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Faecal indices of dietary quality can provide useful knowledge about the general ecology of a species, but only if the measurements are accurate and the results are interpreted with caution. In this article, we evaluated the potential of near-infrared spectroscopy (NIRS) as an analytic tool to derive faecal indices of dietary quality in an omnivorous monogastric species with a wide dietary range, i.e. the brown bear *Ursus arctos*. We also tested the effects of field exposure on faecal constituents (i.e. nitrogen, lignin, crude fiber (CF), ether extracts (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), ash and dry matter (DM)), which are commonly used as faecal indices of dietary quality. We collected 172 faecal samples from 45 GPS-marked brown bears in south-central Sweden between May and October 2010. For each sample, we recorded maximum field exposure time (in hours) and canopy cover (in %). We used multivariate partial least-squares regression with a segmented cross validation procedure to calibrate the NIRS method. We obtained very good ($r^2 \geq 0.9$) NIRS validation results for faecal nitrogen content and NDF, and good ($0.7 \leq r^2 < 0.9$) results for lignin, CF, EE, ADF and ash. Validation results for DM were poor ($r^2 = 0.29$). We found that field exposure time negatively affected faecal nitrogen content, especially during the first 40 hours of exposure. Because CF and NDF are strongly negatively correlated with faecal nitrogen content, concentrations of these two components increase as a consequence of field exposure. Faecal EE content appeared to be stable under field conditions. Our conclusions are twofold. First, NIRS can be an accurate, fast and inexpensive analytical tool to evaluate certain faecal indices of dietary quality, including for omnivorous species. Second, faecal indices of dietary quality can be affected by field exposure and can vary among individual animals. Ignoring individual variance and the effects of field exposure on faecal indices of dietary quality may cause bias in research findings.

Key words: brown bear, diet quality, faeces, field exposure, near-infrared spectroscopy, NIRS, omnivore, Ursus arctos

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Information derived from faeces can provide valuable knowledge about a species' general ecology (Putman 1984). Feeding and nutrition are essential in ecology. Evaluating dietary composition, quantity and quality is, however, extremely difficult and often controversial, because the actual dietary intake of a wild mammal is almost always unknown (Putman 1984, Kohn & Wayne 1997). Dietary composition of faecal samples is commonly assessed using visual estimation methods (for a methodological review, see Klare et al. 2011) or more recently also using genetic techniques such as DNA-barcoding (Valentini et al. 2009). The analysis of diet quality is often carried out with stable isotope analysis on tissue samples (Crawford et al. 2008, Blanco-Fontao et al. 2010) and with standard chemical analyses on faeces (e.g. the Kjeldahl extraction method; Pritchard & Robbins 1990, Gad & Shyama 2011). These qualitative methods are very valuable in ecological research, but are relatively expensive and time consuming as well as technically relatively complicated (Givens & Deaville 1999, Dixon & Coates 2009).

Near-infrared spectroscopy (NIRS) is a nondestructive, fast, accurate and inexpensive technique to estimate the chemical content and composition of analytes (Cen & He 2007). The interactions (i.e. absorption, reflection or transmittance) among electromagnetic radiation at given wavelengths and a given analyte yield a 'spectral signature', which can be recorded with a spectrometer. In combination with reference samples of known content and multivariate statistics, spectral signatures can be used to identify and predict certain characteristics of analytes (Næs et al. 2001). When applied to the $\sim$ 700-2,500 nm part of the electromagnetic spectrum, this method is referred to as NIRS (Cen & He 2007).

NIRS is routinely applied in various fields of research, such as food science (Næs et al. 1996, Cen & He 2007), clinical and pharmaceutical research (Pellicer & Bravo 2011) and animal husbandry (Givens & Deaville 1999). In animal husbandry, NIRS has often been applied to faecal samples, because a strong correlation appears to exist between the chemical composition of forage and faeces derived from that forage (Dixon & Coates 2009). Faecal NIRS has, for example, been used to estimate diet quality, diet compostition and digestibility, ecological impacts of grazing and parasite burden (for a review on the use of faecal NIRS in herbivores, see Dixon & Coates 2009). Commonly used faecal constituents used to derive indices of dietary quality include nitrogen, crude fiber (CF), ether extracts (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin and dry matter (DM; Pritchard & Robbins 1990, Leslie et al. 2008, Dixon & Coates 2009). Although faecal NIRS has proven its potential in wildlife research, it has rarely been used, and if so, almost exclusively in herbivores. For example, faecal NIRS was used to evaluate the dietary quality of free-ranging red deer *Cervus elaphus* and roe deer *Capreolus capreolus* (Kamler et al. 2004), white-tailed deer *Odocoileus virginianus* (Showers et al. 2006) and African elephants *Loxodonta africana* (Greyling 2004), as well as to differentiate between faeces of red deer and fallow deer *Dama dama* (Tolleson et al. 2005) and between the sexes in African elephants (Greyling 2004).

NIRS calibrations are generally less accurate to predict the chemical composition of compound materials compared to raw materials (Givens & Deaville 1999). Because omnivores presumably have a wider dietary niche than herbivores and can consume plant as well as animal material, it is expected that NIRS calibrations perform less well for omnivores than for herbivores. Faecal NIRS has nevertheless been applied to omnivores, such as domestic pigs *Sus scrofa domesticus* under controlled conditions (Zijlstra et al. 2011) and humans (Rivero-Marcotegui et al. 1998). However, no studies apply faecal NIRS to omnivores in the wild.

The use of faecal constituents as indices of dietary quality has been debated and criticised, especially with respect to unstable constituents such as nitrogen (Hobbs 1987, Wehausen 1995 *cf.* Leslie & Starkey 1987, Leslie et al. 2008). In addition to e.g. diet selection, seasonality and individual variation, also environmental exposure (e.g. to sunlight, precipitation and insect activity) and sampling design (e.g. sample freshness) can cause variation in the faecal composition (Putman 1984, Leite & Stuth 1994). Ultimately, this variation can cause bias in research findings. Crucial information that is needed to account for variation in faecal composition is the time and place of defecation and the identity of the

defecating individual. Information on defecation time, place and identity of the defecating individual can only be obtained by direct observation or by tracking individuals with spatio-temporally highly accurate tracking devices such as Global Positioning System (GPS).

Our goal was to assess the suitability of NIRS to obtain faecal indices of dietary quality in an omnivorous carnivore, based on faecal samples collected in the wild. We used the brown bear *Ursus arctos*, an opportunistic omnivore, as our model species. We also evaluated the effects of field exposure time and intensity on the various faecal constituents, based on faeces of GPS-marked brown bears.

Material and methods

We collected faecal samples from free-ranging brown bears carrying GPS-GSM (Global System for Mobile Communications, Vectronic Aerospace GmbH) collars in south-central Sweden during May-October 2010. We refer to Martin et al. (2010) for a detailed study area description and to Arnemo et al. (2006) for bear capture and handling details. Brown bears are opportunistic feeders and their diet changes seasonally according to forage quality and availability (Mattson 1997, Dahle et al. 1998). In our study area, bears feed mainly on graminoids, forbs, ant species *Formica* spp. and *Camponotus herculeanus* and moose *Alces alces* calves during spring and early summer (Dahle et al. 1998). During late summer and autumn, bears feed mainly on berries, i.e. blueberry *Vaccinium myrtillus*, crowberry *Empetrum nigrum hermaphroditum* and cowberry *Vaccinium vitis-idaea* (Dahle et al. 1998).

We scheduled the GPS-collars to provide one location every 30 minutes. We visited sites where individual bears had stayed for ≥ 1.5 hours at a cluster site, i.e. for at least three consecutive GPS locations within a radius of 30 m. We collected faecal samples at cluster sites only if no observations or signs (e.g. tracks of different size and multiple day beds) indicated that other bears might have been present at the same cluster site. For each sample, we recorded the maximum field exposure time (i.e. the time in hours from when the bear entered the cluster site until the time a sample was collected) and canopy cover (% cover, measured with a spherical forest densiometer; Lemmon 1956) as measures of duration and intensity of field exposure. We avoided collecting soil and debris with a sample. After collection, samples were homogenised, dried at 60°C in an oven until the

moisture content was < 5% (measured with HP-9034C wood moisture content meter) and stored dry in a closed container at room temperature until further processing. For further analysis, we reground each sample with an IKA M20 universal grinder (particle size < 1 mm) and subdivided each sample into a reference sample and a prediction sample. We used standard lab procedures (Kjeldahl, Weender and detergent fiber analysis) to obtain measures of faecal constituents (nitrogen, ADF, NDF, lignin, ash, CF, EE and DM) from each reference sample (Nehring 1960, Naumann & Bassler 1976, van Soest et al. 1991). ADF, NDF, lignin, ash, EE, CF and nitrogen were measured relative to the faecal DM content (% of faecal DM). DM content was measured (in %) relative to oven dried sample weight. For each prediction sample, we obtained spectral information in the 780-2,740 nm range with an MPA Multi Purpose FT-NIR spectrometer (Bruker Optik GmbH) with a helium-neon probe. We scanned each prediction sample three times and calculated the arithmetic mean of the three spectra per sample to obtain an optimal, homogenised spectrum per sample. Thus, for each faecal sample, we obtained reference values for the faecal constituents with the standard laboratory procedures as well as spectral information with NIRS. We calculated the standard error of the method (S_{ref}) for each constituent, for which we obtained duplicate measurements in the laboratory analysis, to evaluate how much the error of the NIRS method was explained by error in the reference methods (Næs et al. 2001). S_{ref} was calculated according to the below equation 1, where s_i is the standard deviation of the duplicate measurements, I the total number of samples that were analysed and N is the number of duplicate measurements per sample:

$$S_{ref} = \sqrt{\frac{\sum_{i=1}^{I} s_i^2}{IN}} \qquad (1).$$

We used partial least-squares regression (PLSR) with a NIPALS algorithm for multivariate calibration on the 935-2,670 nm spectral range (Næs et al. 2001) and considered 2nd derivative using Savitzky-Golay smoothing and Extended Multiplicative Signal Correction (EMSC) for spectral preprocessing. Spectral preprocessing methods normalise the spectra and aim to minimise overall scaling effects (e.g. measurement inaccuracy) and to facilitate detection of 'real' variation among the spectra (Næs et al. 2001). We

Table 1. Validation results of near-infrared spectroscopic (NIRS) calibration models to predict the content of nitrogen, lignin, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extracts (EE), ash and dry matter (DM) in 172 faecal samples of brown bears, collected during May-October 2010 in central Sweden. 'Prep.' stands for the type of spectral preprocessing ('EMSC' = Extended Multiplicative Scatter Correction, and 2ND = second derivative). '# outliers' indicates the number of outliers that were removed to obtain the models; '# factors' indicates the number of partial least-square factors that were included in the models; 'RMSECV' = root mean square error of the cross validation; 'r^2' = the coefficient of determination; and 'Reference r^2' = the range of coefficients of determination for NIRS models as reported in the literature review by Dixon & Coates 2009.

Component	Prep.	# Outliers	# Factors	RMSECV	r^2	Reference r^2
Nitrogen	EMSC	2	8	1.54	0.91	0.58-0.98
Lignin	EMSC	4	10	2.40	0.84	0.82-0.94
CF	EMSC	5	14	1.92	0.88	-
NDF	2ND	3	10	4.13	0.86	0.76-0.94
ADF	EMSC	4	15	2.80	0.91	0.79-0.97
EE	2ND	4	9	0.78	0.85	-
Ash	EMSC	5	9	3.29	0.86	0.74-0.97
DM	-	-	5	1.18	0.29	-

used segmented cross validation to validate the calibration models, with each segment assigned to a unique 'bear ID' ('leave-one-bear-out' cross validation). We evaluated the model quality for each of the faecal constituents based on the coefficients of determination (r^2; $r^2 < 0.7$ = poor, $0.7 < r^2 < 0.9$ = good, $r^2 > 0.9$ = excellent; Shenk & Westerhaus 1996), the number of model factors and the root mean-square errors of the cross validation (RMSECV; Næs et al. 2001). We visually evaluated outliers in the reference and predicted concentrations of faecal constituents with predicted vs reference plots. We occasionally removed outliers to improve model fit (maximum 2.9% of all records; Table 1). Assuming normality and no bias, values of 2*RMSECV around the prediction delineate its 95% confidence region (Næs et al. 2001). We used Unscrambler® 10.1 software (Camo software AS) for the multivariate calibration and validation.

We evaluated the effects of field exposure time and canopy cover on the faecal constituents (in % DM) with linear mixed-effect regression models. We used the reference values of each faecal constituent as the response variable. For each model, we included 'bear ID' as a random factor and considered all possible combinations of 'canopy cover', 'exposure time', and the interaction term 'canopy cover*exposure time' as fixed effects (eight combinations including a null model). We evaluated the most parsimonious model for each faecal constituent based on Akaike's Information Criteria scores for small sample sizes (AIC_c) and AIC_c weights (Burnham & Anderson 2002). We used the 'lme4' package (Bates & Maechler 2010) for statistical modelling and generated p values for the fixed effects of the regression models with a Markov Chain Monte Carlo algorithm (package 'LMERConvenienceFunctions'; Tremblay 2011) in R.2.12.0 (R Development Core Team 2009). We considered α = 0.05 as the threshold level for statistical significance.

Results

We collected 172 faecal samples from 45 GPS-marked bears between 10 May and 22 September 2010. Mean field exposure time of the faeces was 46.3 hours (range: 13-104 hours) and mean canopy cover at the collection sites was 75.7% (range: 0-100%). The reference values for each faecal constituent, as extracted by the standard chemical laboratory analysis, are summarised in Table 2.

NIRS calibration
We developed PLSR calibration models to predict the content of nitrogen, lignin, ash, CF and ADF

Table 2. Reference values (in %) of nitrogen, lignin, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extracts (EE), ash and dry matter (DM) in brown bear faeces, collected in south-central Sweden during May-October 2010. DM is expressed in % relative to the weight of oven-dried faeces. The other constituents are expressed as % relative to DM content. 'SD' = standard deviation and 'Sref' = standard error of the method.

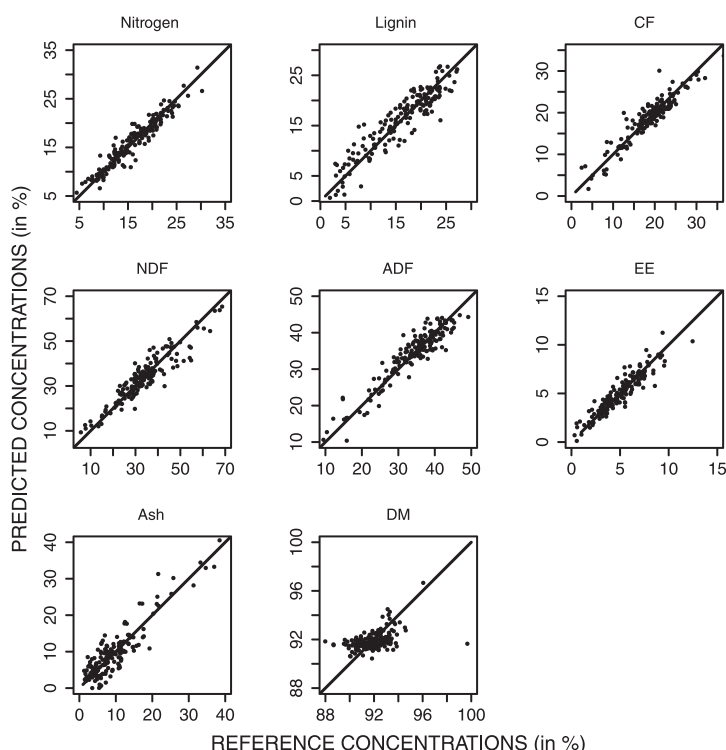|	Mean	Minimum	Maximum	SD	Sref
Nitrogen	16.32	4.55	38.34	5.31	0.189
Lignin	16.13	2.00	27.30	6.32	0.282
CF	18.86	2.57	36.67	5.42	0.210
NDF	34.25	5.88	68.84	10.85	0.571
ADF	33.38	4.88	49.31	8.93	0.431
EE	5.03	0.37	12.51	2.07	na
Ash	10.12	1.44	47.65	8.75	na
DM	91.94	88.03	99.71	1.33	na

Figure 1. Concentrations (in %) of nitrogen, lignin, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extracts (EE), ash and dry matter (DM) predicted by Near-Infrared Reflectance Spectroscopy plotted against reference concentrations based on laboratory extractions (Kjeldah, Weender and detergent fiber analysis) in faeces of brown bears collected in central Sweden during May-October 2010. DM is expressed as % relative to the weight of oven-dried faeces, whereas the other components are measured in % relative to the faecal DM content. See Table 1 for statistical details. The diagonal line represents perfect linear correlation (x = y).

based on EMSC preprocessed spectra. We used Savitzky-Golay 2nd derivative preprocessed spectra to predict the faecal content of EE and NDF. We used unprocessed spectra to develop a calibration equation to predict faecal DM content, because the preprocessing methods did not improve the calibration results (see Table 1). The optimal number of PLS factors varied from five (DM) to 15 (ADF) among the models (see Table 1). The number of removed outliers varied from zero (DM) to five (CF and Ash) among the models (see Table 1). The NIRS-predicted values of the faecal constituents corresponded well with the reference values ($r^2 > 0.84$, all RMSECV between 0.78 and 4.13; Fig. 1, and see Table 1), with the exception of the predicted values for DM. The model to predict faecal DM performed poorly ($r^2 = 0.29$). NIRS validation diagnostics for all models are summarised in Table 1.

Table 3. Outputs of the most parsimonious models to evaluate the effect of field exposure time and intensity on faecal constituents (nitrogen, lignin, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extracts (EE) and ash; in % relative to faecal dry matter content; DM) in brown bear faeces (collected during May-October 2010 in central Sweden) as predicted with near-infrared spectroscopy (NIRS); 'ß' = parameter estimate; 'σ' = standard error; 't' = test statistic; 'p' = p value; and 'σ²' indicates the variance of the random component (Bear ID); 'wAICc' = Akaike's weight for each most parsimonious regression model.

Response variable	Field exposure time*				Bear ID	
	ß	σ	t	p	σ²	wAICc
Nitrogen	-0.067	0.023	-2.856	0.005	4.45	0.95
Lignin	-0.012	0.027	-0.383	0.702	3.667	0.88
CF	0.080	0.026	3.068	0.003	3.405	0.96
NDF	0.419	0.049	1.773	0.003	22.934	0.96
ADF	0.072	0.041	3.022	0.078	< 0.001	0.92
EE	-	-	-	-	< 0.001	0.97
Ash	0.076	0.041	-1.873	0.063	11.793	0.78

*Field exposure time was the only fixed variable that was included in the most parsimonious model to evaluate faecal content of nitrogen, lignin, CF, NDF, ADF and ash. Faecal EE content was best explained by the null model. Each faecal constituent was treated separately as a response variable in a mixed effect regression model.

Field exposure

The most parsimonious models to evaluate the effect of field exposure (time and canopy cover) on the faecal content of nitrogen, lignin, ADF, NDF, ash and CF only contained 'exposure time' as a fixed factor. Field exposure time significantly and negatively affected the faecal content of nitrogen (ß = -0.067, t = -2.856, P = 0.005), and positively affected the faecal content of CF (ß=0.08, t=3.068, P=0.003) and NDF (ß = 0.149, t = 3.022, P = 0.003). Field exposure time had no apparent effect on faecal content of lignin (ß = -0.010, t = -0.383, P = 0.702), ADF (ß = 0.072, t = 1.773, P = 0.078) or ash (ß = -0.076, t = -1.873, P = 0.063; (Table 3). Faecal composition varied among individual bears, especially with regard to faecal NDF (mean = 33.78% DM; random effect σ^2 = 22.934) and ash content (mean = 10.22% DM; random effect σ^2 = 11.793; see Table 3). Faecal EE content was best explained by the null model, suggesting that exposure time and intensity did not affect EE in faecal samples (see Table 3). We validated each most parsimonious model with residual-versus-fit plots (Zuur et al. 2009). We found no trends in the residual-versus-fit plots, suggesting that no model assumptions were violated.

Discussion

The NIRS calibrations for faecal indices of dietary quality for the omnivorous brown bear showed a quality comparable to NIRS calibrations for herbivore faeces as reported in the literature (see Dixon & Coates 2009 for a review). Dixon & Coates (2009) reported coefficients of determination of 0.58-0.94 for nitrogen, 0.82-0.94 for lignin, 0.76-0.94 for NDF, 0.79-0.97 for ADF and 0.74-0.97 for ash. The coefficients of determination obtained in our study fell within the reported ranges and were > 0.84, with the exception of DM. According to the criteria proposed by Shenk & Westerhaus (1996), we obtained excellent calibration results (r^2 > 0.9) for nitrogen and ADF, good precision (0.7 < r^2 < 0.9) for NDF, ash, lignin, CF and EE, but poor calibration results for faecal DM content. The measurement errors of the laboratory analyses (S_{ref}) were relatively low and explained between 10.9% (CF) and 15.4% (ADF) of the RMSECV of the NIRS multivariate calibration.

The use of faecal indices of dietary quality has been heavily debated, because factors such as weather, insect activity and exposure time can affect faecal composition, and thus ultimately research findings

(Putman 1984, Jenks et al. 1990, Robbins et al. 1991, Leslie et al. 2008). Especially indices based on faecal nitrogen (e.g. crude protein and correlated variables such as CF and NDF) may be unreliable, because nitrogen compounds can dissolve from faeces with water or as volatile ammonia (Putman 1984, Leslie et al. 2008). Relatively dry faeces, such as pellets of white-tailed deer and goats *Capra* spp. have been reported to be relatively stable under field conditions (for 2-3 weeks) with respect to the nitrogen content (Jenks et al. 1990, Dixon & Coates 2009). However, Dixon & Coates (2009) reported that moister faeces (such as brown bear faeces) can be expected to be less stable under field conditions. Our results show that exposure time negatively affected the nitrogen content in faecal samples of brown bears (approximately 0.07 (ß) ± 0.023 (σ) % was lost per hour exposed in the field; see Table 3). We plotted the nitrogen content of the reference samples against the field exposure time and it seems that nitrogen loss is most apparent during the first 40 hours of field exposure (Fig. 2). Because CF and NDF are closely related with nitrogen (Pearson's product-moment correlation test CF-nitrogen: correlation coefficient = -0.61, P < 0.001; NDF-nitrogen: correlation coefficient = -0.60, P < 0.001), we could thus also expect a significant effect of field exposure time on CF and NDF. Canopy cover was never included in the models evaluating the stability of faecal constituents, which
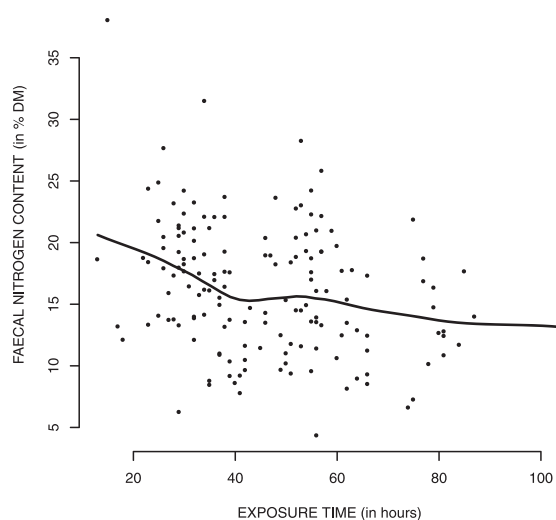

Figure 2. Nitrogen content (in % of faecal dry matter (DM), derived with the Kjeldalh nitrogen extraction method) in brown bear faeces plotted against the time (in hours) a faecal sample was exposed to field conditions. The data were fitted with a LOESS smoother (—) to facilitate interpretation.

suggests that canopy cover *per se* is a poor proximate for exposure intensity. We also found that faecal constituents (especially NDF and ash) can vary considerably among individuals.

Our results show that NIRS can be an accurate tool for the prediction of faecal constituents in omnivorous species with a wide dietary range. Some faecal constituents are, however, affected by the time of exposure to climatic conditions in the field, and may also vary among individual animals. It is therefore advisable to control for these factors in a statistical analysis of faecal constituents as indices of dietary quality.

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