Alternative Proteins in Companion Animal Nutrition

Dale A. Hill, Ph.D., P.A.S., D.A.C.A.N.
ADM Animal Health & Nutrition
Quincy, IL, USA 62301

Presented at the
Pet Food Association of Canada Fall Conference
Toronto, Ontario
October 27, 2004

Background - Over the last two years, protein ingredient costs for human food, companion animal foods, and livestock feeds have been quite volatile, with large upward swings and lesser downward swings. For most pet food companies, it has been interesting times to say the least, and has had significant impacts on profitability. This paper will explore some of the reasons behind protein pricing volatility along with opportunities and challenges of using alternative proteins in companion animal foods.

The two North American cows diagnosed with bovine spongiform encephalopathy (BSE) in the last two years have had a significant impact on protein prices. This was primarily due to the immediate negative response of many foreign governments with the closing of borders to all ruminant-origin products from North America. The impact on human and pet foods was much more significant on the export market than on the domestic market. BSE is still a challenge for North America, not just Canada. Also, many countries have restricted the importation of poultry products for human and companion animal foods due to local/regional outbreaks of Avian Influenza in North America.

The North American soy harvest of 2003 and the South American soy harvest of 2004 was less than predicted. China's booming economy has resulted in a significant increase in demand for soy imports into China. The favorable exchange rate for the U.S. dollar has increased global demand for plant proteins, especially soy concentrates and soy isolates. Global soy stocks are near 50% of typical reserve levels, which may not be fully replenished even with a good North American crop in 2004. Early frost in some northern areas may have reduced yield, but most of the North American soy crop appears to be in good condition as of early September.

Low carbohydrate diets have significantly increased protein demand from all sources. Several speakers at the Institute of Food Technologists meeting in July 2004 predicted the demand for low carbohydrate products to triple in the next three years. There is also increasing consumer awareness of soy as a "healthy ingredient" for neutracuetical properties for human foods.

Fuel costs have increased freight rates more than 7% in the last year and are not expected to fall. Stricter transportation laws in the US require more downtime, resulting in reduced efficiency and higher rates charged by carriers. Natural gas costs have increased significantly over the last 24 months. Natural gas is a large part of processing costs for plant proteins, the same situation faced by pet food manufacturers. Packaging costs have increased approximately 6% in the last year as well.

The major animal protein sources for companion animal formulas include meat and bone meal, poultry byproduct meal, and lamb meal. Fresh beef, pork and poultry are also used many premium formulas. These protein sources will not be discussed further in this presentation.

The major plant proteins include soybean meal and corn gluten meal. Plant protein sources have not been associated with the negative connotations of animal diseases. Prices of plant proteins have certainly been affected by most of the above listed factors. Only protein sources with 20% crude protein or greater will be discussed in this paper. With the exception of soy proteins, there is very little published scientific literature, so much of the contents of this paper is based on experience with dogs, cats and other species.

The North American soybean meal price has jumped approximately 50% over the last two years. Pricing of other proteins, both plant and animal sources, normally follow soy prices. Pet food companies, today more than ever before, are competing with food manufacturers, foreign markets and animal feed manufacturers, for available plant proteins.

Plant Proteins - Unprocessed plant proteins, legume seeds in particular, typically contain anti-nutritional factors that reduce palatability, digestion and nutrient utilization when consumed by animals. This is one of Nature's natural defense mechanisms for plant survival. These factors include trypsin inhibitors, oligosaccharides, saponins, phytoestrogens, lectins and potentially allergenic proteins.

Soy protein has been recognized for several decades for its complementary amino acid pattern when combined with other proteins and grain sources for animal feeds. Soybean meal is the major protein source for animals for meat, milk and egg production. It is one of the commonly used plant proteins for pet foods, with approximately 3% of total US soybean production being used in companion animal foods.

Soybean meal in animal feeds and pet foods must have adequate heat processing to inactivate the trypsin inhibitor, to create the mild, sweet nut taste and flavor, and to increase digestibility and utilization by the animal.

In typical soy processing today, soybeans are cleaned and sorted, then the top-grade beans are dried so the hulls can be removed. The dehulled soybeans are then crushed to form a flake. The flakes are then subjected to solvent extraction to remove the oil. The oil is then further refined. The flakes, after solvent extraction and drying, are then ground to produce soy grits, soy flakes, soy flours and soybean meal, or further processed to produce textured vegetable protein TVP), soy protein concentrate (SPC) and soy protein isolate (SPI). Soybean meal is commonly used in dry extruded dog and cat formulas, while soy flakes and soy flour are typically used in treats to provide specific finished product textures.

Soy flakes can be processed alone or combined with other vegetable proteins to produce TVP. TVP is usually ground to various particle sizes for specific applications. TVP is frequently used in human foods and in canned and pouch pet foods as a meat extender. Once TVP or any other meat analog product has been re-hydrated, it must be handled as any other perishable food item.

Soy flakes can be subjected to extraction methods to remove most of the carbohydrate fraction (including most of the oligosaccharides) to produce SPC. Soy flakes can also be subjected to a multiple step process in which the protein fractions are precipitated and separated to produce SPI. SPC and SPI will not have the limitations associated with soybean meal. Some soy protein concentrates are further processed by additional heat treatment, which further reduces anti-nutritional factors, and also reduces water-holding capacity. SPC is frequently used in dog and cat treat formulas as a protein source, as a fat emulsifier, a water-binding protein and for specific finished product texture.

It has been well established by researchers and pet food manufacturers around the world that adult dogs fed only vegetable proteins maintain normal health. The dog is not a true carnivore (meat eater), but rather an omnivore – a meat and vegetable eater (Morris and Rogers 1989, Meyer and Kienzle 1991). It is common practice among nutritionists to use combinations of ingredients to optimize the amino acid, energy, fat, mineral and vitamin balances. Dogs prefer animal protein as a matter of taste preference. Very recent information suggests that taurine (found only in animal proteins and not plant proteins) may be an essential nutrient for dogs in some situations.

The cat, being a true carnivore, requires the amino acids taurine and arginine. Taurine is routinely supplemented in feline diets. Arginine is typically higher in animal proteins than plant proteins. These are some of the reasons that cats have a higher “protein” requirement than dogs.

Clapper et al (2000) demonstrated that soy proteins (soybean meal, soy flour, soy grits, TVP, SPC and SPI), when formulated as part of a balanced diet with other plant and animal proteins sources to optimize amino acids, can provide a nutritionally balanced, high quality diet for dogs. Research conducted by Kendall and Holme (1982) demonstrated the digestibility of plant materials. Wiernusz et al (1995) found SPC and SPI had higher total tract digestibility than did soy flour or soy grits in canned dog foods. Murray et al (1997) found similar digestibility of soy flour when compared to animal proteins. Zentek and Mischke (1997) reported soy protein isolate digestibility was similar to casein. Bednar et al (2000) found no differences in digestibility of dog food containing soybean meal, defatted soy flour, poultry meal, poultry byproduct meal or beef and bone meal.

Animal protein ingredients have incorrectly been assumed to be more consistent than plant proteins (Lowe 1989, Murray et al 1997). It is reasonable to assume that there is variation in components and processing methods used for both plant and animal protein sources among different suppliers and processing plants. These studies demonstrate that soy is an acceptable protein source for dogs and have similar digestibility as animal proteins in well-balanced canine formulas. The most significant negative factor for soybean meal is related to the naturally occurring carbohydrate fractions consisting primarily of raffinose and stachyose. These two oligosaccharides (amounting to 15-18% by weight) cannot be digested by mammalian enzymes, and thus are not digested and utilized by humans or animals. They can, however, be digested by many intestinal bacteria. When these oligosaccharides are digested (fermented), the typical end products of bacterial fermentation include carbon dioxide, hydrogen sulfide, other gases, lactic acid and other short-chain fatty acids. Flatulence can result from the gases produced, and if excessive, loose stools and occasional diarrhea may occur due to the osmotic imbalance created in the large intestine as a result of excess acid production. While flatulence may not be a major concern for outdoor dogs, it is objectionable to owners of indoor dogs. Loose stools and diarrhea are of significant concern to all pet owners. Fermentation of oligosaccharides, soluble sugars and soluble fiber also increases fecal volume due to greater bacteria excretion and greater water content. For these reasons many consumers and veterinarians have rejected pet foods that use soybean meal as a protein source. While increasing fiber intake is considered positive in the human population, the associated increase in fecal volume is not popular among pet owners who must clean up after their dogs.

Weirnusz et al (1995) found that reducing oligosaccharides by using SPC and SPI in dog foods reduced fecal volume and reduced the moisture level in feces. Results from Zuo et al (1996) show that reduced oligosaccharide levels did not reduce fecal volume compared to soybean meal. The difference between these two studies is that SPC and SPI reduced fiber levels as well as oligosaccharide levels, and this may largely account for the differences observed in these two trials. Higher fiber diets typically result in greater fecal volume in monogastric species due to the water holding capacity of the indigestible fiber fractions. When expressed on a 100% dry matter basis, most differences in fecal mass disappear. Thus, increased stool volume is not necessarily an indication of lower digestibility.

Zenteck (1995) observed that dogs fed meat and bone meal diet had higher concentrations of hydrogen sulfide than did dogs fed a soy-based diet, suggesting that diets containing meat protein only may result in more flatulence than diets with blends of animal and vegetable protein sources. (This is likely due to amino acid imbalance and an excess of sulfur amino acids relative to body needs in high meat protein diets).

Many allergies in dogs and cats are incorrectly associated with food allergies. Food allergies may make up 3-5% of observed allergic sensitivities and may affect 1-3% of the pet population. Heat processing of soybeans to soybean meal, soy grits, and soy flours reduces potential allergens by 65-95% of raw soy values. Further processing of soybean meals to SPC and SPI further reduces potential allergens to 0.01- 0.02% of soybean meal values. SPC and SPI are common ingredients in low allergen formulas for human infants, piglets, calves, and zoo diets.

Court and Freeman (2002) reported on the isoflavones content of commercial cat foods containing soybean meal. No animal feeding trial was conducted, so it is not known if the isoflavones content in typical diets are sufficient to produce a biological effect. The isoflavones concentration in some feline formulas was detected in amounts predicted to have a biological (hyperthyroidism) effect. For this reason, perhaps the maximum inclusion rate of soybean meal should be 10% in cat and reptile formulas. If this theory is correct, the isoflavones concentration in the diet would be reduced using SPC and SPI (as compared to soybean meal) due to processing methods.

Lectins (also known as hemagglutinins) are proteins with blood clot-promoting properties. Lectins are heat-labile and are denatured at lower temperatures than the trypsin inhibitors. Thus, heat treatments applied to inactivate trypsin inhibitors will also inactivate lectins.

Saponins are compounds that consist of either a sterol or a triterpenoid and water-soluble sugar residues. Saponins have been suggested to be anti-carcinogenic (Rao and Sung 1995), but also possess a bitter taste. Unlike saponins from other plant sources, soy saponins have little effect on active transport of nutrients in the small intestine (Johnson et al 1986). Saponins would be significantly reduced in SPC and SPI (as compared to soybean meal) due to processing methods.

From a practical standpoint, most manufacturers limit the amount of soybean meal used in pet food formulas. Inclusion levels of soybean meal at 10-12.5% of the formula usually minimize negative consumer responses to the pet food manufacturers. Due to the high potassium content of most soy ingredients, soy is a major contributor in high dietary cation-anion balance (DCAD) in diets. The exception is SPI, which is relatively low in potassium due to processing methods. Cats appear to be more sensitive to the taste (or other factors) in soybean meal than dogs.

The choice of soy product in a companion animal formula must be based on the purpose in the diet. The negative connotations sometimes associated with soy are mainly related to soybean meal and un-toasted soy flour, soy flakes and soy grits. These negative factors are significantly reduced when toasted soy flours, flakes, and grits or TVP, SPC or SPI are used.

Soy flours are available in several grades that will vary from 50-53% crude protein and 3-15% fat. There will be variable enzyme activity depending on the heat treatment used for each grade. If the formula is to be extruded at relatively high temperature and high shear, most of this enzyme activity will be denatured in the extrusion process.

Defatted, toasted soy grits, soy flakes and soy flours are recommended for most treat applications, depending on desired texture of the finished products. Soy grits will usually provide a courser texture in the finished product than will soy flour. Soy flakes and grits are used primarily in treats, semi-moist, pouch and canned formulas for texture and meat extenders, and secondarily as a protein source. There is some use of soy flour in biscuit manufacturing.

Soy protein concentrates (SPC) are typically 70% crude protein, with most of the soluble sugars and anti-nutritional factors being removed during processing. SPC are used for their high protein solubility, water binding, fat emulsification and bland flavor properties. Depending on the amount of heat applied, there will likely be some anti-nutritional enzyme activity present. However, when heat is applied to reduce the enzyme activity, it also reduces the water binding and fat emulsification (protein functionality) properties. If the SPC is being used in treats, semi-moist, pouch and canned applications, the water binding, fat emulsification and meat-replacement properties are very important. If the SPC application is for protein in biscuits, gravies, bastes or supplements, the protein functionality is much less important.

Soy protein isolates are typically 90% crude protein, have all of the characteristics of SPC, and have gone through additional protein isolation and concentration steps in processing. SPI are used in the same applications as SPC, but where a greater amount of crude protein or protein functionality is needed.

Textured vegetable protein (TVP) is mechanically textured using a combination of vegetable proteins. TVP is used primarily as a meat extender in treats, semi-moist, pouch and canned formulas. Once TVP is rehydrated, it will have a similar texture to meat, and must be handled as meat or other perishable food product. TVP will have a similar nutrient profile as soy flours. TVP is often added to real meat in canned products to enhance both nutrition and palatability.

Corn protein sources include corn gluten meal (60% crude protein, 2% crude fat, 2-3% crude fiber), corn gluten feed (21% crude protein, 3% crude fat, 5-15%

crude fiber), and corn germ meal (24% crude protein, 3% crude fat, 10% crude fiber). Corn protein products are typically low in lysine and tryptophan.

Corn gluten meal (60% crude protein) and **corn germ meal** (24% crude protein) are relatively low in sodium, potassium and chloride (low DCAD balance). **Corn gluten feed** (21% crude protein) is relatively high in crude fiber, depending on source, and higher in potassium, thus providing an intermediate DCAD contribution. These three corn proteins are made in the wet corn milling process, thus will be low in readily digestible carbohydrates. Corn gluten meal and feed are considered to be very palatable for companion animals. A 20% maximum inclusion rate is suggested for corn gluten meal. A 15% maximum inclusion rate is suggested for corn gluten feed and corn germ meal, primarily based on the high fiber and low lysine content.

Dried corn distillers' grains with solubles (25-30% crude protein, 8-10% crude fat, 4-12% crude fiber) is a product of ethanol production. Energy and fiber will be somewhat similar to oats. Corn fiber in these ingredients may be as fermentable as beet pulp and soy hulls. Since ethanol production is a yeast fermentation process, there will be minimal soluble carbohydrates (sugars and starches) in this product, but it will have residual yeast cells. It will be intermediate for DCAD contribution, due to the relatively high potassium content. Distillers' grains with solubles from fuel ethanol production should be tested for mycotoxins prior to acceptance for pet food production. Corn distillers' grains with solubles may be used up to 15% maximum inclusion rate based on the work of Allen et al (1981) and a review by Corbin et al (1980). Typical of corn protein, this product is low lysine and tryptophan. Distillers' grains with solubles will have a distinct "malt" odor and a slight bitter taste that does not appear to be objectionable as most dogs will find this product very palatable. The use of distillers' grains or distillers' solubles is not recommended due to the amino acid imbalances, but the combination as distillers' grains with solubles is acceptable when the formulator pays close attention to amino acid digestibility and balances. Dried distillers' grains with solubles can vary considerably in color due to variable heat processing and should be a golden color. Dark product (noticeable blackened spots to coffee color should be rejected due to heat-damaged protein and reduced digestibility.

The supply of distillers' grains with solubles will likely continue to increase due to increasing demand for ethanol-blended fuels. Corn, wheat, barley, sorghum and rye are also used for ethanol production, so formulators must specify to their purchasing department which grain distillers' product is needed.

Dried brewers' grains (26% crude protein, 7% crude fat, 13-14% crude fiber) is a product of beer and ale production and is primarily barley with less than 3% hops, but may some other grains as well. While there will be some residual yeast cells as not all of the solubles fraction is typically separated, most of the yeast is removed and sold as dried brewers yeast. Palatability of dried brewers grains for other species appear to be reasonably good, but this product is relatively high in phosphorus and sulfur, and low in calcium and potassium. A 10% maximum inclusion rate is recommended.

Wheat Proteins include vital wheat gluten and wheat germ meal. Vital wheat gluten (75-80% crude protein, 1-2% crude fat) is typically used to strengthen biscuits when low-grade flours are used. In treats, semi-moist, pouch and canned formulas, vital wheat glutens are used for texture and as meat extenders. It has high protein functionality, good water binding capacity and good fat emulsification properties. Vital wheat gluten is relatively low in lysine and high in total sulfur amino acids as compared to soybean meal. It is intermediate for DCAD balance. Wheat germ meal has an amino acid pattern that is consistent with wheat grain and has an intermediate DCAD balance. A 10% maximum inclusion rate is recommended for all pet food applications.

Canola meal (36% crude protein, 3.5% crude fat) is similar to corn gluten feed for fiber content, and has a similar amino acid pattern to soybean meal. It has a relatively low DCAD balance, but is somewhat unpalatable for dogs and cats. As with soy, canola must be heat-processed to reduce anti-nutritional factors. Full-fat canola seeds could be used in pet foods, but the hard seed coat must be fractured. The small whole seed size may be a handling problem in many plants. A 5% maximum inclusion rate is recommended for either canola meal or full-fat canola seeds. From a labeling and regulatory note, any oilseed **meal** implies that the oilseed has been fat-extracted.

Sunflower meal (35% crude protein, 1% fat, 20% crude fiber) is low in lysine, threonine, and tryptophan relative to soybean meal, and is intermediate for DCAD balance. It is considered somewhat unpalatable for dogs and cats, so a maximum inclusion rate of 10% is recommended. Whole full-fat sunflower kernels (small and broken kernels -20% crude protein, 40% crude fat) are sometimes used in bird and pet food applications.   As with any full-fat oilseed that has had the cell walls fractured, fat stability is a concern.

Full-fat Flaxseed (22% crude protein, 35% crude fat) is a minor source of protein, but the major source of omega-3 fatty acids in dry pet food applications. The lysine level is relatively low compared to soybean meal, and is intermediate for DCAD balance. A maximum of 5% inclusion rate is recommended for dogs and cats. Early 2004 frost may have damaged some seeds, so monitor quality parameters. Peroxide value of the oil should be less than 5 meg/kg for material coming into your manufacturing facility.

Rice Protein Concentrate (75% CP, 4% fiber) is lower in lysine, higher in total sulfur amino acids, but similar in other amino acids when compared to soy protein concentrates. Rice protein concentrate is available as a non-GM product for products that require non-GMO protein sources.

Peanut meal (45-48% crude protein, 6-12% crude fiber, 2-5% crude at) is ground defatted kernels and hulls. It is slightly lower in lysine, threonine and tryptophan when compared to soybean meal. Due to its distinctive flavor, it is generally used more as a flavoring agent rather than a protein source. Due to a variety of factors, peanuts are more susceptible to aflatoxin contamination than many other protein sources. Due to very low use rate in companion animal products, the incidence of allergic reactions to peanut meal in companion animals is unknown. Allergic reactions to peanuts in the human population are well documented. A practical maximum use rate of 2.5% is recommended. DCAD balance is low to intermediate.

Cottonseed Meal is generally not used in companion animal diets due to its low lysine, high gossypol, high fiber content and it is generally not very palatable. Cottonseed meal is not recommended for use in companion animal diets.

Yeast sources (42-46% crude protein, 1-3% crude fiber, 1-3% crude fat) may include the yeast cells as well as the remaining growth medium or the separated yeast cells. The yeast cells can be used as protein source as they are generally higher in amino acids than soybean meal. Yeast sources are used primarily for palatability enhancement and improving nutrient digestibility. A practical maximum inclusion rate is up to 3%. DCAD balance would be intermediate.

Potato protein (75% crude protein) may be an option for specific dietary applications. Potato protein is sometimes used in low-allergen and specialty diets. Uncooked potatoes should not be fed. Cooked potatoes are usually well tolerated in humans as cooking denatures patatin, the primary storage protein in potatoes. Potato allergies are not common, and when they do occur, appear to be related to uncooked potatoes, potato peelings and dust. It would be advisable to monitor the glycoalkaloid levels (generally below 150 mg/kg), which are potentially toxic in humans; and sulfite levels, as sulfites are sometimes added to potato protein preparations. Potato protein will have a very high DCAD balance due to high potassium level. No published papers describing the use of potato proteins in companion animals were found, but it is known that potato protein is being used in specialty commercial dog food formulas. A 5% maximum inclusion rate is recommended for companion animal diets. Availability may be a limiting factor.

Dried Peas (22% crude protein, 6% crude fiber, 1% crude fat) are sometimes used in specialty, low-allergin, organic and BARF formulas. Being a legume, there is a possibility of low levels of trypsin inhibitors that can be de-natured with heat treatment. Peas are a good quality protein source with a good balance of digestible complementary amino acids. A practical maximum inclusion rate of 15% is recommended due to potential natural estrogenic compounds. DCAD balance would be low to intermediate. Availability may be a limiting factor.

Other plant protein sources may include lentils, lupins, field beans, safflower meal, and sesame meal. These are not in common use, either in pet food manufacturing or in production livestock feeding. If these protein sources are considered, use swine and/or poultry feeding guidelines as a starting point.

Spray dried animal plasma is a very good protein source, being well balanced in amino acids, but high in sodium. There may be BSE issues from a regulatory and export viewpoint with bovine origin plasma, but usually not with porcine origin plasma. It is relatively expensive compared to most other protein sources. Palatability enhancement is observed in many species, so inclusion into a palatability enhancer may be a good option. A 5% maximum inclusion rate is recommended. **Spray dried blood meal** is low in isoleucine and is rather unpalatable, and is not recommended for use in companion animal diets.

Spray dried whole egg is an excellent protein source. Egg protein has been used as the reference protein to which all other proteins have been compared for humans for many years. Egg protein should be cooked to minimize avidin content (binds biotin) and must be salmonella-free. Food-grade egg protein will generally be higher cost compared to other protein sources. Off-spec product may be used in pet food formulas, but pet food manufacturers need to know why it has been downgraded before purchasing.

Several different types and grades of **Fishmeal** (60-70% crude protein) are available, with Menhaden being the most common. Other sources include white fish meal from cod or haddock, and meals from sardines, herring, salmon, tuna, and others. Typical processing is drum-drying under partial vacuum to maintain higher protein quality and amino acid digestibility. Fishmeal should be low in fat (less than 4%), as fat rancidity is a major concern. A maximum inclusion rate of 5% is reasonable for most formulas due to the high ash content (10-15%).

Dried Skim Milk (34% crude protein) may be used in puppy and kitten formulas at 1-2.5%. Dried skim milk is used extensively for human consumption as “non-fat dry milk solids” or ‘defatted milk solids”, so pet food manufacturing will be competing with food manufacturers for the limited supply, making this generally too expensive for most pet food applications. Lactose content may be a problem for some individual animals.

Other animal proteins may include **deer and elk**, but these would have the same ruminant protein restrictions as beef. If considered, use material only from government-inspected slaughter facilities processing animals for human consumption. **Ostrich, emu and duck** have also been used in specialty, Rx and low allergy formulas. Supply of these protein sources may be limited in some areas.

Information on these ingredients may also apply to other animals, including ferrets, small rodents, fur-bearing animals, fish, shrimp and reptiles.

Summary – Several plant and animal protein sources have been reviewed in this presentation. Each source needs to be evaluated relative to nutrition, cost and how it may fit into your company's marketing objectives.

There is increasing competition among food manufacturers, pet food manufacturers, animal feed manufacturers and export interests for available plant protein sources. It is anticipated that there will be market swings in protein costs, but costs will likely continue to be higher than previous years.

References:

Allen, S.E., G.C. Fahey, Jr., J.E. Corbon, J.L. Pugh and R. A. Franklin. 1981. Evaluation of Byproducts Feedstuffs as Dietary Ingredients for Dogs. J. Animal Science 53(6):1538-1544.

Association of American Feed Control Officials. 2004. Official Publication.

Bednar, G.E., S.M. Murray, A.R. Patil, E.A. Flickenger, N.R. Merchen and G.C. Fahey, Jr. 2000. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of illeally cannulated dogs. Arch. An. Nutr. 53:127-140.

Clapper, G.M, C.M. Grieshop, N.R. Merchen, J.C. Russett, J.L. Brent, Jr, and G.C. Fahey, Jr. 2001. Ileal and total tract digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. J. Animal Science 19:1523-1532.

Corbin, Jim., George C. Fahey, Jr., and Judith L. Pugh. 1980. Distillers Dried Grains with Solubles in Digestibility Tests with Dogs. Proceedings Distillers Feed Conference. 35:29-33.

Court, M.H. and L.M. Freeman. 2002. Identification and concentration of soy isoflavones in commercial cat foods. Am J Veterinary Research 63(2):181-185.

Johnson. I.T., J.M. Gee, K. Price, C. Curl and G.R. Fenwick. 1986. Influence of saponins on gut permeability and active nutrient transport. J. Nutrition 116:2270-2277.

Kendall, P.T. and D.W. Holme. 1982. Studies on the digestibility of soya bean products, cereals, cereal and plant by-products in diets of dogs. J. Sci. Food Agric 33:813-822.

Lowe, J.A. 1989. Effective use of new and novel concepts in pet food design. In: Biotechnology in the Feed Industry: Proceedings Alltech Fifth Annual Symposium p 209-221.

Meyer, H. and E. Kienzle. 1991. Dietary protein and carbohydrates: Relationships to clinical disease. In: Proceeding of the Purina International Symposium. St Louis, MO. p 13-26.

Morris, J.G. and Q.R. Rogers. 1989. Comparative aspects of nutrition and metabolism of dogs and cats. In: Nutrition of the dog and cat. Waltham Symposium 7. I.H. Berger and J.P.W. Rivers, editors. Cambridge University Press, Cambridge, U.K.. p 35-66.

Murray, S.M., A.R. Patil, G.C. Fahey, N.R. Merchen and D.M Hughes. 1997. Raw and rendered animal byproducts as ingredients in dog foods. J. Animal Science 75:2497-2505.

Rao, A.V. and M.K. Sung. 1995. Saponins as Anticarcinogens. J Nutrition 125(3S):717S-724S.

Wiernusz, C.J., R. G. Shields, D.J. Van Vlierbergen, P.D. Kigin and R. Ballard. 1998. Canine nutrient digestibility and stool quality evaluation of canned diets containing various soy protein supplements. Vet. Clin. Nutr. 2:49-56.

Zentek, J. 1995. Influence of diet composition on the microbial activity in the gastro-intestinal tract of dogs. III. In vitro studies on the metabolic activities fo the small-intestinal microflora. J. Anim Physiol Anim Nutr. 74:62-73.

Zentek, J. and R. Mischke. 1997. Soya and casein as dietary proteins for dogs. J. Anim Physiol Anim Nutr. 77:139-148.

Zuo, Y., G.C. Fahey, Jr., N.R. Merchen and N.L. Bajjalieh. 1996. Digestion responses to low oligosaccharide soybean meal by ileally-cannulated dogs. J. An. Sci 74:2441-2449.