

ENZYME OUTPUT CAPACITY OF THE PANCREAS IN ADULT RABBIT ACCORDING TO DIET COMPOSITION

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ABSTRACT

Three groups of adult rabbits were fed for 35 days with high starch diet (high starch group, HSG), high protein diet (high protein group, HPG), or high fat diet (high fat group, HFG) compared with a control group (CG) fed with a specific diet. Then pancreatic juice was collected and measured in acute experiments, in two variants of secretion: basal and stimulated by secretin. Pancreatic juice samples were analysed for protein content and amylase, trypsin and lipase activities. Basal values of juice flow (in $\mu\text{L}/10 \text{ min}/\text{Kg b.w.}$) showed no significant differences between any experimental fed groups (overall mean: 33) vs. CG (mean: 27). Secretin stimulated juice flows were increased in all the groups from 3.5 in HFG to 4.8 folds in CG, but the increase was higher only in HSG (180) vs. CG (130) ($P<0.05$). Basal protein flows (in $\mu\text{g}/10 \text{ min}/\text{Kg b.w.}$) of experimental fed groups (overall mean: 29) did not differ significantly vs. CG (mean: 32). In contrast, the stimulated protein output was 39% higher in HSG vs. CG ($P<0.05$). Amylase activities were higher in HSG vs. CG, both in basal (144×10^3 and 52×10^3 amylase units (AU), $P<0.001$) and in the stimulated pancreatic juice (422×10^3 and 162×10^3 AU, $P<0.001$). Moreover, the activities of trypsin and lipase in HSG did not differ significantly vs. CG, nor for basal neither for stimulated juice. Trypsin activity (in nmols benzoyl-arginyl-ethyl-ester decomposed / 10 min / kg bw) was higher for HPG vs. CG, both in basal (62.5 vs. 22.2, $P<0.01$) and in stimulated juices (166.0 vs. 31.5, $P<0.001$). Basal lipase activity (in mequivalents of liberated oleic acid per mg protein per h, 37°C) was higher in HFG vs. CG (122.4 and 86.5, respectively). In the stimulated juice, lipase activity increased to 246.0 in HFG and 184.1 in CG, but no significant differences were found in HFG vs. CG nor for lipase neither for amylase and trypsin.

Key words: Pancreatic juice, nutrition, rabbit.

INTRODUCTION

Many works show the influence of different factors on the rabbit enzymatic digestive system, intestinal glands and the pancreas in main. Gilliland and Glazer (1980) found that enzyme secretion by the rabbit pancreas remained proportional (parallel) after acute stimulation despite a 100% rise in protein output. The researches of Gutierrez *et al.* (2002) indicated that digestive capability of early-weaned rabbits is limited and should be taken into account to establish optimal levels and sources of carbohydrates in diet. Debray *et al.* (2002) found a different development of trypsin, chymotrypsin, amylase and lipase activities into the small intestine contents, not related to changes in pancreatic or intestinal enzymatic profiles but more dependent on quality of dietary ingredients. *In vitro* rabbit pancreas experiments showed that direct bath administration of pancreozymin or acetylcholine produced prompt increases of protein output (Welch and Littman, 1974). The influence of diet on digestive parameters and not only has still many unknowns (Gidenne and Fortun-Lamothe, 2002). The aim of our work is to find the rabbit pancreas ability to change flow ratio of different digestive enzymes in the secreted juice depending to the composition of diet.

MATERIALS AND METHODS

Animals, animal housing, animal grouping, diets

Six months old New Zealand white male rabbits, 3.230 ± 0.12 kg b.w. were used in this experiment. The rabbits were housed in metallic cages (75x70x47 cm), two rabbits per cage, in a naturally lighted room at $24 \pm 3^\circ\text{C}$, and 65% humidity, during September and October. The cages were made of galvanized wire net and equipped with automatic drinkers and manual feeders. The animals were fed *ad libitum* and had free access to water. Four groups of seven rabbits each one were constituted according to the composition of their pelleted diet and housed in four cages per group: a control group (CG) fed with a control diet, a group (HSG) fed with a high starch diet, a group (HPG) fed with a high protein diet, a group (HFG) fed with a high fat diet, according to calculated chemical composition (Table 1). Duration of feeding with experimental prescriptions was 35 days, during which rabbit weight and food intake were also monitored. Main ingredients of the diets were: maize, wheat bran, soybean meal, dehydrated lucerne (*Medicago sativa*), and a vitamin-mineral supplement. The starch content was enriched by addition of maize. Protein content was enriched by addition of soybean meal. Fat content was enriched by addition of flaxseeds.

Table 1. Calculated chemical composition of the experimental diets.

Diets	Dry matter (DM) (g/Kg)	Crude fibre (g/kg DM)	Starch (g/kg DM)	Crude protein (g/kg DM)	Fat (g/kg DM)
Control	877	202	188	123	28
High starch	897	176	261	120	18
High protein	868	196	178	162	27
High fat	870	184	181	120	58

At the end of the experimental feeding period, four or five rabbits of each group were immediately anesthetized with chloralose 1% in a 0.9% saline solution injected intravenously (*v. auricularis*), at a dose of 100 mg/Kg bw, without any starvation or meal delay before surgery. The *vena femoralis* was prepared by inserting a cannula to inject secretin for stimulation of pancreatic juice flow. The abdomen cavity was opened and the main pancreatic duct was spotted. A silver cannula (outer diameter of 0.05 mm) was inserted into the main pancreatic duct just before its opening into the intestinal lumen to collect pure pancreatic juice. A calibrated polyethylene tube (0.01 mL/mm) was attached to the free end of the silver cannula for pancreatic juice flow measurement. Then, the abdominal wall was closed with sutures. The rectal temperature of the animals was maintained at 38°C by a heating lamp.

Sampling of pancreatic juice

Under anaesthesia, the basal pancreatic juice was collected and measured for a period of 50 minutes from the moment of the attachment of the cannula to the main pancreatic duct. The calibrated tube was detached when the period of 50 minutes ended. Another empty calibrated tube was attached immediately to the free end of the silver cannula. Then, a single dose of synthetic secretin (BioVision, San Francisco), $100 \mu\text{g/Kg}$ b.w. was injected to collect stimulated pancreatic juice. Measurement of pancreatic juice flow continued for another 50 minutes. Secretin has hidraletic effect on the pancreas, allowing it to develop its exocrine particularities. The volume of juice was measured on the calibrated tube every 10 minute from the beginning of the collecting, so five periods of measurements were done: 0-10, 10-20, 20-30, 30-40 and 40-50 minute periods both, in basal and stimulated experiments. At the end of the 50 minute periods, all volume of pancreatic juice was measured. The obtained values from each rabbit were used for the calculation of the mean values of the two periods of secretion (0-50 minutes for basal and 0-50 minutes for stimulated secretion). The contents of calibrated tubes from each rabbit and from each sample (basal or secretin stimulated) were separately collected and diluted 10 or 20 times with a buffered saline solution and conserved at -20°C up to biochemical determinations. The anesthetized animals were killed at the end of the experiment, by cutting the *a. carotidis communis*.

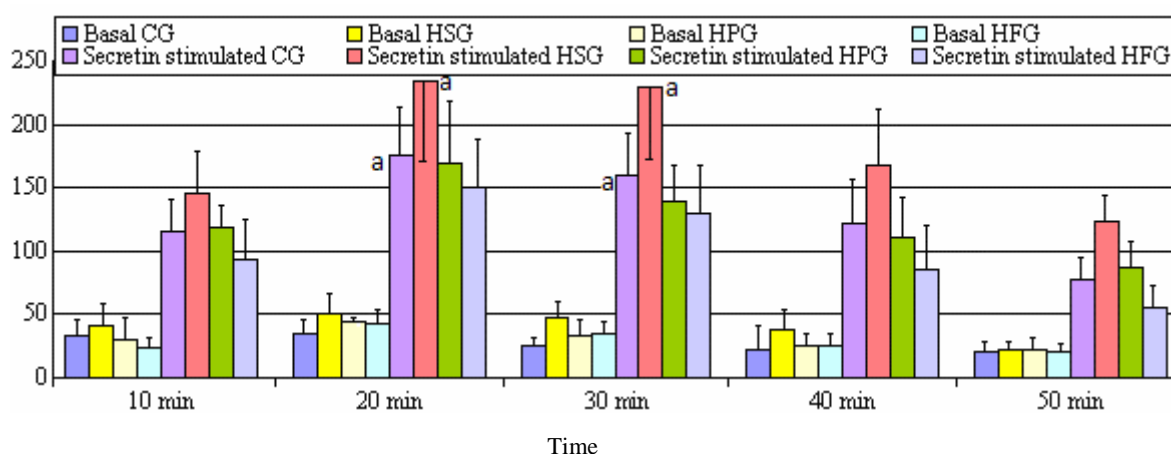
Analyses and statistics

Protein contents, amylase (EC 3.2.1.1), trypsin (EC 3.4.21.4), and lipase (EC 3.1.1.3) activities were measured in each pancreatic juice sample, basal or secretin stimulated, following previous methods mentioned by Dojana *et al.* (2000). The data were statistically analysed and presented as mean \pm standard error of mean (SEM). The significance of differences between control and experimental groups was evaluated using Student's unpaired *t* test. Repeated measures ANOVA were performed to analyse the effect of the diet influence on enzyme activity (STATISTICA v10 QC from StatSoft inc.).

RESULTS AND DISCUSSION

The basal value of pancreatic secretion of the CG averaged 27.0 ($\mu\text{L}/10 \text{ min./kg b.w.}$, CV: 24%) and was relatively constant, ranging between 20 and 35 along the 50 minutes of acute experimental monitoring ($P>0.05$, figure 1). Mean basal values of juice flow of the three experimental groups were similar to control: 40 (CV= 28%) in HSG, 31 (CV= 27%) in HPG and 29 (CV= 31%) in HFG ($P>0.05$).

When stimulated with secretin, a maximal pancreatic secretion was reached within 20 minutes whatever the groups. The peak of secretin was 176 ± 46 in CG ($\mu\text{L}/10 \text{ min./kg b.w.}$), while in HPG and HLG groups, the peaks were 169 ± 49 and 150 ± 64 , respectively, without significant differences with CG. The highest peak of secretion was reached by the HSG, with a value of 234 ± 52 that was higher than CG ($P<0.05$). Mean stimulated pancreatic secretions (over 50min.) were: 130 ± 39 in CG, 180 ± 49 in HSG, 125 ± 31 in HPG and 103 ± 37 in HFG, and HSG differed from CG ($P<0.05$).



unit: $\mu\text{L}/10 \text{ min./Kg bw.}$ Stimulation of pancreatic secretion with a single dose of secretin ($100 \mu\text{g}/\text{Kg bw.}$). Mean values (\pm SEM) from 4 or 5 adult rabbits. Pancreatic secretion was monitored over 50 minutes. ^a: significant difference between HSG and CG.

Figure 1. Basal and stimulated pancreatic secretion in adult rabbit fed either with a high starch, fat or protein diets for 35 days.

It seems that the pancreas of HSG rabbits has undergone some functional changes during or due to feeding with high starch diet since the peak value and mean juice flow of the stimulated secretion in this group were significantly above the CG. Similar results were found by Jakob *et al.* (2000) who reported that potato fibre in the diet in growing pigs tended to stimulate the pancreatic secretion.

No statistical differences were found between groups fed with experimental diets and CG concerning the basal protein output (table 2). Secretin induced a 4-fold increase of protein output in both HSG and HPG, while in CG and HFG the increase was about 3-fold. The stimulated protein output was similar in both HSG and HPG rabbit groups vs. CG ($P>0.05$). Protein outputs induced by the administration of secretin, although smaller, were comparable to those induced by cholecystokinin (Gilliland and Glazer, 1980) or cholecystokinin and methacholine chloride on pancreatic exocrine secretion in rabbits (Adelson *et al.*, 1995). Higher differences respect to the pancreatic protein output were reported by Jakob *et al.* (2000) in growing pigs fed with high potato fibre diet.

Table 2. Protein output and enzyme activities of the pancreatic juice collected as a basal and secretin stimulated secretions in adult rabbit fed with high starch, protein or fat diets.

Group	Protein output ¹		Amylase activity ²		Trypsin activity ³		Lipase activity ⁴		Weight ⁵ (kg)	Mean intake ⁶ (g/d)
	Basal	Stimulated	B	S	B	S	B	S		
CG - Control	32.1	88.9	52.0	162.1	22.2	31.5	86.5	184.1	3.31	189
HSG	35.3	124.3	144.3 **	422.0**	26.5	42.0	90.0	201.0	3.38	176
HPG	28.6	105.4	25.3	144.3	62.5 *	166.0*	71.3	176.5	3.28	195
HFG	25.2	85.6	32.2	156.7	18.7	42.9	122.4	246.0	3.18	181
SEM	8.4	77.7	55.0	173.5	16.6	60.3	42.2	38.1	0.74	44
P level	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS

B = basal secretion, S = secretin stimulated secretion. ¹ : $\mu\text{g} / 10 \text{ min./kg bw}$. The values are expressed as mean from measurement during 50 minutes and from 4 or 5 rabbits. ² : amylase units, $\text{AU} \times 10^3$, mg of starch hydrolyzed in 30 min. at 37°C . ³ : unit= nmol benzoyl-arginyl-ethyl-ester decomposed /kg bw/10 min. ⁴ : unit= mequivalents of liberated oleic acid per mg protein per h, at 37°C , using triolein as a substrate. ⁵ : mean of final weight of the rabbits. ⁶ : mean intake of the last week of the experimental period. * : $P < 0.01$, unpaired Student's *t* test. ** : $P < 0.001$, unpaired Student's *t* test.

Amylase activity was found about 3-fold more increased in basal pancreatic juice of HSG vs. CG ($P < 0.001$). Secretin stimulated pancreatic juice of HSG registered an amylase activity value about 3-fold higher vs. the secretin stimulated amylase activity of pancreatic juice in CG ($P < 0.001$). In the same HSG, the activities of trypsin and lipase did not differ significantly vs. CG ($P > 0.05$), nor for basal neither for secretin stimulated pancreatic juice samples.

Trypsin activity was higher in basal pancreatic juice of HPG compared to control ($P < 0.01$), with a 3:1 ratio. In the same group, the trypsin activity of the secretin stimulated pancreatic juice was 5.3-fold higher vs. CG ($P < 0.001$). On the other hand, amylase and lipase activities values of HPG group were similar to those of CG. According to the results of this experiment, the pancreas could discharge large quantities of trypsin into the small intestine, although total protease activity of the small intestine content could be lower than that of the caecum (Marounek *et al.*, 1995).

Lipase activity was also increased in basal and stimulated pancreatic juice of HLG compared to CG. However, lipase activities in HLG were not significantly different vs. CG ($P > 0.05$), nor for basal nether for stimulated pancreatic juice. Our results were partially in agreement with the results reported by other authors: Debray *et al.* (2003) found in growing rabbit that small intestine activity of lipase was 58% higher ($P < 0.001$) in a high fat than in a low fat diet, but they found that amylase, trypsin and chymotrypsin activities were not influenced by the energetic sources of the diet. Although amylase and trypsin secretion showed parallel increases (3-fold increases each one in our experiment), in the case of lipase, the situation seemed to be different. Lipase activity increased much less than the other two studied enzymes. Other factors than the presence of the fat substrate seemed to play a role. Adelson *et al.* (2005) consider that the nonparallel secretion of the digestive enzymes occurs routinely, among and between basal conditions and the two classical types of stimulant, hormonal and neurotransmitter, seeming to be the rule not the exception. Instead, parallel increase in the mean values of activities of lipase, trypsin and amylase was found by Jakob *et al.* (2000) in growing pigs fed with high potato fibre diet.

CONCLUSION

In conclusion, higher starch, protein or fat diets do not alter specifically the basal or the stimulated pancreatic juice volume flow, or protein output in anesthetised adult rabbits. In contrast, pancreatic enzyme output adapted differently to dietary substrate concentration changes for starch, protein or fat. Further researches could find the velocity of adaptation of pancreatic exocrine secretion to changes in substrate levels and to what degree the pancreas can respond adequately to increased supply of various substrate levels. The results may be used improve the nutritional recommendation of the rabbit.

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