Importance of nutritional and anti-parasite strategies in the foraging decisions of horses: an experimental test

Geraldine Fleurance, Patrick Duncan, Herve Fritz, Jacques Cabaret and Iain J. Gordon

The primary goal of foraging herbivores is to maximise the net rate of intake of digestible energy (or of a limiting nutrient). However, foraging strategies of herbivores are also sensitive to other selective forces (e.g. predation, parasites), which may modify their choice of feeding patches. Horses feed in spatially complex swards, and allocate their time among patches which vary both in terms of their nutritional benefits, and the risk of parasitism. It has long been suggested that horses allocate time among patches principally in relation to the risk of parasitism, though the nutritional costs and benefits of different foraging choices must play some role too. In this study, we investigated the roles of nutritional and anti-parasite factors in foraging decisions by horses. Six naturally parasitized and six unparasitized two-year-old geldings were allowed to graze from pairs of trays (112×72 cm) with swards at two heights (tall: 52 cm, medium: 15 cm) and two levels of contamination (280 g m^{-2} of faeces, no faeces) in a latin square, pair-wise design with six different choices. In nutritional terms the differences between the swards were slight: the tall sward provided larger bite sizes and higher intake rates ($+36\%$). Feeding motivation was not high, and the faecal stimulus was strong. The horses selected uncontaminated swards when the nutritional benefits were identical, and tall swards in virtually all circumstances. Contamination of the preferred tall sward (i.e. a test of the tradeoff between anti-parasite and nutritional strategies) had little effect on its selection by the horses. Their parasite status also had little effect: both groups selected the tall sward in the tradeoff (though this was only a trend for the non-parasitised group in one analysis). We conclude that patch selection by horses in these particular conditions was driven principally by their nutritional strategy.

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Grasslands are generally a mosaic of patches of different structure, where resources for herbivores vary in abundance and quality. An herbivore foraging at random in such a heterogeneous environment ingests less nutrients than a selective one (Illius and Gordon 1993). Since the rate at which animals can extract nutrients from their environment determines their fitness through its effects on growth, reproduction and condition, maximising the net intake rates of energy and nutrients is often a primary goal of foraging individuals (Owen-Smith and Novellie 1982, Stephens and Krebs 1986, Ungar and Noy-Meir 1988).

However, other processes such as predation also have strong potential effects on individual fitness, and

foraging models now incorporate these (Lima and Dill 1990). Gastrointestinal parasites affect the rates of growth, fecundity and even mortality in mammalian herbivores (Gulland 1992), as a result of an increased loss of nitrogen, damage to gastrointestinal tissue (Poppi et al. 1986, Bown et al. 1991) and loss of appetite (Coop and Kyriazakis 1999). Consequently it could be expected that animals evolve foraging strategies that reduce the risk of parasitism, however, the influence of parasites on the foraging strategies of animals has received little attention (Lozano 1991, Norris and Johnstone 1998).

Herbivores expel the eggs of gastrointestinal parasites in their faeces and areas around faeces are often highly contaminated with infective larvae (Sykes 1987). As mammalian herbivores appear not to be able to detect the gastrointestinal parasites themselves (Cooper et al. 2000), avoidance of faeces could be the mechanism involved in reducing parasite infestation. This has been confirmed by recent work on lambs, whose grazing behaviour led to a reduction of the risk of infection (Hutchings et al. 1998, 1999, 2002, Cooper et al. 2000). The parasite status of these lambs influenced their response to risk: parasitised animals were more sensitive to risk than unparasitized ones. However, it is to be expected that animals' choices will depend on the marginal cost of extra parasites, and this could be less for a parasitised individual than for an unparasitised one, so this result may not be general.

Like other large generalist herbivores, horses use patchy grasslands, and spend most of their feeding time on short lawns. This behaviour is particularly marked in pastures, where they defecate in "latrines" or "eliminative areas" which are avoided for feeding; in extensive grazing systems "eliminative areas" are less marked (Lamoot et al. 2004). The highly selective feeding behaviour of horses at pasture has long been interpreted as an anti-parasite strategy (Taylor 1954), though this theory has never been tested. In spite of its importance as a process influencing the impact of horses on pastures, as well as the well-being of the animals themselves, the factors determining patch choice by horses have not received much attention, and no experimental work has been done at all, unlike in other species (e.g. cattle, Wallis De Vries and Daleboudt 1994).

The aim of this experimental study was to investigate the importance of nutritional and anti-parasite strategies in patch choice by horses. The result will clearly depend on the conditions of the experiment, in particular the benefits and costs associated with the exact quantities of faeces and relative nutritional benefits of the swards. In this experiment there were only slight differences in nutritional value of the swards, and the faecal stimuli were strong. Under these conditions we expected the animals' choices to be driven primarily by their anti-parasite strategy, in particular that the horses:

1) would avoid swards where there is a risk of ingesting infective larvae when the nutritional benefit is equal;
2) would select for swards offering higher nutritional benefits when the parasite risk is equal;
3) when offered a tradeoff between nutritional and anti-parasite strategies (the choice between a sward with a higher nutritional benefit but a high risk of parasite ingestion against a sward with a lower nutritional benefit and no parasite risk), the avoidance of faeces would outweigh the attraction of a sward with a higher nutritional benefit.

The cost of parasitism is likely to increase the animals' nutritional requirements and to change the marginal cost of extra parasites, so two groups of animals, parasitized and unparasitized were used. We expected the two groups to:

4) show different responses to the choices offered of nutritional benefits and risks of parasite infection.

Material and methods

The experiments were conducted between 5 June and 6 July 2001 at a field station of the French National Stud, the Station Expérimentale des Haras Nationaux (Chamberet, France).

Animals

Twelve geldings (two-year-olds, Anglo-Arab and French saddle breeds), born at the station and which had not been treated with anthelminthics since 26 December 2000, were divided into two groups (11 May 2001) balanced for liveweight (mean value $=497 \pm 4$ kg $\pm$ se), origin (2 fathers) and faecal egg counts (FEC) of nematodes. The individuals of the two groups had similar incisor breadths (mean value $\pm$ se) $= 6.9 \pm 0.1$ cm; Mann–Whitney test: U $=26.5$, p >0.05, n $=12$) and were all in good condition for growing horses. From 17 April 2001 to 13 May 2001, all the animals were brought in from pasture every afternoon to familiarise them with the experimental protocol.

One group (parasite-free) was dosed orally with Moxidectin (Equest) every 15 days from 11 May 2001, this anthelminthic is effective for two weeks (Perez et al. 1999), while a group, naturally parasitized with nematodes, received water as a placebo. Moxidectin was chosen because small strongyle nematodes are the main parasites affecting horses at pasture (Herd and Gabel 1990, Love et al. 1999) and were the most abundant at the Chamberet station (80% of the infective larvae at pasture, Fleurance et al. unpubl.). These nematodes have developed high rates of resistance to

all commonly used anthelminthics except the avermectin/milbemycin class (Kaplan 2002). The typical impact of these parasites involves an enteropathy which causes protein loss (Love et al. 1999) but a potential fatal syndrome can occur where large numbers of hypobiotic encysted larvae are released from the mucosa of the large intestine, causing rupture of mucosal cells and other tissue damage with extensive fluid and protein loss ('larval cyathostomosis', Lyons et al. 1999). Moxidectin is not totally effective against hypobiotic early third-stage cyathostome larvae (Xiao et al. 1994), so we did not expect the individuals of the unparasitised group to be entirely free of parasites, but we did expect clear differences in the parasite loads of the two groups. Faecal egg counts (FEC) were used as an index of parasite infection (Round 1969, Cabaret et al. 1998, Craven et al. 1998). A faecal sample from each parasitized and unparasitized animal was examined twice a week during the experiment. We used the McMaster technique modified by Raynaud (1970) (ClNa, d = 1.18–1.2, 360 g l^{-1}) with a minimal detection rate of 15 eggs per gram of faeces and we transformed data as log (FEC+1) before statistical analyses.

The choice tests

Two swards of contrasting height and quality were prepared in a pasture sown in 2000 (0.5 ha; 38% English rye grass *Lolium perenne*, 37% tall fescue *Festuca arundinacea*, 13% cocksfoot *Dactylis glomerata*, 12% white clover *Trifolium repens*). The pasture had not been used by horses since sowing and was, therefore, free of parasites. We maintained a medium height sward (14.9 ± 0.2 cm, se, n = 1716) by fertilisation with ammonitrate at a rate of 100 kg ha^{-1}, and mowing to a height of approximately 2 cm above the soil every 15 days. A taller sward (51.6 ± 0.4 cm, se, n = 1691) was allowed to grow for 60 days after cutting. Both swards were of good quality, their growth stages (measured by NDF concentration and culm densities) were similar and the major difference was that the tall one was nutritionally better (N+), as it provided much larger bite sizes and higher intake rates (Table 1).

The day before the tests, rectangles of the two swards including the top 10 cm of soil and roots were cut to the dimensions of the trays, and wedged in the trays with three horizontal metal bars (5 mm diameter) at the soil surface to prevent the horses from pulling the swards out of the trays. We chose not to allow substantial depletion of the sward during grazing, so after preliminary trials the size of the tray was made large enough to allow the animals to take 100 bites without noticeable depletion.

Each tray was composed of four sub-trays (560 × 360 mm, depth ×108 mm), and the risk of parasitism was introduced by placing a single piece of fresh faeces

Table 1. Sward characteristics. N+ =tall, N− =medium height. Data reported are means ±se. No effect of faecal contamination was apparent on the fibrousness of the grass ingested by horses (NDF: neutral detergent fiber, ADF: acid detergent fiber, cellulose, CP: crude protein; Mann−Whitney, p >0.05). DMIIR: instantaneous intake rate of dry matter on medium and tall swards (test N+P − vs N −P −) is calculated for all individuals as they were no differences between the two treatments (parasitised, parasite-free) one-way anova, p >0.05). The instantaneous intake rate of digestible dry matter (DDMIIR) was calculated as the product of DMIIR (dry matter digestibility); and the same principle was used to calculate the instantaneous intake rate of digestible protein (DPIIR). Within columns, means with different superscripts are significantly different at p <0.05 (one-way Anova).

Sward	Height (cm)	Biomass (g$_{DM}$ m^{-2})	NDF (%DM)	ADF (%DM)	Cellulose (%DM)	CP (%DM)	Tiller density (n m^{-2})	Dry matter content (%)	DMD (%)	Bite size (g$_{DM}$ bite^{-1})	Bite rate (n min^{-1})	DMIIR (g min^{-1})	DDMIIR (g min^{-1})	DPIIR (g min^{-1})
N+	51.6±0.4 [a] (n=1691)	1404.4±49[a] (n=49)	52.1±1.2[a] (n=25)	29.6±0.7[a] (n=25)	29.3±1.0[a] (n=25)	10.5±0.5[a] (n=25)	2449±53[a] (n=144)	32.3±1.2[a] (n=25)	53.2±0.6[a] (n=25)	2.9±0.2[a] (n=14)	12.8±0.6[a] (n=14)	36.2±1.4[a] (n=14)	19.2±0.8[a] (n=14)	2.02±0.08[a] (n=14)
N−	14.9±0.2[b] (n=1716)	371.5±20[b] (n=49)	48.5±0.8[b] (n=25)	23.7±0.6[b] (n=25)	21.8±0.7[b] (n=25)	19.9±0.6[b] (n=25)	2725±57[b] (n=143)	27.2±1.6[b] (n=25)	63.6±0.6[b] (n=25)	0.8±0.1[b] (n=30)	34.2±1.2[b] (n=30)	26.6±2.9[b] (n=30)	16.9±1.8[a] (n=30)	3.36±0.36[b] (n=30)

(70 g, obtained from parasitized animals) centrally in each sub-tray (P+; i.e. 4 per tray). The eggs of the parasites did not have the time to develop into infective larvae (at least 3 days are needed for strongyles, Mfitilodze and Hutchinson 1987) but faeces appear to be used as cues to parasite risk by herbivores (Hutchings et al. 1998, Cooper et al. 2000). We chose the quantity of faeces to approximate the amount occurring in normal «eliminative areas», and used fresh faeces, which are known to be a stronger stimulus than old ones in sheep (Hutchings et al. 1998). Faecal production (per ha) of horses at pasture has been estimated at 740 kg dry matter ha^{-1} yr^{-1} (Edwards and Hollis 1982), with a mean dry matter content of 19% in faeces (Fleurance et al. 2001, Ménard et al. 2002), the maximum fresh feces density would have been 390 g m^{-2} in the study of Edwards and Hollis (1982). In a preliminary trial (15 May 2001–30 May 2001) we found that significant rejection was produced by 280 g m^{-2}, which was close to the amount of faeces, (fresh and old) calculated from the results of Edwards and Hollis (1982). We, therefore, used this amount in the experiment (i.e. 70×4 g m^{-2}).

We used the following four treatments: N+P+, N+P−, N−P+, N−P− (where N+ =tall, high nutrient benefit; N− =medium, low nutrient benefit; P+ = contaminated with faeces; P− =no faeces), and used a pairwise, rather than a multiple choice since we wanted to test the tradeoff. The treatments were combined in these six choices: (1) N−P− vs N+P−: a height choice with no risk of parasitism, (2) N−P+ vs N+P+: a height choice with the risk of parasitism constant, (3) N−P− vs N−P+: variable parasite risk in the nutritionally poorer sward, (4) N+P− vs N+P+: variable parasite risk in the better sward, (5) N−P+ vs N+P−: the control, lower nutrient benefits with high risk vs the opposite, (6) N−P− vs N+P+: a choice where the horses had to tradeoff nutritional benefits against a risk of parasitism.

Experimental protocol

The horses of both groups were maintained at pasture together, and brought indoors to individual boxes just before the beginning of the tests at 13:30 h. For the first three days of the experiment, we starved the animals for two hours to ensure that they ate from the trays, but this was stopped as it was unnecessary. In the analyses we tested for an effect of fasting by incorporating a dummy variable into the model.

The experiment consisted of 144 tests (12 animals $\times 6$ choices $\times 2$ replicates). We presented the trays to the animals on a metal table (1 m high, to prevent trampling) with the trays 5 cm apart. The animals were allowed to graze from the trays either until they taken 100 bites, or 20 min had elapsed. To control for lateral biases, the position of the tray with the faeces was always on the right between 5–20 June 2001 (first session); it was reversed between 21 June and 6 July 2001 (second session). This design did not allow the 'session' $\times$ 'position' interaction to be tested because these two factors were confounded.

Within groups and sessions each of the six individuals was presented with each of the six tests twice, in sequences which differed between animals. Three parasitized and three unparasitized animals were tested every day, on the six choices. Two horses (one parasitized, one unparasitized) were brought from their individual boxes to the two test boxes (A, B) and the order of passage of the three pairs tested was alternated on each day of test. During the first session, we tested parasitized and unparasitized animals respectively in boxes A and B and this was reversed for the second session.

Measurements

Prior to each test, we weighed each tray (sum of the four subtrays) and we estimated the mean sward height from 12 measurements per tray (6 on each diagonal) using the HFRO sward stick (Barthram 1985). Tiller density was measured inside a 25×25 cm square placed at chance in one randomly selected sub-tray. We recorded the number of bites taken from each tray, and the order of the sequences of bites taken from the trays. We recorded the time spent feeding (biting, chewing or swallowing) on each tray for each test, and the bite rate was calculated as the number of bites/time spent feeding. We calculated the mean grazed height (height above the soil surface) by taking 20 measures of the height of the remaining grass where bites had been taken. An extra tray from each sward was used in each test to measure the mass loss resulting from evapo-transpiration. We kept these trays in the test area and weighed them at the same time as the trays used by the horses. The mass loss resulting from evapo-transpiration during the test was subtracted from the total mass loss for each sward tray, to give herbage consumption. This was then divided by the number of bites taken from the tray to give the mean bite size (g wet weight).

Each day, we used one tray of each of the swards (N− and N+) to determine the dry matter content (DM), biomass and quality of the swards. In two of the four subtrays, we cut the sward to the mean grazing height measured the day before, contaminated and uncontaminated treatments separately. The two samples were used to determine the quality of the sward ingested by the animals, crude protein (CP, Kjeldahl method), cellulose (Weende method), cell walls (neutral detergent fibre, NDF) and acid detergent fiber (ADF). Dry

matter digestibility (DMD, a proportion) was estimated from the crude protein content of each sward type (CP, g kg_{DM}^{-1}) as: $DMD = 0.417 + 0.0011 \times CP$ (r.s.d. $= 0.043$, $r^2 = 0.525$, n $= 27$; Mésochina et al. 1998). We cut the vegetation to ground level in a 15×15 cm square from the remaining two subtrays to determine dry matter content of the sward and biomass offered.

To determine the effect of sward type on dry matter instantaneous intake rate (DMIIR, g min^{-1}) and digestible dry matter instantaneous intake rate (DDMIIR, g min^{-1}), we estimated intake rates using the following equations for each sward type:

$$DMIIR_i = B_i \times S_i \times pDM_i$$

$$DDMIIR_i = DMIIR_i \times DMD_i$$

where $B_i =$ mean bite rate from bouts of at least 10 consecutive bites taken from sward i (bites min^{-1} in the long or the medium sward, and $S_i =$ mean bite size for sward type i (g $bite^{-1}$, wet mass) and $pDM_i =$ proportion of dry matter in the sward type i.

We determined digestible protein instantaneous intake rate, $DPIIR_i$, from sward i as:

$$DPIIR_i = DDMIIR_i \times CP_i$$

where $CP_i =$ proportion of crude protein in the vegetation ingested by the animals from the sward type i.

Statistical analyses

Proportions were arcsine transformed prior to their use in the statistical analyses (Sokal and Rohlf 1995). The assumptions of analysis of variance were met, so these were used for simple comparisons between swards for their characteristics, and between parasitized animals for their FEC.

To test for the factors determining the selection of swards, the effects of the variables were measured using a residual maximum likelihood procedure (REML, Patterson and Thompson 1971), in a mixed covariance analysis (the backwards variant of the MIXED procedure, SAS 1999). This analysis allows the specific effects of covariates and factors, the "fixed variables", to be tested while accounting for the variance explained by potentially confounding variables which are included as "random variables". This prevents the variance due to these random variables being erroneously incorporated in the error term of the analysis (a typical example is the effects of "individuals"). For the acceptance of the tests (i.e. 100 bites taken in less than 20 minutes) we included Test (= the six modalities), Parasite_status (parasitized/unparasitized) and Test × Parasite_status as fixed effects, and Individuals as a random effects.

To test for the effects of the different variables and their interactions on sward selection by the horses, we also used the same procedure on two sub-sets of the experiments, those where sward quality varied (i.e. $N-P-$ vs $N+P-$, $N-P+$ vs $N+P+$, $N-P+$ vs $N+P-$, $N-P-$ vs $N+P+$) and those where parasite risk varied ($N-P-$ vs $N-P+$, $N+P-$ vs $N+P+$, $N-P+$ vs $N+P-$, $N-P-$ vs $N+P+$). The relative amount of grass eaten in the poorer vs the richer swards (measured as the log ratio of dry matter eaten in $N-/N+$) or the safe vs risky swards ($P-/P+$) was used as the dependent variable. For the trials where sward varied (i.e. $N-$ and $N+$) the following variables were tested as fixed effects: Fasting, $N+$_Laterality (right tray, left tray), $P+$_Laterality (none, right tray, left tray, both trays), First_bite_location (right tray, left tray), First_bite_location $\times N+$_Laterality, First_bite_location $\times P+$_Laterality, First_bite_location $\times P+$, $P+$ (no $P+$, $P+$ on $N-$, $P+$ on $N+$, $P+$ on both swards), Parasite_status, Parasite_status $\times P+$. The model included Day, Individual, Period_of_day (first, second or third test of the day) and Individual $\times$ Period_of_day as random effects.

For the tests where parasite risk varied ($N-P-$ vs $N-P+$, $N+P-$ vs $N+P+$, $N-P-$ vs $N+P+$, $N+P-$ vs $N-P+$), we used the same procedure to estimate the fixed effects of Fasting, $P+$_Laterality (right tray, left tray), $N+$_Laterality (none, right tray, left tray, both trays), First_bite_location (right tray, left tray), First_bite_location $\times P+$_Laterality, First_bite_location $\times N+$_Laterality, First_bite_location $\times N+$, $N+$ no $N+$, $N+$ on $P-$, $N+$ on $P+$, $N+$ on both trays), Parasite_status, Parasite_status $\times N+$. This model included the same random effects as the model above.

We tested for sward selection for each pairwise test separately, by testing the null hypothesis that the amount of grass consumed from the two treatments did not differ. For example, for the test $N-P-$ vs $N-P+$ we calculated the ratio of the amount of dry matter consumed from $N-P-$/amount of dry matter consumed from $N-P+$ The distribution of the values of the log of these ratios did not differ significantly from a normal distribution, so we tested the significance of departures from zero using t-tests (Elston et al. 1996) The sample size is the number of individuals and not the number of tests, to avoid pseudoreplication.

The effects of the sward type ($N+$, $N-$), parasite risk ($P+$, $P-$) and parasite status of the animals on the grazing height above the soil surface were tested using the same procedure. The model included Fasting, Sward type, Parasite_risk, Parasite_status, Sward_type $\times$ Parasite_risk, Sward_type $\times$ Parasite_status, Parasite_risk $\times$ Parasite_status, Sward_type $\times$ Parasite_risk $\times$ Parasite_status as fixed effects and Day. Individual, Period_of_day and Individual $\times$ Period_of_day as random effects.

Results

Parasite status of the animals

The faeces of the unparasitized animals were free of nematode eggs throughout the experiment. No clinical signs of any disease were observed in any animal during the experiment, though in the infected group some horses had high FEC counts. These counts varied strongly (34–1435 eggs per gram of wet faeces, total strongles, one-way anova: $F_{5,47} = 36.138$, $p < 0.0001$), suggesting that the parasite status of the parasitised group was very variable in spite of the fact that the animals had had the same treatment since birth.

Test acceptance

The horses accepted the test procedure well: 131/144 tests lasted less than 20 minutes (four parasitised and three unparasitised horses took <100 bites in one or two tests). There was a slight but significant effect of their parasite status on their feeding motivation (REML, $p = 0.047$): the unparasitised group took on average 99 bites, the parasitised 95 per test. The other variables (Choice [six modalities], Individual [12 individuals]) and the interactions had no effect. For the analyses below, we retained the tests in which the animals took at least 67 bites ($2/3 \times 100$; 8/13 tests) and discarded the remaining five. Of the total number of choices × individuals possible (72), we had at least one replicate for 71 tests.

Sward characteristics

The medium height swards had a third of the biomass (and height), nearly twice the protein content, and were less fibrous than were the tall swards, however, their growth stages were similar (Table 1). The major difference was that bite size on the medium swards was less than a third of that on the tall swards, and though the animals compensated to some extent by increasing their bite rates, the instantaneous dry matter intake rate (DMIIR) on the medium sward was 36% lower. As the predicted digestibility of the medium swards was higher, the instantaneous digestible dry matter intake rate (DDMIIR) was not significantly different from that on the tall swards.

The instantaneous digestible protein intake was much higher in the short swards, but both swards were of such good quality that the horses could easily cover their digestible protein and energy requirements on either. The digestible protein requirement of growing horses of 500 kg is about 500 g day^{-1} (Jarrige and Tisserand 1984); the digestible protein intake on the tall swards

would have been 1890 g day^{-1}, on the medium ones it would have been even higher (assuming they would graze for 65% of the 24 h, which is normal for horses, Duncan 1992, $0.65 \times 24 \times 60$ min $\times 2.02$ g min^{-1}). The difference in nutritional value between the swards was, therefore, slight, the main one being that dry matter intake rates were higher on the tall swards.

Selection of swards when the risk of parasitism varied

In the four tests where the risk of parasitism varied, the only significant effect was N+ (its modalities were: no N+, N+ on P−, N+ on P+, N+ on both trays $F_{3,43} = 18.1$, $p < 0.0001$). The proportion of food taken from 'no risk' trays (P−) therefore depended on the nutritional context. For the tests where the two swards were the same, most of the horses preferred no-risk swards when these were nutritionally poorer (N−, Fig. 1a; 11/12 horses, Fig. 2a); for the swards with the higher nutritional content the trend went in the same direction (N+, Fig. 1b; 7/12 horses, Fig. 2b) but was non-significant. The first prediction was, therefore, confirmed, at least on the poorer swards.

Selection of swards when their quality varied

In the four tests where the sward quality varied, the effect of the interaction Parasite_status × P+ (no P+, P+ on N−, P+ on N+, P+ on both trays) was just significant ($F_{3,36} = 3.62$, $p = 0.0222$; the other variables and their interactions were not, see Statistical analyses). When the parasite risk was constant (tests N−P− vs
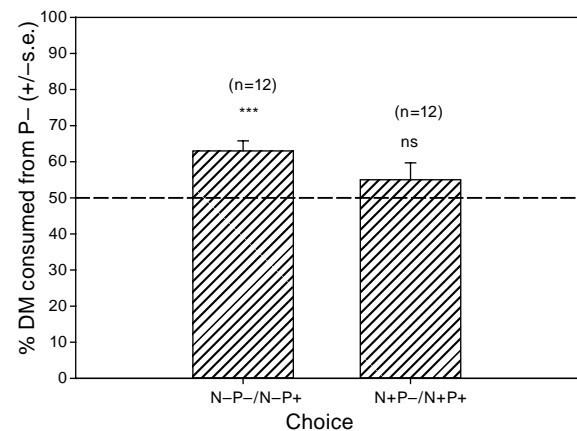

Fig. 1. Patch selection by the horses of both groups, given choices between swards of equal quality (N−: nutritionally poorer, N+: nutritionally better) but variable parasite risk (P+ =contaminated, P− =uncontaminated). Significant selection is noted (t-tests, *p <0.05, **p <0.01, ***p <0.001, n = number of horses).
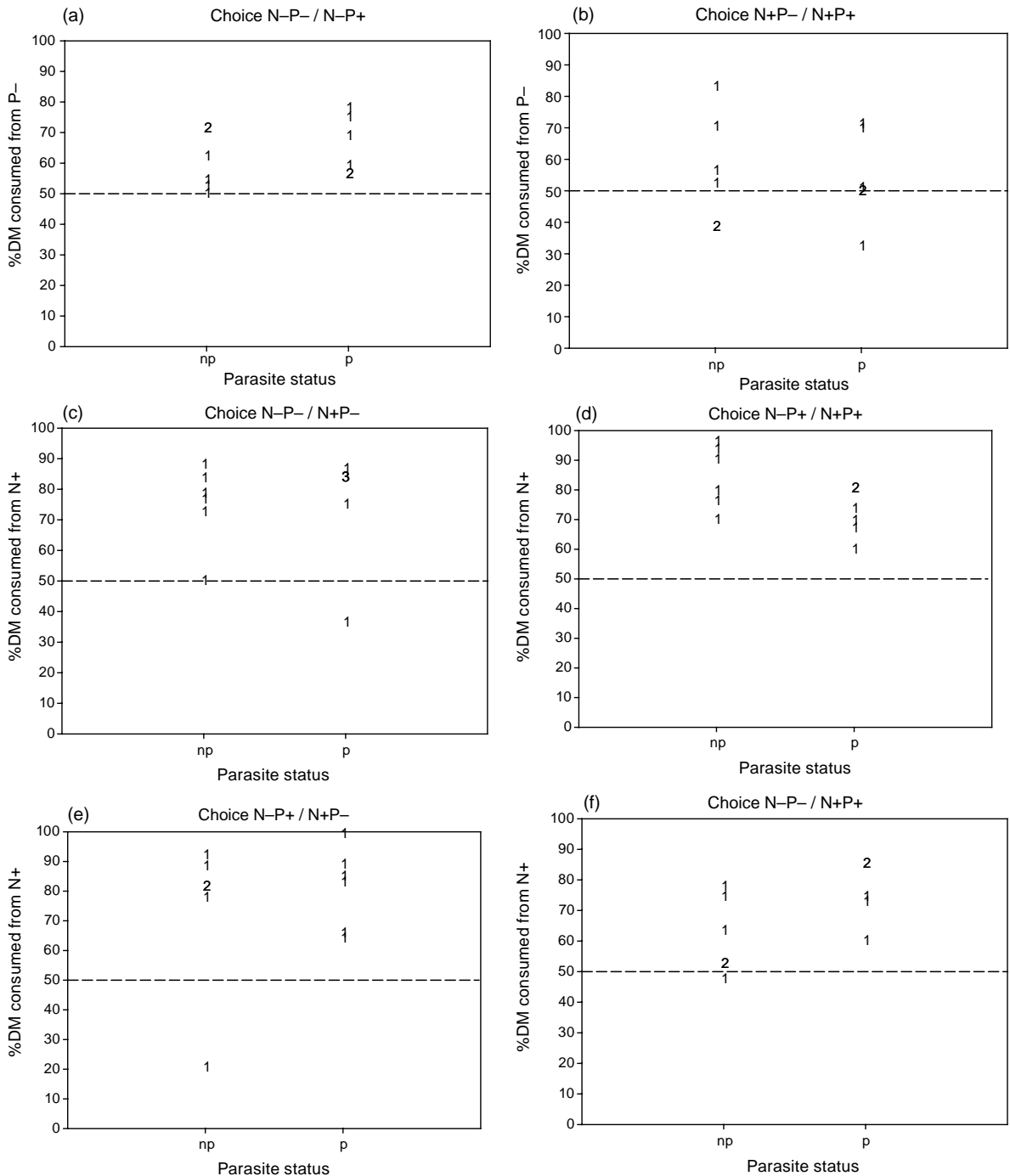
Fig. 2. Feeding site selection by the individual horses (np: unparasitised, p: parasitised) for each of the six tests. The points are the means of the two replicates per individual, except for 16 of the 71 cases where there was only one replicate.

N+P− and N−P+ vs N+P+), the horses ate more from the tall swards (Fig. 3) in virtually all tests (10/12 horses in N−P−/N+P−, 12/12 horses in N−P+/N+P+, Fig. 2c, d). Both groups of horses therefore selected N+ consistently, in accordance with the second prediction.

Tradeoff between anti-parasite and nutritional strategies

When both parasite risk and nutritional quality varied, all but one of the horses selected the nutritionally better, no-risk swards against poorer, risky swards (i.e. control
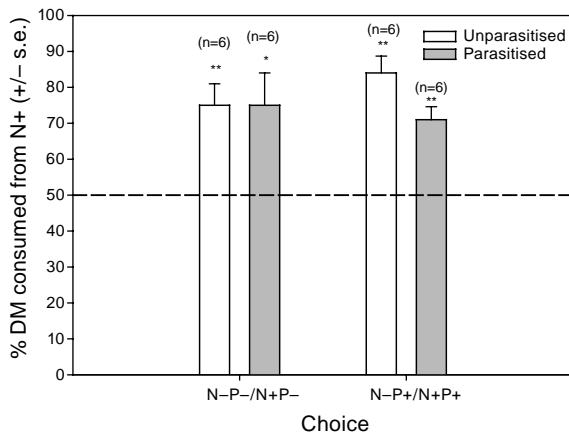
Fig. 3. Feeding site selection by the horses given choices between swards of variable quality (N −: nutritionally poorer, N+: nutritionally better) with an equal risk of parasitism (P+ =contaminated, P − =uncontaminated). Significant selection is noted (t-tests, *p <0.05, **p <0.01, *** p <0.001).

test, N+P − vs N −P+, Fig. 2e, Fig. 4). In the test with a tradeoff, where they were offered nutritionally better but risky swards (N+P+), the parasitised horses clearly selected for the richer grass in spite of the parasite risk (N+P+, 5/5 horses Fig. 2f, Fig. 4). The unparasitised ones showed the same trend, but it was not significant (5/6 horses Fig. 2f, Fig. 4a), hence the Parasite_status ×P+ interaction in the REML for the tests where the nutritional quality of the swards varied. This difference between the two groups of horses with different parasite status, significant at P =0.022, was strongly influenced by one unparasitised individual which ate a great deal more from the poor sward (80%) in the control test (Fig. 2e); unfortunately the replicate for this test failed.

The difference between the two groups of different parasite status did not appear in the test on the four choices where the risk of parasitism varied: the horses selected significantly the nutritionally better sward (N+), whatever the risk of parasitism (Fig. 4b).

To test the robustness of this result we conducted three further analyses:

1) the amount of grass eaten was replaced by time spent eating: there was no difference between parasitised and unparasitised individuals,
2) Parasite_status was replaced with FEC as a covariate, as there was considerable variation among parasitised animals in their FEC (and presumably, the number of parasites they hosted): the interaction was not significant, and
3) separate REMLs were run for each choice: the difference between parasitised and unparasitised individuals did not show the same trend in choices other than the tradeoff (i.e. the parasitised animals sometimes ate less N+ than the non parasitised ones, Fig. 2d).
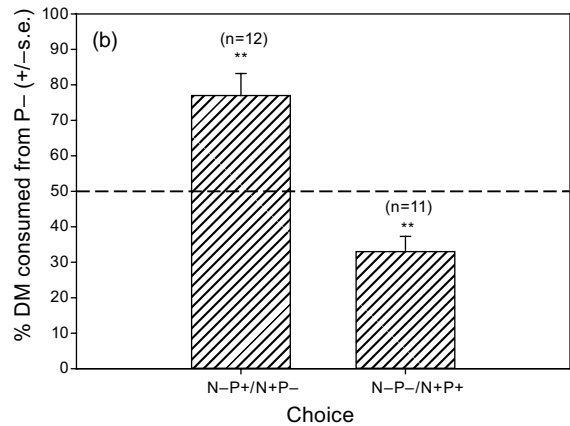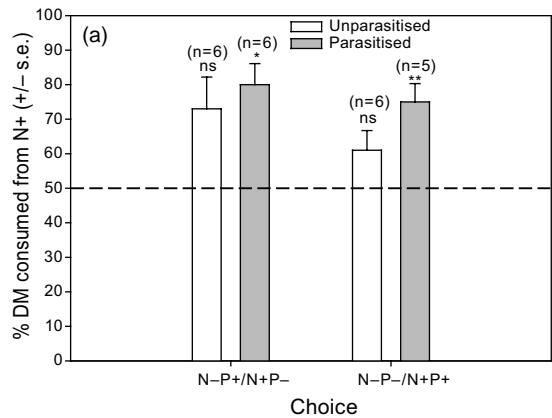

Fig. 4. Feeding site selection by the horses: (a) the proportion of food taken from the nutritionally better patch in the four tests where patch quality varied (N −: nutritionally poorer, N+: nutritionally better), and (b) the proportion of food taken from the patch of lower parasite risk in the four tests where the parasite risk varied (P+ =contaminated, P − =uncontaminated). The two groups of horses (parasitised, unparasitised) are combined here because there was no significant effect of parasite status in this analysis. Significant selection is noted (t-tests, *p <0.05, **p <0.01, ***p <0.001).

Further, there was no difference in grazing height between parasitised and unparasitised individuals. We, therefore, conclude that variations in the parasite status of the animals had little influence on their choices. The great majority of horses selected tall swards, in every test, though the degree of the selection was quite variable (Fig. 2), probably due to random variation in these tests, whose duration was short.

Grazing height

The height at which the horses grazed the sward was affected only by sward type (REML, $F_{1,238} =660.7$, p < 0.0001). The horses grazed higher in the taller swards compared to the medium height ones (18.9 ± 0.5 cm above the soil vs 6.8 ± 0.1 cm). When grazing medium or tall swards contaminated with faeces, the animals did not

graze significantly higher in contaminated than in uncontaminated swards of the same height (REML, $F_{1,237} = 2.86$, p = 0.09) (H − F −: 6.4 ± 0.1 (se) cm, H − F +: 7.2 ± 0.2 cm; H + F −: 18.6 ± 0.8 m, H + F +: 19.2 ± 0.5 cm). There was not even a suggestion that the parasite status of the animals had an effect on the grazing height (REML, $F_{1,236} = 0.32$, p = 0.57).

Discussion

The feeding motivation of the horses was slightly affected by their parasite status, since parasitised animals took about 5% fewer bites in the tests than unparasitised ones. It is well known that parasitism can affect feeding motivation in horses (Coop and Kyriazakis 1999), however since there was no effect of the choice offered on feeding motivation, we do not expect that this decrease in motivation had any effect on the results of the comparisons between swards (tall, medium) or parasite risk (faeces, no faeces).

The first expectation, that the horses would avoid swards contaminated with faeces was confirmed in the medium height swards, when the nutritional benefit of chosing between contaminated and uncontaminated was equal. There was a similar but non-significant trend in the tall swards (tests 1 and 2, Fig. 2). Excessively strong feeding motivation is unlikely to explain the weak effect in tall grass choice, since the horses were feeding in nutritionally comfortable conditions and had been fasted before the trials briefly or not at all (there was no detectable difference between the choices they made in these two periods). In view of the studies conducted in ruminants, sight and smell are likely to be the principal senses involved in the recognition of faeces (Arnold 1966, Dohi et al. 1991) and the smell stimuli used here are likely to be stronger than the stimuli at pasture, so the lack of an effect in the tall swards is unlikely to be due to a weak stimulus.

Both swards were of such good quality that the horses could cover their digestible protein and energy requirements easily on either. The bite sizes and intake rates were higher in the tall sward, and the horses selected this one when the risk of parasite ingestion was equal between the two swards (Fig. 3). The second expectation is, therefore, confirmed, i.e. that the horses would select the sward which offered the greater nutritional benefit. When offered tall, uncontaminated swards, the horses, except one in one test (Fig. 2e), unsurprisingly ate more from N + P − than from N − P + (75%, Fig. 4). When offered N − P − vs N + P + there was a tradeoff between nutritional benefit and parasite avoidance: the horses ate more from the nutritionally better swards, in spite of the risk of parasitism, though this was only a trend for unparasitised individuals in the stastical test on the four choices where the type of sward varied (Fig. 4). In the tradeoff choice, virtually all the horses, whatever their parasite status, selected N + P + (Fig. 2e). These results contradict the third expectation that avoidance of a strong faecal stimulus would outweigh the attraction of a sward with slightly greater nutritional benefits. Overall, selection for the nutritionally better swards overrode selection for swards with no parasite risk in this experiment. This did not result from a high motivation to feed, as the animals were not fasted for most of the experiments.

The interaction between parasite status of the horses and risk could suggest that the parasite status of these horses influenced their choices. This result was not robust as it was obtained in only for one of the two REML analyses, so further experimental comparisons of the influence of their parasite load on the choice of feeding sites by horses are needed. Provisionally we conclude that as in reindeer (van der Wal et al. 2000), but in contrast to lambs with no experience of parasites (Hutchings et al. 1998) the parasite load does not appear to affect their choice of feeding sites in horses. This could be because these animals have no means of evaluating the level of parasites in their gastointestinal tracts, which is unlikely as lambs appear to be able to do so. An alternative explanation is that the horses respond to their history of infestation, over the last few months. Differences among individuals in their experience of parasites could have been the source of the differences in the selection for tall swards in the tradeoff choice, where parasitized individuals took greater risks in order to obtain nutritional benefits than did non-parasitized ones. This experiment should, therefore, be repeated with foals which have never been infected, using two treatments, one free of parasites and the other dosed with a high dose of parasites (Hutchings et al. 1998). This finding does not support the traditional view that the choice of feeding sites by horses is driven primarily by an anti-parasite strategy, but this conclusion is clearly valid only for the specific context of this experiment, with its particular set of costs and benefits associated with the selection of the swards we provided. The conclusions of this experiment clearly need testing in other situations: horses spend most of their time feeding on short grass (e.g. 5–16 cm in summer and 0–4 cm in autumn, Fleurance et al. 2001, Ménard et al. 2002) and on nutritionally poorer swards, so it would be of interest to test their behaviour where the nutritionally better treatment is a short sward, and in contexts where their energy/protein requirements are more difficult to cover. It may be that the costs associated with gastro-intestinal parasites are low compared to the cost of selecting nutritionally sub-optimal food in horses. For a parasite, finding a host is essential to its survival and selection should favour parasites whose costs to their hosts are moderate, below the threshold where selection of an efficient mechanism would be triggered (Combes 2001).

Pathogenicity of small strongyles is low, except when they are present in high numbers (Drudge and Lyons 1966) and this could explain why the horses did not avoid these parasites more strongly.

In this study the horses selected swards with greater nutritional benefits strongly even though the differences between swards in their nutritional benefits were slight. This preference persisted when the swards offering the greater nutritional benefit presented a parasite risk. Grazing decisions by these horses seem, therefore, to have been driven primarily by their nutritional strategy. Again, this conclusion is clearly valid only for the specific context of this experiment, with its particular set of costs and benefits associated with the selection of the swards we provided. However, the fact that the difference in nutritional quality between the two swards was slight, and the density of fresh faeces high compared to the usual conditions at pasture means that the conclusion that horses favour nutritional benefits over anti-parasite ones may well be robust.

References

Arnold, G. W. 1966. The special senses in grazing animals. I. Sight and dietary habits in sheep. – Aust. J. Agric. Res. 17: 531–543.

Barthram, G. T. 1985. Experimental techniques: the HFRO sward stick. – Bienn. Rep. 1984–85, 29–30. Hill Farming Res. Org., Penicuik, UK.

Brown, M. D., Poppi, D. P. and Sykes, A. R. 1991. The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. – Aust. J. Agric. Res. 42: 253–267.

Cabaret, J., Gasnier, N. and Jacquiet, P. 1998. Faecal egg counts are representative of digestive-tract strongyle worm burdens in sheep and goats. – Parasite 5: 137–142.

Combes, C. 2001. Parasitism – the ecology and evolution of intimate interactions. – Univ. of Chicago Press.

Cooper, J., Gordon, I. J. and Pike, A. W. 2000. Strategies for the avoidance of faeces by grazing sheep. – Appl. Anim. Behav. Sci. 69: 15–33.

Coop, R. L. and Kyriazakis, I. 1999. Nutrition–parasite interaction. – Vet. Parasitol. 84: 187–204.

Craven, J., Bjorn, H., Henriksen, S. A. et al. 1998. Survey of anthelmintic resistance on Dansih horse farms, using 5 different methods of calculating faecal egg count reduction. – Equine Vet. J. 30: 289–293.

Dohi, H., Yamada, A. and Entsu, S. 1991. Cattle feeding deterrents emitted from cattle faeces. – J. Chem. Ecol. 6: 1197–1203.

Drudge, J. H. and Lyons, E. T. 1966. Control of internal parasites of the horse. – J. Am. Vet. Ass. 148: 378–383.

Duncan, P. 1992. Horses and grasses: the nutritional ecology of equids and their impact on the Camargue. – Springer-Verlag.

Edwards, P. J. and Hollis, S. 1982. The distribution of excreta on New Forest grassland used by cattle, ponies and deer. – J. Appl. Ecol. 19: 953–964.

Elston, D. A., Illius, A. W. and Gordon, I. J. 1996. Assessment of preference among a range of options using log ratio analysis. – Ecology 77: 2538–2548.

Fleurance, G., Duncan, P. and Mallevaud, B. 2001. Daily intake and the selection of feeding sites by horses in heterogeneous wet grasslands. – Anim. Res. 50: 149–156.

Gulland, F. M. D. 1992. The role of nematode parasites in Soay sheep (*Ovies aries* L.) mortality during a population crash. – Parasitology 105: 493–503.

Herd, R. P. and Gabel, A. A. 1990. Reduced efficacy of anthelmintics in young compared with adults horses. – Equine Vet. J. 22: 164–169.

Hutchings, M. R., Kyriazakis, I., Anderson, D. H. et al. 1998. Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. – Anim. Sci. 67: 97–106.

Hutchings, M. R., Kyriazakis, I., Gordon, I. J. et al. 1999. Tradeoffs between nutrient intake and faecal avoidance in herbivore foraging decisions: the effect of animal parasitic status, level of feeding motivation and sward nitrogen content. – J. Anim. Ecol. 68: 310–323.

Hutchings, M. R., Gordon, I. J., Kyriazakis, I. et al. 2002. Grazing in heterogeneous environments: infra- and supra-parasite distributions determine herbivore grazing decisions. – Oecologia 132: 453–460.

Illius, A. W. and Gordon, I. J. 1993. Diet selection in mammalian herbivores: constraints and tactics. – In: Hughes, R. N. (ed.), Diet selection. An interdisciplinary approach to foraging behaviour, pp. 157–181.

Jarrige, R. and Tisserand, J. L. 1984. Métabolisme, besoins et alimentation azotés du cheval. – In: Jarrige, R. and Martin-Rosset, W. (eds), Le cheval: reproduction, sélection, alimentation, exploitation. INRA, Paris, pp. 277–302.

Kaplan, R. M. 2002. Anthelmintic resistance in nematodes of horses. – Vet. Res. 33: 1–17.

Lamoot, I., Callebaut, J., Degezelle, T. et al. 2004. Eliminative behaviour of free-ranging horses: do they show latrine behaviour or do they defecate where they graze? – Appl. Anim. Behav. Sci. 86: 105–121.

Lima, S. L. and Dill, L. M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. – Can. J. Zool. 68: 619–640.

Love, S., Murphy, D. and Mellor, D. 1999. Pathogenicity of cyathostome infection. – Vet. Parasitol. 85: 113–122.

Lozano, G. A. 1991. Optimal foraging theory: a possible role for parasites. – Oikos 60: 391–395.

Lyons, E. T., Tolliver, S. C. and Drudge, J. H. 1999. Historical perspective of cyathostomes: prevalence, treatment and control programs. – Vet. Parasitol. 85: 97–112.

Ménard, C., Duncan, P., Fleurance, G. et al. 2002. Comparative foraging and nutrition of horses and cattle in European wetlands. – J. Appl. Ecol. 39: 120–133.

Mésochina, P., Martin-Rosset, W., Peyraud, J. L. et al. 1998. Prediction of the digestibility of the diet of horses: evaluation of faecal indices. – Grass Forage Sci. 53: 189–196.

Mfitilodze, M. W. and Hutchinson, G. W. 1987. Development and survival of free-living stages of equine strongyles under laboratory conditions. – Vet. Parasitol. 33: 121–133.

Norris, K. and Johnstone, I. 1998. The functional response of oystercatchers (*Haematopus ostralegus*) searching for cockles (*Cerastoderma adule*) by touch. – J. Anim. Ecol. 67: 329–346.

Owen-Smith, N. and Novellie, P. 1982. What should a clever ungulate eat? – Am. Nat. 119: 151–178.

Patterson, H. D. and Thompson, R. 1971. Recovery of inter-block information when block sizes are unequal. – Biometrica 58: 545–554.

Perez, R., Cabezas, I., Garcia, M. et al. 1999. Comparison of pharmacokinetics of moxidectin (Equest) and ivermectin

(Eqvalan) in horses. – J. Vet. Pharmacol. Therapeutics 22: 174–180.

Poppi, D. P., MacRae, J. C., Brewer, A. et al. 1986. Nitrogen transactions in the digestive tract of lambs exposed to the internal parasite, *Trichostrongylus colubriformis*. – Brit. J. Nutr. 55: 593–602.

Raynaud, J. P. 1970. Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. – Ann. Pathol. Humaine Comparée 85: 321.

Round, M. C. 1969. The prepatent period of some horse nematodes determined by experimental infection. – J. Helminthol. 18: 185–192.

SAS Institute 1999. SAS/STAT user's guide, Cary, USA.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry, 3rd ed. – W. H. Freeman Co.

Stephens, D. W. and Krebs, J. R. 1986. Foraging theory. – Princeton Univ. Press.

Sykes, A. R. 1987. Endoparasites and herbivore nutrition. – In: Hacker, J. B. and Ternouth, J. H. (eds), Nutrition of herbivores. Academic Press, pp. 211–232.

Taylor, E. L. 1954. Grazing behaviour and helmintic disease. – Brit. J. Anim. Behav. 2: 61–62.

Ungar, E. D. and Noy-Meir, I. 1988. Herbage intake in relation to availability and sward structure: grazing processes and optimal foraging. – J. Appl. Ecol. 25: 1045–1062.

van der Wal, R., Irvine, J., Stien, A. et al. 2000. Faecal avoidance and the risk of infection by nematodes in a natural population of reindeer. – Oecologia 124: 19–25.

Wallis De Vries, M. F. and Daleboudt, C. 1994. Foraging strategy of cattle in patchy grassland. – Oecologia 100: 98–106.

Xiao, L., Herd, R. P. and Majewski, G. A. 1994. Comparative efficacy of moxidectin and ivermectin against hypobiotic and encysted cyathostomes and other equine parasites. – Vet. Parasitol. 53: 83–90.

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