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Different methodologies for determining the nutrient metabolism and energy coefficients of a commercial diet for two species of parrots of the genus, *Amazona*

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Abstract

This study aimed to compare two methods of excreta collection: the traditional, total method and a partial method using indigestible neutral detergent fibre (iNDF) as an internal marker for determining the metabolism coefficients of nutrients and energetic value of the excreta for two species of parrots from the *Amazona* genus. Protein, energy, and feed consumption were also analysed using metabolic weight for the two species of parrots. Four pairs of parrots of the turquoise-fronted amazon (*Amazona aestiva*) and four pairs of the orange-winged parrots (*Amazona amazonica*) were used. Each pair stayed in a cage, giving a total of eight experimental units. The data were submitted to analysis of variance and compared using F- and Tukey's tests. Of the collection methods, the total excreta collection substantially improved the energy values and metabolism coefficients of the commercial diet. The %dry matter (DM) feed formula - (%DM excreted × indigestibility factor) underestimated coefficients of apparent metabolism of dry matter (CAMDM) when using partial excreta collection with iNDF as an indicator. No significant difference was found between species when considering protein and crude energy intake of the commercial diet.

Keywords: *Amazona aestiva*, *Amazona amazonica*, excreta collection, intake, metabolizable energy, wild animals

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Introduction

Of the animals trafficked, birds belong to the most wanted group due to their beauty and song (Pereira & Brito, 2005). Parrots stand out for being very sociable and for their ability to imitate human sounds (Lara, 2006). However, according to the Convention on International Trade in Endangered Species (CITES, 2016), species of the genus, *Amazona,* are classified as of little concern.

Since the creation of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) Normative Instruction No. 169 of February 20th, 2008, which regulates the categories of use and management of wild fauna for captivity in Brazilian territory and aims to meet scientific research, species conservation, breeding, reproduction and commercialization goals, the number of commercial wild animal farms registered with IBAMA has been growing. This offers legalized animals a health certificate. These animals still have a higher trade value when compared to animals captured in the wild by traffickers.

Recently, there has been increasing interest in developing alternatives to improve the market of wild animals from legalized commercial breeding sites. Thus, the nutrition of these animals has become an important aspect of study. If properly applied, it becomes a strong ally to reduce the raising costs and increase their productivity (Lara, 2006). To meet the nutritional requirements of each stage of development, feed companies have started to develop several rations specific for

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parrots. As a result, zoos and commercial breeding grounds have begun to replace natural foods by commercially formulated feeds (Werneck, 2016).

Besides containing quality raw materials and being palatable, it is important that commercial diets provide adequate amounts of nutrients and energy to meet the needs of the birds (Machado & Saad, 2000). The drastically decrease in physical activity influences the energy needs between captive and free-living birds, since free-living birds often fly several miles in search of food compared to captive birds. Consequently, the nutritional and energetic values of the foods offered to animals in captivity, as well as the nutritional utilization of these foods by these birds, is important. These characteristics can be understood by considering the coefficients of apparent metabolism measured using excreta collection methods.

The total excreta collection methodology is one of the most common methods for determining the digestibility of nutrients and energy values of food. This method has the principle of measuring the total food consumed and the total excreta produced during a period (Sakomura and Rostagno, 2007). Although the total collection of excreta is the most popular method, it is possible to observe variable results between experiments. This happens because, with each experimental situation, variables such as age of birds, adaptation period, fasting period, and collection period change (Dassi, 2015). An alternative method using the partial collection of excreta using indicators stands out because it is not necessary to measure the total feed intake, or the total amount of excreta produced.

The objective of this study was to compare the methods of total collection of excreta and partial collection of excreta using internal indicator assays in two species of *Amazona* parrots, bred in captivity, to determine the coefficients of apparent metabolism of dry matter, crude protein, and gross energy, as well as metabolizable energy of the commercial diet, to determine which method is the best to be used with parrots. Feed and energy intake using metabolic weight, consumption, waste, and feed costs were determined in the two species.

Materials and Methods

The experiment was approved by the Ethics Committee on Animal Use of the Federal University of Ceara, under the protocol number 67/2017. It was also approved by the Biodiversity Information and Authorization System, granting authorizations for *ex situ* biological samples, under number 57134-1 of 2018.

The metabolic experiments were conducted at the Haras Claro Commercial Farm, registered at IBAMA under registration number, 302352, located in the municipality of Caucaia, in the state of Ceara. Subsequently, the laboratory analysis was performed at the Animal Nutrition Laboratory of the Department of Animal Science of the Federal University of Ceara, located in the city of Fortaleza in the state of Ceara.

For the metabolism test, 16 adult parrots were used, i.e., four pairs of turquoise-fronted amazon (*Amazona aestiva*) and four pairs of orange-winged parrots (*Amazona amazonica*), with an average weight of 400 g. The birds were clinically healthy and adapted to captivity. The mean weight reported in the literature of 400 g (Grespan *et al*., 2014) was used, as we were not allowed to handle the birds to avoid interfering with the reproductive process. A completely randomized, 2 × 2 factorial design (two collection methods and two species of parrots), totalling four treatments. Repetitions were performed over time, with two replications in each of the three distinct periods, totalling six replicates.

The experimental collection period did not have an adaptation phase since the parrots used in the research were already housed in the cages previously detailed. The diet analysed was the one already offered daily. Excreta collection was divided into three periods, five days in March, four days in May, and four days in June. The excreta collections were made in different periods so they did not coincide with the reproductive period of the birds. The excreta collected in the three periods totalled six replications of each treatment in the analysis. Two different excreta collection methods were considered: the traditional, total excreta collection and a partial collection, using indigestible neutral detergent fibre as the internal indicator.

The parrots were distributed in pairs with the purpose of reproductive and commercialization of the parrots. They were housed in galvanized steel cages (202 cm × 103 cm × 67 cm) equipped with trays to collect excreta and food remains, three perches, a drinking fountain, and a feeder. The infrastructure provided was used to distribute the treatments and to carry out the metabolism tests, without the need of transferring the birds from their habitual cages, avoiding any negative influence on the reproductive process.

Temperature and relative humidity of the air were collected by data loggers installed inside and outside the shed, to register the macro- and microclimate. The mean values for minimum, maximum temperatures and humidity were 22.53 ºC, 40.25 ºC, and 72.84%, respectively, referring to the averages for the months of March, May, and June. The diet used in the experiment was an extruded

commercial diet specific to parrots, produced by Megazoo (Juatuba, MG, Brazil), which was already supplied daily to the animals (Table 1).

During the period of the metabolic tests, the animals received food and water *ad libitum* so as not to fast, and early in the morning, the food was replaced for all birds at 07:00. Excreta were collected twice a day at 08:00 and 16:00, independent of the method. Moreover, to achieve better results, the collected excreta were cleaned by removing feathers, feed extrudates, and wood chips from the perch. The excreta were stored in previously marked, individual plastic pots, and kept in a freezer at -10 °C until further laboratory analysis. Total feed intake was obtained by recording the initial weight of the rations offered minus the final weight of the remnants in the feeder and the waste in the excreta collection trays.

The coefficients of apparent metabolism of dry matter (CAMDM), crude protein (CAMCP), and crude energy (CAMCE) of the commercial diet were determined using the equations proposed by Sakomura and Rostagno (2007).

Total excreta collection:

$CAMDM\ (\%) = (DM_{intake} - DM_{excreted} / DM_{intake}) \times 100$

$CAMCP\ (\%) = (CP_{intake} - CP_{excreted} / CP_{intake}) \times 100$

$CAMCE\ (\%) = (CE_{intake} - CE_{excreted} / CE_{intake}) \times 100$

Partial collection of excreta:

CAMDM (%) = (1-IF × 100)

CAMCP (%) = (% CP of diet - (% CP of excreta × IF) /% CP of diet)

CAMCE (%) = (CE of diet - (CE of excreta × IF) / CE of diet)

where IF = Indigestibility factor; IF = iNDF of the diet / iNDF of the excreta; iNDF = Indigestible neutral detergent fibre.

As a comparison, two other formulas available in the literature were used to determine the CAMDM, one by Rodrigues *et al*. (2005):

CAMDM: %DM of feed - (%DM of excreta × IF),

and other by Sakomura and Rostagno (2007):

CAMDM: 100 - [(% indicator in the food / % indicator in the excreta) x (% DM in the excreta /% DM in the diet)] × 100.

Using the data from the total excreta collection, the values of apparent metabolizable energy (AME) and the metabolizable energy corrected for the nitrogen balance (AMEn) were determined, based on dry matter and natural matter, as proposed by Matterson *et al*. (1965):

$AME\ (kcal/kg) = [(CE_{intake} – CE_{excreted})/ DM_{intake}];$

$AMEn\ (kcal/kg) = [(CE_{intake} – CE_{excreted} \pm 8{,}22 \times NB)/ DM_{intake}],$

where NB: nitrogen balance = intake nitrogen – excreted nitrogen.

In contrast, the energy values of the rations calculated by means of the partial excreta collection using internal indicators were calculated as described by Sakomura and Rostagno (2007):

$AME\ (kcal/kg) = [CE\ of\ diet – (CE_{excreted} \times IF)];$

$AMEn\ (kcal/kg) = [CE\ of\ diet – (CE_{excreted} \times IF \pm 8{,}22 \times NB)],$

where NB = intake nitrogen – (excreted nitrogen × IF), and $IF = iNDF_{diet} / iNDF_{excreta}$.

After collection, the samples were homogenized, weighed, and dried in a forced ventilation oven for 72 hours at 55 °C then analysed for dry matter (DM), nitrogen (N) and neutral detergent fibre (NDF). The laboratory analysis of the ration and the excreta were performed as described by Silva and Queiroz (2002). The crude energy (CE) was determined by bomb calorimetry. For partial excreta collection, the indigestible neutral detergent fibre (iNDF) was used as internal indicator, obtained using the methodology proposed by Casali *et al*. (2008). Between the species, it was quantified using metabolic weight ($kcal/kg^{0.75}$/day). The intake of natural matter, dry matter, crude protein, AME, and AMEn was determined according to the methodology described by Souza (2016).

The results were submitted to analysis of variance using the software, Statistical Analysis System, considering a completely randomized design in a 2 × 2 factorial scheme (two collection methods and two species of parrots). The averages were compared by F-test using a 5% probability for significance. Tukey's test (5%) was performed to compare the three formulas used to quantify the coefficient of apparent metabolism of the dry matter of the ration using partial excreta collection.

Table 1 Basic composition of the commercial extruded feed on the label (Megazoo, Parrots AM16)

Commercial diet: extruded, balanced feed for parrots	
Basic composition	
Whole grain milled maize, rice, extruded whole soybeans, soybean meal, oatmeal, dehydrated egg, corn protein, wheat bran, beet pulp, yeast extract (nucleotide source), beer brewer's yeast, tuna flour (*Schizochytrium* sp.), dicalcium phosphate, calcitic limestone, soybean oil, palm kernel oil, salmon oil, sodium chloride (common salt), mannan oligosaccharides, beta Vitamin B12, Vitamin B12, Vitamin C, Vitamin E, Vitamin A, Vitamin B12, Vitamin B12, Vitamin B12 K3, niacin, choline chloride, folic acid, calcium pantothenate, biotin, inositol, zinc amino acid chelate, copper sulphate, copper amino acid chelate, calcium iodate, manganese monoxide, manganese amino acid chelate, zinc, cobalt sulphate), fungistatic additive, chelated iron, beta-carotene, lutein, selenium enriched yeast, natural turmeric dye, coconut aroma, antioxidant additive (BHA).	
Guaranteed levels (expressed as is)	
Humidity (Maximum)	110g/kg
Crude Protein (Minimum)	160 g/kg
Ether extract (Minimum)	50 g/kg
Fibrous matter (Maximum)	35 g/kg
Mineral matter (Maximum)	70 g/kg
Calcium (Maximum)	11 g/kg
Calcium (Minimum)	8.000 mg/kg
Phosphorus (Minimum)	6.000 mg/kg
Sodium (Minimum)	2.300 mg/kg
Mannan oligosaccharides - MOS (Minimum)	400 mg/kg
Beta-glucans (Minimum)	440 mg/kg
Fructo oligosaccharides - FOS (Minimum)	1.000 mg/kg
Omega 3 (Minimum)	3.100 mg/kg
Adsorbents of toxins (Minimum)	1.000 mg/kg
Thistle Extract	0,50 mg/kg
DL-Methionine (Minimum)	4.500 mg/kg
Enrichment per kilogram of the product described on the label	
Vitamin A (8,000.00 IU), beta-carotene (8.00 mg), vitamin D3 (1,300.00 IU), vitamin E (120,00 IU), vitamin K3 (3.0 mg), vitamin C Folic acid (2.00 mg), calcium pantothenate (35.00 mg), choline chloride (1500 mg), vitamin B6 (6.00 mg), vitamin B1 (6.00 mg), vitamin B2 (10.00 mg), vitamin B12 (80.00 mg), niacin (55.00 mg), biotin (0.30 mg), inositol (60.00 mg), chelated copper (3.00 mg), cobalt (0.15 mg), iodine (1.10 mg), chelated iron (25.00 mg), manganese (35.00 mg), chelated manganese mg), zinc (60.00 mg), chelated zinc (30.00 mg), organic selenium (0.30 mg).	

Results

The values of dry matter, crude protein, and crude energy of the commercial feed used in the experiment were determined at the Animal Nutrition Laboratory of the Animal Science Department of the Federal University of Ceara as 93.59%, 19.80%, and 4628 kcal/kg DM, respectively. There was no difference between the two species analysed in the determination of metabolism coefficients for DM, CD, and CE, regardless of the collection method used (Table 2).

However, excreta collection methodologies influenced (P <0.05) metabolization coefficients, since the partial collection of excreta, using the indigestible NDF as indicator, provided lower values when compared to those generated by total collection of excreta.

For the CAMDM performed by partial collection of excreta, the formula that was chosen to be used in the research [CAMDM: (1-FI) × 100, with FI = indigestibility factor], generated an average value of 65.26% for this variable, while of the other two formulae described in the literature, one of them [CAMDM: %DM of the ration - (% of excrements × IF)], was not so precise and generated a lower average value for CAMDM (Table 3).

The method of total collection of excreta showed larger results for AME and AMEn of the commercial diet. However, between species, no significant difference was observed for AME and

AMEn for the commercial diet (Table 5). There were no significant differences in the intake of natural matter, dry matter, protein, and energy by metabolic weight between species when using the commercial diet (Table 6).

Table 2 Coefficients of apparent metabolism of dry matter, crude protein, and crude energy of the experimental ration determined using different methods and species of cage-bred parrots

CAMDM (%)[1]				
Species (SPE)	Methodologies (MET)[2]		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	94.46±1.30	64.36±3.13	79.41A	0.0000
Turquoise-fronted amazona	95.06±1.65	66.16±3.87	80.61A	0.0000
Average	94.76[a]	65.26b		
P-value (SPE within MET)	0.7067	0.2615		
ANOVA[3] *(p- overall value)*				
Species (SPE)	0.2900		Average overall	CV (%)[4]
Methodologies (MET)	0.0000		80.01	3.38
SPE × MET	0.5903			
CAMCP (%)[1]				
Species (SPE)	Methodologies (MET)		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	89.68±3.18	36.11±7.44	62.90A	0.0000
Turquoise-fronted amazona	90.32±3.43	31.67±8.37	60.99A	0.0000
Average	90.00a	33.89b		
P-value (SPE within MET)	0.8587	0.2201		
ANOVA *(p- overall value)*				
Species	0.4517		Average overall	CV (%)
Methodologies	0.0000		61.95	9.80
SPE × MET	0.3187			
CAMCE (%)[1]				
Species (SPE)	Methodologies (MET)		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	95.49±0.93	71.40±2.96	83.45A	0.0000
Turquoise-fronted amazona	96.12±1.42	73.11±2.98	84.61A	0.0000
Average	95.80[a]	72.25b		
P-value (SPE within MET)	0.6406	0.2081		
ANOVA *(P- overall value)*				
Species	0.2239		Average overall	CV (%)
Methodologies	0.0000		84.03	2.70
SPE × MET	0.5653			

[1]CAMDM = Coefficient of apparent metabolism of dry matter; CAMCP = Coefficient of apparent metabolism of crude protein; CAMCE = Coefficient of apparent metabolism crude energy; CAM formulas according to Sakomura and Rostagno (2007)
[2]Traditional method of total collection of excreta; Alternative method of partial collection of excreta using indicator.
[3]Analysis of variance (P <0.05); Different upper-case letters in the columns and lower-case letters in the rows reveal differences using the F-test (P <0.05)
[4] CV = Coefficient of variation

Table 3 Coefficients of metabolism of dry matter of experimental rations determined by partial collection of excreta with different formulas and two species of cage-bred parrots

CAMDM (%)[1]				
Formulas (FOR)[2]	Species (SPE)		Average	*P-value (SPE within FOR)*
	Orange-winged parrot	Turquoise-fronted amazon		
1	64.36±3.14	66.16±3.88	65.26A	0.4083
2	60.85±3.16	62.12±4.17	61.49B	0.5593
3	65.02 ±3.37	66.38±4.46	65.70[a]	0.5329
Average	63.41a	64.89[a]		
P-value (FOR within SPE)	0.1298	0.0987		
ANOVA[3] *(P-value overall)*				
Species (SPE)	0.2436		Average overall	CV (%)[4]
Formulas (FOR)	0.0178		64.15	5.82
SPE × FOR	0.9824			

[1]CAMDM = Coefficient of apparent metabolism of dry matter

[2]Formula 1 for CAMDM: (1-FI) × 100, where IF = indigestibility factor (Sakomura and Rostagno, 2007); Formula 2 for CAMDM: %DM of the ration - (% of excreta × IF), according to Rodrigues *et al.* (2005); Formula 3 of CAMDM, 100 - [(% indicator in feed / indicator% in excreta) × (%DM in excreta /%DM in feed)] × 100 (Sakomura and Rostagno, 2007).

[3]Analysis of variance (P <0.05); Different uppercase letters in the columns and lowercase letters in the rows reveal difference using Tukey's test (*P* <0.05) and F-test (*P* <0.05); respectively.

[4]CV = Coefficient of variation

The results for the indigestibility factor and recovery rate of the indicator used in the partial excreta collection with the different parrot species indicated no significant difference between the equations (Table 4).

Table 4 Indigestibility factors and recovery rates of indigestible neutral detergent fibre used as an indicator in partial excreta collection with two species of cage-bred parrots

Species	Variables[1]	
	IF	Tx Rec
Orange-winged parrot	0.35±0.03	81.31±34.42
Turquoise-fronted amazona	0.33±0.03	97.89±37.88
CV (%)[2]	10.16	40.39
Average	0.34	89.60
ANOVA[3]	*P-value*	
Species	0.3959	0.4459

[1]IF = Indigestibility factor = Indicator (iNDF) in the ration / Indicator in excreta, where iNDF is the indigestible neutral detergent fibre used as indicator; Tx Rec = Indicator recovery rate = (Amount in grams of indicator excreted / amount in grams of indicator ingested) × 100

[2]CV = Coefficient of variation

[3]Analysis of variance (*P* <0.05)

Table 5 Energy values of experimental rations determined using different methods of collecting excreta and two species of parrots kept in cages

AME (kcal / kg DM)[1]				
Species (SPE)	Methodologies (MET)[2]		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	4419±43	3304±137	3861[a]	0.0000
Turquoise-fronted amazona	4448±65	3383±138	3915[a]	0.0000
Average	4433a	3343b		
P-value (SPE within MET)	0.6406	0.2081		
ANOVA[3] *(p-value overall)*				
Species (SPE)	0.2239		Average overall	CV (%)[4]
Methodologies (MET)	0.0000		3888	2.70
SPE × MET	0.5653			
AMEn (Kcal / kg DM)[1]				
Species (SPE)	Methodologies (MET)		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	4185±36	3210±124	3698[a]	0.0000
Turquoise-fronted amazona	4212±57	3300±127	3756[a]	0.0000
Average	4199a	3255b		
P-value (SPE within MET)	0.6273	0.1155		
ANOVA *(p-value overall)*				
Species	0.1461		Average overall	CV (%)
Methodologies	0.0000		3727	2.55
SPE × MET	0.4247			
AMEn (Kcal / kg NM)[1]				
Species (SPE)	Methodologies (MET)		Average	*P-value (MET within SPE)*
	Total collection	Partial collection		
Orange-winged parrot	3917±33	3004±116	3461[a]	0.0000
Turquoise-fronted amazona	3942±53	3089±119	3516[a]	0.0000
Average	3930a	3046b		
P-value (SPE within MET)	0.6273	0.1155		
ANOVA *(p-value overall)*				
Species	0.1461		Average overall	CV (%)
Methodologies	0.0000		3488	2.55
SPE × MET	0.4248			

[1]AME (kcal / kg DM) = Apparent metabolizable energy (expressed as dry matter); AMEn (kcal / kg DM) = Apparent metabolizable energy corrected for nitrogen balance (value expressed as dry matter); AMEn (kcal / kg NM) = Apparent metabolizable energy corrected for nitrogen balance (value expressed in natural matter)
[2]Traditional method of total collection of excreta; Alternative method of partial collection of excreta using indicator
[3]Analysis of variance
[4]CV = Coefficient of variation; Different upper-case letters in the columns and lower-case letters in the rows reveal differences using the F-test (P <0.05)

Table 6 Intake of natural matter, dry matter, crude protein, and energy of the experimental ration as a function of the metabolic weight of different species of cage-bred parrots

Variables[1]	Species of parrot		CV[2] (%)	Average	ANOVA[3] *P-value*
	Orange-winged parrot	Turquoise-fronted amazona			
Intake of natural matter (g NM/kg^{0,75}/day)	58.67±5.23	61.36±6.94	10.24	60.01	0.2956
Intake of dry matter (g DM/kg^{0,75}/day)	54.91±4.90	57.42±6.50	10.24	56.17	0.2956
Intake of crude protein (CP) (g CP/kg^{0,75}/day)	10.87±0.97	11.37±1.29	10.24	11.12	0.2956
Intake of AME (DM) (kcal/kg^{0,75}/day)	211.50±33.07	225.20±43.19	17.61	218.35	0.3922
Intake of AMEn (DM) (kcal/kg^{0,75}/day)	202.56±29.47	216.05±38.89	16.49	209.30	0.3488

[1]An average weight of 0.400 kg of live weight was used for turquoise-fronted amazons and orange-winged parrots
[2]CV = Coefficient of variation
[3]Analysis of variance (P <0.05)

Discussion

The partial collection of excreta, using indigestible NDF as an indicator, provided lower values for all metabolism coefficients when compared to those generated by the total collection of excreta. The partial collection of excreta using this internal indicator must be used with caution in metabolic tests in which it is intended to determine energy values or metabolism of nutrients, since it underestimates the values intended.

In the present study, the partial collection method with iNDF showed lower values than the total collection. The results were somewhat similar to values found in the literature, revealing a certain efficiency of the indigestible NDF as an internal indicator for parrots. Di Santo (2016), by testing different diets for turquoise-fronted parrots, obtained a CAMDM value of 67.65% and therefore validates the results of the current study (65.26% for the same variable). Saad (2003), using the total excreta collection to evaluate rations for turquoise-fronted parrots obtained a similar mean value of metabolism coefficient for DM of 69.86%. However, according to the research carried out, the method of total collection of excreta showed better results (94.76%) and was the best alternative when working with parrots.

The value of the metabolism coefficient of CE using the partial collection of excreta of 72.25% was consistent with Souza (2016), who reported 79.15% for CAMCE. However, for CAMCE using the total collection, it was possible observe much higher values (95.80%), even higher than those reported by Veloso Junior (2011) in studies with macaws (84.49%).

The results from the current study suggest that partial collection of excreta can be used to determine metabolism coefficients without compromising the accuracy of the estimates or causing substantial changes in the determined values. However, the method of total collection of excreta is really the best option. It is worth mentioning that these values were determined for a commercial diet for parrots and no determinations were made for specific feeds. To confirm the theory that these values are extrapolatable to specific feed energy values requires further studies.

The metabolism determination using the total excreta collection method depends on the precise quantification of feed consumption and excreta produced. However, it is possible to obtain information directly from animal metabolism, consequently requiring the use of indicators (Veloso, 2011).

Considering iNDF as an indicator, it is possible to determine the indigestibility factor, which is used to estimate the amount of excreta corresponding to one unit of feed consumed. Subsequently, the amount of nutrients presented in a diet that has been digested and absorbed by the animal can be calculated (Sakomura & Rostagno, 2007).

Rodrigues *et al*. (2005), while investigating the influence of time and collection methods on the metabolization and energetic value of rations for poultry, obtained 76.38% of CAM from DM after 4 d of collection using 0.2% chromic oxide, which was higher than the observed in this study (65,26%) for partial collection with indicator, however, lower than the value of 94.76% from the total collection of excreta. The author stated that there was some inconsistency in the values, regardless of the evaluation of chromium levels at each collection time.

In the present study, no difference (P <0.05) was found for the values of indigestibility factor for both species analysed. For the indicator recovery rate, a similar result was observed for the two

species, showing a mean recovery value of 89.60%. According to Dourado *et al*. (2010), the rate of recovery of indicators should be close to 100%, to indicate better efficiency of the indicator used.

The ideal indicator should present the ability to resist digestion during its passage through the gastrointestinal tract (Oliveira *et al*., 2014). However, the recovery rate of iNDF demonstrated that part of this indicator disappeared during gastrointestinal transit. There was still a low efficiency in the estimates generated for the metabolism coefficients, even though values were lower than those generated by the total collection of excreta, as explained earlier.

The coefficient of variation related to the indicator recovery rate (40.39%), also reported by Zeoula *et al*. (2002) and Ítavo *et al*. (2002), emphasise that the iNDF underestimated the digestibility when presenting high coefficients of variation. The high values can be justified by the size of the particles, the fibre composition in the diet, and/or the mode of incubation. These factors should considered when the iNDF is used as internal indicator.

According to Souza (2016), high coefficients of variation in metabolic tests with parrots may occur due to difficulty in quantification, sampling, contamination of feathered excreta, pieces of scrap from the perch, as well as other rations or excreta that may fall out of the tray. However, in the present study, the coefficients of variation presented were within the acceptable range. In fact, the values obtained using the partial collection of excreta using iNDF as an indicator should not be used with parrots, since they also underestimated the energy values of the feed, giving preference to the total collection of excreta as a method.

The success of a food program in wild birds is related to the dietary energy supply since voluntary consumption of food is regulated by the energy levels in the diet (Simão, 2010). Considering the values obtained and using 4,628 kcal/kg DM CE as a reference value for the ration, the mean values of AME and AMEn of the ration for the total collection method (4,433 and 4,199 kcal/kg DM, respectively) and partial collection (3,343 and 3,255 kcal/kg DM, respectively) indicate that the first methodology (total collection) is more appropriate to estimate these energy values.

For any of the species used, the total excreta method provided lower variations for the feed AMEn (kcal/kg DM). These observations can be explained by its standard deviation, which oscillated by 36 and 57 kcal/kg DM for orange-winged parrot and turquoise-fronted parrots, respectively; for the partial excreta method changed by 124 and 127 kcal/kg DM, for the same species, respectively. The higher variation in the ration energy value for the partial excreta method may affect the food energy value to be supplied, and consequently, the parrots' performance.

Saad (2003), using total collection method, obtained different values of AMEn for different balanced rations for psittacines. The commercial diet was supposed to provide AMEn of 3,628 kcal/kg DM, which was different from that determined in this experiment when total excreta collection was used for Orange-winged (4,185 kcal/kg DM) and turquoise-fronted parrot (4,212 kcal/kg DM).

Rodrigues *et al*. (2005), by studying different times and methods to determine the digestibility and energetic value in rations for roosters, showed variations in AMEn values for the alternative collection method using indicators and time periods from 1 to 5 days of collection. This difference may occur as a result of the weight gain of the birds and, consequently, from different nitrogen balances, whose values are directly related to the calculation of AMEn. Nitrogen retention can be affected by several factors, including consumption and composition of the food supplied.

The present study showed NM and DM intake of 61.36 and 57.42g/kg^{0,75}/day for turquoise-fronted parrots and for orange-winged parrots was 58.67 and 54.91g/kg^{0,75}/day, showing no difference between species. The species also showed identical consumption of 10.87g CP/kg^{0,75}/day in orange-winged species when compared to 11.37g CP/kg^{0,75}/day for the turquoise-fronted parrots. These values agree with those obtained by Lara (2006) in a study on the bioavailability of amino acids in feeds for turquoise-fronted adult parrots, which reported a CP intake of 8.32g CP/ kg^{0,75}/day for an experimental feed and 13.89g CP/ kg^{0,75}/day for a commercial feed, both specific for psittacids. The values quoted were higher than those reported by Di Santo (2016), who obtained 5.79g CP/ kg^{0,75}/day for one tested ration and 5.46g CP/ kg^{0,75}/day for a second ration in turquoise-fronted parrots.

Werneck (2016) reports that in species of granivorous birds, the requirement for protein increases accordingly to body size. For the parrots used in the research, the average protein intake between the two species was 11,12g CP/kg^{0,75}/day. Therefore, larger-sized birds, such as macaws, require higher levels of protein in their diets. In nature, there is a seasonal availability of foods with high protein content, and this is a determining factor in the reproductive performance of these birds. Saidlaja *et al*. (1988) described amino acid nutrition as one of the largest influencers of reproduction in psittacines.

In relation to the daily intake of AME and AMEn, the results showed no statistically significant effect between species (P >0.05). The daily intake of AME obtained (218 kcal/kg^{0,75}/day) are in

agreement with those presented by Souza (2016) of 224.12 kcal/ $kg^{0,75}$/day for turquoise-fronted parrots, and by Veloso (2011) studying macaws (250 kcal/ $kg^{0,75}$/day). Therefore, the results demonstrate that both species present similar energy ingestions when receiving the same type of feed.

However, the intake of AME and AMEn is directly linked to feed intake in dry matter and it is known that the metabolizable energy consumption is well established in adult monogastric animals. Increases in the energy concentration of the diet do not alter the net energy consumption since the dry matter intake of the diet is reduced. Therefore, with a decrease in dietary energy density, it can be assumed that consumption increases. However, other factors may also interfere with the animal's voluntary consumption, overlapping the energy content of the diet (Saad *et al*., 2007).

Conclusions

Of the collection methods, the total excreta collection substantially improved the energy values and metabolism coefficients of the commercial diet. The %dry matter (DM) feed formula - (%DM excreted × indigestibility factor) underestimates coefficients of apparent metabolism of dry matter (CAMDM) when using partial excreta collection, using iNDF as an indicator. No difference was found between species when considering protein and crude energy intake of the commercial diet.

Author contributions

SDTM, TRG, RCN collected the data for this study and analysis; SDTM and GAJN wrote the initial draft of this manuscript; ERF, TRG, PHW, review and editing; GAJN, supervision. All authors have read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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