FINAL REPORT

PROJECT TITLE: **Weanling pigs fed diets containing high-protein corn co-products**

PROJECT NUMBER: **6078-22DD**

PRINCIPAL INVESTIGATOR AND CO-INVESTIGATOR(S): **Hans H Stein, University of Illinois.**

ABSTRACT

Provide a project summary describing an overview of the project including principle findings. Include a statement on how the project was of benefit to corn farmers.

Diets with a high inclusion levels of high-protein distillers dried grain with solubles (**HP-DDGS**) or corn-high protein corn co-product (**HPCP**) may have an excess of dietary Leu, and therefore, have a detrimental effect on growth performance of pigs. However, it was hypothesized that the negative effect of using HPCP in diets for weanling pigs may be overcome if diets are fortified with crystalline sources of Val, Trp, and (or) Ile. A total of 320 weanling pigs [body weight (**BW**): 6.11 ± 0.66 kg] were randomly allotted to one of 10 dietary treatments in a completely randomized design. There were four pigs per pen (i.e., 2 gilts and 2 barrows) and eight replicate pens per treatment. A two-phase feeding program was used with d 1 to 14 as phase 1 and d 15 to 28 as phase 2. Within each phase, a corn-soybean meal (**SBM**) diet was formulated and 2 basal diets based on corn and 10% HPCP or corn and 20% HPCP were used as well. Seven additional diets were formulated by adding Val, Ile, Trp, Val and Ile, Val and Trp, Ile and Trp, or Val, Ile, and Trp to the basal diet with 20% HPCP. Phase 1 diets were fed from day 1 to 14 post weaning and phase 2 diets were fed from day 15 to 28. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and average gain:feed ratio (**G:F**) were calculated for each phase and for the overall experiment. Fecal scores were recorded every other day. Blood samples were collected on d 14, and 28; plasma samples were analyzed for blood urea N, total protein, albumin, peptide YY and, immunoglobulin G. On d 14, ileal scrapings and fecal samples were collected from one pig per pen from the treatments fed the corn-SBM diet and the two basal diets containing 10 or 20% HPCP) to evaluate volatile fatty acids (**VFA**) and ammonium concentration, and microbial protein. Results indicated that inclusion of 10 or 20% HPCP in diets reduced ($P <0.05$) final BW on d 28, ADG and ADFI in phase 2 and for the entire experimental period, and G:F for the entire experiment. However, pigs fed SBM and HPCP supplemented with the 3 amino acids had a greater ($P < 0.05$) final BW and ADG in phase 2 and for the overall experiment than pigs fed the other diets. Fecal scores in phase 2 were reduced ($P < 0.05$) if HPCP was used instead of SBM. On d 28, pigs fed the diet with 20% HPCP and only Val, Val and Trp, or Val, Trp, and Ile had reduced ($P < 0.01$) blood urea N compared with pigs fed the corn-SBM diet or the other HPCP-based diets. There were no effects on peptide YY, immunoglobulin G, ileum morphology, VFA, ammonium concentration, or microbial protein of adding HPCP to the diets. In conclusion, HPCP may be included in diets for weanling pigs without affecting growth performance, gut health, or fermentation rate, if these diets are fortified with extra Val, Trp, and Ile.

INTRODUCTION

Provide background information related to the project including such item as the problem statement, knowledge gaps, and relevant previous work completed on this issue.

High-protein distillers dried grains with solubles (**HP-DDGS**) and other high protein corn co-products (**HPCP**) have been developed in recent years, and most of these ingredients are produced by fractionating corn co-products from the ethanol industry. High protein corn co-products contain between 40 and 50% crude protein, and therefore, may be used in diets for pigs as a source of amino acids (**AA**). Indeed, it is possible to formulate diets for pigs based on corn and HP-DDGS or other corn co-products, but such diets will contain more than twice as much Leu as recommended. Results of recent research indicated that there is a negative relationship between dietary Leu and brain synthesis of serotonin, which results in reduced feed intake of pigs fed diets containing excess Leu. There is also a reduced protein synthesis because of increased Val and Ile metabolism due to excess dietary Leu. As a consequence, pigs often have reduced growth performance if fed diets with high concentrations of HPCP. However, it may be possible to counteract the negative effects of the high Leu concentrations in corn co-products by adding crystalline sources of Trp and Val to the diets and it may, therefore, be possible that HPCP can be used in diets without influencing growth performance or intestinal health of weanling pigs. However, at this time, limited research has been conducted to confirm this hypothesis.

OBJECTIVE AND GOAL STATEMENTS

The objective of this research was to test hypothesis that HPCP may be used as the primary source of AA in diets for weanling pigs if diets are fortified with crystalline AA.

MATERIALS AND METHODS

As appropriate, describe the site(s), experimental design, and other relevant methodology used in completing the project.

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved before the initiation of the experiment. Pigs that were the offspring of Line 800 boars mated to Camborough females were used (Pig Improvement Company, Hendersonville, TN, USA). The HPCP used in the experiment was sourced from Green Plains Energy (Omaha, NE; Table 1), and soybean meal (**SBM**) was sourced from Stutzman's Feed & Supply (Arthur, IL). Ground yellow corn was obtained from the University of Illinois Feed Mill (Champaign, IL).

Diets, animals, and experimental design

A two-phase feeding program was used with day 1 to 14 as phase 1 and day 15 to 28 as phase 2. A total of 320 weanling pigs with an initial body weight (**BW**) of 6.11 ± 0.66 kg were used in 2 blocks of 160 pigs. Within each block, pigs were randomly assigned to 10 dietary treatments in a randomized complete block design. There were two barrows and two gilts in each pen and eight replicate pens per treatment. A control diet based on corn and SBM was formulated and two basal diets were formulated based on corn and 10% HPCP or corn and 20% HPCP (Tables 2 and 3). Seven additional diets were formulated by supplementing the diet with 20% HPCP with crystalline Ile, Trp, and Val as follows: 1) HPCP + 0.10% Ile; 2) HPCP + 0.05% Trp; 3) HPCP + 0.10% Val; 4) HPCP + 0.10% Ile + 0.10% Val; 5) HPCP + 0.10% Ile + 0.05% Trp; 6) HPCP + 0.10% Val + 0.05% Trp; 7) HPCP + 0.10% Ile + 0.10% Val + 0.05% Trp. All diets in phases 1 and 2 were formulated to meet the current estimates for nutrient requirements by nursery pigs (NRC, 2012).

Pigs were housed in pens with fully slatted plastic floors. Each pen was equipped with a feeder and a nipple drinker, and pigs had free access to feed and water throughout the experiment.

Sample and data collection

Individual pig weights were recorded at the beginning of the experiment, on day 14, and at the end of the 28-day experiment. Daily feed allotments were recorded and the weight of feed left in the feeders were recorded on day 14 and on the last day of the experiment to calculate feed consumption. Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea).

On day 14 and on the last day of the experiment, blood samples were collected from one pig in each pen that had a body weight that was closest to the pen average. Two blood samples were collected from pigs in heparinized vacutainers and vacutainers containing EDTA. Blood samples were centrifuged at 4,000 × g at 4 °C for 13 min, and plasma was collected and stored at -20 ºC until analysis. Heparinized plasma samples were analyzed for blood urea nitrogen, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer at the University of Illinois Veterinary Diagnostic Laboratory. Plasma samples treated with EDTA were also analyzed for peptide YY (**PYY**) and immunoglobulin G (**IgG**) using ELISA kits according to the recommendations from the manufacturer (Phoenix Pharmaceuticals Inc., Burlingame, CA, Bethyl Laboratories Inc., Montgomery, TX, respectively).

On d 14, one pig per pen (the pig with a body weight closest to the pen average) in 3 of the 10 dietary treatments (i.e., corn-SBM basal diet and the two basal diets containing 10 and 20% HPCP) was euthanized via captive bolt penetration. Ileal tissue samples between 2 and 3 cm long were collected approximately 80 cm from the ileal-cecal junction. Samples were cut and pinned with the serosa side down on a piece of cardboard. Samples were then fixed in 10% neutral buffered formalin until processing for immunohistochemistry staining and morphological evaluation. After fixation, all tissue samples were sectioned and transferred to slides. 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. Lamina propria thickness were also measured at the midpoint of the villus. Villus height:crypt depth (**VH:CD**) was also calculated.

Fecal samples from these pigs were also collected and analyzed for microbial protein, fecal NH_3, and volatile fatty acids (**VFA**). For microbial protein, 5 to 10 g of feces were collected and stored at -20 °C until analyzed. For VFA and NH_3 analysis, 5 g of feces were placed in 15 mL tubes and samples were stabilized in 2*N* HCl and stored at -20 °C until analyzed. Fecal ammonia concentrations were determined according to the method by Chaney and Marbach (1962) and VFA were determined using previously established procedures (Sunvold et al., 1995). Microbial protein was determined following the procedure described by Espinosa et al., (2019).

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 the subsequent calculation of crude protein as N × 6.25 (Tables 3, 4 and 5). 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). 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].

Statistical analysis

Data were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) for each pen of pigs and for each treatment group at the conclusion of the experiment. Normality of data was verified and outliers were identified using the UNIVARIATE procedure of SAS. Data were analyzed using the PROC MIXED of SAS with the experimental unit being the pen. The model included diet as fixed effect and block and replicate within block as random effects. Treatment means were calculated using the LSMEANS statement. Statistical significance and tendency was considered as $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS AND DISCUSSION

All animals remained healthy throughout the experiment and readily consumed their assigned diets. Inclusion of 10 or 20% HPCP in diets reduced ($P < 0.05$) final BW on d 28, ADG and ADFI in phase 2 and for the entire experimental period, and G:F for the entire experiment (Table 6). Pigs fed the diet containing SBM and HPCP supplemented with all three AA had a greater ($P < 0.05$) final BW and ADG in phase 2 and for the overall experiment than pigs fed diets supplemented with only one or two AA. However, pigs fed diets supplemented with Val, Val and Ile, or Val and Trp had greater ($P < 0.05$) final BW and greater ADG in phase 2 and for the overall experiment than pigs fed diets containing only Ile, only Trp, or Ile and Trp. Pigs fed the control diet or the diet with 20% HPCP and Val, Val and Ile, Val and Trp, or Val, Ile and Trp had greater ($P < 0.05$) ADFI in phase 2 and for the overall experiment than pigs fed diets with 10 or 20% HPCP and no AA or diets with 20% HPCP and Ile and Trp. Pigs fed the control diet had greater ($P < 0.05$) G:F for the entire experiment than pigs fed a diet with HPCP regardless of AA supplementation. However, pigs fed a diet with 10 or 20% and HPCP without AA had a greater

($P < 0.05$) G:F than pigs fed the diet with 20% HPCP and Ile, Trp, or Ile and Val. Likewise, pigs fed the diet with 10% HPCP had greater ($P < 0.05$) G:F than pigs fed the diet with 20% HPCP and Trp and Val, or 20% HPCP and Ile and Val.

One of the reasons for the reductions in growth performance of pigs fed diets containing 10 or 20% HPCP is likely the high concentration of Leu in HPCP because HPCP contains almost twice as much Leu as SBM. This results in diets containing HPCP having a large excess of Leu. Excess Leu in diets for growing pigs influences metabolism of Val by increasing Val catabolism, resulting in a deficiency of Val for protein synthesis, and therefore, reduced growth performance of pigs is usually observed if there is excess Leu in the diet (Cemin et al., 2019a and b; Kwon et al., 2019). High levels of Leu in diets is detrimental to Trp metabolism as well. Tryptophan is a precursor for serotonin, which is a neurotransmitter that influences feed intake regulation (Zhang et al., 2007), and excess dietary Leu may reduce synthesis of serotonin in the brain (Wessels et al., 2016) by preventing Trp from being transported to the brain (Kwon et al., 2019). Reduced serotonin synthesis can result in reduced feed intake and pigs with reduced feed intake due to excess Leu also have reduced growth performance.

The observation that pigs fed the control diet or diets containing HPCP and Val, Ile, and Trp had growth performance that was not different indicates that the negative effects of high-Leu diets can be overcome when HPCP-diets are fortified with crystalline Val, Ile, and Trp. This is in agreement with Kwon et al., (2020) who reported that adding both Trp and Val to a diet high in Leu may be beneficial for preventing detrimental effects of excess Leu on growth performance of pigs. However, according to the present data, there was no advantage of adding Val and Trp rather than only Val, which indicates that the negative effects of Leu mainly is associated with increased Val catabolism. In contrast, it appears that effects of adding only Trp to high-Leu diets are limited.

From d 15 to d 28 and for the overall experiment, fecal scores were reduced ($P < 0.05$) if HPCP was used instead of SBM (Table 7). No differences among experimental diets were observed for blood urea N, total protein, albumin, peptide YY, or IgG on d 14 (Table 8). However, on d 28, pigs fed the diet with 20% HPCP and only Val, Val and Trp, or Val, Trp, and Ile had reduced ($P < 0.01$) blood urea N compared with pigs fed the control diet or the other HPCP-based diets (Table 9). Likewise, pigs fed the control diet or the diet with 10% HPCP had a greater ($P < 0.01$) albumin concentration in plasma compared with pigs fed all other diets except the diet with added Val, Trp, and Ile. Blood urea N is positively correlated with urinary N excretion, and is therefore, an indicator of AA utilization efficiency (Kohn et al., 2005). The observation that pigs fed the HPCP diets with supplemented Val, Val and Trp, or Val, Trp, and Ile had reduced blood urea N, therefore indicates that protein utilization in these pigs became more efficient than for pigs fed only SBM and no HPCP. This is likely a result of these diets having a more balanced AA composition with less excess AA. Specifically, reducing SBM and includign HPCP in the diets reduced excesses of Arg in the diets and also reduced the concentrations of Asp and Glu, which likely contributed tot eh reduced blood urea N in pgis fed the diets containing HPCP.

Albumin binds and transports nutrients in the blood (Bern et al., 2015). Thus, the observation that there was no difference in growth performance of pigs fed the control diet and the diet with 20% HPCP and all three AA may be related to the lack of difference in albumin concentration in plasma of pigs fed these two diets. The lack of difference for IgG among treatments indicate that the inclusion of HPCP did not influence immune response and systemic health of pigs.

No differences in villus height, crypth depth, or VH:CD of pigs were observed among pigs fed the control diet or the 2 basal diets containing HPCP (Table 10). Changes in the morphological structure in weanling pigs, such as villus atrophy and crypt hyperplasia, have been reported if pigs are fed diets that are inadequate in nutrient supply. These changes may result in a decrease in surface area, and a reduction in nutrient absorption. Thus, the lack of differences in morphology of pigs fed experimental diets indicates that inclusion of HPCP in diets does not have a negative impact on nutrient absorption when compared to pigs fed a diet based on SBM.

Inclusion of HPCP in experimental diets did not affect the concentration of microbial protein in feces of pigs (Table 11). Likewise, concentrations of VFA and ammonium in feces were not affected by inclusion of HPCP in the diets. Distiller's dried grains with solubles (**DDGS**) may increase growth of bacteria in the hindgut of pigs (Bindelle, 2008), and increased microbial growth may result in increased synthesis of VFA because of increased fermentation (Espinosa et al., 2019). The lack of a difference among treatments in VFA indicates that the inclusion of HPCP does not impact microbial growth and fermentation.

CONCLUSIONS

Inclusion of HPCP in diets for weanling pigs did not affect immune system, ileum morphology, microbial protein, VFA, or fecal ammonium. Likewise, pigs fed HPCP with added Val, Val and Trp, or Val, Trp, and Ile had a better protein utilization than pigs fed without those AA. However, using HPCP in diets for weanling pigs had a negative impact on growth performance, but this effect could be mostly overcome with supplementation of Val, Trp, and Ile.

Therefore, HPCP with added Val, Trp, and Ile may be included in diets for weanling pigs without affecting growth performance or blood characteristics.

EDUCATION, OUTREACH, AND PUBLICATIONS

Identify conferences, workshops, field days etc. at which project results were presented. Include number of farmers estimated to be present. List articles and/or manuscripts in which project results were published.

Results will be disseminated using the following avenues:

1. A research report will be included in the monthly on-line newsletter from our Monogastric Nutrition Research Laboratory. The newsletter is available for free at http//www.nutrition.ansci.illinois.edu
2. An abstract with results will be presented at the Midwest Animal Science Meeting in Madison, WI in March, 2024.
3. A manuscript for peer reviewed publication will be prepared and submitted to Animal Feed Science and Technology

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Table 1. Analyzed composition of main ingredients, as-fed basis[1]

Item	Soybean meal	High protein corn co-product	Corn
Gross energy, kcal/kg	4,194	4,950	3,847
Crude protein, %	46.98	50.26	6.72
Dry matter, %	89.35	91.72	86.41
Ash, %	12.73	9.34	1.95
Acid hydrolyzed ether extract, %	2.86	5.82	3.53
Indispensable AA, %			
Arg	3.26	2.30	0.29
His	1.35	1.36	0.18
Ile	2.24	2.34	0.24
Leu	3.55	6.51	0.69
Lys	2.66	1.80	0.22
Met	0.62	1.11	0.12
Phe	2.36	2.92	0.30

Thr	1.82	1.96	0.22
Trp	0.62	0.49	0.05
Val	2.25	2.84	0.33
Dispensable AA, %			
Ala	2.00	3.81	0.44
Asp	5.25	3.62	0.42
Cys	0.66	0.93	0.14
Glu	8.33	8.52	1.11
Gly	1.95	2.11	0.27
Pro	2.25	3.99	0.52
Ser	2.13	2.22	0.27
Tyr	1.70	2.19	0.15

[1]High protein corn co-product (Green Plains Energy, Omaha, NE).

Table 2. Ingredient composition of phase 1 experimental diets, as-fed basis[1,2,3,4]

Ingredient, %	Control diet	HPCP[1], 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp
Ground corn	44.70	47.25	48.89	48.79	48.84	48.79	48.69	48.74	48.74	48.64
Soybean meal	20.00	10.00	-	-	-	-	-	-	-	-
Corn protein	-	10.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean oil	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Whey powder	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
ESBM[1]	9.00	6.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	1.04	1.18	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Dicalcium phosphate	0.70	0.65	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
L-Lysine	0.47	0.74	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
DL-Methionine	0.23	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
L-Threonine	0.13	0.17	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Tryptophan	-	0.04	0.07	0.07	0.12	0.07	0.07	0.12	0.12	0.12
L-Valine	0.03	0.04	0.03	0.03	0.03	0.13	0.13	0.03	0.13	0.13
L-Isoleucine	-	-	-	0.10	-	-	0.10	0.10	-	0.10

L-Histidine	-	0.05	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated values										
ME, kcal/kg	3,456	3,443	3,439	3,436	3,438	3,436	3,433	3,434	3,434	3,431
Crude protein, %	19.26	18.42	18.10	18.09	18.10	18.09	18.09	18.09	18.09	18.08
Total Ca, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
P[2], %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Amino acids[4], %										
Arg	1.13	0.92	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
His	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Ile	0.79	0.73	0.69	0.79	0.69	0.69	0.79	0.79	0.69	0.79
Leu	1.45	1.63	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84
Lys	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Met	0.48	0.45	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Met + Cys	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74

Phe	0.82	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Thr	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Trp	0.23	0.23	0.23	0.23	0.28	0.23	0.23	0.28	0.28	0.28
Val	0.86	0.86	0.86	0.86	0.86	0.96	0.96	0.86	0.96	0.96

[1]ESBM = enzyme-treated soybean meal: Hamlet Protein, Findley, OH.; HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

[2]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[3]Standardized total tract digestible P.

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

Table 3. Ingredient composition of phase 2 experimental diets containing high protein corn co-product (HPCP), as-fed basis[1,2,3,4]

Ingredient, %	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp
Ground corn	54.49	54.38	56.97	56.87	56.92	56.87	56.77	56.82	56.82	56.72
Soybean meal	29.00	19.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Corn protein	-	10.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean oil	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	0.95	1.10	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Dicalcium phosphate	0.88	0.77	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
L-Lysine	0.50	0.65	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
DL-Methionine	0.23	0.18	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Threonine	0.17	0.16	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
L-Tryptophan	-	0.02	0.06	0.06	0.11	0.06	0.06	0.11	0.11	0.11
L-Valine	0.08	0.02	0.02	0.02	0.02	0.12	0.12	0.02	0.12	0.12
L-Isoleucine	-	-	-	0.10	-	-	0.10	0.10	-	0.10
L-Histidine	-	0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated values										
ME, kcal/kg	3,382	3,399	3,409	3,406	3,408	3,406	3,402	3,404	3,404	3,401
Crude protein, %	17.68	18.38	17.89	17.89	17.89	17.89	17.88	17.88	17.88	17.88
Total Ca, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
P[2], %	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Amino acids[3], %										
Arg	1.06	0.96	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
His	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Ile	0.69	0.70	0.66	0.76	0.66	0.66	0.76	0.76	0.66	0.76
Leu	1.31	1.60	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lys	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29
Met	0.46	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Met + Cys	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Phe	0.75	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80

Thr	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Trp	0.21	0.21	0.21	0.21	0.26	0.21	0.21	0.26	0.26	0.26
Val	0.82	0.82	0.82	0.82	0.82	0.92	0.92	0.82	0.92	0.92

[1]The high protein corn co-product was sourced from Green Plains Energy, Omaha, NE.

[2]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[3]Standardized total tract digestible P.

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

Table 4. Analyzed nutrient composition of phase 1 diets[1]

Item	Control diet	HPCP[1], 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp
Gross energy, kcal/kg	3,998	4,010	4,001	3,989	4,005	3,994	4,071	4,024	4,063	3,976
Crude protein, %	21.35	20.45	20.18	20.79	21.48	20.18	20.63	20.27	20.43	20.24
Dry matter, %	89.83	89.49	89.92	89.98	89.78	89.86	89.94	89.68	89.91	89.83
Ash, %	9.85	8.41	8.19	8.26	8.87	8.16	8.64	8.20	8.36	8.74
Acid hydrolyzed ether extract, %	4.59	4.63	4.74	4.41	4.85	4.25	4.16	4.34	4.95	4.77
Indispensable amino acids, %										
Arg	1.24	0.99	0.79	0.82	0.80	0.79	0.82	0.80	0.79	0.81
His	0.52	0.51	0.51	0.52	0.52	0.50	0.51	0.51	0.50	0.52
Ile	0.97	0.87	0.79	0.89	0.79	0.79	0.88	0.89	0.77	0.88
Leu	1.69	1.83	2.01	2.05	2.05	2.03	2.05	2.03	2.04	2.05
Lys	1.61	1.62	1.62	1.61	1.59	1.58	1.58	1.60	1.59	1.58
Met	0.4	0.45	0.45	0.45	0.47	0.44	0.47	0.45	0.45	0.45
Phe	0.97	0.92	0.88	0.89	0.89	0.87	0.89	0.89	0.86	0.86

Thr	0.91	0.90	0.92	0.93	0.93	0.90	0.91	0.89	0.90	0.93
Trp	0.24	0.24	0.24	0.24	0.27	0.24	0.24	0.28	0.28	0.27
Val	1.02	1.01	1.00	1.01	1.01	1.10	1.11	1.00	1.10	1.10
Dispensable amino acids, %										
Ala	0.95	1.07	1.20	1.24	1.22	1.18	1.22	1.20	1.20	1.22
Asp	2.13	1.74	1.42	1.46	1.43	1.40	1.44	1.43	1.40	1.44
Cys	0.34	0.34	0.35	0.35	0.35	0.34	0.35	0.35	0.34	0.34
Glu	3.62	3.31	3.10	3.18	3.15	3.05	3.16	3.09	3.01	3.15
Gly	0.79	0.73	0.68	0.70	0.69	0.67	0.68	0.67	0.67	0.69
Pro	1.14	1.22	1.33	1.39	1.37	1.33	1.36	1.35	1.32	1.36
Ser	0.84	0.79	0.76	0.79	0.78	0.76	0.78	0.78	0.76	0.78

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

Table 5. Analyzed nutrient composition of phase 2 diets[1]

Item	Control diet	HPCP[1], 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp
Gross energy, kcal/kg	4,004	4,059	4,087	4,105	4,136	4,104	4,091	4,047	4,112	4,103
Crude protein, %	19.21	19.90	19.18	20.01	19.42	20.81	20.26	20.54	19.55	19.99
Dry matter, %	89.87	89.70	89.89	89.91	89.97	89.84	90.04	89.65	89.10	89.66
Ash, %	10.04	9.37	8.42	9.13	8.61	8.11	8.85	8.59	8.24	8.86
Acid hydrolyzed ether extract, %	4.14	4.69	4.91	4.42	4.14	4.76	4.87	4.65	4.11	4.18
Indispensable amino acids, %										
Arg	1.12	1.08	0.88	0.88	0.87	0.82	0.89	0.89	0.89	0.84
His	0.50	0.51	0.51	0.50	0.54	0.54	0.52	0.54	0.52	0.53
Ile	0.90	0.90	0.82	0.93	0.83	0.83	0.95	0.95	0.85	0.94
Leu	1.63	1.90	2.02	2.04	2.02	2.05	2.09	2.11	2.04	2.08
Lys	1.53	1.52	1.53	1.40	1.47	1.52	1.51	1.44	1.44	1.48
Met	1.42	1.50	1.42	1.42	1.45	1.50	1.50	1.44	1.44	1.44
Phe	0.95	0.95	0.96	0.92	0.91	0.95	0.95	0.98	0.96	0.97

Thr	0.88	0.87	0.86	0.89	0.89	0.89	0.85	0.89	0.89	0.88
Trp	0.22	0.23	0.23	0.23	0.28	0.23	0.23	0.28	0.28	0.28
Val	1.01	1.01	1.03	1.01	1.03	1.13	1.15	1.02	1.17	1.15
Dispensable amino acids, %										
Ala	0.93	1.20	1.20	1.20	1.21	1.24	1.25	1.28	1.27	1.28
Asp	1.93	1.79	1.46	1.47	1.44	1.54	1.50	1.53	1.53	1.51
Cys	0.29	0.33	0.31	0.33	0.32	0.35	0.34	0.36	0.36	0.35
Glu	3.41	3.42	3.15	3.11	3.13	3.25	3.21	3.33	3.28	3.31
Gly	0.75	0.77	0.71	0.71	0.71	0.74	0.72	0.74	0.73	0.74
Pro	1.09	1.26	1.33	1.32	1.38	1.33	1.35	1.36	1.34	1.35
Ser	0.79	0.80	0.77	0.78	0.78	0.80	0.79	0.80	0.80	0.80

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

Table 6. Growth performance of pigs fed experimental diets[1, 2,3,4]

Item	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp	SEM	*P* value
Body weight, kg												
Initial body weight	6.33	6.29	6.34	6.33	6.32	6.30	6.29	6.31	6.31	6.29	0.23	0.154
d 14	7.42	7.26	7.35	7.21	7.20	7.35	7.34	6.97	7.40	7.43	0.28	0.398
d 28	14.22^{a}	12.58^{cde}	12.07^{cdef}	11.80^{ef}	11.93^{def}	13.02^{bcd}	13.08^{bc}	11.44^{f}	13.00^{bcde}	13.77^{ab}	0.54	0.001
ADG, g												
d 1 to 14	78	69	72	62	63	74	75	47	78	81	9.4	0.281
d 15 to 28	485^{a}	379^{cd}	337^{d}	334^{d}	337^{d}	405^{bc}	375^{cd}	319^{d}	399^{bc}	453^{ab}	23.7	0.001
d 1 to 28	281^{a}	224^{cde}	204^{cdef}	198^{ef}	200^{def}	240^{bc}	225^{cde}	183^{f}	238^{bcd}	267^{ab}	14.3	0.001
ADFI, g												
d 1 to 14	141	136	135	128	135	141	136	118	146	157	10.3	0.080
d 15 to 28	513^{a}	361^{c}	355^{c}	378^{c}	391^{bc}	461^{ab}	479^{a}	345^{c}	465^{ab}	513^{a}	140.2	0.001
d 1 to 28	300^{ab}	232^{c}	230^{c}	258^{bc}	263^{bc}	301^{ab}	314^{a}	232^{c}	301^{ab}	335^{a}	64.91	0.001

G:F											
d 1 to 14	0.55	0.44	0.52	0.44	0.45	0.51	0.49	0.35	0.52	0.51	0.05	0.119
d 15 to 28	0.73	0.69	0.66	0.68	0.67	0.70	0.68	0.73	0.69	0.70	0.37	0.349
d 1 to 28	0.77[a]	0.71[b]	0.69[bc]	0.63[d]	0.62[d]	0.67[bcd]	0.64[d]	0.65[cd]	0.66[cd]	0.66[bcd]	0.01	0.004

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

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

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

[4]All pigs were fed phase 1 diets for 14 d post-weaning, and they were then fed phase 2 diets from d 15 to 28 post-weaning.

Table 7. Fecal scores of pigs fed the experimental diets[1,2,3]

Item	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp	SEM	*P* value
d 1 to 14	2.42	0.25	2.12	2.03	2.03	2.17	2.51	2.08	2.21	2.11	0.21	0.295
d 15 to 28	1.67^{a}	1.44^{b}	1.23^{c}	1.23^{c}	1.37^{bc}	1.21^{c}	1.26^{bc}	1.26^{bc}	1.21^{c}	1.33^{bc}	0.13	0.001
d 1 to 28	2.05^{a}	1.84^{abc}	1.67^{bc}	1.63^{c}	1.70^{bc}	1.75^{bc}	1.89^{ab}	1.67^{bc}	1.71^{bc}	1.72^{bc}	0.14	0.030

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

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

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

Table 8. Plasma characteristics on d 14 of pigs fed experimental diets[1,2]

Item	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp	SEM	*P* value
Blood urea nitrogen, mg/dL	8.71	6.75	6.87	6.00	6.12	5.12	5.75	6.54	4.50	5.37	1.06	0.371
Total protein, mg/dL	4.53	4.50	4.31	4.38	4.47	4.42	4.38	4.40	4.41	4.38	0.11	0.858
Albumin, mg/dL	2.71	2.73	0.72	2.66	2.67	2.63	2.56	2.68	2.57	2.66	0.09	0.611
Peptide YY (ng/mL)	1.93	1.81	1.73	1.76	1.84	1.84	1.86	1.72	1.84	1.89	0.12	0.960
Immunoglobulin G (mg/mL)	5.35	3.71	3.92	4.78	3.07	3.17	3.05	4.06	4.29	3.60	0.84	0.290

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

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

Table 9. Plasma characteristics on d 28 of pigs fed experimental diets[1,2]

Item	Control diet	HPCP, 10%	HPCP, 20%	HPCP + Iso	HPCP + Trp	HPCP + Val	HPCP + Ile + Val	HPCP + Ile + Trp	HPCP + Val + Trp	HPCP + Ile + Val + Trp	SEM	*P* value
Blood urea nitrogen, mg/dL	6.25[a]	4.62[abc]	4.50[abc]	4.00[bcd]	5.00[abc]	3.62[cd]	4.62[abc]	5.62[ab]	2.59[d]	2.37[cd]	0.66	0.006
Total protein, mg/dL	4.82	4.86	4.68	4.71	4.75	4.83	4.60	4.66	4.62	4.80	0.10	0.390
Albumin, mg/dL	3.18[a]	3.12[ab]	2.97[abcde]	2.85[cde]	2.83[de]	3.03[abcd]	2.72[e]	2.86[bcde]	2.82[de]	3.11[abc]	0.09	0.009
Peptide YY (ng/mL)	2.69	2.72	2.79	2.75	2.72	2.96	2.96	3.16	2.95	2.94	1.03	0.971
Immunoglobulin G (mg/mL)	6.59	4.59	4.61	5.64	4.93	4.59	4.68	4.70	4.71	3.91	0.91	0.679

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

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

339 **Table 10.** Morphology measurements of ileal sampels on d 14 of pigs fed the three basal diets diets[1,2]

Item	Control diet	HPCP, 10%	HPCP, 20%	SEM	*P* value
Villus height, µm	283.15	260.95	285.18	35.13	0.866
Crypt depth, µm	241.28	224.23	206.91	11.17	0.119
Villus height:crypt depth ratio	1.24	1.33	1.50	0.19	0.534
Lamina propria thickness, µm	67.72	62.49	55.96	5.14	0.3370

340 [1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

341 [2]Data are least square means of 8 observations per treatment.

Table 11. Intestinal microbial protein concentrations (mg/g, dry matter), rate of fermentation of volatile fatty acids, and ammonia concentration in feces on d 14 of pigs fed the three basal diets[1,2]

Item	Control diet	HPCP, 10%	HPCP, 20%	SEM	*P* value
Microbial protein, mg/g	2.87	3.74	3.43	0.34	0.199
Volatile fatty acids, µmol/100 µmol					
Acetate	291.79	279.63	259.89	29.73	0.160
Propionate	109.31	105.35	88.81	9.92	0.299
Butyrate	66.20	58.38	46.28	8.30	0.265
Isobutyrate	14.34	12.11	11.36	1.18	0.205
Isovalerate	23.62	18.61	20.51	2.05	0.221
Valerate	23.75	22.70	19.76	2.73	0.551
Ammonium (NH_4^+), µmol/g dry matter	139.24	98.00	128.31	13.69	0.123

[1]HPCP = high protein corn co-product (Green Plains Energy, Omaha, NE).

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