

Digestive enzyme concentrations and activities in healthy pancreatic tissue of horses

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Objective—To measure concentrations and activities of major digestive enzymes in healthy equine pancreatic tissue.

Animals—7 adult horses with normal pancreatic tissues.

Procedures—Small pieces of pancreatic tissue were collected immediately after euthanasia, immersed in liquid nitrogen, and maintained at -80°C until analyzed. Concentrations and activities of amylase, lipase, chymotrypsin, trypsin, and elastase were determined by use of a microtiter technique. Relative pancreatic protein concentrations were determined by use of bovine serum albumin as the standard. Pancreatic DNA was extracted and concentrations determined by use of the diphenylamine method with calf thymus DNA as the standard.

Results—The pancreatic cellular concentration of each enzyme, expressed as units per milligram of DNA, was consistent among horses. Cellular concentration of lipase ($1,090.8 \pm 285.3$ U/mg of DNA) was highest, followed by amylase (59.5 ± 9.8 U/mg of DNA). Elastase, trypsin, and chymotrypsin were detected in small concentrations (1.9 ± 0.6 , 3.5 ± 1.5 , and 9.6 ± 2.9 U/mg of DNA, respectively). Similar results were obtained for specific activities of the enzymes.

Conclusions and Clinical Relevance—Results were unexpected because, under natural conditions, the predominant energy source for horses is carbohydrate. These results may indicate, in part, the reason horses seem to tolerate large amounts of fat added to their diet. (*Am J Vet Res* 2007;68:1070–1072)

Little is known about equine pancreatic function, in large part because of the relative inaccessibility of the organ for in vivo studies. Nevertheless, results of the few studies that have been performed indicate that its function is quite unique in comparison to other mammalian species. Classic studies were performed by Alexander and Hickson,¹ who found that basal secretion is profuse and continuous; that when volume of secretion is increased by injection of secretin, the volume-related reciprocal change in chloride and bicarbonate concentration of the pancreatic juice, common to other species, is not detected in horses; and that only in horses can gastrin provoke as profuse a secretory rate as secretin, and the total protein concentration in pancreatic juice, which is a reflection of

digestive enzyme content, is low at all secretory flow rates. Furthermore, Alexander and Hickson¹ were only able to measure small amounts of amylase activity by use of a classic starch substrate procedure. In the present authors' (ML-F and AMM) laboratory, the secretagogue effect of gastrin has been detected,² but it has not been possible to measure any tryptic activity by use of a hemoglobin substrate procedure that easily detects that enzyme in the pancreatic juice of other species. The question remains, however, as to what degree the equine pancreas can produce the various digestive enzymes that have high activities, particularly at the lower rates of secretion, in many other species. Because surgically preparing horses for collection of pure pancreatic juice in vivo is difficult, the purpose of the study reported here was to measure the relative concentrations and activities of amylase, lipase, elastase, trypsin, and chymotrypsin in equine pancreatic tissue that was collected at necropsy.

Materials and Methods

Tissue collection—Pancreatic tissue was collected within 30 minutes of euthanasia from 7 adult horses. Four were female and 3 were geldings; ages and body weights ranged from 1 to 23 years and 316 to 744 kg, respectively. Horses were euthanatized via IV injection of pentobarbital solution (1 mL/5 kg of body weight).^a All horses were euthanatized because of problems unrelated to the gastrointestinal tract and had normal gastrointestinal organs at necropsy. All procedures were approved by the animal care and use committees of the institutions involved.

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Table 1—Relative cellular digestive enzyme concentration in equine pancreatic tissue.

Horse	Tissue data			Cellular components concentration (enzyme U/mg DNA)				
	mg Tissue/ mg DNA	mg DNA/ mg tissue	mg protein/ mg DNA	A	L	E	Tr	Ch
1	204.1	4.90	29.8	72.2	889.4	2.24	3.10	6.70
2	226.6	4.41	26.1	59.5	1,187.7	1.98	2.89	8.86
3	228.6	4.38	22.4	57.8	752.8	1.06	2.02	16.5
4	192.3	5.20	16.2	28.3	631.7	1.08	2.17	6.98
5	278.1	3.60	27.8	68.4	1,306.1	1.31	2.17	5.59
6	247.9	4.03	32.0	72.4	1,549.7	3.22	6.94	10.91
7	224.7	4.45	29.9	58.0	1,318.2	2.25	5.17	11.46
Mean	228.9	4.42	26.3	59.5	1,090.8	1.88	3.49	9.57
SEM	19.5	0.4	4.1	9.8	285.3	0.61	1.5	2.9

A = Amylase. L = Lipase. E = Elastase. Tr = Trypsin. Ch = Chymotrypsin.

Prior to euthanasia, 4 of the horses had been fed only a commercial complete pelleted ration, and the other 3 had received a sweet feed and hay diet. After positioning the euthanatized horse in left lateral recumbency, an incision into the abdomen was made along the entire paracostal arch and all the ribs were cut near their articulation with the vertebral column after the skin above the articulations had been incised. The right rib cage was lifted up and forward, with the help of a winch hook attached through the last intercostal space. During that procedure, the right triangular ligament that attaches the liver to the diaphragm was left intact, thus lifting the liver up and away from the underlying structures to reveal the pancreas where it is attached to the duodenum. Numerous small pieces of pancreatic tissue were immediately collected with scissors, encased in aluminum foil, and immersed in liquid nitrogen. Frozen samples were then transferred to a freezer and stored at -80°C . No samples were submitted for histologic examination, but the pancreas of each horse appeared to be normal in appearance and texture.

Tissue processing—The collected tissues were kept at -80°C until analyzed. Pieces of tissue were thawed and homogenized (10% homogenate) in a buffer with pH of 7.4 and composed of TRIS (20mM); NaCl (150mM); Triton X-100 (0.1%); and the protease inhibitors diisopropyl fluorophosphate (1mM), leupeptin (20 $\mu\text{g}/\text{mL}$), and aprotinin (20 $\mu\text{g}/\text{mL}$).³ Homogenates were prepared by 10 passes through a glass-glass homogenizer. Thus, the final concentration of tissue was 10 mg/mL of homogenate.

Enzyme assays—After appropriate dilutions established from the kinetic reaction for each enzyme in the homogenizing buffer, amylase, lipase, chymotrypsin, trypsin, and elastase activities were determined on the same microtiter plate.³ For amylase, the substrate was 2-chloro-4-nitrophenol-alpha-maltotriose, as described.⁴ Chymotrypsin, trypsin, and elastase activities were indicated by the release of p-nitroanilide from specific substrates, with a sensitivity of 25 to 6,500, 15 to 260, and 20 to 600 U/L, respectively. Lipase activity was determined by the clearing of a commercially available stabilized emulsion of triolein that enabled measurement of values from 90 to 3,600 U/L.³ Pancreatic DNA was extracted

Table 2—Specific activities of various enzymes in the pancreas of various species.

Species	A	L	E	Tr	Ch
Adult horse* (n = 7)	2.3 (1.00)	41.5 (18.04)	0.07 (0.03)	0.13 (0.06)	0.36 (0.16)
Adult pig ⁵ (n = 12)	107 (1.00)	49 (0.46)	0.22 (< 0.01)	0.44 (< 0.01)	2.26 (0.02)
Adult ratt (n = 20)	56 (1.00)	39 (0.69)	0.02 (< 0.01)	0.10 (< 0.01)	1.34 (0.02)
Calf† (14 days–6 months old; n = 4)	2.3 (1.00)	11 (4.78)	0.03 (0.01)	0.07 (0.03)	1.51 (0.66)

Values are mean units per milligram of pancreatic protein. Values in parentheses indicate ratio to amylase value.
*Values from the present study. †Values from laboratory of one of the authors (JM).
See Table 1 for remainder of key.

from homogenates as described,⁵ and its concentration was determined by use of the diphenylamine method with calf thymus DNA as the standard.⁶ Homogenate protein concentration was determined,^b with bovine serum albumin as the standard. Each assay was performed in triplicate to check for major variations. Resultant cellular enzyme concentrations were expressed as units per milligram of DNA. From these data, the specific enzyme activity, expressed as units per milligram of pancreatic tissue protein, was calculated by dividing the cellular enzyme concentration (U/mg of DNA) by the tissue protein concentration (mg/mg of DNA). Results are reported as mean \pm SEM values.

Results

For each enzyme, the cellular concentration in the pancreatic tissue was consistent among horses. Compared with other enzymes, lipase was predominant ($1,090 \pm 285.3$ U/mg of DNA), followed by amylase (59.5 ± 9.8 U/mg of DNA). The proteolytic enzymes elastase, trypsin, and chymotrypsin were detected in small concentrations (Table 1). The predominance of lipase contrasted to findings in

other species, particularly with regard to amylase (Table 2).

Discussion

Two variables were calculated in the present study for comparative analysis of exocrine pancreas enzyme activity between horses and other species. Cellular enzyme concentration (U/mg of DNA) is a good indication of the digestive potential of the pancreas. Furthermore, when a particular cellular enzyme concentration is similar in the pancreas of 2 species, enzyme output should be the same if the sensitivities of each species for the given stimulus are identical. Accordingly, if a particular cellular enzyme concentration of one species is higher than that of another species, fewer pancreatic cells in the former species would be required to secrete an equivalent amount of that enzyme, compared with the latter species. The second variable was specific enzyme activity, which is an indication of the efficiency of an enzyme; when specific enzyme activities are comparable in tissues of 2 species, a certain amount of substrate will be hydrolyzed by the 2 species at the same rate.

Results of this study were unexpected because the predominant digestive enzyme in the equine pancreatic tissues was lipase. The traditional view is that horses are herbivores and their primary foodstuff is composed mainly of carbohydrates; fat constitutes a small proportion (2% to 5%) of the diet, especially if human influence is precluded. This is supported by results of investigations of natural feeding habits of ancient and modern horses. The influence of diet on relative and absolute amounts of the various digestive pancreatic enzymes has been well established in other species, particularly rats and swine.⁷⁻¹² Published values reveal considerable variance in relative and absolute amounts, some of which is undoubtedly attributable to the diet fed prior to collection of tissue for analysis. The diet of horses in the present study was either a low-fat hay and grain ration or a complete pelleted feed, which we believe was representative of the species. Thus, the predominance of lipase seems incongruous and requires further investigation.

These results are relevant to the recent trend to provide horses, especially those with high energy requirements, with calories from fat rather than from soluble carbohydrate. Horses can readily assimilate diets that include $\geq 20\%$ fat.¹³⁻¹⁵ It is not yet known whether this ability is primarily attributable to an inherent ability to increase enzymatic lipolytic activity within the upper gastrointestinal tract, an adaptation involving increased

microbial lipolytic activity within the large intestine, or both. Such knowledge might provide useful guidance for formulation of feedstuffs for athletic horses that further supports use of fats, rather than soluble carbohydrates, as the primary source of extra calories.

- a. Beuthanasia, Schering-Plough Animal Health Corp, Kenilworth, NJ.
- b. Pierce Biotechnology Inc, Rockford, Ill.

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