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Effect of soya bean antinutritional proteins on rats and sheep

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ABSTRACT

In the first experiment we showed that isolated soya bean lectin (SBA) and trypsin inhibitor (STI) are unable to suppress food consumption in rats, if administered orally at a level of 100 mg/kg b.w. Other factors connected with raw soya bean may be responsible for suppression. In another experiment defatted raw soya bean flour was administered intraruminally at a level of 10 g/kg b.w. to a sheep fitted with ruminal, duodenal and ileal cannulas. Soya bean lectin (SBA) and trypsin inhibitors appeared in the duodenum within 1 hour; both reached the terminal ileum and had disappeared by the time 24 hours had elapsed. The passage of the lectin along the gut progressively lagged behind that of the inhibitors, which indicates that this lectin binds to the intestinal surface in this species. The inhibitors were neutralised by marked pancreatic hypersecretion resulting in a supraphysiological trypsin level in the intestine. This experiment shows that soya bean antinutritional proteins pass along the digestive tract at different rates, and if administered intraruminally they are not degraded effectively in the forestomachs. It is assumed that this differential passage may also occur in monogastric animals.
(Keywords: lectin, inhibitor, soya bean, rat, sheep)

ZUSAMMENFASSUNG

Einfluss der antinutritiven Eiweiße der Sojabohne auf Ratten und Schafe

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Im ersten Versuch wurde gezeigt, dass das isolierte Lektin (SBA) und der Trypsininhibitor (STI) der Sojabohne nicht fähig sind, die Futteraufnahme der Ratten zu verringern, wenn diesen oral eine Dosis von 100 mg/kg Körpergewicht verabreicht wurde. Für den Rückgang der Futteraufnahme können andere Stoffe der rohen Sojabohne verantwortlich sein. In einem anderen Versuch wurden einem Schaf 10 g/kg Körpergewicht entfettetes rohes Sojamehl intraruminal eingegeben. Dem Schaf waren in einer vorangegangenen Operation Kanülen in Pansen, Duodenum und Ileum eingesetzt worden. Das Lektin (SBA) und die Trypsin-Inhibitoren erschienen innerhalb einer Stunde im Duodenum; beide Stoffe gelangten in den terminalen Ileum und waren nach

24 Stunden verschwunden. Während des Durchlaufes durch den Darmkanal blieb das Lektin immer mehr hinter den Inhibitoren zurück, was darauf hinweist, dass sich bei dieser Tierart das Lektin an die Oberfläche der Darmschleimhaut bindet. Die Inhibitoren wurden durch eine ausgesprochene Pankreas Hypersekretion neutralisiert, was dann ein supraphysiologisches Trypsinniveau im Darm zur Folge hatte. Dieser Versuch zeigt, dass bei intraruminaler Eingabe die antinutritiven Eiweiße der Sojabohne in den Vormägen nicht wirksam genug zerlegt werden und mit unterschiedlicher Geschwindigkeit den Verdauungstrakt durchlaufen. Wir nehmen an, dass dieser differenzierte Durchlauf auch bei monogastrischen Tieren vorkommt.
(Schlüsselwörter: Lektin, Inhibitor, Sojabohne, Ratte, Schaf)

INTRODUCTION

Soya bean proteins are divided into high m.w. storage proteins and a low m.w. albumen fraction. The latter contains mostly antinutritional proteins: soya bean lectin and proteinase inhibitors. The trypsin inhibitor of the soya bean was one of the earliest proteins isolated, this being the so-termed Kunitz inhibitor (STI). Later another proteinase inhibitor was isolated: this was the Bowman-Birk inhibitor (BBI), which is a so-termed double-headed inhibitor active against both trypsin and α-chymotrypsin. Soya bean also contains a lectin, a carbohydrate binding protein specific to galactose and N-acetyl-galactosamine residues in complex carbohydrates (soya bean agglutinin, SBA). This protein is largely, but not completely resistant to proteolysis in the gut (Pusztai et al., 1990), requiring the presence of inhibitors to exert its full activity. SBA binds to glycoproteins on the inner surface of the small intestine, resulting in different biological effects (Pusztai, 1991).

In the first experiments we examined the effect of SBA and STI on voluntary food consumption in rats. In the second experiment the effect of the antinutritional proteins was investigated in a ruminant species, the sheep.

MATERIALS AND METHODS

Wistar, SPF-derived 120 g female rats were kept in cages and fed on commercial chow. In another experiment Scottish Blackface wethers (30 kg) were fitted with chronic ruminal, duodenal, and ileal cannulas. The animals were kept in metabolic cages and fed at maintenance level with a ration composed of grass hay and grass cubes with mineral supplements.

For the first experiment SBA was isolated on guar-gum column according to *Pusztai et al*. (1991). STI and BSA are Sigma products.

For the second experiment raw soya bean was ground, extracted five times with petroleum ether and dried at room temperature. On the basis of dry matter the extracted flour contained 1.2 % STI, 0.4 % BBI and 0.4 % SBA.

In the food consumption experiments the rats were allotted randomly into groups of 4, 5 or 6. After 30 hr fast the animals were transferred to individual cages and had free access to water throughout. Due to the feeding habits of the rats the experiments were started at dusk and terminated in the morning. All manipulations were performed in dim light. The test protein and the control BSA were administered by gastric intubation or intestinal infusion, dissolved in 0.4 ml physiological saline. At the same time intact, pre-weighed cylinders of granulated chow were laid on the feeding grid and removed periodically for weighing to calculate cumulative food consumption over 12, 18 and 24

hours. During the experiment the animals consumed the commercial granulated chow to which they were accustomed.

Trypsin was measured by direct spectrophotometric determination (*Schwert and Takenaka*, 1955) at 253 nm of the hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE). Trypsin inhibitors were determined by measuring the remaining activity after reaction with excess trypsin. SBA was determined with rocket electrophoresis using rabbit anti-SBA IgG (Sigma) with 0.2 % galactose included in the agarose gel.

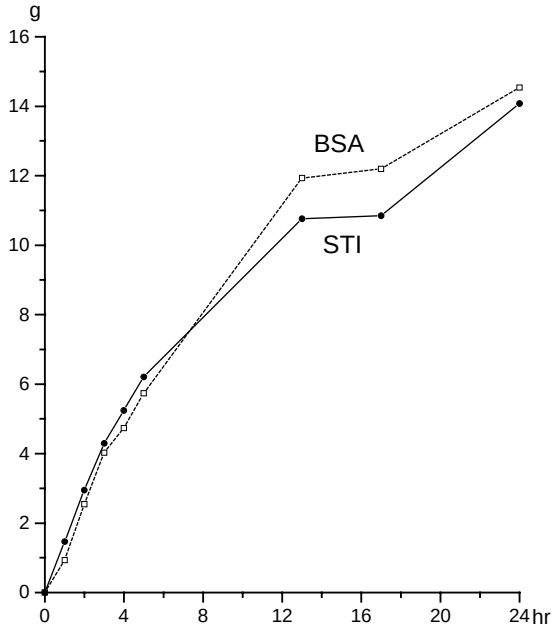
RESULTS AND DISCUSSION

Food consumption

Isolated soya bean lectin (SBA) or trypsin inhibitor (STI) was administered to rats through a gastric tube at a level of 100 mg/kg body weight. Bovine serum albumin (BSA) was used as the control. With both STI and SBA (*Figs. 1 and 2*) the food consumption curves were almost identical in the experimental and the control group. This means that these antinutritional proteins did not suppress food consumption, in spite of their known cholecystokinin (CCK) releasing activity. Observations show that defatted, heat-treated soya bean meal is consumed better than when fed raw; however, the reason for this remains unclear.

Fig. 1

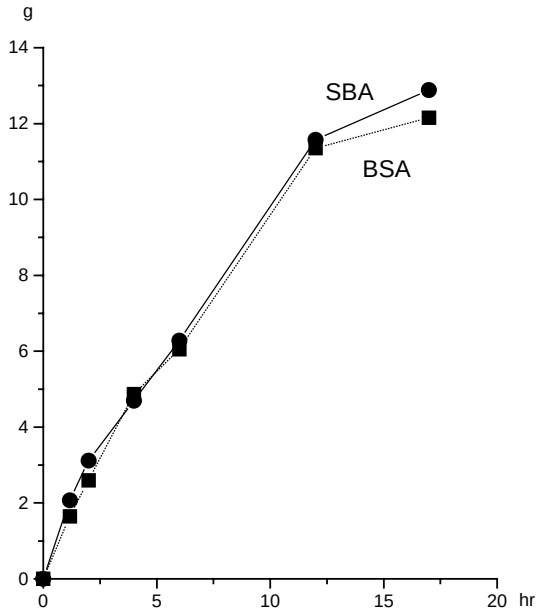
Effect of soya bean trypsin inhibitor (Kunitz-type, STI) on food consumption in rats
Differences between the curves are not significant, n = 4



1. Abbildung: Einfluss des Trypsininhibitors der Sojabohne (Kunitztyp, STI) auf den Futterverbrauch der Ratten
(Die Unterschiede zwischen den Kurven sind nicht signifikant)

Fig. 2

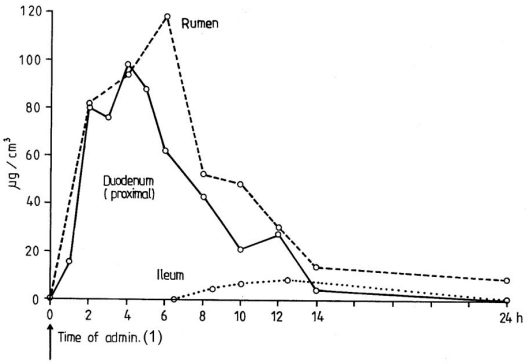
Effect of soya bean lectin (SBA) on food consumption in rats
Differences between the curves are not significant, n = 6



2. Abbildung: Einfluss des Lektins der Sojabohne (SBA) auf den Futterverbrauch der Ratten
(Die Unterschiede zwischen den Kurven sind nicht signifikant)

Fig. 3

Soya bean lectin concentrations in the ovine gastrointestinal tract after intraruminal administration of 10 g/kg b.w. defatted soya bean flour



3. Abbildung: Konzentration des Sojalektins im Verdauungstrakt des Schafes nach intraruminaler Eingabe von 10 g/kg Körpergewicht entfettetem Sojamehl

Zeitpunkt der Eingabe(1)

Effect of defatted soya bean on the sheep

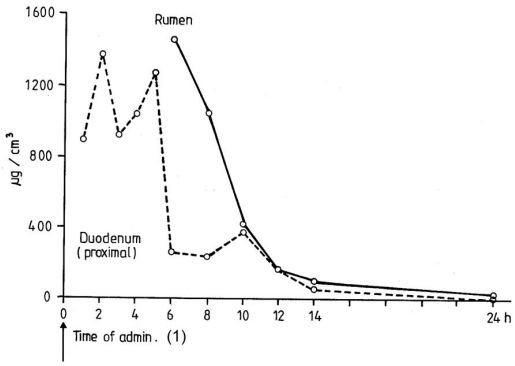
Defatted soya bean flour in the form of a slurry was administered through the rumen cannula at 10 g/kg b.w. (300 g/sheep) in the morning and the daily feed was reduced to half. Samples were taken through the ruminal, duodenal and ileal cannulas for 24 hours and kept frozen until use.

In the rumen several hours were required for the leaching of lectin and inhibitors from the soya bean flour particles and for the homogenisation of rumen content. The concentration of antinutritional proteins then declined steadily (*Figs. 3 and 4*).

The antinutritional proteins appeared within 1 hour in the proximal *duodenum*, but the increase of the lectin was more sluggish than that of the inhibitor, and the decline of the lectin also occurred later. This difference was even more pronounced in the *ileum*, where the lectin appeared just after the disappearance of the inhibitor (*Figs. 3 and 4*). This difference shows that the lectin *binds* to the surface of the small intestinal cells, i.e. to the brush border. Both lectin and inhibitor reached the ileum and had disappeared by the end of 24 hours.

Fig. 4

Trypsin inhibitor concentrations in the ovine rumen and proximal duodenum after intraruminal administration of defatted soya bean flour



4. Abbildung: Konzentration des Trypsininhibitors im Pansen des Schafes und dem proximalen Duodenum nach intraruminaler Eingabe von entfettetem Sojamehl

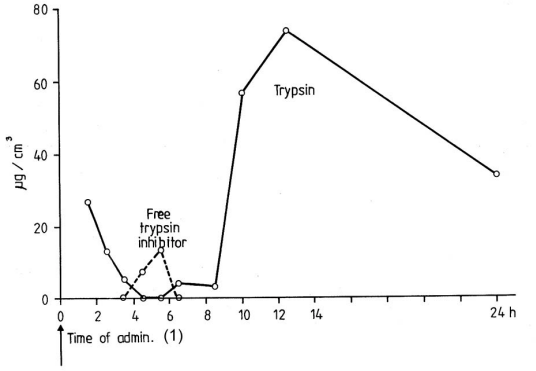
Zeitpunkt der Eingabe(1)

In conclusion, it was ascertained that the two antinutritional proteins (lectin and inhibitor) progressed along the small intestine at *differential rates*, because their interaction with the gut was also different.

In the *ileum* physiological trypsin activity declined to zero in the first hours, due to the appearance of the inhibitor, which came to be in excess above trypsin for a few hours (*Fig. 5*). However, a new surge of trypsin started and was elevated to supraphysiological concentrations after the 8th hour. This finding indicates a strong *pancreatic reaction* that is mediated, obviously, by the CCK-releasing effect of the antinutritional proteins (*Green and Lyman*, 1972; *Brand and Morgan*, 1981; *Calam et al*., 1987; *Jordinson et al*., 1997; *Pusztai et al*., 1997).

Fig. 5

Trypsin and inhibitor concentrations in the ovine ileum after intraruminal administration of defatted soya bean flour



5. Abbildung: Trypsin- und Inhibitorkonzentration im Ileum des Schafes nach intraruminaler Eingabe von entfettetem Sojamehl

Zeitpunkt der Eingabe(1)

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