

The impact of tannins on protein, dry matter, and energy digestion in moose (*Alces alces*)

D.E. Spalinger, W.B. Collins, T.A. Hanley, N.E. Cassara, and A.M. Carnahan

Abstract: Recent work suggests that moose (*Alces alces* (L., 1758)) in the boreal ecoregion may be nutritionally limited by the availability of protein as a consequence of both low nitrogen (N) concentrations and high tannin levels in their principal foods. However, the ability of moose to digest protein in the presence of tannins is unknown. We undertook a series of digestion trials with captive moose to quantify the effects of tannins and compare the digestive capabilities of moose to other browsing cervids. We fed four moose 14 single-species diets including 10 native tannin-containing forages and 4 non-tanniferous foods over four winters. True protein digestibility in moose was 92%, and metabolic fecal N excretion was estimated at 0.389 g N/100 g dry matter (DM) intake. Tannins reduced protein digestion, on average, by 38%, and the rate of reduction in protein digestion was estimated to be 8.28 g protein/100 g DM per unit protein-precipitating capacity. The digestion of protein, neutral detergent fiber, DM, and energy of tanniferous foods was not significantly different between moose and mule (*Odocoileus hemionus* (Rafinesque, 1817)) or white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)). However, our experiments provide refined assays for evaluating the nutritional quality of browses for moose.

Résumé : Des travaux récents laissent croire que l'orignal (*Alces alces* (L., 1758)) dans l'écorégion boréale souffre peut-être de carences nutritionnelles en protéines en conséquence à la fois des concentrations basses d'azote (N) et des teneurs élevées en tannins de ses aliments principaux. Cependant, la capacité des orignaux à digérer les protéines en présence de tannins est inconnue. Nous avons entrepris une série d'essais de digestion chez des orignaux en captivité afin de mesurer les effets des tannins et de comparer les capacités digestives des orignaux à celles d'autres cervidés brouteurs. Au cours de quatre hivers, nous avons nourris four orignaux de régimes contenant 14 espèces individuelles, dont 10 espèces de fourrage indigènes contenant des tannins et 4 aliments sans tannins. La digestibilité véritable des protéines chez l'orignal est de 92 % et l'excrétion métabolique fécale est estimée à 0.389 g N/100 g d'ingestion de matière sèche. Les tannins réduisent la digestion de protéines en moyenne de 38 % et le taux de réduction de digestion des protéines est estimé à 8,28 g protéines/100 g matière sèche par unité de capacité de précipitation des protéines. La digestion des protéines, les fibres neutres solubles au détergent, la matière sèche et l'énergie dans les aliments riches en tannins ne diffèrent pas significativement chez l'orignal et chez le cerf mulot (*Odocoileus hemionus* (Rafinesque, 1817)) et le cerf de Virginie (*Odocoileus virginianus* (Zimmermann, 1780)). Cependant, nos expériences fournissent des analyses précises pour évaluer la valeur nutritionnelle du brouet chez l'orignal.

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Introduction

The boreal and arctic ecoregions are characterized by short growing seasons and cold, unproductive soils, which limit the rates of nitrogen (N) mineralization and N fixation, and contribute to the relative nitrogen deficiency for plant growth (Van Cleve et al. 1983; Callesen et al. 2007; Rohrs-Richey and Mulder 2007; Hobbie and Hobbie 2008). As a

consequence, plants of boreal and arctic regions are potentially N deficient (Aphalo et al. 2006), and the deficiency may be amplified for herbivores because of plant defenses such as condensed tannins. These phenolic polymers are characterized by their ability to bind and precipitate proteins, thus limiting their digestion by herbivores (Robbins et al. 1987a). Tannin-protein effects may be especially important for moose (*Alces alces* (L., 1758)) because woody dicots, particularly the willows (genus *Salix* L.), constitute a large proportion of their diets in the boreal region (Van Ballenberghe et al. 1989; Renecker and Schwartz 1997), and woody dicots are especially rich in tannins (Ayes et al. 1997; McArt et al. 2009). A recent study of two Alaskan sites confirmed that N concentrations of the major forage species of moose declined rapidly with phenological advancement in summer, while simultaneously, tannins increased significantly (McArt et al. 2009). As a consequence, N is likely deficient for moose, particularly in mid- to late summer when moose must regain body condition in preparation for breeding in the fall (McArt et al. 2009).

Moose are the dominant, and perhaps keystone, herbivores of the boreal ecosystem, and they appear to be well-

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adapted to scavenging N from protein in boreal plants by producing salivary binding proteins specific to the tannins of their foods (Hagerman and Robbins 1993; Juntheikki 1996). However, their capability to digest tanniferous forages remains unknown. Assays based on the capability of closely related cervids (mule deer (*Odocoileus hemionus* (Rafinesque, 1817)), white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)), elk (*Cervus elaphus* L., 1758)) to digest plant proteins in the presence of tannins are currently available (Robbins et al. 1987a, 1987b; Hanley et al. 1992; McArt et al. 2006), but recent studies suggest that herbivore species may differ significantly in their capabilities to digest proteins when tannins are present (Shipley and Felicetti 2002). Moreover, the specificity of moose salivary binding proteins (Hagerman and Robbins 1993) suggests that they may be more efficient at digesting protein and perhaps other nutrients in tanniferous foods than their smaller relatives, and they may be adapted specifically to the condensed tannins of the major browse species on which they depend (Ayres et al. 1997).

The principal objectives of this study were to determine the ability of moose to digest protein of native tanniferous forages and to determine the role of tannins in reducing protein availability. Secondly, we tested whether moose are more efficient in digesting protein in tanniferous forages than mule deer, white-tailed deer, and elk (Robbins et al. 1987a, 1987b; Hanley et al. 1992) as suggested by studies of their salivary binding proteins. We examined the possibility that they are adapted to the tannins inherent in their principal foods by feeding a variety of browses that often dominate their diets in the arctic and boreal regions of Alaska. We tested these hypotheses over a 4-year period in a series of digestion experiments with captive moose fed single-forage diets. We then compared the fiber, dry matter (DM), and energy digestion capabilities of moose to those of the other cervids (hereafter called "deer") and examined the role of tannins in the digestion of the components.

Materials and methods

We conducted a series of *in vivo* digestion trials during the winters of 2005, 2006, 2008, and 2009 with moose at our captive animal facilities at the University of Alaska Fairbanks Matanuska Experiment Farm in Palmer, Alaska, USA, following protocols similar to those of Robbins et al. (1987a) and Hanley et al. (1992). Five moose were used in the 4 years of trials (4 female, 1 neutered male). They were aged 1.5 years at the beginning of the trials (128 ± 11 kg) and 5.5 years (340.2 ± 5.5 kg) in the final trial. Between January and March of each winter, a series of trials lasting 14 days each were run consecutively, with each of four moose randomly assigned to one of four different forages in each trial. Each trial consisted of a pretrial period of 7 days in which the animals were adapted to the diets, and the food offered each day was adjusted to match voluntary intake levels. Over the subsequent 7 days, animals were confined to digestion stalls (4 m $\times$ 4 m) equipped with steel floor grating through which all feces and urine fell and were collected below. Feces were collected on a screen immediately below the floor grating, whereas urine passed through this

screen and was collected in a plastic-lined basin and funneled into plastic containers.

Over the course of the 4-year period, we fed 14 forages to the moose, including the current growth of stems of browses collected in winter, leaves of common forages of moose collected in summer, a pelleted ration, and a silaged grass hay (Table 1). We selected forages in an attempt to maximize the variability in DM digestibility and tannin and protein concentrations while maintaining adequate voluntary intake to sustain body condition over the course of the trials each winter. In summer, we collected the tanniferous leaves of seven common foods of moose and preserved them in near-natural state by freezing them in small batches with dry ice immediately after harvesting. The frozen forage was then transported in large insulated boxes to a walk-in freezer where it was kept frozen until trials were conducted the following winter. In late fall, we collected the leaves of balsam poplar (*Populus balsamifera*) after leaf fall and stored them in trash bags in the frozen state until feeding. We collected the current annual growth stems of two willow species in winter and kept them frozen under tarps until feeding. Three brome hay silages were harvested in the summers of 2005, 2006, and 2009 and preserved using plastic wrapping until fed frozen. In winter, the forages were thoroughly mixed before the trials and then fed in the frozen state. During feeding of the brome silage in 2009, we added 4.3% by mass of quebracho tannin (Traditional Tanners, Cave Junction, Oregon, USA) to further examine the role of tannins on protein digestion.

We conducted all digestion trials in the winter for several reasons. First and foremost, conducting digestion trials on summer browse leaves that are high in condensed tannins poses significant experimental and logistical problems. It is extremely difficult to harvest and feed native forages fresh daily to moose in summer because none of the forages grow locally, and all would nutritionally degrade between the time of harvest and consumption (Hove et al. 2003; Wolfe et al. 2008). Likewise, frozen forages fed in summer would be subject to thawing and degradation (from tannin-protein complexation, as well as Maillard reactions) unless fed continuously in small amounts throughout the day. Feeding frozen forages in winter avoided those potential problems. During our winter trials, temperatures remained below freezing, with only occasional thawing periods. However, even in the brief periods of thaw, temperatures remained very close to freezing, humidity was high, and the forages appeared to be unaffected (i.e., they did not discolor).

Second, we chose to conduct all trials in winter to minimize seasonal variation in digestion and intake. Because our principal objective was to examine the effect of tannins on protein digestion in moose for a wide range of forages, minimizing seasonal variability should strengthen our tests. Third, we chose to conduct winter trials because our experience in feeding browse leaves of single species to cervids is that intake is virtually always limited by factors other than appetite (e.g., noxious compounds). Hence, feeding the animals in winter when metabolism is lower may reduce the effects of low intake on body condition throughout the trials.

All orts (uneaten food from the previous day), fecal material, and urine were collected for each trial each day and weighed. Composite grab samples of feed, orts, and fecal

Table 1. Chemical characteristics of the forages, expressed as a percentage of forage dry matter (DM) except where noted, fed to moose (*Alces alces*).

Feed name	NDF ^a	ADF ^b	Lignin + cutin ^c	N ^d	ADFN ^e	PPC ^f	GE ^g
Diamondleaf willow (<i>Salix pulchra</i> Cham.) leaves	24.8	15.7	7.3	2.2	1.2	0.30	21.2
Scouler willow (<i>Salix scouleriana</i> Barratt ex Hook.) leaves	26.8	18.7	7.4	1.7	1.4	0.31	21.1
Barclay's willow (<i>Salix barclayi</i> Anderss.) leaves	28.5	20.1	8.7	2.2	1.3	0.09	20.4
Feltleaf willow (<i>Salix alaxensis</i> (Anderss.) Coville) leaves	30.0	17.8	5.1	2.0	0.7	0.18	19.0
Quaking aspen (<i>Populus tremuloides</i> Michx.) leaves	33.4	21.3	8.3	1.6	1.0	0.18	21.8
Balsam poplar (<i>Populus balsamifera</i> L.) leaves	34.9	24.6	9.8	1.0	1.3	0.14	18.8
Fireweed (<i>Chamerion angustifolium</i> ssp. <i>angustifolium</i> (L.) Holub) (whole plant)	39.9	29.3	5.2	3.0	0.5	0.27	19.6
Paper birch (<i>Betula papyrifera</i> Marsh.) leaves	49.1	31.6	14.3	1.6	0.8	0.18	22.2
Pelleted ration	50.5	22.8	2.7	1.8	0.4	0.00	18.6
Scouler willow stems	52.1	39.4	12.6	1.2	0.5	0.14	21.7
Bromegrass (<i>Bromus inermis</i> Leyss.) silage (2006)	59.3	31.8	2.3	2.3	0.4	0.00	20.9
Feltleaf willow stems	60.5	45.2	10.2	1.1	0.4	0.15	20.6
Bromegrass silage (2005)	64.3	36.8	2.2	2.1	0.4	0.00	20.5
Bromegrass silage (2009)	52.4	47.6	6.5	2.8	0.6	0.02	20.0
Mean	43.3	27.6	7.1	1.9	0.8	0.14	20.4
SD	13.7	8.9	3.8	0.6	0.4	0.11	1.1
High	64.3	45.2	14.3	3.0	1.4	0.31	22.2
Low	24.8	15.7	2.2	1.0	0.4	0.00	18.6

^aNDF is the neutral detergent fiber concentration (g NDF/100 g forage DM).^bADF is the acid detergent fiber concentration (g ADF/100 g forage DM).^cLignin + cutin concentration of forage DM (g lignin + cutin/100 g DM).^dN is the nitrogen concentration (g N/100 g forage DM).^eADFN is the nitrogen concentration of the ADF fiber (g N/100 g ADF fiber).^fPPC is the protein-precipitating capacity of tannins expressed as mg bovine serum albumin (BSA) protein precipitated/mg forage DM.^gGE is the gross energy expressed in kJ/g forage DM.

material were dried at 100 °C for 24 h to estimate DM fed, feed refused, and feces produced. In addition, a fresh sample of each forage, samples of any refused feed from the previous day, and a fresh uncontaminated sample of feces were collected each day and frozen for later chemical analyses. Feed and orts samples for each animal were composited, but fecal samples were analyzed separately for each day.

All feed, orts, and feces were freeze-dried and ground to pass a 1 mm screen prior to chemical analysis. Nitrogen of feed and feces was determined on a Leco™ CHN Analyzer. Crude protein (CP) concentrations were calculated by assuming that the mean protein in food and feces was 16% N (Robbins 1993). Protein-precipitating capacity (PPC) of the tannins in the forages was determined with bovine serum albumin (BSA) according to the method of McArt et al. (2006). Briefly, tannins were extracted in aqueous methanol under high pressure and temperature in an accelerated solvent extractor (Dionex™ ASE-200) and then serially diluted and mixed with a standard solution of BSA in a 2 mL well microplate. The plate was subsequently centrifuged to pelletize the precipitated BSA and a 50 µL aliquot of the remaining solution was transferred to a 350 µL filter plate prepared with Sephadex G-25. The purified extract was then filtered into an optically clear 100 µL microplate by centrifugation. The resulting clear solution was mixed with Bradford Protein Reagent (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and absorbance at 595 nm was read on a UV-Vis microplate spectrometer.

Sequential fiber analysis was conducted on all forages according to the methods of Hanley et al. (1992). We extracted forages in neutral detergent solution (yielding neutral detergent fiber, NDF), with sodium sulfite added for tanniferous forages and without sodium sulfite for the non-tanniferous foods (the pelleted ration and brome-grass silages), as recommended by Hanley et al. (1992). The solubilized fraction of the sample (mostly cell contents) is hereafter termed the neutral detergent solubles (NDS). We extracted NDF residues with acid detergent solution (yielding acid detergent fiber, ADF) and then digested the ADF residue with 72% sulfuric acid (Klason lignin determination) (Goering and Van Soest 1970) to determine the lignin + cutin concentration. We determined the N concentration of the ADF (ADFN) by preparing separate samples of each forage in an identical manner to obtain the ADF residue and then analyzing this residue for N using the Leco CHN analyzer. Fecal NDF residues, as well as the concentration of N in the NDF residue, were determined in a similar manner, but feces were not extracted with sodium sulfite. All results were analyzed and expressed on a DM basis by oven-drying samples at 100 °C for 24 h.

In addition to the above analyses, we determined the potential NDF degradability on all forages in separate in situ nylon bag digestion trials. Two fistulated adult female moose were used in these trials; all forages were incubated singly in each moose in the winter of 2009. The animals were maintained on a mixed diet of native browses (found within the 5 ha paddock in which they were kept) supplemented with a brome-grass silage and approximately 1 kg/day of pelleted ration. Eight samples of each freeze-dried and ground forage were weighed (approximately 0.75 g) into 5 cm × 15 cm nylon bags (50 µm pore size; Ankom

Technology, Macedon, New York, USA), heat sealed, attached with a nylon tie to a stainless steel cable encased in latex tubing, and placed in the rumen of each animal for incubation. Two additional bags of each forage, designated as 0-time bags, were prepared in an identical manner but were not incubated in the animals. During each digestion trial, two sets of 27 bags each were incubated simultaneously in each animal, including the 8 bags of each of six forages and triplicate samples of paper birch (*Betula papyrifera*) leaves (an “internal standard” of our laboratory used to measure intra-animal and intra-trial variation). A single bag of each forage was removed from each animal at 3, 6, 9, 12, 24, 48, 72, and 96 h following insertion into the rumen. Internal standard bags were removed at 12, 24, and 48 h following insertion. Bags were rinsed in tap water and immediately frozen for transport to the laboratory. The samples were then analyzed immediately after thawing by gently rinsing and squeezing in warm water for several minutes and then refluxing in boiling neutral detergent solution with agitation for 1 h. Following the reflux, the bags were rinsed in boiling distilled water twice, followed by two washings in acetone. The bags were then oven-dried at 100 °C overnight and weighed. Loss of NDF was calculated as the difference in fiber remaining in the bag relative to the duplicate 0-time bags. NDF loss was fitted by nonlinear regression (JMP™ statistical software) to a first order kinetics model with time delay:

$$[1] \quad \text{NDF}_t = ae^{(-bt)^{(1-e^{-ct})}} + (1-a)$$

where NDF_t is the NDF remaining at time *t*, and *a*, *b*, and *c* are fitted parameters corresponding to the extent of NDF digestion, the rate of NDF digestion, and the lag-time coefficient, respectively. In all cases, the predicted asymptotic extent of digestion was attained within 48–96 h. We examined between trial and between animal effects using ANOVA on the internal standards removed at 12, 24, and 48 h in each of the three trials. In addition, we compared the 48 h digestibility of the internal standards to a series of 48 h digestions of the same standard over several years (15 trials total) to test for differences between trials. We found no significant difference among animals in any of the trials, although we found that digestion of the internal standards was significantly higher (118% of the long-term mean, *p* < 0.001, root mean squared error (RMSE) = 0.0356) in the third trial than in the first two trials. Hence we corrected the extent of digestion of these forages (paper birch leaves and the pelleted ration) by estimating the extent of digestion at 96 h from the model and multiplying this by 0.849.

We analyzed the effect of tannins on N digestibility in two ways. First, we followed the procedures of Robbins et al. (1987a) and Hanley et al. (1992) by regressing digestible protein (DP) on protein concentration of the foods, then regressed the residuals of this relationship on the PPC of the forages. These simple regressions allowed us to compare the capability of moose to digest protein in tanniferous foods to that of deer from the studies by Robbins et al. (1987a, 1987b) and Hanley et al. (1992). Second, we performed a multiple regression analysis to produce a single model of digestibility and to allow us to examine the interactions between independent variables. We restricted our multiple

regression analysis to the three independent variables that we hypothesized to influence protein digestion: percentage of CP, tannin PPC, and ADFN concentrations. ADFN was included because it is equivalent to indigestible protein in many foods (Licitra et al. 1996) and because ADFN in forages can vary substantially.

All animal protocols were approved by the Institutional Animal Care and Use Committees of the University of Alaska Fairbanks, the University of Alaska Anchorage, and the Alaska Department of Fish and Game.

Results

Forages we fed in these trials varied widely in their chemical composition (Table 1) and their digestibilities (Table 2). Although the low digestibilities and high phenolic concentrations (D.E. Spalinger and W.B. Collins, unpublished data) of some of the forages resulted in low DM intake rates (e.g., as low as 3.39 g DM/day per kg body mass on feltleaf willow (*Salix alaxensis*) leaves), intake during all trials was sufficient to provide reliable digestion data (based on the observed variance between animals; Table 2). Digestible energy intake averaged 20.5 MJ/day (SD = 11.8, range = 6.5–53.8 MJ/day), which was estimated to be 72% of the maintenance energy requirement of the moose (range = 31%–238% of maintenance energy requirement based on maintenance energy requirement reported by Schwartz and Renecker 1997). Hence, although moose lost some body mass during some trials, the random sequence of feeds fed to animals usually insured that animals fed a low-quality food in one trial were able to recover in the subsequent trial because they were fed a relatively high-quality food. All animals completed all trials in good to excellent body condition. In all years, only brief periods of above-freezing weather were encountered, and in those cases, our forages remained near freezing during feeding. There was no indication (based on variance in digestion results between animals or discoloration of the feeds during the trials) that the results were affected by temperature variation. Likewise, there were no statistically significant effects ($p > 0.05$) of animal or year on our analyses of protein and DM digestion.

Protein digestibility and tannin-protein precipitation

The protein concentrations of our forages ranged from 6% (senesced balsam poplar leaves) to 18.7% (fireweed, *Chamerion angustifolium* ssp. *angustifolium*), while the PPC of the tannins varied between 0 (bromegrass (*Bromus inermis*) silage and pelleted rations) to 0.31 mg BSA protein precipitated/mg forage DM in scouler willow (*Salix scouleriana*) leaves (Table 1). Apparent DP varied significantly between forages, ranging from –45% digestibility in the senesced balsam poplar leaves to 62% in the pelleted ration (Table 2). DP was most strongly related to CP concentration ($r^2 = 0.75$), and following the approach of Robbins et al. (1987a), we estimated true DP was $95\% \pm 17\%$ (i.e., the slope of the regression; Robbins 1993). The endogenous excretion rate of N by moose, termed metabolic fecal nitrogen (MFN), was estimated at 1.07 ± 0.32 g N/100 g feed DM intake.

The multiple regression model of DP as a function of CP, PPC, and ADFN explained 99% of the variation in DP in the foods and included a significant ($p = 0.020$) interaction

term between PPC and ADFN (Fig. 1). In this expanded model, MFN was estimated to be 0.389 ± 0.105 g N/100 g food intake and the estimated true digestibility of protein was $92\% \pm 4\%$ of feed CP. The combined effects of protein-precipitating tannins and ADFN lowered protein digestibility by 3.7 g/100 g DM, a mean of 55% (Table 2). Among the tannin-containing forages (excluding the bromegrass hays and pelleted ration), tannins alone reduced protein digestion by 38% (69% of the total protein reduction). ADFN reduced protein digestibility by 17%. The rate of reduction in protein digestion was estimated to be 8.28 g protein/100 g DM per unit PPC and 2.88 g protein/100 g DM per g ADFN. The absolute reduction in DP was higher for low protein – high tannin forages (e.g., a 154% reduction in DP in balsam poplar leaves) than for foods with high protein concentrations (e.g., a 23% reduction in DP in fireweed, in spite of its relatively high PPC level; Table 2).

Digestibility of DM, NDF, and neutral detergent solubles

Dry matter digestibility (DMD) averaged 55% across all forages and ranged from 41% DMD for the scouler willow stems to 69% for the pelleted ration (Table 2). The digestibility of the neutral detergent fiber fraction (DNDF) was highly variable, ranging from –36% for scouler willow leaves to 71% for the bromegrass silage (2009) (mean = 30%, SD = 29%). Negative digestibilities of NDF were observed for two forages: scouler willow leaves fed in 2005 (–36% NDF digestibility) and diamondleaf willow (*Salix pulchra*) leaves fed in 2006 (–2% NDF digestibility).

The digestibility of NDF (% of forage DM) was highly correlated to the lignin + cutin concentration of the NDF, but the regression explained only 82% of the variation in digestible NDF of the foods ($r^2 = 0.82$, RMSE = 0.068). Including the effects of reduced protein digestibility significantly improved the predictive relationship:

$$[2] \quad \text{DNDF} = 60.2 - 12.1 \ln(\text{LC}) - 20.0\text{PR};$$

$$r^2 = 0.89, p < 0.0001$$

where DNDF is digestible NDF (g/100 g forage DM), LC is the lignin + cutin concentration of the NDF (g/100 g NDF), and PR is the protein reduction owing to tannins and ADFN (g of protein lost/100 g forage DM).

In the nylon-bag digestions, we found that the extent of digestible NDF was highly correlated to the lignin + cutin concentration of NDF ($\text{DNDF}_{\text{ex}} = 62.28 - 14.2 \ln(\text{LC})$; $r^2 = 0.90$, RMSE = 0.0385). Furthermore, the relationship between the extent of NDF digestion in nylon bags and the in vivo digestible NDF was found to be slightly better than that between lignin + cutin and digestible NDF (Fig. 2):

$$[3] \quad \text{DNDF} = 11.4 + 75.6 \text{DNDF}_{\text{ex}} - 23.5\text{PR};$$

$$r^2 = 0.91, p < 0.0001$$

where DNDF is the in vivo apparent digestible NDF (g NDF digested/100 g DM), DNDF_{ex} is the extent of digestion of NDF from the in situ nylon bag (g/100 g DM), and PR is the reduction in DP owing to tannins and ADFN (g protein lost/100 g forage DM).

In the case of neutral detergent soluble (NDS), the relationship between digestible NDS and NDS concentration

Table 2. Apparent digestibilities of the forages fed to moose (*Alces alces*) expressed in percentage of dry matter (DM).

Feed name	DM intake (g DM/kg body mass)	Apparent digestibility						Protein reduction ^b (g/100 g DM)
		DM	Energy	Nylon-bag NDF ^a	NDF	Cell solubles	Protein	
Balsam poplar leaves	5.4	41.9 (2.9)	34.9 (4.5)	50.2 (1.9)	19.5 (6.2)	54.0 (4.9)	-39.8 (28.4)	4.7
Scouler willow leaves	6.9	43.4 (9.8)	32.3 (2.6)	58.7 (4.3)	-35.5 (32.0)	72.3 (1.7)	-0.6 (11.9)	8.0
Scouler willow stems	6.8	41.1 (2.3)	42.0 (2.1)	29.6 (1.7)	13.1 (6.2)	71.5 (3.4)	11.9 (31.4)	2.8
Feltleaf willow stems	4.9	43.2 (3.3)	40.0 (6.7)	35.1 (1.1)	27.1 (5.5)	68.0 (2.7)	22.6 (13.2)	2.4
Paper birch leaves	7.4	48.9 (3.2)	47.4 (2.9)	29.8 (1.6)	34.5 (5.1)	62.0 (2.8)	30.6 (7.6)	3.9
Quaking aspen leaves	13.0	53.8 (5.3)	54.5 (1.8)	56.1 (1.2)	27.4 (11.7)	70.0 (3.0)	24.2 (15.9)	4.5
Diamondleaf willow leaves	4.1	58.7 (1.1)	48.1 (15.3)	56.1 (2.8)	-1.8 (7.1)	78.8 (1.5)	27.8 (8.0)	6.6
Barclay's willow leaves	4.5	55.0 (7.7)	49.1 (12.6)	60.9 (1.5)	10.9 (17.1)	72.6 (5.5)	47.2 (9.3)	4.1
Feltleaf willow leaves	3.4	58.2 (5.6)	58.0 (9.0)	56.4 (2.6)	23.1 (13.4)	73.2 (5.5)	37.1 (7.3)	3.5
Bromegrass silage (2005)	16.3	63.3 (8.6)	46.1 (10.7)	79.4 (2.2)	61.6 (8.2)	55.8 (7.2)	59.0 (9.7)	1.8
Fireweed (whole plant)	9.5	63.8 (7.3)	61.4 (8.6)	47.9 (1.2)	55.5 (15.2)	68.5 (7.6)	61.4 (5.6)	3.4
Bromegrass silage (2006)	6.4	64.1 (2.7)	69.1 (1.7)	75.5 (1.3)	63.7 (2.5)	67.6 (1.9)	59.3 (2.9)	1.8
Pelleted ration	27.5	69.2 (2.8)	67.6 (2.9)	57.4 (1.9)	55.6 (4.1)	83.1 (1.4)	62.2 (3.2)	1.8
Bromegrass silage (2009)	11.4	67.0 (2.2)	66.0 (2.5)	77.8 (1.3)	70.7 (1.8)	63.0 (5.0)	62.7 (9.8)	2.2
Mean	9.1	55.1	51.2	55.1	30.4	68.6	33.3	3.7
SD	6.5	9.9	12.0	16.0	29.4	8.0	29.4	1.9

^aNumbers in parentheses are the SD of the mean, with the exception of the nylon-bag NDF (neutral detergent fiber concentration) column, for which the numbers in parentheses are the approximate SE of the estimate of the extent of digestion of NDF from the nonlinear regression.

^bThe estimated reduction in protein digested owing to tannins and nitrogen concentration of the acid detergent fiber (ADFN).

Fig. 1. The relationship between digestible protein (DP) concentration of the feeds and crude protein (CP) concentration, protein-precipitating capacity (PPC), and acid detergent fiber bound nitrogen (ADFN) ($DP = -2.434 + 0.917CP - 8.281PPC - 2.885ADFN - 11.116(ADFN - 0.793)(PPC - 0.140)$; $r^2 = 0.99$, root mean squared error (RMSE) = 0.09, $n = 14$).

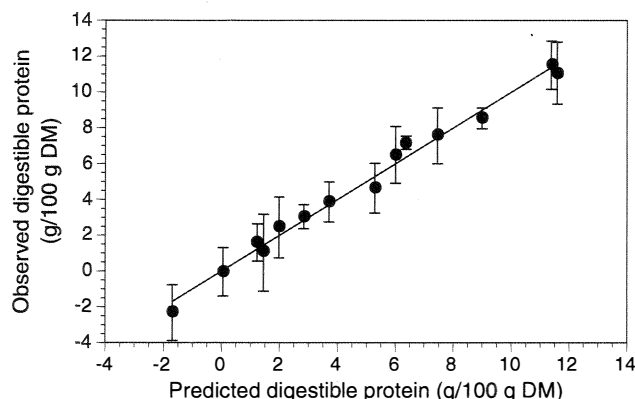
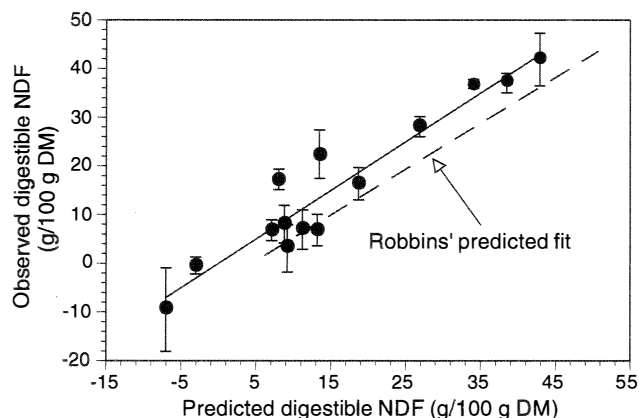


Fig. 2. The relationship between digestible neutral detergent fiber (NDF) (g/100 g forage dry mass (DM)) and the lignin + cutin concentration in the NDF (%) and protein reduction. The Robbins and Hanley line was calculated as the product of the predicted NDF digestibility in deer (from Robbins et al. 1987b) and the NDF concentration of the food. The relationship between digestible NDF (g digestible NDF/100 g DM) and the in situ nylon-bag extent of digestible NDF (g digestible NDF/100 g DM) and protein reduction is identical to that of the lignin + cutin and protein reduction relationship.



was highly significant ($DNDS = 0.831 NDS - 7.0$; $p < 0.001$, $r^2 = 0.94$), but protein reduction did not affect apparent NDS digestion. Because balsam poplar leaves were an outlier from the fitted regression and exhibited strong leverage on the regression (Cook's $D = 9.4$ times the mean Cook's D value), we omitted this observation from the fitted regression. Hence, our moose digested approximately 83% of the NDS available in the forages, on average, and the endogenous fecal soluble excretion was approximately 7 g/100 g of forage consumed, regardless of tannin PPC or ADFN. In spite of the fact that tannins did not affect NDS digestion in our trials, NDS in moose was satisfactorily estimated us-

Fig. 3. The relationship between the predicted digestible NDS concentration (based on Robbins et al.'s (1987) equation for deer fed tanniferous forages) and the observed digestible NDS concentrations in the forages fed to moose (*Alces alces*). The isolated sample in the lower right (balsam poplar (*Populus balsamifera*) leaves; open circle) was not included in the regression equation owing to its high Cook's D value.

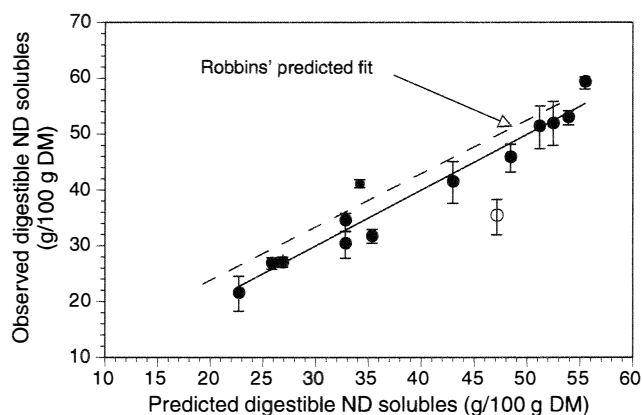
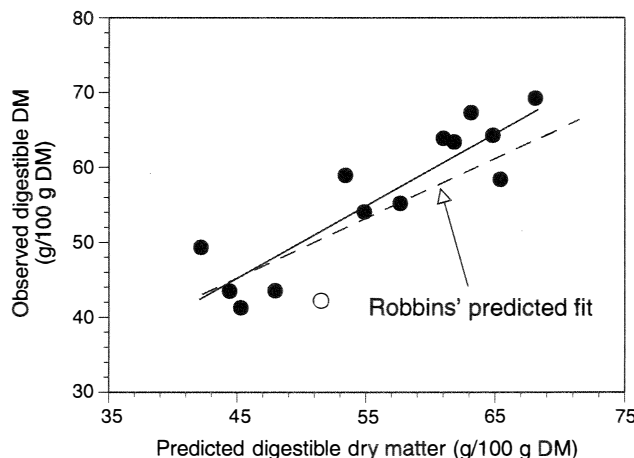


Fig. 4. The relationship between the summative equation of digestible NDF (based on the lignin + cutin predictive equation) and digestible NDS and the observed DDM in moose (*Alces alces*). The open circle (balsam poplar (*Populus balsamifera*) leaves) was not included in the regression.



ing the equation of Robbins et al. (1987b) for tanniferous foods fed to deer (Fig. 3), although their predictive equation underestimated DNDS by approximately 8%.

Theoretically, the digestible dry matter (DDM) (g/100 g DM) of the forages should be the sum of the digestible NDF and the digestible NDS. Comparison of our observed DDM to that predicted by the sum of digestible NDF and digestible NDS (balsam poplar leaves omitted) confirmed this expectation (for DNDF based on nylon-bag digestions: $DDM_{\text{observed}} = 0.89DDM_{\text{predicted}} + 6.42$; $r^2 = 0.77$; for DNDF based on lignin + cutin: $DDM_{\text{observed}} = 0.97DDM_{\text{predicted}} + 1.8$; $r^2 = 0.81$; Fig. 4).

The gross energy concentration of the foods varied only

slightly between foods (coefficient of variation = 0.057), averaging 20.5 KJ/g DM (Table 1). Therefore, the digestible energy (DE) of the foods was highly correlated to DDM (DE (% of DM) = 1.09DDM - 7.44; $r^2 = 0.91$).

Discussion

Protein digestion

Protein digestibility was hypothesized to be a linear function of the CP concentration of the food and the PPC of the tannins, as with previous studies in mule and white-tailed deer and elk (Robbins et al. 1987b; Hanley et al. 1992). Our results were consistent with that hypothesis. The slope of the relationship between observed DP in moose and predicted DP from the deer equation (Robbins et al. 1987a) was 1.08; the intercept (0.186) was not significantly different from 0 ($p = 0.10$). However, the precision of this relationship ($r^2 = 0.93$, RMSE = 0.19) was not as high as found by Robbins et al. (1987a) and Hanley et al. (1992) for deer. We found that including ADFN in the relationship improved the precision of the regression significantly ($r^2 = 0.99$, RMSE = 0.09). Our predicted MFN for moose (3.89 g N/kg feed intake) was lower than that of deer (6.19 g N/kg feed; Robbins et al. 1987a) and slightly lower than that estimated for moose in previous studies (4.58 g N/kg feed intake) (Schwartz et al. 1987) but fell within the range of values for ruminants (3.53–6.98 g N/kg feed intake; Robbins 1993). The estimated true digestibility of protein by moose was 92%, which is very similar to that observed in deer (93%; Robbins et al. 1987a). Our observed reduction in protein digestion as a result of tannins was 8.28 (SE = 1.74) units of protein/unit PPC, compared with 11.82 (SE not given) units of protein/unit PPC in deer.

Also, we found that the fiber-bound nitrogen (ADFN) significantly influenced protein digestion in our trials. Although ADFN has long been associated with protein availability in livestock feeds (Licitra et al. 1996), its role in natural foods of wild herbivores has largely been ignored. That ADFN contributes significantly to protein digestion in wild herbivores such as moose is a new finding. Because many of our forages contained significant levels of ADFN that could not be explained by inadvertent Maillard reactions owing to collection and freezing (i.e., the browse stems collected in winter and the balsam poplar leaves collected after senescence in late fall), this potentially represents a significant source of variation in protein digestion in native foods. ADFN reduced apparent digestible protein by approximately 17%. Accounting for this indigestible protein appropriately partitions indigestible protein from metabolic fecal losses, and hence, should significantly improve estimates of protein metabolism. Hence, the most accurate measure of protein digestibility of browses for moose should include the analysis of both PPC and ADFN in addition to CP concentration. Therefore, we suggest using the following equation for predicting digestible protein (DP) in moose:

$$[4] \quad DP = 5.73N - 2.43 - 8.28PPC - 2.88ADFN \\ - 11.12(ADFN - 0.793)(PPC - 0.140); \\ r^2 = 0.98, \text{RMSE} = 0.09$$

If measures of ADFN are not available, then the alternative

equation of Robbins et al. (1987a) based on only CP concentration and PPC can suffice, although the estimates will not be as accurate or precise.

DM and energy digestion

The digestible cell wall fraction (DNDF) was more variable in moose and not as strongly related to the concentration of lignin and cutin in the NDF ($r^2 = 0.82$) as was found in deer (Robbins et al. 1987b; Hanley et al. 1992). Moreover, digestible NDF in moose averaged 34% less than that predicted from the Robbins equation (Fig. 2). Indeed, in two cases, we found negative values of digestible NDF; a phenomenon that would appear to be impossible, as negative apparent digestibility should occur only when "endogenous sources" of NDF are excreted. However, we hypothesize that those results are not artifacts of experimental error, but rather are a consequence of tannin-protein complexing. These complexes would arise primarily from the cell contents (NDS) of the forage, but subsequently appear as NDF in the feces, thus inflating the apparent digestibility of the NDS and decreasing the apparent digestibility of the NDF. Tannin-protein complexing might also explain why NDF digestion was not as strongly related to the lignin + cutin concentration of the NDF as observed by Robbins et al. (1987b) and Hanley et al. (1992), and why moose appear to be less capable of digesting cell wall than deer. Negative NDF digestibilities have been observed in other studies of ruminants fed high tannin – low quality diets (Barnes 1988; Barnes et al. 1991; Makkar et al. 1995; Reed 1995; Kendrick et al. 2009), and in vitro digestions of high-tannin foods have been shown to result in inflated values for NDF and ADF residues following digestion (Makkar et al. 1997), consistent with this hypothesis and our observations.

In addition, the discrepancy in digestible NDF from that predicted by the lignin + cutin concentration may also involve the ADFN fraction. ADFN can originate from two sources in feeds. It may represent the naturally occurring fiber-bound N of the plant cell wall, of which there is always at least a small amount, or it can arise as an artifact from the curing process when high moisture conditions lead to protein-carbohydrate complexes (Van Soest 1994). In the feed or the feces, these complexes would appear as lignin + cutin in a Klason lignin determination, but unlike true lignins, would not potentially depress the digestion of other cell wall components such as cellulose. We hypothesized that because both ADFN and tannins are apparently responsible for the reduction of protein digestion, and the protein reduction would manifest itself as indigestible NDF in the feces, then the digestible NDF should be significantly related to the amount of protein reduction. We found this to be the case, as the regression was significantly improved by the inclusion of this term.

The extent of NDF digestion, calculated as the asymptote of a time series of nylon-bag digestions (which in all cases was achieved by approximately 72 h residence in the rumen; Table 2), was found to be highly correlated to the lignin + cutin concentration of the NDF, and hence, to the apparent digestible NDF of the feeds (Fig. 2). The prediction equation was only slightly more precise ($r^2 = 0.91$) than that using the lignin + cutin concentration of the NDF, and hence, either analytical method would be acceptable for estimating

digestible NDF of foods for moose. Although nylon bag in situ digestions must be carefully controlled to ensure reliable results, and require the availability of fistulated animals, the method is simple and requires no caustic chemicals that pose waste-disposal problems. Hence, it can be a useful alternative to the lignin + cutin method in determining cell wall digestibility.

Digestibility of the NDS was closely predicted by the equation developed by Robbins et al. (1987b) for deer consuming tannin-containing foods (Fig. 3). However, we found that although digestible NDS was strongly correlated with NDS concentration, tannin PPC did not appear to affect NDS digestion in moose, in contrast to the findings of Robbins et al. (1987b). This apparent discrepancy is a consequence of the differences in methodologies between our study and that of Robbins et al. (1987b). In their study, the effects of tannins on NDS digestion were deduced by comparing NDS digestion between tanniferous and non-tanniferous forages in deer. In our studies, we did not feed a sufficient number of non-tanniferous forages to moose to enable us to mimic this approach. In our studies, it was not surprising that tannins and ADFN did not affect apparent NDS digestibility, as tannin or ADF-bound proteins would subsequently be excreted in a form indistinguishable from NDF (as explained above), not as undigested NDS. Hence, it appears that the Robbins' equation for NDS digestion in the presence of tannins provides a very good approximation for moose, too.

As expected, DDM of our forages was a predictable function of the sum of the digestible NDF and NDS (i.e., $\text{DDM} = \text{digestible NDS} + \text{digestible NDF}$):

$$\begin{aligned} [5a] \quad \text{DDM} &= (0.831\text{NDS} - 6.97) \\ &\quad + (12.76 + 0.732\text{DNDF}_{\text{ex}} - 3.90\text{PR}); \\ r^2 &= 0.77, \text{RMSE} = 4.82 \end{aligned}$$

or

$$\begin{aligned} [5b] \quad \text{DDM} &= (0.831\text{NDS} - 6.97) \\ &\quad + [60.2 - 12.1 \ln(\text{LC}) - 20.0\text{PR}]; \\ r^2 &= 0.81, \text{RMSE} = 4.34 \end{aligned}$$

where DDM is the digestible dry matter of the food (g/100 g DM), NDS is the neutral detergent solubles of the food (g/100 g DM), DNDF_{ex} is the extent of nylon-bag digestion of NDF (g/100 g NDF), LC is the lignin + cutin concentration of the NDF of the food (g/100 g NDF), and PR is the protein reduction owing to tannins and ADFN (g/100 g protein). In spite of the fact that the predictions of DNDF_{ex} were slightly more precise based on the in situ nylon-bag digestion, the lignin + cutin procedure resulted in higher precision for estimating DM digestibility ($r^2 = 0.81$ for the LC equation vs. $r^2 = 0.72$ for the DNDF_{ex} equation; Fig. 4).

Although our observed DDM values were significantly ($p = 0.0045$) related to those predicted from the Robbins et al. (1987b) equation, the predictive power of the Robbins' equation was not high for our forages ($r^2 = 0.54$). We, therefore, suggest caution in using the Robbins et al. (1987b) and Hanley et al. (1992) equation for estimating DDM of foods for moose. Although our equations based on either DNDF_{ex}

or LC are more precise than those of Robbins et al. (1987b) or Hanley et al. (1992), it remains perplexing that DDM in our trials cannot be more precisely estimated using current analytical methods. The higher variance in our results was likely a consequence of the diversity in the kinds and qualities of forages that we were able to feed in our trials, but it is apparent that chemical or nutritional factors other than those that we examined in this study have significant influence on the digestion process in moose.

The ecological consequences of tannins for moose

Our results confirmed that protein digestion in moose is very similar to that in black-tailed deer (*Odocoileus hemionus columbianus* (Richardson, 1829)), white-tailed deer, and elk. Tannins in many of the important boreal browse species are effective at reducing protein digestion; moose are not more or less efficient at digesting protein in the presence of tannins than are the other deer species. The equations for protein digestion derived by Robbins et al. (1987a) and tested by Hanley et al. (1992) were reasonably accurate for predicting protein digestion in moose, although precision was significantly increased by including the effects of ADFN in addition to feed CP concentration and tannin PPC. Similarly, inclusion of protein reduction in the predictive equations for digestible NDF and DDM (through its role in extent of NDF digestion) improved the predictions of both nutritional parameters in moose. Although the Robbins et al. (1987a, 1987b) equations provided a good basis for such predictions, how relevant they were for moose was previously unknown. Now, we know that moose do, indeed, respond more or less similarly to other deer, and we have better equations, specifically for moose. Our results also suggest that the adaptive salivary proteins produced by moose (Hagerman and Robbins 1993) do not appear significantly better at enabling moose to circumvent the digestion-inhibiting effects of tannins in their foods than is the case for other cervids. That the equations for moose do not differ radically from the equations for deer is reassuring of their likely generality for other forages in other regions. We now have a strong basis for evaluating moose ranges based on the nutritional value of their foods (Hobbs and Swift 1985; Hanley and Rogers 1989).

In south-central and interior Alaska, moose ranges appear to be potentially protein-limited relative to adult cow metabolic requirements in spring through fall (McArt et al. 2009). The limitations are a result of declining protein concentrations in virtually all the major browse plants and a simultaneous increase in tannin PPC as summer progresses (McArt et al. 2009). Implications for population recruitment rates (through lactation requirements and in preparation for breeding in the fall) and winter survival (through body growth and reserves) are significant. Tannins play a major role in reducing DP in forages, particularly in late summer and winter (D.E. Spalinger and W.B. Collins, unpublished data). In boreal and arctic ecosystems, alternative foods for moose that alleviate these constraints are few.

Moose are an important consumer in the boreal ecoregion; they directly affect vegetation succession and indirectly affect nutrient cycling (Pastor et al. 1993; Kielland et al. 1997; Kielland and Bryant 1998; Butler and Kielland 2008). The role that N and, particularly, tannins play in the func-

tional dynamics of the boreal region especially relative to the upper trophic levels is still unclear, but feedbacks from moose to plant communities, as well as impacts of changes in plant chemistry, including tannins, to moose and other herbivores are likely (Niemelä et al. 2001; Feng et al. 2009). Our work provides further evidence that tannins are an effective defense against herbivores; it provides a basis for accurately evaluating their role in boreal ecosystem dynamics. We now can incorporate the interactive effects of tannins, N, and gross energy on moose in quantitative analyses of habitat and climate change.

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