THE DETERMINATION OF AMINO ACID DIGESTIBILITY IN POULTRY FEEDSTUFFS

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SUMMARY

Measurements were made on protein supplements in a basal diet, of dry matter, nitrogen and amino acid digestibilities using mature and' adult cockerels. Birds were killed 5h after receiving their diet in a single feed. Analyses were made on digesta from (i) the ileum, (ii) the terminal ileum, (iii) post caeca, and on excreta. In a second experiment adult cockerels were fitted with a simple cannula in the terminal ileum. Cr_2O_3 was used as an indicator in the diet. Nitrogen-free diets were also fed. These contained different amounts of rice hulls.

The results showed that digestibility measurements of nutrients based on ileal contents underestimate the true values. 'Consistently' higher values were found at the terminal ileum for the same diets. Considerable fermentation occurred in the caeca, consequently digestibilities measured on excreta did not always agree with those at the terminal ileum. Nitrogen digestibility of six meat meals varied from 0.79 to 0.67 at the terminal ileum. A similar range was observed for most of the amino acids measured at this site.

Cannulation of the terminal ileum was satisfactory and cannulae remained patent for several months. Lysine digestibilities for the four fish meals varied from 0.85 to 0.94. Corresponding values in excreta were 0.95 to 0.86. With increasing amounts of fibre in the diet there was a significant increase in endogenous nitrogen excreta., Generally there was a corresponding increase in the concentration of several amino acids in excreta.

It was concluded that ileal cannulation was a practical method of measuring amino acid and nutrient digestibilities in fowl. Excreta analysis may be useful for many high quality protein feedstuffs but those which result in large amounts of residue in the hind gut may undergo substantial change in amino acid profile. There is still the possibility of using amino acids in excreta as a means of ranking protein supplements for their digestibility.

INTRODUCTION

The utilisation of amino acid acids by livestock varies markedly for many reasons. Of most interest and of central importance to work reported in this paper is the feed ingredient. Utilisation, as defined here, embraces digestibility, absorption and metabolism. In this paper major emphasis will be placed on the digestibility or disappearance of amino acids from the small intestine of the fowl when offered .a range of feedstuffs.

The concept of amino acid availability was reviewed most recently by McNab (1979a, b) and Thomas (1980). There is some uncertainty about chemical methods used to estimate availability of some amino acids since there is frequently, for many feedstuffs, a poor correlation between

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values determined in vitro and in vivo (Batterham 1979; Batterham et al. 1981; Taverner and Farrell 198.1). Dye-binding techniques using acidazo dyes show some promise (Hurrell et al. 1979). The slope-ratio method is probably the method that has the most direct application (Carpenter and Booth 1973) since it measures incremental growth and feed intake responses to additions to a diet of a test amino acid in a feed ingredient. However, there are still several potential problems with this assay (McNab 1979b) and values for utilization of amino acids may exceed 100% (Varnish and Carpenter 1970). Maintenance of balance of the important essential amino acids when the test amino acid is added in incremental amounts is not always considered. Growth depression due to amino acid imbalance may therefore occur. The availability of free amino acids, when added to test diets is assumed to be 100% and that absorption occurs at the same rate as amino acids in the intact protein.

Recently Sibbald (1979a) described a method of determining amino acid digestibility from concentration in feed and corresponding excreta. This general approach was used previously by Kuiken and Lyman (1948) and Bragg et al. (1969) and is the basis of amino acid digestibilities reported in feed tables (Janssen et al. 1979).

In Sibbald's (1979a) method a correction is made for endosenous amino acids voided in excreta, and a true digestibility of the amino acids in a feedstuff is calculated. The basis of this correction has been questioned (Farrell 1981) since starved birds are used to provide endosenous amino acid output. Moreover it was shown by Farrell (1981) that dietary fibre can increase the output of endoaenous excreta by cockerels. N and amino acid contents may be similarly increased.

Clearly the easiest and most attractive method of determination of amino acid digestibility is that of excreta analysis (Janssen et al. 1979). This method is based-on the unproven assumption that microbial changes to the amino acids in digesta during passage along the large intestine are minimal and do not mask changes in amino acid profile between the terminal ileum and the cloaca. Although the alimentary tract of the fowl has an active microflora (Jayne-Williams and Coates 1969) it is assumed that feed residues pass rapidly through the large intestine thereby reducing the opportunity for major changes in amino acid profile of residues to occur. Observations on pigs, rats and cockerels (Slump et al. 1977) on the same diet would tend to support the concept that changes in amino acid profile of feed residues in cockerels are less than for other species. Salter and Coates (1971) working with germ-free and conventional chicks showed that good and poor quality proteins were digested to the same extent by both groups.

The removal of the ileal contents of birds previously fed a test diet and the determination of amino acids in the feed and corresponding ileal contents was used by Payne et al. (1968), Soares and Kifer (1971), Varnish and Carpenter (1975) and Achinewhu and Hewitt (1979) to estimate disappearance of amino acids from a known input of feed. It is necessary to incorporate chromic oxide (Cr_2O_3) in the diet and to recover the marker quantitatively in ileal digesta in order to estimate total contents from the feed consumed.

The main objective of this paper is to describe research undertaken to evaluate biological methods of measuring amino acid disappearance from the small intestine of poultry. The method used was tedious and involved

slaughter of large numbers of birds. In an attempt to overcome this problem a permanent T piece cannula were inserted in the terminal ileum of adult cockerels. An important aspect of the research described here was to examine changes that occurred in amino acid profile of digesta during transit along the large intestine. The purpose was to determine with poultry if excreta were consistently suitable material for determining amino acid digestibility of a range of feedstuffs. The influence of increments of dietary fibre on N and on amino acids in endogenous excreta was also studied to obtain estimates of true digestibility values.

MATERIALS AND METHODS

Slaughter experiments

(i) Experiment 1 One hundred and four White Leghorn x Black Australorp male chicks, 4 weeks of age, used in a previous experiment, were held in suitable group cages until 8 weeks of age. They were then placed in individual mesh-wire cages and trained to eat their daily feed allowance (about 100 g) in one hour (Farrell 1978). Birds were fed a 'commercial chick-grower diet adequate in all nutrients to allow maximum growth rate. The basal test diet consisted of corn and bone meal (2%) supplemented with minerals and vitamins. The 'basal diet was combined with 50% of a protein supplement from one of the following sources: 6 different meat meals, blood meal, fish meal, sweet lupinseed meal, soybean meal, maize meal, copra meal, cottonseed meal, peanut meal and sunflower meal. A nitrogen-free diet based on starch, glucose, oil bone meal and cellulose was used. Cr_2O_3 was added to all diets (0.1%) which were cold pelleted. Each dietary treatment had 3 replicates each of 3 birds. The test diet was given for 2 d; 3 birds were starved for 48 h and then offered 100 g of the test diet for 1 h. Five hours later the birds were killed by cervical dislocation and digesta collected from (i) the ileum - from Mechel's diverticulum to 2.5 cm anterior to the ileo-caecal junction (S1); (ii) the region immediately post caeca to 1 cm from the cloaca (S3), and (iii) excreta (S4). Excreta were collected from a tray placed beneath each cage.

(ii) Experiment 2 One hundred and twenty six White Leghorn x Black Australorp male chicks were housed and treated similar to that described in experiment 1, except that the fish meal, blood meal and peanut meal-based diets were omitted due to contamination with mould. When the birds were killed, digesta were collected only from the last 10 cm of the ileum (S2).

Digesta and excreta

These were combined for 3 birds for each site and collected in plastic containers and quickly cooled, frozen and freeze-dried to constant weight. The dry material was weighed and milled then stored for analysis.

Ileal cannula experiments

A glass T piece cannula was inserted permanently in the terminal ileum about 2.5 cm anterior to the ileocaecal junction of adult cockerels (2-3 kg) using standard surgical procedures. When collection of digesta was required the screw cap and plug were replaced by a small plastic vial which was attached to the thread on the cannual stem. Digesta slowly entered the vial for about, 30 min. and was removed two or three times

TABLE 1 Apparent dry matter (DM) and nitrogen digestibility coefficients of protein conc in the basal diet and measured in ileal contents (S1), at the terminal ileum (S in excreta (S4) of three groups of birds killed 5 h post-feeding.

Feedstuff	Crude protein (g/kg)	Dry matter digestibility					Nitrogen dig	
		S1	S2	S3	S4	MEAN	S1	S2
Meat meal 12	430	0.52^{c*}	0.75^{a}	0.73^{a}	0.65^{b}	0.66^{DE**}	0.40^{c}	0.70^{a}
13	566	0.57^{d}	0.65^{c}	0.81^{a}	0.70^{b}	0.68^{CD}	0.62^{c}	0.79^{b}
14	491	0.52^{c}	0.59^{b}	0.62^{ab}	0.64^{a}	0.59^{H}	0.61^{b}	0.67^{a}
15	506	0.59^{c}	0.63^{bc}	0.67^{ab}	0.70^{a}	0.64^{EF}	0.63^{c}	0.73^{a}
16	479	0.49^{d}	0.58^{c}	0.80^{a}	0.73^{b}	0.65^{EF}	0.53^{c}	0.77^{a}
17	525	0.62^{b}	0.61^{b}	0.64^{b}	0.71^{a}	0.65^{EF}	0.65^{b}	0.77^{a}
Fish meal	446	0.58^{b}		0.67^{a}	0.65^{a}	0.63^{FG}	0.53^{b}	
Blood meal	904	0.77^{a}		0.70^{b}	0.66^{b}	0.71^{B}	0.86^{a}	
Maize gluten	705	0.78^{b}	0.79^{b}	0.84^{a}	0.77^{b}	0.65^{A} 0.79^{H}	0.84^{b}	0.90^{a}
Lupin seed meal	272	0.53^{c}	0.61^{b}	0.67^{a}	0.61^{b}	0.60^{CD}	0.65^{b}	0.78^{a}
Soybean meal	476	0.64^{c}	0.66^{bc}	0.71^{a}	0.68^{b}	0.67^{I}	0.66^{c}	0.85^{a}
Copra meal	207	0.55^{b}	0.56^{b}	0.58^{a}	0.57^{ab}	0.56^{GH}	0.47^{b}	0.53^{a}
Cottonseed meal	361	0.58^{c}	0.61^{bc}	0.63^{b}	0.68^{a}	0.63^{H}	0.64^{b}	0.75^{a}
Sunflower meal	336	0.57^{b}	0.59^{b}	0.66^{a}	0.67^{a}	0.60^{A}	0.52^{b}	0.73^{a}
Corn	89	0.80^{a}	0.81^{a}	0.79^{a}	0.83^{a}	0.80^{BC}	0.67^{c}	0.81^{a}
Peanut meal	437	0.69^{ab}		0.72^{a}	0.68^{b}	0.70	0.76^{a}	
Mean		0.61^{c} ±0.01	0.65^{b} ±0.08	0.70^{a} ±0.07	0.68^{a} ±0.08	0.67 ±0.02	0.62^{b} ±0.12	0.74^{a} ±0.09
Nitrogen-free diet†		0.87^{a} ±0.01	0.87^{a} ±0.01	0.83^{b} ±0.02	0.87^{a} ±0.02	0.86	2.27 ±1.1	1.42 ±0.5

* Values in the same row with different superscripts (a-d) are significantly different (I

** Values in the same column with different superscripts (A-H) are significantly different

† Nitrogen content (g/100g DM)

TABLE 2 Mean (SEM) values for the coefficient of digestibility of amino acids of animal (n=8) and of plant (n=8) protein sources in a basal diet (50%) and measured in the entire ileum (S1), terminal ileum (S2), in post caecal (S3) digesta and in excreta (S4) of 3 groups of 3 birds killed 5 h post-feeding.

Protein source	Amino acid*	SITE S1	S2	S3	S4	Mean of sites (n=8)	SEM	Combined mean of † sites (n=16)
		**						
Animal	Lysine	0.61^{cdefB}	0.77^{bcdefA}	0.81^{abcA}	0.82^{abcA}	0.75^{efgh}	0.02	
	Arginine	0.57^{efB}	0.70^{efA}	0.75^{cA}	0.80^{bcA}	0.70^{hi}	0.01	
	Threonine	0.57^{efC}	0.68^{fB}	0.81^{abcA}	0.84^{abcA}	0.73^{fghi}	0.03	
	Glutamic A	0.65^{bcdefC}	0.76^{bcdefC}	0.84^{abcAB}	0.87^{abcA}	0.78^{bcdef}	0.01	
	Proline	0.67^{bcdeC}	0.73^{defBC}	0.85^{abcA}	0.79^{cAB}	0.78^{bcdef}	0.03	
	Glycine	0.61^{cdefB}	0.69^{fB}	0.64^{dB}	0.83^{bcA}	0.69^{i}	0.02	
	Methionine	0.58^{efB}	0.80^{bcdeA}	0.81^{abcA}	0.88^{abcA}	0.76^{defg}	0.03	
	Isoleucine	0.55^{fB}	0.80^{bcdeA}	0.81^{abcA}	0.87^{abcA}	0.76^{defg}	0.02	
	Leucine	0.70^{bcB}	0.81^{bcdA}	0.87^{abA}	0.90^{abA}	0.82^{bc}	0.03	
	Mean (n=8)	$0.61^{C} \pm 0.05$	$0.75^{B} \pm 0.05$	$0.80^{AB} \pm 0.06$	$0.85^{a} \pm 0.03$	0.75 ± 0.04		
Plant	Lysine	0.59^{defC}	0.68^{fBC}	0.75^{cAB}	0.83^{abcA}	0.72^{ghi}	0.04	0.74^{ab}
	Arginine	0.64^{aA}	0.86^{abA}	0.89^{aA}	0.91^{aA}	0.88^{ab}	0.01	0.79^{ab}
	Threonine	0.64^{bcdefB}	0.76^{bcdefA}	0.81^{abcA}	0.85^{abcA}	0.77^{cdefg}	0.03	0.75^{ab}
	Glutamic A	0.82^{aA}	0.92^{aA}	0.89^{aA}	0.92^{aA}	0.89^{a}	0.01	0.83^{a}
	Proline	0.07^{bcB}	0.82^{abcdA}	0.83^{abcA}	0.85^{abcA}	0.80^{bcde}	0.03	0.79^{ab}
	Glycine	0.69^{bcdB}	0.74^{cdefAB}	0.77^{bcAB}	0.83^{abcA}	0.76^{defg}	0.02	0.72^{b}
	Methonine	0.69^{bcdB}	0.81^{bcdA}	0.83^{abcA}	0.85^{abcA}	0.80^{bcde}	0.03	0.78^{ab}
	Isoleucine	0.70^{bcB}	0.84^{abcA}	0.85^{abcA}	0.91^{aA}	0.83^{b}	0.02	0.80^{ab}
	Leucine	0.74^{abB}	0.84^{abcB}	0.84^{aAB}	0.89^{abcA}	0.83^{b}	0.03	0.83^{a}
	Mean (n=8)	$0.71^{C} \pm 0.05$	$0.81^{B} \pm 0.05$	$0.83^{AB} \pm 0.04$	$0.87^{A} \pm 0.03$	0.81 ± 0.04		
	Combined Mean (n=16)	$0.66^{C} \pm 0.05$	$0.78^{B} \pm 0.05$	$0.81^{AB} \pm 0.05$	$0.86^{A} \pm 0.03$			

* Mean of 24 observations (8 diets x 3 replicates)

** Values in the same column with different superscripts (a-h) are significantly different ($P < 0.05$)

† Valuesin the same row with different capital superscripts (A-C) are significantly different ($P < 0.05$)

during the collection period. This commenced about 5 h after feeding a single meal (100 g) in 1 h to 5 trained birds. Digesta were cooled rapidly and treated as already described. For analysis of excreta, a total collection was made from 5 birds for 32 h.

'Diets were the same as for experiment 1, with three additional fish meals. The N-free diet was diluted with additions of milled rice, hulls to give incremental levels of ADF. Pure cellulose (Solkafloc) was included in one N-free diet.

Analytical and statistical methods

Chemical analyses were undertaken on finely milled representative samples of diet, digesta and excreta using the following procedures.

Dry matter was determined by heating ground samples at 105^{0}C for 24 hours. Nitrogen was determined using the autoanalyser method of Clare and Stevenson (1964). Acid detergent fibre (ADF) was measured according to the method of Van Soest (1963).

Amino acid analysis was determined on the acid hydrolysates (Spackman et al. 1958) using a T.S.M. Technicon amino acid analysed.

Cr_2O_3 was determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 360) and following the procedures outlined by Williams et al. (1962) except that an acetylene-nitrous oxide flame was used.

Data were combined for experiemnts 1 and 2 and a one-way analysis of variance was undertaken. Differences between means were examined using Duncan's multiple range test (Duncan 1955). To examine more critically differences between measurements made on digesta'from the terminal ileum and the entire ileum (experiments 1 and 2), a separate analysis of variance was made on these data.

RESULTS

Slaughter experiments

The apparent digestibility of dry matter and N at the different sites are given for the experimental diets in Table 1. No correction was made for the contribution of the basal diet to these data or those in Table 2. Although dry matter digestibility differed significantly ($P < 0.05$) between sampling sites for individual feedstuffs, the overall mean's showed no differences ($P > 0.05$) between total ileal (S1) and terminal ileal (S2) contents; differences ($P < 0.05$) were observed between values combined for these two sites and those post-caeca (S3) and excreta (S4). However more ($P < 0.05$) N was digested at S2 than S1, but at S4 there was a large depression in N digestibility due. to the addition of urinary N. For the majority of the meat meal diets dry matter and N digestibilities were lower at S1 than S2. The range of overall apparent digestibilities combined for the four sites for dry matter and for N ranged from 56 and 50 for copra meal, to 79 and 83 for maize gluten respectively. The N-free diet showed a decline ($P < 0.05$) in dry matter digestibility in digesta post caeca; while for N, there were higher concentrations (g/100 g) at S1 than at the other three sites.

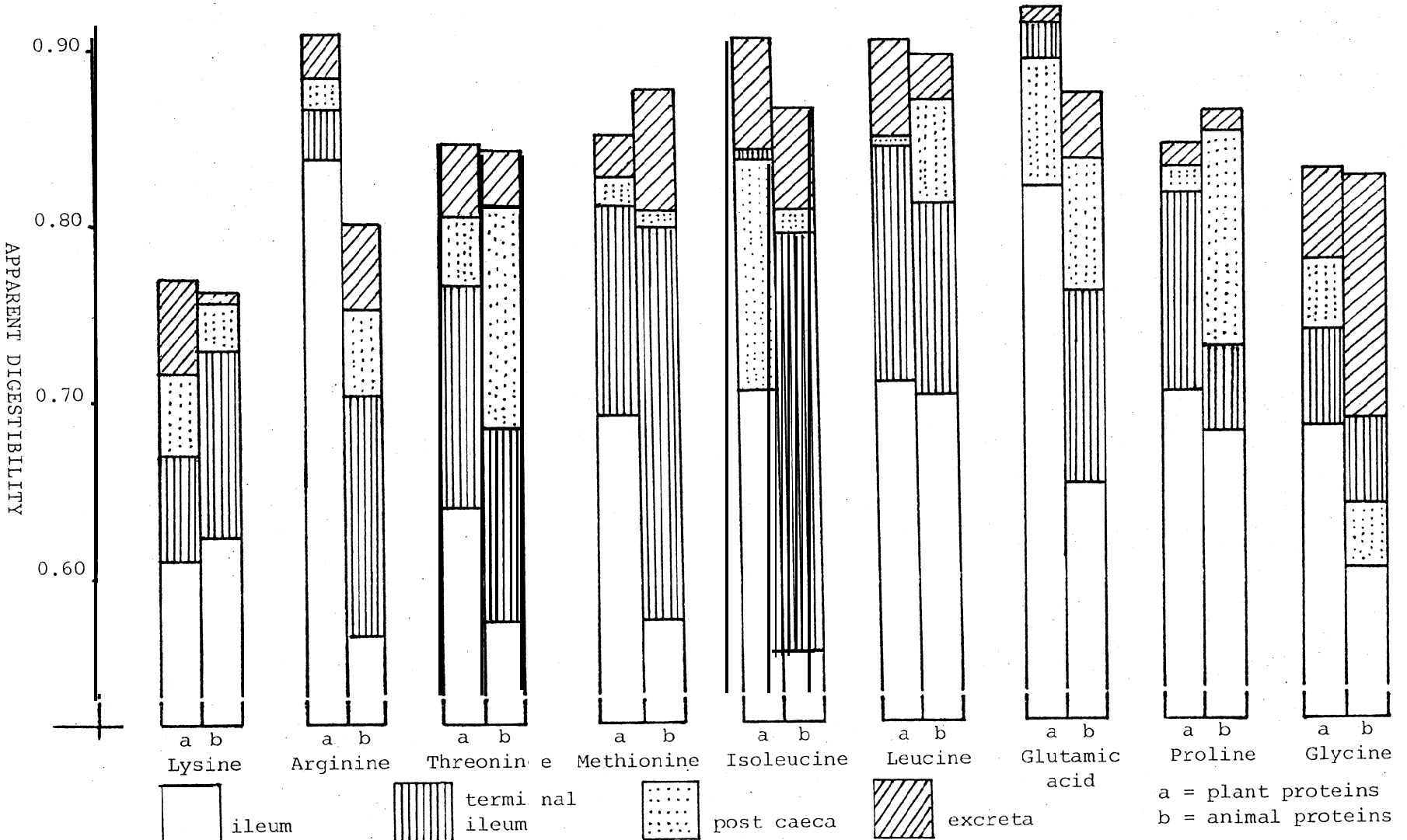

Fig. 1. Mean apparent digestibility of n ine amino acids at four sites (ileum, terminal ileum, post caeca and excreta) in birds killed 5 h after receiv ing diets based on animal and protein supplements.

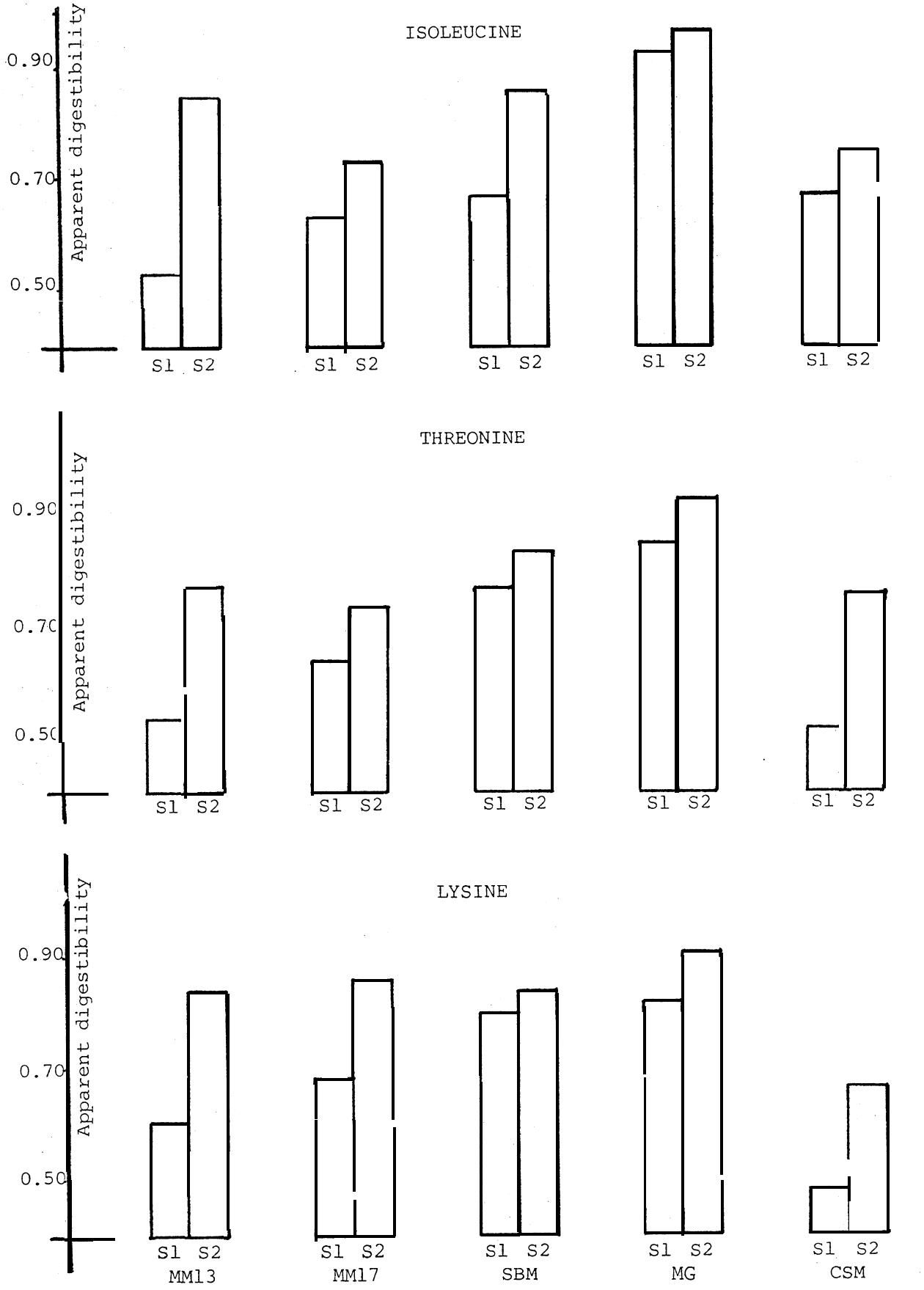

Fig. 2. Mean apparent digestibility of lysine, threonine and isoleucine in digesta of birds fed diets based on meat meals (MM); soybean meal (SBM), maize gluten (MG) and cottonseed meal (CSM) measured in the ileum and the terminal ileum of birds killed 5 h post feeding.

TABLE 3A Mean apparent digestibility coefficients of 3 amino acids in diets containing various protein supplements and measured in the ileum (S1), terminal ileum (S2), post caeca (S3) and excreta (S4) of birds killed 5h after feeding

Amino Acid	Site	PROTEIN SOURCE								
		MM 12	MM 13	MM 14	MM 15	MM 16	MM 17	Fish Meal	Blood Meal	Site
Lysine	S1	0.486^{dc*C**}	0.598^{cC}	0.519^{dC}	0.587^{cC}	0.447^{eD}	0.683^{bC}	0.578^{cC}	0.923^{aA}	0.603^{C}
	S2	0.581^{dB}	0.838^{abB}	0.712^{cB}	0.804^{bA}	0.799^{bC}	0.858^{aA}			0.765^{B}
	S3	0.764^{cA}	0.911^{aA}	0.749^{cB}	0.673^{dB}	0.895^{aA}	0.811^{bB}	0.821^{bA}	0.887^{aAB}	0.814^{AB}
	S4	0.777^{cA}	0.873^{aA}	0.815^{bA}	0.762^{cA}	0.844^{aB}	0.884^{aA}	0.763^{aB}	0.866^{aB}	0.823^{A}
	Mean	0.652^{e}	0.805^{b}	0.699^{d}	0.706^{d}	0.746^{c}	0.805^{b}	0.721^{c}	0.892^{a}	
Isoleucine	S1	0.423^{gD}	0.562^{cdC}	0.480^{efC}	0.515^{deD}	0.450^{fgC}	0.629^{bC}	0.582^{bcB}	0.765^{aA}	0.551^{C}
	S2	0.658^{dC}	0.860^{bB}	0.756^{cB}	0.762^{cB}	0.823^{bB}	0.910^{aA}			0.795^{B}
	S3	0.776^{cdB}	0.925^{aA}	0.749^{deB}	0.713^{eC}	0.904^{aA}	0.818^{bcB}	0.837^{bA}	0.732^{deA}	0.807^{B}
	S4	0.837^{dA}	0.933^{aA}	0.864^{cdA}	0.879^{bcA}	0.911^{abA}	0.905^{abA}	0.833^{dA}	0.764^{eA}	0.866^{A}
	Mean	0.673^{d}	0.820^{a}	0.712^{c}	0.717^{c}	0.772^{b}	0.815^{a}	0.751^{b}	0.754^{b}	
Threonine	S1	0.450^{dC}	0.535^{cC}	0.445^{dD}	0.532^{cC}	0.435^{dD}	0.633^{bD}	0.649^{bB}	0.905^{aA}	0.573^{C}
	S2	0.580^{dB}	0.772^{aB}	0.607^{cC}	0.689^{bB}	0.724^{bC}	0.730^{abC}			0.684^{B}
	S3	0.761^{cA}	0.893^{aA}	0.695^{dB}	0.701^{dB}	0.922^{aA}	0.799^{bcB}	0.825^{bA}	0.893^{aA}	0.811^{A}
	S4	0.762^{dA}	0.900^{aA}	0.827^{bcA}	0.840^{bcA}	0.872^{aB}	0.868^{abA}	0.795^{cdA}	0.867^{abA}	0.842^{A}
	Mean	0.639^{d}	0.775^{b}	0.644^{d}	0.691^{c}	0.738^{bc}	0.758^{b}	0.756^{b}	0.888^{a}	

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* Values in the same row bearing different superscripts (a-e) are significantly different ($P < 0.05$).

** Values in the same column bearing different capital superscripts (A-D) are significantly different ($P < 0.05$).

TABLE 3B Mean apparent digestibility coefficients of 3 amino acids in diets containing various protein supplements and measured in the ileum (S1), terminal ileum (S2), post caeca (S3) and excreta (S4) of birds killed 5h after feeding

Amino Acid	Site	PROTEIN SOURCE								
		Maize gluten	Lupinseed meal	Soyabean meal	Copra meal	Cottonseed meal	Sun-flower meal	Corn	Peanut meal	Site
Lysine	S1	0.824^{a*B**}	0.483^{bC}	0.802^{aC}	0.314^{cD}	0.475^{bB}	0.501^{bC}	0.479^{bC}	0.796^{aAB}	0.597^{C}
	S2	0.913^{aA}	0.789^{bB}	0.840^{bB}	0.384^{eC}	0.662^{cA}	0.599^{dB}	0.614^{cdB}		0.691^{B}
	S3	0.954^{aA}	0.816^{bcA}	0.860^{bAB}	0.569^{eB}	0.705^{dA}	0.597^{eB}	0.639^{eB}	0.760^{cdB}	0.738^{B}
	S4	0.953^{aA}	0.877^{bcA}	0.874^{bcA}	0.753^{dA}	0.686^{eA}	0.811^{cdA}	0.885^{bA}	0.838^{bcA}	0.835^{A}
	Mean	0.911^{a}	0.741^{c}	0.844^{b}	0.505^{e}	0.632^{d}	0.627^{d}	0.654^{d}	0.798^{c}	
Isoleucine	S1	0.894^{aB}	0.726^{cC}	0.778^{bC}	0.319^{fC}	0.578^{eB}	0.673^{dC}	0.792^{bD}	0.870^{aB}	0.704^{C}
	S2	0.969^{aA}	0.904^{bAB}	0.906^{bAB}	0.624^{dB}	0.804^{cA}	0.776^{cB}	0.922^{bB}		0.844^{B}
	S3	0.973^{aA}	0.881^{bB}	0.895^{bB}	0.662^{eB}	0.812^{cdA}	0.775^{dB}	0.868^{bC}	0.844^{bcB}	0.838^{B}
	S4	0.965^{aA}	0.941^{abA}	0.937^{abA}	0.797^{cA}	0.814^{cA}	0.901^{bA}	0.970^{aA}	0.916^{bA}	0.905^{A}
	Mean	0.950^{a}	0.863^{b}	0.879^{b}	0.600^{d}	0.752^{c}	0.781^{c}	0.888^{b}	0.877^{b}	
Threonine	S1	0.847^{aB}	0.606^{cdC}	0.761^{bC}	0.373^{fD}	0.501^{eC}	0.585^{dC}	0.639^{cC}	0.795^{bB}	0.639^{C}
	S2	0.923^{aA}	0.773^{cB}	0.840^{bB}	0.519^{dC}	0.748^{cB}	0.737^{cB}	0.811^{bB}		0.764^{B}
	S3	0.947^{aA}	0.827^{bA}	0.927^{aA}	0.629^{dB}	0.796^{bA}	0.708^{cB}	0.803^{bB}	0.796^{bB}	0.804^{AB}
	S4	0.919^{aA}	0.858^{cdA}	0.888^{bcA}	0.695^{fA}	0.770^{eAB}	0.846^{dA}	0.877^{cdA}	0.898^{bcA}	0.844^{A}
	Mean	0.909^{a}	0.766^{d}	0.854^{b}	0.554^{f}	0.704^{e}	0.719^{e}	0.783^{d}	0.830^{c}	0.002

* Values in the same row bearing different superscript are significantly different ($P < 0.05$).

** Values in the same row bearing different capital superscript are significantly different ($P < 0.05$).

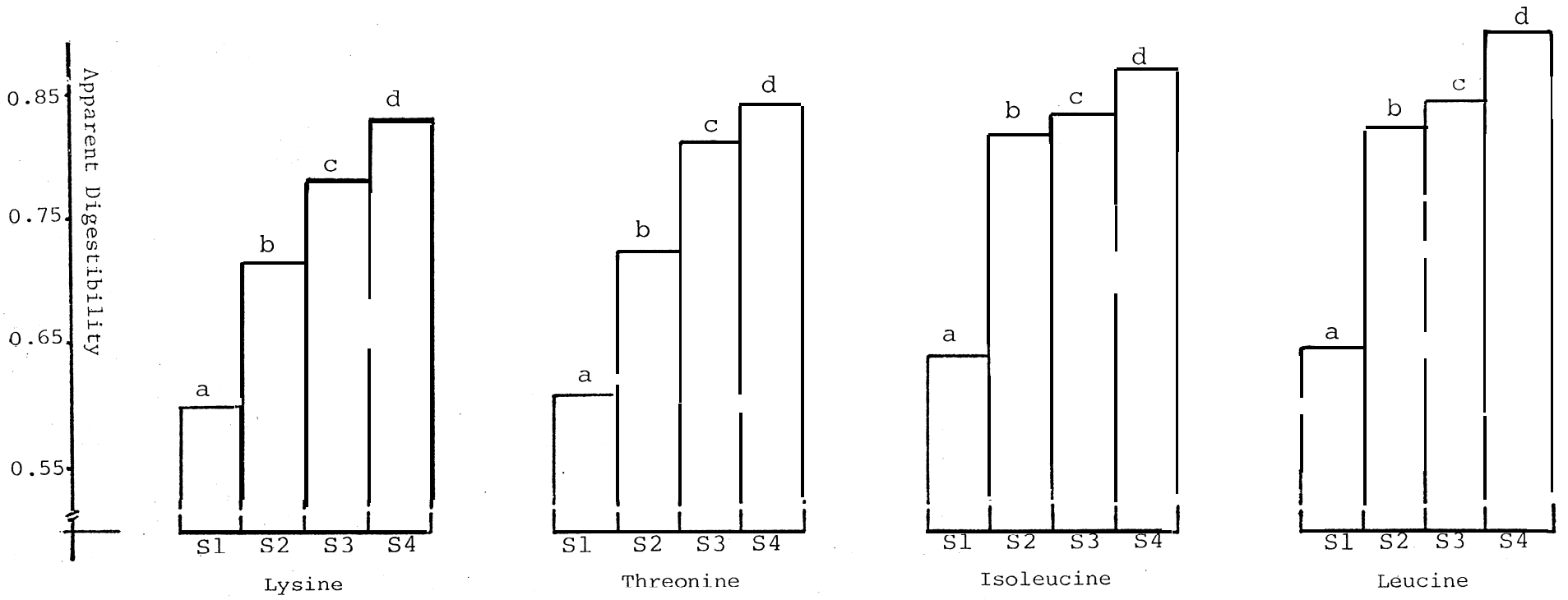

Fig. 3 Mean apparent digestibility of lysine, threonine and isoleucine of 16 diets measured in the ileum (S1), terminal ileum (S2), post caeca (S3) and in excreta (S4) of birds killed 5 h after feeding.

The mean disappearance of amino acids for the 8 plant-protein based diets, and the 8 animal protein diets at the 4 sites is given in Table 2. The mean of the 9 amino acids measured showed differences ($P < 0.05$) for both groups between S1 and S2. Amino acid disappearance increased from S1 through to S4. although this was not always significant. At the terminal ileum (S2) mean disappearance of amino acids for the animal protein-based diet was 0.75 compared with 0.81 for the plant protein-based diets. There was a wide range of values for disappearance among individual amino acids at the four sites. For lysine and threonine disappearance from the important site at the terminal ileum (S2), mean values were 0.77 and 0.68 for the animal proteins and 0.68 'and 0.76 for the plant proteins. Values tended to be greater than 0.80 for all amino acid digestibilities using excreta irrespective of the protein source.

The mean apparent'digestibility of 9 amino acids in the 8 diets based on animal proteins, and those based on plant proteins at the four sites is shown in Figure 1. The major increases occurred between S1 and S2. With the exception of lysine in plant proteins, and glycine in animal proteins, no marked changes occurred in amino acid digestibilities in samples taken post-caeca and from excreta.

Mean digestibility of three essential amino acids, often limiting in poultry diets, for the individual protein sources at the four sites, is given in Tables 3A & B. For the 6 meat meal diets, considerable variation. was observed both between and within sites for all amino acids. Blood meal showed constant and consistently high values at all three sites measured. Within the group of plant proteins, maize gluten and to a lesser extent soybean meal had consistently high values for amino acid digestibilities at the four sites for the three amino acids. Copra meal and cottonseed meal on the other hand gave low values. Shown in a histogram (Fig. 2) is the apparent digestibility of three amino acids at S1 and S2 for two samples of meat meal, soybean meal, maize gluten and cottonseed meal. In all cases there was a considerable increase in digestibility for all feedstuffs at S2 compared with S1.

The mean apparent digestibility of the three important amino acids in the 16 feedstuffs at the four sites is shown in Figure 3. In all cases there was a stepwise increase in digestibility of all amino acids from S1 to S4. Only for lysine and threonine were differences significant ($P < 0.05$) between S1 and S2.

Ileal cannula experiments

Birds recovered rapidly from surgery and normally resumed eating a special high-energy, low residue diet within 36 h. Maintenance of the cannula (Plate 1) was minimal. It remained patent for several months.

Apparent digestibility of 'dry matter, N and four amino acids at S1 and S2 are shown in Table 4. Mean dry matter digestibility was lower ($P < 0.05$) in excreta than at the terminal ileum. Cr_2O_3 estimates of excreta compared favourably with those determined by total collection; . mean values were 0.610 and 0.614 respectively.

Apparent digestibility of amino acids at the two sites varied considerably depending on the protein source. Within the 5 meat meals lysine digestibility varied from 0.71 (MM 16) to 0.85 (MM 13); corresponding values for threonine were 0.70 and 0.73. Interestingly for arginine

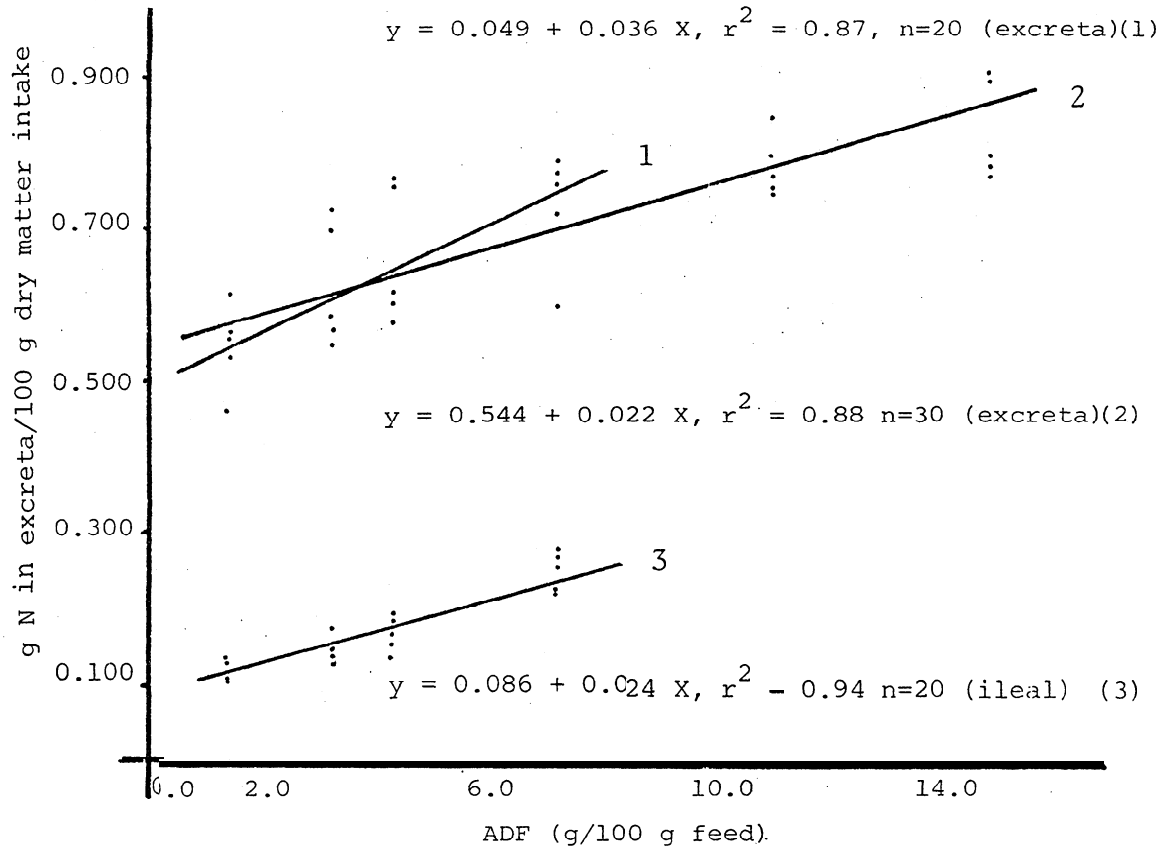

Fig. 4. The relationship between N in excreta (g/100 g feed intake, Y) and acid detergent fibre (ADF) in a N-free diet (g/100 g, X) fed to 5 adult cockerels at each level of ADF intake.

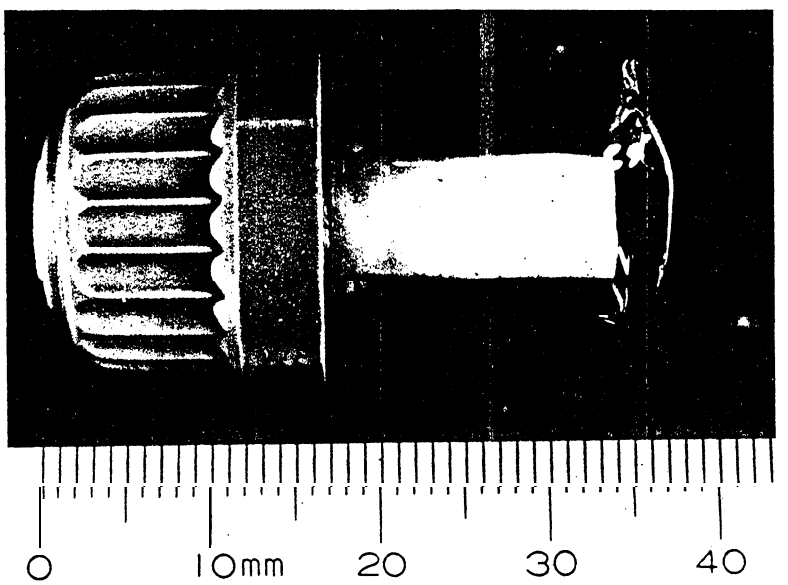

Plate 1. A simple 'T' piece glass cannula with cap and stem used to collect digesta from the terminal ileum of adult cockerels.

TABLE 4 Coefficient of apparent digestibility of amino acids of protein concentrates included at 50% in a basal diet measured at the terminal ileum using a simple cannula, and in excreta

		Meat meal					Soya bean meal	Fish meal				Blood meal	Lupin seed meal	Maize gluten	Copra meal	Cotton seed meal	Corn	Site
	Crude protein of concentrate (g/kg)	13	14	15	16	17		Safcol S.A.	South African	Thai	Danish							
		566	491	506	479	525	476	446	703	664	717	904	278	705	207	361	89	
Arginine	Ileal	0.81^{h*B**}	0.60^{kB}	0.76^{iB}	0.68^{jA}	0.86^{fA}	0.95^{bA}	0.89^{eA}	0.93^{cB}	0.85^{fB}	0.84^{gB}	0.93^{cA}	0.96^{bA}	0.98^{aA}	0.90^{dA}	0.92^{eB}	0.90^{dA}	0.86
	Excreta	0.88^{gA}	0.65^{kA}	0.80^{iA}	0.69^{jA}	0.82^{hB}	0.91^{eB}	0.89^{fA}	0.95^{bA}	0.92^{dA}	0.86^{ghA}	0.87^{gB}	0.90^{fB}	0.98^{aA}	0.87^{gB}	0.93^{eA}	0.88^{gB}	0.86
Lysine	Ileal	0.85^{fA}	0.74^{iA}	0.77^{hA}	0.71^{jA}	0.88^{eA}	0.93^{eA}	0.82^{gA}	0.89^{dA}	0.92^{eA}	0.88^{eA}	0.94^{bA}	0.90^{dA}	0.95^{aA}	0.70^{jA}	0.68^{jA}	0.82^{gA}	0.84
	Excreta	0.80^{eB}	0.71^{hB}	0.77^{fA}	0.69^{iA}	0.79^{eB}	0.85^{bB}	0.71^{hB}	0.86^{bB}	0.84^{eB}	0.79^{eB}	0.81^{dB}	0.73^{gB}	0.95^{aA}	0.41^{kB}	0.61^{jB}	0.61^{jB}	0.75
Threonine	Ileal	0.73^{iB}	0.66^{kB}	0.76^{ghA}	0.70^{jA}	0.81^{fA}	0.88^{cA}	0.78^{gB}	0.84^{eA}	0.85^{dA}	0.83^{eB}	0.93^{aA}	0.81^{fA}	0.91^{bA}	0.66^{kA}	0.71^{jB}	0.75^{hA}	0.79
	Excreta	0.78^{hA}	0.70^{kA}	0.69^{klB}	0.66^{lmB}	0.77^{iB}	0.85^{dB}	0.82^{fA}	0.90^{cB}	0.85^{dA}	0.85^{dA}	0.94^{aA}	0.79^{gB}	0.92^{bA}	0.64^{mA}	0.83^{eA}	0.74^{jA}	0.80
Glycine	Ileal	0.64^{iB}	0.60^{jA}	0.72^{hA}	0.14^{kA}	0.79^{eA}	0.88^{cA}	0.76^{gA}	0.75^{gA}	0.78^{fA}	0.78^{fA}	0.92^{aA}	0.85^{dA}	0.90^{bA}	0.73^{hA}	0.77^{fgA}	0.76^{gA}	0.74
	Excreta	0.89^{eA}	0.60^{eA}	0.62^{eB}	0.10^{jA}	0.69^{cB}	0.70^{cB}	0.38^{hB}	0.57^{fB}	0.65^{dB}	0.33^{iB}	0.73^{bB}	0.72^{bB}	0.92^{aA}	0.43^{gB}	0.61^{eB}	0.57^{fB}	0.58
Dry matter digestibility	Ileal	0.65^{fA}	0.59^{gA}	0.65^{fA}	0.58^{gA}	0.71^{deA}	0.70^{eA}	0.61^{fgA}	0.73^{cdA}	0.70^{eA}	0.75^{cA}	0.77^{cA}	0.58^{gA}	0.86^{aA}	0.61^{gA}	0.65^{fA}	0.81^{bA}	0.68^{A}
	Excreta Cr_2O_3	0.62^{deB}	0.53^{gB}	0.56^{fgB}	0.54^{gB}	0.59^{eB}	0.65^{cB}	0.54^{gB}	0.61^{eB}	0.55^{gB}	0.61^{dB}	0.61^{dB}	0.59^{efA}	0.82^{aB}	0.54^{gB}	0.62^{deA}	0.78^{bA}	0.61^{B}
	Total collection	0.55^{dC}	0.57^{dC}	0.50^{eC}	0.61^{bcA}	0.62^{bcB}	0.62^{bcB}	0.55^{dB}	0.60^{eB}	0.57^{dB}	0.60^{eB}	0.65^{bB}	0.57^{dA}	0.83^{aAB}	0.57^{dB}	0.62^{bcA}	0.81^{aA}	0.61^{B}
Nitrogen digestibility	Ileal	0.71^{ghA}	0.70^{hA}	0.78^{deA}	0.71^{ghA}	0.81^{cdA}	0.90^{aA}	0.76^{efA}	0.87^{abA}	0.84^{bcA}	0.84^{bcA}	0.88^{aA}	0.74^{fgA}	0.88^{aA}	0.81^{cdA}	0.64^{iA}	0.84^{bcA}	
	Excreta	0.67^{cdB}	0.61^{fB}	0.67^{cdB}	0.62^{efB}	0.65^{deB}	0.85^{aB}	0.63^{efB}	0.77^{bB}	0.79^{bB}	0.79^{bB}	0.83^{aB}	0.69^{cB}	0.83^{aB}	0.54^{gB}	0.51^{gB}	0.69^{cB}	

* Values within a row with the same superscripts (a-k) are not significantly different ($P > 0.05$)

** Values within a column with the same superscripts (A-B) are not significantly different ($P > 0.05$) for each amino acid

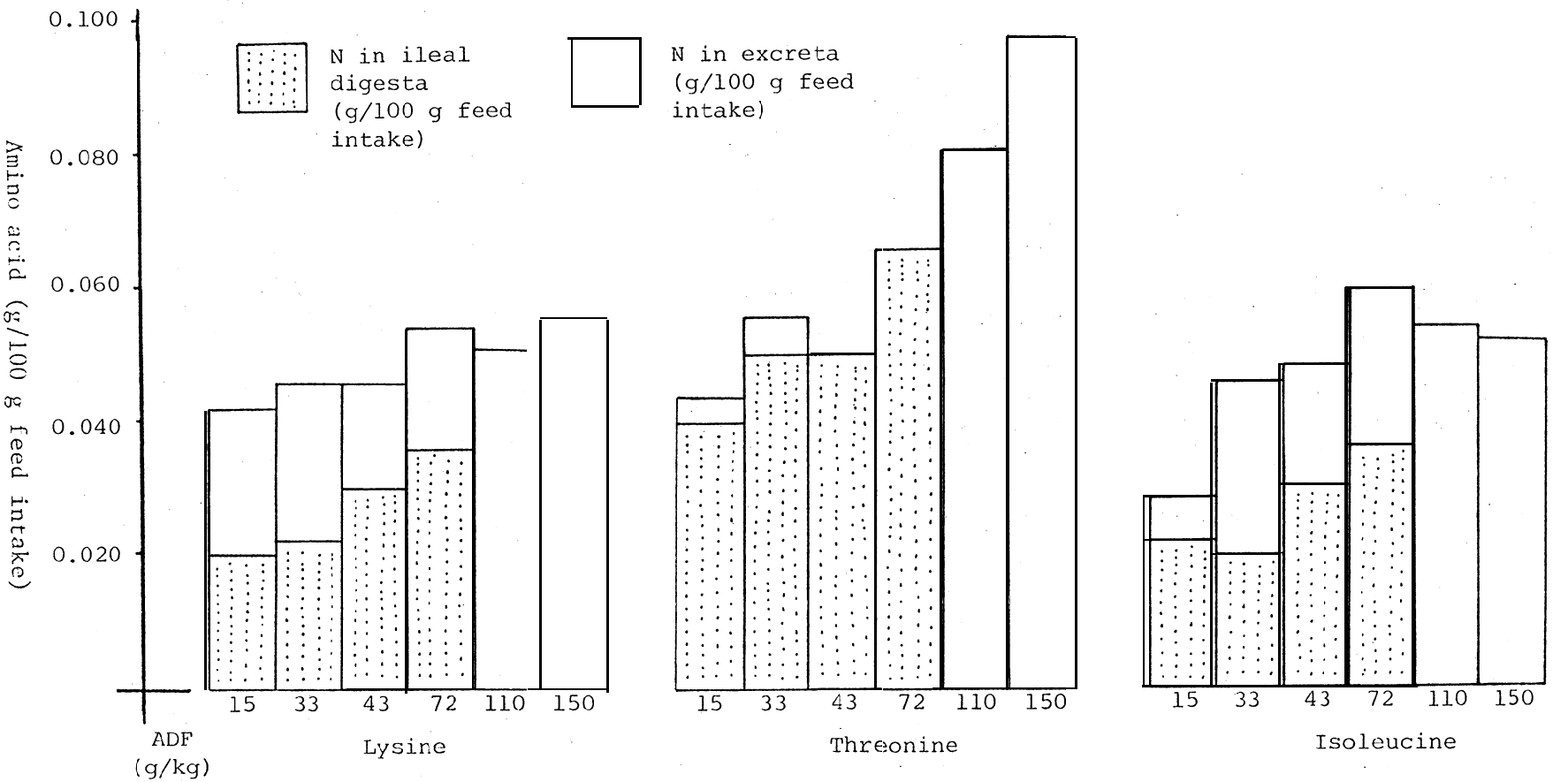

Fig 5 The effect of increments of acid detergent fibre (ADF) in a N-free diet on the amino acid content of excreta and ileal digesta (g/100 g feed intake).

cottonseed meal had a similar ileal digestibility to corn of over 0.90 but for lysine the values were 0.68 and 0.82 respectively. Mean values between the two sites S2 and S4 were generally similar. Exceptions were lysine 0.84 (S2) and 0.75 (S4), and glycine 0.74 and 0.58 respectively.

There was a significant P < 0.01) increase in N output in ileal digesta (S2) and in excreta (S4) with increasing increments of ADF in the 'N-free diet (Fig. 4). At the two highest levels (110 and 150 ADF g/kg) , measurements were made on excreta only. Data for excreta are shown for '4 and 6 observations. The two additional levels of ADF were fed in a different experiment when it was realized that the first four levels did not cover the range of fibre contents'of the test diets containing protein supplements. Similar data for lysine, threonine and isoleucine in digesta and excreta of birds on the N-free diets are shown in Figure 5. Changes in content did not always correspond to changes in dietary ADF.

Dry matter digestibility, estimated at the terminal ileum using Cr_2O_3 in digesta, and in excreta, where total collection values were also calculated, are not given here. Comparisons between total collection of excreta and its estimation using Cr_2O_3 were excellent. Except for the diets containing added cellulose (59g/kg) ileal and excreta digestibilities were similar. For the diet containing cellulose, dry matter digestibility declined from 0.88 to 0.74.

DISCUSSION

The justification for the inclusion of protein supplements at 50% of the basal diet was firstly to attempt to accentuate differences in digestibility between diets, and secondly to minimise the influence of endogenous excreta on the apparent digestibility values.

It is clear from the results' in Tables 1 and 2 that the use of ileal contents, taken below Meckel's diverticulum, underestimates the digestibility of several diets for the component under examination. Thus the report by Payne et al.. (1968) that essentially all of the protein disappears from the gut of the fowl at a point two-thirds of the way along the jejunum is for many proteins incorrect. There was a marked increase in the apparent digestibility of nitrogen (14%) and of amino acids (12%) between the ileum (S1) and the terminal ileum (S2). It should be pointed out that these comparisons were made in two separate experiments since it was necessary to collect all digesta from both sites. This would be impossible in a single experiment using the same birds.

For dry matter, digestibility differences between the two sites may be due to components other than those containing N, and clearly starch and some minerals may be absorbed from the lower part of the ileum. Examination of N digestibilities (Table 1) showed that N was the major component explaining differences between the two sites (0.63 vs. 0.75). For all amino acids measured, there was an increase in digestibility between S1 and S2 (Fig. 2), although this difference was not always significant.

The low value for lysine digestibility of 0.48 found at S1 for cottonseed meal is identical to that of Soares and Kifer (1971) using the same technique; In the present study no correction was made for the

TABLE 5 Endogenous Amino Acids (g/100g dry matter intake) obtained here and from published measurements

Amino Acid	Waring (1969)*	Terpstra (1977)*	Sibbald (1979b)**	Okumura et al. (1978)†				Present experiment			
				In-free diet		casein (200g/kg) diet		ileum	terminal ileum	post caeca	excreta
				GF††	CV††	GF	CV				
Lysine	0.04	0.06	21.4	6.7	19.8	28.2	20.8	0.02	0.04	0.06	0.04
Arginine	0.04	0.05	20.6	23.4	22.6	28.5	16.1	0.02	0.02	0.03	0.05
Aspartic Acid	0.09	0.09	30.2	62.2	64.6	77.2	68.4	0.06	0.07	0.10	0.08
Threonine	0.08	0.06	20.8	57.2	51.8	59.7	39.5	0.04	0.05	0.08	0.05
Serine	0.07	0.07	26.6	88.7	55.8	146.5	120.9	0.03	0.03	0.04	0.05
Gluramic Acid	0.11	0.14	48.4	77.8	86.8	235.8	201.2	0.05	0.09	0.14	0.10
Praline	-	0.06	24.4	61.3	42.5	100.5	64.8	0.03	0.04	0.05	0.04
Glycine	0.05	0.06	-	50.5	44.2	33.7	29.9	0.04	0.03	0.05	0.07
Alanine	0.05	0.06	17.3	31.6	38.6	33.4	37.6	0.02	0.03	0.04	0.05
Valine	0.06	0.06	17.0	50.1	48.8	49.2	51.0	0.02	0.03	0.05	0.04
Methionine	0.02	0.02	5.0	5.2	10.3	12.6	11.8	0.01	0.01	0.02	0.02
Isoleucine	0.04	0.04	13.0	35.6	33.2	70.3	59.4	0.02	0.02	0.04	0.03
Leucine	0.06	0.07	25.5	52.8	43.5	45.5	37.5	0.03	0.05	0.07	0.05
Tyrosine	0.03	0.04	15.0	42.8	43.2	32.4	21.4	0.02	0.02	0.03	0.04
Phenylalanine	0.03	0.05	12.2	44.7	41.8	22.3	17.6	0.06	0.03	0.05	0.03

* Analysis from the excreta of birds fed a protein-free diet.

** Analysis from the excreta collected for 24h from unfed birds (mg/bird).

† (g/160g N) - no data on DM digestibility or total collection.

†† Germ free, conventional chicks.

contribution of the basal diet (50%) although this diet contributed only 4.5% crude protein. There is little doubt that differences between amino, acid digestibility among protein supplements can be identified using ileal analysis (see Table 2). Achinewhu and Hewit (1979) showed differences between heat-damaged and untreated soybean protein to be 0.48 and 0.92 respectively. Values may have been even lower at the terminal ileum.

It is clear that further modification occurred to the drv matter in most diets as digesta passed along the large intestine. Thornburn and Wilcox (1965) showed that with mature cockerels caecal digestion occurred, while Payne et al (1968) observed that caecetomised birds on fish meal-based diets gave lower amino acid digestibilities than those with the caeca intact. However in the present study, for many diets there was a decrease in N digestibility between S2 and S3, but not for many of the individual amino acids (Table 2, Fig. 1, 2 and 3) in these same diets. It would appear that digesta post caeca contained additions of non-protein N contributed probably through microbial. fermentation (Okumura *et al.* 1978). This would suggest that deamination of some amino acids (Table 2) has occurred and that ammonia was released and retained in digesta. The extent of the ammonia liberated and its retention in digesta would depend on the pH of digesta.

It would be expected that N digestibility based on excreta would decline due to the contribution of N in urine (Table 1). This would not be expected for individual amino acids since urine contains only trace amounts of these (Bragg et al. 1969). Further increases in the apparent digestibility of several amino acids occurred between S3 and S4 (Table 2, Fig. 1) indicating further microbial activity.

The amino acid content of the N-free diet is shown in Table 5, and the values (mg/100g dry matter) compare well with published data also given in Table 5. In contrast to the changes in N digestibility between S2 and S3 for the protein-containing diets,. no change occurred on this N-free diet (Table 1). Payne et al. (1968) suggested' that endoqenous amino acids may be absorbed from the caeca. Not only is this highly unlikely, but there is no evidence of this occurring from the data presented here. On the other hand dry matter digestibility did increase at S3 suggesting that some fermentation had occurred. Because N digestibility was lower at S3 (0.72) than at S2 (0.75), there was probably removal of amino acids through deamination and absorption of the fermented residues.

Although the correction for endogenous amino acids is relatively unimportant in the present study because of the high daily intake and high inclusion of the protein supplements in the basal diet such a correction is essential when small amounts of diet are fed. Sibbald (1980) was unable to show any effect of increasing amounts of dietary cellulose and sand on endogenous AA output. This study was criticised by Farrell (1981) because of the unlikely possibility of all the sand. reaching the small intestine from a single input of feed.. Our results here would indicate that pure cellulose may not be a useful'source of fibre in adult birds. It behaved in a different manner to that of milled rice hulls. There was an unexplained decline in dry matter digestibility between S2 and S4 from 0.88 to 0.74, 'This large decline was not seen on the diets with rice hulls. But there was little difference in the N digestibility or amino acid contents of excreta from

this diet with added cellulose compared with diets containing similar additions of ADF from hulls. The effects of dietary fibre (ADF) on endogenous excreta are in agreement with the increased output of energy in endogenous excreta of cockerels (Farrell 1981) and increased endogen-, ous amino acid output in pigs (Taverner et al. 1981).

The use of ileal cannulae in adult cockerels is a viable and simple method of determining amino acid digestibility and that of other nutrients in feedstuffs. The birds are easy to maintain provided they are housed individually in suitable cages. The changes that occur in the amino-acid content of digesta during transit through the hind gut, may mask'differences in digestibility when measured in excreta.' This has already been discussed. There is some uncertainty about the ability of Cr_2O_3 to measure digesta output. Where comparisons were . made excreta dry matter output measured by total collection agreed favourably with that estimated using the indicator Cr_2O_3 (Table 4). This would suggest that ileal digesta output can be estimated using this indicator with sufficient precision to qive reliable values for calculating nutrient digestibility at this site.

A comparison was made of N and amino acid digestibilities in the 16 feedstuffs at the terminal ileum and excreta of slaughtered birds, and of birds prepared with simple: cannulae in Table 6. Although there was a significant ($P < 0.01$) relationship for each amino acid between the two methods, there were significant differences ($P < 0.05$) for some values obtained by the two methods both in digesta at the terminal ileum and in excreta. At the terminal ileum differences were observed only for lysine and methionine but not for N. In excreta, amino acid digestibilities were consistently higher for killed than cannulated birds. In the latter group excreta were on trays for 32 h while for killed birds excreta were collected within about 5 h. Differences may also be explained by age of bird and method used. In the slaughter experiment birds were at least 10 weeks old, while birds prepared with cannulae were at least one year old. Perhaps of greater significance is the shedding of material from the mucosal cells in the gut lumen that occurs when birds are killed and gut contents removed (Badawy 1964; Horzczaruk 1971). This would tend to increase the concentration of some amino acids and N in digesta. As a consequence there would be reduced digestibility but this was generally not the case for selected amino acids in Table 6.

Like many biological techniques, both methods used here to estimate amino acid disappearance have their deficiencies and may therefore be criticised. Removal of contents from killed birds, whether these be from the entire ileum or the terminal portion, may result in contamination as mentioned. Time of killing in relation to feeding time could also influence digesta concentration in different areas of the gut. Although frequently used, five hours after feeding may not be the most appropriate killing time of birds given a single meal- For cannulated cockerels, flow of material past the cannula may not be identical to other areas in the gut, and digesta may not therefore be representative of gut contents. The role of Cr_2O_3 as a suitable marker has been discussed.

In order that data can be presented in a form useful to feed formulators, it is essential to distinguish between' those amino acids that disappeared from the entire diet from those in the protein

TABLE 6 Mean (± standard error of difference, SED) of nitrogen and amino acid digestibilities determined in digesta from the terminal ileum (S2) and excreta (S4) of (i) birds killed 5 h post feeding and (ii) birds with ileal cannulae when offered the same diets (m = 13).

Nutrients	Ileum (S2)			Excreta (S4)		
	Killed	Cannula	SED	Killed	Cannula	SED
Nitrogen	0.77^{a*}	0.75^{a}	0.02	0.60^{b}	0.66^{a}	0.02
Lysine	0.83^{a}	0.79^{b}	0.02	0.83^{a}	0.73^{b}	0.03
Threonine	0.73^{a}	0.76^{a}	0.02	0.86^{a}	0.80^{b}	0.02
Methionine	0.84^{a}	0.54^{b}	0.02	0.89^{a}	0.82^{b}	0.02
Isoleucine	0.77^{a}	0.84^{a}	0.07	0.89^{a}	0.80^{b}	0.01
Glycine	0.71^{a}	0.70^{a}	0.02	0.71^{a}	0.60^{b}	0.04

* Values with the same superscripts are not significantly ($P < 0.05$) different

supplement in the diet. Corrected estimates of some amino acids disappearing at the terminal ileum and through the entire gut, using excreta, are given in Table 7 for a six protein supplements. It is apparent that there is a range of values between amino acids and between protein sources at both sites (S2 and S4). Despite the uncertainty of the method of amino acid analysis of excreta to determine amino acid digestibility of feedstuffs, this technique was used to provide data on amino acid digestibilities of a range of feedstuffs by Janssen et al. (1979).

TABLE 7 Apparent digestibility coefficients of lysine, threonine and isoleucine in some protein supplements (corrected for the basal diet contribution) determined at the terminal ileum (S2) and in excreta (S4) of cockerels prepared with simple cannulae.

		Meat meal		Fish meal		Lupin seed meal	Cottonseed meal
		13	16	Safcol	Thai		
Lysine	S_2	0.86^{bA**}	0.70^{dA}	0.83^{cA}	0.93^{aA}	0.92^{aA}	0.66^{eA}
	S_4	0.82^{bB}	0.71^{eA}	0.74^{dB}	0.87^{aB}	0.77^{cB}	0.63^{fB}
Threonine	S_2	0.73^{dB}	0.69^{eA}	0.78^{cB}	0.86^{aB}	0.82^{bB}	0.69^{eB}
	S_4	0.81^{cA}	0.69^{dA}	0.86^{bA}	0.90^{aA}	0.85^{bA}	0.89^{aA}
Isoleucine	S_2	0.84^{bB}	0.75^{dB}	0.85^{bA}	0.94^{aA}	0.92^{aA}	0.78^{cA}
	S_4	0.87^{bA}	0.78^{dA}	0.83^{cA}	0.95^{aA}	0.88^{bB}	0.79^{dA}

* Values within a row with the same superscripts (a-e) are significantly different ($P < 0.05$)

** Values for each amino acid within a column with the same superscript (A-B) are not different ($P < 0.05$)

On the basis of the experiments reported here it would seem that changes occur in the amino acid profile in digesta as it proceeds from the ileum to the anus of the bird. The extent of these changes appear to depend to some extent on the quality of the protein and on the particular amino acid. For example maize gluten, soybean meal and blood meal were readily digested at S1, and the amino acids in these sources were generally highly digestible. For many of the other proteins which were of poor quality including fish meal with crude protein of 446 g/kg (Safcol, S.A.), considerable differences were observed between S1 and S2 and S4. It follows that if large amounts of protein are entering the large intestine there is opportunity for substantial proteolytic activity to occur. A similar conclusion was made by Varnish and Carpenter (1975). The use of a T piece cannula sited at the terminal ileum appears to be 'a reasonable solution to a different problem.

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