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.

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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
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 cellulose. The analyzed AA composition of diets is presented in meal. The diets were formulated to contain 0, 3%, 6% and 9% 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 (Fiber Sales Development Corp, US). 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 ºC; 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.

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 from 08:00 to 18:00 on each day. Ileal digesta were collected from 08:00 to 18:00.
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 Fentont 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):

\[
\text{IEL} = \frac{A_{d}}{(C_{d}/C_{r})},
\]

\[
\text{AID} = \left(1 - \frac{C_{d}}{C_{r}}\right) \times \left(A_{d}/A_{d'}\right) \times 100,
\]

\[
\text{SID} = \text{AID} + \left(\text{IEL}/A_{d'}\right) \times 100.
\]

where IEL was basal endogenous losses of AA; AID = apparent ileal digestibility; SID = standardized ileal digestibility; \(C_{d}\) was Cr concentration in diets; \(C_{r}\) was Cr concentration in ileal digesta; \(A_{d}\) was AA concentration in diets; and \(A_{d}'\) 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 decreased with increasing dietary cellulose (P < 0.05). Moreover, the AID values ofThr, 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 concentration in the diets and ileal samples was determined according to the procedure of 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).

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

| Item | Levels of supplemented cellulose | Mean | 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 | 0.030 | 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.
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 (Darragh 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 physical-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, resulting 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 (Darragh 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 physical-chemical properties of dietary fiber, especially water-holding capacity and viscosity, were more important than the concentration of dietary fiber.

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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 physico-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 phenylalanine were decreased in increasing soy hulls 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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