

ENZYME OUTPUT CAPACITY OF THE RABBIT EXOCRINE PANCREAS TO ADAPT DIFFERENTLY TO FOOD SUBSTRATE CONCENTRATION CHANGES

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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 analyzed for protein content and amylase, trypsin and lipase activities. Basal values of juice flow showed no significant differences between any experimental fed group vs. CG ($P > 0.05$). Secretin stimulated juice flows were increased in all the groups, but the increase was significant higher only in HSG vs. CG ($P < 0.05$). Basal protein flows of experimental fed groups did not differ significantly vs. CG ($P > 0.05$). In contrast, the stimulated protein output was significant higher in HSG vs. CG ($P < 0.05$). Amylase activities were significant higher in HSG vs. CG, both in basal (144.3×10^3 and, respectively, 52.0×10^3 amylase units (AU), $P < 0.001$) and in the stimulated pancreatic juice (422.0×10^3 and, respectively, 162.1×10^3 AU, $P < 0.001$). Moreover, the activities of trypsin and lipase in HSG did not differ significantly vs. CG ($P > 0.05$), nor for basal neither for stimulated juice. Trypsin activity (in nmols benzoyl-arginyl-ethyl-ester decomposed / 10 min / kg b.w.) increased significantly in HPG vs. CG, both in basal (62.5 vs. 22.2, $P < 0.01$) and in stimulated juices (166.0 vs. 31.5, $P < 0.001$). On the other hand, amylase and lipase activities of HPG group were similar to those of CG. 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 ($P > 0.05$).

Key words: food composition, pancreatic enzymes, 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.

MATERIAL AND METHODS

Six months old New Zealand white male rabbits, 3.230 ± 0.120 kg b.w. were used in this experiment. The rabbits were housed in common metallic cages, two rabbits per cage, in a naturally lighted room at $24 \pm 3^\circ\text{C}$, and 65% humidity. The cages were made of galvanized wire net and equipped with automatic drinkers and manual feeders. The animals were fed *ad libitum* and have free access to water. Four groups of seven rabbits each one were constituted according to the composition of their diet:

- a control group (CG) fed with a specific 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.

Duration of feeding with experimental prescriptions was 35 days. 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. The chemical composition of the diets are presented in Table 1.

Table 1

Chemical composition of the experimental diets

| Diet | Dry matter (DM) (g/Kg) | Crude fiber (g/Kg DM) | Starch (g/Kg DM) | Crude protein (g/Kg DM) | Fat (g/Kg DM) |
|-------------------|------------------------|-----------------------|------------------|-------------------------|---------------|
| Control diet | 877 | 202 | 188 | 123 | 28.3 |
| High starch diet | 897 | 176 | 261 | 120 | 18.0 |
| High protein diet | 868 | 196 | 178 | 162 | 27.1 |
| High fat diet | 870 | 184 | 181 | 120 | 58.3 |

At the end of the experimental feeding period, four or five rabbits of each group were anesthetized with chloralose 1% in a 0.9% saline solution injected intravenously (*v. auricularis*), in dose of 100 mg/Kg b.w. 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.

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 µg/Kg b.w. was injected to collect stimulated pancreatic juice. Measurement of pancreatic juice flow continued for another 50 minutes.

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*.

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 obtained data were statistically analyzed and presented as mean \pm standard error of mean. The significance of differences between control and experimental groups was evaluated using Student's unpaired *t* test.

RESULTS AND DISCUSSION

The evolutions of basal and secretin stimulated juice flow for 50 minute periods are presented in Figure 1. Mean basal value (in microL / 10 minutes / kg b.w.) of juice flow of the CG was 27.0 (not shown in Figure 1) and maintained relatively constant, ranging between 20 and 35 along the 50 minutes of acute experimental monitoring. Mean basal values of juice flow of experimental diet groups along the same period of time were (not shown): 40.2 in HSG, 31.2 in HPG and 29.2 in HFG, with no significant differences vs. CG ($P>0.05$).

Secretin stimulated the juice flows. Peaks of the juice flow were reached in 20 minutes following the moment of secretin administering in all the four groups. The peak of the secretin stimulated pancreatic juice flow (in $\mu\text{L} / 10$ minutes/kg b.w.) was 176 in CG. In HPG and HLG groups, the peaks of secretin stimulated juice flow were 169 and 150, respectively, with no significant differences vs. CG ($P>0.05$). The highest peak of juice secretion was reached by the HSG, with a value of 234 and a significant difference was found vs. CG ($P<0.05$). Mean stimulate values of juice flow (not shown) were: 130 in CG, 180 in HSG, 125 in HPG and 103 in HFG, a significant difference being between HSG and control ($P<0.05$).

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 significant above the CG. Some similar results were found in piglets. Jakob *et al.* (2000) reported that potato fiber in the diet in growing pigs evoked in tendency an increase in the volume of secretion of pancreatic juice.

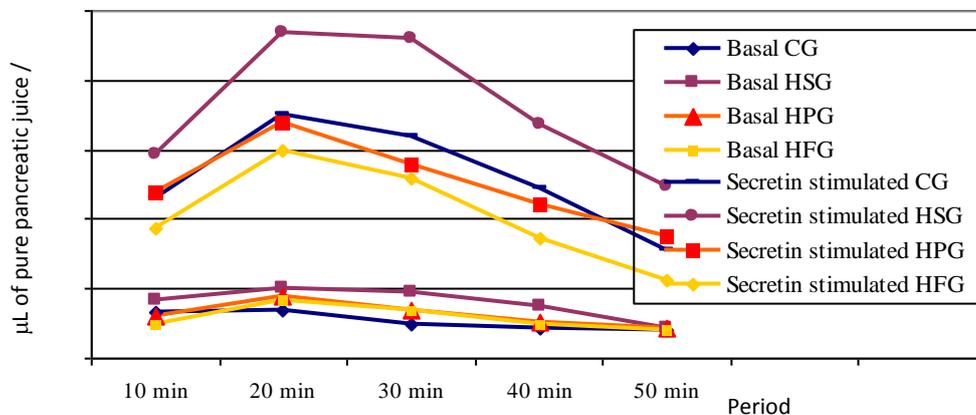


Figure 1. Basal and a single dose of secretin (100 µg/Kg b.w.) stimulated pancreatic juice flow levels over 50 minutes of monitoring periods in adult rabbits fed with high starch, fat or protein diets for 35 days vs. a control rabbit group fed with a specific diet.

Protein contents and enzyme activities of the collected pancreatic juice samples are shown in Table 2.

No statistical differences were found between groups fed with experimental diets and CG concerning the basal *protein* output ($P > 0.05$). Secretin administering 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 higher in both HSG and HPG rabbit groups vs. CG even though the differences were not significant from statistical point of view ($P > 0.05$). Protein outputs induced by the administration of secretin in our experiments, although smaller, are 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). Significant differences regarding pancreatic protein output were reported by Jakob *et al.* (2000) in growing pigs fed with high potato fiber diet vs. control.

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.

Table 2

Protein output (μg protein per 10 min per kg b.w.) and enzyme activities of the pancreatic juice collected during 50 minutes periods as a basal and as a single dose of secretin (100 $\mu\text{g}/\text{Kg}$ b.w.) stimulated secretions in adult rabbits following 35 days of feeding with high starch, protein or fat diets vs. a control group. B = basal secretion, S = secretin stimulated secretion. The values are expressed as mean \pm standard error of mean of four of five animals in acute experiments

| Group | Protein output | | Amylase activity ¹ | | Trypsin activity ² | | Lipase activity ³ | |
|-------|----------------|------------------|-------------------------------|--------------------|-------------------------------|--------------------|------------------------------|------------------|
| | B | S | B | S | B | S | B | S |
| CG | 32.1 \pm 2.3 | 88.9 \pm 17.4 | 52.0 \pm 7.4 | 162.1 \pm 32.2 | 22.2 \pm 4.4 | 31.5 \pm 4.0 | 86.5 \pm 20.0 | 184.1 \pm 42.1 |
| HSG | 35.3 \pm 5.2 | 124.3 \pm 32.1 | 144.3 \pm 33.3** | 422.0 \pm 51.4** | 26.5 \pm 6.0 | 42.0 \pm 10.0 | 90.0 \pm 19.4 | 201.0 \pm 33.2 |
| HPG | 28.6 \pm 4.1 | 105.4 \pm 19.5 | 25.3 \pm 5.5 | 144.3 \pm 4.3 | 62.5 \pm 12.5* | 166.0 \pm 21.5** | 71.3 \pm 18.5 | 176.5 \pm 46.1 |
| HFG | 25.2 \pm 6.2 | 85.6 \pm 22.2 | 32.2 \pm 8.4 | 156.7 \pm 5.0 | 18.7 \pm 5.0 | 42.9 \pm 5.4 | 122.4 \pm 31.3 | 246.0 \pm 61.3 |

CG – control group, rabbit group fed with a specific diet

HSG – rabbit group fed with high starch diet

HPG – rabbit group fed with high protein diet

¹Amylase activity is expressed as amylase units, $\text{AU} \times 10^3$, mg of starch hydrolyzed in 30 minutes at 37°C.

²Trypsin activity is expressed as nmols benzoyl-arginyl-ethyl-ester decomposed / kg b.w./10 min.

³Lipase activity is expressed as mequivalents of liberated oleic acid per mg protein per h, 37°C, using triolein as a substrate.

HFG – rabbit group fed with high fat diet

*P<0.01, unpaired Student's *t* test

** P<0.001, unpaired Student's *t* test

Trypsin activity was found significantly increased in basal pancreatic juice of HPG vs. control (P<0.01), with a 3/1 ratio. In the same group, the trypsin activity of the secretin stimulate pancreatic juice was significant 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 by comparing 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 regarding lipase activity are partially in agreement with the results reported by other authors: Debray *et al.* (2003) found that small intestine activity of lipase was higher in high fat diet fed

rabbits than in low fat diet fed rabbits, but they found that the other pancreatic and intestinal enzyme 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 seems to be different. Lipase activity increased much less than the other two studied enzymes, thus seeming to depend on other reasons, not only the presence of the food fat substrate. Adelson *et al.* (2005) consider that the nonparallel secretion of the digestive enzymes occurs routinely, even during constant stimulation, and is due heterogeneous intrapancreatic sources. 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 fiber diet.

CONCLUSIONS

Higher starch, protein or fat diets do not alter specifically the basal or the stimulated pancreatic juice volume flow, or protein output in rabbits. In contrast, pancreatic enzyme output adapts differently to food 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.

REFERENCES

- Adelson J.W., Clarizio R., Coutu J.A. 1995. Pancreatic digestive enzyme secretion in the rabbit: Rapid cyclic variations in enzyme composition. *Proc. Natl. Acad. Sci.*, 92, 2553-2557.
- Debray L., Le Huerou-Luron I., Gidenne T., Fortun-Lamothe L. 2003. Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. *Comp Biochem Physiol A Mol Integr Physiol.* 135, 3, 443-55.
- Dojana N., Dinischiotu A., Militaru M. 2000. The effect of thyroxin, insulin, hydrocortisone or adrenaline administration on pancreatic exocrine secretion in rabbit. *World Rabbit Science. Journal of World Rabbit Association*, vol. 8, suppl. No. 1, 175-182.
- Gidenne T., Fortun-Lamothe L. 2002. Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Animal Science*, 75, 169-184.
- Gilliland E.L., Glazer G. 1980. Paralel secretion of enzymes by the rabbit pancreas. *J. Physiology*, 303, 33-41.
- Gutierrez I., Espinosa A., Garcia J., Carabano R., De Blas J.C. 2002. Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned. *J. Anim. Science.* 80, 4, 1029-1037.

Jakob S., Mosenthin R., Thaela M.J., Weström B.R., Rehfeld J.F., Olsen O., Karlsson S., Ahrén B., Ohlsson A., Karlsson B.W., Pierzynowski S.G. 2000. The influence of potato fibre on exocrine pancreatic secretions and on plasma levels of insulin, secretin and cholecystokinin in growing pigs. *Archives of Animal Nutrition*, 53, 3, 273-291.

Marounek, M., Vovk S. J. 1995. Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition*, 73, 463-469.

Welch R., Littman A. 1974. Protein output by the in vitro rabbit pancreas. *J. of applied physiology*, 37, 2, 235-23.