THE EFFECT OF OLIGOSACCHARIDES ON THE NUTRITIVE VALUE OF SOYBEAN MEAL

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INTRODUCTION

The oligosaccharides of soybeans have been shown to produce gas in rats, dogs, and man (Steggerda, 1968). Anaerobic bacteria in the lower intestinal tract degrade oligosaccharides to yield gases such as hydrogen, carbon dioxide and small amounts of methane (Rackis, 1975). The oligosaccharides cannot be metabolized in the small intestinal tract by endogenous enzymes from the animal due to the absence of a-1,6-Galactosidase (a-D-Galactosidase,Galactohydrolase E.C.3.2.1.22) activity in intestinal mucosa (Gitzelmann and Auricchio, 1965). The researchers also noted an increased osmotic cathartic effect of raffinose ingested by human subjects. Kuriyama and Mendel (1917) reported that test meals of 3 or 5 grams of raffinose fed to fasting rats resulted in severe diarrhea and evidence of raffinose residues in the feces whereas animals receiving equal weights of sucrose had hard feces containing no or relatively small amounts of invertible non-reducing material. Wagner et al. (1976) noted a decline in hydrogen production from rats when raffinose was fed at levels above 6.7 percent and suggested the high levels of raffinose resulted in osmotic catharsis causing evacuation of a portion of the raffinose before its microbial hydrolysis and fermentation by intestinal microflora could be completed. Wagner and his researchers also noted that the feces of animals receiving the highest levels of raffinose in their diets were wetter and more adhesive than those from animals at zero or lower levels of raffinose ingestion. The previous research indicates the oligosacchrides of soybeans are metabolized by intestinal microflora to produce gases, however, in large quantities the oligosacchrides may produce an osmotic cathartic effect causing an increased evacuation of digesta, wet droppings, diarrhea.

The nutritional consequences of gastrointestinal gas production from soybean oligosacchrides or a potential osmotic cathartic effect producing diarrhea in animals has not been fully studied. Soybean meal has a lower metabolizable energy value for poultry than would be expected based on proximate analysis. The soybean meal metabolizable energy for swine is approximately 20 percent higher than for chicks (McGinnis, 1983). The metabolizable energy of solvent extracted 44 percent protein soybean meal is 3,090 kilocalories per kilogram for swine and 2,230 kilocalories per kilogram for poultry. The metabolizable energy of dehulled solvent extracted 48.5 percent protein soybean meal is 3,485 kilocalories per kilogram for swine and 2,440 kilocalories per kilogram for poultry (NRC 1979; 1984). The differences in metabolizable energy of soybean meal between species may be related to species differences in physiology of digestion of carbohydrates. Fowl and swine have major large intestinal anatomical differences because the fowl has two large cecum and a short colon (Moran, 1982). The ceca in the fowl may assist in the digestion of fine particulate matter, however, course fiber are restricted from the entrance into the ceca by mucosal folds having villi projecting toward the lumen center. The large intestine or colon of fowl is very short and the major intention of this structure is not to perform fermentive and absorptive activities but to convey ileal and cecal digesta. Swine may have a better opportunity to ferment the polysaccharide carbohydrates in soybean meal in the large intestines. Swine have been shown to produce volatile fatty acids that provide up to 12 percent of the metabolizable energy content from diets (Kass et al. 1980). The contribution of energy derived from volatile fatty acid formation in fowl has been reported to be small relative to the total need, however, a failure to obtain major volatile fatty acid energy contributions may have arisen because crude fiber fermentation will not normally occur in the ceca. A possibility exists that fine types of fiber such as found in shorts and red dog milling fractions in some grains and oligosaccharides may be fermented and produce short chain volatile fatty acids in the ceca in the bird (Moran, 1982).

The objectives of the research reported herein are: 1.) to determine if the true metabolizable energy of 44 percent soybean meal can be significantly improved following the removal of oligosacchrides by 80 percent alcohol extraction, 2.) to determine the digestibility of total soluble carbohydrates, oligosaccharides, hemicellulose, and cellulose in 44 percent soybean meal and 44 percent soybean meal extracted with 80 percent alcohol, and 3.) to determine the extent of soybean carbohydrate digestion in the small intestine compared to digestion in the ceca and lower gastrointestinal tract of poultry.

MATERIALS AND METHODS.

Soybean Meal Preparation. Five hundred grams of alcohol extracted soybean meal was obtained by extracting 44 percent soybean meal with 80 percent ethanol using a modified procedure of Tanaka et al. (1975). Fifty grams of 44 percent crude protein soybean meal were ground in a Wiley Mill utilizing a 20 mesh screen and extracted with 500 ml (10 vol/wt) of 80 percent ethanol. The suspension was refluxed for one hour at 75°C, alcohol extract removed and refluxed a second time with another 500 ml of 80 percent ethanol for an equivalent one hour time period. The extracted soybean meal was then filtered through a Whatman No.1 filter paper and the residue of the extracted meal was stirred in 500 ml of distilled water for 30 minutes and filtered again. The soybean residue is then washed with distilled water until the filtrate provides a negative triphenyltetrazolium chloride reaction of the washing as suggested by Horn et al. (1965). The extracted soybean meal residue was freeze dried and kept for feeding experimental birds and for analysis.

True Metabolizable Energy Bioassay and Excreta Collection for Carbohydrate Digestibility Studies. The true metabolizable energy nitrogen-corrected (T.M.E.n) was determined for the soybean test samples by the procedure of Sibbald (1976). Eight adult leghorn roosters previously fasted for a 24-hour period were force-fed either 30 grams of regular soybean meal, 30 grams of alcohol extracted soybean meal, or 30 grams of glucose for a control. The roosters excreta were collected quantitatively for a 48-hour period after feeding. The excreta collected from the glucose fed roosters were utilized to correct for metabolic and endogenous energy losses which occur during the digestion of soybean meal. All excreta collected from roosters were frozen, freeze dried, ground through a 20 mesh screen with a Wiley Mill and weighed. The regular 44 percent soybean meal, alcohol extracted soybean meal and excreta samples were each assayed for nitrogen by a Kjeldahl procedure using A.O.A.C.

methods (1975). The nitrogen was determined in order to correct the true metabolizable energy to a zero nitrogen balance. The soybean samples and excreta were analyzed for gross energy utilizing a Parr adiabatic oxygen bomb calorimeter and the true metabolizable energy determined for each soybean meal sample by utilizing the calculations of Sibbald (1976).

Rooster Ileal Digesta Collection for Carbohydrate Analysis. Thirty adult roosters previously fasted for 24 hours in order to clear the alimentary canal were force-fed with 30 grams of regular 44 percent soybean meal or 30 grams of alcohol extracted soybean meal. The roosters were killed by cervical dislocation five hours after the initial feeding. The ileum of the roosters were immediately removed and the contents flushed into a plastic container with 5 ml of distilled water. The ileum was defined as the portion of intestine extending from the Meckel's Diverticulum to a point 3 cm proximal to the ileo cecal junction. The ileal digesta samples from ten birds were pooled to provide adequate samples for acid insoluble ash and carbohydrate analysis. The pooling of samples from ten birds provided three separate replicates that could be utilized to determine the ileal digestion of carbohydrates. The digesta samples were frozen, freeze dried, ground through a 20 mesh screen and stored for analysis. Acid insoluble ash was determined in the 44 percent soybean meal, alcohol extracted soybean meal, and in the ileal degesta samples collected from the roosters by the method of Keulen and Young (1977) using 2N HCL and double ashing.

Total Soluble Carbohydrates in Soybeans and Digesta Samples. The total carbohydrates were extracted by a procedure presented by Southgate (1969). Five grams of ground 44 percent soybean meal, alcohol extracted soybean meal and excreta were extracted with a 10 to 1 volume/weight of 80 percent methanol for a two hour period at 60°C. Each sample was extracted three times for the two hour duration and the extracts were combined and taken to a 250 ml volume. The sample residue was washed with ethyl ether, dried in an air oven and kept for hemicellulose and cellulose analysis. The supernatant was analyzed for total carbohydrates by the phenol sulfuric acid procedure described by Dubois et al. (1956). A 1 ml aliquot of the supernatant was reacted with phenol (1 ml, 5 percent in water) and 5 ml of concentrated sulfuric acid for 30 minutes. The mixture was allowed to stand 10 minutes at room temperature, then the mixture was shaken and placed for 20 minutes in a water bath at 30°C. The color development of the carbohydrate mixture was measured spectrophotometrically at 490 nm. The absorption was compared to that obtained for known concentrations of glucose. Sugars soluble in hot 80 percent methanol would include monosaccharides and oligosaccharides of the soybean and excreta samples.

Oligosaccharides. The oligosaccharides were extracted by the 80 percent methanol procedure described by Southgate (1969). The oligosaccharides from the alcohol extract were separated by the paper chromatography procedure of Onigbinden and Akinyele (1983). The oligosaccharide sugars were quantitatively determined by using the phenol-sulfuric acid method of Dubois et al. (1956).

Hemicellulose and Cellulose. The hemicellulose and cellulose content was determined on the methanol extracted residue from test soybean samples and excreta. The polysaccharides, hemicellulose and cellulose, were determined by the detergent systems described by Van Soest and Robertson (1980).

RESULTS AND DISCUSSION

The T.M.E.n of the alcohol extracted 44% soybean meal is 3499 kcal/kg compared to 2794 kcal/kg for regular 44% soybean meal (Table 1). The T.M.E.n of regular 44% soybean meal on a dry matter basis is similar to the energy value reported by Sibbald (1986). The 705 kcal/kg additional T.M.E.n for alcohol extracted soybean meal represents a 25% increase in energy because the true dry matter digestibility increases 8.5% and the gross energy of alcohol extracted soybean meal is approximately 300 kcal/kg higher compared to regular 44% soybean meal. The true dry matter digestibility of alcohol extracted soybean meal was 64.75 percent compared to 56.25.

The total soluble carbohydrates of the regular 44% soybean meal were 12.02% compared to .306% for alcohol extracted 44% soybean meal (Table 2). The total soluble carbohydrate fraction of regular 44% soybean meal consist primarily of oligosaccharides. The sucrose, raffinose and stachyose content on a dry matter basis of 44% soybean meal was 5.98, 1.07 and 4.23 percent respectively. Kawamura (1967a,b) determined the oligosaccharide content of six American varieties of defatted soybeans and the average composition was sucrose 6.2%, raffinose 1.4%, and stachyose 5.2%. Delente and Ladenburg (1972) determined the oligosaccharides of defatted soybean meal were 8.9% sucrose, .90% raffinose, and 4.2% stachyose. The amount of sucrose found in soybeans has varied considerably among investigators primarily because hydrolysis of stachyose or raffinose may produce the disaccharide sucrose. Hymowitz et al. (1972) suggests the sucrose content is 60% of the total free carbohydrate fraction of soybeans and raffinose and stachyose represent 4 and 36% respectively. Conkerton et al. (1983) has discovered that a large portion of the stachyose and sucrose carbohydrates may be bound together in a complex compound. The researchers suggested the unidentified compound represented over 90% of the free carbohydrate fraction of soybeans. The syrupy unidentified compound was transformed during storage into stachyose and sucrose suggesting that the stachyose and sucrose content of soybeans is bound together in a complex structure and not in a free form. The preparation of the oligosaccharide-free soybean meal with two 1 hour hot 80% ethanol extractions provided a soybean meal sample containing .306% total soluble carbohydrates representing 2.5% of total soluble carbohydrates in regular 44% soybean meal.

The apparent digestibility of the total soluble carbohydrate fraction of regular 44% soybean meal was 90.4% (Table 2). The digestibilities of specific oligosaccharides such as sucrose, raffinose, and stachyose for regular 44% soybean meal were 94.2, 90.5, and 83.8% respectively. The low concentrations of total soluble carbohydrates, sucrose, raffinose and stachyose in alcohol extracted soybean meal were shown to be less digestible than the oligosaccharides from regular 44% soybean meal. The residual oligosaccharides of alcohol extracted soybean meal may have specific bonding to polysaccharide carbohydrates or other structures thus limiting exposure to microbial a-galactosidase. The 90% digestion of oligosaccharides representing approximately 12% of 44% soybean meal shows the low T.M.E.n of regular soybean meal is not caused by a lack of digestion of oligosaacharides. In fact, the high digestion of oligosaccharides may produce the low T.M.E.n of regular soybean meal by increasing gastrointestinal gas and acidity of the lower-intestinal tract (Cristafaro et al., 1974; Rackis, 1975; Reddy, et al, 1984) and by promoting rapid intestinal transit of digesta (Hellendoorn, 1978; 1979).

The hemicellulose and cellulose content of 44% regular soybean meal was 9.91 and 7.09% respectively (Table 3). Reddy et al. (1984) reported 7.6% hemicellulose in soybeans but no values for cellulose were given. Aspinall et al. (1967) found soybean meal to contain 18% polysaccharides consisting of a complex mixture of acidic polysaccharides, galactomannans, xylan hemicellulose, and cellulose. The hemicellulose and cellulose of alcohol extracted soybean meal was increased to 15.07 and 11.27% respectively. The total polysaccharide content including hemicellulose and cellulose was 9.3% higher in the alcohol extracted soybean meal.

The apparent digestion of hemicellulose and cellulose for regular 44% soybean meal was 9.2% and 0 respectively. The extraction of oligosaccharides from soybean meal with 80% ethanol increased the apparent hemicellulose digestion to 61.6% and the cellulose apparent digestion increased to 35%. The discovery of increased polysaccharide digestion of oligosaccharide-free soybean meal provides support to explain the increased T.M.E.n and true digestibility of alcohol extracted soybean meal. The increase in polysaccharide digestion in alcohol extracted soybean meal may be from increased digestion of fiber in the ceca of the rooster. The lack of oligosaccharides may allow a slower transit of digesta containing complex carbohydrates and an optimum microflora for hydrolyzing polysaccharides to volatile fatty acids in the lower gastrointestinal tract. The combined digestion coefficients of polysaccharides and residual oligo- saccharides of alcohol extracted soybean meal is 49.67% for 26.64% dry matter. The combined digestion coefficients of poly- saccharides and total soluble carbohydrates (oligosaccharides) for 44% regular soybean meal is 40.59% for 29.02% dry matter. The 9% higher digestion of the major carbohydrates of the alcohol extracted soybean meal closely supports the 8.5% increase in true dry matter digestibility of alcohol extracted soybean meal (Table 1).

The apparent digestibility of total soluble carbohydrates was 77.9% for regular soybean meal and approximately zero for alcohol extracted soybean meal when determining the digestibility at the terminal ileum location (Table 4). The rationale for total soluble carbohydrates being 77.9% digested at the terminal ileum compared to 90.4% (Table 2) is directly shown to be associated with the 89% sucrose digestion at this location and very low digestibility values for raffinose and stachyose at the lower small intestinal location. The hemicellulose and cellulose apparent digestibility at the ileum location was approximately zero for both the regular 44% soybean meal and alcohol extracted soybean meal. These findings support the ceca and colon as being the major location for fermentation of hemicellulose and cellulose for both regular 44% soybean meal and alcohol extracted soybean meal. The apparent digestibility of regular 44% soybean meal dry matter was 50.79% and the alcohol extracted soybean meal dry matter was 44.34% digestible at the ileum location. These digestion coefficients for dry matter are associated with the digestion of soybean proteins and sucrose carbohydrates in the small intestinal area. The dry matter true digestibility shown in Table 1 is approximately 6% higher for regular soybean meal and 20% higher for alcohol extracted soybean meal. The reason the true digestibility of dry matter in Table 1 is higher is because the excreta was collected after the digesta passed completely through the rooster which would include the ceca and colon. The 9% digestion of hemicellulose for regular soybean meal will add approximately 1% dry matter digestion for regular 44% soybean meal. The lower gastrointestinal tract becomes a major location for dry matter digestion of the alcohol extracted soybean meal because approximately 13% of actual dry matter digestion (hemicellulose and cellulose) occurred in the lower tract (Table 3). The research with the ileal digesta samples combined with the total collection experiments provided information regarding specific locations for oligosaccharide and polysaccharide digestion.

The ability to increase the metabolizable energy of soybean meal for poultry would increase the economic value of soybean meal to poultry producers substantially. The ability to have soybean meal available for feeding broilers and laying hens that is 25 % higher in metabolizable energy would drastically reduce the amount of added energy required in these diets. The use of higher metabolizable energy soybean meal for broilers and laying hens would also allow a poultryman to utilize potential cheaper grains and feedingstuffs other than corn in formulating diets. The low metabolizable energy values of barley, oats, and other cereal grains mixed with soybean meal provides low energy diets that often need additional calories to produce the same feed efficiency as corn-soybean meal diets. The economic value of increasing the metabolizable energy value of soybean meal (48.5% protein, solvent extracted, and dehulled) from 2440 kcal/kg to 3485 kcal/kg (metabolizable energy value for swine) would be equal to the calories from 3/10 of a pound of animal tallow. A ton of 48.5% soybean meal would require 272 pounds of animal tallow to provide the same calories for poultry compared to the metabolizable energy calories of one ton of the soybean meal for swine. The cost of increasing the calories with animal tallow to make one ton of soybean meal equivalent to the calories available to swine is approximately $53.00 (19.50/pound for animal tallow).

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Table 1. True Metabolizable Energy (T.M.E.n) Nitrogen - Corrected and True Dry Matter Digestibility of 44% Soybean Meal and Soybean Meal Extracted with 80% Ethanol.[1]

	T.M.E.n Kcal/kg	Dry Matter True Digestibility (%)
Soybean Meal (44%CP)	2794±113	56.25±2.83
Soybean Meal - 80% ethanol extracted	3499±68	64.75±5.67

[1]The T.M.E.n and True Digestibility Means ± S.E.M. are on a dry matter basis.

Table 2. The Apparent Digestibility of Total Soluble Carbohydrates and Oligosaccharides of 44% Soybean Meal and Alcohol Extracted Soybean Meal.

Type of Carbohydrates	Soybean Meal (Dry Matter)		Apparent Digestibility Soybean Meal	
	44%CP	Alc.Ext.-44CP	44%CP	Alc.Ext.-44%CP
	(%)			
Total Soluble Carbohydrates	12.02	.306	90.42±.34	0.62±11.49
Sucrose	5.98	.13	94.23±.45	40.17±.86
Raffinose	1.07	.08	90.52±.19	55.56±6.41
Stachyose	4.23	.03	83.81±.55	47.22±1.47

Table 3. The Apparent Digestibility of Cellulose and Hemicellulose of 44% Soybean Meal and Alcohol Extracted Soybean Meal.

Type of Soybean Meal	Hemicellulose	Cellulose	Apparent Digestibility Hemicellulose	Apparent Digestibility Cellulose
	(Dry Matter)		(%)	
44% Soybean Meal	9.91	7.09	9.19±13.79	0.0
Alcohol Ext. Soybean Meal	15.07	11.27	61.64±5.76	34.99±7.88

Table 4. The Apparent Digestibility of Total Soluble Carbohydrates, Oligosaccharides, Hemicellulose, Cellulose and Dry Matter of 44% Soybean Meal and Alcohol Extracted Soybean Meal Determined with Ileal Digesta Samples.

Type of Carbohydrate	Apparent Digestibility Regular 44% Soybean Meal	Apparent Digestibility Alcohol Extracted Soybean Meal
	(%)	
Total Soluble Carbohydrates	77.88±.42	0.0
Sucrose	89.35±.41	47.81±1.20
Raffinose	0.69±.06	0.66±.09
Stachyose	0.80±.01	0.75±.03
Hemicellulose	0.41±.02	0.48±.06
Cellulose	0.47±.02	0.02±.003
Dry Matter	50.79±.82	44.34±2.37