

Effects of soybean agglutinin on body composition and organ weights in rats

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Abstract

An experiment was conducted to assess the effect of soybean agglutinin dosage level on growth, body composition, plasma lipids, glucose, urea nitrogen content and aminotransferase activities in rats. Male and female rats ($n=60$) weaned at 19 d were given a dose of 0, 3.5, 7.0, 10.5, or 14.0 mg soybean agglutinin by gastric infusion once daily for 10 days. With increasing doses of soybean agglutinin, body weight, lipid content of carcass, spleen and kidneys relative dry weights decreased, while small intestine and pancreatic weight, the contents of urea nitrogen and triglyceride, and the activities of aspartate aminotransferase linearly increased in plasma. Though soybean agglutinin decreased plasma insulin content, changes in plasma glucose content due to soybean agglutinin were not detected. It is suggested that dietary soybean agglutinin may affect the secretion of other hormones besides insulin, which modulate blood glucose reserves. In conclusion, consumption of soybean agglutinin resulted in a depletion of lipid and an overgrowth of small intestine and pancreas in rats. Meanwhile, poor growth of spleen and kidneys was observed in the soybean agglutinin-fed rats.

Keywords: *Soybean agglutinin, body composition, lipid, metabolism, rat*

1. Introduction

As one of the main factors responsible for the anti-nutritive effects of soybean (Pusztai et al. 1997), soybean agglutinin is a tetrameric glycoprotein, which binds specifically to terminal N-acetyl-D-glucosamine and D-galactose (Schulze et al. 1995). Soybean agglutinin also has a high binding affinity to intestinal cells containing N-acetyl-D-galactosamine (Koninkx et al. 1996). Soybean agglutinin retained at least 50% of its biological activity during passage through the small intestine (Hajos et al. 1995). Raw soybean products contain 10–20 mg/g of soybean agglutinin (Huisman & Tolman 1992). It is assumed that lectins are almost or completely inactivated during food and feed processing. However, soybean agglutinin is resistant to inactivation by dry heat (de Muelenaere 1964). The residual lectin levels of soybean agglutinin were 0.22–0.67 mg/g in desolventization-toasting processed meals, and residual lectins in soybean-based diets could have a negative effect on performance of production animals (Maenz et al. 1999).

It is very difficult to evaluate anti-nutritional effects of soybean agglutinin from seed or soybean meal, because soybean seeds contain other anti-nutritional factors, which interfere with soybean agglutinin in terms of anti-nutritional effects (Li et al. 2003a). Therefore, it is significant to explore the toxic effects of soybean agglutinin using purified samples. It has been found that soybean agglutinins induce pancreatic enlargement (Crass & Morgan 1982), disrupt the brush border (Oliveira et al. 1989), increase endogenous nitrogen (Schulze et al. 1995), decrease intestinal enzymatic activities (Fasina et al. 2004), and reduce apparent nitrogen digestibility (Li et al. 2003a) in mammals. Furthermore, soybean agglutinins may be taken up into cells by endocytosis and as a result, reactive forms of the soybean agglutinin are distributed in the circulation and internal organs, and may lead to deleterious systemic effects (Vasconcelos & Oliveira 2004). However, those systemic effects of the soybean agglutinin on body metabolism are unknown, and no systematic studies have ever been carried out to determine the systemic effects of soybean agglutinin in rats. Accordingly, based on the previous studies in our laboratory, the objective of this experiment was to determine the effects of different doses of purified soybean agglutinin on body composition and organ weights, as well as plasma lipid, glucose, and insulin contents, and aminotransferase activities in rats, since the contents of lipid, glucose, insulin, and the activities of aminotransferases are the important parameters of changes in body metabolism and organ functions.

2. Materials and methods

2.1. Purification of soybean agglutinin

Soybean agglutinin was purified by affinity chromatography using the combinative methods of Gordon et al. (1972) and Li et al. (2003a). Briefly, raw soybean seeds (wild-type, planted in Northeast China) were crushed, defatted with petroleum ether (10%, w/v), and were then suspended in extracting buffer (0.01 M PBS, pH 7.6) with constant stirring at 4°C. The extract was filtered and centrifuged at 4°C, and the supernatant was added to solid ammonium sulphate to attain 85% saturation. The precipitate was dissolved in the phosphate-buffer and dialyzed extensively against PBS. The dialyzed solution was subjected to a column of epoxy-activated N-acetyl-D-galactosamine-sepharose-6B (A2787, Sigma, USA) that is specific for soybean agglutinin and eluted from the column with phosphate buffer containing D-galactose (36 mg/ml). The elution of soybean agglutinin was dialyzed against distilled water and then freeze-dried and stored at 4°C. Indirect enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of soybean agglutinin. This resulted in 660 mg of soybean agglutinin per 100 g of raw soy flour. The antibody to soybean agglutinin was developed in rabbits as previously described (Li et al. 2003b). Goat anti-rabbit IgG-peroxidase (A6154) and lectin from *Glycine max* (soybean) (L1395) were purchased from Sigma. A hemagglutination assay indicated that the activity of purified soybean agglutinin was similar to the soybean agglutinin product (L1395) of Sigma.

2.2. Animals and diets

Male and female Sprague-Dawley rats ($n = 60$) weaned at 19 d were provided a commercial stock diet and water for 1 week and received then an experimental semi-synthetic diet (see Table I) for 3 d to reach 78–80 g initial weight.

The applied dosages were based on previous studies on the effect of soybean agglutinin on nitrogen metabolism, where amounts between 2.8 mg/d and 14 mg/d were used (Li et al. 2003a, 2003b). In order to study the systemic effects of purified soybean agglutinin, in the

Table I. Composition of semi-synthetic diets.

Ingredient	Contents [g · kg ⁻¹]	Analysed nutrient levels [‡]	
Cornstarch	529.5	Gross energy [MJ · kg ⁻¹]	15.76
Casein	200.0	Dry matter [g · kg ⁻¹]	973.8
Sucrose	100.0	Crude protein [g · kg ⁻¹]	173.4
Soybean oil	70.0	Calcium [g · kg ⁻¹]	14.3
Fiber	50.0	Total phosphate [g · kg ⁻¹]	3.6
Mineral mix*	35.0	Lysine [g · kg ⁻¹]	14.7
Vitamin mix [†]	10.0	Methionine + Cystine [g · kg ⁻¹]	4.8
L-Cystine	3.0	Threonine [g · kg ⁻¹]	6.5
Choline chloride	2.5	Tryptophan [g · kg ⁻¹]	1.8
t-Butylhydroquinone (TBNQ) [mg]	14.0		

*[†]For composition of vitamin and mineral mixes, see Reeves (1997); [‡]Nutritional values were analysed in triplicate.

current study four graded doses of soybean agglutinin were chosen, ranging between 3.5 mg/d and 14.0 mg/d.

According to a 5 × 2 factorial design, i.e., five levels of soybean agglutinin (0, 3.5, 7.0, 10.5 and 14.0 mg/d) and two sexes, the rats were divided into 10 groups of six each, based on body weight and sex after a 10 d adaptation period. The animals were housed individually in a well-ventilated rat metabolism room maintained at 23°C with 65% relative humidity and were on a 12:12h light/dark cycle in the Ministry of Agriculture Feed Safety and Bioavailability Evaluation Center (Beijing, China). The amount of feed for each animal was restricted to 6 g of semi-synthetic diet per day. After feeding rats were intragastrically intubated and received soybean agglutinin (dissolved in 1.5 ml of saline) for 10 d at 0, 3.5, 7.0, 10.5, or 14.0 mg per day and animal, respectively. The controls received saline only. In the morning of the 10th day, blood samples were taken from the heart 2 h after rats were given 2 g of semi-synthetic diet. The rats were then killed with an overdose of ether anesthesia. Their pancreases were removed and freed from blood, fat and other tissues. Hearts, livers, spleen, kidneys, and lungs were excised. The small intestine, caecum and colon were separated according to two fixed anatomical parts. They were then rinsed with saline. All tissues, organs and carcasses were freeze-dried and then weighed. Until analysis for insulin, cholesterol, triglycerides, high density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, and glucose, plasma samples were stored at -20°C. All management and experimental protocol were carried out in strict accordance with the regulations of the China Agricultural University for experimental animals.

2.3. Chemical analysis

Freeze-dried and weighed carcasses were ground to powder and extracted with chloroform-methanol (2:1, v/v) for lipid determination. The defatted carcass samples were analysed for total nitrogen (N) using a Foss Heraeus Macro N automated system (Foss Electric) according to AOAC (1990) methods. Protein content was calculated as N · 6.25. The amino acid content of casein was determined using an automatic amino acid analyzer (Hitachi L-8800, Tokyo, Japan).

Plasma total cholesterol, triglycerides, high density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, and glucose were determined enzymatically according to assay kits 180271, 220771, 200353, 010161, 0021, 280091 and 240401 (Zhongsheng Beikong Bio-technology and Science Inc.), respectively, and an

automated biochemistry analyzer (Technico RA-1000, Barer corporation). Plasma insulin was measured by the method of Bardocz et al. (1996) in Beijing Huaying Biotechnological Institute using an insulin RIA kit. The inter- and intra-assay coefficient of variation was 8.9% and 4.2%, respectively.

2.4. Statistical analyses

All data were subjected to analysis by PROC GLM of SAS (Cary, NC), Version 8.02. The model included soybean agglutinin dose, sex, as well as interaction of soybean agglutinin $\times$ sex. Linear polynomial contrasts were used to determine the dose response of soybean agglutinin. Differences with $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Body weight and weight of internal organs

As one of the major antinutritional factors in soybean, soybean agglutinin has been shown to depress growth (Liener 1953; Pusztai et al. 1990; Douglas et al. 1999; Li et al. 2003a). Our results also showed that rats receiving soybean agglutinin up to 14.0 mg/d reduced weight gain and carcass weight in a dose-dependent manner ($p < 0.001$).

Both the absolute and relative weights of some of the organs and tissues of the rats were affected in a dose-dependent way by soybean agglutinin infusion. This finding agreed with the trend previously found in our laboratory that soybean agglutinin elevated the contents of protein, DNA, RNA and polyamine in the jejunum and induced pancreatic enlargement, therefore resulting in hyperplastic growth of the small intestinal epithelium and pancreas in rats (Li et al. 2003a). Soybean agglutinin markedly increased relative dry weights of pancreas ($p < 0.001$), small intestine ($p < 0.01$) and caecum ($p = 0.01$) (Table II). However, relative dry weights of spleen and kidneys were decreased by soybean agglutinin administration ($p < 0.001$), which disagrees with our previous finding, where these organs were not affected by soybean agglutinin (0.4–2 mg/g) incorporation in diets (Li et al. 2003a). This discrepancy may be related to the differences in dose rate as well as the method of supplementation. In addition to increased soybean agglutinin dose, gastric infusion was used in the present study, which caused abruptly a relative high concentration of soybean agglutinin. However, our findings agreed with de Aizpurua and Russell-Jones (1988), who reported that soybean agglutinins led to severe atrophy of kidneys.

Compared with the control group, relative dry weights were increased for pancreas by 9.94%, 20.2%, 35.6% and 58.9%; for small intestine by 15.3%, 15.3%, 17.5% and 18.2%, and for caecum by 10.6%, 18.1%, 14.1% and 21.3%, for 3.5, 7.0, 10.5 and 14.0 mg soybean agglutinin, respectively. In contrast, the relative dry weight of spleen was reduced by 7.79%, 21.2%, 25.0% and 29.8%, and the dry weight of kidney by 0, 2.1%, 3.3% and 3.3% for the respective treatment.

The effect of soybean agglutinin on pancreas weights may be due to soybean agglutinin stimulation of pancreatic growth as well as pancreatic exocrine secretion in rats (Jordinson et al. 1996). Thus, growth of the pancreas stimulated by soybean agglutinin in the present study may influence exocrine and endocrine functions.

Small intestine and caecum weights increased in our study, which may indicate that a relative low dose of soybean agglutinin was a potential growth factor for the gut. Though the nutritional cost of the compulsive gut growth contribute to the overall antinutritive effects of the soybean agglutinin, this effect of soybean agglutinin may be beneficial to weaning

Table II. Body weight, body composition and relative tissue dry weights in rats fed graded doses of soybean agglutinin (SBA) for 10 d.

	Soybean agglutinin dose [mg/d]					Sex		<i>p</i> -value				
						Male	Female	PSEM*	SBA	Sex	SS [†]	Linear contrast
	0	3.5	7.0	10.5	14.0							
Body weight gain [g]	10.9	4.5	3.9	2.5	1.6	4.7	4.5	0.44	<0.001	0.28	0.88	<0.001
Carcass weight [g]	17.1	15.7	15.6	15.4	15.2	15.9	15.7	0.12	<0.001	0.41	0.78	<0.001
Carcass protein content [%]	76.6	74.1	76.1	75.9	74.4	75.4	75.5	0.36	0.13	0.98	0.96	0.32
Carcass lipid content [%]	11.8	11.2	11.1	10.3	9.4	10.8	10.7	0.14	<0.001	0.61	0.98	<0.001
<i>Organ weight [g · kg carcass⁻¹]</i>												
Pancreas	3.12	3.43	3.75	4.23	4.96	3.87	3.92	0.08	<0.001	0.08	0.001	<0.001
Liver	53.7	54.8	55.1	54.7	54.7	54.4	54.8	0.31	0.69	0.49	0.31	0.37
Spleen	10.4	9.6	8.2	7.8	7.3	8.6	8.7	0.15	<0.001	0.50	0.42	<0.001
Kidneys	24.0	24.4	23.5	23.2	23.2	20.9	23.2	0.21	<0.001	0.94	0.46	<0.001
Heart	4.77	4.77	4.83	4.89	4.90	4.81	4.89	0.03	0.63	0.20	0.54	0.16
Lung	8.40	8.63	8.51	8.60	8.63	8.48	8.62	0.05	0.57	0.18	0.66	0.25
Small intestine	41.8	48.2	48.2	49.1	49.4	47.2	47.4	0.81	0.01	0.89	0.29	0.004
Caecum	4.04	4.47	4.77	4.61	4.90	4.41	4.71	0.08	0.01	0.06	0.87	0.01
Colon	7.33	7.82	7.62	7.47	7.61	7.48	7.66	0.09	0.48	0.30	0.84	0.73
Stomach	8.88	9.41	9.23	9.26	9.02	9.08	9.24	0.07	0.19	0.28	0.96	0.79

*Pooled standard error of the mean; †Soybean agglutinin × sex.

mammals. At the same time, soybean agglutinin supplementation caused atrophy in the spleen and kidneys, which suggests that soybean agglutinin might be particularly damaging for function of the immune and metabolism systems. Study results showed that dietary soybean agglutinin suppressed both the cell-mediated immune and humoral immune functions in rats in our laboratory. No effects of soybean agglutinin on the relative weights of dry heart, lung, colon and stomach were detected. Although body weight and all tissue relative weights were not affected by sex, a positive soybean agglutinin dose $\times$ sex interaction ($p = 0.001$) was observed for the relative dry weight of pancreas in rats (see Table II). It seemed that female rats were more sensitive to soybean agglutinin than male in terms of pancreas growth. This may be related to the different proportions of high- and low-affinity cholecystokinin receptors on pancreatic acinar cells in male and female rats, as soybean agglutinin stimulation of pancreatic growth may be primarily mediated by cholecystokinin (Jordinson et al. 1997). A portion of the protein was utilized for synthesis of the small intestine and pancreas and thus not available for body growth. Therefore, soybean agglutinin reduced protein utilization, which is a potential reason for the observed growth depression.

3.2. Body and plasma compositions

Lipid content of the carcass, and insulin levels in rats were reduced linearly ($p < 0.001$) with increasing doses of soybean agglutinin (Table II). Compared with rats fed the semi-synthesized diets only, the protein content was obviously not changed ($p = 0.13$), but lipid content was reduced by 5.1–20.3%. How soybean agglutinin stimulates lipid loss is unknown. Corresponding to our finding that 1.2 mg and 2.0 mg of soybean agglutinin per g diet decreased insulin content in plasma (Li et al. 2003a), insulin also declined linearly ($p < 0.001$) as supplemental soybean agglutinin increased in this study. Insulin levels fell from 13.0–6.9 $\mu\text{IU/ml}$ in the plasma of rats as soybean agglutinin dosage increased (Table III). Therefore, low insulin levels may result in the mobilization and catabolism of lipids, because it is well known that insulin is a key hormone involved in blood glucose synthesis from carbohydrates, amino acids, and lipids. The exact mechanism of insulin decline in rats given different doses of soybean agglutinin is unknown. It is likely that soybean agglutinin influences the secretion of insulin from the pancreas since insulin is synthesized by the islet cells.

Soybean agglutinin significantly affected blood urea nitrogen and triglycerides, and aspartate aminotransferase activity levels. Blood urea nitrogen is a waste product of protein metabolism in the body. Blood urea nitrogen content increased from 25.7–36.5 mg/dl, and then declined to 32.5 mg/dl at the dose of 14.0 mg/d (linear, $p = 0.003$). This suggests that nitrogen utilization declined with administration of soybean agglutinin from 3.5–10.5 mg/d, however, it is unknown why blood urea nitrogen content declined at a dose of 14.0 mg/d. These results are supported by the increase of aspartate aminotransferase activities by 15.3%, 35.5%, 65.5% and 101.8% after receiving 3.5, 7.0, 10.5 and 14.0 mg soybean agglutinin, respectively. These changes support the theory that soybean agglutinin interfered with amino acid or nitrogen metabolism to a certain degree. Furthermore, the changes of aminotransferase activities may contribute to the influence of soybean agglutinin on liver function. Triglyceride content declined to 104.7 mg/dl then increased at the dose of 7.0 mg/d (linear, $p = 0.02$). This implies that triglycerides were catabolized at soybean agglutinin dosage levels lower than 7.0 mg/d, while it was synthesized at dosage levels higher than 7.0 mg/d by an unknown mechanism. Soybean agglutinin had no significant effects on total cholesterol, high density lipoprotein cholesterol, or glucose levels in plasma (see Table III). This differs from the results of Grant et al. (1987) who noted that plasma insulin levels were reduced, while plasma free fatty acids were elevated in soybean-fed rats.

Table III. Changes in plasma urea nitrogen, lipid levels, insulin content, and aminotransferase activities in rats fed with different doses of soybean agglutinin (SBA) for 10 d.

	Soybean agglutinin dose [mg/d]					Sex		<i>p</i> -value				
	0	3.5	7.0	10.5	14.0	Male	Female	PSEM*	SBA	Sex	SS [†]	Linear contrast
Blood urea nitrogen [mg·dl ⁻¹]	25.7	25.2	31.0	36.5	32.5	29.8	30.5	0.97	<0.001	0.48	0.05	0.0003
Total cholesterol [mg·dl ⁻¹]	90.2	87.8	89.0	88.3	89.2	89.2	88.6	1.53	0.99	0.87	0.30	0.94
Triglyceride [mg·dl ⁻¹]	112.2	107.5	104.7	116.2	118.8	114.4	109.3	1.76	0.01	0.06	0.82	0.02
High density lipoprotein cholesterol [mg·dl ⁻¹]	90.2	87.8	89.0	88.3	89.2	89.2	88.6	0.86	0.99	0.87	0.30	0.28
Alanine aminotransferase [U·l ⁻¹]	31.6	36.5	37.8	38.1	31.0	34.9	35.0	0.99	0.04	0.96	0.55	0.68
Aspartate aminotransferase [U·l ⁻¹]	181.5	209.2	246.0	300.4	366.2	266.6	266.6	12.79	<0.001	0.16	0.99	<0.001
Glucose [mg·dl ⁻¹]	96.2	93.5	98.8	101.6	98.3	97.3	98.0	1.10	0.27	0.77	0.90	0.18
Insulin [μ U·ml ⁻¹]	13.0	10.8	8.2	7.6	6.9	9.3	9.3	0.43	<0.001	1.00	0.94	<0.001

*Pooled standard error of the mean; †Soybean agglutinin $\times$ sex.

Though insulin levels declined, plasma glucose remained stable in rats given different doses of soybean agglutinin. Thus, soybean agglutinin may be responsible for insulin-mimicking effects of lectins (Pusztai & Watt 1974) on body metabolism. At the same time, soybean agglutinin perhaps causes changes in other endocrine hormones, which modulate glucose levels in blood, since soybean agglutinin can react with endocrine cells after binding to intestinal mucosa. Based on the present findings, soybean agglutinin may motivate systemic catabolism, since Pusztai et al. (1998) showed that activated system catabolism could lead to lipid depletion, high urinary urea output, and reduces body N accretion.

4. Implications

Soybean agglutinin induced the growth of gut, depletion of lipid in carcass, atrophy of spleen and kidney, and reduction in circulating insulin but without interruption on glucose levels in blood. Therefore, high levels of soybean agglutinin may produce toxic effects on mammalian organs and the metabolism system. However, modest or low doses of soybean agglutinin provide a potential novel means to prevent and/or cure adiposity and hyperlipidemia, and may induce gut maturation in mammalian. Because there are differences of effects of soybean agglutinin on gut development or lipid metabolism among different species, further research need to be carried out in other mammals.

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