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The TMEn, Proximate Analysis, Amino Acid Content and Amino Acid Digestibility of Glandless and Commercial Cottonseed Meal for Broilers

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Abstract: Cotton seed meal (CSM) is an alternative ingredient in poultry diets but its use is limited due to the presence of gossypol and the potential effects of gossypol on digestibility of nutrients. Glandless cottonseed is available and contains very low gossypol but there has been a limited amount of poultry nutritional studies completed with glandless cottonseed meal (GCSM). The TMEn, proximate analysis, amino acid content and amino acid (AA) digestibility of a glandless (GCSM) and a commercial (CCSM) cottonseed meal were determined with broilers. Thirty 42-day old Cobb 500 male broilers were precision-fed 30g of CCSM, GCSM and glucose and excreta collected during a 48 h period. Glucose was fed to serve as a control (no nitrogen or AA content). The chemical composition, gossypol content, True metabolizable energy (TMEn) and digestibility coefficients for AA were calculated for both meals. The crude protein and fat content of GCSM was higher than the CCSM (54 and 51%, 6 and 2%, respectively). Both meals were similar in calcium, total phosphorus and phytic acid contents. The CCSM had a higher content of total and free gossypol (1.52 and 0.161%, respectively) when compared to GCSM (0.02 and .003%, respectively). The TMEn for the GCSM provided approximately one thousand kcal more per energy/ kg than the CCSM. The essential AA content (g/kg; 90% DM) was determined for both cottonseed meals and was generally higher for GCSM compared to CCSM but both types of CSM contained higher levels of key essential AA than reported values for AA in the literature. The most extreme differences were for methionine and cystine; % methionine content was approximately 2 fold higher than values in the literature and the % cystine was 74 to 93% higher. The true digestibility coefficients for essential AA ranged from the low of 73.9% for isoleucine to 91.8% for arginine, for CCSM; the amino acid digestibility coefficients for GCSM were all higher than 90% for the essential AAs.

Key words: Cottonseed meal, amino acid digestibility, TMEn, gossypol, broilers

INTRODUCTION

Higher prices for corn and soybean meal (SBM) have forced the poultry industry into evaluating alternative ingredients with economic benefits. Alternative feed ingredients may contain lower digestible amino acids, more fiber and less metabolizable energy (ME). For the nutritionist to be able to utilize alternative ingredients such as cottonseed meal (CSM), the ME and digestible amino acids must be determined in order to formulate diets on a digestible nutrient basis for maximum performance. The use of digestible AA for feed formulation has been recognized as being preferable to formulating diets on a total AA basis (Rostagno *et al*., 1995).

Cottonseed meal is a byproduct of the oil extraction from cotton seeds. CSM protein contains deficiencies of methionine, lysine, threonine and valine compared to SBM. The digestibility of CSM is said to be low due to increased amounts of cell walls and the presence of gossypol (Nagalakshmi *et al*., 2007). The presence of gossypol in CSM and low levels of lysine limit the dietary use of this ingredient in poultry diets. Gossypol is a metabolite present in the plant and seeds and has been shown to cause reduced performance and increased mortality in chickens (Henry *et al*., 2001). The inclusion levels of CSM utilized by the poultry industry are dependant of the amount of gossypol in the meal. Gossypol binds iron in the diet, in the bloodstream and in the egg yolks causing iron deficiencies (Watkins *et al*., 1993).

Several techniques have been developed to reduce the gossypol in the meal (solvent extractions, expander-solvent extractions, addition of iron, heat, extrusion). Additionally, glandless varieties of cotton plants have been developed to reduce the concentration of gossypol in the seed. The objective of this research was to determine the TMEn, proximate analysis, amino acid content and amino acid digestibility of a glandless and a commercial variety of cottonseed meal for broilers.

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MATERIALS AND METHODS

Stock and management: Thirty 42-day old Cobb 500 male broilers were individually housed in battery cages with individual feeders and nipple drinkers. The 42 d old broilers where reared in floor pens and fed *ad libitum* during the grow-out period prior to being utilized for digestibility studies. The thirty broilers utilized for the digestibility study were randomly selected and assigned to one of the three different feedstuffs to be tested (glandless CSM, commercial CSM and glucose). The glandless cotton cultivar utilized is known as "Acala GLS". The glanded meal was produced from upland cotton cultivated in Texas from the United States. Both meals were produced with the expander/solvent extraction process, in which the solvent utilized was hexane.

True amino acid and metabolizable energy bioassay: Ten broilers were each precision-fed 30g of commercial CSM, glandless CSM or glucose (Sibbald, 1976). Glucose was fed to broilers to serve as a control because it contains no nitrogen or amino acids and it minimizes endogenous loss. The excreta energy and amino acids from the broilers fed glucose are subtracted from excreta energy and amino acids from test broilers fed the GCSM and CCSM to standardize digestibility coefficients and determine true metabolizable energy (TME) values.

The broilers were fasted for 48 hours prior to the precision feeding of glucose and test samples. Excreta were quantitatively collected for a 48-hour period utilizing a tray placed under each cage. Excreta samples were freeze dried, weighed and ground for further analysis. Collection of feathers and scales were avoided during the excreta collection period.

The proximate analysis of the CSMs was determined according to AOAC approved methods (1990). The energy content of the feedstuffs and excreta was determined using a Parr adiabatic calorimeter by the ANSI/ASTM Method D2015-77 (1978) and the true metabolizable energy was corrected by nitrogen excretion. The nitrogen in the excreta and feed ingredients was measured by AOAC Method 990.03 (1995). The standard amino acids of feed and excreta were determined by AOAC method 982.30a, sulfur amino acids were determined by AOAC method 985.28 and tryptophan was determined by AOAC method 988.15 (1990). The cottonseed meals were also submitted to ARS for determination of total gossypol and free gossypol content [by HPLC and AOCS (1989) method Ba 7-58, respectively]. The phytic acid analysis was performed using the modified method described by Ellis *et al.* (1977).

RESULTS AND DISCUSSION

The proximate analysis for both CSM is shown in Table 1. The protein content of both meals is similar and

Table 1: Chemical composition, total and free gossypol content, DM digestibility and TMEn of glandless and commercial cottonseed meal (on 90% dry matter basis)

	Glandless CSM	Commercial CSM
Crude protein (%)*	53.67	50.67
Fat (%)*	5.99	1.94
Crude Fiber (%)*	13.38	12.88
Ash (%)*	9.06	9.46
Total Phosphorus (%)*	1.79	1.71
Phytate Phosphorus (%)**	1.29	1.30
Phytic acid (%)***	4.57	4.60
Calcium (%)*	0.29	0.24
Gross energy, kcal/kg*	5119	4961
Total Gossypol (%) §	0.02	1.52
(+)-gossypol enantiomer (%) §	54.8	60.0
Free Gossypol % §	0.003	0.161
True DM digestibility % †	97.37[a]	77.27[b]
TME Kcal/kg †	3983[a]	2971[b]
TMEn kcal/kg †	3975[a]	2963[b]

*Analysis performed at the Central Analytical Laboratory of Poultry Science. University of Arkansas. Center of Excellence for Poultry Science, average of 2 samples.

**Calculated assuming a P content of 282 g/kg in phytic acid molecule (Cosgrove, 1966).

***Analysis performed by Eurofins Scientific Inc. Method Reference: Phytic Acid - analog Biochem. 77: 536-539 (1977) (mod.)

§Analysis Performed by the Agricultural Research Service, United States Department of Agriculture, average of 2 samples.

†Calculations based in 10 repetitions for the glandless CSM and 9 repetitions for the commercial CSM.

Means in the same row that do not share a common superscript letter differ significantly (P<0.05).

higher than protein levels reported in the literature, where the values range from 40.9 to 44.7% (Vargas and Lobo, 1992; National Research Council, 1994; LaRue *et al.*, 1985; Cheng *et al.*, 2002). Calcium, total phosphorus, phytate phosphorus and phytic acid contents for both meals were very similar and slightly higher than previous reports. In the literature, the levels for calcium range from 0.10 to 0.22%, for total phosphorus from 1.02 to 1.79%, for phytate phosphorus from 0.54 to 1.02% and for phytic acid from 1.92 to 3.30% (Nwokolo and Bragg, 1977; LaRue *et al.*, 1985; Vargas and Lobo, 1992; National Research Council, 1994; Ravindran *et al.*, 1999b, Selle *et al.*, 2000, Selle and Ravindran, 2007).

Levels of total and free gossypol were measured in both cottonseed meals (Table 1). As expected, the commercial CSM had a higher content of total and free gossypol. The level of total gossypol (1.52% for the commercial CSM) is within the range of 0.39-1.7% total gossypol reported for cotton varieties (Abou-Donia, 1976; Berardi and Goldblatt, 1980; Thacker and Kirkwood, 1990). The low levels of total gossypol for the glandless CSM (0.02%) are also in agreement with reports in the literature (LaRue *et al.*, 1985).

Gossypol is present in CSM as two enantiomers with different optical properties which are called (+) or (-) enantiomers. Enantiomer ratios for CSM vary from 50 to 60 % (+)-gossypol enantiomer in commercial CSM (Bailey *et al.*, 2000). That is in agreement with the results found in this experiment, where the proportion of (+) - gossypol enantiomers were 54.8 and 60% for the glandless and the commercial CSM, respectively (Table 1). The (-) form of gossypol has been reported to be more toxic than the (+) form, causing lower body weights and higher response from the immune system (Blackstaffe *et al.*, 1997; Joseph *et al.*, 1986; Lordelo *et al.*, 2005; Wang *et al.*, 1987).

The dry matter digestibility and TMEn (Table 1) were also determined for both cottonseed meals. The dry matter digestibility of GCSM was significantly higher than the digestibility of CCSM. The GCSM provided approximately one thousand more kcal of TMEn/kg meal than the CCSM which was equal to a 27% difference (P = 0.0356). This can be partially explained by the higher fat content in the GCSM, however the gross energy (GE) was only 200 kcal higher. In a review made by Lusas and Jividen (1987), the authors report that the ME of solvent extracted glandless CSM is approximately 20% higher than that of glanded prepress solvent extracted meal. Reports found in the literature for ME values range from 1857 to 2600 kcal/kg for the commercial CSM (Dale and Fuller, 1987; Fernandez *et al.*, 1995; National Research Councol, 1994; Sibbald, 1986; Thacker and Kirkwood, 1990) which are in disagreement with our higher TMEn values for the commercial CSM (2963 kcal ME/g).

The amino acid content found for the two cottonseed meals is shown in Table 2. The amino acid content of the commercial CSM examined was higher than reported values in the literature. The amino acids were 15 to 60% higher than reported values depending on the amino acid. The most extreme difference was for methionine and cystine. The % methionine content of CCSM was approximately 2 fold higher than the values reported in the literature and the % cystine was 74 to 93% higher (Cheng and Hardy, 2002; Garcia *et al.*, 2007; National Research Council, 1994; Ravindran *et al.*, 1999a, b).

The digestibility coefficients obtained for the two cottonseed meals are shown in Table 3. The AA digestibility determined herein is corrected for endogenous AA found in the excreta. The significance of the determination of these losses is more important when the ingestion of amino acids from the test feeds is low, for example in precision feeding bioassays (Lemme *et al.*, 2004). In this study, the crude protein and amino acid content of CSM is relatively high; but the total dry matter ingested in the precision feeding bioassay per bird was low so the effect of standardizing the digestibility coefficients was important. The true digestibility coefficients for essential AA ranged from a low of 73.9% for isoleucine to a high of 91.8% for arginine for commercial CSM. Chung and Baker (1992) also found that the true digestibility of isoleucine in CSM was the lowest of all the indispensable amino acids.

Table 2: Amino acid content for glandless and commercial cottonseed meal (on 90% Dry Matter basis) †

	Total AA g/kg (CSM)	
	Glandless	Commercial
Essential AA		
Arginine	69.21	61.38
Histidine	13.86	13.05
Isoleucine	16.20	15.39
Leucine	28.80	28.17
Lysine	21.15	21.87
Methionine	10.26	10.62
Phenylalanine	27.09	25.20
Threonine	14.67	15.30
Tryptophan	5.76	4.68
Valine	22.95	21.15
Non-essential		
Alanine	18.63	18.54
Aspartic Acid	45.45	43.65
Cystine	10.26	9.72
Glutamic Acid	94.50	88.20
Glycine	20.16	20.07
Serine	18.90	19.26
Tyrosine	12.78	11.97

† Calculations based on 2 samples of each CSM.

Table 3: Digestibility coefficients of amino acids in glandless and commercial cottonseed meal for broilers (on 90% Dry Matter basis) †

	Digestibility coefficients % (CSM)	
	Glandless	Commercial
Essential AA		
Arginine	97.8[a]	91.8[b]
Histidine	94.1[a]	83.0[b]
Isoleucine	94.1[a]	73.9[b]
Leucine	95.0[a]	76.9[b]
Lysine	92.5[a]	64.6[b]
Methionine	94.9[a]	76.6[b]
Phenylalanine	96.5[a]	84.7[b]
Threonine	93.2[a]	74.9[b]
Tryptophan	96.8[a]	80.9[b]
Valine	94.3[a]	76.1[b]
Non-essential AA		
Alanine	91.4[a]	71.3[b]
Aspartic Acid	94.8[a]	82.1[b]
Cystine	92.5[a]	76.5[b]
Glutamic Acid	96.4[a]	88.0[b]
Glycine	61.8[a]	41.4[b]
Serine	95.9[a]	82.9[b]
Tyrosine	96.9[a]	80.6[b]

† Calculations based in 10 repetitions for the glandless CSM and 9 repetitions for the commercial CSM.

The true digestibility coefficients obtained in this trial for the CCSM are very similar when compared with the literature but in some cases are higher (Garcia *et al.*, 2007; Ravindran *et al.*, 1999a).

There is little information in the literature about the content and digestibility of the amino acids in GCSM. In this trial, the amino acid content and digestibility coefficients were significantly higher for the GCSM when compared to the CCSM. The digestibility coefficients for

GCSM were all higher than 90% for essential amino acids. The differences in amino acid digestibility coefficients can be attributed to the presence of gossypol in the CCSM. Gossypol forms indigestible protein complexes that make them unavailable for the birds to digest and absorb (Thacker and Kirkwood, 1990).

Conclusions: The CCSM had a higher content of total and free gossypol when compared to GCSM. The TMEn for the glandless CSM was 27% higher than the TMEn for commercial CSM. The essential amino acid content (g/kg, 90%DM) was higher for GCSM compared to the CCSM. The % methionine and cystine contents of CCSM were higher than previous reported values. The true digestibility coefficients for essential amino acids ranged from a low of 73.9% for isoleucine to a high of 91.8% for arginine, for CCSM. The amino acid digestibility coefficients for GCSM were all higher than 90% for essential amino acids.

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