Gastrointestinal nematodes and dietary fibre: Two factors to consider when using FN for wildlife nutrition monitoring

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Gastrointestinal nematodes and dietary fibre: Two factors to consider when using FN for wildlife nutrition monitoring

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ABSTRACT

Faecal nitrogen (FN) – the combination of metabolic nitrogen and residual food nitrogen – has been used as a proxy for diet quality in wild and domestic ruminants for over half a century. However, a common misconception in some of these studies is that FN is a direct proxy for dietary N, in spite of experimental evidence that links FN to general diet digestibility. Additionally, gastrointestinal nematodes (GIN) can alter N metabolism and increase FN by various mechanisms. To clarify the role of dietary N, fibre and GIN as a factor in FN excretion, 10 naturally parasitised sheep were fed two different isocaloric diets (LPF: low-protein, low-fibre; HPF: high-protein, high-fibre). One month after these diets began, a single anthelmintic treatment was applied to remove GIN, after which the sheep were kept on the same diet for an additional 2 weeks. Throughout the experiment, individual faecal samples were obtained to estimate both FN and GIN intensity (using faecal egg counts, FEC). In addition, two blood samples were taken before and after deworming to measure serum total protein concentrations (TP) as a proxy for protein absorption. In spite of the difference in dietary protein, FN was higher on an LPF diet, supporting the overall digestibility concept. The influence of GIN on FN was later revealed by the anthelmintic treatment, which led to a decrease of FEC and FN in both dietary groups. Serum total protein showed a slight but non-significant increase in both groups after the anthelmintic treatment. Our study supports not only the concept that FN is a proxy for diet digestibility, and not directly for dietary N, but also that gastrointestinal nematodes limit its use as a proxy for diet quality in ruminants, especially under high parasite loads (e.g., 1000 faecal eggs per gram of faeces). Such limitations should be considered before using FN for wildlife nutrition monitoring. Some recommendations are given to avoid misinterpretations.

1. Introduction

For over half a century, faecal indices have been used as a proxy for food quality in ruminants. One of the best known is faecal nitrogen (FN), an index that was initially proposed as a proxy for metabolic N in rats in 1934 (Schneider, 1934), and 1 year later accepted as an excellent indicator of the nutritional status of a wide variety of mammals (Schneider, 1935).

The use of FN as a proxy for metabolic nitrogen is based on the following formula:

$$FN = MFN + FRFN$$

where FN is the combination of metabolic (MFN) and food residue nitrogen (FRFN) excreted in faeces (Schneider, 1935). In many

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animals, there is a correlation between dietary N and FN, a fact that has favoured its wide use as a proxy for diet crude protein in livestock since the 1940s (Raymond, 1948). Even while the influence of secondary plant compounds, a common constraint on this relationship, is well understood (Leslie et al., 2008), one cannot always assume that dietary protein is directly reflected in the faeces. However, in herbivores, FRFN represents only a minor fraction of FN; the major fraction of FN consists of MFN (Mason and Frederiksen, 1979; Van Soest, 1994; Schwarm et al., 2009; Steuer et al., 2014). As a concentration, FN will be higher if there are less indigestible substances in the diet (e.g., indigestible fibre components) and more substrates on which intestinal microbes can grow (e.g., digestible fibre components and other carbohydrates). Because FRFN is mostly associated with indigestible fibre (Van Soest, 1994), FN will decrease if FRFN increases due to the increased faecal fibre concentration and hence reduced overall digestibility (Schwarm et al., 2009).

Therefore, FN in herbivores should be considered a proxy for diet quality linked to overall diet digestibility (Clauss et al., 2013), which again is often, but not necessarily, higher in diets of higher N. Use of FN as a proxy for digestibility was experimentally validated in domestic and wild ruminants (Lancaster, 1949; Robbins et al., 1987a; Lukas et al., 2005; Wang et al., 2009) and horses (Mésochina et al., 1998). In diets with secondary plant compounds such as tannins, higher FN values occur because tannins make dietary N partly indigestible (Robbins et al., 1987b; Núñez-Hernández et al., 1992; Schlecht and Susenbeth, 2006). Thus, in the absence of secondary plant compounds, it should be possible to achieve a decrease in FN with a diet rich in N if that diet is at the same time less digestible due to high fibre content.

Despite the considerations mentioned above, monitoring FN in wild ruminants is common practice (Leslie et al., 2008). The duration of environmental exposure of faeces in the wild does not seem to affect FN (Kamler et al., 2003) and droppings are easily detected during field surveys even several days after excretion. FN was widely incorporated into wild ruminant studies in the early 1970s (Robbins et al., 1975), becoming the most commonly employed faecal indicator of food quality in wildlife in the 1980s. In fact, since 1984 at least one article using FN as a diet proxy has been published annually, especially in studies of feeding ecology (e.g. Beier, 1987; Leslie and Starkey, 1985), population ecology (Choquenot, 1991; Albon and Langvatn, 1992), or even in long-term monitoring programmes (Blanchard et al., 2003; Hamel et al., 2009) of cervids and bovids.

Gastrointestinal nematodes (GIN), on the other hand, are ubiquitous parasites of herbivores that cause cell damage, cell excretion (Holmes, 1993), reduction in the activity of digestive enzymes (Ritchie et al., 1966; Jones, 1983) and amino acid malabsorption (Poppi et al., 1986, Brown et al., 1991) in the infected hosts. Thereby, GIN are recognised sources of metabolic N in ruminants (Kimambo et al., 1988; MacRae, 1993; Haile et al., 2004). There is no easy way to distinguish microbial (i.e., physiological) and truly endogenous N (such as gut epithelium, enzymes), or nematode tissue in the metabolic N fraction in herbivores (Schwarm et al., 2009). Despite that limitation, no work has evaluated the effect of GIN parasite load on the interpretation of FN in ruminants.

In this work, we conducted an experiment to evaluate the influence of dietary fibre and GIN on FN in sheep (*Ovis aries*). Our objectives were three-fold: [1] to perform a systematic review on the use of FN as a proxy for diet quality in wildlife, expanding the work of Leslie et al. (2008) to the present; [2] to assess the difference in FN in two tannin-free diets, one being high in protein but also high in lignocellulose, the other being low in protein and low in lignocellulose; and [3] to evaluate the effects of deworming on FN measurements.

The experimental results presented in this work underline the importance of considering both gastrointestinal parasite load and dietary fibre content when assessing diet quality of herbivores by means of FN.

2. Materials and methods

2.1. Systematic review on the use of FN as a proxy for diet quality in wildlife

We performed a bibliographic search using specific key words (e.g., "Diet quality indices" OR "Faecal indices" OR "Faecal metabolic nitrogen" OR "Non-dietary faecal nitrogen" OR "Faecal crude protein"), following the recommendations outlined by the Cochrane Handbook for Systematic Reviews (http://www.cochrane.org/). After finding that FN was published together with other faecal indices we performed a second search using other faecal indices such as "Faecal crude protein", "Faecal neutral detergent fibre", "Faecal acid detergent fibre", "Faecal lignin", "Faecal phosphorous" and "Faecal 2,6-diaminopimelic acid", as well as some recent diet-quality indices such as "Faecal chlorophyll" and "Faecal carbon isotopes. In each case, we also performed a search using the American spelling 'fecal' instead of faecal. Only works focused on wildlife species were considered (Table 1).

2.2. Experimental study

This experimental study was performed in the installations of the Veterinary School of the Autonomous University of Barcelona (UAB) from 7 April to 3 June 2011. All animal care activities and study procedures complied with the guidelines of the Good Experimental Practices of the Ethical and Animal Welfare Committee of the UAB.

2.2.1. Animals and diets

The experiment was carried out on ten 2–3-year-old female sheep (Manchega × Ripollesa crossbreeds) from a local flock. Once the presence of spontaneous GIN infection had been confirmed by coprological examination, selected animals were transported to the Veterinary School and randomly assigned to three experimental groups.

A randomised block design was used (Pinheiro and Bates, 2000). For the first 6 days, sheep were fed an adaptation diet (500 g barley + 250 g alfalfa + straw ad libitum) with low protein levels designed to prevent *Clostridium* – related digestive upset. We analysed the ingredients – barley, alfalfa hay and straw – to ensure that all the diets contained the protein levels that had been assigned to each group (Table 2). Nutrient analyses were performed following guidelines of the Association of Official Analytical Chemist (2000): Dry matter (DM) (AOAC 934-01), ash (AOAC 942-05), crude protein (CP) analysed as nitrogen (N) using the DUMAS combustion method (kit Leco® TruSpec N, AOAC 977-02), gross energy (GE) in an adiabatic bomb calorimeter C 4000. Crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the ANKOM method (220 Fibre Analyzer ANKOM).

Subsequently, on day 6 the sheep were randomly assigned to one of two different diets at an n of 5 animals per diet: low-protein, low-fibre (LPF; 1000 g barley + 1100 g straw per animal and day); and high-protein, high-fibre (HPF; 1600 g alfalfa + 1100 g straw). Neither barley, alfalfa nor straw are rich in secondary plant compounds (such as tannins) and hence there is no effect on gastrointestinal nematode control. The mean amount of straw ingested was approximately 1 kg per animal and day in LPF, and 0.5 kg per day in HPF diets, which resulted in a difference in nutrient content of the ingested diets in CP, ash, CF and ADF (and the not analysed soluble carbohydrates, Table 2). To guarantee the complete consumption of all the provided food, each daily portion was divided into two different rations. Before adding new food, straw fallen

Table 1
Systematic review on the use of FN as a proxy for diet quality in vertebrates. The first column indicates the taxonomic group in which the study was carried out. The numbers in the column 'Source' correspond to the numbered list of references shown in the electronic supplementary Material S1. * no species differentiation.

Taxonomic group	Source
Artiodactyla	
Bovidae	
Buffalo (*Syncerus caffer*)	[15,26,51,52,121,122,153,169,170]
Hartebeest (*Alcelaphus buselaphus*)	[15,48]
Eland (*Taurotragus oryx*)	[15,48]
Thomson's gazelle (*Eudorcas thomsonii*)	[15,48]
Grant's gazelle (*Nanger granti*)	[15]
Duiker (*Sylvicapra grimmia*)	[48]
Dik-dik (*Madoqua* sp.)	[15]
Klipspringer (*Oreotragus oreotragus*)	[15]
Impala (*Aepyceros melampus)*	[15,22,51,79,80,107]
Bison (*Bison bison*)	[55,96]
Kudu (*Tragelaphus strepsiceros*)	[51,52,78]
Springbok (*Antidorcas marsupialis*)	[46,161,165]
Dorcas gazelle (*Gazella dorcas*)	[54]
Mongolian gazelle (*Procapra gutturosa*)	[91]
Blackbuck (*Antelope cervicapra*)	[89]
Blue wildebeest (*Connochaetes taurinus*)	[51,52,79,161]
Blue duiker (*Philantomba monticola*)	[76]
Muskox (*Ovibos moschatus*)	[27,28]
Japanese serow *(Capricornis crispus)*	[21]
Bighorn sheep (*Ovis canadensis*)	[2,3,6,14,33,88,155]
Desert bighorn sheep (*Ovis canadensis nelsoni*)	[95]
Mountain goat (*Oreamnos americanus*)	[14,154]
Pyrenean chamois (*Rupicapra pyrenaica*)	[11,167,172]
Nyala (*Tragelaphus angasii*)	[107]
Roan antelope (*Hippotragus equines*)	[111]
Sable antelope (*Hippotragus niger*)	[160,162,170]
Waterbuck (*Kobus ellipsiprymnus*)	[15,63]
Sheep (*Ovis aries*)	[4,8,29,48,99,100,125,148,164]
Cattle/steer (*Bos taurus* or *Bos indicus*)	[4,8,29,48,64,65,70,83,102,103,117,118,121,130,139,164]
Goat (*Capra aegagrus*)	[70]
Cervidae	
Red deer (*Cervus elaphus*)	[16,31,35,36,40,43,50,55,61,62,67,68,85,86,87]
Sika deer (*Cervus nippon*)	[5,7,34,90,142,145,147]
Fallow deer (*Dama dama*)	[25,128,129,136]
Roosevelt elk (*Cervus canadensis*)	[13]
Sambar (*Cervus unicolor*)	[143]
Mule deer (*Odocoileus hemionus)*	[9,38,44,69,93,138]
White-tailed deer (*Odocoileus virginianus)*	[10,17,35,37,41,42,45,47,49,56,58–60,71–73,75,104,106,127]
Black-tailed deer (*Odocoileus h. columbianus*)	[13,66,87,92]
Reindeer (*Rangifer tarandus*)	[81]
Moose (*Alces alces*)	[41,150]
Roe deer (*Capreolus capreolus*)	[20,40,61,112,124,152,154]
Hippopotamidae	
Hippopotamus (*Hippopotamus amphibious*)	[30]
Antilocapridae	
Pronghorn (*Antilocapra americana*)	[24]
Musk deer (*Moschus moschiferus*)	[53]
Giraffidae	
Giraffe (*Giraffa camelopardalis*)	[51]
Proboscidea	
Elephantidae	
African elephant (*Loxodonta africana*)	[77]
Perissodactyla	
Rhinocerotidae	
Rhinoceros (*Rhinoceros unicornis*)	[82]
Equidae	
Asiatic wild ass (*Equus hemionus*)	[54]
Zebra (*Equus zebra*)	[51,79,170]
Horse (*Equus ferus*)	[97]
Camelidae	
Camel (*Camelus dromedarius*)	[1]
Llama (*Lama glama*)	[151]
Suidae	
Warthog (*Phacochoerus africanus*)	[107]
Wild boar (*Sus scrofa*)	[163]
Pig (*Sus domesticus*)	[123]

Table 1 (*Continued*)

Taxonomic group	Source
Diprotodontia	
Macropodidae	
Grey kangaroo (*Macropus giganteus*)	[101]
Wallaroo (*Macropus robustus*)	[108]
Red kangaroo (*Macropus rufus*)	[140]
Petauridae	
Sugar glider (*Petaurus breviceps*)	[74]
Northern brown bandicoot (*Isoodon macrourus)*	[157]
Phascolartidae	
Koala (*Phascolarctos cinereus*)	[19,110]
Pseudocheiridae	
Greater glider (*Petauroides volans*)	[115]
Possum (*Trichosurusvulpecula*)	[114,115,173]
Rodentia	
Cricetidae	
Muskrat (*Ondatra zibethicus*)	[126]
Geomyidae	
Pocket gopher (*Thomomys bottae*)	[133]
Muridae	
Rat (*Rattus rattus*)	[98,130]
Caviidae	
Guinea pig (*Cavia porcellus*)	[134]
Chinchillidae	
Plains Viscacha (*Lagomostomus maximus*)	[84]
Erethizontidae	
Porcupine (*Erethizon dorsatum*)	[113,116]
Lagomorpha	
Leporidae	
Hare (*Lepus europaeus*)	[144]
Carnivora	
Musteloidea	
Mink (*Neovison vison*)	[119]
Procyonidae	
Raccoon (*Procyon lotor*)	[166]
Ursidae	
Black bear (*Ursus americanus*)	[105]
Brown bear (*Ursus arctos*)	[168,171]
Primates	
Cercopithecidae	
Leaf monkey (*Trachypithecus obscurus*)	[19]
Birds	
Sturnidae	
European starling (*Sturnus vulgaris*)	[39]
Phasianidae	
Red grouse (*Lagopus lagopus scotica*)	[18]
Actinopterygii	
Salmonidae	
Rainbow trout (*Oncorhynchus mykiss*)	[141]
Ictaluridae	
Channel catfish (*Ictalurus punctatus)*	[131]
Chichilidae	
Hybrid tilapia (*Oreochromis mossambicus x O.niloticus)*	[131]
Pomacentridae	
Reef-dwelling jewel damselfish (*Plectroglyphldodon lacrymatus)*	[146]
Sparidae	
Black porgy (*Acanthopagrus schlegeli)*	[158]
Sauropsida	
Trionychidae	
Chinese soft-shelled turtle (*Pelodiscus sinensis*)	[130]
General	
Review	[32,57,125,132]
Wildlife*	[23,147]
Wildlife + Livestock*	[94,109,135,137,156]

Table 2
Chemical composition of the diet fed to sheep. DM = dry matter, CP = crude protein, CF = crude fibre, NDF = neutral detergent fibre, ADF = acid detergent fibre, GE = gross energy. Diet nutrient content assuming complete consumption of 1 kg barley and 1 kg straw for LP, and 1.6 kg alfalfa and 0.5 kg straw for HP. Data are expressed in %.

	$DM_{as\ fed}$	CP	Ash	CF	NDF	ADF	GEKcal/100 g
Nutrient content on dry basis							
Barley	90.43	11.05	2.59	5.35	15.89	5.54	461.35
Alfalfa	90.03	19.62	10.66	30.96	39.73	31.12	442.97
Straw	92.38	5.21	8.48	43.74	77.94	48.55	449.25
LP diet	91.41	8.10	5.57	24.75	47.25	27.27	455.24
HP diet	90.59	16.12	10.13	34.06	49.01	35.35	444.49

on the floor was completely removed. Before the feeding of the assigned diets was initiated, a *t*-test (for FN and total serum protein) and a Mann–Whitney test for faecal egg count were used to check that the FN ($t = 0.06$, $P = 0.95$), the TP ($t = 0.36$, $P = 0.72$) and the FEC ($U = 15$, $P = 0.67$) did not differ between groups (Table 3). In addition, on day 6 we performed a coproculture, maintaining a wet faecal mixture collected from individuals from each group at 20 °C for a minimum of 15 days (Pereira Lima and Delgado, 1961), to confirm that LPF (64% of larvae belonged to *Ostertagia* and 36% to other *Trichostrongylus* species, most of which were *Nematodirus*) and HPF animals (62% of larvae belonged to *Ostertagia* and 38% to other *Trichostrongylus* species) were infected with the same genus of nematodes.

2.2.2. Experimental design

Faecal samples were collected directly from the rectum of each sheep on experimental days 0 (arrival at the experimental facility), 6 (start of diet), 19, 26, 33, 34, 35, 36, 37, 38, 39, 40, 46, 47, 48, 49, 50 and 51. Even though GIN egg output shows no circadian pattern (Rinaldi et al., 2009), samples were collected in the morning. On day 33, all sheep were dewormed with a subcutaneous injection of ivermectin (Ivomec®) with a dosage of 200 μg/kg body-weight. Blood samples were collected on days 19, 33, 46 and 51.

2.3. Laboratory analysis

Quantitative and qualitative coprological analyses were performed according to MAFF (1980). In brief, we performed a flotation in 33% zinc sulphate solution, and a faecal egg count (FEC) of the GIN eggs was performed with the modified McMaster method. For each 3-g faecal sample, all strongyle-like eggs observed in the two chambers of a McMaster slide were counted and then multiplied by 50 to determine the number of eggs per gram of faeces. FEC techniques remain the most common and reliable approach for indirectly estimating GIN infection intensity (Cringoli et al., 2004; Villanúa et al., 2006; Rinaldi et al., 2009).

A sample of individual faecal samples was frozen for FN determination. These frozen faeces were thawed and oven-dried at 60 °C to a constant weight (24 h) and subsequently ground with a laboratory mill with 1 mm pitch (Cyclotec 1093, FOSS Tecator, Höganäs, Sweden). Subsamples were used in duplicate to determine DM, N and ADF concentrations as described above. Although to our knowledge, faecal ADF concentrations have not been established as markers of digestibility, we assume higher levels to reflect a lower diet digestibility in this study.

Blood samples were collected from the jugular vein and serum was stored at −20 °C until biochemical analyses were performed. Serum was analysed with an OLYMPUS AU400® (Olympus, Tokyo, Japan) automated chemistry analyser to obtain serum total protein concentration (TP) based on the Biuret reaction (Gornall et al., 1949). Due to its relationship to body reserves, TP can be used for monitoring body condition in wild ungulates (Serrano et al., 2008).

2.4. Statistical analysis

2.4.1. Systematic review

We used linear models to explore the trend in the number of publications on FN since 1981, when publications on FN became common.

2.4.2. FN, faecal ADF and FEC in LPF and HPF diets

For describing FN, faecal ADF and FEC patterns in sheep on different diets, we used generalised mixed models (GLMM) including FN, faecal ADF or FEC as response variables and diet treatment, experimental days (ED) and their interaction as explanatory variables. To test for an effect of FEC on FN, we performed a randomised block design where the response variable was the FN measured from day 19 (13 days after diet assignment) until the administration of the anthelmintic treatment (day 33). In the fixed part of the model the response variables were the single effects of both FEC (as a proxy for GIN load) and the diet treatment (i.e., LPF and HPF diets) and their interactions. In addition, to control for the potential effect of diet digestibility on FN estimates, faecal ADF was included as a covariate in the fixed part of our mixed models. Finally, given that we took repeated measurements from the same individuals, we included individual sheep as a blocking factor in the random part of the GLMM. We chose the optimal random structure (e.g., the random intercept, results not shown) in the statistical modelling as recommended by Zuur et al. (2009).

2.4.3. Changes in FN and TP due to anthelmintic treatment

In this analysis, we tested whether or not the suppression of gastrointestinal nematodes influenced FN excretion and TP concentration in sheep on the two different diets by comparing FN measurements from before (days 6–34 for FN comparisons and on days 19–33 for TP analysis) and after (days 40–50 for FN and 46–51 for TP analysis) the anthelmintic treatment.

In the fixed part of this randomised block design, we included the single effects of drug administration, diet treatment and their interaction. As in the previous analysis, faecal ADF contents were also included as a covariate in the fixed part of our mixed models for

Table 3
Percentage of faecal nitrogen (FN); gastrointestinal parasite faecal egg counts (FEC); serum total protein concentrations (TP); low-protein (LPF), and high-protein (HP) diets. Adaptation: time until beginning of diet (6 days). Treatment: time that sheep were on their specific diets (33 days). Dewormed: time from the anthelmintic application onwards.

		FN (%)			ADF (%)			FEC (egg/g faeces)				TP (g/L)		
		Mean	Min	Max	Mean	Min	Max	Mean	Median	Min	Max	Mean	Min	Max
LPF	Adaptation	1.68	1.51	1.93	37.2	29.88	40.54	229.8	100	49	700	67.02	56	74.9
	Treatment	2.41	1.99	2.91	28.76	19.9	36.61	1390	1125	200	3050	66.94	51.3	79.5
	Dewormed	1.95	1.12	2.59	27.94	13.06	34.45	3.33	0	0	50	71.59	62.8	81.2
HPF	Adaptation	1.68	1.51	1.86	35.01	23.98	42.53	240	300	50	500	68.7	61.9	81.9
	Treatment	1.97	1.68	2.26	40.45	29.94	47.86	275	175	100	600	70.07	53.8	79.6
	Dewormed	1.88	1.61	2.31	40.34	34.51	47.5	136.7	100	0	450	78.31	70.7	88.7

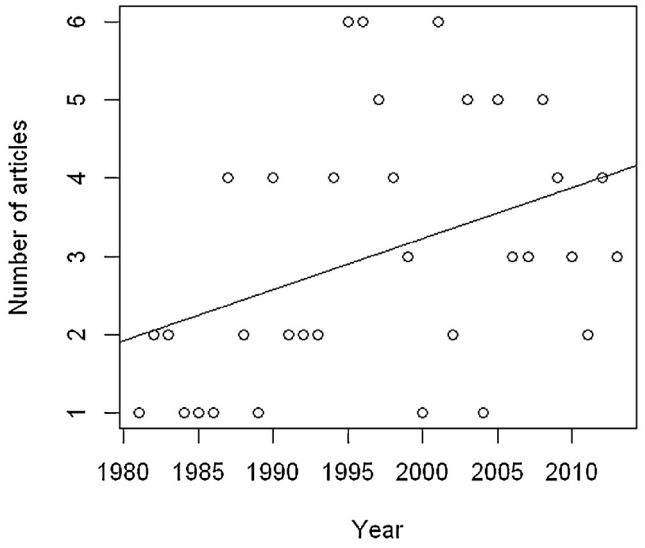

Fig. 1. Trend in the number of articles published from 1980 to 2013 that use faecal nitrogen as a proxy for diet quality in vertebrates. Though the first record appeared in the 1940s, only works published from 1980 to 2013 were considered in this analysis.

FN. Following the same rationale, individual sheep were included as a blocking factor in the random part of the GLMM.

Furthermore, for both mixed models we tested whether or not the inclusion of the initial value of FN (measured during the adaptation period before assignment of the diets, to characterise the digestive efficiency of individual sheep) as a covariate reduced the residual error. To avoid underestimating the variance components (Pinheiro and Bates, 2000), we used restricted maximum likelihood to fit both statistical models. Before model interpretation, we also checked for the lack of a residual pattern and for normality using graphical procedures. Thus, for the first statistical modelling procedures FEC was log-transformed to reduce the residual pattern. The residual error associated with our mixed models was not reduced by the inclusion of the initial values of FN as a covariate, either in the model exploring the relationships between FEC and FN (L-ratio test = 2.16, $P = 0.14$, after comparing the model with and without initial FN values) or in the model for exploring the effects of parasite suppression on FN (L-ratio test = 2.41, $P = 0.12$). Thus, the initial FN was not taken into consideration in our statistical models. Mixed models were fitted using the 'nlme' package (Pinheiro et al., 2012) from R 3.1.1 (R Development Core Team, 2014).

3. Results

3.1. Use of FN for diet quality assessment in wildlife

Table 1 shows the results of a thorough search of the literature contained in Thomson Reuters Web of Knowledge (covering the period 1905–2013). Since the 1980s, an average of three articles per year were published (min = 1, max = 6), using FN as a proxy for diet quality in a broad range of vertebrate species. On year later, the number of articles using FN in vertebrates increased every year ($\beta = 0.06$, SE = 0.02, $P = 0.02$, Fig. 1). FN has been used to assess the diet quality of fish, reptiles, birds and mammals, and in particular in artiodactyls.

3.2. FN, faecal ADF and FEC in the different diets

Our mixed model revealed that FN ($F_{1,38} = 6.05$, $P = 0.01$, for the ED × Diet treatment interaction), ADF ($F_{1,38} = 10.30$, $P = 0.002$; for the ED × Diet treatment) and FEC ($F_{1,38} = 5.06$, $P = 0.03$; for the ED × Diet treatment) before anthelmintic treatment (day 33) depended on the diet assigned. In fact, from day 19 onwards, FN in the animals on a LPF diet began to exceed that of their counterparts fed a HPF diet (Figs. 2A and 3A for ED 19, $\text{mean}_{LPF} = 2.17$, min = 1.77, max = 2.47, $\text{mean}_{HPF} = 1.86$, min = 1.6, max = 2.07). However, significant differences only occurred beginning on ED 28 ($t = 2.56$; $P = 0.036$, $\text{mean}_{LPF} = 2.41$, min = 1.99, max = 2.77 vs. $\text{mean}_{HPF} = 1.97$, min = 1.71, max = 2.26). The concentration of faecal ADF decreased over time in the LPF diet ($\beta_{\text{LogFEC} \times \text{LPF}} = -0.6$, SE = 0.18, t-value = −3.21, $P = 0.002$) but increased in HPF (Fig. 2). In fact, ADF was lower in the LPF diet than in the HPF diet from day 19 onwards ($\text{mean}_{LPF} = 30.41$, min = 28.95, max = 32.20 vs. $\text{mean}_{HPF} = 36.92$, min = 29.94, max = 43.78) but statistical differences only occurred from ED 28 on ($t = 4.55$; $P = 0.003$, $\text{mean}_{LPF} = 28.17$, min = 23.51, max = 32.91 vs. $\text{mean}_{HPF} = 41.91$, min = 36.65, max = 47.86). Likewise, FEC was also higher in the LPF diet on ED 28 ($w = 23$; $P = 0.036$, $\text{median}_{LPF} = 1070$, min = 200, max = 2300, vs. $\text{median}_{HPF} = 280.4$, min = 100, max = 500).

FEC was correlated with FN ($\beta = 0.28$, SE = 0.12, t-value = 2.33, $P = 0.02$), especially in the LPF diet (Table 2, Fig. 2A and B). The influence of FEC on FN was 0.44 units higher in the LPF diet than in the HPF diet ($\beta_{\text{LogFEC} \times \text{HPF}} = -0.44$, SE = 0.21, t-value = −2.09, $P = 0.043$, Figs. 2 and 3). In fact, animals on the LPF diet excreted on average 1.2 times more FN than animals on the HPF diet. The FEC effect on FN excretion was no longer significant when FN was corrected for faecal ADF ($\beta_{\text{LogFEC} \times \text{HPF}} = -0.32$, SE = 0.2, t-value = −1.58, $P = 0.12$). In these analyses, 40% of the observed variability in the relationship between FEC, faecal ADF, diet group and FN was due to inter-individual variation.

3.3. Effects of anthelmintic treatment on FN excretion and serum TP

Ivermectin treatment resulted in a clear reduction in GIN load in the LPF ($W = 6$, $P < 0.001$) and HPF ($W = 150$, $P < 0.01$) diets (Table 3, Figs. 2B and 3B). In contrast, faecal ADF levels were not affected by deworming in either the LPF ($t = 1.73603$, $P = 0.08$, Table 3, Fig. 2A) or HPF ($t = 0.238$, $P = 0.81$, Fig. 3A) diet. On the other hand, mixed modelling showed that the anthelmintic application resulted in a reduction in FN ($F_{1,79} = 7.02$, $P = 0.009$, for the Anthelmintic × Diet treatment interaction). This interaction was also significant when FN was corrected for faecal ADF ($F_{1,78} = 8.83$, $P = 0.003$). Averaged over all animals, FN was 1.1 times higher before deworming than after. However, FN diminished more in the LPF diet (1.23 times, Table 3) than the HPF diet (1.04 times, Table 3) after the anthelmintic treatment. The individual sheep effect on the variability in the observed pattern was 14.2%.

Finally, although no significant differences were found between the groups, the highest TP in serum was observed in the HPF diet (Table 3). Both groups increased their TP to the same degree after the anthelmintic treatment ($F_{2,28} = 20.22$, $P = 0.0001$).

4. Discussion

Faecal nitrogen (FN) is still widely used as a proxy for diet quality in a broad range of vertebrate species, especially in the assessment of habitat quality in free-ranging herbivores. Our results support this practice, but provide important evidence that the widespread simplistic interpretation of a direct link between dietary protein content and FN should be reconsidered. The most frequent caution in using FN as a proxy for dietary N is related to the influence of secondary plant compounds, which may lead to an increase in FN at similar dietary N levels (reviewed by Leslie et al., 2008). This approach may erroneously assume a direct link between dietary N and FN, ignoring modifications by secondary plant compounds. However, in the absence of such metabolites, the low-protein diet

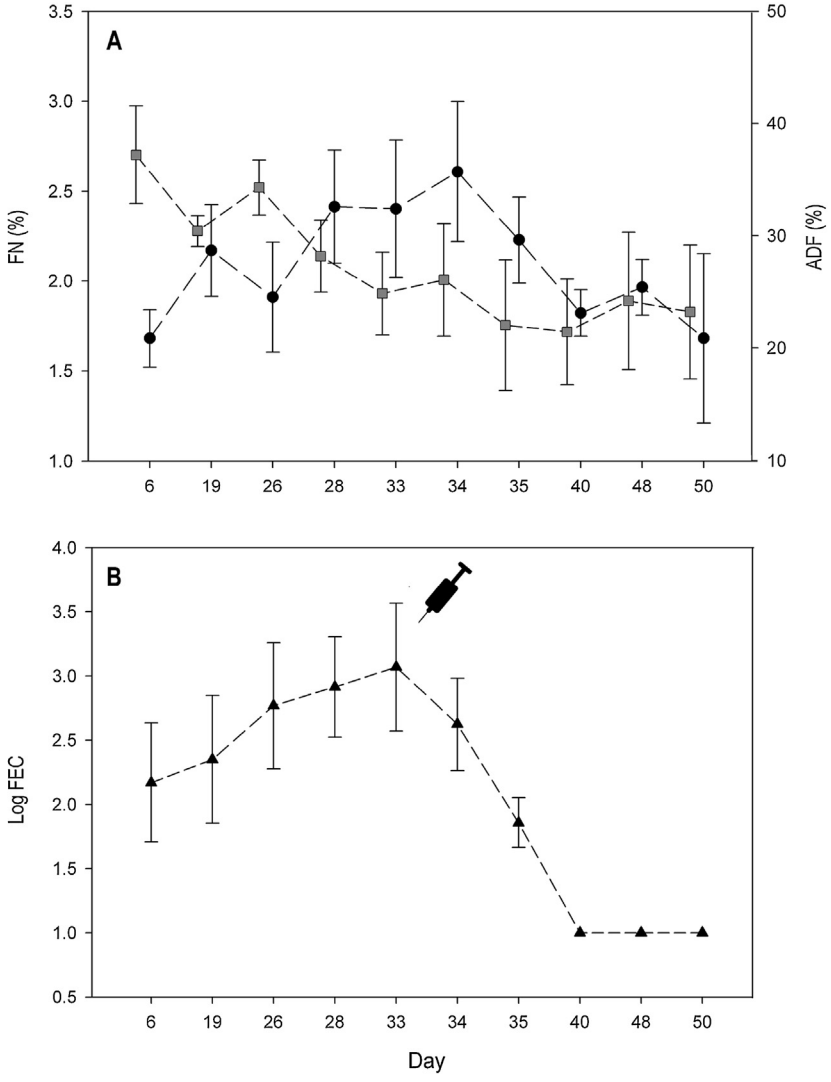

Fig. 2. The plot (A) shows the mean percentage of faecal nitrogen (black circles) and acid detergent fibre (grey squares) in five sheep maintained on a low-protein, low-fibre diet. (B) shows changes in log-transformed gastrointestinal faecal egg counts over time in the same group of sheep. The syringe icon indicates that ewes were given an anthelmintic treatment (ivermectin) on day 33. *X* axes have been shifted slightly to avoid overlap. Bars represent the standard deviation of the mean.

(LPF) in our study (at a protein content of about 8% DM) led to higher FN values than the high-protein diet (HPF) (which had twice the protein content at about 16% DM).

This effect is mostly explained by the higher content of lignocellulose (ADF) in the HPF diet and the higher content of easily digestible carbohydrates, mainly starch, in the LPF diet. The LPF diet used in our study was clearly artificial due to the inclusion of grain, and will have no equivalent in the natural environment of grazing ruminants, for which a decrease in dietary N is usually linked to an increase in fibre and a decrease in digestibility. Nevertheless, it serves to illustrate that FN does not always directly reflect dietary N, but rather is the result of two different biological effects: diets higher in N are usually (in the absence of secondary plant compounds) more digestible, and more digestible diets lead to higher FN in herbivores.

Gastrointestinal helminths also influenced FN excretion. Endoparasites are recognised sources of protein losses in ruminants (Parkins and Holmes, 1989), but their potential impact on FN estimates has usually been ignored by wildlife ecologists. In the first part of our manipulation, the increased FN and FEC in the group on the LPF diet was best explained by the high digestibility of this diet, and the corresponding lower proportion of diluting fibre in the faeces. However, the influence of helminths on FN was later demonstrated by the decrease in FN once parasites had been removed by anthelmintic treatment. Despite the fact that sheep on the LPF diet had a higher FN excretion, they also had numerically lower concentrations of serum TP, which highlights the absence of an association between FN and nutritional condition in cases of severe parasitism. Once dewormed, sheep probably lost less nitrogen due to protein catabolism and specific immune response, as suggested by the increase in TP.

The decrease in FN excretion after the anthelmintic treatment (even when dietary fibre was corrected for) was probably due to the reduction of damage in the digestive tract caused by gastrointestinal nematodes (Fox, 1997) leading to the increase in metabolic nitrogen losses (Parkins and Holmes, 1989). In addition, the increased efficiency of digestive enzymes in the absence of parasites (Jones, 1983) may also explain the decrease in FN as a result of a decrease in residual dietary N. The loss of proteins in the gastrointestinal tract due to parasites is associated with the loss of plasma and erythrocytes, exfoliated epithelial cells and mucus in the gastrointestinal tract (Parkins and Holmes, 1989). In infections associated with gastrointestinal haemorrhage, such as *Haemonchus contortus*, *Oesophagostomun* spp. or *Trichurus colubriformis*, a

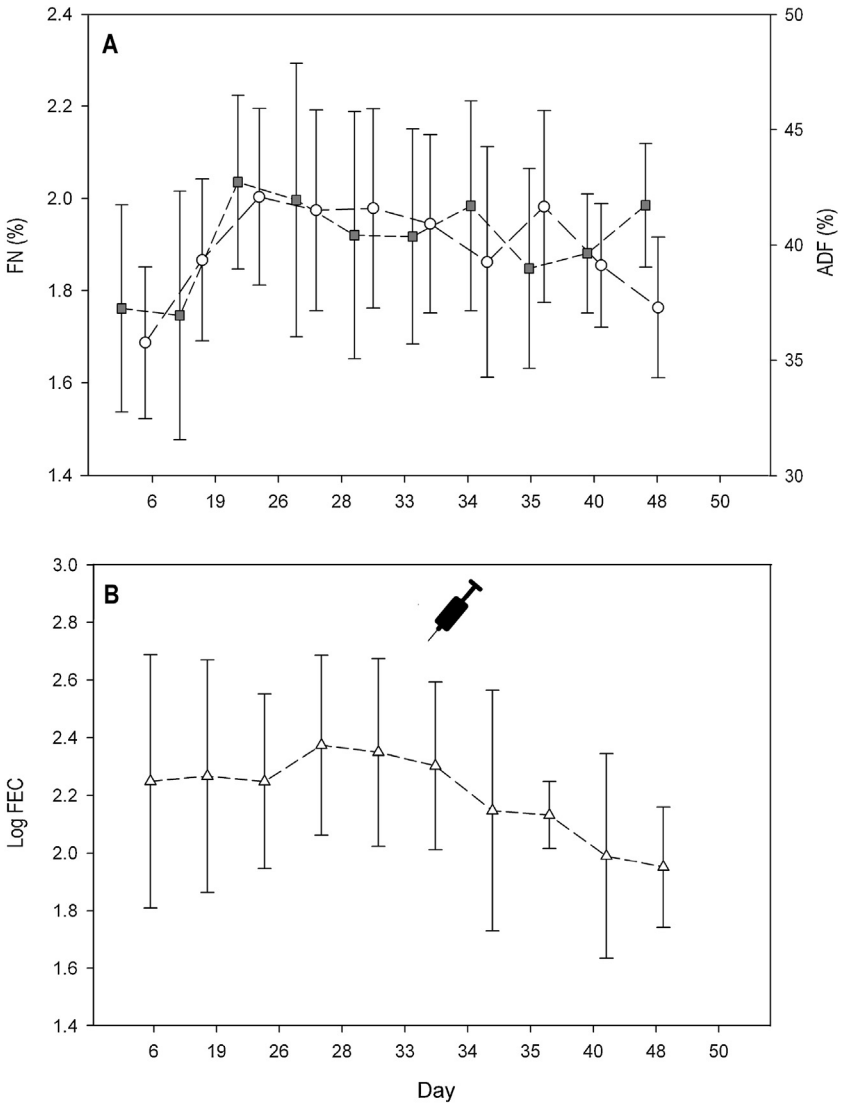

Fig. 3. The plot (A) shows the mean percentage of faecal nitrogen (open circles) and acid detergent fibre (grey squares) in five sheep maintained on a high-protein, high-fibre diet. (B) shows changes in log-transformed gastrointestinal faecal egg counts over time in the same group of sheep. The syringe icon indicates that ewes were given an anthelmintic treatment (ivermectin) on day 33. X axes have been shifted slightly to avoid overlap. Bars represent the standard deviation of the mean.

massive loss of erythrocytes can be partially absorbed in the ileum (Rowe et al., 1982) and the small intestine (Poppi et al., 1981). In fact, small FN increases due to blood losses in ewes experimentally infected with these parasite species had been reported in the late 1970s (Sykes and Coop, 1977; Symons et al., 1981). These FN increases due to endogenous protein losses would increase in the case of infections by nematodes infecting both the abomasums and the small intestine (Haile et al., 2004), as in the current study.

4.1. Implications for population monitoring

Long-term assessment of FN has become common (Blanchard et al., 2003; Hamel et al., 2009; Gálvez-Cerón et al., 2013) in programmes of wild ungulate population monitoring. FN should be used with the understanding that it is a proxy for digestibility but not directly related to diet protein content. Researchers working in feeding ecology should take gastrointestinal parasite load into account when assessing diet quality by means of FN. Other faecal indicators of diet quality should also be incorporated into programmes as a means of avoiding the effects of GINs on FN (see Christianson and Creel, 2009). Further research is still needed to explore how these results might be applied to other ungulate species with other types of foraging strategies (e.g., browsers such as roe deer) and diets with higher levels of tannins, which act as parasiticides (Hoste et al., 2006) and limit protein absorption.

Conflicts of interest

The authors declare no conflicts of interest.

Supporting information

Literature published since 1905 on the use of faecal nitrogen as a proxy for diet quality in wildlife and livestock.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2014.11.020.

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