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Possible Nutritional Deficiency of the Red Deer (*Cervus elaphus*) Population in the Wanda Mountains, Northeast China

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Abstract.- Foraging analyses of red deer (*Cervus elaphus*) inhabiting the Wanda Mountains of Northeast China have indicated that the major food component of the population is leaf browse, with a low proportion of grass cropping. This form of feeding might complicate the transfer of energy and dry matter supply, possibly leading to nutritional deficiency and population decline. We measured the allantoin to creatinine ratio (A:C) of urine excreted in snow by free-ranging individuals of this population. In parallel, we used equations to calculate the digestible dry matter intake (DDMI) and metabolizable energy intake (MEI) of captive adult female red deer to evaluate the nutritional condition of the population. Between-year differences were found in the A:C ratio, DDMI, and MEI, which were 30.14%, 20.51%, and 35.39% higher in 2010 than in 2009. The DDMI in both sampling years was below the minimum wintering requirement. In 2009, the MEI ranged from 0.845 to 1.18 MJ ME/kg $BM^{0.75}$, which would have satisfied the red deer energy requirement for approximately 70% of the adult females, indicating a slight shortage of energy supply. This variation appeared to be correlated with snow depth, which was lower in 2010 compared to 2009. Overall, the A:C ratio, DDMI, and MEI showed a significant negative correlation with snow depth. Food supplementation might be needed to fulfill the minimum nutritional requirement of red deer population, especially when snow depth reaches a threshold.

Key words: Allantoin, Digestible dry matter intake, Digestible energy intake, Red deer, Winter nutrition

INTRODUCTION

For ungulates, winter is a period of chronic under-nutrition with catabolism of lean muscle and body fat because forage availability and quality are low, whereas energetic demands are relatively high (Moen, 1976; Torbit *et al.*, 1985; DelGuidice *et al.*, 1990). The intake of digestible dry matter varies within and across winters, as increased snow levels and decreased temperature limit forage availability, reduce foraging efficiency, and increase energy expenditure of movements (Gates and Hudson, 1979; Skovlin, 1982; Parker *et al.*, 1984; Wickstrom *et al.*, 1984). However, more recent studies suggest that ungulates substantially reduce energy expenditure during winter (Arnold *et al*., 2004, 2006; Signer *et al*., 2011; Turbill *et al*., 2011). Due to differences in protein and energy content of food, deer preferentially browse on woody plants or graze on forbs and graminoids (*e.g*., grasses, sedges, and rushes) to meet survival demands (Van, 1994; Hoffman, 1989; Christianson and Creel, 2007). Deer possibly optimize the balance between grazing and browsing based on their current stores of energy and protein. If a population were under predation risk, a trade-off between safety and harvesting would be another factor that might affect the selection of food type (Pierce *et al*., 2004). Metabolizable energy is maximized by grazing (*i.e*., grasses, rushes, sedges), whereas dietary protein intake is generally maximized by browsing (*i.e*., conifers, deciduous trees, shrubs) (Robbins, 1993; Van, 1994). To fulfill nutritional requirements, White *et al*. (2008) showed that red deer might selectively eat more browsing materials with higher protein content than grazing materials during the deep winter, with a reversal of this trend in spring.

Purine derivatives (PD) are the products of nucleic acid catabolism, and arise from tissue turnover (endogenous origin) or the digestion of feed and ruminal microbial matter within the small intestine (exogenous origin) (Mcallan and Smith, 1973a,b; Puchala and Kulasek, 1992). The assessment of PD could be used to indicate the nutritional and ruminal microbial condition of ruminants. Allantoin(A) is the principal PD metabolite excreted by red deer, therefore, the excretion of this substance reflects total PD excretion accurately (Vagnoni *et al*., 1996). In

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previous studies, urinary PD: C ratios (C refers to creatinine) were found to be constant for 24 hours after feeding (range = 2.19–2.31, CV = 2.5%; Chen *et al*., 1992). In another study, allantoin to creatinine ratio (A:C ratio) was found to respond relatively quickly to changes in diet, providing an index of the dietary intake of animals for a period of several days immediately prior to voiding of urine (Garrott *et al*., 1997). Moreover, equations have been developed to evaluate the relationships of digestible dry matter intake and metabolizable energy intake with the urinary A:C ratio, which have proved to be useful diagnostic tools for the study of free-ranging red deer (Vagnoni *et al*., 1996).

From the 1970s to early 1990s, red deer (*Cervus elaphus*) were widespread in the mountainous areas of Heilongjiang Province in Northeast China. The Wanda Mountain area had the highest red deer population density of this region, with an average annual population increase of 2.81% from 1975 to 1990 (Xu *et al*., 2000; Li *et al*., 2008). However, human activities aimed to boost economic development (such as road construction and logging) and illegal hunting (for medical use and meat consumption) have altered habitats, leading to an 83% decline in the red deer population in the late 1990s (Jiang *et al*., 2005; Liu, 2005; Liu *et al*., 2007). Over the last three to four years, steel cable snares and poisons, which had been previously used in illegal hunting, were prohibited and removed from this mountain region by staff from the local forestry bureau and forestry farm. Occasional poaching is still occurring, but kept to a small scale according to the local forestry bureau and forestry farm. However, even following such actions, red deer populations still declined in the Wanda Mountain area (Tian *et al*., 2010), which led us to explore other causes of the population decline.

There have been extensive studies on habitat selection and suitability of the red deer population in the Wanda Mountains, including assessments of habitat quality, food abundance, biomass and environmental carrying capacity, distribution of forage sites, climate, and geophysical characteristics (Jiang *et al*., 2006; Zhou *et al*., 2006; Jiang *et al*., 2007; Li *et al*., 2008). Such habitat suitability evaluations focus on extrinsic factors, describing the reality of habitat selection by individuals and the potential use of nearby habitats (Hanley, 1997). However, these studies do not describe whether the selected habitat provides sufficient digestible nutrients for resident deer. To obtain a full understanding of the actual living conditions of red deer in this region, intrinsic factors must be evaluated, including skull morphology and dentition, digestive physiology, and nutrient requirements (Hanley, 1982; Hofmann, 1989). In the Wanda Mountains, the results of previous studies analyzing microhistological plant fragments from red deer fecal pellets and feeding sites indicated that deciduous trees and shrubs are the major wintering food-type of red deer populations in the Wanda Mountains (Li and Zhang, 2005; Li, 2008; Zhang and Liu, 2008). Therefore, we sought to determine if nutritional shortage was at least partially responsible for the population decline of the red deer population.

In this paper, we evaluated digestive physiology from the aspect of purine derivatives. Using urine samples deposited in snow by red deer, we quantified the A:C ratio, then estimated digestible dry matter intake (DDMI) and metabolizable energy intake (MEI) of red deer through their curvilinear relationships (Vagnoni *et al*., 1996). By comparing the calculated DDMI and MEI values against the daily needs of captive individuals, we then evaluated whether the nutritional (dry matter and energy) supply to free-ranging red deer is sufficient for the population inhabiting the Wanda Mountains of Northeast China. Finally, we correlated A:C ratio, DDMI, and MEI with average snow depth during the sampling period, to evaluate how snow cover influences dry matter and energy intake, and hence nutritional condition.

MATERIALS AND METHODS

Study area

Our study was conducted at the Wupao Forestry Farm (E127°04’07”–127°16’45” and N46°27’31”–46°38’60”) of the Yingchun Forestry Bureau, which is located in the Wanda Mountains of northeastern China. Natural and plantation forests surround the 155.6-km^2 farm, except for crop fields in the east. The distance to the nearest town is about 10 km. The farm is located in the lower elevations of the Wandashan Mountains, at an altitude of 300–500

m. Long, cold winters and short hot summers characterize the weather of this region. The annual average temperature is 2.2°C, with extreme temperatures ranging from -34.8 in winter to –34.6°C in summer. Average annual precipitation ranges from 500 to 800 mm; from late November/early December to late March/early April, precipitation is represented by snowfall, while for the remainder of the year it is represented by rain.

The major forest types in the study area are coniferous-deciduous and deciduous forests. Meadows and farmlands are also an important component of the vegetation. The dominant overstory tree species are *Pinus korainensis*, *Fraxinus mandsburica*, *Tilia amurensis*, *Pdendron amurense*, *Ulmus* spp., *Populus* spp., *Betula* spp., *Salix* spp., *Lalix olgensis*, and *Acer mono*. The dominant understory shrubs include *Corylus mandshurica*, *Deutzia* spp., *Lonicera* spp., *Syringa amurensis*, and *Acanthopanas senticosus*. The dominant species of the herbaceous layer include sedges (*Carex* spp.), *Gramineae* spp., *Urtica* spp., and *Aegopodium alpestre*.

Samples

Samples were collected at two-week intervals from January to February in winter 2009 and 2010, when snow cover is thickest and air temperature is lowest. Each collection period spanned seven to ten days, during which time we collected red deer urine samples that were freshly deposited in the snow (as indicated by the backward tracing of fresh foot-print chains of animals near to bedding sites).. Spot-sampling was conducted in place of the collection of total pure urine samples (Nsahlai *et al*., 2000; Nurca *et al*., 2006; George *et al*., 2006a). In the event that urination was directly observed, we collected the most concentrated portion of the urine-soaked snow. As soon as two to three snow-urine samples had been collected from the same foot chain of one individual, the tracing was stopped. Snow-urine samples were transferred to zip-lock plastic bags by a researcher wearing disposable gloves. All samples from the same foot chain were mixed, as we assumed they were from the same individual. Six to eight drops of sulfuric acid were added to the snow-urine samples to retain a pH of below three. Samples were stored at -20°C. During the two research years, snow depth (in cm) was measured at sampling points when tracing each foot chain.

Experiments and statistics

We used a Waters 600E high performance liquid chromatography system, which was equipped with Waters 2487 UV spectrophotometry, to analyze urine samples for nutritional indices. The wavelength was set at 220 nm for allantoin and creatinine. Waters Empower chromatography working stations are used for calculating areas of peak wavelength. Before the analyses, the samples were thawed in a 50°C water bath, filtered through 0.2 um membranes, the pH was adjusted to 7.0 with saturated sodium hydroxide, and centrifuged under 12000 r/min for 10 min. Supernatant was collected for high performance liquid chromatography detection. Standard solutions (200 mg/ml) containing two components of allantoin was prepared every 48 h from allantoin powder dissolved in a water bath at 55°C. Creatinine powder was dissolved in water at room temperature, without a water bath or heating. The two components were separately prepared up to a 1 mg/ml solution, and then equal volumes were mixed together and diluted to 200 mg/ml. The pH was immediately adjusted to 7.0 with sulfuric acid before determination. A calibration curve was prepared over the concentration range of 0.0010–0.2000 mg/ml. The content of each PD component and creatinine were calculated from the calibration curve. In addition, tests of precision, stabilization recurrence, and recovery rate were carried out following the method described by Zhang *et al*. (2009). We optimized the chromatographic conditions described by George *et al*. (2006b). Separation was performed at a temperature of 25°C on a thermo-scientific C_{18} reversed-phase column (250 × 4.60 mm, 0.5 μm particle size). The mobile phase was 0.01 mol/L potassium dihydrogen phosphate solution. A standard sample was injected between two snow-urine samples to identify the PD and creatinine content, and to avoid misinterpretation that may be caused by other component in snow-urine samples. We used the following equations to calculated DDMI and MEI by solving the equations described by Garrott *et al*. (1997) and Vagnoni *et al*. (1996)

using Matlab.

(1) the relationship between the urinary A:C ratio and DDMI:

$$A:C = 0.3088 - 0.1046*DDMI + 0.0537*DDMI^2$$

(2) the relationship between the urinary A:C ratio and MEI:

$$A:C = 0.1121 + 0.0004*MEI$$

The equations used in the current study were developed from experiments on captive adult female individuals, and thus were not suitable for data from calves and bulls that have different nutritional requirements and metabolism. It would have been inappropriate to use the A:C ratio of the whole population to calculate DDMI and MEI values. Because there are difference in mean A:C ratios between cows and calves, A:C ratios from populations of these cohorts would have separate distributions. Mean A:C ratios for bulls were higher than those for cows, and some calves have extreme A:C values, so trimming could censor bull measurements and calves' extreme values from a distribution of randomly collected samples (Pils *et al*., 1999). Therefore, according to Pils *et al*. (1999), we trimmed 15% from the right tail and 20% from the left tail of the ordered A:C ratio distribution, to attempt to exclude the A:C ratio from most calves and male individuals in other words, the influence from different sex-age classes was reduced, and the remaining data represented primarily the adult female individuals in the population.

We then compared the estimates of mean DDMI and MEI with the minimum winter daily nutrient requirement that was derived from free-ranging red deer in the Wanda Mountains. Minimum DDMI requirements of free-ranging red deer was calculated by feeding captive adult female red deer individuals in Yingchun forestry bureau in Wanda Mountains from November to March of next year-winter in northeast China using the method described by Papageorgiou *et al*. (1981) and Zhang and Liu (2008). Metabolizable energy of food was evaluated by methods described by Schwartz *et al*. (1988). Variance analysis (ANOVA) was used to test differences in the measures of the A:C ratio, DDMI, and MEI across the two-year period. In addition, the A:C ratio, DDMI, and MEI were correlated with monthly snow depth measurements.

RESULTS

We collected 20–25 mixed samples from free-ranging during each visit (n=4) in the Wupao Forestry Farm of Yingchun Forestry Bureau in the Wanda Mountains over the two-year study period for comparative analysis. In total, 95 snow-urine samples were collected, 46 samples in 2009 and 49 samples in 2010. Allantoin and creatinine were detected successfully in all samples. Three A:C ratios of >1.0 were considered to be exceptional values, and were omitted from the analysis before trimming.

Across years, differences were found in the mean A: C ratio ($F = 162.2$, $P<0.001$), DDMI ($F = 228.77$, $P<0.001$), and MEI ($F = 199.7$, $P<0.001$), whereby the values of all three parameters were significantly higher in 2010 compared to 2009, but no differences were found between months ($P > 0.05$). The mean A:C ratio, DDMI, and MEI increased significantly from 0.51 to 0.73, from 3.13 kg/d to 3.92 kg/d, and from 0.9953 MJ ME/kg $BM^{0.75}$ to 1.5404 MJ ME/kg $BM^{0.75}$, respectively, from 2009 to 2010. We compared the calculated DDMI and MEI values with the minimum requirement of red deer in the Wanda Mountains to evaluate whether their nutrition was below or above the maintenance level for each winter. Previously published literature has shown that the minimum winter daily MEI requirement of adult female red deer in the Wanda Mountains is 0.949 MJ ME/kg $BM^{0.75}$, and that the requirement of DDMI is 4.41 kg/d (Zhang and Liu, 2008). In our study, we obtained a DDMI range from 2.86 to 3.43 in 2009, and from 3.48 kg/d to 4.29 kg/d (Fig. 1a) in 2010 (Fig. 1b); hence, DDMI in both sampling years was below the minimum wintering requirements. The MEI ranged from 0.845 to 1.18 MJ ME/kg $BM^{0.75}$ in 2009 (Fig. 2a), which suggests that many adult females would have been below the minimum energy requirement. This result indicates that there was a slight shortage in energy supply during the winter of 2009. This part of the results is consistent with that of Zhang and Liu (2008), indicating that energy is in short supply and should be considered as a critical factor influencing the carrying capacity in the Wanda Mountain area. The situation was better in 2010 (Fig. 2b), when MEI met minimum energy requirements of female adults.

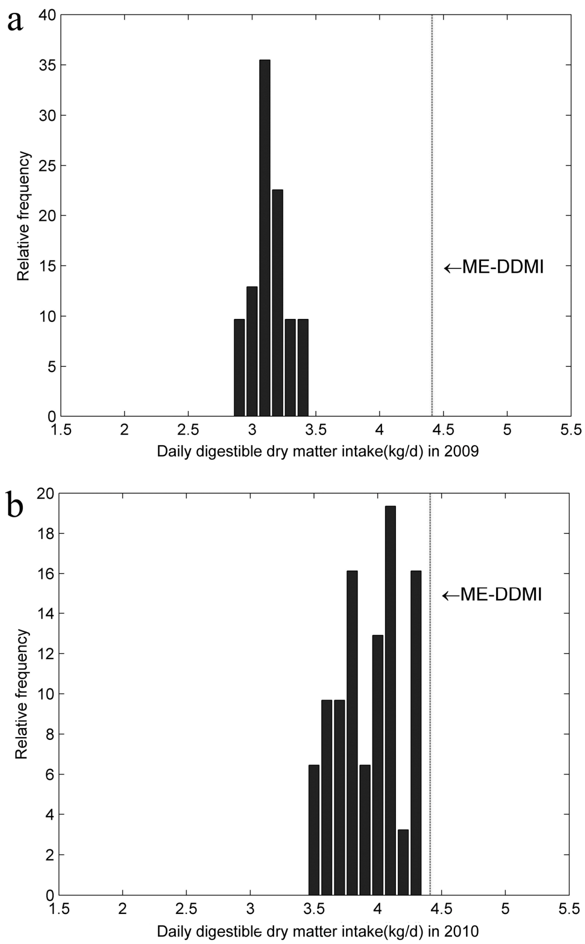

Fig. 1. Relative frequency of estimated daily DDMI of red deer in the Wupao forest farm at the Wanda Mountains in China during January and February of 2009 (a) and 2010 (b). Compared with the minimum requirement of digestible dry matter intake (ME-DDMI) (dotted line), the daily DDMI was below the minimum requirement level in both years.

The average snow depth decreased from 0.311 m in 2009 to 0.171 m in 2010, without significant changes between months in each of the two years. All three physiological indices (A: C ratio, DDMI, and MEI) were significantly negatively correlated with snow depth (Fig. 3), producing r = -0.9966, -0.9689, -0.9788 and P = 0.0034, 0.0311, 0.0314, respectively.

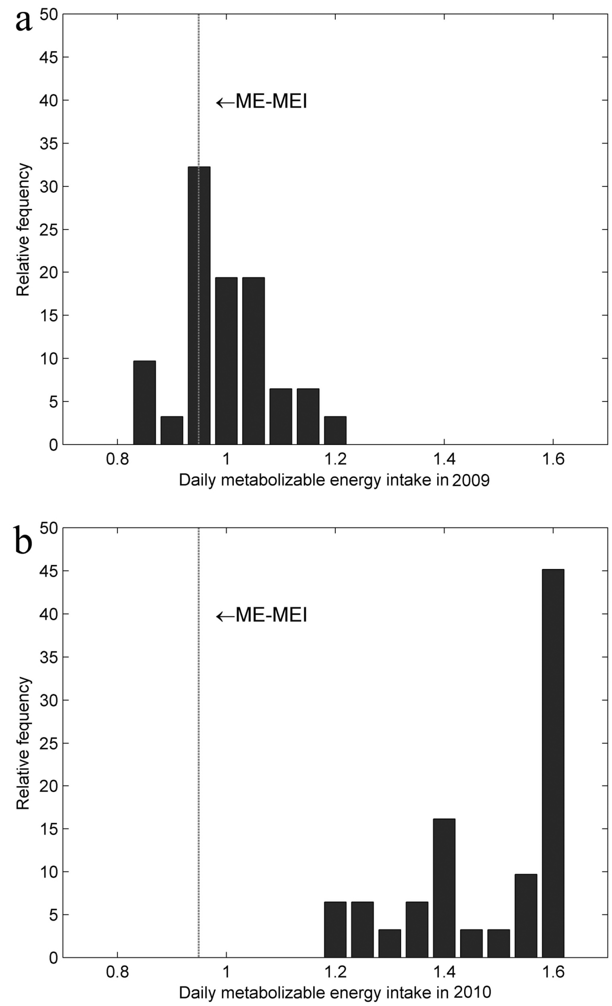

Fig. 2 Relative frequency of estimated daily MEI in the Wupao forest farm at the Wanda Mountains in China during January and February of 2009 (a) and 2010 (b). Compared with the minimum requirement of metabolizable energy intake (ME-DDMI) (dotted line), 70% and 100% of individual red deer daily MEI met the minimum requirement level in 2009 and 2010, respectively.

DISCUSSION

Energy and protein requirements during reproductive seasons (usually summer) are much higher than those required in winter; hence, winter may not be the most limiting season for temperate ungulates, and should not be considered in isolation (Tollefson *et al.*, 2010). However, there is no evidence

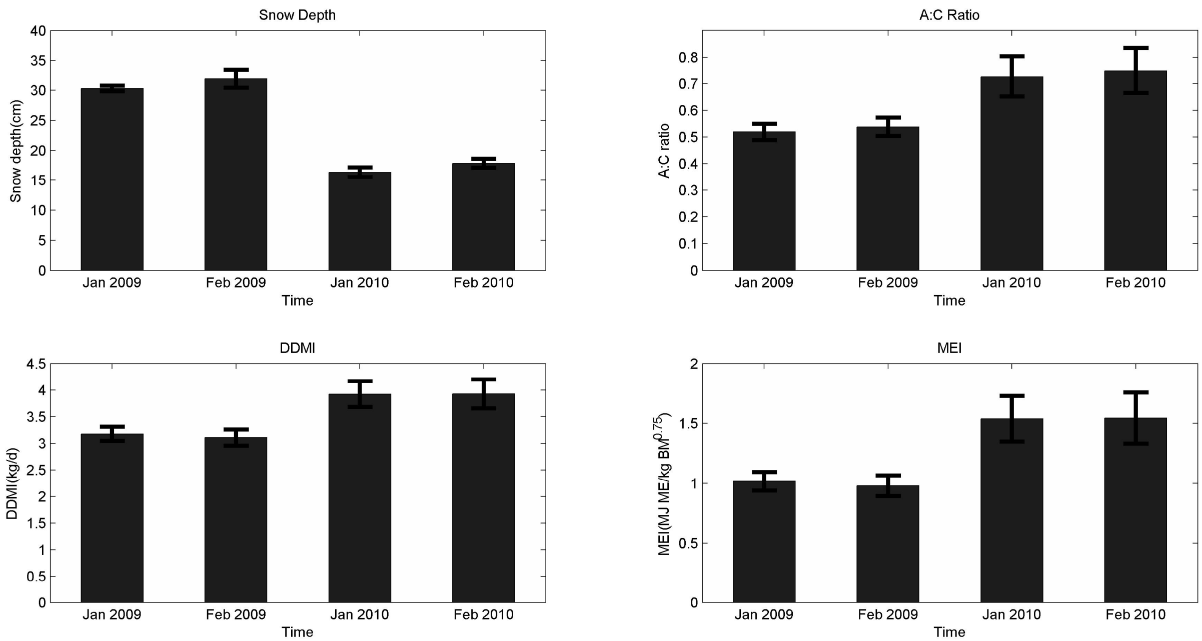

Fig. 3. Changes in the urine allantoin:creatinine (A:C) ratio and DDMI (a) and MEI (b) with snow depth in 2009 and 2010.

indicating that red deer inhabiting the Wanda Mountains suffer from food or nutritional deficiency in summer. Even assuming that nutritional deficiency exists in summer, first we should prove that the winter nutritional supply is sufficient in the Wanda Mountains, indicating that other factors are limiting red deer access to these resources. Our current study showed that dry matter was insufficient to meet the energy requirements of red deer inhabiting the Wanda Mountains, contradicting the study by Zhang and Liu (2008) estimated by feeding captive adult females. Snow level varied between the winters of 2009 and 2010 (the period of study). In this period, although the analysis of urine samples indicated that MEI was nearly sufficient in both years, that of DDMI was below the minimum requirement in both years.

In polygynous animals, such as ungulates, adult females are essentially the reproductive unit of the population. Therefore, winter nutrition of adult females, which can represent for a large part of the whole population needs (Pils *et al*., 1999), has an important effect on population dynamics. An estimate of environmental carrying capacity by Liu (2008) suggested that DDMI supply to red deer in this region was sufficient, whereas the MEI supply was not. In contrast, our results showed that more DDMI was required in both years. Estimates of MEI primarily met the population needs in 2010, but were slightly below the minimum requirement in 2009. These results indicate that there were complications in the supply of nutritional components.

Supporting the studies by Parker *et al*. (1984), Sweeney and Sweeney (1984) and Telfer and Kelsall (1984), we suspect that heavy snow limits deer access to the necessary resources for survival during these long cold periods, which in turn leads to increased energy requirement. Snow reduces the ease with which red deer move (Parker *et al*., 1984), and might prevent individuals from moving to suitable habitats. The greater energy requirement of moving in heavier snow also increases as a curvilinear function of snow depth and density. When snow accumulates to a threshold of 40 cm, energy expenditure increases six-fold compared to an area without snow (Parker *et al*., 1984).

Red deer in the Wanda Mountains mainly feed on plant organisms that are above the level of snow cover; therefore, heavier snow reduces food availability, particularly of grass-like plants. This limitation, in turn, increases energy consumption due to the necessity to move further afield in snow (Mautz, 1978; Tucker *et al*., 1991). Furthermore, the negative correlation between the physiological indices (*i.e*., A: C ratio, DDMI, and MEI) and snow depth observed in this study demonstrate that increased snow depth leads to a decrease in nutritional component intake. Hence, the poorer nutritional condition of red deer recorded in 2009 was probably caused by heavier snowfall in 2009 compared to 2010. Animals might compensate for energy deficiency by losing weight in winter. Fat comprises about 15% of red deer body mass (Hobbs *et al*., 1982; Moore and Christie, 1984), with individuals potentially obtaining 6 kcal of energy by catabolizing 1 gram of fat (Mautz, 1978). In addition, dry matter was obviously in short supply in both years. Therefore, we considered that the deer met their minimum energy requirements in part from their MEI, and in part from fat catabolism leading to weight loss.

In conclusion, we recommend provision of additional food with high protein content to fulfill the minimum dry matter requirements of the red deer population in their winter habitat. In addition, when snow depths reach, or have the potential to reach, thresholds of 40 cm in certain regions that are potentially red deer winter habitats, food with high energy content should be supplied by forestry managers.

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