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Non ruminant nutrition

Nutritional value of a new source of cheese coproduct fed to weanling pigs

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Abstract

Three experiments were conducted to test the hypothesis that values for standardized ileal digestibility (**SID**) of amino acids (**AA**) and metabolizable energy (**ME**) in a the cheese coproduct are greater than in fish meal or enzyme-treated soybean meal (**ESBM**). The second objective was to test the hypothesis that pigs fed a diet containing cheese coproduct will have growth performance that is not different from that of pigs fed other sources of protein. In experiment 1, eight ileal-cannulated barrows (11.0 ± 0.4 kg) were allotted to a replicated 4 × 4 Latin square design with four diets and four periods and two pigs per diet in each period. The four diets included an N-free diet and three diets that contained ESBM, fish meal, or the cheese coproduct as the source of AA. Results indicated that the cheese coproduct had greater ($P < 0.05$) SID of most AA compared with ESBM and fish meal. In experiment 2, 32 weanling barrows (14.0 ± 1.1 kg) were housed individually in metabolism crates and randomly allotted to one of four diets. A corn-based diet and three diets that contained corn and ESBM, fish meal, or cheese coproduct were formulated. Feces and urine were collected quantitatively. The ME in cheese coproduct was greater ($P < 0.05$) than in ESBM and fish meal. In experiment 3, 128 weaned pigs (6.2 ± 0.6 kg) were allotted to a randomized complete block design with four treatments and 8 replicate pens per diet. Phase 1 diets that contained 0%, 6.65%, 7.35%, or 14% cheese coproduct were fed from days 1 to 14 and a common phase 2 diet without cheese coproduct was fed from days 15 to 28. Individual pig weights were recorded at the beginning of the experiment, on days 14 and 28, and daily feed allotments were also recorded. Two blood samples were collected from 1 pig per pen on day 14 to analyze for blood urea N, albumin, total plasma protein, peptide YY, immunoglobulin G, tumor necrosis factor-α, interleukin-6, and interleukin-10. No differences were observed in average daily gain among treatments, but there was a tendency ($P < 0.10$) for total protein on day 14 to increase as cheese coproduct increased in the diets. In conclusion, the cheese coproduct used in this experiment has a greater SID of AA and greater ME than ESBM and fish meal and the cheese coproduct may be included in prestarter diets for weanling pigs without negatively impacting growth performance or indicators of intestinal health.

Lay Summary

Milk proteins are highly digestible and have an excellent balance of indispensable amino acids (AA), but the price of dairy products are expensive compared with other protein sources. However, cheese that cannot be used for human consumption may be used in diets for pigs, but there is limited information about the nutritional value of cheese coproducts. Therefore, three experiments were conducted to determine the standardized ileal digestibility of AA, and the metabolizable energy (ME) of a cheese coproduct and effects of inclusion of different levels of cheese coproduct in phase 1 (1 to 14 d postweaning) diets for weanling pigs. Results demonstrated that the cheese coproduct has an excellent digestibility of AA and due to its greater ME than fish meal and enzyme-treated soybean meal, cheese coproduct can be used to increase the energy density of diets for weanling pigs without affecting the health or growth performance of pigs.

Key words: amino acids, cheese coproduct, digestibility, growth, metabolizable energy, pigs

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; DE, digestible energy; ESBM, enzyme treated soybean meal; G:F, gain to feed ratio; ME, metabolizable energy; SID, standardized total tract digestibility; STTD, standardized total tract digestibility

Introduction

Animal proteins such as milk protein, blood protein, and fish meal are often included in diets for weanling pigs as sources of amino acids (**AA**), because these ingredients have a high digestibility of nutrients and are free of antinutritional factors (Rojas and Stein, 2015). Unlike older pigs, weanling pigs are negatively affected if fed exclusively with protein from soybean meal, because soybean meal contains trypsin inhibitors and nondigestible oligosaccharides, which negatively affect nutrient digestibility and intestinal health of young pigs (Pieterse et al., 2000; Limbach et al., 2021). Soybean meal can be processed to remove the antinutritional factors, and fermented soybean meal, enzyme-treated soybean meal (**ESBM**), and soy protein concentrate may, therefore, be used in diets for weanling pigs (Jones et al., 2010; Kim et al., 2010; Rojas and Stein, 2013). However, processed soybean products did

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not support pig growth performance equal to that from feeding dried skim milk, especially during the first week following weaning (Coffey et al., 1995).

Milk proteins are highly digestible and have an excellent balance of indispensable AA, and inclusion of milk byproducts in diets for pigs improve average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**; Grinstead et al., 2000; Arevalo et al., 2016). However, milk proteins are expensive compared with other proteins and weaned pig diets containing high levels of milk byproducts are, therefore, expensive (Yoo et al., 2018).

Cheese that cannot be used for human consumption may be used in the feeding of animals (Sohn and Maxwell, 1991), but there is limited information about the nutritional value and the effects of replacing other dietary proteins by cheese coproducts in diets fed to weanling pigs. Therefore, three experiments were conducted to test the hypothesis that values for standardized ileal digestibility (**SID**) of AA and concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) in a cheese coproduct are greater than in fish meal or ESBM. The second objective was to test the hypothesis that pigs fed diets containing cheese coproduct will have growth performance that is not different from that of pigs fed traditional sources of protein.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for three experiments. Pigs that were used in the three experiments were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA). The cheese coproduct that was used in all experiments (Pro 88) was produced by Keys Manufacturing Co. Inc., Paris, IL, USA. The cheese coproduct was mixed with soybean meal (30% to 40% of the final product) because soybean meal was used as a carrier to facilitate the drying process of the cheese. Before drying, the cheese and soybean meal were pulverized and then dried at 40.5 °C using a drum drier until the product reached a moisture concentration lower than 10%.

Experiment 1: Amino Acid Digestibility

Animals and treatments

Eight weanling pigs with an average initial body weight of 11.0 ± 0.4 kg that had a T-cannula installed in the distal ileum (Stein et al., 1998) were allotted to a replicated 4 × 4 Latin square design with four diets and four 7-d periods in each square (Kim and Stein, 2009). There were two pigs per diet in each period for a total of 8 observations per treatment. Pigs were placed in individual pens (1.2 m × 1.5 m) that were equipped with a self-feeder, a nipple waterer, and a fully slatted tri-bar floor.

Fish meal (Omega protein), enzyme treated soybean meal (**ESBM**; Hamlet Protein, Findlay, OH), and cheese coproduct were the three protein sources used (Table 1). Three diets were based on each of these ingredients as the only source of AA. A N-free diet was included in the experiment to determine basal endogenous losses of crude protein and AA (Table 2). Thus, a total of four diets were used. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

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

Item	ESBM[1]	Fish meal[2]	Cheese coproduct[3]
Dry matter, %	95.19	92.20	96.91
Gross energy, kcal/kg	4,512	4,124	5,279
Crude protein, %	56.07	64.00	43.65
Acid hydrolyzed ether extract, %	2.49	2.46	17.90
Insoluble dietary fiber, %	16.6	–	13.5
Soluble dietary fiber, %	0.6	–	1.3
Total dietary fiber, %	17.2	–	14.8
Ash, %	7.92	7.11	8.63
Indispensable AA, %			
Arg	3.83	3.73	2.60
His	1.45	1.38	1.21
Ile	2.71	2.59	2.19
Leu	4.24	4.22	3.63
Lys	3.07	4.84	2.98
Met	0.77	1.68	0.82
Phe	2.90	2.43	2.31
Thr	2.09	2.41	1.62
Trp	0.70	0.64	0.62
Val	2.77	2.92	2.41
Dispensable AA, %			
Ala	2.32	3.89	1.62
Asp	6.04	5.43	4.14
Cys	0.79	0.51	0.45
Glu	9.57	7.86	7.98
Gly	2.28	4.65	1.44
Pro	2.77	2.93	2.96
Ser	2.27	2.00	1.90
Tyr	2.05	1.91	1.93

[1]ESBM, enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA).
[2]Fish meal sourced from Omega protein, Reedville, VA, USA.
[3]Cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA.

Experimental procedures

Pigs were limit fed to 3.2 times their estimated maintenance requirement for ME, which was calculated as 197 kcal/kg body weight$^{0.60}$ (NRC, 2012), but throughout the experiment, pigs had free access to water. The first 5 d of each period was considered the adaptation period to the diet, whereas ileal digesta were collected for 8 h on days 6 and 7 of each period. A 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Digesta were stored at −20 °C immediately after collection to prevent bacterial degradation of AA in the digesta.

Table 2. Composition (as-is basis) of diets used in experiment

Ingredient, %	Diet			
	ESBM[1]	Fish meal[2]	Cheese coproduct[3]	N-free
ESBM	36.00	–	–	–
Fish meal	–	29.05	–	–
Cheese coproduct		–	40.00	
Milk, lactose	20.00	20.00	20.00	20.00
Cornstarch	38.75	50.00	35.00	67.90
Soybean oil	2.00	0.00	2.00	4.00
Solca flok	–		–	4.00
Dicalcium phosphate	1.30	0.00	1.05	2.15
Limestone	1.00	0.00	1.00	0.50
Chromic oxide	0.40	0.40	0.40	0.40
Magnesium oxide	–	–	–	0.10
Potassium carbonate	–	–	–	0.40
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin-micromineral premix[3]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Analyzed composition, %				
Dry matter	93.11	92.27	94.13	92.09
Crude protein	19.00	18.79	17.63	0.11
Indispensable AA				
Arg	1.38	1.16	1.02	0.01
His	0.52	0.45	0.51	0.00
Ile	0.95	0.86	0.88	0.02
Leu	1.54	1.44	1.53	0.02
Lys	1.15	1.66	1.25	0.01
Met	0.28	0.56	0.33	0.00
Phe	1.05	0.80	0.96	0.01
Thr	0.78	0.82	0.70	0.01
Trp	0.26	0.19	0.24	0.02
Val	0.97	0.95	0.98	0.01
Dispensable AA				
Ala	0.86	1.26	0.68	0.01
Asp	2.22	1.81	1.74	0.01
Cys	0.28	0.16	0.19	0.00
Glu	3.62	2.73	3.54	0.02
Gly	0.85	1.40	0.60	0.01
Pro	1.02	0.90	1.31	0.01
Ser	0.88	0.73	0.85	0.01
Tyr	0.66	0.51	0.71	0.01

[1]ESBM, enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA).
[2]Fish meal sourced from Omega Protein, Reedville, VA, USA.
[3]Cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA.
[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 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 thiamin mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 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.

Sample analyses

At the conclusion of the experiment, ileal digesta were thawed and mixed within animal and diet, and a subsample was collected for analysis. Samples of all diets and of each of the AA-containing ingredients were also collected. Ileal digesta were lyophilized and finely ground before analysis. Samples of diets, ileal digesta, and ingredients were analyzed for dry matter (method 930.15; AOAC Int., 2019) and N was analyzed (method 990.03; AOAC Int., 2019) on an FP628 protein analyzer (Leco Corporation, St. Joseph, MI). Crude protein was calculated as N × 6.25. These samples were also analyzed for AA on a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard (method 982.30 E [a, b, c]; AOAC Int., 2019). Ingredients were analyzed for acid-hydrolyzed ether extract (Method AM 5-04; AOAC Int, 2019), which was analyzed by acid hydrolysis using 3*N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology). Gross energy in ingredients was analyzed using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL), and ingredients were analyzed for ash as well (method 942.05; AOAC Int., 2019). Ingredients were also analyzed for total dietary fiber (method 991.43; AOAC Int., 2019) using standard procedures as described by Navarro et al. (2018). Diets and ileal digesta samples were also analyzed for chromium (method 990.08; AOAC Int., 2019). All analyses were conducted in duplicates.

Calculation and statistical analyses

The apparent ileal digestibility (**AID**) and SID of crude protein and AA were calculated for the three diets containing ESBM, fish meal, or cheese coproduct (Stein et al., 2007). Values calculated for these three diets also represented the values for each ingredient. The basal endogenous losses of crude protein and AA were calculated from pigs fed the N-free diet (Stein et al., 2007).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Normality of residuals was confirmed using the UNIVARIATE procedure, and outliers were identified as individual means that deviated from the treatment mean by more than three times the interquartile range. One outlier from the cheese coproduct diet was removed. Data for AID and SID of crude protein and AA were analyzed using a model that included diet as fixed effect and pig and period as random effects. The pig was the experimental unit and differences were considered significant at $P < 0.05$ and considered a trend at $P \leq 0.10$.

Experiment 2: Energy Measurements

Animals and treatments

A corn-based diet and three diets containing a mixture of corn and cheese coproduct, fish meal, or ESBM were formulated (Table 3). The three diets with the protein supplements were formulated to an equal concentration of crude protein. Vitamins and minerals were included in all diets to meet requirement estimates (NRC, 2012). Thirty-two pigs with an initial body weight of 14.0 ± 1.1 kg were allotted to a randomized complete block design with four diets and 8 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped

Table 3. Composition (as-is basis) of experimental diets in experiment

	Diet			
Ingredient, %	Corn	ESBM[1]	Fish meal[2]	Cheese coproduct[3]
Ground corn	96.85	76.00	71.45	67.25
ESBM	–	21.15	–	–
Fish meal	–	–	28.00	–
Cheese coproduct	–	–	–	30.00
Dicalcium phosphate	1.80	1.40	–	1.30
Ground limestone	0.80	0.90	–	0.90
Sodium cloride	0.40	0.40	0.40	0.40
Vitamin micromineral premix [3]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
analyzed composition				
Dry matter,%	86.97	88.36	88.11	88.94
Gross energy, kcal/kg	3,649	3,832	3,941	4,137

[1]ESBM, enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA).
[2]Fish meal sourced from Omega Protein, Reedville, VA, USA.
[3]Cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA.
[4]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 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamin 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 mg as copper chloride; Fe, 123 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.

with a self-feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials.

Experimental procedures

Feed was supplied in meal form and pigs were limit fed daily at 3.2 times the ME requirement for maintenance. Daily feed provisions were divided into two equal meals that were provided at 0800 and 1600 hours. Water was available at all times. Pigs were fed experimental diets for 12 d. The initial 5 d were considered an adaptation period to the diet. Fecal markers were fed on day 6 (chromic oxide) and day 11 (ferric oxide) and fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at –20 °C immediately after collection. Urine collections were initiated on day 6 at 1600 hours and ceased on day 11 at 1600 hours. Urine buckets were placed under the metabolism crates to permit total collection. Buckets were emptied every morning and a preservative of 50 mL of 6 *N* HCl was added to each bucket when they were emptied. The weight of the collected urine was recorded and a 10% subsample was stored at –20 °C. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was lyophilized before analysis.

Sample analyses

Fecal samples were thawed and mixed within pig and diet, and then dried at 65 °C using a forced air drying oven. Samples were then ground through a 1-mm screen using a Hammermill (model MM4; Schutte Buffalo, NY, USA) before analysis. Urine samples were prepared for analysis according to Kim et al. (2009). Diets and fecal samples were analyzed for dry matter and diets, fecal samples, and urine samples were analyzed for gross energy as explained for experiment 1. All samples were analyzed in duplicate.

Calculation and statistical analyses

Apparent total tract digestibility (**ATTD**) of gross energy and dry matter was calculated for each diet. The DE and ME of corn were calculated by dividing the DE and ME of the corn diet by the inclusion rate of corn in that diet. The contribution of DE and ME from corn to the DE and ME in the diets containing cheese coproduct, fish meal, or ESBM was subtracted from the DE and ME of these diets, and the DE and ME of each ingredient was calculated by difference (Widmer et al., 2007). The ATTD of gross energy and dry matter in cheese coproduct, fish meal, and ESBM was calculated using the same procedure. Data were analyzed as explained for experiment 1.

Experiment 3: Growth Performance and Blood Characteristics

Animals and treatments

A total of 128 newly weaned pigs (initial body weight: 6.2 ± 0.6 kg) were allotted to a randomized complete block design with body weight as the blocking factor. There were four diets and 8 replicate pens per diet for a total of 32 pens, with 4 pigs per pen. A two-phase feeding program was used with days 1 to 14 as phase 1 and days 15 to 28 as phase 2. Pigs were fed one of four experimental diets during phase 1, whereas all pigs were fed a common diet in phase 2. Thus, a total of five diets were used (Table 4). The four experimental diets used in phase 1 consisted of: 1) a positive control diet based on corn and soybean meal with 3% spray dried plasma and 8% ESBM; 2) corn and soybean meal, 6% cheese coproduct, 8% ESBM, and no spray dried plasma; 3) corn and soybean meal, 7% cheese coproduct, 3% spray dried plasma, and no ESBM; and 4) corn and soybean meal, 14% cheese coproduct, and no spray dried plasma or ESBM. The cheese coproduct replaced ESBM or spray dried plasma on an equal SID Lys basis. The common diet used in phase 2 was based on corn and soybean meal. All phase 1 diets also contained 15% whey powder, and the phase 2 diet contained 10% whey powder and 3% spray dried plasma. All diets were formulated to meet nutrient requirements (NRC, 2012).

Experimental procedures

Individual pig weights were recorded at the beginning of the experiment and on days 14 and 28. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on days 14 and 28. At the conclusion of the experiment, data were summarized to calculate ADG, ADFI, and G:F within each pen and treatment group. Data were summarized for each phase, and over the entire experiment.

Table 4. Composition of experimental diets, as-fed basis, used in experiment 3[1]

Ingredient	Control diet	6.65 % cheese coproduct	7.35 % cheese coproduct	14 % cheese coproduct	Common diet Phase 2
Ground corn	45.54	41.85	46.11	42.45	51.66
Soybean meal	22.00	22.00	22.00	22.00	29.00
Cheese coproduct	–	6.65	7.35	14.00	–
ESBM	8.00	8.00	–	–	–
Spray dried plasma	3.00	–	3.00	–	3.00
Whey powder, dried	15.00	15.00	15.00	15.00	10.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Limestone	1.10	1.00	1.20	1.02	1.10
Dicalcium phosphate	0.95	1.05	0.90	1.05	0.90
L-Lysine HCl, 78%	0.33	0.34	0.33	0.34	0.28
DL-Met	0.12	0.13	0.13	0.15	0.11
L-Threonine	0.06	0.08	0.08	0.09	0.05
Salt	0.40	0.40	0.40	0.40	0.40
Vit-mineral premix[2]	0.50	0.50	0.50	0.50	0.50
Analyzed composition					
Dry matter, %	88.34	88.55	88.15	88.65	86.29
Gross energy, kcal/kg	4,003	3,926	3,923	4,146	3,867
Crude protein, %	20.34	20.65	19.12	19.19	13.41
Acid hydrolyzed ether extracted, %	3.26	5.03	4.72	6.29	2.75
Insoluble dietary fiber, %	11	11.8	10.3	8.5	10.1
Soluble dietary fiber, %	1.1	1.7	1.5	1	0.3
Total dietary fiber, %	12.1	13.5	11.8	9.5	10.4
Ash, %	10.56	9.06	8.19	7.69	4.91
Calculated composition[3]					
Metabolizable energy, Kcal/kg	3,449	3,425	3,418	3,391	3,423
Lys, %	1.43	1.43	1.43	1.43	1.34
Ile, %	0.87	0.93	0.83	0.89	0.80
Met, %	0.41	0.45	0.43	0.47	0.39
Met + Cys, %	0.79	0.78	0.78	0.78	0.76
Thr, %	0.84	0.84	0.83	0.83	0.79
Trp, %	0.27	0.27	0.26	0.24	0.26
Val, %	0.97	0.97	0.95	0.95	0.91

[1]ESBM, enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA); cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA; spray dried plasma = Apetin 901 (American Protein Corporation, Johnsonville, IA, USA).
[2]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 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamin 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 mg as copper chloride; Fe, 123 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.
Amino acids are indicated on the basis of standardized ileal digestible amino acids.
[3]Amino acids indicated as standardized ileal digestible amino acids.

At the end of phase one (day 14), two blood sample were collected from one pig in each pen from the jugular vein via vena puncture. One blood sample was collected in vacutainers that contained heparin, and the other sample was collected in vacutainers that contained ethylenediaminetetraacetic acid. Both samples were stored on ice immediately after collection and then centrifuged (Model Sorvall ST8, Thermo Fisher Scientific, Waltham, MA, USA) at 4,000 × *g* for 13 min to recover the serum and plasma, respectively. Pigs were not fasted before bleeding and, therefore, effects on blood characteristics of fasting the pigs prior to bleeding were not determined.

Sample analyses

All diet samples and ingredients were ground through a 1-mm screen using a Hammermill (model MM4; Schutte Buffalo, NY, USA) before analysis. Diets were analyzed for dry matter, crude protein, gross energy, and acid-hydrolyzed ether extract as explained for experiment 1. Diets were also analyzed for ash and insoluble and soluble dietary fiber as explained for the ingredients in experiment 2.

Heparinized blood samples were analyzed for blood urea nitrogen, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea,

CA). Plasma samples collected in the vacutainer with ethylenediaminetetraacetic acid were analyzed for tumor necrosis factor-α, interleukin 6, and interleukin 10 (R&D Systems, Inc., Minneapolis, MN), immunoglobulin G (Bethyl Laboratories, Inc., Montgomery, TX), and peptide YY (Phoenix Pharmaceuticals, Inc., Burlingame, CA) using ELISA kits according to recommendations from the manufacturers. All samples were analyzed in duplicate.

Calculation and statistical analyses

Pen was considered the experimental unit, and data were summarized for each treatment group. ADG, ADFI, and G:F were calculated for each of the 2 phases as well as for the entire experiment. Data were analyzed as explained for experiment 1.

Results

Experiment 1: amino acid digestibility

Pigs readily consumed their assigned diets and remained healthy throughout the experiment. The analyzed crude protein and Lys were 56.07% and 3.07%, 64.99% and 4.84%, and 43.65% and 2.98 % for ESBM, fishmeal, and cheese coproduct, respectively.

No difference was observed between ESBM and fish meal for AID of crude protein and most AA, but the cheese coproduct had greater ($P < 0.05$) AID of crude protein and most AA compared with ESBM and fish meal (Table 5). Likewise, the SID of crude protein and most AA was greater ($P < 0.05$) in the cheese coproduct than in ESBM and fish meal, but differences between ESBM and fish meal were not observed.

Experiment 2: energy measurements

Pigs remained healthy during the experiment and no problems with feed consumption were observed. The daily dry matter intake was greater ($P < 0.01$) for pigs fed diets containing the cheese coproduct, ESBM, or fish meal compared with pigs fed the corn diet (Table 6), and the daily gross energy intake was greater ($P < 0.01$) for pigs fed diets containing cheese coproduct or fish meal compared with pigs fed the corn diet. Pigs fed the cheese coproduct also had a greater ($P < 0.05$) gross energy intake than pigs fed the ESBM diet. Likewise, gross energy excretion in feces and urine was greater ($P < 0.01$) for pigs fed diets containing cheese coproduct, ESBM, or fish meal compared with pigs fed the corn diet.

The ATTD of dry matter was less ($P < 0.01$) in the fish meal diet than in cheese coproduct or corn diets, but not different from the ESBM diet. The ATTD of gross energy was

Table 5. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids in ESBM, fish meal, and cheese coproduct in experiment 1[1,2,3]

Item, %	AID					SID				
	ESBM	Fish meal	Cheese coproduct	Pooled SEM	*P*-value	ESBM	Fish meal	Cheese coproduct	Pooled SEM	*P*-value
Crude protein	72.8[b]	70.18[b]	80.89[a]	2.30	0.009	82.0[b]	79.41[b]	90.93[a]	2.30	0.005
Indispensable AA										
Arg	88.9	86.9	91.1	1.14	0.067	92.8[a,b]	91.5[b]	96.4[a]	1.14	0.024
His	81.6[b]	79.1[b]	88.3[a]	1.64	0.002	86.5[b]	84.7[b]	93.3[a]	1.64	0.003
Ile	81.4[b]	81.2[b]	86.9[a]	1.77	0.019	86.3b	86.5[b]	92.2[a]	1.77	0.015
Leu	83.0[b]	83.7[b]	89.3[a]	1.57	0.004	86.6[b]	87.6[b]	93.0[a]	1.57	0.005
Lys	76.6[b]	82.1[a,b]	86.6[a]	2.24	0.011	81.1[b]	85.2[a,b]	90.8[a]	2.24	0.013
Met	83.4[b]	85.7[b]	90.4[a]	1.32	0.002	87.7[b]	87.9[b]	94.1[a]	1.32	0.001
Phe	83.5[b]	79.4[b]	88.2[a]	1.58	0.001	87.9[b]	85.2[b]	93.1[a]	1.58	0.001
Thr	69.0[b]	76.0[a]	77.1[a]	2.03	0.017	79.5[b]	85.9[a,b]	88.9[a]	2.03	0.014
Trp	79.4[b]	81.0[a,b]	85.9[a]	1.57	0.029	85.4[b]	89.3[a,b]	92.5[a]	1.57	0.021
Val	76.9[b]	78.6[b]	85.0[a]	1.87	0.005	83.4[b]	85.2[b]	91.6[a]	1.87	0.005
Dispensable AA										
Ala	72.6[b]	80.2[a]	78.9[a,b]	2.24	0.044	80.1[b]	85.3[a,b]	88.5[a]	2.24	0.041
Asp	76.7[a,b]	74.5[b]	81.7[a]	1.81	0.013	80.8[b]	80.3[b]	87.8[a]	1.81	0.006
Cys	52.2	52.7	61.9	4.21	0.196	63.6[b]	72.5[a,b]	78.9[a]	4.21	0.048
Glu	80.5[b]	80.7[b]	87.7[a]	2.23	0.043	83.8	85.1	91.1	2.23	0.054
Gly	56.0[b]	73.3[a]	65.3[a,b]	3.57	0.010	71.9[b]	82.8[a,b]	88.1[a]	3.57	0.017
Pro	74.8[a,b]	64.3[b]	79.5[a]	5.29	0.036	96.1	88.2	96.3	5.29	0.2607
Ser	76.7[b]	78.4[a,b]	83.6[a]	1.77	0.029	84.3[b]	87.4[a,b]	91.5[a]	1.77	0.026
Tyr	81.1[b]	78.1[b]	88.9[a]	1.74	0.001	86.7[b]	85.3[b]	94.2[a]	1.74	0.002

[a,-b]Least squares within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Each least squares mean represents 8 observations per diet, except for cheese coproduct for which means represent 7 observations.
[2]Values for SID were calculated by correcting the values for apparent ileal digestibility for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of dry matter intake) as crude protein, 18.80; Arg, 0.58; His, 0.27; Ile, 0.50; Leu, 0.79; Lys, 0.56; Met, 0.13; Phe, 0.50; Thr, 0.88; Trp, 0.17; Val, 0.68; Ala, 0.69; Asp, 1.13; Cys, 0.34; Glu, 1.29; Gly, 1.45; Pro, 2.34; Ser, 0.72; and Tyr, 0.40.
[3]ESBM, enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA); Fish meal sourced from Omega Protein, Reedville, VA, USA; Cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA.

Table 6. Apparent total tract digestibility (ATTD) of gross energy and dry matter, and digestible energy (DE) and metabolizable energy (ME) in corn, cheese coproduct, fish meal, and ESMB, experiment 2[1,2]

Item	Corn	ESBM	Fish meal	Cheese coproduct	SEM	*P*-value
Dry matter intake, g/d	584[b]	692[a]	696[a]	716[a]	22.15	<0.001
Gross energy intake, kcal/d	2,524[d]	3,001[c]	3,116[b]	3,404[a]	82.88	<0.001
Gross energy in feces, kcal/d	280[b]	334[a]	347[a]	356[a]	22.58	0.012
ATTD, %						
Dry matter	90.88[a]	90.08[a,b]	88.23[b]	90.44[a]	0.53	0.004
Gross energy	89.51	88.28	88.90	89.34	0.65	0.545
DE, diet, kcal/kg	3,267[d]	3,384[c]	3,504[b]	3,696[a]	24.66	<0.001
GE in urine, kcal/d	59.2[b]	109[a]	118[a]	111[a]	11.61	0.008
ME, diet, kcal/kg	3,174[c]	3,238[c]	3,332[b]	3,556[a]	28.81	<0.001
Ingredient						
ATTD, gross energy, %	89.51	86.36	87.62	89.21	1.56	0.433
ATTD, dry matter, %	90.88[a]	85.61[a,b]	81.76[b]	89.65[a]	1.48	0.004
As-fed basis						
DE, kcal/kg	3,375[c]	3,887[b]	3,910[b]	4,763[a]	71.55	<0.001
ME, kcal/kg	3,278[c]	3,634[b]	3,723[b]	4,608[a]	83.07	<0.001
Dry matter basis						
DE, kcal/kg	3,888[c]	4,083[b,c]	4,241[b]	4,915[a]	75.82	<0.001
ME, kcal/kg	3,777[c]	3,817[b,c]	4,038[b]	4,754[a]	88.56	<0.001

[ab,c,d]Least squares within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Data are means of 6 or 8 observations per treatment.
[2]ESBM, = enzyme-treated soybean meal (HP 300, Hamlet Protein, Findlay, OH, USA). Fish meal sourced from Omega Protein, Reedville, VA, USA; Cheese coproduct (Pro 88) was sourced from Keys Manufacturing Co., Inc., Paris, IL, USA.

not different among diets, but the cheese coproduct diet had greater ($P < 0.01$) DE and ME than the other diets.

The ATTD of dry matter in the cheese coproduct and corn was not different from the ATTD of dry matter in ESBM, but greater ($P < 0.05$) than in fish meal. Concentrations of DE and ME on an as-fed basis as well as on a dry matter basis were greater ($P < 0.01$) in the cheese coproduct than in the other ingredients.

Experiment 3: growth performance and blood characteristics

All animals were healthy and easily consumed their diets throughout the experiment. No differences among treatments were observed for ADFI or ADG in phase 1, phase 2, or during the overall experimental period (Table 7). Final body weight of pigs at the end of phase 1 and at the end of the experiment were also not different among treatments. However, ADFI in phase 2 and during the overall experiment tended to increase ($P < 0.10$) as cheese coproduct increased in the diets. Blood urea nitrogen was less ($P < 0.05$) in pigs fed the diet with 7.3% cheese coproduct than in diets with 0% or 6.65% cheese coproduct, whereas total protein tended to increase ($P < 0.10$) in plasma of pigs as cheese coproduct increased in the diets (Table 8). However, no differences among diets were observed for albumin, peptide YY, immunoglobulin G, tumor necrosis factor-α, interleukin-6, or interleukin-10.

Discussion

Postweaning diets for pigs are commonly formulated to contain highly digestible protein sources to avoid excess protein in the hindgut of pigs, which may result in diarrhea (Kil and Stein, 2010; Limbach et al., 2021). Because large quantities of soybean meal has a negative impact on intestinal health of young pigs due to its content of oligosaccharides and other antinutritional factors, fish meal, ESBM, or milk proteins are often used as protein sources in postweaning diets due to high digestibility and palatability of these ingredients (Cervantes-Pahm and Stein, 2010; Kim et al., 2016; Jones et al., 2018).

The AID and SID of crude protein and AA for fish meal and ESBM that were calculated in experiment 1 were within the range of values reported from previous experiments with weanling pigs (Cervantes-Pahm and Stein, 2010; Rojas and Stein, 2013; Sulabo et al., 2013; Hossain et al., 2016; Ma et al., 2019). Likewise, the SID of AA in the cheese coproduct was in agreement with values for skim milk powder (NRC, 2012). The reason cheese coproduct had greater SID of AA compared with ESBM, may be that this ingredient has a lower concentration of total dietary fiber than ESBM. Dietary fiber increases specific endogenous losses of AA, and therefore reduces SID of AA (Schulze et al., 1994), which may contribute to the lower SID of AA in ESBM compared with the cheese coproduct. The fact that the cheese coproduct had greater concentration of acid hydrolyzed ether extract than fish meal and ESBM may also have contributed to the greater SID of AA by reducing passage rate of digesta in the intestinal tract, which allow more dietary AA to be digested and absorbed (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). Thus, in agreement with other milk based ingredients, it appears that the AA in the cheese coproduct have an excellent digestibility.

Concentrations of DE and ME in corn used in this study were in agreement with values previously reported (NRC, 2012; Cristobal et al., 2020), which is important because this value was used to calculate the DE and ME in the other ingredients. The values calculated for DE and ME in fish meal and

Table 7. Growth performance of pigs fed phase 1 diets containing increasing concentrations of cheese coproduct, experiment 3[1]

Item	Control	6.65% cheese coproduct	7.35% cheese coproduct	14% cheese coproduct	SEM	*P*-value
Body weight, kg						
Initial body weight	6.16	6.18	6.17	6.17	–	–
Day 14	8.00	7.79	8.12	8.10	0.30	0.343
Day 28	15.77	14.86	15.76	15.99	0.52	0.133
ADG, g						
Days 1 to 14	131	115	139	138	10.27	0.323
Days 14 to 28	554	505	547	563	21.51	0.191
Days 1 to 28	343	310	343	350	13.94	0.127
ADFI, g						
Days 1 to 14	180	165	193	176	9.72	0.183
Days 14 to 28	716	658	719	733	24.31	0.077
Days 1 to 28	448	412	456	454	15.36	0.084
G:F						
Days 1 to 14	0.72	0.68	0.72	0.77	0.02	0.180
Days 14 to 28	0.77	0.76	0.75	0.76	0.01	0.892
Days 1 to 28	0.76	0.75	0.75	0.76	0.01	0.621

[1]Each least squares mean represents 8 observations for each treatment.

Table 8. Plasma analyses from pigs consuming phase 1 diets containing increasing levels of cheese coproduct, experiment 3[1]

Item	Control	6.6% cheese diet	7.3% cheese diet	14% cheese diet	SEM	*P*-value
Blood urea N, mg/dL	11.62[a]	12.14[a]	6.25[b]	9.42[a,b]	0.92	<0.001
Total protein, g/dL	4.07	3.97	4.05	4.27	0.07	0.085
Albumin, g/dL	2.32	2.35	2.27	2.42	0.06	0.354
Peptide YY, ng/mL	2.37	2.05	2.52	2.07	0.35	0.591
Immunoglobulin G, mg/mL	5.90	5.74	4.71	6.75	0.75	0.326
Tumor necrosis factor-α, pg/mL	229.77	209.59	252.57	240.07	18.54	0.434
Interleukin-6, pg/mL	24.10	24.12	20.08	29.20	5.56	0.536
Interleukin-10, pg/mL	3.77	5.48	6.78	4.55	1.05	0.218

[1]Each least squares mean represents 8 observations for each treatment.

ESBM were also within the range of previous values (Goebel and Stein, 2011; NRC, 2012; Sulabo et al., 2013). The greater concentration of gross energy and acid hydrolyzed ether extract in the cheese coproduct than in the other ingredients and the increased SID of AA are most likely the reason for the greater DE and ME in the cheese coproduct than in fish meal or ESBM. As a consequence, the ME of diets will increase if fish meal or ESBM is replaced by cheese coproduct, and pigs will, therefore, get a more energy-dense diet if cheese coproduct is used unless adjustments in other ingredients are made to maintain a constant ME.

The observation that there were no differences in growth performance in phase 1 among pigs fed the four experimental diets is in agreement with data indicating that cheese coproduct can substitute spray dried plasma partially or completely without compromising growth performance of pigs (Baidoo et al., 2020). Inclusion of spray dried plasma in diets for weanling pigs usually has a positive impact on diet palatability (Ruckman et al., 2020), but because ADFI was not negatively impacted by inclusion of the cheese coproduct instead of plasma, it is likely that cheese coproduct also has a positive impact on palatability. Because growth performance was also not different among treatments in phase 2, when all pigs were fed the same diet, there was no indication that phase 1 diet composition impacted growth performance in phase 2. These observations indicate that if the cheese coproduct replaces spray dried plasma protein and ESBM in phase 1 diets for weanling pigs, no difference in growth performance will be observed.

Blood urea nitrogen may be used as an indicator of AA utilization efficiency (Espinosa et al., 2020; Limbach, et al., 2021), and the observed reduction in blood urea nitrogen as the concentration of cheese coproduct increased in the diets, indicates that the cheese coproduct increased the efficiency of nitrogen utilization in pigs. This is most likely a result of the greater SID of AA in cheese coproduct than in ESBM, and may also indicate that AA were more balanced in the cheese coproduct diets. However, the greater ME in the cheese coproduct, which resulted in a reduced AA to ME ratio may also have contributed to the lower blood urea nitrogen in pigs fed the cheese coproduct diet. The observation that none of the immune indicators (i.e., immunoglobulin G and interleukins)

were changed by substituting ESBM and protein plasma by cheese coproduct indicate that under the conditions of this experiment, pig health was not affected by diet composition.

Conclusion

The SID of AA and DE and ME in the cheese coproduct used in these experiments were greater than in fish meal or ESBM, demonstrating that AA digestibility and diet energy will increase if cheese coproduct is used in diets for weanling pigs instead of ESBM or fish meal. Therefore, if diets are formulated to constant SID AA and ME, concentrations of soybean meal and dietary fat may be reduced if the cheese coproduct is used, which may reduce diet cost. No differences were observed for growth performance or blood characteristics for pigs fed diets in which the cheese coproduct replaced ESBM and (or) protein plasma without adjustment for the increased ME in the cheese coproduct. Therefore, the cheese coproduct can be used as a source of AA and energy in diets for weanling pigs without compromising pig growth performance.

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Conflict of Interest Statement

At the time the experiments were conducted, D.A.L. was an employee at Keys Manufacturing Co., Inc., Paris, IL, USA. The other authors have no conflicts of interest.

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