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n-Alkane and long-chain alcohol recovery in moose (*Alces alces*), a browsing herbivore

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Abstract

Habitat management for herbivores often depends on an understanding of the food habits of animals. Plant cuticular waxes containing nearly indigestible complex mixture of n-alkanes and long-chain alcohols (LCOHs) have recently shown promise for diet analyses, but the accuracy of the technique depends strongly on the efficiency of recovery of the markers in feces. Fecal recovery of n-alkanes and LCOHs from ten browse stems or leaves and two ensiled grass hays fed to moose (*Alces alces* (L., 1758)) during *in vivo* digestion trials was investigated. n-Alkanes and LCOHs were extracted using a single-step accelerated solvent extraction technique, and the recovery of these cuticular components was calculated from the feces of the animals. n-Alkane recoveries from feces averaged 0.82, but ranged from a low of 0.58 (haylage) to a high of 0.95 (browse stems). Long-chain alcohol recoveries averaged 0.92 across all forages, ranging from 0.80 (haylage) to a high of 1.13 (browse stems). n-Alkane and LCOH fecal recovery increased with increasing chain length, similar to findings in other studies. Although fecal recovery of n-alkanes and LCOHs were variable, we conclude that they are inversely related to forage digestibility, are consistent within forage classes, and are therefore predictable markers for use in assessing herbivore diets.

Keywords Alaska, diet analysis, fecal recovery, herbivores, long-chain alcohols, moose (*Alces alces* L., 1758), n-alkanes, plant cuticular waxes

Introduction

Many aspects of wildlife management depend on accurate determination of the food habits or foraging behavior of wild herbivores, yet few techniques exist that are both reliable and cost effective for this purpose. Since feces of herbivores are often easy to collect, techniques that reliably determine diet composition from fecal indicators or markers of individual diet components have long been sought.

Many complex chemical classes of plant materials can potentially be used as dietary markers, provided they (1) are easily identified and quantified; (2) are sufficiently diverse or distinct between plant species; and (3) pass undigested, or are recovered predictably from the gastrointestinal (GI) tract of herbivores. Several types of hydrocarbons found in the cuticular layer of plants may fit these criteria as potential forage markers. These include very long-chain fatty acids (VLCFA), long-chain alcohols (LCOH), and n-alkanes. These hydrocarbons have been recently used for predicting diet composition of herbivores (Dove et al. 1996; Ali et al. 2005; Dove and Mayes 2006), measuring forage intake (Ferreira et al. 2004; Dove and Mayes 2005; Smith et al. 2007; Hu et al. 2014), and digestive tract passage rate (Hatt et al. 2002; Dove and Mayes 2006; Giraldez et al. 2006). n-Alkanes, LCOHs, and VLCFAs can be readily extracted from plants using either a process of saponification and esterification (Dove and Mayes 2006) or accelerated solvent extraction (ASE; Carnahan et al. 2013), then analyzed using gas chromatography. The ease of extracting these three classes of hydrocarbons using the same analytical process (using either saponification and esterification or ASE) greatly enhances the utility and attractiveness of this method. The variety of chain lengths in these three classes of hydrocarbons composing the cuticular waxes of plants can be sufficiently diverse to be used as diet markers for herbivores with a limited selection of dietary components.

Many studies have examined the fecal recovery of n-alkanes in cattle (*Bos taurus* (L., 1758)) (Brosh et al. 2003; Ferreira et al. 2007c), goats (*Capra hircus* (L., 1758)) (Olivan et al. 2007; Ferreira et al. 2009a), horses (*Equus caballus caballus* (L., 1758)) (Ferreira et al. 2007c), sheep (*Ovis aries* (L., 1758)) (Ali et al. 2004; Lin et al. 2009), and yaks (*Bos grunniens* (L., 1766)) (Ding and Long 2010), and also the recovery of LCOHs in sheep (Ali et al. 2004; Lin et al. 2009). The consensus of these studies is that both n-alkanes and LCOHs are incompletely passed through the digestive tract of animals, and require correction factors to adjust for incomplete fecal recovery in diet composition analysis; no correction factor is needed for intake rates. In general, n-alkane and LCOH extent of recovery increases with carbon chain length (Dove and Mayes 2006; Lin et al. 2009) and decreases with increasing forage digestibility (Ferreira et al. 2005). Additionally, fecal recovery of n-alkanes seems to differ among herbivore species (Ferreira et al. 2007c) and plant species (Lin et al. 2009), and is lower in plants with relatively low concentrations of plant waxes (Brosh et al. 2003; Elwert et al. 2008).

Despite potentially incomplete recoveries, n-alkanes and LCOHs have been used successfully in many foraging studies of domestic ruminants. For foraging studies of wild browsing ruminants, the extent of fecal recovery has usually been estimated from studies of domestic species. For example, Hatt et al. (1998) used the extent of fecal recovery rates of sheep from Mayes and Dove (2000) as surrogates for fecal recovery rates in giraffe (*Giraffa camelopardalis* (L., 1758)). Gedir and Hudson (2000) used Controlled-Release Devices (CRD) with C₃₂ and C₃₆ n-alkanes to estimate digestibility and fecal recovery in wapiti (*Cervus elaphus* (L., 1758)). Neither study, however, used total fecal collection to determine digestibility and FR, making it difficult to compare their results with complete digestion trials conducted with domestic animals.

Moreover, most studies in which fecal recoveries were directly measured were conducted with animals fed mixed diets, thus potentially confounding individual plant n-alkane recoveries. In only one study (Elwert et al. 2008) was the diet mixing effect examined, finding no significant mixing effect. Fecal recovery of n-alkanes and LCOHs have not been examined in wild herbivores. This consideration is particularly germane to assessment of diets of browsing ruminants such as moose (*Alces alces* (L., 1758)), whose foods vary considerably in cuticular waxes, particularly between summer and winter, and whose digestibility is generally lower and potentially affected by secondary plant defenses (McArt et al. 2009). We assessed fecal recovery of n-alkanes and LCOHs in moose fed a series of single-species diets of native browse and ensiled grass hay (haylage) in complete digestion trials to determine their applicability in identifying moose diets.

Materials and methods

Study area

A series of single-species *in vivo* digestion trials with ten forages commonly consumed by moose (*Alces alces*) in Alaska (MacCracken and Van Ballenberghe 1993) were conducted. We conducted these trials at the University of Alaska Fairbanks Matanuska Experiment Farm (MEF) in Palmer, AK, on four captive adult female moose and one captive adult male moose between January and March during the winters of 2005, 2006, 2008, and 2009 (Spalinger et al. 2010). Moose were aged 1.5 years at the beginning of the experiment (128 ± 11 kg) and 5.5 years (340.2 ± 5.5 kg) by the final trial. All methods were approved by University of Alaska Anchorage (Protocol # 181596-2) and University of Alaska Fairbanks (Protocol # 182744-2) Institutional Animal Care and Use Committees. All procedures involving animals were

performed in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Experimental design

Leaves of common summer forages of moose, current year's growth of browse stems (collected in winter), a pelleted ration, and a silaged grass hay were fed in *in vivo* digestion trials each winter, with the ensiled grass hay being replicated in 2006 and 2009. Leaves were collected over a two week period in summer and immediately frozen on dry ice to eliminate enzymatic browning or protein degradation by tannins. Once collected, leaves were transported in insulated boxes and stored in a walk-in freezer until immediately prior to feeding. All trials were conducted during the period January to March to ensure that feeds remained frozen and retained their nutritional quality after being placed in feed troughs. The leaves of balsam poplar (*Populus balsamifera* L.) were collected after leaf fall and stored frozen in trash bags until feeding. Current annual growth stems of two willow species were collected in winter and kept frozen under tarps until feeding. Two brome hay silages (*Bromus inermis* Leysser) were harvested from single cuttings in the summers of 2006 and 2009 and ensiled by wrapping bales in plastic and likewise fed frozen.

We conducted separate trials of four forages each year. During each trial, each moose was randomly assigned one of the four different forages in a latin-square design; over four trials, each moose would be fed each forage. Each trial lasted two weeks, during which animals were acclimated to a single forage during a pre-trial period of seven days, followed by seven days in a metabolic stall (4 m X 4 m) for total fecal collection. Animals were offered an intake of 1% of their body weight once a day and allowed access to food throughout the day; temperatures remained below freezing with a few exceptions. During these exceptions, temperatures remained

near freezing, and there was no evidence that forage quality was affected (e.g., no discoloration in the forages over time, and the variance in protein digestion between trials was not elevated (Spalinger et al. 2010)).

All orts, feces, and urine were collected daily for each moose and weighed. Duplicate composite samples of food, orts, and fecal material were collected; one sample was dried at 100° C for 24 hrs to estimate dry matter fed, food refused, and feces produced, while the second was frozen for chemical analyses. Frozen samples were later freeze-dried and ground to pass through a 1 mm screen. We used forage and fecal samples from eleven of these forages including ten browse leaves or stems from 2005, 2006, 2008, and 2009 and two hay cuttings from 2006 and 2009. For simplicity, we categorized these forages into three classes: grass haylage, browse leaves, and browse stems (Table 1).

Digestible dry matter determinations

Digestible dry matter (DDM) was calculated from food and fecal samples collected during *in vivo* digestion trials using equation 1:

$$\text{DDM} = [(I - \text{FO}) / I] * 100$$

Equation 1

where *I* is total forage intake (kg DM/day) and FO is total fecal output (kg DM/day).

n-Alkane and LCOH analysis

n-Alkanes and free LCOHs were extracted from samples of forages fed and feces collected from each *in vivo* digestion trial following methods described by Carnahan et al. (2013). Samples were extracted in hexane (Sigma-Aldrich, St. Louis, Missouri, USA) using accelerated solvent extraction which uses heat and high pressure (Carnahan et al. 2013) instead of chemical saponification (Dove and Mayes 2006). ASE extraction was followed by derivatization of

LCOHs with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) + 1% TMCS catalyst) (Sigma-Aldrich, St. Louis, Missouri, USA). The extracts were analyzed on a Hewlett Packard 5890 Series II+ GC (Hewlett-Packard, Wilmington, Delaware, USA) fitted with a Flame Ionization Detector (FID) under the following conditions: Varian CP-SIL8 fused silica column (30M X 0.25mm ID; 0.25 μ m film thickness) (Agilent Technologies, Santa Clara, California, USA), 1:10 injection split, injector at 320° C, oven temperature initially at 80° C and then increased at 11° C/min to 325° C, with a hold at 325° C for five minutes. We integrated peaks using ChemStation software (Agilent Technologies, Santa Clara, California, USA) and converted the raw counts to mg n-alkane/kg sample using a standard curve of serial dilutions (LCOH standards were also derivatized using BSTFA).

Fecal recovery calculation

We calculated fecal recovery of n-alkanes and LCOHs from forage and fecal samples of the *in vivo* digestion trials using the following formula:

$$FR = [A_{\text{fecal}} / (A_{\text{forage}} / (1 - \text{DDM}))]$$

Equation 2

where FR is the proportion of fecal n-alkanes or LCOHs to the calculated recovery of n-alkanes or LCOHs, DDM is the percent digestible dry matter, A_{forage} is the n-alkane or LCOH concentration (mg/kg) of the forage, and A_{feces} is the n-alkane or LCOH concentration (mg/kg) of feces.

Statistical analysis

We performed statistical analyses using program R (2013). We examined the effects of digestibility (%DDM), forage class, and chain length and their interactions on fecal recovery of each alkane and LCOH using the linear model (LM) routine fitting explanatory variables in a

stepwise process. Model selection was guided using Akaike's Information Criteria (AIC, although we confined the number of explanatory variables allowed in the model to 2 because of the limitations of our sample size. We also analyzed effects of nutritional constraints including dry matter intake (DMI), metabolizable energy intake (MEI), digestible nitrogen (DN), digestible nitrogen intake (DNI), and neutral detergent fiber (NDF) digestion rate (rate at which ruminal microbes degrade the NDF fraction) for each food and their interactions on fecal recovery using the same approach.

Results

Plant n-alkane profiles

The forages fed to moose in digestion trials contained from three (fireweed, *Chamerion angustifolium* L.) to 12 n-alkanes (paper birch, *Betula papyrifera* Marshall), ranging from C₂₂ to C₃₃ in chain length (Table 1). Total n-alkane concentrations ranged from 80 mg/kg (fireweed) to 6 425 mg/kg (balsam poplar). On average, odd-chain n-alkanes made up 95% of the total n-alkanes for all forage species (Table 1).

Fecal recovery of n-alkanes

Based on measured *in vivo* DDM, which ranged from 35.4% DDM in Scouler willow (*Salix scouleriana* Barratt) to 69.3% DDM in fireweed (Table 1), fecal recovery of n-alkanes averaged 0.82 across all forages and chain lengths, ranging from 0.54 in haylage (*Bromus inermis* Leysser, 2006) to 0.97 in diamondleaf willow (*Salix pulchra* Chamisso) leaves (Table 2).

To explore the potential causes of variation in fecal recoveries, we examined a series of nutritional factors that theoretically could influence fecal recovery. We examined the effects of fecal recovery for each food using a linear model with a stepwise process against dry matter intake (DMI), metabolizable energy intake (MEI), digestible nitrogen (DN), digestible nitrogen

intake (DNI), and neutral detergent fiber (NDF) digestion rate (rate at which ruminal microbes degrade the NDF fraction) for each food. We found some indication that fecal recovery was influenced by MEI (all forages, $r^2=0.14$, coefficient= -4.3×10^{-5} , $SE=1.1 \times 10^{-5}$, $p < 0.01$), and that fecal recovery was influenced by DNI (all forages, $r^2=0.18$, coefficient= -8.1×10^{-3} , $SE=2.0 \times 10^{-3}$, $p < 0.01$). We found one 2-factor model that indicated fecal recovery was correlated ($r^2 = 0.51$) to MEI/DNI (coefficient= 8.6×10^{-5} , $SE=1.6 \times 10^{-5}$, $p < 0.01$) and NDF digestion rate (coefficient= -5.7×10^{-1} , $SE=6.7 \times 10^{-2}$, $p < 0.01$).

Although average fecal recovery was weakly correlated to MEI, DNI, and perhaps the ratio of MEI/DNI and fiber digestion rate, n-alkanes are variably recovered, and that recovery was affected by chain length. To examine this relationship, we analyzed the correlation of fecal recovery against n-alkane chain length, with forage class as a factor. We found that fecal recovery was positively related to chain length ($r^2=0.49$, coefficient= 2.4×10^{-2} , $SE=4.8 \times 10^{-3}$, $p < 0.01$). We also found that fecal recovery was lower in the grass silages than in either browse leaf or browse stem forages (Fig. 1). Within forage classes, fecal recovery was correlated to digestibility (Fig. 1) and increased with decreasing digestibility ($r^2=0.18$, coefficient= -0.79 , $SE=0.18$, $p < 0.01$). On average, browse stems exhibited the lowest digestibility (DDM = $42.4 \pm 2.2\%$), and the highest fecal recovery (0.95 ± 0.01), whereas browse leaves exhibited slightly higher digestibility (DDM = $51.8 \pm 9.9\%$) with a lower fecal recovery (0.85 ± 0.09). Grass haylage exhibited the highest digestibilities (DDM = $67.6 \pm 1.8\%$) and the lowest fecal recovery (0.57 ± 0.03) as compared to browse stems and leaves.

Plant long-chain alcohol (LCOH) profiles

The forages fed to moose in digestion trials contained from two (haylage) to seven (paper birch) LCOHs, ranging from C₂₂ to C₂₈ in chain length (Table 3). Concentrations of LCOHs were

substantially higher in all forages compared to n-alkane concentrations, ranging from a low of 1 386 mg/kg in haylage to a high of 6 103 mg/kg in paper birch (Table 3). On average, even-chain LCOHs made up 56.8% of the total LCOHs for all forage species.

Fecal recovery of long-chain alcohols (LCOH)

Based on measured *in vivo* DDM, fecal LCOH recovery averaged 0.92 across all forages and chain lengths, and ranged from a low of 0.80 in haylage to a high of 1.13 in feltleaf willow stems (*Salix alaxensis* (Andersson) Coville, Table 4). Fecal LCOH recoveries generally increased with carbon chain length (Table 4, Fig. 2), though not significantly ($r^2=0.13$, $p > 0.05$) and increased with decreasing digestibility ($r^2=0.44$, coefficient=-1.39, SE=0.34, $p < 0.01$).

Discussion

A technique of diet quantification for wild herbivores has long been sought to aid with managing herbivores and their habitats. Microhistological analysis, where plant species are identified from microscopic fragments found in feces, has long been the standard for diet analysis. However, this method often produces biased results toward species that are not easily digested, as highly digestible species often leave little trace in the feces. Although advances in genetic-based diet assessment have improved in recent years, it can remain cost prohibitive (Czernik et al. 2013), have problems qualitatively distinguishing between forage species (Czernik et al. 2013), and be quantitatively problematic even with advances in Next Generation Sequencing (Pompanon et al. 2012). Unless further advances with genetic-based diet analysis are made, chemical analysis of diets is likely to be the best quantitative and cost effective method.

The usefulness and accuracy of dietary determinations using plant chemical markers such as n-alkanes and long-chain alcohols measured in forages and feces depend more on the relative recovery of the markers in the feces rather than absolute recoveries. The predictable nature of the

relative recoveries of n-alkanes and LCOHs make them robust markers for dietary estimation. Our examination of fecal recovery of n-alkanes and LCOHs in a series of *in vivo* digestion trials involving a variety of browse leaves, browse stems and haylage consumed by moose illustrates that fecal recovery of n-alkane for these markers varies from an average high of 0.97 for browse leaves to a low of 0.54 for grass haylage (Table 2). Within forage classes (browse leaves vs. browse stems), we found that fecal recovery of n-alkanes of winter browse stems averaged 0.95 ± 0.01 , whereas summer browse leaves averaged 0.85 ± 0.08 .

Fecal recovery of LCOHs in moose is generally higher than those of n-alkanes (Table 2 and Table 4). On average, LCOH recovery was 0.92; however, recoveries ranged from a high of 1.13 in fettleaf willow stems to as low as 0.80 in haylage (Table 4). Our fecal recoveries >1.0 are likely related to the variance associated with measuring digestibility when feeding single-species diets of very low-quality food. Under these conditions, animals exhibit voluntary intake rates which are very low or may vary across a seven-day trial. The samples analyzed were also collected from a single day, while species digestibility is calculated across a seven-day period. Although fecal recovery of LCOHs was quite variable across all forages examined, it was less variable within forage classes. Fecal recovery for summer browse leaves, for example, averaged 0.89 ± 0.05 , while fecal recovery for winter browse stems averaged 1.13 ± 0.04 . Others have found that fecal recovery of LCOHs in sheep averaged 0.76 – 0.79 (Ali et al. 2004; Lin et al. 2009) and Lopez et al. (2015) found LCOH recovery in cattle and horses averaged ~ 1.0 , with animals fed diets having woody species tending to have higher LCOH fecal recovery. While other studies indicate that fecal recoveries of LCOHs increase with chain length, there are not enough published data with which to meaningfully compare our results. Furthermore, we extracted our LCOHs using a different method than other published studies, which may confound

comparisons. For example, Carnahan et al. (2013) found that our ASE extraction method was more efficient at extracting LCOHs, yet yielded lower concentrations because the ASE extraction recovers only free LCOHs.

Our results showed that fecal recovery of n-alkanes and long-chain alcohols in moose fed a single diet is variable, and often less than 1.00. These results were consistent with other studies, although fecal recoveries in moose eating woody dicots are generally higher than fecal recoveries for livestock eating dicot or monocot vegetation (Fig. 3), primarily due to the low digestibility for most woody dicots. However, when moose are offered a highly digestible monocot forage (i.e. haylage), their fecal recoveries are much lower (Fig. 3). For example, Ferreira (2009b) found that fecal recovery in goats, sheep, cattle, and horses fed herbage diets (perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.)) differed significantly among herbivore species (fecal recovery of 0.60, 0.69, 0.82, and 0.99 respectively). These differences may be confounded by digestive efficiency since fecal recovery appeared to be related to forage digestibility (e.g., DDM ranged from 0.67-0.78 in that study). Ferreira et al. (2007b) suggested that fecal recovery may differ between ruminant and non-ruminant herbivores; our comparison among herbivores suggests the relationship is likely related to diet digestibility as opposed to gut morphology. Fecal recovery in the only non-ruminant herbivore tested, the horse, does not appear to deviate from the expected relationship between diet digestibility and fecal recovery within ruminants (Fig. 3).

The variance in fecal recovery of n-alkanes and LCOHs between plants fed to moose in our study appears to be negatively related to diet digestibility as has been shown in other species (Fig. 3). Under anaerobic conditions, there is theoretically minimal biological degradation of n-alkanes. However, Bian et al. (2015) found that fumarate can act as an electron acceptor for the

efficient splitting of the C-C bond and subsequent conversion to alkylsuccinates and ultimately to LCOHs or other products. Grossi et al. (2008) also found that n-alkanes seem to be degraded in conjunction with the production of fumarate in the rumen. Nitrate and sulfate substrates and methanogens may also act as electron acceptors in the rumen environment as they do in anoxic environments elsewhere (Grossi et al. 2008; Morris et al. 2013). Although the site of degradation in the GI tract has not been identified definitively in ruminants, Mayes (1988) observed that the main site for n-alkane loss in sheep was the small intestine, whereas Ohajuruka and Palquist (1991) found that some n-alkane loss was occurring within the rumen in cattle. However, Keli et al. (2008) found no synthesis or degradation of n-alkanes by rumen bacteria through *in vitro* manipulations using sheep ruminal fluid. Degradation of n-alkanes may be secondarily dependent upon a suite of other conditions in the rumen, including rumen passage rate, microbial efficiency, which depends on both energy and N supplies to the rumen, and the presence of plant microbial toxins such as terpenes. Nevertheless, this study and others (Grossi et al. 2008) have shown there is predictable degradation of n-alkanes.

Evidence of microbial degradation of n-alkanes in our animals is also corroborated by studies of the patterns of microbial degradation of n-alkanes in other systems. We found that fecal recoveries of n-alkanes and LCOHs increased as chain length increased (Fig. 1 and 2). Similarly, others have found that n-alkane degradation by naturally occurring microbes is more rapid for n-alkanes of shorter chain length (Mohanty and Mukherji 2008).

Although further study of rumen microbial degradation of n-alkanes and LCOHs may prove insightful, we have found that n-alkane recovery rates are similar among browse species of similar phenology (e.g., summer or winter) (Table 2), suggesting that a causal understanding of the factors determining fecal recovery may not be necessary for the accurate estimation of diet

composition. If n-alkane degradation is a result of a syntrophic consortium in the rumen or lower GI tract, or reliant on alternative sources of electron acceptors (e.g., nitrates or sulfates from foods), it is likely that recoveries of individual plant species n-alkanes in a mixture within the rumen would be virtually identical, since conditions for microbial degradation would be very similar throughout the rumen.

Additionally, we found that DDM has the most significant effect on fecal recoveries (Tables 2,4; Fig. 3). Ferreira et al. (2007c) noted that differences in fecal recovery between species of herbivores fed the same species of plants might not be due to differences in classes of herbivores, but in the efficiency at which each species can digest a particular forage. When domestic species are used as surrogates for wildlife, especially browsing herbivores, it is important to verify that both species of herbivores are similarly efficient at digesting the same plants.

For managers to make informed decisions about wild herbivore populations and how they relate to range or habitat conditions, an accurate and efficient method for determining the diets of wild herbivores is needed. The use of n-alkanes and LCOHs as markers for dietary analysis is increasingly applied for such assessments, but its application to dietary analysis for wild herbivores remains relatively untested. The ease of extraction and analysis of these markers makes them an appealing alternative to microhistological analysis. As with most internal nutritional markers (e.g., lignin), the reliability of the technique depends on an ability to recover the marker completely in feces, or alternately, understanding the factors that reduce its complete recovery. Our study of single-species diets in moose indicates that n-alkanes and LCOHs are efficiently recovered from the feces, and that recovery is related more to forage digestibility than forage class. Hence, we suggest managers use forage digestibility as a metric for estimating fecal

recoveries of n-alkanes and LCOHs in mixed diets. n-Alkane and LCOH analysis will likely become a more widely accepted component of wildlife habitat management for species having limited dietary components.

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Table 1. n-Alkane content (mg/kg DM) of forages from *in vivo* digestion trials performed in metabolic stalls with moose using single-species diets.

Forage	n-Alkane Chain Length												Total	%DDM ^a
	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33		
Grass Haylage														
Haylage 2006 ^b				31.1		45.0		56.0		58.6		61.8	252.5	66.3
Haylage 2009 ^b				11.1		30.4	21.8	79.2		83.6		18.0	244.1	68.9
Browse Leaves														
Paper birch ^c	18.9	442.3	61.8	877.0	84.2	674.6	13.7	150.6	10.0	283.3	9.7	48.5	2 674.3	49.1
Fireweed ^d				16.1		36.2		27.7					80.0	69.3
Balsam poplar ^e	9.4	330.9	83.3	1 505.8	137.2	2 387.4	76.1	1 726.7	10.3	146.5		11.8	6 425.3	45.1
Quaking aspen ^f	17.5	201.6	91.3	870.7	150.9	956.2	10.7	62.1		12.7			2 373.6	58.1
Feltleaf willow ^g		24.6	22.4	252.4	53.6	1 809.5	31.7	216.5		12.1			2 422.7	53.9
Barclay's willow ^h	8.7	53.0	30.7	294.9	40.4	624.4	21.6	160.5					1 234.2	54.2
Diamondleaf willow ⁱ	6.8	40.9	20.8	177.6	33.5	572.6	18.4	71.9					942.5	49.0
Scouler willow ^j		74.3	23.9	360.5	53.1	1 734.7	21.4	179.6					2 447.5	35.4
Browse Stems														
Feltleaf willow ^g		22.0	9.3	230.9	16.5	1 067.7	8.9	107.4					1 462.7	44.0
Scouler willow ^j		112.8	19.1	695.0	35.9	2 615.5	26	369.7					3 873.9	40.8

^aDDM is digestible dry matter^b*Bromus inermis* Leysser^c*Betula papyrifera* Marshall^d*Chamerion angustifolium* L.^e*Populus balsamifera* L.^f*Populus tremuloides* Michaux^g*Salix alaxensis* (Andersson) Coville^h*Salix Barclayi* Anderssonⁱ*Salix pulchra* Chamisso^j*Salix scouleriana* Barratt

Table 2. Fecal recovery (FR) of n-alkanes from *in vivo* digestion trials performed in metabolic stalls with moose fed single-species diets. Fecal recovery is calculated by: $FR = \frac{\text{Mean fecal n-alkane}}{\text{Mean plant n-alkane} / (1 - \% \text{ DDM})}$ where DDM is digestible dry matter

Forage	n-Alkane Chain Length												AVG FR	% DDM ^a
	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33		
Grass Haylage														
Haylage 2006 ^b				0.34		0.35		0.56		0.71		0.75	0.54	66.3
Haylage 2009 ^b				0.63		0.58	0.42	0.53		0.62		0.74	0.59	68.9
Browse Leaves														
Paper birch ^c	0.51	0.60	0.54	0.72	0.66	0.83	0.83	0.92	0.92	0.95	0.97	1.06	0.79	49.1
Fireweed ^d				0.95		0.89		0.83					0.89	69.3
Balsam poplar ^e	0.75	0.54	0.59	0.67	0.70	0.78	0.77	0.82	0.93	0.97		1.10	0.78	45.1
Quaking aspen ^f	1.10	0.96	0.96	0.82	0.91	0.83	0.88	0.89		0.81			0.91	58.1
Feltleaf willow ^g		0.59	0.59	0.68	0.66	0.72	0.71	0.73		1.00			0.71	53.9
Barclay's willow ^h	0.85	0.81	0.72	0.83	0.81	0.90	0.94	1.07					0.86	54.2
Diamondleaf willow ⁱ	0.91	0.92	0.86	1.00	0.96	1.01	1.04	1.06					0.97	49.0
Scouler willow ^j		0.92	0.96	0.84	0.95	0.82	1.00	0.89					0.91	35.4
Browse Stems														
Feltleaf willow ^g		0.76	1.16	0.87	1.06	0.95		0.91					0.95	44.0
Scouler willow ^j		0.84	0.92	0.95	0.91	0.99	0.99	1.02					0.94	40.8
Average FR	0.82	0.77	0.81	0.78	0.84	0.81	0.84	0.85	0.93	0.85	0.97	0.91	0.82	
StDEV FR	0.22	0.16	0.21	0.18	0.15	0.17	0.19	0.17	0.01	0.15		0.19	0.14	

^aDDM is digestible dry matter
^b*Bromus inermis* Leysser
^c*Betula papyrifera* Marshall
^d*Chamerion angustifolium* L.
^e*Populus balsamifera* L.
^f*Populus tremuloides* Michaux
^g*Salix alaxensis* (Andersson) Coville
^h*Salix Barclayi* Andersson

ⁱ*Salix pulchra* Chamisso

^j*Salix scouleriana* Barratt

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Table 3 Long-chain alcohol (LCOH) concentrations (mg/kg DM) of forages from *in vivo* digestion trials with moose fed single-species diets

Forage	LCOH Chain Length							Total even	Total	% DDM ^a
	C22	C23	C24	C25	C26	C27	C28			
Grass Haylage										
Haylage 2009 ^b					617.0		768.9	1 386.0	1 386.0	68.9
Browse Leaves										
Paper birch ^c	1 017.5	669.2	848.2	1 182.2	611.0	981.5	703.3	3 180.0	6 012.8	49.1
Fireweed ^d			2 028.0	608.7	422.9	723.9		2 450.9	3 783.4	69.3
Quaking aspen ^e	281.4	775.7	518.0	570.7	701.4	662.6	1 109.5	2 610.4	4 619.3	58.1
Feltleaf willow ^f	982.4			1 562.6	794.3	1 068.8	949.2	2 726.0	5 357.5	53.9
Browse Stems										
Feltleaf willow ^f				993.6	675.1	541.2	513.6	1 188.7	2 726.3	44.0

^aDDM is digestible dry matter
^b*Bromus inermis* Leysser
^c*Betula papyrifera* Marshall
^d*Chamerion angustifolium* L.
^e*Populus tremuloides* Michaux
^f*Salix alaxensis* (Andersson) Coville

Table 4 Fecal recovery (FR) of long-chain alcohols (LCOH) from *in vivo* digestion trials with moose fed single-species diets. Fecal recovery is calculated by: $FR = [\text{Mean fecal LCOH}] / [\text{Mean plant LCOH} / (1 - \% \text{ DDM})]$

Forage	LCOH Chain Length							AVG FR	% DDM ^a
	C22	C23	C24	C25	C26	C27	C28		
Grass Haylage									
Haylage 2009 ^b					0.80		0.79	0.80	66.2
Browse Leaves									
Paper birch ^c	0.80	0.84	0.90	0.94	0.94	1.00	0.95	0.91	49.1
Fireweed ^d			0.52	1.01	0.80	1.01		0.83	69.3
Quaking aspen ^e	0.93	0.91	1.02	0.92	0.97	0.92	0.98	0.95	58.1
Feltleaf willow ^f	0.95			0.80	0.90	0.78	0.97	0.88	53.9
Browse Stems									
Feltleaf willow ^f				1.06	1.11	1.16	1.19	1.13	44.0
Average FR	0.89	0.87	0.81	0.95	0.93	0.97	0.96	0.92	
StDEV FR	0.08	0.05	0.26	0.10	0.11	.014	0.17	0.12	

^aDDM is digestible dry matter

^b*Bromus inermis* Leysser

^c*Betula papyrifera* Marshall

^d*Chamerion angustifolium* L.

^e*Populus tremuloides* Michaux

^f*Salix alaxensis* (Andersson) Coville

Figure captions

Figures were created using SigmaPlot v. 12.0.

Fig. 1 Fecal recovery of n-alkanes in browse stems, browse leaves, and grass haylage fed to moose. Only odd-chain n-alkanes are included here because even-chain n-alkanes were in low concentrations with relatively high standard deviations

Fig. 2 Fecal recovery of long-chain alcohols (LCOH) in browse leaves and browse stems fed to moose. Grass silage contained only two LCOH peaks and were not included

Fig. 3 The relationship between fecal recovery of n-alkanes and digestible dry matter in ruminants and horses (Data from Ferreira et al. 2005; Ferreira et al. 2007a; Ferreira et al. 2007b; Ferreira et al. 2007c; Olivan et al. 2007; Sun et al. 2008; and this study)

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76x57mm (300 x 300 DPI)

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