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DIFFERENCES BETWEEN SHEEP AND RED DEER IN *IN VITRO* APPARENT AND TRUE DIGESTIBILITY OF COMMONLY USED RED DEER FEEDS

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Differences between sheep and red deer in in vitro apparent and true digestibility of commonly used red deer feeds

The nutritive value of red deer feeds is frequently determined by sheep despite the ultimate arbitrator of the nutritive value of any feed is the host animal. The objective of the trial was to determine the influence of rumen fluid donor (sheep *vs* red deer) on *in vitro* dry matter (DMD), neutral-detergent fibre (NDFD) and true digestibility (*iv*TD) of eleven substrata, naturally occurring in Slovenian forests (chestnut fruits, acorns of common and sessile oak, two fresh grasses) and those frequently used in supplemental red deer feeding (two grass hays and two grass silages, apple pomace and sugar beet roots). Only the fresh grass from Jelendol had greater ($p < 0.05$) DMD (646 *vs* 508 g/kg) when incubated in red deer inoculum. The NDFD and *iv*TD were always numerically greater when substrates were incubated in red deer inocula, however the NDFD and *iv*TD were significantly greater ($p < 0.05$) only when fresh grass from Jelendol (590 *vs* 343 g/kg and 801 *vs* 681 g/kg, respectively), grass silage from Kokra (541 *vs* 359 g/kg and 742 *vs* 639 g/kg, respectively) and apple pomace (428 *vs* 328 g/kg and 704 *vs* 653 g/kg, respectively) were incubated in the inoculum prepared from red deer rumen contents. These results indicate that rumen fluid from sheep can be used to predict *in vitro* digestibility in red deer and that these parameters can be used in the formulation of deer diets.

Key words: animal nutrition; red deer; sheep; rumen; feed evaluation; *in vitro* digestibility; supplementary feeding

Razlike med ovcami in navadnimi jeleni v in vitro navidezni in pravi razgradljivosti v prehrani navadnega jelena običajno uporabljene krme in krmil

Hranilno vrednost krme in krmil, namenjeno prehrani navadnega jelena, pogosto ocenjujemo s pomočjo ovc, čeprav je edini pokazatelj hranilne vrednosti katerega koli krmila lahko samo žival, ki ji je krmilo namenjeno. Namen pričujoče raziskave je bil ugotoviti, kako vrsta vampovega soka (ovce *vs* navadni jelen) vpliva na *in vitro* razgradljivost suhe snovi (DMD) in v nevtralnem detergentu netopne vlaknine (NDFD) ter na *in vitro* pravo prebavljivost suhe snovi (*iv*TD) enajstih krmil, ki jih imajo na razpolago navadni jeleni v slovenskih gozdovih (plodovi kostanja in gradna ter želod in dva vzorca sveže trave) in krmila, ki jih pogosto uporabljamo pri njihovem zimskem dokrmljevanju (po dva vzorca mrve in travne silaže, jabolčne tropine in koreni sladkorne pese). Sveža trava iz Jelendola je imela večjo ($p < 0,05$) DMD (646 *vs* 508 g/kg), če smo jo inkubirali v inokulumu, pripravljenem iz jelenovega vampovega soka. NDFD in *iv*TD sta bila vedno numerično večji ob inkubaciji krmil v inokulumu, pripravljenem iz vampovega soka navadnega jelena, vendar so bile vrednosti značilno večje ($p < 0,05$) le pri inkubaciji sveže trave iz Jelendola (NDFD: 590 *vs* 343 g/kg in *iv*TD: 801 *vs* 681 g/kg), travne silaže iz Kokre (NDFD: 541 *vs* 359 g/kg in *iv*TD: 428 *vs* 328 g/kg) in jabolčnih tropin (NDFD: 742 *vs* 639 g/kg in *iv*TD: 704 *vs* 653 g/kg) v inokulumu, pripravljenem iz vampovega soka navadnega jelena. Dobljeni rezultati so pokazali, da vampov sok ovc lahko uporabimo za določanje *in vitro* prebavljivosti krme in krmil, ki jih uporabljamo v prehrani navadnega jelena ter da lahko na ta način dobljeno hranilno vrednost uporabimo pri sestavljanju njihovih obrokov.

Ključne besede: prehrana živali; navadni jelen; ovce; vamp; hranilna vrednost; *in vitro* prebavljivost; dokrmljevanje

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1 INTRODUCTION

The Alpine region of Slovenia is characterised by large areas of mixed coniferous and deciduous forests with small areas of pastures available for red deer. This area is also characterised by wet and cold winters. Under such environmental conditions, the herbage availability for grazing deer is characterised by a low quantity and a poor quality of herbage during late autumn and during the winter. Such limitations together with large herds of red deer in some areas create a need for supplementary feeding as a common strategy to improve animal condition, trophy quality and reduction of winter mortality and is also accepted as an efficient biological method to reduce the bark browsing caused by red deer (Rajský *et al.*, 2008).

To obtain these goals it is essential to know the nutritive value of feed ingredients, especially for those feeds which are commonly used for the supplementary feeding of red deer and those who are commonly found in their environment. Currently, the nutritive value of feeds determined in sheep is used as guideline despite the fact that the ultimate arbitrator of the nutritive value of any feed is the host animal (Hervás *et al.*, 2004; Ru *et al.*, 2002). The use of domestic ruminants rather than deer greatly facilitates the *in vitro* digestion technique in that it eliminates problems associated with the deer as living rumen fluid donor or the necessity of sacrificing an animal (Palmer *et al.*, 1976).

Most approaches to feed characterisation are intended to meet the needs of rationing systems and, because digestibility is the principal cause of variation of the metabolizable energy content, the energy value of feeds is commonly predicted from *in vitro* estimates of digestibility. With the increased use of *in vitro* techniques to evaluate ruminant feeds, it is of great importance to identify whether the species from which the rumen fluid inoculum is prepared has a significant influence on the obtained results.

This trial was carried out to determine the influence of rumen fluid donor (sheep *vs* red deer) on *in vitro* digestibility of several substrates naturally occurring in Slovenian forests and those frequently used in supplemental red deer feeding.

2 MATERIAL IN METHODS

Eleven feeds were used as substrates. In the areas of north Slovenia, Jelendol (community of Tržič) and Kokra (community of Preddvor), which are on average 767 and 940 m above the sea level, respectively, we collected samples of two fresh grasses, horse chestnut fruits (*Aesculus hippocastanum*) and acorns of common (*Quercus robur*) and sessile (*Quercus petraea*) oak. Other feeds (grass hays, grass silages, fresh sugar beets roots, apple pomace) were purchased and served as winter supplemental feeds for red deer. Samples of dried feeds were analysed for crude protein (CP), ash, ether extract (EE), crude fibre (CF) according to Neumann and Bassler (1986) and for neutral detergent fibre according to Goering and Van Soest (1970) using ANKOM^{220} Fibre Analyser (Ankom Technology, Macedon, NY). Their chemical composition is given in Table 1.

Sheep rumen fluid was derived from two mature castrated Jezersko Solčavska × Romanovska rams (*Ovis aries*) weighing on average 70 kg and fitted with permanent rumen cannula. They received a daily ration containing approximately 1.0 kg average quality hay *ad libitum* and 0.5 kg of commercial compound feed (180 g/kg CP), supplemented with mineral and vitamin mix (25 g) once a day in the morning after rumen fluid collection. The diet composition was calculated according to the German metabolizable energy and utilizable protein requirements (nXP; DLG, 1997) to cover the energy and protein requirements for maintenance and to balance the energy-to-protein ratio in the rumen. Four red deer (*Cervus elaphus*) hinds aged 2 to 4 years were shot in areas of Jelendol and Kokra in September and October 2011 during the deer-stalking season according to the hunting action plan. Hinds did not show any signs of illness and were in body condition typical for the time in the season. Immediately after the hinds were shot the entire rumen was removed from the animal, placed in a warm polystyrene box and transported into the laboratory in no more than 45 minutes. Sheep rumen fluid and deer rumen were collected on different days because of equipment limitations.

In vitro incubations were carried out by using ANKOM *in vitro* fermentation system Daisy^{II} (ANKOM Technology, Macedon, NY, USA). Inoculum from each rumen fluid donor was prepared according to Menke and Steingass (1988). In the laboratory red deer rumen fluid was obtained by squeezing the rumen contents manually through four layers of cheese-cloth, while sheep rumen fluid was strained through four layers of cheesecloth. Obtained rumen fluids were diluted into the reduced buffer medium in the proportion 1-to-2 (v/v). Approximately 450 mg of ground air-dry sample milled through 1 mm screen was weighed into ANKOM F57 filter bag (ANKOM Technology, Macedon, NY, USA), heat sealed and placed in incubation jars. Each substrate was weighed into four bags, which were placed into 2 jars (2 bags/jar). Each jar contained 24 bags (2 bags/substrate + two blanks). Two litres of buffered rumen fluid dispensed under CO_2 were poured into each jar, which was

then deposited into the rotating incubator at 39 °C for 24 h. At the completion of incubation, bags were rinsed thoroughly with cold tap water until the water was clear, dried and weighed to determine DM disappearance (DMD). Bags were then treated with boiling neutral detergent solution for 1 h using an ANKOM^{220} fibre analyser (ANKOM Technology, Macedon, NY, USA), washed with distilled water, dried and weighed to determine both neutral detergent fibre disappearance (NDFD) and *in vitro* true DM digestibility (*iv*TD). Incubations and treatments with sheep inoculum were performed in two repetitions. The time from collection to completion of the inoculation was less than 2 hours as recommended by Schwartz and Nagy (1972).

One-way analysis of variance using the general linear model (GLM) procedure of the statistical package SAS/STAT version 9.4 (SAS Institute Inc., 2015) was used to compare the differences between animal species (sheep and red deer) for each substrate. Data are presented as least square means. Statistical significance was declared at $p \leq 0.05$, whereas trends were discussed when $0.05 < p < 0.10$.

3 RESULTS AND DISCUSSION

Chemical composition (Table 1) varies greatly between used substrates especially between forages (fresh grasses, grass hays and grass silages) and other used substrates (apple pomace, sugar beet roots, chestnut fruits and oak acorns).

The values of DMD, NDFD and *iv*TD (Table 2) show the expected variation depending on the substrate incubated, however, they show also some significant differences related to the species of the rumen inoculum donor (deer *vs* sheep). The considerably greater variation could exist among digestibility values from inoculums obtained from sacrificed red deer hinds, which could be a consequence of large variability of natural diets, who range from highly digestible fruits and seeds to poorly digestible browse.

In vitro DM disappearance was higher ($p < 0.05$) only for fresh grass from Jelendol when incubated in the inoculum obtained from deer. Crawford and Hankinson (1984) and Gordon *et al.* (2002) also did not find any differences in *in vitro* DMD using the inoculums prepared from white-tailed deer and cattle and white-tailed deer and cattle, respectively. However, Hervás *et al.* (2004) found that *in vitro* DMD were higher ($p < 0.05$) in sheep than in red deer of five out of eight studied forages.

The Tilley and Terry (1963) two-stage method for estimation of DM digestibility was used by Palmer *et al.* (1976) and Ru *et al.* (2002) to compare DM digestibility using inoculum from white-tailed deer, red deer and cattle. While Palmer *et al.* (1976) did not establish any differences in DM digestibility by using white-tailed deer and cattle inoculum, Ru *et al.* (2002) found that the substrates were generally better ($p < 0.05$) digested in inoculum obtained from red deer. They claimed that if the samples are incubated under the same conditions and the same diet was fed to animals before the rumen fluid was obtained then the differences in rumen digestibility are influenced only from differences in bacterial activity. However, in the present trial, the *iv*TD should represent total tract digestibility similar to Tilley and Terry (1963) method. The *iv*TD were always numerically higher when feeds were incubated in red deer inoculum; however, the differences were significant ($p < 0.05$) only for fresh grass

Table 1: *Chemical composition of the feeds used for in vitro digestibility determination (g/kg DM)*

Feeds	DM	CP	EE	CF	Ash	NFE	NDF	NFC
Fresh grass (Jelendol)	202	203	29	197	64	506	440	171
Fresh grass (Kokra)	282	134	24	278	70	494	600	91
Grass silage (Jelendol)	387	128	31	340	97	404	640	42
Grass silage (Kokra)	586	194	28	237	107	434	517	72
Grass hay (Jelendol)	867	107	18	304	97	472	543	178
Grass hay (Kokra)	843	93	19	282	70	537	563	192
Apple pomace	153	58	28	218	27	669	482	338
Sugar beet roots (fresh)	202	68	5	61	27	840	141	695
Chestnut fruits	372	85	16	143	25	730	389	422
Sessile oak acorns	579	52	36	130	22	760	278	542
Common oak acorns	508	53	37	134	22	753	291	532

DM – dry matter; CP – crude protein; EE – ether extract; CF – crude fibre; Ash – crude ash; NFE – nitrogen-free extract (NFE = DM – (Ash + CP + EE + CF)); NDF – neutral detergent fibre, NFC – nonfibre carbohydrates (NFC = DM – (Ash + CP + EE + NDF))

Table 2: *In vitro dry matter (DMD; g/kg) and neutral-detergent fibre (NDFD; g/kg) disappearances and in vitro true dry matter digestibility (ivTD; g/kg) of different substrates incubated in the inoculum prepared from sheep and red deer rumen fluid*

	DMD			NDFD			*iv*TD		
Feeds	deer	sheep	RMSE	Deer	sheep	RMSE	deer	sheep	RMSE
Fresh grass (Jelendol)	646[a]	508[b]	75.0	590[a]	343[b]	95.1	801[a]	681[b]	46.1
Fresh grass (Kokra)	454	410	56.7	404	325	57.9	611	560	37.8
Grass silage (Jelendol)	491	400	67.4	423*	289*	76.9	606*	515*	52.5
Grass silage (Kokra)	600	474	117.1	541[a]	359[b]	71.6	742[a]	639[b]	40.3
Grass hay (Jelendol)	540	520	36.1	426	375	49.5	670	641	28.5
Grass hay (Kokra)	464	393	51.9	374*	257*	68.3	624*	554*	41.0
Apple pomace	497	464	30.8	428[a]	328[b]	40.6	704[a]	653[b]	21.0
Sugar beet roots (fresh)	934	897	30.7	737	576	117.3	960	936	17.6
Chestnut fruits	408	431	39.7	338	328	51.4	725	721	21.4
Sessile oak acorns	523	546	30.4	221	215	27.9	767	765	8.4
Common oak acorns	506	502	57.5	213	176	45.5	755	745	14.1

RMSE = residual mean square error; [a, b] = means with different superscripts within the parameter differ significantly ($p < 0.05$); * = means within the parameter show trends ($0.05 < p < 0.10$)

from Jelendol, grass silage from Kokra and apple pomace. There was a trend ($0.05 < p < 0.10$) that grass silage from Jelendol and grass hay from Kokra had higher *iv*TD when incubated in inoculum obtained from red deer. On the contrary, Hervás *et al.* (2004) found that *iv*TD were higher ($p < 0.05$) in sheep than in red deer of five out of eight studied forages, while *iv*TD of barley and wheat grains were lower ($p < 0.05$) in sheep than in red deer.

There was no possibility to control the red deer diets. They consumed feeds naturally occurring in their environment, while sheep consumed the balanced diet prepared from conserved feeds and yet only a few substrates show differences ($p < 0.05$) in the DM digestibility.

It was assumed that the diet nutrient composition did not differ substantially between red deer and sheep, because red deer hinds inhabit sites with higher-quality forages than males (Barbosa and Bowyer, 2000), because Adamič (1990; cited by Jerina, 2007) noted that grasses comprise between 50 and 70 % of red deer diet in the vegetative season and because in the fall of 2011 (at the time of study) oaks, common beech and chestnut cropped heavily in the areas of Jelendol and Kokra (D. Veternik, personal communication, January 25th, 2012). The later are rich sources of non-fibre carbohydrates (NFC, Table 1), mainly starch. Therefore, in this study, the red deer diet and sheep diet supplemented with compound feed had similar physical and chemical composition and thus should have also the comparable activity of rumen microorganisms in both studied species. These observations are supported also by the observation of Gordon *et al.* (2002) who reported that red deer rumen microorganisms adapted to high-quality diets digest these more efficiently than when low-quality diets are fed.

Similar to *iv*TD also the NDFD were always numerically higher when feeds were incubated in red deer inoculum (Table 2) and the differences were significant ($p < 0.05$) or tended to be significant ($0.05 < p < 0.10$) when comparing the two inoculums within the same feed. Hervás *et al.* (2004) also found that NDFD differed ($p < 0.05$) between inoculums for the same feed as did *iv*TD. The reason of identical statistical significancies is linked to the use of the neutral detergent solution in the determination of both, *iv*TD and NDFD.

The difficulty of generalizing the results on the digestion capacity of sheep and red deer imposed by the interaction between animal species and feed ingredients has been pointed out in the literature (Ru *et al.*, 2002). Comparisons of digestion by sheep and deer have resulted in differences in favour of the deer or sheep and sometimes even in no differences depending on the feed studied.

4 CONCLUSIONS

Small differences in digestibility parameters, especially in *in vitro* DM disappearance (DMD) between sheep and red deer suggest that sheep inoculum could be used to predict DMDs of naturally occurring red deer feeds and feeds used frequently in their supplementary feeding. However, *in vitro* NDF disappearance (NDFD) and *in vitro* true DM digestibility (*iv*TD) were always higher when feeds were incubated in red deer inoculum,

being significant ($p < 0.05$) or tended to be significant ($0.05 < p < 0.10$) for almost one-half of tested feeds. These differences are more prominent for forages than for high non-fibre carbohydrate (NFC) feeds, suggesting the higher fibrolytic activity of red deer rumen microorganisms, which could be a consequence of different microbial species inhabiting rumen of red deer (Henderson *et al.*, 2015). Further research on this subject is needed in which the comparisons between deer and sheep are performed where sheep are fed with diets of different composition.

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