

# A note on ash as indigestible dietary marker to determine digestibility of seeds in adult granivorous birds

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## ABSTRACT

A study has been conducted to evaluate the possible use of ash as indigestible dietary marker to determine energy metabolizability, nitrogen retention and digestibility of dry matter, organic matter and crude fat in seeds fed to adult granivorous birds. Digestibility values of whole maize and peas for adult domestic pigeons, and sunflower seed for adult African Grey parrots, were compared between the use of the ash ratio in seeds and excreta and the method of total collection of feed intake and excreta output. A temperature of 400°C was found to be optimal to obtain ash recovery values of near to 100 % in both species for all seeds evaluated. Similar means and variation in values indicate that the ash ratio method could be a simple alternative to the laborious method of total collection to determine digestibility of seeds for adult granivorous bird. Precautions in the use of the ash ratio method are discussed.

KEY WORDS: ash, digestibility, seeds, granivorous birds

## INTRODUCTION

Reliable measurements of the digestibility of various nutrients in feed are critical for effective animal nutrition research (De la Noüe and Choubert, 1986). Disadvantages attributed to total collection of feed intake and excreta output, the most widely accepted method to determine digestibility, have been described in detail by Sales and

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Janssens (2003a,b). Difficulty in collection of all wasted food and excreta is especially prominent in birds with active feeding and husking habits, such as parrots (Sales et al., 2004). Furthermore, confinement of free-flying birds such as pigeons to small enclosures might have an influence on behaviour (Sales, personal observation).

The use of an indigestible marker, where only samples of food and excreta are needed to calculate digestibility (Bjorndal, 1985), could eliminate some of the above problems. The use of whole seeds in nutrition of granivorous birds rendered the use of external markers, that have to be mixed with the diet, unsuitable (Sales and Janssens, 2003c; Sales et al., 2004). Furthermore, extremely low contents of some widely used indigestible markers inherent to feed ingredients, such as acid-insoluble ash and lignin, occur in seeds commonly fed to granivorous birds. This caused analytical errors that result in unrealistic nutrient digestibility values when used in both pigeons (Sales and Janssens, 2003c) and parrots (Sales et al., 2004).

Due to simplicity, several studies with invertebrates, fish, reptiles and mammals (Bjorndal, 1985) have applied the ash ratio technique since its introduction in 1966 by Conover. However, among the several reasons why ash is denied as dietary marker, especially in domesticated species, is that it contains several minerals that are digested by the animal's body for growth and maintenance. Some of the minerals are excreted back into the intestines and will not interfere with the use of ash as marker. Another disadvantage is that ash can be dissolved and lost in either a gas or liquid phase during passage through the digestive system (Bjorndahl, 1985). No information could be obtained regarding the use of ash as indigestible marker in avian species. A study has been conducted to evaluate the possible use of ash as dietary marker in adult granivorous birds that are fed on seeds.

## MATERIAL AND METHODS

Apparent metabolizable energy ( $AME_n$ ) corrected for total nitrogen retained or lost from body tissue to nitrogen (N) equilibrium using a factor of 34.39 kJ/g N retained (Hill and Anderson, 1958), N retention and apparent digestibility of dry matter (DM), organic matter, and crude fat in whole maize (88.94% DM; 7.56% crude protein on DM basis) and peas (86.44% DM; 19.91% crude protein on DM basis) were determined with 20 adult domestic pigeons through the method of total collection of feed intake and excreta output, as described in detail by Sales and Janssens (2003c). A similar experiment on nutrient digestibility of sunflower seed (92.83% DM; 17.09% crude protein on DM basis) has been conducted with six adult African Grey parrots, as described by Sales et al. (2004). As parrots dehusked sunflower seed before swallowing, intake was calculated from contents in feed offered and feed refusals (hulls).

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

Despite slight modifications to accommodate the calculation of  $AME_n$  and N retention, apparent digestibility coefficients were calculated according to standard formulas for the total collection and marker methods (Maynard and Loosli, 1969):

Total collection

$$\text{Digestibility} = (\text{Nutrient intake} - \text{Nutrient output}) \times \text{Nutrient intake}$$

Marker method (ash ratio)

$$\text{Digestibility} = 1 - \frac{\% \text{ ash in feed}}{\% \text{ ash in excreta}} \times \frac{\% \text{ nutrient in excreta}}{\% \text{ nutrient in feed}}$$

Ash was determined by combustion of a about 2 g freeze-dried sample of feed or excreta for a period of 12 h in a muffle furnace. Dry matter, organic matter, nitrogen and crude fat contents were determined according to the AOAC (1980), whereas gross energy was determined using an IKA C-7000<sup>2</sup>-type adiabatic bomb calorimeter.

Paired t-tests were used to compare between values obtained by total collection and the ash ratio.

## RESULTS

In a preliminary study an ashing temperature of 400°C was found to give ash recoveries in whole peas and sunflower seeds of near 100% for pigeons and parrots, respectively (Table 1).

Table 1. Recovery of ash (%; mean  $\pm$  SD) at different ashing temperatures

	Temperature, °C		
	400	600	800
<i>Pigeons</i>			
whole peas (n = 10)	103.98 $\pm$ 2.10	98.41 $\pm$ 4.10	95.80 $\pm$ 1.67
<i>Parrots</i>			
sunflower seed (n = 6)	100.59 $\pm$ 1.74	85.34 $\pm$ 1.22	79.02 $\pm$ 1.14

Despite a similar ash recovery rate at 600 than 400°C in peas for pigeons, the former has resulted in increased variation. Furthermore, comparative results at

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400°C in sunflower seed for parrots, has led to the acceptance of the latter ashing temperature.

Although digestibility coefficients were different between methods for whole maize in pigeons ( $P < 0.05$ ), these differences had similar variation (Table 2) and were of limited practical value.

Table 2. Values (mean  $\pm$  SD) determined with either total collection or the ash ratio

	Total collection	Ash ratio
<i>Pigeons</i>		
whole maize (n = 10)		
AME <sub>n</sub> , MJ/kg	15.81 $\pm$ 0.05	15.85 $\pm$ 0.04
N retention, mg/g diet N	3.69 <sup>b</sup> $\pm$ 0.26	4.25 <sup>a</sup> $\pm$ 0.23
digestibility, %		
dry matter	84.17 <sup>b</sup> $\pm$ 0.27	85.23 <sup>a</sup> $\pm$ 0.20
organic matter	85.32 <sup>b</sup> $\pm$ 0.25	86.31 <sup>a</sup> $\pm$ 0.19
crude fat	90.03 <sup>b</sup> $\pm$ 0.74	90.72 <sup>a</sup> $\pm$ 0.64
whole peas (n = 10)		
AME <sub>n</sub> , MJ/kg	13.04 $\pm$ 0.07	13.10 $\pm$ 0.06
N retention, mg/g diet N	2.55 $\pm$ 0.62	3.67 $\pm$ 0.52
digestibility, %		
dry matter	63.78 $\pm$ 0.52	65.11 $\pm$ 0.39
organic matter	65.64 $\pm$ 0.50	66.90 $\pm$ 0.38
crude fat	78.82 $\pm$ 0.73	79.58 $\pm$ 0.83
<i>Parrots</i>		
sunflower seed (n = 6)		
AME <sub>n</sub> , MJ/kg	25.94 $\pm$ 0.18	26.14 $\pm$ 0.08
N retention, mg/g diet N	7.77 $\pm$ 0.92	9.42 $\pm$ 0.52
digestibility, %		
dry matter	67.07 $\pm$ 0.41	67.22 $\pm$ 0.61
organic matter	70.65 $\pm$ 0.41	70.35 $\pm$ 0.64
crude fat	96.93 $\pm$ 0.13	96.98 $\pm$ 0.14

AME<sub>n</sub> = apparent metabolizable energy corrected for nitrogen retention

<sup>a,b</sup> values with different superscripts a,b in the same row are significantly different ( $P < 0.05$ )

## DISCUSSION

The possible use of ash as indigestible diet marker in digestibility studies with adult granivorous birds fed on seeds has been demonstrated in this study. Despite a small increase in values determined through the ash ratio method compared to total collection, the latter should not be applied as the standard in digestibility studies. The use of the ash ratio technique resulted in similar variation than obtained through the method of total collection.

The use of ash as indigestible marker is based on the assumption that ash is either indigestible, or the study animal is in mineral balance, therefore mineral intake will equal mineral output (Bjorndahl, 1985). Present results indicate that birds have been either in mineral balance (ash recovery around 100%), or that excretion of minerals back into the intestines was slightly higher than absorption (> 100%). A lower ash recovery rate at ashing temperatures of 600 and 800°C could be ascribed to volatilization of certain minerals (Misra et al., 1993), such as phosphorus, selenium and potassium, at high temperatures.

Application of the above technique warrants further investigation. The accuracy of a marker varies among species and diets fed to the same species (Bjorndahl, 1985). The physiological condition of the species, such as stage of maturing and reproductive status that result in mineral digestion, needs consideration. Contamination during analysis should be avoided, and all analytical protocols, such as ashing temperature, kept constant. Further studies are needed on the chemical and thermal stability of the individual minerals in ash contents during passage through the digestive tract, especially in diet ingredients high in ash content. The possibility to relate digestibility to seed selection in mixtures presents a challenging opportunity.

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