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Original article

Soybean impairs Na^+-dependent glucose absorption and Cl^- secretion in porcine small intestine

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Abstract — Recent evidence indicates that soybean, which is widely used in animal nutrition, could directly alter intestinal ion and nutrient transport. However, the mechanisms involved are still unknown. The aim of the study was to investigate the effect of three differently treated soybean products on the glucose and Cl^- transport capacity in porcine small intestine by the Ussing chamber technique. Jejunal and ileal piglet epithelial tissues were pre-incubated with extracts of raw soybean flour (RSF), heated soybean flour (HSF), or ethanol heat-treated soybean protein concentrate (SPC). The Na^+-dependent glucose co-absorption capacity was then measured as an increase in the short-circuit current (I_{SC}) after luminal addition of D-glucose. The effect of the soybean products on cAMP-dependent Cl^- secretion was measured as the increase in I_{SC} after the addition of the phosphodiesterase inhibitor, theophylline, while nervous regulation of Cl^- secretion was investigated by the addition of the enteric neurotransmitters; 5-hydroxytryptamine (5-HT), substance P and vasoactive intestinal polypeptide (VIP). Incubation with RSF and HSF induced a 30% decrease of the Na^+-dependent glucose absorption capacity in the jejunum. The effect was similar for RSF in the ileum. Theophylline-induced secretion was decreased by 30% after incubation with RSF, HSF and SPC but only in the jejunum. 5-HT-, substance P- and VIP-induced secretion were not altered by incubation with soybean extracts except in the HSF-incubated where the substance P-induced secretion was significantly reduced. In conclusion, soybean contains ethanol-sensitive heat-insensitive compounds impairing Na^+-dependent glucose absorption in the jejunum and ileum, and ethanol- and heat-insensitive compounds causing an acute impairment of cAMP-dependent jejunal secretion.

soybean / Ussing chambers / intestinal secretion / glucose absorption / nutrition / electrolyte transport

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1. INTRODUCTION

Soybean is widely used in human and animal food because of its high protein content [1–4]. In animal production, low cost plant proteins are often preferred to more expensive animal protein, especially after the ban on animal products like meat and bone meal or blood meal. Therefore, soybean constitutes a useful substitute for milk proteins in calf and pig nutrition [2, 3], and partially or completely replaces fish meal in aquaculture [5]. However, the highest nutritional values of soybean are only obtained when it is highly processed [2–4]. This lower digestibility of raw soybean is often attributed to the presence of anti-nutritional factors (ANF) such as heat-labile protease inhibitors, lectins, oligosaccharides, heat-resistant antigenic proteins, phenolic compounds or phytate [6]. The presence of ANF leads to alterations in pancreatic and intestinal morphology and function [7–1], and thus to a reduction in the digestibility of raw soybean.

A recent study demonstrated that soybean meal decreases carrier-mediated transport of nutrients in the distal intestines of fish [12]. Such a decrease in the absorption capacity, if confirmed in mammals, could hence be a new and interesting explanation for the lower digestive utilization of raw soybean. Moreover, soybean seems to disturb electrolytes and water transport in piglets [13]. Since the balance between absorption and secretion provides an aqueous medium for a proper digestion and absorption of intraluminal nutrients, the lowest digestibility of raw soybean could also be due to electrolyte secretion disturbances. The aim of the present study was to verify these effects of soybean on the porcine small intestine and try to understand some of the mechanisms involved. We investigated the effect of soybean on intestinal glucose absorption and on intestinal Cl^- secretion in piglets using the Ussing chamber, a technique which allows a direct contact of the mucosa with soluble compounds of soybean together with a measurement of nutrient and electrolyte transport.

2. MATERIAL AND METHODS

2.1. Animals and Ussing chambers

Danish 6–8 week-old crossbred Landrace × Yorkshire fully weaned pigs (12–15 kg) were used. The animals were deprived of food overnight before the experiment but were permitted free access to water containing 300 mM glucose. The pigs were stunned by a boltpistol, and then killed by exsanguination. A section of 20 cm of tissue was removed 30 cm distal to the ligament of Treitz in the jejunum, and 60 cm proximal to the ileocaecal junction in the ileum.

Jejunal and ileal segments were stripped of outer muscle layers and immediately mounted as sheets in Ussing chambers (opening area 1.0 cm^2) with an O-ring placed on the mucosal side to minimize tissue edge damage. The bathing medium was a bicarbonate Ringer solution (in mM: 25 $NaHCO_3$, 120 NaCl, 1 $MgSO_4$, 6.3 KCl, 2 $CaCl_2$, 0.32 phosphate buffer; pH 7.4), continuously oxygenated (95% O_2 / 5% CO_2), and maintained at 38 °C.

After correction for solution resistance, the transepithelial potential difference (PD) was clamped to a value of 0 mV by an external current (short-circuit current, I_{SC}) through Ag/AgCl electrodes. PD was recorded through Ag/AgCl electrodes connected to the chamber with agar bridges (3% in Ringer solution) in a saturated KCl solution. The transepithelial resistance R was determined from current deflections in response to ± 3 mV transepithelial voltage pulses for 0.3 s generated by the voltage clamp set-up every 30 s. R and the corresponding open-circuit PD were calculated by the Ohm law and, together with I_{SC}, recorded every 10 s [14, 15].

2.2. Soybean extracts

Soybean extracts were obtained from three commercial products: defatted raw soybean flour (RSF, Société Industrielle des Oléagineux, Bougival, France), heated soybean flour (HSF, Société Industrielle des Oléagineux) and soybean protein concentrate (SPC, Aarhus Oliefabrik, Aarhus, Denmark). HSF was prepared from dehulled seed by lipid extraction with hexane and heat treatment to denature trypsin inhibitors [16]. SPC was obtained from RSF by hot aqueous ethanol treatment, which eliminated oligo-saccharides and denatured most of the proteins. These soybean products were stirred in bicarbonate Ringer solution (10% w/v) at room temperature for one hour then centrifuged (4000 rpm, 10 min, room temperature). Protein content of the supernatants was adjusted to obtain a final concentration of 1 $mg \cdot mL^{-1}$ in the chambers. Soybean extracts or 18 mM D-mannitol (control) were usually (except experiment 1) added to the mucosal side 5 min after mounting the tissues. The increase in osmolality on the mucosal side induced by these additions was compensated for by the addition of 18 mM D-glucose on the serosal side.

Final osmolality, ion, amino acids, genistein and daidzein concentrations of the mucosal bath after the addition of soybean extracts are presented in Table I. Osmolality was obtained by freezing point depression (Advanced Wide-Range Osmometer 3W2, Advanced Instruments, Boston, Ma, USA). Na^+ and K^+ by flame photometry (FLM3 Photometer, Radiometer, Copenhagen, Denmark) and Cl^- by colorimetric titration (CMT 10 Chloride Titrator, Radiometer, Copenhagen, Denmark). Total free amino-acid concentrations were determined by chromatographic separation on Biotronik LC5001 analyzer (Biotronik, Pusheim Bahnhof, Germany). Genistein and daidzein concentrations were obtained according to the ELISA method described by Le Houérou et al. and Bennetau-Pelissero et al. [17, 18].

2.3. Experiment 1: The ionic basis of soybean extracts-induced increase of I_{SC}

Twelve adjacent sheets of jejunum from three piglets were mounted randomly in the Ussing chambers. The Ringer bicarbonate solution bathing the tissues contained 16 mM D-glucose on the serosal side and 16 mM D-mannitol on the mucosal side. Tissue samples were exposed to the Na^+-K^+-$2Cl^-$-cotransporter inhibitor bumetanide (0.1 mM) on the serosal side for 30 min or to the SGLT-1 inhibitor phloridzin (0.5 mM) on the mucosal side for 5 min before addition of soybean extracts or served as

Table I. Final osmolality, ions, free amino acids, genistein and daidzein concentrations of the mucosal bath after the addition of soybean extracts.

	Control	RSF	HSF	SPC
Osmolality ($mOsmol \cdot kg\ H_2O^{-1}$)	310	302	307	310
Na^+ ($mmol \cdot L^{-1}$)	145	144	143	139
Cl^- ($mmol \cdot L^{-1}$)	128	128	128	130
K^+ ($mmol \cdot L^{-1}$)	6.3	7.8	10.7	19.1
Total free amino acids ($\mu mol \cdot L^{-1}$)	0	68	312	498
Genistein ($\mu g \cdot mL^{-1}$)	0	2.1	3.4	0.1
Daidzein ($\mu g \cdot mL^{-1}$)	0	2.4	3.9	0.1

RSF: raw soybean flour, HSF: heated soybean flour, SPC: soybean protein concentrate. Mannitol was used as the control.

untreated controls. HSF and SPC extracts were then added to the mucosal side and the increase in I_{SC} was compared between the control and inhibitor-treated tissues.

2.4. Experiment 2: The effect of soybean on glucose-absorption and Cl^- secretion

Four adjacent sheets of the jejunum and four adjacent sheets of the ileum from twelve piglets were mounted randomly in Ussing chambers. The Ringer bicarbonate solution bathing the tissues contained 16 mM D-glucose on the serosal side and 16 mM D-mannitol on the mucosal side. Soybean extracts were immediately added on the mucosal side and tissues were left incubating for 45 min. After this, the glucose-absorption capacity was measured as the increase in I_{SC} (ΔI_{SC} glucose) before and after the addition of 16 mM D-glucose on the mucosal side, which was osmotically balanced on the serosal side by 16 mM D-mannitol. After 10 min, the Cl^- secretion capacity was measured by adding 2.5 mM theophylline to the mucosal and serosal side and the increase in I_{SC} (ΔI_{SC} theo) was measured.

2.5. Experiment 3: 5-HT, substance P and VIP-induced secretion

The results of the second experiment led us to perform a third experiment involving the neurocrine regulatory pathways of Cl^- secretion. This experiment was performed on the jejunum only, since theophylline-induced secretion was impaired by soybean extracts only in the jejunum. Another set of twelve pigs was used. For each pig, twelve adjacent segments of the jejunum were mounted randomly in Ussing chambers. The Ringer bicarbonate solution bathing the tissues contained 16 mM D-glucose on both sides. Soybean extracts were immediately added to the mucosal side and tissues were incubated for 45 min. After this incubation period, either 5-hydroxytryptamine- (5-HT), substance P- or vasoactive intestinal polypeptide (VIP) – induced secretion were measured as the increase in I_{SC} (i.e. ΔI_{SC} 5-HT, ΔI_{SC} SP, and ΔI_{SC} VIP, respectively) before and after the addition of 0.1 mM 5-HT (Sigma Chemical Co, St Louis, MO, USA), 10 μM substance P (Peninsula Laboratories Europe Ltd, St Helens, Merseyside, UK) or 100 nM VIP (Peninsula Laboratories Europe Ltd, St Helens, Merseyside, UK), respectively to the serosal side.

2.6. Statistical analysis

Statistical analysis was performed using the General Linear Model procedure of SAS (SAS Institute, Cary, NC, USA). Since the different treatments studied were tested for each animal, the treatment effect was tested against an intra-animal error. The differences were considered significant for a p value less than 0.05. Data are presented as least-square means (lsmeans) ± SEM with n corresponding to the number of animals.

3. RESULTS

3.1. Ionic basis of soybean extract effects on Isc

The addition of soybean extracts induced an increase in I_{SC} (Tab. II), with no effect on the transepithelial resistance (data not shown).

The increase in I_{SC} induced by HSF or SPC in the jejunum was not inhibited when the tissues were pre-treated with either bumetanide or phloridzin (Fig. 1), indicating that the soybean addition effect was neither Na^+-dependent glucose absorption (no effect of phloridzin) nor Cl^- secretion (no effect of bumetanide).

Table II. The effect of the addition of soybean extracts on jejunal and ileal short-circuit current (ΔI_{SC}; $\mu A \cdot cm^{-2}$) measured in Ussing chambers.

	Jejunum (ΔI_{SC}; $\mu A \cdot cm^{-2}$)		Ileum (ΔI_{SC}; $\mu A \cdot cm^{-2}$)	
Control	–2.9 ± 1.0	a	–4.4 ± 4.5	a
RSF	3.8 ± 1.1	b	10.8 ± 5.4	b
HSF	6.7 ± 1.0	c	36.5 ± 4.5	c
SPC	10.9 ± 1.0	d	34.3 ± 4.5	c

RSF: raw soybean flour, HSF: heated soybean flour, SPC: soybean protein concentrate. Mannitol was used as the control. Data are Lsmeans ± SEM. Different letters within a column are significantly different ($P < 0.05$, $n = 12$).

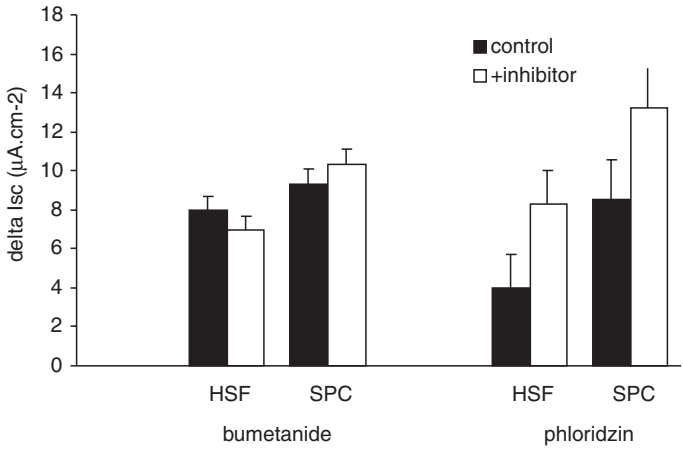

Figure 1. Pharmacological blockade of a soybean extract-induced increase in the jejunal short-circuit current (ΔI_{SC}) measured in Ussing chambers. HSF: heated soybean flour, SPC: soybean protein concentrate. The increase in I_{SC} induced by HSF and SPC was not inhibited by either phloridzin nor by bumetanide ($n = 3$). This indicates that this effect was probably neither glucose absorption nor chloride secretion.

3.2. Na^+-dependent glucose absorption

Na^+-dependent glucose absorption capacity was significantly decreased in the RSF-incubated jejunum and ileum (Fig. 2). Incubation with HSF also induced a significant decrease in the Na^+-dependent glucose absorption capacity but only in the jejunum. By contrast, incubation with SPC did not alter the Na^+-dependent glucose absorption capacity. These results indicate that RSF and HSF contained ethanol-sensitive components that impaired the Na^+-dependent glucose absorption capacity since the ethanoltreated soybean product (SPC) did not induce any change in glucose absorption.

3.3. Theophylline-induced secretion

Incubation with RSF, HSF or SPC induced a significant decrease in theophylline-induced secretion in the jejunum but not in the ileum (Fig. 3). This indicates that soybean extracts contained ethanol- and

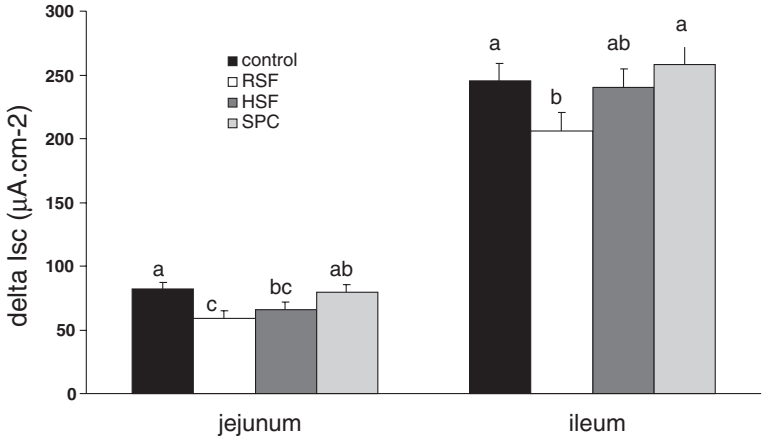

Figure 2. Na^+-dependent glucose absorption of the jejunum and ileum incubated with soybean extracts measured as the change in the short-circuit current (ΔI_{SC}) in Ussing chambers. RSF: raw soybean flour, HSF: heated soybean flour, SPC: soybean protein concentrate. Within an intestinal site, bars with different letters are significantly different ($P < 0.05$, $n = 12$). Incubation of the jejunum and ileum with RSF impaired glucose absorption. Incubation with HSF had the same effect on the jejunum.

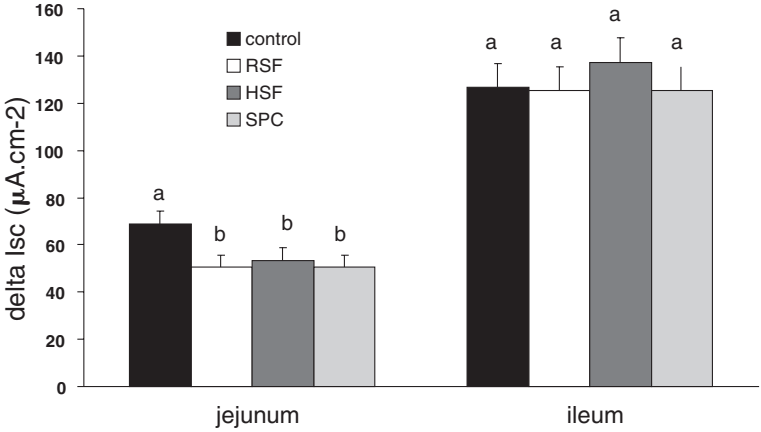

Figure 3. Theophylline-induced Cl^- secretion of the jejunum and ileum incubated with soybean extracts measured as the change in the short-circuit current (ΔI_{SC}) in Ussing chambers. RSF: raw soybean flour, HSF: heated soybean flour, SPC: soybean protein concentrate. Within an intestinal site, bars with different letters are significantly different ($P < 0.05$, $n = 12$). Incubation of the jejunum but not the ileum with the three soybean product extract impaired theophylline-induced secretion.

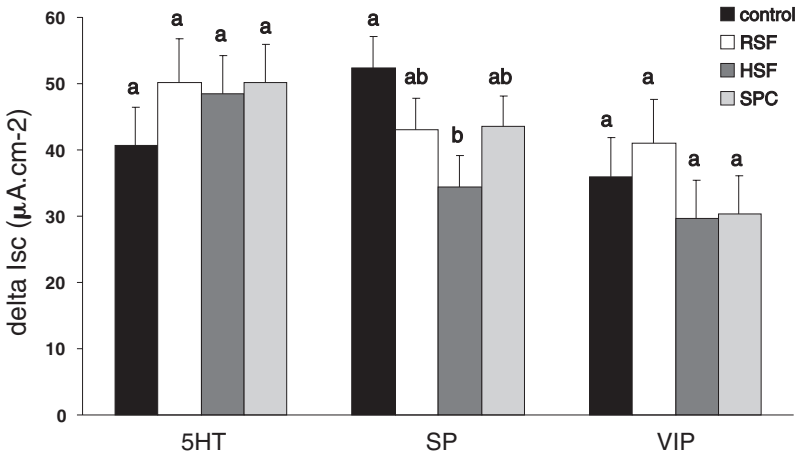

Figure 4. 5-HT-, substance P- and VIP-induced Cl^- secretion of the jejunum incubated with soybean extracts measured as the change in the short-circuit current (ΔI_{SC}) in Ussing chambers. RSF: raw soybean flour, HSF: heated soybean flour, SPC: soybean protein concentrate. Within a secretagogue, bars with different letters are significantly different ($P < 0.05$, $n = 12$). The incubation of the jejunum with soybean product extracts had no effect on the 5-HT-, substance P- or VIP-induced secretion except HSF which decreased substance P-induced secretion.

heat-resistant components that impaired the jejunal cAMP-induced secretion capacity.

3.4. 5-HT, substance P and VIP-induced secretion

Jejunal 5-HT and VIP-induced secretion were not altered by incubation with soybean products (Fig. 4). HSF-incubated jejunum exhibited a lower response to substance P compared to control tissues whereas this response was not impaired in RSF and SPC-incubated tissues.

4. DISCUSSION

We demonstrated that soybean contains components which impair jejunal and ileal Na^+-dependent glucose absorption and jejunal cAMP-mediated secretion. The components that inhibit the glucose absorption were sensitive to the ethanol treatment but not to the thermal treatment in contrast to the compounds that inhibited the cAMP-induced secretion, which was resistant to the heat and to the ethanol treatment.

4.1. Direct effect of soybean extracts

The addition of soybean extracts induced a minor increase in I_{SC}, with no effect on the transepithelial resistance, that was bumetanide and phloridzin-insensitive. This increase was therefore not due to Na^+-dependent glucose absorption or to Cl^- secretion. Our hypothesis is that the increase in I_{SC} was due to the presence of free amino acids in the soybean products whose absorption is Na^+-dependent [19]. This hypothesis is consistent with (i) the higher amino acid concentrations in SPC and HSF compared to RSF and (ii) the higher I_{SC} increase induced by the soybean extracts in the ileum compared to the jejunum, since amino acids induce a higher electrogenic Na^+-absorption in the distal part of the small intestine than in the proximal part [20].

4.2. Impaired glucose absorption

The incubation of the jejunum and ileum with raw or heated soybean extracts impaired the carrier-mediated glucose absorption capacity of the intestinal tissues as described for carrier-mediated transport in the distal intestine of fish fed soybean meal for 3 weeks [12]. Although this effect was demonstrated after 3 weeks in fish, we were able to observe an acute inhibitory effect of soybean on glucose absorption in the piglet. Such rapid changes in the glucose absorption capacity are not uncommon since increases in glucose absorption have already been reported after a 30-min exposure to glucose [21], 1-hour exposure to EGF [22] or immediately after exposure to CCK [23].

The presence of particular compounds in soybean including flavonoids, saponins or phenolic compounds could explain this effect. Indeed, an isoflavonoid-containing soybean extract has been shown to inhibit glucose uptake in rabbit intestinal brush-border membrane vesicles in vitro [24]. Similarly, saponins decrease active nutrient transport in vitro [25] and phenolic compounds (tannic acid and chlorogenic acid) inhibit glucose uptake in rat intestinal brush border membranes [26]. Among compounds, isoflavonoids could be incriminated. This hypothesis is consistent with the virtual absence of genistein and daidzein in the SPC incubation media compared to RSF and HSF. The mechanisms involved could be an alteration of enterocyte membrane fluidity [27, 28], a direct interaction of the compounds with the cotransporter SGLT-1 [29, 30] or intracellular mechanism alterations such as the inhibition of the tyrosin kinase activity by genistein [22, 31].

4.3. Impaired cAMP-mediated secretion

Incubation of the jejunum but not the ileum with the three soybean extracts impaired cAMP-mediated secretion. The compounds responsible for this effect are heat- and ethanol-resistant and therefore cannot be flavonoids, otherwise known for their prosecretory effect [32]. Neurocrine regulation mechanisms are minimally altered, suggesting that the effect is mainly at the enterocyte level. Lastly, the mechanism involved is regional specific.

Previous in vitro observations of theophylline-induced secretion in piglets fed for 3 weeks with cowpea showed the same heat-treatment insensitivity of a putative compound in cowpea inducing higher responses to theophylline [33]. Regional intestinal differences were also observed since the ileum was not responsive. Regarding the acute effect of soybean incubation on intestinal secretory physiology, our observations appear contradictory with those of Nabuurs and coworkers obtained in vivo on a ligated loop model. They showed an enteropooling effect of soya peptone in piglets that was probably linked to an increased biosynthesis of prostanoids [34]. It is, however, difficult to compare in vivo and in vitro studies. Nabuurs and coworkers used piglets on the day of weaning with a model of ligated loops [13] where soy was in contact with the mucosa for up to 24 h. In our present study, we used older piglets, shorter incubation periods and an in vitro method.

The mechanism involved in the interaction of soybean extracts with Cl^- secretion is hypothetical. A role of the Cl^- channel CFTR is possible since it could explain the different response between the jejunum and ileum. Indeed CFTR expression exhibits a proximal-distal decreasing gradient along the intestine [35]. The mechanism may therefore involve a membrane fluidity modification, as previously proposed for SGLT-1, and subsequent alteration of CFTR functioning.

The lack of effect of soybean extracts on 5-HT, substance P or VIP-induced Cl^- secretion suggests that the effect of soybean is at the enterocyte level and would be significant in vivo only for the basal level of secretion necessary for proper digestion. Soybean

would not be beneficial in cases of stimulated secretion, i.e. in response to toxins such as the cholera toxin or *E. coli* toxins (LT and STa) whose mechanisms of action involve the neuro-endocrine mediators tested [36].

In conclusion, we demonstrated that soybean impairs carrier-mediated glucose absorption and c-AMP dependent Cl^- secretion in vitro. However, in vivo experiments are needed to confirm these effects. The knowledge of the mechanisms of action as well as the compounds involved would help explain the lower digestive utilization of raw soybean compared to ethanol-treated products.

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