Evaluation of Energy Digestibility and Prediction of Digestible and Metabolizable Energy from Chemical Composition of Different Cottonseed Meal Sources Fed to Growing Pigs

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ABSTRACT: The present experiment was conducted to determine the digestible energy (DE), metabolizable energy (ME) content, and the apparent total tract digestibility (ATTD) of energy in growing pigs fed diets containing one of ten cottonseed meals (CSM) collected from different provinces of China and to develop *in vitro* prediction equations for DE and ME content from chemical composition of the CSM samples. Twelve growing barrows with an initial body weight of 35.2±1.7 kg were allotted to two 6×6 Latin square designs, with six barrows and six periods and six diets for each. A corn-dehulled soybean meal diet was used as the basal diet, and the other ten diets were formulated with corn, dehulled soybean meal and 19.20% CSM. The DE, ME and ATTD of gross energy among different CSM sources varied largely and ranged from 1,856 to 2,730 kcal/kg dry matter (DM), 1,778 to 2,534 kcal/kg DM, and 42.08 to 60.47%, respectively. Several chemical parameters were identified to predict the DE and ME values of CSM, and the accuracy of prediction models were also tested. The best fit equations were: DE, kcal/kg DM = 670.14+31.12 CP+659.15 EE with R^2 = 0.82, RSD = 172.02, p<0.05; and ME, kcal/kg DM = 843.98+25.03 CP+673.97 EE with R^2 = 0.84, RSD = 144.79, p<0.05. These results indicate that DE, ME values and ATTD of gross energy varied substantially among different CSM sources, and that some prediction equations can be applied to predict DE and ME in CSM with an acceptable accuracy. (**Key Words:** Cottonseed Meals, DE, ME, Growing Pigs, Prediction Equations)

INTRODUCTION

Cotton is not only a major fibrous crop, but also the second most important oil and protein crop after soybean in the world. For every 100 kg of cotton fiber produced, the cotton plant also yields approximately 160 kg of cottonseed. China is the world's largest cotton and cottonseed producing country. Cottonseed meal (CSM) is a co-product of the cottonseed oil processing industry. With processing, typical yields from cottonseed are 50% meal, 22% hulls, 16% oil, 7% linters, with a 5% loss. Cottonseed meal is the third most widely traded protein ingredient after soybean meal and rapeseed meal, which represents a 7.10% of the world protein meal production, reaching 14.45 million metric tons in 2004 and 2005 (Ash and Dohlman, 2006). With the increasingly severe shortage of protein resources, cottonseed meal as a nontraditional protein feed ingredient

has drawn more and more attention from livestock producers. Although much of the cottonseed meal is utilized in ruminant diets (Brown and Pate, 1997; Cranston et al., 2006; Winterholler et al., 2009), the price of cottonseed meal compared with other high-protein feedstuffs could provide an excellent opportunity for pork producers to reduce feed costs (Tanksley, 1990). In previous studies, cottonseed meal has been used effectively at low levels in the growing pig diets (Robison, 1931; Papadopoulos et al., 1987; Fombad and Bryant, 2004). However, due to the different cotton varieties, planting environment, and cottonseed processing methods, the nutrient levels vary largely among different sources of cottonseed meals, especially with regard to crude protein and crude fiber. In addition, due to the different temperature and organic solvent used in cottonseed processing, there is also a big difference in the content of free gossypol which is the main anti-nutritional factor in cottonseed meal. All these factors seriously restrict the reasonable application of cottonseed meal in animal feed. Therefore, it is necessary to determine

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the actual nutrient content in cottonseed meal, especially for available nutrient content.

As a common protein supplement source of animal feed, there have been many reports on the protein and amino acid digestibility of cottonseed meal (Tanksley et al., 1981; Knabe et al., 1989; Batterham et al., 1990). However, limited data were found on the energy value of cottonseed meal in pigs. Only Husby and Kroening (1971) gave a relatively detailed study on the energy value of cottonseed meal for pigs. Therefore, in order to make better use of cottonseed meal raw materials and to achieve the accurate preparation of feed, it is necessary to evaluate the energy value of cottonseed meal in pigs.

At present, the evaluation of the energy value of feed ingredients is made mainly through the traditional digestion and metabolism experiment, which is time consuming, labor intensive, and expensive. Therefore, it is essential to establish a rapid and accurate method to measure the energy value of feed ingredients. Given these challenges, however, prediction equations are a useful tool in estimating the energy value of feed ingredients utilized in the livestock industry. Although some models were established for complete diets (Noblet and Perez, 1993), barley (Fairbairn et al., 1999), sweet lupins (Kim et al., 2009), wheat-DDGS (Cozannet et al., 2010), and corn co-products (Anderson et al., 2012), to our knowledge, there have been no such equations generated for predicting digestible energy (DE) and metabolizable energy (ME) values of CSM fed to growing pigs. Therefore, the objectives of this study were to determine the energy digestibility and prediction of DE and ME value based on the chemical composition of CSM fed to growing pigs.

MATERIALS AND METHODS

All procedures used in the present experiment were approved by the Animal Care and Use Committee of China Agriculture University (Beijing, China).

General procedures

Ten CSM samples were obtained from different regions located in the main cotton producing areas of China and were all produced from cottonseed by pre-press extraction in local cottonseed oil processing plants. The chemical composition of different CSM sources was showed in Table 1.

The metabolic experiment was conducted in Metabolism Laboratory of Ministry of Agriculture Feed Industry Center (China Agricultural University, Beijing, China). Pigs were housed individually in stainless steel metabolism cages (1.4×0.45×0.6 m) in an environmentally controlled room (22±2°C). Twelve growing crossbred barrows [(Yorkshire×Landrace)×Duroc] with initial body weight (35.2±1.7) kg and 90 d of age were allotted to two 6×6 Latin square designs, with six barrows and six periods and six diets for each. At the end of this experiment, the final body weight of the pigs was (74.8±2.3) kg.

All the diets used in this experiment were prepared based on the chemical composition of feed ingredients. The basal diet was formulated to meet or exceed NRC (1998) nutrient requirements for growing pigs. Ten experimental diets were formulated based on the difference method (Adeola, 2001), and contained 61.84% corn, 14.88% dehulled soybean meal and 0.08% L-lysine-HCl, which was equal to 80% of these ingredients in the basal diet, and 19.2% CSM which replaced 20% of the energy supplied by

Table 1. Analyzed composition of the ten cottonseed meal (CSM) sources (as dry matter basis)

Items[2]	CSM source[1]									
	1	2	3	4	5	6	7	8	9	10
Moisture (%)	9.18	9.30	8.97	9.17	8.43	8.98	8.68	8.83	10.67	8.53
Crude protein (%)	50.85	34.35	46.66	46.35	40.78	41.65	47.11	47.22	49.64	54.01
Ether extract (%)	0.30	0.03	0.16	0.15	0.39	0.04	0.47	0.30	0.60	0.50
NDF (%)	29.56	46.94	33.40	30.37	33.71	38.12	31.51	32.31	30.11	27.90
ADF (%)	18.04	31.24	21.78	20.13	21.47	25.24	21.51	20.60	20.22	16.49
ADL (%)	8.81	16.88	11.81	10.98	12.03	11.28	9.35	9.33	8.23	6.11
Ash (%)	6.88	7.06	6.93	6.54	6.80	6.96	6.75	6.69	6.72	6.67
Calcium (%)	0.21	0.19	0.29	0.30	0.23	0.25	0.22	0.26	0.31	0.32
TP (%)	0.42	0.36	0.45	0.46	0.71	0.96	0.97	0.98	0.96	1.03
Phy-P (%)	0.30	0.27	0.29	0.31	0.48	0.69	0.68	0.70	0.66	0.73
FG (mg/kg)	214.3	265.47	268.45	222.22	279.98	244.69	395.05	346.60	336.02	302.32
GE (kcal/kg)	4,543	4,445	4,532	4,445	4,455	4,486	4,565	4,517	4,514	4,602

[1] Source 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were collected from Shandong, Shanxi, Hubei, Hunan, Sichuan, Henan, Hebei, Xinjiang, Anhui, and Liaoning province of China, respectively.

[2] NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; TP = Total phosphorus; Phy-P = Phytate phosphorus; FG = Free gossypol; GE = Gross energy.

corn, dehulled soybean meal and L-lysine-HCl in the basal diet. The composition and nutrient levels of experimental diets were showed in Table 2 and 3.

The daily feed allowance was equivalent to 4% of body weight at the beginning of each period (Adeola, 2001). It was divided into two equal parts and fed at 08:00 and 17:00 h in mash form. Water was available *ad libitum* through a drinking nipple. The pigs were weighed individually at the beginning of each period and the amount of feed supplied each day was recorded, as well as any feed refusals. During each period, d 1 to 5 was adaptation phase and d 6 to 10 was collection phase in which feces and urine were collected totally. The collection and sample preparation of feces and urine were conducted according to the methods described by Song et al. (2003). Feces and urine samples were thawed and mixed uniformly for each pig at the end of the experiment, and representative subsamples were taken for chemical analysis. Feces samples were dried in a vacuum-freeze dryer (Tofflon Freezing Drying Systems, Minhang District, Shanghai, China), ground through a 1 mm screen, and thoroughly mixed before a subsample was collected for chemical analysis.

Chemical analysis

The ingredients used in this experiment were analyzed for dry matter (DM) (AOAC procedure 4.1.06, 2000), crude protein (CP) (AOAC procedure 990.03, 2000), Kjeldahl N (Thiex et al., 2002), ether extract (EE) (Thiex et al., 2003), ash (AOAC procedure 3.4.11, 2000), calcium (Ca) (AOAC procedure 4.8.03, 2000), total phosphorus (TP) (AOAC procedure 3.4.11, 2000), acid detergent lignin (ADL) (AOAC procedure 973.18, 2000). Phytate phosphorus was determined by colorimetric method of Wheeler and Ferrel (1971) as modified by Reddy et al. (1978). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using filter bags and fiber analyzer equipment (Fiber Analyzer, Ankom Technology, Macedon, NY, USA)

Table 2. Composition of the experimental diets (%, as-fed basis)

Items	Basal diet	Basal diet+CSM[1]
Corn	77.30	61.84
Dehulled soybean meal	18.60	14.88
CSM[1]	0.00	19.20
L-lysine-HCl	0.10	0.08
Medical stone[2]	0.90	0.90
Dicalcium phosphate	0.90	0.90
Limestone	0.90	0.90
Sodium chloride	0.30	0.30
Vitamin and mineral premix[3]	1.00	1.00
Total	100.00	100.00

[1] CSM = Cottonseed meal.
[2] Medical stone consisted of silicon oxide and aluminium oxide, purchased by YiXian BeiQiao Tou Ore Company Limited.
[3] Provided per kg of complete diet: Vit A, 6,000 IU; Vit D_3, 2,400 IU; Vit E, 21.6 IU; Vit K_3, 2 mg; Vit B_1, 0.96 mg; Vit B_2, 5.2 mg; Vit B_6, 2 mg; Vit B_{12}, 12 µg; Nicotinic acid, 22 mg; Pantothenic acid, 11.2 mg; Folic acid, 0.4 mg; Biotin, 40 µg; Choline chloride, 0.4 g; Iron, 120 mg; Copper, 140 mg; Zinc, 100 mg; Manganese, 16 mg; Iodine, 0.24 mg; Selenium, 0.4 mg; Calcium, 7.2 g; Phosphorus, 0.8 g; Sodium chloride, 4.4 g.

following a modification of the procedure of Van Soest et al. (1991). Free gossypol was determined according to the method described by Pons and Guthrie (1949). The gross energy (GE) in CSM, diets, feces, and urine samples was analyzed via adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL, USA).

Calculations

The energy lost in feces and urine was determined for each diet, and the DE and ME values of ten different CSM diets were calculated. The DE and ME in the basal diet was then divided by 0.96 to calculate the DE and ME in the energy-contributing ingredients (including corn, dehulled soybean meal and L-lysine-HCl) according to Gottlob et al. (2006). After that, the DE and ME values contributed by

Table 3. Analyzed nutrient composition of the experimental diets (%, as-fed basis)

Items[2]	Cottonseed meal source[1]										
	Basal diet	1	2	3	4	5	6	7	8	9	10
Dry matter	88.24	89.58	89.04	89.03	89.66	89.94	89.83	89.25	89.49	87.94	88.31
Crude protein	15.18	20.55	17.43	19.83	21.18	19.40	20.81	20.87	21.17	20.83	20.63
Ether extract	0.97	1.05	0.91	1.06	1.39	1.34	1.08	1.08	1.25	1.82	1.76
Crude fiber	1.89	4.12	5.42	4.77	4.35	4.83	5.32	5.29	4.86	4.52	3.64
NDF	8.01	12.10	14.13	13.86	12.56	6.34	14.30	14.52	13.96	13.91	12.54
ADF	2.31	5.88	6.96	6.66	5.77	7.48	6.98	7.06	6.46	7.43	5.85
Calcium	0.65	0.68	0.68	0.71	0.68	0.83	0.69	0.66	0.70	0.75	0.78
Phosphorus	0.41	0.55	0.50	.58	0.57	0.54	0.53	0.52	0.55	0.57	0.56
Ash	5.11	5.88	5.80	6.12	5.78	6.33	6.12	5.97	6.08	5.55	5.65
GE (kcal/kg)	3,850	3,800	3,770	3,790	3,830	3,820	3,800	3,820	3,810	3,800	3,780

[1] Source 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were collected from Shandong, Shanxi, Hubei, Hunan, Sichuan, Henan, Hebei, Xinjiang, Anhui, and Liaoning province of China, respectively.
[2] NDF = Neutral detergent fiber, ADF = Acid detergent fiber; GE = Gross energy.

each CSM sample were calculated by subtracting the DE and ME values contributed by the basal energy-contributing ingredients according to the difference procedure (Adeola, 2001). N-balance of each diet was also calculated. The apparent total tract digestibility (ATTD) of energy and N of each diet and each source of CSM were calculated by the method of Adeola (2001).

Statistical analysis

Data were analyzed statistically using the Proc GLM procedure of SAS (SAS Inst. Inc., NC, USA). Each pig was considered as an experimental unit. The data from ten CSM sources were compared by ANOVA using the MIXED procedure of SAS (Littell et al., 1998). Mean comparisons were calculated using the PDIFF option of the LSMEANS procedure. A probability of p<0.05 was accepted as statistically significant. The relationship between chemical composition and DE, ME were analyzed using the CORR and REG procedures of SAS (SAS, 1999).

RESULTS

Chemical composition of CSM sources

The chemical composition of ten CSM sources was showed in Table 1. On a dry matter basis, the concentration ranged from 34.35 to 54.01%, 0.03 to 0.60%, 29.56 to 47.90%, 16.49% to 31.24%, 6.11 to 16.88%, 5.73 to 7.06%, 0.19 to 0.32%, 0.36 to 1.03% for CP, EE, NDF, ADF, ADL, ash, Ca, and TP, respectively. The content of GE was similar among different sources, while the content of free gossypol ranged from 214.30 to 395.05 mg/kg. Small differences in nutrient levels were observed between sources 3 and 4, as well as between sources 7 and 8. The sources 4 and 8 were chosen as the validation samples to test the accuracy of prediction models, while the remaining 8 sources were used as calibration samples.

Energy and nitrogen digestibility

The amount of energy excreted from feces of the diet containing source 6 was 1,148 kcal, which was higher (p<0.0001) than that of any other CSM diets (Table 4). However, there were no significant differences in the amount of energy excreted from urine. The DE, ME and ATTD of GE were similar among the diets containing different CSM sources.

The GE intake and amount of energy excreted from urine for pigs fed basal diet were not different from those of pigs fed CSM diets. However, pigs fed CSM diets excreted more (p<0.0001) energy from the feces than that of pigs fed the basal diet. On the contrary, the DE, ME and ATTD of GE of basal diet were greater (p<0.05) than those of CSM diets.

Although the amount of N intake (p<0.0001) and N excreted from feces (p<0.05) were different among the diets containing different CSM sources, no differences in the amount of N excreted from the urine, ATTD of N, and N retention were observed. However, pigs fed basal diet had lower (p<0.0001) N excretion from feces than that of pigs fed CSM diets, and they also had a tendency to lower (p = 0.0577) N excretion from urine and greater (p = 0.0535) ATTD of N.

The DE, ME, and ATTD of GE were all different (p<0.05) among the ten CSM collected from different regions of China, which ranged from 1,856 to 2,730 kcal/kg DM, 1,778 to 2,534 kcal/kg DM, and 41.37 to 60.47%, respectively (Table 5). However, there was no difference in the ratio of ME to DE among the ten CSM sources.

Correlations and prediction equations

Fiber and ash had a negative correlation with DE and ME, while the CP and EE content were positively correlated with DE and ME (p<0.05) (Table 6). The best single predictor for DE and ME was the CP content, having the highest correlation coefficient (0.73 and 0.72 for DE and

Table 4. Daily energy and N balances for growing pigs fed basal diet and cottonseed meal (CSM) diets (as-fed basis)

Items[2]	CSM diet[1]										Basal diet	CSM (ten sources)		CSM×B[2]	
	1	2	3	4	5	6	7	8	9	10		SEM[3]	p-value	SEM[3]	p-value
GE intake (kcal)	6,336	6,270	6,415	6,506	6,376	6,423	6,502	6,482	6,791	6,639	6,400	49.68	0.4404	87.80	0.6857
GE in feces (kcal)	895[c]	1,070[ab]	1,025[abc]	1,002[abc]	874[c]	1,148[a]	1,060[ab]	973[bc]	1,134[ab]	1,114[ab]	600	16.46	<0.0001	64.43	<0.0001
GE in urine (kcal)	171	125	179	168	161	138	127	140	118	94	151	11.56	0.8470	10.19	0.6876
DE of the diet (kcal/kg)	3,262	3,125	3,185	3,238	3,298	3,117	3,201	3,240	3,162	3,145	3,486	25.72	0.8534	68.00	0.0296
ME of the diet (kcal/kg)	3,159	3,050	3,080	3,139	3,202	3,036	3,127	3,158	3,096	3,091	3,395	22.74	0.8836	65.03	0.0259
ATTD of GE (%)	85.88[ab]	82.89[cd]	84.01[bcd]	84.62[abc]	86.26[a]	82.12[d]	83.65[cd]	85.02[abc]	83.30[cd]	83.22[cd]	90.52	0.23	<0.0001	1.04	0.0005
N intake (g)	49.51[cd]	46.39[d]	53.68[bc]	57.61[ab]	51.77[c]	56.33[ab]	56.77[ab]	57.58[ab]	59.62[a]	57.98[ab]	40.42	0.69	<0.0001	1.85	<0.0001
N in feces (g)	7.27[b]	7.14[b]	7.74[ab]	7.85[ab]	7.69[ab]	8.83[a]	8.69[a]	7.91[a]	8.92[a]	8.00[ab]	4.30	0.14	0.0145	0.54	<0.0001
N in urine (g)	19.15	16.87	15.23	18.30	14.46	15.88	16.24	19.33	24.38	22.69	12.15	1.49	0.9009	1.62	0.0577
N digested (g)	42.24[cd]	39.25[d]	45.95[abc]	49.76[a]	44.08[bc]	47.51[ab]	48.07[ab]	49.68[a]	50.70[a]	49.99[a]	36.12	0.63	<0.0001	1.51	<0.0001
N retained (g)	23.09	22.38	30.72	31.46	29.62	31.63	31.83	30.35	26.32	27.30	23.97	1.42	0.7944	1.43	0.1175
ATTD of N (%)	85.30	84.52	85.59	86.37	85.11	84.31	84.60	86.26	85.04	86.20	89.18	0.24	0.4854	1.01	0.0535
N retention (%)	46.66	49.57	57.61	54.86	57.18	56.38	56.22	52.62	44.11	47.06	59.42	2.59	0.9567	3.08	0.2540

[1] Each CSM diet contained one of the ten CSM sources. Source 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were collected from Shandong, Shanxi, Hubei, Hunan, Sichuan, Henan, Hebei, Xinjiang, Anhui, and Liaoning province of China, respectively.

[2] GE = Gross energy; DE = Digestible energy; ME = Metabolizable energy; ATTD = Apparent total tract digestibility; B = Basal diet.

[3] SEM = Standard error of means. Means within the same row lacking a common superscript letter differ significantly (p<0.05).

Table 5. Energy content and ATTD of GE in ten different cottonseed meals (CSM) sources

Items[2]	CSM source[1]										SEM[3]	p-value
	1	2	3	4	5	6	7	8	9	10		
DE (kcal/kg as-fed)	2,453[a]	1,697[c]	1,973[bc]	2,255[ab]	1,990[bc]	1,690[c]	2,092[ab]	2,263[ab]	2,438[a]	2,353[ab]	44.12	<0.0001
DE (kcal/kg DM)	2,701[ab]	1,870[d]	2,167[cd]	2,482[abc]	2,173[cd]	1,856[d]	2,291[bc]	2,482[abc]	2,730[a]	2,573[abc]	49.05	<0.0001
ME (kcal/kg as-fed)	2,301[a]	1,690[b]	1,834[ab]	2,078[ab]	1,910[ab]	1,618[b]	2,063[ab]	2,102[ab]	2,254[a]	2,294[a]	47.00	0.0008
ME (kcal/kg DM)	2,534[a]	1,863[b]	2,015[ab]	2,288[ab]	2,086[ab]	1,778[b]	2,259[ab]	2,306[ab]	2,524[a]	2,509[a]	52.07	0.0007
ATTD of GE (%)	59.45[a]	42.08[c]	47.81[bc]	55.85[ab]	48.78[bc]	41.37[c]	50.18[bc]	54.96[ab]	60.47[a]	55.91[ab]	1.06	<0.0001
ME/DE (%)	93.81	99.58	92.93	92.39	97.05	95.24	98.61	93.07	92.13	97.26	1.23	0.8613

[1] Source 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were collected from Shandong, Shanxi, Hubei, Hunan, Sichuan, Henan, Hebei, Xinjiang, Anhui, and Liaoning province of China, respectively.

[2] GE = Gross energy; DE = Digestible energy; ME = Metabolizable energy; ATTD = Apparent total tract digestibility; ME/DE = The ratio of ME to DE.

[3] SEM = Standard error of means. Means within the same row lacking a common superscript letter differ significantly (p<0.05).

ME, respectively). Dietary fiber was also a good predictor for DE and ME. Dietary fiber was estimated from different analytical procedures, so NDF, ADF and ADL were tested separately in the regression analysis (Table 7). Among the three fiber fractions, NDF had the highest accuracy to predict DE and ME. Meanwhile, DE and ME could also be predicted based on the EE content. The accuracy of the equations was improved with the inclusion of both the CP and EE factors. The best fit equations were obtained for DE and ME, which were: DE, kcal/kg DM = 670.14+31.12 CP +659.15 EE with $R^2 = 0.82$, RSD = 172.02, p<0.05; and ME, kcal/kg DM = 843.98+25.03 CP+673.97 EE with $R^2 = 0.84$, RSD = 144.79, p<0.05. The suitability of these two prediction models was tested by validation samples, which consisted of source 4 and 8 (Table 8). The maximum absolute difference and relative deviation between *in vivo* DE determinations and predicted DE values was 271 kcal/kg and 5.77%, respectively, while these two values for ME were 183 kcal/kg and 4.17%, respectively.

DISCUSSION

Chemical composition of CSM sources

The chemical composition of CSM sources was quite variable for all criteria in the current study. One reason may be that the soil and environment conditions and the cultivars of cotton plants were different in these regions of China, which led to a large variation in the quality of cottonseed, and when the cottonseed meals were produced from these cottonseeds by proper processing, differences in their quality may be observed. Although the same pre-press extraction method was used, there may be some differences in the treatment temperature and solvent used during the production of cottonseed oil among the cottonseed meal processing plants, also leading to the variation in the quality of cottonseed meals (Lea and Hannan, 1949; Anderson-Hafermann et al., 1993).

The NDF, ADF, ADL content varied largely among the CSM sources. It can be partly explained by the different levels of hulls added to the final cottonseed meal products (Stein et al., 2006). The content of CP was relatively high while the contents of cell wall fractions were low, agreeing with previous reports (Tanksley et al., 1981; Batterham et al., 1990). The concents of CP in sources 1, 9, and 10 (50.85, 49.64, and 54.01%, respectively) were a little higher than previously published values (Tanksley et al., 1981; Batterham et al., 1990; NRC, 1998; Prawirodigdo et al., 1998; Li et al., 1999). The contents of ether extract in all of the CSM were much lower than excepted values (Tanksley et al., 1981; NRC, 1998), which indicates that more

Table 6. Correlation coefficients between chemical composition and DE and ME of calibration cottonseed meal (CSM) samples[1]

Item	CP	EE	NDF	ADF	ADL	Ash	GE	DE	ME
EE	0.69								
NDF	- 0.93	- 0.79							
ADF	- 0.93	- 0.74	0.98						
ADL	- 0.95	- 0.76	0.94	0.92					
Ash	- 0.76	- 0.95	0.85	0.81	0.86				
GE	0.90	0.54	- 0.77	- 0.77	- 0.83	- 0.66			
DE	0.86	0.81	- 0.83	- 0.83	- 0.80	- 0.73	0.63		
ME	0.85	0.84	- 0.82	- 0.82	- 0.81	- 0.77	0.68	0.98	
FG	0.21	0.64	- 0.28	- 0.15	- 0.30	- 0.61	0.35	0.20	0.27

[1] CP = Crude protein, EE = Ether extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, GE = Gross energy, DE = Digestible energy, ME = Metabolizable energy, FG = Free gossypol; Correlation whose absolute value is above 0.69 is different from zero (p<0.05).

Table 7. Prediction equations of digestible and metabolizable energy (kcal/kg DM) from chemical composition of calibration cottonseed meal samples (CSM) (%, as dry matter basis)[1]

No.	Equation	R^2	RSD^2	p-value
1	DE = 171.64+46.54 CP	0.73	192.46	0.01
2	DE = 1,893.05+1,291.80 EE	0.65	220.54	0.02
3	DE = 3,890.43-47.05 NDF	0.70	205.61	0.01
4	DE = 3,679.86-62.95 ADF	0.69	207.71	0.01
5	DE = 3,194.74-85.17 ADL	0.64	223.45	0.02
6	DE = 670.14+31.12 CP+659.15 EE	0.82	172.02	0.01
7	DE = 1,303.01+32.95 CP-15.09 NDF	0.74	207.20	0.03
8	DE = 1,142.27+34.18 CP-18.49 ADF	0.74	207.73	0.03
9	DE = 3,861.78+38.79 CP-487.39 Ash	0.75	204.69	0.03
10	DE = 3,102.28+628.83 EE-29.58 NDF	0.75	202.49	0.03
11	DE = 2,944.90+682.11 EE-39.19 ADF	0.77	194.60	0.02
12	DE = 2,560.99+750.67 EE-47.29 ADL	0.73	210.68	0.04
13	ME = 334.27+40.80 CP	0.72	174.40	0.01
14	ME = 1,827.81+1,182.93 EE	0.70	181.63	0.01
15	ME = 3,579.52-40.80 NDF	0.67	189.85	0.01
16	ME = 3,405.92-55.00 ADF	0.67	188.70	0.01
17	ME = 2,998.56-75.98 ADL	0.65	194.66	0.02
18	ME = 843.98+25.03 CP+673.97 EE	0.84	144.79	0.01
19	ME = 1,062.02+32.06 CP-9.70 NDF	0.73	189.39	0.04
20	ME = 1,109.61+30.93 CP-14.77 ADF	0.73	188.87	0.04
21	ME = 5,460.12+30.04 CP-677.01 Ash	0.76	177.71	0.03
22	ME = 2,687.90+711.39 EE-21.04 NDF	0.76	175.51	0.03
23	ME = 2,944.90+682.11 EE-39.19 ADF	0.79	165.78	0.02
24	ME = 2,372.24+741.87 EE-38.55 ADL	0.77	174.07	0.03

[1] CP = Crude protein, EE = Ether extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, DE = Digestible energy, ME = Metabolizable energy.

[2] RSD which is the root mean square of the error that applies to the whole model (Cozannet et al., 2010).

effective solvent had been used to extract the oil from cottonseed more entirely, and, as a result, the GE of the CSM were lower than the values obtained by Batterham et al. (1990). With a high content of Phy-P, the availability of the P in these CSM was low, in accordance with Eeckhout and Paepe (1994) and NRC. (1998). With the proper treatment temperature and solvent used during the processing of CSM, the free gossypol contents of the CSM sources were relatively low, ranging from 214.30 to 395.05 mg/kg. The ethanol vapor used as a solvent could reduce free gossypol to a safe level in CSM for feeding as a general animal feed protein source (Hron et al., 1996). Cooking temperature could aslo influence the free gossypol content

in CSM. Reduction in free gossypol during cooking was due to binding with other components while that occurring during pre-pressing and solvent extraction resulted mainly from removal of gossypol in the pre-pressed and solvent-extracted oil (Pons et al., 1955).

Energy and nitrogen digestibility

It has been suggested that the presence of fiber could increase the endogenous secretions of CP and fat associated with the increasing microbial mass, as a result, the digestion of CP and fat was reduced (Sauer and Ozimek, 1986; Noblet and Perez, 1993). With greater fiber contents in the CSM diets compared with the basal diet, more energy

Table 8. Comparison of DE and ME contents in validation cottonseed meal (CSM) samples determined by using *in vivo* method and prediction models (as dry matter basis)[1]

CSM source[2]	Determined DE (kcal/kg)	DE = 670.14+31.12 CP+659.15 EE			Determined ME (kcal/kg)	ME = 843.98+25.03 CP+673.97 EE		
		Predicted DE (kcal/kg)	Difference, (kcal/kg)	Relative deviation (%)		Predicted DE (kcal/kg)	Difference (kcal/kg)	Relative deviation (%)
4	2,482	2,211	271	5.77	2,288	2,105	183	4.17
8	2,482	2,337	145	3.01	2,306	2,228	78	1.72

[1] CP = Crude protein, EE = Ether extract, DE = Digestible energy, ME = Metabolizable energy.

[2] Source 4 and 8 were collected from Hunan and Xinjiang province of China, respectively.

excreted from feces was expected, thus making the energy values greater in the basal diet than that of CSM diets (Ren et al., 2011). It was also reported that energy digestibility was negatively affected by the dietary fiber content, especially for NDF (Noblet and Perez, 1993), which may be the reason that the basal diet had greater energy digestibility than CSM diets. A similar situation was also observed among the diets containing different CSM sources.

The results of current experiment indicate that the DE and ME content in the ten CSM sources ranges from 1,856 to 2,730 kcal/kg DM and 1,778 to 2,534 kcal/kg DM, respectively. The average DE and ME contents (2,332 and 2,198 kcal/kg DM, respectively) for the ten CSM sources are lower than the values recommended by NRC (1998), the reason for this may be the extremely low EE levels in the CSM sources used in this experiment (Noblet and Perez, 1993; Ren et al., 2011). Meanwhile, because of the differences in CP and cell well fractions levels, the DE and ME contents were also different among the ten CSM sources (Noblet and Perez, 1993).

The ME to DE ratios varied from 92.13 to 99.58% among the ten CSM sources. The average ME to DE ratio (95.21%) is similar with the previously published value of 96% (Noblet and Perez, 1993; Fairbairn et al., 1999). Thus, because it is difficult to collect the urine entirely and determine the energy value of urine accurately in the digestion-metabolism experiment, we suggest that the ME value can be replaced by the DE value in the energy system of pigs to some extent.

Correlations and prediction equations

The results of this study indicated that the variability of chemical composition in CSM collected from different regions of China could contribute to the difference of DE and ME content. A number of prediction equations for DE and ME contents of feed ingredients and diets have been successfully developed (Just et al., 1984; Noblet and Perez, 1993; Fairbairn et al., 1999; Kim et al., 2009; Olukosi and Adeola, 2009; Cozannet et al., 2010; Anderson et al., 2012). However, the models obtained from one hundred and fourteen diets (Noblet and Perez, 1993) are unsuitable when applied to a certain ingredient. The best equations for DE and ME in barley (Fairbairn et al., 1999) and wheat-DDGS (Cozannet et al., 2010) contained fiber component, while the prediction equations for ME in meat and bone meal used other factors, such as GE, CP, P, Ca, and ash (Olukosi and Adeola, 2009). Therefore, the prediction equations for estimating DE and ME contents in CSM fed to growing pigs should be established based on the measured data of CSM.

With the correlation and regression analysis, the prediction models were mainly established according to the significant linear relationships between DE, ME and the chemical composition of CSM (Table 7). The results in Table 6 clearly showed that CP and EE content were positively correlated with DE and ME, while ash and fiber content had a negative effect. In this study, CP was the most significant factor affecting the DE and ME variation. It was likely because CSM was usually used as a protein source in swine feed with a high protein level and the negative correlation between CP and fiber was also very high as shown in Table 6. As a fraction of dietary fiber, NDF was also significant factor affecting the DE and ME variation. Simple analysis suggests that for every 1% increase in NDF content, the DE content would decrease by 1.21%, slightly higher than that of Noblet and Le Goff (2001). ADF also displayed a significantly negative correlation with DE and ME. The reason for this may be that the insoluble fiber such as NDF and ADF are hardly digested by pigs, and thus, lower the energy content by replacing the digestible nutrients. The amount of CP and EE are negatively affected by the fiber content in this study, which agrees with Noblet and Perez (1993) and Noblet and Le Goff (2001).

The results of the current study demonstrate that it is possible to estimate the DE and ME of CSM samples from their chemical composition. Considering the cost and repeatability of the required chemical analysis, Equations 6 and 18 (Table 7) are chosen as the best two equations with an acceptable accuracy when estimating DE and ME content of CSM. However, in order to improve the accuracy of the prediction models, more representative CSM sources should be used in further study.

IMPLICATIONS

An improved understanding of the energy variation and the ability to estimate energy content in individual feed ingredients would make the formulation of swine more precise. This study indicated that DE and ME contents of cottonseed meal could be predicted based on several chemical characteristics with an acceptable accuracy. A practical method for accurately estimating the energy values in cottonseed meal also would reduce feed costs and decrease amount of wasted nutrients.

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