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BINDING OF LIPASE, AMYLASE AND PROTEASE TO INTESTINAL EPITHELIUM AS AFFECTED BY CARBOHYDRATES AND LECTINS IN VITRO

By

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SANDHOLM, M. and M. L. SCOTT: *Binding of lipase, amylase and protease to intestinal epithelium as affected by carbohydrates and lectins in vitro.* Acta vet. scand. 1979, *20*, 329—342. — Intraluminal digestive enzymes were shown to bind to chick intestinal epithelial surface (glycocalyx). Affinity of the intestinal epithelium for the enzymes decreased in the order, lipase > amylase > protease. The plant hemagglutinins, Con A, phytohemagglutinin, pokeweed mitogen and raw soybean effectively released lipase and amylase from the glycocalyx. Based on specific inhibition of binding by sugars, such as fucose and N-acetylated sugars, lipase and amylase appeared to be bound to blood group antigen-like sugar moieties on the glycocalyx of the microvilli.

amylase; lipase; protease; pancreatic hydrolases; hemagglutinins; lectins; intestinal epithelium; glycocalyx.

The assimilation of food substances in the gut is an integration of three different stages: intraluminal digestion, membrane-contact digestion and nutrient absorption. The intraluminal digestion and the membrane-contact digestion have been proved to be combined processes. Membrane-contact digestion of starches, proteins and triglycerides may occur when pancreatic hydrolases are adsorbed to the intestinal lining. The epithelial binding may promote the hydrolytic activity. Bound pancreatic hydrolases apparently are better protected from proteolytic damage than the intraluminal enzymes. The adsorption of intraluminal hydrolases

onto the epithelial surface probably means that much of the intraluminal digestion takes place at the epithelial lining. This ensures that the digestion products are released structurally near the next stage of digestion which is due to membrane-bound enzymes such as disaccharidases and peptidases. A co-ordination of different stages of digestion and absorption has been suggested by *Ugolev* (1974).

Previous evidence has indicated that the intestinal surface glycocalyx is the site of binding of pancreatic hydrolases. However, the mechanism of the enzyme attachment and the nature of the binding forces have not yet been elucidated. The present investigation was conducted to determine the degree of binding for each group of digestive enzymes and to discover the nature of the binding structures. Because the surface coat (glycocalyx) of the enterocytes is known to be the site of enzyme trapping, experiments by competitive inhibition of binding were undertaken to determine the receptor moieties in the glycocalyx. Hemagglutinins (known to combine with certain oligosaccharides on cell surfaces) would also interfere with the trapping mechanism of the epithelial lining. Although the degree of enzyme binding might be analyzed more directly by comparing digestive properties of the mucosa in the presence and absence of bound enzymes, the method used in this investigation involved a study of desorption kinetics and re-adsorption of native intraluminal enzymes. The amylolytic, caseolytic and lipolytic activities were measured from sequential elutions of everted intestinal loops by an enzyme diffusion in agar-gel technique.

MATERIALS AND METHODS

The materials are listed below*. Although sources of materials appeared to be unimportant, it was found that calcium paracaseinate from other sources were not digested completely by proteases and were therefore unsuitable for protease determination.

* Agarose: — Sigma Chemical Co., St. Louis, MO 63178, USA (No. A-6014). Ca-paracaseinate: — Baker Chemical Co., Phillisburg, NJ 08865, USA (No. E282) (can be prepared by rennet precipitation of skimmed milk in the presence of Ca). Corn oil (Mazola): — Best Foods,, Englewood Cliffs, NJ 07632, USA. Cornstarch: — A. E. Staley Mfg. Co., Oak Brook, IL 60521, USA. Na-deoxycholate: — Sigma Chemical Co. (No. 6750).

Enzyme determination by enzyme diffusion in substrate containing agar gels

Preparation of the diffusion gels:

1. Starch plates for amylase determination. One g agarose was dissolved in 90 ml phosphate buffered saline (PBS)*, pH 7.4, in a bath of boiling water. One g corn starch and 0.1 g sodium azide were suspended in 10 ml PBS at 20°C. The starch suspension was mixed into the hot, dissolved agarose. The mixture was cooled to 60°C and poured on to glass plates or into Petri dishes to form a 2 mm layer.

2. Calcium paracaseinate plate for protease determination. One g agarose was melted in 50 ml PBS in a bath of boiling water. One g Ca-paracaseinate and 0.1 g sodium azide were suspended in another 50 ml PBS at 20°C. When the agarose was dissolved, the mixtures were combined, mixed and poured on to glass plates or into Petri dishes to form a 2 mm layer.

3. Corn oil plate for lipase determination. One g agarose was dissolved in 100 ml PBS in a bath of boiling water, 50 µl corn oil and 250 mg Na-deoxycholate were mixed into the hot agarose using a Sorvall Omni-mixer** for 1 min. The emulsion was then poured on to glass plates or into Petri dishes to form a 2 mm layer.

Assay. The plates were allowed to solidify in a humid chamber at room temperature. When the agar gel was completely solidified, holes of 5.5 mm diameter were punched out of the gel with a cork borer, and the resultant wells were emptied by suction. The wells were filled with the intestinal contents or intestinal elutions under study, allowed to diffuse in a humid chamber at room temperature (22°C) for 24 h and investigated by an overhead projector. The diameters of the cleared zones were measured (Fig. 1).

Experimental design. Six-week old chickens, fed a commercial diet, were sacrificed and their small intestines were removed, everted on 16 cm applicator sticks, ligated at both ends. Each

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* Phosphate buffered saline (PBS), pH 7.4 (Dulbecco's solution): 8.0 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.1 g $CaCl_2$, 0.1 g $MgCl_2 \cdot 6\ H_2O$, make up to 1000 ml with H_2O.

** Sorvall Omni-mixer, I. Sorvall, Inc., Norwalk, CN 06850, USA.

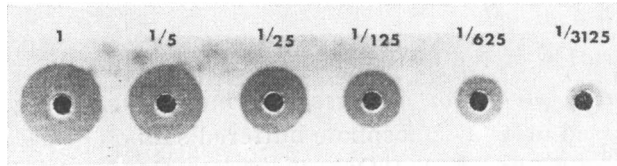

Figure 1. Radial diffusion of intestinal amylase into the agarose gel containing 1 % starch. The first dilution represents the whole chicken intestinal contents eluted in 50 ml PBS. Incubation time 24 h.

segment was then transferred with its contents to a test tube containing 5 ml PBS and mixed under vertical rotation for 15 min. To determine the extent of enzyme binding, each segment was then serially transferred into sequential tubes containing 5 ml PBS and the elution was repeated nine times. An aliquot of each washing (50 µl) was then analyzed by radial diffusion.

Experiment 1. Desorption — re-adsorption of amylase, protease and lipase. The whole small intestine of a 6-week old chicken was everted and ligated on 10 applicator sticks (16 cm). The segments were eluted together in 10 sequential tubes containing 50 ml PBS. An aliquot (50 µl) of each washing was then

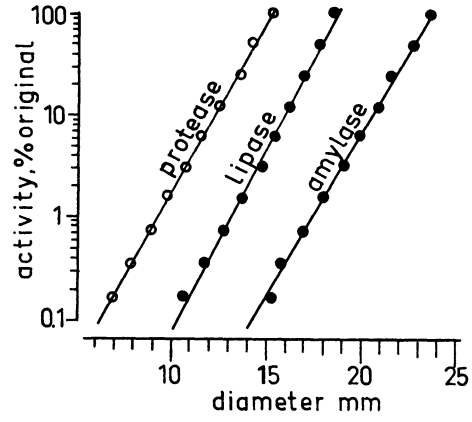

Figure 2. Correlation of the enzyme dilution with the diameter of the clear zone in protease, amylase and lipase plates. The 100 % value represents the diameter obtained with intestinal contents suspended in 50 ml PBS, other determinations are from double dilutions of the initial dilution. Incubation time 24 h.

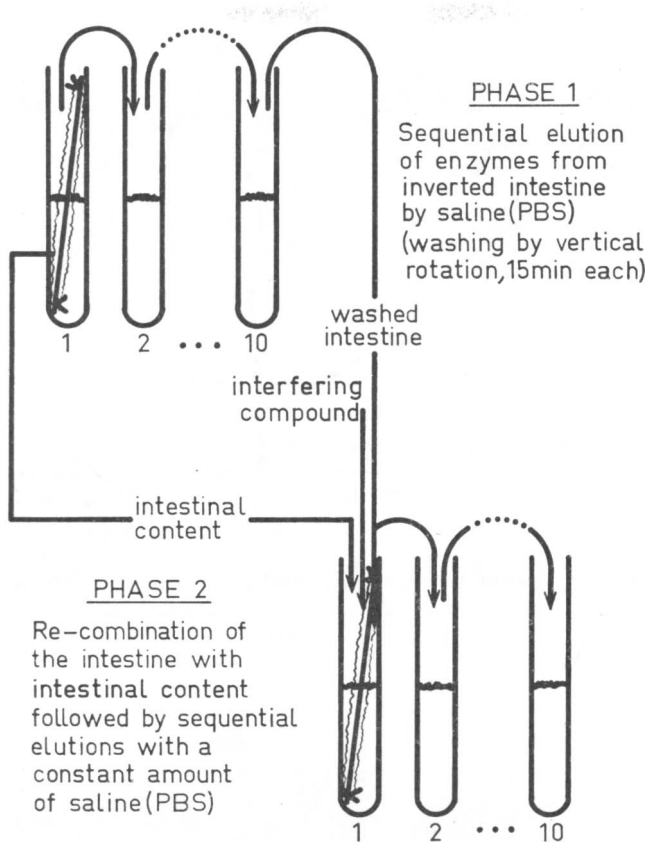

Figure 3. The sequential elution of hydrolases from everted intestinal segments. Phase 1 shows standard elution procedure. Phase 2 shows elution following re-exposure of intestinal segment to the first washing.

analyzed by radial enzyme diffusion (Fig. 1). After the last elution the washed intestinal segments were re-combined with the first washing (intestinal content) and the elution procedure repeated (Figs. 3 and 4).

Experiment 2. Effect of sugars and lectins on the binding of intraluminal enzymes on the intestinal epithelium,

Method A. The 10 everted segments of small intestine were placed together in 50 ml PBS and mixed for 15 min. After equalization of the initial enzyme levels, each segment was subjected to a separate series of sequential elutions (5 ml each, 10×

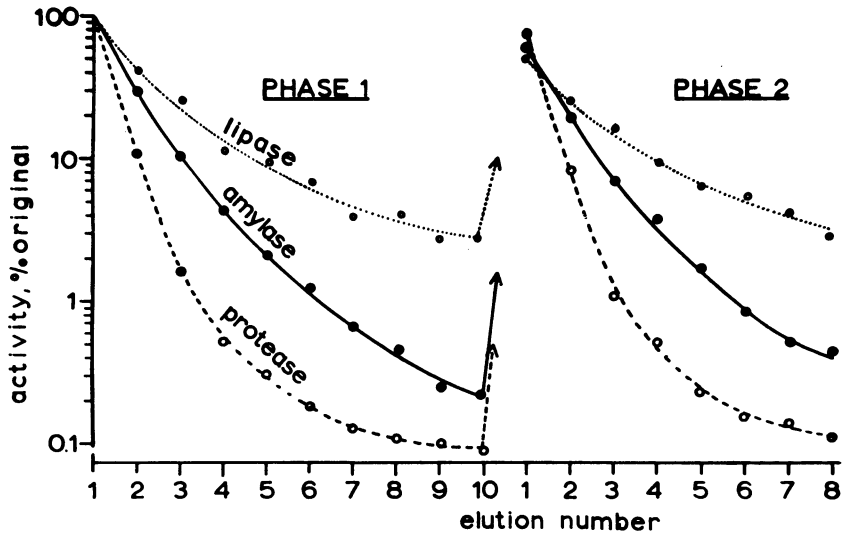

Figure 4. Elution patterns of standard washings (Phase 1), and elutions after re-exposure of intestinal segments to first washings (Phase 2), (Method A).

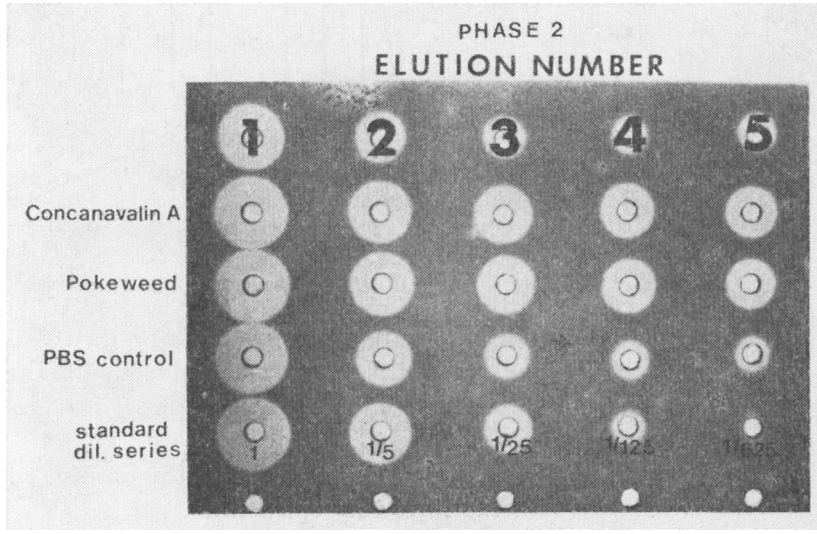

Figure 5. Elution patterns according to Method A (see Fig. 3, Phase 2) of amylase in the presence and absence of lectins. Note that both Concanavalin A and pokeweed mitogen caused a greater and more prolonged release of enzyme than occurred with the PBS control.

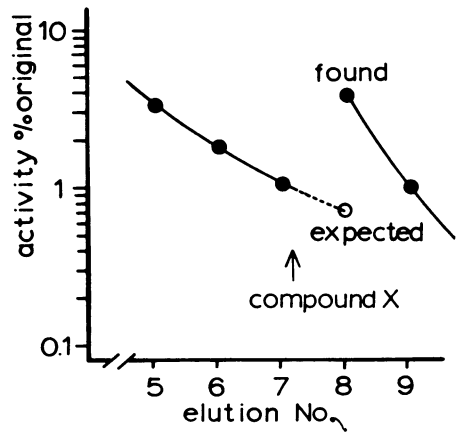

Figure 6. Principle of Method B showing elution pattern of amylase during sequential elutions and following addition of an interfering compound. The ratio found/expected was calculated (Tables 2 and 3).

15 min). After the 10 elutions each two consequtive segments were each re-combined with 5 ml of the first elution pool (intestinal content) in the presence or absence of the sugar* or lectin** (1 % concentrations). The segments were then subjected to further serial elutions. Each elution was then analyzed for its amylase, protease and lipase content by the enzyme diffusion method.

Method B. The initial enzyme levels of different segments of the small intestine were equalized as described in Method A, above. After this, each segment was eluted sequentially with 5 ml PBS. After seven elutions the sugar or lectin suspected of interfering with the enzyme binding was included at a level of 1 % in the PBS used for the eighth elution; the ninth and tenth in plain PBS. All the elutions were then analyzed by enzyme diffusion (Fig. 6).

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* All carbohydrates were from Sigma Chemical Co., St. Louis, MO 63178, USA.

** Lectin sources: PHA (from kidney bean) Type 3: — Sigma Chemical Co., St. Louis, MO 63178, USA. Con A (from jack bean): — Nutritional Biochemicals Co., Cleveland, OH 44128, USA. Pokeweed mitogen: — Grand Island Biological Co., Grand Island, NY 14072, USA.

RESULTS

The effect of the intestinal elution on the release of digestive enzymes from the epithelial lining is shown in Fig. 4 (the whole small intestine eluted). It appears that the elution pattern can be reproduced after re-combining the washed intestine with the original intestinal content.

The fact that the three different enzyme activities are not released at the same rate during sequential elutions indicate that various pancreatic hydrolases do have differing affinities to the intestinal epithelial surface in the following order: lipase > amylase > protease. If the concentration of each enzyme present in the first washing of the everted intestine is regarded as the original intraluminal concentration, and the enzyme activities released during the subsequent nine washings represent the "bound" fraction, the percentage distribution can be calculated (Table 1). However, it appears that much of the lipase and amylase can not be washed out even by 10 15-min elutions, which means that a certain fraction of these enzymes is bound quite firmly.

Table 1. Estimation of the epithelially adsorbed and intraluminal portions of amylolytic, proteolytic and lipolytic activity from results obtained by the sequential elution method (Figs. 3 and 4). The concentration of each enzyme activity in the first washing was taken as the intraluminal activity, and the activities released during the subsequent nine washings represent the "bound fraction".

	Protease	Amylase	Lipase
Bound	12	33	52
Free	88	67	48

The plant lectins (hemagglutinins) effectively interfered with the binding of both lipase and amylase. A picture of the amylase releasing effect of the lectins is shown in Fig. 5. The interference by lectins also became clear by the extrapolation method (Fig. 6, Table 2); in general the lectins released bound amylase and lipase from the intestinal lining. The phytohemagglutinin (PHA) from the kidney bean produced an increased binding of amylase probably indicating a cross linking effect by this lectin. PHA did not, per se, inactivate amylase.

Table 2. Effect of lectins on release of bound amylase and lipase from intestinal epithelium.

	Amylase (units found/expected)	Lipase
Concanavalin A (Canavalia ensiformis = Jack bean)	2.8	5.9
Pokeweed mitogen (Phytolacca americana)	2.5	5.2
Phytohemagglutinin (Phaseolus vulgaris = kidney bean)	0.3	5.0
Raw whole soybean (Glycine max)	1.9	7.0

Table 3. Effect of carbohydrate on amylase release from intestinal epithelium.

	Units found/expected
α-D-fucose	9.0
Mucin	4.6
N-acetyl-β-D-mannosamine	3.8
N-acetyl-D-galactosamine	3.6
N-acetyl-D-glucosamine	3.0
D-glucosamine	2.5
D-ribose	2.2
D-xylose	2.1
D-raffinose	2.0
D-mannitol	2.0
D-arabinose	1.0
β-D-fructose	1.8
2-deoxy-D-ribose	1.6
α-D-glucose	1.6
α-lactose	1.6
D-galactose	1.5
D-mannosamine	1.4
D-mannose	1.4
D-maltose	1.4
D-galactosamine	1.1
Cellulose	1.0
Glycogen (oyster)	0.9
N-acetyl neuraminic acid	0.6
Corn starch	0.5

The effect of various carbohydrates on the release of amylase as measured by the extrapolation method (Fig. 6) is shown in Table 3. The most effective amylase releasing sugars were fucose and N-acetylated sugars. One notable exception was N-acetyl neuraminic acid (sialic acid) which delayed the release of amylase but resulted in an increased release of lipase. Corn starch also increased the binding of free amylase to the intestinal lining.

DISCUSSION

Epithelial binding of amylase and lipase appears to be a specific process influenced by the composition of the intestinal contents, especially dietary carbohydrates, and by the presence or absence of interfering substances.

The luminal surface of the enterocyte is known to be covered by polysaccharide-rich material (the glycocalyx) which must be the site for the adsorption of pancreatic hydrolases. Efforts to determine the chemical nature of this intestinal surface coat has been unsuccessful because the polysaccharide material is difficult to isolate in pure form. When the surface coat was labeled with radioactive materials by in vitro incubation of epithelial cells, it was found that glucose, glucosamine, galactose, mannose, serine, sulphate, acetate and fucose were incorporated into the glycocalyx. The surface coat has been identified as a dynamic cell component continuously synthetized by the enterocyte (*Ito & Revel* 1968, *Forstner* 1969, *Bennett & Le Blond* 1970, *Lojda* 1974). It appears likely, however, that much of the muco-substance adhering to the epithelial surface originates from the secretions of the goblet cells. The brush border of the intestine can be visualized as containing many structurally bound enzymes such as sucrase, maltase, isomaltase, lactase, trehalase, β-D-glucosidase, alkaline phosphatase, ATPase, leucyl-β-naphtylamidase, peptidases, enterokinase, cholesterol- and retinol ester hydrolases, palmitate thiokinase, mono- and diglyceride acylases, sphingomyelinases and ceraminase (*Lojda*). In addition to these structurally bound enzymes, the epithelial surface is able to adsorb intraluminal enzymes (*Ugolev* 1974).

The results described in the present report confirm and extend this finding. Assuming the glycocalyx is the site of adsorption of intraluminal enzymes and that certain carbohydrate moieties of the surface coat are involved as the determinants for enzyme

binding, their introduction in free form should result in occupation of the enzyme-binding site, thus inhibiting the binding and releasing of the enzyme. This approach has been used extensively to study the structures of blood group antigens (*Kabat* 1976). When the effects of a variety of carbohydrates were studied, the same sugar moieties which act as blood group antigen determinants (fucose and N-acetylated amino sugars) were also found to affect enzyme binding (Table 3). These results indicated that the intestinal glycocalyx may react similarly to the surface of blood cells. To test this possibility, the effects of the plant lectins (hemagglutinins), known to interfere with cell surface moieties, such as the blood group antigens were studied (*Jaffe* 1969, *Lis & Sharon* 1973, *Cohen* 1974, *Kabat*). The lectins were found to effectively release bound lipase and amylase, indicating that they interfered with the binding of these intraluminal pancreatic hydrolases.

As evidenced by the marked enzyme release occurring throughout 10 sequential elutions, the lectins appeared to become bound firmly, whereas the effect of competitive sugars lasted only for one or two subsequent elutions.

The effective removal of bound amylase and lipase from the epithelial glycocalyx by the plant lectins has intriguing nutritional implications. It has been demonstrated that plant lectins, such as wheat germ agglutinin, can pass the digestive tract in a biologically intact form after oral ingestion in man (*Brady et al.* 1978). Harmful effects of plant hemagglutinins have been demonstrated in many feeding experiments, but it has never been understood how blood cell-agglutinating factors can be harmful in the gut. *Jaffe* observed that bean lectins became bound when shaken with homogenized intestinal tissue, and assumed that this combination interfered with the normal activity of the intestinal cell. *Etzler* (1974) reported that the rat stomach and duodenal epithelium were capable of binding labeled lectins. The toxicity of food hemagglutinins may be due to this intestinal binding. Many feeds, particularly legumes, contain high amounts of lectins. For example, the protein of jack beans contains 2—3 % Concanavalin A (*Sumner & Howell* 1936), and hemagglutinin represents 1—1.5 % of the soybean protein (*Liener & Pallansch* 1952).

The digestion of food substances occur sequentially on the intestinal epithelial surface. The terminal stages of digestion are

closely associated with absorption of the products by the mucosal cells. A cooperation of all enzymes and carriers is required for optimum digestion and absorption of nutrients at the brush border (*Ugolev*). Epithelial binding of the intraluminal enzymes appears be be an important mechanism which preserves intestinal enzymes and increases the efficacy of digestion. The adverse nutritional effects of plant lectins may be explained by the flushing of the membrane-bound pancreatic hydrolases into the intestinal contents and subsequent escape in the feces.

The present investigation indicates that plant lectins do interfere with digestion because they release pancreatic hydrolases from their binding sites into the intestinal contents and feces. The feeding of raw soybean has been shown to result in a greater need for pancreatic hydrolases, with consequent pancreatic hypertrophy (*Nitsan & Liener* 1976). The antitrypsin in raw soybeans, for the most part, has been blamed for this effect. Following the feeding of raw soybeans, an almost immediate increase in fecal amylase has been demonstrated (*Nitsan & Liener*). The findings agree with the results of the present investigation showing that raw soybeans effectively flush out epithelial-bound amylase. Low efficiency of feed utilization and nitrogen retention usually occur in animals fed plant hemagglutinins. An increased demand for fat-soluble vitamins has been observed (*Jaffe*). These effects may also be explained by the flushing of epithelial-bound enzymes into the intestinal contents and feces, thus decreasing the capacity to utilize these nutrients.

The average life span of the enterocyte is some 72 h, after which it is desquamated into the lumen. Since lectins are shown to be bound quite firmly to the epithelial surface, it is tempting to speculate that this desquamation of intestinal epithelial cells may be a phenomenon which developed to rid the intestinal glycocalyx of harmful lectins.

The phytohemagglutinins are heat-labile proteins whose harmful effects are destroyed by normal cooking. Destruction of lectins by heat treatment is known to enhance the nutritional values of lectin-containing feedstuffs. Further studies may disclose means of improving digestibility of many feedstuffs now labeled as being "poorly digested".

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SAMMANFATTNING

Adsorption av tarminnehållets lipas, amylas och proteas till tarmepitelet studerad med hjälp av sockerarter och hemagglutininer in vitro.

Tarminnehållets pankreashydrolaser adsorberades på ut- och invända (inverterade) tarmsegmenter. När segmenterna eluerades i en serie provrör med buffrade koksaltlösningar, frigjordes den proteolytiska aktiviteten lättare än den amylolytiska eller lipolytiska aktiviteten. Rangordningen för affiniteten måste därför vara: lipas > amylas > proteas.

För att påvisa, att den epiteliala adsorptionen av pankreashydrolaser är en specifik process, gjordes försök att förhindra adsorptionen eller att frigöra den adsorberade enzymaktiviteten med olika sockerarter (som finns i den normala epiteliala kolhydratsubstansen, glycocalyx). Fucos och N-acetylerade sockerarter frigjorde lipas och amylas aktivitet. Växt hemagglutininer, som reagerar med sockerarter på cellytorna, frigjorde effektivt enzymaktivitetet.

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