

## CARBOHYDRASE ACTIVITY IN THE PANCREAS AND SMALL INTESTINE OF MOOSE AND CATTLE

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**ABSTRACT:** Moose (*Alces alces*) are difficult to keep in captivity and often die of apparent digestive problems. It has been hypothesized that some of the problem may stem from an inability to produce adequate quantities of the enzymes necessary to digest the starch contained in cereal grains formulated into synthetic diets. We tested this hypothesis by quantifying concentrations of the important enzymes found in the pancreas and small intestine of 2 moose. We compared these values to a grain fed steer. Pancreatic  $\alpha$ -amylase concentrations were higher in moose ( $4,228 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ) than the steer ( $1,104 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ), and intestinal maltase concentrations were similar between moose ( $0.33 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ) and the steer ( $0.47 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ). Additionally, moose produced concentrations of isomaltase ( $0.6 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ) at a rate similar to values published for cattle ( $0.4\text{-}0.6 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ). Only lactase values were lower in moose than cattle. Although our sample size was small, these data suggest that moose are quite capable of producing the enzymes necessary for the breakdown of both starch and disaccharides.

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It has long been recognized that moose are difficult to keep in captivity (Speidel 1965): noninfectious disease and accidents account for about 67% of all reported mortalities (Schwartz 1992). The single greatest cause of deaths seems to be associated with poor nutrition and digestive upsets. Afflicted animals develop chronic diarrhea, eventually lose body condition, and become emaciated and dehydrated. Symptoms can last for weeks to many months, but inevitably result in death. The syndrome has been referred to as "chronic wasting". This term has also been used to describe similar but seemingly unrelated diseases in mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*) (Williams and Young 1993), and moose (Rehbinder *et al.* 1991, Merza *et al.* 1994). Moose maintained in most facilities for more than a few years inevitably develop "chronic wasting" (Schwartz 1992), but the etiology is not understood.

"Chronic wasting" has been associated with the feeding of hay and/or crop residue to moose (Schwartz 1992). Eliminating them from diets of captive moose was one reason why a sawdust-based ration was developed in the late 1970s (Schwartz *et al.* 1980, 1985). Over the years there have been modifications of this ration: one involved the replacement of cereal grain with sucrose, additional molasses, and beat pulp as the energy sources (Schwartz 1992, Purina, Saint Louis, Mo). This diet was based on an untested hypothesis that moose cannot digest starch. Supposedly, undigested starch in the lower gut fermented causing chronic diarrhea. No quantitative data are available to support these claims, and the diet has not been on the market long enough for complete evaluation. Here, we test this hypothesis by quantifying concentrations of enzymes found in the pancreas and small intestine of 2 moose. We compared these values to a grain fed steer.

## MATERIALS AND METHODS

### Tissue Sampling and Preparation

We obtained tissue samples from two adult female moose slaughtered for body composition studies (Jan. 1994) at the Moose Research Center, Kenai Peninsula, Alaska. Moose #1 was in peak body condition (age 4 years, 510 kg), whereas #2 was older (age 9 years, 310 kg) and debilitated (she showed signs of wasting). Animals were maintained on a sawdust based ration (Schwartz 1985). We obtained samples of the pancreas and small intestine (duodenum, jejunum, and ileum) immediately after slaughter. Duodenum samples included the first 15cm of tissue distal to the pylorus, whereas ileal tissue was the last 15 cm of small intestine before the ileocecal junction. Jejunum samples were from the middle of the small intestine. Digesta within the lumen of the intestine were expelled, and excess fluids were removed with paper towels. Samples were stored in plastic bags, frozen at  $-20^{\circ}\text{C}$ , and shipped to the laboratory in Kentucky on dry ice. Before analysis, tissues were thawed on ice, stripped of mesenteric fat and rinsed with saline.

Bovine tissues were collected immediately after slaughter from an animal maintained at the livestock facilities at the University of Kentucky, Lexington on a high-concentrate diet for at least 60 days before slaughter. Samples were treated as for the moose but they were stored at  $-80^{\circ}\text{C}$ .

Once thawed, a 2.5 g sample of pancreatic tissue from each species was combined with 10 g of 0.15 M saline in a 50 ml plastic tube (Kreikemeier *et al.* 1990). This mixture was homogenized (Polytron Homogenizer, Brinkman Instruments, Westbury, NY) at 70% maximum speed for approximately 2 minutes. One and two hundred mg samples of homogenate were mixed with 1 g bovine plasma and 18.9 g saline and tested for  $\alpha$ -amylase activity.

Thawed intestine samples were sectioned longitudinally, rinsed with saline to remove

digesta, and gently blotted to remove excessive saline. Mucosa was removed from the intestine by scraping the lining with a glass microscope slide (Kreikemeier *et al.* 1990). We mixed 2.5 g of mucosa with 10 g 0.15 M saline and homogenized this mixture at 70% maximum speed in a 50 ml plastic tube. Then, 25 and 50 mg samples of the homogenate were mixed with 1 g bovine plasma and 18.9 g saline and tested for maltase, isomaltase (moose only), lactase and sucrase (moose only) activity.

### Enzymatic Analysis

Pancreatic homogenate was analyzed for  $\alpha$ -amylase activity following procedures from Walker and Harmon (1996). The amylase substrate used was amylopectin mixed with 0.05M Tris (pH 7.0) which provided 0.75 g amylopectin /dl. The amylopectin and Tris were homogenized (Polytron Homogenizer) at approximately 55% maximum speed for 1-2 minutes. A 10.6 mM potassium ferricyanide solution was used to stop the enzyme reaction followed by heating. Absorbency was measured at 420 nm (Shimadzu, model UV-160, Tokyo, Japan).

Mucosa from each intestinal site was assayed for maltase and lactase in steer and moose tissues, whereas sucrase was assayed only in moose. The mid-jejunum in the moose was the only site assayed for isomaltase. Lactase and isomaltase activity were analyzed according to Kreikemeier *et al.* (1990). Maltase and sucrase were analyzed by mixing 100  $\mu\text{l}$  homogenate, 500  $\mu\text{l}$  substrate, and 500  $\mu\text{l}$  buffer. Maltase and sucrase substrates were 60 mM maltose and sucrose, respectively; 50 mM potassium phosphate buffer at pH 6.3. Samples were incubated for 15 min at  $37^{\circ}\text{C}$ . Following incubation, enzyme activity was quenched by placing samples in a waterbath at  $90^{\circ}\text{C}$  for two minutes. Samples were then put on ice. Liberated glucose was measured with hexokinase (Cobas Fara II, Roche Diagnos-

tic, MontClair, NJ; Sigma Kit #16) and activity expressed as  $\mu\text{mol}$  product (glucose) produced per minute.

The small number of animals precluded statistical tests.

### RESULTS AND DISCUSSION

Pancreatic concentrations of  $\alpha$ -amylase in moose were higher than in cattle (Table 1). Comparisons of  $\alpha$ -amylase activity with other published results are difficult because of differences in methods and units of expression. Diet and intake level both influence  $\alpha$ -amylase production (Kreikemeier *et al.* 1990) although there is disagreement in the literature whether grain or forage based diets elevate  $\alpha$ -amylase more (Clary *et al.* 1969, Janes *et al.* 1985, Kreikemeier *et al.* 1990). Kreikemeier *et al.* (1990) suggested that increases in  $\alpha$ -amylase production are probably more closely associated with increased energy intake rather than with grain feeding per se. These results have been confirmed in a report by Walker and Harmon (1995)

Maltase activities in the small intestine of moose (Table 1) were similar to that of the steer. The highest maltase activities oc-

curred in the jejunum, followed by the ileum and duodenum. These results agree with maltase activities in bovine calves fed both forage and grain diets at 1 and 2-times net energy at maintenance (Kreikemeier *et al.* 1990) and agree with the distribution of enzyme activity in the intestine of steers. Activities tended to be higher in moose #1, (young and healthy) than #2.

Isomaltase activity was estimated for one moose in jejunal tissue. The concentration ( $0.59 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ , Table 1) was nearly identical to data presented for bovine calves ( $0.38$ - $0.58 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ; Kreikemeier *et al.* 1990).

We also measured lactase and sucrase activities in the small intestine of the moose. Concentrations of lactase ( $0.044$ - $0.085 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ , Table 1) were 4 to 10 times lower than values presented by (Kreikemeier *et al.* 1990) for bovine calves ( $0.1$  to  $2.6 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ) at 3 points along the jejunum. Their duodenal activities were  $1.2 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$  compared to  $0.0625 \mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$  in our moose. These data suggest that the moose were producing lower concentrations of lactase com-

Table 1. Pancreatic and small intestinal carbohydrases in moose and cattle. All values are  $\mu\text{moles}$  of product hydralyzed per gram tissue per minute( $\mu\text{mol}\cdot\text{g tissue}^{-1}\cdot\text{min}^{-1}$ ).

Enzyme	Tissue	Moose 1	Moose 2	Bovine
$\alpha$ -amylase	pancreas	4,156	4,300	1,104
Maltase	duodenum	0.353	0.306	0.404
	jejunum	2.015	0.541	0.618
	ileum	0.952	0.186	0.812
Isomaltase	Jejunum	0.593		
Lactase	duodenum	0.080	0.045	
	jejunum	0.085	0.064	
	ileum	0.044	0.044	
Sucrase	duodenum	0.096	0.015	
	jejunum	0.038	0.048	
	ileum	0.020	0.019	

pared to cattle. This could be the result of advanced age in our moose, as lactase activities decline with age in most species (St. Jean *et al.* 1989). We also detected low levels of sucrase activity measured in our moose (Table 1). This is unique in that cattle have no measurable activity (Kreikemeire *et al.* 1990) and is indicative of differential expression of carbohydrase activity between cattle and moose.

Based on the results from this pilot study, it appears that moose produce concentrations of starch digesting enzymes similar to those in domestic cattle. They differ in that they may possess low levels of sucrase activity. How the two species compare in their ability to transport glucose remains the missing link in a comparison of the abilities of moose and cattle to assimilate starch in the small intestine. Although our sample size is small, we must reject the hypothesis regarding the lack of starch digestion enzymes in moose.

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#### REFERENCES

- CLARY, J. J., G. E. MITCHELL, Jr., C. O. LITTLE, and N. W. BRADLEY. 1969. Pancreatic amylase activity from ruminants fed different rations. *Can. J. Physiol. Pharmacol.* 47:161-164.
- JANES, A. N., T. E. C. WEEKES, and D. G. ARMSTRONG. 1985. Carbohydrase activity in the pancreatic tissue and small intestine mucosa of sheep fed dried-grass or ground maze-based diets. *J. Agric. Sci. (Camb.)* 104:435-443.
- KREIKEMEIER, K. K., D. L. HARMON, J. P. PETERS, K. L. GROSS, C. K. ARMENDARIZ, and C. R. KREHBIEL. 1990. Influence of dietary forages and feed intake on carbohydrase activities and small intestinal morphology of calves. *J. Anim. Sci.* 68:2916-2929.
- MERZA, M. E. LARSSON, M. STÉEN, and B. MOREIN. 1994. Association of a retrovirus with a wasting condition in the Swedish moose. *Virology* 202:956-961.
- REHBINDER, C., E. GIMENO, K. BELÁK, S. BELÁK, M. STÉEN, E. RIVERA, and T. NIKKILÄ. 1991. A bovine viral diarrhoea/mucosal disease-like syndrome in moose (*Alces alces*): investigations on the central nervous system. *Veterinary Record* 129:552-554.
- SCHWARTZ, C. C. 1992. Techniques of moose husbandry in North America. *Alces Suppl.* 1:177-192.
- \_\_\_\_\_, W. L. REGELIN, and A. W. FRANZMANN. 1980. A formulated ration for captive moose. *Proc. N. Am. Moose Conf. Workshop* 16:82-105.
- \_\_\_\_\_, W. L. REGELIN, and A. W. FRANZMANN. 1985. Suitability of a formulated ration for moose. *J. Wildl. Manage.* 49:137-141.
- SPEIDEL, G. 1965. Care and nutrition of moose (*Alces alces americana*) in captivity. *Inter. Zoo Yearbook* 6:88-90.
- St. JEAN, G. D., M. RIGS, L. M. SCHMALL, G. F. HOFFSIS, and B. L. HULL. 1989. Jejunal mucosal lactase activity from birth to 3 weeks in conventionally raised calves. *A. J. Vet. Res.* 50:1496-1498.
- WALKER, J. A. and D. L. HARMON. 1995. Influence of ruminal or abomasal starch hydrolysate infusion on pancreatic exocrine secretion and blood glucose and insulin concentration in steers. *J. Anim. Sci.* 73:3766.
- WALKER, J. A., and D. L. HARMON. 1996. Technical Note: a simple, rapid assay for  $\alpha$ -amylase in bovine pancreatic juice. *J. Anim. Sci.* 74:658.
- WILLIAMS, E. S. and S. YOUNG. 1993.



Neuropathology of chronic wasting disease of mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus nelsoni*). Vet. Pathol. 30:36-45.