EFFECT OF DIGESTIVE ACTIVITY AND STARCH INTAKE ON AMYLASE ACTIVITY IN SALIVA
AND PANCREATIC JUICE OF RABBIT

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INTRODUCTION

Ingestion of starch has been closely related to the enteritis syndrome, frequent in growing rabbits (Cheeke and Patton, 1980; Morisse, 1982; Morisse *et al.*, 1985). A primary cause should be the inability of the enzymatic system, mainly of pancreatic origin, to digest large amounts of this carbohydrate in the small intestine.

The activity of pancreatic amylase has sometimes been assessed, but there is little work about amylase activity in saliva, *succus entericus* and milk, which could have a relative importance as it has been demonstrated in humans.

The present work tried to evaluate amylase activity in saliva and pancreatic juice of adult rabbits, fasting and after feeding, when two diets with a different starch content were supplied, in order to achieve an appropriate sampling for a following study about age-related changes in these enzymatic activities.

MATERIAL AND METHODS

Sampling. Eight adult male rabbits were used to obtain saliva. Three samples were taken from each one: a first basal sample after fasting one day and the others 10 and 60 minutes after offering the diets. Before starting the experiment, half of the animals were fed *ad libitum* for 15 days on a low starch pelleted diet (15.2% DM) and the rest of them on a high starch diet (31% DM). Samples of 0.1 to 0.3 ml were collected did aspirated through a soft plastic cateter, centrifugated and deep-frozen until assay.

Samples of pancreatic juice were obtained from twelve adult male rabbits either in basal condition, fasted for one day, or 150 minutes since pellets were offered. Animals were previously distributed in two groups and fed for 15 days on the diets mentioned before. Rabbits were anaesthetized with sodium thiopental, 20 mg/Kg liveweight, and a cannula of 0.8 mm internal diameter was implanted in the pancreatic duct, near the duodenum. Samples were kept frozen until analyzed.

Amylase assay. Amylase was determined by the method of Bernfeld (1955) and the results are expressed as the amount of maltose formed during the hydrolisis of a starch solution incubated at 37 °C and 6.9 pH (μM/min). Protein in biological fluids was measured using the method of Lowry *et al.* (1951).

Statistical methods. An analysis of variance with two factors (feeding *status* and dietary starch) was carried out, using the test of Duncan to compare means.

<div align="center">RESULTS AND DISCUSSION</div>

Saliva. The effect of feeding and starch content of the diet is shown in Table 1. A large variation was observed for the results. No significant part of this variation could be explained by those factors or their interaction. Large differences between individuals have also been described in human saliva (Lieberman *et al.*, 1977).

Table 1. Amylase activity and protein content of the saliva in several feeding *status* with diets differing in starch content.

	Fasting		10' after feeding		60' after feeding		Mean (se)	p_{tail}		
								feeding *status*	dietary starch	interaction
	Low	High	Low	High	Low	High				
Total activity (U/ml)	146	155	115	143	126	161	141 (9)	p>0.05	p>0.05	p>0.05
Protein (mg/ml)	7.3	6.4	5.2	7.1	5.6	6.1	6.3 (0.4)	p>0.05	p>0.05	p>0.05
Specific activity (U/mg protein)	21.1	28.1	27.2	20.9	25.3	27.0	24.9 (2.2)	p>0.05	p>0.05	p>0.05

The effect of food ingestion on amylase concentration seems to be related to flow rate of saliva, but this relationship is not adequately explained (Dawes, 1970).

Regarding to the influence of dietary starch, the results are contradictory. Lieberman *et al.* (1977) did not find any relationship, while Wesley-Hadzija and Pigon (1972) found an appreciable increase in amylase activity of the saliva when a high starch diet was consumed.

No data on the amylase present in saliva of rabbits are available. Saliva of herbivores probably has a low amylase activity compared to 500 U/ml and 600 U/mg protein reported in human (Wesley-Hadzija and Pigon, 1972; Behall *et al.*, 1973). Our results would support that conclusion, although the great variability, already commented, has to be considered, together with the absence of standardization of the amylase assays.

Pancreatic juice. The results are shown in Table 2. No significant differences in total amylase activity and protein content were observed after ingestion of food or in animals fed the high starch diet. These variables showed a great residual variation, but were positively correlationed within each experimental group and this contributed to the low residual variation of the specific amylase activity. Thus, results were clearer when specific amylase activity is considered as unit, because it increased after ingestion of both diets, but specially for the high starch diet.

Table 2. Amylase activity and protein content of the pancreatic juice in several feeding *status* with diets differing in starch content.

	Fasting		150' after feeding		Mean (se)	Prob. feeding *status*	dietary starch	interaction
	Low	High	Low	High				
Total activity (U/ml)	4320	2780	3220	6380	4180 (660)	p>0.05	p>0.05	p>0.05
Protein (mg/ml)	25.7	15.5	11.8	13.9	16.7 (2.9)	p>0.05	p>0.05	p>0.05
Specific activity (U/mg protein)	158ᵃ	177ᵃ	254ᵇ	454ᶜ	261 (10)	p<0.001	p<0.001	p<0.05

a, b, c Means followed by different letter differ with p<0.05

The effect of digestive activity on secretion of pancreatic amylase has not been well studied in rabbits. Wu *et al.* (1985) found that the flow of pancreatic juice varied throughout the day, showing a peak between 3 and 6 hours after ingestion. Using parasimpatic stimulants or gastrointestinal hormones increased the flow and protein output (Murillo and López, 1971; López *et al.*, 1976).

The pancreas gland would be stimulated by digesta in duodenum, activating the secretion of juice and increasing its specific amylase activity, which in turn would increase amylase output. This hypothesis has been demonstrated in pigs (Simoes-Nunes and Corring, 1979).

The influence of dietary starch has been insufficiently studied. Corring *et al.* (1972) described the amylase content of the pancreas gland in young rabbit. It increases for the first six weeks of life, being linked to physiological development itself, but the feeding schedule can alter the level of response.

Although the flow of pancreatic juice has not been determined, this study suggests that the ingestion of starch activates the secretion of pancreatic amylase in adult rabbits, as in other species. The adaptation period to starchy concentrates has been found to be less than 15 days, like those reported for rats and broilers (Huland and Bird, 1972; Johnson *et al.*, 1977), although Simoes-Nunes and Corring (1979) showed a much faster response in pigs.

The only available data on amylase activity of pancreatic juice of rabbits were obtained by Courtot and Baradat (1973) from 18-days old animals and are not comparable with our results. The figures obtained here suggest, from comparisons with other species, that adult rabbits present high amylase activity in the pancreatic secretion, as monogastric. Within this group, total amylase activity and protein content seem to vary with the intensity of metabolism, increasing when size of the species decreases, whereas specific amylase activity presents smaller differences between species (Hulan and Bird, 1972; Simoes-Nunes and Corring, 1979).

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SUMMARY

Amylase activity in saliva and in pancreatic juice was determined in
rabbits fed on diets containing 15.2 and 31% starch. Samples were collected
from animals fasting for 1 day or after feeding. Both factors, starch percentage
of the diet and feeding status of animals, had no influence on the activity of
amylase in saliva, but they clearly affected to amylase activity in pancreatic
juice. Mean fasting values for pancreatic juice were 150 and 180 U/mg protein
when animals were fed on diets with 15.2 and 31% starch content respectively;
these figures increased after feeding to 250 and 450 for the two diets
mentioned above.

RESUMEN

Se estudió la actividad amilásica de la saliva y del jugo pancreático del
conejo en diferentes fases de actividad digestiva con dietas de distinto
contenido en almidón. La actividad amilásica salivar no presentó cambios
importantes en función de la mayor o menor actividad digestiva o del contenido
en almidón del pienso consumido. La actividad amilásica del jugo pancreático
varió ostensiblemente con el grado de actividad digestiva y la riqueza en
almidón de la dieta ingerida, si bien, en virtud del diseño experimental, estas
variaciones sólo se pusieron de manifiesto claramente cuando se expresó como
actividad amilásica específica; así, se obtuvieron valores medios de 150-180
U/mg de proteína en las muestras tomadas en condiciones basales,
independientemente del contenido en almidón del pienso consumido, mientras que
en las muestras tomadas durante el periodo postprandial se registraron valores
medios de 250 ó 450 U/mg de proteína según de trató respectivamente de un
pienso pobre o rico en almidón.

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