Ingredients						Phase 1 diet		Phase 2 diet
	SDP	Corn	Soybean meal	Whey powder	Soy protein concentrate	Fish meal	0% SDP	6% SDP	0% SDP
Dry matter, %	93.91	86.82	88.49	89.38	89.92	91.14	88.82	89.29	89.06
Ash, %	8.46	1.09	6.98	7.49	6.04	20.10	6.19	6.35	6.24
AEE, %	0.47	3.15	1.25	1.31	0.77	6.48	4.48	5.02	4.85
Crude protein, %	79.26	7.21	45.97	12.19	58.31	62.54	20.01	21.43	23.80
Gross energy, kcal/kg	4,914	3,840	4,113	3,677	4,331	4,244	3,954	4,050	4,035
Starch, %	N/A[2]	69.71	N/A	N/A	N/A	N/A	27.77	31.21	33.15
Indispensable AA, %									
Arg	4.66	0.32	3.26	0.38	4.23	3.65	1.28	1.22	1.29
His	2.48	0.19	1.21	0.25	1.49	1.20	0.51	0.55	0.53
Ile	2.48	0.24	2.29	0.60	2.92	2.56	0.98	0.89	0.97
Leu	7.56	0.76	3.56	1.15	4.6	4.22	1.71	1.84	1.75
Lys	7.24	0.24	2.95	0.92	3.63	4.59	1.58	1.56	1.43

Table 3.2 (cont.)

Met	0.90	0.13	0.61	0.17	0.75	1.59	0.51	0.38	0.45
Phe	4.26	0.33	2.42	0.42	3.17	2.40	1.01	1.03	1.03
Thr	5.33	0.24	1.79	0.69	2.24	2.44	0.88	1.03	0.86
Trp	1.44	0.04	0.59	0.18	0.79	0.55	0.26	0.3	0.26
Val	5.49	0.31	2.26	0.63	2.89	2.89	1.03	1.12	1.02
Total	41.84	2.80	20.94	5.39	26.71	26.09	9.75	9.92	9.59
Dispensable AA, %									
Ala	3.93	0.48	2.02	0.54	2.55	3.95	0.97	1.03	1.07
Asp	7.96	0.44	5.15	1.11	6.56	5.36	2.17	2.08	2.11
Cys	2.67	0.15	0.66	0.26	0.85	0.48	0.34	0.47	0.31
Glu	11.26	1.18	8.33	1.80	10.58	8.19	3.63	3.41	3.61
Gly	2.78	0.27	1.96	0.28	2.46	4.73	0.82	0.79	0.96
Pro	4.32	0.57	2.33	0.62	3.05	2.99	1.16	1.18	1.17
Ser	5.32	0.31	1.96	0.54	2.53	2.18	0.88	0.97	0.89
Tyr	4.16	0.22	1.61	0.29	2.09	1.80	0.69	0.75	0.68
Total	42.40	3.62	24.02	5.44	30.67	29.68	10.66	10.68	10.80

Table 3.2 (cont.)

Total AA, %	84.24	6.42	44.96	10.83	57.38	55.77	20.41	20.60	20.39

[1]AA, amino acid; AEE, acid hydrolyzed ether extract; SDP, spray dried plasma.

[2]N/A, not analyzed.

Table 3.3. Analyzed nutrient composition of ingredients and diets (as-fed basis), Exp. 2[1]

Item	Ingredients					Phase 1 diet		Phase 2 diet
	SDP	Corn	Soybean meal	Whey powder	Soy protein concentrate	0% SDP	6% SDP	0% SDP
Dry matter, %	93.84	85.97	88.15	90.04	90.52	88.81	88.83	88.39
Ash, %	9.35	1.14	6.14	8.15	6.77	5.76	6.08	5.23
Crude protein, %	79.75	6.48	46.90	10.76	63.13	21.49	22.12	18.96
Gross energy, kcal/kg	4,929	3,822	4,223	3,614	4,378	4,032	4,016	4,060
Starch, %	N/A[2]	60.20	N/A	N/A	N/A	25.56	23.62	28.86
Insoluble dietary fiber, %	2.90	8.60	15.90	N/D	18.70	10.40	8.70	10.20
Soluble dietary fiber, %	3.40	N/D[3]	0.40	N/D	7.00	0.30	0.10	0.10
Total dietary fiber, %	6.20	8.60	16.30	N/D	25.80	10.70	8.90	10.50
Calcium, %	0.12	0.01	0.31	0.48	0.39	0.85	0.97	1.24
Phosphorus, %	1.64	0.25	0.55	0.53	0.58	0.55	0.58	0.48
Indispensable AA, %								
Arg	4.54	0.35	3.21	0.22	4.49	1.28	1.12	1.21

Table 3.3 (cont.)

His	2.42	0.21	1.15	0.18	1.65	0.52	0.51	0.50
Ile	2.5	0.26	2.22	0.63	3.12	0.98	0.83	0.91
Leu	7.48	0.76	3.59	1.01	4.94	1.74	1.73	1.64
Lys	7.25	0.29	2.86	0.83	4.00	1.61	1.50	1.41
Met	0.96	0.15	0.61	0.13	0.87	0.48	0.42	0.38
Phe	4.16	0.33	2.32	0.32	3.23	1.00	0.94	0.94
Thr	5.10	0.25	1.72	0.64	2.41	0.95	0.94	0.82
Trp	1.54	0.06	0.63	0.2	0.86	0.29	0.32	0.26
Val	5.58	0.35	2.31	0.58	3.22	1.01	1.07	0.98
Total	41.53	3.01	20.62	4.74	28.79	9.86	9.38	9.05
Dispensable AA, %								
Ala	3.94	0.51	1.96	0.49	2.74	0.98	0.98	0.95
Asp	7.95	0.51	5.08	1.04	7.10	2.20	1.94	2.01
Cys	2.75	0.17	0.61	0.24	0.89	0.33	0.40	0.30
Glu	11.31	1.23	8.16	1.73	11.43	3.69	3.22	3.43
Gly	2.77	0.31	1.93	0.20	2.66	0.82	0.75	0.80

Table 3.3 (cont.)

Pro	4.05	0.61	2.26	0.58	3.18	1.14	1.10	1.11
Ser	5.01	0.31	1.86	0.41	2.66	0.89	0.89	0.84
Tyr	3.83	0.16	1.64	0.20	2.19	0.67	0.71	0.63
Total	41.61	3.81	23.50	4.89	32.85	10.72	9.99	10.07
Total AA, %	83.14	6.82	44.12	9.63	61.64	20.58	19.37	19.12

[1]AA, amino acid; SDP, spray dried plasma.

[2]N/A, not analyzed.

[3]N/D, not detected.

Table 3.4. Apparent ileal digestibility of acid hydrolyzed ether extract (AEE), starch, crude protein, and amino acids (AA) in phase 2 diet, Exp. 1[1,2]

Item, %	Phase 1 diet by % SDP			
	0	6	Pooled SEM	*P* - value
AEE	75.7	74.1	1.69	0.520
Starch	91.6	90.6	0.60	0.272
Crude protein	72.7	72.2	1.44	0.845
Indispensable AA				
Arg	84.8	85.5	0.85	0.573
His	77.9	77.9	1.29	0.996
Ile	77.7	77.4	1.06	0.858
Leu	77.9	76.8	1.24	0.557
Lys	75.6	76.3	1.28	0.697
Met	85.1	84.2	0.78	0.409
Phe	77.4	76.6	1.21	0.621
Thr	67.6	66.9	1.32	0.739

Table 3.4 (cont.)

Trp	78.7	78.6	1.04	0.961
Val	74.9	73.8	1.34	0.563
Mean	77.5	77.2	1.10	0.834
Dispensable AA				
Ala	71.6	70.2	1.41	0.494
Asp	70.8	71.0	1.09	0.897
Cys	58.6	58.3	2.43	0.923
Glu	77.3	78.9	7.83	0.888
Gly	60.1	60.4	2.33	0.934
Ser	73.8	74.4	1.10	0.711
Tyr	72.7	72.6	1.25	0.927
Mean	78.6	77.5	1.03	0.439
Total AA	72.7	72.4	1.61	0.905

[1]Data are least squares means of 8 observations for pigs fed the phase 1 diet without SDP and 7 observations for pigs fed the phase 1 diet with SDP.

[2]SDP, spray dried plasma protein.

Table 3.5. Body weight and feed intake of pigs fed phase 2 diet without spray dried plasma (SDP; as-fed basis), Exp. 2[1,2]

Item	Phase 1 diet by % SDP		Pooled SEM	*P* - value
	0	6		
Initial BW, kg (d 0)	9.2	9.7	0.40	0.416
Final BW, kg (d 15)	16.9	17.2	0.54	0.695
Feed intake, g/12 d	7,804	7,912	248.10	0.760
ADG, g/d	512	500	13.19	0.551
ADFI, g/d	650	659	20.62	0.762

[1]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight.

[2]Data are least squares means of 12 observations for pigs fed the phase 1 diet without SDP and 11 observations for pigs fed the phase 1 diet with SDP.

Table 3.6. Apparent total tract digestibility (ATTD) of gross energy (GE), Ca and P, and digestible energy (DE) and metabolizable energy (ME) in phase 2 diet (as-fed basis), Exp. 2[1,2]

Item	Phase 1 diet by % SDP		Pooled SEM	*P* - value
	0	6		
Intake				
Feed intake, g/d	670	679	17.71	0.728
GE, Mcal/d	2.72	2.76	0.07	0.731
Ca, g/d	8.31	8.41	0.22	0.739
P, g/d	3.23	3.27	0.09	0.728
Insoluble dietary fiber, g/d	68.32	69.21	1.81	0.731
Soluble dietary fiber, g/d	0.67	0.68	0.02	0.668
Total dietary fiber, g/d	70.32	71.24	1.86	0.730
Fecal excretion				
Dry feces output, g/d	60.3	63.3	2.25	0.359
GE, kcal/d	284	298	10.94	0.377
Ca, g/d	1.31	1.41	0.06	0.290

Table 3.6 (cont.)

P, g/d	1.44	1.47	0.05	0.663
Insoluble dietary fiber, g/d	18.27	18.64	0.64	0.692
Soluble dietary fiber, g/d	1.83	1.74	0.22	0.782
Total dietary fiber, g/d	20.12	20.38	0.75	0.810
ATTD, %				
GE	89.6	89.1	0.36	0.394
Ca	84.1	83.0	0.92	0.416
P	55.4	54.7	1.34	0.696
Insoluble dietary fiber	73.2	73.1	0.67	0.869
Soluble dietary fiber	-179.9	-151.8	32.91	0.553
Total dietary fiber	71.3	71.4	0.82	0.906
DE in diet, kcal/kg	3,636	3,619	14.45	0.394
Urine output, kg/d	2.30	2.72	0.30	0.334
Urinary GE output, kcal/d	100	116	9.85	0.246
ME in diet, kcal/kg	3,488[a]	3,449[b]	12.53	0.041

Table 3.6 (cont.)

[1]SDP, spray dried plasma.

[2]Data are least squares means of 12 observations for pigs fed the phase 1 diet without SDP and 11 observations for pigs fed the phase 1 diet with SDP.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 3.7. Nitrogen balance of pigs fed a phase 2 diet without spray dried plasma (SDP) during a 7-d collection period (as-fed basis), Exp. 2[1]

Item	Phase 1 diet by % SDP			
	0	6	Pooled SEM	*P* - value
Feed intake, g/7 d	4,688	4,750	124.04	0.731
N intake, g/7 d	142	144	3.76	0.731
N output in feces, g/7 d	17.8	19.6	0.86	0.165
N output in urine, g/7 d	21.9	21.2	1.19	0.686
ATTD[2] of N, %	87.5	86.3	0.60	0.181
N retention, g/7 d	102	103	2.72	0.838
N retention, %	72.1	71.7	0.56	0.641
Biological value[3], %	82.4	83.1	0.62	0.412

[1]Data are least squares means of 12 observations for the phase 1 diet without SDP and 11 observations for phase 1 diet with SDP.

[2]ATTD, apparent total tract digestibility.

[3]Biological value was calculated as (N retained / [N intake – N output in feces]) × 100 (Rojas and Stein, 2013).

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CHAPTER 4: Inclusion of spray dried plasma in diets fed to young pigs increases the ileal digestibility of starch, crude protein, and amino acids of some, but not all, ingredients in the diet

Abstract

An experiment was conducted to test the hypothesis that inclusion of spray dried plasma (SDP) in diets for young pigs increases apparent ileal digestibility (AID) of starch and the AID and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) of other ingredients, which may result in a lack of additivity in such diets. Thirty ileal cannulated barrows (body weight: 9.30 ± 0.63 kg) were randomly allotted to a triplicated 10 × 3 Youden square 2 wk post-weaning with 10 diets and three 7-d periods during which ileal digesta were collected for 9 h on d 6 and 7. Eight diets contained ingredients used in commercial swine diets in the U.S.A. [corn and soybean meal (SBM)], Canada (wheat, barley, SBM, and fermented SBM), the European Union (corn, SBM, wheat, and barley), and Asia (corn, SBM, broken rice, and fermented SBM). Four of these diets did not contain SDP and 4 diets contained 6% SDP. A diet containing SDP as the sole source of AA was also formulated, and a N-free diet was used in the calculation of SID of CP and AA. Differences between measured and predicted values for SID of CP and AA in the mixed diets with SDP were also calculated. The AID of starch in diets with SDP tended to be greater ($P < 0.10$) compared with diets without SDP. The AID of CP, Arg, His, Thr, Val, and most dispensable AA, and the SID of Gly was greater ($P < 0.05$) in the European Union diet with SDP than without SDP, and the AID and SID of CP and all AA, except Lys, was greater ($P < 0.05$) in the Canada diet with SDP than without SDP, but for the U.S.A. and Asia

diets, no impact of SDP on AID or SID of CP and AA, except Glu and Pro, was observed (interaction, $P < 0.05$). The measured AID of starch was greater ($P < 0.05$) than the predicted value for the U.S.A. and the European Union diets with SDP. The measured SID of CP and AA was consistent with predicted values for the European Union and Asia diets containing SDP. The measured SID of CP, Arg, Trp, and most dispensable AA was less ($P < 0.05$) than predicted for the U.S.A. diet, and the measured SID of CP and all AA, except Cys and Glu, was greater ($P < 0.05$) than predicted in the Canada diet. In conclusion, addition of 6% SDP to diets based on wheat and barley may increase the AID of starch and the AID and SID of CP and AA from other ingredients in the diet, and therefore, the SID of CP and AA in diets containing SDP is not always additive in wheat and barley based diets.

Key words: additivity, spray dried plasma, standardized ileal digestibility, weaned pigs

Abbreviations

AA	amino acid
AID	apparent ileal digestibility
BW	body weight
CP	crude protein
IAA	indispensable amino acid
SBM	soybean meal
SDP	spray dried plasma
SID	standardized ileal digestibility

Introduction

Weaning is a stressful time for pigs often resulting in a temporary drop in feed intake leading to physiological changes in the structure and function of the intestinal tract (Pluske et al., 1997; Lallès et al., 2004; Campbell et al., 2013). After weaning, villus atrophy occurs in the small intestine, the activity of brush border digestive enzymes is reduced, and there are greater rates of intestinal protein turnover, resulting in reduced capacity for digestion and absorption of amino acids (**AA**; Pluske et al., 1997; Lallès et al., 2004; Leterme and Théwis, 2004). Due to these changes and their negative effect on AA utilization, diets balanced with indispensable AA (**IAA**) and formulated with highly digestible protein sources are recommended during the post-weaning period (Pluske et al., 2018). Animal proteins have greater AA digestibility and contain fewer anti-nutritional factors compared with plant proteins (Yun et al., 2005), and animal proteins are, therefore, used to provide highly digestible AA to newly weaned pigs.

Spray dried plasma (**SDP**) is an animal protein source that may be included at 3 to 8% in diets for newly weaned pigs (van Dijk et al., 2001). Inclusion of SDP in diets improves average daily feed intake and average daily gain during the initial 2 wk post-weaning (van Dijk et al., 2001), and AA in SDP have a high digestibility when measured in both young pigs (Chae et al., 1999; Mateo and Stein, 2007; Almeida et al., 2013) and growing pigs (Gottlob et al., 2006; Wu et al., 2018). Addition of SDP to weaned pig diets improves intestinal barrier function and reduces intestinal inflammation (Peace et al., 2011). The improvement in intestinal health may lead to an increase in the apparent ileal digestibility (**AID**) of starch and in the AID and standardized ileal digestibility (**SID**) of crude protein (**CP**) and AA originating from other ingredients in the diet.

The SID of AA determined in individual ingredients usually are additive in mixed diets fed to growing pigs (Stein et al., 2005; Xue et al., 2014); however, there are no data demonstrating the additivity of AA in mixed diets containing SDP, and additivity may not always be expected if SDP improves intestinal health, and therefore, increases the SID of AA from other ingredients in the diet. Therefore, this experiment was conducted to test the hypothesis that inclusion of SDP increases the AID of starch and the AID and SID of CP and AA originating from other ingredients in mixed diets for weanling pigs, which may result in a lack of additivity in such diets.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Animals, diets, and feeding

Thirty newly weaned barrows (20 ± 2 d of age) that were the offspring of PIC Camborough females and PIC Line 359 males (Pig Improvement Company, Hendersonville, TN, USA) with an initial body weight (**BW**) of 7.21 ± 0.55 kg were housed in groups of 5 and fed a phase 1 diet containing 6% SDP for 2 wk post-weaning. After 2 wk, barrows (BW: 9.30 ± 0.63 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998). Following surgery, pigs were randomly allotted to a triplicated 10 × 3 Youden square design (Kim and Stein, 2009) with 10 diets and three 7-d periods. Therefore, there were 9 replicate pigs per diet. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors. A feeder and a nipple drinker were also installed in each pen. During

the post-surgery recovery period, all pigs were fed the phase 1 diet, but feeding of experimental diets started 18 d post-weaning.

Ten experimental diets were formulated (Tables 4.1 and 4.2). Four diets contained ingredients used in commercial swine diets in the U.S.A. [corn and soybean meal (**SBM**)], Canada (wheat, barley, SBM, and fermented SBM), the European Union (corn, SBM, wheat, and barley), and Asia (corn, SBM, broken rice, and fermented SBM). These diets did not contain SDP. However, 4 additional diets were formulated by mixing 94% of the previous 4 diets with 6% SDP. A diet containing SDP as the sole source of CP and AA and a N-free diet were also formulated. Vitamins and minerals were included in all diets to meet or exceed current nutrient requirement estimates for 7 to 11 kg pigs (NRC, 2012). All diets, except the phase 1 diet, contained 0.40% chromic oxide as an indigestible marker, and all diets were provided in meal form. At the time of diet mixing, samples of all diets and the main ingredients were collected and used for chemical analysis. Pigs were fed their assigned diets on an ad libitum basis and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment.

Sample collection

Experimental periods were 7 d with the initial 5 d being the adaptation period, whereas ileal digesta were collected for 9 h (from 0800 to 1700 h) on d 6 and 7 following standard procedures (Stein et al., 1998). In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 minutes, and immediately frozen at –20°C to prevent bacterial degradation of the AA in the digesta. At the completion of one experimental period, animals were deprived of feed overnight and the following morning, the new experimental diet was offered. At the conclusion

of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and finely ground prior to analysis.

Chemical analysis

All ingredients were analyzed in duplicate for dry matter (method 930.15, AOAC Int., 2019), ash (method 942.05; AOAC Int., 2019), and N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA), and CP was calculated as N × 6.25. All ingredients were also analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int., 2019], and gross energy was determined using an isoperibol bomb calorimeter (Model 6400; Parr Instruments, Moline, IL, USA) with benzoic acid as the standard for calibration. Acid hydrolyzed ether extract was determined using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). Cereal grains were analyzed for starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019). Samples of diets and ileal digesta were analyzed for dry matter, CP, AA, and starch as indicated for the ingredients. All diets, except the phase 1 diet, and ileal digesta samples were also analyzed for chromium (method 990.08; AOAC Int., 2019).

Calculations

Using equations published by Stein et al. (2007), values for AID of CP and AA in SDP and AID of CP, AA, and starch in the mixed diets were calculated using the direct procedure. The basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet and these data were used to calculate the SID of CP and AA in SDP and the mixed diets. The predicted AID of

CP and AA in the mixed diets containing SDP were calculated according to the following equation (Stein et al., 2005; Xue et al., 2014):

$$AID_P = [(AA_{SDP} \times AID_{SDP}) + (AA_M \times AID_M)] / (AA_{SDP} + AA_M),$$

where AID_P is the predicted AID for an AA (%) in the mixed diet with SDP; AA_{SDP} and AA_M are the concentrations (%) of that AA contributed by SDP and mixed diets without SDP (i.e., U.S.A., The European Union, Canada, or Asia), respectively, which were calculated by multiplying the concentration of that AA (%) in SDP or the mixed diets without SDP by 6 or 94%, respectively. The AID_{SDP} and AID_M are the measured AID (%) for that AA in SDP and the mixed diets without SDP, respectively. The AID of CP and starch in the mixed diets with SDP was predicted using the same equation, and the predicted SID of CP and AA in mixed diets with SDP were also calculated using this equation.

In the calculation for predicted AID and SID values in mixed diets containing SDP, the predicted values for each diet were obtained from mean values of measured AID and SID for AA, CP, or starch in SDP and mixed diets without SDP. The difference between measured and predicted AID and SID values was then calculated by subtracting the single predicted AID or SID value from the measured AID or SID values determined for the 9 replicate pigs per treatment.

Statistical analysis

At the conclusion of the experiment, normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if the value deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). Data for AID of starch, CP, and AA and SID of CP and AA in the regional diets were analyzed as a 2 × 4 factorial arrangement of

treatments using PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA), with 2 levels of SDP and 4 regions. The pig was the experimental unit for all analyses. The model included dietary concentration of SDP, region, and the interaction between SDP and region as fixed effects and pig and period as random effects. Additivity of AID and SID values was analyzed using a 2-sided *t* test within each of the 4 regional diets with added SDP; the null hypothesis that the difference between measured and predicted values for AID and SID was equal to 0 was tested (She et al., 2018). The main effect of period was also analyzed using contrast statements in PROC MIXED of SAS to determine linear and quadratic effects of period on the SID of CP and AA in all diets. Treatment means were calculated using the LSMeans statement in SAS, and if significant, means were separated using the PDIFF option in PROC MIXED procedure. Statistical differences were established at $P < 0.05$, whereas $0.05 \leq P < 0.10$ was considered a trend.

Results

The CP in SDP was 80.6% (as-is basis) and CP in SBM, fermented SBM, soy protein concentrate, and fish meal was 44.2, 49.2, 61.8, and 65.9%, respectively (Table 4.3). Broken rice contained 77.6% starch (as-is basis), whereas corn, wheat, and barley contained 65.1, 62.1, and 60.4% starch, respectively.

The AID of CP in SDP was 84.7% and the AID was greater than 85% for all AA except Cys, Gly, and Pro (i.e., 90.1, 87.3, 85.5, and 89.4% for Lys, Met, Thr, and Trp, respectively; Table 4.4). The AID of starch in the SDP diet was 99%. The SID of CP in SDP was 92.5% and the SID was greater than 91% for all AA, except Cys (i.e., 93.3, 93.5, 91.3, and 93.6% for Lys, Met, Thr, and Trp, respectively).

There was an interaction between inclusion of SDP and region for the AID of CP and all AA, but not for the AID of starch (Table 4.5). The AID of starch in diets with SDP tended to be greater ($P < 0.10$) compared with diets without SDP, and the AID of starch was greater in the Asia diet than the U.S.A. and the European Union diets. The AID of CP and IAA did not differ between U.S.A. or Asia diet without and with SDP, but when SDP was included in the European Union diet, the AID of CP, Arg, His, Thr, Val, and most dispensable AA was greater ($P < 0.05$) than the European Union diet without SDP. For the Canada diet, the AID of CP and all AA, except Lys, was greater ($P < 0.05$) if SDP was included in the diet than if SDP was not used.

An interaction between inclusion of SDP and region for the SID of CP and all AA was observed (Table 4.6). The SID of Glu and Pro was greater ($P < 0.05$) in the U.S.A. diet without SDP compared with SDP and the SID of Lys and Gly was greater ($P < 0.05$) in the European Union diet containing SDP than the diet not containing SDP. Except for Lys, Pro, Glu, and Gly, the SID of CP and all other AA did not differ between the U.S.A., the European Union, or Asia diets without and with SDP. The SID of CP and all AA was greater ($P < 0.05$) in the Canada diet with SDP compared with the Canada diet without SDP.

The measured AID of starch was greater ($P < 0.05$) than the predicted value in the U.S.A. and the European Union diet with SDP (Table 4.7). The measured AID of CP was greater ($P < 0.05$) than predicted in the Canada diet with SDP, but the measure AID of CP was consistent with the predicted value in the other diets with SDP. The measured AID of Arg, Lys, Thr, Asp, Glu, Gly, Pro, and Ser was greater ($P < 0.05$) than the predicted value in the European Union diet with SDP, and the measured AID of CP and all AA, except Glu, was greater ($P < 0.05$) than the predicted value in the Canada diet with SDP. The measured SID of CP and AA was consistent with predicted values for the European Union and Asia diets containing SDP. The measured SID

of CP, Arg, Trp, and most dispensable AA was less ($P < 0.05$) than the predicted value for the U.S.A. diet with SDP. The measured SID of CP and AA in the Canada diet with SDP was greater ($P < 0.05$) than the predicted SID value for all AA, except Cys and Glu. The SID of CP and all AA linearly increased ($P < 0.05$) from period 1 to period 3 as the BW of pigs increased from 10.54 to 18.28 kg (Table 4.8).

Discussion

The chemical composition of all ingredients was generally in agreement with expected values (NRC, 2012). Diets formulated for the European Union and Canada were based on wheat and barley, which are the cereal grains used as a dietary source of energy in swine diets in western Canada and northern Europe (Nasir et al., 2015; Menegat et al., 2019). However, barley is the third-most produced grain in Canada after wheat and corn and approximately 65% of barley is included in animal feed (Zhou et al., 2016); therefore, barley was included at a greater rate in the Canada diet than in the European Union diet. Wheat is also produced in the U.S.A. and has a greater CP and AA content than corn (NRC, 2012), but because the U.S.A. is the leading global producer of corn, wheat is usually not competitively priced with corn (FAOSTAT, 2019; Menegat et al., 2019). Therefore, corn was included as the only cereal grain in the U.S.A. diet. The large production of corn and its high concentration of energy makes it a favorable ingredient in swine diets (Kim et al., 2021), but instead of relying heavily on imports of corn from other countries, rice, the primary cultivated grain in southern China and most countries in Asia, often replaces corn in swine diets in Asia (Zhang et al., 2002; Kim et al., 2021). Therefore, rice was included in high concentrations in the Asia diet in the current study.

The AID of starch in the SDP only diet was 99.3%; however, because SDP does not contain starch, all starch in this diet was from corn starch, which has a high digestibility (Li et al., 2015). The AID of CP and IAA in SDP were less than published values from Gottlob et al. (2006) and Almeida et al. (2013), and the SID of CP and IAA in SDP were less than published values from Almeida et al. (2013), but in agreement with values from Gottlob et al. (2006). However, the AID and SID of CP and all AA in SDP determined in the current study are in agreement with values from Mateo and Stein (2007) who used pigs with an initial BW of 5.0 kg. Gottlob et al. (2006) and Almeida et al. (2013) used pigs with a greater initial BW (37.6 and 11.5 kg, respectively) compared with the initial BW of the pigs used in the current experiment (9.3 kg). The BW of pigs and the AID and SID of AA are correlated because increased BW results in greater AID and SID of AA (Leterme and Théwis, 2004; Nitrayová et al., 2006; Urbaityte et al., 2009). The greater AA digestibility in older pigs compared with younger pigs may be a result of lower basal endogenous losses of AA (Leterme and Théwis, 2004; Pedersen et al., 2016). Thus, the linear increase in SID values as BW of the pigs increased in this experiment is in agreement with previous data and demonstrates that as young pigs grow, the ability to digest protein and absorb AA is gradually increased. However, this increase in protein digestibility is most pronounced from weaning to around 20 kg, whereas the SID of AA is constant in pigs after 20 kg (Pedersen et al., 2016).

The ileal digestibility of starch among diets varied regardless of the inclusion of SDP, which is a result of the different sources of starch used in the diets. The large inclusion of rice in the Asia diet resulted in greater AID of starch compared with the other diets, because rice has a greater concentration of total starch compared with corn, barley, and wheat (Cervantes-Pahm et al., 2014). The AID of starch in rice is also greater than the AID of starch in corn and barley, but

does not differ from the AID of starch in wheat (Cervantes-Pahm et al., 2014), which resulted in no differences between the AID of starch in the Asia and Canada diets. The European Union and Canada diets were formulated with wheat and barley, but corn was not included in the Canada diet and the concentration of barley was greater compared with the European Union diet. Therefore, the observation that the AID and SID of CP and most AA were less in the Canada diet may reflect the lesser digestibility of AA in barley compared with the digestibility of AA in wheat and corn (Wang et al., 2017). Additionally, the AID and SID of most AA were greater for the diets formulated without wheat or barley. Wheat and barley have higher concentrations of non-starch polysaccharides, particularly arabinoxylans and β-glucans, than corn and rice, which may have antinutritive properties and increase the digesta viscosity and endogenous AA losses via increased mucin secretion (Li et al., 1996; Barrera et al., 2004; Cervantes-Pahm et al., 2014). Therefore, diets with high concentrations of non-starch polysaccharides have reduced ileal digestibility of AA. Supplementing wheat and barley based diets with phytase, xylanase, or β-glucanase increased the AID of AA by pigs due to improved digestion of these antinutritional factors (Li et al., 1996; Barrera et al., 2004; Dersjant-Li and Dusel, 2019). However, the content of arabinoxylans or β-glucans in wheat and barley were not determined in this experiment.

The SID of Pro was greater than 100% for most diets fed in the current experiment, and this is likely due to an overestimation of the endogenous loss of Pro from pigs fed a N-free diet (Stein et al., 2005). The endogenous loss of Pro is generally increased for pigs fed a diet with low AA concentrations (Pedersen et al., 2002; Stein et al., 2005), and digestibility values greater than 100% are not physiologically possible. Therefore, the calculation for SID of Pro >100% demonstrates overestimation of the endogenous losses of Pro when measured using a N-free diet,

and is in agreement with previous data (Dilger et al., 2004; Moter and Stein, 2004; Stein et al., 2005).

Although starch in SDP is negligible, the AID of starch tended to increase if 6% SDP was included in the diets, which is in agreement with increased digestibility of energy observed by dogs fed kibble with 1 to 3% SDP (Quigley et al., 2004). Cereal grains are the main sources of energy in diets due to their high starch concentration and high inclusion rate in diets for swine (Velayudhan et al., 2015). The activity of amylase and maltase, enzymes that aids in starch digestion, in the jejunal digesta and mucosa, respectively, increase in pigs fed a diet with SDP (Zhang et al., 2015). Therefore, the greater AID of starch in diets with SDP compared with diets without SDP may be due to an increase in enzymatic activity in the small intestine.

Spray dried plasma is a source of highly bioavailable and digestible AA (Almeida et al., 2013). The digestibility of AA in SDP is greater than 90% (Mateo and Stein, 2007), therefore, the observed improvement in AID and SID of AA in diets was expected when SDP was included in the diet. Spray dried plasma of porcine or avian origin included in a diet fed to weanling pigs increased the activity of trypsin in the jejunal digesta compared with pigs fed a control diet without SDP (Zhang et al., 2015). Trypsin is needed for protein digestion, and increased trypsin activity may be the reason for the increased AID and SID of CP and AA in diets with SDP compared with diets without SDP. However, the enzymatic activity of trypsin also increases after weaning as pigs adapt to changes in the concentration of protein, fat, or carbohydrates in solid feed (Zhang et al., 2015).

Spray dried plasma also has a high concentration of immunoglobulin G that acts as a functional protein in the intestinal tract of pigs (Pierce et al., 2005; Zhang et al., 2016). Immunoglobulin G in SDP may act in preventing pathogenic bacteria from colonizing on the

mucosal layer of the small intestine (Pierce et al., 2005; Zhang et al., 2015), thereby reducing villus blunting and maintaining the integrity of the mucosa (Torrallardona, 2010). Pigs fed a diet with SDP have increased villus height, increased villus height to crypt depth ratio, and increased villus surface area (Pierce et al., 2005), indicating improved maintenance of the intestinal barrier post-weaning, which is critical for nutrient digestion and absorption (Campbell et al., 2010). Therefore, SDP may increase the AID and SID of CP and AA of diets by improving the immunocompetence of the young pig.

The improvement in CP and AA digestibility in the Canada diet with SDP compared with the diet without SDP resulted in greater measured AID and SID values than what was expected based on values predicted from the mixture of SDP and the diet without SDP, indicating that AID or SID values determined for individual ingredients in the Canada diet are not additive in a mixed diet with 6% SDP. Additivity of digestible nutrients in ingredients is a fundamental assumption when formulating diets for swine (Stein et al., 2005). Apparent ileal digestibility values obtained for individual ingredients are less additive in a mixed diet compared with SID values for those ingredients (Stein et al., 2005). This is due to the underestimation of AID values for low-protein ingredients, such as cereal grains, caused by a greater loss of endogenous AA (Stein et al., 2005). Therefore, correcting AID values for basal endogenous losses of AA to calculate SID values results in greater additivity of individual AA in a mixed diet (Stein et al., 2005), which was observed for the European Union and Asia diets, but not for the Canada and U.S.A. diets with SDP fed in the current trial.

It has been demonstrated that SID values for corn, SBM, and canola meal are additive in mixed diets (Stein et al., 2005), but the addition of corn distillers' dried grains with solubles to a corn-SBM diet results in greater measured AID of AA than predicted values (Xue et al., 2014).

Data from the current experiment indicate that some IAA provided by corn and SBM may not be additive when SDP is supplemented in the mixed diet. The AID for mixed diets formulated with less digestible cereal grains may underestimate the digestibility of AA due to greater endogenous losses of AA (Fan et al., 1994; Xue et al., 2014). However, even after correcting the AID values for endogenous losses of AA, the measured SID values were less than predicted values in the U.S.A. diet, which is in agreement with previous data for mixed diets based on corn-SBM (Xue et al., 2014). The greatest difference between measured and predicted values for AA was observed for the Canada diet. This diet contained greater concentrations of wheat and barley, which are cereal grains with low concentrations of protein, but the greater inclusion rate of these ingredients in the Canada diet may also have resulted in greater concentrations of non-starch polysaccharides and, therefore, antinutritional factors further leading to increased endogenous AA losses (Barrera et al., 2004; Stein et al., 2007). However, even after correcting AID values for endogenous AA losses, the measured SID values were greater than predicted values in the Canada diet. This indicates that SDP may have improved the digestibility of other ingredients in the diet resulting in digestibility of the diet being greater than expected when SDP was added.

In conclusion, SDP is a highly digestible animal protein source for young pigs, and when included in complete diets formulated with high levels of cereal grains with low AA digestibility, such as wheat and barley, SDP may increase the SID of CP and most AA in the mixed diet. As a consequence, the SID of CP and AA in mixed diets based on wheat and barley may not always be additive if SDP is included in the diets.

Tables

Table 4.1. Ingredient composition of experimental diets[1]

Item, %	Phase 1	N-free	SDP	U.S.A.		European Union		Canada		Asia	
				-SDP	+SDP	-SDP	+SDP	-SDP	+SDP	-SDP	+SDP
SDP	6.00	–	18.00	–	6.00	–	6.00	–	6.00	–	6.00
Corn, ground	43.16	–	–	55.13	51.83	25.06	23.57	–	–	10.93	10.28
Wheat, ground	–	–	–	–	–	20.00	18.80	30.00	28.20	–	–
Barley, ground	–	–	–	–	–	15.00	14.10	30.00	28.20	–	–
Rice, ground	–	–	–	–	–	–	–	–	–	45.00	42.30
Soybean meal	25.00	–	–	15.00	14.10	9.00	8.46	9.02	8.49	10.00	9.40
Fermented soybean meal	–	–	–	–	–	–	–	7.00	6.58	7.00	6.58
Fish meal	–	–	–	–	–	–	–	–	–	5.00	4.70
Whey powder, dried	20.00	–	–	15.00	14.10	15.00	14.10	15.00	14.10	15.00	14.10
Soy protein concentrate	–	–	–	7.00	6.58	8.00	7.52	–	–	–	–
Corn starch	–	57.45	40.00	–	–	–	–	–	–	–	–
Solka floc	–	4.00	4.00	–	–	–	–	–	–	–	–
Lactose	–	15.00	15.00	–	–	–	–	–	–	–	–

Table 4.1 (cont.)

Sucrose	–	15.00	15.00	–	–	–	–	–	–	–	–
Soybean oil	3.10	4.00	4.00	4.00	3.76	4.00	3.76	5.00	4.70	4.00	3.76
Ground limestone	1.20	0.40	1.35	1.00	0.94	1.20	1.13	1.30	1.22	0.90	0.85
Dicalcium phosphate	0.80	2.70	1.20	1.20	1.13	1.00	0.94	0.80	0.75	0.50	0.47
Sodium chloride	0.10	0.40	0.40	0.45	0.42	0.45	0.42	0.45	0.42	0.45	0.42
L-Lys HCl	0.29	–	–	0.37	0.35	0.45	0.42	0.58	0.55	0.40	0.38
DL-Met	0.15	–	–	0.13	0.12	0.12	0.11	0.13	0.12	0.10	0.09
L-Thr	0.05	–	–	0.10	0.09	0.10	0.09	0.10	0.09	0.10	0.09
Magnesium oxide	–	0.10	0.10	–	–	–	–	–	–	–	–
Potassium carbonate	–	0.40	0.40	–	–	–	–	–	–	–	–
Chromic oxide	–	0.40	0.40	0.45	0.42	0.45	0.42	0.45	0.42	0.45	0.42
Vitamin-mineral mix[1]	0.15	0.15	0.15	0.17	0.16	0.17	0.16	0.17	0.16	0.17	0.16

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate;

Table 4.1 (cont.)

I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 4.2. Analyzed nutrient composition of experimental diets (as-fed basis)[1]

Item, %	Phase 1	N-free	SDP	U.S.A.		European Union		Canada		Asia	
				-SDP	+SDP	-SDP	+SDP	-SDP	+SDP	-SDP	+SDP
Dry matter	88.42	92.86	93.68	89.20	88.94	89.04	89.28	89.55	89.36	89.45	89.40
Crude protein	19.73	0.38	16.68	18.54	21.32	16.92	21.26	17.31	20.87	18.25	21.54
Starch	26.73	46.37	36.70	36.47	35.45	35.40	36.06	34.17	35.98	44.72	42.89
Indispensable AA											
Arg	1.30	0.00	0.80	1.10	1.21	0.89	1.19	0.92	1.09	1.12	1.31
His	0.57	0.00	0.44	0.45	0.54	0.37	0.52	0.38	0.49	0.43	0.52
Ile	0.97	0.01	0.45	0.80	0.84	0.69	0.85	0.74	0.80	0.85	0.89
Leu	1.92	0.02	1.41	1.53	1.82	1.24	1.72	1.22	1.59	1.43	1.70
Lys	1.68	0.01	1.36	1.25	1.64	1.18	1.57	1.45	1.62	1.32	1.60
Met	0.43	0.01	0.17	0.38	0.45	0.40	0.36	0.31	0.39	0.50	0.47
Phe	1.07	0.01	0.79	0.88	1.02	0.74	1.01	0.76	0.96	0.83	1.01
Thr	1.05	0.00	0.97	0.82	1.09	0.69	1.00	0.69	0.98	0.80	1.02
Trp	0.33	0.02	0.29	0.21	0.29	0.21	0.28	0.21	0.25	0.22	0.28

Table 4.2 (cont.)

Val	1.20	0.01	0.98	0.84	1.08	0.75	1.09	0.81	1.07	0.93	1.11
Total	10.52	0.07	7.66	8.26	9.98	7.16	9.59	7.49	9.24	8.43	9.91
Dispensable AA											
Ala	1.07	0.01	0.75	0.89	1.03	0.72	0.97	0.69	0.89	0.92	1.07
Asp	2.22	0.02	1.54	1.87	2.13	1.48	2.01	1.46	1.87	1.78	2.16
Cys	0.46	0.00	0.52	0.31	0.46	0.29	0.47	0.32	0.46	0.30	0.42
Glu	3.74	0.03	2.14	3.30	3.50	3.05	3.81	3.32	3.68	3.05	3.53
Gly	0.84	0.01	0.53	0.72	0.79	0.62	0.80	0.66	0.76	0.86	0.96
Pro	1.13	0.01	0.81	1.08	1.21	1.03	1.30	1.01	1.26	0.85	1.08
Ser	0.99	0.01	0.87	0.80	1.00	0.63	0.92	0.63	0.89	0.70	0.94
Tyr	0.80	0.01	0.58	0.58	0.74	0.47	0.68	0.49	0.66	0.56	0.73
Total	11.25	0.10	7.74	9.55	10.86	8.29	10.96	8.58	10.47	9.02	10.89
Total AA	21.77	0.17	15.40	17.81	20.84	15.45	20.55	16.07	19.71	17.45	20.80

[1]AA, amino acid; SDP, spray dried plasma.

Table 4.3. Analyzed nutrient composition of ingredients (as-fed basis)[1]

Item	SDP	Corn	Soybean meal	Fermented soybean meal	Wheat	Barley	Soy protein concentrate	Rice	Fish meal	Whey powder
Dry matter, %	94.11	86.23	88.13	89.22	87.52	87.65	92.28	87.36	91.79	90.94
Crude protein, %	80.56	6.27	44.18	49.15	11.45	9.06	61.76	7.19	65.88	10.67
Ash, %	8.75	0.73	5.77	6.89	1.25	2.30	6.37	0.08	19.44	8.04
GE, kcal/kg	4,915	3,833	4,202	4,306	3,840	3,772	4,412	3,729	4,379	3,618
AEE, %	0.21	2.65	1.84	0.77	1.61	1.70	1.39	1.53	8.97	0.53
Starch, %	N/A[2]	65.09	N/A	N/A	62.07	60.35	N/A	77.55	N/A	N/A
Indispensable AA, %										
Arg	4.53	0.36	3.34	3.33	0.51	0.48	4.51	0.51	3.62	0.24
His	2.41	0.20	1.21	1.28	0.25	0.21	1.64	0.17	1.36	0.20
Ile	2.49	0.26	2.30	2.53	0.42	0.36	3.18	0.32	2.51	0.68
Leu	7.49	0.75	3.61	3.93	0.74	0.65	4.94	0.60	4.10	1.09
Lys	7.24	0.28	2.96	2.85	0.34	0.41	3.97	0.28	4.64	0.90
Met	0.95	0.14	0.63	0.70	0.17	0.15	0.86	0.18	1.64	0.15

Table 4.3 (cont.)

Phe	4.15	0.34	2.39	2.60	0.51	0.44	3.24	0.38	2.33	0.34
Thr	5.12	0.25	1.77	1.91	0.30	0.31	2.37	0.24	2.35	0.68
Trp	1.55	0.07	0.65	0.70	0.13	0.11	0.89	0.08	0.74	0.21
Val	5.60	0.34	2.40	2.67	0.49	0.50	3.27	0.44	2.87	0.61
Total	41.53	2.99	21.26	22.50	3.86	3.62	28.87	3.20	26.16	5.10
Dispensable AA, %										
Ala	3.95	0.48	2.03	2.25	0.39	0.43	2.74	0.41	3.98	0.53
Asp	7.90	0.50	5.21	5.59	0.56	0.63	7.08	0.63	5.29	1.10
Cys	2.69	0.16	0.66	0.73	0.28	0.24	0.89	0.17	0.52	0.26
Glu	11.29	1.23	8.49	9.13	3.23	1.94	11.53	1.31	7.82	1.85
Gly	2.77	0.30	2.00	2.24	0.45	0.41	2.67	0.32	4.88	0.22
Pro	3.84	0.56	2.19	2.41	1.01	0.79	3.03	0.32	2.82	0.58
Ser	4.90	0.30	1.89	2.03	0.43	0.34	2.51	0.32	1.92	0.42
Tyr	3.96	0.21	1.70	1.82	0.28	0.23	2.19	0.16	1.76	0.25
Total	41.30	3.74	24.17	26.20	6.63	5.01	32.64	3.64	28.99	5.21
Total AA, %	82.83	6.73	45.43	48.70	10.49	8.63	61.51	6.84	55.15	10.31

Table 4.3 (cont.)

[1]AA, amino acids; AEE, acid hydrolyzed ether extract; GE, gross energy; SDP, spray dried plasma.

[2]N/A, not analyzed.

Table 4.4. Apparent ileal digestibility (AID) of starch and AID and standardized ileal digestibility (SID) of crude protein and amino acids (AA) in spray dried plasma[1]

Item, %	Spray dried plasma	
	AID	SID
Starch	99.3	–
Crude protein	84.7	92.5
Indispensable AA		
Arg	90.5	96.1
His	88.3	92.7
Ile	84.7	92.2
Leu	89.5	93.3
Lys	90.1	93.3
Met	87.3	93.5
Phe	89.3	93.5
Thr	85.5	91.3
Trp	89.4	93.6
Val	85.4	90.9
Mean	88.3	93.0
Dispensable AA		
Ala	86.5	93.2
Asp	85.8	90.8
Cys	83.8	88.1
Glu	87.8	92.1

Table 4.4 (cont.)

Gly	75.2	95.5
Pro	76.8	103.6
Ser	85.0	90.5
Tyr	88.1	92.9
Mean	84.7	93.0
Total AA	86.5	93.0

[1]Data are least squares means of 8 observations.

Table 4.5. Apparent ileal digestibility (AID) of starch, crude protein, and amino acids (AA) in experimental diets[1]

Item, %	U.S.A.		European Union		Canada		Asia		Pooled	P-value		
SDP[2]	–	+	–	+	–	+	–	+	SEM	SDP	Region	SDP*Region
Starch	96.2	97.3	96.3	97.8	97.4	97.1	97.9	98.2	0.57	0.078	0.097	0.385
Crude protein	81.0^{a}	79.3^{ab}	75.3^{b}	80.2^{a}	69.9^{c}	79.2^{ab}	79.1^{ab}	82.1^{a}	1.88	0.001	0.001	0.011
Indispensable AA												
Arg	90.0^{a}	89.4^{ab}	85.8^{c}	88.8^{ab}	78.3^{d}	86.7^{bc}	88.8^{abc}	90.2^{a}	1.43	<0.001	<0.001	<0.001
His	84.3^{a}	84.0^{a}	79.6^{b}	83.8^{a}	73.5^{c}	83.8^{a}	83.9^{a}	86.1^{a}	1.77	<0.001	<0.001	<0.001
Ile	84.0^{a}	82.2^{ab}	80.2^{b}	82.6^{ab}	73.3^{c}	81.7^{ab}	84.0^{a}	85.1^{a}	1.91	0.006	<0.001	0.001
Leu	84.5^{ab}	84.6^{a}	80.9^{b}	84.3^{ab}	72.9^{c}	84.5^{ab}	84.2^{ab}	86.5^{a}	1.90	<0.001	<0.001	<0.001
Lys	84.6^{xy}	85.9^{x}	82.2^{y}	86.2^{x}	78.5^{z}	85.1^{xy}	83.9^{xy}	86.6^{x}	1.39	<0.001	0.004	0.081
Met	90.2^{a}	89.9^{a}	89.8^{ab}	87.5^{b}	80.5^{c}	88.8^{ab}	89.6^{ab}	89.8^{ab}	1.29	0.021	<0.001	<0.001
Phe	85.1^{a}	84.4^{ab}	81.2^{b}	84.3^{ab}	72.9^{c}	83.7^{ab}	83.4^{ab}	86.2^{a}	1.88	<0.001	<0.001	<0.001
Thr	79.6^{a}	80.1^{a}	74.1^{b}	80.1^{a}	65.3^{c}	80.1^{a}	79.4^{a}	82.5^{a}	2.08	<0.001	<0.001	<0.001
Trp	83.2^{ab}	83.6^{ab}	80.4^{b}	83.0^{ab}	72.2^{c}	81.5^{ab}	83.4^{ab}	85.8^{a}	1.96	0.002	<0.001	0.038
Val	79.6^{ab}	79.9^{a}	75.0^{b}	79.6^{a}	66.3^{c}	80.1^{a}	80.9^{a}	83.0^{a}	2.45	<0.001	<0.001	<0.001
Mean	84.5^{a}	84.4^{a}	80.8^{b}	84.1^{ab}	73.6^{c}	83.6^{ab}	84.1^{ab}	86.2^{a}	1.77	<0.001	<0.001	<0.001

Table 4.5 (cont.)

Dispensable AA												
Ala	79.8^{ab}	79.1^{b}	73.9^{c}	78.3^{bc}	63.1^{d}	78.2^{bc}	80.1^{ab}	83.9^{a}	2.47	<0.001	<0.001	<0.001
Asp	81.1^{ab}	78.6^{b}	74.7^{c}	79.6^{ab}	69.2^{d}	79.1^{b}	80.4^{ab}	83.1^{a}	1.84	<0.001	<0.001	<0.001
Cys	73.3^{ab}	69.5^{bc}	68.3^{bc}	76.5^{a}	63.9^{c}	75.9^{a}	72.8^{ab}	76.9^{a}	3.05	0.002	0.189	0.008
Glu	86.4^{a}	81.7^{bc}	83.9^{abc}	86.5^{a}	80.7^{c}	84.6^{ab}	84.7^{ab}	87.0^{a}	1.85	0.258	0.075	0.005
Gly	71.4^{ab}	67.1^{b}	60.1^{c}	72.0^{ab}	57.1^{c}	68.9^{ab}	73.9^{a}	75.3^{a}	2.99	0.004	<0.001	0.003
Pro	82.9^{abc}	80.4^{c}	80.4^{bc}	84.2^{a}	74.8^{d}	83.9^{ab}	79.7^{c}	82.9^{abc}	1.75	<0.001	0.142	<0.001
Ser	82.2^{a}	81.1^{a}	75.3^{b}	81.2^{a}	67.3^{c}	80.2^{a}	79.5^{a}	83.4^{a}	1.86	<0.001	<0.001	<0.001
Tyr	83.4^{a}	84.2^{a}	78.4^{b}	83.8^{a}	69.5^{c}	83.0^{a}	81.4^{ab}	85.8^{a}	2.22	<0.001	<0.001	0.002
Mean	82.3^{ab}	79.2^{bc}	77.6^{c}	82.1^{ab}	72.6^{d}	81.0^{abc}	80.9^{abc}	83.7^{a}	1.96	0.002	0.002	0.001
Total AA	83.3^{a}	81.7^{ab}	79.1^{b}	83.0^{a}	73.0^{c}	82.2^{ab}	82.5^{ab}	84.9^{a}	1.84	<0.001	<0.001	<0.001

[1]Data are least squares means of 9 observations for U.S.A. + SDP treatment; 8 observations for the European Union + SDP, Canada + SDP, and Asia + SDP treatments; and 7 observations for U.S.A., the European Union, Canada, and Asia treatments.

[2]SDP, spray dried plasma.

[a,b,c,d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[x,y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 4.6. Standardized ileal digestibility (SID) of crude protein and amino acids (AA) in experimental diets[1,2]

Item, %	U.S.A.		European Union		Canada		Asia		Pooled	P-value		
SDP[3]	–	+	–	+	–	+	–	+	SEM	SDP	Region	SDP*Region
Crude protein	87.6[a]	85.0[ab]	82.6[b]	86.0[ab]	77.0[c]	85.1[ab]	85.9[ab]	87.8[a]	1.88	0.018	0.003	0.016
Indispensable AA												
Arg	93.9[a]	92.9[ab]	90.6[b]	92.4[ab]	82.9[c]	90.6[b]	92.6[ab]	93.4[ab]	1.43	0.003	<0.001	0.001
His	88.4[a]	87.4[ab]	84.6[b]	87.3[ab]	78.4[c]	87.6[ab]	88.2[a]	89.6[a]	1.77	<0.001	<0.001	<0.001
Ile	87.9[ab]	85.9[ab]	84.8[b]	86.4[ab]	77.6[c]	85.7[ab]	87.8[ab]	88.6[a]	1.91	0.019	<0.001	0.002
Leu	87.8[ab]	87.4[ab]	85.1[b]	87.3[ab]	77.1[c]	87.7[ab]	87.8[ab]	89.6[a]	1.90	<0.001	<0.001	<0.001
Lys	87.8[ab]	88.4[ab]	85.6[b]	88.8[a]	81.4[c]	87.6[ab]	87.0[ab]	89.2[a]	1.40	<0.001	0.002	0.050
Met	92.8[a]	92.1[ab]	92.3[ab]	90.3[b]	83.8[c]	91.3[ab]	91.6[ab]	92.0[ab]	1.29	0.040	<0.001	<0.001
Phe	88.7[ab]	87.5[ab]	85.4[b]	87.4[ab]	77.0[c]	87.0[ab]	87.2[ab]	89.3[a]	1.88	<0.001	<0.001	<0.001
Thr	86.1[ab]	85.0[ab]	81.8[b]	85.4[ab]	73.1[c]	85.6[ab]	86.1[ab]	87.8[a]	2.08	<0.001	<0.001	<0.001
Trp	88.7[a]	87.6[a]	85.9[a]	87.1[a]	77.7[b]	86.1[a]	88.7[a]	89.9[a]	1.96	0.031	<0.001	0.024
Val	85.7[ab]	84.6[ab]	81.8[b]	84.3[ab]	72.7[c]	84.9[ab]	86.4[ab]	87.6[a]	2.45	0.002	<0.001	<0.001
Mean	88.6[ab]	87.8[ab]	85.6[b]	87.7[ab]	78.2[c]	87.3[ab]	88.2[ab]	89.6[a]	1.77	0.001	<0.001	<0.001
Dispensable AA												
Ala	85.2[abc]	83.7[bc]	80.6[c]	83.2[bc]	70.1[d]	83.6[bc]	85.4[ab]	88.4[a]	2.47	<0.001	<0.001	<0.001

Table 4.6 (cont.)

Asp	85.0^{ab}	82.0^{bc}	79.6^{c}	83.2^{abc}	74.3^{d}	83.0^{abc}	84.5^{ab}	86.5^{a}	1.84	0.004	<0.001	<0.001
Cys	80.3^{ab}	74.2^{bc}	75.7^{abc}	81.1^{a}	70.6^{c}	80.6^{a}	80.0^{ab}	82.1^{a}	3.05	0.082	0.129	0.008
Glu	89.0^{a}	84.1^{bc}	86.7^{abc}	88.8^{a}	83.3^{c}	87.0^{ab}	87.6^{ab}	89.5^{a}	1.86	0.448	0.058	0.007
Gly	85.6^{a}	80.1^{ab}	76.6^{bc}	84.8^{a}	72.7^{c}	82.4^{ab}	85.8^{a}	86.0^{a}	2.99	0.070	0.011	0.008
Pro	102.0^{ab}	97.4^{cd}	100.4^{abc}	100.1^{bc}	95.3^{d}	100.3^{bc}	104.0^{a}	102.0^{ab}	1.75	0.614	0.002	0.004
Ser	88.0^{a}	85.7^{ab}	82.5^{b}	86.2^{ab}	74.6^{c}	85.4^{ab}	86.1^{ab}	88.3^{a}	1.86	<0.001	<0.001	<0.001
Tyr	88.0^{ab}	87.8^{ab}	84.1^{b}	87.7^{ab}	75.0^{c}	87.0^{ab}	86.2^{ab}	89.5^{a}	2.22	<0.001	<0.001	0.003
Mean	88.7^{ab}	84.8^{c}	85.0^{bc}	87.7^{abc}	79.7^{d}	86.8^{abc}	87.7^{abc}	89.3^{a}	1.96	0.051	0.004	0.002
Total AA	88.6^{ab}	86.2^{ab}	85.3^{b}	87.7^{ab}	79.0^{c}	87.1^{ab}	87.9^{ab}	89.5^{a}	1.84	0.009	<0.001	0.001

[1]Data are least squares means of 9 observations for U.S.A. + SDP treatment; 8 observations for the European Union + SDP, Canada + SDP, and Asia + SDP treatments; and 7 observations for U.S.A., the European Union, Canada, and Asia treatments.

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of dry matter intake) AA were as follows: crude protein, 13.80 Arg, 0.48; His, 0.21; Ile, 0.36; Leu, 0.58; Lys, 0.46; Met, 0.11; Phe, 0.35; Thr, 0.60; Trp, 0.13; Val, 0.58; Ala, 0.54; Asp, 0.83; Cys, 0.24; Glu, 0.97; Gly, 1.15; Pro, 2.31; Ser, 0.51; Tyr, 0.30.

[3]SDP, spray dried plasma.

[a,b,c,d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4.7. Differences[1] between measured and predicted apparent ileal digestibility (AID) values for starch, crude protein, and amino acids (AA) and for standardized ileal digestibility (SID) of crude protein and AA in mixed diets containing spray dried plasma (SDP) from the U.S.A., European Union, Canada, and Asia[2,3]

Item, %	U.S.A.		European Union		Canada		Asia	
	AID	SID	AID	SID	AID	SID	AID	SID
Starch	1.2*	–	1.4*	–	-0.2	–	0.3	–
Crude protein	-2.5	-3.6*	2.8	1.3	6.1*	4.7*	1.6	0.4
Indispensable AA								
Arg	-0.7	-1.4*	2.1*	0.7	5.7*	4.7*	0.9	0.0
His	-1.3	-2.0	1.9	0.6	6.4*	5.4*	0.7	-0.1
Ile	-1.9	-2.7	1.8	0.5	6.7*	5.8*	0.6	-0.1
Leu	-1.1	-1.7	1.3	0.2	7.2*	6.4*	0.7	0.1
Lys	-0.2	-0.9	1.9*	1.2	3.9*	3.5*	0.9	0.3
Met	0.1	-0.8	-1.8	-2.0	7.3*	6.2*	0.3	0.0
Phe	-1.6	-2.3	1.3	0.2	6.9*	6.0*	1.1	0.4
Thr	-1.2	-2.6*	2.6*	0.8	8.6*	6.9*	1.1	-0.1

Table 4.7 (cont.)

Trp	-1.5	-2.6*	-0.1	-1.0	4.0*	3.6	0.3	-0.5
Val	-1.4	-2.6	1.7	0.0	8.3*	7.0*	0.5	-0.4
Mean	-1.0	-1.9	1.5	0.4	6.5*	5.6*	0.8	0.0
Dispensable AA								
Ala	-2.2	-3.3	1.5	-0.2	9.2*	7.7*	2.0*	1.0
Asp	-3.5*	-4.2*	2.4*	1.1	5.9*	4.8*	1.3	0.4
Cys	-7.5*	-8.9*	3.0	1.3	5.5*	4.3	-0.1	-1.1
Glu	-5.0*	-5.4*	2.2*	1.4	3.0	2.4	1.5	0.8
Gly	-5.0*	-7.5*	8.8*	4.3*	8.4*	5.4*	0.9	-1.8
Pro	-1.3	-4.9*	4.7*	-0.7	9.0*	3.7*	3.6*	-2.1
Ser	-1.9	-3.0*	3.0*	1.3	7.3*	5.7*	1.9	0.6
Tyr	-0.6	-1.7	2.3	0.9	7.4*	6.2*	2.0*	0.9
Mean	-3.6*	-4.8*	3.1*	1.1	5.9*	4.3*	1.7	0.2
Total AA	-2.3	-3.4*	2.4*	0.7	6.2*	4.9*	1.2	0.1

[1]Difference is calculated by subtracting predicted AID of starch, crude protein, or individual AA from measured value. Likewise, for the difference between predicted value for SID of crude protein or individual AA from measured value.

Table 4.7 (cont.)

[2]Data are least squares means of 7 to 9 observations per treatment.

[3]* = $P < 0.05$. Ileal digestibility was underestimated when the difference between measured and predicted ileal digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

Table 4.8. Effect of period on standardized ileal digestibility of starch, crude protein, and amino acids (AA) in diets fed to weaned pigs[1]

Item, %	Period[2]			Pooled	*P*-value		
	1	2	3	SEM	Period	Linear	Quadratic
Crude protein	84.3	84.4	88.0	1.60	0.010	0.008	0.150
Indispensable AA							
Arg	90.0	91.6	93.6	1.35	0.002	<0.001	0.780
His	84.9	87.2	89.5	1.46	<0.001	<0.001	0.957
Ile	84.0	86.3	88.9	1.48	<0.001	<0.001	0.858
Leu	84.6	87.1	89.5	1.59	<0.001	<0.001	0.992
Lys	86.0	87.6	89.5	1.19	0.002	<0.001	0.858
Met	89.5	91.0	92.8	1.09	0.003	<0.001	0.800
Phe	84.7	87.1	89.4	1.60	<0.001	<0.001	0.966
Thr	82.5	84.5	87.2	1.85	0.005	0.001	0.756
Trp	85.4	87.0	89.5	1.62	0.012	0.003	0.682
Val	81.3	84.2	87.7	1.86	<0.001	<0.001	0.815
Mean	85.2	87.3	89.7	1.47	<0.001	<0.001	0.873
Dispensable AA							
Ala	80.6	83.8	86.9	2.25	<0.001	<0.001	0.981
Asp	81.0	83.5	85.3	1.69	0.004	<0.001	0.701
Cys	75.4	80.5	81.9	2.06	0.006	0.003	0.269
Glu	85.2	88.0	89.6	1.17	0.004	0.001	0.571
Gly	80.4	83.0	86.9	2.46	0.005	0.001	0.666

Table 4.8 (cont.)

Pro	97.9	100.8	103.1	1.32	0.009	0.002	0.831
Ser	83.2	85.3	87.4	1.68	0.005	0.001	0.995
Tyr	83.8	86.5	89.3	1.82	0.001	<0.001	0.991
Mean	84.5	87.3	89.4	1.43	0.001	<0.001	0.740
Total AA	84.8	87.3	89.5	1.42	<0.001	<0.001	0.900

[1]Data are least squares means of 22 observations for period 1 and 3, and 25 observations for period 2.

[2]Pigs had an average body weight of 10.54 ± 2.08, 13.95 ± 3.24, and 18.28 ± 4.06 in period 1, 2, and 3, respectively.

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CHAPTER 5: Inclusion of spray dried plasma to diets based on different ingredient combinations increases the digestibility of energy, fiber, Ca, and P by young pigs

Abstract

An experiment was conducted to test the hypothesis that the inclusion of spray dried plasma (SDP) to diets increases the apparent total tract digestibility (ATTD) of gross energy (GE), N, insoluble-, soluble-, and total-dietary fiber (IDF, SDF, TDF), Ca, and P and the standardized total tract digestibility (STTD) of P of other ingredients in diets for young pigs. Eighty barrows (body weight: 9.30 ± 0.97 kg) housed in individual metabolism crates were allotted to 1 of 10 diets in a randomized complete block design with 8 replicate pigs per diet. Four diets were prepared without SDP and contained ingredients commonly used in the U.S.A., Canada, the European Union, and Asia. Four additional diets were prepared by mixing 94% of the previous 4 diets and 6% SDP. A diet containing SDP as the sole source of P and a P-free diet were also formulated to measure basal endogenous losses of P. Differences between measured and predicted values for ATTD of GE, N, IDF, SDF, TDF, Ca, and P and the STTD of P in the diets with SDP were also calculated and the *t* test was used to analyze the additivity of ATTD and STTD values. An interaction was observed between SDP and region for the ATTD of SDF where the digestibility decreased ($P < 0.05$) for pigs fed the U.S.A. diet with 6% SDP compared with 0% SDP, but did not differ from the other regional diets without or with SDP. There was no interaction for the ATTD of GE, N, IDF, TDF, Ca, and P or the STTD of P, but the ATTD and STTD values, except for TDF, were greater ($P < 0.05$) when 6% SDP was included in the diet

compared with diets with 0% SDP regardless of region. The ATTD of TDF tended to increase ($P < 0.10$) for pigs fed a diet with 6% SDP compared with pigs fed a diet with 0% SDP. Additionally, the ATTD of GE, IDF, TDF, and P and the STTD of P was greater ($P < 0.05$) for the Asia diet compared with the other diets regardless of inclusion of SDP. The measured ATTD of IDF and TDF was greater ($P < 0.05$) than the predicted for the U.S.A. and European Union diets, and the measured ATTD of GE, N, Ca, and P and the STTD of P was greater ($P < 0.05$) than the predicted for the Asia diet compared with the other diets. In conclusion, the addition of 6% SDP to a diet will increase the ATTD of energy and nutrients and the STTD of P regardless of the diet formulation, and therefore, the measured ATTD of energy and nutrients or the STTD of P by pigs fed diets containing SDP may be greater than the predicted.

Key words: additivity, apparent total tract digestibility, endogenous phosphorus losses, spray dried plasma

Abbreviations

ATTD	apparent total tract digestibility
DE	digestible energy
EPL	endogenous phosphorus loss
GE	gross energy
IDF	insoluble dietary fiber
ME	metabolizable energy
SDF	soluble dietary fiber
STTD	standardized total tract digestibility
SDP	spray dried plasma

Abbreviations (cont.)

TDF	total dietary fiber

Introduction

Spray dried plasma (**SDP**) has been used in diets for newly weaned pigs to promote feed intake and growth due to the high palatability and high amino acid digestibility of SDP (Ermer et al., 1994; Gottlob et al., 2006; Mateo and Stein, 2007). The concentration and digestibility of energy in SDP is also high (91% digestibility of gross energy) and similar to yeast products and other animal protein sources (Gottlob et al., 2006; Wu et al., 2018). In addition, SDP is a source of completely digestible and bioavailable P when fed to both pigs and poultry and SDP can be included in diets to reduce P excretion in the manure (Almeida and Stein, 2011; Munoz et al., 2020). Calcium is often measured in conjunction with P and when SDP was included in a corn-soybean meal diet fed to pigs, the apparent total tract digestibility (**ATTD**) of Ca in the diet increased (Zhang et al., 2015), indicating that SDP may increase the total tract digestibility of Ca originating from other ingredients in the diet. Additionally, when SDP was included in kibble fed to dogs, the digestibility of crude fiber and total dietary fiber (**TDF**) was increased compared with a diet without SDP (Quigley et al., 2004), but SDP is not a source of fiber, so this indicates that SDP positively influenced the digestibility of TDF in the other dietary ingredients. Spray dried plasma is a source of highly digestible crude protein, and therefore, an increase in N digestibility by pigs or dogs fed diets with SDP can be explained (Bosi et al., 2001; Quigley et al., 2004). However, the improvement of the digestibility of other nutrients in a diet containing SDP is hypothesized to be due to the biologically active peptide immunoglobulin G that acts in the intestinal tract to prevent pathogens from colonizing on the mucosal membrane, which leads

to an improvement in the immunocompetence and enteric health of the animal (Pierce et al., 2005; Torrallardona, 2010). However, data are limited on the effect of SDP on total tract digestibility of energy, P, or fiber originating from other ingredients in the diet. Therefore, the objectives of this research were 1) to determine the ATTD of energy, N, fiber, Ca, and P, and the standardized total tract digestibility (**STTD**) of P in SDP; and 2) to test the hypothesis that inclusion of SDP in diets increases the ATTD of energy, N, fiber, Ca, and P and the STTD of P originating from other ingredients in the diet.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Barrows that were offspring of PIC Camborough females and PIC Line 359 males (Pig Improvement Company, Hendersonville, TN, USA) were used in the experiment.

Diets, animals, and experimental design

Spray dried plasma (Appetein B) was sourced from APC Inc., Ankeny, IA, USA, and the same batch was used in all diets containing SDP. A common phase 1 diet containing 6% SDP and 10 phase 2 diets were formulated (Tables 5.1, 5.2, and 5.3). Four phase 2 diets were formulated with ingredients used in commercial swine diets in the U.S.A., European Union, Canada, and Asia, respectively. These diets did not contain SDP, however, 4 additional diets were formulated by mixing 94% of the previous 4 diets and 6% SDP. In addition, a diet containing SDP as the sole source of P and a P-free diet were formulated to measure basal endogenous losses of P. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement

estimates of nursery pigs (NRC, 2012). A sample of the main ingredients and of all diets were collected at the time of diet mixing and used for chemical analysis.

Eighty pigs were weaned at 20 ± 2 d when they had a body weight of 6.53 ± 0.59 kg and were allotted to a randomized complete block design with 2 blocks of 40 pigs, and the blocking factor was weaning date. Within each block, the 40 pigs were housed in groups of 5 and fed the phase 1 diet for 2 wk immediately post-weaning. After 2 wk, when pigs had a body weight of 9.30 ± 0.97 kg, they were moved to individual metabolism crates that were equipped with a feeder, a nipple drinker, fully slatted floor, a screen floor, and a urine tray, which allowed for the total, but separate, collection of feces and urine. Pigs were randomly allotted to the 10 phase 2 diets, resulting in 4 replicate pigs per diet per block for a total of 8 replicate pigs per diet.

Feeding and sample collection

All pigs were provided feed at a daily level of 3.3 times the maintenance energy requirement (i.e., 197 kcal/kg $BW^{0.60}$; NRC, 2012). The daily feed allowance was provided in 2 equal meals at 0700 and 1600 h and feed consumption was recorded daily. Water was available at all times throughout the experiment. All pigs were fed experimental diets for 11 d, with the initial 5 d of the experiment being the adaptation period to the diet followed by 4 d of total collection of feces and urine according to the marker-to-marker procedure (Adeola, 2001). Fecal collection began when the first marker (i.e., indigo carmine), fed in the morning meal on d 6, appeared in the feces, and ceased when the second marker (i.e., ferric oxide), fed in the morning meal on d 10, appeared in the feces. In addition, orts were collected and weighed daily to determine feed intake.

All pigs were weighed at the beginning of the experiment (at weaning), prior to moving into metabolism crates, and at the end of both the adaptation and collection period. During the

collection period, feces were collected twice daily and stored at -20°C as soon as collected, and urine was collected over a preservative of 50 mL of 6 *N* HCl in buckets placed under the metabolism crates. The urine buckets were emptied once daily, the weight of the collected urine was recorded, and 20% was stored at -20°C. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet and 2 subsamples were collected. One subsample was lyophilized and the other subsample was stored at -20°C until analysis for N.

Chemical analysis

All collected fecal samples were dried at 65°C in a forced air oven (Metalab Equipment Corp., Hicksville, NY, USA) and finely ground using a 500G stainless steel mill grinder (RRH, Zhejiang, China) prior to chemical analysis. The lyophilized urine samples, fecal samples, and all diet and ingredient samples were analyzed in duplicate for concentrations of gross energy (**GE**) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Diet and fecal samples and the urine samples that were not lyophilized were analyzed for N using the Kjeldahl method (method 984.13; AOAC Int., 2019) on a Kjeltec™ 8400 (FOSS Inc., Eden Prairie, MN, USA) with subsequent calculation of crude protein by a conversion factor of 6.25. Fecal, diet, and ingredient samples were also analyzed in duplicate for dry matter by oven drying at 135°C for 2 h (method 930.15, AOAC Int., 2019), dry ash at 600°C for 3 h (method 942.05; AOAC Int., 2019), and insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**; method 991.43; AOAC Int., 2019) using the Ankom TDF Analyzer (Ankom Technology, Macedon, NY, USA). Fecal, diet, and ingredient samples were also analyzed for Ca and P using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing (method 985.01 A, B and C; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental

Protection Agency, 2000). All diet and ingredient samples were analyzed for amino acids on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int., 2019] and for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019) at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA).

Calculations

Using the direct procedure, the ATTD of P in all diets was calculated using the following equation **[5.1]** (Almeida and Stein, 2010; NRC, 2012):

$$\text{ATTD of P (\%)} = [(P_i - P_f) / P_i] \times 100 \quad \textbf{[5.1]}$$

where ATTD of P is the apparent total tract digestibility of P (%), P_i is the P intake (g) from d 6 to 10; and P_f is the P output (g) in the feces originating from the feed that was fed from d 6 to 10. The same equation was used to calculate the ATTD of fiber, N, Ca, and GE in the diets. The digestible energy (**DE**) and metabolizable energy (**ME**) in all diets was calculated by subtracting the GE in feces and the GE in feces and urine, respectively, from GE in the diet (NRC, 2012).

The basal endogenous P losses (**EPL**; mg/kg of DMI) were measured from pigs fed the P-free and also from pigs fed the SDP diet using the following equation **[5.2]** (NRC, 2012):

$$\text{EPL (mg/kg DMI)} = [(P_f / F_i) \times 1{,}000 \times 1{,}000] \quad \textbf{[5.2]}$$

where EPL is the endogenous P loss and F_i is the total feed (g) intake from d 6 to 10.

The STTD of P was calculated using the following equation **[5.3]** (NRC, 2012):

$$\text{STTD of P (\%)} = \{[P_i - (P_f - EPL)] / P_i\} \times 100 \quad \textbf{[5.3]}$$

where STTD (%) is the standardized total tract digestibility of P.

The predicted ATTD of P in the regional diets containing SDP was calculated according to the following equation **[5.4]** (Stein et al., 2005; Xue et al., 2014):

$$ATTD_P = [(P_{SDP} \times ATTD_{SDP}) + (P_D \times ATTD_D)] / (P_{SDP} + P_D), \quad \textbf{[5.4]}$$

where $ATTD_P$ is the predicted ATTD for P (%) in the regional diet with SDP; P_{SDP} and P_D are the concentrations (%) of P contributed by SDP and regional diets without SDP, respectively, which were calculated by multiplying the concentration of P (%) in that ingredient by the proportion (%) of that ingredient in the regional diet with SDP; and $ATTD_{SDP}$ and $ATTD_D$ is the measured ATTD (%) for P in SDP and the regional diets without SDP, respectively. The predicted STTD of P in regional diets containing SDP was also calculated using this equation.

The retention of N for each pig was calculated using the following equation **[5.5]** (Pedersen et al., 2007):

$$Nr = \{[N_i – (N_f + N_u)] / N_i\} \times 100 \quad \textbf{[5.5]}$$

where Nr is the retention of N (%), N_i is the N intake (g) from d 6 to 10, N_f and N_u are N output (g) in feces and urine originating from the feed that was fed from d 6 to 10, respectively.

The biological value of the protein in the diet was also calculated by expressing Nr as a percentage of the difference between N intake and N output in feces (Rojas and Stein, 2013).

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if the value deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). Data were analyzed as a 2 × 4 factorial arrangement of treatments using PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) with 2 levels of SDP and 4 regions. The pig was the experimental unit for all analyses. The model included dietary concentration of

SDP, region, and the interaction between SDP and region as fixed effects and block and replicate within block as random effects. Data from pigs fed the P-free and SDP diet were analyzed using PROC MIXED procedure of SAS where the statistical model included diet as the fixed effect and block and replicate within block as random effects. Treatment means were calculated using the LSMEANS statement in SAS, and if significant, means were separated using the PDIFF option in PROC MIXED procedure. An alpha value of 0.05 was used to assess significance among means.

Additivity of ATTD and STTD values was analyzed using the *t* test within each of the 4 regional diets with added SDP and for the sum of all diets with added SDP; the null hypothesis that the difference between measured and predicted values for ATTD and STTD was equal to 0 was tested (She et al., 2018). Statistical differences were established at $P < 0.05$, and $0.05 \leq P < 0.10$ was considered a trend.

Results

The ATTD of GE in SDP was 94.3% and 94.1% for the ATTD of N in SDP (Table 5.4). For Ca and P, the ATTD values in SDP were 77.2 and 90.6%, respectively, and the STTD of P in SDP was 96.5%.

The ATTD of dry matter was greater ($P < 0.05$) for pigs fed the P-free diet compared with the SDP diet (Table 5.5). All other values, including feed intake, P intake, fecal excretion, P in feces, fecal P excretion, and basal EPL, were greater ($P < 0.05$) for pigs fed the diet with SDP as the sole source of P compared with the P-free diet.

Pigs fed the European Union diet with 6% SDP had greater ($P < 0.05$) feed intake than pigs fed the European Union diet without SDP (Table 5.6), but feed intake of pigs fed the other

regional diets was not increased if 6% SDP was included (interaction, $P < 0.05$). Intake of GE was greater ($P < 0.05$) for pigs fed the European Union diet with 6% SDP compared with pigs fed the same diet without SDP, but there was no difference in GE intake among pigs fed the other diets without or with 6% SDP (interaction, $P < 0.05$). The intake of Ca, TDF, and IDF was increased ($P < 0.05$) for pigs fed the European Union diet with 6% SDP compared with pigs fed the European Union diet without SDP, but intake of Ca, TDF, and IDF was less ($P < 0.05$) for pigs fed the Canada or Asia diet with 6% SDP than for pigs fed those diets with 0% SDP, and the intake of those nutrients did not change if SDP was included in the U.S.A. diet (interaction, $P < 0.05$). In contrast, the intake of P increased ($P < 0.05$) for pigs fed the U.S.A., European Union, or Asia diet with 6% SDP compared with pigs fed those diets without SDP, but that was not the case for pigs fed the Canada diet (interaction, $P < 0.05$). The intake of SDF decreased ($P < 0.05$) for pigs fed the U.S.A. or Canada diets with 6% SDP compared with pigs fed those diets with 0% SDP, and increased ($P < 0.05$) for pigs fed the European Union diet with 6% SDP compared with pigs fed the European Union diet without SDP, but did not differ for pigs fed the Asia diet with 0 or 6% SDP (interaction, $P < 0.05$).

Pigs fed the Canada diet with 0% SDP had a tendency for greater ($P < 0.10$) fecal output than pigs fed the Canada diet with 6% SDP, but the fecal output of pigs fed the other regional diets did not differ if 0 or 6% SDP was included in the diet (interaction, $P < 0.05$). There was no interaction between inclusion of SDP and region for the output of urine, GE, Ca, P, IDF, SDF, and TDF. The DE in diets with 6% SDP was increased ($P < 0.05$) compared with diets without SDP, but the increase in DE of the diet was greater when 6% SDP was included in the Asia diet compared with the other diets (interaction, $P < 0.05$), and DE was greater ($P < 0.05$) for the Canada diet than the U.S.A. and European Union diet when 6% SDP was included. The ME in

the European Union, Canada, and Asia diet increased ($P < 0.05$) when 6% SDP was included compared with diets without SDP, but ME in the U.S.A. diet without SDP was not different from the ME in the diet with 6% SDP (interaction, $P < 0.05$).

There was no interaction between inclusion of SDP and region for the ATTD of GE, Ca, P, TDF, or IDF and the STTD of P (Table 5.7); however, an interaction between SDP and region was observed for the ATTD of SDF where digestibility decreased ($P < 0.05$) for pigs fed the U.S.A. diet with 6% SDP compared with 0% SDP, but the ATTD of SDF did not differ for the other regional diets without or with SDP. The ATTD of DM, GE, Ca, P, and IDF and the STTD of P was greater ($P < 0.05$) when 6% SDP was included in the diet compared with 0% SDP regardless of region, and the ATTD of TDF tended to increase ($P < 0.10$) for pigs fed a diet with 6% SDP compared with a diet with 0% SDP. Additionally, the ATTD of DM, GE, P, TDF, and IDF and the STTD of P was greater ($P < 0.05$) for the Asia diet compared with the other diets regardless of inclusion of SDP.

Intake of N was greater in all regional diets if 6% SDP was included (Table 5.8) compared with 0% SDP in the diet, but the increase was greater for the U.S.A. and European Union diets than for the Canada and Asia diets (interaction, $P < 0.05$). Pigs fed diets with 6% SDP had greater N retention during the 4-d collection period than pigs fed diets without SDP, but the increase tended to be greater for pigs fed the European Union or Asia diets than for pigs fed the other diets (interaction, $P < 0.10$). Pigs fed a diet with 6% SDP had greater ($P < 0.05$) ATTD of N compared with pigs fed a diet without SDP regardless of region, and pigs fed the Asia diet had greater ($P < 0.05$) ATTD of N than pigs fed the Canada or European Union diet regardless of SDP inclusion. There was a tendency for the biological value of N to increase ($P < 0.10$) for pigs fed the U.S.A. diet compared with the Asia diet regardless of SDP inclusion.

The measured ATTD of GE was greater ($P < 0.05$) than the predicted value in the Canada and Asia diet with SDP (Table 5.9), and the measured value for ATTD of N, Ca, and P and the STTD of P was greater ($P < 0.05$) than the predicted value in the Asia diet with SDP. The measured ATTD of TDF was greater ($P < 0.05$) than predicted in the U.S.A. and European Union diet with SDP, and the measured ATTD of IDF was greater ($P < 0.05$) than the predicted value in the U.S.A., European Union, and Canada diet with SDP. The measured ATTD of SDF was greater ($P < 0.05$) than predicted in the European Union and Asia diet with SDP, but the measured value was less ($P < 0.05$) than predicted in the U.S.A. and Canada diet with SDP. When calculating the overall effect of SDP in diets fed to pigs, it was observed that the measured ATTD of GE, N, Ca, P, TDF, and IDF, but not the ATTD of SDF or STTD of P, was greater ($P < 0.05$) than predicted values when SDP was included in the diet.

Discussion

When comparing the digestibility results from the current experiment with published data on the digestibility of energy and nutrients in SDP, the ATTD of GE is in agreement with values observed by Wu et al. (2018) and the ATTD and STTD of P in SDP is in agreement with values by Almeida et al. (2011). The ATTD of N in SDP has not been reported, but the apparent ileal digestibility of N in SDP was approximately 82% (Jeong et al., 2016), which is less than the 94.1% ATTD of N observed in the current experiment indicating absorptions of N from the hindgut (Stein, 2017). To our knowledge, the ATTD of Ca, TDF, IDF, or SDF in SDP has not been reported because the concentration of these nutrients in SDP is negligible. In comparison with blood meal, the ATTD and STTD of P in SDP is greater (Almeida et al., 2011), but the ATTD of Ca and TDF in other blood products has not been reported.

The digestibility of GE in rice is greater compared with corn, wheat, or barley (Cervantes-Pahm et al., 2014), which likely is the reason for the greater ATTD of GE and greater concentrations of DE and ME in the Asia diets compared with the other diets. The negative values that were observed for the ATTD of SDF in both the Asia and U.S.A. diets are a result of microbial material in the feces that was analyzed as TDF, and negative values for the ATTD of SDF have been previously reported (Jørgensen et al., 1996; Wilfart et al., 2007; Cervantes-Pahm et al., 2013). The amount of TDF analyzed in the Canada and European Union diets was greater than in the U.S.A. and Asia diets because of the inclusion of wheat and barley in these diets. Wheat and barley have greater concentrations of soluble fibers, specifically β-glucans and arabinoxylans, compared with corn and rice, which increases viscosity of digesta in the small intestine and leads to a reduction in nutrient digestion and absorption due to decreased interactions between nutrients and digestive enzymes (Cervantes-Pahm et al., 2014). Therefore, the increased ATTD of P, TDF, and IDF in the Asia diet compared with the other diets may be due to the reduced concentration of SDF in this diet.

The observation that if 6% SDP was included in the diet, the ATTD of energy and nutrients and STTD of P increased, regardless of the diet formulation, is in agreement with data from Zhang et al. (2015). The reason for the increase in ATTD and STTD of P in diets with SDP is due to the high STTD of P in SDP (Almeida et al., 2011; Munoz et al., 2020).

To determine the STTD of P, values for the ATTD of P are corrected for basal EPL, which can be determined by feeding a P-free diet (Petersen and Stein, 2006). Values for the basal EPL that were determined by feeding a P-free diet in this experiment (193 mg/kg DMI) are in agreement with the average for EPL from a large number of experiments of 190 mg/kg DMI (NRC, 2012). Basal EPL were also measured in this experiment by feeding a diet with 20% SDP

as the only source of P, which is a common method used in Brazil because P in SDP is believed to be 100% digestible, and therefore, all P excreted in feces from pigs fed a diet with SDP is believed to be of endogenous origin (Bünzen et al., 2012; Alves et al., 2016). However, basal EPL measured from the SDP diet (370 mg/kg DMI) was greater than basal EPL measured from the P-free diet, which indicates that the absorption of P from SDP is not 100%. Excretion of P in feces and ATTD of P in diets in which all P was from SDP was greater in a diet with 30% SDP compared with a diet with 10% SDP (Alves et al., 2016), further indicating that the digestibility of P in SDP is high, but not 100%. As a consequence, estimating EPL from a diet containing SDP results in an overestimation of basal EPL. To correct this overestimation, the indigestible fraction needs to be considered in the calculation of basal EPL (Alves et al., 2016), or the inclusion of SDP in the diet should be reduced to limit undigested dietary P in the feces.

The observed increase in ATTD of N in diets containing SDP compared with diets without SDP is in agreement with data from mice and pigs (Thomson et al., 1995; Pan et al., 2019). An increase in ATTD of dry matter and N was also observed if SDP was included in a diet fed to pigs compared with a diet containing wheat gluten (Pendergraft et al., 1993). The improvement in N digestibility and retention by pigs fed a SDP-containing diet is due to the high bioavailability and digestibility of amino acids in SDP (Mateo and Stein, 2007). The improvement in dietary protein utilization by pigs fed a diet with SDP may also be due to reduction in intestinal amino acid catabolism as a result of an improvement in immunocompetence (Zhang et al., 2015; Pan et al., 2019). Spray dried plasma has a high concentration of immunoglobulin G that acts as a functional protein in preventing the colonization of pathogenic bacteria on the surface of the intestinal mucosa and subsequently prevents overstimulation of the immune system (Torrallardona, 2010; Zhang et al., 2016). The

reduced activation of the immune system in the intestinal lymphoid tissue of pigs fed a diet with SDP may improve dietary energy absorption (Nofrarías et al., 2006), and therefore, may explain the increased ATTD of GE and increased concentrations of DE and ME in diets containing 6% SDP compared with diets without SDP. The ATTD of GE in extruded kibble that included 1 to 3% SDP was observed to linearly increase (Quigley et al., 2004), further demonstrating the positive impact of SDP on digestibility of GE.

The increased ATTD of IDF and TDF in diets with SDP compared with diets without SDP is in agreement with results from dogs fed kibble with 1 to 3% SDP (Quigley et al., 2004). However, there is no fiber in SDP, indicating that the improvements in digestion of fiber in SDP containing diets are independent of the fiber in SDP (Quigley et al., 2004). However, microbial populations in the large intestine, specifically species within the *Lactobacillus* genera that are often developed as probiotic supplements, may be increased in mice and pigs fed a diet with SDP (Torrallardona et al., 2003; Moretó et al., 2020). The density of lamina propria cells in the colon was also decreased in pigs fed a diet with SDP and a decrease in leukocytic infiltration in the intestinal mucosa was observed as well (Nofrarías et al., 2006; Nofrarías et al., 2007). These observations indicate a reduced activation of the immune system and improved maintenance of the intestinal mucosa layer in pigs fed a diet containing SDP compared with pigs fed no SDP, which may have resulted in improved nutrient absorption (Campbell et al., 2019).

The mostly positive differences between measured and predicted ATTD and STTD of energy and nutrients in diets containing SDP indicate that the nutrient and energy digestibility measured in pigs fed a diet with 6% SDP was greater than what was calculated from the individual components. A difference between measured and predicted values for ATTD or STTD indicates that the quantities of digestible nutrients provided by a mixed diet is greater than the

sum of nutrients from the individual ingredients (She et al., 2018). It therefore appears that SDP may confer synergistic effects on nutrient digestibility, which results in actual digestibility of some nutrient being greater than predicted. The reason for this observation may be that SDP in the diet reduced intestinal inflammation and maintained the integrity of the intestinal mucosa (Zhang et al., 2015; Campbell et al., 2019). The observation that diets with greater concentrations of fiber had a greater increase in ATTD of TDF than calculated for the rice based diet may have been a result of SDP increasing microbial concentrations in the large intestine, thereby increasing the ATTD of fiber (Torrallardona et al., 2003). However, the rice based diet had less fiber than the corn-soybean meal or wheat-barley based diets, which may not have provided enough substrate to generate the prebiotic effect. However, the fact that inclusion of 6% SDP to the rice based diet resulted in ATTD of GE, N, Ca, and P and STTD of P in the diet being greater than the predicted values indicates that the activity of brush-border enzymes may have been improved by SDP because of the improved integrity of the intestinal mucosa stimulated by SDP in the diet (Zhang et al., 2015). This effect may be greater in diets with low concentrations of fiber and reduced digesta viscosity than in diets with more fiber (Cervantes-Pahm et al., 2014). However, data for the interaction of fiber and SDP and their effect on intestinal health and nutrient digestibility are limited and further research is warranted.

In conclusion, addition of 6% SDP to a diet increases the ATTD of energy and nutrients and the STTD of P regardless of diet formulation. However, inclusion of 6% SDP to diets with low fiber ingredients has a greater synergistic effect on energy and nutrient digestibility of those ingredients resulting in a greater measured digestibility of the complete diet compared with predicted values. Therefore, the ATTD of energy and nutrients and the STTD of P for individual ingredients are not always additive in a diet containing SDP.

Tables

Table 5.1. Ingredient composition of experimental diets

Item, %	Phase 1	P-free	SDP	U.S.A.		European Union		Canada		Asia	
				-SDP	+SDP	-SDP	+SDP	-SDP	+SDP	-SDP	+SDP
SDP[1]	6.00	–	20.00	–	6.00	–	6.00	–	6.00	–	6.00
Corn, ground	43.16	–	–	55.58	52.25	25.51	23.98	–	–	11.38	10.70
Wheat, ground	–	–	–	–	–	20.00	18.80	30.00	28.20	–	–
Barley, ground	–	–	–	–	–	15.00	14.10	30.00	28.20	–	–
Rice, ground	–	–	–	–	–	–	–	–	–	45.00	42.30
Soybean meal	25.00	–	–	15.00	14.10	9.00	8.46	9.47	8.90	10.00	9.40
Fermented soybean meal	–	–	–	–	–	–	–	7.00	6.58	7.00	6.58
Fish meal	–	–	–	–	–	–	–	–	–	5.00	4.70
Whey powder, dried	20.00	–	–	15.00	14.10	15.00	14.10	15.00	14.10	15.00	14.10
Soy protein concentrate	–	–	–	7.00	6.58	8.00	7.52	–	–	–	–
Gelatin	–	20.00	–	–	–	–	–	–	–	–	–
Corn starch	–	26.70	29.01	–	–	–	–	–	–	–	–

Table 5.1 (cont.)

Solka floc	–	4.00	4.00	–	–	–	–	–	–	–	–
Lactose	–	20.00	20.00	–	–	–	–	–	–	–	–
Sucrose	–	20.00	20.00	–	–	–	–	–	–	–	–
Soybean oil	3.10	4.00	4.00	4.00	3.76	4.00	3.76	5.00	4.70	4.00	3.76
Limestone, ground	1.20	0.80	1.35	1.00	0.94	1.20	1.13	1.30	1.22	0.90	0.85
Dicalcium phosphate	0.80	–	–	1.20	1.13	1.00	0.94	0.80	0.75	0.50	0.47
Sodium chloride	0.10	0.40	0.40	0.45	0.42	0.45	0.42	0.45	0.42	0.45	0.42
L-Lys HCl	0.29	0.70	0.12	0.37	0.35	0.45	0.42	0.58	0.55	0.40	0.38
DL-Met	0.15	0.30	0.28	0.13	0.12	0.12	0.11	0.13	0.12	0.10	0.09
L-Thr	0.05	0.40	0.02	0.10	0.09	0.10	0.09	0.10	0.09	0.10	0.09
L-Trp	–	0.20	–	–	–	–	–	–	–	–	–
L-His	–	0.30	–	–	–	–	–	–	–	–	–
L-Ile	–	0.40	0.17	–	–	–	–	–	–	–	–
L-Leu	–	0.75	–	–	–	–	–	–	–	–	–
L-Val	–	0.40	–	–	–	–	–	–	–	–	–
Magnesium oxide	–	0.10	0.10	–	–	–	–	–	–	–	–

Table 5.1 (cont.)

Potassium carbonate	–	0.40	0.40	–	–	–	–	–	–	–	–
Vitamin-mineral mix[2]	0.15	0.15	0.15	0.17	0.16	0.17	0.16	0.17	0.16	0.17	0.16

[1]SDP, spray dried plasma.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 5.2. Analyzed nutrient composition of experimental diets (as-fed basis)[1]

Item	Phase 1	P-free	SDP	U.S.A.		European Union		Canada		Asia	
SDP[2], %	6	0	20	0	6	0	6	0	6	0	6
Dry matter, %	88.30	92.71	92.18	87.34	87.53	88.36	88.31	89.05	89.61	89.26	89.25
Crude protein, %	21.15	22.83	15.91	17.10	20.63	16.88	20.38	16.77	20.24	17.36	20.94
Ash, %	5.99	1.53	3.23	5.55	5.51	5.38	5.52	5.18	5.38	5.30	5.14
Gross energy, kcal/kg	3,985	4,109	4,097	3,956	4,015	3,919	4,036	3,983	4,121	4,001	4,074
Insoluble dietary fiber, %	8.30	6.30	3.80	9.00	9.10	10.00	10.00	10.60	10.00	5.30	4.60
Soluble dietary fiber, %	1.00	N/D[3]	0.10	0.30	0.10	0.70	1.00	2.20	1.00	0.10	0.10
Total dietary fiber, %	9.40	6.30	4.00	9.30	9.20	10.70	10.90	12.80	11.00	5.40	4.70
Starch, %	24.40	16.96	21.73	33.06	29.18	31.22	29.42	29.75	30.62	35.54	35.50
Ca, %	0.89	0.33	0.57	0.75	0.70	0.78	0.75	0.88	0.77	0.84	0.79
P, %	0.61	0.01	0.30	0.57	0.63	0.56	0.63	0.59	0.61	0.55	0.61
Indispensable amino acids, %											
Arg	1.22	1.47	0.87	1.06	1.17	0.98	1.17	0.89	1.27	1.13	1.30
His	0.55	0.31	0.47	0.44	0.54	0.41	0.52	0.37	0.54	0.43	0.53
Ile	0.90	0.49	0.61	0.79	0.86	0.74	0.83	0.69	0.90	0.81	0.88
Leu	1.85	1.33	1.48	1.54	1.84	1.37	1.69	1.20	1.73	1.45	1.76

Table 5.2 (cont.)

Lys	1.61	1.20	1.53	1.29	1.58	1.34	1.73	1.20	1.82	1.36	1.78
Met	0.44	0.42	0.39	0.41	0.41	0.38	0.37	0.37	0.41	0.44	0.44
Phe	1.03	0.41	0.84	0.86	1.00	0.82	0.99	0.76	1.09	0.86	1.02
Thr	1.03	3.32	1.04	0.80	1.00	0.74	0.95	0.75	1.04	0.80	1.03
Trp	0.34	0.27	0.33	0.24	0.30	0.23	0.32	0.22	0.29	0.25	0.32
Val	1.16	0.68	1.09	0.88	1.11	0.84	1.09	0.79	1.17	0.94	1.18
Total	10.13	9.90	8.65	8.31	9.81	7.85	9.66	7.24	10.26	8.47	10.24
Dispensable amino acids, %											
Ala	1.03	1.63	0.78	0.85	1.02	0.76	0.93	0.67	0.95	0.93	1.04
Asp	2.12	1.13	1.62	1.79	2.05	1.60	1.92	1.42	2.05	1.83	2.11
Cys	0.43	0.03	0.54	0.30	0.43	0.30	0.44	0.30	0.52	0.30	0.43
Glu	3.53	1.96	2.19	3.13	3.45	3.30	3.76	3.34	4.13	3.02	3.38
Gly	0.80	4.45	0.58	0.67	0.77	0.67	0.79	0.64	0.87	0.87	0.90
Pro	1.26	2.58	0.88	1.08	1.21	1.19	1.34	1.24	1.48	1.08	1.16
Ser	1.00	0.61	0.94	0.77	0.98	0.73	0.95	0.68	1.01	0.78	0.96
Tyr	0.79	0.27	0.77	0.65	0.75	0.59	0.75	0.54	0.80	0.70	0.90
Total	10.96	12.66	8.30	9.24	10.66	9.14	10.88	8.83	11.81	9.51	10.88

Table 5.2 (cont.)

Total amino acids, %	21.09	22.56	16.95	17.55	20.47	16.99	20.54	16.07	22.07	17.98	21.12

[1]The phase 1 SDP diet was formulated based on the requirements of 5 to 7 kg pigs, and all other diets, except the P-free and SDP diet, were formulated based on the requirements of 7 to 11 kg pigs (NRC, 2012).

[2]SDP, spray dried plasma.

[3]N/D, not detected.

Table 5.3. Analyzed nutrient composition of ingredients (as-fed basis)[1]

Item	SDP	Corn	Soybean meal	Fermented soybean meal	Wheat	Barley	Soy protein concentrate	Rice	Fish meal	Whey powder	Gelatin
Dry matter, %	91.71	84.81	88.22	89.72	87.53	87.77	88.63	87.69	91.93	90.92	89.94
Crude protein, %	79.62	6.94	45.15	51.63	11.38	10.56	59.46	7.40	62.78	12.38	100.87
Ash, %	7.42	1.06	6.59	7.40	1.55	1.71	6.52	0.50	19.80	8.25	0.08
Gross energy, kcal/kg	4,826	3,721	4,127	4,337	3,841	3,863	4,238	3,746	4,416	3,654	4,656
Insoluble dietary fiber, %	4.80	8.80	15.10	9.10	11.00	14.00	17.20	2.00	4.00	0.20	0.10
Soluble dietary fiber, %	N/D	0.30	1.30	5.10	0.30	4.50	0.70	N/D	0.50	N/D	N/D
Total dietary fiber, %	4.80	9.20	16.40	14.10	11.30	18.60	17.90	2.00	4.50	0.20	0.10
Starch, %	N/A	57.88	N/A	N/A	55.42	39.55	N/A	69.18	N/A	N/A	N/A
Ca, %	0.13	0.01	0.29	0.55	0.03	0.11	0.45	0.01	5.31	0.75	N/D
P, %	1.58	0.24	0.63	0.78	0.35	0.69	0.80	0.12	3.28	0.95	N/D
Indispensable amino acids, %											
Arg	4.52	0.35	3.25	3.35	0.57	0.57	4.39	0.58	3.56	0.28	7.82

Table 5.3 (cont.)

His	2.44	0.21	1.19	1.30	0.27	0.25	1.59	0.18	1.42	0.22	0.90
Ile	2.52	0.27	2.18	2.50	0.40	0.39	2.95	0.32	2.55	0.73	1.32
Leu	7.44	0.86	3.53	3.98	0.77	0.74	4.76	0.62	4.19	1.20	2.81
Lys	7.11	0.25	2.92	2.93	0.36	0.43	3.94	0.28	4.47	1.04	3.94
Met	0.94	0.16	0.62	0.73	0.19	0.18	0.86	0.19	1.67	0.21	0.93
Phe	4.18	0.36	2.36	2.62	0.51	0.56	3.16	0.40	2.31	0.37	1.94
Thr	5.09	0.27	1.76	2.01	0.33	0.37	2.43	0.26	2.38	0.77	1.66
Trp	1.52	0.06	0.65	0.72	0.12	0.11	0.82	0.09	0.60	0.22	0.02
Val	5.65	0.37	2.32	2.66	0.51	0.55	3.02	0.45	2.98	0.69	2.32
Total	41.41	3.16	20.78	22.80	4.03	4.15	27.92	3.37	26.13	5.73	23.64
Dispensable amino acids, %											
Ala	3.83	0.53	1.97	2.31	0.43	0.44	2.64	0.42	3.94	0.58	8.20
Asp	7.83	0.50	5.10	5.72	0.64	0.69	6.91	0.66	5.27	1.24	5.60
Cys	2.60	0.16	0.65	0.76	0.28	0.23	0.87	0.17	0.49	0.29	0.12
Glu	10.78	1.33	8.22	9.08	3.07	2.53	11.29	1.36	7.72	2.03	9.80
Gly	2.74	0.29	1.92	2.28	0.48	0.45	2.54	0.33	4.68	0.24	20.80

Table 5.3 (cont.)

Pro	4.07	0.62	2.43	2.72	1.06	1.13	3.17	0.37	3.01	0.70	12.31
Ser	4.55	0.34	1.99	2.26	0.47	0.42	3.06	0.36	2.01	0.49	2.95
Tyr	3.82	0.27	1.70	1.83	0.34	0.30	2.16	0.25	1.80	0.28	0.76
Total	40.22	4.04	23.98	26.96	6.77	6.19	32.64	3.92	28.92	5.85	60.54
Total amino acids, %	81.63	7.20	44.76	49.76	10.80	10.34	60.56	7.29	55.05	11.58	84.18

[1]N/A, not analyzed; N/D, not detected; SDP, spray dried plasma.

Table 5.4. Apparent total tract digestibility (ATTD) of gross energy (GE), Ca, P, and fiber, and standardized total tract digestibility (STTD) of P in spray dried plasma[1,2]

Item, %	Spray dried plasma
ATTD	
GE	94.3
N	94.1
Ca	77.2
P	90.6
TDF	41.5
IDF	41.2
SDF	-4.9
STTD[2]	
P	96.5

[1]IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

[2]Data are least squares means of 8 observations.

[2]The STTD of P in spray dried plasma was calculated by correcting ATTD of P for basal endogenous P loss that was obtained from pigs fed the P-free diet = 193 mg/kg dry matter intake.

Table 5.5. Basal endogenous P loss (EPL) and apparent total tract digestibility (ATTD) of dry matter and P in a P-free diet and diet with spray dried plasma (SDP) as the sole source of P fed to pigs[1]

Item	P-free	SDP	Pooled SEM	*P*-value
Feed intake, g/d	482.0	577.1	18.36	0.017
P intake, g/d	0.05	1.74	0.03	<0.001
Fecal excretion, g/d	16.96	28.92	0.80	<0.001
P in feces, %	0.45	0.71	0.02	<0.001
Fecal P excretion, g/d	0.06[b]	0.16	0.01	<0.001
ATTD of dry matter, %	97.2	95.9	0.08	<0.001
Basal EPL, mg/kg DMI	192.6	370.3	10.12	<0.001

[1]Data are least square means of 6 to 8 observations per treatment.

Table 5.6. Nutrient intakes and outputs by pigs fed regional diets without or with spray dried plasma (SDP; as-fed basis)[1,2]

Item	U.S.A.		European Union		Canada		Asia		Pooled	*P*-value		
SDP, %	0	6	0	6	0	6	0	6	SEM	SDP	Region	Interaction
Intake												
Feed, g/d	617^{b}	633^{ab}	609^{b}	674^{a}	662^{a}	632^{ab}	638^{ab}	631^{ab}	22.22	0.297	0.522	0.022
GE intake, Mcal/d	2.44^{cd}	2.54^{bcd}	2.39^{d}	2.72^{a}	2.64^{ab}	2.61^{abc}	2.55^{abcd}	2.57^{abc}	0.09	0.019	0.245	0.023
Ca, g/d	4.64^{ef}	4.46^{f}	4.74^{def}	5.09^{bc}	5.80^{a}	4.87^{cde}	5.36^{b}	5.01^{cd}	0.17	0.002	<0.001	<0.001
P, g/d	3.52^{c}	3.98^{b}	3.41^{c}	4.24^{a}	3.91^{b}	3.86^{b}	3.54^{c}	3.83^{b}	0.13	<0.001	0.139	<0.001
TDF, g/d	57.4^{d}	58.3^{d}	65.1^{c}	73.5^{b}	84.7^{a}	69.6^{bc}	34.5^{e}	29.7^{f}	2.18	0.026	<0.001	<0.001
IDF, g/d	55.5^{e}	57.6^{de}	60.9^{cd}	67.4^{ab}	70.2^{a}	63.3^{bc}	33.8^{f}	29.0^{g}	2.02	0.489	<0.001	<0.001
SDF, g/d	1.85^{d}	0.63^{e}	4.26^{c}	6.74^{b}	14.56^{a}	6.32^{b}	0.64^{e}	0.63^{e}	0.20	<0.001	<0.001	<0.001
Output												
Fecal, g/d	67.2^{y}	65.5^{y}	69.8^{y}	72.6^{y}	85.2^{x}	71.1^{y}	47.5^{z}	39.6^{z}	4.18	0.031	<0.001	0.075
Urine, kg/d	1.86	2.53	1.83	2.28	2.10	3.08	2.54	2.70	0.42	0.059	0.433	0.790
GE in feces, kcal/kg	4,795	4,818	4,740	4,710	4,690	4,666	4,647	4,629	37.44	0.597	<0.001	0.832
GE output, kcal/d	322	316	331	342	399	341	221	183	21.68	0.052	<0.001	0.164
GE in urine, kcal/kg	51.6	46.9	44.1	41.1	46.2	39.5	36.3	38.1	6.48	0.425	0.213	0.897

Table 5.6 (cont.)

Urine GE output, kcal/d	73.6	103.6	68.6	98.7	85.9	107.9	93.0	88.8	9.01	0.002	0.450	0.148
Ca in feces, %	1.96	1.98	2.04	1.94	2.04	1.91	3.57	3.16	0.14	0.136	<0.001	0.482
Ca output, g/d	1.30	1.29	1.42	1.24	1.74	1.35	1.71	1.24	0.12	0.002	0.100	0.171
P in feces, %	2.11	2.14	2.02	2.01	1.95	1.97	2.60	2.66	0.08	0.691	<0.001	0.985
P output, g/d	1.12	1.12	1.12	1.03	1.33	1.11	1.00	0.84	0.08	0.030	0.002	0.534
TDF in feces, %	31.1	30.5	34.3	33.3	36.7	37.2	20.6	20.7	1.06	0.662	<0.001	0.846
TDF output, g/d	20.8	19.9	23.9	23.8	31.3	26.4	9.8	8.1	1.21	0.013	<0.001	0.103
IDF in feces, %	27.6	27.5	31.9	30.5	34.2	34.6	16.9	18.2	0.89	0.955	<0.001	0.384
IDF output, g/d	18.6	18.0	22.1	21.9	29.1	25.3	8.5	7.1	1.10	0.029	<0.001	0.253
SDF in feces, %	3.41	3.08	2.46	2.78	2.89	2.60	2.26	2.48	0.30	0.905	0.030	0.571
SDF output, g/d	2.27	1.96	1.72	1.96	2.47	1.84	1.31	0.97	0.20	0.073	<0.001	0.199

[1]IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

[2]Data are least square means of 6 to 8 observations per treatment.

[a,b,c,d,e,f,g]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[x,y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 5.7. Concentrations of digestible energy (DE) and metabolizable energy (ME), apparent total tract digestibility (ATTD) of gross energy (GE), Ca, P, and fiber, and standardized total tract digestibility (STTD) of P in regional diets without or with spray dried plasma (SDP; as-fed basis)[1,2]

Item	U.S.A.		European Union		Canada		Asia		Pooled	*P*-value		
SDP, %	0	6	0	6	0	6	0	6	SEM	SDP	Region	Interaction
DE in diet, kcal/kg	3,435^{e}	3,519^{d}	3,376^{e}	3,531^{d}	3,381^{e}	3,594^{c}	3,655^{b}	3,784^{a}	26.88	<0.001	<0.001	0.039
ME in diet, kcal/kg	3,314^{ef}	3,338^{de}	3,265^{ef}	3,385^{cd}	3,251^{f}	3,423^{c}	3,509^{b}	3,627^{a}	29.75	<0.001	<0.001	0.040
ATTD, %												
GE	86.8	87.6	86.2	87.5	84.9	87.2	91.4	92.9	0.67	<0.001	<0.001	0.568
Ca	71.6	70.9	70.0	72.5	70.9	74.5	68.2	75.3	2.11	0.041	0.894	0.303
P	68.0	71.9	67.2	72.4	67.7	71.8	72.0	78.2	1.60	<0.001	<0.001	0.832
TDF	63.9	66.2	63.4	67.7	63.1	62.1	71.7	72.6	1.35	0.085	<0.001	0.214
IDF	66.7	68.9	63.7	67.7	58.6	61.2	75.0	75.4	1.34	0.018	<0.001	0.558
SDF	-22.3^{b}	-191.0^{d}	59.5^{a}	70.8^{a}	83.3^{a}	71.0^{a}	-102.8^{c}	-54.9^{bc}	22.71	0.063	<0.001	<0.001
STTD[3], %												
P	71.0	74.5	70.2	75.2	70.6	74.7	75.1	81.0	1.60	<0.001	<0.001	0.848

[1]IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

Table 5.7 (cont.)

[2]Data are least square means of 6 to 8 observations per treatment.

[3]The STTD of P in diets was calculated by correcting ATTD of P for basal endogenous P loss that was obtained from pigs fed the P-free diet = 193 mg/kg dry matter intake.

[a,b,c,d,e,f]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 5.8. Nitrogen balance of pigs fed regional diets without or with spray dried plasma (SDP) during a 4-d collection period (as-fed basis)[1]

Item	U.S.A.		European Union		Canada		Asia		Pooled	*P*-value		
SDP, %	0	6	0	6	0	6	0	6	SEM	SDP	Region	Interaction
N intake, g/4 d	67.5^{cd}	83.6^{ab}	65.7^{d}	88.0^{a}	71.0^{c}	81.9^{b}	70.9^{c}	84.6^{ab}	2.63	<0.001	0.681	0.018
N output in feces, g/4 d	11.4^{yz}	11.9^{xy}	11.7^{yz}	12.8^{xy}	13.6^{x}	11.6^{xyz}	11.5^{yz}	9.9^{z}	0.74	0.295	0.032	0.057
N output in urine, g/4 d	6.8	10.9	7.5	11.6	8.8	11.4	10.3	12.1	1.10	<0.001	0.018	0.292
ATTD of N[2], %	83.1	85.8	82.3	85.5	80.7	85.1	83.8	88.3	0.81	<0.001	0.003	0.578
N retention, g/4 d	51.3^{y}	60.5^{wx}	46.6^{z}	63.6^{w}	48.0^{yz}	58.4^{x}	49.1^{yz}	62.6^{wx}	2.14	<0.001	0.269	0.073
N retention, %	73.0	72.3	70.8	73.3	67.5	71.0	69.3	74.1	1.73	0.003	0.020	0.101
Biological value[3], %	87.8	84.3	85.9	84.6	84.4	83.4	82.8	84.0	1.79	0.152	0.089	0.250

[1]Data are least square means of 7 or 8 observations per treatment.

[2]ATTD, apparent total tract digestibility.

[3]Biological value was calculated as (N retained / [N intake – N output in feces]) × 100 (Rojas and Stein, 2013).

[a,b,c,d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[w,x,y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 5.9. Differences[1] between measured and predicted apparent total tract digestibility (ATTD) values for gross energy, N, Ca, P, and fiber and for standardized total tract digestibility (STTD) of P in regional diets with spray dried plasma from the U.S.A., European Union, Canada, and Asia[2,3]

Item	U.S.A.	European Union	Canada	Asia	Overall[4]
ATTD, %					
Gross energy	0.14	0.71	1.65*	1.29*	3.36*
N	0.12	0.47	1.29	2.20*	2.36*
Ca	-0.83	2.45	3.56*	6.95*	3.10*
P	0.44	1.79	0.92	3.31*	2.77*
TDF	2.76*	4.89*	-0.52	2.41	3.48*
IDF	2.89*	4.70*	3.10*	2.19	5.56*
SDF	-167.60*	9.44*	-13.06*	71.05*	-0.82
STTD, %					
P	-0.26	1.03	0.41	2.62*	1.64

[1]Difference is calculated by subtracting predicted ATTD of gross energy, N, Ca, or P from measured value. Likewise, for the difference between predicted value for STTD of P from measured value.

Table 5.9 (cont.)

[2]Data are least square means of 6 to 8 observations, except for the calculation of overall which are least square means of 30 to 32 observations.

[3]* = $P < 0.05$. Total tract digestibility was underestimated when the difference between measured and predicted total tract digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

[4]Overall was calculated as sum of the differences from all the diets with spray dried plasma.

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CHAPTER 6: Inclusion of spray dried plasma in low crude protein diets does not affect growth performance or diarrhea incidence, but the combination decreases circulating cytokines of weanling pigs

Abstract

The hypothesis that dietary SDP complements low CP in phase 1 diets by improving growth performance, decreasing diarrhea incidence, reducing immune system activation, and maintaining intestinal health of weanling pigs was tested. One-hundred and sixty weaned pigs (body weight: 5.89 ± 0.39 kg) were allotted to a randomized complete block design with 4 diets and 2 blocks with weaning group as the blocking factor (8 pens/diet; 5 pigs/pen). Four phase 1 diets were formulated: 2 diets with 23.0% CP without or with 6% SDP and 2 diets with 18.5% CP without or with 6% SDP; and a common phase 2 diet without SDP was used. Growth performance parameters were recorded; diarrhea incidence was scored every other day; blood samples were collected on d 7, 14, and 28; and intestinal tissue and mucosa were collected on d 14. Results indicated that pigs fed 23% dietary CP had greater ($P < 0.05$) growth performance parameters than pigs fed 18.5% dietary CP, and pigs fed 6% SDP had greater ($P < 0.05$) growth performance parameters than pigs fed no dietary SDP. There was a reduction ($P < 0.05$) in diarrhea scores for pigs fed 18.5% dietary CP compared with 23% dietary CP, and diarrhea scores were not influenced by dietary SDP. Villus height in the ileum and mucosa width in the colon increased if SDP was included in the diet with 23% CP, but that was not the case if SDP was included in the diet with 18.5% CP (interaction, $P < 0.05$). Jejunal mucosa interleukin- (IL-) 12 tended to increase if 6% SDP was included in the diet with 23% CP, but inclusion of SDP in

the 18.5% CP diet did not affect IL-12 (interaction, $P < 0.10$). Whereas in the ileum, IL-12 was reduced if 6% SDP was included in the 23% CP diet, but increased if 6% SDP was included in the 18.5% CP diet (interaction, $P < 0.05$). Circulating cytokine IL-2 decreased (interaction, $P < 0.05$) and interferon gamma, IL-6, and IL-18 tended to decrease (interaction, $P < 0.10$) if 6% SDP was included in the 18.5% CP diet, but the concentration of these cytokines was not affected by dietary SDP if pigs were fed the 23% CP diet. In conclusion, the combination of reduced dietary CP and inclusion of SDP did not further improve growth performance or reduce diarrhea incidence of weaned pigs, but resulted in decreased circulating pro-inflammatory cytokines, indicating a complementary relationship between dietary SDP and reduced CP in reducing inflammatory response in pigs.

Key words: cytokines, inflammation, reduced crude protein, spray dried plasma, weanling pigs

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CP	crude protein
G:F	gain to feed ratio
IFN-γ	interferon-gamma
IgA	immunoglobulin A
IL-	interleukin-
IL-1Ra	interleukin-1 receptor antagonist

Abbreviations (cont.)

PUN	plasma urea N
SDP	spray dried plasma
sIgA	secretory immunoglobulin A
TNF-α	tumor necrosis factor-α

Introduction

During the initial 2 wk post-weaning, when pigs are experiencing dietary, environmental, and social stressors, low feed intake and post-weaning diarrhea may result in decreased growth (van Dijk et al., 2001; Campbell et al., 2013). Dietary approaches may be implemented to alleviate effects of weaning stress on pigs (van Dijk et al., 2001). Spray dried plasma (**SDP**) has greater palatability compared with dried milk and is a highly digestible animal protein with standardized ileal digestibility values that are greater than 96% for indispensable amino acids (**AA**; Ermer et al., 1994; Almeida et al., 2013). Therefore, inclusion of up to 6% SDP in diets for weaned pigs may increase feed intake, which is associated with increased growth (van Dijk et al., 2001). Inclusion of SDP in diets may ameliorate intestinal barrier dysfunction and mucosal inflammation by reducing ileal and colonic permeability and pro-inflammatory cytokine concentrations during the first wk post-weaning (Peace et al., 2011), and as a result, over-stimulation of the immune system may be prevented and post-weaning diarrhea can be reduced.

Reducing crude protein (**CP**) in diets for weaned pigs is also a dietary approach to alleviate post-weaning diarrhea (Heo et al., 2008; Yue and Qiao, 2008; Kil and Stein, 2010). By reducing CP in diets for newly weaned pigs, the concentration of undigested protein and AA entering the large intestine is reduced, which may reduce fermentation of CP by microorganisms

and subsequently decrease diarrhea (Wang et al., 2018). A reduction in dietary CP may negatively affect growth performance (Hansen et al., 1993; Nyachoti et al., 2006); however, there are no data for the combination of reduced dietary CP and addition of SDP. Therefore, the current experiment was conducted to test the hypothesis that SDP complements low CP concentrations in phase 1 diets by improving growth performance, reducing diarrhea, reducing stimulation of the immune system, and maintaining intestinal health of newly weaned pigs.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in this experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and experimental design

Five diets were prepared (Tables 6.1, 6.2, and 6.3). Two phase 1 diets with normal concentrations of CP (i.e., approximately 23.0%) and 2 phase 1 diets with reduced CP (i.e., approximately 18.5%) were formulated without or with 6% SDP (Appetein B) that was sourced from APC Inc., Ankeny, IA, USA. A common phase 2 diet without SDP was also formulated. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement estimates of nursery pigs (NRC, 2012).

One-hundred and sixty pig were weaned at 20 ± 2 d with an initial body weight (**BW**) of 5.89 ± 0.39 kg and randomly allotted to 1 of the 4 phase 1 diets in a randomized complete block design. Wean group was used as the blocking factor. Two blocks were used for a total of 32 pens with 5 pigs per pen and 8 split-sex pens per treatment (3 barrows and 2 gilts or 2 barrows and 3

gilts in each pen). The 4 phase 1 diets were fed for 14 d after weaning and pigs were allowed *ad libitum* access to feed and water throughout the experiment. After 14 d, all pigs were fed the common phase 2 diet for an additional 14 d.

Sample and data collection

Diarrhea scores were assessed visually every other day by 2 independent observers using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Individual pig weights were recorded at the beginning of the experiment and at the end of each phase. Daily feed allotments were recorded and feed left in the feeders was weighed at the end of each phase. Data collected for pig weights and feed allowance were summarized and used to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) for each pen and treatment group. Data were summarized for each phase and over the entire experiment.

At the beginning of the experiment, the pig in each pen with the BW closest to the pen average was identified with 4 barrows and 4 gilts selected per dietary treatment, and 2 blood samples were collected from the jugular vein of this pig on d 7, 14, and 28. One blood sample was collected in vacutainers with ethylenediaminetetraacetic acid, and these samples were stored on ice immediately after collection and were delivered to the University of Illinois Veterinary Diagnostic Laboratory for white blood cell, neutrophil, and lymphocyte cell counts. After whole blood analysis, samples were centrifuged (Model Sorvall ST8, Thermo Fisher Scientific, Waltham, MA, USA) at 4,000 × g for 13 min to recover the plasma. Plasma samples were stored at -20^{o}C until analyzed at the University of Illinois Metabolomics Center for free AA using the Vanquish system on a TSQ Altis Mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The second blood sample was collected in heparinized vacutainers and centrifuged at

4,000 × g for 13 min to recover the plasma. Plasma samples were stored at -20°C until analyzed at the University of Illinois Veterinary Diagnostic Laboratory for plasma urea N (**PUN**), albumin, and total protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Globulin was calculated by subtracting albumin from total protein and then the albumin:globulin ratio was calculated. Heparinized plasma samples were also analyzed for immunoglobulin A (**IgA**) using ELISA kits according to the recommendations of the manufacturer (catalog # E101-102; Bethyl Laboratories, Inc., Montgomery, TX, USA), and for the following cytokines: interferon-gamma (**IFN-γ**), interleukin- (**IL-**) 1α, IL-1β, IL-1 receptor antagonist (**IL-1Ra**), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor-α (**TNF-α**) using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

On d 14, all pigs were weighed and the pig in each pen with the BW closest to the pen average was euthanized with sex balanced for each dietary treatment. If the pig with the BW closest to the pen average was the same pig being used for blood sample collection, the pig with the next closest BW to the pen average was euthanized via captive bolt stunning for tissue and mucosa collection.

Intestinal morphology

Tissue samples of jejunum, ileum, and proximal colon were collected (about 5 cm in length); jejunum and ileal tissue samples were collected approximately 150 cm distal to the pylorus and 80 cm caudal to the ileal-cecal junction, respectively. All intestinal samples were opened longitudinally along the mesenteric attachment, rinsed with phosphate buffered saline, pinned serosa side down on a piece of cardboard (Nabuurs et al., 1993), and then fixed by immersion in

10% neutral buffered formalin until analysis. After fixation, all tissue samples were delivered to Veterinary Diagnostic Pathology, LLC (Fort Valley, VA, USA) where they were sectioned and transferred to slides. Slides were examined on a Meiji 5300 microscope using 40x magnification and photographed with a mounted 17-megapixel Canon Rebel 3 Ti camera, and for each slide, 10 intact villi and the associated crypts were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA), with the line tool calibrated from pixels to micrometers using an AmScope MR400 calibration slide. Villus height was measured from the villus tip to the base, and the crypt depth was measured from the crypt-villus junction to the base of the crypt. Villus and lamina propria widths were also measured at the midpoint of the villus. Neutrophils were counted in 5 fields in a 10 × 10 grid with 250 mm per side at approximate equidistant points along the slide, using a Leica DM 3000 microscope at 400 × magnification, and expressed as neutrophils/mm^2 for each pig (Li et al., 2016; Zhu et al., 2017).

Secretory immunoglobulin A and cytokine analysis

On d 14, samples of jejunum, ileum, and proximal colon mucosa were collected with jejunal and ileal samples collected approximately 150 cm from the pylorus and 80 cm from the ileal-cecal junction, respectively. Mucosa samples were washed with phosphate buffered saline, snap frozen in liquid N, and stored at -80^{o}C until analysis. Intestinal mucosa samples were homogenized in phosphate buffered saline containing protease inhibitors (SKU, P8340; Sigma-Aldrich, St. Louis, MO, USA). The supernatant was collected and used for determination of secretory IgA (**sIgA**) using an ELISA kit according to the manufacturer's recommended procedures (catalog # E101-102; Bethyl Laboratories, Inc., Montgomery, TX, USA). Concentrations of sIgA were expressed on a per mg protein basis and all values were normalized with total protein concentration in each sample quantified by a Pierce bicinchoninic acid Protein Assay Kit (Thermo Scientific,

Woltham, MA, USA). The supernatant was also used to determine concentrations of the following cytokines: IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and IL-18 using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

Chemical analysis

All diet and ingredient samples were analyzed in duplicate for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with the subsequent calculation of CP as N × 6.25. Dry matter was analyzed in all diet and ingredient samples by oven drying at 135ºC for 2 h (method 930.15, AOAC Int., 2019) and dry ash (method 942.05; AOAC Int., 2019) was analyzed as well. All diet and ingredient samples were analyzed for acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). At the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Columbia, MO, USA, minerals (i.e., Ca, P, Na, Cl, and K) were analyzed using inductively coupled plasma optical emissions spectrometry (method 985.01 A, B, and C; AOAC Int., 2019). Diets and ingredients were also analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019] and all diets were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019).

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if

they were located outside the lower and upper far fences, which are located at 3 × the interquartile range (Tukey, 1977). Data for growth performance, diarrhea scores, tissue morphology and mucosa were analyzed by ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC, USA) in a randomized complete block design with weaning group as the blocking factor and pen as the experimental unit. The model was a 2 × 2 factorial and included the fixed effects of CP, SDP, and the interaction between CP and SDP, and the random effects of block and replicate within block. Blood samples were collected from the same pig each collection day, therefore, data were analyzed as repeated measures with unstructured variance based on the likelihood ratio test using PROC MIXED procedure of SAS. The model included the fixed effects of CP, SDP, and the interaction between CP and SDP, day as the time effect, the random effects of block and replicate within block, and pig was the subject. Contrast statements were used with coefficients for unequally spaced treatments being generated using the PROC IML statement in SAS to determine linear and quadratic effects of day on blood cell counts and plasma AA. Treatment means were calculated using the LSMEANS statement, and if an interaction was significant, means were separated using the PDIFF option in the PROC MIXED procedure. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Growth performance

There were no interactions between dietary CP and inclusion of SDP in the phase 1 diet for growth performance parameters (Table 6.4). At the end of phase 1, greater ($P < 0.05$) BW was observed for pigs fed the 23.0% CP diet compared with pigs fed the 18.5% CP diet, and greater

($P < 0.05$) BW was observed for pigs fed the diet containing 6% SDP compared with pigs fed the diet without SDP. During phase 1, pigs fed the diet with 23.0% CP had greater ($P < 0.05$) ADG, ADFI, and G:F compared with pigs fed the diet with 18.5% CP. A greater ($P < 0.01$) ADG, ADFI, and G:F was also observed for pigs fed the diet containing 6% SDP compared with pigs fed a diet without SDP.

During phase 2, when pigs were fed the common diet without SDP, no differences were observed for ADG, ADFI, and G:F between pigs that had been fed the phase 1 diet with 18.5 or 23.0% CP, but pigs that had been fed the phase 1 diets without SDP had greater ($P < 0.01$) ADG and G:F in phase 2 than pigs that had been fed the phase 1 diets with 6% SDP. At the end of phase 2, there was a tendency for greater ($P < 0.10$) BW of pigs fed the phase 1 diets with 23.0% CP compared with pigs fed the phase 1 diets with 18.5% CP. During the overall experiment, there was a tendency for greater ($P < 0.10$) ADG and ADFI of pigs that had been fed the phase 1 diets with 23.0% CP compared with pigs that had been fed the phase 1 diets with 18.5% CP. Pigs fed phase 1 diets without SDP had greater ($P < 0.05$) overall G:F compared with pigs fed the phase 1 diets with 6% SDP, regardless of CP concentration.

Diarrhea scores

There was no interaction between dietary CP and inclusion of SDP in the phase 1 diet for diarrhea scores, and only main effects are, therefore, presented (Table 6.5). During the initial 6 d post-weaning, there was a tendency ($P < 0.10$) for a reduction in diarrhea scores for pigs fed diets with 18.5% CP compared with pigs fed diets with 23.0% CP, but no differences were observed in diarrhea scores for pigs fed diets without or with 6% SDP. From d 8 to 14, a tendency for a reduction ($P < 0.10$) in diarrhea scores was observed for pigs fed the diets with 18.5% CP compared with the 23.0% CP diets, and there was also a tendency for a reduction ($P <$

0.10) in diarrhea scores for pigs fed the diets without SDP compared with pigs fed the diets with 6% SDP. Overall in phase 1, there was a reduction ($P < 0.05$) in diarrhea scores for pigs fed the diets with 18.5% CP compared with diets with 23.0% CP.

Tissue morphology

Pigs fed diets with 6% SDP had greater ($P < 0.05$) villus height and crypt depth in the jejunum on d 14 compared with pigs fed diets without SDP, and pigs fed diets with 23.0% CP tended to have greater ($P < 0.10$) villus height and crypt depth in the jejunum compared with pigs fed diets with 18.5% CP (Table 6.6). Villus height in the ileum increased if SDP was included in the diet with 23.0% CP, but that was not the case if SDP was included to the diet with 18.5% CP (interaction, $P < 0.05$). The mucosa width in the colon was greater for pigs fed the 23.0% CP diet with 6% SDP, but no effect of SDP was observed when SDP was included in the 18.5% CP diet (interaction, $P < 0.05$). However, pigs fed the diets with 23.0% CP had greater ($P < 0.05$) mucosa width in the colon than pigs fed the diets with 18.5% CP.

Secretory immunoglobulin A and mucosal cytokines

In the jejunum mucosa on d 14, sIgA concentration was not influenced by SDP inclusion in phase 1 (Table 6.7), but sIgA concentration decreased ($P < 0.05$) in the jejunum of pigs fed the diets with 18.5% CP compared with pigs fed the diets with 23.0% CP. The mucosal concentration of IL-12 tended to increase if 6% SDP was included in the diet with 23.0% CP, but inclusion of SDP in the 18.5% CP diet did not affect IL-12 (interaction, $P < 0.10$). Pigs fed the diets with 18.5% CP had decreased ($P < 0.05$) mucosal concentration of IL-1β and IL-6 concentration also tended to decrease ($P < 0.10$) if 6% SDP was included in the diet compared with the diet without SDP, regardless of CP concentration. In the ileum mucosa on d 14, IL-12 concentration was reduced if 6% SDP was included in the 23.0% CP diet, whereas if 6% SDP

was included in the 18.5% CP diet, IL-12 concentration increased (interaction, $P < 0.05$). The concentration of IL-1β also had a tendency to decrease if 6% SDP was included in the 23.0% CP diet, but increased in the diet with 18.5% CP (interaction, $P < 0.10$). In contrast, IL-4 increased if 6% SDP was included in the 23.0% CP diet, but decreased in pigs fed the diet with 18.5% CP (interaction, $P < 0.05$). In the colon, the mucosal concentration of IL-2 and IL-6 tended to decrease ($P < 0.10$) if 6% SDP was included in the diet, regardless of CP concentration.

Blood parameters

Circulating IgA on d 7 and 14 was not influenced by dietary CP or SDP (Table 6.8). Whereas circulating concentrations decreased ($P < 0.05$) for IL-2 and tended to decrease ($P < 0.10$) for IFN-γ, IL-6, IL-10, and IL-18 if 6% SDP was included in the diet with 18.5% CP, the concentration of these cytokines was not affected by dietary SDP if pigs were fed the 23.0% CP diet (interaction, $P < 0.05$). In contrast, the concentration of TNF-α on d 7 and 14 tended to increase if 6% SDP was included in the diet with 23.0% CP, but this was not observed if 6% SDP was included in the diet with 18.5% CP (interaction, $P < 0.10$). If 6% SDP was included in the diet, regardless of dietary CP, concentrations of IL-1α, IL-1β, and IL-4 decreased ($P < 0.05$) compared with the diets without SDP. Concentrations of circulating cytokines also changed over time with most cytokines decreasing ($P < 0.05$) from d 7 to d 14, but IL-12 and IgA increased ($P < 0.05$) from d 7 to 14.

White blood cells, and the neutrophils and lymphocytes that make up white blood cells, were not influenced by dietary CP or SDP on d 7, 14, or 28 post-weaning (Table 6.9). Plasma urea N was reduced in the 18.5% CP diet without SDP compared with the 23.0% CP diet without SDP, but if SDP was included in these diets, PUN did not differ (interaction, $P < 0.05$). In contrast, total protein in plasma was not influenced by dietary CP, but total protein was reduced

($P < 0.05$) if 6% SDP was included in the diet compared with the diet without SDP. These blood cell counts also changed from d 7 to 28 post-weaning, with white blood cells and neutrophils having the greatest concentration on d 14 (quadratic, $P < 0.05$), but lymphocytes, albumin, and the ratio between albumin and globulins had the least concentration on d 14 (quadratic, $P < 0.05$), and PUN linearly decreased ($P < 0.05$) from d 7 to 28.

For plasma AA from d 7 to 28 post-weaning, the concentration of Ile decreased if 6% SDP was included in the diet with 23.0% CP, but dietary SDP did not affect Ile concentration if the diet was formulated to 18.5% CP (interaction, $P < 0.05$). Plasma Met decreased if 6% SDP was included in the 18.5% CP diet, but this was not observed for the diet with 23.0% CP (interaction, $P < 0.05$). In contrast, Gln increased if 6% SDP was included in the diet with 23.0% CP, but inclusion of SDP in the 18.5% CP diet did not influence plasma Gln (interaction, $P < 0.05$). Valine and Ser in plasma increased if 6% SDP was included in the diet with 18.5% SDP, but this was not observed if SDP was included in the 23.0% CP diet (interaction, $P < 0.05$). Regardless of CP concentration in the diet, plasma Arg tended to decrease ($P < 0.10$) with inclusion of 6% dietary SDP, and plasma Gly tended to increase ($P < 0.10$) with 6% SDP in the diet. The concentration of plasma AA also changed throughout the post-weaning period with His and Ala having the greatest concentration on d 14 (quadratic, $P < 0.05$) and Ile, Met, and Trp having the least concentration on d 14 (quadratic, $P < 0.05$). Whereas plasma concentrations of Arg, Leu, Lys, Thr, Asp, Glu, Gln, Gly, Pro, Tyr, total AA, and citrulline linearly increased ($P < 0.05$) from d 7 to d 28, the concentration of Val linearly decreased ($P < 0.05$) during this time.

Discussion

The post-weaning period is often characterized by a high occurrence of intestinal disturbances with increased diarrhea incidence and depressed growth performance of pigs (Heo et al., 2013). Diarrhea caused by bacterial or viral pathogens is a vital post-weaning problem leading to economic losses due to pig mortality, morbidity, and decreased efficiency (Wang et al., 2011). Reducing CP in diets fed to weanling pigs is a strategy used to decrease post-weaning diarrhea by reducing the amount of N from undigested protein and AA reaching the hindgut for fermentation (Stein and Kil, 2006; Wang et al., 2018). However, reductions in dietary CP may result in dispensable AA becoming limiting, resulting in decreased growth performance (Nyachoti et al., 2006). Inclusion of SDP in diets for weanling pigs increases post-weaning performance and improves intestinal function of pigs (van Dijk et al., 2001; Zhang et al., 2016). Dietary SDP may also reduce inflammation throughout the intestine (Peace et al., 2011), which may result in reduced post-weaning diarrhea. However, research on the synergistic effects of low dietary CP and inclusion of SDP in diets is limited.

The observed decrease in diarrhea incidence for pigs fed the low CP diets is in agreement with previous data indicating that reducing dietary CP to 17% decreased post-weaning diarrhea and improved fecal consistency (Lordelo et al., 2008; Yue and Qiao, 2008; Bhandari et al., 2010). Addition of 5% SDP to a diet reduces diarrhea of pigs challenged with *E. coli* (Zhang et al., 2015). The influence of dietary SDP on intestinal health is greater when pigs are exposed to increased pathogen loads (Campbell et al., 2010). Therefore, the observation that diarrhea incidence during the initial 2 wk post-weaning generally was low and not influenced by dietary SDP indicates that pigs used in this experiment were of high health status.

A reduction in dietary CP can result in dispensable AA becoming limiting in the diet, which negatively affects growth performance of pigs (Hansen et al., 1993; Nyachoti et al., 2006; Wang et al., 2018). Despite addition of some indispensable AA, if CP was reduced to 17.2%, ADG and G:F of weanling pigs decreased (Yue and Qiao, 2008). Therefore, the observed reduction in growth performance of pigs fed the low CP diet the initial 2 wk post-weaning is in agreement with previous data. In contrast, SDP included in diets from 2 to 8% results in a linear increase in ADG, ADFI, and G:F of pigs during the initial 2 wk post-weaning (Torrallardona, 2010). The observed improvement in growth performance of pigs fed 6% dietary SDP is in agreement with previous data; however, the greater phase 2 efficiency of pigs fed no dietary SDP in phase 1 may indicate compensatory weight gain. Pigs adapt to increased stress by reducing feed intake immediately after weaning, and once they have adapted, protein turnover increases leading to compensatory growth (Remus et al., 2013).

Villus atrophy and local inflammation are characteristic of weaning (Lallès et al., 2004). A decrease in villus height indicates less surface area for nutrient absorption (Yin et al., 2020), and inflammation of the intestine may result in increased intestinal permeability (Peace et al., 2011). Dietary SDP can reduce villus atrophy and mucosal cell production of pro-inflammatory cytokines by inhibiting pathogen colonization on the mucosal membrane (Peace et al., 2011; Tran et al., 2014). The observed increase in villus height in the jejunum of pigs fed SDP is in agreement with previous data (Torrallardona, 2010; Zhang et al., 2015). Reducing dietary CP to 19% increased the villus height to crypt depth ratio (Opapeju et al., 2008), whereas a decrease in the villus height to crypt depth ratio were reported for pigs fed diets where CP was reduced from 18 to 15% (Chen et al., 2018). Previous data are inconclusive on the effect of reducing dietary CP on intestinal morphology of pigs, but reductions of dietary CP more than 3% compared with

NRC (2012) can have detrimental effects on intestinal morphology (Yu et al., 2019). The observed reduction in villus height in the jejunum of pigs fed diets with reduced CP is in agreement with Yue and Qiao (2008), and may be associated with reduced protein synthesis due to an insufficient supply of AA required to maintain the structure of the intestinal epithelium (Gu and Li, 2004; Wang et al., 2018). The increase in mucosa width of the colon for pigs fed 6% SDP may indicate increased inflammation in the intestine (Fernandes et al., 2014). However, SDP may reduce intestinal wall thickness (Jang et al., 2016), and with a tendency for a reduction in IL-2 and IL-6 in the colon of pigs fed 6% SDP, the increase in colonic mucosa width that was observed is not indicative of increased inflammation.

The mucosa membrane lining the intestine is important in protecting the host against pathogens (Xun et al., 2018). Additionally, sIgA, secreted by cells in the mucosa, functions in protecting the membrane by preventing the adhesion and colonization of pathogens (Hansen et al., 2019), and synthesis of pro-inflammatory cytokines is regulated by sIgA (Hansen et al., 2019). Pigs fed a diet with 5% SDP had decreased sIgA in the ileum compared with pigs fed a diet without SDP (Zhang et al., 2016), but sIgA in the jejunum and ileum mucosa was not influenced by dietary SDP in the current experiment. However, decreased sIgA in the jejunum of pigs fed reduced dietary CP may indicate that local inflammation was reduced, which is in agreement with Limbach et al. (2021). Decreased inflammation in the jejunum of pigs fed reduced dietary CP is also indicated by the decrease in pro-inflammatory cytokines IL-1β and IL-6. Decreased expression of pro-inflammatory cytokines in the intestine of pigs fed low CP diets has also been observed in the past (Limbach et al., 2021; Wang et al., 2021). Spray dried plasma improves intestinal barrier function of pigs (Peace et al., 2011), and maintains local immune homeostasis by suppressing synthesis of pro-inflammatory cytokines IL-6, IFN-γ, and TNF-α, or

the anti-inflammatory cytokines IL-4 and IL-10 that regulate pro-inflammatory cytokine production (Pérez-Bosque et al., 2010; Zhang et al., 2016). Inconsistencies in the response of cytokine synthesis to dietary SDP have also been reported (Peace et al., 2011), which was observed in the current experiment as well. Spray dried plasma is hypothesized to mainly elicit its effects in the intestinal tract of pigs (Campbell et al., 2019), and therefore, cytokine synthesis by intestinal mucosa have been more widely researched compared with circulating cytokines for pigs fed SDP. However, cytokines may act in the brain where they may reduce feed intake (Johnson, 1997), and therefore, the increased feed intake observed with inclusion of dietary SDP may be a result of decreased circulating cytokines. Additionally, the observation that plasma anti- and pro-inflammatory cytokines decreased for pigs fed the combination of reduced CP and inclusion of SDP indicates that stimulation of the systemic immune response was reduced in pigs fed this diet.

Conclusion

Inclusion of SDP to diets for newly weaned pigs improved growth performance parameters, whereas reduced dietary CP decreased efficiency of pigs during the initial 2 wk post-weaning. Reducing dietary CP from 23.0% to 18.5% decreased diarrhea incidence of weanling pigs, but also decreased villus height in the jejunum unless pigs were fed a combination of 18.5% CP and 6% SDP, in which case villus height in the ileum was maintained. Reduced dietary CP decreased synthesis of pro-inflammatory cytokines and sIgA in the jejunum, whereas inclusion of SDP did not influence intestinal cytokine production. However, the combination of reduced dietary CP and inclusion of SDP in the diet resulted in decreased circulating pro-inflammatory cytokines,

indicating a synergistic relationship between dietary SDP and reduced CP in decreasing systemic inflammation of pigs.

Tables

Table 6.1. Ingredient composition of experimental diets

Item, %	18.5% crude protein		23.0% crude protein		Phase 2
Spray dried plasma, %:	0.0	6.0	0.0	6.0	0.0
Spray dried plasma	–	6.00	–	6.00	–
Corn, ground	53.15	54.01	41.19	43.44	48.65
Soybean meal, 46% crude protein	12.70	14.20	25.00	25.00	25.00
Whey powder, dried	20.00	20.00	20.00	20.00	15.00
Soy protein concentrate	8.00	–	8.00	–	5.00
Soybean oil	3.10	3.10	3.10	3.10	3.50
Limestone, ground	0.91	1.17	0.95	1.20	0.99
Dicalcium phosphate	1.20	0.88	1.00	0.70	1.00
Sodium chloride	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.40	0.25	0.29	0.18	0.36
DL-Met	0.16	0.12	0.16	0.13	0.16
L-Thr	0.11	0.02	0.06	–	0.09
L-Val	0.02	–	–	–	–
Vitamin mineral premix[1]	0.15	0.15	0.15	0.15	0.15

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg;

Table 6.1 (cont.)

D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 6.2. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	18.5% crude protein		23.0% crude protein		Phase 2
Spray dried plasma, %:	0.0	6.0	0.0	6.0	0.0
Dry matter, %	87.52	87.59	88.20	87.96	87.30
Crude protein, %	18.96	18.91	23.17	22.93	21.17
Ash, %	5.36	5.36	5.82	5.75	5.32
Acid hydrolyzed ether extract, %	4.20	4.07	4.13	3.72	4.78
Gross energy, kcal/kg	3,935	3,969	3,990	4,031	3,974
Starch, %	28.92	26.18	24.83	24.69	27.31
Minerals, %					
Ca	0.89	0.84	0.92	0.91	0.84
P	0.57	0.60	0.60	0.63	0.56
Na	0.22	0.36	0.25	0.39	0.17
Cl	0.40	0.50	0.40	0.40	0.30
K	1.10	0.97	1.47	1.25	1.16
Indispensable amino acids, %					
Arg	1.00	0.95	1.42	1.28	1.30
His	0.43	0.46	0.58	0.58	0.53
Ile	0.83	0.76	1.12	0.99	0.99
Leu	1.55	1.65	1.94	2.00	1.78
Lys	1.33	1.41	1.61	1.63	1.51
Met	0.39	0.24	0.42	0.48	0.40
Phe	0.80	0.84	1.09	1.06	1.00

Table 6.2 (cont.)

Thr	0.80	0.88	0.98	1.04	0.91
Trp	0.14	0.19	0.27	0.29	0.23
Val	0.90	1.00	1.17	1.23	1.03
Total	8.17	8.38	10.60	10.58	9.68
Dispensable amino acids, %					
Ala	0.88	0.91	1.09	1.11	1.00
Asp	1.75	1.71	2.41	2.23	2.17
Cys	0.29	0.38	0.35	0.51	0.34
Glu	3.14	2.96	4.18	3.86	3.82
Gly	0.66	0.63	0.90	0.83	0.82
Pro	1.00	1.02	1.22	1.25	1.15
Ser	0.82	0.90	1.10	1.15	1.01
Tyr	0.54	0.62	0.75	0.78	0.69
Total	9.08	9.13	12.00	11.72	11.00
Total amino acids, %	17.25	17.51	22.60	22.30	20.68

Table 6.3. Analyzed nutrient composition of ingredients (as-fed basis)

Item	Spray dried plasma	Corn	Soybean meal	Whey powder	Soy protein concentrate
Dry matter, %	93.27	83.71	87.65	90.08	90.82
Crude protein, %	82.44	7.05	46.99	13.37	64.65
Ash, %	9.70	0.81	6.15	8.12	6.67
Acid hydrolyzed ether extract, %	0.18	2.97	1.43	0.51	0.96
Gross energy, kcal/kg	4,931	3,763	4,176	3,616	4,394
Minerals, %					
Ca	0.13	0.01	0.28	0.63	0.44
P	1.46	0.24	0.52	0.68	0.70
Na	2.40	< 0.02	< 0.02	0.86	< 0.02
Cl	0.70	< 0.10	< 0.10	1.50	< 0.10
K	0.16	0.31	1.90	2.21	2.17
Indispensable amino acids, %					
Arg	4.63	0.32	3.34	0.30	4.55
His	2.46	0.20	1.21	0.24	1.65
Ile	2.48	0.27	2.27	0.81	3.14
Leu	7.53	0.81	3.56	1.31	4.97
Lys	7.27	0.24	2.98	1.14	4.01
Met	0.90	0.14	0.60	0.17	0.83
Phe	4.18	0.33	2.33	0.41	3.28

Table 6.3 (cont.)

Thr	5.26	0.25	1.79	0.83	2.46
Trp	1.19	0.04	0.64	0.22	0.84
Val	5.71	0.35	2.35	0.77	3.23
Total	41.61	2.95	21.07	6.20	28.96
Dispensable amino acids, %					
Ala	3.92	0.50	2.00	0.64	2.76
Asp	7.90	0.47	5.18	1.31	7.05
Cys	2.58	0.17	0.65	0.30	0.87
Glu	11.37	1.25	8.63	2.20	11.76
Gly	2.80	0.28	1.95	0.25	2.65
Pro	4.18	0.55	2.29	0.72	3.15
Ser	5.31	0.31	2.21	0.57	3.07
Tyr	4.07	0.20	1.66	0.29	2.20
Total	42.13	3.73	24.57	6.28	33.51
Total amino acids, %	83.74	6.68	45.64	12.48	62.47

Table 6.4. Main effects of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets on growth performance parameters of weaned pigs[1,2]

Item	CP, %		SDP, %		Pooled	*P*-value[3]	
	18.5	23.0	0.0	6.0	SEM	CP	SDP
d 1 to 14							
Initial BW, kg	5.88	5.89	5.89	5.88	0.12	0.464	0.659
ADG, g	92[b]	118[a]	86[b]	123[a]	10.61	0.003	<0.001
ADFI, g	158[b]	180[a]	153[b]	185[a]	5.66	0.010	<0.001
G:F	0.56[b]	0.65[a]	0.55[b]	0.66[a]	0.08	0.039	0.009
Final BW, kg	7.19[b]	7.56[a]	7.12[b]	7.63[a]	0.12	0.003	<0.001
d 15 to 28							
ADG, g	430	439	463[a]	406[b]	12.19	0.591	0.002
ADFI, g	548	575	574	549	16.65	0.160	0.176
G:F	0.78	0.76	0.80[a]	0.74[b]	0.03	0.311	<0.001
Final BW, kg	13.72[z]	14.25[y]	14.11	13.86	0.26	0.079	0.388
d 1 to 28							
ADG, g	261[z]	278[y]	275	264	9.02	0.095	0.318
ADFI, g	353[z]	377[y]	364	367	10.91	0.071	0.792
G:F	0.73	0.73	0.75[a]	0.72[b]	0.04	0.934	0.031

[1]Data are least square means of 16 observations for all treatments.

[2]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight, G:F, gain to feed ratio.

Table 6.4 (cont.)

[3]There was no interaction between level of CP and SDP for any of the growth performance parameters, therefore, the interaction term was removed from the final model and only main effects are shown.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 6.5. Main effects of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets on diarrhea scores of weaned pigs[1]

Item	CP, %		SDP, %		Pooled	*P*-value[2]	
	18.5	23.0	0.0	6.0	SEM	CP	SDP
Diarrhea score[3]							
d 1 to 6	1.89[z]	2.11[y]	2.05	1.94	0.23	0.092	0.398
d 8 to 14	2.34[z]	2.60[y]	2.33[z]	2.60[y]	0.64	0.086	0.071
d 1 to 14	2.14[b]	2.39[a]	2.21	2.33	0.46	0.025	0.260

[1]Data are least square means of 16 observations for all treatments.

[2]There was no interaction between level of CP and SDP for any of the growth performance parameters, therefore, the interaction term was removed from the final model and only main effects are shown.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 6.6. Morphology of the jejunum, ileum, and colon of pigs fed low crude protein (CP) or normal CP phase 1 diets without or with spray dried plasma (SDP)[1]

Item	18.5% CP		23.0% CP		Pooled	*P*-values		
Spray dried plasma, %:	0.0	6.0	0.0	6.0	SEM	CP	SDP	CP × SDP
Jejunum								
Villus height, µm	255	306	304	332	21.82	0.053	0.047	0.543
Villus width, µm	135	131	129	134	4.95	0.803	0.907	0.372
Crypt depth, µm	323	339	331	401	22.88	0.061	0.027	0.148
Villus height:crypt depth ratio	0.83	0.92	0.97	0.88	0.07	0.410	0.974	0.190
Lamina propia thickness, µm	80.9	74.0	74.9	79.7	4.34	0.976	0.786	0.147
Neutrophils, cells/mm^2	13.1	13.3	12.0	16.3	2.34	0.647	0.304	0.350
Ileum								
Villus height, µm	297^{ab}	254^{b}	268^{b}	337^{a}	27.69	0.247	0.564	0.019
Villus width, µm	137	137	137	141	6.34	0.728	0.743	0.759
Crypt depth, µm	278	281	284	323	16.13	0.107	0.151	0.207
Villus height:crypt depth ratio	1.10	0.93	0.94	1.08	0.11	0.952	0.865	0.118

Table 6.6 (cont.)

Lamina propia thickness, µm	77.0	79.5	78.8	85.0	4.81	0.442	0.356	0.691
Neutrophils, cells/mm^2	28.8	26.1	29.6	33.8	3.86	0.279	0.854	0.385
Colon								
Mucosa width, µm	410^b	408^b	423^b	501^a	25.02	0.004	0.029	0.021
Lamina propia thickness, µm	62.9	53.5	61.1	58.7	3.75	0.647	0.132	0.356
Neutrophils, cells/mm^2	3.6	1.8	1.9	2.9	0.94	0.703	0.655	0.131

[1]Data are least square means of 8 observations for all treatments, with the exception that the 18.5% CP diet with 6.0% SDP was least square means of 7 observations.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 6.7. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets fed to weaned pigs on intestinal mucosa concentrations of secretory immunoglobulin A (μg/mg of protein) and cytokines (ng/mL)[1,2]

Item	18.5% CP		23.0% CP		Pooled	*P*-value		
Spray dried plasma, %:	0.0	6.0	0.0	6.0	SEM	CP	SDP	CP × SDP
Jejunum								
sIgA	2.06	2.42	2.90	3.82	0.68	0.034	0.211	0.576
IL-1α	0.09	0.11	0.10	0.11	0.02	0.616	0.110	0.832
IL-1β	1.99	1.97	2.99	3.34	0.41	0.001	0.610	0.578
IL-1Ra	0.55	0.56	0.60	0.66	0.09	0.178	0.552	0.579
IL-2	0.04	0.04	0.06	0.04	0.01	0.608	0.241	0.127
IL-4	0.03	0.03	0.03	0.03	0.01	0.789	0.222	0.823
IL-6	0.03	0.01	0.06	0.03	0.01	0.052	0.057	0.410
IL-8	32.40	19.54	19.21	19.87	6.55	0.125	0.144	0.108
IL-10	0.04	0.04	0.05	0.05	0.01	0.215	0.885	0.722
IL-12	0.16^{yz}	0.16^{yz}	0.13^{z}	0.20^{y}	0.03	0.849	0.094	0.098
IL-18	15.71	15.75	16.69	14.32	0.95	0.818	0.228	0.214

Table 6.7 (cont.)

Ileum								
sIgA	2.58	1.70	2.33	2.26	0.64	0.807	0.445	0.518
IL-1α	0.11	0.14	0.12	0.13	0.02	0.953	0.153	0.552
IL-1β	3.39^{yz}	4.32^{y}	3.55^{yz}	2.85^{z}	0.40	0.112	0.774	0.051
IL-1Ra	0.62	0.69	0.61	0.60	0.05	0.326	0.477	0.382
IL-2	0.05	0.05	0.05	0.05	0.01	0.780	0.301	0.608
IL-4	0.05^{ab}	0.04^{b}	0.04^{ab}	0.06^{a}	0.01	0.656	0.996	0.033
IL-6	0.04^{z}	0.07^{yz}	0.07^{y}	0.06^{yz}	0.01	0.245	0.609	0.061
IL-8	22.44	19.80	22.14	20.14	2.22	0.992	0.304	0.886
IL-10	0.05	0.06	0.07	0.07	0.01	0.101	0.828	0.651
IL-12	0.24^{ab}	0.29^{a}	0.28^{ab}	0.18^{b}	0.03	0.290	0.449	0.045
IL-18	12.75	12.51	11.69	11.74	0.83	0.107	0.859	0.797
Colon								
IL-1α	0.34	0.31	0.42	0.31	0.24	0.722	0.536	0.745
IL-1β	3.61	2.18	3.76	2.77	2.46	0.726	0.252	0.830
IL-1Ra	0.61	0.55	0.66	0.60	0.16	0.616	0.583	0.986

Table 6.7 (cont.)

IL-2	0.04	0.03	0.04	0.03	0.01	0.278	0.060	0.522
IL-4	0.04	0.04	0.04	0.04	0.01	0.444	0.779	0.779
IL-6	0.03	0.02	0.03	0.02	0.01	0.669	0.095	0.650
IL-8	2.56	2.73	2.46	2.36	0.54	0.656	0.947	0.800
IL-10	0.02	0.02	0.04	0.02	0.01	0.353	0.224	0.288
IL-12	0.05	0.03	0.05	0.04	0.03	0.906	0.337	0.648
IL-18	4.73	5.03	4.66	5.89	2.43	0.735	0.516	0.690

[1]Data are least square means of 6 to 8 observations per treatment.

[2]IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; sIgA, secretory immunoglobulin A.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 6.8. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) to phase 1 diets fed to weaned pigs on circulating concentrations of immunoglobulin A (g/mL) and cytokines (ng/mL)[1,2]

Item	18.5% CP		23.0% CP		Pooled	*P*-value[3]			Day		Pooled	
SDP, %:	0.0	6.0	0.0	6.0	SEM	C	S	C × S	7	14	SEM	*P*-value[4]
IgA	145.8	112.1	148.0	158.8	19.8	0.175	0.517	0.215	124.9	157.5	14.57	0.028
Cytokines												
IFN-γ	39.65^{y}	8.08^{z}	14.35^{yz}	12.01^{yz}	8.29	0.209	0.051	0.090	28.27	8.77	4.42	<0.001
IL-1α	0.22	0.10	0.17	0.11	0.03	0.396	<0.001	0.136	0.23	0.07	0.03	<0.001
IL-1β	0.72	0.15	0.45	0.15	0.10	0.169	<0.001	0.161	0.45	0.29	0.06	0.008
IL-1Ra	1.33	1.49	1.25	0.94	0.16	0.056	0.648	0.141	1.42	1.09	0.11	0.044
IL-2	1.19^{a}	0.15^{b}	0.64^{b}	0.16^{b}	0.13	0.044	<0.001	0.040	0.62	0.45	0.10	0.215
IL-4	4.07	0.62	2.45	0.88	0.63	0.281	<0.001	0.140	3.01	1.00	0.42	<0.001
IL-6	0.50^{y}	0.10^{z}	0.31^{yz}	0.13^{z}	0.07	0.230	<0.001	0.084	0.37	0.15	0.05	<0.001
IL-8	0.25	0.19	0.17	0.24	0.04	0.687	0.967	0.153	0.33	0.10	0.02	<0.001
IL-10	1.89^{y}	0.40^{z}	1.19^{yz}	0.42^{z}	0.22	0.121	<0.001	0.090	1.24	0.71	0.16	0.008
IL-12	0.87	0.92	0.91	0.72	0.09	0.290	0.301	0.102	0.80	0.91	0.07	0.035

Table 6.8 (cont.)

IL-18	2.89^{y}	0.77^{z}	1.87^{yz}	0.85^{z}	0.30	0.134	<0.001	0.079	2.06	1.13	0.21	0.001
TNF-α	0.85^{y}	0.63^{yz}	0.49^{z}	0.83^{y}	0.18	0.630	0.737	0.089	1.23	0.17	0.12	<0.001

[1]Data are least square means of 6 to 8 observations per treatment.

[2]IFN-γ, interferon-gamma; IgA, immunoglobulin A; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; TNF-α, tumor necrosis factor-α.

[3]*P*-values were calculated to test the main effect of crude protein concentration (C) and inclusion of spray dried plasma (S) and the interaction between crude protein and spray dried plasma (C × S).

[4]*P*-value was calculated to test the main effect of day on circulating immune parameters.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

Table 6.9. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets fed to weaned pigs on blood cell counts and plasma amino acids (AA; µM/mL)[1,2]

Item	18.5% CP		23.0% CP		Pooled	*P*-value[3]			Day			Pooled	*P*-value[4]	
SDP, %:	0.0	6.0	0.0	6.0	SEM	C	S	C × S	7	14	28	SEM	Linear	Quadratic
White blood cells	16.21	15.94	15.92	16.17	0.83	0.966	0.990	0.739	10.54	19.81	17.82	0.76	<0.001	<0.001
Neutrophils	35.21	35.29	30.37	35.85	4.06	0.432	0.310	0.322	28.14	47.80	26.60	3.66	0.028	<0.001
Lymphocytes	57.03	57.36	61.14	56.43	4.06	0.579	0.447	0.382	63.69	44.74	65.54	3.62	0.037	<0.001
Plasma urea N	8.25^{b}	10.77^{ab}	11.58^{a}	9.45^{ab}	0.71	0.167	0.788	0.003	11.53	10.88	7.63	0.57	<0.001	0.337
Albumin	2.80	2.80	2.92	2.78	0.06	0.377	0.252	0.284	2.99	2.58	2.90	0.05	0.842	<0.001
Total protein	4.97	4.77	5.08	4.79	0.11	0.556	0.029	0.693	4.86	4.81	5.03	0.08	0.050	0.212
AGR[5]	1.33	1.61	1.38	1.44	0.13	0.627	0.205	0.391	1.63	1.19	1.51	0.10	0.821	<0.001
Indispensable AA														
Arg	84.2	72.8	101.3	79.1	13.3	0.230	0.091	0.571	55.9	64.5	132.7	12.5	<0.001	0.105
His	15.9	19.5	18.2	19.0	2.8	0.529	0.117	0.319	18.5	22.6	13.3	2.7	<0.001	<0.001
Ile	195^{ab}	189^{b}	226^{a}	171^{b}	10.8	0.489	0.004	0.020	186	164	236	9.9	<0.001	<0.001
Leu	150^{ab}	174^{a}	158^{ab}	145^{b}	11.4	0.145	0.433	0.016	149	148	173	11.6	0.022	0.324

Table 6.9 (cont.)

Lys	609[ab]	519[b]	545[ab]	650[a]	39.7	0.365	0.846	0.014	503	541	698	31.9	<0.001	0.398
Met	49.5[a]	25.9[b]	42.7[a]	48.8[a]	4.4	0.061	0.045	0.002	39.6	28.1	57.5	4.3	<0.001	0.001
Phe	86.4	82.3	91.6	84.6	4.1	0.322	0.153	0.705	82.5	90.0	86.1	4.2	0.699	0.228
Thr	294	348	240	177	44.5	0.011	0.914	0.151	139	282	373	45.5	<0.001	0.227
Trp	24.9	30.0	29.9	30.0	2.2	0.263	0.261	0.278	27.7	22.9	35.6	1.9	<0.001	0.003
Val	177[b]	237[a]	208[ab]	187[b]	23.7	0.357	0.062	<0.001	222	215	170	24.7	0.005	0.538
Total	1,685	1,670	1,652	1,580	95.3	0.488	0.619	0.745	1,409	1,569	1,962	85.1	<0.001	0.797
Dispensable AA														
Ala	920	980	895	1,100	152.8	0.569	0.119	0.384	938	821	1,162	144.4	0.003	0.016
Asp	54.2	50.5	55.3	50.2	4.1	0.932	0.283	0.860	42.4	47.2	68.0	3.2	<0.001	0.307
Glu	407	351	437	445	42.4	0.127	0.539	0.412	333	410	486	33.7	<0.001	0.430
Gln	172[ab]	147[b]	152[b]	182[a]	11.9	0.523	0.810	0.024	141	152	196	9.2	<0.001	0.410
Gly	1,226	1,458	1,276	1,387	226.4	0.916	0.091	0.539	1,167	1,199	1,644	223.9	<0.001	0.284
Pro	331	335	337	348	15.2	0.468	0.567	0.812	278	317	419	14.3	<0.001	0.603
Ser	71.0[b]	92.9[a]	75.3[ab]	71.4[b]	8.6	0.110	0.095	0.020	71.5	66.8	94.6	8.4	<0.001	0.050
Tyr	69.1	69.1	81.1	71.2	6.9	0.274	0.435	0.436	47.3	60.2	110.3	6.3	<0.001	0.255

Table 6.9 (cont.)

Total	3,239	3,480	3,304	3,633	378.7	0.549	0.126	0.807	3,014	3,065	4,164	368.0	<0.001	0.089
Total AA	4,915	5,140	4,947	5,202	414.6	0.843	0.318	0.951	4,414	4,625	6,115	396.0	<0.001	0.153
Citrulline	36.1[z]	38.9[yz]	41.5[y]	36.1[z]	3.1	0.563	0.589	0.093	32.0	37.6	44.7	2.8	<0.001	0.515

[1]Data are least square means of 6 to 8 observations per treatment.

[2]Units for the blood cell counts: white blood cells, ×10^3 per µL; neutrophils, % of white blood cells; lymphocytes, % of white blood cells; plasma urea N, mg per dL; albumin, g per dL; total protein, g per dL.

[3]*P*-values were calculated to test the main effect of crude protein concentration (C) and inclusion of spray dried plasma (S) and the interaction between crude protein and spray dried plasma (C × S).

[4]*P*-values were calculated to test the linear and quadratic effects of day.

[5]AGR, albumin to globulin ratio.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).

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CHAPTER 7: Addition of spray dried plasma to phase 2 of nursery improves growth performance, reduces diarrhea incidence, and decreases mucosal pro-inflammatory cytokines of nursery pigs

Abstract

An experiment was conducted to test the hypothesis that pigs fed a low crude protein (CP) diet with 6% spray dried plasma (SDP) during phase 1 will have improved growth performance and intestinal health if the phase 2 diet is supplemented with 2.5% SDP. Three-hundred weaned pigs [body weight (BW): 6.36 ± 0.78 kg] were randomly allotted to an incomplete 2 × 2 × 2 factorial arrangement with 2 levels of SDP in phase 1 (0 or 6%), 2 levels of SDP in phase 2 (0 or 2.5%), and 2 levels of CP in phase 2 (normal or low). Growth performance was recorded; diarrhea incidence was scored every other day; blood samples were collected on d 7, 14, and 28; and intestinal tissue and mucosa were collected on d 28. Pigs that were fed the phase 1 diet with 6% SDP had greater average daily feed intake (ADFI), average daily gain (ADG), and final BW in phase 2 if 2.5% SDP was included in the normal CP diet, but not in the low CP diet (interaction, $P < 0.10$ for ADFI and $P < 0.05$ for ADG and BW). Diarrhea incidence during phase 2 was less ($P < 0.05$) if 2.5% SDP was included in the phase 2 diet compared with the diet without SDP, and diarrhea incidence was less ($P < 0.01$) for pigs fed the low CP phase 2 diet than for pigs fed the normal CP diet. Villus height in the jejunum on d 28 was not influenced by SDP in phase 1 or phase 2 or by CP in phase 2. Pigs that were fed the phase 1 diet with 6% SDP had a reduced villus height to crypt depth ratio in the jejunum if, in phase 2, 2.5% SDP was included in the normal CP diet, but not in the low CP diet (interaction, $P < 0.05$). Ileal mucosa interleukin- (IL-)

1α and IL-1β decreased ($P < 0.05$) and IL-6 and IL-12 tended to decrease ($P < 0.10$) if pigs were fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP. Addition of 2.5% SDP to the phase 2 diet decreased ($P < 0.05$) IL-1β in the ileal mucosa compared with the phase 2 diet without SDP. In conclusion, growth performance was improved, but the villus height to crypt depth ratio of the jejunum was reduced, if pigs were fed a diet with low CP with SDP in phase 1 and then a diet with normal CP with SDP in phase 2. Whereas the combination of SDP and low CP did not affect intestinal health in phase 2, diarrhea incidence was decreased throughout the experiment for pigs fed diets with SDP and pro-inflammatory cytokines were decreased throughout the small intestine of pigs fed dietary SDP in phase 1 or phase 2 or if pigs were fed the low CP diet.

Key words: cytokines, low crude protein, nursery pigs, spray dried plasma

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CP	crude protein
G:F	gain to feed ratio
IL-	interleukin-
SDP	spray dried plasma
sIgA	secretory immunoglobulin A
SPC	soy protein concentrate

Introduction

Spray dried plasma (**SDP**) is commonly included in diets fed to pigs during the initial 14 d post-weaning (Torrallardona, 2010; Balan et al., 2021); however, pigs fed a diet with SDP during the initial 7 d post-weaning have a greater improvement in feed intake and daily gain compared with pigs fed a diet with SDP from d 8 to 14 post-weaning (Balan et al., 2021). Additionally, no lasting positive carryover effects of feeding SDP on growth performance are observed for pigs fed a diet with SDP longer than 14 d post-weaning (van Dijk et al., 2001). Improvements in growth performance of pigs fed a diet with SDP is greater when pigs are under stress (Torrallardona, 2010; Balan et al., 2021) because of change in diet or being provided insufficient nutrition. Adequate nutrition is vital to pigs post-weaning as pigs are exposed to greater pathogen loads, which can up-regulate the immune system resulting in slightly greater amino acid (**AA**) requirements for cytokine and other immune cell synthesis (Klasing, 1988). However, data are lacking on the effect of feeding SDP for a longer period of time to pigs under increased nutritional stress. Therefore, this experiment was conducted to test the hypotheses that 1) pigs fed a low crude protein (**CP**) diet containing 6% SDP during phase 1 of the nursery period continue to have increased growth performance and improved intestinal health if fed a low CP diet during phase 2 compared with pigs fed a diet without SDP in phase 1; and 2) pigs fed a low CP diet with 6% SDP during phase 1 have improved growth performance and intestinal health if the phase 2 diet is supplemented with 2.5% SDP.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used.

Diets, animals, and experimental design

Spray dried plasma (Appetein B) was sourced from APC Inc., Ankeny, IA, USA. Seven diets were prepared (Tables 7.1, 7.2, and 7.3). Two phase 1 diets with reduced CP were formulated with 8% soy protein concentrate (**SPC**) or with 6% SDP. Four phase 2 diets were formulated with low CP and with either 2.5% SPC or 2.5% SDP, or with normal CP and either 4.0% SPC or 2.5% SDP. A common phase 3 diet was formulated at normal CP concentration without SDP. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement estimates of weanling pigs (NRC, 2012).

Three-hundred pigs were weaned at approximately 20 ± 2 d with an initial body weight, (**BW**) of 6.36 ± 0.78 kg and randomly allotted to an incomplete 2 × 2 × 2 factorial arrangement with 6 dietary treatments: 1) phase 1, 8% SPC and phase 2, 4% SPC with normal CP; 2) phase 1, 8% SPC and phase 2, 2.5% SPC with low CP; 3) phase 1, 6% SDP and phase 2, 4% SPC with normal CP; 4) phase 1, 6% SDP and phase 2, 2.5% SPC with low CP; 5) phase 1, 6% SDP and phase 2, 2.5% SDP with normal CP; and 6) phase 1, 6% SDP and phase 2, 2.5% SDP with low CP. There were 5 pens of barrows and 5 pens of gilts per dietary treatment with 5 pigs per pen for a total of 60 pens in 2 blocks where the blocking factor was weaning group. Phase 1 diets were fed from d 1 to 14 post-weaning, phase 2 diets were fed from d 15 to 28 post-weaning, and the phase 3 common diet was fed from d 29 to 42. All pigs were allowed *ad libitum* access to feed and water throughout the experiment.

Sample collection

Diarrhea scores were visually assessed every other day for 42 d by 2 independent observers using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Individual pig weights were recorded at the beginning of the experiment and at the end of each phase. Daily feed allotments were recorded and feed left in the feeders was weighed at the end of each phase. Data collected for pig weights and feed allowance were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed (**G:F**) ratio for each pen and dietary treatment group. Data were summarized for each phase and over the entire experiment.

At the beginning of the experiment, one pig in each pen with the BW closest to the pen average was identified, and 2 blood samples were collected on d 7, 14, and 28 from the jugular vein of this pig. Therefore, blood samples were collected from 5 barrows and 5 gilts per dietary treatment. One blood sample was collected in vacutainers with ethylenediaminetetraacetic acid, and the other blood sample was collected in heparinized vacutainers. Blood samples were stored on ice immediately after collection, and then ethylenediaminetetraacetic acid blood samples were delivered to the University of Illinois Veterinary Diagnostic Laboratory for analysis of white blood cell, neutrophil, and lymphocyte cell counts in the whole blood. Following analysis, samples were centrifuged at 4,000 × g for 13 min to recover the plasma, which was stored at -20^{o}C until analysis for free AA. The blood collected in heparinized vacutainers was centrifuged at 4,000 × g for 13 min to recover the plasma, which was stored at -20^{o}C until analysis for plasma urea N, albumin, and total plasma protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Globulin was calculated as the difference between total protein and albumin and then the albumin:globulin ratio was calculated. At the end

of phase 2, the pig that was used for blood sampling was euthanized via captive bolt stunning and intestinal tissue and mucosa were collected.

Intestinal morphology

Tissue samples from the jejunum were collected approximately 150 cm from the pylorus on d 28 for morphology analysis. Tissue samples were approximately 5 cm in length. All intestinal samples were opened longitudinally along the mesenteric attachment, rinsed with phosphate buffered saline, pinned serosa side down on a piece of cardboard (Nabuurs et al., 1993), and then fixed by immersion in 10% neutral buffered formalin until analysis. After fixation, jejunum samples were sent to Veterinary Diagnostic Pathology, LLC (Fort Valley, VA, USA) where they were sectioned (5 mm thick cross-sections) and embedded in paraffin for slide preparation. For each sample, 3 to 4 transverse sections were stained with hematoxylin and eosin for histological analysis. Slides were then scanned using a 2.0-HT NanoZoomer (Hammatsu, Bridgewater, NJ, USA), and for each slide, 10 intact villi and the associated crypts were measured using NDP.View2 (Hammatsu, Bridgewater, NJ, USA). Villus height was measured from the villus tip to the base and crypt depth was measured from the crypt-villus junction to the base of the crypt. Then villus height to crypt depth ratio was calculated. Villus width and lamina propria width were measured at the midpoint of the villus. Villus width was measured at the third top of the villus and at the level of the crypt-villus junction to calculate villus surface area.

Secretory immunoglobulin A and cytokine analysis

On d 28, scrapings of jejunum and ileum mucosa were collected approximately 150 cm from the pylorus and 80 cm from the ileal-cecal junction, respectively. Mucosa samples were washed with phosphate buffered saline, snap frozen in liquid N, and stored at -80^{o}C until analysis. Intestinal mucosa samples were homogenized in phosphate buffered saline containing protease inhibitors

(SKU, P8340; Sigma-Aldrich, St. Louis, MO, USA). The supernatant was collected and used for analysis of secretory immunoglobulin A (**sIgA**) using an ELISA kit according to the manufacturer's recommended procedures (catalog # E101-102; Bethyl Laboratories, Inc., Montgomery, TX, USA). Concentrations of sIgA were expressed on a per mg protein basis and all values were normalized with total protein concentration in each sample quantified by a Pierce bicinchoninic acid Protein Assay Kit (Thermo Scientific, Woltham, MA, USA). The supernatant was also used to determine concentrations of the following cytokines: interleukin- (**IL-**) 1α, IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and IL-18 using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex-multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

Chemical analysis

All diet and ingredient samples were analyzed in duplicate for concentrations of gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA) and N by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with the subsequent calculation of crude protein as N × 6.25. Dry matter was also analyzed in diet and ingredient samples by oven drying at 135^{o}C for 2 h (method 930.15, AOAC Int., 2019) and these samples were also analyzed for dry ash (method 942.05; AOAC Int., 2019). Minerals (i.e., Ca, P, Na, K, and Mg) were analyzed in diets and ingredients using inductively coupled plasma optical emissions spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600^{o}C for 4 h (method 985.01 A, B and C; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). All diet and ingredient samples were analyzed for acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl

Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). All diet and ingredient samples were analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019] and diets were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019) at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Columbia, MO, USA.

Statistical analysis

Normality of residuals were verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if they were located outside of the lower and upper far fences, which are located at 3 × the interquartile range (Tukey, 1977). Data for mucosa inflammation were log2 transformed before statistical analysis to obtain normal distribution. Data for growth performance, diarrhea scores, tissue morphology, and mucosa inflammation were analyzed by ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC, USA) in a randomized complete block design with weaning group as the blocking factor and pen as the experimental unit. The model included the fixed effect of dietary treatment and the random effects of block and replicate within block. The experimental design was an incomplete 2 × 2 × 2 factorial, but due to the incomplete factorial, contrast statements were used to determine effects of inclusion of SDP in phase 1 and phase 2 diets and level of dietary CP in phase 2. Contrasts included 1) interaction within phase 1 diets with SDP: phase 2 SDP × phase 2 CP; 2) interaction within phase 2 diets without SDP: phase 1 SDP × phase 2 CP; 3) main effect of phase 1 SDP; 4) main effect of phase 2 SDP; and 5) main effect of phase 2 CP. Blood samples were collected from the same pig each collection day, therefore, data were analyzed as repeated measures with unstructured variance

based on the likelihood ratio test using the PROC MIXED procedure of SAS. The model included the fixed effects of dietary treatment and day and the interaction between dietary treatment and day. The time effect of day, the random effect was block and replicate within block, and pig was the subject. The interaction between dietary treatment and day was not significant, therefore, contrast statements were used with coefficients for unequally spaced treatments being generated using the PROC IML statement in SAS to determine linear and quadratic effects of day on blood variables. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option in the PROC MIXED procedure. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Growth performance and diarrhea scores

There was no difference in initial BW of pigs among treatments (Table 7.4), but at the end of phase 1, pigs fed the diet with 6% SDP had greater ($P < 0.01$) BW than pigs fed the diet without SDP. Pigs fed the phase 1 diet with 6% SDP had greater ($P < 0.01$) ADG, ADFI, and G:F during the initial 14 d post-weaning than pigs fed the phase 1 diet without SDP. Pigs that were fed the phase 1 diet with 6% SDP had greater ADFI and ADG during phase 2 and greater BW at the end of phase 2 if 2.5% SDP was included in the normal CP phase 2 diet than if the normal CP diet was without SDP, but inclusion of 2.5% SDP in low CP diets did not influence ADFI, ADG, or BW in phase 2 (interaction, $P < 0.10$ for ADFI and $P < 0.05$ for ADG and BW). Pigs that were fed the phase 1 diet without SDP had greater ADG in phase 2 if diets had normal concentration of CP than low concentration of CP, but if pigs were fed the phase 1 diet with 6% SDP, dietary

CP concentration did not affect ADG (interaction, $P < 0.10$). Inclusion of SDP in phase 1 or phase 2 diets did not influence G:F in phase 2, but pigs fed the diet with normal CP had greater ($P < 0.01$) G:F than pigs fed the low CP diet.

In phase 3, where all pigs were fed the common diet without SDP, ADG of pigs that were fed the phase 1 diet with 6% SDP was greater if fed the low CP phase 2 diet without SDP than the normal CP diet without SDP, but ADG during phase 3 was not influenced by CP concentration in phase 1 if 2.5% SDP was included in the phase 2 diet (interaction, $P < 0.10$). In contrast, ADFI during phase 3 and final BW of pigs fed the phase 1 diet with 6% SDP was not influenced by dietary CP in phase 2, but pigs fed the normal CP diet had greater ADFI and final BW if 2.5% SDP was also included in the diet than if the phase 2 diet did not contain SDP (interaction, $P < 0.10$ for ADFI and $P < 0.05$ for BW). The G:F of pigs in phase 3 was not influenced by SDP inclusion in the phase 1 diet, but G:F increased ($P < 0.10$) for pigs fed the phase 2 diet without SDP compared with pigs fed the diet with SDP, and G:F also increased ($P < 0.05$) for pigs fed the low CP diet compared with pigs fed the normal CP diet.

The overall ADG and ADFI from phase 1 to phase 3 was greater for pigs fed the phase 1 diet with 6% SDP if 2.5% SDP was included in the normal CP diet in phase 2 compared with the normal CP diet without SDP, but this was not the case if 2.5% SDP was included in the low CP diet in phase 2 (interaction, $P < 0.05$). Overall ADFI tended to be greater for pigs fed the phase 1 diet with 6% SDP if pigs were fed the phase 2 low CP diet without SDP compared with pigs fed the phase 1 diet without SDP and the phase 2 low CP diet without SDP, but inclusion of SDP in phase 1 did not affect ADFI if phase 2 diets without SDP had normal CP concentration (interaction, $P < 0.10$). Overall G:F of pigs was not influenced by inclusion of SDP in the phase 1 diet, but pigs fed the phase 2 diet with SDP had decreased ($P < 0.05$) overall G:F compared

with pigs fed the phase 2 diet without SDP, and pigs fed the normal CP diet in phase 2 had greater ($P < 0.05$) overall G:F than pigs fed the low CP diet in phase 2.

Diarrhea scores during the initial 6 d post-weaning were reduced ($P < 0.10$) for pigs fed the diet with 6% SDP compared with pigs fed the diet without SDP (Table 7.5). During phase 2 (d 16 to 28), diarrhea scores were reduced ($P < 0.05$) for pigs fed the diet with 2.5% SDP compared with pigs fed the diet without SDP, and diarrhea scores were less ($P < 0.01$) for pigs fed the low CP diet than for pigs fed the normal CP diet. During phase 3 (d 30 to 42), pigs previously fed the phase 1 diet with 6% SDP had reduced ($P < 0.05$) diarrhea scores compared with pigs previously fed the phase 1 diet without SDP, and diarrhea scores were also reduced ($P < 0.05$) for pigs previously fed the phase 2 diet with 2.5% SDP compared with pigs fed the phase 2 diet without SDP.

Tissue morphology

Villus height, villus width, and lamina propria thickness of the jejunum on d 28 was not influenced by phase 1 or phase 2 inclusion of SDP or phase 2 dietary CP (Table 7.6). Pigs that were fed the phase 1 diet with 6% SDP had greater crypt depth in the jejunum if fed the normal CP phase 2 diet with 2.5% SDP than if pigs were fed the normal CP phase 2 diet without SDP, but inclusion of 2.5% SDP to the low CP phase 2 diet did not influence crypt depth in the jejunum (interaction, $P < 0.01$). Pigs that were fed the phase 1 diet with 6% SDP had decreased villus height to crypt depth ratio in the jejunum if 2.5% SDP was included in the normal CP diet compared with the normal CP diet without SDP, but villus height to crypt depth ratio was not influenced by addition of 2.5% SDP to the low CP phase 2 diet (interaction, $P < 0.05$). Pigs fed the phase 1 diet with 6% SDP had greater villus height to crypt depth ratio in the jejunum if fed the normal CP phase 2 diet without SDP than the low CP diet without SDP, but if pigs were fed

the phase 1 diet with 6% SDP, CP concentration in the phase 2 diet without SDP did not affect villus height to crypt depth ratio (interaction, $P < 0.05$). Villus surface area in the jejunum was greater ($P < 0.10$) for pigs that were fed the phase 1 diet with 6% SDP than the phase 1 diet without SDP, but villus surface area was not influenced by inclusion of SDP or dietary CP in phase 2.

Secretory immunoglobulin A and mucosal cytokines

Secretory IgA concentration in the jejunal mucosa on d 28 was not influenced by SDP inclusion in phase 1 or phase 2 or by dietary CP in phase 2 (Table 7.7). Pigs that were fed the phase 1 diet with 6% SDP had greater jejunal mucosa IL-10 on d 28 if fed the normal CP phase 2 diet than if pigs were fed the low CP phase 2 diet, but if pigs were fed the phase 1 diet without SDP, no impact of CP concentration in phase 2 was observed for IL-10 (interaction, $P < 0.10$). Mucosal concentrations of IL-1α and IL-18 in the jejunum were greater ($P < 0.10$) for pigs fed the normal CP diet in phase 2 than pigs fed the low CP diet, regardless of SDP inclusion in phase 2. Mucosal concentration of IL-4 was not influenced by dietary CP in phase 2, but was greater ($P < 0.05$) if pigs were fed the phase 1 diet with 6% SDP than if fed the phase 1 diet without SDP, and IL-4 concentration was greater ($P < 0.05$) for pigs fed the phase 2 diet with 2.5% SDP compared with pigs fed the phase 2 diet without SDP. Pigs that were fed the phase 1 diet with 6% SDP had greater concentration of sIgA in the ileal mucosa if fed the normal CP phase 2 diet without SDP compared with pigs fed the low CP diet without SDP, but sIgA did not differ between pigs fed the normal or low CP phase 2 diet if 2.5% SDP was included (interaction, $P < 0.05$). If pigs were fed the phase 1 diet without SDP, IL-10 in ileal mucosa was greater for pigs fed the low CP diet without SDP in phase 2 compared with pigs fed the normal CP diet without SDP, but dietary CP in phase 2 did not affect mucosal IL-10 concentration if pigs were fed the phase 1 diet with 6%

SDP (interaction, $P < 0.05$). If pigs had been fed the phase 1 diet with 6% SDP, ileal mucosa concentration of IL-18 tended to increase if the normal CP diet without SDP was fed in phase 2 compared with pigs fed the low CP diet without SDP in phase 2, but IL-18 was not influenced by dietary CP in phase 2 if pigs had been fed the phase 1 diet without SDP (interaction, $P < 0.10$). The concentration of IL-1α and IL-1β in the ileal mucosa decreased ($P < 0.05$) and IL-6 and IL-12 tended to decrease ($P < 0.10$) if pigs were fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP. Likewise, pigs fed the phase 2 diet with 2.5% SDP had decreased ($P < 0.05$) concentration of IL-1β, but tended to have increased ($P < 0.10$) concentration of IL-4, in the ileal mucosa compared with pigs fed the phase 2 diet without SDP, regardless of dietary CP in phase 2 or SDP inclusion in phase 1. Pigs fed the normal CP diet in phase 2 had greater ($P < 0.01$) mucosal IL-8 concentration compared with pigs fed the low CP diet in phase 2, regardless of SDP inclusion in the phase 1 or phase 2 diet.

Blood parameters

The concentration of white blood cells, neutrophils, and lymphocytes and the albumin to globulin ratio from d 7 to 28 was not influenced by inclusion of SDP in phase 1 or phase 2 diets or by dietary CP in phase 2 (Table 7.8). If pigs had been fed the phase 1 diet without SDP, albumin concentration in plasma from d 7 to 28 had a tendency to increase if the normal CP diet without SDP was fed in phase 2 compared with pigs fed the low CP diet without SDP, but albumin was not influenced by dietary CP in phase 2 if pigs had been fed the phase 1 diet with 6% SDP (interaction, $P < 0.10$). The concentration of plasma urea N from d 7 to 28 was less ($P < 0.05$) for pigs fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP. Total protein concentration in plasma of pigs was not influenced by inclusion of SDP in phase 1 or phase 2 diets, but total protein was increased ($P < 0.01$) for pigs fed the normal CP

diet compared with pigs fed the low CP diet in phase 2. Blood concentrations of white blood cells and neutrophils increased and then decreased from d 7 to 28 (quadratic, $P < 0.05$), whereas concentrations of lymphocytes, plasma urea N, albumin, and total protein, and the albumin to globulin ratio decreased and then increased from d 7 to 28 (quadratic, $P < 0.05$).

For pigs fed the phase 1 diet with 6% SDP, plasma Met tended to decrease from d 7 to 28 if pigs were fed the normal CP phase 2 diet with 2.5% SDP compared with pigs fed the normal CP phase 2 diet without SDP (Table 7.9), but plasma Met tended to increase for pigs if 2.5% SDP was included in the low CP diet compared with pigs fed the low CP diet without SDP (interaction, $P < 0.10$). Pigs fed the phase 1 diet with 6% SDP had increased ($P < 0.01$) concentrations of plasma Lys, Trp, Val, and Tyr from d 7 to 28 compared with pigs fed the phase 1 diet without SDP, regardless of dietary treatment in phase 2. In contrast, plasma Ile decreased ($P < 0.01$) from d 7 to 28 for pigs fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP, and plasma Ile was also less ($P < 0.01$) for pigs fed the phase 2 diet with 2.5% SDP compared with pigs fed the phase 2 diet without SDP. Pigs fed the normal CP diet in phase 2 had decreased ($P < 0.10$) concentration of plasma Asn from d 7 to 28 compared with pigs fed the low CP diet, whereas the concentration of ornithine was greater ($P < 0.10$) for pigs fed the normal CP diet compared with pigs fed the low CP diet in phase 2, regardless of SDP inclusion in phase 1 or phase 2. The concentration of most plasma AA, except for His, Met, Asp, and Cys, increased (quadratic, $P < 0.05$) for pigs from d 7 to d 28.

Discussion

At weaning pigs face normal production challenges such as changes in environment, diet, and social structure. These changes can activate the immune response leading to inflammation

throughout the intestinal tract, post-weaning diarrhea, reduced feed intake, and decreased growth; thus the subsequent development of the pig is negatively affected (Lallès et al., 2004). High quality ingredients are justified in post-weaning diets to ameliorate stress caused by weaning, and SDP is included in post-weaning diets to improve pig performance and reduce intestinal inflammation (Torrallardona, 2010; Peace et al., 2011). Dietary SDP can maintain immune homeostasis in the intestinal tract by preventing pathogen colonization in the mucosa (Peace et al., 2011; Campbell et al., 2019), thereby reducing post-weaning diarrhea (Peace et al., 2011). Reducing the concentration of CP in post-weaning diets can also decrease the prevalence of diarrhea by reducing excess N being fermented in the large intestine (Stein and Kil, 2006; Wang et al., 2018). The combination of low dietary CP and inclusion of SDP has not previously been tested, but this combination may have a synergistic effect on diarrhea prevention due to the different modes of action of these strategies. The observation that inclusion of SDP to the low CP diet decreased diarrhea incidence during the initial 6 d post-weaning is in agreement with Heo et al. (2008) and Peace et al. (2011), who reported that a low CP diet or a diet with SDP is effective in reducing diarrhea during the initial wk post-weaning. In wk 3 and 4 post-weaning of the current experiment, continuing to feed a diet with SDP or low CP was effective in decreasing diarrhea scores, which is in contrast with Le Bellego and Noblet (2002) in which feeding reduced CP diets for 5 wk post-weaning did not affect the occurrence of diarrhea, but N excretion was reduced. Diets with low CP are generally fed the initial 1 or 2 wk post-weaning when pigs are most susceptible to diarrhea, and continuing to feed low CP diets more than 1 or 2 wk post-weaning may lead to losses in growth performance (Wang et al., 2018).

The impact of feeding a low CP diet on growth performance parameters such as ADG, ADFI, and G:F of pigs is generally the greatest concern for producers (Yu et al., 2019). Results

of several studies indicate that growth performance of pigs was unchanged or improved if pigs were fed low CP diets (Le Bellego and Noblet, 2002; Htoo et al., 2007; Heo et al., 2008); however, reductions in dietary CP can negatively affect ADFI, feed efficiency, or final BW of pigs during the nursery period (Hansen et al., 1993; Nyachoti et al., 2006; Yue and Qiao, 2008).

Inclusion of SDP to diets has consistently been reported to improve growth performance of weanling pigs (Torrallardona, 2010; Balan et al., 2021), which was also observed in the current experiment when SDP was included in phase 1 and phase 2 diets. Inclusion of SDP to a low CP diet may, therefore, ameliorate reductions in growth performance when feeding diets low in CP for longer periods post-weaning. However, the observation that ADG, ADFI, and d 28 BW were increased when SDP was included in the normal CP diet, but not if SDP was included in the low CP diet, indicates that inclusion of SDP did not improve growth performance of pigs fed a low CP diet. Crystalline Lys, Met, and Thr were supplemented to the low CP diet in the current experiment, but decreased growth performance has been reported for pigs fed low CP diets supplemented with these AA (Yue and Qiao, 2008). In contrast, supplementing branched chain AA to low CP diets increases feed intake and protein deposition in the muscle of pigs (Powell et al., 2011; Zheng et al., 2016), indicating that branched chain AA may be next limiting in low CP diets (Wang et al., 2018). Indispensable AA were decreased by 15% compared with the requirement (NRC, 2012) in the current experiment, which indicates that dietary AA were insufficient to support maximum growth of the pig (Menegat et al., 2019).

Pigs fed a low CP diet immediately post-weaning followed by a diet with greater CP concentration can compensate for the poor performance observed on the low CP diet (Libal and Wahlstrom, 1976). The reduction in G:F observed for pigs fed low CP diets in phase 2 compared with pigs fed greater CP concentrations is in agreement with previous data (Nyachoti et al.,

2006), but reductions in growth performance can be compensated by improved efficiency in the subsequent phases (Stein and Kil, 2006), which was also observed in the current experiment. Pigs adapt to reduced feed intake immediately after weaning, and once they have adapted, protein turnover increases leading to compensatory growth (Remus et al., 2013). Data from the current experiment indicate that pigs fed either the low CP diet or a diet without SDP in phase 2 exhibited compensatory growth when all pigs were on the same plane of nutrition in phase 3. However, the final BW was greatest for pigs fed both phase 1 and phase 2 diets with SDP indicating that supplementation with SDP for longer periods can further improve pig BW, which is in agreement with previous data (Balan et al., 2021). However, as SDP is included in diets for pigs up to 40 d post-weaning, improvements in ADG, ADFI, or feed efficiency tend to be less significant (Balan et al., 2021).

Intestinal morphology may be maintained if dietary CP is reduced within 3% of NRC (2012) recommended CP concentration (Yu et al., 2019); but reducing dietary CP without AA supplementation may be associated with reduced villus height and crypt depth due to an insufficient supply of AA required to maintain the structure of the intestinal epithelium (Gu and Li, 2004; Wang et al., 2018). The observation that pigs fed low CP diets had a reduced villus height to crypt depth ratio is in agreement with Chen et al. (2018), whereas reducing dietary CP from 21 to 19% increased the villus height to crypt depth ratio (Opapeju et al., 2008). Including 6% SDP to the low CP diet resulted in the villus height to crypt depth ratio not differing for pigs fed the normal CP diet without SDP, indicating that inclusion of SDP to a low CP diet may maintain the intestinal barrier. Dietary SDP can increase villus height and decrease crypt depth in the intestine resulting in a greater villus height to crypt depth ratio compared with pigs fed a diet without SDP (Zhang et al., 2015). The greater crypt depth observed for pigs fed the normal CP

diet with SDP compared with pigs fed the diet without SDP is not in agreement with data demonstrating no effect of dietary SDP on crypt depth throughout the small intestine (Torrallardona et al., 2003; Nofarias et al., 2006; Corl et al., 2007; Tran et al., 2014). A deeper crypt can indicate poorer performance (Pluske et al., 1997), whereas a shallower crypt may imply less rapid cell turnover for villus renewal (Yin et al., 2020). Therefore, the ratio of villus height to crypt depth is a more important parameter to evaluate intestinal barrier function than the crypt depth by itself (Montagne et al., 2003).

Activation of the immune system may increase AA requirements, but generally protein deposition is reduced and AA are used for the synthesis of immune cells rather than for growth of the pig (Goodband et al., 2014). Therefore, the improved growth performance observed for pigs fed diets with SDP may indicate reduced immune cell synthesis. The observed decrease in mucosal pro-inflammatory cytokines IL-1α, IL-1β, IL-6, and IL-12 in the ileum of pigs fed SDP in phase 1 is in agreement with previous data indicating dietary SDP can maintain intestinal mucosa immune homeostasis by suppressing synthesis of pro-inflammatory cytokines (Pérez-Bosque et al., 2010; Zhang et al., 2016). The pro-inflammatory cytokine IL-1β is responsible for priming and amplifying the subsequent intestinal immune response (Pérez-Bosque et al., 2016). As consequence, the reduction in IL-1β in the ileum for pigs fed SDP in both phase 1 and phase 2 indicates a lasting positive effect of dietary SDP on intestinal inflammation. No antagonistic or synergistic effects were observed between dietary SDP and low CP, but the pro-inflammatory cytokines IL-1α and IL-18 in the jejunum and IL-8 in the ileum were observed to decrease for pigs fed low CP diets, which is in agreement with previous data (Limbach et al., 2021; Wang et al., 2021). Reduced pro-inflammatory cytokines synthesized in the mucosa throughout the small

intestine indicates reduced intestinal inflammation, thereby contributing to reduced incidence of diarrhea in pigs fed low CP diets (Limbach et al., 2021).

Plasma urea N is an indicator of protein utilization due to the positive correlation between plasma urea N and N excretion in the urine (Kohn et al., 2005). Therefore, the observed decrease in plasma urea N on d 7, 14, and 28 when 6% SDP was included in the diet from d 1 to 14 may indicate improved efficiency of protein utilization contributing to the better performance of pigs fed dietary SDP, which is in agreement with previous data (Jiang et al., 2000; Hernández et al., 2010; Weaver et al., 2014). In contrast, the observation that pigs fed diets with low CP had decreased albumin in the plasma from d 7 to 28 is in agreement with Limbach et al. (2021). The observed reduction in albumin may be related to the reduction in growth performance observed for pigs fed the low CP diet, because albumin functions in binding and transporting nutrients in the blood (Quinlan et al., 2005; Francis, 2010).

Conclusion

Inclusion of SDP in low CP diets fed to weanling pigs for 4 wk post-weaning did not improve growth performance parameters, but including 6% SDP to normal CP diets resulted in greater ADG and ADFI of pigs. Supplementing diets with SDP for 28 d resulted in greater BW of pigs on d 42 post-weaning. The combination of reduced dietary CP and inclusion of SDP in the diet did not affect diarrhea incidence of pigs, but dietary SDP or diets low in CP were effective in reducing diarrhea 3 to 4 wk post-weaning. This may be associated with decreased mucosal pro-inflammatory cytokine synthesis throughout the intestine of pigs fed diets with SDP or low in CP indicating decreased intestinal inflammation.

Tables

Table 7.1. Ingredient composition of experimental diets[1]

Item, %	Phase 1		Phase 2				
	Low crude protein		Low crude protein		Normal crude protein		Phase 3
Spray dried plasma, %:	0.0	6.0	0.0	2.5	0.0	2.5	0.0
Spray dried plasma	–	6.00	–	2.50	–	2.50	–
Corn, ground	52.85	53.71	59.14	59.28	49.70	51.22	62.50
Soybean meal, 46% crude protein	12.70	14.20	22.00	22.00	25.00	25.00	32.00
Whey powder, dried	20.00	20.00	10.00	10.00	15.00	15.00	–
Soy protein concentrate	8.00	–	2.50	–	4.00	–	–
Soybean oil	3.10	3.10	3.10	3.10	3.10	3.10	2.50
Limestone, ground	0.91	1.17	0.90	1.03	0.93	1.05	1.00
Dicalcium phosphate	1.20	0.88	1.30	1.15	1.07	0.95	0.90
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys HCl	0.40	0.25	0.36	0.28	0.41	0.40	0.35
DL-Met	0.16	0.12	0.07	0.08	0.11	0.13	0.11

Table 7.1 (cont.)

L-Thr	0.11	0.02	0.08	0.03	0.11	0.10	0.09
L-Val	0.02	–	–	–	0.02	–	–
Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1]Phase 1 diets were fed from d 1 to 14 post-weaning; phase 2 diets were fed from d 15 to 28 post-weaning; and the phase 3 diet was fed from d 29 to 42 post-weaning.

[2]The vitamin-micromineral premix will provide the following quantities of vitamins and microminerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 7.2. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	Phase 1		Phase 2				
	Low crude protein		Low crude protein		Normal crude protein		Phase 3
Spray dried plasma, %:	0.0	6.0	0.0	2.5	0.0	2.5	0.0
Dry matter, %	88.96	89.61	88.75	88.31	89.13	88.6	87.49
Crude protein, %	18.02	18.24	17.77	17.70	19.60	19.48	20.11
Ash, %	5.50	5.38	5.24	5.62	5.64	5.89	4.59
Acid hydrolyzed ether extract, %	4.17	4.53	5.43	5.71	4.03	4.92	5.57
Gross energy, kcal/kg	3,947	4,029	4,001	4,034	4,019	4,012	3,999
Starch, %	32.80	34.57	34.34	32.54	31.15	28.79	36.17
Minerals, %							
Ca	0.90	0.93	0.75	0.79	0.73	0.88	0.89
P	0.63	0.68	0.64	0.70	0.66	0.70	0.71
K	0.84	1.13	0.96	0.98	1.15	1.19	1.15
Mg	0.11	0.14	0.14	0.16	0.15	0.16	0.20
Na	0.23	0.69	0.25	0.33	0.29	0.43	0.08

Table 7.2 (cont.)

Indispensable amino acids, %							
Arg	1.01	0.97	1.04	1.02	1.15	1.15	1.22
His	0.43	0.46	0.44	0.45	0.48	0.49	0.50
Ile	0.80	0.77	0.80	0.77	0.91	0.87	0.87
Leu	1.52	1.66	1.51	1.56	1.64	1.69	1.64
Lys	1.29	1.37	1.23	1.24	1.50	1.59	1.32
Met	0.36	0.36	0.35	0.30	0.37	0.39	0.41
Phe	0.85	0.90	0.88	0.89	0.96	0.98	1.01
Thr	0.79	0.88	0.75	0.78	0.85	0.90	0.79
Trp	0.22	0.25	0.21	0.27	0.26	0.27	0.25
Val	0.89	1.02	0.87	0.92	0.99	1.02	0.96
Total	8.16	8.64	8.08	8.20	9.11	9.35	8.97
Dispensable amino acids, %							
Ala	0.85	0.90	0.86	0.88	0.92	0.95	0.95
Asp	1.75	1.77	1.72	1.72	1.97	1.97	1.98
Cys	0.26	0.38	0.28	0.31	0.31	0.36	0.30

Table 7.2 (cont.)

Glu	3.03	2.91	3.03	2.93	3.37	3.31	3.42
Gly	0.66	0.65	0.70	0.69	0.76	0.76	0.81
Pro	0.97	1.00	0.96	0.98	1.04	1.05	1.07
Ser	0.76	0.84	0.74	0.80	0.82	0.86	0.84
Tyr	0.57	0.64	0.59	0.63	0.63	0.68	0.66
Total	8.85	9.09	8.88	8.94	9.82	9.94	10.03
Total amino acids, %	17.01	17.73	16.96	17.14	18.93	19.29	19.00

Table 7.3. Analyzed nutrient composition of ingredients (as-fed basis)

Item	Spray dried plasma	Corn	Soybean meal	Whey powder	Soy protein concentrate
Dry matter, %	90.75	86.81	87.98	89.96	92.23
Crude protein, %	82.11	7.27	45.93	11.36	63.44
Ash, %	7.32	0.69	6.73	7.50	6.97
Acid hydrolyzed ether extract, %	0.36	3.67	2.30	0.31	0.74
Gross energy, kcal/kg	4,847	3,867	4,158	3,636	4,415
Minerals, %					
Ca	0.07	0.01	0.33	0.61	0.38
P	0.89	0.28	0.61	0.75	0.91
K	0.12	0.30	1.81	2.55	2.37
Mg	0.02	0.08	0.24	0.14	0.36
Na	1.31	0.01	0.08	0.70	0.01
Indispensable amino acids, %					
Arg	4.53	0.35	3.08	0.26	4.57

Table 7.3 (cont.)

His	2.41	0.20	1.13	0.21	1.65
Ile	2.57	0.25	2.12	0.70	3.04
Leu	7.49	0.77	3.39	1.14	4.83
Lys	7.20	0.25	2.76	0.90	4.03
Met	0.95	0.14	0.61	0.18	0.88
Phe	4.26	0.35	2.34	0.38	3.28
Thr	5.09	0.25	1.67	0.71	2.39
Trp	1.56	0.05	0.65	0.22	0.83
Val	5.67	0.34	2.19	0.67	3.14
Total	41.73	2.95	19.94	5.37	28.64
Dispensable amino acids, %					
Ala	3.84	0.50	1.88	0.54	2.58
Asp	7.89	0.48	4.85	1.14	6.89
Cys	2.62	0.15	0.62	0.26	0.90
Glu	10.84	1.20	7.85	1.89	11.33
Gly	2.77	0.30	1.85	0.24	2.43

Table 7.3 (cont.)

Pro	3.97	0.56	2.12	0.63	3.03
Ser	4.74	0.31	1.97	0.47	2.74
Tyr	3.74	0.23	1.60	0.27	2.20
Total	40.41	3.73	22.74	5.44	32.10
Total amino acids, %	82.14	6.68	42.68	10.81	60.74

Table 7.4. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets fed to weaned pigs on growth performance parameters[1,2,3,4]

Phase 1 SDP:	0.0%		6.0%					
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%		
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	Pooled SEM	Contrasts[5,6]
d 1 to 14								
Initial BW, kg	6.39	6.33	6.37	6.37	6.33	6.35	0.22	–
ADG, g	132	114	176	167	163	163	8.36	c**
ADFI, g	182	176	231	217	218	231	17.10	c**
G:F	0.72	0.66	0.77	0.77	0.75	0.73	0.05	c**
d 14 BW, kg	8.16	8.02	8.82	8.67	8.58	8.72	0.25	c**
d 15 to 28								
ADG, g	440	529	485	460	508	572	28.73	a*, b, c, d, e**
ADFI, g	656	696	733	720	686	763	26.69	a, b, c*, d*
G:F	0.67	0.75	0.66	0.64	0.74	0.76	0.03	e**
d 28 BW, kg	14.40	15.58	15.69	15.27	15.76	16.85	0.47	a*, c**, d*, e**

Table 7.4 (cont.)

d 29 to 42								
ADG, g	670	636	693	654	631	661	19.50	a, e
ADFI, g	877	899	919	898	862	951	29.74	a
G:F	0.76	0.71	0.75	0.73	0.74	0.70	0.01	d, e*
Final BW, kg	23.11	23.79	24.74	23.75	23.94	25.45	0.65	a*, c*, d
d 1 to 42								
ADG, g	412	426	452	426	434	466	12.46	a*, c**
ADFI, g	570	588	628	610	591	648	18.16	a*, b, c**, d*
G:F	0.72	0.73	0.72	0.70	0.74	0.72	0.02	d*, e*

[1]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; G:F, gain to feed ratio.

[2]Data are least square means of 9 or 10 observations per treatment.

[3]Phase 1 diets were fed from d 1 to 14 post-weaning, phase 2 diets were fed from d 15 to 28 post-weaning, and the common phase 3 diet was fed from d 29 to 42 post-weaning.

[4]Growth performance parameters were based on 5 pigs per pen from d 1 to 28, and 4 pigs per pen from d 29 to 42.

Table 7.4 (cont.)

[5]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

[6]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

Table 7.5. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets on diarrhea scores for weaned pigs[1]

Phase 1 SDP:	0.0%		6.0%					
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%	Pooled	
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	SEM	Contrasts[2,3]
Diarrhea score[4]								
d 1 to 6	2.28	1.87	1.67	2.10	2.00	1.80	0.19	c
d 8 to 14	2.37	2.43	2.57	2.38	2.51	2.78	0.13	–
d 1 to 14	2.33	2.20	2.15	2.31	2.29	2.30	0.10	–
d 16 to 28	1.92	2.15	2.08	1.76	2.22	2.04	0.10	d*, e**
d 30 to 42	1.32	1.43	1.27	1.27	1.37	1.19	0.05	c*, d*
Overall	2.28	1.87	1.67	2.10	2.00	1.80	0.19	–

[1]Data are least square means of 8 to 10 observations per treatment.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

Table 7.5 (cont.)

[3]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

[4]Diarrhea score: 1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; 5 = watery diarrhea.

Table 7.6. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets fed to weaned pigs on jejunum morphology[1]

Phase 1 SDP:	0.0%		6.0%					
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%	Pooled	
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	SEM	Contrasts[2,3]
Villus height, µm	509	494	520	512	526	533	22.41	–
Villus width, µm	133	135	142	143	141	135	6.63	–
Crypt depth, µm	361	339	367	349	329	384	14.02	a**
Villus height:crypt depth ratio	1.44	1.35	1.43	1.55	1.70	1.46	0.09	a*, b*, c*
Lamina propria thickness, µm	85.9	88.2	90.2	95.0	90.3	84.9	5.34	–
Villus surface area, mm	213,665	213,883	241,304	224,739	234,857	227,974	12,344.82	c

[1]Data are least square means of 8 to 10 observations per treatment.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

[3]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

Table 7.7. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets fed to weaned pigs on jejunal mucosa concentrations of secretory immunoglobulin A (μg/mg of protein) and cytokines (ng/mL)[1,2,3]

Phase 1 SDP:	0.0%		6.0%					
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%	Pooled	
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	SEM	Contrasts[4,5]
Jejunum								
sIgA	5.07	5.16	4.78	3.90	3.81	4.74	1.534	–
IL-1α	0.08	0.10	0.08	0.07	0.10	0.08	0.012	e
IL-1β	2.09	2.95	1.92	2.22	2.51	1.71	0.546	–
IL-1Ra	0.52	0.51	0.55	0.52	0.55	0.48	0.116	–
IL-2	0.08	0.08	0.07	0.09	0.08	0.07	0.015	–
IL-4	0.07	0.06	0.07	0.10	0.08	0.08	0.012	c*, d*
IL-6	0.02	0.02	0.02	0.02	0.03	0.02	0.004	–
IL-8	23.74	23.50	26.23	22.99	25.94	29.52	2.963	–
IL-10	0.05	0.04	0.04	0.05	0.05	0.04	0.004	b
IL-12	0.17	0.19	0.19	0.19	0.18	0.14	0.030	–

Table 7.7 (cont.)

IL-18	24.05	29.35	22.02	23.04	25.47	26.68	6.780	e
Ileum								
sIgA	3.57	3.33	2.34	3.60	4.36	3.11	0.630	a*, b*
IL-1α	0.12	0.14	0.11	0.09	0.10	0.11	0.034	c*
IL-1β	4.33	5.02	3.42	2.47	3.50	3.33	1.662	c*, d*
IL-1Ra	0.58	0.64	0.61	0.48	0.55	0.57	0.145	–
IL-2	0.07	0.08	0.07	0.08	0.08	0.08	0.009	–
IL-4	0.06	0.04	0.05	0.07	0.06	0.07	0.010	d
IL-6	0.05	0.05	0.04	0.05	0.04	0.04	0.007	c
IL-8	19.87	32.41	27.77	22.28	32.18	32.12	4.079	e**
IL-10	0.08	0.05	0.05	0.06	0.05	0.06	0.007	b*, e
IL-12	0.30	0.34	0.27	0.26	0.25	0.27	0.036	c
IL-18	15.37	12.60	9.71	9.35	16.56	8.69	4.836	b, c, d*

[1]IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; sIgA, secretory immunoglobulin A.

[2]Values were Log10 transformed before analysis to obtain a normal distribution, but data are shown as back-transformed least square means.

Table 7.7 (cont.)

[3]Data are least square means of 8 to 10 observations per treatment.

[4]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

[5]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

Table 7.8. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets fed to weaned pigs on blood cell counts[1,2]

Phase 1 SDP:	0.0%		6.0%											
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%	Pooled		Day			Pooled	*P*-value[5]	
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	SEM	Contrasts[3,4]	7	14	28	SEM	Linear	Quadratic
White blood cells	17.42	17.78	17.11	19.22	18.38	17.65	2.29	–	12.56	25.10	16.12	2.22	<0.001	<0.001
Neutrophils	43.69	39.90	38.90	44.98	41.53	40.44	3.76	–	37.25	50.98	36.49	3.11	0.682	<0.001
Lymphocytes	49.95	54.54	54.88	48.96	50.85	51.92	3.99	–	56.07	42.00	57.47	3.36	0.433	<0.001
Plasma urea N	7.43	8.32	6.50	7.33	5.57	7.17	0.64	c*	9.36	5.92	5.88	0.47	<0.001	0.001
Albumin	2.45	2.74	2.56	2.50	2.62	2.63	0.07	b, e**	2.78	2.36	2.61	0.04	<0.001	<0.001
Total protein	4.23	4.52	4.26	4.24	4.41	4.40	0.08	e**	4.42	4.25	4.36	0.05	0.208	<0.001
AGR[6]	1.45	1.56	1.56	1.52	1.50	1.55	0.07	–	1.74	1.28	1.55	0.05	0.002	<0.001

[1]Units for the blood cell counts: white blood cells, ×10^3 per µL; neutrophils, % of white blood cells; lymphocytes, % of white blood cells; plasma urea N, mg per dL; albumin, g per dL; total protein, g per dL.

[2]Data are least square means of 8 to 10 observations per treatment.

[3]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

Table 7.8 (cont.)

[4]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

[5]*P*-values were calculated to test the linear and quadratic effects of day.

[6]AGR, albumin to globulin ratio.

Table 7.9. Influence of inclusion of spray dried plasma (SDP) and concentration of crude protein (CP) in phase 1 or phase 2 diets fed to weaned pigs on concentrations of plasma amino acids (AA; μM/mL)[1]

Phase 1 SDP:	0.0%		6.0%											
Phase 2 SDP:	0.0%	0.0%	0.0%	2.5%	0.0%	2.5%	Pooled			Day		Pooled	*P*-value[4]	
Phase 2 CP:	Low	Normal	Low	Low	Normal	Normal	SEM	Contrasts[2,3]	7	14	28	SEM	Linear	Quadratic
Indispensable AA														
Arg	70	76	64	69	70	66	21.34	–	42	54	111	20.98	<0.001	<0.001
His	33	31	30	32	31	28	6.66	–	33	30	30	6.43	0.095	0.246
Ile	234	230	170	169	167	155	42.29	c**, d**	166	126	271	42.08	<0.001	<0.001
Leu	248	232	238	240	242	231	45.96	–	214	206	295	45.40	<0.001	<0.001
Lys	425	394	452	463	461	447	49.99	c**	385	394	542	48.26	<0.001	<0.001
Met	97	88	82	92	92	82	14.58	a, b	100	71	95	14.20	0.453	<0.001
Phe	138	133	134	141	135	126	13.54	–	121	124	157	13.27	<0.001	0.010
Thr	257	298	321	290	328	307	32.04	–	209	238	454	22.23	<0.001	<0.001
Trp	43	46	55	52	57	57	11.94	c**	44	37	75	11.87	<0.001	<0.001
Val	255	276	315	333	330	302	62.22	c**	303	274	329	60.81	0.157	0.010
Total	1,818	1,783	1,862	1,896	1,967	1,797	299.4	–	1,622	1,572	2,368	294.5	<0.001	<0.001
Dispensable AA														

Table 7.9 (cont.)

Ala	2,340	2,114	2,222	2,083	2,118	2,140	371.6	–	1,908	2,045	2,556	359.3	<0.001	0.133
Asn	55	58	54	55	61	58	7.31	e	32	46	93	7.06	<0.001	<0.001
Asp	109	109	109	113	112	111	6.16	–	115	107	109	5.89	0.022	0.021
Cys	9	9	10	9	9	9	0.45	–	9	9	10	0.31	0.152	0.072
Gln	299	286	311	324	324	313	41.87	c*	264	281	383	40.82	<0.001	0.002
Glu	220	237	256	208	213	223	56.05	a, b*	195	256	227	54.91	0.017	<0.001
Gly	1,117	1,076	1,253	1,072	1,212	1,134	235.0	–	1,056	940	1,436	231.1	<0.001	<0.001
Pro	323	308	330	320	328	312	53.62	–	262	271	428	52.94	<0.001	<0.001
Ser	100	93	114	99	106	108	23.19	c*	76	85	148	22.96	<0.001	<0.001
Tyr	126	132	148	141	155	152	26.21	c**	98	114	215	25.74	<0.001	<0.001
Total	4,707	4,426	4,808	4,408	4,729	4,558	814.1	–	4,041	4,165	5,612	803.6	<0.001	<0.001
Total AA	6,525	6,243	6,668	6,308	6,696	6,358	1,104.4	–	5,681	5,737	7,981	1,092.4	<0.001	<0.001
Ornithine	53	57	53	53	59	56	7.12	e	30	44	91	6.87	<0.001	<0.001

[1]Data are least square means of 8 to 10 observations per treatment.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = interaction within phase 1 diets with 6% SDP, phase 2 SDP × phase 2 CP; b = interaction within phase 2 diets without SDP, phase 1 SDP × phase 2 CP; c = main effect of phase 1 SDP; d = main effect of phase 2 SDP; e = main effect of phase 2 CP.

Table 7.9 (cont.)

[3]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

[4]*P*-values were calculated to test the linear and quadratic effects of day.

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CHAPTER 8: Spray dried plasma included at 8% in the diet improves growth performance, increases amino acid utilization, and supports the immune system of weaned pigs housed in a sanitation challenged environment

Abstract

This experiment aimed at testing the hypothesis that as inclusion of spray dried plasma (SDP) increases in the diet for newly weaned pigs, growth performance and intestinal morphology are improved and inflammation is reduced. Four-hundred weaned pigs (body weight: 6.05 ± 0.80 kg) were allotted to a randomized complete block design with 5 diets and 2 blocks (16 pens/diet; 5 pigs/pen). Pens were not cleaned between groups to create a sanitation challenge. Phase 1 diets containing 0, 2, 4, 6, or 8% SDP were formulated. One pig per pen was sacrificed on d 14 and samples of intestinal tissue and mucosa were collected. During phase 1, growth performance parameters and body weight of pigs on d 14 increased (linear, $P < 0.05$) with increasing dietary SDP. Villus width in the jejunum of pigs on d 14 tended to increase (linear, $P < 0.10$) with increasing inclusion of SDP, and villus height to crypt depth ratio was greatest (quadratic, $P < 0.10$) for pigs fed a diet with 8% SDP. The jejunal mucosa concentration of interleukin- (IL-) 2 tended to be least (quadratic, $P < 0.10$) at 8% inclusion of SDP and IL-8 tended to increase (linear, $P < 0.10$) as SDP inclusion increased in the diet. Secretory immunoglobulin A in the ileal mucosa was greatest at 2% inclusion of SDP to the diet and then decreased with increasing dietary SDP (quadratic, $P < 0.05$), and IL-10 concentration tended to be least at 4 and 6% dietary SDP, but increased with 8% dietary SDP (quadratic, $P < 0.10$). Activated T cells and the ratio of

activated to regulatory-T cells tended to be greatest at 4% dietary SDP but then decreased as SDP increased in the diet (quadratic, $P < 0.10$), whereas circulating lymphocytes linearly decreased ($P < 0.05$) as SDP increased in the diet. The concentration of plasma urea N also linearly decreased ($P < 0.05$) as dietary SDP increased, indicating greater amino acid utilization with greater dietary SDP. In conclusion, the optimal inclusion of SDP in diets for weanling pigs was 8% as indicated by improvements in growth performance and utilization of amino acids, but data for intestinal morphology and mucosal and systemic inflammation did not result in a conclusive optimum concentration of dietary SDP.

Key words: cytokines, immunology, growth performance, sanitation challenge, spray dried plasma, weanling pigs

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CD	cluster of differentiation
EDTA	ethylenediaminetetraacetic acid
FoxP3	forkhead box protein 3
G:F	gain to feed ratio
IL-	interleukin-
IL-1Ra	interleukin-1 receptor antagonist
PUN	plasma urea N

Abbreviations (cont.)

SDP	spray dried plasma
sIgA	secretory immunoglobulin A

Introduction

The optimum concentration of spray dried plasma (**SDP**) in phase 1 diets fed to weanling pigs is approximately 6% (van Dijk et al., 2001; Torrallardona, 2010). This inclusion level of SDP was determined by evaluating improvements in growth performance parameters of weaned pigs fed diets with graded levels of SDP (van Dijk et al., 2001; Torrallardona, 2010). However, the mode of action of SDP is hypothesized to be related to its immunoglobulin concentration (Torrallardona, 2010). Immunoglobulins protect and maintain the function of the intestinal mucosa by identifying and neutralizing pathogens on the membrane (Torrallardona, 2010), thereby preventing para-cellular diffusion of pathogens, decreasing intestinal inflammation, and reducing villus atrophy in the small intestine post-weaning (Pierce et al., 2005; Pérez-Bosque et al., 2016b). Because the antibody functions in preventing pathogen colonization on the mucosal membrane, SDP has a greater effect on improving intestinal barrier function when fed to pigs exposed to greater pathogen loads (Campbell et al., 2010). Feeding a diet with SDP increases anti-inflammatory cytokines and decreases pro-inflammatory cytokines in the mucosa (Pérez-Bosque et al., 2016b; Zhang et al., 2016), indicating that dietary SDP reduces the activation of the immune system (Nofrarías et al., 2006). However, in most previous experiments, one level of SDP, usually around 6%, was used, and data for effects of graded levels of SDP in the diet on intestinal morphology and inflammation in weaned pigs are lacking. Therefore, this experiment was conducted to test the hypothesis that pigs exposed to greater pathogen loads have reduced

prevalence of diarrhea, decreased concentrations of mucosal pro-inflammatory cytokines, and improved intestinal morphology and growth performance as inclusion rate of SDP in the diet increased.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in this experiment were offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and experimental design

Spray dried plasma (Appetein B) was sourced from APC Inc., Ankeny, IA, USA and the same batch was used in all diets containing SDP. Six diets were prepared (Tables 8.1, 8.2, and 8.3). A basal diet was formulated based on corn, soybean meal, and soy protein concentrate, and 4 additional diets were formulated by including 2, 4, 6, or 8% SDP in the basal diet at the expense of soy protein concentrate. A common phase 2 diet without SDP was also formulated. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement estimates of weaned pigs (NRC, 2012).

Four-hundred pigs were weaned at approximately 20 ± 2 d with an initial body weight (**BW**) of 6.05 ± 0.80 kg and randomly allotted to 1 of the 5 dietary treatments. Wean group was used as the blocking factor, and there were 2 blocks of 40 pens with 5 pigs per pen. For each dietary treatment there were 4 pens of barrows and 4 pens of gilts in each block, therefore, there were a total of 16 replicate pens per dietary treatment. Pigs were placed into uncleaned pens using a sanitation challenge model and pens remained uncleaned throughout the experiment (Adewole et al., 2016). The 5 treatment diets were fed for 14 d and pigs were allowed *ad libitum*

access to feed and water throughout the experiment. On d 14, one pig per pen was euthanized and all remaining pigs were fed the common phase 2 diet until d 28 post-weaning.

Sample collection

Diarrhea scores were assessed visually every other day for 28 d by 2 independent observers using a score from 1 to 5 (1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea). Diarrhea frequency was obtained by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100. Individual pig weights were recorded before weaning and at the end of each phase. Daily feed allotments were recorded and feed left in the feeders was weighed at the end of each phase. Data collected for pig weights and feed allowance were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed (**G:F**) ratio for each pen and treatment group. Data were summarized for each phase and over the entire experiment.

The day before weaning, pigs were allotted to dietary treatments and 2 pigs per pen with a BW closest to the pen average were identified for blood collection. All blood samples were collected from the jugular vein of each pig using vacutainers. On the day before weaning, one blood sample from each pig was collected, but on d 7 and 14 post-weaning, 2 blood samples from each pig were collected. One blood sample was collected in vacutainers with ethylenediaminetetraacetic acid (**EDTA**) and stored on ice immediately after collection. Samples were then analyzed for white blood cells, neutrophils, and lymphocytes at the University of Illinois Veterinary Diagnostic Laboratory, Urbana, IL, USA. Following this analysis, samples were then centrifuged at 4,000 × g for 13 min to recover the plasma, which was stored at -20^{o}C until analysis for free amino acids (**AA**). The second blood sample was collected in heparinized vacutainers and centrifuged at 4,000 × g for 13 min to recover the plasma, which was stored at -

20°C until analysis for plasma urea N (**PUN**), albumin, and total plasma protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA), globulin was calculated as the concentration of albumin subtracted from total protein, and then the albumin:globulin ratio was calculated. The third blood sample was collected in sodium heparinized vacutainers (BD Biosciences, San Jose, CA, USA) and peripheral blood monocyte cells were isolated by centrifugation at 1,650 RCF for 20 min at 25°C. The peripheral blood monocyte cells were analyzed for toll-like receptor 4 and choline acetyltransferase at Michigan State University (East Lansing, MI, USA). The fourth blood sample was collected directly into sodium citrate mononuclear cell preparation vacutainers (BD Biosciences, San Jose, CA, USA) and centrifuged at 1,650 RCF for 20 min at 25°C. The peripheral blood monocyte cells were re-suspended in the plasma layer by gentle inversion of the tube and samples were shipped overnight to Iowa State University (Ames, IA, USA) for staining for cluster of differentiation (**CD**) 4, forkhead box protein 3 (**FoxP3**), CD25, and CD8alpha and CD8beta. This enabled the identification of the following subsets in pigs: 1) Th naïve ($CD4^+CD8alpha^-$); 2) Th effector or memory ($CD4^+CD8alpha^+$ with or without $FoxP3^+CD25^+$ to indicate current activation status); 3) Cytotoxic T cells ($CD8beta^+$ with or without $FoxP3^+CD25^+$ to indicate current activation status); 4) Regulatory T cells (CD4+$CD8alpha^+$, $CD4^+CD8alpha^-$, and $CD8beta^+$ with $FoxP3^+CD25^+$); and 5) Activated T cells (CD4+$CD8alpha^+$, $CD4^+CD8alpha^-$, and $CD8beta^+$ without $FoxP3^+CD25^+$). The third and fourth blood samples were collected only on d 7 and 14 post-weaning, but not on the day before weaning. On the last day of phase 1, one of the pigs identified at the beginning of the experiment for blood sampling was euthanized via captive bolt stunning and samples for intestinal morphology assessment and secretory immunoglobulin A (**sIgA**) and cytokine analysis were collected.

Intestinal morphology

Samples of jejunum (about 5 cm in length) were collected approximately 150 cm from the pylorus on d 14 post-weaning. All intestinal samples were opened longitudinally along the mesenteric attachment, rinsed with phosphate buffered saline, pinned serosa side down on a piece of cardboard (Nabuurs et al., 1993), and then fixed by immersion in 10% neutral buffered formalin. After fixation, jejunum samples were sent to Veterinary Diagnostic Pathology, LLC (Fort Valley, VA, USA) where they were sectioned (5 mm thick cross-sections) and embedded in paraffin for slide preparation. For each sample, 3 to 4 transverse sections were stained with hematoxylin and eosin for histological analysis. Slides were then scanned using a 2.0-HT NanoZoomer (Hammatsu, Bridgewater, NJ, USA), and for each slide, 10 intact villi and the associated crypts were measured using NDP.View2 (Hammatsu, Bridgewater, NJ, USA). Villus height was measured from the villus tip to the base and crypt depth was measured from the crypt-villus junction to the base of the crypt. Then villus height to crypt depth ratio was calculated. Villus width and lamina propria width were measured at the midpoint of the villus. Villus width was measured at the third top of the villus and at the level of the crypt-villus junction to calculate villus surface area. Inflammatory cell infiltration, edema, and misshaped tips of the villi were also evaluated on the transverse sections of tissue. A range of scores from 0 to 5 (0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe) was considered to evaluate the severity of morphological damages.

Secretory immunoglobulin A and cytokine analysis

Samples of jejunum and ileum mucosa scrapings were collected approximately 150 cm from the pylorus and 80 cm from the ileal-cecal junction, respectively. Mucosa samples were washed with phosphate buffered saline, snap frozen in liquid N, and stored at -80^{o}C until analysis. Intestinal

mucosa samples were homogenized in phosphate buffered saline containing protease inhibitors (SKU, P8340; Sigma-Aldrich, St. Louis, MO, USA) and the supernatant was collected and used for determination of sIgA using an ELISA kit according to the manufacturer's recommended procedures (catalog # E101-102; Bethyl Laboratories, Inc., Montgomery, TX, USA). Concentrations of sIgA were expressed on a per mg protein basis, and all values were normalized with total protein concentration in each sample quantified by a Pierce bicinchoninic acid Protein Assay Kit (Thermo Scientific, Woltham, MA, USA). The supernatant was also used to determine concentrations of the following cytokines: interleukin- (**IL-**) 1α, IL-1β, IL-1 receptor antagonist (**IL-1Ra**), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and IL-18 using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

Chemical analysis

All diet and ingredient samples were analyzed in duplicate for concentrations of gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of crude protein as N × 6.25. Diets and ingredients were also analyzed for dry matter by oven drying at 135^{o}C for 2 h (method 930.15, AOAC Int., 2019), dry ash (method 942.05; AOAC Int., 2019), and acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA), followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). In addition, all diet samples were analyzed for insoluble- and soluble-dietary fiber (method 991.43; AOAC Int., 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA), and

then total dietary fiber was calculated as the sum of insoluble- and soluble-dietary fiber. Minerals (i.e., Ca, P, K, Mg, Na, Fe, Mn, and Zn) were analyzed in diet samples using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600ºC for 4 h (method 985.01 A, B and C; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). All diet and ingredient samples were also analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int., 2019], and diets and corn were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019). Amino acids and starch were analyzed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA), but all other analyses were conducted at the University of Illinois.

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if they were located outside of the lower and upper far fences, which are located at 3 × the interquartile range (Tukey, 1977). Data for growth performance, diarrhea scores, tissue morphology, and mucosa inflammation were analyzed by ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC, USA) in a randomized complete block design with wean group as the blocking factor and pen as the experimental unit. Contrast statements were used with coefficients for equally spaced treatments to determine linear and quadratic effects of inclusion of SDP in the diet on performance, diarrhea, tissue, mucosa, and blood data. The

statistical model included the fixed effect of dietary treatment and the random effects of block and replicate within block. Data for tissue histological scores were analyzed using PROC GLIMMIX with Poisson distribution. Data for the frequency of T regulatory cells were log2 transformed before statistical analysis to obtain a normal distribution and data were analyzed using PROC MIXED because blood samples for this analysis were not collected from pigs before weaning. For all other blood parameters, baseline measurements were collected from pigs before weaning and used as a covariate. The differences between baseline and the subsequent days were calculated and analyzed as repeated measures using the PROC MIXED procedure of SAS. The model included diet, day, and the interaction between diet and day as the fixed effects, day as the time effect, block and replicate within block as the random effects, and pig as the subject. However, the interaction between diet and day was not significant and was, therefore, removed from the final model. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option in the PROC MIXED procedure. The chi-squared test was used to analyze frequency of diarrhea among treatments, but contrast statements were not included in this analysis. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Growth performance and diarrhea scores

There was no difference among treatments for the initial BW of pigs (Table 8.4), but at the end of phase 1 there was a linear increase ($P < 0.05$) in pig BW as the inclusion of SDP in the diet increased. There was also a linear increase ($P < 0.05$) in ADG, ADFI, and G:F of pigs in phase 1 as the inclusion of SDP in the diet increased. In phase 2, there was no difference among

treatments in ADG, ADFI, G:F, or final BW. However, there was a trend for a linear increase ($P < 0.10$) in the overall ADG from d 1 to 28 of pigs as SDP in phase 1 diets increased, but no differences were observed for the overall ADFI and G:F of pigs. There was no effect of dietary treatment on diarrhea scores or the frequency of diarrhea of pigs in phase 1 or phase 2 (Table 8.5).

Tissue morphology

Villus width on d 14 tended to increase ($P < 0.10$) in pigs as the inclusion of SDP in the diet increased (Table 8.6), and villus height to crypt depth ratio had a tendency to decrease (quadratic, $P < 0.10$) as SDP inclusion increased with the least value observed in pigs fed the diet with 6% SDP. Histological scores of the jejunum of pigs were not influenced by inclusion level of SDP in the diet.

Secretory immunoglobulin A and mucosal cytokines

In the jejunum mucosa on d 14 post-weaning, sIgA concentration was not influenced by dietary SDP (Table 8.7). The mucosal concentration of IL-2 tended to increase (quadratic, $P < 0.10$) as the level of SDP in the diet increased, with the greatest concentration observed in pigs fed diets with 4 or 6% SDP. Mucosal concentration of IL-8 also tended to increased (linear, $P < 0.10$) as dietary SDP increased, whereas the concentration of IL-12 tended to decrease (linear, $P < 0.10$) as dietary SDP increased.

In the ileum mucosa on d 14 post-weaning, there was a quadratic increase ($P < 0.05$) in sIgA, with the greatest concentration observed in pigs fed the diet with 2% SDP. A quadratic response was observed for IL-1β ($P < 0.05$) and IL-10 ($P < 0.10$) with concentrations in the mucosa being least in pigs fed the diet with 4 or 6% SDP. The concentration of IL-6 in ileal mucosa tended to increase (linear, $P < 0.10$) as the level of SDP in the diet increased.

Blood parameters

For T regulatory cells in the blood of pigs on d 7 post-weaning (Table 8.8), the frequency of CD8beta$^+$ T cells and CD4$^+$CD8alpha$^+$ T cells tended to increase (quadratic, $P < 0.10$) for pigs with the greatest value observed at 2% SDP in the diet. The frequency of CD4$^+$CD8alpha$^+$ FoxP3negCD25$^+$ cells also increased (quadratic, $P < 0.05$) as SDP was added to the diet with the greatest value observed at 4% SDP. Subsequently, the sum of activated T cells and the ratio of activated to regulatory T cells increased (quadratic, $P < 0.10$) as dietary SDP increased with the greatest value observed for pigs fed diets with 4 or 6% SDP. There were no differences among regulatory T cells in the blood collected from pigs on d 14 post-weaning (data not shown).

The change in white blood cells from weaning to d 7 and 14 was not influenced by inclusion of SDP in the diet (Table 8.9). The difference from weaning to d 7 and 14 in neutrophil concentration increased (linear, $P < 0.05$) as SDP inclusion in the diet increased, whereas a linear decrease was observed in the difference from weaning to d 7 and 14 for lymphocytes and PUN ($P < 0.05$) and a tendency for a linear decrease in albumin ($P < 0.10$) was observed as the inclusion of dietary SDP increased. Concentrations of these blood parameters also changed over time with white blood cells, neutrophils, PUN, total protein, and albumin:globulin ratio being greater ($P < 0.05$) for pigs on d 14 than on d 7, whereas the concentration of lymphocytes and albumin were less ($P < 0.05$) for pigs on d 14 than on d7.

Blood collected from pigs the day before weaning was used as a baseline measurement to calculate the change in plasma AA concentrations of pigs fed diets with increasing levels of SDP during phase 1 (Table 8.10). For AA concentrations in plasma (Table 8.11), a quadratic increase ($P < 0.05$) was observed for differences from weaning to d 7 and 14 of Gly and Ser with the greatest concentrations in the plasma of pigs fed the diet with 4% SDP, and the change in

concentration of Pro and total AA from weaning to d 7 and 14 tended to increase (quadratic, $P < 0.10$) as SDP was added to the diet. The change in concentration of Cys from weaning to d 7 and 14 increased (linear, $P < 0.05$) and the change in concentrations of Lys, Ala, and Tyr tended to increase (linear, $P < 0.10$) as dietary SDP increased, but the change in concentration of plasma Ile decreased ($P < 0.05$) as SDP was added to the diet. The concentration of most AA in plasma were greater ($P < 0.05$) on d 14 than on d 7, but the concentration of Met was less ($P < 0.05$) for pigs on d 14 than on d 7.

Discussion

Weaning exposes the animal to many stressors, such as environmental, social, and dietary changes, that may result in anorexia or infections that negatively affect the health and performance of the animal (Pluske et al., 1997; Lallès et al., 2004). Including SDP in diets fed to pigs immediately after weaning stimulates feed intake, and therefore, improves growth performance (Torrallardona, 2010). Improvements in growth performance have also been reported in dogs (Quigley et al., 2004), calves (Henrichs et al., 2021), broiler chickens (Campbell et al., 2019), and mice (Thomson et al., 1995) fed a diet with SDP compared with animals fed a diet without SDP. A review of trials with pigs where SDP was included in a dose-dependent manner concluded that 6% SDP inclusion in diets for pigs was most consistent in improving gain to feed ratio and increased ADG and ADFI (van Dijk et al., 2001). However, growth performance continues to increase as SDP inclusion increases in the diet (Torrallardona, 2010), which was also observed in the current experiment. Diets have been supplemented with up to 25% SDP (Torrallardona, 2010), but including more than 10% SDP in a diet may result in an imbalance of AA, because SDP is low in Met and Ile, or in a high salt content (Kats et al., 1994;

Torrallardona, 2010). Therefore, inclusion of 2 to 8% inclusion of SDP in the diet has been recommended (Torrallardona, 2010).

Spray dried plasma is often included in diets for pigs for 2 wk after weaning, and after those 2 wk, improvements in growth performance are usually not observed for pigs fed a diet with SDP (Torrallardona, 2010). Therefore, in the current experiment, diets with SDP were fed to pigs for 2 wk after weaning and then a common diet without SDP was provided, and results of the experiment are in agreement with van Dijk et al. (2001) who demonstrated that growth performance of pigs was not further improved in wk 3, 4, or 5 after weaning. However, the tendency for an improved ADG that was observed for the overall post-weaning period may be a result of the sanitation challenge environment in which the pigs were housed. Pigs experiencing greater antigen exposure have greater improvements in feed intake, weight gain, and feed efficiency if fed a diet with SDP than pigs not exposed to antigens (Coffey and Cromwell, 1995; Torrallardona, 2010).

Post-weaning diarrhea is a concern for pigs, which may be a result of the underdeveloped intestinal tract producing insufficient amounts of digestive enzymes. Therefore, digestion and absorption are incomplete in the small intestine resulting in greater undigested material in the large intestine that is fermented by microorganisms causing diarrhea (Zhang et al., 2015; Balan et al., 2021). Feeding pigs a diet with SDP has been observed to decrease the incidence of post-weaning diarrhea (Gatnau and Zimmerman, 1991), but no differences among treatments were observed in frequency of diarrhea in the current experiment. Spray dried plasma may not be as effective in reducing *E. coli* concentrations in the small intestine compared with the antibiotic colistin (Pérez-Bosque et al., 2016b), but when fed in combination, an interaction between SDP and antimicrobials have been observed (Torrallardona et al., 2003; Bikker et al., 2004). In

contrast, SDP included in a diet fed to pigs challenged with rotavirus reduced the incidence of diarrhea compared with challenged pigs fed a diet with soy protein (Campbell et al., 2010), indicating that SDP may be more effective in protecting pigs against viral pathogens than bacterial pathogens.

The increased villus width as SDP inclusion increased may be a result of the increased ADFI that was observed, because post-weaning anorexia may be a primary factor in the alteration of tissue morphology often observed after weaning (Lallès et al., 2004). Feeding a diet with 5% SDP to weaned pigs increased villus height and villus height to crypt depth ratio in the duodenum and increased the ratio of villus height to crypt depth in the jejunum, but decreased crypt depth compared with pigs fed a diet without SDP (Tran et al., 2014; Zhang et al., 2015). However, villus height and crypt depth in the jejunum, ileum, and colon were not influenced by 6% SDP in the diet (Nofrarías et al., 2007). The tendency for an increase in the ratio of villus height to crypt depth that was observed at the greatest inclusion of SDP is, therefore, in agreement with pervious data (Torrallardona, 2010).

An increase in crypt hyperplasia post-weaning results in a greater number of immature cells, and therefore, pathogens can more easily migrate through the intestinal barrier (Pluske et al., 1997; Lallès et al., 2004; Zhang et al., 2016). Maintenance of the intestinal barrier is important for nutrient absorption and prevention of para-cellular diffusion of toxins and microorganisms to reduce over-stimulation of the immune system (Pérez-Bosque et al., 2016b). The immune system is also activated by weaning (Nofrarías et al., 2006), which may result in greater allocation of nutrients to support the immune response than to stimulate growth of pigs (Goodband et al., 2014; Campbell et al., 2019). However, the continued improvement in growth performance of pigs observed as SDP was included in the diet indicates that SDP may have

prevented over-stimulation of the immune system, and one of the hypothesized modes of action for SDP is the modulation of intestinal immune response (Campbell et al., 2019). In contrast to results of the current experiment, mice fed a diet with 6% SDP or rats fed a diet with 8% SDP had increased synthesis of anti-inflammatory cytokine IL-10 in the intestinal mucosa (Nofrarías et al., 2006; Pérez-Bosque et al., 2010). Expression of the pro-inflammatory cytokine IL-8 in the jejunal mucosa of rats or pigs fed 6% dietary SDP and expression of IL-6 in the jejunal mucosa of rats fed 8% dietary SDP was reduced compared with animals fed a diet without SDP (Bosi et al., 2004; Pérez-Bosque et al., 2010; Pérez-Bosque et al., 2016b). However, Peace et al. (2011) reported that IL-10 and IL-6 in the ileal and colonic mucosa of pigs were not influenced by 2.5 or 5% dietary SDP, although growth performance of pigs fed 2.5 or 5% dietary SDP was greater than of control pigs. Therefore, even though the present data indicate an increase in a few pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines in the jejunal and ileal mucosa of pigs fed increasing dietary SDP, the immune cell infiltration in the villi of the jejunum were minimal to mild in severity, indicating that the local inflammatory response may not have been over-stimulated, which is in agreement with Bosi et al. (2004). The pro-inflammatory cytokine IL-1β functions in the development and prolongation of intestinal inflammation (Al-Sadi et al., 2012), and an increase in IL-1β in the mucosa may result in an increase in intestinal tight junction permeability (Rawat et al., 2020). Tight junction proteins were increased in the jejunum, ileum, and colon of pigs fed 5% dietary SDP compared with pigs fed a diet without SDP (Peace et al., 2011; Zhang et al., 2016). Therefore, reduction in IL-1β as dietary SDP increased in the current experiment may indicate reduced para-cellular permeability of pathogens, which is in agreement with previous data (Moretó and Pérez-Bosque, 2009).

Secretory IgA is a component of the adaptive immune system with decreased concentrations in the mucosa correlated with decreased activation of the adaptive immune response (Mantis et al., 2011). Secretory IgA is secreted by cells in the lamina propria and can prevent pathogen attachment to epithelial cells protecting intestinal barrier function (Ushida et al., 2008; Mantis et al., 2011). In contrast with data from the current experiment, a reduction in lamina propria cell density and reduced sIgA concentration in the intestinal mucosa of pigs fed a diet with 5% SDP have been reported (Peace et al., 2011; Zhang et al., 2016). Neutrophils are a component of the innate immune system and are among the initial cells recruited to sites of inflammation to function in phagocytosis and inflammation (Jones et al., 2016). Increased neutrophils in the blood of pigs fed increased dietary SDP are in agreement with data from Nile tilapia fed graded levels of SDP (Araújo et al., 2017). The increase in neutrophils may be a result of the increase in IL-8, which can stimulated neutrophils to migrate to the sites of inflammation (Bosi et al., 2004). However, increased neutrophil concentration with increased dietary SDP in the current experiment indicates activation of the innate immune system, which can lead to the subsequent activation of the adaptive immune system consisting of T lymphocytes (Moretó and Pérez-Bosque, 2009).

Regulatory T cells are responsible for maintaining immune homeostasis and suppressing intestinal inflammation (Pérez-Bosque et al., 2016a). However, IL-2 can activate regulatory T cells in pigs (Käser et al., 2011), and once activated, T cells can stimulate the release of pro-inflammatory cytokines (Pérez-Bosque et al., 2016a). Therefore, changes in IL-2, activated T cells, and pro-inflammatory cytokines observed in the current experiment are consistent with previous data (Käser et al., 2011; Pérez-Bosque et al., 2016a). In contrast with data from the current experiment, supplementation of dietary SDP has also been observed to reduce the percent

of activated T lymphocytes and to reduce release of IL-2 (Pérez-Bosque and Moretó, 2015). However, dietary SDP may also reduce circulating lymphocyte concentration (Pérez-Bosque and Moretó, 2015), which was observed in the current experiment.

Increased activation of the immune response, innate or adaptive, results in repartitioning of amino acids from protein gain to synthesis of immune cells (Goodband et al., 2014). Plasma urea N is positively correlated with urinary N excretion and therefore, PUN is an indicator of amino acid utilization efficiency (Kohn et al., 2005). Decreased PUN observed with increasing dietary SDP in the current experiment indicates that protein utilization of the pig became more efficient contributing to the better performance of pigs fed dietary SDP, which is in agreement with previous data (Jiang et al., 2000; Hernández et al., 2010; Weaver et al., 2014).

Conclusions

Increasing inclusion of dietary SDP is positively correlated with improvements in growth performance of weanling pigs, indicating that inclusion of up to 8% SDP in diets for weanling pigs from d 1 to 14 post-weaning, linearly increases ADFI, ADG, G:F, and final BW. However, increasing levels of dietary SDP may stimulate the innate immune system through increased pro-inflammatory cytokine synthesis and increased sIgA concentration in the intestinal mucosa, as well as increased circulating neutrophils. In contrast, the adaptive immune response was reduced when 8% SDP was included in the diet as observed by reduced circulating lymphocytes and reduced frequency of activated T cells.

Tables

Table 8.1. Ingredient composition of experimental diets

Item, %	Basal					Phase 2
Spray dried plasma, %:	0	2	4	6	8	0
Spray dried plasma	–	2.00	4.00	6.00	8.00	–
Corn, ground	39.24	39.81	39.91	40.49	41.03	48.30
Soybean meal, 45% crude protein	25.00	25.00	25.00	25.00	25.00	25.00
Whey powder, dried	20.00	20.00	20.00	20.00	20.00	15.00
Soy protein concentrate	9.50	7.00	5.00	2.50	–	5.00
Soybean oil	3.14	3.14	3.14	3.14	3.14	3.50
Limestone, ground	0.95	1.00	1.10	1.17	1.26	0.99
Dicalcium phosphate	1.08	1.00	0.89	0.79	0.69	1.00
Sodium chloride	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.31	0.28	0.22	0.18	0.15	0.36
DL-Met	0.11	0.12	0.12	0.13	0.13	0.16
L-Thr	0.07	0.05	0.02	–	–	0.09

Table 8.1 (cont.)

Vitamin mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 mg; vitamin D_3 as cholecalciferol, 1,660 mg; vitamin E as selenium yeast, 66.0 mg; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_D$-pantothenic acid as $_D$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper chloride; Fe, 123.0 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 8.2. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	Basal					Phase 2
Spray dried plasma, %:	0	2	4	6	8	0
Dry matter, %	91.28	91.17	91.20	91.17	91.00	89.67
Crude protein, %	23.01	23.45	23.67	23.88	23.97	19.69
Ash, %	5.88	5.77	5.74	5.90	5.61	5.35
Acid hydrolyzed ether extract, %	3.65	3.20	3.48	3.44	3.35	4.04
Gross energy, kcal/kg	4,109	4,117	4,127	4,128	4,150	4,052
Insoluble dietary fiber, %	10.60	10.50	10.50	10.40	10.40	12.70
Soluble dietary fiber, %	0.20	0.20	0.30	0.40	0.20	0.75
Total dietary fiber, %	10.80	10.70	10.80	10.80	10.60	13.45
Starch, %	27.93	28.31	28.44	30.98	28.31	33.65
Minerals, %						
Ca	0.86	0.90	0.88	0.95	0.92	0.84
P	0.75	0.74	0.73	0.76	0.71	0.65
K	1.47	1.47	1.44	1.37	1.30	1.21
Mg	0.18	0.18	0.17	0.17	0.15	0.17

Table 8.2 (cont.)

Na	0.23	0.25	0.35	0.38	0.44	0.16
Fe	0.02	0.02	0.02	0.02	0.02	0.02
Mn	0.01	0.01	0.01	0.01	0.01	0.01
Zn	0.02	0.02	0.02	0.01	0.01	0.02
Indispensable amino acids, %						
Arg	1.40	1.37	1.40	1.39	1.35	1.23
His	0.56	0.57	0.60	0.61	0.61	0.52
Ile	1.07	1.06	1.07	1.04	1.01	0.93
Leu	1.88	1.91	2.01	2.04	2.06	1.70
Lys	1.56	1.71	1.59	1.68	1.70	1.46
Met	0.39	0.40	0.40	0.42	0.48	0.40
Phe	1.08	1.09	1.14	1.14	1.14	0.96
Thr	0.97	1.02	1.05	1.09	1.13	0.83
Trp	0.30	0.31	0.32	0.30	0.32	0.26
Val	1.14	1.18	1.25	1.29	1.31	1.00
Total	10.35	10.62	10.83	11.00	11.11	9.26

Table 8.2 (cont.)

Dispensable amino acids, %						
Ala	1.06	1.06	1.12	1.13	1.14	0.96
Asp	2.35	2.36	2.43	2.42	2.38	2.06
Cys	0.35	0.39	0.42	0.46	0.50	0.34
Glu	4.02	3.97	4.10	4.00	3.91	3.61
Gly	0.89	0.87	0.90	0.89	0.87	0.79
Pro	1.23	1.22	1.27	1.26	1.28	1.08
Ser	0.96	0.99	1.07	1.08	1.10	0.87
Tyr	0.73	0.73	0.78	0.81	0.83	0.70
Total	11.59	11.59	12.09	12.05	12.01	10.40
Total amino acids, %	21.94	22.21	22.92	23.05	23.12	19.65

Table 8.3. Analyzed nutrient composition of ingredients (as-fed basis)

Item	Spray dried plasma	Corn	Soybean meal	Whey powder	Soy protein concentrate
Dry matter, %	90.59	87.11	87.97	89.86	92.15
Crude protein, %	80.47	7.12	45.20	11.66	62.62
Ash, %	7.08	0.93	6.30	7.20	6.40
Acid hydrolyzed ether extract, %	0.12	2.88	1.90	0.11	0.53
Gross energy, kcal/kg	4,831	3,868	4,152	3,634	4,391
Starch, %	N/A[1]	64.25	N/A	N/A	N/A
Indispensable amino acids, %					
Arg	4.56	0.34	3.17	0.26	4.46
His	2.47	0.20	1.17	0.21	1.65
Ile	2.49	0.25	2.12	0.70	3.07
Leu	7.46	0.79	3.43	1.13	4.81
Lys	7.13	0.24	2.87	0.91	3.99
Met	0.90	0.17	0.58	0.18	0.84

Table 8.3 (cont.)

Phe	4.19	0.33	2.26	0.36	3.22
Thr	5.14	0.24	1.75	0.71	2.38
Trp	1.58	0.06	0.66	0.22	0.89
Val	5.59	0.33	2.22	0.66	3.19
Total	41.51	2.95	20.23	5.34	28.5
Dispensable amino acids, %					
Ala	3.87	0.50	1.94	0.54	2.69
Asp	7.96	0.47	5.05	1.16	6.99
Cys	2.78	0.17	0.67	0.29	0.94
Glu	11.11	1.22	8.34	1.94	11.49
Gly	2.77	0.29	1.90	0.24	2.61
Pro	4.05	0.57	2.15	0.62	3.11
Ser	4.71	0.31	2.11	0.46	2.68
Tyr	4.11	0.25	1.70	0.27	2.27
Total	41.36	3.78	23.86	5.52	32.78
Total amino acids, %	82.87	6.73	44.09	10.86	61.28

Table 8.3 (cont.)

[1]N/A, not analyzed

Table 8.4. Growth performance of weaned pigs fed phase 1 diets with increasing inclusion of spray dried plasma[1,2,3]

Item	Basal					Pooled	*P*-value	
Spray dried plasma, %:	0	2	4	6	8	SEM	Linear	Quadratic
d 1 to 14								
Initial BW, kg	6.05	6.05	6.04	6.05	6.04	0.39	0.422	0.692
ADG, g	76	99	107	116	125	29.08	<0.001	0.315
ADFI, g	152	179	180	178	188	24.78	0.004	0.219
G:F	0.47	0.54	0.57	0.64	0.65	0.09	<0.001	0.376
Final BW, kg	7.06	7.44	7.54	7.67	7.79	0.80	<0.001	0.192
d 15 to 28[4]								
ADG, g	520	526	519	519	508	24.10	0.485	0.637
ADFI, g	607	623	631	611	616	54.66	0.905	0.411
G:F	0.86	0.85	0.83	0.86	0.83	0.04	0.209	0.771
Final BW, kg	14.26	14.91	14.75	14.94	14.56	1.17	0.520	0.142
d 1 to 28								
ADG, g	296	313	318	317	317	26.95	0.097	0.210

Table 8.4 (cont.)

ADFI, g	380	398	407	395	402	40.24	0.221	0.295
G:F	0.78	0.79	0.79	0.81	0.79	0.01	0.225	0.300

[1]Data are least square means of 15 or 16 observations per treatment.

[2]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; G:F, gain to feed ratio.

[3]All pigs were fed phase 1 diets for 14 d post-weaning, and they were then fed the common phase 2 diet with no spray dried plasma from d 15 to 28 post-weaning.

[4]Growth performance parameters were based on 5 pigs per pen from d 1 to 14. One pig per pen was euthanized on d 14, and therefore, growth performance parameters from d 15 to 28 were based on 4 pigs per pen.

Table 8.5. Diarrhea scores and frequency of diarrhea for weaned pigs fed phase 1 diets with increasing inclusion of spray dried plasma[1]

Item	Basal					Pooled	*P*-value		
Spray dried plasma, %:	0	2	4	6	8	SEM	Diet	Linear	Quadratic
Diarrhea score[2]									
d 1 to 6	2.10	2.22	2.29	2.21	2.24	0.19	–	0.195	0.176
d 8 to 14	2.89	2.85	3.03	2.92	2.83	0.16	–	0.902	0.304
d 1 to 14	2.57	2.58	2.71	2.64	2.58	0.07	–	0.742	0.185
d 16 to 28	1.84	1.93	1.73	1.90	1.81	0.05	–	0.499	0.948
d 1 to 28	2.21	2.26	2.24	2.29	2.19	0.05	–	0.945	0.170
Frequency of diarrhea									
d 1 to 6									
Pen days[3]	48	48	48	48	48				
Frequency[4]	27.08	31.25	20.83	25.00	29.17	–	0.813	–	–
d 8 to 14									
Pen days	64	64	64	64	64				

Table 8.5 (cont.)

Frequency	60.94	53.13	56.25	64.06	53.13	–	0.648	–	–
d 1 to 14									
Pen days	112	112	112	112	112				
Frequency	3.57	2.68	4.46	6.25	4.46	–	0.751	–	–
d 16 to 28									
Pen days	112	112	112	112	112				
Frequency	46.43	43.75	41.07	47.32	42.86	–	0.878	–	–
d 1 to 28									
Pen days	224	224	224	224	224				
Frequency	25.00	23.21	22.77	26.79	23.66	–	0.863	–	–

[1]Data are least square means of 15 or 16 observations per treatment.

[2]Diarrhea scores were visually assessed every other day by 2 independent observers for 28 days. Diarrhea score: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea.

[3]Pen days = number of pens × the number of days assessing diarrhea scores.

[4]Frequency = (number of pen days with diarrhea scores ≥ 3 / pen days) × 100.

Table 8.6. Morphology measurements and histology scores of the jejunum of pigs fed phase 1 diets with increasing inclusion of spray dried plasma[1]

Item	Basal					Pooled	*P*-value	
Spray dried plasma, %:	0	2	4	6	8	SEM	Linear	Quadratic
Villus height, µm	349	335	347	336	371	14.83	0.330	0.154
Villus width, µm	124	122	131	127	132	4.51	0.085	0.861
Crypt depth, µm	320	320	331	333	317	11.07	0.805	0.253
Villus height:crypt depth ratio	1.12	1.07	1.09	1.05	1.20	0.06	0.355	0.051
Lamina propria thickness, µm	81.4	80.8	87.5	84.0	86.5	5.47	0.167	0.720
Villus surface area, mm	138	129	144	136	154	8.81	0.143	0.346
Histological scores[2]								
Lymphoid infiltration	1.5	1.6	1.9	1.4	1.6	0.31	0.998	0.624
Neutrophilic infiltration	1.1	1.1	1.2	1.1	1.1	0.27	0.880	0.802
Edema	0.1	0.1	0.1	0.3	0.1	0.08	0.815	0.562
Misshapen tips	0.3	0.6	0.9	0.6	0.7	0.20	0.213	0.175

[1]Data are least square means of 14, 15, or 16 observations per treatment.

[2]A score of 0 to 5 was assigned: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe.

Table 8.7. Concentrations of secretory immunoglobulin A (μg/mg of protein) and cytokines (ng/mL) in jejunal and ileal mucosa of pigs fed phase 1 diets with increasing inclusion of spray dried plasma[1]

Item	Basal					Pooled	*P*-value	
Spray dried plasma, %:	0	2	4	6	8	SEM	Linear	Quadratic
Jejunum								
sIgA	2.50	1.89	1.39	2.89	1.66	0.370	0.541	0.569
IL-1α	0.146	0.109	0.136	0.136	0.136	0.016	0.875	0.416
IL-1β	4.04	3.87	3.45	3.25	3.63	0.522	0.379	0.497
IL-1Ra	0.632	0.613	0.648	0.745	0.670	0.072	0.136	0.766
IL-2	0.075	0.078	0.081	0.081	0.074	0.004	0.989	0.080
IL-4	0.060	0.064	0.061	0.063	0.068	0.004	0.257	0.696
IL-6	0.022	0.019	0.024	0.019	0.020	0.003	0.768	0.797
IL-8	15.88	15.15	17.01	17.17	17.91	1.697	0.096	0.770
IL-10	0.051	0.050	0.051	0.051	0.047	0.004	0.420	0.496
IL-12	0.196	0.238	0.175	0.157	0.179	0.027	0.058	0.944
IL-18	35.31	33.25	38.05	37.76	36.41	4.573	0.154	0.509

Table 8.7 (cont.)

Ileum								
sIgA	1.03	1.68	1.48	1.24	1.11	0.190	0.654	0.030
IL-1α	0.129	0.093	0.118	0.128	0.127	0.013	0.463	0.264
IL-1β	6.12	4.53	3.57	5.26	4.86	0.752	0.353	0.031
IL-1Ra	0.608	0.531	0.522	0.594	0.555	0.092	0.713	0.271
IL-2	0.062	0.066	0.065	0.062	0.061	0.005	0.591	0.510
IL-4	0.064	0.060	0.059	0.058	0.064	0.005	0.892	0.124
IL-6	0.033	0.035	0.038	0.034	0.041	0.005	0.068	0.715
IL-8	17.78	18.60	18.14	19.28	18.86	1.378	0.407	0.825
IL-10	0.060	0.057	0.053	0.053	0.061	0.006	0.966	0.091
IL-12	0.244	0.272	0.227	0.236	0.268	0.058	0.892	0.533
IL-18	25.61	24.47	25.15	25.33	22.81	1.418	0.245	0.495

[1]Data are least square means of 10 to 15 observations per treatment.

[2]IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; sIgA, secretory immunoglobulin A.

Table 8.8. Frequency of T regulatory cells in the peripheral blood monocyte cell layer of blood collected from pigs fed phase 1 diets with increasing inclusion of spray dried plasma on d 7 post-weaning[1,2]

Item	Basal					Pooled	*P*-value	
Spray dried plasma, %:	0	2	4	6	8	SEM	Linear	Quadratic
$FoxP3^{+}CD25^{+}$ cells	1.55	1.54	1.46	1.57	1.45	0.130	0.629	0.956
$CD8beta^{+}$ T cells	5.08	6.55	5.83	5.97	5.46	0.513	0.848	0.094
$CD8beta^{+}$ $FoxP3^{+}CD25^{+}$ cells	0.04	0.06	0.05	0.05	0.05	0.008	0.904	0.408
$CD8beta^{+}$ $FoxP3^{-}CD25^{+}$ cells	0.01	0.01	0.01	0.01	0.01	0.014	0.956	0.404
$CD4^{+}CD8alpha^{+}$ T cells	2.31	2.13	2.22	2.17	2.61	0.213	0.373	0.185
$CD4^{+}CD8alpha^{+}$ FoxP3+$CD25^{+}$ cells	0.37	0.35	0.31	0.35	0.33	0.364	0.562	0.515
$CD4^{+}CD8alpha^{+}$ $FoxP3^{-}CD25^{+}$ cells	0.01	0.01	0.01	0.01	0.01	0.007	0.549	0.965
$CD4^{+}CD8alpha^{-}$ T cells	21.46	24.96	20.85	20.84	18.43	1.275	0.011	0.095
$CD4^{+}CD8alpha^{-}$ $FoxP3^{+}CD25^{+}$ cells	0.39	0.36	0.32	0.42	0.40	0.434	0.560	0.289
$CD4^{+}CD8alpha^{-}$ $FoxP3^{-}CD25^{+}$ cells	0.12	0.12	0.14	0.13	0.09	0.033	0.421	0.030
Regulatory T cells[3]	1.29	1.31	1.23	1.38	1.26	0.118	0.984	0.942
Activated T cells[4]	0.15	0.15	0.17	0.17	0.13	0.045	0.492	0.090

Table 8.8 (cont.)

Activated:Regulatory T cell	0.12	0.12	0.14	0.12	0.10	0.027	0.460	0.071

[1]Data are least square means of 14, 15, or 16 observations per treatment.

[2]Values were Log10 transformed before analysis to obtain a normal distribution, but data are shown as back-transformed least square means.

[3]Regulatory T cells are the sum of $CD4^{+}CD8alpha^{+}$, $CD4^{+}CD8alpha^{-}$, and $CD8beta^{+}$ with $FoxP3^{+}CD25^{+}$.

[4]Activated T cells are the sum of $CD4^{+}CD8alpha^{+}$, $CD4^{+}CD8alpha^{-}$, and $CD8beta^{+}$ without $FoxP3^{+}CD25^{+}$.

Table 8.9. Differences[1] from baseline for blood parameters of weaned pigs fed phase 1 diets with increasing inclusion of spray dried plasma[2]

Item	Basal					Pooled	*P*-value		Day		Pooled	*P*-value[3]
Spray dried plasma, %:	0	2	4	6	8	SEM	Linear	Quadratic	7	14	SEM	
White blood cells, ×10^3/µl												
Baseline	10.07	11.00	10.73	9.71	9.74							
Difference	7.63	6.73	7.14	8.86	8.70	4.01	0.134	0.420	0.17	15.46	3.96	<0.001
Neutrophils, % of WBC[4]												
Baseline	34.96	38.79	34.31	30.97	33.44							
Difference	4.82	3.95	7.58	13.98	10.22	5.39	0.023	0.780	-0.79	17.01	4.81	<0.001
Lymphocytes, % of WBC												
Baseline	58.31	53.86	60.06	62.10	59.03							
Difference	-5.52	-3.94	-11.14	-14.84	-10.72	5.67	0.009	0.372	0.20	-18.66	5.25	<0.001
Plasma urea N, mg/dL												
Baseline	5.44	6.08	5.33	5.53	6.33							
Difference	9.00	9.97	6.00	7.69	5.19	1.82	0.004	0.747	6.49	8.65	1.60	0.004
Albumin, g/dL												

Table 8.9 (cont.)

Baseline	2.77	2.69	2.90	2.78	2.88							
Difference	0.06	0.04	-0.09	0.01	-0.13	0.15	0.093	0.994	0.11	-0.16	0.13	<0.001
Total protein, g/dL												
Baseline	4.49	4.24	4.58	4.48	4.47							
Difference	0.14	0.28	-0.01	0.11	0.03	0.19	0.227	0.946	0.23	-0.01	0.17	<0.001
Albumin:Globulin												
Baseline	1.64	1.84	1.76	1.68	1.89							
Difference	-0.06	-0.26	-0.15	-0.10	-0.29	0.08	0.125	0.850	-0.08	-0.27	0.06	<0.001

[1]Baseline blood samples were collected the day before pigs were fed the treatment diet. Baseline samples were used to calculate the difference between d 7 and baseline and between d 14 and baseline. The d 7 and d 14 differences were then analyzed using repeated measures of SAS.

[2]Data are least square means of 15 or 16 observations per treatment.

[3]*P*-value was calculated to test the main effect of day on blood parameters.

[4]WBC, white blood cells.

Table 8.10. Baseline plasma amino acid concentrations (μM/mL) of pigs prior to being fed treatment diets with increasing inclusion of spray dried plasma[1]

Item	Basal				
Spray dried plasma, %:	0	2	4	6	8
Indispensable amino acids					
Arg	115.0	105.2	107.6	112.4	114.7
His	32.9	29.2	29.2	31.4	31.4
Ile	224.5	216.1	213.8	235.7	269.9
Leu	276.5	274.5	278.5	306.7	297.9
Lys	366.3	404.3	391.9	386.9	394.5
Met	127.3	130.2	125.1	127.1	121.9
Phe	124.8	129.4	118.6	119.8	129.5
Thr	258.8	276.6	221.9	258.9	270.0
Trp	76.5	83.5	83.7	96.8	91.8
Val	540.1	584.2	530.7	530.3	613.1
Total	2,200	2,233	2,104	2,179	2,329

Table 8.10 (cont.)

Dispensable amino acids					
Ala	1,304.8	1,300.0	1,276.3	1,258.6	1,383.1
Asn	68.3	68.1	73.3	69.8	74.7
Asp	77.9	76.6	72.9	68.5	74.6
Cys	11.2	11.7	12.2	10.2	10.4
Gln	239.8	266.3	259.3	262.7	266.1
Glu	86.6	75.0	76.0	74.8	91.3
Gly	1,027.0	921.9	923.3	840.7	903.6
Pro	998.6	965.8	896.5	935.3	989.0
Ser	147.8	132.3	122.8	134.0	148.2
Tyr	314.0	333.5	342.6	364.7	341.9
Total	4,335	4,206	4,080	4,072	4,353
Total amino acids	6,534	6,439	6,185	6,386	6,682
Ornithine	72.3	72.6	75.8	74.3	80.8
Citrulline	69.8	78.0	79.8	86.5	79.5

[1]Data are least square means of 13 to 16 observations per treatment.

Table 8.11. Differences[1] from baseline of plasma amino acid (AA) concentrations (μM/mL) of pigs fed phase 1 diets with increasing inclusion of spray dried plasma (SDP)[1,2]

Item	Basal					Pooled	*P*-value		Day		Pooled	*P*-
SDP, %:	0	2	4	6	8	SEM	Linear	Quadratic	7	14	SEM	value[3]
Indispensable AA												
Arg	-24.5	-12.7	-13.7	-13.7	-24.2	8.40	0.985	0.151	-38.8	3.3	3.87	<0.001
His	8.7	12.0	9.7	4.7	6.2	2.64	0.106	0.478	4.8	11.7	1.38	<0.001
Ile	176.2	182.5	144.6	71.7	34.2	42.08	<0.001	0.134	117.5	126.1	30.94	0.477
Leu	95.5	122.5	114.2	73.3	86.8	36.39	0.324	0.456	75.7	121.3	26.06	0.002
Lys	-40.0	19.2	-17.1	34.1	4.8	19.64	0.087	0.215	-11.0	11.4	8.81	0.019
Met	-34.7	-30.6	-20.8	-27.5	-24.2	36.83	0.450	0.628	-14.1	-41.0	29.25	<0.001
Phe	33.2	45.7	50.4	55.1	49.3	10.10	0.139	0.272	38.8	54.7	5.56	0.002
Thr	-2.7	0.0	37.5	43.3	42.5	92.64	0.338	0.814	-47.8	96.0	69.12	<0.001
Trp	-25.9	-18.8	-13.6	-16.3	-15.8	5.02	0.146	0.247	-21.0	-15.1	2.26	0.011
Val	180.7	207.9	292.4	206.5	259.0	114.93	0.329	0.523	165.6	293.0	87.29	<0.001
Total	367.4	518.2	601.9	431.2	418.6	251.46	0.973	0.282	273.2	661.8	179.57	<0.001
Dispensable AA												

Table 8.11 (cont.)

Ala	-292.6	-183.4	-145.2	-60.8	-101.7	128.47	0.098	0.477	-135.3	-178.2	81.84	0.423
Asn	-25.4	-22.8	-23.5	-21.9	-26.1	3.53	0.951	0.347	-34.6	-13.3	1.81	<0.001
Asp	-2.5	5.9	-1.4	-12.8	-13.4	12.28	0.233	0.583	-6.6	-3.1	6.41	0.177
Cys	-1.0	-0.8	-0.4	4.3	2.3	1.65	0.004	0.956	-0.8	2.5	1.04	<0.001
Gln	-17.7	20.7	-2.0	18.9	1.5	14.72	0.383	0.172	-9.8	18.3	7.66	<0.001
Glu	52.0	54.8	69.5	60.8	56.9	14.77	0.705	0.458	26.6	91.0	8.45	<0.001
Gly	25.5	172.7	268.1	210.3	79.8	93.63	0.585	0.029	187.5	115.1	52.51	0.273
Pro	-642.5	-580.8	-537.7	-522.9	-635.8	140.90	0.684	0.071	-636.5	-531.3	107.99	<0.001
Ser	-70.1	-46.8	-34.6	-51.8	-70.8	21.70	0.845	0.006	-62.6	-47.0	16.05	<0.001
Tyr	-175.0	-145.9	-140.5	-133.2	-120.1	21.24	0.072	0.708	-174.9	-111.0	8.44	<0.001
Total	-1,158.5	-732.2	-601.8	-502.0	-827.4	211.41	0.182	0.054	-870.0	-658.8	98.46	0.123
Total AA	-782.5	-214.0	-18.3	-70.8	-408.8	377.93	0.361	0.077	-604.1	6.4	223.40	<0.001
Ornithine	-29.1	-26.8	-28.5	-25.5	-30.7	3.90	0.868	0.449	-39.2	-17.0	1.89	<0.001
Citrulline	-35.1	-38.7	-39.2	-44.3	-32.2	6.04	0.989	0.142	-42.6	-33.2	3.54	<0.001

Table 8.11 (cont.)

[1]Baseline blood samples were collected the day before pigs were fed the treatment diet. Baseline samples were used to calculate the difference between d 7 and baseline and between d 14 and baseline. The d 7 and d 14 differences were then analyzed using repeated measures of SAS.

[2]Data are least square means of 14 to 16 observations per treatment.

[3]*P*-value was calculated to test the main effect of day on plasma amino acids.

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CHAPTER 9: Effect of spray dried plasma on oxidative stress, inflammation, and litter performance of sows subjected to heat stress and the subsequent growth performance of pigs weaned from the sows

Abstract

An experiment was conducted to test the hypotheses that 1) during heat stress, sows fed a diet with spray dried plasma (SDP) in late gestation and throughout lactation have improved reproductive and litter performance and reduced inflammation; and 2) pigs weaned from sows fed 0.5% SDP have reduced diarrhea incidence and improved growth performance during the initial 14 d of the nursery period regardless of dietary SDP. On d 107 of gestation, 79 sows were allotted to a randomized complete block design with 4 blocks and 4 treatment groups. Two diets were formulated without or with 0.5% SDP and there were 2 parity groups (young, parity 1 and 2; and mature, parity ≥ 3). Three blood samples were collected from each sow on d 1, 10, and 20 after farrowing. At weaning, 8 or 10 pigs from each sow were split into 2 groups and allotted to a split-plot design with sow treatment as the main plot and nursery diet (a phase 1 diet without or with 6% SDP) as the sub-plot. Results indicated that there was no effect of SDP on number of total pigs born or pigs born alive, but there was a tendency for fewer ($P < 0.10$) mummified pigs born from sows fed the diet with 0.5% SDP than from sows fed the diet without SDP. The percent of low vitality or starved pigs during lactation was less ($P < 0.05$) from sows fed 0.5% dietary SDP compared with sows fed the diet without SDP. Dietary SDP did not influence oxidative stress markers in the plasma of sows, but serum cytokines increased ($P < 0.05$) in sows

fed the diet with 0.5% SDP compared with sows fed the diet without SDP. Pigs weaned from young sows fed no dietary SDP or from mature sows fed no or 0.5% SDP had a greater gain to feed ratio when fed the phase 1 diet with 6% SDP compared with pigs fed the diet without SDP, but the gain to feed ratio of pigs weaned from young sows fed 0.5% dietary SDP was not affected by dietary SDP in phase 1 (interaction, $P < 0.05$). Regardless of sow treatment, pigs fed the phase 1 diet with 6% SDP had greater ($P < 0.05$) growth performance parameters than pigs fed the phase 1 diet without SDP, and pigs fed the phase 1 diet with 6% SDP had reduced ($P < 0.05$) diarrhea incidence in phase 1. In conclusion, feeding 0.5% dietary SDP to sows may reduce the number of mummified pigs and increase pig vitality during lactation, but adding 0.5% SDP to sow diets during lactation did not further improve post-weaning performance of pigs fed a starter diet with 6% SDP.

Key words: cytokines, heat stress, lactation, weanling pigs, spray dried plasma, sows

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
EDTA	ethylenediaminetetraacetic acid
G:F	gain to feed ratio
GPX1	glutathione peroxidase 1
IL-	interleukin-
IFN-γ	interferon-gamma
MDA	malondialdehyde
SDP	spray dried plasma

Abbreviations (cont.)

TNF-α	tumor necrosis factor-α

Introduction

Sows kept in areas with high temperatures are under greater stress compared with sows located in regions with lower average temperatures (Lucy and Safranski, 2017). Increased stress during gestation can result in reduced feed intake, and therefore, the sow may enter a negative energy balance before farrowing (Lucy and Safranski, 2017; Feyera et al., 2018). This can lead to increased number of still born pigs, reduced litter size, and reduced pig birth weight. The inflammatory response of the sow may also increase during farrowing if sows are in negative energy balance (Lucy and Safranski, 2017). Increased stress during early lactation can result in increased oxidative stress (Zhao and Kim, 2020), reduced litter weight gain, reduced pre-weaning survival, and decreased weaning weight of pigs (Crenshaw et al., 2007; Carter et al., 2018).

Sows that are in a high stress environment had greater feed intake and a more efficient immune response if spray dried plasma (**SDP**) was included in the diet (Crenshaw et al., 2021), and inclusion of 0.5 or 2.5% SDP in diets fed to gestating sows 4 d before parturition reduced the number of still born pigs (Crenshaw et al., 2021). Lactating sows fed a diet with 0.5% SDP had increased feed intake and increased weaning weight of pigs (Carter et al., 2018). However, data are lacking on the effect of dietary inclusion of SDP on sow oxidative stress and inflammatory response, especially cytokine synthesis, and subsequent performance of the offspring from sows fed a diet with SDP. Feeding weanling pigs a diet with up to 6% SDP increases daily gain and feed intake during the initial 2 wk post-weaning (van Dijk et al., 2001). However, research to

determine if growth performance of weanling pigs can be further improved if they are weaned from sows fed a diet containing SDP is limited.

Therefore, this experiment was conducted to test the following hypotheses: 1) addition of 0.5% SDP to diets fed to sows subjected to heat stress in late gestation and throughout lactation reduces the number of stillborn pigs, increases litter weight at birth and at weaning, and reduces blood cytokines and biomarkers of oxidative stress throughout lactation; and 2) pigs weaned from sows fed 0.5% SDP have reduced diarrhea incidence and improved growth performance during the initial 14 d of the nursery period regardless of whether or not the diet fed after weaning contains SDP.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Animals, diets, and experimental design

At the University of Illinois, a total of 79 Camborough sows were bred to Line 800 boars (Pig Improvement Company, Hendersonville, TN, USA), and on d 107 of gestation, sows were moved into the lactation facility. Sows were randomly assigned to dietary treatments in a randomized complete block design with date of breeding used as the blocking factor. There were 2 dietary treatments (a diet with 0 or 0.5% SDP) and 2 parity groups (young, parity 1 and 2; and mature, parity ≥ 3); therefore, there were a total of 39 and 40 replicate sows receiving each of the 2 dietary treatments, and between 17 and 23 replicate sows per diet within parity group. The nursery part of the experiment, where weaned pigs remained with their litter mates, used a split-plot design with sow treatment as the main plot and nursery diet (a phase 1 diet with 0 or 6%

SDP) as the sub-plot. Wean group was used as the blocking factor for the nursery experiment, and there were, therefore, 8 treatment groups in the nursery with 15 to 19 replicate pens of 4 or 5 pigs per pen for each of the 4 sow treatment groups.

Spray dried plasma (Appetein B) was sourced from APC Inc., Ankeny, IA, USA, and the same batch was used in the sow and post-weaning diets. Five diets were prepared (Tables 9.1 and 9.2); 2 diets, without or with 0.5% SDP, were fed to sows from d 107 of gestation and until weaning. In addition, 2 phase 1 diets, formulated without or with 6% SDP, were fed to pigs for 14 d after weaning, and 1 common phase 2 diet without SDP was fed to all pigs for an additional 22 days. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement estimates of sows or nursery pigs (NRC, 2012). A sample of the main ingredients and of all diets were collected at the time of diet mixing and these samples were used for chemical analysis.

Feeding and sample collection

Sows were moved from the gestation facility to individual farrowing crates (2.1 × 1.5 m) in the farrowing unit on d 107 of gestation. All sows were subjected to heat stress with the ambient temperature controlled in the lactation facility at 26.6 ± 4.8^{o}C. The humidity was not governed in the facility but recorded at 65.9 ± 13.9%. From d 107 of gestation to farrowing, all sows were fed their assigned diet at 2.5 kg per d, which was provided in 2 equal meals, but prior to each morning feeding, feed left from the previous day was removed and the weight was recorded. After farrowing, sows were fed their assigned diet on an *ad libitum* basis and feed was added to feeders twice daily (at 0615 and 1400 h) until pigs were weaned at 20.9 ± 0.3 d. Feed refusals were collected and weights were recorded on d 10 of lactation and on the day of weaning to calculate average daily feed intake (**ADFI**) from d 1 to 10, d 10 to weaning, and for the overall

lactation period. Sows were weighed at the beginning of the experiment, on d 1 and d 10 of lactation, and on the day of weaning to calculate average daily gain (**ADG**).

Respiration rates (breaths per minute) were measured for all sows by measuring the number of flank movements per minute. Measurements were collected manually using a digital stopwatch and tally counter by one observer every 2 d (between 0900 and 1000 h) from when the sow moved into the lactation facility and until weaning. Sows were induced to farrow on d 114 of gestation and data for litter performance were recorded. On d 1 of lactation, the number of total born pigs, pigs born alive, stillborn pigs, and mummified pigs was recorded from each sow. Pigs underwent routine processing, including umbilical cord and needle teeth clipping, tail docking, castration of male pigs, administration of iron dextran (Uniferon 200, Pharmacosmos A/S, Holbaek, Denmark) and centiofur antibiotic (Excede, Zoetis, Parsippany, NJ, USA), and ear notching within 24 h after birth. Cross-fostering was completed immediately after processing and only within treatments groups to normalize litter size between sows based on teat capacity. Following normal farm procedures, pigs weighing less than 0.8 kg at birth were euthanized. Weights of pigs that died during the lactation period, as well as the reason for death (i.e., crushed by sow, low vitality/starved, or rupture) were recorded. All pigs were weighed on d 10 of lactation and the day prior to weaning. All litters were offered a standard creep feed with 4.75% SDP at d 13 post-farrowing, according to normal farm procedures. Creep feed disappearance was measured by recording the amount provided each day and the amount left in the feeder on the day of weaning. On d 1 and 10 of lactation, and at weaning, the individual weight of all pigs was recorded.

At weaning, pigs remained with their litter mates and 8 or 10 pigs from each sow, depending on the number of pigs weaned, were randomly selected and moved to the nursery

facility. Pigs were then allotted to the phase 1 diet without or with 6% SDP and housed in mixed sex pens in groups of 4 or 5 pigs per pen, and sex was balanced within treatments. Pigs were fed the phase 1 diets for 14 d post-weaning and all pigs were supplemented with Gentamicin Sulfate (Bimeda Inc., Le Sueur, MN, USA) via the water supply for 3 d at a dosage of 0.5 mg/lb per d starting on d 7 post-weaning due to an outbreak of rotavirus in the facilities. Pigs were then fed the common phase 2 diet without SDP for an additional 22 d. All pigs were allowed *ad libitum* access to feed and water. Diarrhea scores were assessed visually every other day for 36 d by 2 independent observers using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Diarrhea frequency was calculated by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100. Individual pig weights were recorded at the beginning of the nursery period and on d 7, 14, and 36. Daily feed allotments were recorded and feed left in the feeders was weighed on d 7, 14, and 36.

Blood sampling and chemical analysis

Three blood samples were collected from the jugular vein of each sow on d 1 and 10 after farrowing, and at weaning. Two of the 3 blood samples were collected in vacutainers with ethylenediaminetetraacetic acid (**EDTA**). These samples were stored on ice immediately after collection and 1 of the EDTA vacutainers was delivered to the University of Illinois Veterinary Diagnostic Laboratory for counts of white blood cells, neutrophils, and lymphocytes. The second EDTA vacutainer was centrifuged at 4,000 × g for 13 min to recover the plasma and stored at -20^{o}C until analyzed for malondialdehyde (**MDA**) and glutathione peroxidase 1 (**GPX1**) using ELISA kits according to the recommendations from the manufacturer (MyBioSource, Inc., San Diego, CA, USA). The third blood sample was collected in vacutainers without EDTA and blood

serum was obtained from this sample by centrifugation at 1,500 × g at 4°C for 15 min. Serum samples were stored at -20°C until analysis for the following cytokines: interferon-gamma (**IFN-γ**), interleukin- (**IL-**) 1α, IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor-α (**TNF-α**). Cytokines were analyzed using a MILLIPLEX kit (EMD Millipore Corporation, Billercia, MA, USA) in a MagPix instrument with ProcartaPlex-multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

All diets and the SDP ingredient were analyzed in duplicate for concentrations of gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and for N by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as N × 6.25. Dry matter was analyzed in all diets and in SDP by oven drying at 135°C for 2 h (method 930.15, AOAC Int., 2019) and dry ash was analyzed as well (method 942.05; AOAC Int., 2019). Concentrations of Ca and P in diets and SDP were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600°C for 4 h (method 985.01 A, B and C; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). All diets and SDP were also analyzed for insoluble- and soluble-dietary fiber (method 991.43; AOAC Int., 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA), and total dietary fiber was calculated as the sum of insoluble- and soluble-dietary fiber. Acid hydrolyzed ether extract was analyzed using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY, USA). At the Agricultural Experiment Station Chemical Laboratories at the University of

Missouri (Columbia, MO, USA), all diets and the SDP ingredient were analyzed for amino acids on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int., 2019], and all diets were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int., 2019).

Calculations and statistical analysis

At the conclusion of the experiment, data for sow body weight (**BW**) loss, ADFI, and pig mortality during lactation (calculated as the percentage of live born pigs that died before weaning after adjusting for cross-fostering) were calculated. Total live litter birth weight, live litter birth weight after cross fostering, litter weight on d 10 of lactation and at weaning, and litter ADG were calculated as well. Average pig weights at birth, at d 10 of lactation, and at weaning were calculated, as well as pig ADG during lactation and creep feed disappearance. During the nursery period, data collected for pig weights and feed allowance were summarized and ADG, ADFI, and gain to feed ratio (**G:F**) were calculated for each pen and sow treatment group.

Normality of residuals were verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures of SAS, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if the value deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). Data analysis was carried out in a split-plot design with sow treatment (diet within parity) as the main plot and nursery diet as the sub-plot. For the sow portion of the experiment, data were analyzed as a 2 × 2 factorial arrangement of treatments using SAS PROC GLIMMIX with negative binomial distribution for data related to pig mortality or Poisson distribution for data related to number of pigs per litter, and PROC MIXED was used for all other data analyses. The sow was the experimental unit for all analyses and date of

breeding was the blocking factor. For both PROC GLIMMIX and PROC MIXED, the statistical model included the fixed effects of diet, parity, and parity by diet interaction and the random effect of block. Blood samples were collected from the same sows during the experiment, therefore, data for blood analyses were analyzed as repeated measures using the PROC MIXED procedure of SAS. The model included diet, parity group, and day, and all 2- and 3-way interactions as main effects, day as the time effect, and sow as the subject. However, the interactions between diet and day, parity and day, and diet, parity, and day were not significant, therefore, contrast statements were used with coefficients for equally spaced treatments to determine linear and quadratic effects of day on blood variables. For the nursery part of the experiment, wean group was the blocking factor and pen was the experimental unit for all analyses. The model included the fixed effect of sow treatment (main plot), nursery diet (sub-plot), and the interaction between sow treatment and nursery diet and the random effect of block and block by sow treatment.

Treatment means were estimated for each mortality-related variable using the LSMEANS statement with the inverse link option in PROC GLIMMIX. Data for plasma MDA and GPX1 and serum cytokines were transformed using base-10 log prior to analysis in PROC MIXED to obtain a normal distribution. Treatment means were reported for all other variables using the LSMEANS statement in PROC MIXED, and if an interaction was significant, means were separated using the PDIFF option. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Four sows were removed from the experiment: 2 young sows fed the diet with 0.5% SDP were removed before farrowing; and 1 mature sow fed the diet with 0.5% SDP and 1 young sow fed the diet without SDP were removed within 2 d after farrowing.

Part 1: Lactation

Respiration rates measured for young sows fed the diet with 0.5% SDP were greater 7 d prior to farrowing compared with young sows fed the diet without SDP (Figure 9.1), but respiration rates for mature sows were not influenced by diet (interaction, $P < 0.05$). Respiration rates 5 d after farrowing were not influenced by diet for either sow parity group, but mature sows fed the diet without SDP had greater respirations per minute than young sows fed the diet without SDP, whereas when SDP was included in the diet, no difference between young and mature sows was observed (interaction, $P < 0.05$).

The parity of young sows was less ($P < 0.05$) than the parity of mature sows (Table 9.3), but within the 2 parity groups, parity did not differ between dietary treatments. Body weight of sows was not affected by dietary treatment at the initiation of the experiment, at farrowing, or at weaning. However, mature sows had greater ($P < 0.05$) BW throughout the experiment compared with young sows. From d 10 to 20 of lactation, young sows fed the diet with 0.5% SDP had less BW loss compared with young sows fed the diet without SDP, but BW loss for mature sows was not affected by diet (interaction, $P < 0.05$). During the entire lactation period (d 1 to 20), sows fed the diet with 0.5% SDP tended to have less ($P < 0.10$) BW loss than sows fed the diet without SDP, regardless of parity. Mature sows had less ($P < 0.05$) BW loss than young sows from d 1 to 10 and from d 1 to 20 of lactation. The ADFI of sows was not affected by diet, but the ADFI of mature sows was greater ($P < 0.05$) than the ADFI of young sows.

There was no effect of diet on number of total pigs born or pigs born alive (Table 9.4), but there was a greater ($P < 0.05$) number of total pigs born from mature sows compared with young sows. There was a tendency for a reduced number of still born pigs from young sows fed the diet without SDP than with SDP, but the number of still born pigs from mature sows did not differ between treatments (interaction, $P < 0.10$). There was a tendency for fewer ($P < 0.10$) mummified pigs born from sows fed the diet with 0.5% SDP compared with sows fed the diet without SDP, regardless of parity. No differences between treatments were observed for total litter weight or individual pig weight at birth, but pigs born from sows fed the diet without SDP tended to have greater ($P < 0.10$) BW at weaning and had greater ($P < 0.05$) ADG during lactation compared with pigs born from sows fed the diet with 0.5% SDP. Creep feed disappearance during lactation was greater ($P < 0.05$) for pigs from mature sows fed the diet without SDP compared with sows fed the diet with 0.5% SDP, but creep feed disappearance did not differ between treatments for young sows (interaction, $P < 0.05$). Dietary treatment did not affect the proportion of pigs from mature sows that died before weaning, but young sows fed the diet with 0.5% SDP tended to have reduced pig mortality compared with mature sows (interaction, $P < 0.10$). There was a tendency for increased ($P < 0.10$) percent of pigs crushed by mature sows compared with young sows, and there was a greater ($P < 0.05$) percent of low vitality or starved pigs from mature sows than young sows, but the percent of low vitality or starved pigs was less ($P < 0.05$) from sows fed the diet with 0.5% SDP than from sows fed the diet without SDP, regardless of parity group.

There were no interactions between sow treatment and day for any of the analyzed blood parameters (Table 9.5). White blood cell counts did not differ between dietary treatment groups, regardless of sow parity group, but young sows fed the diet with 0.5% SDP had greater white

blood cell counts than mature sows fed the diet with 0.5% SDP (interaction, $P < 0.05$). Neutrophils as a percent of white blood cells were greater ($P < 0.05$) in mature sows than young sows, whereas lymphocytes as a percent of white blood cells were less ($P < 0.05$) in mature sows than young sows. No difference was observed for the oxidative stress marker MDA among treatments, whereas, GPX1 was less ($P < 0.05$) in mature sows than young sows. All serum cytokines increased ($P < 0.05$) in sows fed the diet with 0.5% SDP compared with sows fed the diet without SDP, and the concentration of all cytokines, except IFN-γ, was greater ($P < 0.05$) in mature sows compared with young sows. The day when blood samples were collected influenced blood parameters, regardless of sow parity or diet. Concentrations of white blood cells, MDA, and GPX1 increased from d 1 to d 10 and from d 10 to d 20 of lactation (quadratic, $P < 0.05$). Serum concentrations of IL-1α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNF-α linearly increased ($P < 0.05$) from farrowing to weaning.

Part 2: Nursery

Pigs weaned from mature sows fed the diet without SDP had greater ADG from d 7 to 14 post-weaning if they were fed the SDP diet than the diet without SDP (Table 9.6), but the ADG of pigs weaned from the other sow groups was not influenced by post-weaning diet (interaction, $P < 0.10$). In phase 1, pigs weaned from young sows fed the diet without SDP or from mature sows fed the diet without or with 0.5% SDP had greater G:F when fed the diet with 6% SDP compared with pigs fed the diet without SDP, but the G:F of pigs weaned from young sows fed the diet with 0.5% SDP was not affected by phase 1 dietary treatment (interaction, $P < 0.05$). In phase 2, pigs weaned from young sows fed the diet with 0.5% SDP had reduced G:F if fed a phase 1 diet with SDP compared with pigs fed a phase 1 diet without SDP, whereas phase 1 diet did not affect G:F of pigs weaned from the other sow treatments (interaction, $P < 0.05$). Regardless of

sow treatment, pigs fed the phase 1 diet with 6% SDP had greater ($P < 0.05$) ADG, ADFI, and BW at the end of phase 1 and greater ($P < 0.05$) ADFI and final BW at the end of phase 2 than pigs fed the phase 1 diet without SDP. During the overall nursery period, pigs fed the phase 1 diet with 6% SDP had greater ($P < 0.05$) ADG and ADFI than pigs fed the phase 1 diet without SDP, but the overall G:F was not affected by phase 1 diet.

Pigs weaned from mature sows had less diarrhea in phase 2 when fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP (Table 9.7), but the incidence of diarrhea in phase 2 for pigs weaned from young sows was not affected by phase 1 dietary treatment (interaction, $P < 0.05$). Regardless of sow treatment, pigs fed the phase 1 diet with 6% SDP had reduced ($P < 0.05$) diarrhea in phase 1 and phase 2 compared with pigs fed the phase 1 diet without SDP. The frequency of diarrhea in phase 2 and overall was less ($P < 0.05$) for pigs fed the phase 1 diet with 6% SDP compared with pigs fed the phase 1 diet without SDP.

Discussion

Pigs lack functional sweat glands and are, therefore, inefficient in thermoregulation and highly susceptible to heat stress (Cottrell et al., 2015). Heat stress costs the United States swine industry approximately $299 million per year (St-Pierre et al., 2003), with loss in sow productivity alone estimated at $113 million per year (St-Pierre et al., 2003). The temperature used in the current experiment to stimulate heat stress is in agreement with published data, where sows used in heat stress experiments were housed at temperatures between 27 to 32ºC (Bjerg et al., 2020). Physiological responses to heat stress include increases in respiration rate and body temperature, with respiration rate being a more sensitive indicator of heat stress than body temperature (Lucy and Safranski, 2017). Sows housed in a thermoneutral environment generally had a respiration

rate of approximately 30 breaths per minute, whereas the respiration rate of heat stressed sows ranged between 50 and 80 breaths per minute (Lucy and Safranski, 2017). Respiration rates measured in the current experiment are in agreement with the values by Lucy and Safranski (2017).

Heat stress is also characterized by decreased feed intake of sows (Lucy and Safranski, 2017), leading to greater weight loss, which can have a negative effect on farrowing and litter performance (Bjerg et al., 2020). Sows of parity 1 and 2 fed a diet with 0.25 or 0.50% SDP had increased feed intake during summer months compared with sows fed a diet without SDP (Crenshaw et al., 2007), but data from the current experiment are in agreement with Carter et al. (2018) who reported that feed intake of parity 1 to 3 sows was not affected by dietary SDP. During lactation, sows often need to mobilize body reserves to support milk production, leading to increased weight loss of the sow (Kim et al., 2021). Results from the current experiment indicating that weight loss tended to be reduced during lactation of sows fed dietary SDP is in agreement with previous data (Kim et al., 2021). Because there was no impact of SDP on ADFI of sows, it is speculated that addition of SDP to the diets resulted in improved digestibility or utilization of energy or nutrients in the diets. Inclusion of SDP in diets for weanling pigs increases digestibility and absorption of nutrients (Pendergraft et al., 1993; Zhang et al., 2015), but this has not been demonstrated for sows.

Crenshaw et al. (2007) reported decreased still born pigs from sows fed 0.5 or 2.5% SDP. Litter size, pig birth weight, sow BW, and sow parity can impact the number of still born pigs (Crenshaw et al., 2021), and as sow parity increases the probability of still born pigs increases (Le Cozler et al., 2002), which was also observed in the current experiment. The observation that ADG of pigs during lactation decreased when sows were fed 0.5% dietary SDP is a result of the

numerical increase in the number of pigs weaned from sows fed SDP, because litter daily gain was not influenced by SDP. This is in contrast with results indicating an increase in growth rate of pigs during lactation from sows fed a diet supplemented with 1% SDP (Kim et al., 2021). Vitality of pigs from sows fed dietary SDP in the current experiment was increased, and Kim et al. (2021) indicated that milk production increased, and thus milk consumption by pigs increased, if sows were fed a diet containing SDP. However, milk production from sows was not measured in either experiment. Overall, our data are in agreement with Frugé et al. (2009) and Carter et al. (2018) indicating limited effects of dietary SDP on litter performance, but further research is needed to elucidate the mechanisms of improved pig vitality during lactation from sows fed dietary SDP.

Spray dried plasma contains immunoglobulins that are hypothesized to have immunomodulatory effects on pigs, which is more important during periods of increased stress than in periods without stress (Torrallardona, 2010). Including 1% SDP in diets fed to sows during late gestation and throughout lactation reduced serum TNF-α 7 d after farrowing (Kim et al., 2021), although data from Crenshaw et al. (2021) indicated no influence of 0.5 or 2.5% dietary SDP on serum cytokines for sows before or after farrowing. Thus, results from the current experiment indicating that both pro- and anti-inflammatory serum cytokines increased for sows fed 0.5% dietary SDP are in contrast with previous data (Crenshaw et al., 2021). Age is a significant factor affecting cytokine production, and serum cytokines were greater for mature sows than young sows, which is in agreement with de Groot et al. (2005), but pigs have high individual variation in cytokine production (de Groot et al., 2005). Cytokines are secreted by innate immune cells, such as white blood cells, in response to various stimuli related to inflammation and infection in the animal (Burger and Dayer, 2002), but this is unlikely to have

influenced concentrations of cytokines because white blood cells were not affected by dietary SDP. Increased cytokine production activates the immune system, which increases nutrient requirements to maintain immune cell synthesis and leaves fewer nutrients available for growth of the animal (Goodband et al., 2014; Campbell et al., 2019), but sow performance in the current experiment was not reduced due to dietary SDP. There was, therefore, no negative impact of the increased concentration of cytokines on sow performance.

Spray dried plasma is often included in diets for weanling pigs and at greater concentrations in the diet compared with sow diets (Torrallardona, 2010). Including SDP in diets fed to weanling pigs improves growth performance of pigs and reduces diarrhea incidence (Peace et al., 2011; Balan et al., 2021), and data from the current experiment are in agreement with these observations. Dietary SDP is usually included in diets for weanling pigs during the initial 1 to 2 wk post-weaning, because improvements in growth performance are not significant thereafter (van Dijk et al., 2001). Thus the increased growth performance in phase 1 and the increased ADG for the overall experimental period is in agreement with previous data. The observation that final BW at the end of phase 2 was greater for pigs fed the phase 1 diet with SDP compared with pigs fed the phase 1 diet without SDP demonstrates that the benefits of feeding SDP in phase 1 were maintained in phase 2.

The effect of supplementing sow diets with SDP on the subsequent performance of their offspring post-weaning has only recently been evaluated (Kim et al., 2021). Pigs fed diets without SDP and weaned from sows fed 1% SDP had greater ADG than pigs weaned from sows fed a diet without SDP (Kim et al., 2021). Thus, the numerical increase in post-weaning ADG observed in the current experiment for pigs fed a diet without SDP and weaned from sows fed SDP compared with pigs weaned from sows fed a diet without SDP is in agreement with the

results from Kim et al. (2021). However, the observation that ADG did not differ between pigs fed a diet without SDP and with SDP when weaned from sows fed SDP, indicates that inclusion of SDP in lactation diets improved performance of pigs after weaning, which is in agreement with previous data (Kim et al., 2021). The reason for these changes in weanling pig performance may be that sows fed a diet containing SDP during lactation may secrete more immune cells in milk and thereby prepare pigs for the stress of weaning. However, research to address this hypothesis needs to be conducted.

Diarrhea caused by bacterial and viral pathogens is a vital problem post-weaning, leading to economic losses due to pig mortality, morbidity, and decreased efficiency (Wang et al., 2011). The inclusion of SDP in post-weaning diets has been reported to reduce diarrhea incidence and improve intestinal function of weanling pigs (Campbell et al., 2010; Torrallardona, 2010), and the observation that, regardless of SDP inclusion in the sow diet, pigs fed SDP post-weaning had reduced diarrhea incidence is in agreement with Torrallardona (2010). Dietary SDP is also more effective in improving intestinal function and performance of pigs when housed in an environment undergoing a sanitation challenge (Campbell et al., 2010). The reduction in diarrhea incidence observed for pigs fed SDP was carried over into the subsequent phase when dietary SDP was not fed to pigs, indicating that SDP maintained the function of the intestinal barrier of pigs challenged with rotavirus, which is in agreement with Corl et al. (2007). Increasing feed intake and limiting weight loss of the sow during lactation can result in improvements in milk yield, which benefits litter performance (Alexopoulos et al., 2004). Additionally, including probiotics in sow diets has been reported to modify the intestinal ecosystem of the pigs, resulting in less incidence of diarrhea and improvements in intestinal barrier function post-weaning (Hayakawa et al., 2016). However, data on the effect of SDP fed to sows on their pigs post-

weaning performance have not been previously reported. Thus the observation that diarrhea incidence did not differ between pigs fed a diet without SDP and with SDP when weaned from young sows fed SDP, indicates that inclusion of SDP in lactation diets improved the intestinal barrier function of pigs after weaning.

In conclusion, feeding 0.5% dietary SDP to sows may reduce number of mummified pigs born and increase pig vitality during lactation, but this does not appear to be a result of an increase in circulating cytokines of sows fed 0.5% dietary SDP. Feeding a diet with 0.5% SDP to sows during lactation did not improve post-weaning performance of pigs, but 6% dietary SDP improved growth performance parameters and decreased diarrhea incidence of pigs.

Tables

Table 9.1. Ingredient composition of experimental diets

Item, %	Lactation period			Nursery period		
	Basal		Creep feed	Phase 1		Phase 2
Spray dried plasma, %:	0.0	0.5	4.75	0.0	6.0	0.0
Spray dried plasma	–	0.50	4.75	–	6.00	–
Corn, ground	67.41	66.97	41.97	40.65	42.81	48.30
Soybean meal, 45% crude protein	25.00	25.00	22.00	25.00	25.00	25.00
Soybean hulls	10.00	10.00	–	–	–	–
Whey powder, dried	–	–	25.00	20.00	20.00	15.00
Soy protein concentrate	–	–	–	8.00	–	5.00
Soybean oil	4.00	4.00	–	3.10	3.10	3.50
Choice white grease	–	–	2.00			
Limestone, ground	0.78	0.81	1.35	0.95	1.20	0.99
Dicalcium phosphate	1.69	1.65	0.45	1.10	0.80	1.00
Sodium chloride	0.40	0.40	0.20	0.10	0.10	0.10

Table 9.1 (cont.)

Choline	0.10	0.10	–	–	–	–
L-Lys HCl	0.11	0.07	0.35	0.38	0.29	0.36
DL-Met	–	–	0.15	0.12	0.15	0.16
L-Thr	0.01	–	0.08	0.10	0.05	0.09
Zinc oxide	–	–	0.40	–	–	–
Vitamin mineral premix[1]	0.50	0.50	–	0.50	0.50	0.50
Vitamin premix[2]	–	–	0.20	–	–	–
Trace mineral premix[3]	–	–	0.35	–	–	–
Pulmotil 18[4]	–	–	0.75	–	–	–

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,136 mg; vitamin D_3 as cholecalciferol, 2,208 mg; vitamin E as DL-alpha tocopheryl acetate, 66 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 9.1 (cont.)

[2]The vitamin premix provided the following quantities of vitamins per kg of complete diet: vitamin A, 681,818 mg; vitamin D_3, 68,181 mg; vitamin E, 9,091 mg; vitamin K, 454.5 mg; vitamin B_{12}, 3.64 mg; riboflavin, 909.1 mg; d-pantothenic acid, 2,500 mg as calcium pantothenate; niacin, 3,409 mg; and choline, 29,523 mg.

[3]The trace mineral premix provided the following quantities of minerals per kg of complete diet: Fe, 257 mg as ferrous sulfate; Zn, 286 mg as zinc sulfate; Mn, 5,710 mg as manganous oxide; Cu, 2,290 mg as copper sulfate; I, 100 mg as calcium iodate; and Se, 85.7 mg as sodium selenite.

[4]Pulmotil® 18, tilmicosin phosphate, sourced from Elanco Animal Health, Indianapolis, IN, USA.

Table 9.2. Analyzed nutrient composition of experimental diets, creep feed, and spray dried plasma (as-fed basis)

Item	Lactation period			Nursery period			
	Basal		Creep feed	Phase 1		Phase 2	Spray dried
Spray dried plasma, %:	0.0	0.5	4.75	0.0	6.0	0.0	plasma
Dry matter, %	88.15	87.93	89.39	88.43	88.16	88.60	89.53
Crude protein, %	16.72	16.87	20.37	22.84	22.34	19.47	80.91
Ash, %	5.47	5.42	6.73	6.02	5.74	5.56	6.92
Acid hydrolyzed ether extract, %	4.78	5.65	3.40	4.16	4.31	3.96	2.39
Gross energy, kcal/kg	3,956	3,972	3,886	3,939	3,976	3,957	4,710
Insoluble dietary fiber, %	17.20	17.90	9.10	9.80	9.50	10.40	1.50
Soluble dietary fiber, %	1.30	1.20	0.40	1.20	0.80	0.90	0.50
Total dietary fiber, %	18.50	19.30	9.50	11.00	10.30	11.30	2.00
Starch, %	26.09	27.12	29.25	26.76	28.28	31.38	–
Ca, %	0.79	0.72	1.44	1.20	1.17	1.23	0.13
P, %	0.70	0.65	0.97	1.09	1.14	1.05	1.52

Table 9.2 (cont.)

Indispensable amino acids, %							
Arg	1.06	1.08	1.09	1.45	1.32	1.18	4.45
His	0.44	0.45	0.49	0.58	0.58	0.49	2.35
Ile	0.73	0.74	0.86	1.04	0.93	0.90	2.41
Leu	1.40	1.45	1.69	1.91	1.93	1.64	7.18
Lys	1.04	1.04	1.55	1.61	1.65	1.42	7.05
Met	0.26	0.25	0.39	0.43	0.46	0.42	0.93
Phe	0.82	0.85	0.90	1.11	1.07	0.92	4.08
Thr	0.65	0.67	0.98	0.99	1.07	0.83	4.89
Trp	0.17	0.20	0.28	0.30	0.33	0.26	1.57
Val	0.81	0.84	1.05	1.11	1.16	0.97	5.50
Total	7.38	7.57	9.28	10.53	10.50	9.03	40.41
Dispensable amino acids, %							
Ala	0.82	0.85	0.94	1.09	1.08	0.93	3.75
Asp	1.70	1.74	1.95	2.41	2.28	1.99	7.75
Cys	0.27	0.28	0.38	0.36	0.46	0.31	2.66

Table 9.2 (cont.)

Glu	2.90	2.99	3.22	4.06	3.74	3.41	10.65
Gly	0.73	0.75	0.71	0.89	0.83	0.75	2.68
Pro	0.96	1.00	1.09	1.30	1.26	1.11	4.04
Ser	0.73	0.78	0.82	1.01	1.06	0.78	4.41
Tyr	0.56	0.58	0.64	0.76	0.79	0.64	3.88
Total	8.67	8.97	9.75	11.88	11.50	9.92	39.82
Total amino acids, %	16.05	16.54	19.03	22.41	22.00	18.95	80.23

Table 9.3. Performance of young and mature sows fed a diet without or with 0.5% spray dried plasma during lactation[1]

Item								
Parity[2]:	Young		Mature		Pooled	*P*-value[3]		
Spray dried plasma, %:	0.0	0.5	0.0	0.5	SEM	D	P	D × P
Parity	1.52	1.48	4.35	4.44	0.20	0.928	<0.001	0.746
Body weight, kg								
d 107 gestation	227	224	254	246	3.93	0.142	<0.001	0.632
d 1 lactation	214	210	240	235	4.30	0.274	<0.001	0.930
d 10 lactation	205	202	234	234	5.84	0.648	<0.001	0.712
d 20 lactation	193	194	226	222	4.61	0.740	<0.001	0.706
Average daily gain, kg								
d 1 to 10 lactation	-0.92	-0.86	-0.60	-0.13	0.27	0.114	0.002	0.236
d 10 to 20 lactation	-1.37[b]	-0.78[a]	-0.98[ab]	-1.05[ab]	0.32	0.031	0.624	0.006
d 1 to 20 lactation	-1.07	-0.83	-0.72	-0.58	0.12	0.065	0.005	0.612
Average daily feed intake, kg								
d 1 to 10 lactation	3.92	4.14	4.50	4.63	0.28	0.301	0.003	0.790

Table 9.3 (cont.)

d 10 to 20 lactation	5.45	5.23	6.05	6.13	0.17	0.697	<0.001	0.380
d 1 to 20 lactation	4.68	4.69	5.28	5.43	0.18	0.590	<0.001	0.649

[1]Data are least square means of 15 to 21 observations per treatment.

[2]Young, parity 1 and 2; and mature, parity ≥ 3.

[3]*P*-values were calculated for the main effects of diet (D) and parity (P) and the interaction between diet and parity (D × P).

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 9.4. Performance of litters from young and mature sows fed a diet without or with 0.5% spray dried plasma during lactation[1]

Item								
Parity[2]:	Young		Mature		Pooled	*P*-value[3]		
Spray dried plasma, %:	0.0	0.5	0.0	0.5	SEM	D	P	D × P
Pigs per litter, n								
Total born	14.52	14.62	17.56	16.33	0.95	0.581	0.014	0.509
Born alive	14.00	13.86	15.88	14.56	0.90	0.434	0.158	0.538
After cross-fostering	14.33	14.05	15.00	15.00	0.89	0.869	0.365	0.869
Still born	0.31^{z}	0.65^{yz}	1.14^{y}	0.81^{yz}	0.26	0.504	0.011	0.071
Mummified	0.19	0.05	0.47	0.13	0.11	0.052	0.177	0.965
At d 10 of lactation	12.43	12.76	12.76	12.81	0.83	0.818	0.815	0.862
At weaning	12.24	12.85	12.24	12.44	0.83	0.625	0.805	0.808
Litter weight, kg								
Live at birth	19.11	19.63	21.52	19.93	0.90	0.506	0.095	0.199
After cross-fostering	19.72	20.01	20.33	20.70	0.76	0.570	0.262	0.950
At d 10 of lactation	44.14	44.85	42.91	43.51	1.83	0.586	0.285	0.965

Table 9.4 (cont.)

At weaning	69.49	70.14	67.90	67.24	3.08	0.998	0.254	0.739
Litter average daily gain, kg	2.48	2.50	2.48	2.33	0.13	0.425	0.306	0.310
Creep feed disappearance[4], g/d	72.35^{ab}	100.98^{a}	102.43^{a}	69.04^{b}	35.46	0.823	0.931	0.007
Individual pig weight, kg								
Live at birth	1.37	1.44	1.42	1.41	0.05	0.608	0.797	0.428
At d 10 of lactation	3.49	3.47	3.51	3.41	0.08	0.454	0.881	0.622
At weaning	5.60	5.49	5.76	5.45	0.13	0.092	0.612	0.421
Pig average daily gain, kg	0.21	0.20	0.22	0.20	0.01	0.037	0.649	0.539
Pig mortality[5], %								
Died prior to weaning	14.74^{yz}	9.10^{z}	18.37^{y}	19.24^{y}	3.30	0.162	0.002	0.088
Crushed by sow	7.35	5.63	8.01	10.90	2.12	0.905	0.080	0.176
Low vitality/starved	6.11	3.00	8.96	6.21	2.17	0.034	0.030	0.513
Rupture	0.33	0.34	0.78	0.83	0.47	0.963	0.312	0.981

[1]Data are least square means of 15 to 21 observations per treatment.

[2]Young, parity 1 and 2; and mature, parity ≥ 3.

[3]*P*-values were calculated for the main effects of diet (D) and parity (P) and the interaction between diet and parity (D × P).

Table 9.4 (cont.)

[4]Creep feed was fed from d 13 of lactation until weaning.

[5]Mortality was calculated as the percentage of live born pigs that died before weaning after adjusting for cross-fostering.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($P < 0.10$).

Table 9.5. Blood cell counts, indicators of oxidative stress, and serum immune response parameters of young and mature sows fed a diet without or with 0.5% spray dried plasma during lactation[1,2]

Item														
Parity[3]:	Young		Mature		Pooled	*P*-value[4]			Day of lactation			Pooled	*P*-value[5]	
SDP, %:	0.0	0.5	0.0	0.5	SEM	D	P	D × P	1	10	20	SEM	Linear	Quadratic
WBC, ×10^3/µl	12.19[ab]	13.06[a]	11.82[ab]	10.72[b]	0.58	0.799	0.003	0.031	10.29	13.30	12.26	0.50	<0.001	<0.001
Neutrophils[6]	51.43	52.51	56.87	56.24	1.72	0.893	0.010	0.622	53.32	53.84	55.62	1.43	0.240	0.716
Lymphocytes[6]	39.18	36.43	31.81	33.80	1.57	0.808	0.002	0.135	36.12	35.74	34.06	1.27	0.230	0.672
Oxidative stress[7]														
MDA, nmol/mL	1.45	1.64	1.49	1.46	0.16	0.282	0.388	0.160	1.26	1.69	1.62	0.16	<0.001	0.005
GPX1, ng/mL	0.43	0.41	0.35	0.31	0.04	0.453	0.019	0.777	0.32	0.42	0.37	0.03	0.013	<0.001
Cytokines[7], ng/mL														
IFN-γ	34.78	67.02	36.84	68.75	15.67	0.002	0.837	0.938	44.51	52.12	51.62	11.32	0.003	0.053
IL-1α	0.33	0.75	0.69	1.22	0.16	<0.001	<0.001	0.506	0.62	0.69	0.72	0.08	<0.001	0.499
IL-1β	1.64	4.77	3.88	8.12	1.29	<0.001	0.002	0.465	3.83	3.99	4.07	0.55	0.110	0.729
IL-1Ra	1.86	4.23	3.90	7.22	1.03	<0.001	<0.001	0.552	4.63	3.35	3.70	0.62	0.002	<0.001
IL-2	1.78	4.63	3.80	7.23	1.03	<0.001	0.002	0.421	3.55	4.00	4.12	0.49	<0.001	0.198
IL-4	9.60	28.93	27.46	62.19	10.77	<0.001	<0.001	0.583	22.73	27.59	28.81	4.23	<0.001	0.105
IL-6	0.97	2.14	1.90	4.35	0.58	<0.001	0.001	0.918	1.90	2.05	2.17	0.21	0.001	0.800

Table 9.5 (cont.)

IL-8	0.17	0.23	0.20	0.47	0.06	0.003	0.031	0.173	0.42	0.18	0.19	0.03	<0.001	<0.001
IL-10	4.37	9.93	8.46	17.73	2.30	<0.001	0.002	0.835	8.53	8.91	9.53	0.90	0.015	0.755
IL-12	1.76	3.28	2.91	5.51	0.66	<0.001	0.002	0.961	2.91	3.12	3.27	0.35	<0.001	0.661
IL-18	7.25	17.46	15.11	32.21	4.49	<0.001	0.001	0.762	14.42	16.08	16.86	1.97	<0.001	0.354
TNF-α	0.13	0.37	0.24	0.69	0.13	<0.001	0.041	0.985	0.28	0.30	0.31	0.05	0.050	0.907

[1]Data are least square means of 13 to 21 observations per treatment.

[2]GPX1, glutathione peroxidase 1; IFN-γ, interferon-gamma; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; MDA, malondialdehyde; SDP; spray dried plasma; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

[3]Young, parity 1 and 2; and mature, parity ≥ 3.

[4]*P*-values were calculated for the main effects of diet (D) and parity (P) and the interaction between diet and parity (D × P).

[5]*P*-values were calculated to test the linear and quadratic effects of day.

[6]Neutrophils and lymphocytes are a % of white blood cells measured in the whole blood.

[7]Values were Log10 transformed before analysis to obtain a normal distribution, but data are shown as back-transformed least square means.

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 9.6. Influence of sow treatment group and phase 1 diet on growth performance of weaned pigs[1,2,3]

Item												
Parity[4]:	Young				Mature				Pooled	*P*-value[5]		
Sow SDP, %:	0.0		0.5		0.0		0.5					
Nursery SDP, %:	0.0	6.0	0.0	6.0	0.0	6.0	0.0	6.0	SEM	S	N	S × N
d 1 to 7												
Initial BW, kg	5.74	5.70	5.45	5.51	5.69	5.85	5.70	5.61	0.17	0.441	0.808	0.851
ADG, g	35	109	37	111	8	101	25	95	11.09	0.377	<0.001	0.557
ADFI, g	105	157	111	163	90	155	105	147	9.88	0.610	<0.001	0.618
G:F	0.27	0.62	0.34	0.68	0.19	0.66	0.12	0.56	0.07	0.233	<0.001	0.646
d 7 BW, kg	5.91	6.48	5.67	6.23	5.77	6.53	5.85	6.27	0.20	0.700	<0.001	0.799
d 7 to 14												
ADG, g	171^{yz}	188^{yz}	188^{yz}	195^{yz}	129^{z}	202^{y}	181^{yz}	210^{y}	19.26	0.314	<0.001	0.051
ADFI, g	231	236	240	252	205	253	243	271	17.22	0.239	0.004	0.209
G:F	0.70^{ab}	0.76^{ab}	0.81^{a}	0.81^{a}	0.62^{b}	0.82^{a}	0.74^{ab}	0.78^{ab}	0.04	0.216	<0.001	0.008
d 14 BW, kg	7.10	7.82	6.98	7.67	6.69	7.84	6.98	7.59	0.26	0.911	<0.001	0.597

Table 9.6 (cont.)

d 1 to 14												
ADG, g	104	148	112	150	70	146	98	154	12.75	0.344	<0.001	0.115
ADFI, g	169	196	173	206	147	199	169	210	11.81	0.460	<0.001	0.477
G:F	0.58^{bcd}	0.74^{a}	0.63^{abc}	0.72^{ab}	0.44^{d}	0.71^{ab}	0.52^{cd}	0.72^{ab}	0.04	0.206	<0.001	0.021
d 14 to 36												
ADG, g	488	511	499	477	494	520	504	509	16.82	0.693	0.341	0.151
ADFI, g	693	744	708	727	710	784	721	755	24.88	0.648	<0.001	0.445
G:F	0.71^{ab}	0.69^{abc}	0.71^{a}	0.66^{c}	0.70^{abc}	0.67^{bc}	0.70^{ab}	0.68^{abc}	0.01	0.354	<0.001	0.026
Final BW, kg	17.95	18.76	17.96	18.18	17.64	19.38	18.13	18.86	0.54	0.895	0.006	0.383
Overall												
ADG, g	341	363	349	352	332	374	344	367	12.53	0.987	0.002	0.246
ADFI, g	481	517	498	517	488	548	503	541	17.54	0.666	<0.001	0.530
G:F	0.69	0.70	0.70	0.68	0.69	0.69	0.69	0.69	0.01	0.905	0.506	0.204

[1]Data are least square means of 13 to 19 observations per treatment.

[2]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; G:F, gain to feed ratio; SDP, spray dried plasma.

Table 9.6 (cont.)

[3]All pigs were fed a diet without or with 6% SDP for 14 d post-weaning, and then fed a common diet with no SDP from d 14 to 36 post-weaning.

[4]Young, parity 1 and 2; and mature, parity ≥ 3.

[5]*P*-values were calculated for the main effects of sow treatment (S) and nursery diet (N) and the interaction between sow treatment and nursery diet (S × N).

[a,b,c,d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript letter differ ($P < 0.10$).

Table 9.7. Influence of sow treatment group and phase 1 diet on diarrhea score and frequency of diarrhea of weaned pigs[1,2]

Item												
Parity[3]:	Young				Mature							
Sow SDP, %:	0.0		0.5		0.0		0.5		Pooled	*P*-value[4]		
Nursery SDP, %:	0.0	6.0	0.0	6.0	0.0	6.0	0.0	6.0	SEM	S	N	S × N
Diarrhea score[5]												
d 1 to 6	1.82	1.90	1.95	1.90	2.05	1.87	1.94	2.07	0.09	0.481	0.946	0.195
d 8 to 14	2.93	2.92	3.23	2.93	3.03	2.90	3.06	2.80	0.10	0.351	<0.001	0.112
d 1 to 14	2.47	2.48	2.69	2.49	2.59	2.49	2.57	2.47	0.08	0.499	0.012	0.215
d 16 to 36	2.36^{abc}	2.32^{abc}	2.37^{abc}	2.34^{abc}	2.33^{ab}	2.18^{c}	2.41^{a}	2.21^{bc}	0.05	0.359	<0.001	0.028
d 1 to 36	2.40	2.38	2.49	2.40	2.43	2.30	2.47	2.32	0.05	0.491	<0.001	0.156
Frequency of diarrhea[6]												
d 1 to 6	8.77	12.28	19.30	5.26	18.75	25.00	20.00	22.22			0.068	
d 8 to 14	59.21	67.11	78.95	65.79	64.06	59.38	71.67	63.33			0.206	
d 1 to 14	37.59	43.61	53.38	39.85	44.64	44.64	49.52	45.71			0.243	
d 16 to 36	17.22	16.75	20.10	18.18	19.89	7.39	21.21	13.94			0.015	

Table 9.7 (cont.)

d 1 to 36	25.15	27.19	33.04	26.61	29.51	21.88	32.22	26.30	0.042

[1]Data are least square means of 13 to 19 observations per treatment.

[2]SDP, spray dried plasma.

[3]Young, parity 1 and 2; and mature, parity ≥ 3.

[4]*P*-values were calculated for the main effects of sow treatment (S) and nursery diet (N) and the interaction between sow treatment and nursery diet (S × N).

[5]Diarrhea scores were visually assessed every other day by 2 independent observers for 36 days. Diarrhea score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.

[6]Frequency = (number of pen days with diarrhea scores ≥ 3 / pen days) × 100.

[a,b,c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Figure

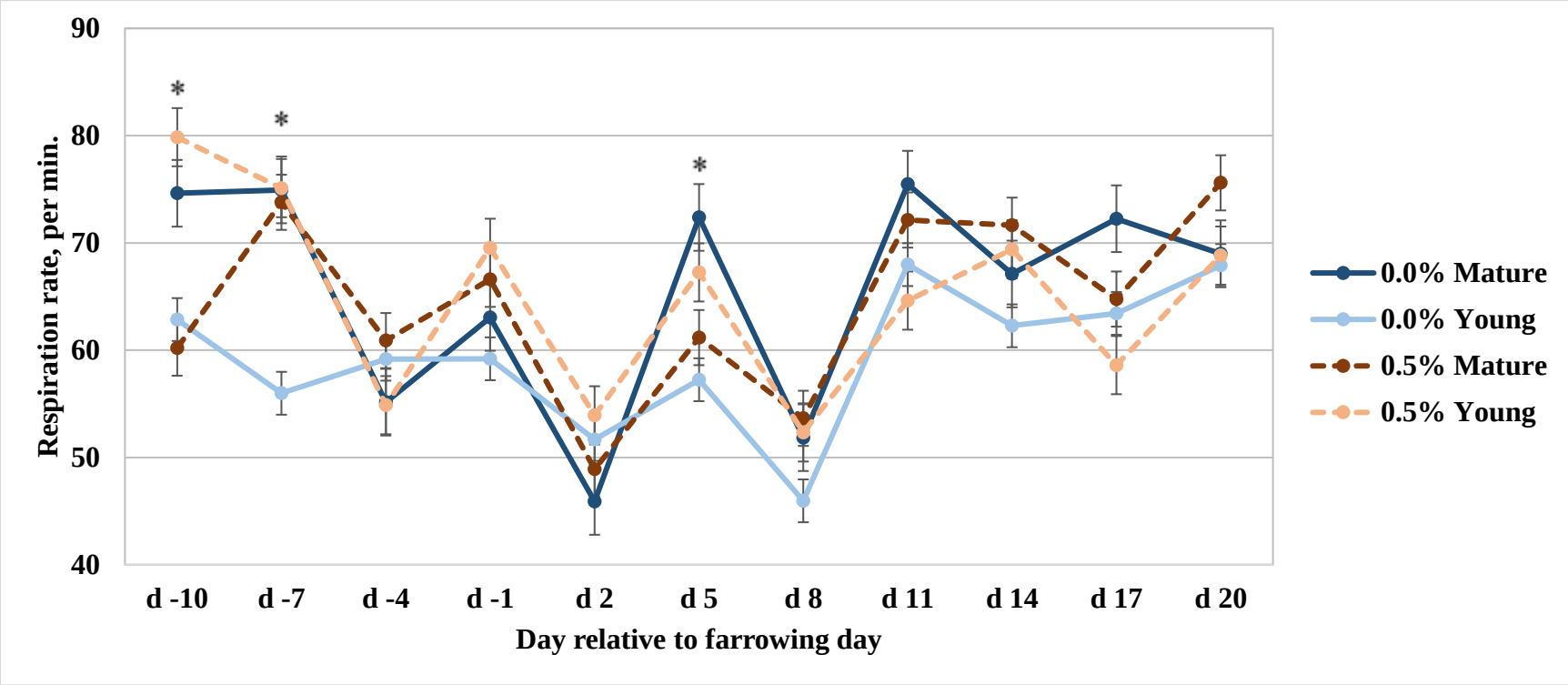

Figure 9.1. Effect of dietary spray dried plasma on respiration rate of young or mature sows housed at 29.0°C on sow respiration rates (number of respirations per minute). *$P < 0.05$ for the interaction between inclusion of SDP and parity group.

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CHAPTER 10: Conclusions

Weaning is accompanied by environmental, nutritional, and social stressors that result in intestinal and immune system dysfunctions leading to reduced feed intake, decreased digestion and absorption of nutrients, post-weaning diarrhea, and thus a lag in growth of the pig. Spray dried plasma (**SDP**) is a widely accepted ingredient included in diets for weanling pigs that can improve growth performance, decrease post-weaning diarrhea, and reduce inflammation. The overall focus of this dissertation was to determine mechanisms of dietary SDP in improving growth performance of weanling pigs. It was also the intent to demonstrate effects of SDP on performance and immune response of weanling pigs and lactating sows upon exposure to environmental and nutritional challenges.

It was established that indispensable amino acids of SDP have an average standardized ileal digestibility of 93%, and therefore, it was assumed that inclusion of SDP to a diet would contribute more digestible amino acids for the pig. However, results of this research indicate that addition of 6% SDP to diets formulated with high levels of cereal grains with low AA digestibility, such as wheat and barley, may improve the digestibility of crude protein (**CP**) and amino acids of the diet. Additionally, inclusion of 6% SDP in a diet formulated with high levels of cereal grains low in fiber, such as rice, resulted in increased apparent total tract digestibility of energy, N, Ca, and P in the mixed diet. It therefore appears that SDP may confer synergistic effects on nutrient digestibility, which results in actual digestibility of some nutrients being greater than predicted. The reason for this observation may be that SDP in the diet reduced intestinal inflammation and maintained the integrity of the intestinal mucosa.

Including 6% SDP in diets fed to pigs the initial 14 d post-weaning was not as effective in reducing diarrhea as diets formulated with low CP. However, results indicated that continuing

to feed SDP at 2.5% inclusion in the diet for an additional 2 wk was as effective as low CP diets in reducing diarrhea throughout the nursery period. The reduction in diarrhea incidence may be associated with the observed reductions in circulating and intestinal pro-inflammatory cytokines for pigs fed a diet with 6% SDP during the initial 2 wk post-weaning or for pigs that continued to be fed a diet with 2.5% SDP during wk 3 and 4 post-weaning.

Responses in average daily gain, average daily feed intake, and the gain to feed ratio of pigs fed dietary SDP increase with an inclusion rate of up to 8% SDP in the diet. Additionally, plasma urea N linearly decreased with increasing inclusion of SDP in the diet, indicating that protein utilization was more efficient when pigs were fed a diet with greater inclusion of SDP. These improvements in growth performance parameters were continually observed for pigs fed a diet with 6% SDP. However, to test the hypothesis that similar improvements in intestinal inflammation can be obtained with greater inclusion of SDP, an experiment was designed to investigate the effect of increasing inclusion rate of SDP in diets fed to newly weaned pigs on intestinal mucosa cytokine synthesis. It was observed that responses in anti- and pro-inflammatory cytokine synthesis throughout the intestine were not consistent with increasing inclusion of SDP to the diet. However, the adaptive immune response was not stimulated when 8% SDP was included in the diet as observed by reduced circulating lymphocytes and less activated T cells compared with regulatory T cells in the blood of pigs.

The performance and health of the sow can influence the subsequent performance and survivability of her offspring, and this is imperative during the summer months when sows are more susceptible to heat stress. Therefore, it was hypothesized that including 0.5% SDP to diets fed to lactating sows exposed to high ambient temperatures will reduce systemic inflammation and improve litter performance, resulting in improved post-weaning performance of her

offspring. Results indicated that circulating cytokines were increased throughout lactation if sows were fed 0.5% SDP compared with sows fed the diet without SDP, but sow performance was not negatively impacted. In contrast, body weight loss of sows fed 0.5% dietary SDP was reduced compared with sows fed the diet without SDP. Inclusion of SDP did not affect total number of pigs born from sows, but the vitality of pigs during lactation was improved if sows were fed 0.5% SDP. However, improvement in pig vitality during lactation was not carried over to the nursery period where pig post-weaning performance was not affected by sow diet, but improved if 6% SDP was included in the post-weaning diet compared with the diet without SDP. This indicates that inclusion of 6% SDP in diets for weanling pigs was more effective than including 0.5% SDP to diets for lactating sows in terms of improving post-weaning performance of the pig.

In summary, dietary SDP increases the ileal and total tract digestibility of energy and nutrients from some other ingredients originating in the diet. Dietary SDP improves average daily gain, average daily feed intake, the gain to feed ratio, and body weight of pigs if fed for 14 or 28 d post-weaning and reduces weight loss of sows if fed throughout lactation. Dietary SDP also increases circulating cytokines in lactating sows, but if SDP is fed to weanling pigs in combination with low CP for 14 or 28 d post-weaning, intestinal and circulating pro-inflammatory cytokines are decreased.