

Excretal Near Infrared Reflectance Spectrometry to monitor the nutrient content of diets of grazing young ostriches (*Struthio camelus*)

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Abstract

Feeding systems in which young ostriches feed on pasture but have access to concentrates provide better welfare than confined systems but are sustainable only if nutrition is carefully controlled. This study was conducted to evaluate the potential of "excretal NIRS", a methodology that associates excretal spectral information in the near infrared (NIR) region with dietary attributes, in predicting dietary quality and nutrient intake in grazing ostrich chicks. Sixty sets of excretal and dietary information from chicks fed only concentrate or also grazing lucerne, barley, sulla or natural pastures, were used. The coefficient of determination (R^2) and the standard error of cross validation (SECV) served to evaluate calibration quality. The prediction of dietary concentrate content ranging 420 to 1000 g/kg of DMI, was highly linear ($R^2 = 0.96$), with SECV of 63 g/kg. Similar R^2 values were noted for the dietary contents of crude protein (CP), acid detergent fibre (ADF) and ash; that for the prediction of neutral detergent fibre (NDF) was lower (0.87). Ash, CP, NDF and ADF were predicted with SECV values of 14.8, 5.0, 8.9 and 10.7 g/kg DM diet, respectively. The calibration for apparent total organic matter digestibility was of poor quality. Good ($R^2 = 0.95$) and acceptable ($R^2 = 0.86$) calibrations were obtained for the daily intakes of pasture and concentrate, respectively, with SECVs of 75 and 131 g/d. Predictions of ash ($R^2 = 0.85$, SECV = 11 g/d) and ADF ($R^2 = 0.80$, SECV = 19 g/d) intakes had mediocre accuracy, and calibrations for CP and NDF intakes were even poorer. These results suggest that excretal NIRS may be useful to predict dietary intake and composition for grazing ostriches when applied to a known nutritional environment attended with calibration standards.

Keywords: Ratites, faecal NIRS, nutrition; pasture, herbivory

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Introduction

Although ostriches are natural herbivores, the commercial diets of growing ostrich chicks in most Israeli farms consist mainly of concentrates formulated for turkey chicks. The design of the gastro-intestinal tract (GIT) of ostriches, and especially of the hindgut, enables them to utilize fibre-rich vegetation. In their natural habitat ostriches are adaptable grazers and will consume mainly green annual grasses, new leaves and shoots from shrubs and trees (Milton *et al.*, 1994; Angel, 1996). The most prominent feature of the ostrich GIT is the size of the colon (i.e. 9 m), which represents approximately 57% of the entire GIT (Angel, 1996). A suitable environment for fermentative microflora exists in the enlarged hindgut (Swart *et al.*, 1993).

Neglect of the needs of ostriches for dietary fibre is associated with mutual feather pecking that impairs bird welfare and reduces the quality of hides. Degen *et al.* (1989) reported that ostriches spent only 46 min/d at eating when fed a concentrate diet. Insufficient time spent by ostriches at eating was identified by Sambraus (1995) as the cause of mutual feather pecking. A feeding system for fattening ostriches that integrates grazing of pastures of lucerne (*Medicago sativa*) with supplementation with a concentrate is used in irrigated regions of South Africa (Shanawany & Dingle, 1999). In Israel, young ostriches reduced their voluntary intake of concentrate by 40% without any effect on growth performance when green, lush pasture of lucerne, sulla (*Hedysarum coronarium*), common vetch (*Vicia sativa*) or a natural pasture, mainly of annual forbs, was available. Green barley (*Hordeum vulgare*) pasture, however, did not sustain maximal growth (Nitzan *et al.*, 2002). The prerequisite for a successful feeding system is that composition of diets consumed as pasture is monitored, in order to optimize the formulation of concentrates provided as supplement.

The importance of the chemical composition of faeces to the understanding of nitrogen and energy status was demonstrated in grazing ruminants by Nunez-Hernandez *et al.* (1992). The concept that the chemical information concealed in the near-infrared spectra of faeces, obtained by near infrared spectrometry (NIRS), can be used to predict the dietary composition of grazing animals was demonstrated in cattle by Lyons & Stuth (1992). The technology, termed "faecal NIRS", was based on mathematical modelling of the relationship between the dietary composition and NIRS spectra of faeces, followed by a validation process. A typical database for faecal NIRS consists of sets of faecal spectra and nutrient analyses (reference values, in NIRS jargon) of the diets consumed by the animals. A good estimate of intake is easily obtained with confined animals, but it is more of a challenge with grazing ones. Principles established by Penning & Hooper (1985) to measure herbage intake of sheep can be successfully applied to ostriches (Nitzan *et al.*, 2002).

The nutrient composition of diets has been successfully predicted by using faecal NIRS for grazing beef cows (Lyons & Stuth, 1992; Coates, 1999) and goats (Landau *et al.*, 2005). Coates (1999) claimed that not only dietary composition, but also the intake of nutrients can be predicted by means of faecal NIRS in beef cows. No attempts to use faecal NIRS to assess dietary attributes in birds have been reported, maybe because faeces and urine cannot be easily separated, even though, in ostriches, they are voided via distinct ducts and their excretion is not synchronous (Duke *et al.*, 1995). However, the principles of faecal NIRS are empirical, and there is no theoretical reason to think that the chemical information encompassed in a mixture of faeces and urine has less value than that of faeces alone in predicting dietary attributes.

Using sets of individual excretal samples and data for intake and diet quality, the potential of NIRS of excreta to predict the dietary proportion of concentrate or pasture and contents of crude protein (CP), ash, neutral (NDF) and acid detergent fibre (ADF), and apparent total organic matter digestibility (TOMD) was evaluated.

Material and Methods

The study was carried out in Eshel Hanassi (31°22'N, 34°25'E) in the Northern Negev Desert of Israel. The climate is Mediterranean, featuring no rain before November or after the beginning of April. Rainfall was 240 mm and irrigation provided an additional 110 mm. Four types of pastures were sown at mid-November and grazed sequentially for 6 - 14 days each during five experimental periods. Grazed material was a sward of lush green lucerne (*M. sativa*, grazed 1 - 14 January and 11 - 17 February, i.e. two periods), barley (*H. vulgare*, grazed 15 - 27 January), natural pasture (grazed 28 January - 4 February) or sulla (*H. coronarium*, grazed 5 - 10 February).

All the procedures used in the determination of individual chick diets have been described previously (Nitzan *et al.*, 2002). Here, the information necessary to understand how a database consisting of 60 sets of excretal and dietary information, to be used as reference data for excretal NIRS, is presented.

Twelve 8-week-old ostrich chicks, averaging 21.9 (s.e. 0.55) kg were housed in individual pens measuring 2.4 × 1.2 m. The first 16 days were an adaptation period when all chicks received a commercial concentrate (Nir Oz, Israel) at 30 g/kg (days 1 - 8) and 40 g/kg (days 9 - 16), based on group average body weight (BW). Thereafter, chicks were allotted to one of three groups and stayed in the same group throughout the experiment. Group 1 was fed on concentrates only, provided at 40 g/kg BW (n = 4); groups 2 and 3 were fed concentrate, provided at 20 (n = 4) and 30 (n = 4) g/kg BW, respectively, and also grazed for 4-6 h daily as one flock (Table 1). Concentrate allowance was adjusted weekly on the basis of individual BW.

Ostriches grazed for 14, 13, 8, 6, 7 days on monocultures of lucerne, barley, natural pasture, sulla and lucerne, respectively, and pasture intake was evaluated during the last two days of each grazing period (Table 1). In order to assess dry matter intake (DMI), a modified Penning & Hooper (1985) procedure (PHP) based on animal BW before and after grazing, adapted for ostriches (Nitzan *et al.*, 2002), was used. Excreta were collected in bags tied to the harnessed animals. Intake was estimated as the difference between initial and final BW, adjusted for individual time-weighted insensible weight loss (IWL) that was estimated during a fasting session. The PHP sessions lasted 6 h and consisted of two grazing sessions of 2 h, each followed by 1 h of IWL measurement. The assessment of IWL during fasting was achieved by covering the birds' heads with a cloth sleeve to deny access to food. The whole procedure was repeated on two successive days for each pasture species. The digital scale used in the experiment recorded the weight 100 times per second (Merav 2000, Shekel Scales, Rosh Ha'Ayin, Israel) and was equipped with software including an algorithm designed to interrupt weighing once the standard error reaches the level of accuracy of the scale (10 g). Five

25 × 25 cm² pasture samples were harvested at the beginning and end of each grazing session. The DM content of ingested material was assumed to be similar to that calculated from the analysis of these samples. The PHP procedure accounted for 91% of the variation in intake (Nitzan *et al.*, 2002).

Full excreta collection from the harnessed ostriches was done for 48 h at each PHP session. The excreta collection devices were coated with disposable diapers and were emptied every 6 h. The excreta of each ostrich were kept separately in plastic bags, and frozen to -17 °C.

Vegetation, concentrate and excreta samples were dried in a ventilated oven for 72 h at 60 °C and ground to pass through a 1.0 mm sieve (Roetsch, Germany). Six-hour excretal samples were then pooled within ostrich for a grazing period (48 h). An aliquot was ashed at 520 °C for 4 h (Carbolite, ESF 12/23). The ash content was subtracted from DM to calculate the organic matter (OM) content. Analyses for neutral (NDF) and acid (ADF) detergent fibre were carried out according to Goering & Van Soest (1970) in micro-bags, with an automated apparatus (Ankom Technology Corp., U.S.A). Crude protein (CP) was assayed according to AOAC (1984) in a semi-automated Kjehldal procedure (Tecator, Sweden). The apparent digestibility of OM from the total diet (TOMD) was calculated for each bird within each of the five grazing periods.

The whole procedure yielded 60 sets of excretal and nutritional data through five experimental periods. Twenty sets were from chicks kept on all-concentrate diets, and 40 sets from chicks kept on pasture-and-concentrate diets (Table 1).

Table 1 Time schedule of the experiment showing dietary treatments, pasture species and grazing periods for the three groups of chicks

Group (n = 4)	1	2	3
Concentrate allowance	40 g/kg BW	20 g/kg BW	30 g/kg BW
Grazing			
1-14 January	no grazing	Lucerne	Lucerne
15-27 January	no grazing	Barley	Barley
28 January - 4 February	no grazing	Natural	Natural
5-10 February	no grazing	Sulla	Sulla
11-17 February	no grazing	Lucerne	Lucerne

Before scanning, samples were dried in an oven at 50 °C for 2 h to stabilize moisture content, and were placed in a desiccator for 1 h to cool to ambient temperature. Ground dry excreta samples were packed into sample cells with a near-infrared-transparent quartz cover glass and scanned between 1104 and 2492 nm in 2-nm increments with a Foss NIRSystems 5000 NIR reflectance monochromator spectrometer (Foss Tecator, Hoganas, Sweden), which yielded NIR spectra as log (1/R) where R = reflectance. Before development of calibration equations, raw spectral data were transformed with the Standard Normal Variance (SNV) and detrend procedures to remove the non-linearity that results from light scattering (Barnes *et al.*, 1989). Mathematical treatments used to enhance spectral differences were "1, 4, 4, 1" or "2, 6, 6, 2", where the numbers represent the derivative, gap width over which the derivative is calculated and the number of points in a moving average (i.e. smoothing procedure), respectively (ISI, 1999).

In order to assess whether NIR spectra of excreta can be used in monitoring the feeding management system (i.e. confinement *vs.* grazing), and whether chicks kept on similar management regimens have excreta of similar spectral characteristics, the Mahalanobis spectral (H) distance was used. The H distance (Naes *et al.*, 2002) was calculated between individual spectra and a population centroid by using an average computed

spectrum according to the score routine of WinISI II that is based on six best components in principal component analysis (PCA; ISI, 1999).

Calibration equations in which dietary DM composition data (percentages of pasture and concentrate, ash, CP, and NDF, shown in Table 2) served as reference values were developed on the basis of the mathematically treated spectral data, by using the modified partial least-squares (MPLS) routine of the WinISI II software. Before final calibration equations were calculated, outlier passes were made to remove observations with $T > 2.5$, as recommended in ISI (1999).

Table 2 Range of values of dietary attributes encompassed in the 60 sets of excreta and diets used in excretal NIRS calibrations

	avg	min.	max.	s.d.
Dietary content (g/kg DM)				
Concentrate proportion	779	420	1000	184
Pasture proportion	220	0	580	184
Neutral detergent fibre (NDF)	301	274	353	22
Acid detergent fibre (ADF)	101	63	189	32
Crude protein	198	181	232	15
Ash	74	35	200	36
Total OM apparent digestibility (g/kg DM)	841	711	963	45
Intake (g/d)				
Concentrate intake	815	423	1394	222
Pasture intake	221	0	641	196
Total DM intake	1036	638	1415	206
NDF intake	294	175	412	55
ADF intake	99	42	180	35
Crude protein intake	195	115	284	45
Ash intake	71	31	177	34

The predictive quality of the equations was evaluated according to the coefficient of determination (R^2), i.e. the proportion of variability in the reference data accounted for by the regression equation and the standard error of calibration (SEC), which represents the variability in the difference between predicted values and reference values. The accuracy of calibrations was evaluated with the aid of cross-validation against the whole dataset (with SECV as estimate of quality), i.e. dividing the whole set of samples into six subsets, and calibrating in five subsets while validating equations on the remaining subset. The SECV was calculated as the average of the six s.e.s. of validation obtained by this procedure.

Results

Average excretal NIR spectra are shown in Figure 1. Because the diets differed primarily in fibre characteristics, it was decided to zoom on first-derivatized spectra in the 2106- and 2140-nm regions, featuring C-O-O and CH stretch, where starch and fibre chemistry are best expressed, in order to identify spectral differences between group average spectra (Figure 1). Even though spectra from the different groups look different, their identification by PCA was not straightforward. Figure 2 depicts the Mahalanobis distances calculated for each individual spectrum, relative to its own nutritional group and to other group average spectra.

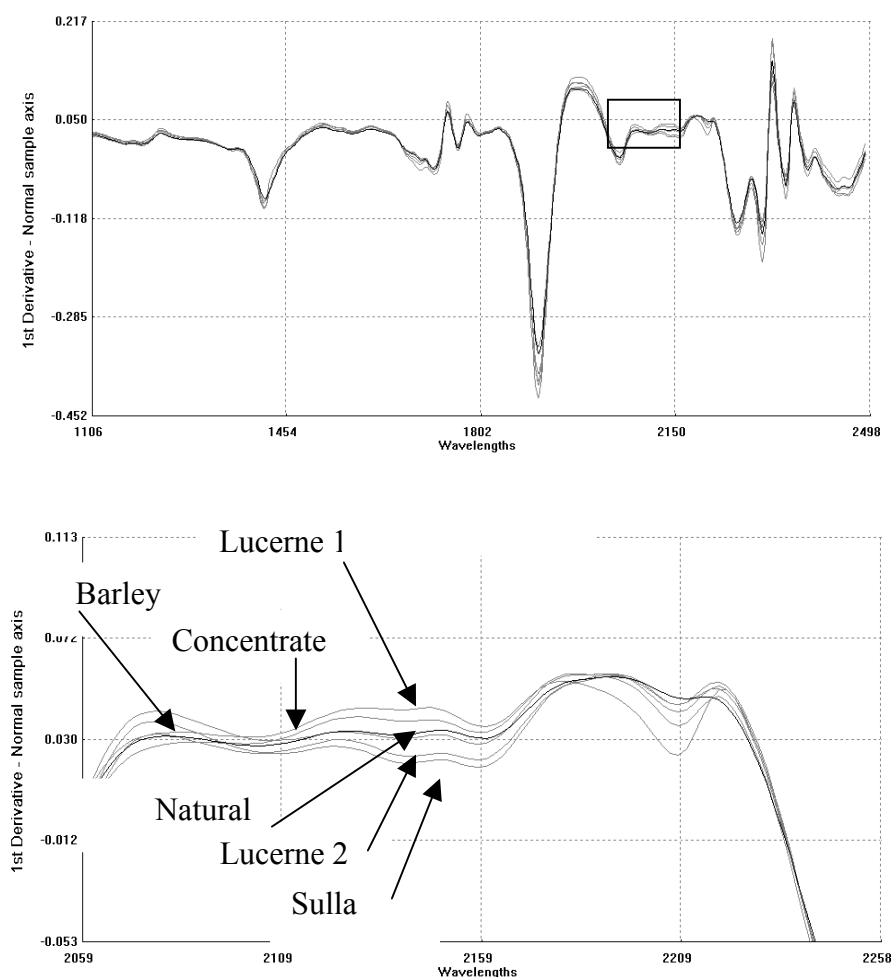


Figure 1 First-derivative-transformed NIR spectra of the excreta of ostrich chicks fed concentrate only, or fed concentrate plus pasture of lucerne in January (Lucerne 1), barley, sulla, natural pasture, or lucerne in February (Lucerne 2). Spectra were averaged within treatments

If any individual faecal spectrum had a distance less than 0.47 in H, from an average nutritional group spectrum, this individual belonged to the group. However, individuals on similar diets did not always have spectra with small H distances between them. Spectra from some groups, such as chicks feeding on lucerne in February, or chicks feeding on natural pasture, shared high spectral identity. In contrast, the lowest level of spectral identity was found for chicks fed all-concentrate diets. The proportion of chicks whose diet could be identified on the basis of spectral distance from the average ranged from 1/8 for all-concentrate-fed ostriches to 7/8 for their counterparts that grazed lucerne in February or natural pasture.

Optimal calibrations are shown in Table 3. The NIRS equations enabled attribution of 96% of the variation of dietary concentrate proportion, ranging from 420 to 1000 g/kg of DM, with accuracy (SECV) of ± 63 g/kg. Similar R^2 values were clearly noted for the pasture proportion of the diet, and also for the dietary contents of CP, ADF and ash; R^2 for the prediction of NDF content was lower (0.86). Dietary contents of ash, CP, NDF and ADF were predicted with SECVs of 14.8, 5.0, 8.9, and 10.7 g/kg DM, respectively. The calibration for apparent TOMD was of poor quality, with low R^2 (0.35) and high SECV. Therefore, no attempt was made to calibrate equations for the intake of organic matter digested. Good ($R^2 = 0.95$) and acceptable ($R^2 = 0.86$) calibrations were obtained for the intakes of pasture and concentrate, with respective SECVs of 75 and 131 g/d, respectively. Lower R^2 values were found for the prediction of ash ($R^2 = 0.85$, SECV = 18 g/d) and ADF ($R^2 = 0.80$, SECV = 19 g/d) intakes. The calibrations of CP and NDF intakes were even poorer, with $R^2 = 0.71$ and 0.58, respectively, and SECVs of 28 and 39 g/d, respectively.

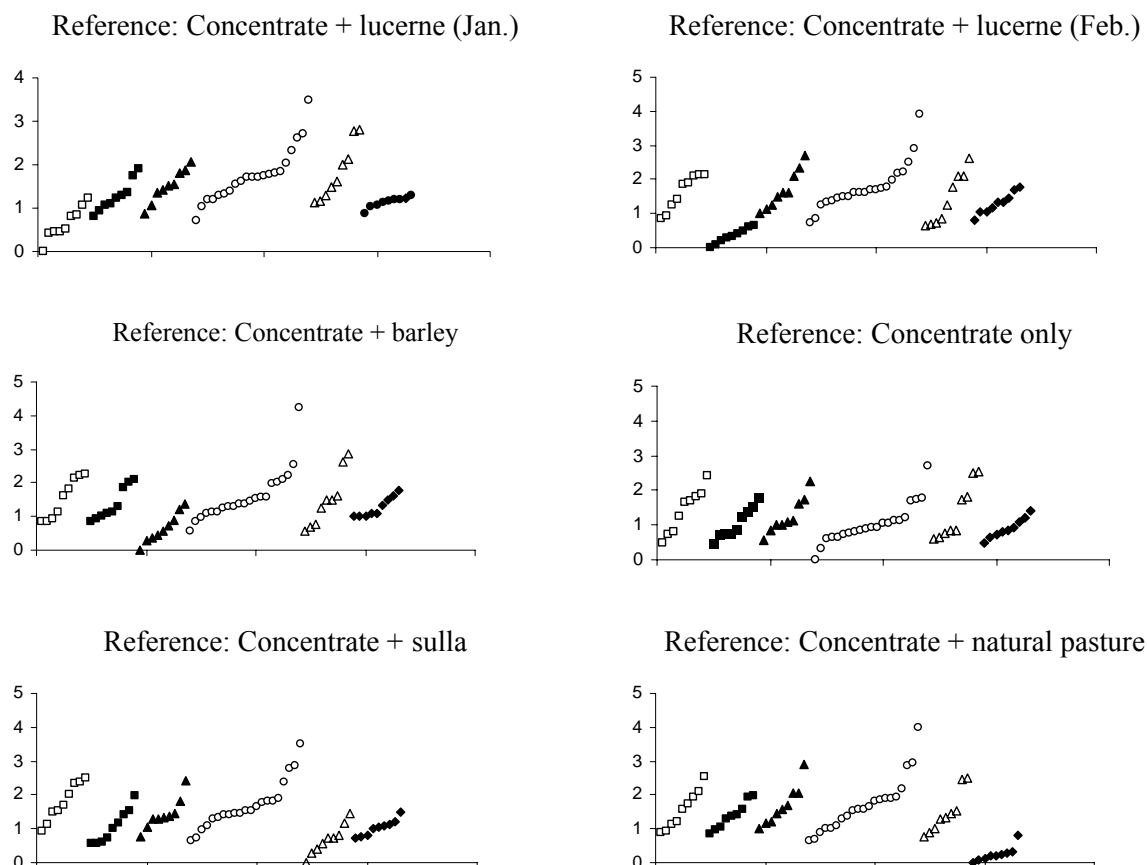


Figure 2 Mahalanobis distances between individual chick faecal NIR spectra and average group spectra taken as reference with $H = 0$: concentrate + lucerne (January, \square), concentrate + lucerne (February, \blacksquare), concentrate + barley (\blacktriangle), concentrate only (\circ), concentrate + sulla (\triangle) and concentrate + natural pasture (\bullet)

Discussion

Even though NIRS is a potent tool for statistical classification (Naes *et al.*, 2002), not all dietary groups of ostriches were easily classified by principal component analysis on the basis of spectral characteristics of their excreta. Landau *et al.* (2005) reported that a spectral distance (H) less than 0.50 confirmed with 95% confidence that goats consumed similar browse diets. This finding was backed by the present results with ostriches, which showed that a spectrum with H less than 0.47 to a group average spectrum warrants membership of the group. This could be utilized for nutritional clustering of populations of ostriches in the wild.

Overall, diets resulted in sufficient spectral variability of excreta to enable NIRS determination of CP, ADF and NDF contents in the diet of ostriches. The R^2 values obtained in the present study are similar to those reported for faecal NIRS with grazing cattle (Lyons & Stuth, 1992; Coates, 1999) and goats (Landau *et al.*, 2005). The SECV for dietary CP content found in the present study (5 g/kg DM) is similar to that reported for confined (5 g/kg; Landau *et al.*, 2004) and grazing goats (5.3 g/kg DM; Landau *et al.*, 2005), and for confined (5 g/kg DM; Boval *et al.*, 2004) and ranging cattle (8.6 g/kg DM; Lyons & Stuth, 1992; and 8.7 g/kg DM; Coates, 1999). The accuracy of excretal NIRS-based predictions of CP obtained in the present study almost matched that of direct predictions of CP in feeds that typically yield SECV ranging between 4.0 and 6.0 g/kg DM (Landau *et al.*, 2006). Our present data seem to confirm that for ostriches, the dietary content of CP can be predicted by excretal NIRS calibrations as accurately as it is in ruminants by faecal NIRS calibrations. Our calibration of dietary NDF content gave even lower SECV than a calibration developed with confined goats ($R^2 = 0.94$, SECV = 15.3 g/kg DM; Landau *et al.*, 2004) or cattle ($R^2 = 0.80$, SECV = 12.2 g/kg; Boval *et al.*, 2004). In other words, the NIRS of ostrich excreta seems to be potentially

useful in monitoring the intake of roughage – information that is important in ensuring normal behaviour and welfare in captive ostriches.

Table 3 Statistics of Optimal Calibrations, including standard errors of calibration (SEC) and of cross-validation (SECV). N represents the number of data pairs in calibration after removal of outliers

	N	Method	R ²	SEC	SECV
Dietary content (g/kg DM)					
Concentrate proportion	55	1,4,4,1	0.96	38	63
Pasture proportion	55	1,4,4,1	0.96	38	63
Neutral detergent fibre (NDF)	53	1,4,4,1	0.86	7.6	8.9
Acid detergent fibre (ADF)	55	2,6,6,2	0.95	7.2	10.7
Crude Protein	54	2,6,6,2	0.96	2.9	5.0
Ash	56	2,6,6,2	0.95	7.1	14.8
Total OM apparent digestibility (g/kg)	57	1,4,4,1	0.35	37	39
Intake (g/d)					
Concentrate intake	57	2,6,6,2	0.86	75	131
Pasture intake	57	2,6,6,2	0.95	45	72
Total DMI	52	1,4,4,1	0.71	87	111
NDF intake	52	1,4,4,1	0.58	31	39
ADF intake	56	2,6,6,2	0.80	16	19
Crude Protein intake	53	1,4,4,1	0.71	22	28
Ash intake	54	2,6,6,2	0.85	11	18

It was not possible to accurately calibrate equations for TOMD (Table 3), a major dietary attribute that determines the energy content of diets for animals. As with all regression procedures, calibrations based on MPLS can be established only from data sets of wide variability. The range of TOMD values in the present study was quite narrow, because the young herbage grazed was as digestible as the concentrate (Nitzan *et al.*, 2002). However, the narrow range of TOMD data cannot be incriminated because CP and NDF ranges were just as narrow, but their calibration was successful.

A survey of the literature on faecal NIRS calibrations of digestibility indicated that digestibility in ruminants is generally evaluated by using *in vitro* procedures. Faecal NIRS calibrations of *in vitro* digestibility feature high R² and low SECV values (0.97 and 21 g/kg DM, respectively; Landau *et al.*, 2005; 2006). However, in cattle, Coates (1999) reported lower R² and higher SEC values for faecal NIRS calibrations of DM digestibility measured *in vivo* than for those measured *in vitro*, and Boval *et al.* (2004) reported mediocre R² (0.72) for *in vivo* OM digestibility. *In vivo* digestibility differs from *in vitro* digestibility because it accounts for individual variability. Similarly, including urine in the excretal samples increases the contribution of individual bird metabolism, as opposed to "microbial" hindgut metabolism. The hypothesis that the higher the digestive contribution of microbes (in the rumen or the hindgut) the more accurate and precise is excretal or faecal NIRS, is supported by the higher spectral identity of excreta in grazing than in non-grazing chicks (Figure 2), and this hypothesis deserves further research.

The idea of relating direct feed NIR spectra to ruminants' intake has accompanied the NIRS technology from its very first days (Norris, 1976). Our calibration model predicted 86 and 95%, respectively, of the variation in DMI from concentrate and pasture. This compares well with 61% for confined cattle (Boval *et al.*, 2004). When expressed on a metabolic weight basis (28 to 40 kg BW), the SECV for DMI in Table 2 is equivalent to 6.8 to 8.5 g/kg BW^{0.75}. The error associated with the prediction of the forage intake of sheep from direct NIR spectra of their feeds was reported to be 6 g/kg BW^{0.75} by Paul *et al.* (2001) and

6.3 - 10.6 g/kg BW^{0.75} by Coleman (1999). In other words, the level of accuracy of excretal NIRS in predicting intake in ostriches resembles that of faecal NIRS in ruminants and that of direct NIRS analysis of feeds. This level of accuracy is comparable with standard errors in the predictions of intake in sheep based on the *in vivo* digestibility and on the nitrogen content of forages given as sole food (5.4 to 11.8 g/kg BW^{0.75}; Poppi, 1996).

The present results with excretal NIRS in ostrich chicks seem to support those of Coates (1999) and Boval *et al.* (2004), which were obtained from faecal NIRS with cattle, and demonstrate the potential to predict not only dietary contents, but also intake, in terms of g/d.

Conclusions

This study presents novel information on the potential of excretal NIRS to monitor important dietary attributes, such as dietary CP, NDF and ADF contents, and possibly, intake, in grazing young ostriches. The accuracy of faecal NIRS must be assessed in larger datasets, and validations must encompass the effects of year, location, gender and, importantly, the specific food source and nutrient status of the feeding environment, in order to achieve maximal robustness.

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