THE USE OF INTERNAL MARKERS TO DETERMINE METABOLIZABLE ENERGY AND DIGESTIBILITY OF DIETS IN THE AFRICAN GREY PARROT (*PSITTACUS ERITHACUS*)

Het gebruik van interne merkers om de metaboliseerbare energie en verteerbaarheid van voeders te bepalen bij de Grijze Roodstaartpapegaai (Psittacus erithacus)

J. Sales, L. de Schutter, G.P.J. Janssens

Laboratory of Animal Nutrition
Department of Animal Nutrition, Genetics, Breeding and Ethology
Faculty of Veterinary Medicine, Ghent University
Heidestraat 19, B-9820 Merelbeke, Belgium
jsales@umd.edu

ABSTRACT

Acid-insoluble ash (AIA) and acid-detergent lignin (ADL) were evaluated as internal markers in digestibility studies with African Grey parrots (*Psittacus erithacus erithacus*). Apparent metabolizable energy, corrected for nitrogen (N) retention (AME_n), nitrogen retention (NR), and apparent digestibility of dry matter, organic matter, crude protein and crude fat of a commercial seed mixture, sunflower seed, and a commercial pelleted parrot diet were determined using either the method of total collection of feed and excreta or using the marker technique. Both AIA and ADL presented unrealistic negative digestibility values for whole seed diets due to the higher concentration of marker found in the calculated feed intake than in the excreta. This study illustrates the necessity to determine nutrient concentration in feed refusals in digestibility studies with parrots due to the feeding habits of these birds. However, AIA as marker presented digestibility values that, although higher ($P < 0.05$), showed similar or less variation than the method of total collection when applied to pelleted parrot diets in which feed refusals contain a similar nutrient concentration as the feed offered.

SAMENVATTING

Zuuronoplosbare as en acid detergent lignin werden geëvalueerd als interne merkers in verteerbaarheidsstudies bij Grijze Roodstaartpapegaaien (*Psittacus erithacus erithacus*). De metaboliseerbare energie, gecorrigeerd voor stikstofretentie (AME_n), stikstofretentie en schijnbare verteerbaarheid van drogestof, organische stof, ruw eiwit en ruw vet van een commerciële zadenmengeling, zonnebloempitten en een commercieel gepelleteerd papegaaienvoeder werden bepaald met zowel de methode van totale collectie van voeder en excreta, als met de merkertechniek. Zowel zuuronoplosbare as als acid detergent lignin gaf onrealistische negatieve verteerbaarheidswaarden bij gehele zaden als voeding aangezien een hogere concentratie van de merker werd gevonden in de berekende voederopname dan in de excreta. Deze studie illustreerde de noodzaak van de bepaling van nutriëntgehalten in voederresten bij verteerbaarheidsstudies met papegaaien vanwege de voedingsgewoonten van deze vogels.

Desalniettemin leverde zuuronoplosbare as als merker toch verteerbaarheidswaarden op die, ook al waren ze hoger ($P < 0,05$), een vergelijkbare of lagere variatie vertoonden dan de methode met totale collectie bij gekorrelde papegaaienvoeders, waarbij de voederresten een gelijkaardige nutriëntenconcentratie hebben als het aangeboden voeder.

INTRODUCTION

The formulation of diets with accurate metabolizable energy (ME) and nutrient digestibility values, and the evaluation of the effect on digestibility of dietary treatments such as conditioning or additives requires reliable methods for obtaining these values. The most general method for determining digestibility involves the collection of all food eaten and all excreta produced, the so-called total collection method. The difficulty of collecting the above with parrots could have been a contributory factor to the lack of information on the digestibility of diets and diet ingredients for these birds. Parrots often select individual seeds from seed mixtures. Furthermore, being very active birds, parrots often waste feed by knocking it out of their feeders. The mixing of wasted feed with excreta will increase experimental error because feed intake and excreta weight are both overestimated (Hagen 1999).

In comparison with the total collection method, the use of markers to determine ME and digestibility values avoids errors associated with the inaccurate measurement of feed intake and excreta output and the contamination of excreta (Sibbald 1987). With the marker method, either an indigestible added component (external marker, a known concentration of which is mixed into the diet) or an indigestible natural dietary component (internal marker) is used to calculate digestibility. When a marker is used, only samples of food and excreta are needed in order to calculate digestibility by the ratio of marker concentrations in food and excreta (Bjorndal, 1985). The use of markers to determine digestibility in avian species has been reviewed in detail by Sales and Janssens (2003a).

The aim of the present study was to compare ME and nutrient digestibility values of different diets in African Grey parrots (*Psittacus erithacus erithacus*) derived using either the measurements of total feed intake and excreta output or using the internal markers acid-insoluble ash (AIA) and acid detergent lignin (ADL).

MATERIALS AND METHODS

Animal Husbandry and Collection Procedures

Six African Grey parrots, aged 5 months and with a mean body weight of 447.2 g, were used in three successive trials to evaluate the apparent metabolizable energy, corrected for nitrogen (N) retention (AME_n), nitrogen retention (NR), and apparent digestibility of dry matter, organic matter, crude protein and crude fat of (1) a commercial parrot diet consisting of a mixture of several seeds fortified with protein and vitamin/mineral pellets, (2) sunflower seed as sole diet ingredient, and (3) a commercial pelleted parrot diet. The parrots were housed individually in wire cages placed in a room under a 12L:12D photoperiod and room temperature of 16 to 19 °C. Fresh water was available at all times. The birds were fed a diet *ad libitum* for two weeks. For the following six days, *ad libitum* feeding was continued and the daily feed intake and excreta production were measured. All hulls and spilled feed were carefully separated from excreta during collection and, together with feed remaining at the end of the experiment, described as 'feed refusals'. The excreta were scraped off the floor of the cages as soon as dropped during the daytime, whereas the night excreta were collected at 0800 h. Daily excreta collections were pooled for each bird and stored at –20 °C until further processing.

The experimental setup and housing conditions in all trials were approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University.

Analytical Procedures

Determination of the nitrogen (N), organic matter and crude fat (ether extract) contents of oven dried feed and feed refusals and freeze dried excreta was done according to the methods described by the Association of Official Analytical Chemists (AOAC) (1980), whereas the gross energy was determined using an IKA C-7000-type adiabatic bomb calorimeter. Separation of the N content of excreta into N of urinary and N of fecal origin was done following the method of Terpstra and De Hart (1974). Crude protein was calculated from N x 6.25 (AOAC, 1980). The AIA content of the feed and of the excreta was determined using the procedure of Van Keulen and Young (1977) as adapted by Atkinson *et al.* (1984) for high fat content diets. The hydrolytic (sulfuric acid) ADL was determined according to the method of Van Soest (1965).

Digestibility Coefficient Calculations and Statistical Analyses

The intake of dry matter, organic matter, crude protein, crude fat, AIA and ADL in whole seed diets was

calculated from determinations of the contents in the feed offered and in the feed refusals. This step was omitted for the pelleted diet because the pellets offered and the pellets refused naturally had the same nutrient composition. The AME_n, the NR and the apparent digestibility coefficients of the nutrients were calculated using standard formulas for the total collection and the marker methods (Maynard and Loosli 1969). The formulas used to determine digestibility, including slight modifications to accommodate the calculation of AME_n, NR and dry matter digestibility, are as follows:

Total collection

$$\text{Digestibility} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}}$$

Marker method

$$\text{Digestibility} = 1 - \left(\frac{\%\text{ marker in feed}}{\%\text{ marker in excreta}} \times \frac{\%\text{ nutrient in excreta}}{\%\text{ nutrient in feed}} \right)$$

The above is described as 'apparent' digestibility, as it does not account for material of metabolic origin, such as sloughed intestinal cells or the digestive enzymes found in the excreta.

The AME was corrected to N equilibrium for the total nitrogen retained or lost from body tissue using a factor of 34.39 kJ/g N retained. This represents the energy equivalent of uric acid per gram of nitrogen (Hill and Anderson, 1958). Paired t-tests were used to compare values derived using the total collection method, either taking refusals into account or disregarding them, as well as to compare values derived either from total collection or from the marker technique with the pelleted diet.

RESULTS

The feed intake and excreta production was 13.30 ± 0.781 and 3.99 ± 0.205 g/d, respectively, for the mixed whole seed diet, and 15.22 ± 0.549 and 5.02 ± 0.232 g/d, respectively, for the sunflower seed diet. The corresponding values for the pelleted diet were 28.21 ± 0.939 and 9.49 ± 0.356 g/d, respectively.

The nutrient concentrations in the feed offered, in the feed refusals, and in the excreta for diets consisting of whole seeds are presented in Table 1.

Because of the higher concentration of marker found in feed than in excreta, Table 1 clearly shows that the use of the marker technique (AIA and ADL) produces negative digestibility values, regardless of whether feed refusals are taken into account or disregarded. Therefore digestibility was calculated only according to the total collection method, and a comparison was made between values obtained, with either taking into account and disregarding feed refusals (Table 2).

The values for all the evaluated nutrients differed ($P < 0.05$) when the nutrients in the feed refusals with sunflower seed as sole diet ingredient were taken into account. The dry matter, organic matter and crude fat digestibility of the seed mixture presented comparable ($P > 0.05$) values.

Although different ($P < 0.05$) from the method of total collection, the use of markers with a pelleted diet (95.56 % organic matter, 13.82 % crude protein, 16.35 % crude fat, 0.19 % AIA, and 1.17 % ADL, on a dry matter basis) produced positive digestibility values (Table 3). The highest values ($P < 0.05$) were derived through the use of AIA as marker, whereas ADL as marker produced the lowest ($P < 0.05$) values. However, the differences between values derived with AIA as marker and the method of total collection were small, with similar or less variation found with AIA.

DISCUSSION

This study illustrates the difficulty of using markers to evaluate the digestibility of diets in parrot nutrition. The use of traditionally fed seed mixtures not only eliminates the use of external markers due to the impracticality of mixing the marker into the diet, but the dehulling of seeds by the parrots to ingest the contents and the preferential selection of certain seeds changes the concentration of nutrients and internal markers in the actual intake as compared to the feed offered. The only option for determining the nutrient and marker concentrations in the actual feed intake when seed or seed mixtures are fed is to make calculations based on the measurements of the weight and composition of the total feed offered and the feed refusals.

The internal markers used in the present study (AIA and ADL) presented higher concentrations in the feed intake than were found in the excreta with seeds, leading to unrealistic negative digestibility values. The failure of AIA and ADL in the present study when whole seeds were fed might partly be attributed to analytical error due to a low excreta marker content. Mueller (1956) described relatively great variation in percentage of lignin recovered due

Table 1. Nutrient concentrations in feed offered, feed refusals and excreta, and calculated nutrient concentration in feed intake for whole seed diets (dry matter basis, means ± SE, n = 6).

Component	Feed offered	Refusals	Excreta	Calculated nutrient concentration in feed intake[1]
Seed mixture				
Gross energy (MJ/kg)	20.48	19.06 ± 0.125	14.71 ± 0.060	27.13 ± 0.876
Nitrogen (%)	1.88	1.71 ± 0.022	9.67 ± 0.146	2.67 ± 0.103
Crude protein (%)	11.75	10.70 ± 0.135	7.84 ± 0.278	16.67 ± 0.641
Organic matter (%)	94.83	94.46 ± 0.208	88.26 ± 0.175	96.65 ± 1.065
Crude fat (%)	11.47	11.57 ± 0.415	4.97 ± 0.131	11.11 ± 1.988
Acid detergent lignin (%)	10.17	7.46 ± 0.266	1.39 ± 0.045	22.89 ± 1.837
Acid-insoluble ash (%)	1.05	1.18 ± 0.057	0.10 ± 0.010	0.81 ± 0.538
Sunflower seed				
Gross energy (MJ/kg)	25.65	22.82 ± 0.160	14.14 ± 0.071	30.62 ± 0.164
Nitrogen (%)	2.73	1.75 ± 0.054	11.21 ± 0.101	4.47 ± 0.056
Crude protein (%)	17.09	10.92 ± 0.335	70.06 ± 0.632	27.93 ± 0.353
Organic matter (%)	96.79	97.27 ± 0.043	89.44 ± 0.051	95.96 ± 0.043
Crude fat (%)	36.30	22.22 ± 0.938	5.69 ± 0.270	61.01 ± 1.240
Acid detergent lignin (%)	9.51	13.77 ± 0.370	0.65 ± 0.032	2.12 ± 0.230
Acid-insoluble ash (%)	0.15	0.10 ± 0.019	0.07 ± 0.026	0.26 ± 0.040

[1] $\frac{\text{(Feed offered x Nutrient concentration) - (Feed refused x Nutrient concentration)}}{\text{(Feed offered – Feed refused)}} \times 100$

Table 2. Apparent metabolizable energy, corrected for nitrogen retention (AME_n), nitrogen retention (NR), and digestibility of dry matter, organic matter, crude protein and crude fat determined with the total collection method (means ± SE, n = 6).

Component	Seed mixture		Sunflower seed	
	Account for refusals	Disregard refusals	Account for refusals	Disregard refusals
AME_n (MJ/kg)	22.97 ± 0.773[b]	16.06 ± 0.043[a]	25.94 ± 0.175[b]	20.99 ± 0.054[a]
NR (mg/g diet N)	-2.42 ± 0.482[b]	-10.29 ± 0.719[a]	-45.25 ± 3.112	-62.60 ± 3.476[a]
Digestibility(%)				
Dry matter	69.94 ± 0.327		67.07 ± 0.411	
Organic matter	72.54 ± 0.380	72.03 ± 0.257	69.31 ± 0.374[a]	69.57 ± 0.376[b]
Crude protein	85.66 ± 0.524[b]	80.04 ± 0.870[a]	92.56 ± 0.162[b]	87.98 ± 0.236[a]
Crude fat	82.78 ± 4.804	81.23 ± 0.680	96.93 ± 0.133[b]	94.85 ± 0.187[a]

Values with different superscripts (a, b) in the same line within diet are significantly different ($P < 0.05$)

Table 3. Apparent metabolizable energy, corrected for nitrogen retention (AME_n), nitrogen retention (NR), and digestibility of dry matter, organic matter, crude protein and crude fat determined with the total collection method (TC), acid-insoluble ash (AIA) and acid detergent lignin (ADL) as markers with a commercial pelleted parrot diet (means ± SE, n = 6)

Component	TC	AIA	ADL
AME_n (MJ/kg)	15.64 ± 0.057[b]	15.86 ± 0.044[c]	13.04 ± 0.278[a]
NR (mg/g diet N)	1.93 ± 0.232[b]	3.18 ± 0.325[c]	-9.69 ± 1.367[a]
Digestibility (%)			
Dry matter	66.35 ± 0.356[b]	68.46 ± 0.344[c]	47.01 ± 2.076[a]
Organic matter	69.50 ± 0.360[b]	71.41 ± 0.351[c]	51.97 ± 1.924[a]
Crude protein	70.11 ± 1.828[b]	71.37 ± 1.471[c]	51.90 ± 2.659[a]
Crude fat	91.86 ± 0.350[b]	92.38 ± 0.291[c]	87.25 ± 0.451[a]

Values with different superscripts (a, b) in the same line are significantly different ($P < 0.05$)

to small deviations in sulfuric acid concentration from the prescribed 72 %. Therefore the same batch of chemicals was used in the present study to determine ADL. However, studies with poultry (Mueller 1956) and ruminants (Fahey and Jung 1983) conceded that changes occur in the lignin molecule with passage through the digestive tract, suggesting that the lignin in the diet and the lignin in the excreta were not chemically identical entities. Furthermore, the 72 % sulfuric acid detergent lignin method, as used in the present study, measures cutin and Maillard-type browning products such as lignin, whereas some of the true lignin may be destroyed (Goering and Van Soest 1970). This might partly explain the unrealistic ADL content of 23 % calculated in feed intake with the mixed seed diet.

However, in a pelleted diet both AIA and ADL as markers presented realistic positive digestibility values, with AIA resulting in similar values with similar or less variation than the method of total collection. Although the total collection method is the most widely used method for determining the digestibility of feeds in animal species, the reliability of this method is questionable, especially in avian species due to the loss of feed or excreta during collection that would change the digestibility values (Sales and Janssens 2003*a*; *b*). Further studies are needed to investigate whether AIA as marker and the total collection method might be equally able to detect differences between treatments.

It can be concluded from the results presented in this study that the internal markers AIA and ADL are unsuitable for determining the digestibility of diets consisting of whole seeds in parrots with feeding habits similar to those of the African Grey parrot, and further studies are needed in the search for a suitable marker. Preliminary results (Sales and Janssens, unpublished results) have produced evidence that crude ash as a marker with seeds of low mineral content shows potential with adult avian species and warrants further investigation. However, AIA has proven to be a suitable marker with relatively low variation between animals for determining the digestibility of pelleted parrot diets.

ACKNOWLEDGEMENTS

Versele-Laga nv, Kapellestraat 70, B-9800 Deinze, Belgium, is gratefully acknowledged for the provision of feed during this study.

LITERATURE

Association of Official Analytical Chemists (AOAC) (1980). Official Methods of Analysis of the Association of Official Analytical Chemists. 13th ed.

Association of Official Analytical Chemists, Arlington, VA, USA.

Atkinson J.L., Hilton J.W., Slinger S.J. (1984). Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences 41*, 1384-1386.

Bjorndal K.A. (1985). Use of ash as an indigestible dietary marker. *Bulletin of Marine Science 36*, 224-230.

Fahey G.C. Jr., Jung H.G. (1983). Lignin as marker in digestion studies: a review. *Journal of Animal Science 57*, 220-225.

Goering H.K., Van Soest P.J. (1970). *Forage Fiber Analyses (apparatus, reagents, procedures and some applications)*. USDA Agricultural Handbook 379, Washington DC, USA.

Hagen M. (1999). The metabolizable energy of sunflower seed kernel in Goffin's cockatoo (*Cacatua goffini*) and a review of the amino acid and fatty acid composition of oil seeds eaten by parrots. http://shell.pubnix.net/~mhagen/docu/papers.html.

Hill F.W., Anderson D.L. (1958). Comparison of metabolizable energy and productive energy determinations with growing chickens. *Journal of Nutrition 64*, 587- 603.

Maynard L.A., Loosli J.K. (1969). *Animal Nutrition*, 6th ed. McGraw-Hill, New York, USA.

Mueller W.J. (1956). Feasibility of the chromic oxide and the lignin indicator methods for metabolism experiments with chickens. *Journal of Nutrition 58*, 29-36.

Sales J., Janssens G.P.J. (2003a). The use of markers to determine energy metabolizability and nutrient digestibility in avian species. *World's Poultry Science Journal 59*, 314-327.

Sales J., Janssens G.P.J. (2003b). Acid-insoluble ash as a marker in digestibility studies: a review. *Journal of Animal and Feed Sciences 12*, 383-401.

Sibbald I.R. (1987). Estimation of bioavailable amino acids in feedingstuffs for poultry and pigs: A review with emphasis on balance experiments. *Canadian Journal of Animal Science 67*, 221-300.

Terpstra K., N. De Hart N. (1974). The estimation of urinary nitrogen and faecal nitrogen in poultry excreta. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 32*, 306-320.

Van Keulen J., Young B.A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science 44*, 282-287.

Van Soest P.J. 1965. Use of detergents in analyses of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Agricultural Chemists 46*, 829-83