Determination of nutrient digestibility in corn and soybean meal using the direct and substitution methods as well as different basal diets fed to growing pigs

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ABSTRACT
Two experiments were conducted to compare methods of determination of the apparent total tract digestibility (ATTD) of various chemical constituents in corn and soybean meal (CS) when fed to growing pigs. Expt. 1, eighteen barrows (34.3 ± 1.1 kg) were randomly allotted to 1 of 3 diets. The three diets were Corn, CS-Basal, and CS-Test. The ATTD of DM, GE, CP, and organic matter (OM) in corn determined by the direct method were not different from those determined by the substitution method. Expt. 2, twenty-four barrows (36.2 ± 1.4 kg) were randomly allotted to 1 of 4 diets. The four diets were Basal diet 1 (97.5% corn), Test diet 1 (replaced corn in Basal diet 1 with 15% SBM), Basal diet 2 (72.5% corn and 25% SBM), and Test diet 2 (58% corn and 39.5% SBM). The ATTD of GE and CP in SBM were greater (p < 0.05) determined by the Corn-Basal diet method than Corn-SBM-Basal diet method. In summary, there was no difference in the ATTD of nutrients in corn when determined using direct or substitution method, but the ATTD of GE and CP in SBM determined using the Corn-Basal diet method were greater than using Corn-SBM-Basal diet method.

1. Introduction
The cost of feed is about 70% of the total cost of pig production. It is important to estimate precisely the nutrients value of feeds, either for least-cost formulation purposes or for adapting feed supply to nutrient requirements of pigs (Noblet and Henry 1993). The digestion study is defined that quantifying the intake of a specific component of an ingredient or feed, the fecal output from the undigested portion of that component of the ingredient or feed, and the difference between intake and output (Adeola 2001). The apparent total tract digestibility (ATTD) calculated by a digestion study provides an important reference to estimate the nutrients value of feed or ingredients (Young et al. 1991).

The ATTD of nutrients of a test ingredient is determined either by the direct or the substitution methods (Stefanello et al. 2016). With the direct method, the diet is formulated such that all of the component of interest is supplied by the test ingredient alone. Diets formulated as such are offered to pigs, and the ATTD of the nutrients is determined (Bolariniwa and Adeola 2012). The substitution method is defined that the basal diet was replaced by one or several designed proportion of the test ingredient. The substitution method is usually used to determine the ATTD of nutrients under these situations that the test ingredient cannot be fed for a long enough period or the diets could not be formulated with the test ingredient alone to supplying all the component of interest (Adeola 2001). The ATTD of nutrients determined using the substitution method are based on the assumption that there is no interaction between the basal diet and the included test ingredient that could influence the results of the determination (Wiseman and Cole 1985).

Although a study for determining the energy value of ingredients fed to growing pigs using several methods had just finished in our laboratory (Liu et al. 2014), comparisons of the direct or substitution methods, as well as the effects of different basal diet on ingredient nutrient digestibility have not been extensively presented in the literature. Therefore, the aim of the present work was to compare the ATTD of nutrients in ingredients using the direct and substitution methods (Exp. 1), as well as using different basal diets (Exp. 2) fed to growing pigs.

2. Material and methods
2.1. General
The animal protocols used in these experiments were approved by the China Agricultural University Institutional Animal Care and Use Committee (Beijing, China). All pigs used were Durouch × (Landrace × Large White) crossbred barrows. In Exp. 1 and 2, pigs were individually housed in 1.2 × 0.7 × 0.96 m³ stainless steel metabolism cages located in an environmentally controlled room (22 ± 2°C). Pigs were fed 2400 kJ ME/kg BW0.65 d−1, which is approximately ad libitum (Zhang et al. 2014). Feed was divided into two equal portions at 07:30 and 15:30 h and fed in mash form. Water was provided ad libitum through a nipple drinker. The amount of feed supplied each day was recorded as well as any feed refusals.
2.2. Nutrient digestibility of corn determined directly or by the substitution method

A total of 18 growing barrows with an initial BW of 34.3 ± 1.1 kg were used to determine the ATTD of dry matter (DM), gross energy (GE), crude protein (CP), and organic matter (OM) in corn using both the direct and substitution methods. Pigs were randomly allotted to a randomized block design with 3 dietary treatments (Table 1) and 3 experimental periods. The first dietary treatment (Corn) contained 97.5% corn, which was used to determine the ATTD of nutrients in corn directly. The second dietary treatment (CS-Basal) contained 72.5% corn and 25% soybean meal, which was used as the basal diet, while the third dietary treatment (CS-Test) substituted 40% of the energy supplied by soybean meal in the CS-Basal diet with corn and this diet was used as the test diet to determine the ATTD of nutrients in corn using the substitution method.

Each experimental period consisted of a 7-d adaptation period followed by a 5-d total collection of feces. During the 5-d collection period, a total, but separate, collection of feces was conducted according to the methods described by Song et al. (2003). Feces were collected, sealed in plastic bags, and stored at −20°C. At the end of the experiment, fecal samples were oven-dried for 72 h at 65°C, ground through a 1-mm screen and thoroughly mixed before a sub-sample was collected for chemical analysis.

Samples of feces, diets, and ingredients were analyzed and the ATTD of nutrients in each diet was then calculated. The ATTD of nutrients in corn was then multiplied by 85% and 75% in order to calculate the contribution from the Corn-Basal diet to the ATTD of nutrients in corn because corn was the only energy supplying feedstuff in the diet using the direct method. However, to calculate the ATTD of nutrients in corn using the substitution method, the ATTD of nutrients in the CS-Basal diet was multiplied by 60% to calculate the contribution from the CS-Basal diet to the ATTD of nutrients in the CS-Test diet. The ATTD of nutrients in corn was then calculated by difference (Kong and Adeola 2014).

Table 1. Composition of the experimental diets used in Exp. 1 (% as-fed).

| Item                        | Direct method Corn diet | Substitution method CS-Basal diet | Substitution method CS-Test diet |
|-----------------------------|-------------------------|-----------------------------------|---------------------------------|
| **Ingredients**             |                         |                                   |                                 |
| Corn                        | 97.50                   | 72.50                             | 82.50                           |
| Soybean meal                | –                       | 25.00                             | 15.00                           |
| Limestone                   | 0.75                    | 0.75                              | 0.75                            |
| Dicalcium phosphate         | 0.90                    | 0.90                              | 0.90                            |
| Salt                        | 0.35                    | 0.35                              | 0.35                            |
| Vitamin-mineral premix a    | 0.50                    | 0.50                              | 0.50                            |
| **Chemical content (analyzed value)** |                     |                                   |                                 |
| Dry matter                  | 86.76                   | 87.69                             | 87.63                           |
| Crude protein               | 8.38                    | 17.19                             | 13.76                           |
| Starch                      | 59.86                   | 46.63                             | 51.12                           |
| Ether extract               | 2.01                    | 1.94                              | 1.99                            |
| Neutral detergent fibre     | 7.87                    | 9.18                              | 7.84                            |
| Acid detergent fibre        | 1.68                    | 2.96                              | 2.13                            |
| Ash                         | 2.86                    | 4.13                              | 3.64                            |
| Gross energy, MJ/kg         | 15.53                   | 16.03                             | 15.82                           |

aVitamin-mineral premix supplied the following nutrients per kilogram of diet:
- vitamin A, 5512 IU; vitamin D₃, 2200 IU; vitamin E, 30 IU; vitamin K₂, 2.2 mg; vitamin B₁₂, 27.6 μg; riboflavin, 4 mg; pantothenic acid, 14 mg; niacin, 30 mg; choline chloride, 400 mg; folic acid, 0.7 mg; thiamine, 1.5 mg; pyridoxine, 3 mg; biotin, 44 μg; Mn (MnO), 40 mg; Fe (FeSO₄·H₂O), 75 mg; Zn (ZnO), 75 mg; Cu (CuSO₄·5H₂O), 100 mg; I (KI), 0.3 mg; Se (Na₂SeO₃), 0.3 mg.

2.3. Nutrient digestibility of soybean meal determined using different basal diets

A total of 24 barrows (initial BW = 36.2 ± 1.2 kg) were used to determine the ATTD of nutrients in soybean meal using different basal diets. Pigs were randomly allotted to a randomized block design with 4 dietary treatments (Table 2) and 4 experimental periods, in which each period lasted 12 d. The feeding and treatment of pigs in each experimental period was similar to Exp. 1. The diets included a 97.5% corn basal diet (Basal diet 1) and test diet (Test diet 1) that replaced corn with 15% soybean meal and a second basal diet (Basal diet 2) which contained 72.5% corn and 25% soybean meal and a second test diet (Test diet 2) that contained 58% corn and 39.5% soybean meal, these diets were used to determine the ATTD of nutrients in soybean meal using the corn basal diet (Corn-Basal) or the corn-soybean meal basal diet (Corn-SBM-Basal) method, respectively. The Basal diet 1, Basal diet 2, and Test diet 1 were the corn diet, CS-Basal diet, and CS-Test diet used in Exp. 1, respectively.

Diets, feces, and ingredient sample collections were similar to Exp. 1. For the Corn-Basal diet method, the ATTD of nutrients in the Corn-Basal diet was multiplied by 85% and 75% in order to calculate the contribution from the Corn-Basal diet to the ATTD of nutrients in the soybean meal test diet. The ATTD of nutrients in soybean meal was then calculated by difference (Adeola 2001). The calculation method for the Corn-SBM-Basal diet method is similar to that of the Corn-Basal diet method, but was only multiplied by 80% when calculating the ATTD of nutrients in soybean meal test diets.

2.4. Chemical analysis and calculations

Corn, soybean meal, diets, and feces were analyzed for DM (method 930.15, AOAC 2007), ash (method 942.05, AOAC 2007), Kjeldahl N (Thiex et al. 2002), and ether extract (Thiex et al. 2003). Gross energy in corn, soybean meal, diets, and feces were determined using a Parr 6400 bomb calorimeter (Parr Instruments, Moline, IL).
The ATTD of DM, GE, CP, and OM in diets and ingredients were calculated by the direct method using the equation below: ATTD = [(Fi – Ff)/Fi] × 100%, where ATTD is the apparent total tract digestibility of DM, GE, CP, and OM; Fi is the total intake of DM (g), GE (MJ), CP(g), or OM (g) during the 5-d collection period; and Ff is the total fecal output of DM (g), GE (MJ), CP (g), or OM (g) originating from the feed that was fed during the 5-d collection period (Pedersen et al. 2007). The ATTD of DM, GE, CP, and OM in diets and ingredients were calculated by the substitution method using the equation below:

\[ \text{AD}_{\text{n}} = \left(\frac{\text{AD}_{\text{test}} - (100\% - X\%) \times \text{AD}_{\text{basal}}}{X\%}\right) \]

where AD\(_{\text{n}}\) is the ATTD of a nutrient in the test ingredient (%), AD\(_{\text{test}}\) is the ATTD of the nutrient in the test diet (%), AD\(_{\text{basal}}\) is the ATTD of the nutrient in the basal diet, and X is the proportion that the basal diet was replaced by the test ingredient (Kong and Adeola 2014).

2.5. Statistical analysis

Data were analyzed on the basis of a randomized complete block design by the MIXED procedure of SAS (Version 8.2, Cary, NC, 2001). Diet was treated as the only fixed effect, and period as random effects. The LSMEANS statement was used to separate mean values. The differences among means were determined by Tukey’s Multiple Range Test. Results were considered significant at \( p \leq 0.05 \) and considered a trend at \( p \leq 0.10 \).

3. Results

3.1. Chemical composition of corn and soybean meal samples

Corn and soybean meal used in Exp. 1 and 2 were from a single batch, and analyzed for chemical composition (Table 3). The CP and starch content were 8.31% and 61.03% in corn and 45.01% and 3.74% in soybean meal, respectively. Ether extract content was similar in corn and soybean meal. The neutral detergent fibre (NDF), acid detergent fibre (ADF), and GE content in soybean meal were greater than that of corn.

3.2. Nutrient digestibility of corn determined directly or by the substitution method

No difference was observed in the ATTD of DM, GE, and OM among the three experimental diets. However, pigs fed the CS-Basal and CS-Test diets had a greater \(( p < 0.01)\) ATTD of CP compared with pigs fed the Corn diet (Table 4). The ATTD of nutrients in corn determined by the substitution method was not different compared with the direct method (Table 5).

3.3. Nutrient digestibility of soybean meal determined using different basal diets

Pigs fed Test diet 1, Basal diet 2 and Test diet 2 had a greater \(( p < 0.01)\) ATTD of CP compared with pigs fed Basal diet 1, but there were no differences in the ATTD of DM, GE, and OM among pigs fed the four experimental diets (Table 6). Different basal diets had no effect on the ATTD of DM and OM in soybean meal. However, the ATTD of GE and CP in soybean meal determined by the Corn-SBM-Basal diet method was lower \(( p < 0.05)\) compared with the Corn-Basal method (Table 7).

4. Discussion

4.1. Nutrient digestibility of corn determined directly or by the substitution method

A basic assumption in the use of the substitution method is that the ingredient supplying the same component in the total diet do not interact with one another to enhance or depress the digestibility of that component (Wiseman and Cole 1985; Adeola 2001). In the current study, the ATTD of DM, GE, and OM in the three experimental diets were not affected by

| Table 3. Chemical composition of corn and soybean meal used in Exp. 1 and 2 (as-fed basis, %)\(^{a}\)|
|---------------------------------------------|
| **Corn** | **Soybean meal** |
| Dry matter | 86.62 | 88.69 |
| Crude protein | 8.31 | 45.01 |
| Starch | 61.03 | 3.74 |
| Ether extract | 2.16 | 1.54 |
| Neutral detergent fibre | 7.21 | 11.38 |
| Acid detergent fibre | 1.58 | 6.08 |
| Ash | 1.06 | 5.84 |
| Gross energy, MJ/kg | 16.02 | 17.33 |

\(^{a}\)All data are the results of a chemical analysis conducted in duplicate.

| Table 4. Effects of the direct and substitution methods on the apparent total tract digestibility of nutrients (%) in diets fed to growing pigs (Exp. 1)\(^{a}\). |
|---------------------------------------------|
| **Direct method** & **Substitution method** & **SEM** & **p value** |
| | Corn diet & CS-basal diet & CS-test diet & |
| Dry matter | 90.86 & 91.15 & 91.30 | 0.19 & 0.67 |
| Gross energy | 91.31 & 91.40 & 91.28 | 0.20 & 0.29 |
| Crude protein | 87.97\(^{b}\) & 90.60\(^{a}\) & 89.56\(^{b}\) | 0.30 & <0.01 |
| Organic matter | 92.26 & 92.63 & 92.68 | 0.17 & 0.63 |

\(^{a}\)Means in the same row with different superscripts differ \(( p \leq 0.05)\).

| Table 5. Effects of the direct and substitution methods on the apparent total tract digestibility of nutrients (%) in corn fed to growing pigs (Exp. 1)\(^{a}\). |
|---------------------------------------------|
| **Direct method** & **Substitution method** & **SEM** & **p value** |
| | Corn diet & CS-basal diet & CS-test diet & |
| Dry matter | 93.19 & 92.13 | 0.41 & 0.29 |
| Gross energy | 90.01 & 88.76 | 0.53 & 0.26 |
| Crude protein | 88.17 & 88.70 | 0.55 & 0.76 |
| Organic matter | 94.62 & 93.27 | 0.37 & 0.16 |

\(^{a}\)Data are means of 6 observations.

| Table 6. Effects of different basal diets on the apparent total tract digestibility of nutrients (%) in diets fed to growing pigs (Exp. 2)\(^{a}\). |
|---------------------------------------------|
| **Basal diet** & **Test diet** & **Basal diet** & **Test diet** & **SEM** & **p value** |
| | 1 & 1 & 2 & 2 & |
| Dry matter | 90.86 & 91.30 & 91.15 & 91.38 | 0.18 & 0.70 |
| Gross energy | 91.31 & 91.28 & 91.39 & 91.81 | 0.19 & 0.11 |
| Crude protein | 87.97\(^{b}\) & 89.55\(^{a}\) & 90.60\(^{b}\) & 91.45\(^{a}\) | 0.26 & <0.01 |
| Organic matter | 92.26 & 92.68 & 92.63 & 92.92 | 0.16 & 0.52 |

\(^{a}\)Means in the same row with different superscripts differ \(( p \leq 0.05)\).

\(^{a}\)Data are means of 6 observations.
soybean meal inclusion. This may be attributed to the fact that corn DM, GE, and OM digestibility was similar to that of the soybean meal (Graham et al. 2014). Bolarinwai and Adeola (2012) reported that there were also no significant difference among the ATTD values obtained for the DM in four experimental diets. The CP content in the CS-Basal and CS-Test diets was much greater than the CP content in the Corn diet and this may be the reason why pigs fed the CS-Basal and CS-Test diets had a greater ATTD of CP compared with pigs fed the Corn diet. These results are in agreement with previous research that has shown that pigs fed diets high in CP have a relatively greater ATTD of CP compared with pigs fed diets with adequate levels of CP (Lloyd and Crampton 1955; Li et al. 1993). The ATTD of the nutrients in corn was not different between the direct and substitution methods. This may be attributed to the fact that corn nutrient digestibility was similar to that of the Corn diet, which was formulated to meet the requirements for growing pigs (Sauber and Owens 2001). However, Wu and Ewan (1979) reported a linear improvement in DM and energy digestibility of diets fed to pigs with increasing wheat inclusion. Compared to the high SBM level in experimental diets of Wu’s, the test ingredient in the present study contains lower level of anti-nutritive factors, which indicated little effects on energy and nutrients digestibility (Veum and Odle 2001).

### 4.2. Nutrient digestibility of soybean meal determined using different basal diets

An important point with regard to compare the ATTD of nutrients in ingredients using different basal diets is that the nutrient composition of the basal diet in relation to the tested ingredient (Villamide 1996). In the present study, there were no significant difference among the ATTD values obtained for the GE, DM and OM in four experimental diets. However, Su et al. (2016) reported that the ATTD of GE, DM, and OM of each experimental diet were significantly affected by the type of basal diets, which was explained by the interaction between the soybean oil and inherent fat in the basal diet. The results in the current experiment may indicated that there were little interaction between the corn and soybean meal.

The ATTD of GE and CP in SBM were greater (p < 0.05) in the Corn-Basal diet than in the Corn-SBM-Basal diet and this may be attributed to the fact that the ATTD of GE and CP in soybean meal are greater than the Corn-Basal diet and were similar to that of the Corn-SBM-Basal diet (Li et al. 1993). In addition, compared with the Corn-Basal diet, the Corn-SBM-Basal diet replaced by SBM could result in a higher CP level, which may cause a higher incidence of diarrhea in pigs (Ball and Aherne 1987). Therefore, the Corn-Basal diet method was a better choice than a Corn-SBM-Basal diet method to determine the nutrient digestibility of the feedstuff when the test ingredient has a greater CP content.

### 5. Conclusion

The ATTD of nutrients in corn determined using the direct method were not different from those determined using the substitution method. Different basal diets did not affect the ATTD of DM and OM in soybean meal. However, the ATTD of GE and CP of soybean meal determined by the Corn-SBM-Basal diet method was lower compared with the Corn-Basal method.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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