RESEARCH ARTICLE

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Identifying the optimal ratios for replacing spray-dried plasma protein with hydrolyzed porcine intestinal protein in weaning pig

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Abstract

This study aimed to evaluate the effects of replacing spray-dried plasma protein (SDPP) with hydrolyzed porcine intestinal protein (HP) in weaning pigs and determine the optimal replacement ratio. Ninety-six crossbred weaning pigs (initial body weight 7.35 ± 0.67 kg) were used for five weeks and assigned to four dietary treatments: HP0 (100% SDPP), HP25 (25% HP), HP50 (50% HP), and HP100 (100% HP), with 6 replicates of 4 pigs per treatment. The HP0 and HP25 diets significantly increased ($p < 0.05$) average daily gain (ADG) and feed efficiency (G:F) compared with HP100 at weeks 3 to 5 and over the entire study period. Increasing levels of HP replacement linearly decreased ($p < 0.05$) ADG and G:F. At week 3, HP0 and HP25 diets significantly increased crude protein (CP) digestibility compared to HP50 and HP100, with similar results observed at week 5. Additionally, HP0 and HP25 diets led to significantly lower ($p < 0.05$) total protein (TP) and blood urea nitrogen (BUN) levels than HP50 and HP100 at week 5. TP and BUN levels increased linearly as HP levels increased. The HP25 diet notably increased *Lactobacillus* counts in feces compared to HP50 and HP100 at week 5. Total weight gain was significantly higher ($p < 0.05$) in the HP0 and HP25 groups compared to HP100, and the HP25 diet significantly reduced feed cost per kg gain (FCG) compared with HP100. Moreover, during the study, the HP25 diet showed a trend ($p = 0.087$) towards lower FCG compared with HP0. In conclusion, replacing 25% of SDPP with HP provides optimal benefits in growth performance, nutrient digestibility, and feed cost efficiency without negative impacts on weaning pigs.

Keywords: Hydrolyzed porcine intestinal protein, Spray-dried plasma protein, Weaning pig

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Ethics approval and consent to participate
The experimental protocol was approved (CBNUA-2185-23-02) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

INTRODUCTION

Weaning stress caused by separation of the sow and its adaptation into solid feed can trigger physiology, gastrointestinal microbiology, and immunology changes [1]. These changes can cause damage to the intestinal villi and reduce absorption due to immaturity of the piglet's digestive system, leading to diarrhea and reduced growth performance [1,2]. To cope with this problem, numerous studies have been conducted on protein sources that could be easily digested by weaning pigs [3–5].

Spray-dried plasma protein (SDPP) manufactured from porcine blood has been reported to contain highly digestible protein with balanced amino acid profile, which could improve the performance of early weaning pigs [6,7]. SDPP can also prevent binding of pathogens to gut wall and reduce the incidence of diarrhea in weaning pigs due to its abundance of bioactive compounds, such as immunoglobulin G (IgG) [8,9]. Previous studies have reported that supplementation of SDPP (2.5% and 5.0%) can improve average daily gain (ADG), feed efficiency (G:F), and serum IgG levels (4.44 mg/mL) of weaning pigs [9,10].

However, SDPP is approximately eight times more expensive than other protein sources such as soybean meal (SBM) [11–14]. Cho et al. [15] have pointed out that there were no significant differences on growth performance between the SDPP and hydrolyzed porcine intestinal protein (HP) diets in weaning pigs for 28 days. As feed costs constitute more than 60% of the total cost of swine production, an alternative strategy is needed to increase profit by reducing feed costs [16]. Therefore, numerous studies have been conducted to identify alternative cost-effective protein sources for replacing SDPP in weaning pigs [17,18].

HP is a small molecular-weight peptone protein obtained by hydrolyzing pig intestinal mucosa. It can be broken down into small-chain peptides during the hydrolysis process [19]. Due to their low molecular-weight peptide characteristics, HP can be absorbed more rapidly than non-hydrolyzed protein sources [20]. Previous studies have demonstrated that supplementation of hydrolyzed porcine intestinal product (2.5% and 5%, respectively) can increase ADG and G:F during the first 14 days after weaning [21,22]. In addition, HP is approximately 2.9 times cheaper than SDPP [23]. Kim et al. [24] have reported that when replacing SDPP with HP, approximately more than 0.09 USD/kg of feed could be reduced without impairing growth performance compared to the SDPP diet.

Therefore, we hypothesized that replacing SDPP with HP could reduce feed cost without impairing growth performance, nutrient digestibility, diarrhea, or immune system in weaning pigs. Thus, the objective of this study was to investigate effects of replacing SDPP with HP on growth performance, nutrient digestibility, diarrhea scores, blood profiles, bacteria count in feces, and economic evaluation.

MATERIALS AND METHODS

Preparation of HP and SDPP

The chemical composition of HP and SDPP are presented in Table 1. HP and SDPP were supported by a commercial company. According to the supplier, the HP (Palbio 50 RD, Bioiberica) is a coproduct of the heparin industry obtained from clean endothelial and mucosal digestive tissues of pigs free of digesta content. The fresh tissues are washed with hot water before heparin extraction, and then the mucosa was digested, solubilized, and sterilized via an enzymatic process under controlled conditions of time, temperature, and pH. The hydrolyzed mucosa was sprayed into high-protein SBM at an approximate proportion of 30% to facilitate handling. Then, the commercial

Table 1. Chemical composition of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP; as-fed-basis)

Items (%)	SDPP	HP
GE (kcal/kg)	4,862	4,010
DM	91.50	91.46
CP	78.20	50.88
CF	0.10	2.71
EE	8.60	2.16
Ash	12.20	11.53

GE, gross energy; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract.

product was dried using a fluid bed system that prevents structural damages of the protein fraction. Also, according to the supplier, the SDPP was produced by spinning porcine plasma at high speeds to separate the plasma and red cells, and then spray dried where it is transformed into powder.

Experimental design, animals, and housing

A total of ninety-six crossbred weaning pigs ([Landrace × Yorkshire] × Duroc) with an initial body weight (BW; 7.35 ± 0.67 kg) were used for 5 weeks. Pigs were randomly assigned to four dietary treatments, with 6 replicates of 4 pigs per treatment in a randomized complete block design. All pigs were housed in an environmentally controlled room (30 ± 1℃). Each pen was equipped with a one-sided stainless steel self-feeder and a nipple drinker. Dietary treatments were as follows: HP0, a basal diet based on SDPP; HP25, replacing 25% of SDPP with HP; HP50, replacing 50% of SDPP with HP; and HP100, replacing 100% of SDPP with HP. All diets were formulated to meet or exceed the National Research Council [25] requirement and fed during the experiment in 3 phases: phase 1 (0 to 1 weeks), phase 2 (1 to 3 weeks), phase 3 (3 to 5 weeks), and each phase contains SDPP for 5.0, 2.5, and 1.0% (Tables 2, 3, and 4). Each pig had *ad libitum* access to water.

Growth performance and diarrhea score

All pigs were individually weighed, and feed intake was measured to calculate the average daily feed intake (ADFI) and G:F at initial, 1, 3, and 5 weeks. ADG, ADFI, and G:F were calculated for each period (0 to 1 weeks, 1 to 3 weeks, 3 to 5 weeks, and 0 to 5 weeks). The diarrhea scores were individually recorded at 08:00 h and 17:00 h by the same person during the entire experimental period. The diarrhea score was assigned as follows: 0, Normal feces; 1, Soft feces; 2, Mild diarrhea; 3, Severe diarrhea. Scores were calculated as the average diarrhea score for each period per treatment group by summing the average daily diarrhea scores of each pig. The frequency of diarrhea was calculated by counting pen days in which the average diarrhea score of individual pigs in each pen was ≥ 2.

Nutrient digestibility

To estimate the digestibility, 0.2% chromium oxide (Cr_2O_3) was supplemented with the diets as an indigestible marker. Fresh fecal samples from each treatment with 6 replicates are collected by rectal massage at 1, 3, and 5 weeks to determine the apparent total tract digestibility (ATTD) of dry matter (DM), CP, and gross energy (GE). Fresh fecal and feed samples were stored in a freezer at –20℃ immediately after collection. At the end of the experiment, fecal samples were dried at 70℃ for 72 h and then crushed on a 1 mm screen. The procedures utilized for the determination of DM and CP digestibility were conducted with the methods by the AOAC [26] and for GE using a bomb calorimeter (Parr 6400, Parr Instruments). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu) using the Williams et al. [27] method. For

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Table 2. Compositions of basal diet and feeding experimental diets (as-fed-basis; phase 1/0–1w)

Items	HP0	HP25	HP50	HP100
Ingredients (%)				
Corn	37.92	37.92	37.92	37.92
Extruded corn	15.00	15.00	15.00	15.00
Lactose	10.00	10.00	10.00	10.00
Dehulled soybean meal (47% CP)	10.00	10.00	10.00	10.00
Soy protein concentrate (65% CP)	9.00	9.00	9.00	9.00
SDPP	5.00	3.75	2.50	-
HP	-	1.25	2.50	5.00
Whey	7.00	7.00	7.00	7.00
Soy oil	2.10	2.10	2.10	2.10
Monocalcium phosphate	1.22	1.22	1.22	1.22
Limestone	1.10	1.10	1.10	1.10
L-lysine-HCl (78%)	0.40	0.42	0.44	0.46
DL-methionine	0.16	0.14	0.12	0.10
Choline chloride (25%)	0.10	0.10	0.10	0.10
Vitamin premix[1)]	0.25	0.25	0.25	0.25
Trace mineral premix[2)]	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated value				
ME (kcal/kg)	3,428	3,428	3,428	3,428
CP (%)	20.48	20.47	20.46	20.44
Lysine (%)	1.51	1.51	1.51	1.51
Methionine (%)	0.44	0.44	0.44	0.44
Ca (%)	0.80	0.80	0.80	0.80
P (%)	0.65	0.65	0.65	0.65

[1)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K_3, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 mg.

[2)]Provided per kg of complete diet without Zinc: Cu (as $CuSO_4$•$5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as Na_2SeO_3•$5H_2O$), 0.15 mg.

SDPP, spray-dried plasma protein; HP, hydrolyzed porcine intestinal protein; CP, crude protein; ME, metabolize energy; Ca, calcium; P, phosphorus.

calculating the ATTD of the nutrients, we used the following Equation:

$$\text{Digestibility} = 1 - [(Nf \times Cd) / (Nd \times Cf)] \times 100,$$

where Nf = concentration of nutrient in fecal, Nd = concentration of nutrient in the diet, Cd = concentration of chromium in the diet, and Cf = concentration of chromium in the fecal.

Blood profiles

Blood samples were obtained from the jugular vein of six pigs per each treatment at 1, 3, and 5 weeks. At the time of collection, blood samples were collected into vacuum tubes containing K_3EDTA for complete blood count analysis and nonheparinized tubes for serum analysis, respectively. After collection, blood samples were centrifuged (12,500×g for 20 min at 4℃). The white blood cells, red blood cells, lymphocyte, neutrophil, eosinophil, and monocyte in whole blood

Table 3. Compositions of basal diet and feeding experimental diets (as-fed-basis; phase 2/1–3w)

Items	HP0	HP25	HP50	HP100
Ingredients (%)				
Corn	41.45	41.45	41.45	41.45
Extruded corn	15.00	15.00	15.00	15.00
Lactose	8.00	8.00	8.00	8.00
Dehulled soybean meal (47% CP)	13.50	13.50	13.50	13.50
Soy protein concentrate (65% CP)	9.00	9.00	9.00	9.00
SDPP	2.50	1.87	1.25	-
HP	-	0.63	1.25	2.50
Whey	5.00	5.00	5.00	5.00
Soy oil	1.80	1.80	1.80	1.80
Monocalcium phosphate	1.29	1.29	1.29	1.29
Limestone	1.05	1.06	1.06	1.05
L-lysine-HCl (78%)	0.30	0.30	0.31	0.33
DL-methionine	0.11	0.10	0.09	0.08
Choline chloride (25%)	0.10	0.10	0.10	0.10
Vitamin premix[1)]	0.25	0.25	0.25	0.25
Trace mineral premix[2)]	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00
Calculated value				
ME (kcal/kg)	3,406	3,406	3,406	3,406
CP (%)	20.24	20.23	20.22	20.20
Lysine (%)	1.35	1.35	1.35	1.35
Methionine (%)	0.39	0.39	0.39	0.39
Ca (%)	0.80	0.80	0.80	0.80
P (%)	0.65	0.65	0.65	0.65

[1)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K_3, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 mg.

[2)]Provided per kg of the complete diet without Zinc: Cu (as $CuSO_4$•$5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as Na_2SeO_3•$5H_2O$), 0.15 mg.

SDPP, spray-dried plasma protein; HP, hydrolyzed porcine intestinal protein; CP, crude protein; ME, metabolize energy; Ca, calcium; P, phosphorus.

were measured using an automatic hematology analyzer (XE2100D, Sysmex). The total protein (TP) and blood urea nitrogen (BUN) in the blood were measured using a fully automated chemistry analyzer (Cobas C702, Hoffmann-La Roche).

Bacteria counts in feces

Microbial analysis was immediately carried out according to the method described by Hu et al. [28]. After fresh fecal sample collection, they were placed on ice and transported directly to the lab, and 1 g of a fecal sample from each treatment was diluted in 9 mL of 1 × phosphate-buffered saline (PBS; GenDEPOT) and then homogenized. Then, viable bacteria were counted in fecal samples by placing serial 10-fold dilutions on MacConkey agar plates (KisanBio) for *Escherichia coli* and de Man, Rogosa and Sharpe (MRS) agar (KisanBio). The MacConkey agar plates were incubated for 24 h at 37℃, and the MRS agar plate were then incubated for 48 h at 39℃ under anaerobic conditions. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the

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Table 4. Compositions of basal diet and feeding experimental diets (as-fed-basis; phase 3/3–5w)

Items	HP0	HP25	HP50	HP100
Ingredients (%)				
Corn	63.15	63.15	63.15	63.15
Extruded corn	5.00	5.00	5.00	5.00
Lactose	3.00	3.00	3.00	3.00
Dehulled soybean meal (47% CP)	15.70	15.70	15.70	15.70
Soy protein concentrate (65% CP)	8.00	8.00	8.00	8.00
SDPP	1.00	0.75	0.50	-
HP	-	0.25	0.50	1.00
Soy oil	0.80	0.80	0.80	0.80
Monocalcium phosphate	1.12	1.12	1.12	1.12
Limestone	0.99	0.99	0.99	0.99
L-lysine-HCl (78%)	0.27	0.27	0.27	0.28
DL-methionine	0.07	0.07	0.07	0.06
Choline chloride (25%)	0.10	0.10	0.10	0.10
Vitamin premix[1)]	0.25	0.25	0.25	0.25
Trace mineral premix[2)]	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated value				
ME (kcal/kg)	3,387	3,387	3,387	3,387
CP (%)	19.90	19.89	19.88	19.86
Lysine (%)	1.24	1.24	1.24	1.24
Methionine (%)	0.36	0.36	0.36	0.36
Ca (%)	0.70	0.70	0.70	0.70
P (%)	0.60	0.60	0.60	0.60

[1)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K_3, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 mg.

[2)]Provided per kg of complete diet without Zinc: Cu (as $CuSO_4{\cdot}5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3{\cdot}5H_2O$), 0.15 mg.

SDPP, spray-dried plasma protein; HP, hydrolyzed porcine intestinal protein; CP, crude protein; ME, metabolize energy; Ca, calcium; P, phosphorus.

incubator and expressed as the logarithm of colony-forming units per gram (Log CFU/g).

Economic evaluation

The economic evaluation of replacing SDPP with a different ratio of HP was determined based on feed costs without considering other deductible costs. The prices were expressed in dollars (USD). For each experimental phase, total feed intake (TFI, kg) and total weight gain (TWG, kg) were calculated. Also, feed cost per kg gain (FCG, kg) is represented by: TFI × feed cost / TWG. The feed cost was obtained from a feed company (DH Vital Feed). The feed costs per treatment were as follows: Phase 1, HP0: 1.22 USD/kg; HP25: 1.19 USD/kg; HP50: 1.15 USD/kg; HP100: 1.09 USD/kg; Phase 2, HP0: 1.08 USD/kg; HP25: 1.06 USD/kg; HP50: 1.04 USD/kg; HP100: 1.01 USD/kg; Phase 3, HP0: 0.94 USD/kg; HP25: 0.94 USD/kg; HP50: 0.93 USD/kg; HP100: 0.92 USD/kg.

Statistical analysis

All data except for the frequency of diarrhea were statistically analyzed using the general linear model's procedure of SAS (Statistical Analysis System 9.1, SAS Institute), using each pen as the experimental unit. Orthogonal polynomial contrasts were used to analyze the significance of the linear or quadratic effects of replacing SDPP with different ratios of HP. The frequency of diarrhea was compared with a chi-square test, using the FREQ. Differences between treatment means were determined using Tukey's multiple range test. The variability in the data was expressed as the pooled standard error. A probability level of $p < 0.05$ was indicated to be statistically significant, and a level of $0.05 \leq p < 0.10$ was considered to have such a tendency.

RESULTS

Growth performance

The effect of replacing SDPP with different ratios of HP in the diet on growth performance is presented in Table 5. At 5 weeks, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) BW. At 0 to 1 weeks, the HP100 diet significantly decreased ($p < 0.05$) ADG compared with the HP0 diet. Also, the HP50 and HP100 diets significantly decreased ($p < 0.05$) G:F compared with the HP0 diet. Increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) ADG and G:F. In contrast, increasing levels of replacing SDPP with HP significantly increased (linear, $p < 0.05$) ADFI. At 3 to 5 weeks, the HP0 and HP25 diets

Table 5. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on growth performance of weaning pigs

Items	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
BW (kg)								
Initial	7.31	7.36	7.39	7.33	0.299	0.998	-	-
Weeks 1	8.43	8.46	8.42	8.35	0.289	0.993	0.818	0.867
Weeks 3	12.83	12.96	12.76	12.67	0.304	0.917	0.618	0.717
Weeks 5	19.80	19.94	19.44	18.83	0.312	0.087	0.024	0.245
Weeks 0–1								
ADG (g)	160.00[a]	157.14[ab]	146.90[ab]	145.48[b]	3.609	0.021	0.003	0.845
ADFI (g)	276.00	274.05	284.67	289.29	5.360	0.179	0.048	0.547
G:F	0.58[a]	0.57[ab]	0.52[bc]	0.50[c]	0.015	0.002	< 0.001	0.822
Weeks 1–3								
ADG (g)	314.05	321.43	310.12	308.57	4.868	0.275	0.217	0.370
ADFI (g)	585.50	584.67	590.33	589.33	6.430	0.903	0.557	0.990
G:F	0.54	0.55	0.53	0.52	0.012	0.375	0.215	0.560
Weeks 3–5								
ADG (g)	497.86[a]	498.33[a]	477.14[ab]	440.24[b]	15.077	0.042	0.009	0.229
ADFI (g)	923.33	916.17	918.33	924.83	7.233	0.811	0.839	0.356
G:F	0.54[a]	0.54[a]	0.52[ab]	0.48[b]	0.014	0.013	0.003	0.103
Weeks 0–5								
ADG (g)	356.76[a]	359.33[a]	344.29[ab]	328.62[b]	4.959	0.001	< 0.001	0.081
ADFI (g)	658.73	655.14	660.40	663.52	3.560	0.430	0.232	0.357
G:F	0.54[a]	0.55[a]	0.52[ab]	0.50[b]	0.007	<0.001	< 0.001	0.035

[a–c]Means within a row with different letters are significantly different at $p < 0.05$.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; BW, body weight, ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; SE, standard error.

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significantly increased ADG and G:F compared with the HP100 diet. Also, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) ADG and G:F. At 0 to 5 weeks, the HP0 and HP25 diets significantly increased ($p < 0.05$) ADG and G:F compared with the HP100 diet. Also, increasing levels of replacing SDPP with HP significantly increased (linear, $p < 0.05$) ADG and G:F. Moreover, increasing levels of replacing SDPP with HP significantly affected (quadratic, $p < 0.05$) the G:F.

Diarrhea scores

The effect of replacing SDPP with different ratios of HP in the diet on diarrhea scores is presented in Table 6. There was no significant difference ($p > 0.05$) in diarrhea scores and frequency of diarrhea during the experimental phases.

Nutrient digestibility

The effect of replacing SDPP with different ratios of HP in the diet on nutrient digestibility is presented in Table 7. At 1 weeks, the HP100 diet significantly decreased ($p < 0.05$) CP digestibility compared with the HP0 diet. Also, at 3 weeks, the HP0 and HP25 diets significantly increased ($p < 0.05$) CP digestibility compared with the HP50 and HP100 diets. Moreover, at 5 weeks, the HP0 and HP25 diets significantly increased ($p < 0.05$) CP digestibility compared with the HP100 diet. Furthermore, at 1, 3, and 5 weeks, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) CP digestibility.

Blood profile

The effect of replacing SDPP with different ratios of HP in the diet on the blood profile is presented in Table 8. At 1 and 3 weeks, increasing levels of replacing SDPP with HP significantly decreased ($p < 0.05$) lymphocyte levels. At 5 weeks, the HP0 and HP25 diets significantly decreased ($p < 0.05$) TP levels compared with the HP50 diet. The HP0 and HP25 diets significantly decreased ($p < 0.05$) BUN levels compared with the HP100 diet. At 3 and 5 weeks, increasing levels of replacing SDPP with HP significantly increased ($p < 0.05$) TP and BUN levels.

Bacteria counts in feces

The effect of replacing SDPP with different ratios of HP in the diet on fecal microflora is presented in Table 9. At 5 weeks, the HP25 diet significantly increased ($p < 0.05$) the counts of *Lactobacillus*

Table 6. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on diarrhea score of weaning pigs

Items	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
Diarrhea score[1]								
Weeks 0–1	1.60	1.67	1.66	1.63	0.031	0.396	0.614	0.106
Weeks 1–3	1.70	1.70	1.70	1.74	0.050	0.897	0.580	0.867
Weeks 3–5	1.38	1.36	1.38	1.41	0.020	0.324	0.174	0.280
Weeks 0–5	1.55	1.60	1.60	1.62	0.029	0.394	0.123	0.441
Frequency of diarrhea[2] (%)	27.62	33.82	35.72	32.62	-	0.269	-	-

[1]Diarrhea score was determined as follow: 0, Normal feces; 1, Soft feces; 2, Mild diarrhea; 3, Severe diarrhea.

[2]Frequency of diarrhea (%) = (number of pigs with diarrhea / number of pen days) × 100.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; SE, standard error.

Table 7. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on nutrient digestibility of weaning pigs

Items (%)	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
Weeks 1								
DM	85.22	84.96	84.99	84.76	0.217	0.517	0.174	0.946
CP	81.74[a]	81.43[ab]	80.28[ab]	79.95[b]	0.431	0.021	0.003	0.985
GE	81.95	81.91	81.93	81.74	0.459	0.986	0.766	0.863
Weeks 3								
DM	83.59	83.48	83.04	83.34	0.172	0.153	0.136	0.244
CP	81.14[a]	81.12[a]	80.20[b]	80.11[b]	0.211	0.002	< 0.001	0.855
GE	80.46	80.39	79.92	80.23	0.178	0.174	0.156	0.297
Weeks 5								
DM	82.58	82.70	81.98	81.79	0.601	0.655	0.263	0.801
CP	80.14[a]	80.06[a]	79.46[ab]	79.06[b]	0.241	0.014	0.002	0.511
GE	80.19	80.12	79.62	79.44	0.794	0.885	0.449	0.944

[a,b]Means within a row with different letters are significantly different at $p < 0.05$.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error.

in feces compared with the HP50 and HP100 diets. Also, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) the counts of *Lactobacillus* in feces.

Economic evaluation

The effect of replacing SDPP with different ratios of HP in the diet on economic evaluation is presented in Table 10. At 0 to 1 weeks, the HP100 diet significantly decreased ($p < 0.05$) TWG compared with the HP0 diet. Also, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) TWG. At 3 to 5 weeks and the experimental periods, the HP0 and HP25 diets significantly increased ($p < 0.05$) TWG compared with the HP100 diet. In addition, increasing levels of replacing SDPP with HP significantly decreased (linear, $p < 0.05$) TWG. At 3 to 5 weeks, the HP25 diet significantly decreased ($p < 0.05$) FCG compared with the HP100 diet. Also, during the experimental periods, the HP25 diet showed a tendency ($p = 0.087$) for FCG to be lower than the HP0 diet and increasing levels of replacing SDPP with HP showed a tendency (linear, $p = 0.083$) for FCG to be decreased.

DISCUSSION

In this study, increasing levels of replacing SDPP with HP in diets linearly decreased ADG and G:F during the 0 to 5 weeks. This result was in agreement with previous studies showing, that pigs fed with increasing levels of replacing SDPP with HP (50% and 100%, respectively) have impaired ADG and G:F compared with HP0 diets (based on SDPP) in weaning pigs [24,29]. According to Kazimierska and Biel [30], SDPP possesses a higher CP content (75.43 vs. 71.42, %) and metabolizable energy content (342.69 vs. 339.85, kcal/100 g) than HP. It is well-documented that dietary CP and energy levels are major factors for regulating ADG and G:F in pigs, which could support the linear decrease of growth performance in this study [31,32].

In contrast, the HP25 diet did not significantly affect ADG or G:F unlike HP0 diet (Table 5). Moreover, HP25 diet increased ADG and G:F compared with the HP100 diet (Table 5). Previous

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Table 8. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on blood profiles of weaning pigs

Items	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
Weeks 1								
RBC (10^6/μL)	7.01	7.02	7.04	7.06	0.023	0.356	0.080	0.909
TP (g/dL)	4.97	4.67	5.02	5.00	0.141	0.281	0.362	0.207
BUN (mg/dL)	8.53	7.85	8.50	8.18	0.264	0.253	0.953	0.222
WBC (10^3/μL)	18.90	18.44	18.74	18.38	0.281	0.527	0.421	0.535
Lymphocyte (%)	35.22	35.00	33.77	32.85	0.760	0.129	0.022	0.843
Neutrophil (%)	57.28	56.85	57.57	59.15	0.766	0.197	0.081	0.324
Eosinophil (%)	1.12	1.07	1.02	1.08	0.048	0.536	0.453	0.287
Monocyte (%)	5.53	5.58	5.98	5.95	0.232	0.397	0.109	0.858
Weeks 3								
RBC (10^6/μL)	6.87	6.81	6.97	6.93	0.081	0.536	0.278	0.758
TP (g/dL)	4.72	4.75	5.10	5.07	0.122	0.068	0.013	0.798
BUN (mg/dL)	9.17	9.67	10.67	11.17	0.580	0.091	0.013	0.788
WBC (10^3/μL)	18.64	18.80	18.75	18.30	0.214	0.366	0.297	0.262
Lymphocyte (%)	43.15	42.07	41.62	41.58	0.484	0.111	0.030	0.179
Neutrophil (%)	46.33	47.15	47.90	47.48	0.578	0.296	0.111	0.276
Eosinophil (%)	8.38	8.28	8.25	8.23	0.076	0.513	0.178	0.452
Monocyte (%)	1.67	1.68	1.98	2.12	0.165	0.173	0.032	0.850
Weeks 5								
RBC (10^6/μL)	6.85	6.86	7.04	6.83	0.081	0.239	0.597	0.394
TP (g/dL)	5.33[c]	5.37[bc]	5.98[a]	5.87[ab]	0.128	0.002	0.001	0.656
BUN (mg/dL)	9.17[b]	9.33[b]	11.00[ab]	11.50[a]	0.603	0.027	0.003	0.931
WBC (10^3/μL)	18.56	18.89	18.23	18.53	0.202	0.184	0.276	0.579
Lymphocyte (%)	52.25	51.10	49.90	47.92	2.339	0.606	0.197	0.966
Neutrophil (%)	38.22	39.33	40.40	42.57	2.308	0.595	0.196	0.993
Eosinophil (%)	1.23	1.27	1.28	1.38	0.063	0.399	0.130	0.870
Monocyte (%)	7.90	7.65	7.97	7.83	0.121	0.313	0.638	0.350

[a–c]Means within a row with different letters are significantly different at $p < 0.05$.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; WBC, white blood cell; RBC, red blood cell; TP, total protein; BUN, blood urea nitrogen; SE, standard error.

studies have reported that pigs fed with HP (supplementation with 2.5% and 5%, respectively) diets increased the ADG and G:F during the first 14 days after weaning [21,22]. Consistently, Joo and Chae [33] have indicated that substitution of SDPP (up to 50%) with HP did not impair ADG in weaning pigs. Sun et al. [17] have also demonstrated that the substitution of SDPP (50%) with hydrolyzed protein product showed the same effects on ADG and G:F in weaning pigs.

Also, no significant differences in growth performance between the HP25 and HP0 diets might be derived from the same levels of CP digestibility in this study (Table 5). Numerous studies have showed that the positive correlation between improved ATTD of CP and enhanced growth performance in pigs [34,35]. Consistently, we observed similar CP digestibility with HP0 (based on SDPP) and HP25 diets. According to Kim et al. [24], replacing SDPP (up to 50%) with the HP diet did not show a difference in CP digestibility in weaning pigs. For these reasons, it seems that replacing SDPP with 25% of the HP diet is adequate for providing sufficient nutrients for promoting ADG and G:F with increased CP digestibility, thereby improving growth performance

Table 9. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on fecal microflora of weaning pigs

Items (Log CFU/g)	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
Weeks 1								
Escherichia coli	6.40	6.42	6.44	6.46	0.028	0.495	0.131	0.925
Lactobacillus	7.33	7.31	7.28	7.30	0.021	0.448	0.198	0.390
Weeks 3								
Escherichia coli	6.32	6.32	6.36	6.37	0.030	0.470	0.124	0.944
Lactobacillus	7.34	7.35	7.26	7.28	0.036	0.251	0.098	0.908
Weeks 5								
Escherichia coli	6.21	6.18	6.25	6.24	0.028	0.326	0.170	0.575
Lactobacillus	7.38[ab]	7.40[a]	7.29[b]	7.28[b]	0.025	0.004	0.001	0.505

[a,b]Means within a row with different letters are significantly different at $p < 0.05$.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; CFU, colony forming unit; SE, standard error.

Table 10. Effect of different ratios of spray-dried plasma protein (SDPP) and hydrolyzed porcine intestinal protein (HP) in the diet on economic evaluation of weaning pigs

Items	HP0	HP25	HP50	HP100	SE	*p*-value		
						Diet	Linear	Quadratic
Weeks 0–1								
TWG (kg/pig)	1.12[a]	1.10[ab]	1.03[ab]	1.02[b]	0.025	0.021	0.003	0.845
TFI (kg/pig)	1.93	1.92	1.99	2.02	0.038	0.179	0.048	0.541
FCG (USD/kg gain)	2.11	2.08	2.25	2.17	0.067	0.289	0.242	0.739
Weeks 1–3								
TWG (kg/pig)	4.40	4.50	4.34	4.32	0.068	0.275	0.217	0.370
TFI (kg/pig)	8.20	8.19	8.26	8.25	0.090	0.903	0.560	0.978
FCG (USD/kg gain)	2.02	1.93	2.08	1.93	0.043	0.367	0.280	0.718
Weeks 3–5								
TWG (kg/pig)	6.97[a]	6.98[a]	6.68[ab]	6.16[b]	0.211	0.042	0.009	0.229
TFI (kg/pig)	12.93	12.83	12.86	12.95	0.101	0.811	0.836	0.356
FCG (USD/kg gain)	1.76[ab]	1.73[b]	1.80[ab]	1.94[a]	0.054	0.047	0.018	0.114
Weeks 0–5								
TWG (kg/pig)	12.49[a]	12.58[a]	12.05[ab]	11.50[b]	0.174	0.001	< 0.001	0.081
TFI (kg/pig)	23.06	22.93	23.11	23.22	0.125	0.430	0.231	0.350
FCG (USD/kg gain)	1.95	1.89	1.96	2.00	0.028	0.087	0.083	0.115

[a,b]Means within a row with different letters are significantly different at $p < 0.05$.

HP0, basal diet on SDPP; HP25, basal diet with 25% replacement of SDPP with HP; HP50, basal diet with 50% replacement of SDPP with HP; HP100, basal diet with 100% replacement of SDPP with HP; TWG, total weight gain; TFI, total feed intake; FCG, feed cost per kg gain; SE, standard error.

of weaning pigs.

Major concerns for weaning pigs are pathogenic microbes' infection, which can induce intestinal damage and diarrhea [36–38]. In this study, although we replaced the SDPP with a different ratio of HP, there were no significant differences in diarrhea scores or increases in counts of *Lactobacillus*. In other words, replacing SDPP with HP did not impair microorganisms in intestinal tracts of pigs. Correlated with this study, Hossain et al. [39] have reported that replacing fish meal with HP (1.5%)

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increased the counts of *Lactobacillus* in weaning pigs. Also, Sun et al. [17] have demonstrated that replacing SDPP with hydrolyzed protein sources (1%–2%) did not show differences in diarrhea rate in weaning pigs. Supplementation of HP can increase the number of goblet cells [40] and the expression of tight junction protein in the intestine [41,42], thereby enhancing intestinal barrier function, intestinal mucosa permeability, and gut health [36,43].

SDPP is a protein source that can improve immune regulation through its immunoglobulin fractions in weaning pigs [44,45]. Previous studies have demonstrated that supplementation of SDPP (2%–5%) can enhance the immunity system in weaning pigs [9,46]. In this study, replacing SDPP with HP linearly decrease on lymphocytes and a linear increase on monocytes, TP, and BUN. As lymphocytes in the whole blood provide specific cellular with humoral immune responses [38] and monocytes activate the specific immune response by presenting antigens and releasing cytokines [47], changes in these levels indicate a decline in the immune system of weaning pigs. Supportably, Kim et al. [29] have indicated that higher inclusion levels of HP with SDPP diets are associated with lower lymphocyte and monocyte in weaning pigs. However, HP25 diets decreased TP and BUN levels compared with HP50 and HP100 diets. TP in the blood is used as an indicator of liver-protein metabolism [48,49]. BUN is also used as an index of protein and amino acid catabolism, which indicates nitrogen absorption and protein utilization in the body [50,51].

The major objective of replacing SDPP with different ratios of HP in weaning phase was to meet the cost-effective substitute protein source, due to its high cost. In this study, replacing SDPP with 25% of the HP diet numerically decreased FCG compared with the CON (based on SDPP) diet. Generally, profit margin was determined by the BW, feed intake, and feed cost [52]. As mentioned above, replacing SDPP with 25% of HP had no effect on ADG or CP digestibility, thereby causing no difference in TWG. Correlated with this study, Joo and Chae [33] have reported that replacing SDPP with HP can decrease feed cost by 0.09 USD/kg. As feed costs represent 65%–75% of total production costs, alternative protein resources are required for a cost-effective production [23,53]. Therefore, this study's results suggest the possibility of an economical feeding strategy without showing adverse effects by replacing 25% of SDPP with HP in weaning pigs.

CONCLUSION

The result of this study supports the possibility of replacing SDPP with HP in weaning pigs. In this study, replacing SDPP with 25% of HP diets did not show significant differences between SDPP diets. Also, replacing SDPP with 25% of HP diets showed enhanced growth performance, nutrient digestibility, blood profile, and fecal microflora in weaning pigs.

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