An Ileal Amino Acid Digestibility Assay for the Growing Meat Chicken-Effect of Feeding Method and Digesta Collection Procedures

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ABSTRACT : The objective was to evaluate method of feeding (free access or intubation), method of slaughter (carbon dioxide gas or barbiturate) and digesta flushing medium (distilled water or physiological saline), in the development of an ileal amino acid digestibility assay for 4 week-old broiler chickens. Three diets were used (commercial (C), semi-synthetic meat-and-bone meal (MBM) or wheat (W)). For the coarser C and W diets but not for the MBM diet, feeding method had a significant effect on concentrations of chromium (Cr), nitrogen (N), acid detergent fibre (ADF) and neutral detergent fibre (NDF) in the crop contents at a set time after a meal. There appeared to be a selection of food particles under free-access feeding. For birds receiving the wheat diet there was an effect ($p < 0.05$) of sampling time after feeding on the concentrations of Cr, N, ADF and NDF/Cr in the crop contents. Flushing ileal digesta with distilled water or saline led to similar apparent ileal N digestibility coefficients. Birds given the MBM diet, and killed by inhalation of CO_2, had significantly ($p < 0.05$) lower apparent ileal N digestibility coefficients (73 versus 80%) than those killed by barbiturate overdose.

(Key Words: Chicken, Digestibility, Intubation, Ileal, Nitrogen)

INTRODUCTION

Several methods have been developed with poultry to allow determination of nitrogen and amino acid digestibility (Papadopoulos, 1985; Low, 1990). These procedures may involve the sampling and analysis of either the ileal digesta or excreta from intact or caecectomised birds. A major criticism of methods based on excreta collection is that the gut microflora, found mainly in the caeca and colon, may influence the amino acid composition of the excreta (Austic, 1983; Thomas and Crissey, 1983; van Weerden, 1989; Whitacre and Tanner, 1989; ten Doeschate, et al., 1993). Hindgut microbial activity is reduced following caecectomy but may not be eliminated (Whitacre and Tanner, 1989). Also, the effect of removing the caeca on the general functioning of the digestive tract is unknown. To minimise the effect of the gut microbes, Payne et al. (1968) sampled digesta from the terminal ileum. Subsequently, other workers (Soares and Kifer, 1971; Varnish and Carpenter, 1975; Achinewhu and Hewitt, 1979; Bielorai et al., 1982; Raharjo and Farrell, 1984; Johns et al., 1986) have also used this technique. The ileal assay has the added advantage of not being affected by urinary amino acid and nitrogen excretion. The latter confound digestibility measures based on excreta.

Conventionally, ileal digesta have been collected from chickens using the slaughter method. With this method, however, there are a number of technical aspects such as feeding method, time and method of killing, means of removing digesta and length of ileum sampled, which may influence the results and should be considered. There has been little experimental work conducted to evaluate these factors and allow specification of a standard procedure. The aim of this study was to evaluate the method of feeding (free access to meal for 1 hour period *versus* intubation), slaughter method (carbon dioxide gas or barbiturate) and the type of digesta flushing medium (distilled water or physiological saline) in the development of a broiler assay for ileal nitrogen and amino acid digestibility.

MATERIALS AND METHODS

Study 1-feeding method

Animals, housing and allocation to treatments

Male and female broiler chickens of the Ross strain (23-days-old) were allocated at random to a battery of suspended wire cages. The birds were located in an insulated environmentally-controlled room (temperature 20±2℃; 14 h light: 10 h dark cycle). The birds

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underwent a 7 day acclimatisation period to enable the birds to adjust to cage conditions, whereby they were fed a commercially-formulated broiler starter meal and had unlimited access to fresh water. At 30 days-of-age birds were allocated at random to the treatments. The study comprised two parts. In the first part, 36 birds were allocated to six treatments (n=6); three diets (a compound diet, a meat-and-bone meal based diet and a wheat diet) and two methods of feeding (free access and intubation). There were equal numbers of males and females on each treatment. This part of the study aimed to evaluate whether under free access feeding, birds selected food particles (by comparison of concentrations of chemical components in crop contents of free access and intubated birds). In the second part of the study, 24 birds which were given free access to a meat-and-bone meal based diet were allocated to four times (n=6) for the sampling of crop contents (1, 2, 3 or 4 h after the start of feeding). A further 24 birds were given the wheat diet by intubation and were also equally allocated to four crop sampling times (0.5, 2, 3 or 4 h after intubation). A 0.5 h time delay was chosen in the intubation procedure to mimic as closely as possible the time of meal ingestion found under free access feeding. There were equal numbers of males and females on each treatment. This part of the study aimed to evaluate whether there was a differential rate of removal of ingested food particles from the crop over time (by comparison of the concentrations of chemical components in crop contents at different times after feeding).

Table 1. Ingredient composition (g/kg air dry weight) of the experimental diets

Ingredient	Compound diet	Meat and bone meal diet	Wheat diet
Maize	450.0	–	–
Barley	205.0	–	–
Soyabean meal	212.3	–	–
Meat-and-bone meal	100.0	500.0	–
Wheat	–	–	908.5
Maize starch	–	317.0	–
Sucrose	–	80.0	–
Maize oil	12.0	50.0	50.0
Purified cellulose	–	40.0	–
Potassium carbonate	–	3.0	–
Sodium chloride	2.5	2.5	4.0
Dicalcium phosphate	–	–	20.0
Calcium carbonate	10.0	–	10.0
DL-methionine	0.7	–	–
Vitamin, mineral Premix[1]	4.5	4.5	4.5
Chromic oxide	3.0	3.0	3.0

[1] Technik Products broiler/starter vitamin and mineral Premix (Technik Products, Auckland, New Zealand).

Diets

The ingredient compositions of the experimental diets are given in table 1. The diets were blended in a mechanical mixer and had chromic oxide added as an indigestible marker. These were given to the birds as a dry mash. The maize, barley and wheat were ground with a hammer-mill (4 mm sieve size) before inclusion in the diets. The wheat diet was chosen to represent a mix of fine and coarse particles, the meat-and-bone meal based diet had a relatively fine particle mix and the compound diet was included to provide information relevant to a typical commercial diet.

Experimental procedure

At 30 days-of-age the birds received their respective test diet *ad libitum* for 4 days. Then following a 24 h fast, the treatments were applied. In the first part of the study, birds on the free access treatments were given unrestricted access to their diet for a 1 h period (08:00 to 09:00 h), follwing which they were asphyxiated with carbon dioxide gas. Birds on the intubation treatments were removed and individually force-fed 25 g (air-dry weight) of their diet. The force-feeding was accomplished using a stainless steel funnel with a 17.2 cm long stem and a 17.7 cm long plunging rod. The external and internal diameters of the stem were 0.90 and 0.75 cm, respectively. The end of the stem was inserted into the crop and food delivered directly to the crop. The intubated birds were gassed half an hour after the completion of intubation. Following euthanasia the crop of each bird was dissected from the body and the contents were emptied into a container, with residual material being flushed from the crop using distilled water. The crop contents were freeze-dried and stored at −20℃ while awaiting chemical analysis.

Chemical analysis

Samples of the diets and crop contents were finely ground (1 mm sieve) before chemical analysis. The diets and crop contents were analysed in duplicate for chromium, nitrogen, acid detergent fibre and neutral detergent fibre.

Total nitrogen was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1975).

Chromium was determined by atomic absorption spectrophotometry following the method of Costigan and Ellis (1987). Acid and neutral detergent fibre were determined by the method of James and Theander (1981).

Statistical analysis

For comparison between the three diets of the effect of free access feeding versus intubation on the concentrations of various chemical components in the crop contents, a linear model which included terms for diet, method of feeding and diet × method was fitted to the data for each chemical measure separately. Reduction in sums of squares was used to determine levels of significance. Where a significant interaction was found, the effect of method was examined within diet. For investigation of the effect of time of sampling on the composition of the crop contents, the data were subjected to a one-way analysis of variance for each measure separately (within diet and within method of feeding). Where appropriate, treatment means were compared using orthogonal contrasts (Snedecor and Cochran, 1982).

Study 2 - Effect of slaughter method and digesta sampling procedures on apparent nitrogen digestibility

Animals, housing and allocation to treatments

Twenty-four broiler chickens of the Ross strain (23-days old) were housed as described above. The birds underwent a 6-day acclimatisation period before being allocated to the treatments. The 24 birds were allocated to four treatments (two methods of slaughter × two digesta flushing media) such that there were 3 males and 3 females (n = 6) per treatment.

Experimental procedure

Following the acclimatisation period the birds received a meat-and-bone meal-based diet (table 1) *ad libitum* for four days. The birds were fasted for 24 h and then given free-access to the meat-and-bone meal diet for 1 h (08:00 to 09:00 h). At four hours after the start of feeding, birds were either asphyxiated with CO_2 gas in a wooden chamber or were killed by a 1 ml intra-cardial injection of sodium pentobarbitone (Anathal, 300 mg sodium pentobarbitone/ml South Island Chemicals Ltd., Christchurch). When the birds were fully immobilised the blood vessels of the neck were severed and the abdomen opened. The terminal 15 cm of ileum was removed and digesta gently flushed out using either distilled water or physiological saline (8.5 mg NaCl/ml distilled water) from a plastic syringe. The ileal digesta samples were freeze-dried and stored frozen (−20°C) while awaiting chemical analysis.

Chemical analysis

The diet and ileal digesta samples were subjected to duplicate analysis for total nitrogen and chromium, as described above. Apparent digestibility coefficients were calculated by reference to the chromium concentrations in the diet and digesta.

Statistical analysis

A linear model which included terms for method of slaughter, flushing medium and method × medium was fitted to the digestibility data and reduction in sums of squares used to determine levels of significance.

RESULTS

Study 1 - Feeding method

The concentrations of the chemical components in the crop contents after intubation are compared with the dietary concentrations in table 2. There was close

Table 2. Comparison of the mean (±SE) concentrations[1] of chemical components in the crop contents after intubation and the corresponding dietary concentrations for chickens given a meat-and-bone meal diet, a wheat diet or a compound diet

	Crop contents	Diet
Meat-and-bone meal diet		
Cr[2]	0.254 (0.010)	0.258 (0.003)
N	4.191 (0.117)	4.213 (0.020)
Wheat diet		
Cr	0.288 (0.007)	0.286 (0.002)
N	1.991 (0.018)	1.982 (0.001)
NDF	10.237 (0.236)	10.220 (0.317)
ADF	3.005 (0.044)	3.034 (0.060)
Compound diet		
Cr	0.278 (0.009)	0.287 (0.006)
N	3.739 (0.074)	3.732 (0.084)
NDF	12.460 (0.631)	12.772 (0.472)
ADF	2.956 (0.209)	3.031 (0.092)

[1] g/100 g dry matter, 6 birds intubated per diet and 6 sub-samples of diet each analysed in duplicate.
[2] Cr = chromium.
N = total nitrogen.
NDF = neutral detergent fibre.
ADF = acid detergent fibre.

agreement between the crop contents and the diet, indicating that the intubated birds were a satisfactory control.

There was no significant interaction ($p > 0.05$) between diet and feeding methods for the ratio of total nitrogen (N) to chromium (Cr), the concentration of neutral detergent fibre (NDF), the ratio of NDF to Cr or the ratio of ADF to Cr, in the crop contents (table 3). There was no significant effect of method of feeding on the ratio of N to Cr in the crop contents but the concentration of NDF and the ratios of NDF and ADF to Cr were significantly ($p < 0.01$) higher for free access versus intubation.

Table 3. Overall effects of method of feeding and type of diet on the mean concentrations[1] or ratios of concentrations of chemical components in the crop contents of growing broiler chickens

Crop Component[2]	Diet			Method		Overall SE	Significance[3]	
	Compound	Wheat	Meat-and-bone meal	Free	Intubation		Diet	Method
N/Cr	14.0[a]	7.5[b]	16.5[c]	13.0	12.3	0.27	***	NS
NDF	12.7	10.6	–	11.9	11.4	0.12	***	**
NDF/Cr	48.8	38.8	–	47.3	40.2	0.70	***	***
ADF/Cr	11.3	11.7	–	12.4	10.6	0.22	NS	***

[1] g/100 g dry matter, n = 6.
[2] N = total nitrogen.
Cr = chromium.
NDF = neutral detergent fibre.
ADF = acid detergent fibre.
[3] NS non significant; ** $p < 0.01$; *** $p < 0.001$.
[a,b,c] Values without a letter in common are significantly different.

For the crop concentrations of Cr, N and ADF, there were significant ($p < 0.05$) interactions between diet and method of feeding. For the latter measures, means for the feeding methods within each of the three diets are given in table 4. For the meat-and-bone meal based diet there was no effect of method of feeding on the concentrations of elements in the crop, but for the compound and wheat diets there were significant ($p < 0.01$) differences in the measures between free access feeding and intubation. Birds having free access to the compound diet had lower crop concentrations of Cr and N but similar levels of ADF. Birds having free access to the wheat diet had lower Cr concentrations in the crop contents, but higher levels of N and ADF.

Table 4. Effect of method of feeding on the mean concentrations[1] of chemical components in the crop contents of growing broiler chickens

Crop Component[2]	Compound diet		Wheat diet		Meat-and-bone meal diet		Overall SE
	Free access	Intubation	Free access	Intubation	Free access	Intubation	
Cr	0.25	0.28**[3]	0.26	0.29***	0.27	0.25^{NS}	0.032
N	3.54	3.74**	2.07	1.99***	4.3	4.2^{NS}	0.063
ADF	2.94	2.96^{NS}	3.33	3.01***	–	–	0.062

[1] g/100 g dry matter, n = 6.
[2] Cr = chromium.
N = total nitrogen.
ADF = acid detergent fibre.
[3] Significance of method determined within each diet. NS non significant; ** $p < 0.01$; *** $p < 0.001$.

The effect of time after feeding on the concentrations of chemical components in the crop contents of birds given a meat-and-bone meal based diet (free access) or a wheat diet (intubation) is shown in table 5. For the birds given free access to the meat-and-bone meal based diet there was no significant ($p > 0.05$) effect of time of sampling on the crop contents. For the wheat diet, however, there was a significant ($p < 0.05$) effect of time for several of the chemical components. There was no clear overall trend in the data. For the ratios of components to Cr, the effect of time was either non significant (ADF) or the ratio at 3 h was significantly higher than at the other sampling times. The amount of dry matter recovered from the crop expressed as a percentage of the ingested dietary dry matter, decreased from 85% at 1 h to 7% at 4 h for birds given free access to the meat-and-bone meal diet. For birds given the wheat diet by intubation 90% of the meal dry matter was recovered at the 0.5 h sampling time and 6% at 4 h.

Table 5. The effect of time after feeding birds a meat-and-bone meal (MBM) or wheat diet on the mean concentrations[1] of chemical components in the crop contents of growing broiler chickens

	Time after feeding (hours) for crop sampling						sign.[3]
	0.5	1	2	3	4	overall SE	
MBM diet							
Cr[2]	–	0.27	0.26	0.26	0.25	0.006	NS
N	–	4.32	4.30	4.20	4.09	0.066	NS
N/Cr	–	16.1	16.3	15.9	16.7	0.458	NS
Wheat diet							
Cr	0.28^{a}	–	0.28^{a}	0.24^{b}	0.25^{b}	0.028	*
N	2.00^{a}	–	1.97^{ab}	1.91^{bc}	1.88^{c}	0.025	*
NDF	10.3	–	10.1	10.1	10.0	0.200	NS
ADF	2.78^{ab}	–	2.99^{a}	2.67^{b}	2.74^{b}	0.080	*
N/Cr	7.17^{a}	–	7.06^{a}	7.95^{b}	7.46^{ab}	0.201	*
NDF/Cr	36.79^{a}	–	36.38^{a}	41.95^{b}	39.89^{ab}	1.298	*
ADF/Cr	9.95	–	10.74	11.16	10.79	0.431	NS

[1] g/100 g dry matter, n = 6.
[2] Cr = chromium.
N = total nitrogen.
NDF = neutral detergent fibre.
ADF = acid detergent fibre.
[3] NS non significant; * $p < 0.05$.
[a,b,c] Values without a letter in common are significantly different.

Study 2 - Effect of slaughter method and digesta-sampling procedures on apparent nitrogen digestibility

There was no significant ($p > 0.05$) interaction between method of slaughter and flushing medium for the apparent ileal digestibility of N in meat-and-bone meal. The overall means and levels of significance for the main effects are presented in table 6. There was no significant ($p > 0.05$) effect of the flushing solution used to recover the ileal digesta but apparent ileal N digestibility was significantly ($p < 0.05$) lower when birds were gassed with carbon dioxide rather than being euthanased using the barbiturate.

DISCUSSION

For birds in the present study given free access to the relatively fine meat-and-bone meal based diet, there was no indication of particle selection. However, for the compound and wheat diets, which were coarser than the meat-and-bone meal diet, evidence for particle selection was found. There was a general tendency for fibrous material to be at a higher concentration in the crop contents after a single meal, for the birds having free

Table 6. Overall effects of slaughter procedure[1] and flushing solution[2] on the apparent ileal digestibility of total nitrogen for growing chickens fed a meat-and-bone meal based diet

	Slaughter method		Flushing medium		Overall SE	Significance[3]	
	Carbon dioxide	Barbiturate	Distilled water	Saline		Slaughter method	Flushing medium
Apparent digestibility (%)	72.9	80.2	76.5	76.6	2.11	*	NS

[1,2] Birds were euthanased with either carbon dioxide gas or by an intra-cardial injection of barbiturate. Ileal digesta were removed using distilled water or physiological saline. There were 6 birds per treatment.
[3] NS non-significance; *$p < 0.05$.

access to their food. The differences in crop content concentrations between the free access and intubated birds were particularly marked for the ratios of neutral detergent fibre or acid detergent fibre and chromium, and for the indigestible marker element, chromium, the concentration of which tended to be lower with free access feeding. It appears that with free access the birds may have selected coarser more fibrous particles in perference to the finer chromium-containing material. It is concluded that with feedstuffs containing a mixture of coarse and fine particles, intubation would be the preferred technique for administering food in a digestibility assay. However, for relatively fine semi-synthetic diets, free access feeding will not lead to marked particle separation. Sibbald (1987) emphasised that the intubation technique ensures complete intake of a precise amount of food at a known time. The technique prevents birds from selecting preferred dietary components, avoids the problem of food spillage and overcomes food intake variation. In the present work, the free access feeding procedure was found to be easier to implement than intubation and it was noted that with intubation the crop contents tended to be drier than for those ingested under free access conditions. The possible effect that this may have on nutrient digestibility should be determined.

The emptying of material from the crop, post-feeding, was also evaluated here. If different food components were emptied from the crop at the same rate post-feeding, then the concentration of chemical components in the crop contents would be expected to remain relatively constant over time. For the meat-and-bone meal based diet there was no significant effect of time post-feeding on the concentrations of the chemical components examined. However, for the wheat-based diet, the concentrations of the various chemical components differed significantly ($p < 0.05$) over time. Chromium in particular was found at a significantly lower concentration at 3 h as opposed to 1 h post-feeding. It is possible that there was a progressive dilution over time of chromium by endogenous material entering the crop, though it appears more likely, given that a similar decline was not observed with meat-and-bone meal, that components of the crop material emptied at different rates. This may have implications in regard to an optimal time for sampling ileal contents in an ileal digestibility assay and should be investigated. That an effect of time was observed for the wheat-based diet but not for the meat-and-bone meal diet may be related to the coarser texture and greater range in particle size for the wheat diet.

In the second study, there was no effect on the apparent ileal digestibility of nitrogen consequent upon using distilled water as opposed to physiological saline as the flushing medium. This was recently reported to also be the case for the growing pig (Butts, 1991). Distilled water is preferred for digesta collection as saline increases the ion content of the digesta sample which results in hygroscopic dried digesta samples and potentially can interfere with amino acid analysis (Ambler, 1981). There was a significant ($p < 0.05$) effect of the method used for killing the birds, with a considerably lower mean apparent digestibility coefficient being found with asphyxiation in carbon dioxide gas. Carbon dioxide inhalation was accompanied by struggling in the birds and in some cases, defaecation and regurgitation of crop contents were observed. The struggling may have influenced the passage of digesta in the gut, thus altering the digesta N content. Such an effect was reported by Bolton (1964) who found that cervical dislocation caused agonal spasms of the intestine with consequent digesta movement in the gut. It is also possible that there was a shedding of small intestinal mucosal cells with the gas treatment. Use of a barbiturate minimises mucosal cell shedding at death (Badawy et al., 1958). In the present study, birds killed

with the barbiturate were immobilised immediately and lapsed quickly into unconsciousness. It is concluded that ethically and operationally, an intra-cardial injection of sodium pentobarbitone is the preferred method for killing birds in the ileal digestibility assay. It should be noted, however, that Summers and Roblee (1985) found no difference in apparent ileal amino acid digestibility between birds sampled under halothane anaesthesia or following death by cervical dislocation.

Overall, it is concluded that for the ileal digestibility assay in broiler chicks, food should be administered by intubation, though this may not be necessary for fine semi-synthetic diets. The birds should be killed by administration of a barbiturate and digesta flushed from the terminal ileum using distilled water. There was evidence for a differential rate of emptying over time of different food components from the crop of birds fed a wheat-based diet. The effect of time after intubation for sampling of the ileal digesta on apparent ileal nitrogen digestibility needs to be studied, as does the effect of the length of ileum sampled. Once a standardised ileal digestibility assay has been developed it should be compared with the traditional excreta assays and validated on the basis of accuracy of prediction of bird growth performance. The slaughter technique for determining ileal digestibility has the advantage of being rapid and involves minimal physiologcal disturbance prior to the time of digesta sampling.

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