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Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*)

I. Energy intake

Regina Kuntz, Christina Kubalek, Thomas Ruf, Frieda Tataruch and Walter Arnold*

Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Savoyenstraße 1, 1160 Vienna, Austria

*Author for correspondence (e-mail: walter.arnold@vu-wien.ac.at)

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Summary

Large ruminants respond to changing plant phenology during winter by decreasing voluntary food intake, increasing gut passage time and utilizing body fat reserves. It is uncertain, however, how other large mammals with a non-ruminant digestive physiology cope with winter forage conditions. Therefore, we investigated seasonality of energy intake in a large herbivorous wild mammal, the Przewalski horse (*Equus ferus przewalskii*). Throughout all seasons we used the n-alkane method to measure daily dry matter intake (DMI), diet composition and digestion, and determined an index of gut passage time in horses living under close to natural conditions.

DMI correlated positively with its content of crude protein and nitrogen-free extract. Independent of these effects, DMI further varied seasonally with a peak in autumn and a nadir in late winter. Fluctuations of DMI corresponded to the annual change in body condition, which decreased during winter while energy reserves were depleted, and increased during the fattening period. Gut passage time varied in the course of the year and was longer during winter when the diet was high in crude fibre. Nevertheless, changes in gut passage time occurred rather independently of changes in forage composition and DMI, suggesting endogenous control for timely adaption of the digestive strategy to meet predictable changes in forage quality.

Key words: annual rhythm, body mass, dry matter intake, food composition, digestibility, gut passage time, n-alkane method.

Introduction

In seasonal environments, herbivores are confronted during winter with a nutritional bottleneck caused by the cessation of herbage growth, and by low digestibility of the remaining scarce standing crop because of a high content of crude fibre (Arnold et al., 2004). As an adaptation to this seasonal change in food availability and quality, ruminants have evolved pronounced cycles of voluntary food intake and body mass (Barry et al., 1991; Crete et al., 1993; Ellenberg, 1978; Holand, 1994; Holand and Staaland, 1992; Loudon et al., 1989; Loudon, 1991; Weber and Thompson, 1998). An anabolic growth phase with an accumulation of body energy reserves is timed to summer when food is abundant, whereas winter physiology is characterized by catabolic metabolism and anorexia. These adjustments are controlled by an endogenous rhythm entrained to the natural photoperiod that enables anticipation of the seasonal environmental changes and timely adaptation (Lincoln et al., 2001; Lincoln et al., 2003; Lincoln et al., 2005).

Whether horses show similar physiological seasonal adaptation is uncertain. Horses could be less seasonal and use an alternative feeding strategy to cope with winter conditions because of their different digestive physiology. Equids can live on extraordinarily poor forage with the help of cellulose-fermenting symbionts in their large hindgut (Meyer, 2002). Although hindgut fermentation is less efficient, in terms of digestibility, than the foregut fermentation of ruminants, horses compensate for this by high food intake and shorter gut passage time. Compared with ruminants of similar size, horses are able to absorb more nutrients per day not only from food with a high fibre content but from a whole range of forage qualities (Duncan et al., 1990; Menard et al., 2002). Therefore, horses may respond to the lower quality of food plants during winter in a completely different manner to ruminants, and may in fact increase, rather than decrease their daily dry matter intake (DMI).

Previous observations of the foraging behaviour of horses and estimates of DMI have so far furnished inconsistent and contradictory evidence. Highest values of the time spent feeding were recorded during autumn (herd 1) or winter (herd 2) in free-living Przewalski horses in Mongolia (Van

Dierendonck et al., 1996), at the end of winter in Camargue horses (Duncan, 1985) or during spring in ponies in Southern England, and in Przewalski horses kept in a semi-natural reserve in Germany (Pratt et al., 1986; Berger et al., 1999). By total collection of faeces and using the crude protein content of faeces as an indicator of food digestibility, Duncan found in Camargue horses higher food intake during winter (January, February; *N*=1) compared with spring (March–June, lowest values in May; *N*=2) (Duncan, 1992), whereas Fleurance and Duncan reported particularly high levels of food intake in horses during autumn (Fleurance and Duncan, 2001).

In order to clarify the issue, we investigated seasonality of DMI, diet composition, gut passage time and nutrient uptake of Przewalski horses (*Equus ferus przewalskii* Poljakow) kept under close to natural conditions with the n-alkane method, a relatively precise tool to estimate DMI and diet composition in free-roaming herbivores (Dove and Mayes, 1996; Mayes et al., 1986). Przewalski horses, or Takhi, are the ancestral wild form of domestic horses. They are typical steppe herbivores of Central Asia and are exposed in their natural habitat to aridity and most severe winter conditions. They became extinct in nature in the middle of the 20th century and only a zoo population survived (Boyd and Houpt, 1994). By studying Przewalski horses in a natural setting, we attempted not only to enlarge our knowledge about seasonal foraging strategies in large non-ruminant herbivores living in strongly seasonal environments, but also to facilitate the current reintroduction of the species into its original habitat in Mongolia by a better understanding of the horses' ecophysiology and nutritional requirements.

Materials and methods

Study area and animals

The study was performed between 2002 and 2004 in an approximately 3 km^2 enclosure in the Nationalpark Neusiedlersee-Seewinkel in Austria (47°45′N, 16°48′E). The enclosure was approximately 4 km long and 750 m wide, and was bordered by an electrical fence on three sides and Lake Neusiedl in the west. The vegetation cover was natural, with three spatially distinct types of habitat: a lake-side reed belt (1.5 km^2), followed by a land-side reed belt (0.67 km^2), and a salt meadow (0.73 km^2). The horses depended entirely on this vegetation throughout the year. At the northern end there was an artificial water supply and an open shelter (5 m × 9 m) to provide shade during the typically hot and dry summer. Air temperatures and precipitation were recorded every 15 min at a weather station located approximately 500 m south of the enclosure.

The study animals belonged to the Zoo Vienna (Austria) and were part of a herd of Przewalski horses kept in the enclosure since 1999 to protect through grazing the national park's unique halophytic plant communities against invasion of the reed belt. All animals were part of the 'European Breeding Program for Przewalski Horses (EEP)'. As a consequence, the composition of the herd changed during the study because of the removal of the stallion, the integration of a two-year-old mare, births and deaths. Three of the five mares studied reproduced successfully during the study period, two of them twice (Table 1).

Time spent feeding

The horses were well habituated to the presence of humans, which enabled us to follow and record their feeding behaviour from a distance of approximately 1 m without disturbing the animals. Foraging activity was quantified every other month from May 2003 to January 2004, for a total of 356 h. We observed focal animals (Altmann, 1974) within the 14 days around an experiment to determine DMI (see below). For each of these sampling periods, the proportion of time spent feeding per day was calculated for three to four horses by combining for each focal animal three one-hour periods of observations, distributed over the day to cover the whole period of daylight.

Body condition

We assessed the body condition of the horses from standardized photographs taken at least once a month from August 2003 to August 2004. Body condition was scored on an ordinal scale from 0 (very thin) to 5 (very fat) according to the shape of the rump (from concave to convex) and the visibility of pelvis and ribs (Rudman and Keiper, 1991). To calibrate body-condition scores, we weighed animals immobilized for veterinary treatments during the period of visual assessment to the closest kg using a portable balance.

Forage composition and DMI

From July 2002 to January 2004 we investigated the amount of food intake of adult horses and its composition using the n-alkane method (Mayes et al., 1986; Dove and Mayes, 1996). This method takes advantage of the specific properties of natural n-alkanes, components of plant cuticulas that are essentially indigestible for mammalian herbivores. Alkanes of odd chain length are typically found in plants at much higher concentrations than those of even chain length. Furthermore, the concentrations of alkanes of different chain lengths vary remarkably between plant species. To a much lesser extent n-alkanes vary within a species throughout the growing season and between different parts of a plant. DMI can be estimated with the n-alkane method by feeding a known quantity of an artificial even-chain alkane and comparing the faecal concentration of this external marker with the faecal concentration of an internal marker, i.e. an odd-chain alkane of close chain length contained in food plants. The major advantage of the n-alkane method is that it renders total faeces collection unnecessary and allows for simultaneous inference of forage composition from faecal material (Dove and Mayes, 1996). The n-alkane technique was initially developed to estimate DMI of domestic ruminants, but feeding experiments corroborated its reliability for horses (Gudmundsson and Thorhallsdottir, 1998; Stefanon et al., 1999; Stevens et al., 2002). Furthermore, the n-alkane technique has been successfully applied to determine DMI or diet composition in

Table 1. *Composition of the Przewalski herd in the Nationalpark Neusiedlersee-Seewinkel*

Individual[a]	Sex	Date of birth	Arrival	Removal	Death
Dino[b]	Male	9 May 1993	3 October 1999	10 May 2003	
Viktoria[b]	Female	24 June 1973	3 October 1999		14 January 2003[c]
Gobi[b]	Female	9 July 1985	3 October 1999		
Barbara	Female	5 March 2001	From birth	6 August 2003	
Illmitz	Female	15 May 2002	From birth		
Prinz	Male	10 May 2003	From birth		
Tosca[b]	Female	23 March 1992	3 October 1999		
Gina	Female	20 June 2001	From birth	6 August 2003	
Hank	Male	17 May 2002	From birth		
Colt	Male	9 May 2003			Stillbirth
Urgli[b]	Female	13 April 1991	3 October 1999		
Hengst	Male	23 October 2001	From birth		19 November 2002[d]
Holle	Female	30 August 2002	From birth		
Bruno	Male	4 August 2003	From birth		
Okasvenja[b]	Female	1 June 2001	15 May 2003		

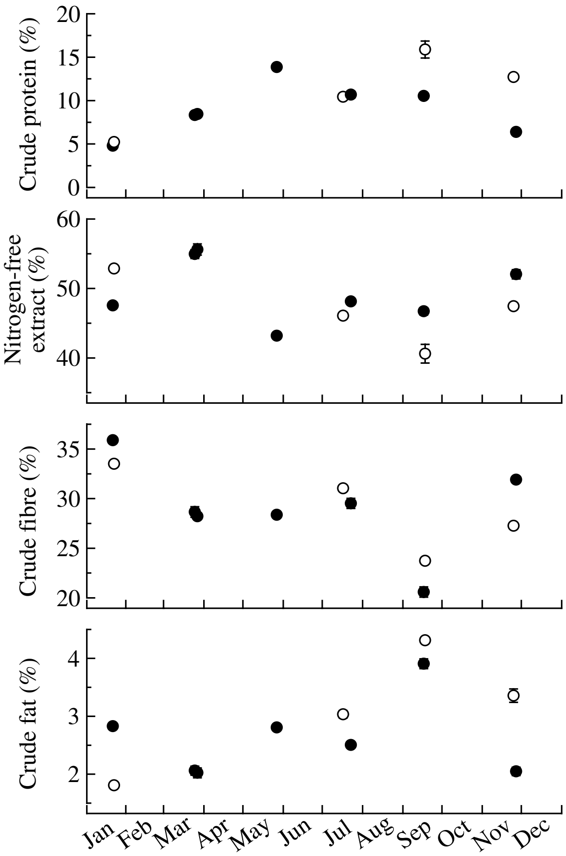
[a] Offspring of a mare are listed in subsequent rows in italics.

[b] Study animals for estimation of dry matter intake and diet composition.

[c] Death because of old age.

[d] Death because of trauma.

wild herbivores living free or under semi-natural conditions in enclosures (Bugalho et al., 2001; Bugalho and Milne, 2003; Gedir and Hudson, 2000; Hulbert et al., 2001; Martins et al., 2002; Perez et al., 1997).



Experiments to determine DMI were performed once every other month, and twice within one week in March 2003. Each of the total 40 experiments lasted for two consecutive days. On day one of an experiment, we hand-fed an external marker four times to each study horse at approximately equal time intervals between 13.00 h and 16.00 h. The most suitable dose of the external marker and the best method of administration to reach a marker equilibrium in faeces after 24 h were determined during preceding tests. As an external marker, we used n-dotriacontane (C32). For oral marker administration, we prepared pieces of bread. On each piece we spread 1 g of n-dotriacontane powder on top. Stable attachment of the powder was achieved by heating the pieces in a microwave for 3 min at 350 W. After marker administration, we followed the grazing herd, noted which plant species were consumed by the horses, roughly estimated an order of preference, and collected the 10 most frequently eaten plant species. These species covered approximately 80% of the enclosure area. During the second day, we collected five faecal samples from each experimental animal over a period of eight hours, on average 245 g of dry matter per sample (s.e.m.=7). In the laboratory, plant and faecal samples were dried at 60°C to constant mass, ground to a particle size of approximately 1 mm, and analysed quantitatively for n-alkanes by gas chromatography (Autosystem XL; PerkinElmer, Norwalk, CT, USA) according to Mayes et al. (Mayes et al., 1986).

Fig. 1. Seasonal changes of nutrients in dry matter intake (percentage of dry matter intake; values are means ± s.e.m., missing error bars represent s.e.m. smaller than symbol size). Filled symbols represent data from the first year of dry matter intake measurements (July 2002–May 2003), open symbols represent data from the second year (July 2003–January 2004).

Altogether, 10 different n-alkanes (odd-chain C21–C35 and even-chain C28 and C30) could be identified, a number sufficient for estimating the dietary proportions of the 10 most frequently eaten food plants (Dove and Mayes, 1996). Diet composition was estimated with a maximum-likelihood optimisation procedure (nlminb in S-Plus. 6.2) finding the relative proportions of the 10 food plants that best explained the n-alkane concentrations found in a faecal sample (Newman et al., 1995).

DMI (kg) was calculated according to Dove and Mayes (Dove and Mayes, 1996):

$$\mathrm{DMI} = \frac{\frac{Fi}{Fj} \times Dj}{\left(Hi - \frac{Fi}{Fj} \times Hj\right)}, \quad (1)$$

where Hj is the concentration of external marker (C32) in the ingested plant material (virtually zero in most plants) (Dove and Mayes, 1996). Hi is the concentration of internal marker in the ingested material. As internal marker, we used the mean of concentrations of C25–C33 odd chain length n-alkanes because the amounts of C31 (n-hentriacontane) and C33 (n-tritriacontane) alone were too low in some plant species to give reliable estimates. Prior studies with equids indicated that faecal recoveries of n-alkanes were independent of their chain lengths (O'Keefe and McMeniman, 1998; Ordakowski et al., 2001; Stevens et al., 2002). Therefore, it was not necessary, unlike in ruminants, to use adjacent pairs of dosed and natural alkanes (e.g. C32/C31 or C32/C33) to reliably estimate intake (Dove and Mayes, 1996). Fj is the concentration of external marker in faeces. A peak concentration of the external marker was reached 18–24 h after its administration. We calculated Fj by averaging the three highest C32 concentrations found in faecal samples collected from an individual during day two of a trial. Fi is the concentration of internal marker in the same faecal samples used to calculate Fj. Dj is the administered quantity of external marker.

Nutrient intake and output

We further analysed the plant and faeces samples for the content of crude protein (Kjedahl method), crude fat (Soxhlet method), nitrogen-free extract (NFE), crude ash and crude fibre by Weender analyses (Nehring, 1960). The total intake of nutrients was calculated for each horse and trial from the respective DMI, the percentages of various food plant species in the ingested material, and the percentages of nutrients in these plants.

Gut passage time

Common measures of the rate of passage of food residues through the gastrointestinal tract, such as transit time (i.e. the time from first dosing to the first appearance of the marker in the faeces) or mean retention time, were impossible to determine for two reasons. First, the marker substance n-dotriacontane also occurred naturally in plants and was therefore always present in faeces. Second, consecutive faeces samples from dosed individuals were too fragmentary to permit reliable estimation of mean retention time. Therefore, we used the time period between the first marker administration in an experiment and the occurrence of peak marker concentration in faeces as an index of gut passage time that could be determined unequivocally. This index represents transit time plus the ascending portion of the marker concentration curve (Van Soest, 1994).

Data analyses

Statistical tests were performed using S-Plus 6.2. for Windows (Insightful Corporation, Seattle, WA, USA). Linear mixed-effect models with random-effect 'individual' were used for repeated-measurement analyses (Pinheiro and Bates, 2000). We tested for seasonal variation by entering a sine (t) and cosine (t) term into the linear model, with t representing day of year in radians. Sums of squares of these terms were added to obtain a single F- and P-value for the periodic function. To ease interpretation, coefficients of the sine and cosine term were then used to algebraically compute the amplitude (A) and phase (ϕ) of the seasonal function. Confidence limits of A and ϕ were determined by bootstrapping. To compare regression models, we used Akaike's Information Criterion (Akaike, 1973), which is proportional to the residual sum of squares penalized by the number of parameters in the model.

The most energetically challenging periods of time during reproduction for mares are the last two months before parturition and the first month of lactation (Boyd and Houpt, 1994; National Research Council, 1989). Only six experiments with three Przewalski mares were performed during these periods of time. With this small sample, we did not find any significant effects of gestation or lactation on the variables measured and therefore neglected gestation and lactation in statistical analyses.

Results

Nutrient content of DMI

The nutrient content of DMI changed remarkably over the year. The content of crude protein was high during the vegetative period, crude fat peaked in autumn, whereas crude fibre and NFE were the major DMI components during winter (Fig. 1). In addition to season, precipitation influenced food plant phenology. Rainfall during July 2003 was particularly low, with a monthly total of only 64 mm compared with 112 mm in July 2002. Hence, the vegetation in the enclosure became dryer during summer 2003, though the nutrient content of DMI remained largely unaffected (Fig. 1). Presumably, a further effect of the severe drought during summer 2003 was the particularly intense second peak of vegetation growth during autumn 2003, with accordant nutrient composition of DMI (Fig. 1).

Food intake

Mean DMI of the horses studied was 10.0±0.48 kg day^{-1} (s.e.m.), but varied significantly between months of measurement (Fig. 2; $F_{5,29}$=5.8, P<0.001). DMI was particularly low during January in both study years with a mean of only 7.2±0.22 kg day^{-1}. DMI determined during July was considerably lower in 2003 compared with 2002, whereas DMI determined during November was higher in 2003 (Fig. 2).

The reliability of our DMI assessment was verified by two lines of evidence. First, two measurements of DMI within four days during March 2003 resulted in virtually identical values (11.0±0.5 kg day^{-1}, N=3 versus 10.5±1.48 kg day^{-1}, N=4; paired Student's t-test: t=0.74, P=0.54). Second, the estimated changes in DMI agreed well with the proportion of time spent feeding per day determined during several days around the respective experiment for measuring DMI (Fig. 3; $F_{1,12}$=7.6, P=0.017).

Among the nutrients tested, only the content of crude protein and NFE influenced DMI significantly, and both had a stimulating effect on DMI (Table 2). The residual variation of DMI after adjusting for the influence of nutrients was not random. Adding a periodic function to the regression model improved the fit significantly (Table 2). This nutrient-independent periodic variation of DMI peaked in autumn and had a nadir during late winter, with an annual average amplitude of 2.6 kg (95% confidence limits 1.4 kg and 4.0 kg, respectively; Fig. 2, line).

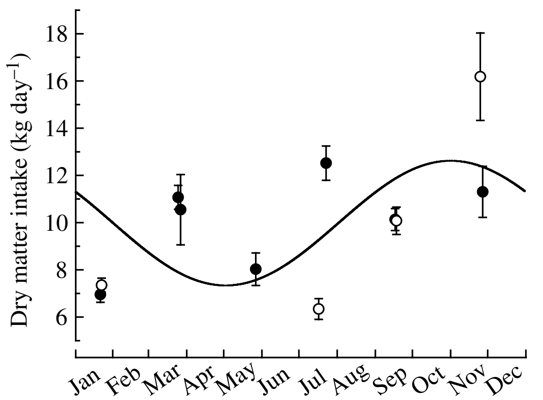


Fig. 2. Seasonal variation of dry matter intake (values are means ± s.e.m.). Filled symbols represent data from the first year of study (July 2002–May 2003), open symbols represent data from the second year (July 2003–January 2004). Line: periodic fit to residual variation of dry matter intake adjusted for the influence of nutrients (for statistics, see Table 2).

Gut passage time

Gut passage time, as indicated by the time period between the first marker administration in an experiment and the occurrence of peak marker concentration in faeces, varied throughout the year in an approximately sinusoidal shape (Fig. 4; mean time period was 21.6±0.32 h).

The annual course of the index of gut passage time mirrored that of DMI and was shortest during the autumn fattening period when DMI was highest (cf. Figs 2, 4). However, in a multiple regression including total DMI, the proportions of all nutrients in DMI and a periodic function, the periodic function was the most significant predictor of the index of gut passage time (AIC=168). A model containing only the periodic function explained the index of gut passage time almost better than the full model {AIC=165, difference between models, P=0.183; test for difference to zero for the amplitude of the periodic fit [$\cos(\text{Julian day}/365\times 2\pi-\phi)$], $F_{2,32}$=10.2, P<0.001; amplitude 1.1 h, 95% confidence interval, 0.4–2.1; acrophase, 8 March, 95% confidence limits, 20 January, 22 May. This result suggests that gut passage time varied rather independently of DMI and its composition, but periodically over the year.

Nutrient extraction

Comparison of the estimated proportions of crude protein and crude fibre in DMI and in faeces demonstrated that both

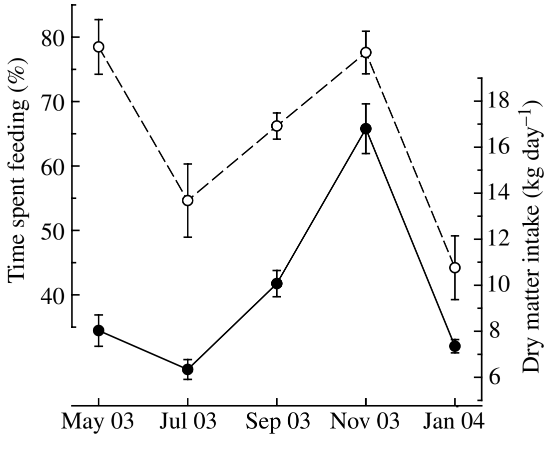


Fig. 3. Variation of dry matter intake (filled symbols, solid line, values are means ± s.e.m.) and average time spent feeding per day (open symbols, broken line, values are means ± s.e.m.). Data for both parameters were simultaneously collected from three to four Przewalski horses during five experiments.

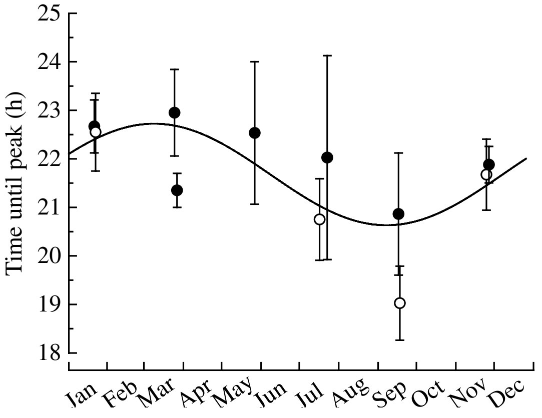


Fig. 4. Seasonal changes in the time period between administration of the first marker and occurrence of peak concentration of the marker in faeces (values are means ± s.e.m.). Filled symbols represent data from the first year of study (July 2002–May 2003), open symbols represent data from the second year (July 2003–January 2004). Line: periodic fit.

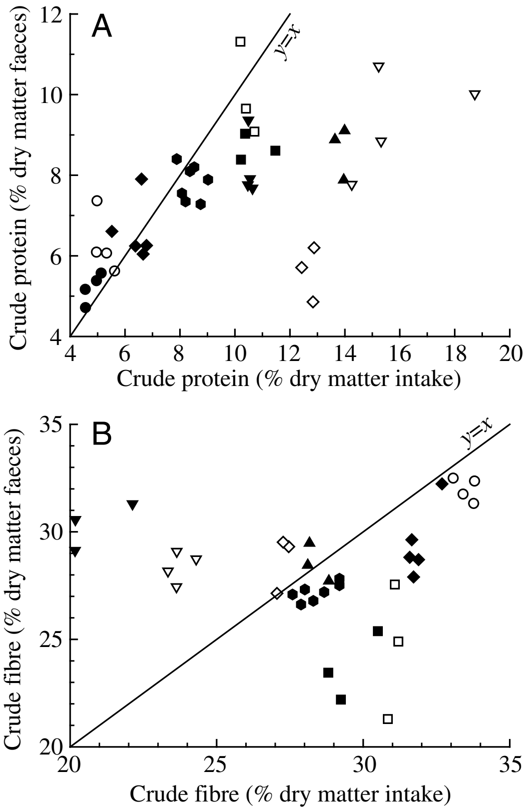


Fig. 5. Concentrations of crude protein (A) and crude fibre (B) in dry matter intake and in faecal material. Different symbols indicate different months of measurement (circle: January; hexagon: March; triangle: May; square: July; inverted triangle: September; diamond: November). Filled symbols represent data from the first year of study (July 2002–May 2003), open symbols represent data from the second year (July 2003–January 2004).

nutrients were extracted from DMI with higher efficacy when present in high concentrations (Fig. 5).

This result corresponded to differences in gut passage time. According to single-predictor regression models, the index of gut passage time correlated positively with crude-fibre content ($F_{1,33}$=7.6, P=0.010), but negatively with crude protein content of DMI ($F_{1,33}$=6.0, P=0.019). As expected, digestibility of crude protein decreased with gut passage time, whereas digestibility of crude fibre increased (difference between slopes of regression of crude-fibre extraction and crude-protein extraction with the index of gut passage time, respectively, $F_{1,71}$=4.70, P=0.034).

Body condition

Changes in DMI during the course of the year corresponded to changes in visually assessed body-condition scores. Body condition was lowest in April after the winter fast and increased thereafter to reach the annual peak at the end of October {Fig. 6; test for difference to zero for the amplitude of the periodic fit [cos(Julian day/365×2π–ϕ)], $F_{(2,83)}$=52.7, P<0.001}.

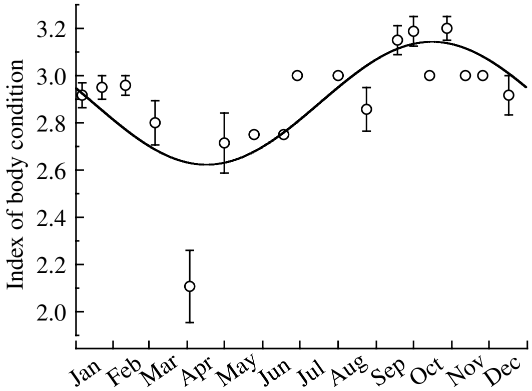


Fig. 6. Seasonal variation of visually assessed index of body condition (values are means ± s.e.m., missing error bars represent sample sizes too small to calculate s.e.m.; line, periodic fit).

The actual measured body weights correlated positively with simultaneously assessed body-condition scores (Table 3; $F_{1,6}$=41.7, P<0.001). Hence, changes in visually estimated body-condition scores reflected to a significant degree changes in the amount of body fat stored, and repeated weighing of two mares in autumn and the subsequent spring confirmed a high mass-loss during winter of, on average, 18% (Table 3) (Gobi and Urgli).

Discussion

Variation of DMI and underlying mechanisms

The most important result of this study was that dry matter and hence energy intake varied considerably during the year. The nutrient-independent DMI, which showed an annual difference of approximately 5 kg, was approximately two-thirds higher at the autumn peak compared with the late-winter nadir (Fig. 2). This finding clarifies hitherto inconsistent results about seasonal changes in the feeding behaviour of free-ranging horses (Duncan, 1985; Duncan, 1992; Fleurance and Duncan, 2001; Van Dierendonck et al., 1996). Przewalski horses responded to changing plant phenology like ruminants and substantially decreased DMI during winter.

At first glance, the low value of DMI in January is rather surprising. In both study years January was the measurement period with lowest air temperatures (monthly mean during January 2003=0.2°C, during January 2004=–1.5°C). Thus, because of higher costs for thermoregulation an increase in DMI was to be expected rather than the observed drop. Since in both years January was the only month with a snow cover present in the enclosure, one could speculate that the low DMI during January simply reflected limited accessibility of food. However, snow covered only parts of the study area in both years and was never thick enough to render the vegetation inaccessible for the horses. We carefully checked the enclosure every second day during time periods with snow cover and always had the impression that the horses used only a fraction of the accessible food. The total area available for foraging

Table 2. *Regression coefficients and analysis of variance statistics from a linear model of dry matter intake (DMI) as a function of its content of nutrients and a periodic annual change*

Fixed effect	Coefficient	s.e.m.	$F_{1,28}$	*P*-value
Crude protein (% of DMI)	1.053	0.236	19.9	<0.001
Nitrogen-free extracts (% of DMI)	0.752	0.240	9.8	0.004
Crude fibre (% of DMI)	0.218	0.206	1.1	0.300
Crude fat (% of DMI)	0.435	1.357	0.1	0.751
Amplitude of unexplained seasonal effect $[\cos(\text{Julian day}/365\times2\pi-\phi)]^a$			13.4	<0.001

[a] No common coefficient computable for this fit of phase and amplitude of a periodic function, d.f.=2,28 for the tested combined term.

further indicated the existence of a surplus of natural food throughout the year. Even during winter, when the horses avoided the lake-side reed belt, an area of on average 0.16 km^2 was left for each horse. In comparison, Duncan has shown in unmanaged Camargue horses that an area of 0.06 km^2 per horse is sufficient to guarantee survival without supplementary food and without long-term damage to the vegetation (Duncan, 1992). Therefore, it is most likely that our horses reduced food intake during winter voluntarily.

A lower DMI might indicate an increased use of body fat reserves to fuel metabolism. The significant decrease in body condition of our study horses during winter suggests the existence of such a shift towards a catabolic metabolism. A seasonal change in body mass is typical for Przewalski horses. It was also found in animals kept under semi-natural conditions in an enclosure in the Schorfheide, Germany (Scheibe and Streich, 2003). The winter mass-loss of our study animals was in the range reported for other large herbivores over-wintering without supplemental food (Giacometti et al., 1997; Knaus and Schröder, 1983; Parker et al., 1993). During summer and autumn, the horses built up the body energy reserves to be consumed during winter and therefore had much higher DMI. The highest DMI values found resembled 5.1% of body mass, and were approximately two times more than the recommended DMI for domestic mares at peak energy demand during early lactation (2.5–3% of body mass) (National Research Council, 1989).

A reduction of DMI during winter and a shift towards body energy reserves as the major metabolic fuel is adaptive if the energetic and time costs of foraging and digestion outweigh the possible energy uptake. A potential mechanism leading to such a response is the control of DMI by food compounds like crude protein indicating quality and digestibility of the forage (Duncan, 1992; Mésochina et al., 1997). Our results suggest that such a mechanism exists in Przewalski horses. A high content of crude protein was the best predictor for high DMI at any time of the year (Table 2). The second nutritional compound stimulating DMI was the content of NFE representing to a certain extent easily digestible carbohydrates.

The differences in DMI found between study years for July and November were presumably because of different weather conditions. The vegetation became particularly dry during July 2003 because of the low amount of precipitation, and dry forage might have been less attractive for the horses. However, the nutrient content of DMI found in July was similar in both years (Fig. 1). Thus, it seems more likely that the lower foraging activity and DMI during July 2003 was a result of the higher heat load experienced by the horses during the day of measurement of DMI in July 2003 (Figs 2, 3; mean air temperature on 23 July 2002, 20.8°C; on 17 July 2003, 24.5°C). In both study years plant growth resumed after the summer drought, although this resumption was considerably more intense in 2003. As a consequence, fresh and green plant material with a more attractive nutrient composition was available for longer during autumn 2003, compared with 2002 (Fig. 1), presumably leading to the higher DMI of November 2003 (Fig. 2).

Changing proportions of nutrients in DMI did not explain the whole range of annual DMI variation. A significant part was clearly seasonal, but food-independent (Table 2; Fig. 2). A candidate cue governing this change is the natural photoperiod presumably entraining an endogenous seasonal rhythm of appetite. It remains an open question as to whether the appetite cycle itself was the regulated entity or whether it reflected the energy demands arising from other seasonal cycles (Barry et

Table 3. *Body masses and simultaneously assessed body-condition scores*

Individual	Date of measurement	Body mass (kg)	Body-condition score
Gobi	26 August 2003	366	3
	28 November 2003	350	3
	02 April 2004	286	1
Urgli	17 October 2003	350	3
	02 April 2004	290	1
Tosca	02 April 2004	277	1
Okasvenja	26 August 2003	241	3
	02 April 2004	208	2
Illmitz	26 August 2003	245	3
	02 April 2004	238	2
	24 May 2004	282	3
Hank	02 April 2004	265	3
Holle	02 April 2004	239	2
	24 May 2004	268	3

al., 1991). However, our results from simultaneously recorded heart rates, subcutaneous body temperatures and behaviour support the latter view. During winter the study horses substantially decreased locomotor activity, energy expenditure for thermoregulation and eventually metabolic rate (Arnold et al., 2006).

Seasonal changes in digestive strategies

In order to maximize the rate of energy uptake from forage of a given quality, animals should retard food passage when feeding on a highly fibrous diet and accelerate it when feeding on food rich in crude protein (Stevens and Hume, 1995). The Przewalski horses studied apparently employed such a strategy. Gut passage time was longer during winter when DMI contained high concentrations of crude fibre, but shorter during the autumn fattening period when relatively high concentrations of crude protein were found in DMI, in both cases improving nutrient absorption. However, multiple regression analysis suggested that gut passage time was rather independent of both the concentrations of nutrients in DMI and total DMI. A likely interpretation of this result is again the existence of an endogenous cycle entrained by the photoperiod that regulates gut passage time [and possibly, gut size as well as nutrient transport capacity (Arnold et al., 2006)] independent of actual forage composition. Such a mechanism can adapt gut function and hence the digestive strategy in a timely manner to meet the highly predictable changes in food quality in a seasonal environment.

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