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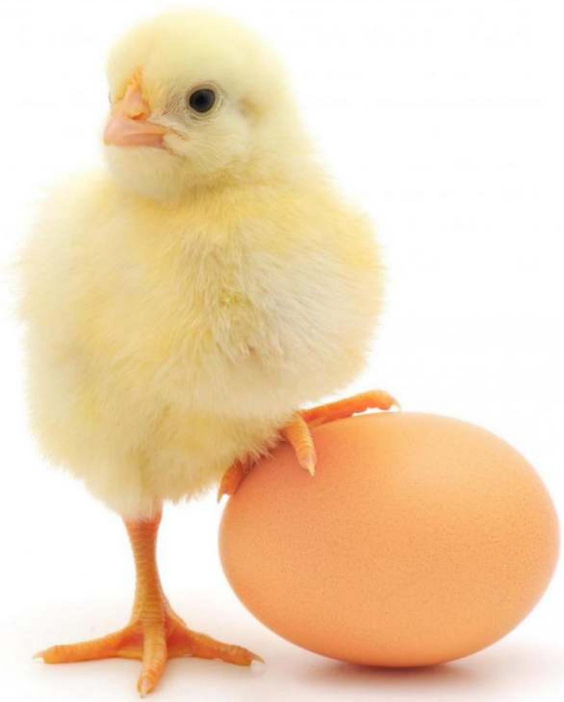

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Ileal Amino Acid Digestibility Assay for the Growing Meat Chicken - Assessment of a New Ileal Amino Acid Digestibility Assay for Broiler Chickens

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Abstract: The aim of the study was to evaluate the accuracy of ileal amino acid digestibility coefficients in dietary formulation for broiler chickens. Two relatively poor quality protein sources, a meat-and-bone meal and a dried blood meal were used. The meals were subjected to the ileal assay and respective mean coefficients of digestibility of 0.58 and 0.67 were found for the first-limiting amino acid methionine+cysteine. Subsequently, each of the meals was the sole source of protein in semi-synthetic maize starch based diets. A casein containing semi-synthetic maize starch based control diet was also formulated. The two test diets and the casein diet contained equal amounts of methionine+cysteine, which was clearly first-limiting. Methionine+cysteine was supplied at 60% of the NRC requirement for the growing bird. Two further semi-synthetic maize starch-based diets were formulated, which contained either the meat-and-bone meal or the blood meal as the main sources of protein, but synthetic methionine was added so that the digestible methionine+cysteine content equaled that in the casein-based control diet. All amino acids other than methionine+cysteine were supplied in moderate excess of the requirements of the growing bird. The experimental diets were fed to broiler chickens in a conventional growth trial. Daily feed intakes were adjusted to achieve comparable feed and metabolizable energy intakes across all the diets. The significantly lower growth performance for birds on the unsupplemented meat-and-bone meal and blood meal diets indicated that the actual digestible methionine+cysteine level was lower in those diets compared to the control. That supplementation with synthetic methionine to give equal amounts of dietary ileal digestible methionine+cysteine restored parity in growth performance is evidence for the accuracy of the new ileal digestibility assay.

Key words: Ileal, amino acid, digestibility, casein, meat, bone meal, broiler

Introduction

Amino acid digestibility is widely accepted as a useful measure to estimate amino acid availability in poultry (Parsons, 1986; Johns *et al.,* 1986). Several methods have been developed with poultry to allow determination of dietary amino acid digestibility (Papadopulos, 1985; Low, 1990, Kadim *et al.,* 2002). These procedures involve the sampling and analysis of either the ileal digesta or excreta. Ileal measurement is preferred to the faecal method as a means of determining amino acid digestibility in chickens because of the possible modifying action of the microflora in the large intestine (Raharjo and Farrell, 1984; Van Weerden, 1989; Whitacre and Tanner, 1989; Kadim *et al.,* 2002). It is considered to provide a satisfactory estimate of protein digestibility because uptake of amino acids takes place before or in the ileum (Webb, 1990).

It is surprising that there has been little work demonstrating the practical application of digestible amino acids in diet formulation. Green (1986) compared broiler diets based on different oil seed meals formulated to equal levels of either total or digestible lysine. The advantage of using digestible amino acids was demonstrated, but there was not identical performance on all the oil seed meals. Similarly, Rostagno *et al.* (1995) and Wang and Parsons (1998) could overcome most of the gap in performance between two broiler diets with high or low digestibility, when synthetic lysine and methionine were added to make up for the difference in digestible lysine and Methionine contents.

A method has been developed, in which ileal digestibility is determined in broiler chickens using a slaughter technique (Kadim and Mouhgan, 1997a,b; Yap *et al.,* 1997; Kadim *et al.,* 2002). The present study was undertaken using broiler chickens to evaluate the accuracy of results from the new assay developed by Kadim and Moughan, 1997a,b) when used in practical dietary formulation. Different broiler diets were formulated to contain equal amounts of methionine+cysteine on a total or digestible basis. Similar growth performance on the iso-digestible methionine+cysteine diets would lend support to the usefulness of the assay.

Table 1: Ingredient contents (%, air-dry basis) of the experimental diets used in the digestibility study

	Diets[1]					
Ingredient	Basal (B)	B+MBM	B+MBM	B+BM	B+BM	B+BM
Soyabean meal	35.00	17.50	17.50	17.50	17.50	17.50
Maize	57.85	28.90	28.90	28.90	28.90	28.90
Meat-and-bone meal	-	20.50	20.50	-	-	-
Blood meal	-	-	-	12.50	12.50	12.50
Soyabean oil	3.00	2.50	2.50	4.00	4.00	4.00
Starch	-	23.10	23.10	26.00	26.00	26.00
Sucrose	-	4.00	4.00	4.80	4.80	4.80
Cellulose	-	0.75	0.75	2.00	2.00	2.00
Potas. Carbonate	-	0.15	0.15	0.20	0.20	0.20
Sodium chloride	0.40	0.35	0.35	0.35	0.35	0.35
Dical. phosphate	2.00	1.00	1.00	2.00	2.00	2.00
Calcium carbonate	1.00	0.50	0.50	1.00	1.00	1.00
Premix	0.45	0.45	0.45	0.45	0.45	0.45
Chromic oxide	0.30	0.30	0.30	0.30	0.30	0.30

[1]MBM:two different processing plant for meat and bone meal products. BM: three different processing plant for blood meal products.

Table 2: Determined gross amino acid compositions (mg/mg dry matter) of the selected meat-and-bone and blood meals

Amino Acid	Meat-and -bone meal	Blood meal
Threonine	1.60	4.65
Serine	1.92	4.04
Glycine	7.40	3.82
Valine	1.99	8.00
Methionine	0.76	1.11
Cysteine	0.28	1.00
methionine+cysteine	1.04	2.11
Isoleucine	1.27	0.90
Leucine	2.94	12.10
Tyrosine	1.01	2.86
Phenylalanine	1.57	6.58
Histidine	0.94	7.22
Lysine	2.17	8.32
Arginine	3.50	3.66

Materials and Methods

Preliminary digestibility study: The digestibility and bioavailability of amino acids in meat and bone meals and blood meals may vary greatly due to different processing conditions. A preliminary trial was therefore conducted using two meat-and-bone meals and three blood meals collected from different processing plants to determine the apparent ileal digestibility of nitrogen. The latter information was used to select a low quality meat-and-bone meal and a low quality blood meal to be used in the subsequent growth study.

Birds and housing: One hundred and forty-four (23-day-old) male and female Ross strain meat chickens were allocated equally and at random to 36 suspended wire cages with 2 males and 2 females per cage. Six diets were allocated randomly to the cages of birds, such that there were six cages each of four birds/diet (24 birds/diet). The birds were housed in an environmentally controlled room (18°C) and received 16 h of light daily and were allowed 7 days acclimatization on a standard commercial broiler diet before assay procedures commenced at 30 days of age. Access to fresh water was *ad-libitum*.

Diets: The ingredient compositions of the six diets are given in Table 1. The soyabean/maize diet was used as a base for formulation of the test diets. The basal diet made up half the dry matter of each test diet. The diets were subjected to cold pelleting (<60°C, 4 mm die-ring). Feeding procedure.

From day 8, the birds were fed their respective experimental diets *ad libitum* for 4 days and were then fasted for 24 hrs. The birds were then allowed to consume the pelleted test diets for a 1 hr period. Subsequently, the birds were sacrificed by an intra-cardial injection of sodium pentobarbitone, 4 hrs after the start of feeding. When the birds were completely immobilized, the body cavity was opened, the ileum removed and digesta were flushed from the ileum (terminal 15 cm, adjacent to the ileo-caecal junction) using distilled water from a syringe. Digesta were removed and pooled by cage group (4 birds/sample) to provide sufficient material for chemical analysis. The material was immediately frozen (-20°C) and subsequently freeze - dried, finely ground and frozen (-20°C) while awaiting chemical analysis.

Chemical analysis: Duplicate determinations of total nitrogen, dry matter and chromium were made on each diet and ileal digesta sample. Nitrogen was determined by the Kjeldahl technique (AOAC, 2000). Chromium determinations were made by atomic absorption spectrophotometry following the method of Costigan and Ellis (1987). Where the differences between duplicates for nitrogen or chromium exceeded 2% of the lower value, samples were reanalyzed. Known amounts of ferrous ammonium sulphate and chromic oxide were included in each nitrogen and chromium run as standards, to check for the complete recovery of nitrogen and chromium.

Table 3: Mean (±SE) determined apparent ileal digestibility values (%) for selected amino acids in a meat-and-bone meal and a blood meal

Amino acids	Meat-and-bone meal	Blood meal
Threonine	53.2±1.02	68.7±2.12
Serine	45.9±1.15	67.8±1.98
Glutamic acid	50.2±0.92	68.8±1.68
Glycine	61.0±1.19	68.2±2.01
Alanine	61.1±0.89	71.3±1.93
Valine	61.7±0.89	67.5±2.23
Methionine	62.6±0.73	72.9±1.64
Cysteine	53.2±1.17	61.9±1.79
methionine+cysteine	57.9±0.95	67.4±1.72
Isoleucine	51.8±0.86	68.0±1.79
Leucine	52.1±0.84	69.8±2.05
Tyrosine	46.8±0.98	68.7±1.85
Phenylalanine	54.6±0.83	70.7±2.00
Histidine	50.0±1.42	69.2±2.45
Lysine	54.9±0.84	73.0±1.88
Arginine	60.4±0.82	64.7±1.67
Mean	54.8	68.7

Amino acid contents were determined in duplicate 5 mg digesta and quadruplicate 5 mg diet samples using a Waters ion-exchange HPLC system, with post-column ninhydrin derivatisation and fluorescence detection, following hydrolysis in 6M glass distilled HCl containing 0.1% phenol for 24 h at 110±2^{o}C in evacuated sealed tubes. For analysis of the sulphur amino acids, methionine+cysteine were oxidized using performic acid to methionine sulphone and cysteic acid respectively.

Digestibility calculation: The flow of nitrogen at the terminal ileum was calculated using the following equation:

$$\frac{\text{Nitrogen concentration in sample X diet chromium}}{\text{digesta chromium}}$$

Apparent ileal nitrogen (N) digestibility was calculated using the following equation:

$$\frac{\text{Dietary N intake - Ileal N flow}}{\text{Dietary N intake}} \times 100$$

For the diets which included the maize/soyabean basal component, the calculated values for apparent N digestibility of the test ingredient were determined using the following formula:

$$Nd_{test} = \frac{(2 \text{ X } DN_{basal+test}) - (DN_{basal})}{(2 \text{ X } TN_{basal+test}) - (Tn_{basal})}$$

Where: ND_{test} = nitrogen digestibility in test protein; $DN_{basal+test}$ = digested nitrogen (g/kg) in basal+test diet; DN_{basal} = digested nitrogen (g/kg) in basal diet; $TN_{basal+test}$ = total nitrogen in basal+test diet; TN_{basal} = total nitrogen in basal diet.

Table 4: Ingredient compositions (%) of four test diets and a casein-based control diet formulated on either a total or digestible amino acid basis

	Daiet[1]				
Ingredient	Casein	MBMT	MBMD	BMT	BMD
Casein	12.00	-	-	-	-
M&BM	-	30.00	30.00	-	-
Blood meal	-	-	-	15.00	15.00
Starch	46.55	30.55	29.65	43.15	43.10
Maize	30.00	30.00	30.00	30.00	30.00
Soyabean Oil	02.00	05.00	05.00	04.00	04.00
Cellulose	03.60	02.00	02.00	02.00	02.00
Potas. Carbonate	00.40	00.40	00.40	00.40	00.40
Sod. chloride	00.60	00.60	00.60	00.60	00.60
Dica. Phosphate	02.00	-	-	02.00	02.00
Cal. carbonate	01.50	-	-	01.50	01.50
Premix	00.40	00.40	00.40	00.40	00.40
Syn. Threonine	00.21	00.17	00.27	-	00.04
Syn. Valine	-	00.11	00.18	-	-
Syn. Isoleucine	00.10	00.27	00.31	00.51	00.44
Syn. Leucine	-	-	00.11	-	-
Syn. Lysine	00.07	00.28	00.38	-	-
Syn. Arginine	00.58	-	00.14	00.44	00.42
Syn. Methionine	-	-	00.13	-	00.10
Syn. Phenylalanine	-	00.06	00.15	-	-
Syn. Tyrosine	-	00.17	00.23	-	-
Syn. Histidine	-	-	00.06	-	-

[1]MBMT: Meat-and-bone meal diet formulated to the same level of total methionine+cysteine as in the casein-based control diet. MBMD: Meat-and-bone meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet. BMT: Blood meal diet formulated to the same level of total methionine+cysteine as in the casein-based control diet. BMD: Blood meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet.

The same formula was used to calculate ileal amino acid digestibility coefficients.

Growth trial: A growth trial was conducted to evaluate the accuracy of the apparent ileal digestibility coefficients for methionine determined in the preliminary trial as predictors of absorbed methionine. Four test diets and a lactic casein-based control diet (two of the test diets contained the same amount of methionine+cysteine as the casein diet on a total basis while the other two contained the same amount of methionine+cysteine as the casein diet on a digestible basis) were fed to broiler chickens and growth performance was recorded.

Birds and housing: One hundred and eighty 15-day-old male and female Ross chickens were allocated equally and at random to 30 suspended cages (62 X 62 X 37cm) with 3 males and 3 females per cage. Five diets were allocated equally and randomly to the cages of birds, such that there were six groups each of six birds for each diet. The birds were housed in an environmentally controlled room (18^{o}C) and received 16 h of light daily and were allowed 5 days acclimatization on a standard commercial broiler diet before the experiment commenced at 20 days of age. Access to fresh water was *ad-libitum*.

Table 5: Chemical compositions[1] (%) of two test diets and a casein-based control diet formulated on a total amino acid basis

	Diet[2]		
Component	Casein	MBMT	BMT
Energy (ME Kcal/Kg)	3373	3327	3371
Protein%	13.0	15.0	12.2
Fat%	3.0	9.2	5.2
Fibre%	4.4	2.8	2.8
Threonine	0.74(1)3	0.74(1)	0.79(1.07)
Serine	0.70	0.70	0.73
Valine	0.82(1)	0.82(1)	1.32(1.6)
Methionine	0.31(.81)	0.25(0.66)	0.22(0.57)
Cysteine	0.12	0.18	0.21
Methionine+cysteine	0.43(0.59)	0.43(0.59)	0.43(0.59)
Isoleucine	0.73(1)	0.73(1)	0.73(1)
Leucine	1.39(1.28)	1.31(1.2)	2.13(1.95)
Phenylalanine	0.71(1.09)	0.65(1)	1.11(1.7)
Tyrosine	0.67	0.57	0.53
Histidine	0.45(1.40)	0.36	1.16
Lysine	1.00(1)	1.00(1)	1.32(1.3)
Arginine	1.10(1)	1.16(1.06)	1.10(1)

[1]Based on tabulated values. Casein protein assumed to be completely digested and absorbed. Tabulated values used to correct ileal digestibility of maize. MBMD: Meat-and-bone meal diet formulated to the same level of digestible methionine+cysteine as in casein-based control diet. BMD: Blood meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet. [3]Express as a proportion of the NRC requirement in digestible units of 0.80.

Table 6: Chemical compositions[1] (%) of two test diets and a casein-based control diet formulated on a digestible amino acid basis

	Diet[2]		
Component	Casein	MBMD	BMD
Energy (ME Kcal/Kg)	3373	3302	3372
Protein%	13.0	15.0	12.2
Fat%	3.0	9.2	5.2
Fibre%	4.4	2.8	2.8
Threonine	0.72(1.22)3	0.59(1)	0.59(1)
Serine	0.68	0.37	0.50
Valine	0.81(1.23)	0.66(1)	0.92(1.4)
Methionine	0.30(1)	0.30(0.98)	0.27(0.88)
Cysteine	0.11	0.12	0.15
Methionine+cysteine	0.41(0.72)	0.41(0.72)	0.41(0.72)
Isoleucine	0.72(1.24)	0.58(1)	0.58(1)
Leucine	1.38(1.58)	0.87(1)	1.57(1.8)
Phenylalanine	0.70(1.34)	0.52(1)	0.38(1)
Tyrosine	0.67	0.46	0.810
Histidine	0.43(1.67)	0.26(1)	0.81(3.1)
Lysine	0.99(1.24)	0.80(1)	0.97(1.2)
Arginine	1.09(1.24)	0.80(1)	0.880(1)

[1]Based on tabulated values. [2]Casein protein assumed to be completely digested and absorbed. Tabulated values used to correct ileal digestibility of maize. MBMD: Meat-and-bone meal diet formulated to the same level of digestible methionine+cysteine as in casein-based control diet. BMD: Blood meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet. [3]Express as a proportion of the NRC requirement in digestible units of 0.80.

Diets: The ingredient compositions of the five experimental diets are given in Table 4. The calculated chemical composition of the experimental diets formulated on a total or digestible amino acid basis are given in Tables 5 and 6, respectively. The diets were formulated to have either casein, meat-and-bone meal or blood meal as the main protein source. Based on mean apparent ileal methionine+cysteine digestibility coefficients for the meat-and-bone meal (0.58) and blood meal (0.67), five experimental diets were formulated: A *casein-based control diet* (assumed amino acids were completely digested and absorbed), a *meat-and-bone meal diet* (MBMT: Formulated diet to the same level of total methionine+cysteine as in the casein diet), a *meat-and-bone meal diet* (MBMD: formulated diet supplemented with DL-methionine to give the same level of digestible methionine+cysteine as in the casein control diet), a *blood meal diet* (BMT:formulated diet to the same level of total methionine+cysteine as in the casein diet) and a *blood meal diet* (BMD: Formulated diet supplemented with DL-methionine to give the same level of digestible methionine+cysteine as in the casein control diet). Maize (30% in each diet) was included in all the diets to improve palatability. Soyabean oil was added to each diet to ensure an adequate supply of essential fatty acids and equal metabolizable energy contents. A mineral / vitamin supplement was included in each diet so that minerals and vitamins were supplied in excess of requirements. Purified cellulose was added to give similar dietary crude fibre contents and similar metabolizable energy contents across all the diets. The diets which were provided in mash form were blended thoroughly in a Hobart mixer for 45 minutes.

The casein diet was first-limiting in methionine+cysteine as compared to NRC requirement for methionine+cysteine (0.60 of the requirement). Such a control diet can be used as a base for comparing bird performance on the meat-and-bone meal or blood meal containing diets. All amino acids other than methionine+cysteine were supplied in moderate excess to the requirements of the growing bird.

Feeding procedure: Feed consumption was a critical factor in the present study. Therefore, special attention was given to ensuring that all groups of birds ate the same amounts of feed. This was achieved by adjusting daily feed intakes based on the observed feed intake of the lowest intake group. The test diets were introduced gradually to the birds from day one (15-d old) to day five to eliminate any reductions in feed intake and, therefore, growth performance of the birds due to a sudden change from one diet to another. On day six, the birds were weighed as groups of six birds and 300 g of each test diet was fed to each group of birds. The next day, the residual feed for each group was weighed and the difference was recorded. The amount of feed given to each group was adjusted based on the lowest feed consumption group. However, if the feed was finished by all the groups of birds, the amount of feed was increased by 10% for the next day. This procedure was adopted daily throughout the trial (14 days) to ensure

Table 7: Broiler feed intakes and growth performance as affected by diet

	Diet[1]					
	Casein	MBMT	MBMD	BMT	BMD	SEM
Growth data, 20-26 days of age						
Feed intake (g)	2258	2290	2305	2260	2259	73
MEI (kcal/kg)5	7617	7618	7611	7618	7618	91
Initial weight (g)	4433	4423	4442	4323	4467	69
Final weight (g)	5350[b]	5142[a]	5358[b]	5035[a]	5400[b]	51
Weight gain (g)	917[b]	718[a]	917[b]	712[a]	933[b]	39
Growth rate (g/d)	131[b]	103[a]	131[b]	102[a]	133[b]	6.5
Feed/gain	2.5[b]	3.2[a]	2.5[b]	3.2[a]	2.4[b]	0.1
Growth data, 27-34 days of age						
Feed intake (g)	3701	3734	3758	3721	3731	73
MEI (kcal/kg)	12485	12421	12410	12543	12581	33
Initial weight (g)	5350[b]	5142[a]	5358[b]	5035[a]	5400[b]	61
Final weight (g)	6842[b]	6208[a]	6817[b]	6175[a]	6883[b]	67
Weight gain (g)	1492[b]	1067[a]	1458[b]	1140[a]	1483[b]	51
Growth rate (g/d)	213[b]	152[a]	208[b]	169[a]	212[b]	12
Feed/gain	2.5[b]	3.5[a]	2.6[b]	3.3[a]	2.5[b]	0.1
Growth data, 20-34 days of age						
Feed intake (g)	5959	6024	6064	5981	5990	73
MEI (kcal/kg)	20102	20039	20021	20162	20199	34
Initial weight (g)	4433	4423	4442	4323	4467	69
Final weight (g)	6842[b]	6208[a]	6817[b]	6175[a]	6883[b]	67
Weight gain (g)	2408[b]	1785[a]	2375[b]	1852[a]	2417[b]	45
Growth rate (g/d)	172[b]	128[a]	170[b]	132[a]	173[b]	09
Feed/gain	2.5[b]	3.38[a]	2.55[b]	3.24[a]	2.48[b]	0.05

[ab]Means in the same row with different superscripts were significantly different (P<0.05). [1]MBMT: Meat-and-bone meal diet formulated to the same level of total methionine+cysteine as in the casein based control diet. MBMD: Meat-and-bone meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet. BMT: Blood meal diet formulated to the same level of total methionine+cysteine as in the casein-based control diet. BMD: Blood meal diet formulated to the same level of digestible methionine+cysteine as in the casein-based control diet.

that similar amounts of feed were eaten across all groups of birds. Feed consumption was recorded daily, while the weight of birds and feed conversion data were recorded every second day during the experimental period.

Data analysis: The data were subjected to analysis of variance (SAS, 1993) to compare the effect of the five treatments on the performance of the broiler chickens. Statistical significance of differences among treatment means were assessed using the least significant difference procedure.

Results

Mean apparent ileal nitrogen digestibility values for the two sources of meat-and-bone meal were 50.1% and 60.2% and for the three sources of blood meal were 71.7%, 93.3% and 61.4%. Because the objective of the growth study was to evaluate the accuracy of apparent ileal amino acid digestibility coefficients and as large a difference in digestibility as possible was desired, the two lowest digestibility meals were selected to be used in the growth study. The amino acid contents and the apparent ileal digestibility values for selected amino acids for the two lowest quality protein sources were determined and are shown in Tables 5 and 6, respectively. The overall mean apparent ileal amino acid digestibility coefficients for the low digestibility meat-and-bone and blood meals were 0.55 and 0.69, respectively. The experimental diets for the growth study were formulated based on these determined digestibility values.

Feed consumption, body weight and feed conversion data for the broilers given the experimental diets are given in Table 7. During the first 24-h period, the average feed consumption by birds for each group were 297, 300, 278, 284 and 260 g for casein, MBMT, MBMD, BMT and BMD, respectively. The next day, 260 g of each experimental diet was fed to each group and all groups managed to consume all their feed. On the third day, 286 g of the respective diets were consumed by all groups. As a result of this adjustment procedure, feed intake was similar among the five groups of birds (Table 7). As expected, performance was significantly influenced by diet. The lower amount of digestible methionine+ cysteine in the MBM-based and BM-based diets resulted in lower bird performance. Adding DL-methionine to the two test diets to give equal digestible methionine+cysteine to that in the control diet restored performance to that of birds on the casein control diet. The same trend was found in each period of the study (20-26 and 27-34 d).

Discussion

Limiting methionine+cysteine to 60% of the NRC (1994) recommendation resulted in crude protein concentrations in the experimental diets (Table 3) lower than those normally used in commercial practice. Nevertheless, the birds grew rapidly and had normal feed intakes. The validity of the approach adopted in this study is reliant upon the assumptions that methionine+cysteine was the first-limiting amino acid in the MBM-based and BM-based diets on a total basis. Amino acids from the casein were completely digested and absorbed. All birds received equal levels of digestible energy. Minerals, vitamins and essential fatty acids were supplied in excess of the bird's requirements. The first assumption was tested here by determining bird performance after supplementing either the MBM-based or BM-based diets with synthetic methionine. A distinct and significant improvement in growth and feed efficiency was found upon addition of the methionine, demonstrating that methionine+cysteine was first limiting.

The methionine-cysteine relationship is significant if there is a cysteine deficiency in the diet, because of the conversion of methionine to cysteine and the difference in molecular weight between methionine (149) and cysteine (240) (Creek, 1968). In the present study, cysteine and methionine levels were similar and the percentage of both amino acids in the diets were considerably below the bird's requirements. Therefore, it is probable that there is no conversion from methionine to cysteine in this case and the contribution of both methionine and cysteine to methionine+cysteine deficiency across the diets was equal.

Evidence for the complete digestion of casein protein and absorption of the constituent amino acids has been documented for the pig (Kies *et al.,* 1985), for the pig and rat (Rutherfurd and Moughan, 2007, personal communication) and for the chicken (NRC, 1994). The metabolizable energy contents of the test diets used in the present study were similar and adjustments to the daily levels of feed intake were made, to ensure equal intakes of digestible energy across all diets. Finally, the chickens estimated intakes of minerals and vitamins and amino acids other than methionine+cysteine were in excess of estimates (NRC, 1994) of requirements.

In accordance with the above experimental conditions being met, the similar growth rates of chickens fed equal amounts of the casein-based, MBM-based and BM-based diets on a digestible basis indicate that similar amounts of methionine+cysteine were digested and absorbed and that the newly developed ileal digestibility assay accurately describes digestibility at least for the amino acid methionine. These results support the findings of (Green, 1986; Fernadez, *et al.,* 1995; Douglas and Parsons, 1999; Wang and Parsons, 1998), who formulated diets to equal levels of either total or digestible lysine. He found that diet formulation based on total lysine resulted in poorer growth and feed efficiency than when diets were formulated on digestible lysine. Similarly, Rostagno *et al.* (1995) demonstrated that adding methionine and lysine to the low digestibility diet to overcome the difference in digestible methionine and lysine essentially equalized performance with the control diet.

The present findings show, that at least for the ingredients examined, apparent ileal amino acid digestibility should be useful in dietary formulation for indicating the levels of dietary amino acids available for broiler growth. They also suggest that apparent ileal digestibility coefficients are accurate measures of the degree of amino acid digestion and absorption in the growing chicken. A negative growth response occurred in broilers when diets were formulated on a total amino acid basis. Similarly, Douglas and Parsons (1999) and Wang and Parsons (1998) found that weight gain and feed efficiency of chicks fed diet formulated on a total amino acid were significantly ($P<0.05$) less than those based on digestible amino acids. Added DL-methionine could compensate for the difference in digestible methionine+cysteine content of the diets. A similar conclusion was stated by Fernandez *et al.* (1995) and Rostagno *et al.* (1995).

Conclusion: Formulating broiler diets based on an apparent ileal digestible amino acid basis using the new ileal digestibility assay gives a better prediction of dietary protein quality and bird performance than using total amino acids.

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