In vitro dry matter disappearance using roe deer inocula from summer and winter

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Abstract: In vitro dry matter disappearance (IVDMD) of 12 forages were determined using ruminal inocula from 10 European roe deer (*Capreolus capreolus* L.) collected in summer and winter. There was significant difference in the ability of winter and summer inocula to digest winter and summer forages respectively. Each of the 6 summer forages had a significantly higher IVDMD in ruminal inocula of animals collected in summer versus winter. However, no significant difference in IVDMD of winter versus summer inocula was observed for each of the 6 winter forages. These results suggest adaption, although limited, by ruminal microorganisms in roe deer to winter forages or a potential problem in standard *in vitro* laboratory procedures when using animals on a high-fiber diet as inocula donors.

Key words: European roe deer, *Capreolus capreolus, in vitro* dry matter disappearance, rumen inocula

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Introduction

The European roe deer has been classified as a concentrate selector (Hofmann *et al.* 1976) dependent upon consumption of high quality forage to meet nutritional requirements. When snow depth exceeds 0.5 m, roe deer are apparently forced to browse twigs and buds from deciduous and evergreen trees (Cederlund *et al.* 1980) low in digestibility.

Studies with domestic ruminants have shown that the composition of rumen microbial populations adapt to dietary changes which enables fermentation of the major components of the diet (Hungate 1966). Rumen microbial adaptations to seasonal dietary changes have been suggested as an important nutritional strategy for wild ruminants facing a seasonal environment (Felber 1968; Thomas and Kroeger 1980; Trudell *et al.* 1980; Cederlund and Nystrøm 1981; Boomker 1984). This study tests the hypothesis that the ruminal microbial population of European roe deer adapts to seasonal dietary changes. This was accomplished by assessing the ability of summer and winter collected ruminal inocula to digest summer versus winter forages. Summer forages, low in fiber, were expected to be more completely digested by ruminal inocula collected during summer than by ruminal inocula collected during winter. Winter forages, high in fiber were expected to be digested more completely by winter collected inocula than by summer collected inocula.

Study area

The study was conducted in southeastern Norway, in the vicinity of the Agricultural University of Norway. The area was within the boreonemorale zone (Abrahamsen *et al.* 1977). Long-term mean temperature in January and July were -5.2 and 16.8°C respectively and average snow depth in February was 35 cm, with a range of approximately 0 to 110 cm (Heldal 1975).

Methods

In vitro dry matter disapperance (IVDMD) of 12 typical forages of roe deer were determined using inocula from all portions of the rumen of 5 roe deer collected in June 1987 and 1988 and 5 animals collected in February 1987. One *in vitro* trial was run immediately after each collection. Two of the animals collected in summer were road-kills.

Samples of typical summer forages of roe deer in Fennoscandia (Huseby 1976; Cederlund *et al.* 1980) consisted of leaves from 4 herbs and 2 deciduous trees, and typical winter forages (Hagen 1958; Cederlund *et al.* 1980) consisted of green shoots from 1 dwarf shrub, needles from 2 evergreen trees and current-year growth of stems (<2 mm in diameter) from 3 deciduous trees. Forages were collected in June 1986 and February 1987.

Plant samples were dried at 50°C and ground to pass through a 1 mm mesh sieve. Representative samples of whole rumen contents were taken, freeze-dried to a constant weight and ground. Forages and rumen samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest and Wine 1967).

A two stage *in vitro* dry matter disappearance (IVDMD) technique (Tilley and Terry 1963) was used. Immediately after being sacrified or reported as road-killed (within 1 hour after estimated death) ruminal fluid from an animal was strained through 4 layers of cheesecloth and added to preheated buffer solution (McDougall 1948). Inocula was maintained at 39°C and flushed with CO_2 for 15 minutes. Duplicates of all plant materials, three blanks and 3 different standards in triplicates were incubated in each run. Mean *in vivo* dry matter digestibility of standards by sheep were 80.5 %, 73.4 % and 68.5 % (unpublished data).

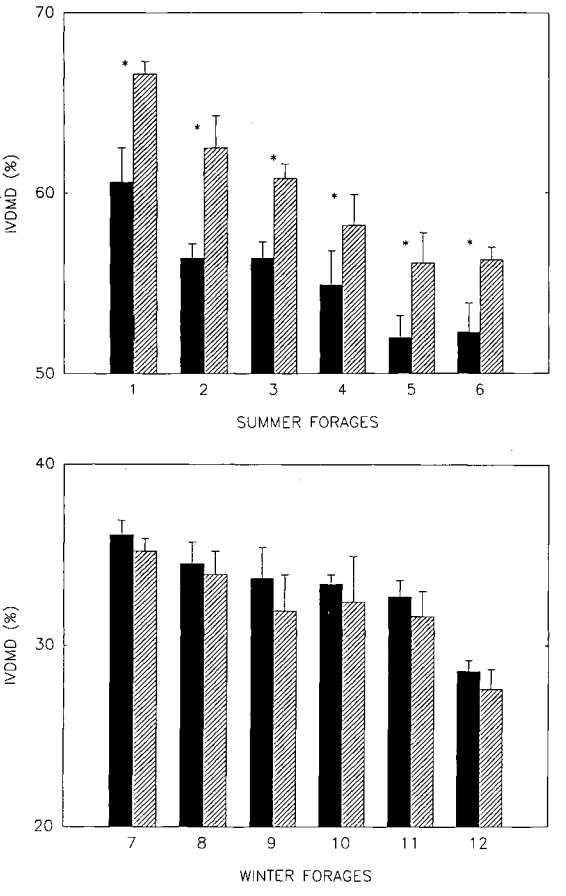

Fig. 1. Mean *in vitro* dry matter disappearance (IVDMD ± SD) of 6 summer and 6 winter forages using inocula of roe deer collected in February (filled bars) and in June and August (hatched bars). Asterisks indicate significant seasonal difference for each forage.

Numbers of forages refer to summer forages: 1: *Oxalis acetosella*, 2: *Filipendula ulmaria*, 3: *Chamaenerion angustifolium*, 4: *Sorbus aucuparia*, 5: *Salix caprea*, 6: *Rubus idaeus* and winter forages, 7: *Vaccinium myrtillus*, 8: *Pinus sylvestris*, 9: *Salix caprea*, 10: *Sorbus aucuparia*, 11: *Picea abies* and 12. *Betula pubescens*.

Deer were grouped in two distinct groups according to time of collection and were a representative subsample of the population. Paired-sample t-test (Zar 1984) was performed to test average difference in IVDMD between the two groups of animals, for summer forages and winter forages. In addition Student's t-test (Zar 1984) was performed to test differences in mean IVDMD between the two groups of animals, for each of the 12 forages separately. Student's t-test was also carried out to test differences in

chemical characteristics (i.e., hemicellulose, cellulose and lignin) of rumen contents sampled in summer and winter and of summer and winter forages. Significance levels were set to $P<0.05$ for all tests.

Results and discussion

The summer forages ranged between 50–70 % IVDMD, and winter forages ranged between 30–40 % (Fig. 1). Seasonality in forage quality was reflected in the significantly higher cellulose and lignin content of winter forages than summer forages (Table 1) and significantly greater cellulose and lignin content of rumen content from winter compared to summer collected animals (Table 2). The hypothesis of microbial adaptation to dietary changes in roe deer therefore may be tested by assessing the ability of summer and winter collected inocula to digest summer versus winter forages.

Seasonal adaptations of rumen microorganisms resulted in positive effects on digestibility of forages both in summer and winter. There was significant difference in the ability of winter and summer inocula to digest winter and summer forages respectively. The IVDMD of each of the 6 summer forages was significantly higher with ruminal inocula from deer collected in summer versus winter (Fig. 1). However, no significant difference in IVDMD of winter versus summer inocula was observed for each of the 6 winter forages (Fig. 1).

Table 1. Mean composition of cell wall fraction (% of dry matter) (±SD) of summer (n=6) and winter (n=6) forages used as substrate for *in vitro* trials.

	Winter forages	Summer forages	
	------- % of dry matter ------		
Hemicellulose	12.2±1.4	16.8±2.9	*
Cellulose	19.5±3.0	11.5±1.4	*
Acid deter. lignin	20.6±2.0	11.4±2.1	*

* Seasonal significant difference ($P<0.05$).

Table 2. Mean composition of cell wall fraction (% of dry matter) of rumen samples (±SD) of roe deer used as donors of inocula.

	Winter (n=5)	Summer (n=5)	
	------- % of dry matter ------		
Hemicellulose	16.8±2.3	12.8±5.8	NS
Cellulose	20.1±3.2	12.5±4.3	*
Acid deter. lignin	16.9±1.6	7.3±2.61	*

* Seasonal significant difference ($P<0.05$).

Faster fermentation rates in summer than in winter have been reported in various northern and temperate cervids (e.g., Felber 1968; Short 1971; Langer 1974). These differences were most pronounced for highly digestible forages and was probably an effect of higher quality forages and higher food intake in summer which can support a larger microbial population. However, different forages may approach the asymptotic maximum *in vitro* digestibility at different times (e.g., Troelsen and Hanel 1966; Milchunas *et al.* 1978; Trudell *et al.* 1980), and a given forage may approach the same end point in different ways depending on the microbial environment (Tilley and Terry 1963, Troelsen and Hanel 1966, Trudell *et al.* 1980). Normally, 48 hours after incubation the *in vitro* fermentation process is complete (Tilley and Terry 1963; Milchunas *et al.* 1978). End-point digestibility does not measure rate of digestion, but rate of digestion is related to *in vivo* rate of passage. Total mean retention time for roe deer fed a high quality diet was about 20 hours versus 30 hours when fed a low quality winter diet high in fiber (Holand unpublished data). Further, there are time lags observed for bacterial populations to adapt to a switch in substrate quality (Van Soest 1982). Both rate of digestion and lag times in microbial population adaptation are factors not accounted for in end-point *in vitro* digestion. Therefore, my estimates of the ability of roe deer to adapt to seasonal dietary changes is probably conservative.

The pool size of cellulolytic organisms may limit the initial breakdown of diets rich in fiber (Van Soest 1982). Roe deer have a sparse ruminal population of cellulolytic bacteria compared to larger cervids (*Cervus elaphus* and *Dama dama*) (Brüggeman *et al.* 1967; Prins and Geelen 1971). Based on *in vitro* techniques, Prins and Geelen (1971) record that cellulose fermentation by European roe deer is low, in fact the lowest *in vitro* cellulose disapperance rate out of 11 ruminants (Prins *et al.* 1984). In contrast, Dissen

and Hartfiel's (1985) *in vivo* studies indicate a relatively high digestibility of crude fiber by roe deer, however the forages tested were low in crude fiber compared to a typical winter browse diet. The more likely results are by Drozdz (1979) who found an *in vivo* digestibility coefficient of natural winter food (30–40 % crude fiber) of 40 %; again indicating low cellulolytic activity. In both groups of studies proximate food analysis was used to assess fiber and this technique does not given a uniform composition of crude fiber (Robbins 1983) and therefore makes comparison difficult.

Variations in the rate of cellulolysis with the changes in diet are well documented (Prins *et al.* 1984). There are some indications that IVDMD of cellulose-rich plant material is highest during winter in European roe deer (Felber 1968; Cederlund and Nystrøm 1981). The higher IVDMD of winter forages in inocula collected during winter may indicate higher cellulolytic activity. However, no evidence of higher activity was found considering that for each of the 6 winter forages the cell soluble fraction was higher than its IVDMD. This makes a sound biological interpretation difficult, but may suggest some component of the soluble fraction is prevented from being digested, perhaps by plant compounds (White and Trudell 1980; Person *et al.* 1980).

Microbes that digest fiber adhere to the particulate phase of the rumen content (e.g., Akin and Amos 1975; Patterson *et al.* 1975). Greater amounts of fiber in the rumen contents collected in winter than in summer may have resulted in the discarding of a greater proportion of cellulolytic microorganisms from rumens collected in winter. This may have contributed to the small differences in IVDMD of winter forages using ruminal inocula collected in summer versus winter. Agitation of rumen contents from animals on high fiber diets may be necessary to dislodge cellulolytic microorganisms before filtering and use as *in vitro* inocula (Milchunas and Baker 1982).

The inoculum preparation stage of *in vitro* disappearance trials using wild ruminants as donors may influence disappearance coefficients, because of difficulties in maintaining the microbial activity of the ruminal fluid (Schwartz and Nagy 1972). Prins and Geelen (1971) found that storing rumen fluid up to 2 hours did not significantly change cellulolytic or amylolytic activity. However, data from Milchunas and Baker (1982) indicated that if inoculum temperatures dropped to 29°C or if incubation was delayed for 2 hours fermentation was significantly reduced. In this study, incubations were started within 1.5 hours after the animals were sacrified or estimated road-killed, to maintain viable inocula. Fermentation was evideneed by sustained gas production.

Although a conclusion possibly limited due to laboratory technique, my results based upon standard IVDMD techniques does not conclusively support the contention that a high degree of rumen microbial adaptation to diet is an important mechanism by which roe deer improve their survival in harsh winter conditions.

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References

Abrahamsen, J., Jakobsen, N. K., Dahl, E., Kalliola, R., Wilborg, L. and **Påhlson, L.** 1977. Naturgeografisk region-inndeling av Norden. – *Nordiske utredninger B 1977:* 34, Stockholm.

Akin, D. E. and **Amos, H. E.** 1975. Rumen bacterial degradation of forage cell wall investigated by electron microscopy. – *Appl. Microbiol.* 29: 692–701.

Boomker, E. A. 1984. Seasonal variation of in vitro digestibility in the kudu, *Tragelaphus strepsiceros.* – *Can. J. Anim. Sci. 64 (Suppl):* 208–209.

Brüggeman, J., Giesecke, D. and **Walser-Kärst, K.** 1967. Verfahren zur Untersuchung mikrobieller Leitungen im Panseninhalt wildelebender Wiederkauer post mortem. – *In: XIth Int. Congr. of Game Biologist, Stockholm,* 525–532.

Cederlund, G., Ljunguist, H., Markgren, G. and **Stalfelt, F.** 1980. Foods of moose and roe deer at Grimsø in Central Sweden; results of rumen content analyses. – *Swed. Wildl. Res.* 11(4): 169–247.

Cederlund, G. and **Nystrøm, A.** 1981. Seasonal differences between moose and roe deer in ability to digest browse. – *Holartic Ecology* 4:59–65.

Dissen, J. and **Hartfiel, W.** 1985. Beobachtungen zum Äsungsver halten sowie Untersuchungen zum Nährstoffverdaulichkeit von Rehwild. – *Z. Jagdwiss.* 31: 83–91.

Drozdz, A. 1979. Seasonal Intake and Digestibility of Natural Foods by Roe-deer. - *Acta Theriol.* 24: 137-170.

Felber, L. 1968. *Studien in vitro über die Aktivität der Mikro-organismen aus dem Pansen von Rothirsh (Cervus elaphus) und Reh (Capreolus capreolus).* - Inaugural-Dissertation der Tierärztlichen Fakultät der Ludwig-Maximillians Universitat, München.

Hagen, Y. 1958. Vinterernæringen hos rådyr og elg. - *Jeger og Fisker* 10: 563-464.

Heldal, B. 1975. Jorda i Ås.p. 30-44. - *In:* Semb, G. (ed.). *Klimaet i Ås.* Landbrusforlaget, Oslo.

Hofmann, R. R. 1982. Vergleich der einzelnen Wildwiederkauer. - *Deutsche Jagd-Zeitung* 2: 4-5.

Hofmann, R. R., Geiger, G. and **Kønig, R.** 1976. Vergleichend-anatomische Untersuchungen and der vormagenschleimhaut von Rehwild (Capreolus capreolus) und Rotwild (Cervus elaphus). - *Z. Saugertierk.* 41: 167-193.

Hungate, R. E. 1966. *The rumen and its microbes.* Acad. Press Inc., New York.

Huseby, K. 1976. *Rådyrets ernæring.* - Thesis Agric. Univ. of Norway.

Langer, P. 1974. Stomach evolution in the Artiodactyla. - *Mammalia* 38: 295.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. - *Biochem. J.* 43: 99-109.

Milchunas, D. G., Dyer, M. Y., Wallmo, O. C. and **Johnson, D. E.** 1978. In-vivo/in-vitro relationships of Colorado mule deer forages. - *Colorado Division of Wildlife. Special Report 43.*

Milchunas, D. G. and **Baker, D. L.** 1982. In Vitro Digestion Sources of Within - and Between - Trial Variability. - *J. Range Manage.* 35: 199-203.

Patterson, H., Irving, R., Costerton, J. W. and **Cheng, K. J.** 1975. Ultrastructure and adhesion properties of Ruminococcus albus. - *J. Bacteriol.* 12: 278-287.

Person, S. J., Pegau, R. E., White, R. G. and **Luick, J. R.** 1980. In vitro and nylon bag digestibilities of reindeer and caribou forages. - *J. Wildl. Manage* 44: 613-622.

Prins, R. A. and **Geelen, M. J. H.** 1971. Rumen characteristics of red deer, fallow deer and roe deer. - *J. Wildl. Manage.* 36: 1341-1343.

Prins, R. A., Lankhorst, A. and **Van Hoven, W.** 1984. Gastrointestinal fermentation in herbivores and the extent of plant cell-wall digestion. - *In: Proc. Int. Symp. Herbivore Nutrition in the Subtropic and Tropics, Pretoria.* Eds.: Gilchrist, F. M. C. and Mackie, R. I. The Science Press, Craighall, pp. 408-434.

Robbins, C. T. 1983. *Wildlife feeding and nutrition.* Acad. Press Inc., New York.

Schwartz, C. C. and **Nagy, J. G.** 1972. Maintaining deer rumen fluid for *in vitro* digestion studies. - *J. Wildl. Manage.* 36: 1341-1343.

Short, H. L. 1971. Forage digestibility and diet of deer on southern upland range. - *J. Wildl. Manage.* 35: 698-706.

Thomas, D. C. and **Kroeger, P.** 1980. In vitro digestibilities of plants in rumen fluids of Peary caribou. - *Arctic* 33: 757-767.

Tilley, J. M. A. and **Terry, R. A.** 1963. A two-stage technique for the *in vitro* digestion of forage crops. - *J. British Grassland Soc.* 18: 104-111.

Troelsen, J. E. and **Hanel, D. J.** 1966. Ruminant digestion *in vitro* as affected by inoculum donor, collection day and fermentation time. - *Can. J. Anim. Sci.* 46: 149-156.

Trudell, J., White, R. G., Jacobsen, E., Staaland, H., Ekern, K., Kildemo, K. and **Gaare, E.** Comparison of some factors affecting the *in vitro* digestibility estimate of reindeer forages. - *In: Proc. 2nd. Int. Reindeer/Caribou Symp., Røros, Norway.* Eds.: Reimers, E., Gaare, E. and Skjennerg, S. Direktoratet for vilt og fersvannfisk, Trondheim. pp. 262-273.

Van Soest, P. J. 1982. *Nutritional ecology of the ruminants.* O & B Books, Corvallis, Oregon.

Van Soest, P. J. and **Wine, R. H.** 1967. Use of detergents in the analysis of fibrous feed. IV. Determination of plant cell wall constituents. - *J. Assoc. Off. Anal. Chem.* 50: 50-55

White, R. G. and **Trudell, J.** 1980. Habitatpreference and forage consumption by reindeer and caribou near Atkasook, Alaska. - *Arct. Alp. Res.* 12 (4): 511-530.

Zar, J. H. 1984. *Biostatistical Analysis (2nd Ed.)* Prentice-Hall, Int. Inc., London.

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