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In vitro Viscosity as a Function of Guar Meal and ß-Mannanase Content of Feeds

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Abstract: Guar meal, a high protein by-product of guar gum production can be separated into germ and hull fractions. Guar meal at high concentrations in the diet depresses growth and feed efficiency of chickens. The gum, a galactomannan polysaccharide increases intestinal viscosity and thereby decreases nutrient absorption. Guar gum residue is the presumptive anti-nutritive factor in guar meal. An *in vitro* procedure was developed to measure the impact of guar meals and endo-ß-mannanase (Hemicell®) on feed viscosity. ß-mannanase was added to diets containing 0, 2.5, 5.0, 7.5 and 10.0% germ and hull guar meal fractions. ß-mannanase was added into each diet at ten different concentrations ranging from 1/32-100 times the recommended dose (1.09×10^5 units/kg). Feed samples were mixed with water and enzyme solution (1:5 w/v) and incubated. Subsequently, samples were centrifuged and supernatant viscosity measured. Supernatant viscosity increased in direct proportion to guar meal concentration. Hull fraction increased viscosity more than the germ fraction. ß-mannanase significantly reduced the viscosity of all diets except the 2.5% germ fraction diet. An inverse relationship was observed between enzyme concentration and sample viscosity from 0 to the recommended enzyme level. Enzyme concentrations exceeding the recommended level did not further reduce viscosity of feed supernatant. These results are consistent with feed viscosity as the basis of feed efficiency improvement with enzyme feeding. Increased viscosity with guar meal inclusion and reduced viscosity with enzyme inclusion is consistent with the hypothesis that feed viscosity is the anti-nutritive effect of guar meal feeding.

Key words: Guar, galactomannan, mannanase, feed, viscosity, chicken

INTRODUCTION

Extraction of guar gum from guar beans yields a mixture of germ and hull fractions as a by-product. Various fractions and mixtures of these guar meals contain approximately 35-45% protein (Nagpal *et al*., 1971; Lee *et al*., 2004) and 14-18% residual gum (Anderson and Warnick, 1964; Nagpal *et al*., 1971). Guar gum is a galactomannan polysaccharide consisting of a 1-4 ß-mannose backbone with galactose bound at position six on alternate mannose sugars. Mixing the gum with water produces a highly viscous solution.

Inclusion of guar gum in poultry diets reduces broiler chicken growth 25-30% (Vohra and Kratzer, 1965). Most researchers believe that gum residue, not trypsin inhibitor causes the anti-nutritive effects of guar meal. Growth depressing effects of guar meal attributed to a trypsin inhibitor by Couch *et al*. (1966) contradicts Bochers and Ackerson (1950) who state that guar beans contain no trypsin inhibitor. Quantitation of trypsin inhibitor activity in guar beans, guar meal, soybeans, and soybean meal proved that less trypsin inhibitor occurs in guar meal than in heat-treated commercially processed soybean meal (Lee *et al*., 2004). However, residual gum in guar meal increases intestinal viscosity in chickens, which reduces growth and feed efficiency (Burnett, 1966; Lee *et al*., 2003a).

Excessive intestinal viscosity detrimentally affects growth and feed efficiency. Increased viscosity severely compromises the ability of the gut to physically mix digesta (Edwards *et al*., 1988). Impaired mixing has severe implications for fat digestion since fat emulsification requires vigorous intestinal mixing and excessive viscosity impairs diffusion and convective transport of digestive enzymes within the gastrointestinal tract of young chickens (Almirall *et al*., 1995). Edwards *et al*. (1988) demonstrated *in vitro* that convective transport of glucose and sodium declines in a viscous environment. Increased viscosity also may reduce contact intensity between potential nutrients and their respective digestive secretions, thereby reducing diffusion to the epithelial surface (Choct and Annison, 1992). Rainbird *et al*. (1984) using isolated porcine jejunal loops demonstrated that guar gum significantly reduced net absorption of glucose and maltose solutions from 74.2-41.4% and 71.1-35.0%, respectively. A significant increase in intestinal viscosity leads to increased weights and lengths of intestinal segments, and decreases digestibilities of lipid, starch and nitrogen (Smits *et al*., 1997). Highly viscous diets cause pasty feces and depresses growth and feed efficiency in chickens (Vohra and Kratzer, 1964a).

Isolation and use of enzymes for degradation of indigestible substrates occur frequently in scientific literature. Enzyme supplementation of diets containing guar meal and other highly viscous ingredients such as barley and wheat improve growth and feed utilization in

chickens (Vohra and Kratzer, 1964b; Vohra and Kratzer, 1965; Choct *et al*., 1995; Steenfeldt *et al*., 1998; Lee *et al*., 2003b). Improvement of nutrient availability from feed ingredients such as wheat and barley by enzyme supplementation is attributed to a decrease in intestinal viscosity (Burnett, 1966; Rotter *et al*., 1990). Effective enzymes hydrolyze polysaccharides from these ingredients and reduce viscosity, thereby improving digestibility coefficients, growth and feed efficiency.

This research report describes an *in vitro* method that measured the viscosity of feeds. *In vitro* viscosity of feed formulations with varying concentrations of germ and hull guar meals were measured with and without addition of endo-ß-mannanase. The primary enzymatic activity of this ß-mannanase is degradation of galactomannan polymers. Degrading a viscous polysaccharide within the gastrointestinal tract allows increased movement of digesta through the small intestine and improves macronutrient digestibility (Almirall *et al*., 1995). Therefore, a direct relationship between feed viscosity and guar gum source and concentration in feed and an inverse relationship between feed viscosity and ß-mannanase concentration was hypothesized.

MATERIALS AND METHODS

Experimental design: An industry type broiler starter diet (21% CP; 3000 kcal/kg ME) was used as a control diet or substituted with varying amounts of germ and hull fractions of guar meal[1]. Guar germ and hull diets contained 2.5, 5.0, 7.5 and 10.0% of either fraction and were mixed on an isocaloric and isonitrogenous basis (Table 1). The protein contents of fractions used in this experiment were 42% for the germ fraction and 34% for the hull fraction (Lee *et al*., 2004).

Beta-mannanse enzyme[2] solutions were added to diet samples to measure enzyme impact on *in vitro* viscosity of the feed sample. Enzyme stock solutions were diluted to different concentrations with phosphate buffer (pH 7.0) for addition to the control and guar starter diets. Stock solutions ranged in concentration from 1/32-100 times the recommended dose for poultry rations (1.09×10^5 units/kg as determined by the manufacturer). Enzyme solutions were added to the feed solution at 1.0 mL per feed sample. Enzyme-free samples included 1.0 mL phosphate buffer without enzyme.

Viscosity procedure: Viscosity of each guar meal fraction was determined in quadruplicate by mixing different amounts (0.25, 0.5, 1.0, 2.0 and 4.0 g) of each guar fraction with 7.5 mL distilled water and incubating in a water bath at 40°C for one h. Quadruplicate samples were then centrifuged for 10 min (1000 x g). An aliquot of supernatant (0.5 mL) was placed in a cone and plate viscometer with a CPE-40 spindle[3] and run for 30s before viscosity data was recorded.

Feed solution viscosities were determined by a procedure similar to the procedure that was used to determine viscosities of guar meal fractions. Quadruplicate aliquots of each feed sample (2.5 g) were

Table 1: Diet composition[1] of control and experimental diets

	Germ Fraction (%)					Hull Fraction (%)			
Ingredients	0	2.5	5	7.5	10	2.5	5	7.5	10
Guar meal fraction									
Germ	0	2.5	5.0	7.5	10.0	0	0	0	0
Hull	0	0	0	0	0	2.5	5.0	7.5	10.0
Corn	59.4	59.3	59.1	59.0	58.8	58.2	57.1	58.9	54.7
Dehulled soybean meal	34.0	31.7	29.5	27.1	24.9	32.4	31.1	29.1	27.4
Fat A and V blend	2.5	2.4	2.3	2.2	2.1	2.8	3.1	3.4	3.7
DL-methionine	0.22	0.23	0.23	0.24	0.24	0.22	0.22	0.22	0.22
L-lysine HCl	0.09	0.11	0.13	0.16	0.18	0.10	0.12	0.14	0.15
Limestone	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mono-dicalcium PO_4	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium chloride	0.46	0.46	0.43	0.38	0.33	0.46	0.46	0.43	0.39
Sodium bicarbonate	0.0	0.0	0.05	0.12	0.19	0.0	0.0	0.05	0.10
Coban 60	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

[1]Calculated analysis of all diets were as follows: Crude Protein, 22%; Metabolizable Energy, 3050 kcal/kg; Methionine, 0.56%; Lysine, 1.23%; Calcium, 0.89%; Available Phosphorous, 0.43%.

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Vitamin premix added at this rate yields 11,023 IU Vitamin A, 3,858 IU Vitamin D, 46 IU Vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls

each mixed with 6.5 mL distilled water and 1.0 mL enzyme solution. Samples were incubated for one h in a water bath (40°C) then transferred to an ice bath (4°C) for 15 min to reduce enzymatic activity. Subsequently, solutions were centrifuged for 10 min (1000 x g). A 0.5 mL aliquot of supernatant was placed into the viscometer and run for 30s before the viscosity was recorded.

Statistical analysis: Linear models were developed on each guar meal containing feed. Regression analysis also was performed on viscosity of feed samples as a function of enzyme concentration. Each guar meal concentration was modeled as a single feed treatment that was modified by enzyme supplementation. Linear models for diets containing each guar fraction at different concentrations were developed as a function of enzyme supplementation. Slope similarities and differences among treatments were determined by a test of heterogeneity of regression.

RESULTS AND DISCUSSION

Supernatants from the hull fraction of guar meal were more viscous than supernatants from the germ fraction. As the mass of the guar meal fraction that was extracted increased, the viscosity of the supernatant increased in a linear manner (Fig. 1). Viscosity increased by approximately two centipoise (cP) units per gram of meal extracted and the slopes of the germ and hull regressions were not significantly different. These data suggest that the agent responsible for the viscosity from the two fractions is the same agent and perhaps is of different concentrations in the two fractions.

Every diet containing guar meal of either fraction had an increased viscosity when compared to that of the corn-soy diet (Fig. 2). As concentrations of hull and germ fractions increased in the diets, the viscosities of the solutions without enzyme increased with the exception of the 2.5% germ fraction. The 2.5% germ fraction had a higher viscosity than other germ fraction diets. Enzyme reduced the viscosity of all guar meal treatments except 2.5% germ. The viscosity and the enzyme response of the 2.5% germ fraction was inconsistent with other data in the experiment and therefore is considered an aberration. Regression analysis of the viscosity response to increasing concentrations of enzyme in the diets indicated that the increasing the enzyme concentration above 1.09×10^5 units/kg was ineffective at further reducing viscosity. Slopes of the regression of viscosity on enzyme concentration in all diets including guar fractions were significantly different from zero except for the 2.5% germ diet (Table 2). As the concentrations of guar fractions increased in the diets, slopes of the viscosity response due to the addition of the enzyme became more negative. The viscosity of the control diet was not affected by addition of enzyme at any level.

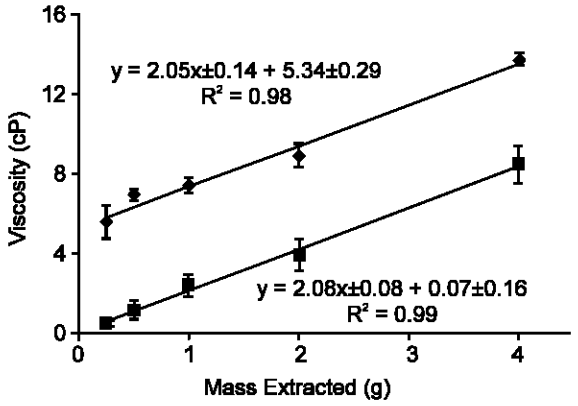

Fig. 1: Viscosity of supernatants extracted from different amounts of germ (■) and hull fractions (◆). Vertical lines associated with data points indicate standard error of the mean

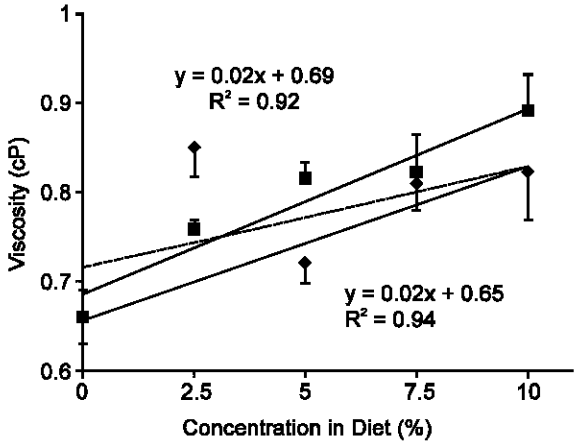

Fig. 2: Viscosity as a function of guar fraction concentration in broiler starter diets (germ --◆--, germ excluding 2.5% data point -◆- , or hull -■-). Vertical lines associated with data points indicate standard error of the mean

Slopes of the viscosities of guar germ and hull were parallel, therefore not significantly different. Increased amounts of guar fractions added to diets, increased the viscosity of the diet. These results suggest that the growth depression in broiler chickens associated with guar hull containing diets as compared to diets only containing the germ fraction (Lee *et al*., 2005) was due to an increased intestinal viscosity. Analysis of feed samples including different concentrations of guar meal fractions indicated that the viscosity of the feed increased as the gaur fraction content of the feed increased (Fig. 2). The elevated viscosity of the 2.5% germ fraction diet and the diet's failure to respond to enzyme treatment suggested that the diet was aberrant. Therefore, the weight given to data supplied from the 2.5% germ diet was minimal. The increase in viscosity

Table 2: Regression of viscosities of feed sample extracts as a function of b-mannanase concentration added to feeds containing different concentrations of guar meal germ and hull fractions

Guar content of feed	Slope	Intercept	R^2
(%)			
0	-0.05±0.04	0.66±0.02	0.90
Germ fraction			
2.5	-0.05±0.03	0.83±0.01	0.82
5.0	-0.08±0.04*	0.69±0.02	0.79
7.5	-0.12±0.03*	0.76±0.01	0.86
10	-0.08±0.03*	0.77±0.01	0.56
Hull fraction			
2.5	-0.08±0.02*	0.72±0.01	0.69
5.0	-0.12±0.04*	0.71±0.02	0.64
7.5	-0.12±0.05*	0.71±0.02	0.55
10	-0.18±0.04*	0.77±0.02	0.75

*Slope is significantly different from zero at p<0.05

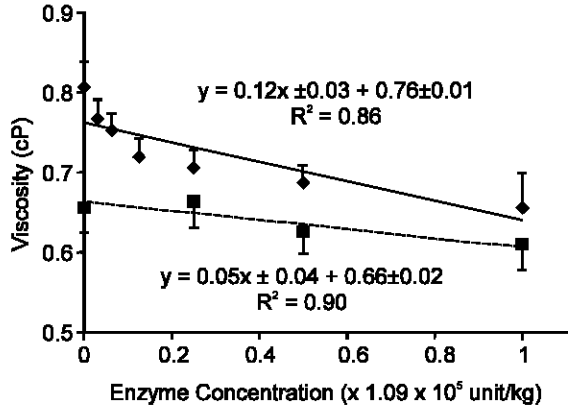

Fig. 3: Viscosity as a function of ß-mannanase concentration in a corn-soy diet formulated with (■) and without (◆) 7.5% guar germ fraction. Vertical lines associated with data points indicate standard error of the mean

of the feed that was observed as the concentration of guar fraction increased is consistent with the hypothesis that increased viscosity is the cause of growth depression in broiler chickens consuming guar-meal containing diets. Research shows a direct relationship between concentration of guar meal in the ration and the degree of growth depression observed (Sathe and Bose, 1962; Vohra and Kratzer, 1964a; Couch *et al*., 1967; Lee *et al*., 2003a).

Addition of ß-mannanase reduced the *in vitro* viscosity of feed samples containing guar meal. Viscosity of the extracted supernatant from the feed sample decreased (Fig. 3) as the concentration of the enzyme increased in the feed until the enzyme concentration exceeded 1.09 x 10^5 units/kg. Enzyme supplementation had no effect ($p > 0.05$) on a typical industry corn-soy based diet (Fig. 3). As the concentration of enzyme increased in the diet, reduction of viscosity as evidence by the negative slope became more pronounced, which when fed to broiler chickens by others results in increased body weights (Burnett, 1966; Almirall *et al*., 1995; Choct *et al*., 1995; Steenfeldt *et al*., 1998). Burnett (1966) used barley as his model for increasing the intestinal viscosity in broilers to evaluate the relationship between growth and intestinal viscosity. The significant increases observed in intestinal viscosity when barley was included in broiler diets was cited as the probable factor associated with reduced growth. Enzymes reduced intestinal viscosity and improved growth and feed efficiency. Addition of ß-mannanase ameliorated the increased viscosity associated with inclusion of guar meal in broiler diets.

Conclusion: Inclusion of certain ingredients in broiler diets is concentration sensitive due to the deleterious effects directly associated with the increased intestinal viscosity that follows (Lee *et al*., 2003a). Increased intestinal viscosity depresses the ability of the gut to physically mix gut contents (Edwards *et al*., 1988), which may be responsible for reducing digestibility coefficients of macronutrients (Choct and Annison, 1992; Almirall *et al*., 1995) thereby inhibiting growth and feed efficiency. Addition of enzymes to highly viscous compounds increase apparent and true metabolizable energy for broilers (Rotter *et al*., 1990).

Inclusion of guar meals containing the hull fraction at concentrations exceeding 2.5% of broiler diets depresses growth in broiler chickens at six weeks of age (Lee *et al*., 2005). Depressed growth resulting from feeding hull fraction-containing guar meals is attributed to the presence of a residual guar gum in the meal (Anderson and Warnick, 1964; Vohra and Kratzer, 1964b). This *in vitro* assay could be used to detect viscous compounds in feeds that inhibit broiler growth. This assay likewise could predict the effectiveness of enzymes that ameliorate deleterious effects associated with viscosity in broiler diets.

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REFERENCES

Almirall, M., M. Francesch, A.M. Perez-Vendrell, J. Brufau, and E. Esteve-Garcia, 1995. The difference in intestinal viscosity produced by barley and ß-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. J. Nutr., 125: 947-955.

Anderson, J.O. and R.E. Warnick, 1964. Value of enzyme supplements in rations containing certain legume seed meals or gums. Poult. Sci., 43: 1091-1097.

Bochers, R. and C.W. Ackerson, 1950. The nutritive value of legume seeds. J. Nutr., 41: 339-345.

Burnett, G.S., 1966. Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. Br. Poult. Sci., 7: 55-75.

Choct, M. and G. Annison, 1992. The inhibition of nutrient digestion by wheat pentosans. Br. Poult. Sci., 67: 123-132.

Choct, M., R.J. Hughes, R.P. Trimble, K. Angkanaporn, and G. Annison, 1995. Non-Starch polysaccharide degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. J. Nutr., 125: 485-492.

Couch, J.R., C.R. Creger and Y.K. Bakshi, 1966. Trypsin inhibitor in guar meal. Proc. Soc. Exp. Biol. Med., 123: 263-265.

Couch, J.R., Y.K. Bakshi, T.M. Ferguson, E.B. Smith and C.R. Creger, 1967. The effect of processing on the nutritional value of guar meal for broiler chicks. Br. Poult. Sci., 8: 243-250.

Edwards, C.A., I.T. Johnson and N.W. Read, 1988. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection. Eur. J. Clin. Nutr., 42: 306-312.

Lee, J.T., C.A. Bailey and A.L. Cartwright, 2003a. Guar meal germ and hull fractions differently affect growth performance and intestinal viscosity of broiler chickens. Poult. Sci., 82: 1589-1595.

Lee, J.T., C.A. Bailey and A.L. Cartwright, 2003b. ß-mannanase ameliorates viscosity-associated depression of growth in broiler chickens fed guar germ and hull fractions. Poult. Sci., 82: 1925-1931.

Lee, J.T., S. Conner-Appleton, A.U. Haq, A. Cartwright, and C. Bailey, 2004. Quantitative measurement of negligible trypsin inhibitor activity and nutrient analysis of guar meal fractions. J. Agric. Food Chem., 52: 6492-6495.

Lee, J.T., S. Connor, A. Cartwright and C. Bailey, 2005. Effects of Guar Meal By-Product with and without ß-mannanase Hemicell on Broiler Performance. Poult. Sci., 84: 1261-1267.

Nagpal, M.L., O.P Agrawal and I.S. Bhatia, 1971. Chemical and biological examination of guar-meal (*Cyampsis tetragonoloba* L.). Ind. J. Anim. Sci., 4: 283-293.

Rainbird, A.L., A.G. Low and T. Zebrowska, 1984. Effect of guar gum on glucose and water absorption from isolated loops of jejunum in conscious growing pigs. Br. J. Nutr., 52: 489-498.

Rotter, B.A., O.D. Friesen, W. Guenter and R.R. Marquardt, 1990. Influence of enzyme supplementation on the bioavailable energy of barley. Poult. Sci., 69: 1174-1181.

Sathe, B.S. and S. Bose, 1962. Studies on the utilization of industrial and farm by-products in growing poultry rations. Ind. J. Vet. Sci., 32: 74-84.

Smits, C.H.N., A. Veldman, M.W.A. Verstegen and A.C. Beynen, 1997. Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr., 127: 483-487.

Steenfeldt, S., A. Mullertz and J. Jensen, 1998. Enzyme supplementation of wheat-based diets for broilers. 1. Effect on growth performance and intestinal viscosity. Anim. Feed Sci. Technol., 75: 27-43.

Vohra, P. and F.H. Kratzer, 1964a. Growth inhibitory effect of certain polysaccharides for chickens. Poult. Sci., 43: 1164-1170.

Vohra, P. and F.H. Kratzer, 1964b. The use of guar meal in chicken rations. Poult. Sci., 43: 502-503.

Vohra, P. and F.H. Kratzer, 1965. Improvement of guar meal by enzymes. Poult. Sci., 43: 1201-1205.

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