SEASONAL VARIATION OF PHENOLS, NITROGEN, FIBER, AND IN VITRO DIGESTIBILITY IN SWEDISH MOOSE

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ABSTRACT: Understanding how different components of food are processed and digested within the compartments of the digestive tract of large herbivores has important implications in their foraging behaviour, nutritional ecology, and techniques for measuring diet composition and nutritional quality of forage. Analysis of contents from different compartments of the digestive tract of moose in central Sweden showed that neutral detergent fiber (NDF) and nitrogen (N) content varied throughout the digestive tract and among individual moose (*Alces alces*). Total phenols (TP) had an inconsistent pattern throughout the digestive tract, possibly reflecting variation in diet composition and phenol patterns. The study moose were divided into 2 groups; the winter group had low N in the digestive tract and high NDF and dry matter content, and the summer group had high levels of N and low NDF and dry matter content. The phenol platyphyllane, indicative of consumption of dormant silver birch (*Betula pendula*), was detected throughout the contents of the digestive tract in 2 animals in the winter group. The winter moose had higher NDF than summer moose, indicating the seasonal change in diet quality. *In vitro* organic matter digestibility (IVOMD) was not different between the summer and winter diets. The effect of birch phenols on IVOMD was concentration-dependent; differences between seasons were apparent at only the highest concentration. The 2 groups had marked differences in digestive content of major nutrients, NDF, and ability to digest forage which were consistent with typical variation in seasonal diet quality.

ALCES VOL. 48: 7-15 (2012)

Key words: *Alces alces*, digestion, fiber, gastrointestinal tract, plant phenol, rumen function.

The digestive tract is a dynamic system that changes throughout the year as the diet varies with seasonal change in forage availability (Weckerly 1989, Cork and Foley 1991, Pehrson et al. 1997, Hume 1999). Diet selection of browsing herbivores is complex because diet composition among individuals can be substantial and diets vary both seasonally and geographically (Palo and Wallin 1996). Seasonal variation in the capacity of rumen microorganisms to digest fiber and handle secondary compounds is also expected (Palo et al. 1985, Pehrson and Faber 1994).

Digestibility is critical in evaluating food utilization by herbivores because it accounts for both passage time of food in the digestive tract and nutritional benefit from forage (Clauss et al. 2007). In ruminants, breakdown of ingested plants is facilitated by mastication and microbial degradation that act as sequential processes that reduce particle size enabling passage from the rumen (Duncan and Poppi 2008). Thus, the rate of and maximal digestibility may be limited by plant metabolites that depress microbial growth, hence reduce substrate extraction from food. Further, tannins in the diet may reduce maximal protein digestibility by reducing absorption in the lower digestive tract and eventual loss in faeces (Robbins et al. 1987).

A critical factor affecting food intake rate and digestibility is neutral detergent fiber (NDF) that can reduce intake as its content increases in forage (Meyer et al. 2010). Phenols in plants may depress digestibility of NDF and organic matter thereby slowing fermentation and supply of nitrogen for microbial growth (Lundberg and Palo 1993, Duncan and Poppi 2008, Meyer et al. 2010).

Moose are commonly classified as a concentrate selector (Hoffman 1989, Illius and Gordon 1991, Robbins et al. 1995). Year-round they consume birch (*Betula* spp.) and willow (*Salix* spp.) as staple foods in northern Sweden, but also consume ~40 other plant species (Palo and Wallin 1996). Scots pine (*Pinus sylvestris*) in winter and bilberry (*Vaccinium myrtillus*) in autumn are considered important foods (Cederlund et al. 1980, Palo and Wallin 1996). The *in vitro* digestibility of these plant species varies seasonally due to changes in chemical composition (Palo et al. 1985, Pehrson and Faber 1994, Stolter 2008). For example, birch species vary in phenolic concentrations by altitude, individual trees, tissues within trees, and tree height (Palo et al. 1992, Santamour and Lundgren 1996, Hodar and Palo 1997, Rousi et al. 1997, Nordengren et al. 2003). Similar seasonal patterns but with other chemical components are found in Scots pine, willow, alder (*Alnus* spp.), and aspen (*Populus* spp.) (Palo 1984, Bryant et al. 1987, Sunnerheim and Hämäläinen 1992). In birch, a majority of the phenol compounds are composed of glycosides with fairly low molecular weight as compared to tannins (Santamour and Lundgren 1996, Sunnerheim et al. 1988). Platyphylloside is the predominant phenol compound in winter twigs of silver birch (*B.pendula*), and it inhibits *in vitro* and *in vivo* digestibility in moose, goats (*Capra capra*), rabbits (*Oryctolagus cuniculus*), and hares (*Lepus* spp.) (Palo 1985, 1987, Sunnerheim et al. 1988, Iason and Palo 1991, Palo et al. 1997, Bratt and Sunnerheim 1999).

Plant species composition in the rumen of moose is not a good predictor of digestibility variation among individuals (Pehrson and Faber 1994). Changes in the species composition of rumen microorganisms by season and food type, and their digestive capacity may also be critical factors in food utilization and digestibility. Changes in fiber, nitrogen, and phenols are the components that most drastically change with season and between plant species (Palo et al. 1985, Risenhoover 1989). Thus, understanding their changes in the digestive process is important to further interpret the digestive process and nutritional benefits of forage.

The hypothesis of this investigation was that moose show diminishing nitrogen (N) concentrations from rumen to rectum, and conversely, higher proportion of NDF towards the rear of the digestive tract due to digestion and absorption in the intestine. Further, it could be expected that the level of N in the rumen is decisive for the ability of rumen microorganisms to digest food and metabolize plant phenols. We expected that moose in winter have higher NDF and lower N concentrations throughout the digestive tract, and less ability to handle phenols in the diet as compared with other seasons.

MATERIALS AND METHODS

We studied 6 free-ranging moose shot in April-November at Grimsö Wildlife Research Station in central Sweden; live body weight ranged from 115-189 kg and ages were estimated at 6-13 months. Strictly regulated hunting outside of the November hunting season precluded a larger sample size and limited us to a general trend analysis. The functional segments of the digestive tracts were separated and their content was isolated by binding with a thread (Fig. 1). Gut material was sampled/collected in triplicate from the isolated parts of the digestive tract (Staaland et al. 1992, Pehrson et al. 1997); material was not present (collected) from each compartment from all animals.

The intestinal samples were dried at 70° C for 48 h and stored until later analysis for phenols, N, and fiber. For phenol analyses, the dry content was eluted with EtOH, cleaned through a Sepac C18 cartridge (Merck Inc.), and the remaining EtOH phase was dried in an evaporator and dissolved in distilled water. The dilution of extracts for the analysis was 660x from the crude extract. This procedure optimizes the analysis of total water soluble phenols by the Folin-Ciocalteau method (Palo 1985, Stolter 2008). This method measures the concentrations of low molecular phenol aglycones and quantifies the amount as with more advanced chemical methods (Sunnerheim et al. 1988, Hodar and Palo 1997, Bratt and Sunnerheim 1999). The Folin-Ciocalteu reaction was measured in a spectrophotometer after reaction for 2 h at 740 nm and at room temperature as described by Palo et al. (1985). The EtOH extract of content from different digestive compartments was analysed for the presence of the compounds platyphyllone and platyphyllane by Thin Layer Chromatography (TLC) (Merck Silica gel HF-254). These compounds are major phenol metabolites of silver birch twigs in the winter and have been found in the rumen of moose (Palo 1987, Sunnerheim et al. 1988, Sunnerheim and Bratt 2004). The extract from the gut content was run on TLC using the solvent chloroform:methanol:water (80:15:1 v/v). Phenols were detected by spraying the TLC plates with diazotized sulphanilic acid (Sigma-Aldrich) in 10% Sodium carbonate (w/v), followed by 50% sulphuric acid (v/v). Platyphyllone has a Rf = 0.53 and platyphyllane a Rf = 0.68 on TLC with the solvent used.

For NDF measurements, 0.5 g of dried gut content from 5 animals was put in glass filter tubes with a pore size of 0.2 mm and incubated with NDF solution according to Van Soest and Wine (1967). After incubation with solvents, the tubes were dried at 105° C, weighed, and the material combusted at 600° C. After cooling, the tubes were weighed again to calculate ash weight; NDF is expressed as the ash free weight. From one animal, only colon and rectum samples were analysed. The gut content was analysed for total N according to the Kjeldahl method using a CN- analyser.

In vitro dry matter digestibility (IVOMD) was measured with fresh rumen content from the collected moose. Immediately after killing, rumen contents were removed and transferred to thermos flasks and saturated with CO_2. Within 1 h of sampling, the content was filtered through a cloth and mixed with a McDoughal buffer stock solution 1:50 (Palo 1985). For IVOMD measurements, 0.5 g of dried and milled timothy (*Phleum pratense*) was put in glass filter tubes with a pore size of 0.2 mm. The EtOH extract from birch (as described above) was added at concentrations 1, 3, and 6 times that naturally found in 2-6 mm birch twigs in winter. The tubes were dried at 70° C over night, filled with stock buf-

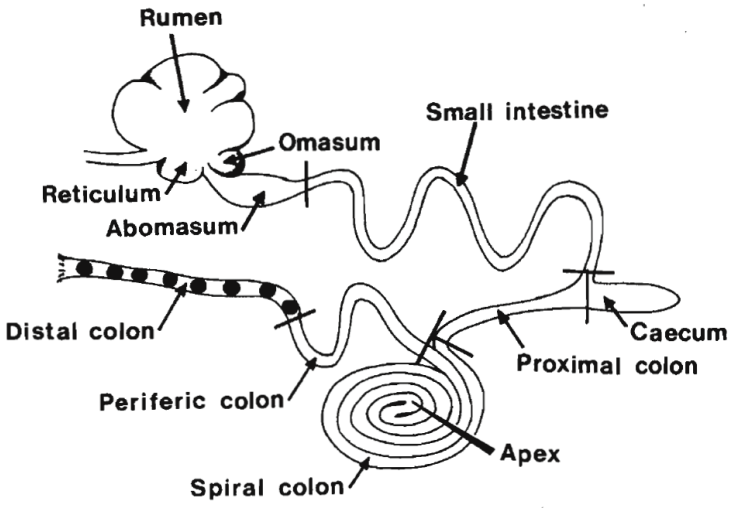

Fig. 1. Demarcation of the functional compartments of the moose digestive tract used in this study (from Pehrson et al. 1997).

fer solution, saturated with CO_2, and incubated for different times up to 96 h in a water bath at 37° C. The tubes were shaken twice daily during the incubation. After digestion, the tubes were washed with distilled water and rinsed with acetone. They were then dried at 105°C, weighed, and the material combusted at 600°C; after cooling the tubes were reweighed to obtain the ash weight. The organic matter disappearance was compared to those of a control that consisted of timothy treated with pure ethanol and dried. The IVOMD was calculated as:

$$(IVOMD_{control} - IVOMD_{sample}) / IVOMD_{control}$$

ANOVA and Student *t*-statistics was used for analysis of the data.

RESULTS

Based on the mean N concentrations throughout the digestive tract, 2 distinct groups of moose were evident; a summer group with high N concentration ($\bar{x}$ = 4.55, SD = 1.55, n = 3) and a winter group with low N concentration ($\bar{x}$ = 2.65, SD = 2.3, n = 3) (student *t*-test, *P* = 0.038, df = 19). The summer group consisted of moose shot in June and early November, and the winter group had moose shot in late November and April. The mean concentration of N in the individual compartments of the digestive tract was somewhat stable at ~4 mg/g in the summer group and ~2 mg/g in the winter group, with the exception of the highest levels at ~9 mg/g in the duodenum in both seasons (Fig. 2). These differences occurred in all parts of the digestive tract except the duodenum (Fig. 2).

The phenol concentration varied among compartments but no difference was found between the groups (Spearman rank r = 0.71, *P* <0.1, df = 4; Fig. 3). Platyphyllane (from platyphylloside found in silver birch twigs) occurred throughout the digestive tract of 2 winter moose only; these animals had similar amounts of total phenols as those without

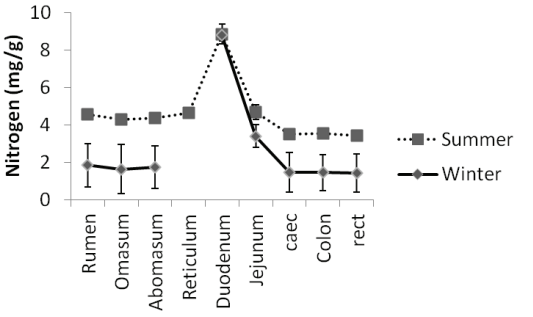

Fig. 2. Nitrogen (N) concentration (mg/g) of gut contents in compartments of the moose digestive tract by season in Sweden (x and SD).

platyphyllane. Dry matter content was high in the omasum, declined to the jejunum, and then increased to the rectum (Fig. 4), indicating major water absorption in the omasum and lower digestive tract. Dry matter in the rectum ranged from 15-30% among individuals (Fig.5), and dry matter and N concentration were inversely related between the summer and winter groups.

The % NDF varied (5-60%) throughout the digestive tract with similar seasonal patterns; concentration was highest in the front and rear compartments and lowest in the duodenum (Fig. 5). However, NDF was lower in summer (~40%) than in winter (~60%) (Fig. 5). Therefore, the summer group was characterized by high N concentration and low dry matter and NDF; the opposite described the winter group.

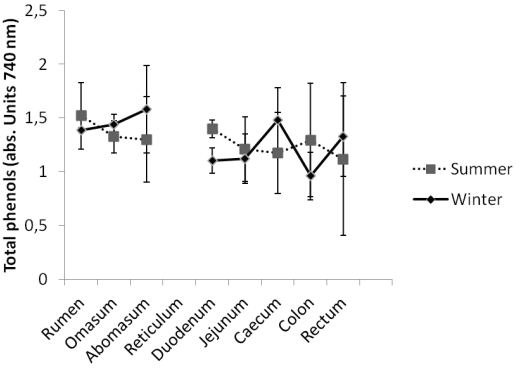

Fig. 3. Concentration of total phenols of gut contents in compartments of the moose digestive tract by season in Sweden; absolute units (740 nm) ($\bar{x}$ and SD).

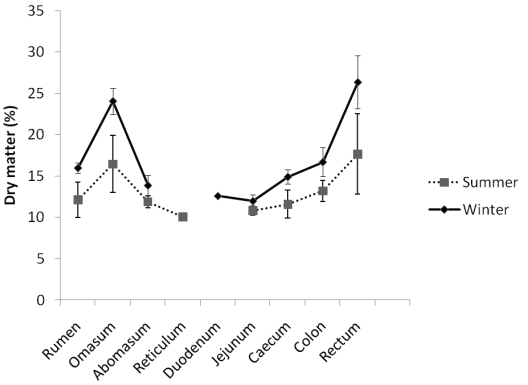

Fig. 4. Dry matter content (%) of gut contents in compartments of the moose digestive tract by season in Sweden (x and SD).

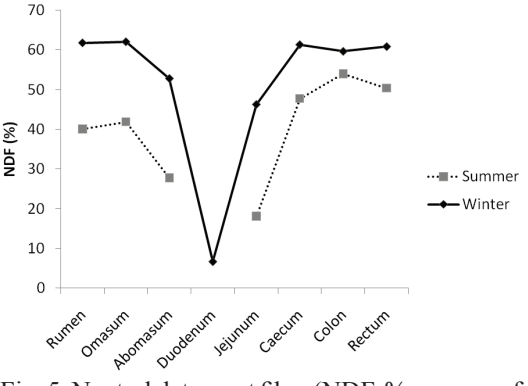

Fig. 5. Neutral detergent fiber (NDF, % average of 2 animals) of gut contents in compartments of the moose digestive tract by season in Sweden.

Digestibility of birch twigs did not differ between seasons (winter (low N) $\bar{x}$ = 23.49%, SD = 7.16; summer (high N) $\bar{x}$ = 23.78%, SD = 0.97), nor did digestibility of hay. Addition of phenols reduced IVOMD for both birch twigs and hay, but differences between seasons were only apparent at the highest concentration (F = 134.4, P <0.001, df = 2; Fig. 6).

DISCUSSION

We have shown that the concentrations of N, NDF, phenols, and dry matter vary within the compartments of the digestive tract and with season. Moose collected in winter, versus summer, generally had lower concentrations of N and higher concentrations of NDF and dry matter. Further, IVOMD was reduced by the concentration of phenols in the diet, but the effect was independent of season except at the highest concentration of phenols. These results corroborate with previous research on moose indicating that rumen content varies seasonally in dry matter content, plant species composition, and digestibility (Cederlund and Nyström 1981, Schwartz et al. 1984, Palo and Wallin 1996, Pehrson et al. 1997). In particular, NDF and digestibility reducing compounds (e.g., tannins) influence digestion and passage rate, hence rate of food intake (Robbins et al. 1987, Clauss et al. 2007, Spalinger et al. 2010).

The concentration of N was highest in the duodenum reflecting excretion of endogenous enzymes such as cellulase and nuclease, and was consistent with digestive studies indicating that N compounds are rapidly absorbed in the jejunum (Leng and Nolan 1984). Overall, N concentration in rumen content and faeces reflects the dietary intake of N and supports the use of faecal output as an indicator of range quality (Renecker and Hudson 1985, Staaland et al. 1992, Massey et al. 1994, Leslie et al. 2008, Palo and Olsson 2009). However, moose collected in November were represented in both the high and low N groups, an apparent contradiction. One possible explanation is that moose are in a seasonal transition of diet

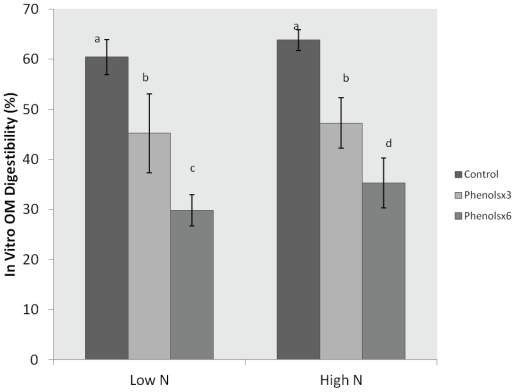

Fig. 6. In vitro dry matter digestibility (IVOMD) of hay with summer and winter moose rumen inocula; birch phenols were added at 1-6 X that occurring naturally in winter birch twigs (x and SD). Different letters denote significant differences among groups (P <0.05).

which could vary individually and locally in November.

Plant secondary compounds greatly influence food selection and nutritional value of plants because they reduce digestibility or impose a cost for detoxification (Palo 1985, Robbins et al. 1987, Iason and Palo 1991, Sunnerheim and Bratt 2004). Moose have few forage options in northern areas during winter and often consume plants low in palatability, high in secondary metabolites, and of low nutritional value. For example, birch is considered of low quality due to low IVOMD and high phenol concentration (Palo et al. 1992, Rousi et al. 1997). We found that silver birch twigs were in the winter diet because of the presence of platyphyllane that is an inhibitory substance only present in winter twigs of silver birch (Palo et al. 1992). Because birch could be consumed in other seasons, it is only applicable as a chemical marker in winter.

Total phenolic glycosides and free phenols varied in the compartments of the digestive tract, but faecal analysis revealed 2 groups with high and low phenol content. The high phenol group included moose collected in April and probably reflects high phenol intake associated with consumption of birch, however, this group also included one animal collected in June. It is possible that the method used to measure total phenols is compromised; for example, if an animal is excreting excess free aromatic amino acids such as tyrosine and tryptophane, the Folin-Ciocalteau method might overestimate phenol content (Folin and Ciocalteau 1927). No analysis of amino acid composition was performed to control for this possibility. Another explanation is that tannins are more common in the diet in June and would bind to salivary or dietary proteins that would be excreted in faeces as indicated by higher phenol concentrations in faeces (Hagerman and Robbins 1993). Diet composition of moose varies more in summer than winter which is reflected in the phenol concentration patterns, and diet composition may also vary widely within season and locally (Pehrson and Faber 1994, Palo and Wallin 1996).

The analysis of NDF was done on only 5 animals, but 2 distinct groups of high (winter) and low NDF concentration (June and early November) was apparent. Presumably, low sample size accounted for the lack of statistical difference between the groups. The range of NDF contents (30-60%) indicates that intake of fibrous foods is high during all seasons and individuals; these values were similar to those measured in feeding trials with several domestic and captive wild ruminants including moose (Palo et al. 1985, Renecker and Hudson 1985, Lechner et al. 2010, Meyer et al. 2010). High NDF impairs food intake in most herbivores, yet selective feeding on smaller diameter, more digestible twigs is likely an important behaviour to relax this constraint (Vivås et al. 1991, Palo et al. 1992, Hodar and Palo 1997). Based on the equation of Van Soest (1994) as modified by Meyer et al. (2010) for the relationship between dry matter intake and NDF in cervids, the estimated daily food intake is in the range 52-62 g DM $Kg^{-0.75}d^{-1}$. Since the animals in this study had rumen fill that corresponded to 79 g DM $Kg^{-0.75}$, it could be argued that food intake was in the range of 66-78% of rumen fill, with higher intake in summer (Pehrson et al. 1997). Since Pehrson et al. (1997) found no seasonal difference in the wet weight of gut content in moose, NDF would appear to be the single most important factor affecting dry matter intake by moose (Meyer et al. 2010).

ACKNOWLEDGEMENTS

This paper is in honour of my co-authors and research friends; Professor emeritus Peter A Jordan on his 80-years anniversary in 2010, Dr. Åke Pehrson who is retiring in 2011, and in memory of Professor Hans Staaland. We greatly appreciated the service from the hunting team at Grimsö Research Station and permit to hunt moose outside the legal season. Ms. Emilia Fuchs assisted in the laboratory

at The Swedish Agricultural University at Uppsala. Comments by Dr. M. Clauss, S. Öhmark, and 2 anonymous reviewers on an earlier draft of this manuscript were greatly appreciated.

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