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Dietary Content and Gastrointestinal Function of Soybean Oligosaccharides in Monogastric Animals

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1. Introduction

Soybean is a major ingredient in non-ruminant animal diets throughout the world. There is an extensive body of information suggesting that soybean is an excellent source of high quality protein, whereas less attention has been paid to soybean oligosaccharides. Soybean oligosaccharides, also referred to as α -galacto-oligosaccharides, oligosaccharides of the raffinose family or simply α -galactosides, are water-soluble, low-molecular weight carbohydrates raffinose, stachyose and verbascose. In maturing seeds, oligosaccharides are formed by successive addition of galactosyl moieties to a sucrose primer. Alpha-galactosides are characterized by the presence of $\alpha(1\rightarrow6)$ linkages between galactose moieties which are bonded via $\alpha(1\rightarrow3)$ to terminal sucrose. Unlike other oligosaccharides, soybean α -galactosides can be extracted directly from the raw material and do not require enzymatic manufacturing processes.

Soybean oligosaccharides comprise approximately 4% of the soybean dry matter (DM) and during processing in the preparation of soybean meal (SBM) they are not removed or destroyed. Therefore, in SBM, α -galactosides represent approximately 5-6% but could be as high as 8% DM. Other processed soybean products, however, may contain significantly less oligosaccharides than SBM. The oligosaccharide content of soy protein concentrates (SPC) is as low as 3% DM while soy protein isolates (SPI) contain only trace amounts of oligosaccharides.

Soybean oligosaccharides appear to be indigestible in the upper intestinal tract of monogastric animals due to the absence of α -galactosidase enzyme. However, they are easily fermented by the lower gut microflora, resulting in the production of various gases and short-chain fatty acids. Studies have shown considerable microbial fermentation of α -galactosides in the small intestine with some authors referring to soybean oligosaccharides as bifidogenic factors which stimulate the growth of beneficial bacteria and others claiming that increased consumption of oligosaccharides may lead to negative effects in the large intestine of mammals, such as flatulence, diarrhea, and excessive dietary protein decay.

The content of α -galactosides in animal diets usually ranges from 0.5 to 3% and, since SBM is a rich source of oligosaccharides, an increase in the SBM content of the ration results in an increase in the concentrations of α -galactosides. A trend for increased inclusion of SBM in animal diets has been observed in recent decades due to the EU ban on the use of meat-and-bone meals and a decrease in fishmeal production. In animals whose nutrient requirements are high, such as young meat-type turkeys, the SBM content of the diet may be as high as 50%. Therefore, the objective of our research presented in this chapter is to determine whether excessive amounts of α -galactosides in turkey diets may increase the risk of diarrhea and result in reduced growth performance.

2. Chemical properties and occurrence of α -galactosides

Oligosaccharides, next to sucrose, are the most widely distributed water-soluble carbohydrates in the plant kingdom (Han & Baik, 2006). Oligosaccharides (the name is derived from the Greek word *oligos*, meaning a few) are compounds that yield only monosaccharide units upon complete hydrolysis (Kadlec et al., 2001). Depending on the number of monosaccharide residues, oligosaccharides are classified as trisaccharides, tetrasaccharides and so forth. The main group of oligosaccharides present in SBM are the raffinose family oligosaccharides (RFOs), so named after the first member of this homologous series of α -galactosides, which are characterized by the presence of $\alpha(1\rightarrow6)$ links between the galactose moieties (Han & Baik, 2006). In addition to raffinose, this group comprises stachyose, verbascose and ajugose, which consist of 1, 2 and 3 $\alpha(1\rightarrow6)$ linked units of galactose bonded through $\alpha(1\rightarrow3)$ to terminal sucrose (Kadlec et al., 2001). The remaining α -galactosides are galactosyl cyclitols, mainly ciceritol (Barnabé et al., 1993) and unnamed longer-chain oligosaccharides up to nonasaccharides (Cerning-Beroard & Filiatre-Verel, 1976). According to the International Union of Pure and Applied Chemistry, raffinose is a trisaccharide (α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside) composed of fructose, glucose and galactose. Stachyose (a tetramer) consists of two α -D-galactose units, one α -D-glucose unit, and one β -D-fructose unit sequentially linked as α -D-Galp-(1 \rightarrow 6)- α -D-Galp-(1 \rightarrow 6)- α -D-Glup(1 \rightarrow 2)- β -Fru. Verbascose is a pentasaccharide with a longer chain of galactose units joined to sucrose as α -D-Galp-(1 \rightarrow 6)- α -D-Galp-(1 \rightarrow 6)- α -D-Galp-(1 \rightarrow 6)- α -D-Glup(1 \rightarrow 2)- β -D-Fru.

Chemically, α -galactosides are low molecular weight non-reducing carbohydrates that are soluble in water and aqueous alcohol solutions (Arentfot et al., 1993). They are associated with the onset of desiccation tolerance during seed development, and with seed storability (Blackman et al., 1992; Horbowicz & Obendorf, 1994; Obendorf et al., 1998). The synthesis of α -galactosides is also affected by growing conditions and the rate of seed maturation. Obendorf et al. (1998) found that the axes of seed matured at 25°C accumulated higher concentration of sucrose and raffinose, whereas stachyose content remained unchanged.

Among the grain legume crops grown in Europe, faba bean and lentil seeds are characterized by a low α -galactoside content, while pea seeds contain a moderate and lupin seeds contain a high level of α -galactosides. (Table 1). Although lupine seeds have a relatively high α -galactoside content they play a limited role in intensive animal farming. Pea, lentil and faba bean seeds are used in animal diets but not as extensively as soybean. Soybean α -galactosides comprise approximately 4% of the soybean dry matter (Karr-Lilienthal et al., 2005). During SBM processing, α -galactosides are not removed or destroyed, therefore, in toasted SBM α -galactosides may account for 5% (Seve et al., 1989;

	Lentil	Pea	Faba bean	White lupine	Narrow leaf lupine	Yellow lupine
Raffinose	0.3	0.8	0.2	0.7	1.4	2.2
Stachyose	1.7	2.5	0.9	6.6	5.5	6.9
Verbascose	0.4	1.7	1.4	0.5	2.2	2.8
Total	2.4	5.0	2.5	7.8	8.6	11.9

Table 1. The α -galactoside content of the seeds of legume crops (%) (Kozłowska et al., 2001)

Coon et al., 1990), 6-7% (van Kempen et al., 2006) or even 8% of DM (Grieshop et al., 2003). Some authors reported a lower, i.e., below 3% α -galactoside content of SBM, DM (Smirickey et al., 2002). Similar and considerably lower α -galactoside levels were observed in the seeds of selected and genetically modified soybean lines (Kerr et al. 1993; Parsons et al., 2000; Neus et al., 2005). Low-oligosaccharide SBM obtained from the seeds of the improved lines would contain only 0.2-0.5% α -galactosides (Parsons et al., 2000). In the future, such soybean lines could be grown on a large scale providing yields comparable to that of conventional varieties.

Soy products, mainly SBM, have superior nutritional characteristics in terms of a high protein content and amino acid profile, which is why SBM is a major protein source in swine (Cromwell, 2000) and poultry diets (Baker, 2000; Grieshop et al., 2000). In addition to protein, SBM contains over 30% total carbohydrates (Grieshop et al., 2003). Approximately one third of which are non-structural low molecular weight carbohydrates, including oligosaccharides (Karr-Lilienthal et al., 2005). Diets for growing pigs with an average content of SBM of 16% contain less than 1% α -galactosides (Kozłowska et al., 2001). The α -galactoside content of poultry diets usually ranges from 0.5 to 3%, with the main sources, in decreasing order, being: soybean meal (6% DM), pea (5%), faba beans (4%), rapeseed meal (3%) and sunflower meal (2%) (Carré et al., 1984).

The seeds of soybean and other legume species intended for human consumption are processed by soaking, cooking, irradiation, fermentation and enzymatic treatment to decrease the α -galactoside content (Machaiiah & Pednekar, 2002; Gote et al., 2004; Egounlety & Awort, 2003; Yoo & Hwang, 2008).

Table 2 shows changes in the levels of raffinose and stachyose in raw and cooked soybean seeds subjected to standard soaking, soaking under ultrasound and soaking under high hydrostatic pressure. After 3 h soaking, the raffinose and stachyose contents of uncooked seeds decreased from 6.01 to 4.01% and from 3.50 to 1.87%, respectively. A more significant decrease in the levels of raffinose and stachyose was observed in seeds soaked under ultrasound and under high hydrostatic pressure. The lowest content of raffinose (1.03%) and stachyose (1.30%) was noted in cooked seeds soaked under ultrasound for 3 h (Han & Baik, 2006). Cooked seeds soaked under high hydrostatic pressure contained the largest amounts of oligosaccharides.

The α -galactoside content of SBM used in poultry and swine diets may be decreased either by the extraction with ethanol (Seve et al., 1989; Coo et al., 1990; Leske et al., 1993; Irish et al., 1995; Leske et al., 1999a, b) or by the development of genetically modified soybean varieties (Frias et al., 1999; Parsons et al., 2000; Grieshop et al., 2003; van Kempen et al., 2006). The use of SPC and SPI represents another means of reducing dietary soybean α -galactosides.

Table 3 shows selected chemical components of various soybean products used in turkey diets (Jankowski et al., 2009). The content of soluble sugars, including α -galactosides, was

over two-fold lower in SPC than in SBM. Somewhat smaller differences between SBM and SPC were noted for dietary fiber fractions. Soy protein isolate had the lowest content of soluble sugars and structural carbohydrates while hulls were the richest source of fiber components resulting in limited use of these latter product in animal diets.

Sample	Treatment	Raffinose ¹	Stachyose ¹
Uncooked seeds	Soaked, 3 h	4.01	1.87
	Soaked, 12 h	2.63	1.53
	Soaked under ultrasound, 1.5 h	3.21	3.11
	Soaked under ultrasound, 3 h	2.66	2.50
	Soaked under high hydrostatic pressure, 0.5 h	4.10	3.48
	Soaked under high hydrostatic pressure, 1 h	3.97	3.24
Cooked seeds	Unsoaked	6.93	5.48
	Soaked, 3 h	3.62	2.97
	Soaked, 12 h	2.96	2.43
	Soaked under ultrasound, 1.5 h	3.35	3.10
	Soaked under ultrasound, 3 h	1.03	1.30
	Soaked under high hydrostatic pressure, 0.5 h	3.24	2.99
	Soaked under high hydrostatic pressure, 1 h	2.64	2.40

¹The initial raffinose and stachyose content of seeds was 6.01% and 3.50%, respectively.

Table 2. The raffinose and stachyose content of soybean seeds processed by various methods (Hab & Baik, 2006)

Component	Soybean product			
	Soybean meal	Protein concentrate	Protein isolate	Hulls
Crude protein	47.0	64.9	85.9	12.7
Soluble sugars				
Monosaccharides	0.9	0.5	0.7	1.3
Sucrose	5.7	1.0	0.1	1.5
Oligosaccharides	5.3	2.5	0.1	0.8
Dietary fiber fractions				
Crude fiber	3.5	2.8	0.2	36.4
Acid detergent fiber	5.5	4.4	nd	42.3
Neutral detergent fiber	7.7	7.1	0.2	55.0
Non-starch polysaccharides (NSP)				
Total NSP	12.6	12.9	1.3	46.1
Water-soluble NSP	2.0	1.9	0.3	6.6
Water-insoluble NSP	10.5	11.0	1.0	39.5

Table 3. The content of crude protein and carbohydrate fractions (%) in different soybean products (Jankowski et al., 2009)

The utility of selected soybean products (including SPC and SPI) in the nutrition of baby pigs and chickens has been demonstrated (Coon et al., 1990; Sohn et al., 1994; Russett, 2002; Batal & Parsons, 2003). Swick (2007) indicated that the major advantage to the use of SPC and SPI is that diets higher in density and lower in water-soluble carbohydrates may be formulated and also the proteins are less allergenic.

3. Physiological properties of α -galactosides in the gastrointestinal tract and any potential antinutritive effects of their presence in the diet

Because of the lack of appropriate mucosal enzymes in the small intestine of monogastric animals, α -galactosides are considered as non-digestible carbohydrates. However, the oligosaccharides pass into the lower gut and are fermented by the intestinal microflora (Saini & Gladstone, 1986; Veldman et al., 1993; Price et al., 1988). Alpha-galactosides can also be fermented, to some extent, by bacterial populations in the distal ileum of non-ruminants (Liyang et al., 2003). This fermentation pattern may result in both positive (bifidogenic) and negative (antinutritional) effects (Karr-Lilienthal et al., 2005).

For many years α -galactosides have been considered as antinutritional factors. Kuriyama and Mandel (1917) were the first to report that a meal containing 3 or 5 g raffinose resulted in severe diarrhea in rats. Such response can be explained by the fact that the intestinal mucosa of humans and monogastric animals lacks the enzyme α -galactosidase required to cleave $\alpha(1\rightarrow6)$ linkages (Gitzelman & Auricchio, 1965). As a result, dietary raffinose and stachyose may produce diarrhea resulting in an increased digesta passage rate and decreased digestion and absorption of dietary nutrients (Wiggings, 1984).

For the past two decades increased attention has been paid to the antinutritional effects of α -galactosides in intensively fed animals, including fast-growing broiler chickens. It was found that the concentration of metabolizable energy in SBM was low in comparison to the gross energy content (Pierson et al., 1980), and subsequently the presence of α -galactosides in SBM was implicated as the major reason for the low metabolizability of energy (Coon et al., 1990). The above finding was validated by the results of experiments in which chickens were fed ethanol-extracted SBM with no raffinose and stachyose (Coon et al., 1990; Leske et al., 1999a, b). Leske et al. (1995) found that when fed to chicks ethanol-extracted SBM contributed to better protein utilization and amino acid availability than did non-extracted SBM. The negative effects of dietary oligosaccharides on nutrient utilization as manifested in reduced energy digestibility (Leske et al. 1993) and reduced ileal digestibility (Bedford, 1995) have been related to a reduction of up to 50% in intestinal digesta passage rate and to elevated hygroscopic properties of excreta. Careé et al. (1995) found an apparent α -galactoside digestibility of 87% in broiler chickens and 99% in adult cockerels, which suggested extensive (increasing with age) microbial fermentation in the lower part of the gastrointestinal tract. In this regard, the efficiency of energy utilization from the products of microbial fermentation is lower compared with the utilization of energy from carbohydrates directly absorbed from the small intestine. It has also been demonstrated that the ethanol extraction of SBM decreased energy loss due to a reduced amount of hydrogen gas produced by the chicks fed ethanol-extracted SBM in comparison to control meal (Leske et al., 1999b).

In a study with cannulated piglets, it was found that 3 h after feeding 39% of α -galactosides disappeared from the stomach and small intestine (Gdala et al., 1997). The relatively high digestion of α -galactosides in the upper gut may be attributed to the presence of

endogenous plant and microbial α -galactosidase. Although the results have been inconclusive, attempts have been made to alleviate the adverse effect of α -galactosides by the addition of fungal α -galactosidase to diets. In one of the first studies on the use of fungal α -galactosidase in piglet diets it was demonstrated that the addition of this enzyme did not alleviate the adverse effects of α -galactosides in legume-based diets. It was concluded that an increase in fermentable substrates in the lower part of the digestive tract may disturb the microbial balance, thus increasing the risk of diarrhea (Veldman et al., 1993). In another experiment it was shown that the addition of pectinase and α -galactosidase to broiler diets tended to improve growth performance and increased ($P=0.06$) apparent metabolizable energy content, from 12.13 to 12.55 MJ/kg (Igbasen et al., 1997). In another study dietary supplementation with α -galactosidase significantly increased the cumulative feed intake in chickens, without any apparent effect on α -galactoside digestibility (Daveby et al., 1998). Addition of exogenous α -galactosidase has shown no beneficial effect on the ME content of SBM (Irish et al., 1995).

Relatively few studies on the physiological properties of α -galactosides have been conducted in the last decade. In one experiment, the average true metabolizable energy content of SBM with low total raffinose, stachyose and galactitol content (0.7% and 0.25%) was 9.8% higher ($P<0.05$) compared with conventional SBM (Parsons et al., 2000). Positive results were reported for an enzyme cocktail containing multi-activities, including α -galactosidase, α -amylase, β -glucanase, protease, xylanase, and cellulase. Broilers fed diets supplemented with the enzyme cocktail showed a better feed conversion ratio, although no effects on growth, immunity, or carcass attributes were noted (Kidd et al., 2001). In another experiment, application of α -galactosidase reduced the stachyose and raffinose content of enzyme-treated SBM by 69% and 54%, respectively, and decreased the concentrations of these oligosaccharides in the excreta ($<0.1\text{mg/g}$), but it did not influence the growth of chickens (Graham et al., 2002). In a similar study, although significant oligosaccharide hydrolysis in the chicken gut was achieved (57%), no improvement in growth performance was noted (Slominski et al., 2006).

In a study with cannulated young pigs, soy oligosaccharides reduced nutrient digestibility, but the reduction was small, ranging from 1.1 to 7.4 percentage units (Smiricky et al., 2002). The results of another experiment with growing pigs indicated that the ileal digestibility of α -galactosides added to a semi-purified diet was higher than 75% (Smiricky-Tjardes et al., 2003). According to some authors (Liyang et al., 2003), oligosaccharides at the concentrations found naturally in a typical corn-SBM diet may have little effect on nutrient digestibility. However, it has been demonstrated that nutritionally relevant variability does exist in soy varieties and that a low stachyose content is important for maximizing energy utilization (van Kempen et al., 2006). In addition, soybean meal obtained from low-oligosaccharide varieties may have higher concentrations of most essential amino acids than the conventional SBM. The results of the above experiments and similar studies (Iji and Tivey, 1998) indicate that soybean oligosaccharides can be regarded as a factor capable of decreasing the health status and growth rate of animals.

4. Soybean oligosaccharides as potential prebiotics

Prebiotics are dietary ingredients, typically oligosaccharides and polysaccharides with defined properties, administered intentionally to improve and stimulate the growth and activity of intestinal microflora, and thereby reduce the risk of disease. To be considered as a prebiotic, a compound must conform to the following: (1) it must be resistant to digestion in

the upper gastrointestinal tract (remain unaltered through hydrolytic-enzymatic digestion), (2) it must selectively stimulate one or a limited number of beneficial microbiota and (3) it must benefit host health by improving colonic microbiota composition (Roberfroid et al., 1998). According to Gibson et al. (2004), a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microflora, that confer benefits upon host well-being and health.

It is well known that the most important characteristic of non-digestible carbohydrates is their fermentability by bacteria in the large intestine of animals and humans. The main products of the fermentation processes are short-chain fatty acids (SCFAs) and the gases: H₂, CO₂, and CH₄ (Krause et al., 1994). The health status of the gastrointestinal tract is significantly affected by the amounts and proportions of SCFAs (acetate, propionate and butyrate), bacterial enzyme activity (e.g. pro- or anti-carcinogenic activity), the content of different bacterial metabolites in feces (e.g. phenols, cresols, products of bacterial breakdown of protein and urea) as well as by the amount and bulking of stool (Salminen et al., 1998, Loo et al., 1999). All SCFAs are rapidly absorbed from the hindgut where they stimulate salt and water absorption. The SCFAs are metabolized principally by the gut epithelium, the liver and muscles, with virtually none appearing in urine and only small amounts present in feces (Salminen et al., 1998). These compounds play a very important role in the function of the large bowel as an energy source for the colonic epithelium. Particularly important for large bowel health is butyrate, which regulates epithelial cell growth and differentiation (Miller & Wolin, 1996).

The name "prebiotics" may be given to the saccharides which selectively enhance the populations of beneficial microflora, primarily endogenous lactic acid bacteria and *Bifidobacteria* (Delzene and Roberfroid, 1994; Gibson and Roberfroid, 1995; Walker and Duffy, 1998). Such properties have been demonstrated for fructans (inulin and oligofructose) obtained from plant sources (mainly chicory root) and the products of biotechnological processing (enzymatic transglycosylation of sucrose). Fructans are among the most popular prebiotic supplements, available with 60% of the publications on the topic of prebiotic supplementation being devoted to fructans (Barry et al., 2009). Reports on the use of soybean α -galactosides as potential prebiotics are scarce, comprising only 9% of the prebiotic literature available. According to one of the first publication dealing with the production of commercial prebiotic preparations, the estimated production of soybean oligosaccharides (based on data obtained by surveying major manufactures of food-grade oligosaccharides) was 2000 tons (Crittenden and Playne, 1996). However, relevant data for subsequent years are not available. The above work is cited in a recent paper (Wang, 2009). Under laboratory and semi-technical conditions, α -galactosides are also extracted from the seeds of legume species other than soybean. The physiological properties of those products may vary. Depending on the plant source and the degree of purification, α -galactoside preparations contain different amounts of stachyose, raffinose, verbascose and sucrose (Table 4.)

In one of the very first experiments it was found that α -galactosides obtained from SBM were well utilized by beneficial bacteria, and also reduced the activity of enzymes specific to pathogenic bacteria (Masai et al., 1987). The ingestion of 10 g of soybean oligosaccharide extract (23% stachyose and 7% raffinose) significantly increased the counts of bifidobacteria in six healthy adult males (Hayakawa et al., 1990). A recent study on young female volunteers showed that a soy oligosaccharide intake of 3 g/day was enough to increase fecal bifidobacteria counts, short-chain fatty acids concentration, and fecal lipid output (Bang et al., 2007).

Component	Soybean		Pea		Lupine	
	Semi-pure ¹	Pure α -G ¹	Semi-pure ²	Pure α -G ³	Semi-pure ²	Pure α -G ³
Dry matter	97.0	95.0	95.6	95.3	94.0	97.0
Sucrose	44.0	2.0	24.5	20.3	35.0	9.1
Raffinose	7.0	20.0	12.3	4.0	3.1	30.2
Stachyose	23.0	71.0	40.9	51.5	32.7	47.0
Verbascose	-	-	-	19.5	-	10.7
Other	23.0	2.0	17.9	-	23.2	-

¹Masai et al., 1987, ²Juskiewicz et al., 2003, ³Gulewicz et al. 2002

Table 4. Average content of α -galactosides and sucrose in α -galactoside (α -G) preparations obtained from soybean, pea and lupine seeds

In an *in vitro* experiment, the addition of soybean oligosaccharides to a growth medium increased the counts of bifidobacteria, measured as a proportion of total viable counts in the culture. Azoreductase, β -glucuronidase and β -glucosidase activities decreased, but only the activity of azoreductase was lowered significantly by soybean oligosaccharide treatment (Saito et al., 1992). In an experiment with young women who received 1.5 or 3 g/day of soy oligosaccharides for 30 days (LSO and HSO group, respectively), the counts of bifidobacteria in feces increased significantly in the HSO group. Significantly higher concentrations of fecal SCFAs, propionate and butyrate, were also noted in this group.

In an experiment with young pigs, dietary supplementation with 1% stachyose increased the counts of lactobacilli in the ileum as well as bifidobacteria in the cecum and colon. However, the weight gains of piglets were lower compared with the control group (Liyang et al., 2003). A higher content of stachyose in the diet (2%) had a negative effect on body weight gain of piglets and lactobacilli and bifidobacteria counts in the cecum. The data indicate that at least a portion of the growth depression observed when soybean is included in the diet of weaning pigs can be attributed to the presence of α -galactosides (mainly stachyose). In a similar experiment, the dry matter content of cecal digesta was significantly lower when compared to control and the activities of bacterial β - and α -galactosidase, α -glucosidase and β -glucuronidase were significantly higher in rats fed diets containing oligosaccharides extracts from pea or lupine seeds (see Table 4) (Juśkiewicz et al., 2003). Compared with cellulose, the total production of SCFAs in the cecum was significantly higher when a diet contained 4.9% of α -galactosides and it tended to be lower when α -galactoside content decreased (3.9%). According to Lan et al. (2004), soybean oligosaccharides can increase the survival rates of lactic acid producing bacteria in broiler chickens infected with *E. tenella*. Furthermore, in a subsequent study Lan et al. (2007) revealed that soybean oligosaccharides can increase the population of a group of lactic acid bacteria (of the genera *Lactobacillus*, *Pedicoccus*, *Weissella* and *Leuconostoc*) in the cecal contents of young broiler chickens. The authors concluded that soybean oligosaccharides show promise for use as a product which may promote competitive exclusion of potential pathogens in young broiler chickens. However, they emphasized that the selective proliferation of *Lactobacillus* by soybean oligosaccharides could not be confirmed.

The results of experiments with chickens have indicated that the physiological effect of α -galactosides would depend on their concentration in the diet; with the level of 0.4% not affecting metabolizable energy and amino acid digestibilities, but level above 0.8% potentially depressing energy utilization (Biggs et al., 2007).

5. The physiological effects of α -galactosides in turkey diets

Little is known how turkeys respond to a different content of α -galactosides in their diets, particularly when SBM is added at a relatively high amount to replace meat and meat-and-bone meals. Meat-type turkeys have high protein and essential amino acid requirements. Starter diets from 1 to 4 weeks of age, should contain at least 27% of protein (NRC, 1994). In the absence of alternative high-protein components, the SBM content of turkey diets is often very high, approaching 50%, with the α -galactoside level exceeding 2.5%. As shown in Table 5, the α -galactoside content of diets for young growing turkeys may be decreased provided that SBM is partially or entirely replaced with other high-protein soy products, including SPC and SPI (Jankowski et al., 2009). In this study, the use of SPC as a replacer for SBM had no effect on the concentrations of non-starch polysaccharides (NSP). The content of water-insoluble NSP was considerably lower in a diet containing SPI, compared with the other groups (8% vs. 10%). The results of an experiment in which turkeys were fed diets from 0 to 8 weeks of age are presented in Table 6.

Ingredient, %	Starter diets from 1 to 4 weeks of age			
	SBM	SBM-SPC	SPC	SPI
Soybean meal (SBM)	44.3	28.3	3.9	-
Soybean protein concentrate (SPC)	-	10	25.3	-
Soybean protein isolate (SPI)	-	-	-	19.5
Soybean hulls	2.5	2.8	3.2	4.8
Soybean oil	5.5	3.6	0.7	-
Wheat	42.3	49.9	61.5	69.9
Minerals, amino acids, vitamins	5.4	5.4	5.4	5.8
Crude protein	27.0	27.0	27.0	27.0
Crude fiber	3.4	3.5	3.4	3.2
Oligosaccharides	2.6	2.0	0.9	0.2
Non-starch polysaccharides	10.2	10.2	10.1	8.0

Table 5. Composition of turkey diets containing different soybean products (Jankowski et al., 2009)

In the second phase of the experiment (4 to 8 wks) the diets contained lower amounts of soybean products, proportionally to the lower concentration of total protein (25.5%), and therefore the α -galactoside content decreased to 2.3, 1.7, 0.9 and 0.05%, respectively. When substituted for SBM, SPC and SPI increased in the weight and water content of cecal digesta in turkeys of 4 weeks of age. No increase in the activity of microbial β -glucosidase was noted, whereas the activity of β -glucuronidase produced by the potentially pathogenic bacteria increased in turkeys fed SPC and SPI. There were no differences in the quantities of SCFAs produced in the ceca. In turkeys of 8 wks of age, the concentration of SCFAs produced in the ceca was proportional to the dietary α -galactoside content. In comparison with other dietary groups, the average body weight of turkeys fed SPI was significantly lower. A reduction in the dietary α -galactoside content in the second phase of the experiment did not result in any beneficial effects. It was found that partial or almost complete replacement of SBM with SPC in turkey diets, which was associated with a decrease in dietary oligosaccharide content, suppressed the fermentation process in the

Item	Main protein source in the diet			
	SBM	SBM-SPC	SPC	SPI
Cecal parameters at 4 weeks of age				
Cecal digesta weight, g/kg of BW	1.91 ^c	2.19 ^{bc}	2.94 ^a	2.83 ^{ab}
Dry matter of digesta, %	14.7 ^c	14.6 ^c	19.4 ^a	18.8 ^{ab}
β -glucosidase activity, U/g	0.20	0.14	0.27	0.28
β -glucuronidase activity, U/g	0.34 ^{bc}	0.39 ^c	0.50 ^a	0.45 ^{ab}
SCFA pool, mol/kg of BW	208.6	208.2	232.1	204.5
Cecal parameters at 8 weeks of age				
Cecal digesta weight, g/kg of BW	2.98	2.71	2.48	2.52
Dry matter of digesta, %	15.8 ^b	17.9 ^b	17.3 ^b	21.6 ^a
β -glucosidase activity, U/g	0.47	0.43	0.43	0.42
β -glucuronidase activity, U/g	0.98	0.69	0.65	0.60
SCFA pool, mol/kg of BW	380.6 ^a	255.5 ^b	218.0 ^b	214.7 ^b
Body weight of turkeys, kg				
4 weeks of age	1.074 ^a	1.078 ^a	1.075 ^a	1.038 ^b
8 weeks of age	4.324 ^b	4.452 ^a	4.459 ^a	4.282 ^b
Feed conversion ratio, kg/kg				
0 to 4 weeks of age	1.416 ^a	1.469 ^a	1.416 ^a	1.368 ^b
0 to 8 weeks of age	1.760 ^a	1.787 ^a	1.742 ^a	1.667 ^b

Table 6. Selected results of an experiment in which different soybean products were fed to turkeys (Jankowski et al., 2009)

cecum but increased the body weight of 8-week-old turkeys. The concentrations and the pool of SCFAs in the cecum were positively correlated with the content of dietary oligosaccharides. An almost complete removal of oligosaccharides from the diet, due to the use of SPI as a substitute for SBM, improved the average feed conversion ratio, but decreased the average body weight of 4-week-old turkeys and had no effect on the overall growth rate of turkey up to 8 weeks of age (Jankowski et al., 2009).

In another experiment, turkeys were fed four diets containing SBM or SPI and different levels of soybean hulls, to increase the crude fiber content of the ration (Juskiewicz et al., 2009). The results of this study are presented in Tables 7 and 8.

Dietary treatment	Fecal DM		LMI	
	4 weeks	8 weeks	4 weeks	8 weeks
α -galactoside content of the diet				
High (2.44%)	21.4 ^b	19.4 ^b	1.96 ^a	2.39 ^a
Low (0.15%)	25.9 ^a	21.4 ^a	1.25 ^b	1.57 ^b
Crude fiber content of the diet				
Low (3.5%)	23.6	19.6	1.54	2.00
High (5.3%)	23.7	21.2	1.68	1.96

Values within each row with the same superscript letter are not different at $P < 0.05$

Table 7. Fecal dry matter content (DM, %) and litter moistness index (LMI, points) (Juskiewicz et al., 2009)

The use of SPI as a substitute for SBM reduced the α -galactoside content of diets from 2.44 to 0.15%, thus significantly increasing the dry matter content of excreta (Table 7) with greater differences observed for turkeys at 4 wks of age. The water content of excreta was well correlated with the moisture content of a litter. It would appear that high content of dietary α -galactosides may affect excreta moisture content, with no obvious symptoms of diarrhea. There were no differences when turkeys were fed diets with a different crude fiber content (3.5 and 5.3%). There was no interaction between the levels of α -galactosides and crude fiber.

The differences in the α -galactosides and crude fiber contents affected some duodenal mucosal structures in turkeys (Table 8).

Parameter	4 weeks				8 weeks			
	α -galactosides		Crude fiber		α -galactosides		Crude fiber	
	High	Low	Low	High	High	Low	Low	High
Villus height, mm	2.01	2.03	2.00	2.04	2.41	2.25	2.51 ^a	2.16 ^b
Villus width, mm	0.14	0.15	0.15	0.13	0.14	0.14	0.13	0.15
GL thickness ¹ , mm	0.30	0.27	0.28	0.29	0.30	0.33	0.28 ^b	0.35 ^a
Crypt depth, mm	0.19 ^a	0.17 ^b	0.19	0.18	0.19 ^b	0.21 ^a	0.18 ^b	0.22 ^a
VCR ¹	10.6	11.9	10.5	11.3	12.7 ^a	10.7 ^b	13.9 ^a	9.82 ^b
Number of GC ²	6.28	5.94	6.37	5.89	5.90	6.45	6.75 ^a	5.60 ^b

¹Glandular layer, ²Villus height/crypt depth ratio, ³Number of goblet cells per 150 μ m of the villus area

Table 8. Duodenal mucosal structures in turkeys fed diets with different levels of α -galactosides and crude fiber at 4 and 8 weeks of age (Juskiewicz et al., 2009)

The effect of dietary oligosaccharides on the duodenal epithelial surface and nutrient utilization depended on the age of turkeys. In contrast to older turkeys (8 weeks of age), younger 4 wks old birds responded differently to dietary treatments with lower α -galactoside levels. Different concentrations of dietary crude fiber affected the turkeys' response to α -galactosides, as reflected in changes in the duodenal crypt depth (interaction $P=0.093$) and in the number of duodenal goblet cells (interaction $P<0.05$) in birds at the age of 4 and 8 weeks, respectively. A high dietary content of α -galactosides decreased crypt depth and increased the villus height/crypt depth ratio in 8-week-old turkeys. However, the results of the above studies indicate that the physiological effect of α -galactosides on mucosal structures in the small intestine was not significantly influenced by different levels of crude fiber. The presence of α -galactosides in diets for young turkeys should be considered as a factor positively affecting the development of the duodenal mucosa. It could be concluded that a high content of α -galactosides in the diet increased the hydration of the intestinal contents, but had no significant effect on DM digestibility and nitrogen, calcium and phosphorus utilization (Juśkiewicz et al., 2009).

The third experiment was conducted to investigate the effect of dietary α -galactoside and crude fiber levels on gastrointestinal functions and the growth performance of young turkeys fed diets with a different content of SBM, SPC, SPI and soybean hulls. Table 9 shows the chemical composition of experimental diets at the second stage of growth (5 - 8 weeks). SBM-based diets contained approximately 2.3% α -galactosides, and a partial replacement of SBM with SPC decreased α -galactoside levels to 1.7%. α -Galactoside content was low

(0.94%) in diets where the predominant high-protein component was SPC. Trace amounts of α -galactosides (below 0.1%) were noted in diets in which SBM was completely replaced with SPI. The initial crude fiber content of approximately 3.5% was increased to over 5% by the addition of soybean hulls. The effects of both experimental factors are summarized in Table 10.

Diet composition, %	Main protein source and low (LF) and high (HF) crude fiber content of the diet							
	SBM _{LF}	SBM _{HF}	SBM-PC _{LF}	SBM-PC _{HF}	SPC _{LF}	SPC _{HF}	SPI _{LF}	SPI _{HF}
Soybean meal (SBM)	39.4	40.5	30.5	29.9	6.2	5.7	-	-
Soy protein concentrate (SPC)	-	-	5.6	6.6	20.8	21.8	-	-
Soy protein isolate (SPI)	-	-	-	-	-	-	17.4	17.9
Soybean hulls	2.6	9.0	2.7	9.1	3.1	9.6	4.6	11.1
Soybean oil	4.6	7.1	3.6	5.8	0.8	3.0	-	2.1
Wheat	48.8	39.0	53.0	43.9	64.4	55.3	72.9	64.1
Minerals, amino acids, vitamins	4.6	4.4	4.6	4.7	4.7	4.6	5.1	4.8
Crude protein	25.4	25.4	25.5	25.4	26.0	25.9	25.6	25.3
Crude fiber	3.68	5.23	3.61	5.38	3.69	5.45	3.69	5.17
α -galactosides	2.27	2.36	1.70	1.68	0.93	0.94	0.05	0.07

Table 9. Composition of experimental diets for turkeys at 5-8 weeks of age (Zdunczyk et al., 2010)

Item	Soybean product				CF level	
	SBM	SBM-PC	SPC	SPI	Low	High
Intestinal tissue mass, g/kg BW	40.5 ^a	40.2 ^a	37.2 ^b	32.2 ^c	35.8	39.3
Viscosity of intestinal digesta, MPas	1.91 ^b	2.08 ^{ab}	2.44 ^a	2.53 ^a	2.28	2.20
Cecal digesta, g/kg BW	2.90	2.57	2.54	2.54	2.47	2.55
Dry matter of cecal digesta, %	17.4 ^{bc}	16.4 ^c	18.4 ^b	20.8 ^a	18.3	18.2
SCFA concentrations, μ mol/g	127.8 ^a	100.7 ^b	89.3 ^b	88.2 ^b	102.3	100.8
SCFA pool, μ mol/kg BW	367.5 ^a	243.8 ^b	223.5 ^b	223.6 ^b	267.2	262.0
Body weight at 8 weeks, kg	4.32 ^{bc}	4.36 ^b	4.82 ^a	4.27 ^c	4.38	4.35
FCR for 0-8 weeks, kg/kg	1.75 ^b	1.76 ^b	1.75 ^b	1.65 ^a	1.74	1.74

Table 10. Selected parameters of the gastrointestinal tract function and growth performance of turkeys fed diets containing various soybean products and different crude fiber levels for 8 weeks (Zdunczyk et al., 2010)

The use of SPC and SPI, which reduced the α -galactoside content of diets, decreased the weight of small intestinal wall and digesta in turkeys. This may have resulted from increased digesta viscosity and a slower rate of transit through the gastrointestinal tract. The weight of cecal digesta was comparable in all groups. Diets containing SPC and SPI

increased the dry matter content of cecal digesta. A decrease in the α -galactoside content of diets resulted in a reduction in the production of cecal SCFAs. Statistically significant differences were found between the group fed the SBM-based diet and the groups fed diets with SBM substitutes with decreased α -galactoside contents. Different crude fiber concentrations in experimental diets had no effect on any parameter investigated.

After 8 weeks of experiment, the highest body weight was observed for the group fed the SPC-based diet containing approximately 1% α -galactosides, while the lowest body weight was noted in the group receiving the SPI-based diet with the α -galactoside content of 0.1%. The latter group had the best feed conversion ratio, which suggests that feeding diets with a reduced α -galactoside content may improve growth performance.

6. Conclusions

Proportionally to the SBM content of diets for monogastric animals, α -galactoside concentrations vary within a broad range of 0.5% to over 2.5%. The results of experiments with chickens and piglets indicate that the physiological effects of α -galactosides are determined by the concentrations of these carbohydrates in the diet. According to some studies, high α -galactoside levels may produce antinutritional effects, e.g. disturb the intestinal passage of digesta and the digestibility of some nutrients. Based on many studies, however, there is little evidence that the oligosaccharides at a normal dietary level pose a nutritional concern and may be even considered as potential prebiotics, although the mechanism of this effect requires further research.

Neither too high (2.3-2.6%) nor too low (0.05-0.1%) α -galactoside content of diets is recommended for young growing turkeys. The best production results have been reported in turkeys fed SPC which allowed for the reduction of dietary α -galactoside content of diets to 1%. A high content of α -galactosides in the diet enhances fermentation processes within the intestines (increased production of SCFAs) and increases the hydration of the intestinal contents, thus increasing the risk of diarrhea. A decrease in α -galactoside levels below 0.1% significantly increases the viscosity of the intestinal contents and has a negative influence on the development of duodenal structures. The physiological effects of α -galactosides, administered at high or low concentrations, are not influenced by different levels of crude fiber in turkey diets.

In view of the development and physiology of the gastrointestinal tract as well as the growth of birds, it may be concluded that a total withdrawal of soybean α -galactosides from turkey diets does not seem to be advisable from a nutritional viewpoint. Thus, SPC, in contrast to SPI, could be considered as an effective SBM substitute.

7. References

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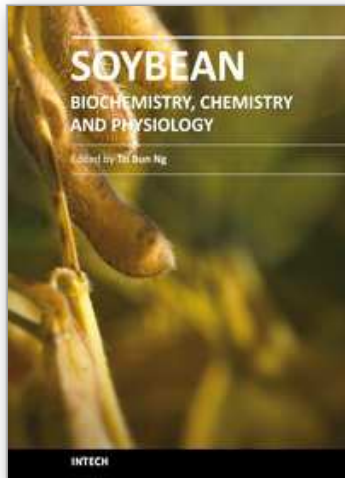
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