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Short communication

Concentration of digestible and metabolizable energy in L-threonine and L-valine biomass products fed to weanling pigs

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ABSTRACT

An experiment was conducted to test the hypothesis that concentrations of digestible energy (DE) and metabolizable energy (ME) in threonine and valine biomass products are not different from those in fish meal. Two threonine biomass products (i.e., Thr-BM1 and Thr-BM2) and 1 valine biomass product (Val-BM) were obtained from the production of crystalline L-threonine and L-valine. The biomass products consist of the fermentation biomass that is left after the crystalline amino acids have been harvested at the end of fermentation. Forty weanling barrows (11.25 ± 0.65 kg) were placed individually in metabolism crates and allotted to a completely randomized design with 5 diets and 8 replicate pigs per diet. A bakery meal-based diet consisting of 973.5 g/kg bakery meal was formulated. Four additional diets containing a mixture of bakery meal and Thr-BM1, Thr-BM2, Val-BM, or fish meal were also formulated. Feces and urine samples were collected for 5 days after a 5-day adaptation period. Results indicated that the apparent total tract digestibility of gross energy was less ($P < 0.05$) in Val-BM than in Thr-BM1, fish meal, and bakery meal. The concentration of ME in bakery meal, Thr-BM1, Thr-BM2, Val-BM, and fish meal was 15.73, 16.59, 16.98, 17.04, and 16.39 MJ/kg (dry matter basis), respectively, and these values were not different. In conclusion, the concentration of ME in threonine and valine biomass products was not different from the ME in bakery meal or fish meal, which indicates that these ingredients may be used as energy sources in diets fed to weanling pigs.

1. Introduction

Increased availability of crystalline amino acids (AA) allows for a reduction in the concentration of crude protein in diets for pigs and crystalline L-threonine and L-valine may now be added to swine diets to provide adequate levels of AA. These crystalline AA are produced via direct fermentation using specific strains of microbes that have been engineered to be efficient in synthesis of a specific AA. In the production of these crystalline AA, the specific bacteria are incubated with carbohydrates and nitrogen in fermentation facilities, and the crystalline AA are produced by harvesting the AA from the fermentation biomass at the conclusion of the fermentation period. However, the fermentation biomass that remains after the target AA has been harvested, contains crude protein

Abbreviations: AA, amino acids; ATTD, apparent total tract digestibility; CP, crude protein; DE, digestible energy; GE, gross energy; ME, metabolizable energy; SID, standardized ileal digestibility; Thr-BM1, threonine biomass 1; Thr-BM2, threonine biomass 2; Val-BM, valine biomass

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Table 1
Analyzed nutrient composition (as-fed basis) of bakery meal, 2 threonine biomass products (Thr-BM1 and Thr-BM2), valine biomass (Val-BM), and fish meal.

Item	Bakery meal	Thr-BM1[1]	Thr-BM2[1]	Val-BM[1]	Fish meal
Gross energy, MJ/kg	17.70	21.48	22.87	21.98	17.97
Dry matter, g/kg	903.2	946.8	938.6	905.5	921.7
Crude protein, g/kg	136.1	810.8	803.0	716.5	630.2
Amino acids, g/kg					
Arginine	5.9	37.9	44.2	26.0	36.6
Histidine	3.3	13.3	15.5	10.5	12.7
Isoleucine	5.0	31.6	35.8	24.3	24.2
Leucine	10.6	55.2	63.6	41.0	42.3
Lysine	4.3	38.2	43.6	25.9	47.0
Methionine	2.0	16.3	19.0	8.7	16.5
Phenylalanine	6.2	26.9	30.8	21.8	22.9
Threonine	4.6	236.0	153.9	26.5	24.5
Tryptophan	1.4	8.4	9.9	3.7	6.4
Valine	6.3	40.0	45.7	172.3	28.3
Alanine	6.2	45.1	51.5	99.2	38.2
Aspartate	7.7	62.0	72.7	52.4	3.9
Cysteine	2.6	4.6	4.8	2.2	4.6
Glutamate	33.5	77.1	88.7	77.2	79.3
Glycine	5.7	32.4	34.3	24.3	45.3
Proline	11.9	22.5	26.2	21.0	29.2
Serine	6.3	22.1	26.3	19.8	22.6
Tyrosine	3.8	21.1	23.0	14.6	17.9
Total amino acids	127.3	790.7	789.5	671.4	552.4

[1] Evonik Nutrition & Care, GmbH, Hanau, Germany.

(CP), AA, and also gross energy (GE) (Sulabo et al., 2013; Almeida et al., 2014). The leftover biomass, therefore, represents a source of digestible energy (DE) that potentially can be utilized by pigs. Indeed, the digestibility of energy in threonine biomass generated from the production of crystalline L-threonine is greater than the digestibility of energy in corn or fish meal (Almeida et al., 2014). Growth performance of weanling pigs is also improved if valine biomass obtained by spray drying the entire biomass, is added to the diets (Oliveira et al., 2019), and it is possible that the biomass has beneficial nutrients other than AA from fermentation. However, there is no information about the concentration of DE and metabolizable energy (ME) in valine biomass produced after crystalline L-valine has been extracted, and there is also no information about the concentration of DE and ME in threonine biomass products derived from production using a non-pathogenic strain of *Escherichia coli* K12. Therefore, the objective of this research was to test the hypothesis that DE and ME in 2 biomass products obtained from production of crystalline L-threonine (i.e., Thr-BM1 and Thr-BM2) and in a biomass product obtained after production of crystalline L-valine (Val-BM) are not different from the DE and ME in fish meal when fed to weanling pigs.

2. Materials and methods

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

2.1. Ingredients, animals, diets, housing, and experimental design

The Val-BM, Thr-BM1, and Thr-BM2 used in this experiment were provided by Evonik Nutrition & Care GmbH (Hanau, Germany; Table 1). The Val-BM was produced via fermentation using a strain of *Corynebacterium glutamicum* DSM 25,202, whereas the 2 sources of threonine biomass were derived from a production strain of a non-pathogenic *Escherichia coli* K12 DSM 25,086, which is a safe production strain that has been widely utilized in microbial fermentation. Bakery meal was obtained from Quality Plus Feeds St. Paul, IA, USA, and the fish meal was sourced from Omega Protein, Houston, TX, USA.

Forty weanling barrows (initial body weight: 11.25 ± 0.65 kg) were allotted to a completely randomized design with 5 diets and 8 replicate pigs per diet. The diets were formulated following the principles for generating DE and ME values in individual feed ingredients using the difference procedure (Adeola, 2001). These principles have also been used in previous experiments to determine the DE and ME in biomass products (Sulabo et al., 2013; Almeida et al., 2014). A basal diet containing 973.5 g/kg bakery meal was formulated. Three additional diets containing 823.5 g/kg bakery meal and 150 g/kg Thr-BM1, Thr-BM2, or Val-BM were also formulated, and the fish meal diet contained 813.0 g/kg bakery meal and 180 g/kg fish meal (Table 2). Vitamins and minerals were included in all diets to meet or exceed the requirement for weanling pigs (NRC, 2012). Pigs were housed individually in metabolism crates that were equipped with a feeder and a nipple drinker. The amount of feed supplied daily to the pigs was calculated as 3 times the estimated requirement for maintenance energy (i.e., 0.824 MJ per kg body weight$^{0.60}$ NRC, 2012). Water was available at all

Table 2
Composition of experimental diets (as-fed basis) containing bakery meal, 2 threonine biomass products (Thr-BM1 and Thr-BM2), valine biomass (Val-BM), and fish meal.

	Bakery meal	Thr-BM1[1]	Thr-BM2[1]	Val-BM[1]	Fish meal
Ingredient, g/kg					
Bakery meal	973.5	823.5	823.5	823.5	813.0
Thr-BM1	–	150.0	–	–	–
Thr-BM2	–	–	150.0	–	–
Val-BM	–	–	–	150.0	–
Fish meal	–	–	–	–	180.0
Dicalcium phosphate	9.0	9.0	9.0	9.0	–
Ground limestone	10.5	10.5	10.5	10.5	–
Sodium chloride	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[2]	3.0	3.0	3.0	3.0	3.0
Analyzed composition					
Gross energy, MJ/kg	17.20	17.77	17.94	17.87	17.58
Dry matter, g/kg	912.8	913.1	916.0	913.3	906.8
Crude protein, g/kg	139.2	235.1	229.1	219.6	230.0
Calculated values					
Standardized ileal digestibility (SID) of Lysine, g/kg	3.10	8.17	8.96	6.38	9.86

[1] Evonik Nutrition & Care, GmbH, Hanau, Germany.
[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

times. Individual pig body weights were recorded at the beginning and at the end of the experiment, and the amount of feed supplied each day was also recorded.

Pigs were fed experimental diets for 12 days. The initial 5 days were considered an adaptation period to the diets. Fecal markers were fed in the morning meal on day 6 and in the morning meal on day 11, and fecal collections were initiated when ferric oxide appeared in the feces and ceased when chromic oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at -20 °C immediately after collection. Urine buckets were placed under the metabolism crates to allow total collection. Urines buckets were emptied every morning during the collection period and a preservative of 50 mL of 6*N* HCL was added to each bucket when they were emptied. The collected urine was weighed and a subsample was stored at -20 °C.

2.2. Chemical analyses

All samples were analyzed in duplicate. After completing sample collections, urine samples were thawed and mixed within animal and diet, and a subsample was collected for analysis. Fecal samples were dried at 65 °C in a forced-air oven and ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ, USA) before analyses. Urine samples were prepared and lyophilized before energy analysis as previously described (Kim et al., 2009). Diets and ingredients were analyzed for dry matter (Method 930.15; AOAC Int., 2007) and CP using the combustion procedure (Method 999.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ, USA). Ingredients were also analyzed for AA [Method 982.30 E (a, b, c); AOAC Int., 2007]. Diets, ingredients, fecal samples, and urine samples were analyzed for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL, USA).

2.3. Calculations and statistical analyses

Following analysis, the apparent total tract digestibility (ATTD) of GE was calculated for each diet. Energy values that were determined from the excretion of GE in the feces and in the urine were subtracted from the intake of GE to calculate DE and ME for each diet (Adeola, 2001). The concentration of DE and ME in the bakery meal diet was divided by 0.9735 to calculate the DE and ME in bakery meal. The contribution of DE and ME from bakery meal to the DE and ME in the diets containing Thr-BM1, Thr-BM2, Val-BM, or fish meal was subtracted from the DE and ME of these diets. The concentrations of DE and ME in the test ingredients were then calculated by difference (Adeola, 2001).

Data were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA) with diet as the fixed effect and replicate as the random effect. Least squares means were used to calculate mean values for each independent variable, and the PDIFF option was used to separate means if differences were detected. Homogeneity of variances among treatments was confirmed using the HOVTEST of SAS. Outliers were identified as values that deviated from the treatment mean by more than 1.5 times the interquartile range using the UNIVARIATE procedure of SAS (Devore and Peck, 1993). Three outliers were detected and removed from the data set (the

Table 3
Concentration of digestible energy (DE) and metabolizable energy (ME), and apparent total tract digestibility (ATTD) coefficient of gross energy (GE) in bakery meal, 2 threonine biomass products (Thr-BM1 and Thr-BM2), valine biomass (Val-BM), and fish meal[1].

Item	Bakery meal	Thr-BM1[2]	Thr-BM2[2]	Val-BM[2]	Fish meal	Pooled SEM	*P*-value
Diets							
GE intake, MJ/day	7.17[b]	6.58[b]	7.92[b]	6.84[b]	10.87[a]	0.75	< 0.01
GE in feces, MJ/day	1.11[b]	0.99[b]	1.21[b]	1.21[b]	1.59[a]	0.13	< 0.01
GE in urine, MJ/day	0.28[b]	0.37[ab]	0.46[a]	0.27[b]	0.45[a]	0.05	< 0.01
ATTD of GE	0.845	0.848	0.845	0.825	0.854	0.01	0.14
DE, MJ/kg	14.53[b]	15.06[a]	15.16[a]	14.74[ab]	15.01[a]	0.14	< 0.01
ME, MJ/kg	13.83	14.05	14.09	14.01	14.27	0.15	0.12
Ingredients							
ATTD of GE	0.845[a]	0.854[a]	0.826[ab]	0.742[b]	0.870[a]	0.03	0.03
DE, MJ/kg	14.92[b]	18.49[a]	19.17[a]	16.31[b]	16.00[b]	0.66	< 0.01
DE, MJ/kg, dry matter basis	16.52[c]	19.53[ab]	20.43[a]	18.01[bc]	17.36[c]	0.71	< 0.01
ME, MJ/kg	14.21	15.71	15.94	15.43	15.11	0.62	0.17
ME, MJ/kg, dry matter basis	15.73	16.59	16.98	17.04	16.39	0.67	0.50

[1]Data are means of 7 or 8 observations per treatment.
[2]Evonik Nutrition & Care, GmbH, Hanau, Germany.
[a−c]Means within a row lacking a common letter are different ($P < 0.05$).

outliers were from each of the 3 biomass diets). The pig was the experimental unit for all analyses and an alpha level of 0.05 was used to assess significance among means.

3. Results

On an as-fed basis, the concentration of CP was 136.1, 810.8, 803.0, 716.5, and 630.2 g/kg in bakery meal, Thr-BM1, Thr-BM2, Val-BM, and fish meal, respectively. The GE was 17.70, 21.48, 22.87, 21.98, and 17.97 MJ/kg in bakery meal, Thr-BM1, Thr-BM2, Val-BM, and fish meal, respectively.

Gross energy intake was greater ($P < 0.01$) in pigs fed the fish meal diet than in pigs fed the other diets (Table 3). Pigs fed the fish meal diet also had greater ($P < 0.01$) fecal excretion of GE than pigs fed other diets, but urine excretion was greater ($P < 0.01$) from pigs fed the Thr-BM2 or fish meal diets than from pigs fed the Val-BM or bakery meal diets. The ATTD of GE was not different among experimental diets. The DE (as-fed basis) was greater ($P < 0.01$) in the fish meal diet than in the bakery meal diet, but the DE in the 3 diets containing ThrBM1, ThrBM2, or Val-BM was not different from the other diets. The ME (as-fed basis) was not different among experimental diets.

The ATTD of GE was less ($P = 0.03$) in Val-BM than in Thr-BM1, fish meal, and bakery meal, but not different compared with Thr-BM2. The DE concentration (as-fed basis) was greater ($P < 0.01$) in both threonine biomass products than in Val-BM, fish meal, and bakery meal. However, on a dry matter basis, the concentration of DE was greater ($P < 0.01$) in Thr-BM2 than in Val-BM, fish meal and bakery meal, but not different from the DE in Thr-BM1. The ME concentration (as-fed and dry matter basis) was not different among ingredients.

4. Discussion

The concentrations of CP and GE in fish meal used in this experiment are in close agreement with reported values (NRC, 2012; Rojas and Stein, 2013; González-Vega et al., 2015). The concentrations of CP and GE in bakery meal used in the experiment were greater than values reported by Rojas et al. (2013), but in agreement with other published values (NRC, 2012; Stein et al., 2016; Liu et al., 2018). Concentrations of CP and GE in Thr-BM1 and Thr-BM2 were in general in agreement with values reported by Almeida et al. (2014) for a different threonine biomass product. To our knowledge, no data have been reported for concentrations of CP and GE in Val-BM. However, Oliveira et al. (2019) reported that the valine fermentation biomass (64.5 % valine) obtained by spray drying the entire biomass contains 21.15 MJ/kg and 67.5 % CP, and values obtained for Val-BM used in this experiment are close to those values. Because there are only a few values published at this time for the nutritional value of biomass products generated from production of crystalline AA, there is a lack of data demonstrating how uniform the composition of biomass from different production batches is. However, the observation that the composition of Thr BM1 and Thr BM2 were similar in terms of concentration of gross energy, CP, and AA and also had a composition that was in agreement with the composition of a threonine biomass product generated using a different production process, indicates that the final biomass products likely are relatively uniform in composition.

Commercial production of L-threonine and L-valine using large-scale fermentation technologies has increased in recent years and industrial production of L-threonine and L-valine may be accomplished using a number of microorganisms. At the conclusion of fermentation, crystalline L-threonine or L-valine are extracted from the fermentation biomass, and the remaining biomass is dried by spray dried, and the final product is granulated. However, the high concentration of threonine in both threonine biomass sources, and of valine in valine biomass indicates that extraction from the biomass of the crystalline AA that is a result of the fermentation process is not 100 % efficient. The observation that the biomass also contains significant proportions of other indispensable AA indicates that

synthesis of other AA than threonine or valine may occur during the fermentation process, which is the reason the co-product biomass that is generated after extraction of the crystalline AA have a high nutritional value.

Values for DE and ME in bakery meal obtained in this experiment concur with previous values (NRC, 2012; Rojas et al., 2013). Likewise, the digestibility of energy, DE, and ME that was calculated for fish meal are in close agreement with previously reported values (NRC, 2012; Sulabo et al., 2013; Almeida et al., 2014). Because of the high concentration of CP in the biomass products, DE and ME values could be determined only using the difference procedure, which has also been used for the calculation of DE and ME in biomass products in previous experiments (Sulabo et al., 2013; Almeida et al., 2014). To obtain accurate results using the difference procedure, it is critical that the DE and ME obtained in the basal diet is correct and the observation that the DE and ME obtained in the bakery meal concurs with previous values gives confidence that the DE and ME calculated for the test ingredients are also accurate.

The observation that the concentration of DE is greater in Thr-BM1 and Thr-BM2 than in Val-BM, fish meal, and bakery meal is most likely a result of greater concentration of GE in the 2 sources of threonine biomass than in the other ingredients. These observations are in agreement with data indicating that dried fermentation biomass from production of crystalline lysine or threonine contained more DE than fish meal (Sulabo et al., 2013; Almeida et al., 2014). The energy in AA biomass products mainly originates from the feedstock used for fermentation and energy from the microbial biomass that is included in the product (Sulabo et al., 2013).

5. Conclusion

The 2 sources of threonine biomass used in the experiment contained more DE than Val-BM, fish meal, and bakery meal, but the concentration of ME was not different among ingredients. Therefore, the threonine and valine biomass products used in this experiment may be used as energy sources in diets fed to weanling pigs, and if they replace fish meal in the diets, the ME of the diets will not be changed. However, further research is warranted to determine digestibility of phosphorus and AA and effects of these biomass co-products on growth performance of pigs.

Author statement

JKH and HHS conceptualized the experiment; OJR and JDB conducted the experiment, analyzed data, and wrote the first draft of the manuscript; MSFO and CDE assisted with data interpretation and wrote the final version of the manuscript; HHS supervised the project and edited the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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References

Adeola, O., 2001. Digestion and balance techniques in pigs. In: Lewis, A.J., Southern, L.L. (Eds.), Swine Nutrition. CRC Press, Washington, D.C, pp. 903–916.
Almeida, F.N., Sulabo, R.C., Stein, H.H., 2014. Amino acid digestibility and concentration of digestible and metabolizable energy in a threonine biomass product fed to weanling pigs. J. Anim. Sci. 92, 4540–4546.
AOAC Int, 2007. Official Methods of Analysis of AOAC. int. AOAC Int., Gaithersburg, MD, USA.
Devore, J.L., Peck, R., 1993. Statistics: the Exploration and Analysis of Data. Belmont, CA, USA. .
González-Vega, J.C., Walk, C.L., Stein, H.H., 2015. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93, 2255–2264.
Kim, B.G., Petersen, G.I., Hinson, R.B., Allee, G.L., Stein, H.H., 2009. Amino acid digestibility and energy concentration in a novel source of high-protein distillers dried grains and their effects on growth performance of pigs. J. Anim. Sci. 87, 4013–4021.
Liu, Y., Jha, R., Stein, H.H., 2018. Nutritional composition, gross energy concentration, and in vitro digestibility of dry matter in 46 sources of bakery meals. J. Anim. Sci. 96, 4685–4692.
NRC, 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl. Acad. Press, Washington, D.C.
Oliveira, M.S.F., Htoo, J.K., González-Vega, J.C., Stein, H.H., 2019. Bioavailability of valine in spray-dried L-valine biomass is not different from that in crystalline L-valine when fed to weanling pigs. J. Anim. Sci. 97, 4227–4234.
Rojas, O.J., Stein, H.H., 2013. Concentration of digestible, metabolizable, and net energy and digestibility of energy and nutrients in fermented soybean meal, conventional soybean meal, and fish meal fed to weanling pigs. J. Anim. Sci. 91, 4397–4405.
Rojas, O.J., Liu, Y., Stein, H.H., 2013. Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn coproducts, and bakery meal fed to growing pigs. J. Anim. Sci. 91, 5326–5335.
Stein, H.H., Lagos, L.V., Casas, G.A., 2016. Nutritional value of feed ingredients of plant origin fed to pigs. Anim. Feed Sci. Tech. 218, 33–69.
Sulabo, R.C., Mathai, J.K., Usry, J.L., Ratliff, B.W., McKilligan, D.M., Moline, J.D., Xu, G., Stein, H.H., 2013. Nutritional value of dried fermentation biomass, hydrolyzed porcine intestinal mucosa products, and fish meal fed to weanling pigs. J. Anim. Sci. 91, 2802–2811.