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The effect of soy polysaccharide fiber on fecal weight and humidity in growing rats

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Abstract

Objective: To compare the effect of soy polysaccharide on fecal weight and humidity in growing rats in relation to cellulose and a soy formula without dietary fiber.

Methods: Twelve, 21-day-old, Wistar male rats, were distributed into three groups and fed AIN-93G ration with varying amounts of dietary fiber: Polysaccharide Group, 5% fiber in the form of soy polysaccharide; Soy Formula Group, 5% soy formula without fiber; and Cellulose Group, 5% fiber in cellulose form. Feces were collected for three 72-hour periods beginning on days 7, 17 and 27. They were weighed while humid and then dried at 105 ºC until a constant weight. The humidity was calculated using the formula [(humid fecal weight - dry fecal weight)/humid fecal weight] X 100.

Results: The total humid fecal weight for Polysaccharide, Soy Formula and Cellulose groups was: 17.372±4.743 g; 6.045±0.619 g; and 16.012±2.600 g, (p = 0.001), respectively. There was no statistically significant difference between the Cellulose and Polysaccharide groups, but a statistically significant difference was observed between theses two groups and the soy formula group. Values for dry weight, in the same order, were: 6.463±1.177 g; 2.909±0.277 g; and 10.068±1.085 g, (p < 0.001), with a statistically significant difference between each of the groups.

Conclusion: Animals that received soy formula presented lower humid and dried fecal weight than the other two groups, while the soy polysaccharide caused higher fecal humidity than did cellulose, probably due to greater fermentation.

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Introduction

Practically all authors writing on the treatment of pediatric chronic functional constipation recommend increasing the consumption of dietary fiber.[1-7] Some studies have also shown that insufficient dietary fiber consumption is associated with an increased risk of constipation in children.[8-9] Notwithstanding, systematic literature reviews published in recent years point out that much of the conduct recommended for constipation treatment is based on the opinion of specialists and is not founded on evidence from adequately planned clinical trials.[3,5] There are few studies of dietary fiber for the treatment of chronic constipation in infancy. A study of the treatment of children with chronic functional constipation found that the median increase in dietary fiber consumption, when the source is items from the habitual diet, while statistically significant, did not reach the minimum recommended in published literature, despite good clinical progress, in a scenario of a treatment program including disimpaction, educational measures and mineral oil in addition to dietary intervention.[10]

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We performed, at our unit, a randomized, double-blind clinical trial to evaluate the efficacy of a soy polysaccharide fiber supplement for the treatment of chronic functional constipation in children, using a soy formula with no fiber as placebo. Because the preliminary results of that study did not confirm the expected efficacy of polysaccharide fiber for chronic constipation treatment,[11] the current experimental project was designed in order to compare, in rats, the effects of soy polysaccharide on fecal weight and humidity and compare them with the effects of cellulose and a soy formula with no dietary fiber.

Methods

Twelve male rats from the Wistar-EPM lineage, 21 complete days old, mean body weight of 50 g, were randomly distributed into three groups of four animals each: Soy polysaccharide, Soy formula and Cellulose. All of the animals were fed on rations recommended by the American Institute of Nutrition, AIN-93G[12] (Table 1), containing the nutrients necessary for adequate growth and development in rats and differing only in terms of the source of dietary fiber. When rations were prepared for the Soy polysaccharide and Soy formula groups, the quantities of proteins, lipids and carbohydrates were taken into account and subtracted from the total amount of cornstarch, casein and soy oil recommended by the AIN-93G standard. Therefore the groups were fed as follows:

1. Soy polysaccharide group: the cellulose was substituted with soy polysaccharide from a dietary fiber module (Pró-fibra®, 71.4 g/kg), so that the ration contained 50 g of fiber/kg of ration;
2. Soy formula group: addition was made from isolated soy protein (Soyac®), at the same proportion (71.4 g/kg) as in the soy polysaccharide group. No dietary fiber was added to this ration;
3. Cellulose Group: ration prepared with cellulose at 50 g/kg, as recommended by the AIN-93G standard.

The compositions of the three rations is to be found in Table 2.

During the experiment the animals received filtered water and rations *ad libitum*. Animals were kept in individual metabolic cages fabricated in acrylic and stainless steel (Nalgene-metabolic cages 650-0100) that permit the collection of feces unmixed with urine.

Seven days after the rations were started, 0.1 g of carmine pink was added to the rations of each animal in order to establish the moment at which fecal collection should start. This was the point at which color changed (red feces). All feces eliminated by the animals were collected for three consecutive days. Seventy-two 72 hours after carmine pink was added, another coloring agent, aniline blue (INLAB-water soluble) was added to the rations to define the end of collection. Collection ended when blue feces began to be eliminated. This procedure was repeated three times during the experiment, starting on the 7th, 17th and 27th days after the start of the experimental rations.

The feces collected over the three days were stored in a freezer (-20 ºC). After the third day of collection the humid fecal weight was obtained on an analytical electronic balance (Metler Toledo – model AB204), with 0.0001 g sensitivity. Next the feces were dried in an oven at 105 ºC. After 22 hours, weighings were begun at 30-minute intervals until two consecutive weights had differences of less than 1.0 mg. The fecal humidity was calculated using the formula [(humid fecal weight – dry fecal weight)/ humid fecal weight] X100.

Table 1 - Compounds of the AIN-93G growth diet for rats

Ingredients	g/kg diet
Corn starch	529.486
Casein	200.000
Sucrose	100.000
Soybean oil	70.000
Cellulose	50.000
L-cystine	3.000
Choline bitartrate	2.500
Tert-butylhydroquinone	0.014
Mineral Mix[A]	35.000
Vitamin Mix[B]	10.000

A: Composition in mg. Minerals: iron, 35; calcium, 5000; phosphorus, 1561; potassium, 3600; sulfur, 300; sodium, 1019; chlorine, 1571; magnesium, 507; zinc, 30 ; manganese, 10; copper, 6; iodine, 0.2; molybdenum, 0.15; selenium, 0.15. Potentially beneficial minerals: silicium, 5; chromium, 1; fluorine, 1; nickel, 0.5; boron, 0.5; lithium, 0.1; vanadium, 0.1.

B: Composition in mg: nicotinic acid, 30; pantothenate, 15; pyridoxine, 6; thiamin, 5; riboflavin, 6; folic acid, 2; composition in g: vitamin k, 750; D-biotin, 200; vitamin B12, 25; vitamin A, 4000; vitamin D3, 1000; vitamin E, 75.

Table 2 - Composition of the three rations for the soy polysaccharide, soy formula and cellulose groups

Ingredients	Soy polysaccharide	Soy formula	Cellulose
Corn starch	518.05 g	531.06 g	529.40 g
Casein	190.71 g	190.00 g	200.00 g
Sucrose	100.00 g	100.00 g	100.00 g
Soybean oil	69.30 ml	57.00 ml	70.00 ml
Cellulose	0	0	50.00 g
Soy formula	0	71.43 g	0
Soy polysaccharide	71.43 g	0	0
L-cystine	3.00 g	3.00 g	3.00 g
Choline bitartrate	2.50 g	2.50 g	2.50 g
Tert-butylhydroquinone	0.014 g	0.014 g	0.014 g
Mineral Mix[A]	35.00 g	35.00 g	35.00 g
Vitamin Mix[B]	10.00 g	10.00 g	10.00 g

Composition of macronutrients of soy formula, in g/100 g of product: carbohydrates, 56.8 g; proteins, 17.0 g; lipids, 18.2 g.
Composition of macronutrients of soy polysaccharide, in g/100 g of product: carbohydrates, 74 g; proteins, 13 g; lipids, 1 g; eating fiber, 70 g.
A: Composition in mg. Essential minerals: iron, 35; calcium, 5000; phosphorus, 1561; potassium, 3600; sulfur, 300; sodium, 1019; chlorine, 1571; magnesium, 507; zinc,30 ; manganese,10; copper, 6; iodine, 0.2; molybdenum, 0.15; selenium, 0.15. Potentially beneficial minerals: silicium, 5; chromium, 1; fluorine, 1; nickel, 0.5; borium, 0.5; lithium, 0.1; vanadium, 0.1.
B: Composition in mg: nicotinic acid, 30; pantothenate, 15; pyridoxine, 6; thiamin, 5; riboflavin, 6; folic acid, 2; composition in g: vitamin k, 750; D-biotin, 200; vitamin B12, 25; vitamin A, 4000; vitamin D3, 1000; vitamin E, 75.

Results were expressed in mean±standard deviation. Analysis of variance (ANOVA) was used to compare the groups. When statistically significant differences were found, analysis was complemented by the Tukey test. The significance level, adopted was 5%. Calculations were performed using Jandel Sigma Stat software. Sample size was calculated based on the results of a previous study performed at our laboratory,[13] in which it was observed that rats receiving rations with cellulose exhibited mean±standard deviation for humid fecal weight over three days of 10.7±3.5 g, while the group that received rations without fiber eliminated 1.9±1.2 g of feces during the same period.

Assuming a difference of 9.5 g between the two groups and setting a test power of 80% and alpha error of 0.05, the number of animals according to Jandel Sigma Stat would be small (n = 2 assuming standard deviation = 1.2 g and n = 4 if standard deviation = 3.5 g). For the current study, at the time the experiment was performed there were 12 metabolic cages available, giving four animals per group. At the end of this phase, a statistically significant difference was detected, as expected, between the three types of ration, permitting the experimental phase to be ended. The proposal for this article was approved by the *Universidade Federal de São Paulo - Escola Paulista de Medicina* Committee for Ethics in Research.

Results

The total quantity of feces excreted during the three 72-hour collection periods after ingestion of rations with cellulose, soy formula and soy polysaccharide are displayed in Table 3.

When humid fecal samples from the first collection were compared, statistically significant differences ($p < 0.05$) were found between the soy polysaccharide and soy formula groups and between the cellulose groups and soy formula. In turn, there were no statistically significant differences observed between the groups fed with rations containing cellulose or soy polysaccharide. When dry feces were compared, statistically significant differences ($p < 0.05$) were found between all three groups. The soy polysaccharide group had greater fecal humidity than the soy formula and cellulose groups, which difference was statistically significant. In turn, a comparison between the soy formula group and the cellulose group did not reveal any statistically significant difference.

The total quantity of feces (g), humid and dry, ratified the values found in the first collection, with just a general increase in fecal weight equivalent to the animals' growth. In this case, fecal humidity in the polysaccharide and soy formula groups did not differ statistically, in contrast with what is observed during the first collection with significant

Table 3 - Humid weight (g), dry weight (g) and humidity (%) of feces during the three 72-hour collection periods beginning on the 7th, 17th and 27th days after ingestion of rations

Feces collection periods	Soy polysaccharide (n = 4)	Soy formula (n = 4)	Cellulose (n = 4)	p
7th to 9th day				
Humid weight (g)	4.690±1.132[a]	1.384±0.147[b]	4.327±1.096[a]	0.00
Dry weight (g)	1.691±0.244[a]	0.773±0.0882[b]	2.642±0.319[c]	< 0.001
Humidity (%)	62±11[a]	44±1[b]	38±7[b]	< 0.001
17th to 19th day				
Humid weight (g)	5.508±1.802[a]	1.818±0.157[b]	5.231±0.732[a]	0.002
Dry weight (g)	2.175±0.541[a]	0.952±0.0902[b]	3.533±0.423[c]	< 0.001
Humidity (%)	59±7[a]	47±5[a]	32±8[b]	< 0.001
27th to 29th day				
Humid weight (g)	7.174±2.147[a]	2.844±0.649[b]	6.455±0.855[a]	0.004
Dry weight (g)	2.595±0.605[a]	1.184±0.214[b]	3.893±0.419[c]	< 0.001
Humidity (%)	63±5[a]	58±2[b]	40±2[b]	< 0.001
Total in the three periods				
Humid weight (g)	17.372±4.743[a]	6.045±0.619[b]	16.012±2.600[a]	0.001
Dry weight (g)	6.463±1.177[a]	2.909±0.277[b]	10.068±1.085[c]	< 0.001
Humidity (%)	61±4[a]	49±2[b]	36±5[c]	< 0.001

* Values with different letter: $p < 0.05$ according to Tukey test.

differences found only between these two groups and the cellulose group.

The comparison between fecal samples collected during the third collection period after 27 days consuming their respective rations maintained the same characteristics as the first collection. Fecal humidity, however, maintained the characteristics of the second collection.

The total weight of feces collected over the three periods revealed total humid weight and total dry weight that maintained the characteristics found when the three collection periods were analyzed individually, i.e. there was a statistically significant difference in humid weight when the soy polysaccharide and soy formula groups were compared and also when the cellulose and soy formula groups were compared, while there was no such difference between the soy polysaccharide and cellulose groups ($p < 0.05$). The total dry weight revealed statistically significant differences between all groups ($p < 0.05$).

Humidity of the sum of all three collections revealed statistically significant differences between all groups ($p < 0.05$).

Visually, differences in fecal volume are obvious between the groups.

Discussion

The diet used in this experimental project was AIN-93G,[12] formulated to meet the nutritional requirements of growing rats, with its source of fiber either unchanged, modified or removed. This being so, the observed differences in fecal weight and humidity may be explained by changes to the composition of the diets. IN line with expectations, the results reveal statistically significant differences in all analyses in which they were expected. The possibility of a beta error (failure to identify a difference that exists) occurring was very small because of the sample size.

The primary objective of the study was to investigate the effects of soy polysaccharide, cellulose and soy formula on the characteristics of rat feces. The soy formula was included because it had been used as placebo in a clinical trial to test the effects of soy polysaccharide in the treatment of constipation in children. The results of the current study showed that, in relation to cellulose and soy polysaccharide, the soy formula was associated with reduced dry and humid weight, which difference was statistically significant when compared with the other two groups. Thus, this experimental evaluation has confirmed that the soy formula produces lower fecal weights than the groups that received dietary fiber.

When the soy polysaccharide and cellulose groups were compared it was found that humid weight had no statistically significant differences although the feces from the soy polysaccharide group demonstrated a mild superiority when compared with those from the cellulose group. However, after drying in an oven, the dry weight of the cellulose group's feces became statistically superior to that of the soy polysaccharide group's feces, which signifies that the polysaccharide group's feces contained greater humidity than the cellulose group.

Evidence in published literature demonstrates that the structure and the physical and chemical characteristics of dietary fibers define their effects on the digestive system and also that the mechanisms by which they act in the colon can be influenced by innumerable factors such as physical and chemical heterogeneous nature of the fiber and the degree of fermentation and its products, which vary depending on the type of fiber.[14]

Digestion of non-cellulose polysaccharides appears to be more efficient than cellulose digestion due to the extensive degradation of non-cellulose polysaccharide components within the gastrointestinal tract.[15] Studies have shown that, on average, 80% of non-cellulose polysaccharides from many different sources are degraded within the gastrointestinal tract, while cellulose components present in the same food sources are degraded less.[16] Plant walls cells resist being broken down by colonic microflora better, because of the lignin, as occurs with bran or cellulose, exercising a physical effect which is added to the water retention.[17]

Thus, one study observed that, on average, 57.2% of carbon-14 marked cellulose administered orally was excreted unbroken, although a significant quantity of carbon-14 was detected in expired air.[18]

The factors described above may explain the greater dried weight of the cellulose group feces, although it was obvious that the water content was greater in the soy polysaccharide group feces, which must be related to the extensive degradation of the fiber by micro-intestinal flora. Bacteria play an important role in changes to fecal weight due to their ability to resist dehydration (despite being almost 80% water themselves), competing against the absorptive forces of the colonic mucosa to retain water in the gastrointestinal tract. Bacteria are, therefore, an important part of human feces.[19]

This being the case, although soy polysaccharide has between 75 and 85% insoluble fiber with low viscosity and that is either resistant to fermentation or only partially fermented (less than 40%, in the colon, is excreted almost unbroken in feces),[19] *in vitro* studies demonstrate that soy polysaccharide is relatively well fermented, which property is attribute to its small particle size.[20] *In vivo*, this more complete fermentation is reproduced, due to the increased intestinal microbial flora stimulation.[20]

The location in which soy polysaccharide fermentation occurs should also be considered. In this case it occurs in the ascending colon,[17] where it is more intense, similar to what is observed in humans, where fermentation is superior at the level of the cecum and ascending colon.[14]

The group that received a diet with no fiber exhibited lower humid and dry fecal weights than the other two, which can be clearly explained by the functions performed by fiber in the gastrointestinal tract, as described above.

In general, the results of our study agree with the results of a literature review[17] that collected and compared a number of different studies that gave a variety of types of fiber to groups of healthy men and demonstrated an increase in humid fecal weight proportional to the fermentation of the dietary fiber.

In conclusion, this study has shown that a soy formula with no dietary fiber is associated with reduced humid and dry fecal weight when compared with the other two groups. The soy polysaccharide was associated with lower dry weight, greater humidity and humid weight similar to the cellulose group, probably due to being more completely fermented at the level of the colon.

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