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Quantitative Measurement of Negligible Trypsin Inhibitor Activity and Nutrient Analysis of Guar Meal Fractions

JASON T. LEE, STACEY CONNOR-APPLETON, AKRAM U. HAQ, CHRISTOPHER A. BAILEY, AND AUBREY L. CARTWRIGHT*

Poultry Science Department, Texas A&M University, College Station, Texas 77843-2472

A complete nutrient characterization of three possible products of guar bean processing does not apparently exist in the literature. Guar meal is a high-protein byproduct produced during extraction of galactomannan gum from the guar bean. During the extraction process, two fractions are produced (germ and hull). Germ and hull fractions are usually combined to form the marketed product, guar meal. Analyses characterized the nutrient, trypsin inhibitor, and galactomannan gum content of the three guar meal byproducts to determine which fraction is more valuable as an ingredient in poultry diets. Analyses indicated that the germ fraction is most appropriate for inclusion in poultry diets. Trypsin inhibitor activity previously reported as an antigrowth factor associated with guar meal was negligible and not considered to be a significant factor limiting its use in poultry feeds.

KEYWORDS: Guar meal; trypsin inhibitor; galactomannan; nutrient analysis; poultry

INTRODUCTION

Extraction of galactomannan gum from guar beans yields both germ and hull residues, which are usually recombined to form guar meal. Although this byproduct meal contains protein concentrations that range from 37 to 47% (*1*, *2*), early research with guar meal revealed deleterious effects on growth, feed consumption, and feed conversion in poultry (*1*, *3*). These effects were attributed to toxic factors such as trypsin inhibitor, saponins, and residual gum (*4*−*10*).

Growth inhibition due to increasing guar meal concentrations in broiler chicken diets first was attributed to the presence of a trypsin inhibitor (*11*). These authors indicated that heating guar meal for 60 min reduced trypsin inhibitor activity by 80%, although they did not directly assay the inhibitor itself. Couch et al. (*12*) specified that heating guar meal to 110 °C for 1 h with 15 min of superheated steam injection after the cooker reached 110 °C alleviated most deleterious effects caused by the guar meal trypsin inhibitor. However, most researchers attribute the major antinutritive effects of guar meal to residual guar gum rather than trypsin inhibitor (*4*, *5*, *7*, *9*, *10*, *13*). Burnett (*6*) attributed these negative effects to increased viscosity of digesta, which interferes with absorption, thereby decreasing growth and efficiency. More recently, a correlation between excessive viscosity of intestinal contents and growth depression was demonstrated in chickens (*14*). Intestinal viscosity was directly proportional to guar gum concentration of the feed. More definitively, these growth depression and intestinal viscosity effects were ameliorated by treatment of guar gum containing feed with a gum-digesting β-mannanase (*15*).

During guar meal manufacture, the germ fraction first is separated by a set of 14-mesh sieves after seed attrition. Thereafter, passing through a 93.3−105 °C rotary furnace, the hull fraction is separated from the galactomannan gum containing endosperm by 18-mesh sieves. Current practice combines the germ and hull fractions at an approximate ratio of 25% germ to 75% hull to yield commercial guar meal.

Although limited data exist regarding the nutrient composition for guar meal (*10*, *16*), no published data exist on the germ and hull fractions that comprise the meal. Because guar germ and hull byproducts with potentially different compositions are separated fairly easily during gum production, separation for complete nutrient analysis is accomplished easily.

This research defines the basic nutrient compositions of germ, hull, and combined guar byproduct fractions. Amino acid composition, trypsin inhibitor, protein solubility, protein dispersibility, and residual galactomannan gum also were determined.

MATERIALS AND METHODS

Proximate Analysis. Compositions of germ, hull, and combined guar meal fractions (Rhodia Inc., Vernon, TX) were determined by proximate analysis. Random samples from each meal were gathered, combined, mixed, subsampled, and then divided into an appropriate number of sample replicates for each assay. Crude protein was determined according to American Oil Chemists' Society (*17*) method Ba 4d-90 for Kjeldahl nitrogen and method Ba 4e-93 for combustion nitrogen using a LECO (LECO FP-2000 nitrogen analyzer model 602-600-400, St. Joseph, MI) analyzer. Each sample was analyzed using ∼0.5 g samples in triplicate for Kjeldahl nitrogen determinations. Five replications of ∼0.2 g samples of each meal were analyzed using the LECO analyzer.

Ash and moisture values were determined for triplicate samples according to modified methods Ba 5a-49 and Ba 2a-38, respectively

* Author to whom correspondence should be addressed [telephone (979) 845-4319; fax (979) 845-1921; e-mail a-cartwright@tamu.edu].

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(*17*). The ashing procedure was modified in that samples were heated for 24 h at 800 °C. For moisture determination samples were heated at 130 °C for 2 h before cooling to room temperature in a vacuum desiccator. Ash and moisture were determined by weight difference. Ether extract was determined in triplicate according to procedure Ba 3-38 (*17*). Crude fiber was determined by the Office of the Texas State Chemist (College Station, TX). Heat of combustion was also determined by adiabatic bomb calorimetry (LECO FP-2000 nitrogen analyzer model 602-600-400).

Protein Solubility and Dispersibility Assays. Protein solubility and dispersibility were determined on each of the three fractions of guar meal. Although these assays were developed for soybean meal, protein solubility and chick growth are directly correlated (*18*). Each fraction was ground through a 0.8 mm screen (Tecator grinder 3260, 1093 sample mill, Hoganas, Sweden). The protein solubility assay was performed according to method Ba 11-65 (*17*). Triplicate 1.0 g samples were stirred with 50 mL of 42 mM KOH for 20 min. Subsequently, samples were centrifuged (2150*g*) at 4 °C for 20 min. Crude protein of the supernatant was determined according to two methods. Supernatant (15 mL) was pipetted into a Kjeldahl flask for protein determination according to method Ba 4d-90 (*17*). A second protein solubility assay was conducted using the same method except that 2 mL of supernatant was analyzed for crude protein content according to method Ba 4e-93 using a LECO analyzer (*17*). Protein dispersibility was determined in triplicate according to method Ba 10-65 (*17*). The protein dispersibility assay utilized a Hamilton Beach Drinkmaster blender that was modified to accommodate a Waring blender cup. The blender was standardized with 300 mL of water at 8500 rpm with a tachometer before each use. The same setting was utilized for each sample. Samples (20 g) were weighed and blended with exactly 300 mL of water for 10 min. The slurry was poured into centrifuge tubes and centrifuged (1750*g* at 24 °C) for 10 min. The upper layer of supernatant was poured into a beaker and allowed to settle for 1 h. Then the upper layer from the beaker was poured into centrifuge tubes and centrifuged (2000*g*) at 24 °C for 15 min. Crude protein of the supernatant was determined as described previously.

Amino Acid Composition. Amino acid analysis was conducted on triplicate 1.0 g samples of freeze-dried test meals at the Texas A&M Protein Chemistry Laboratory. Amino acid analysis consisted of standard liquid-phase HCl−phenol hydrolysis (110 °C for 24 h). Phenol was added to limit halogenation of tyrosine residues. Norvaline (5 nmol) was added as the internal standard for primary amino acids, and sarcosine (5 nmol) was added as the internal standard for secondary amino acids. This technique included automated precolumn derivatization of the hydrolyzed primary amino acids with *o*-pthalaldehyde and secondary amino acids with 9-fluoromethyl-chloroformate. Derivatized amino acids were separated by reverse phase high-pressure liquid chromatography (HPLC) and detected by a photodiode array detector at 338 nm. Amino acids were identified on the basis of retention time using a set of known standards. Cysteine, cystine, and tryptophan were destroyed by hydrolysis and therefore were not quantified in this assay. Amino acids were also independently determined by DeGussa Huls Corp (Applied Technology Chemical Group, Allendale, NJ).

Trypsin Inhibitor Assay. Trypsin inhibitor activity was measured using method Ba 12-75 (*17*) with the following modifications. After the 3 h mixing period in NaOH, solutions were centrifuged (2000*g*) at 22 °C for 10 min before aliquots were taken for dilution; each sample was activated by benzoyl-DL-arginine-*p*-nitroanalide hydrochloride, and trypsin solutions remained in the water bath for 15 min instead of 10 min. Samples were filtered with 0.2 μm membrane filters instead of Whatman no. 2 or 3 filters.

The assay was performed on duplicate samples of each of the three guar meal fractions, raw guar beans, raw guar splits (the endosperm portion that contains the guar gum), dehulled soybean meal, and defatted soybeans. Each meal sample was ground through a 0.8 mm screen in the Cyclotech grinder. Each fraction was diluted with distilled water to achieve the concentrations specified by method Ba 12-75 (*17*). Guar meal samples were diluted 2 parts deionized water to 1 part guar meal supernatant (v/v). Dehulled soybean meal was diluted 3 parts deionized water to 1 part soybean meal supernatant (v/v). Defatted raw soybeans were diluted 20 parts deionized water to 1 part soybean supernatant (v/v).

Table 1. Nutrient Characterization of Germ, Hull, and Combined Guar Meal Fractions

	germ[a]	hull[a]	combined[a]
crude protein,[b] %	45.9 ± 0.6a	36.9 ± 0.8b	38.5 ± 1.0b
crude protein,[c] %	45.5 ± 0.5a	36.3 ± 0.3b	38.3 ± 1.3b
dry matter,[d] %	93.18 ± 0.07c	95.41 ± 0.09a	94.78 ± 0.15b
ash, %	4.64 ± 0.06	4.62 ± 0.13	4.51 ± 0.31
ether extract, %	5.83 ± 0.19a	2.79 ± 0.18c	4.48 ± 0.30b
crude fiber,[e] %	8.4	13.5	11.4
gum, %	7.42 ± 1.50b	13.08 ± 0.21b	11.55 ± 0.21b
gross energy, kcal/kg	4912 ± 12a	4629 ± 38b	4641 ± 17b
protein solubility, %	72.32 ± 1.98b	67.30 ± 0.64c	79.49 ± 3.17a
protein dispersibility, %	19.79 ± 0.01a	15.89 ± 0.49c	17.46 ± 0.96bc

[a] Values within a row without a common letter are different ($P < 0.05$). Standard deviations are reported. [b] Crude protein determined by Kjeldahl method. [c] Crude protein determined by combustion method. Crude protein was 43.69, 35.38, and 38.31% for germ, hull, and combined fractions, respectively, as determined by Degussa Corp. [d] Dry matter was 91.24, 94.08, and 93.09% for germ, hull, and combined fractions, respectively, as determined by Degussa Corp. [e] Values determined by the Texas State Chemists Office.

Urease Assay. Urease activities of guar meals and control samples were determined using method Ba 9-58 (*17*). The urease assay was conducted with duplicate samples of the three guar meal fractions and a positive control of raw defatted soybeans. Each meal sample was ground through a 0.8 mm screen in the Cyclotech grinder.

Galactomannan Gum Assay. Residual guar gum was analyzed in duplicate for each guar byproduct fraction as described by Hansen et al. (*19*) with minor modifications. After drying, 3 g of each guar sample was placed into extraction thimbles and refluxed in ethanol for 8 h to remove free sugars. Subsamples (100 μg) were combined with 10 μg of *myo*-inositol (internal standard) and hydrolyzed with 4 mL of 2 M trifluoroacetic (TFA) acid in a Pyrex tube capped under nitrogen at 121 °C for 90 min. The TFA was evaporated under a stream of nitrogen, and the residue was reconstituted in 1 mL of water and then deionized by filtering through a 2 cm^3 mixed bed ion-exchange resin column. Samples were filtered using a 0.2 μm membrane filter and the monosaccharides separated by HPLC using a Supelcogel Pb 300 × 7.8 mm column at 85 °C. Samples were eluted with pure water at a constant flow rate of 0.7 mL/min. Eluting carbohydrates were detected using a Hewlett-Packard and 1037A refractive index detector. Residual guar gum was reported as the percentage of the sum of galactose plus mannose.

Statistical Analysis. Data were analyzed using SPSS version 11.0 for Windows (*20*). Statistical significance was determined by one-way analysis of variance. Means were separated by Duncan's multiple-range test. The threshold for statistical significance was $P \leq 0.05$.

RESULTS AND DISCUSSION

Proximate Analysis. Crude protein, dry matter, ash, ether extract, crude fiber, and gum content were determined for three guar meal fractions (**Table 1**). Crude protein value for the guar germ fraction was higher than those for the guar hull and combined fractions as determined by Kjeldahl nitrogen and combustion methods. Crude protein values for hull and combined guar fractions were not statistically different. The *Novus Raw Material Compendium* (*21*) reports 30% crude protein in guar germ and between 30 and 41.3% for mechanically extracted and solvent-extracted guar germ meal, which are lower than the crude protein content that was determined in the germ fraction. Nagpal et al. (*16*) reported 29.43% protein in guar seed and 38.78% protein in guar meal, which are similar to the crude protein concentration determined in our combined guar meal. Variations observed among literature values can be attributed

to different strains of guar seed, changes of processing techniques, and variation in analytical procedures among laboratories. The crude protein content of combined guar meal as determined in this study is within the range of published values.

The dry matter content of each of the three guar meal fractions was higher than typical literature values (**Table 1**). Dry matter contents were 93.18, 95.41, and 94.78% for germ, hull, and combined guar byproducts, respectively. Dry matter analysis conducted by Degussa-Huls Corp. in association with an independent amino acid analysis obtained 91.24, 94.08, and 93.09% values, respectively, for germ, hull, and combined guar meals. The *Novus Raw Material Compendium* (*21*) reports a 4% ash content of guar germ. Ash values determined in this study (**Table 1**) revealed a lower value for combined guar meal than those found by Nagpal et al. (*16*) and Verma and McNab (*10*). Nagpal et al. (*16*) reported 3.84% ash in guar seed and 5.47% ash in guar meal. Verma and McNab (*10*) indicated the ash content of guar meal fractions similar to the three samples of guar meal in the present study to be 6.83, 5.68, and 5.37%, respectively.

Ether extract and crude fiber concentrations of the three guar fractions were consistent with values reported in the literature by other researchers (**Table 1**). Whistler and Hymowitz (*22*) report that guar seed contains 4% oil, whereas the *Novus Raw Material Compendium* (*21*) reports values between 3 and 6% for mechanically extracted guar germ meal. Nagpal et al. (*16*) report higher ether extract values for guar seed (5.24%) and guar meal (7.19%). Verma and McNab (*10*) found lower ether extract levels in three samples of guar meal (5.54, 5.74, and 5.89%). The crude fiber content of the germ fraction (8.4%) was lower than crude fiber values for the hull fraction (13.5%) and combined (11.4%) guar fractions. Nagpal et al. (*16*) reported a 10.52% crude fiber value for guar seed and 11.75% crude fiber value for guar meal. Similarly, Verma and McNab (*10*) found the crude fiber content of three guar samples to be 10.22, 9.91, and 10.12%. The *Novus Raw Material Compendium* (*21*) reports crude fiber values of 8.81% for mechanically extracted guar germ meal.

Nagpal et al. (*16*) and Anderson and Warnick (*1*) indicate that the gum content of guar meal is within the range of 18−20%. Our data based on the total galactose and mannose after free sugar extraction suggest concentrations of 12.8 and 14.2% for the hull and combined fractions, respectively, and only 5.8% for the germ fraction (**Table 1**).

Protein Solubility and Protein Dispersibility. Protein solubility and protein dispersibility are often used as measures of protein quality. Protein solubilities of germ and combined guar fractions were within the specified optimum range established for dehulled soybean meal, whereas protein solubility of the hull fraction was below the optimum range (*18*). This observation may result from the heating treatment the hull guar fraction received during the process that separates hull from endosperm. All guar meals had protein dispersibilities (**Table 1**) within the optimal range specified for soybean meal (*18*).

Amino Acid Composition. No attempt was made to protect methionine by the TAMU Protein Chemistry Laboratory during hydrolysis, which resulted in less assayed methionine versus the Degussa assay in which methionine was protected by conversion to methionine sulfoxone. Amino acids of particular interest to poultry nutritionists are methionine, lysine, threonine, and perhaps arginine. None of the guar byproduct fractions are a particularly good source of methionine or lysine (**Table 2**) when compared to dehulled soybean meal (*23*). Nagpal et al. (*16*) reported methionine and lysine values of 1.05 and 6.95% of protein in guar meal, respectively. The methionine and lysine concentration as a percentage of crude protein can be calculated from the values reported in **Tables 1** and **2**. For the combined guar meal lysine values were 4.21 and 4.28% of crude protein for TAMU and Degussa Corp. assays, respectively. The lysine values reported by Nagpal et al. (*16*) are considerably higher than these values. Nagpal's values for methionine as a percentage of crude protein were similar to the 0.89 and 1.17% values observed by TAMU and Degussa laboratories, respectively.

Table 2. Amino Acid Profiles of Three Guar Meal Fractions As Determined by Two Independent Laboratories

	% dry matter					
amino acid	TAMU[a]			Degussa-Huls[b]		
	germ	hull	combined	germ	hull	combined
Asp	5.26	4.12	4.40	4.81	3.88	3.99
Glu	9.88	7.58	8.02	9.59	7.42	7.84
Ser	3.40	2.78	2.90	2.32	1.88	1.99
His	0.98	0.69	0.80	1.30	1.00	1.05
Gly	4.97	3.98	4.24	2.54	2.09	2.16
Thr	1.79	1.37	1.51	1.32	1.08	1.13
Ala	3.07	2.58	2.53	1.86	1.52	1.58
Arg	5.05	3.77	4.03	6.58	4.94	5.26
Tyr	1.22	1.00	1.01			
Val	2.00	1.65	1.64	1.80	1.53	1.48
Met	0.44	0.32	0.36	0.58	0.48	0.48
Phe	1.71	1.30	1.39	1.90	1.49	1.58
Ile	1.51	1.17	1.26	1.44	1.13	1.18
Leu	3.09	2.39	2.55	2.69	2.13	2.22
Lys	2.10	1.53	1.70	2.19	1.64	1.76
Pro	2.54	2.04	2.17	1.63	1.30	1.34
Cys				0.64	0.71	0.54

[a] Texas A&M University Protein Chemistry Laboratory, College Station, TX 77843. [b] Degussa-Huls Corp., Applied Technology Chemical Group, Allendale, NJ 07401

Table 3. Trypsin Inhibitor Activities in Guar and Soybean Samples

sample	trypsin inhibitor,[a] TIU/g[b]
dehulled soybean meal	6840 ± 1214c
defatted raw soybeans	66550 ± 501d
germ fraction	3510 ± 825b
hull fraction	3250 ± 352b
combined guar meal	3510 ± 825b
raw guar beans	2980 ± 20b
raw guar splits	1140 ± 66a

[a] Different letters within a column indicate significant differences ($P < 0.05$). [b] TIU/g = trypsin inhibitor units per gram.

Trypsin Inhibitor. Trypsin inhibitor activities in all guar products were significantly less than trypsin inhibitor activities in soybean meal and raw soybeans (**Table 3**). No urease activity was found in any guar meal fraction, whereas the positive control of defatted raw soybeans produced a pH change of 2.07 units. Previous reports of Anderson and Warnick (*1*), Couch et al. (*11*, *12*), and Verma and McNab (*10*) reported trypsin inhibitor activity in raw guar meal and suggested that trypsin inhibitor activity contributed to poor performance of poultry. Sathe and Bose (*3*), Anderson and Warnick (*1*), and Thakur and Pradhan (*24*) documented reduced feed efficiency, feed consumption, and body weight of poultry fed diets containing raw guar meal. Trypsin inhibitors in guar meal were cited as a possible cause of poor performance. None of these researchers quantified trypsin inhibitor activity in the guar meal.

Prior to this study, no experiment quantified trypsin inhibitor activity in guar meals. The quantified amount of trypsin inhibitor

activity in guar meal was substantially lower than that of heat-treated dehulled soybean meal. The dehulled soybean meal trypsin inhibitor activity assayed in this study was ~90% less than that measured in raw soybeans. Assay of whole unprocessed guar beans and guar splits (endosperm), which are separated to refine the guar gum, indicated still lower amounts of trypsin inhibitor activity. These findings suggest that the low trypsin inhibitor activity of the guar bean is concentrated in the meal, which is composed of the germ and hull fractions. It is interesting to note that similar trypsin inhibitor activity levels are indicated for each of the fractions of guar meal even though the hull fraction is heated in the separation process and the germ fraction is not.

This research indicates that the three fractions of guar meal contain less trypsin inhibitor activity than dehulled soybean meal commonly used in most poultry diets. Therefore, in our view, the poor performance of poultry fed diets containing guar meal is not due to trypsin inhibitor activity. The findings of this research support previous suggestions by Vohra and Kratzer (*4*, *5*), Nagpal et al. (*16*), Verma and McNab (*9*), and Patel and McGinnis (*8*) that attribute the negative effects of guar meal in poultry diets to the gum residue in guar meal. Furthermore, research in our laboratory confirms by experimental evidence that high intestinal viscosity resulting from excessive consumption of guar gum causes the growth depression observed by other researchers (*14*, *15*).

In summary, it is clear that significant differences exist in the nutrient content of guar germ and hull byproducts. The germ fraction, containing significantly more protein and less residual galactomannan gum, appears to be more suited for inclusion in feeds for poultry and swine than either the hull or combined guar byproducts. Trypsin inhibitor activity does not appear to be a significant factor limiting the use of guar meal in poultry.

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