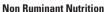
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Evaluation of standardized ileal digestibility of amino acids in corn, barley, wheat bran fed to primiparous sows during gestation, lactation, and post-weaning period

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Abstract

This study determined the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) of corn, barley, and wheat bran in primiparous sows. Four physiological stages of primiparous sows were examined: a gestation stage where sows were restricted-fed, a lactation stage where sows were fed ad libitum, and then two post-weaning stages, an ad libitum-fed phase followed by a restricted-fed phase. A total of 8 primiparous sows fitted with T-cannulas in the distal ileum were assigned to an 8 x 3 Youden square design with 4 diets (corn, barley, wheat bran, and nitrogen [N]-free diet) and three periods, resulting in a total of 6 replicates per treatment. The basal endogenous losses of CP and AA were determined after feeding an N-free diet. For barley, the AID of His, Ile, Lys, and Thr was higher in gestating sows compared to restricted-fed post-weaning sows. For wheat bran, the AID of Ile, Leu, Thr, Val, Ala, Asp, Glu, Gly, Ser, and Tyr was higher in lactating sows compared to gestating sows (P < 0.05). For corn, the AID of CP and most AA did not differ across physiological states. Regarding the SID of barley in gestating sows, only the SID of His was higher than in lactating sows and restricted-fed post-weaning sows (P < 0.01), and the SID of Gly was higher than in lactating sows (P < 0.05). For wheat bran, the SID of Glu, Ser, and Tyr in gestating sows was lower than in lactating sows (P < 0.01). The SID of Val and Tyr in lactating sows was higher than in ad libitum access post-weaning sows (P < 0.05). For corn, the SID of Lys, Ala, and Gly in gestating sows was higher than in lactating sows (P < 0.05). The SID of Ile, Lys, Phe, Thr, and Ala in gestating sows was higher than in ad libitum post-weaning sows (P < 0.05). The findings indicate that similar SID values are found for barley in different physiological stages of primiparous sows under the same diet and feed regime, whether ad libitum or restricted. However, for wheat bran, which has a high fiber content, lactating sows exhibited higher AID values and SID values compared to gestating sows for part of AA. For corn, which has a low fiber content, gestating sows exhibited higher SID values compared to lactating sows and ad libitum post-weaning sows.

Lay Summary

Accurate evaluation of amino acid digestibility is essential for optimizing precision feeding strategies in sows. This study demonstrated that the digestibility of crude protein and amino acids in corn, barley, and wheat bran varies across different physiological stages in primiparous sows. The findings suggest that standardized ileal digestibility values remain stable for barley, while wheat bran, which has higher fiber content, exhibits higher digestibility in lactating sows. In contrast, corn, which contains low fiber content, shows higher standardized ileal digestibility values in gestating sows. These results provide valuable data for formulating diets tailored to different reproductive stages, potentially improving nutrient utilization and feeding efficiency in primiparous sows.

Keywords: amino acids, feed evaluation, feedstuffs, physiological stages, sow, standardized ileal digestibility

Abbreviations: AA, amino acids; ADF, acid detergent fiber; AID, apparent ileal digestibility; BEL, basal endogenous loss; CF, crude fiber; CP, crude protein; DM, dry matter; ENL, endogenous nitrogen loss; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber; SID, standardized ileal digestibility.

Introduction

Accurate evaluation of amino acid (AA) digestibility is essential for precision feeding in sows. Standardized ileal digestibility (SID) is considered the reliable value. However, SID values in the current guidelines (NRC, 2012) are mostly derived from growing pigs. Considering the physiological differences across reproductive stages, establishing sowspecific SID values is necessary to improve diet formulation and feeding efficiency (Jondreville et al., 1995; Rademacher et al., 1999; NRC, 2012). In 1999, Stein et al. conducted a study on the apparent ileal digestibility (AID) of AA in ingredients such as corn, barley, wheat, and meat and bone meal, comparing the differences between gestating sows, lactating sows, and growing pigs. Their findings indicated that the AID values obtained from growing pigs do not always represent those in gestating and lactating sows (Stein et al., 1999a). In 2001, a study on the SID of protein and AA was conducted in sows and growing pigs. The results revealed that gestating sows had significantly higher SID values for both protein and AA than growing pigs (Stein et al., 2001). Moreover, Zhuo et al. found that the SID of dispensable AA (Cys, Gly, Pro, and Tyr) from cottonseed meal differed significantly between gestating and post-weaning sows under the same diet and feed intake (Zhuo et al., 2023). These studies revealed that there are variations in the SID of AA across different physiological stages of pigs. Consequently, applying data from growing pigs directly to sows may be imprecise, as different physiological states of sows can exhibit varying SID values for AA. Primiparous sows have not fully matured in terms of weight and physiological status compared to multiparous sows, and their nutritional needs are higher, as they must support both their own growth and fetal development. Currently, there is a lack of data on the SID of AA in feed ingredients for primiparous sows. It is unclear whether primiparous sows have the same AA digestibility for feed ingredients as multiparous sows. Furthermore, the effects of various physiological stages and feeding levels on the AID and SID of AA in primiparous sows are not well-documented. Corn, barley, and wheat bran are utilized as feed ingredients for sows; however, the SID of AA values for these ingredients in primiparous sows remains undocumented. Therefore, the objective of this experiment was to evaluate and compare the SID of CP and AA in typi-

cal feedstuffs (barley, wheat bran, and corn) across different physiological stages (gestation, lactation, and post-weaning period) in primiparous sows.

Materials and methods

The animal trials were performed at the Tianpeng experimental farm, located in Lang Fang, Hebei province, China. The animal protocol for this research was approved by the Animal Care and Use Committee of the Institute of Feed Research of the Chinese Academy of Agricultural Sciences (IFR-CAAS20230410).

Ingredients, animals, and experimental design

Corn and wheat bran were purchased from Hebei Kangda Co., Ltd. (Langfang, China). Barley was purchased from Guangdong Haida Co., Ltd. (Guangzhou, China). Analysis of the chemical composition of corn, barley, and wheat bran samples was conducted before the start of the experiment (Table 1).

A total of 12 primiparous sows (Landrace × Yorkshire; body weight = 156.5 ± 5.2 kg) were fitted with a T-cannula in the distal ileum according to a previous method (Stein et al., 1998). Approximately 15 d later, after the wounds had fully healed, T-cannula-fitted sows had a mixture of petroleum jelly and zinc oxide applied to their wounds to help them recover.

 Table 1. Analyzed nutrient and amino acid composition of barley, wheat bran, and corn (%, as-fed basis)

Items	Barley	Wheat bran	Corn
Dry matter	90.39	88.38	91.20
Gross energy, MJ/g	16.54	18.22	17.32
Crude protein	9.37	19.27	8.61
Ether extract	1.56	4.29	3.63
Organic matter	88.47	83.40	90.10
Crude fiber	3.01	5.85	1.03
Neutral detergent fiber	23.02	37.27	11.52
Acid detergent fiber	6.86	11.84	3.98
Ash	1.92	4.98	1.10
Indispensable amino acids			
Arginine	0.45	1.34	0.36
Histidine	0.14	0.19	0.17
Isoleucine	0.29	0.44	0.25
Leucine	0.67	1.19	1.03
Lysine	0.32	0.74	0.24
Methionine	0.12	0.21	0.16
Phenylalanine	0.47	0.69	0.41
Threonine	0.12	0.17	0.26
Tryptophan	0.12	0.24	0.05
Valine	0.41	0.80	0.37
Dispensable amino acids			
Alanine	0.42	1.01	0.66
Aspartate	0.63	1.32	0.57
Cysteine	0.11	0.17	0.10
Glutamate	2.23	3.18	1.68
Glycine	0.40	1.14	0.34
Serine	0.44	0.79	0.46
Tyrosine	0.24	0.45	0.21

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After recovery from surgery, sows were subjected to a synchronized estrus treatment followed by artificial insemination. Out of the 12 sows, 8 were successfully conceived and subsequently used in the experiment. Four trials were conducted successively to measure AID and SID values for CP and AA under restricted feeding during gestation, ad libitum feeding during lactation, ad libitum feeding during post-weaning, and restricted feeding during post-weaning. 8 sows were randomly allotted to an 8 × 3 Youden square design with 4 diets and 3 periods. Each period included 2 replicates, resulting in 6 replications per diet. During the mid-term of pregnancy, one pregnant sow exhibited pyrexia and was transferred for medical intervention to prevent miscarriage. Consequently, data from this sow were excluded for that period, resulting in 5 replicates for the corresponding treatment during gestation.

Diets and feeding

Diets included a corn starch-based nitrogen (N)-free diet to calculate endogenous losses of protein and AA (Adeola et al., 2016), and 3 experimental diets, including a corn diet, barley diet, and wheat bran diet. The corn diet contained 95.6% corn, and the barley diet contained 95.6% barley as the only source of AA. The wheat bran diet contained 50% wheat bran as the sole source of AA, along with 45.6% corn starch to enhance palatability. The dietary composition and analyzed nutrient content are shown in Tables 2 and 3. The same feed formulation was applied for sows across all physiological stages. Chromium trioxide (Cr) was added to each diet at a level of 0.3% as an indigestible index. The minerals and vitamins were designed to meet or exceed the nutrient requirements as suggested (NRC, 2012). In the non-experimental period, sows were fed a standard commercial diet. Sows in gestation and post-weaning periods were individually housed in stainless-steel metabolism crates (1.80 m \times 0.62 m \times 1.30 m) that were equipped with a nipple drinker, and sows in the lactation period were transferred into the delivery rooms. Sows in gestation (57 \pm 5 d) were fed 2.5 kg/d of the experimental

diets, which were provided in two meals offered at 0800 and 1400 h. This feeding level was chosen to reflect common commercial practice, as gestating sows are typically limit-fed to control body condition and ensure optimal reproductive performance. Sows in lactation had free access to the experimental diet and water. Sows during the post-weaning period were divided into 2 feeding regimens: initially, they were fed ad libitum, followed by restricted feeding at 2.5 kg/d. The room temperature was maintained steadily at 22 ± 2 °C. Notably, while post-weaning sows are typically fed ad libitum in practice, a group of post-weaning sows in this experiment was subjected to restricted feeding to better assess the influence of feed intake and physiological stage on digestibility.

Sample collection

Every different stage (gestation, lactation, ad libitum-fed post-weaning, and restricted-fed post-weaning) included three periods, each period covering a 5-d acclimation to the diet and a 2-d sample collection period on the sixth and seventh days. Sows were weaned after the completion of the 21-d lactation trial, specifically on 22-d postpartum. Ileal digesta samples were collected continuously for 12 h from 0700 to 1900 h in each experimental period. During each collection, plastic bags were attached to the T-cannula. The bags were replaced every 15 min when the ileal digesta was about to fill the plastic bags. Digesta samples were transferred into containers and frozen at -20 °C immediately after collection. The collected digesta samples from each sow were naturally thawed for 2 d, then mixed evenly before being placed in a freeze-drying machine for drying. After grinding, measurements were conducted.

Chemical analysis and calculations

Dry matter (DM; method 930.15), crude protein (CP; method 990.03), ether extract (method 920.39), and crude ash (Ash; method 942.15) were analyzed as described previously in the literature (AOAC, 2007). Crude fiber (CF), neutral detergent

Table 2. Ingredient composition of experimental diets (%, as-fed basis)

Ingredient	Nitrogen-free diet	Barley diet	Wheat bran diet	Corn diet
Corn	-			95.60
Barley	-	95.60		
Wheat bran	-		50.00	
Corn starch	72.50		45.60	
Sucrose	15.00			
Cellulose acetic acid	4.00			
Soybean oil	3.00			
Monocalcium phosphate	2.32	1.50	1.50	1.50
Potassium carbonate	0.30			
Magnesium oxide	0.10			
Limestone	1.03	1.15	1.15	1.15
Salt	0.45	0.45	0.45	0.45
Chromium trioxide	0.30	0.30	0.30	0.30
Premix ¹	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

 $^{^{1}}$ Premix supplied per kg of diet: vitamin A, 12075 IU; vitamin D₃, 2450 IU; vitamin E, 67 IU; vitamin K₃, 2.98 mg; vitamin B₁, 1.78 mg; vitamin B₂, 3.64 mg; vitamin B₄, 4.46 mg; vitamin B₁₂, 40 µg; Niacin, 42 mg; Calcium pantothenate, 25 mg; Folic acid, 6.92 mg; Biotin, 0.70 mg; Zinc (ZnSO₄·H₂O), 79 mg; Copper (CuSO₄·5H₂O), 23 mg; Iron (FeSO₄·H₂O), 150 mg; Cobalt (CoCl₂), 1.00 mg; Manganese (MnSO₄·H₂O), 100 mg; Iodine (Ca(IO₃)₂), 0.23 mg; Selenium (Na,SeO₃), 0.50 mg.

Table 3. Compositional analysis of experimental diets (%, as-fed basis)

Items	Nitrogen-free diet1	Experimental diets ²				
		Barley diet	Wheat bran diet	Corn diet		
Dry matter	90.48	88.88	89.31	90.01		
Crude protein	0.75	9.03	8.70	8.20		
Gross energy, MJ/g	15.99	15.65	15.91	16.29		
Indispensable amino acids						
Arginine	-	0.75	0.57	0.31		
Histidine	-	0.38	0.28	0.47		
Isoleucine	-	0.30	0.35	0.25		
Leucine	0.01	0.64	0.73	0.86		
Lysine	0.03	0.50	0.48	0.35		
Methionine	-	0.13	0.22	0.20		
Phenylalanine	0.02	0.42	0.49	0.34		
Threonine	0.03	0.29	0.56	0.41		
Tryptophan	-	0.13	0.06	0.06		
Valine	0.02	0.35	0.37	0.58		
Dispensable amino acids						
Alanine	0.02	0.54	0.53	0.57		
Aspartate	0.03	0.78	0.74	0.56		
Cysteine	-	0.20	0.23	0.11		
Glutamate	-	0.78	0.57	0.34		
Glycine	0.08	2.37	3.06	1.51		
Serine	0.01	0.45	0.50	0.32		
Tyrosine	-	0.22	0.27	0.21		

¹Nitrogen-free diet, a cornstarch-based diet.

fiber (NDF), and acid detergent fiber were analyzed as described previously (Van Soest et al., 1991). Gross energy was assessed using an automatic oxygen and N calorimeter. We determined the content of AA using high-performance liquid chromatography (Dai et al., 2014). Chromium determination was performed using atomic absorption spectroscopy (Williams et al., 1962). Basal endogenous loss (BEL) was calculated from sows fed the N-free diet, and the AID and SID values of CP and AA were calculated according to the method (Stein et al., 2007).

The formula used for calculating AID was as follows: AID (%) = [1 – (AA $_{\rm digesta}/AA_{\rm dier}) \times (Cr_{\rm dier}/Cr_{\rm digesta})] \times 100$. The concentrations of AA and chromium (Cr) in ileal digesta were denoted as AA $_{\rm digesta}$ and Cr $_{\rm digesta}$, respectively (g/kg), while their concentrations in the diet were represented as AA $_{\rm diet}$ and Cr $_{\rm dier}$, respectively (g/kg). The formula used for calculating ileal endogenous loss of AA (IAA $_{\rm end}$) was: IAA $_{\rm end}$ = (AA $_{\rm diegesta}$) \times (Cr $_{\rm dier}/Cr_{\rm digesta}$). In which IAA $_{\rm end}$ referred to the ileal endogenous loss of AA expressed as grams per kilogram of DM intake. Concentrations of amino acids (AA $_{\rm digesta}$) and chromium (Cr $_{\rm digesta}$) in ileal digesta from pigs fed an N-free diet were measured and expressed in g/kg. The chromium content in the diet (Cr $_{\rm diet}$) was expressed in g/kg DM. The IAA $_{\rm end}$ was referred to as nonspecific endogenous AA loss and was regarded as BEL of AA. The formula used for calculating SID was: SID (%) = AID + (IAA $_{\rm end}/AA_{\rm dier}) \times 100$.

Statistical analysis

All data analyses were performed using SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). The normality of data distribution and

the presence of outliers were assessed using the UNIVARI-ATE procedure. The main statistical analysis was conducted using PROC MIXED, with individual sows as the experimental units. Between different physiological phases, a variance analysis was performed using physiological phase as a fixed effect and sows and periods as random effects (Wang et al., 2023a). The LSMEANS statement was used to calculate the least squares means for each treatment, and statistical differences were separated by Tukey's multiple range tests. For all statistical analyses, significance was set at P < 0.05, and tendencies were considered when $0.05 \le P < 0.10$.

Results

Endogenous protein and AA losses in sows

The BEL of CP and AA is shown in Table 4. Compared to gestating sows, lactating sows exhibited a lower BEL of CP (P < 0.01). The BEL of AA in lactating sows (including Arg, Thr, Ala, Asp, Cys, Glu, Gly, Ser, and Tyr) was lower (P < 0.05) than that of gestating sows. When compared to ad libitum-fed post-weaning sows, gestating sows exhibited higher BEL of AA, with differences (P < 0.05) observed in Arg, Leu, Ala, Asp, Cys, Glu, Gly, Ser, and Tyr. Between gestating and restricted-fed post-weaning sows, no differences in AA losses were observed, except for Glu and Ser (P < 0.05). However, restricted-fed post-weaning sows displayed higher BEL compared to ad libitum-fed post-weaning sows, with differences in the BEL of His, Ile, Leu, Thr, Trp, and Cys (P < 0.05).

²Barley, wheat bran, and corn were used separately as the only source of amino acids in three test diets.

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Table 4. Basal ileal endogenous losses (mg/kg dry matter intake) of crude protein and amino acids in primiparous sows

Items	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed		
Crude protein	16797ª	6966 ^b	11458ab	14254 ^{ab}	1186	0.014
Indispensable amino	acids					
Arginine	510a	197 ^b	273 ^b	403^{ab}	57	0.004
Histidine	207^{ab}	105 ^b	130 ^b	275ª	25	0.007
Isoleucine	304 ^{ab}	169 ^b	185 ^b	317ª	37	0.008
Leucine	610 ^a	303 ^b	333 ^b	523ª	51	0.003
Lysine	350 ^{ab}	206 ^b	235^{ab}	467ª	52	0.048
Methionine	83	71	85	157	20	0.062
Phenylalanine	307	179	196	274	36	0.096
Threonine	751 ^{ab}	401°	459bc	1060^{a}	95	< 0.001
Tryptophan	88 ^{ab}	35 ^b	47 ^{bc}	95ª	10	< 0.001
Valine	654	385	332	566	58	0.057
Dispensable amino	acids					
Alanine	595ª	308 ^b	337 ^b	459ab	42	0.003
Aspartate	1039ª	478 ^b	583 ^b	724 ^{ab}	56	< 0.001
Cysteine	287ª	99 ^b	121 ^b	261ª	25	< 0.001
Glutamate	1471ª	657 ^b	797 ^b	876 ^b	86	< 0.001
Glycine	1585a	526 ^b	602 ^b	1017^{ab}	149	< 0.001
Serine	765ª	275 ^b	316 ^b	454 ^b	40	< 0.001
Tyrosine	257ª	122°	144 ^{bc}	219^{ab}	16	0.002

 $^{^{}a,b,c}$ Different superscripts indicate that means in the same row differ (P < 0.05).

Apparent ileal digestibility of primiparous sows

The AID of CP of barley in lactating sows had the lowest value but was not different from the other physiological states (Table 5). The AID of most AA of barley was not different in different physiological states, but the AID of His in gestating sows was higher than lactating and restricted-fed post-weaning sows (P < 0.01). The AID of Ile in restricted-fed post-weaning sows was lower than in gestating sows, lactating sows, and ad libitum-fed post-weaning sows (P < 0.05). The AID of Lys in gestating sows and ad libitum-fed post-weaning sows was higher than in restricted-fed post-weaning sows was higher than in restricted-fed post-weaning sows was higher than in restricted-fed post-weaning sows was higher than in gestating sows and lactating sows (P < 0.05). The AID of Glu in restricted-fed post-weaning sows was higher than in gestating sows and lactating sows (P < 0.05).

No difference in the AID of CP in wheat bran was observed among gestating, lactating, and post-weaning sows (Table 6). The AID of Ile, Leu, Thr, Val, Ala, Asp, Glu, Gly, Ser, and Tyr in lactating sows was higher than in gestating sows (P < 0.05). For gestating sows compared to restricted-fed post-weaning sows, the AID value of Trp was higher, but Ala, Asp, Glu, and Ser were lower (P < 0.05). For lactating sows compared to ad libitum-fed post-weaning sows, the AID of most AA of wheat bran did not differ except for Val (P < 0.05). For lactating sows compared to restricted-fed post-weaning sows, the AID of most AA of wheat bran was not different except for Ile, Lys, and Thr (P < 0.05). For ad libitum-fed post-weaning sows compared to restricted-fed post-weaning sows, the AID of most AA of wheat bran was not different except for Thr (P < 0.05).

The AID of CP and most AA of corn was not different among different physiological states in Table 7; however, the

AID of Arg in lactating sows was higher than in gestating sows and restricted-fed post-weaning sows (P < 0.05). The AID of Thr in gestating sows and lactating sows was higher than in restricted-fed post-weaning sows (P < 0.01). The AID of Trp in lactating sows was higher than in gestating sows and restricted-fed post-weaning sows (P < 0.01).

Standardized ileal digestibility of primiparous sows

The SID of CP and AA of barley is shown in Table 8. The SID of CP in gestating sows was higher than in lactating sows and ad libitum-fed post-weaning sows (P < 0.05). The SID of CP in restricted-fed post-weaning sows was higher than in lactating sows (P < 0.05). The SID of His in gestating sows was higher than in lactating sows and restricted-fed post-weaning sows, and the SID of Gly in gestating sows was higher than in lactating sows (P < 0.05). The SID of Asp and Glu in restricted-fed post-weaning sows was higher than in lactating sows (P < 0.05).

The SID of CP and AA of wheat bran is shown in Table 9. The SID of Glu, Ser, and Tyr in gestating sows was lower than in lactating sows (P < 0.05). The SID of Met and Trp in gestating sows was higher than in ad libitum access postweaning sows, and the SID of Glu in gestating sows was lower than in restricted-fed post-weaning sows (P < 0.05). The SID of Val and Tyr in lactating sows was higher than in ad libitum-fed post-weaning sows (P < 0.05). The SID of AA in lactating sows showed no differences with those in restricted-fed post-weaning sows, and the SID of AA in restricted-fed post-weaning sows showed no differences with those in ad libitum-fed post-weaning sows.

The SID of CP and AA of corn is shown in Table 10. For gestating sows, the SID of Lys, Ala, and Gly was higher

Table 5. Apparent ileal digestibility values for crude protein and amino acids in barley for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed		
Crude protein	68.91	59.27	62.81	65.74	2.98	0.264
Indispensable amino	acids					
Arginine	82.00	78.95	85.34	80.09	2.42	0.385
Histidine	87.02a	70.81 ^b	77.50 ^{ab}	69.88 ^b	2.76	0.005
Isoleucine	77.70a	78.73 ^a	77.48a	70.72 ^b	1.54	0.021
Leucine	78.95	80.25	78.53	77.02	1.61	0.708
Lysine	80.01 ^a	72.83 ^{ab}	75.14 ^a	65.03 ^b	1.56	0.008
Methionine	85.51	81.16	81.34	77.75	1.85	0.094
Phenylalanine	81.15	80.28	79.55	78.50	1.64	0.812
Threonine	78.67a	72.01^{ab}	71.46 ^{ab}	61.31 ^b	3.57	0.031
Tryptophan	78.34	74.84	68.71	70.45	2.89	0.261
Valine	70.47	70.55	76.40	66.21	3.80	0.394
Dispensable amino	acids					
Alanine	75.58	69.94	77.34	74.12	1.83	0.165
Aspartate	71.53	66.32	74.80	74.09	1.97	0.061
Cysteine	74.87	77.90	80.34	75.81	2.20	0.431
Glutamate	86.35 ^b	85.01 ^b	87.83 ^{ab}	89.52a	0.86	0.026
Glycine	66.98	60.03	66.95	66.23	4.25	0.693
Serine	74.57	74.50	79.86	76.62	2.22	0.355
Tyrosine	78.78	76.85	82.27	78.38	1.85	0.364

 $^{^{}a,b}$ means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

Table 6. Apparent ileal digestibility values for crude protein and amino acids in wheat bran for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value	
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed			
Crude protein	63.00	74.10	65.56	74.49	4.10	0.155	
Indispensable amino	acids						
Arginine	85.04	89.47	88.28	86.02	1.19	0.102	
Histidine	83.09	86.71	81.70	81.49	1.97	0.274	
Isoleucine	72.00 ^b	81.96 ^a	77.95^{ab}	72.68 ^b	1.90	0.009	
Leucine	72.96 ^b	83.04ª	79.82ab	78.10 ^{ab}	1.85	0.015	
Lysine	75.97^{ab}	81.10 ^a	77.11 ^{ab}	70.34 ^b	2.38	0.039	
Methionine	86.70a	79.52^{ab}	75.44 ^b	72.19 ^{ab}	2.62	0.017	
Phenylalanine	76.37	84.11	79.73	78.97	1.73	0.067	
Threonine	55.34 ^b	71.33 ^a	71.22 ^a	50.12 ^b	2.88	< 0.001	
Tryptophan	78.39a	72.65^{ab}	67.55ab	64.99 ^b	2.66	0.021	
Valine	62.30 ^b	74.33 ^a	62.23 ^b	67.61 ^{ab}	2.88	0.046	
Dispensable amino	acids						
Alanine	70.25 ^b	79.63ª	76.94ª	78.18 ^a	1.56	0.005	
Aspartate	66.14 ^b	79.45ª	78.56a	79.64ª	2.02	0.001	
Cysteine	72.99	81.60	75.05	73.45	2.21	0.065	
Glutamate	81.87 ^b	91.05ª	89.38a	91.53a	0.86	< 0.001	
Glycine	69.94 ^b	81.19 ^a	79.47ª	77.13 ^{ab}	1.70	0.003	
Serine	63.18 ^b	81.13 ^a	79.72ª	80.52a	1.71	< 0.001	
Tyrosine	70.82 ^b	81.23 ^a	75.06ab	77.67ab	1.83	0.010	

 $^{^{}a,b}$ means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

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Table 7. Apparent ileal digestibility values for crude protein and amino acids in corn for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed		
Crude protein	63.78	65.57	64.64	63.86	4.11	0.989
Indispensable amino	o acids					
Arginine	66.06 ^b	77.10 ^a	69.14 ^{ab}	69.71 ^b	2.59	0.046
Histidine	79.40	82.30	83.74	85.49	2.15	0.503
Isoleucine	71.70	67.97	62.96	64.77	2.56	0.194
Leucine	82.10	81.55	83.10	78.62	2.09	0.537
Lysine	72.54	63.24	64.78	60.84	2.56	0.053
Methionine	85.06	83.72	82.34	82.11	2.64	0.875
Phenylalanine	76.53	74.34	72.52	70.60	1.70	0.185
Threonine	77.13 ^a	75.56 ^a	64.73ab	51.34 ^b	4.18	0.002
Tryptophan	64.33 ^b	79.69a	73.08ab	65.89b	2.22	< 0.001
Valine	80.13	71.64	74.23	73.67	3.93	0.593
Dispensable amino	acids					
Alanine	76.67	68.27	68.33	76.10	2.89	0.131
Aspartate	63.60	65.13	64.64	66.00	3.46	0.974
Cysteine	65.81	72.86	73.24	66.31	3.18	0.068
Glutamate	73.74	78.76	79.18	81.31	2.08	0.160
Glycine	48.44	52.10	60.63	50.97	4.16	0.238
Serine	61.03	62.05	65.89	63.21	3.87	0.861
Tyrosine	73.04	65.78	68.77	71.27	3.43	0.603

^{a,b} means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

Table 8. Standardized ileal digestibility values for crude protein and amino acids in barley for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value	
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed			
Crude protein	86.94ª	70.64°	75.98 ^{bc}	82.13 ^{ab}	3.46	0.015	
Indispensable amino	acids						
Arginine	87.63	87.77	87.28	87.14	2.65	0.408	
Histidine	92.47ª	81.18 ^b	80.50 ^{ab}	79.70 ^b	2.91	0.005	
Isoleucine	85.17	84.72	81.60	79.71	1.75	0.408	
Leucine	85.96	85.35	82.27	84.21	1.85	0.716	
Lysine	85.61	70.19	79.52	74.78	1.95	0.098	
Methionine	88.56	88.39	84.22	84.80	2.01	0.387	
Phenylalanine	86.34	85.57	82.70	84.07	1.95	0.734	
Threonine	92.22	83.47	77.92	77.31	3.11	0.062	
Tryptophan	81.99	77.64	72.30	77.77	2.83	0.463	
Valine	83.50	84.77	84.06	80.24	3.75	0.916	
Dispensable amino	acids						
Alanine	84.51	77.71	82.26	82.76	2.19	0.081	
Aspartate	81.71 ^{ab}	73.39 ^b	80.80 ^{ab}	83.93a	2.27	0.036	
Cysteine	86.67	82.44	85.71	87.15	2.50	0.564	
Glutamate	90.44 ^{ab}	88.79 ^b	89.67 ^{ab}	92.38a	1.04	0.035	
Glycine	90.00ª	74.16 ^b	74.60 ^{ab}	83.95 ^{ab}	5.17	0.025	
Serine	87.43	80.62	84.95	85.74	2.34	0.298	
Tyrosine	87.90	92.10	93.21	86.49	1.90	0.213	

 $^{^{}a,b,c}$ means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

Table 9. Standardized ileal digestibility values for crude protein and amino acids in wheat bran for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value	
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed			
Crude protein	86.63	81.82	78.25	88.72	3.76	0.406	
Indispensable amino	acids						
Arginine	91.73	93.84	90.15	91.38	1.21	0.570	
Histidine	88.23	90.61	83.87	88.75	1.96	0.235	
Isoleucine	82.18	83.89	82.34	81.47	2.20	0.180	
Leucine	82.46	86.24	83.59	89.90	1.67	0.050	
Lysine	83.11	84.81	81.06	85.36	2.58	0.517	
Methionine	93.55ª	85.40 ^{ab}	80.01 ^b	84.73 ^{ab}	2.18	0.044	
Phenylalanine	84.09	87.14	83.07	88.39	1.69	0.136	
Threonine	84.22	75.49	79.38	86.41	3.34	0.377	
Tryptophan	86.82ª	77.44 ^{ab}	71.59 ^b	79.91 ^{ab}	2.84	0.010	
Valine	80.80 ^{ab}	81.51 ^a	71.40 ^b	83.78 ^{ab}	2.97	0.019	
Dispensable amino	acids						
Alanine	81.20	84.24	81.29	86.64	1.75	0.079	
Aspartate	79.64	86.88	83.56	88.88	1.91	0.065	
Cysteine	86.90	89.40	80.62	86.06	2.42	0.062	
Glutamate	88.27 ^b	93.28 ^a	91.61 ^{ab}	95.23a	1.15	< 0.001	
Glycine	88.82	90.78	84.53	90.18	1.93	0.261	
Serine	80.04 ^b	88.18 ^a	83.33 ^{ab}	90.70 ^a	1.77	0.004	
Tyrosine	82.37 ^b	91.59a	86.67 ^b	87.67ab	2.04	0.003	

 $^{^{}a,b}$ means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

compared to lactating sows, and the SID of Ile, Lys, Phe, Thr, and Ala was higher compared to ad libitum-fed post-weaning sows (P < 0.05). Only the SID of Thr was higher in gestating sows than in restricted-fed post-weaning sows (P < 0.05). There were no differences in the SID value between lactating sows and restricted-fed or ad libitum-fed post-weaning sows.

Discussion

To our knowledge, only Stein et al. (1999a) reported AID values for CP and AA of barley and corn across different physiological stages of sows, such as gestation and lactation. In our study, the AID of CP and most AA in barley fed to gestating sows and lactating sows was higher or similar to those reports (Stein et al., 1999a; Zhang et al., 2024). Conversely, the AID of CP and most AA in corn fed gestating sows were lower compared to Stein et al.'s findings, except for Lys, Phe, Thr, and Val. For corn fed to lactating sows, the AID of CP and certain AA (Ile, Lys, Phe, Thr, Val, Cys, and Gly) was lower than those reported in Stein et al.'s research, whereas for the other AA, these values were higher (Stein et al., 1999a). Unfortunately, we did not find any data on AID values for wheat bran in sows. It is noteworthy that the current study focused on primiparous sows, whereas Stein et al.'s earlier investigations did not specify the parity of the sows used, which hinders the comparability of results. Additionally, several factors may influence AID values, such as variations in the feed ingredients, the physiological status of the sows, and differences in feed-intake levels (Moter and Stein, 2004).

The AID and SID data currently provided in the NRC (2012) are based on growing pigs. In our study, the mean

AID values of most AA in barley and wheat bran across different physiological stages of sows (gestating, lactating, and post-weaning) were generally similar to or higher than those reported in the NRC (2012). Likewise, when compared to the data from growing-finishing pigs by Zhang et al. and Stein et al., the mean AID values for most AA in barley for sows at various physiological stages were also higher (Stein et al, 1999b; Zhang et al., 2024). For corn, the mean AID values for Arg, Ile, Phe, Gly, and Ser were lower across different physiological stages of sows compared to the NRC (2012) values, whereas Met and Trp were higher, and other AA were similar (NRC, 2012). The higher AID of AA for barley and wheat bran in gestating and restricted-fed post-weaning sows compared to growing pigs can be attributed to their more efficient digestive system and larger, more developed gastrointestinal tract (Bridges et al., 1986). The increased intestinal volume in sows may result in a longer residence time for digesta, allowing for greater nutrient absorption (Varel, 1987). In lactating and ad libitum-fed post-weaning sows, the higher AID values of AA for barley and wheat bran compared to growing pigs can also be due to different feed-intake levels. Previous studies have suggested that feed intake has limited or no direct effects on AID (Sauer et al., 1982; Haydon et al., 1984; Albin et al., 2001). However, other studies have indicated that increased feed intake, particularly in lactating sows under ad libitum feeding conditions, may influence AID indirectly. For example, Stein et al. (1999b) observed that higher feed intake may reduce the relative contribution of endogenous IAA_{end}, thus leading to an apparent increase in AID values. Similarly, Moter and Stein (2004) reported that higher dietary AA concentrations decrease the proportion of IAA_{end} in the digesta, Wei et al.

Table 10. Standardized ileal digestibility values for crude protein and amino acids in corn for gestating, lactating, and post-weaning primiparous sows

Items, %	Gestating sows	Lactating sows	Post-weaning sows	Post-weaning sows	SEM	P-value
	Restricted-fed	Ad libitum intake	Ad libitum intake	Restricted-fed		
Crude protein	84.26	74.06	78.61	81.24	4.27	0.237
Indispensable amino	acids					
Arginine	82.33	81.97	79.99	81.55	2.44	0.951
Histidine	81.64	84.09	85.53	91.35	1.80	0.321
Isoleucine	84.30a	73.87 ^{ab}	68.86 ^b	77.66ab	2.56	0.010
Leucine	89.19	84.70	86.26	84.69	2.30	0.505
Lysine	82.83a	69.27 ^b	70.81 ^b	72.78ab	2.64	0.017
Methionine	89.30	86.90	85.51	90.74	2.86	0.570
Phenylalanine	86.13 ^a	78.92 ^{ab}	77.10 ^b	78.69ab	1.52	0.014
Threonine	95.17a	84.34 ^{ab}	73.50 ^b	74.88 ^b	4.52	0.018
Tryptophan	82.88	85.86	82.43	84.92	1.83	0.706
Valine	88.53	77.92	80.51	86.82	4.13	0.425
Dispensable amino	acids					
Alanine	87.08 ^a	72.86 ^b	72.91 ^b	85.20 ^{ab}	3.18	0.007
Aspartate	82.53	73.04	72.55	78.97	3.69	0.208
Cysteine	87.00	82.55	84.73	87.02	2.82	0.427
Glutamate	83.75	82.49	82.91	87.11	2.04	0.448
Glycine	85.48a	65.08 ^b	73.61 ^{ab}	81.01 ^{ab}	4.21	0.023
Serine	84.80	70.06	73.90	77.57	4.06	0.131
Tyrosine	85.00	79.76	82.75	80.88	3.81	0.188

a,b means within a row with different letters differ significantly (P < 0.05) in different physiological stages.

resulting in higher AID values, while lower AA levels may lead to reduced AID.

In the present study, we compared the effects of physiological differences on the AID of AA in barley, wheat bran, and corn. For wheat bran, the AID value of most AA (Ile, Leu, Thr, Val, Ala, Asp, Glu, Gly, Ser, and Tyr) in lactating sows was higher than that in restricted-fed gestating sows. Moter and Stein (2004) demonstrated that such increases in AID with rising feed intake are partially due to a reduced contribution of IAA end to the total outflow at higher feed intakes. As feed intake increases, the proportion of IAA end decreases, resulting in higher AID estimates. Although differences in feed intake may contribute to the observed variation, other physiological factors associated with lactation, such as increased body weight and digestive capacity, are likely to be the factors of improved AA digestibility (Stein et al., 1999b; Moter and Stein, 2004). Regarding barley and corn, no differences were observed with varying intake levels, likely due to the high fiber content in wheat bran. Piel et al. indicated that fiber content can alter the viscosity and passage rate of digesta, thereby affecting endogenous AA losses (Piel et al., 2005). However, there is a lack of research on how cellulose levels influence the determination of basal IAA end. For barley and corn, the AID value of most AA showed no differences across physiological stages. However, during the gestating period, sows had higher AID values in barley for His, Lys, and Thr, and higher values for Lys and Thr in corn compared to restricted-fed post-weaning sows. Zhuo et al. and Wang et al. also observed differences in AID values between gestating and nonpregnant sows for cottonseed meal and soybean meal (Wang et al., 2023a; Zhuo et al., 2023). To date, very few studies have elucidated the mechanisms underlying the physiological differences in nutrient digestibility between pregnant and nonpregnant sows. Changes in sex hormones, as well as alterations in the gut microbiota and intestinal permeability, may be factors affecting these differences (Yeo et al., 2022).

To more effectively illustrate that the SID values obtained in this study are within a reasonable range, we compare the mean SID values of the three feed ingredients across various physiological stages of sows with the corresponding values reported in NRC (2012) and the nutrient requirements of swine (GB/T 39235-2020), as presented in Table 11. In the current study, the SID value of CP and most AA in barley and corn for primiparous sows (gestating, lactating, and post-weaning period) was similar to the SID values reported by Stein et al. for gestating and lactating sows (Stein et al., 2001). This suggests that parity may not significantly affect the SID values for barley and corn, indicating that both multiparous and primiparous sows could utilize the same SID values in practical production. In our results, we compared the SID values for barley in restricted-fed gestating sows with those of ad libitum-fed lactating sows, as well as with those from nonpregnant sows in the post-weaning phase, both undergoing feed restriction or fed ad libitum. Differences were only observed in the SID of His for barley when comparing restricted-fed gestating and post-weaning sows. No differences were found between lactating and nonpregnant sows under ad libitum feeding conditions. This aligns with Stein et al.'s conclusion that the physiological stage does not affect most SID values for barley, and Stein et al. (2001) stated that lactating sows and growing pigs can share the same SID values under the same diets and ad libitum feeding conditions (Stein et al., 2001). When comparing our barley's average SID values across different physiological stages with Spindler et

Table 11. Comparison of the standardized ileal digestibility mean value of gestating, lactating, and post-weaning with the values of NRC (2012) and nutrient requirements of swine (GB/T 39235-2020).

Items, %	Barley	Wheat bran	Corn	Barley		Wheat b	ran	Corn	
	This study ¹	This study ²	This study ³	GB/T	NRC	GB/T	NRC	GB/T	NRC
СР	77.72 ± 2.00	83.85 ± 2.09	79.54 ± 1.99	77	79	72	78	88	80
Indispensable amin	o acids								
Arginine	87.45 ± 1.44	91.77 ± 0.63	81.46 ± 1.34	82	85	88	90	84	87
Histidine	83.46 ± 2.00	87.86 ± 1.09	85.65 ± 1.86	86	81	91	76	85	83
Isoleucine	82.80 ± 1.11	82.47 ± 1.16	76.17 ± 1.76	83	79	82	75	77	82
Leucine	84.45 ± 1.05	85.55 ± 1.15	86.21 ± 1.12	86	81	86	73	86	87
Lysine	77.53 ± 1.57	83.59 ± 1.35	73.92 ± 1.70	82	75	80	73	69	74
Methionine	86.49 ± 0.99	85.92 ± 1.73	88.11 ± 1.41	74	82	86	72	90	83
Phenylalanine	84.67 ± 1.11	85.68 ± 1.13	80.21 ± 1.12	86	81	82	83	85	85
Threonine	82.73 ± 1.95	81.38 ± 1.47	81.97 ± 2.82	80	76	63	64	71	77
Tryptophan	77.43 ± 2.04	78.94 ± 1.68	84.02 ± 1.16	79	82	79	73	63	80
Valine	83.14 ± 2.28	79.37 ± 2.00	83.44 ± 2.44	79	80	68	79	77	82
Dispensable amino	acids								
Alanine	81.81 ± 1.46	83.34 ± 0.93	79.51 ± 2.08	77	73	74	58	78	81
Aspartate	79.96 ± 1.60	84.74 ± 1.26	76.77 ± 1.91	78	75	76	66	74	79
Cysteine	85.49 ± 1.26	85.75 ± 1.24	85.33 ± 1.67	84	81	80	77	81	80
Glutamate	90.32 ± 0.72	92.10 ± 0.71	84.06 ± 1.11	86	87	88	84	83	84
Glycine	80.68 ± 2.68	88.58 ± 1.05	76.29 ± 2.54	76	82	68	67	69	84
Serine	84.69 ± 1.50	85.56 ± 1.22	76.58 ± 2.24	78	80	79	73	79	82
Tyrosine	89.93 ± 1.24	87.07 ± 1.40	82.10 ± 2.07	87	78	89	56	92	79

¹Standard error of the mean of barley in the different physiological periods.

al.'s SID values for growing pigs, only CP and certain AA (Ile, Phe, Trp, and Ser) exhibited differences exceeding 5%. This may be attributed to variations in research methodologies, as Spindler et al. employed regression analysis (Spindler et al., 2016). These observations suggest that the SID values for barley may apply to growing pigs, as well as to both primiparous and multiparous sows across different physiological stages, provided that the diets and feeding conditions are similar. In our experiment, we found that corn has a higher SID for certain AA during gestation compared to lactation, ad libitum post-weaning period, and restricted-fed post-weaning period. With little difference in AID, this variation is mainly related to differences in endogenous N loss. Furthermore, Wang et al. indicated that different physiological stages of sows did not affect the SID of CP and most AA in extruded full-fat soybeans (Wang et al., 2023c). However, Zhuo et al. noted that for cottonseed meal, most SID values of AA were differentially affected by the physiological phase (Zhuo et al., 2023). This suggests that the different feed ingredients may result in varying effects of physiological stages on SID values.

To our knowledge, this study is the first to publish SID values for wheat bran in sows across different physiological stages. Our experimental results showed that some SID values for wheat bran in lactating sows were higher than those in gestating sows, with Glu, Ser, and Tyr showing significant differences. This suggests that sows during lactation may exhibit higher digestibility for certain AA. Rapeseed or canola, which also contains a high fiber content, showed similar results as Deepak et al. found that the SID values of certain AA in canola for lactating sows were greater than in gestating

sows, despite the higher feed intake (Velayudhan et al., 2019). Tiwari et al. noted differences in the in vitro digestibility of starchy and fibrous feedstuffs (Tiwari et al., 2022). Wang et al. found that extruded full-fat soybeans with relatively higher levels of NDF and CF tended to have lower SID values for AA (Wang et al., 2023c). High fiber content can hinder the binding of proteases to proteins, negatively affecting protein digestion (Dégen et al., 2007; Li et al., 2021). Casas et al. (2022) demonstrated that lactating sows have greater digestibility of CF than gestating sows, which may partly explain the elevated SID values observed during lactation.

Additionally, this study compared the BEL of AA in gestating, lactating, ad libitum-fed post-weaning, and restricted-fed post-weaning sows. Endogenous nitrogen loss (ENL) encompasses nonspecific ENL and specific ENL, which mainly include digestive enzymes, mucin protein, sloughed intestinal epithelial cells, and bacterial protein. Nonspecific ENLs are regarded as BEL (Adeola et al., 2016). Nyachoti et al. (1997) indicated that primary factors influencing ENL include DM intake, body weight, and age. Mariscal-Landín et al. found that pigs of 48 kg had significantly higher ENL at a DM intake of about 60 g/kg metabolic body weight compared to 39 kg pigs consuming about 70 g/kg metabolic body weight (Mariscal-Landín et al., 1995). Wang et al. found that BEL is negatively correlated with feed intake, with higher feedintake levels associated with lower BEL (Wang et al., 2023b). Overall, the BEL observed in gestating sows totaled 16.8 g/kg DM intake, comparable to the 17.8 g/kg DM intake reported by Stein et al., with similar levels of AA losses (Stein et al., 1999a). However, our findings on BEL during gestation were

²Standard error of the mean of wheat bran in the different physiological periods.

³Standard error of the mean of corn in the different physiological conditions.

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higher than those reported by Wang et al. for the same stage (Wang et al., 2023a), likely due to feed-intake differences, as our restricted feeding provided 2.5 kg/d compared to Wang et al.'s 3.0 kg/d. In lactating sows, BEL was lower than that previously reported by Stein et al., likely due to differences in sow body weight and feed intake. Our findings during both gestation and lactation were also lower than those reported by Velayudhan et al., yet aligned with the results by Li et al. and Wang et al. These discrepancies may result from differences in methodologies used to measure BEL, particularly regarding whether casein was included in the N-free diet (Velayudhan et al., 2019; Wang et al., 2023c; Li et al., 2024). Notably, Adeola et al. demonstrated that including casein in the diet elevates endogenous AA losses (Adeola et al., 2016). In our study, primiparous sows exhibited higher BEL during gestation compared to lactation, with higher losses in restricted-fed post-weaning sows than in their ad libitum-fed counterparts. The similarity in BEL during lactation and post-weaning periods aligns with expected feed-intake variations in feed intake. Additionally, our findings on BEL in primiparous sows are comparable to those reported for multiparous sows, suggesting minimal differences in BEL between primiparous and multiparous sows (Stein et al., 1999b; Wang et al., 2023c).

Conclusions

Our experimental results provide AID and SID values for CP and AA in barley, wheat bran, and corn for primiparous sows at different physiological stages (gestation, lactation, and post-weaning phase). The current findings provided a cornerstone for the precise use of barley, wheat bran, and corn in primiparous sows' diets. Based on the current findings in the present study, we can indicate that similar SID values can be used for barley in different physiological stages of primiparous sows under the same diet and feed regime. However, for wheat bran, which has a high fiber content, lactating sows show higher AID and SID values for certain AA. For corn, which has a low fiber content, gestating sows exhibited higher SID values compared to lactating sows and ad libitum post-weaning sows. This indicates that differences in fiber content among feed ingredients may lead to variation in the AID and SID of AA and protein across different physiological stages of sows.

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Author contributions

Zixi Wei (Conceptualization, Data curation, Investigation, Writing—original draft), Lei Xu (Formal analysis, Investigation, Writing—review & editing), Jiaqi Yang (Investigation), Martine Schroyen (Writing—review &

editing), Xianren Jiang (Funding acquisition, Writing—review & editing), Sheng Cui (Writing—review & editing), Xilong Li (Conceptualization, Funding acquisition, Project administration, Writing—review & editing), and Yu Pi (Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review & editing)

Conflict of interest statement

The authors have read the journal's guidelines and have the following competing interests: We declare that this coauthor did not bias the research based on his employment status. The other authors have no competing interests.

Data availability

No data was deposited in an official repository. The study findings are available on request.

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