Nutritional Investigations and Management of Captive Moose

Einav Shochat,[1] Charles T. Robbins,[2*] Steven M. Parish,[3] Paul B. Young,[4] Thomas R. Stephenson,[5] and Alma Tamayo[6]

[1]*Department of Zoology, Washington State University, Pullman, Washington*
[2]*Departments of Natural Resource Sciences and Zoology, Washington State University, Pullman, Washington*
[3]*College of Veterinary Medicine, Washington State University, Pullman, Washington*
[4]*Veterinary Sciences Division, Department of Agriculture for Northern Ireland, Belfast, Northern Ireland, UK*
[5]*Moose Research Center, Alaska Department of Fish and Game, Soldotna, Alaska*
[6]*Gastrointestinal Laboratory, College of Veterinary Medicine, College Station, Texas*

Historically, moose have been difficult to maintain in captivity when on diets of grass or legume hays and grain due to enteritis that frequently leads to chronic diarrhea/wasting disease. The development of wood-fiber diets has increased the lifespan of moose in captivity, but these diets do not completely prevent chronic wasting. Purina Mills (St. Louis, MO) hypothesized that captive moose are unable to digest starch that escapes the rumen, and therefore abnormal bacterial fermentation in the hindgut causes chronic diarrhea. An earlier study found no evidence of a digestive problem, so we tested the hypothesis that moose have difficulty metabolizing excess propionate produced from the fermentation of starch found in traditional cervid rations and high-grain wood-fiber diets. When challenged with an i.v. propionate load, moose metabolized propionate similar to healthy mule deer and domestic livestock. We then tested the hypothesis that grass forage is an initiating factor to chronic diarrhea/wasting and further hypothesized that grass, alfalfa, and other agriculture-based forages in association with an anaerobic bacteria produce inflammatory bowel disease (IBD) in moose. Captive moose that had ad libitum access to a wood-fiber pelleted moose diet and grazed in grass pastures developed chronic wasting symptoms at 2–4 years of age and died at 4.7 ± 0.3 years unless restricted from grass before the development of advanced symptoms. We isolated *Bacteroides vulgatus* in the feces and successfully treated a moose with chronic diarrhea/wasting disease with long-term metronidazole therapy, suggesting that the chronic enteritis causing wasting disease arises from a bacteria-associated defective immunosuppressive response similar to IBD in other species. Further support for the IBD cause of wasting in moose is that this animal will relapse within hours if the metronida-

zole treatment is discontinued even after many months. We developed a highly palatable high-fiber, low-starch moose ration that can be fed as the sole source of nourishment, although additional research and dietary improvements are required. Zoo Biol 16:479–494, 1997.   © 1997 Wiley-Liss, Inc.

Key words: wasting disease, chronic diarrhea, vitamin B$_{12}$, folate, inflammatory bowel disease, metronidazole

INTRODUCTION

Moose are relatively rare in zoos. Most zoos no longer attempt to exhibit moose because they die prematurely. Although wild moose can live 15–20 years like other wild or captive cervids, 70% of all captive moose die during their first year and 90% die by 6 years of age [Kock, 1985; Syroechkovsky et al., 1989; Schwartz, 1992a]. This is largely due to their sensitivity to disease and the difficulty of providing an adequate diet. Historically, the only successful long-term diet has been browse, tree, and shrub leaves and stems, but few zoos have wooded pens large enough for moose to meet their entire dietary requirement through browsing, and hand-cutting browse is costly and time-consuming. Although virtually all other cervids do well on alfalfa, grass, and grains [Oftedal et al., 1996], moose fed these diets ultimately die due to nonspecific enteritis, chronic diarrhea, and loss of body condition [Schwartz et al., 1980, 1985; Schwartz, 1992a]. To our knowledge, no pathogenic agent has ever been identified, nor has a reliable, scientifically based treatment been developed.

Schwartz et al. [1980, 1985] made a major breakthrough in moose husbandry when they developed a pelleted diet (Moose Research Center ration, MRC) in which the alfalfa or grass was replaced with aspen sawdust. They hypothesized that wood-fiber was necessary for moose to maintain a healthy gastrointestinal tract and that moose fed large quantities of grasses and forbs become bulk limited. The diet also contained 57.5% corn, oats, and barley. Although most moose initially thrive on the MRC diet, many ultimately develop chronic diarrhea/wasting and die within 3–8 years [Schwartz, 1992a]. Purina Mills (Mazuri moose diet, PMI Feeds, St. Louis, MO) recently modified the MRC diet based on the untested hypothesis that the enteritis is caused because moose have difficulty digesting starch escaping from the rumen into the lower tract. They suggest that evolutionarily moose would have been exposed to minimal starch in their browse diet. The Mazuri diets replace the grains in the MRC diet with beet pulp and sucrose.

Schwartz et al. [1996] recently tested a portion of this hypothesis by examining the concentrations of carbohydrases in the pancreas and small intestine of moose and grain-fed cattle. The starch-digesting enzyme levels in the tissues were as high in moose as in cattle, which suggests that moose are as capable as any other ruminant of digesting starch escaping into the intestine. However, even though the initial hypothesis was rejected, moose that have begun wasting on the MRC diet will frequently recover when fed the Mazuri diets [K. Petrini, Minnesota Zoological Garden, pers. comm.; Robbins, pers. comm.]. The improvement is rarely permanent as the animals invariably relapse. At necropsy, many of these moose have a nonspecific enteritis and copper and cobalt deficiencies, as judged by domestic animal tissue standards and a temporary positive response to supplementation.

We initiated our studies by further testing the hypothesis that moose have difficulty metabolizing starch. We hypothesized that the defect is in internal metabolism

in which moose might have difficulty handling the higher levels of propionate produced from starch fermentation as compared to acetate from fiber fermentation [Clemens et al., 1983; Van Soest, 1994]. Acetate is readily coverted to energy via acetyl-CoA and oxidized in the citric acid cycle. However, propionate must be converted to succinate, which is a vitamin B_{12} (cobalt)-dependent process. Rumen bacteria produce vitamin B_{12} and inactive analogs from dietary cobalt. The fermentation of large concentrations of starch can shift the distribution of cobamides toward inactive analogs [Sutton and Elliot, 1972]. Therefore, we hypothesized that moose fed high-grain diets become vitamin B_{12} limited in metabolizing propionate. To further test the hypothesis, we compared the serum concentrations of vitamin B_{12} and folic acid, the two vitamins associated with single-carbon transfer, in wild and captive moose.

We then tested the hypothesis that grass consumed by captive moose as fresh pasture during the spring, summer, and fall is an initiating factor in chronic diarrhea/ wasting. Although the diets of wild moose with abundant access to browse average <2% grass [Schwartz, 1992b], captive moose held in large pastures often readily consume grass in preference to either the MRC or Mazuri diets [Robbins, pers. obs.]. Schwartz et al. [1980] and Schwartz [1992a] previously stated that "(moose) dying from long-term chronic diarrhea and loss of body condition generally are fed for several years on diets containing grass hays or a formulated ration containing crop residues or hay—grass or alfalfa hays—are not suitable as moose foods." Subsequently, we hypothesized that these forages either as hay or in fresh form in conjunction with an as yet unidentified bacteria produce inflammatory bowel disease (IBD) in moose irrespective of the pelleted diet being used and that wasting disease in captive moose is not due to the hypothesized bulk limitation [Schwartz et al., 1980; Hofmann and Nygren, 1992] of grass or alfalfa-based diets. Consequently, the successful maintenance of moose depends on the development of nonirritating pelleted diets that moose prefer over other fresh forages. Finally, once IBD develops, we hypothesize that successful management of this disease requires both an antibiotic and an anti-inflammatory agent [Sartor, 1995].

MATERIALS AND METHODS

Moose have been maintained at the Washington State University (WSU) captive ungulate facility since 1989. Calves are bottle-raised according to the protocol of Shochat and Robbins [1997]. Weaned calves are fed a high-fiber diet patterned after the Mazuri diet (Table 1). All moose are regularly wormed every 4–6 weeks with fenbendazole (5 mg/kg, once orally) or doramectin (200 µg/kg once orally or subcutaneously). Periodic fecal analyses are conducted to assess the effects of the worming programs and to identify the development of resistant strains.

Propionate, Folate, and Vitamin B_{12} Studies

Blood samples were collected from healthy captive and wild moose, domestic cows fed on alfalfa, and captive mule deer (*Odocoileus hemionus*) and woodland caribou (*Rangifer tarandus*) fed high-grain pelleted diets. For blood sampling, animals were either immobilized physically in a squeeze chute (cows) or sedated with xylazine hydrochloride (captive moose, mule deer, and caribou) or carfentanil citrate/xylazine hydrochloride (wild moose). Blood was collected in heparinized and plain vacuum tubes. Serum cobalamin and folate and red blood cell folate were mea-

TABLE 1. Composition of WSU moose rations

Original high-fiber ration		Improved orange/high-fiber ration	
Ingredient	%	Ingredient	%
Sawdust[a]	22.5	Sawdust[a]	22.5
Canola meal	20.0	Beet pulp	20.0
Beet pulp	18.8	Canola meal	20.0
Alfalfa meal	10.0	Alfalfa meal	10.0
Molasses (dry)	8.0	Sucrose	5.5
Sucrose	7.5	Soybean meal	5.0
Wheat bran	5.0	Dried citrus peel[b]	5.0
Bone meal	5.0	Bone meal	5.0
Animal fat	0.75	Molasses (dry)	3.68
Trace mineral salt[c]	0.75	Trace mineral salt[c]	0.75
Lime	0.50	Lime	0.50
Dicalcium phosphate	0.05	Dicalcium phosphate	0.50
Vitamin premix[d]	0.25	Vitamin premix[d]	0.25
Vitamin A premix	0.225	Vitamin A premix	0.225
Se premix (0.02% Se)	0.150	Se premix (0.02% Se)	0.150
Vitamin E premix (500 IU/g)	0.070	Vitamin B_{12} premix (1.3 mg/g)	0.100
Vitamin K premix (0.14 g/g)	0.005	Vitamin E premix (500 IU/g)	0.062
		Copper sulfate	0.020
		Vitamin K premix (0.14 g/g)	0.005
		Liquid molasses coating[e]	

[a]Aspen wood #3, American Excelcior, Yakima, WA.
[b]Sun-dried citrus peel, Coast Grain Co., Ontario, CA.
[c]Trace mineral salt: 96–96.5% NaCl, 0.35% Zn, 0.20% Mn, 0.20% Fe, 0.15% Mg, 0.03% Cu, 0.007% I, and 0.005% Co.
[d]Vitamin premix: 880,000 IU A/kg, 66,000 IU D_3/kg, 4,400 IU E/kg, 792 mg B_2/kg, 2,640 mg niacin/kg, 2,640 mg d-pantothenate/kg, 99,000 mg choline/kg, 4 mg B_{12}/kg, 198 mg menadione sodium bisulfite/kg, 220 mg folic acid /kg, 20 mg d-biotin/kg, and 10,000 mg ethoxyquin/kg.
[e]Coat pellets with 18.1 kg/ton.

sured using a radioassay based on heat denaturation (boiling) and charcoal separation (Dualcount Charcoal Boil assay kit, Diagnostic Products, Los Angeles, CA). Samples were refrigerated, kept in a dark container, and analyzed within 48 hr.

The ability of moose and mule deer to handle propionate was tested in propionate-loading experiments. Mule deer were chosen as the control because they routinely do well in captivity when fed high-grain diets. Both species were given intravenous propionate loads while feeding on the WSU orange/high-fiber moose diet (Table 1) and a high-grain diet. The high-grain diet for the moose was the MRC ration (Table 2). Because the mule deer would not eat the MRC ration, their high-grain diet was the WSU high-grain cervid ration (Table 2). Animals were fed each diet for at least 30 days, after which the animals were immobilized with xylazine hydrochloride (0.3 mg/kg, moose; 0.6 mg/kg, deer) and fitted with 14-gauge × 140-mm jugular catheters. The catheters were sutured to the skin and covered by an elastic bandage. Sedation was reversed with tolazoline hydrochloride (0.1 mg/kg) in moose and yohimbine hydrochloride (0.09 mg/kg) in deer within 30 min of the initial xylazine injection. To minimize the effects of sedation on blood parameters and propionate metabolism, propionate loading was delayed for 14 hr. At that time, blood samples were taken from the catheters for folate and vitamin B_{12} analyses and followed by an intravenous infusion of filter-sterilized sodium propionate (Aldrich, Mil-

TABLE 2. Composition of MRC moose ration and WSU high-grain cervid ration

MCR moose ration[a]		WSU high-gran cervid ration	
Ingredient	%	Ingredient	%
Corn, ground yellow	30.0	Oats	30.0
Sawdust	25.0	Corn	20.0
Oats, ground	15.0	Soybean meal	15.0
Barley, ground	12.5	Alfalfa	10.0
Cane molasses, dry	7.5	Beet pulp	10.0
Soybeam meal	6.3	Wheat bran	10.0
Dicalcium phosphate	1.1	Molasses	3.0
Sodium chloride	0.5	Dicalcium phosphate	1.0
Vitamins A-D-E[b]	0.3	SWC vitamin[d]	0.5
Trace minerals and flavors[c]	tr.	Iodized salt	0.3
		Trace mineral salt[e]	0.1
		Se premix (0.02% Se)	0.1

[a]Ration was prepared without two ingredients: Pelaid, pelleting enhancer and Mycoban, mold inhibiter. For complete recipe, see Schwartz et al. [1985] or Schwartz [1992a].
[b]Each kilogram feed contained 5,000 IU vitamin A, 13,000 ICU D_3, and 44 IU vitamin E.
[c]Added trace minerals and flavors (mg/kg) Zn, 55.2; Fe, 30.5; Mn, 16.8; Cu,7.6; I, 1.3; and anise, 125.
[d]Vitamin premix: 880,000 IU A/kg, 66,000 IU D_3/kg, 4,400 IU E/kg, 792 mg B_2/kg, 2,640 mg niacin/kg, 2,640 mg d-pantothenate/kg, 99,000 mg choline/kg, 4 mg B_{12}/kg, 198 mg menadione sodium bisulfite/kg, 220 mg folic acid/kg, 20 mg d-biotin/kg, and 9,990 mg ethoxyquin/kg.
[e]96–96.5% NaCl, 0.35% Zn, 0.20% Mn, 0.20% Fe, 0.15% Mg, 0.03% Cu, 0.007% I, and 0.005% Co.

waukee, 2.5 mmol/ml, 7.4 mmol/kg$^{0.75}$) over 2 min. This dose rate has been used previously in propionate metabolism studies of domestic sheep [Kennedy et al., 1991]. Blood samples were then collected from the catheters at intervals of 15 min (first 2 hr), 30 min (next 2 hr), and 1 hr (last 2 hr).

Propionate metabolism was monitored by measuring methylmalonic acid (MMA). MMA is an inappropriate, nonoxidizable metabolite that is produced when propionate metabolism is impaired, particularly by a vitamin B_{12} deficiency [O'Harte et al., 1989]. Plasma MMA concentration was determined following solvent extraction and butylation using gas chromatography and high-resolution mass spectrometry [Young et al., 1995].

Grass as Inflammatory Bowel Disease Initiating Factor

Six moose (four females and two males) were kept from birth in either a 0.3 or 2.1 ha pasture and provided with shelter, water, and the high-fiber WSU moose ration ad libitum (Table 1). Both pastures had dense stands of smooth brome (*Bromus inermis*) and orchardgrass (*Dactylis glomerata*). Pastures were mowed periodically during the growing season to ensure an ad libitum availability of succulent new growth. Our initial hypothesis was that moose would benefit from increased ingestion of long fiber as fresh forage. For the management purpose of avoiding destruction of the grass pastures, all animals were moved into gravel-covered pens during the winter, where the only food available was the high-fiber WSU moose diet. Half of the animals were treated like this until death. However, in an unsuccessful although extensive effort to save the lives of these animals, numerous hypotheses regarding the cause of the disease were ultimately excluded. The only remaining hypothesis was that grass consumption is an initiating factor to IBD and wasting in

captive moose even when a wood-fiber diet is offered ad libitum. Subsequently, the remaining three moose, which were 2 and 3 years younger, were removed from the grass pasture as soon as early signs of enteritis (poor fecal consistency) and poor body condition (particularly a dull hair coat) were noted. These three moose were then maintained year-round on the high-fiber WSU moose ration and never permitted to feed on grass. For these latter animals, both serum and urinary MMA were monitored once they were moved off the grass pasture to determine whether there was any change in propionate metabolism associated with grass feeding.

Development of Improved, More Palatable Moose Ration

If grass, alfalfa, and other common forages available to captive moose in large pens are an initiating factor for IBD, then the successful maintenance of captive moose partly depends on the development of pelleted diets that are more palatable than these other forages. Because captive moose avidly relish treats like unpeeled bananas and citrus fruits, we explored the use of citrus pulp as an attractant to moose. Air-dried citrus pulp was obtained from Coast Grain (Ontario, CA). Wheat bran in the original WSU high-fiber ration was replaced with citrus pulp. To determine the relative palatability of this new WSU orange/high-fiber ration, we tested the preference of this ration relative to the Mazuri moose breeder diet when fed to six moose ranging in age from bottle-fed calves to 5.5-year-old adults. Each moose was offered ad libitum quantities of both rations for at least 30 days prior to the trial and then isolated during the 7-day trials in which the amount of each diet consumed daily was measured. Placement of the diets was switched daily between two feeders to eliminate the chance of a preference for a specific feed container.

Total collection, 7-day digestion trials were also run with the WSU orange/high-fiber moose diet. Feed and fecal samples were dried at 100°C to determine dry matter content and were analyzed for gross energy (adiabatic bomb calorimetry), nitrogen (macro-kjeldahl), and neutral detergent fiber [Hanley et al., 1992] contents.

Treatment of Inflammatory Bowel Disease in Moose With Chronic Diarrhea/Wasting

No attempt was made to initiate enteritis and wasting following the pasture trials with captive moose. However, we did decide that if a moose developed chronic diarrhea/wasting, we would attempt to establish that is was a typical antibiotic-resistant form of enteritis before developing a new treatment procedure. Initial diagnostics would include viral isolation, aerobic bacterial culture (e.g., *Escherichia coli* or *Salmonella sp.*) and parasite screening. If no pathogens were identified, initial treatment would include the use of broad spectrum antibiotics in the following order: oxytetracycline (10 mg/kg i.m., once daily), penicillin (15,000 units/kg s.c., once daily) and ceftiofur (1.1 mg/kg i.m., twice daily), and sulfadimethoxine (50–75 mg/kg, orally once daily). If those treatments were not successful, we would culture for anaerobic bacteria that could be linked to IBD (such as *Bacteroides vulgatus*). If an overgrowth of an anaerobe was identified, we planned to use metronidazole (15 mg/kg orally, twice daily), as it has been useful in treating IBD in other animals, including humans, and sulfasalazine (7.5 mg/kg orally, twice daily), the most commonly used intestinal anti-inflammatory [Sartor, 1995]. The animal also would be given an

oral 3.5-g probiotic bolus (Probios, Pioneer Hi-bred International, West Des Moines, IA) daily in an attempt to alter gut flora. Fecal cultures, mineral analyses, and necropsies were performed at the Washington Disease Diagnostic Laboratory (Pullman, WA).

Statistical Analyses

In the propionate loading trial, preinjection and peak plasma MMA levels were analyzed via analysis of variance [PROC GLM: SAS, 1996]. These analyses were based on a split-plot experimental design [Steel et al., 1997]. Species and diet were equivalent to the whole plot and subplot, respectively. All other experiments were analyzed using analysis of variance [PROC GLM: SAS, 1996], with protected Fisher's least significant differences used for pairwise comparisons of means. Differences in means were considered significant at $\alpha < 0.05$. All means are reported ± 1 SD.

RESULTS

Propionate, Folate, and Vitamin B_{12} Studies

Serum vitamin B_{12} and folate concentrations were not different between captive moose consuming the WSU pelleted diets and wild or captive moose consuming browse (Fig. 1, $P > 0.05$), but the averages for folate in all cervids were lower than for domestic cows (Table 3). Moose had lower levels of vitamin B_{12} than caribou, but levels were similar to domestic cows. Plasma MMA increased and reached a peak within 65 min of propionate loading in both moose and mule deer. There were no significant differences between animal species or diet in preinjection levels of plasma MMA and cobalamin or peak plasma MMA after propionate loading (Fig. 2, $P > 0.05$).

Grass as Inflammatory Bowel Disease Initiating Factor

Captive moose with access to grass pastures and high-fiber moose pellets exhibited normal seasonal growth patterns during their first 3 years. The adult weights peaked at 500 and 402 kg for the males and averaged 368 ± 19 kg for the females. Fecal consistency was generally solid with well-formed pellets throughout the animals' first year, but deteriorated during subsequent summers. In winter, fecal consistency improved relative to summer norms until the fourth winter when feces were amorphous masses. The three moose left on grass each summer died of chronic diarrhea/wasting at 4.7 ± 0.3 years after losing $32 \pm 9\%$ of their peak weight. Signs included poor, rough hair coat, severe emaciation, low liver copper content (<10 ppm), hemosiderosis of the spleen, nonspecific enteritis with elevated plasma cells, eosinophils, macrophages, and lymphocytes in the lamina propria of the intestinal tract. No significant mucosal necrosis or hemorrhage was seen. There was no long-term response to broad-spectrum antibiotic therapy, copper or cobalt supplementation, rumen transfaunation from a domestic cow, or ad libitum access to willow branches, but there were transient improvements to sulfadimethoxine therapy, copper and cobalt supplementation (dietary content increased to as high as 60 and 75 ppm, respectively), and cyanocobalamin (10,000 μg i.m., biweekly) injections.

The remaining three moose were pulled from the grass pastures either when 2 (n = 1) or 3 (n = 2) years old. These three moose continue to do well on only the WSU high-fiber pelleted ration with the two oldest moose now having lived significantly longer (6 years old) than the moose that died of chronic diarrhea/wasting. As a potential indication
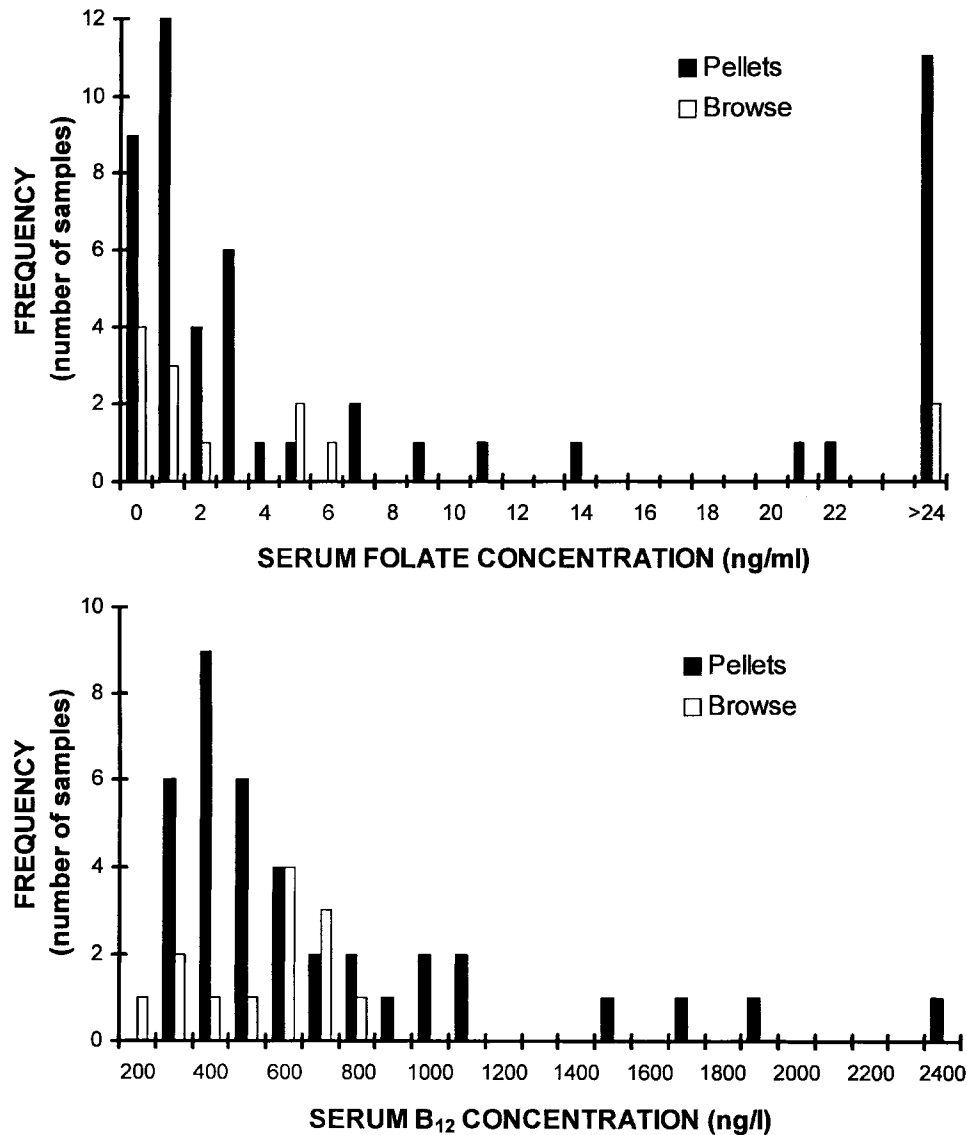
Fig. 1.   Serum folate (**top**) and vitamin B_{12} (**bottom**) concentrations for moose fed the WSU orange/ high-fiber ration (n = 51 and n = 38, respectively) and wild or captive moose feeding on browse (n = 13).

of early signs of enteritis followed by a long-term healing process, urinary MMA dropped to baseline levels after >10 months of having no access to grass (Fig. 3).

Development of Improved, More Palatable Moose Ration

The WSU orange/high-fiber ration (Table 1, Table 4) was greatly preferred by all moose over the Mazuri moose breeder ration. When given ad libitum access to both rations, 97.2 ± 2.9% of the daily intake was the WSU ration with only 2.8% being the Mazuri ration. The cost of producing 1 ton of the WSU orange/high-fiber ration, including ingredients, shipping, and labor, is $348.

TABLE 3. Serum and red blood cell folate and serum cobalamin values for moose, woodland caribou, mule deer, and domestic cow

Species	Serum folate (ng/ml)*	Red blood cell folate (ng/ml)*	Serum cobalamin (ng/l)*
WSU moose[a]	2.03 ± 1.14*	5.31 ± 3.24*	585.7 ± 189.1*
Browse moose[b]	2.93 ± 2.19*	2.92 ± 2.69*	524.5 ± 206.4*
Caribou[c]	1.45 ± 0.64*	6.28 ± 2.67*	2727.9 ± 354.6**
Mule deer[c]	3.13 ± 3.70*	3.41 ± 4.93*	459.6 ± 141.4*
Domestic cow[d]	19.27 ± 1.97**	247.77 ± 214.03**	450.1 ± 292.6*

*,**Values are means ± 1 SD. Different superscripts denote differences in means ($P < 0.05$; ANOVA, Fisher's LSD).
[a]Captive moose fed WSU orange/fiber diet (Table 1). Two animals with outlying serum (210 and 225 ng/ml) and red blood cell folate (256 and 324 ng/ml) values were excluded from the mean.
[b]Semi-captive moose feeding on browse for at least 6 months.
[c]Captive animals fed WSU high-grain cervid ration (Table 2).
[d]Domestic cows fed alfalfa.

Treatment of Inflammatory Bowel Disease in Moose With Chronic Diarrhea/Wasting

One 3–4-month-old calf that had grown normally (874 g/day) developed a persistent diarrhea after being treated 3 successive days with fenbendazole (5 mg/kg). Over the next 3 months, this calf lost 10 kg while her cohort gained 64 kg (Fig. 4). All antibiotic treatments were ineffective except that of sulfadimethoxine, which resulted in dramatic but very temporary (2 days) recovery of appetite and fecal consistency. These signs were very similar to those observed in advanced stage grass-induced IBD in captive moose. Anaerobic culturing of the feces identified an overgrowth of *Bacteroides vulgatus* relative to moose with normal feces. Because this animal was a bottle-raised calf and our desire was to reduce inflammation in the intestinal tract, metronidazole was given orally in an electrolyte solution via a nursing bottle. Over the first 7 days of metronidazole treatment, fecal consistency changed from diarrhea to well-formed pellets. The calf then gained 650 g/day. Once taken off metronidazole at 14 days, the calf relapsed within 3 days, lost 1.36 kg/day, and fecal consistency deteriorated to pretreatment form. Within 36 hr of re-initiating metronidazole treatment, fecal consistency returned to well-formed pellets and the rate of gain increased to 918 g/day during the next 28 days. The treatment was then changed to sulfasalazine in which fecal consistency reverted to soft, poorly formed feces, but the calf was able to gain 130 g/day. Within 24 hr of switching the calf back to metronidazole, feces returned to well-formed pellets and the rate of gain increased to 833 g/day. Thus far, all efforts to wean this animal off metronidazole have been unsuccessful. Because treatment has continued beyond the time that the animal would nurse from a bottle, we are currently administering metronidazole (20 mg/kg, twice daily), alone or with sulfasalazine (15 mg/kg, twice daily), orally in a piece of fruit. Although fecal consistency varies, this animal continues to gain weight during treatment.

DISCUSSION

Domestic ruminants are considered marginally cobalt deficient when serum vitamin B_{12} ranges from 200–400 ng/l [Fisher and MacPherson, 1990]. A significant
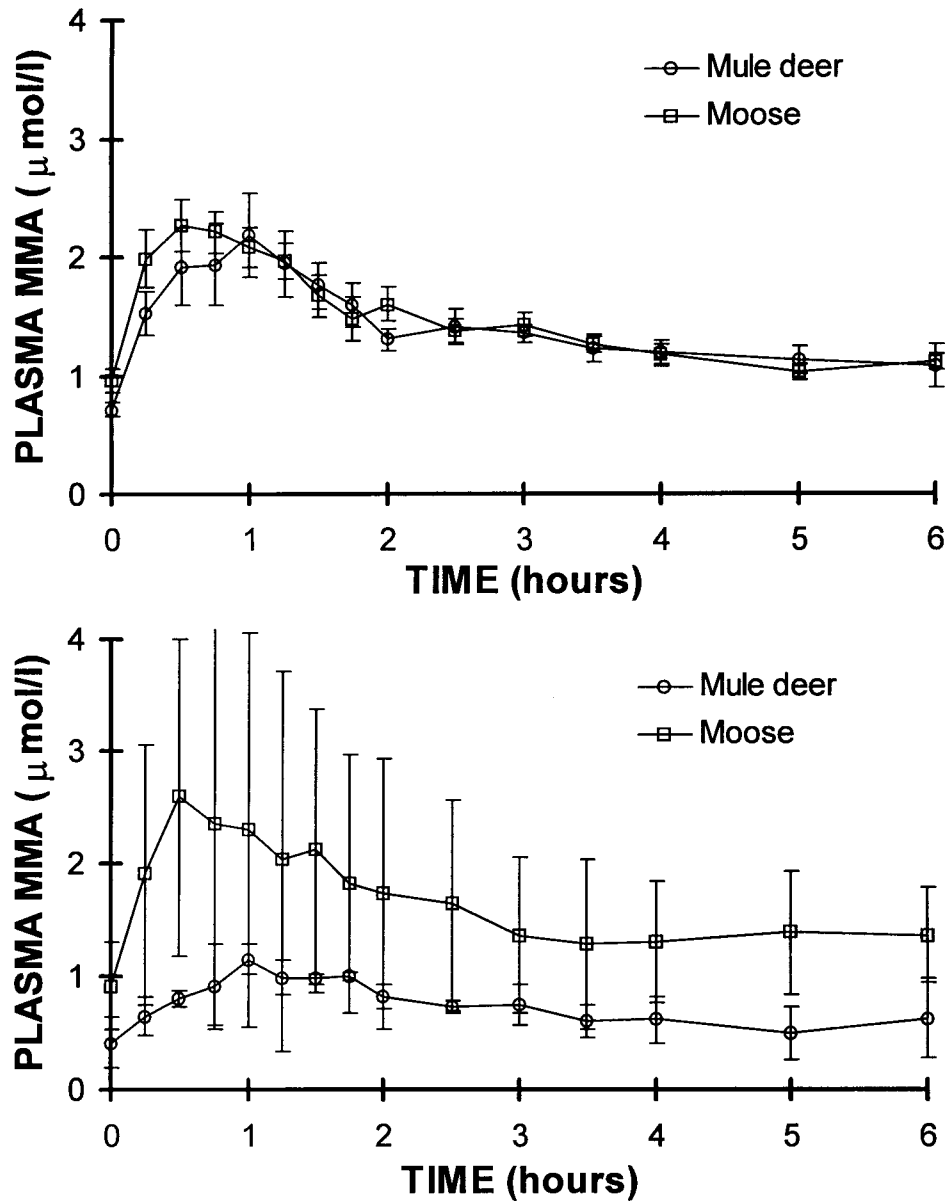
Fig. 2. Plasma methylmalonic acid concentrations in moose (n = 4) and mule deer (n = 3) fed high-grain (**top**) and high-fiber pelleted (**bottom**) diets following and i.v. propionate load.

number of wild and captive moose have serum B_{12} levels within this range. However, plasma MMA levels, even when captive moose receive loading doses of sodium propionate, do not exceed normal levels for healthy domestic livestock [Fisher and MacPherson, 1990; Paterson and MacPherson, 1990; Kennedy et al., 1991]. Serum folates of moose and other cervids are much lower than in domestic cows, ranging from 8.7 to 23.3 ng/ml with a mean of 14 ng/ml [Arbeiter and Winding, 1973; Girard et al., 1989 a,b; Tremblay et al., 1991], but folate levels in cervids are similar
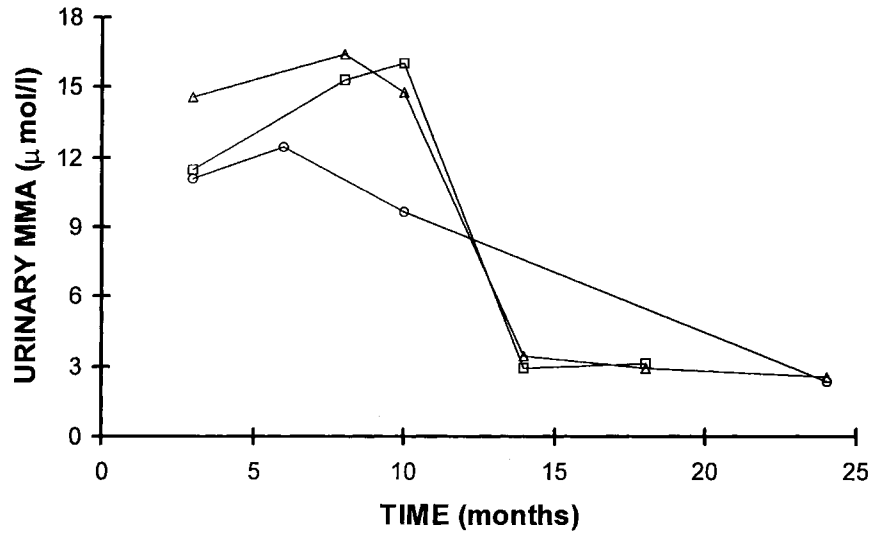
Fig. 3.   Urinary methylmalonic acid concentrations in three moose consuming the WSU high-fiber ration after the removal of animals from grass pastures.

to those in domestic goats [Ford et al., 1972]. Plasma homocysteine concentrations, an indicator of folate and B_{12} deficiency, in both healthy and wasting moose fall within the range considered normal for domestic sheep [Kennedy et al., 1992; Shochat and Young, unpub. data]. Thus there is no clinical evidence that captive or wild moose are deficient in either vitamin B_{12} or folate. Similarly, healthy moose appear to be as capable as any other ruminant of digesting and metabolizing starch. Conse-

TABLE 4.   **Chemical composition and apparent digestibility of the WSU orange/ high-fiber ration**[†]

Analysis	Amount and units
Dry matter	93.3%
Crude protein	13.5%
Neutral-detergent fiber	42.7%
Gross energy	4.20 kcal/g
Calcium	28,000 ppm
Potassium	11,000 ppm
Sulfur	3,700 ppm
Sodium	6,300 ppm
Phosphorus	12,000 ppm
Magnesium	3,100 ppm
Manganese	59.0 ppm
Iron	48.0 ppm
Zinc	81.0 ppm
Copper	47.0 ppm
Cobalt	0.52 ppm
Dry matter digestibility*	$59.1 \pm 3.0\%$**
Crude protein digestibility*	$67.0 \pm 4.3\%$**
Neutral-detergent fiber digestibility*	$41.0 \pm 4.2\%$**

[†]Data expressed on dry weight basis.
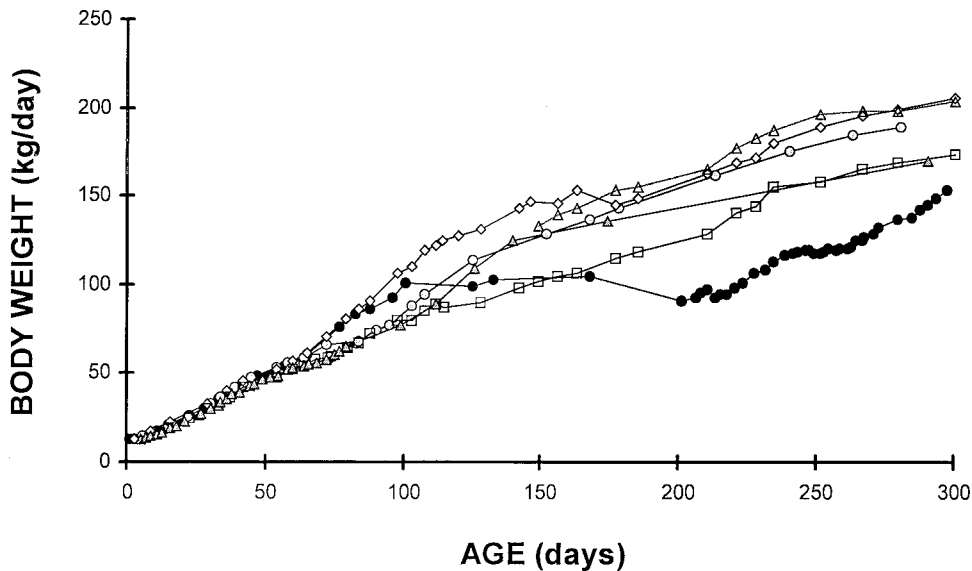*In vivo (n = 3); **mean $\pm$ 1 SD.

Fig. 4. Growth rates of six moose calves. The dark circles denote the body weights of a calf that developed chronic diarrhea and was treated with metronidazole (days 193–207, 213–242, 262–300), sulfasalazine (days 243–261), and probiotics (days 193–300).

quently, we conclude that neither dietary starch nor deficiencies of vitamin B_{12} or folate are causative agents of chronic diarrhea/wasting in moose.

Captive moose frequently have been given grass or legume hays or access to fresh pasture in addition to the wood-based pelleted diets in the belief that long fiber is necessary for a ruminant to maintain a healthy gastrointestinal tract. In our case, moose given access to lush, fresh pasture as well as their normal pelleted diet grew well for up to 3 years. However, a continual decline in fecal consistency was readily noted with each succeeding summer. Even wild moose fed grass or legume hays in "emergency feeding" programs produce amorphous feces [C.C. Schwartz, pers. comm.]. Thus we hypothesize that grasses and legume hays act either as an allergen or alter the intestinal microflora to initiate inflammatory bowel disease in moose and that, although healing partially occurs during the winter when the moose were restricted to pellets, there is an increasing sensitization or accumulation of tissue damage that ultimately leads to uncontrollable intestinal inflammation. For this reason, the last remaining source of grass fiber (wheat bran) was replaced with orange pulp in the WSU high-fiber ration. Whereas a small amount of alfalfa (10%) remains in the pellet, our current research is focused on finding a suitable replacement for the alfalfa component. Elevated urinary MMA levels detected in moose for >10 months following removal from grass may be an indicator of the time course necessary for complete metabolic recovery from malabsorption due to midstage intestinal inflammation. Moose appear similar to some humans in that a wide range of enteric infections, toxins, or dietary allergens are capable of activating an inflammatory cascade that results in chronic intestinal injury when susceptible individuals are unable to downregulate the inflammatory process [Sartor, 1995].

Our induction of chronic diarrhea/wasting in a moose calf with fenbendazole indicates the susceptibility of moose to IBD. Persistent, chronic diarrhea of unknown

etiology is common among moose <1 year of age [Schwartz, 1992a]. Although many of these cases are due to poor husbandry and inadequate bottle-raising protocols, excellent programs also encounter calves with antibiotic-resistant chronic diarrhea that often results in death [Schwartz et al., 1980; Schwartz, 1992a]. Our isolation of *B. vulgatus* and the successful treatment with metronidazole of an otherwise antibiotic-resistant diarrhea suggest that at least some of these cases of diarrhea are due to a bacteria-associated defective immunoregulatory response to ubiquitous gut antigens. Unlike other antibiotics, metronidazole has both antibiotic and anti-inflammatory properites [ASHP, 1996]. Because metronidazole is specific for anaerobic gram-negative bacilli and gram-positive cocci including *Bacteroides* and *Clostridium,* the use of probiotics containing *Lactobacillus spp.* and *Enterococcus spp.* may be useful in establishing competing bacteria.

The successful display of healthy, captive moose depends on the development of diets that effectively simulate browse in maintaining a healthy gastrointestinal tract and that are preferred over other forages. Because of the chronicity of advanced IBD in moose, efforts should be focused toward improving diets and management techniques that prevent development of the disease. The currently proposed WSU orange/high-fiber diet is greatly preferred when compared to the commercially available Mazuri moose diets, has no grass fiber that might be a causative agent of IBD, and has sustained healthy moose when offered as the only food for 3 years. Because liver copper at necropsy is frequently as low as 3–4 ppm in captive moose that have died of wasting relative to wild moose with >100 ppm [Hyvarinen and Nygren, 1993], we recommend that dietary copper levels be elevated from the 14–16 ppm in the current Mazuri diets to ±40 ppm. We have used this level for the past 4 years with moose of all ages without adverse effects. One 2-year-old moose raised entirely on this diet had a liver copper concentration of 200 ppm. To minimize grass feeding, we confine our moose to small pens (30×30 m) and mow at weekly intervals during the growing season. Similarly, we do not feed any grass or legume hays. We hypothesize that a weakness of the MRC diet may be the oat and barley hulls that, like grass, lead to IBD. If one wishes to provide long fiber, only browses should be fed.

Schwartz [1992a] hypothesized that secondary plant compounds that are prevalent in browses, such as tannins, may be important in maintaining the health of a moose's gastrointestinal tract. Many of the preferred forages of wild moose, such as willow, aspen, and birch, are rich sources of salicylates and other potentially anti-inflammatory/anti-diarrheal compounds [Palo, 1984]. 5-Aminosalicylic acid is the active constituent of sulfasalazine, a compound that can be useful in managing the inflammatory response of the gastrointestinal tract [Sartor, 1995]. Salicylic acid fed to ruminants is absorbed intact without bacterial degradation [Short et al., 1990] and is presumably pharmacologically active when wild moose consume their preferred foods. The limited success of the sulfasalazine treatment in our calf with chronic diarrhea/wasting disease was similar to that of *B. vulgatus*-induced IBD animal models [Oestrieicher et al., 1991]. This suggests that salicylates may play a role in controlling intestinal inflammation in healthy animals, but can not reverse the excessive intestinal damage in moose with chronic wasting. Captive moose diets may ultimately require the incorporation of salicylates or other secondary plant compounds if moose through evolutionary time have become dependent on them to regulate gut function.

As for disease management, moose are very susceptible to ivermectin-resistant *Trichuris* (whipworm) infections. Untreated whipworm infections will result in chronic

diarrhea/wasting and death. Whipworms should always be suspected in pens where moose or other wild ungulates have been held for several years. Currently, we are testing the effectiveness of doramectin, as fenbendazole produces a transient diarrhea in many moose that in rare cases can become chronic. Because moose are very susceptible to malignant catarrhal fever, a nonpathogenic virus carried by virtually all domestic and wild sheep and goats [Syroechkovsky et al., 1989; Warsame and Steen, 1989; Schwartz, 1992a; Shochat, Robbins and Parish, unpub. data], moose should be physically separated from sheep and goats, and personnel caring for moose should not care for sheep and goats.

In summary, moose can remain healthy and productive for many years in captivity with improved diets and medical attention. Bottle-raised moose imprint for life on their caregivers and can be readily trained to being led on a leash, to loading in trailers, to blood sampling, or to any other protocol necessary for their care [Shochat and Robbins, 1997]. Although breeding males are as difficult and dangerous as any other male cervid, bottle-raised females will ultimately identify the specific people that raised them as their own calves and will therefore defend them against other moose or strange humans. Thus moose are a very rewarding species and quite fun irrespective of their size.

CONCLUSION

1. Neither dietary starch nor deficiencies of vitamin B_{12} or folate are responsible for enteritis, chronic diarrhea, and wasting in captive moose.

2. Grasses and agriculturally produced legumes, either as fresh forages or hay, are contributing factors to enteritis, chronic diarrhea, and wasting in captive moose. Moose should never be fed such hays or allowed to graze on pastures. Management of captive moose involves either mowing their pens to prevent grazing or confining them to smaller grass-free pens during the growing season. For large, drive-through zoos, moose can free-range in large pens once the grasses have cured and are no longer palatable. However, moose should not be included in mixed species exhibits if alfalfa, clover, or grass-based diets fed to other species are available.

3. A pelleted diet (WSU orange/high-fiber moose diet) has been developed that is much more palatable than the commercially available moose diets and can be used as the sole source of nourishment.

4. Moose are very susceptible to ivermectin-resistant *Trichuris* (whipworm) infections and malignant catarrhal fever. Fenbendazole as a 1-day treatment or, potentially, doramectin should be used to worm captive moose at 4–6 week intervals. Moose should be physically separated from all wild or domestic sheep and goats, and curators should not be assigned to both moose and sheep or goats.

5. Moose can be healthy and productive for many years in captivity with improved diets and appropriate medical attention. Continued research is needed to understand better the dietary needs of captive moose.

ACHKNOWLEDGMENTS

ton State University Lab Animal Care and Use Committee (IACUC #2193). W. Testa provided serum samples from wild moose. We thank T. Rohweder, S. Bosley, H. Degrow, M. Jacoby, and G. Hilderbrand, for their assistance in animal care and data collection. The contributions of S. Farley, G. Kennedy, and D. Williams are greatly appreciated. We are especially grateful to C. Schwartz for his valuable insight and support throughout the study.

REFERENCES

ASHP. AHFS DRUG INFORMATION 96. American Society of Health-System Pharmacists, Bethesda, MD, 1996.

Arbeiter, K; Winding, W. Estimation of folate serum of cattle with special reference to fertility. WIENER-TIERÄRZTLICHE-MONATS-SCHRIFT 60:323–326, 1973.

Clemens, E.T.; Maloiy, G.M.O.; Sutton, J.D. Molar proportions of volatile fatty acids in the gastrointestinal tract of East African wild ruminants. COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY 76A:217–224, 1983.

Fisher, G.E.J.; MacPherson, A. Serum vitamin B_{12} and methylmalonic acid determinations in the diagnosis of cobalt deficiency in pregnant ewes. BRITISH VETERINARY JOURNAL 146:120–128, 1990.

Ford, J.E.; Knaggs, G.S.; Salter, D.N.; Scott, K.J. Folate nutrition in the kid. BRITISH JOURNAL OF NUTRITION 27:571–583, 1972.

Girard, C.L.; Matte, J.J.; Roy, G.L. Serum folates in young dairy heifers. BRITISH JOURNAL OF NUTRITION 61:595–600, 1989a.

Girard, C.L.; Matte, J.J.; Tremblay, G.F. Serum folates in gestating and lactating dairy cows. JOURNAL OF DAIRY SCIENCE 72:3240–3246, 1989b.

Hanley, T.A.; Robbins, C.T.; Hagerman, A.E.; McArthur, C. Predicting digestible protein and dry matter in tannin-containing forages consumed by ruminants. ECOLOGY 73:537–541, 1992.

Hofmann, R.R.; Nygren, K. Morphophysiological specialization and adaptation of the moose digestive system. ALCES SUPPLEMENT 1:91–100, 1992.

Hyvarinen, H.; Nygren, T. Accumulation of copper in the liver of moose in Finland. JOURNAL OF WILDLIFE MANAGEMENT 57:469–474, 1993.

Kennedy, D.G.; Blanchflower, W.J.; Scott, J.M.; Weir, D.G.; Molloy, A.M.; Kennedy, S.;Young, P.B. Cobalt-vitamin B-12 deficiency decreases methionine synthase activity and phospholipid methylation in sheep. JOURNAL OF NUTRITION 122:1384–1390, 1992.

Kennedy, D.G.; O'Harte, F.P.M.; Blanchflower, W.J.; Rice, D.A. Sequential changes in propionate metabolism during the development of cobalt/vitamin B_{12} deficiency in sheep. BIOLOGICAL TRACE ELEMENT RESEARCH 28:233–241, 1991.

Kock, R.A. Health and nutrition of the moose (*Alces alces*) at Whipsnade Park. ERKRANKUNGEN DER ZOOTIERE, INTERNATIONALES SYMPOSIUM ÜBER DIE ERKRANKUNGEN DER ZOOTIERE 27:101–106, 1985.

Oestreicher, P.; Nielsen, S.T.; Rainsford, K.D. Inflammatory bowel disease induced by combined bacterial immunization and oral carrageenan in guinea pigs. Model development, histopathology, and effects of sulfasalazine. DIGESTIVE DISEASES AND SCIENCES 36:461–470, 1991.

Oftedal, O.T.; Baer, D.J.; Allen, M.E. The feeding and nutrition of herbivores. Pp. 129–138 in WILD MAMMALS IN CAPTIVITY: PRINCIPLES AND TECHNIQUES. D.G. Kleiman, ed. Chicage, University of Chicago Press, 1996.

O'Harte, F.P.M.; Kennedy, D.G.; Blanchflower, W.J.; Rice, D.A. Methylmalonic acid in the diagnosis of cobalt deficiency in barley fed lambs. BRITISH JOURNAL OF NUTRITION 62:729–738, 1989.

Palo, R.T. Distribution of birch (*Betula spp.*), willow (*Salix spp.*), and poplar (*Populus spp.*) secondary metabolites and their potential role as chemical defense against herbivores. JOURNAL OF CHEMICAL ECOLOGY 10:499–520, 1984.

Paterson, J.E.; MacPherson, A. A comparison of serum vitamin B_{12} and serum methylmalonic acid as diagnostic measures of cobalt status in cattle. VETERINARY RECORD 126:329–332, 1990.

Sartor, R.B. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. GASTROENTEROLOGY CLINICS OF AMERICA 24:475–507, 1995.

Schwartz, C.C. Techniques of moose husbandry in North America. ALCES SUPPLEMENT 1:177–192, 1992a.

Schwartz, C.C. Physiological and nutritional adaptations of moose to northern environments. ALCES SUPPLEMENT 1:139–155, 1992b.

Schwartz, C.C.; Harmon, D.L.; Hundertmark, K.J.; Robbins, C.T.; Lintzeñich, B.A. Carbohydrase activity in the pancreas and small intestine of moose and cattle. ALCES 32:1–5, 1996.

Schwartz, C.C.; Regelin, W.L.; Franzmann, A.W. A formulated ration for captive moose. PROCEEDINGS OF THE NORTH AMERICAN MOOSE CONFERENCE WORKSHOP 16:82–105, 1980.

Schwartz, C.C.; Regelin, W.L.; Franzmann, A.W. Suitability of a formulated ration for moose.

JOURNAL OF WILDLIFE MANAGEMENT 49:137–141, 1985.

Shochat, E.; Robbins, C.T. Nutrition and behavioral management of bottle-raised moose calves. ZOO BIOLOGY 1997.

Short, C.R.; Hseih, L.C.; Malbrough, M.S.; Barker, S.A.; Neff-Davis, C.A.; Davis, L.E.; Koritz, G.; Bevill, R.F. Elimination of salicylic acid in goats and cattle. AMERICAN JOURNAL OF VETERINARY RESEARCH 51: 1267–1270, 1990.

Statistical Analysis Systems Institute. SAS/STAT GUIDE FOR PERSONAL COMPUTERS, VERSION 6.11. SAS Institute, Cary, NC, 1996.

Steel, R.G.D.; Torrie, J.H.; Dickey, D.A. PRINCIPLES AND PROCEDURES OF STATISTICS: A BIOMETRICAL APPROACH. New York, McGraw-Hill, 1997.

Sutton, A.L.; Elliot, J.M. Effect of ratio of roughage to concentrate and level of feed intake on ovine ruminal vitamin B_{12} production. JOURNAL OF NUTRITION 102:1341–1346, 1972.

Syroechkovsky, E.E.; Rogacheva, E.V.; Renecker, L.A. Moose husbandry. Pp. 367–386 in WILDLIFE PRODUCTION SYSTEMS: ECONOMIC UTILIZATION OF WILD UNGULATE SYSTEMS. R.J. Hudson; K.R. Drew; L.M. Baskin, eds. Cambridge, Cambridge University Press, 1989.

Tremblay, G.F.; Girard, C.L.; Bernier-Cardou, M.; Matte, J.J. Nycterohemeral variations of concentration of serum folates in dairy cows. CANADIAN JOURNAL OF ANIMAL SCIENCE 71: 919–923, 1991.

Van Soest, P.J. NUTRITIONAL ECOLOGY OF THE RUMINANT. Ithaca, New York, Cornell University Press, 1994.

Warsame, I.Y.; Steen, M. Malignant catarrhal fever in wild Swedish moose (*Alces alces* L.). RANGIFER 9:51–57, 1989.

Young, P.B.; Blanchflower, W.J.; Hewitt, S.A.; Price, J.; Kennedy, D.G. Sensitive gas chromatographic-high resolution mass spectrometric method for the determination of methylmalonic acid in bovine plasma. ANALYST 120:2199–2201, 1995.