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Original research article

Effects of dietary cellulose levels on the estimation of endogenous amino acid losses and amino acid digestibility for growing pigs

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Two experiments were conducted to investigate the effects of dietary cellulose levels on the determination of the ileal endogenous losses (IEL) of amino acids (AA), apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA in corn-soybean meal diets for growing pigs. In the first experiment, 28 pigs (BW, 45.1 ± 2.0 kg) that were fitted with simple T-cannulas at the distal ileum were fed 4 nitrogen-free diets consisting of 4 dietary cellulose levels (0, 3%, 6% and 9%) in a randomized complete block design. In the second experiment, 28 pigs (BW, 45.6 ± 2.0 kg) fitted with simple T-cannulas at the distal ileum were fed 4 corn-soybean meal diets consisting of 4 dietary cellulose levels (0, 3%, 6% and 9%) in a randomized complete block design. There were 7 replicates per diet with 1 pig as a replicate in each treatment. Both experiments consisted of a 7-d adjustment period and a 2-d ileal digesta collection period on d 8 and 9. Chromic oxide was used as an indigestible marker to calculate IEL and digestibility of AA. The results showed that the IEL of AA for growing pigs was not influenced by dietary cellulose supplementation ($P > 0.05$). The AID of Thr, Ser, Glu, Cys, Ile, Tyr, Phe, Lys and His decreased with increasing cellulose supplementation levels for pigs fed corn-soybean meal diets ($P < 0.05$). The SID of Thr, Ser, Cys, Val, Ile, Tyr, Phe, Lys and His decreased with increasing cellulose supplementation levels in corn-soybean meal diets ($P < 0.05$). In summary, dietary cellulose levels had no effect on the estimation of IEL of AA for growing pigs. The AID and SID of most AA in corn-soybean meal diets decreased with increasing levels of dietary cellulose supplementation.

1. Introduction

The basal ileal endogenous losses (IEL) of amino acids (AA) represent AA that are present in endogenous proteins secreted into the intestinal lumen of the pig and not digested and reabsorbed before reaching the distal ileum (Tamminga et al., 1995). True digestible AA and standardized ileal digestibility (SID) of AA in feed ingredients and diets were based on the basal IEL of AA (Stein et al., 2007). The SID is more accurate than the apparent ileal digestibility (AID) for determination of AA availability because the SID were corrected for basal IEL of AA using nitrogen-free diets (NFD) (Stein et al., 2007; NRC, 2012). Therefore, it is necessary to accurately assess the basal IEL of AA for growing pigs.

Previous studies showed that ileal AA digestibility for growing pigs significantly decreased with increasing dietary crude fiber content. However, the effect was not obvious for the normal range of dietary crude fiber concentration (Glover and Duthie, 1958; Liu et al., 2008; Wang et al., 2011). In these studies, the anti-nutritional factors were increased as dietary crude fiber levels were increased, thus we could not distinguish the effect of dietary crude fibers and the anti-nutritional factors for AA digestibility. Additionally, the concentration of cellulose in NFD and experimental diets varied across studies (Sauer et al., 1991; Dilger et al., 2004; Moter and Stein, 2004; Kong et al., 2014), which may

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influence estimation of basal IEL and determination of nutrient digestibility. Therefore, the objective of the current experiment was to determine the effects of different cellulose contents on the IEL of AA in the NFD diets and on the AID and SID of AA in corn-soybean meal diet for growing pigs.

2. Materials and methods

Two experiments were conducted in accordance with the Chinese guidelines for animal welfare, and all protocols were approved by the Chinese Academy of Agricultural Sciences Animal Care and Use Committee of the State Key Laboratory of Animal Nutrition at the Chinese Academy of Agricultural Science.

2.1. Animals, housing, and experimental design

Four NFD experimental diets and 4 corn-soybean meal diets were prepared (Tables 1 and 2). The NFD were mainly based on cornstarch and sucrose. The diets were formulated to contain 0, 3%, 6% and 9% cellulose (Fiber Sales Development Corp, US). The corn-soybean meal diets were mainly based on corn and soybean meal. The diets were also formulated to contain 0, 3%, 6% and 9% cellulose. The analyzed AA composition of diets is presented in Table 3. Chromic oxide was added as an indigestible marker in each diet.

In both experiments, 28 barrows (Duroc × Landrace × Yorkshire; initial BW 45.1 ± 2.0 kg or 45.6 ± 2.0 kg, respectively) fitted with simple T-cannulas at the distal ileum were fed the above 4 experimental diets which consisted of 4 dietary levels of cellulose (0, 3%, 6% and 9%) in a randomized complete block design. There were 7 replicates per diet with 1 pig as a replicate in each treatment. All pigs were housed in stainless-steel metabolism crates (1.2 m × 1.5 m) equipped with feeders and low pressure waterers. After a 7-d adaptation period, pigs were surgically fitted with a simple T-cannula at the distal ileum as described by Dilger et al. (2004). Following the surgery, pigs were allowed to recuperate for 14 d. All pigs were housed in 2 environmentally controlled rooms (ambient temperature at 20 ± 2 °C; relative humidity at 50 ± 10%). Pigs received a daily feed allowance that was equivalent to 4% of the BW of the heaviest pig in each block. The ration was divided into 2 equal amounts and fed to pigs at 08:00 and 18:00.

Table 1
Composition and nutrient levels of diets used in Exp. 1 (air-dry basis).

Item	Levels of supplemented cellulose			
	0	3%	6%	9%
Ingredients, ‰				
Cornstarch	775.5	745.5	715.5	685.5
Chromic dioxide	5.0	5.0	5.0	5.0
Choline	1.0	1.0	1.0	1.0
Premix[1]	2.5	2.5	2.5	2.5
Cellulose	0.0	30.0	60.0	90.0
Limestone	8.0	8.0	8.0	8.0
NaCl	3.0	3.0	3.0	3.0
Sucrose	200.0	200.0	200.0	200.0
Potassium carbonate	4.0	4.0	4.0	4.0
Magnesium oxide	1.0	1.0	1.0	1.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Nutrient levels,[2] %				
Dry matter	90.72	87.94	90.47	89.93
Digestible energy, MJ/kg	14.99	15.12	14.96	15.05
Crude protein	2.54	2.87	2.86	2.71
NDF	0.27	1.88	4.36	7.35
ADF	0.03	0.04	0.05	0.06
Calcium	0.32	0.34	0.32	0.31
Total phosphorus	0.079	0.077	0.074	0.073

NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] Premix provided per kilogram of diet: Cu (as $CuSO_4 \cdot 5H_2O$) 8 mg, Fe (as $FeSO_4 \cdot 7H_2O$) 80 mg, Mn (as $MnSO_4 \cdot H_2O$) 20 mg, Zn (as $ZnSO_4 \cdot H_2O$) 80 mg, Se (as Na_2SeO_3) 0.3 mg, I (as KI) 0.4 mg, VA 12,000 IU, VD_3 6,000 IU, VE 60 IU, VK_3 3.6 mg, VB_1 2 mg, VB_2 6 mg, VB_6 4 mg, VB_{12} 0.02 mg, biotin 0.2 mg, pantothenic acid 10 mg, niacin 80 mg, folic acid 1 mg.
[2] Nutrient level values were analyzed except digestible energy.

Table 2
Composition and nutrient levels of diets used in Exp. 2 (air-dry basis).

Item	Levels of supplemented cellulose			
	0	3%	6%	9%
Ingredients, ‰				
Corn	620.0	620.0	620.0	620.0
Cornstarch	150.0	100.0	50.0	0.0
Soybean meal	200.0	200.0	200.0	200.0
Chromic dioxide	5.0	5.0	5.0	5.0
Soy oil		20.0	40.0	60.0
Choline	1.0	1.0	1.0	1.0
Premix[1]	2.5	2.5	2.5	2.5
Cellulose	0.0	30.0	60.0	90.0
Limestone	8.0	8.0	8.0	8.0
Dicalcium phosphate	10.5	10.5	10.5	10.5
NaCl	3.0	3.0	3.0	3.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Nutrient levels,[2] %				
Dry matter	87.36	88.11	88.59	89.11
Digestible energy, MJ/kg	15.88	16.47	17.07	17.54
Crude protein	14.14	14.11	14.29	14.24
NDF	7.47	10.54	12.81	14.63
ADF	1.68	1.87	1.98	2.18
Calcium	0.62	0.64	0.65	0.61
Total phosphorus	0.533	0.536	0.549	0.545

NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] Premix provided per kilogram of diet: Cu (as $CuSO_4 \cdot 5H_2O$) 8 mg, Fe (as $FeSO_4 \cdot 7H_2O$) 80 mg, Mn (as $MnSO_4 \cdot H_2O$) 20 mg, Zn (as $ZnSO_4 \cdot H_2O$) 80 mg, Se (as Na_2SeO_3) 0.3 mg, I (as KI) 0.4 mg, VA 12,000 IU, VD_3 6,000 IU, VE 60 IU, VK_3 3.6 mg, VB_1 2 mg, VB_2 6 mg, VB_6 4 mg, VB_{12} 0.02 mg, biotin 0.2 mg, pantothenic acid 10 mg, niacin 80 mg, folic acid 1 mg.
[2] Nutrient level values were analyzed except digestible energy.

Table 3
Analyzed amino acids composition (mg/kg) of diets used in Exp. 2.

Item	Levels of supplemented cellulose			
	0	3%	6%	9%
Asp	1,425	1,534	1,370	1,479
Thr	586	627	558	570
Ser	709	757	703	698
Glu	2,379	2,480	2,317	2,320
Gly	636	663	590	656
Ala	822	837	772	783
Cys	234	254	248	287
Val	752	750	659	699
Met	184	190	125	184
Ile	614	662	537	599
Leu	1,672	1,567	1,413	1,522
Tyr	591	612	554	571
Phe	846	817	731	783
Lys	854	821	721	756
His	450	443	394	434
Arg	817	1,010	918	851
Pro	2,625	1,864	1,724	2,131

2.2. Sample collection

Each experiment contained 9 days. Barrows adapted for diets 7 days. During the following 2 days, the ileal digesta of the barrows were collected from 08:00 to 18:00 on each day. Ileal digesta were collected in plastic bags (approximately 250 mL) which were attached to the barrels of the cannulas with rubber bands. Samples were stored in a freezer at −20 °C. At the end of each experiment, all the samples from the same pig were pooled and subsampled for freeze-drying.

2.3. Chemical analyses

All freeze-dried ileal digesta samples and diets were ground, using a mill grinder to pass through a 0.5-mm screen before analysis. Analyses for DM and CP were assayed according to methods of the AOAC (2007). The concentrations of AA in experimental diets and ileal digesta were determined according to the procedure of AOAC (2007). The samples were hydrolyzed by 6 mol/L HCl at 110 °C for 24 h, and then they were analyzed for 15 AA with an automatic amino acid analyzer (Model L-8900 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). After cold performic acid oxidation overnight and hydrolysis using 7.5 mol/L HCl at 110 °C for 24 h, methionine and cystine were analyzed as methionine sulfone and cysteic acid using the automatic amino acid analyzer (Model L-8900 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). Tryptophan content was not measured. The chromium (Cr) concentration in the diets and ileal samples was determined according to the procedures of Fenton and Fenton (1979).

2.4. Calculations and statistical analyses

The basal IEL, AID and SID of AA and CP were calculated according to the equations described by Dilger et al. (2004):

$$\text{IEL} = \text{AA}_i \times (\text{Cr}_d/\text{Cr}_i),$$

$$\text{AID}\ (\%) = [1 - (\text{Cr}_d/\text{Cr}_i) \times (\text{AA}_i/\text{AA}_d)] \times 100,$$

$$\text{SID}\ (\%) = \text{AID} + (\text{IEL}/\text{AA}_d) \times 100,$$

where IEL was basal endogenous losses of AA; AID = apparent ileal digestibility; SID = standardized ileal digestibility; Cr_d was Cr concentration in diets; Cr_i was Cr concentration in ileal digesta; AA_d was AA concentration in diets; and AA_i was AA output in ileal digesta. All values used were expressed as milligrams per kilogram of DM, except the IEL was expressed as milligrams per kilogram of DMI.

Data were statistically analyzed using the Proc GLM procedure of SAS 9.2 software package (SAS Inst. Inc., Cary, NC, USA). A probability of $P < 0.05$ was accepted as statistically significant.

3. Results

The pigs remained healthy and consumed their daily allowances throughout the experiment.

3.1. Effects of dietary cellulose level on IEL of amino acids for growing pigs

Table 4 shows that the cellulose supplement concentration in the NFD had no effect on the IEL of AA for growing pigs ($P > 0.05$).

3.2. Effects of dietary cellulose levels on AID and SID of amino acid in corn-soybean meal based diets

Table 5 illustrates that the AID values of Thr, Ser, Glu, Cys, Val, Ile, Tyr, Phe, Lys and His linearly deceased with increasing dietary cellulose ($P < 0.05$). Moreover, the AID values of Thr, Glu, Val, and Ile were quadratically affected by cellulose supplements ($P < 0.05$). The AID values of Thr, Glu, Val, and Ile were the lowest for 6% cellulose supplemental level among all groups. The supplement of dietary cellulose had no effect on AID values of Asp, Gly, Ala, Met, Leu, Arg and Pro for growing pigs ($P > 0.05$). The SID values of AA for growing pigs are presented in Table 6. As shown in Table 6, the SID values of Thr, Ser, Cys, Val, Ile, Tyr, Phe, Lys and His linearly deceased with increasing dietary cellulose level ($P < 0.05$). The levels of dietary cellulose supplements did not influence the SID coefficients of Asp, Glu, Gly, Ala, Met, Arg and Pro for growing pigs ($P < 0.05$).

Table 4
Ileal endogenous amino acid losses (mg/kg DMI) of pigs fed diets supplemented with different levels of cellulose.

Item	Levels of supplemented fiber				Mean	SEM	*P*-value	
	0	3%	6%	9%			Linear	Quadratic
N	3,339	2,933	3,061	2,159	2,842	50.3	0.978	0.648
Asp	166	126	140	87	130	34.7	0.908	0.586
Thr	126	93	112	79	102	28.1	0.956	0.520
Ser	108	91	99	74	93	22.3	0.994	0.695
Glu	210	164	170	100	161	45.7	0.789	0.693
Gly	352	307	369	214	310	64.7	0.667	0.559
Ala	122	93	113	64	98	24.3	0.889	0.482
Cys	292	34	120	30	119	123.2	0.809	0.330
Val	134	97	120	89	110	31.7	0.908	0.501
Met	81	11	28	8	32	34.7	0.713	0.376
Ile	82	58	72	38	62	18.3	0.942	0.476
Leu	127	107	110	73	104	29.7	0.855	0.787
Tyr	86	68	81	51	72	17.3	0.863	0.533
Phe	82	65	72	45	66	17.8	0.964	0.619
Lys	63	60	64	48	59	18.2	0.921	0.887
His	31	33	30	22	29	18.3	0.850	0.771
Arg	79	105	109	61	88	17.2	0.493	0.649
Pro	1,066	855	1,110	684	929	141.7	0.456	0.252

N = nitrogen.

Table 5
Apparent ileal digestibility (AID) (%) of amino acid in pigs fed corn–soybean meal based diets supplemented with different levels of cellulose.

Item	Levels of supplemented cellulose				SEM	*P*-value	
	0	3%	6%	9%		Linear	Quadratic
N	71.34	72.40	68.42	72.82	1.27	0.067	0.171
Asp	76.40	79.34	80.12	79.11	2.79	0.567	0.785
Thr	71.69	74.31	53.54	71.29	3.74	0.002	0.038
Ser	77.43	79.67	73.36	78.03	1.61	0.032	0.070
Glu	80.90	83.93	77.70	82.90	1.53	0.041	0.044
Gly	53.12	61.59	46.79	55.41	4.21	0.103	0.075
Ala	74.65	77.01	70.43	75.72	2.01	0.069	0.128
Cys	64.18	57.78	44.57	63.81	4.95	0.028	0.626
Val	75.97	76.85	66.64	75.71	1.89	0.002	0.049
Met	88.79	72.74	75.40	89.81	6.02	0.530	0.280
Ile	79.45	81.59	72.06	79.34	1.58	0.001	0.016
Leu	84.32	84.16	81.23	84.71	1.61	0.197	0.546
Tyr	83.83	83.96	79.19	83.30	1.06	0.004	0.113
Phe	83.38	83.75	78.04	83.25	1.31	0.007	0.109
Lys	83.92	83.73	76.96	81.69	1.55	0.005	0.143
His	85.28	85.95	80.56	85.86	1.31	0.012	0.112
Arg	85.34	88.81	88.77	88.09	1.52	0.443	0.426
Pro	54.45	51.87	43.93	42.62	6.95	0.342	0.780

N = nitrogen.

4. Discussion

4.1. Effects of dietary cellulose level on the basal IEL of AA

The influence of dietary cellulose level on the basal endogenous nitrogen excretion of AA for pigs deserved discussion. There

Table 6
Standardized ileal digestibility (SID) (%) of amino acids in pigs fed corn–soybean meal based diets supplemented with different levels of cellulose.

Item	Levels of supplemented cellulose				SEM	*P*-value	
	0	3%	6%	9%		Linear	Quadratic
N	92.56	93.67	89.42	93.89	1.27	0.049	0.147
Asp	86.84	89.01	90.84	88.99	2.79	0.463	0.966
Thr	91.61	92.82	74.18	91.42	3.74	0.003	0.073
Ser	92.45	93.55	88.30	92.99	1.61	0.049	0.170
Glu	88.65	91.29	85.54	90.67	1.53	0.054	0.066
Gly	109.06	114.77	106.26	108.69	4.21	0.369	0.262
Ala	88.30	90.29	84.76	89.71	2.01	0.122	0.196
Cys	122.51	110.99	98.66	110.27	4.95	0.017	0.953
Val	92.71	93.47	85.48	93.32	1.89	0.009	0.114
Met	108.67	91.86	104.36	109.29	6.02	0.632	0.098
Ile	91.02	92.16	85.08	91.01	1.58	0.008	0.076
Leu	91.43	91.71	89.54	92.33	1.61	0.378	0.592
Tyr	97.59	97.13	93.65	97.30	1.06	0.021	0.318
Phe	92.31	92.88	88.23	92.75	1.31	0.026	0.165
Lys	91.83	91.84	86.20	90.40	1.55	0.018	0.206
His	92.66	93.37	88.87	93.33	1.31	0.035	0.169
Arg	97.66	98.67	99.58	99.72	1.52	0.519	0.982
Pro	94.96	108.42	104.76	91.48	6.95	0.749	0.377

N = nitrogen.

were different results about the influences from different researchers. Schulze et al. (1994) reported that IEL of AA was linearly increased with increasing dietary neutral detergent fiber level. In addition, Furuya and Kaji (1992) reported that, the basal IEL of AA significantly increased with increasing dietary crude fiber level from 7% to 11%. The increasing amounts of fiber in the diet resulted in the increasing amount of basal IEL of nitrogen. This effect can be explained as following reasons. Dietary fibers may directly stimulate the secretion of digestive enzymes, and the endogenous AA may hardly be reabsorbed because of the presence of dietary fibers (Darraph et al., 1990; Souffrant, 2001). However, Sève et al. (1994) found that the basal IEL of AA significantly increased as dietary crude fiber level increased from 3% to 6%, and then the basal IEL of AA kept stable. In addition, the current experiment showed that dietary cellulose levels do not affect basal IEL for AA and CP. Studies with inconsistent results might result from the obvious difference of physical and chemical properties between the synthetic cellulose added to the diets and the fiber that diets contained. In the studies of Souffrant (2001), Leterme and Thewis (2004), their results showed that the physic-chemical properties of dietary fiber, especially water-holding capacity and viscosity, were more important than the concentration of dietary fiber.

However, animals were in non-physiological conditions when NFD method was used, which might influence the normal body protein metabolism (Millward et al., 1976). As the experimental period was extended for feeding animals NFD, the protein that was required to maintain normal body activities cannot be obtained from NFD. Thus, animals had to use their tissue to release lots of AA, in which glutamine was the maximum. Glutamine can be decomposed into glutamic acid and proline (Mariscal-Landín et al., 1995). When pigs were fed NFD, high content of glycine in the ileal digesta may be related to the secretion of bile and saliva, because saliva and bile contain plenty of glycine and proline (Low, 1982). As indicated by Butts et al. (1993) and Donkoh et al. (1995), a NFD may lack the stimulatory effect on endogenous gut protein secretions. Therefore, this may lead to an overestimation of IEL of glycine and proline and an underestimation of basal IEL overall at the distal ileum (De Lange et al., 1989; Leterme et al., 1996; Butts et al., 1993; Donkoh et al., 1995).

4.2. Effects of dietary cellulose levels on amino acids digestibility

Generally speaking, dietary fiber level in diets above a certain level will retrain the growth of pigs (Yang and Qiao, 2000; Noblet and Le Goff 2001). Compared with other nutrients, dietary fiber possesses complex compositions and different physic-chemical properties. Dietary fibers from different resource with different content have different impact on the digestion and absorption of nutrients for pig (Souffrant, 2001; Leterme and Thewis, 2004).

A large number of investigations have been carried out to study the effect of dietary fiber on the ileal digestibility of AA and CP in pigs. But the results reported by different researchers are not always consistent. The studies of Sauer et al. (1991) showed that the diets added 10% purified cellulose or barley straw had no effect on the AID of most AA except for leucine and glycine compared with basal diets. According to the study of Li et al. (1994), young pigs (weaned at 3 weeks of age) were fed 4 cornstarch-soybean meal basal diets which was containing 4.3%, 7.3%, 10.3% and 13.3% cellulose, respectively, and no difference in the AID of crude protein was observed. Liu et al. (2008) and Wang et al. (2011) reported that the AID value of AA was the maximum when dietary crude fiber level was at 6% or 5%, and dietary fiber levels that ranged from 3% to 6% did not affect the AID of AA. However, the AID of essential AA significantly decreased as dietary crude fiber levels increased from 6% to 12%. Additionally, Dilger et al. (2004) showed that the ileal digestibility of some AA such as Lys, Arg, His and Phe were decreased with increasing soyhulls level when pigs were fed the diets which were formulated to contain graded levels of soyhulls at 0, 3%, 6% and 9%.

The current experiments used purified cellulose to study the effects of different cellulose levels on the digestion and absorption of dietary AA under the same conditions of energy and CP levels. In the current study, the decreases of the SID of Thr, Ser, Cys, Val, Ile, Tyr, Phe, Lys and His resulted from the increase of dietary cellulose levels. The reason may be that high cellulose levels increased the small intestine motility, reduced the time and the possibility of digesta in contact with the gastrointestinal tract and decreased the SID of AA for pigs.

5. Conclusion

The current experiments showed that the dietary cellulose level of NFD had no influence on the determination of basal IEL by growing pigs. For growing pigs fed corn–soybean meal diets, the AID and SID of AA were significantly decreased with increasing dietary cellulose supplement levels. In addition, it is necessary to formulate the dietary fiber levels in an appropriate range to determine ileal AA digestibility in the diets for growing pigs. Furthermore, different experimental diets should be formulated to have similar levels of dietary crude fiber to study the effects of dietary factors on AA ileal digestibility for growing pigs.

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