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Research Paper

Variation of natural diet of free ranging mouflon affects their ruminal protozoa composition

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ABSTRACT

The mouflon's traditional classification as a grazer has recently been questioned, therefore new insight into the species' feeding and digestion is needed. The presence and share of different ciliate genera in the rumen may contribute to an understanding of a species' feeding physiology, therefore we analysed the composition of mouflon rumen ciliates and correlated their density with the percentage of different food types eaten by the animals. We found that the dominant ciliates were *Entodinium* spp., with a small proportion of *Anoplodinium denticulatum, Eudiplodinium maggii, Epidinium ecaudatum caudatum* and *Dasytricha ruminantium*. The main food types taken by the animals were deciduous tree leaves, grass and bramble. The density of ciliates from the *Entodinium* genus rose with an increase in deciduous leaves in the diet and fell with an increase of graminoids in the diet. The density of ciliates from the *Anoplodinium* and *Eudiplodinium* sub-genera increased with a rise in tree shoots and was reduced with a rise of deciduous leaves in the diet. These results confirm that the protozoal fauna vary with current food composition in intermediate feeders.

1. Introduction

According to the traditional classification of ruminants by feeding type, the mouflon is counted as a grazer (Hofmann, 1989; Clauss et al., 2011). However, recently it has been demonstrated in a meta-analysis that the mouflon can be considered an intermediate feeder (Marchand et al., 2013). Moreover a new concept has recently been introduced of classifying ungulates based on several criteria including their food's mechanical structure (Clauss et al., 2003) and rumen physiology (Clauss et al., 2010), as opposed to previously used criteria of their food's chemical composition and rumen morphology (Hofmann, 1989). Apart from various measures of morphophysiology of the digestive tract, the composition of the ruminal protozoal microfauna has also been linked to the feeding niche of wild ruminants (Dehority, 2003; Clauss et al., 2011). Interestingly, in contrast to many morphophysiological characteristics that appear to be overlapping between intermediate feeders and grazers, the protozoal microfauna of strict grazers apparently differs from that of intermediate feeders (Clauss et al., 2011). Particular types of rumen protozoa prefer different nutritive conditions – *e.g.*, the ciliates *Diplodinium* and *Epidinium* prefer cellulose while *Entodinium* and *Isotricha* prefer starch (Coleman, 1986; Williams and Coleman, 1992) or their numbers are apparently reduced by foods with high cellulose content (Clauss et al., 2011). With respect to the above, a high proportion of protozoa that are tolerant of starch, low rumen pH and low-cellulose food may indicate the selection of corresponding food items. On the other hand, a high proportion of fibrolytic protozoa indicates an intake of food of high cellulose content, such as grass.

A majority of research to date on microfauna of the rumen has concerned cattle and sheep species (review in Clauss et al., 2011). There is significantly less information on the composition of protozoa populations in the rumens of wild ruminants. In particular, knowledge of the microfauna of the mouflon is scarce. Existing publications point to a prevalence of representatives of ciliates from the *Entodinium* genus (70.9–99.0%), with a significantly smaller proportion of specimens from the *Diplodinium* (1.0–11.3%) and *Epidinium* (0.0–17.5%) genera and trace amounts of representatives of the *Isotrichidae* family (0.0–0.3%) (Drescher-Kaden and Seifelnasr, 1977; Trabalza-Marinucci et al., 2005). However, using a limited sample size of 11 animals from two different mouflon populations, the study of Drescher-Kaden and

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Seifelnasr (1977) indicated that in a higher-quality habitat more *Entodinium* spp. and in a low-quality habitat more *Diplodinium* spp. occurred.

Given the high variability of the diet of mouflon (Marchand et al., 2013), this species represents an ideal test case to study the effect of different natural diets on the composition of the ruminal protozoal microfauna. Hence, the aim of this study was to determine the taxonomic composition of rumen protozoa of mouflon as well as to establish the relationship between the number of protozoa taxa and the type of food eaten by mouflon living in the forests of Central Europe. In particular, it could be expected that differences between feeding types at an inter-specific level (Clauss et al., 2011) should be reflected at the intra-specific level in mouflon that had ingested different diets. Moreover, a better understanding of the feeding habits of this species may contribute to better management of its populations, and in particular more appropriate selection of its living locations, as the question of how much the specific environmental conditions affect feeding behaviour is still open. This is especially true, given the fact that the purpose and significance of the mouflon's presence in Central Europe as an alien species of unclear anthropogenic provenance – particularly in ecosystems far different from its native climate zone – has of late incited a heated debate (*e.g.*, Dérioz and Grillo, 2006; Nowakowski et al., 2009; Solarz, 2011; Szczęśniak, 2011; Marchand et al., 2013; Nasiadka et al., 2015). To date, reports of its negative impact on ecosystems are scarce and involve small areas (Graczyk and Bereszyński, 1992; Chapuis et al., 1994; Cermak and Mrkva, 2003; Szczęśniak, 2011). However, the observed increase of the wild population due to both new introductions and spontaneous dispersion (Solarz, 2011) encourages a more in-depth study of this species' ecology.

2. Material and methods

The material was collected in the Sowie Mountains, located at a height of 300–1015 m amsl in the Central Sudetes in SW Poland (Fig. 1). The geological base of the region is composed of gneiss raised during the Variscan orogeny, covered mostly by initial brown cambisols (Kondracki, 2002). The mean yearly temperature ranges from 7.5 °C in the foothills to 3.8 °C on the mountaintops. July is the warmest month, with a mean temperature ranging from 12.2 °C to 16.5 °C, and January is the coldest month, with a mean temperature ranging from −1.7 °C to −4.1 °C. Mean yearly precipitation ranges from 700 mm in the foothills to 1000 mm on the mountaintops. The vegetation period lasts between 170 and 210 days, and the snow cover remains from 60 to 130 days, depending on the height of its location (Woś, 1999). The actual vegetation of the region consists of anthropogenic spruce coniferous forests as well as mixed spruce-beech forests, beech woods, and subalpine ash riparian forests (Kuras and Świerkosz, 2014).

Norway spruce and common beech forests with admixture of silver fir, sycamore maple, European ash and silver birch dominate in the dwelling place of the analysed population of mouflon. The ages of these stands are diversified from saplings to old growth, with a prevalence of matured stages. In older stages, undergrowth of beech, spruce, fir, sycamore, ash, and alder, as well as rowan, hazel, and common elder is present. Self-regenerating beech predominates in undergrowths up to 800 m amsl, and spruce above this height. Herb layer type depends on the canopy age. Dwarf shrubs, grasses and forbs dominate in saplings stands, but in places of close tree cover only litter without plants is present. In pole stands, usually only litter without plants is present. Dwarf shrubs, grasses, forbs and tree seedlings are present in older stands.

Mouflon utilize these resources in different ways in the course of the year. In the spring, mouflon move into upper areas and feed in forest clearings and older tree stands, where forbs and grasses are available on the forest floor. The ratio of time spent in the forest to time spent outside it is approximately 50%:50%. During summer, mouflon prefer upper areas of the range, where closed spruce and beech saplings serve as diurnal shelter for them. In late afternoons they feed in older stands and in the evenings go out into mid-forest and close to forest meadows, where they stay until morning. Sometimes they also go into farmlands. The ratio of time spent in forests to time spent outside of it is approximately 60%:40%. In the autumn mouflon eagerly eat fallen sycamore and ash leaves as well as oak acorn and beech mast (if it is a mast year). The ratio of time spent in the forest to time spent outside of it is approximately 70%:30%. In wintertime mouflon move down into

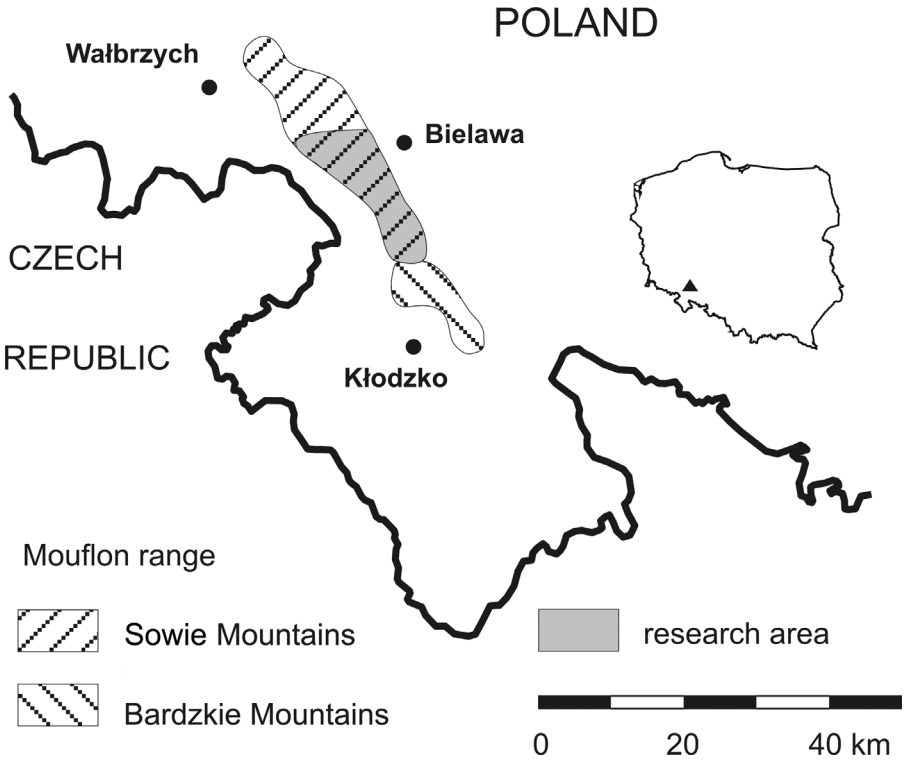

Fig. 1. Study area.