Methods to Determine Metabolizable Energy and Digestibility of Feed Ingredients in the Domestic Pigeon (*Columba livia domestica*)

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ABSTRACT The influence of length of excreta collection period (1, 3, 6, 10, 14 d) and prefeeding protocol (7 d either individual feeding in collection cages or group feeding in housing cages) on AME_n, nitrogen retention (NR), and apparent DM, organic matter and ether extract digestibility of corn and peas were evaluated in domestic pigeons (*Columba livia domestica*). In addition, the use of internal markers [acid-insoluble ash (AIA) and acid detergent lignin (ADL)] to determine AME_n, NR, and apparent digestibility was compared with the method of measuring total feed input and excreta output. A quadratic ($y = a + bx + cx^2$) trend in the CV for AME_n, NR, and apparent digestibility coefficients found over collection periods with corn presented evidence that excreta collection for a period of 3 d will produce a CV of 5% less than the minimum CV. Although no trend could be detected in CV for peas, a 3-d excreta collection period resulted in relatively low variation. Both AIA and ADL, when used as internal markers, resulted in AME_n, NR, and digestibility values below ($P < 0.05$) those obtained with total collection with corn. However, values between markers were comparable ($P > 0.05$) for all components evaluated. The ADL was unsuccessful as marker with peas. Group prefeeding of pigeons in housing cages resulted in lower feed intake, excreta output, NR, and apparent digestibility than when birds were adapted individually to collection cages. This study presents evidence that the method of measuring total feed intake and excreta output for a period of 3 d, with individual adaptation of birds to collection cages, resulted in the most reliable values for AME_n, NR, and apparent digestibility of DM, organic matter and ether extract of feed ingredients in pigeons.

(*Key words*: digestibility, internal marker, pigeon, total excreta collection period)

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INTRODUCTION

Formulation of diets with accurate ME, digestibility values, and evaluation of the effects of treatments, such as conditioning or the addition of enzymes, requires reliable methods to obtain these values. Introduction of rapid bioassays for determining ME and digestibility in poultry in the late 1970s requires birds to be starved, and this has proved to be controversial. Traditional assays, still the most widely accepted method, use ad libitum feeding (McNab, 2000). In the latter type of assay, the diet is fed to birds individually housed in collection cages for a period of 3 d to establish a state of digestive equilibrium followed by a collection period of 4 d (Vohra, 1972; Farrell, 1999). The objective must be to collect a sample representative of that produced from the feed being consumed, thus uncontaminated by residues from other kinds of feed or of the same kind but from a different rate of intake. This requires a preliminary period and a collection period long enough to reduce to negligible terms the errors of irregular excreta collection at the beginning and end (Schneider and Flatt, 1975).

In comparison with the total collection method, the use of markers to determine ME and digestibility values avoids errors associated with inaccurate measurement of feed intake, excreta output, and contamination of excreta (Sibbald, 1987). In the 1960s, the use of chromic oxide as a marker to determine ME content, digestibility of feeds, and feed ingredients was the method commonly used in poultry (Vohra, 1966). However, problems, such as the reproducibility of the assay for chromium oxide (Halloran, 1972) and hazardous effects because of its potential carcinogenicity (Peddie et al., 1982), has led to the acceptance of the total collection method as the most applicable method.

In the domestic pigeon (*Columba livia domestica*), a social free-flying bird with a lively temperament and a high metabolic rate, confinement of individual birds into small

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Abbreviation Key: ADL = acid detergent lignin; AIA = acid-insoluble ash; NR = nitrogen retention.

collection cages might have an influence on feeding behavior (Hatt et al., 2001). It was found in poultry (Sugahara and Kubo, 1995), horses (Katsuki et al., 1998), and camels (Nagpal et al., 2000) that digestibility has been altered by exercise. Furthermore, the use of compound pellets in pigeon feeding is still marginal. Most commercial pigeon feeds are based on mixtures of grains, mainly because this is traditionally preferred by the owners (Janssens et al., 2000; Sales and Janssens, 2003). Selection for certain ingredients in seed mixtures occurs (Janssens et al., 2002), and the use of external markers to determine digestibility of feed ingredients is eliminated due to the impracticality of mixing the external marker into the feed. Few digestibility studies, varying from feeding ad libitum with different lengths of prefeeding and excreta collection (Fekete et al., 1999; Hullar et al., 1999; Janssens et al., 2000) to fasting, force-feeding, and excreta collection in bags secured around the birds' cloaca (Dublecz et al., 1999), were conducted in the domestic pigeon. Hatt et al. (2001) concluded that *n*-alkanes might be used as internal markers for the estimation of apparent digestibility of feeds for pigeons. However, this study was based on protein digestibility with a limited number (n = 4) of birds. Furthermore, digestibility values have to be corrected for incomplete recovery rate of *n*-alkanes.

In order to find the most appropriate method to determine metabolizability and digestibility of feeds and feed ingredients in the domestic pigeon, the present study aimed to evaluate the influence of length of excreta collection period, total collection vs. internal marker [acid-insoluble ash (AIA) and acid detergent lignin (ADL)] method, and prefeeding protocol, on AME_n, nitrogen retention (NR) and apparent digestibility of DM, organic matter, and ether extract of different feed ingredients.

MATERIALS AND METHODS

Experiment 1

Whole corn (88.94% DM; 7.56% CP on DM-basis) was fed ad libitum as the sole feed ingredient to 25 adult (1 to 13 yr old, 452.08 ± 10.15 g BW) pigeons. Pigeons were housed individually in collection cages suitable for quantitative measurement of feed intake and excreta produced, situated in a room under natural daylight conditions and temperature of 15 to 18°C. Fresh water was available at all times. Cages were randomized according to excreta collection period (1, 3, 6, 10, 14 d). Pigeons were adapted for a period of 7 d in the cages to the diet, whereafter feed intake measurements and total excreta collection were performed. Excreta were collected at 0800 h and kept frozen. Daily excreta collections were pooled for each bird and stored at –20°C until further processing.

After termination of the trial, pigeons were kept for 2 wk under conditions commonly used at this institute (eight birds per cage) and fed a commercial pigeon diet,[2] after which the experiment was repeated with whole peas (86.44% DM; 19.91% CP on DM basis) as sole feed ingredient.

Experiment 2

Ten pigeons (five per treatment) from the group used in experiment 1 were adapted in groups on the corn and peas used in experiment 1 under conditions commonly applied in this institute for a period of 7 d, whereafter total collection was performed for 3 d as described in experiment 1.

The experimental arrangement and housing conditions in both experiments were approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University.

Analytical Procedures

Nitrogen, organic matter, and ether extract contents of freeze-dried feed and excreta were done according to the AOAC (1980), whereas gross energy was determined using an IKA C-7000[3]-type adiabatic bomb calorimeter. The AIA content of feed and excreta collected for 6, 10, and 14 d in experiment 1 was determined using the procedure of Van Keulen and Young (1977), as adapted by Atkinson et al. (1984), and hydrolytic (sulfuric acid) ADL according to Van Soest (1965).

Digestibility Coefficient Calculations and Statistical Analyses

The AME_n, NR, and apparent digestibility coefficients of feed ingredients were calculated according to standard formulas for the total collection and marker methods (Maynard and Loosli, 1969). The AME was corrected to N equilibrium using a factor of 8.22 kcal/g N retained (Hill and Anderson, 1958).

Data derived in experiment 1 for different collection periods were analyzed according to one-way ANOVA, whereas nonlinear regression equations were fitted through the CV derived for AME_n, NR, and digestibility values in each collection period. Paired *t*-tests were used to compare values obtained by total collection and markers, whereas unpaired *t*-tests were used in experiment 2 to evaluate different adaptation protocols.

RESULTS

Experiment 1

No differences ($P > 0.05$) were found between excreta collection periods for feed intake, excreta output, AME_n, NR, or digestibility values evaluated in either corn or peas (Table 1).

Evaluation of the CV at different collection periods presented evidence that a quadratic regression ($y = a + bx + cx^2$) was the most suitable to describe the trend of CV over time for values derived with corn (Table 2). With

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TABLE 1. AME_n, nitrogen retention (NR), and digestibility of DM, organic matter (OM), and ether extract on a dry matter basis as influenced by length of excreta collection (means ± SE, n = 5)

	Corn					Peas				
Days	1	3	6	10	14	1	3	6	10	14
Feed intake (g/d)	22.68 ± 1.95	25.45 ± 2.07	26.11 ± 1.74	26.97 ± 2.13	25.59 ± 2.25	31.62 ± 2.13	28.60 ± 1.33	32.89 ± 3.21	29.23 ± 3.08	32.56 ± 1.91
Excreta output (g/d)	3.67 ± 0.30	4.12 ± 0.32	4.05 ± 0.26	4.25 ± 0.32	4.07 ± 0.37	11.24 ± 0.49	10.68 ± 0.50	11.86 ± 0.95	10.39 ± 1.05	11.96 ± 0.62
AME_n (kcal/kg)	3,751 ± 38	3,756 ± 19	3,785 ± 6	3,784 ± 16	3,775 ± 21	3,125 ± 35	3,076 ± 13	3,113 ± 31	3,135 ± 7	3,097 ± 31
NR (mg/g diet N)	3.29 ± 0.50	3.20 ± 0.34	3.72 ± 0.50	4.01 ± 0.31	3.38 ± 0.39	4.66 ± 0.89	2.78 ± 0.30	3.06 ± 1.31	2.57 ± 0.96	2.52 ± 0.90
Digestibility (%)										
DM	83.72 ± 0.84	83.78 ± 0.28	84.49 ± 0.14	84.21 ± 0.36	84.12 ± 0.45	64.25 ± 0.85	62.64 ± 0.41	63.66 ± 0.83	64.37 ± 0.29	63.18 ± 0.98
OM	84.80 ± 0.81	84.92 ± 0.31	85.56 ± 0.13	85.44 ± 0.29	85.20 ± 0.44	66.04 ± 0.81	64.46 ± 0.40	65.52 ± 0.80	66.21 ± 0.27	65.07 ± 0.94
Ether extract	86.10 ± 1.91	85.91 ± 0.97	88.73 ± 1.09	90.30 ± 0.99	89.76 ± 1.20	77.62 ± 2.33	80.33 ± 0.99	80.00 ± 2.25	78.43 ± 1.25	79.21 ± 0.88

the exception of NR, the 95% confidence interval to derive a minimum CV for AME_n and digestibility were all within the range of 3 to 20 d. However, the quadratic regression did not fit the CV obtained with NR well.

No specific trend could be detected in CV from values derived with peas due to an unexpected high value derived at 6 d of excreta collection. Although metabolizability and digestibility in corn determined with either AIA or ADL presented lower ($P < 0.05$) values compared to the total collection method, no difference ($P > 0.05$) was found between values determined with either AIA or ADL (Table 3). Values determined with ADL presented unrealistic, often negative, values with peas. Therefore, these values were omitted in Table 3. Although not significant, values determined with AIA for peas tended to be lower than when determined through total collection. With both feed ingredients, standard errors were smaller for values determined with total collection in comparison to the marker technique.

Experiment 2

Feed intake (20.77 ± 3.06 g/d), AME_n (2,963 ± 39 kcal/kg), NR (–2.51 ± 1.54 mg/g diet N), apparent digestibility of DM (59.25 ± 1.14%), organic matter (61.34 ± 1.07%), and ether extract (70.82 ± 1.75 %) were lower ($P < 0.05$) than those found during the 3-d collection period in exp. 1 (Table 1) for peas but not for corn (16.44 ± 3.66 g/d; 3,750 ± 21 kcal/kg; –2.98 ± 2.35 mg/g diet N; 76.47 ± 4.75%; 82.42 ± 1.32% and 83.56 ± 0.42%, respectively). Excreta output for both corn (2.57 ± 0.49 g/d) and peas (7.22 ± 1.14 g/d) presented lower ($P < 0.05$) values than when pigeons were individually adapted for 7 d in collection cages (Table 1).

DISCUSSION

Present results indicate that an excreta collection period of 3 d would be suitable to determine ME content and digestibility of feed ingredients in the domestic pigeon. This was confirmed by a CV derived at 3 d of collection for AME_n content and digestibility of DM, organic matter, and ether extract that resulted in values 5% less than the minimum CV. A contributing factor was that enough excreta could be collected within 3 d for appropriate analyses of a wide range of components. In agreement with present results, Tyler (1958), according to variation of dry matter excreta production from laying hens, suggested an excreta collection period of 3 d for full-fed poultry.

The lower ($P < 0.05$) AME_n, NR, and apparent digestibility values derived through the use of the internal markers AIA and ADL with corn, greater standard errors found with markers in comparison to total collection, and failure of ADL as a marker with peas found in this study, might partly be attributed to analytical error due to a low dietary marker content. Thonney et al. (1985) recommended that the AIA content should exceed 0.75% on a DM-basis in order to get accurate measurements, whereas values were

TABLE 2. Parameters derived from $y = a + bx + cx^2$ fitted to the CV of AME_n, nitrogen retention (NR), and digestibility of DM, organic matter (OM), and ether extract over days of excreta collection (x) with corn

Component	a	b	c	R^2	Sum of squares	$S_{y,x}$	$X_1 - X_{max}$ (d)
AME_n	2.502	–0.4859	0.02898	0.8071	0.3622	0.4255	2 – 32
NR	35.70	–2.931	0.1538	0.4700	87.49	6.614	5 – 12
DM	2.362	–0.4798	0.02928	0.7100	0.5851	0.5409	3 – 22
OM	2.344	–0.4902	0.02967	0.8271	0.3155	0.3972	1 – 39
Ether extract	5.051	–0.6496	0.03652	0.7191	1.207	0.7768	3 – 20

0.06 and 0.04% for corn and peas respectively, used in the present study.

Differences in type of fiber between corn and peas could also be involved in unrealistic values derived with peas. According to Mueller (1956) a relatively great variation in percentage of lignin recovered was caused by small deviations in sulphuric acid concentration from the prescribed 72%. Also, in their study lignin content of the diet and the lignin content of the excreta were affected differently by changes of the sulfuric acid concentration, suggesting that the lignin in the diet and the lignin in the excreta were not chemically identical entities, and it was therefore concluded that the lignin indicator method was of restricted value for digestion experiments in poultry. Furthermore, the 72% sulfuric acid detergent lignin method measures cutin and Maillard-type browning products as lignin, whereas some of the true lignin may be destroyed (Goering and Van Soest, 1970). Despite the intensive use of ADL as marker in digestibility studies with ruminants, where most researchers concede that changes occur in the lignin molecule with passage through the digestive tract (Fahey and Jung, 1983), this internal marker has rarely been used in studies with avian species (Mueller, 1956; Nizza and di Meo, 2000; Moniello et al. 2001). No explanation could be found for the identical values derived with both markers for corn.

Inherent problems attached to the total collection method included adherence of droppings to the birds' plumage, contamination of excreta with scurf and feathers, changes in chemical composition of excreta due to fermentation, excreta losses during removal and transfer from trays to containers, birds excreting away from the tray, droppings being contaminated with regurgitated feed (McNab, 2000), and the possibility of differences between birds in the amounts of DM in the digestive tract at the beginning and end of the assay period when ad libitum feeding is practiced. However, relatively little variation between birds was derived with this method in the present study. Most studies in which AIA (Tillman and Waldroup, 1988a, b; Nizza and Meo, 2000; Moniello et al., 2001) was compared to the total collection method in avian species (chickens, partridges, ostriches) obtained higher digestibility coefficients in the former, whereas ADL gave comparable results (Nizza and Meo, 2000; Moniello et al., 2001). However, it must be emphasized that the former studies evaluated AIA according to the single ashing method of Vogtmann et al. (1975) that was proved to result in higher digestibility values than the double ashing method as used in the present study (Van Keulen and Young, 1977).

Group prefeeding of the test ingredient to pigeons housed together caused significantly different digestibility values with more variation than when birds were adapted individually in collection cages. All birds fed on peas presented negative NR values when not adapted to cages. This illustrates the need to adapt birds to collection cages. The minimum length of this adaptation warrants further investigation.

Irrespective of differences in origin of feed ingredients and animals, and housing and experimental procedures, comparable values, taking variation into account, for feed intake (22.7 and 33.0 g/d), AME_n (3,527 and 3,348 kcal/kg), and digestibility of DM (81.25 and 71.71%), organic matter (82.38 and 71.20%), and ether extract (82.33 and 82.12%), have been reported for corn and peas, respectively, by Hullar et al. (1999), when determined in domestic pigeons.

TABLE 3. AME_n, nitrogen retention (NR), and digestibility of DM, organic matter (OM), and ether extract calculated using total collection (TC), acid-insoluble ash (AIA) and acid detergent lignin (ADL) (means ± SE, n = 15)

	Corn			Peas	
Component	TC	AIA	ADL	TC	AIA
AME_n (kcal/kg)	3,781[a] ± 9	3,409[b] ± 92	3,423[b] ± 51	3,115 ± 15	2,937 ± 108
NR (mg/g diet N)	3.70[a] ± 0.23	–1.27[b] ± 1.28	–1.46[b] ± 1.10	2.72 ± 0.58	–1.39 ± 2.78
Digestibility (%)					
DM	84.28[a] ± 0.19	74.82[b] ± 2.41	74.98[b] ± 1.47	63.74 ± 0.43	58.27 ± 3.64
OM	85.40[a] ± 0.17	76.60[b] ± 2.27	76.75[b] ± 1.38	65.60 ± 0.41	60.41 ± 3.46
Ether extract	89.60[a] ± 0.61	83.37[b] ± 1.83	83.37[b] ± 1.38	79.21 ± 0.86	75.58 ± 2.74

[a,b]Means within a row within feed lacking a common superscript differ ($P < 0.05$).

It can be concluded that, although this study does not prove the total collection method as correct, reliable and repeatable results were obtained through this method to determine ME and digestibility of feed ingredients in the domestic pigeon. An excreta collection period of 3 d was found as suitable to obtain minimum variation. However, the length of time required to adapt the birds in individual collection cages still has to be investigated.

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