

# Nutritive Value of the Meat and Bone Meals from Cattle or Pigs in Broiler Diets

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**ABSTRACT** The nutritive value of meat and bone meals (MBM) was assessed for broilers. The MBM was produced according to the revised (pressure) processing system ordered by the European Union (EC 96/449). Three batches of MBM from cattle (MBM<sub>cattle</sub>) and three from pigs (MBM<sub>pig</sub>) with different ash contents (224, 306, 387, and 209, 293, 430 g/kg, respectively) were tested for digestibility at a 10% inclusion level. The MBM<sub>cattle</sub> and MBM<sub>pig</sub> with the lowest ash (224 and 209 g/kg, respectively) were tested also at 20% inclusion. A basal diet (corn-soybean meal) was used as a control. Two-week-old broiler chickens were used in four replicates per treatment (14 to 32 d of age). The AME<sub>n</sub> of MBM was high (10.51 to 13.04 MJ/kg DM). Species origin had no significant effect, whereas

more ash and a higher inclusion level decreased the AME<sub>n</sub>. The factors investigated showed no significant effect on the excretal digestibility of CP or on total AA. Excretal digestibility of total amino acids (AA) ranged from 60 to 65%. The ileal digestibility of CP and AA of MBM<sub>pig</sub> with 209 g/kg ash was also tested at 10 and 20% inclusion. Excretal digestibility was significantly higher than ileal digestibility of CP (63.8 and 55.8%, respectively) and total AA (60.9 and 56.2%, respectively). The 20% inclusion level resulted in a lower digestibility for both methods. The digestibility of CP was measured by four different in vitro techniques, based on pepsin digestibility. The data showed a large variation and did not correlate at all with the in vivo digestibility values.

(Key words: meat and bone meal, amino acid, excretal and ileal digestibility, metabolizable energy, broiler)

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## INTRODUCTION

Large quantities of by-products from animal origin (i.e., from abattoirs, renderers industry) are utilized as a well-appreciated feedstuff in livestock production. However, due to the risk of bovine spongiform encephalitis contamination, the processing conditions have been re-evaluated in the European Union, and the agreed regulations have been published. The processing procedure for mammalian animal waste was revised by EC (96/449): maximum particle size, 50 mm; temperature, 133 C; pressure, 3 bar by steam for 20 min. These modifications may affect the quality of meat and bone meals (MBM) but primarily the protein and energy values. As the MBM are widely used in pig and poultry nutrition, it is necessary to investigate the effects of changed processing on the nutritive value.

The ME content and the nutrient digestibility of MBM are affected by many factors, for example, the origin and

the processing of the product, the level of feeding, or the method of digestibility measurement (Johns et al., 1986a; Ravindran and Bryden, 1999). Two of these factors have been studied in more detail. The processing treatment and its conditions, such as the temperature, the pressure, and the period of maintaining these conditions may have substantial effects.

A higher processing temperature (110 vs. 140 C) with a moderate time of 15 to 20 min in a cooker generally yielded in a significantly lower amino acid (AA) digestibility and also had a negative effect on TME<sub>n</sub> content of the MBM (Wang and Parsons, 1998). Also the length of processing time (15 to 20 min vs. 180 to 240 min) indicated at least a partial reduction of AA digestibility with the longer processing time.

The other important factor may be the source of the raw material, i.e., the species origin and the ash content. Johnson and Parsons (1997) found that the protein efficiency ratio of poultry by-product meals was generally higher than of lamb meals, whereas this ratio of the latter was higher than in most of the MBM originating from

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**Abbreviation Key:** AA = amino acid; MBM = meat and bone meal; MBM<sub>cattle</sub> = MBM originating from cattle; MBM<sub>pig</sub> = MBM originating from pigs.

beef or pork as observed in trials with broiler chickens. On the other hand, Johnson et al. (1998) did not find any consistent effect of species origin on AA digestibility in roosters. Concerning the ash content, Johnson et al. (1998) reported no consistent differences in AA digestibility between MBM containing 24 or 34% ash, or poultry co-product meals containing 7 or 16% ash in roosters.

The inclusion level also may have an important impact on the nutrient content of MBM. The  $ME_n$  content of MBM was significantly lower at 40% inclusion than at 20% (Martosiswoyo and Jensen, 1988), whereas Dolz and De Blas (1992) showed a decrease of the average  $AME_n$  content of MBM by 5.5% at increased inclusion levels from 6 to 12%, but the differences were not significant ( $P > 0.05$ ). Esteve-Garcia et al. (1994) reported a lower ME content in the MBM at a higher level of inclusion from 5 or 10% to 20%.

In addition to these factors, the digestibility procedure is an important factor too. In poultry, complete separation of feces and urine is rather difficult, and so assessing N digestibility of droppings may be biased. At present, the excretal digestibility (also termed fecal digestibility by many authors) and the ileal digestibility are most commonly used with broiler chickens. Amino acid digestibility assays in poultry should be based on the analysis of digesta from the terminal ileum rather than excreta, because of the variable and modifying effects by hindgut microflora (Ravindran and Bryden, 1999).

On the basis of the European Union requirements, the subsequent changes in the processing conditions, and the lack of knowledge on the effect of species origin on the nutritive value, the European Renderers Association<sup>2</sup> asked for an evaluation of MBM. In addition, they wanted to quantify variation in nutritive value due to variation in chemical parameters, predominantly ash content, because the latter is mostly a specification of the products offered for animal nutrition. Therefore, this experiment was planned to elucidate four questions:

1. Is there a difference in nutritive value of MBM from cattle ( $MBM_{cattle}$ ) and from pigs ( $MBM_{pig}$ ) (i.e. species origin)?
2. Is there an effect of increasing ash content of MBM on its nutritive value?
3. Is there an effect of the level of inclusion of MBM in the diet on its nutritive value?
4. Is there an effect of inclusion level of MBM on the difference between ileal and excretal digestibility?

## MATERIALS AND METHODS

### MBM

Six batches of MBM were examined, three MBM originating from cattle ( $MBM_{cattle}$ ) and three from pigs ( $MBM_{pig}$ ), each with ash contents of 200, 300, and 400 g/

kg. The raw MBM materials were treated separately in the wet pressing process by DAKA.<sup>3</sup> In the process, the minced raw materials—maximum size of 19 mm—were heated to 85 to 90 C. Because a lot of water and fat was retained in the coagulated material, it was mechanically separated into a press cake and a press liquid. The former product was heated and dried for 4 h at a temperature of at least 110 C. Thereafter, the press cake was sterilized for 20 min at 133 C under 3 bar of pressure. Subsequently, the product was dried and ground through a 4-mm sieve to enhance the uniformity of the products.

### Animals and Housing

The experiment was performed with 360 2-wk-old female broiler chickens (Ross 208) that were housed in three-tier battery cages (10 broilers per cage of 0.45 m<sup>2</sup>). The light was supplied by an alternate light regimen until 21 d of age, in which 1 h of light was alternated with 3 h of darkness. Thereafter, continuous light (24 h daily) was given to establish a regular eating pattern. The environmental temperature was 26 C in the first week of experimental period and decreased by 2 C each following week. The relative air humidity was maintained at a minimum of 55%.

### Diets and Feeding

Nine treatments were applied using a basal diet and the six batches of MBM. The bulk of the basal diet was maize (50%), extracted soybean meal (26%), heat-treated soybean (7.8%), tapioca meal (7.9%), and soybean oil (4.5%); the minor parts were synthetic AA, minerals, trace minerals, and vitamins. All MBM batches were tested at 10% inclusion (six treatments). Furthermore,  $MBM_{cattle}$  with 224 g/kg ash and  $MBM_{pig}$  with 209 g/kg ash were also tested at 20% inclusion (two treatments). The experimental diets are presented in Table 1.

In the experimental diets, part of the basal diet was replaced with MBM by formulation but still met the animal nutrient requirements (CVB, 1999b). Formulation was used to decrease the Ca, P, and NaCl supplementation in the minor part of the basal diet, because of the high concentrations in MBM. The supplementation with synthetic AA was decreased to achieve a better balance of AA supply to meet at least the CVB standards in the Netherlands (CVB, 1999b). Vitamins and trace minerals supplementation was 0.5% in all diets. This procedure resulted in a replacement of the basal diet by MBM for approximately 7% for the bulk and 3% for the minor parts at 10% inclusion. Figures for 20% inclusion were 17 and 3%, respectively. An inert marker ( $Cr_2O_3$ ) was added to the basal diet (Diet 1) and Diets 5 and 9 to determine ileal digestibility from representative samples of ileal chyme. The chemical composition and major nutrient contents of the MBM and the basal diet are presented in Table 2. Feed and water were supplied ad libitum during the whole experimental period. The basal diet and all experimental diets were fed as pellets ( $\varnothing$  2.5 mm).

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<sup>3</sup>DAKA, a.m.b.a., P.O. Box 51, DK-8723 Løsning, Denmark.

TABLE 1. Experimental diets: two types of animal species origin, three types of ash content, and two levels of inclusion

Diet no.	Basal diet (%)	Meat and bone meal			Digestibility method	
		Inclusion (%)	Origin	Ash (g/kg)	Excretal	Ileal
1	100	–	–	–	+	+
2	90	10	Cattle	200	+	–
3	90	10	Cattle	300	+	–
4	90	10	Cattle	400	+	–
5	90	10	Pigs	200	+	+
6	90	10	Pigs	300	+	–
7	90	10	Pigs	400	+	–
8	80	20	Cattle	200	+	–
9	80	20	Pigs	200	+	+

The experiment was carried out according to a randomized block design. The nine experimental treatments were distributed randomly per block over the experimental units. Blocks were broilers of similar live weights at the start of the measurements.

### Experimental Procedure

The nine experimental diets were fed to the broilers between 14 to 32 d of age, after a pre-experimental period of 14 d on a standard broiler diet. The AME<sub>n</sub> content and the excretal digestibility of organic matter, crude fat, CP (= N × 6.25), and individual AA were measured in all diets

(Diets 1 to 9). Droppings were collected quantitatively on trays placed under each cage for 4 d, from Days 25 to 29 once every 4 h during daytime (0800 to 1600 h) as an analytical sample and once during the nighttime (1600 to 0800 h) to calculate dry matter excreta production. The correction for urinary excretion in the droppings is given below in the calculation section.

The ileal digestibilities of CP and AA were determined in the basal diet as well as in the diets with MBM<sub>pig</sub> with 209 g/kg ash at 10 and 20% inclusion, respectively. The ileal chyme samples were collected at 32 d of age. The broilers were killed by an intravenous injection of T61 into the wing vein. After the abdomen and chest cavities

TABLE 2. Analyzed chemical composition and amino acid content of meat and bone meals and of the basal diet (g/kg product)<sup>1</sup>

Component	Meat and bone meal (origin; ash content, g/kg)						Basal diet
	Cattle, ash 200	Cattle, ash 300	Cattle, ash 400	Pigs, ash 200	Pigs, ash 300	Pigs, ash 400	
Dry matter	957	959	961	978	984	983	881
Ash	224	306	387	209	293	430	54
CP	592	530	468	669	586	444	203
Crude fat	116	102	87	112	112	107	89.9
Ca	61	95	128	53	98	151	7.1
P	27	45	64	29	50	80	6.1
K	6.8	5.6	4.5	9.9	5.7	4.2	...
Na	8.8	8.6	8.3	13.6	9.2	8.5	...
Cl	10.0	8.4	6.9	15.7	9.0	6.6	...
Amino acid (AA)							
Lysine	31.2	26.8	22.4	31.2	28.0	19.9	12.0
Methionine	9.2	7.6	6.0	7.1	7.4	5.0	5.0
Cystine	5.1	4.8	4.4	4.6	3.4	2.2	3.6
Threonine	20.2	17.4	14.6	19.9	16.8	11.6	8.6
Tryptophan	4.3	3.4	2.5	2.8	2.7	1.6	2.2
Isoleucine	19.5	16.5	13.4	17.8	15.2	10.3	8.7
Arginine	35.0	32.6	30.1	41.1	38.1	30.8	13.4
Phenylalanine	20.9	18.0	15.0	19.3	17.8	12.8	10.0
Histidine	13.3	11.0	8.6	11.4	9.9	6.4	5.2
Leucine	38.2	32.8	27.4	37.7	31.9	22.3	17.1
Tyrosine	15.9	13.5	11.1	14.7	13.0	8.6	7.8
Valine	24.7	21.9	19.0	25.5	21.2	15.3	10.5
Alanine	35.3	33.4	31.5	41.8	36.6	30.2	10.0
Aspartic acid	43.1	37.9	32.7	45.1	39.0	28.6	20.5
Glutamic acid	63.7	57.5	51.2	74.5	61.4	47.0	34.9
Glycine	46.8	46.9	46.9	64.3	53.6	47.2	8.4
Proline	39.9	37.9	35.8	47.9	49.6	37.7	11.4
Serine	21.4	19.4	17.4	28.2	21.0	15.9	10.4
Sum of AA	488	439	390	535	467	353	200

<sup>1</sup>Analyses were made by ID-Lelystad, except for Na, K, and Cl, which were analyzed by Cehave, Veghel, The Netherlands.

were opened, the chyme was taken from the last 20 cm preceding 1 cm proximal to the ileo-cecal junction (Van der Klis, 1993). The excreta and chyme samples were frozen, freeze-dried, and ground (1-mm sieve) for chemical analysis.

The broilers were weighed at the start, at Day 14, and at 29 d of age. Furthermore, feed intake was measured from 14 to 25 d of age and from 25 to 29 d of age; water intake was measured from 22 to 26 d of age.

### Calculations

The digestibility coefficients for the MBM were calculated by difference based on the analyzed values in the complete diets. The excretal protein digestibility was corrected for urinary N excretion (uric acid, urea, and  $\text{NH}_3$ ). The organic matter digestibility was corrected for urinary N ( $3.41 \times$  total N in urine), according to Terpstra and Bisalsky (1976). Furthermore, the synthetic AA supplementation was taken into consideration for the calculations, assuming a digestibility of 100% for these AA. The AME content was corrected to nitrogen equilibrium.

Comparison of ileal and excretal digestibility was performed on total diet basis. Water intake was calculated as the ratio between water and feed intake (g/g).

### Chemical Analysis

The chemical analysis of the batches of  $\text{MBM}_{\text{cattle}}$  and  $\text{MBM}_{\text{pig}}$ , the nine experimental diets, and the chyme and excretal samples were performed at the laboratory of ID-Lelystad.<sup>4</sup> Dry matter and ash contents were determined according to the Association of Official Analytical Chemists (1984). Nitrogen was assayed by the Dumas method (Association of Official Analytical Chemists, 1984). Crude fat was determined by the Berntrop method (ISO 6492). Gross energy content was determined in an adiabatic bomb calorimeter (ISO 9831). The AA, except for methionine, cystine, and tryptophan, were assayed using ion-exchange column chromatography after hydrolysis for 23 h in HCl (6 M). Cystine and methionine were determined as cysteic acid and methionine-sulfone after oxidation with performic acid prior to hydrolysis (Schram et al., 1954). Tryptophan was determined according to Sato et al. (1984). Ca, K, and Na were analyzed by atomic absorption spectrophotometry after drying ash at 550 C for 4 h (Association of Official Analytical Chemists, 1984). Total P was determined using the vanado-molybdate procedure (Association of Official Analytical Chemists, 1984). Chromium was assayed according to Williams et al. (1962). The K, Na, and Cl contents in the MBM were analyzed

by Cehave.<sup>5</sup> Chloride was assayed by the potentiometric method. Salamon & Seaber Ltd.<sup>6</sup> also analyzed CP and AA of the six batches of MBM. The in vitro CP digestibility (pepsin-HCl) of the MBM was analyzed by LABCO B.V.<sup>7</sup> according to EC method (1972), by Saria Industries,<sup>8</sup> by Cehave (EC method, 1972), and according to Babinszky et al. (1990).

### Statistical Analysis

The experimental data were subjected to the analysis of variance according to a randomized (complete) block design using the GENSTAT-5 software (Payne et al., 1993).

For additional analyses and easier interpretation of the treatment effects, they were split into the following seven first-degree of freedom contrasts: usual main factorial effects of the origin of MBM (O;  $\text{MBM}_{\text{cattle}}$  vs.  $\text{MBM}_{\text{pig}}$ ), the inclusion level (I; 10 vs. 20%), and their interaction ( $\text{O} \times \text{I}$ ); orthogonal linear (A(lin)) and quadratic (A(quad)) components of the ash content (200, 300, and 400), and their interactions with the origin of MBM ( $\text{O} \times \text{A}(\text{lin})$ ,  $\text{O} \times \text{A}(\text{quad})$ ). The last four contrasts were tested only at 10% inclusion. Therefore, the final model for nutritive value of MBM and performance of broilers was

$$Y_{ijk} = F + \text{block}_i + \text{O}_j + \text{I}_k + \text{O} \times \text{I} + \text{A}(\text{lin}) + \text{A}(\text{quad}) + \text{O} \times \text{A}(\text{lin}) + \text{O} \times \text{A}(\text{quad}) + e_{ijk} \quad [1]$$

The analysis of data on excretal and ileal digestibilities was also done with the ANOVA procedure of GENSTAT-5 software by using the following usual factorial model:

$$Y_{ikl} = F + \text{block}_i + \text{In}_k + \text{M}_l + (\text{In} \times \text{M})_{kl} + e_{ikl} \quad [2]$$

where  $Y_{ikl}$  = digestibility response to CP and total AA,  $\text{block}_i$  = replicate ( $i = 1 \dots 4$ ),  $\text{In}_k$  = main effect of inclusion levels (0, 10, and 20%;  $k = 1 \dots 3$ ),  $\text{M}_l$  = main effect of method of digestibility measurements (excretal and ileal;  $l = 1, 2$ ), and  $(\text{In} \times \text{M})_{kl}$  = interaction between inclusion level and method of digestibility.

Additionally, significant differences between treatment means were tested with the Student's *t*-test. The above-defined contrasts, factorial effects, and differences between treatment means were declared significant at  $P < 0.05$ .

## RESULTS

### Chemical Analysis

**MBM.** The nutrient contents of the batches of  $\text{MBM}_{\text{cattle}}$  and  $\text{MBM}_{\text{pig}}$  are summarized in Table 2. The ash content of MBM agreed well with the expected values. The CP content of the MBM with the same ash content was higher from pigs than from cattle except for 400 g/kg ash. The  $\text{MBM}_{\text{pig}}$  with 200 g/kg ash had the highest CP content (669 g/kg). The crude fat content was nearly the same in all MBM, except for the  $\text{MBM}_{\text{cattle}}$  with 400 g/kg ash,

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<sup>5</sup>Cehave, P.O. Box 107, NL-5460 AC Veghel, The Netherlands.

<sup>6</sup>Salamon & Seaber Ltd., London, England, U.K.

<sup>7</sup>LABCO BV, Laboratory Services, Elbeweg 141, NL-3198 LC Europort, Rotterdam, The Netherlands.

<sup>8</sup>Saria Industries, P.O. Box 230, 93523, Saint-Denis Cedex, France.

being 87 g/kg. The Ca and P contents of the MBM agreed well with their ash content. The AA content was similar for both the laboratories of ID-Lelystad and Salamon & Seaber, Ltd. The sum of N of all AA represented 80 to 83% of total N of the MBM. This value was lower than in the Dutch Feedstuff Table (CVB, 1999a) (approximately 93.0%). The difference in total AA and CP of MBM was less than 50 g/kg in an experiment by Ravindran et al. (1999). Just et al. (1982) found differences that, on average, were 17.3% for MBM from different rendering plants, but the CP content agreed very well with the total AA content in a study conducted by Yamazaki and Kamata (1986). The reason for this large difference is not clear, but non-protein nitrogen proportion is responsible for at least a part of it. A part was identified as ammonia-N (4.6 to 5.8% of total N), which is no more than one-quarter or one-third. Also hydroxyproline was not analyzed. The latter can be a substantial proportion in the collagen tissue in bones (Kling and Wöhlbier, 1977).

The analyzed chemical composition of the complete diets showed that the mixing of the diets had been successful, because the differences were small compared to the expected values.

### **Performance of Broilers**

Although not designed as a performance trial, the feed intake and feed conversion of the broilers were recorded during the experimental period. Compared with practice, the broiler chickens showed good performance, and only three birds died during the experimental period. Feed intake from 14 to 29 d of age ranged from 87 to 92 g/d. Water intake ranged from 180 to 227 g/d. Average daily gain was between 64 and 70 g. Due to the balancing of the experimental diets with minerals and supplementary AA, all diets met the CVB standards. Because this trial was not full performance, with only 2 wk of measurement, no statistical analyses would be appropriate and firm conclusions could not be drawn.

### **Nutritive Value of the Complete Experimental Diets**

The AME<sub>n</sub> content of the complete diets was rather high and ranged from 13.8 to 14.6 MJ/kg DM (Table 3). Obviously, it was higher in the diets with MBM with 200 or 300 g/kg ash at 10% inclusion than in the other diets. Excretal digestibility of CP ranged from 75.0 to 85.0% but was not significantly affected by species origin of MBM. The excretal digestibility of CP in the complete diets with the higher inclusion level was lower than in the other diets. If the digestibility of MBM at 10% inclusion was used, by assuming no effect of a higher inclusion level, the calculated values for the digestibility coefficients of CP for Diets 8 and 9 were 75.4 and 74.4, respectively. This digestibility value was 0.4 and -0.9 units higher than the measured value, respectively. This comparison does not indicate any significant reducing effect of the higher inclusion level on the digestibility of protein of MBM.

Excretal digestibility of CP was lower than of total AA (Table 4). Excretal digestibility of total AA in the diets ranged from 76.2 to 87.7% with a low SD. A significantly higher digestibility of CP and total AA was obtained for excretal compared to ileal digestibility (Table 4) and a higher inclusion level of MBM<sub>pig</sub> in the diets significantly ( $P < 0.05$ ) decreased these values. A similar tendency was found for most individual AA, but for lysine, histidine, alanine and glycine these results were not affected by the method of measurement (results not shown). Cystine digestibility values for MBM were considered not reliable (Table 5) due to a large variation within diets between blocks and between diets, probably caused by the low concentration of cystine and the low proportion of cystine from MBM in the total diet.

### **Nutritive Value of the MBM**

The AME<sub>n</sub> content of the MBM ranged from 2,511 to 3,115 kcal/kg (10.51 to 13.04 MJ/kg) DM (Table 6). The AME<sub>n</sub> content in the MBM<sub>pig</sub> with 200 and 300 g/kg ash was significantly higher ( $P < 0.05$ ) than with an ash content of 400 g/kg, whereas species origin had no effect (Table 6). The AME<sub>n</sub> values of the six batches MBM were positively and linearly correlated with the protein content in these MBM ( $r = 0.9$ ). In the Diets 2, 5, 8, and 9 mean AME<sub>n</sub> for MBM<sub>pig</sub> were significantly higher than for MBM<sub>cattle</sub> [2,917 vs. 2,742 kcal/kg (12.21 vs. 11.48 MJ/kg) DM]. AME<sub>n</sub> was significantly better at inclusion of 10% than 20% in the diet [2,986 vs. 2,673 kcal/kg (12.50 vs. 11.19 MJ/kg) DM]. The organic matter digestibility was significantly better in MBM<sub>pig</sub> than MBM<sub>cattle</sub> (71.2 vs. 66.3%). Inclusion level had a significant effect on the organic matter digestibility.

The excreta digestibility of CP of the MBM was significantly affected by inclusion level. The results of the crude fat digestibility were statistically not evaluated because the contribution (less than 12%) of this nutrient was too small from MBM in the experimental diets. The ileal digestibility of AA in the MBM<sub>pig</sub> with 209 g/kg ash at 10 and 20% inclusion in the diet is shown in Table 5. Statistical evaluation showed that for only methionine was a significant interaction found between the digestibility method and the inclusion level. Although the digestibility of most individual AA was higher at the excreta level than at ileal, only the coefficients for threonine, arginine, aspartic acid, glutamic acid, glycine, proline, and serine were significantly higher. At 20% inclusion of MBM<sub>pig</sub>, the digestibilities of methionine, histidine, and proline were significantly lower, whereas in the case of tyrosine it was higher ( $P < 0.05$ ).

### **Evaluation of In Vitro Digestible CP analysis**

There was a large variation among laboratories concerning in vitro methods both in digestibility and ranking of the MBM. All in vitro methods showed a higher digest-

**TABLE 3. The AME<sub>n</sub> content (MJ/kg DM) and excretal digestibility (%) of organic matter, CP, total amino acids, and crude fat of the diets<sup>1</sup>**

Diet, basal or MBM inclusion	Energy/nutrient					
	AME <sub>n</sub>		Organic matter	Crude protein	Total AA	Crude fat
	kcal/kg	MJ/kg DM				
Diet 1, basal diet	3,488 <sup>a</sup>	14.60 <sup>a</sup>	78.0 <sup>a</sup>	85.0 <sup>a</sup>	87.7 <sup>a</sup>	92.0 <sup>a</sup>
MBM						
Diet 2: cattle, 10% ash 200	3,421 <sup>bc</sup>	14.32 <sup>bc</sup>	76.5 <sup>d</sup>	79.9 <sup>bc</sup>	82.0 <sup>bc</sup>	90.6 <sup>bc</sup>
Diet 3: cattle, 10% ash 300	3,430 <sup>b</sup>	14.36 <sup>b</sup>	76.9 <sup>cd</sup>	80.1 <sup>bc</sup>	82.6 <sup>b</sup>	90.9 <sup>abc</sup>
Diet 4: cattle, 10% ash 400	3,399 <sup>cd</sup>	14.23 <sup>cd</sup>	77.1 <sup>bcd</sup>	80.7 <sup>bc</sup>	82.5 <sup>b</sup>	90.5 <sup>c</sup>
Diet 5: pigs, 10% ash 200	3,447 <sup>b</sup>	14.43 <sup>b</sup>	76.6 <sup>d</sup>	79.6 <sup>c</sup>	81.3 <sup>c</sup>	90.2 <sup>cd</sup>
Diet 6: pigs, 10% ash 300	3,438 <sup>b</sup>	14.39 <sup>b</sup>	77.7 <sup>ab</sup>	81.2 <sup>b</sup>	82.7 <sup>b</sup>	91.7 <sup>ab</sup>
Diet 7: pigs, 10% ash 400	3,380 <sup>d</sup>	14.15 <sup>d</sup>	77.5 <sup>abc</sup>	81.2 <sup>b</sup>	82.8 <sup>b</sup>	89.3 <sup>d</sup>
Diet 8: cattle, 20% ash 200	3,304 <sup>e</sup>	13.83 <sup>e</sup>	75.1 <sup>e</sup>	75.0 <sup>d</sup>	77.8 <sup>d</sup>	89.2 <sup>d</sup>
Diet 9: pigs, 20% ash 200	3,321 <sup>e</sup>	13.90 <sup>e</sup>	75.7 <sup>e</sup>	75.3 <sup>d</sup>	76.2 <sup>e</sup>	90.2 <sup>cd</sup>
SED		0.057	0.32	0.62	0.55	0.55
F probability		<0.001	<0.001	<0.001	<0.001	<0.001

<sup>a-e</sup>Means with different superscripts within a column differ significantly (*P* < 0.05).

<sup>1</sup>AA = amino acid; MBM = meat and bone meal; SED = standard error of the difference.

ibility of CP in the MBM<sub>pigs</sub> independent of the ash content (Table 7).

**DISCUSSION**

**AME<sub>n</sub> Content in MBM**

Comparison of the energy values of MBM in the literature and feed tables is difficult because apparent (AME) or true metabolizable energy (TME) values are reported. Generally only intact or cecectomized adult cockerels are used for the determination of AME<sub>n</sub> or TME<sub>n</sub> values (Martosiswoyo and Jensen, 1988; Dolz and De Blas, 1992; Parsons et al., 1997; Johnson et al., 1998), whereas few AME values have been reported for broilers (Kiiskinen and Huida, 1984; Dale and Fuller, 1982; Wang and Parsons, 1998). Most feed tables do not present separate values for

broilers and adult poultry (NRC, 1994; Feedstuffs, 1999), although the physiological state may cause differences and other sources of variation may interfere (Pesti and Edwards, 1983).

The AME<sub>n</sub> content of the MBM in our study with broilers ranged from 2,511 to 3,115 kcal/kg (10.51 to 13.04 MJ/kg) DM and agreed well with the AME<sub>n</sub> content for chickens reported in Feedstuffs (1999) and by Kiiskinen and Huida (1984) and Wang and Parsons (1998) of a MBM with similar ash and protein contents and similar processing conditions as in our study. Similar TME values, as measured with adult cockerels and if corrected for the difference between TME and AME, were reported by Dolz and De Blas (1992), Martosiswoyo and Jensen (1988), Parsons et al. (1997), and Wang and Parsons (1998). In our experiment, as was expected, the AME<sub>n</sub> content of the MBM decreased significantly (linear and quadratic

**TABLE 4. Comparison of the excreta and ileal digestibilities of CP and total AA in the basal diet and in the diets with MBM<sub>pigs</sub> with 200 g/kg ash at 10 and 20% inclusion level<sup>1</sup>**

Method	CP (%)		Total AA (%)	
	Excretal <sup>2</sup>	Ileal <sup>2</sup>	Excretal	Ileal
Diet/inclusion level				
Diet 1: basal diet	85.0 <sup>a</sup>	83.8 <sup>a</sup>	87.7 <sup>e</sup>	86.2 <sup>e</sup>
Diet 5: 10%, ash 200	79.6 <sup>b</sup>	76.5 <sup>c</sup>	81.3 <sup>f</sup>	79.0 <sup>g</sup>
Diet 9: 20%, ash 200	75.3 <sup>c</sup>	71.4 <sup>d</sup>	76.2 <sup>h</sup>	73.5 <sup>i</sup>
SED				
M		0.54		0.51
In		0.66		0.62
In × M		0.60		0.88
F probability				
M		<0.001		<0.001
In		<0.001		<0.001
In × M		0.32		0.60

<sup>a-i</sup>Values within and between columns for the same nutrient with different superscripts are significantly different (*P* < 0.05).

<sup>1</sup>AA = amino acid; MBM<sub>pigs</sub> = meat and bone meal originating from pigs; M = method of digestibility as a percentage; In = inclusion level of MBM; SED = standard error of difference.

<sup>2</sup>Excreta and ileal refer to the digestibility method, from fecal or from ileal digesta samples; digestibility as a percentage.

TABLE 5. The excretal and ileal digestibility of N and amino acids (AA) in the basal diet and in meat and bone meals (MBM) (%)

	Meat and bone meals (origin, inclusion level, and ash content in g/kg)											
	Diet 1 basal diet		Diet 2 cattle, 10%, ash 200,	Diet 3 cattle, 10%, ash 300,	Diet 4 cattle, 10%, ash 400,	Diet 5 pigs, 10%, ash 200		Diet 6 pigs, 10%, ash 300,	Diet 7 pigs, 10%, ash 400,	Diet 8 cattle, 20%, ash 200,	Diet 9 pigs, 20%, ash 200	
	Excreta <sup>1</sup>	Ileal <sup>1</sup>	Excreta	Excreta	Excreta	Excreta	Ileal	Excreta	Excreta	Excreta	Excreta	Ileal
Lys	88.7	88.7	60.5	62.7	56.9	60.1	55.7	60.7	56.3	62.3	60.3	57.7
Met	89.3	91.6	65.5	68.9	68.5	71.1	73.7	68.6	64.7	67.9	69.3	65.0
Cys	75.9	77.2	NR <sup>2</sup>	NR	NR	NR	NR	NR	NR	NR	NR	NR
Thr	82.0	79.1	61.4	63.2	48.2	56.3	50.5	61.5	65.4	63.6	56.8	50.0
Try	86.1	83.0	58.5	59.9	62.6	48.1	48.2	64.2	57.5	58.1	45.7	42.7
Ile	88.9	87.6	65.1	60.7	59.2	64.6	63.3	64.6	52.2	68.3	62.2	59.8
Arg	91.6	90.8	75.3	74.1	71.0	77.0	71.9	80.4	76.8	75.9	77.7	72.5
Phe	89.8	87.9	65.8	63.9	60.7	63.7	66.5	68.5	58.4	67.8	67.5	65.5
His	86.9	87.3	69.7	69.6	74.1	73.3	63.4	77.9	72.3	69.2	60.2	61.7
Leu	89.8	87.6	64.8	63.1	61.1	65.0	64.7	67.7	59.6	67.8	65.6	61.9
Tyr	89.3	87.3	55.6	57.4	56.1	51.0	57.4	61.2	48.4	64.7	62.1	60.0
Val	86.2	84.2	57.7	57.6	49.5	58.9	65.1	59.8	49.9	62.3	61.1	58.9
Ala	86.4	86.9	70.3	69.7	72.0	71.4	66.7	74.3	72.1	68.7	66.8	65.1
Asp	86.2	83.0	35.6	38.9	30.0	25.3	10.2	24.3	27.0	36.2	22.1	13.9
Glu	90.3	88.7	62.3	61.1	54.4	62.6	56.1	59.0	55.5	61.0	58.4	54.1
Gly	76.3	81.1	77.3	76.5	77.7	71.9	61.4	80.1	76.3	68.5	70.6	60.7
Pro	85.7	84.4	67.2	73.0	73.0	69.0	61.9	73.0	68.4	65.6	62.1	57.6
Ser	86.3	84.7	55.3	57.9	40.6	51.7	43.7	47.1	48.5	55.7	51.6	46.1
Total AA	87.7	86.2	62.2	63.3	60.1	61.7	56.8	64.8	61.4	63.0	60.1	55.6
Total N	85.0	83.8	64.8	64.1	65.6	64.3	55.5	66.5	66.4	60.7	63.2	55.9

<sup>1</sup>Excreta and ileal refer to the digestibility method, from fecal or from ileal digesta samples; digestibility as a percentage.

<sup>2</sup>NR = value of cystine not reliable, because coefficients were obtained outside the range possible.

effect) at a higher ash content, which was also observed by Johnson et al. (1998) for the TME<sub>n</sub> values of MBM and by Parsons et al. (1997). However, in our study, the MBM<sub>cattle</sub> with 200 g of ash showed a remarkable low AME<sub>n</sub> content compared to MBM with 300 and 400 g of ash, but an explanation could not be found. In NRC (1994),

the AME<sub>n</sub> content of the MBM for poultry increased with higher CP content (9.68 and 9.99 MJ/kg DM for CP of 50.4 and 54.4%, respectively), whereas in the Feedstuffs (1999) table, these values are also higher with more CP [2,582 and 2,721 kcal/kg (10.81 and 11.39 MJ/kg) DM for 49 and 54% CP, respectively].

TABLE 6. The AME<sub>n</sub> content and excretal digestibility (%) of organic matter, CP, total amino acids, and crude fat of the basal diet and of meat and bone meal (MBM)<sup>1,2</sup>

Diet, basal or MBM inclusion	Energy/nutrient					
	AME <sub>n</sub>		Organic matter	Crude protein	Total AA	Crude fat
kcal/kg	MJ/kg DM					
Diet 1, basal diet	3,488	14.60	78.0	85.0	87.7	92.0
MBM						
Diet 2: cattle, 10% ash 200	2,857 <sup>bc</sup>	11.96 <sup>bc</sup>	64.1 <sup>a</sup>	64.8 <sup>b</sup>	62.2 <sup>ab</sup>	83.3
Diet 3: cattle, 10% ash 300	2,955 <sup>cd</sup>	12.37 <sup>cd</sup>	66.8 <sup>ab</sup>	64.1 <sup>ab</sup>	63.3 <sup>ab</sup>	84.9
Diet 4: cattle, 10% ash 400	2,661 <sup>ab</sup>	11.14 <sup>ab</sup>	68.2 <sup>ab</sup>	65.6 <sup>b</sup>	60.1 <sup>a</sup>	80.0
Diet 5: pigs, 10% ash 200	3,115 <sup>d</sup>	13.04 <sup>d</sup>	65.8 <sup>a</sup>	64.3 <sup>ab</sup>	61.7 <sup>ab</sup>	79.9
Diet 6: pigs, 10% ash 300	3,024 <sup>cd</sup>	12.66 <sup>cd</sup>	75.2 <sup>c</sup>	66.5 <sup>b</sup>	64.8 <sup>b</sup>	89.7
Diet 7: pigs, 10% ash 400	2,511 <sup>a</sup>	10.51 <sup>a</sup>	72.5 <sup>bc</sup>	66.4 <sup>b</sup>	61.4 <sup>ab</sup>	69.2
Diet 8: cattle, 20% ash 200	2,628 <sup>a</sup>	11.00 <sup>a</sup>	63.1 <sup>a</sup>	60.7 <sup>a</sup>	63.0 <sup>ab</sup>	81.5
Diet 9: pigs, 20% ash 200	2,716 <sup>ab</sup>	11.37 <sup>ab</sup>	66.7 <sup>a</sup>	63.2 <sup>ab</sup>	60.1 <sup>a</sup>	84.7
SED		0.459	2.78	1.85	1.98	NA
F probability						
Origin		0.375	0.007	0.419	0.494	NA
Ash-linear		0.001	0.012	0.265	0.391	NA
Ash-quadratic		0.006	0.064	0.980	0.037	NA
Origin × ash-linear		0.015	0.522	0.632	0.520	NA
Origin × ash-quadratic		0.916	0.134	0.342	0.644	NA
Inclusion		0.009	0.025	0.006	0.523	NA
Origin × inclusion		0.420	0.211	0.204	0.164	NA

<sup>a-d</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>The MBM nutritive values were calculated by difference from the experimental diets and the basal diet.

<sup>2</sup>NA = not analyzed; SED = standard error of the difference.

TABLE 7. Digestibility of CP by in vitro methods with the meat and bone meal (MBM) (%) and in vivo with the excreta and ileal levels

Method of	MBM (origin, ash content in g/kg)					
	Cattle, ash 200	Cattle, ash 300	Cattle, ash 400	Pigs, ash 200	Pigs, ash 300	Pigs, ash 400
LABCO (EC) <sup>1,2</sup>	81.9	82.3	82.7	90.7	90.3	93.0
Saria Industries <sup>3</sup>	94.5	92.8	91.0	98.5	97.6	98.8
Cehave (EC) <sup>1,4</sup>	86.4	85.8	85.1	96.7	94.8	95.8
Babinszky et al. (1990)	70.2	67.0	63.8	92.2	84.5	77.0
This experiment	64.8	64.1	65.6	64.3	66.5	66.4
This experiment (ileal)	...	...	...	55.5	...	...

<sup>1</sup>EC = Method as described in EC, PB no L123, dated 29-5-1972, p. 11-12.

<sup>2</sup>LABCO BV, Rotterdam, The Netherlands.

<sup>3</sup>Saria Industries, Saint-Denis, Cedex, France.

<sup>4</sup>Cehave, Veghel, The Netherlands.

In the Dutch table (CVB, 1999a), the AME<sub>n</sub> content of the MBM reported for broilers are 7.52 and 9.56 MJ/kg DM, depending on the fat content (lower or higher than 10%), but no discrimination is made among differences in ash content.

In our studies, the AME<sub>n</sub> content was higher in MBM<sub>pig</sub> than in MBM<sub>cattle</sub>. Dale (1997) reported similar results (average 11.7 and 9.7 MJ/kg in pork and beef meals, respectively), and he speculates that the difference seems reasonable considering the lower percentage of bone in the pork meal samples.

Higher inclusion, at 20%, yielded a significantly lower AME<sub>n</sub> in our study and was similar to differences reported by Esteve-Garcia et al. (1994) comparing AME<sub>n</sub> at 20% inclusion [2,379 kcal/kg (9.96 MJ/kg) DM] with 5 and 10% inclusions [3,151 and 2,946 kcal/kg (13.19 and 12.33 MJ/kg) DM, respectively] and to TME values reported by Martosiswoyo and Jensen (1988) with broiler chickens, at 20 and 40% inclusion. However, Dolz and De Blas (1992) reported no significant deviations from linearity in the relationship between dietary AME<sub>n</sub> and inclusion level of the MBM samples from 6 to 24%. Part of an explanation for the lower AME<sub>n</sub> content of MBM at 20% inclusion in our diets may be the significant increase in the amount of uric acid and fecal nitrogen excreted with higher levels of dietary MBM, as reported by Martosiswoyo and Jensen (1988).

### Excretal and Ileal Digestibility of CP and AA

Ileal digestibility of protein and AA is preferred over excretal digestibility, because excretal digestibility may include substantial changes due to microbial fermentation in the hindgut (Ten Doesschate et al., 1993; Kadim and Moughan, 1997b; Ravindran and Bryden, 1999). Microbial fermentation and endogenous excretion is related to age and body weight (Johns et al., 1986a), and therefore, a comparison between broilers and adult poultry may be biased. Few studies have been conducted to compare the excretal versus ileal AA digestibility in chickens (Ravindran and Bryden, 1999).

Amino acid digestibilities in animal protein meals, except for highly digestible blood meal and fish meal, are

consistently overestimated by excreta analysis (Ravindran and Bryden, 1999). They concluded that even in chickens, the hindgut microflora may be substantial, and less accurate digestibilities may be obtained by the excretal method.

Our excretal digestibility values for protein are lower than those in the Dutch feed table for poultry (CVB, 1999a), but the data from CVB (1999a) are derived from intact adult cockerels. A high ash content tended to decrease excretal AA digestibility (quadratic effect of ash, A(quad)) in our trial, but no detrimental effect was observed by Johnson et al. (1998), whereas Wang and Parsons (1998) noted a significant negative correlation between ash content and digestible sulfur AA per unit of CP. These results suggest a minor effect of the ash concentration on AA digestibility.

Processing temperature of MBM showed variable effects on AA digestibility (Wang and Parsons, 1998), but results of Johns et al. (1986b) with intact and adult cockerels, were similar to our digestibility values, although their MBM were heated at a higher temperature of 150 C for 1, 3, and 5 h. The excretal digestibility coefficient of AA in MBM in our study agreed well with the results of Ravindran et al. (1999) from an experiment with broiler chickens on a diet containing a MBM with 49% CP. They also examined two other types of meat meal with 55.7 and 54.6% CP, respectively. Despite the similar CP content, rather large differences between these two meat meals in the digestibility coefficient of AA were observed.

When comparing the ileal AA digestibility from growing intact chickens with cannulated adult cockerels, Johns et al. (1986a) found significantly lower values from cannulated cockerels. They attributed the significantly lower values from cockerels to an increase in endogenous nitrogen with age and body size. In our experiment, the ileal digestibility of the AA was also lower than the excretal values (Table 5). However, for three AA (Phe, Tyr, and Val) these differences were not significant. Marked differences ( $P < 0.05$ ) were observed with seven AA, as well as total AA and CP. Ravindran et al. (1999) used the same methods to measure digestibility, and they also observed a higher digestibility of AA in MBM by 8% with excreta analysis, but Johns et al. (1987) found no significant ileal-excreta difference in digestibility of lysine with young chickens.

Parsons et al. (1997) observed an even greater variability in MBM than reported in the earlier studies. However, part of the variability can be attributed to differences in methods and techniques of killing animals and collecting the ileal digesta samples (Esteve-Garcia et al., 1994; Kadim and Moughan, 1997a,b; Yap et al., 1997). The above studies demonstrate the rather large differences between batches, and that this difference can be increased by the different processing systems and measuring techniques for digestibility.

### ***In Vitro Digestible Protein Analyses***

In vitro methods are often used for prediction of the nutritive value. They are quick and inexpensive and may help the producer to control the quality of individual batches. For MBM, the in vitro methods are concentrated on protein. These methods are supposed to discriminate rather well among differences in batches of MBM, but, in this experiment, the predictive capabilities of all methods were very poor. The results of the different in vitro protein digestibility methods were substantially higher compared with the in vivo values. Moughan et al. (1989) also concluded from their experiments with MBM on rats, that none of the in vitro assays applied could be used to predict the in vivo digestibility of CP. Maybe the differences among in vivo digestibilities of protein are too small to show the differences in vitro. All in vitro methods showed a higher digestibility value for MBM from pigs compared with cattle, which may be related to bone or collagen content (Kling and Wöhlbier, 1977).

In conclusion, the AME<sub>n</sub> content of the MBM for broilers is high (10.5 to 13.04 MJ/kg DM). Higher ash did not decrease AME<sub>n</sub> substantially, although significantly lower values were noted in MBM with 400g/kg ash at 10% inclusion. There was no significant effect on AME<sub>n</sub> content of the species origin. A higher inclusion at 20% decreased AME<sub>n</sub> significantly in a diet with MBM of 200g/kg ash. Ash content of MBM showed little effect on the digestibility of total AA (range from 60 to 65%), although a significant quadratic effect was observed. The excretal digestibility of CP and total AA was significantly higher than the ileal digestibility in the MBM<sub>pigs</sub>.

AME<sub>n</sub> values in MBM originating from cattle and pigs in this experiment were higher, but CP and AA digestibilities were lower than in several feed tables. However, the comparison with previous digestibility data cannot really be made due to differences in the methods applied for digestibility measurement. Our values are considered valid for broilers in good practical circumstances. Unfortunately, the prediction of protein digestibility failed by the in vitro methods tested and seemed not to be usable in practice.

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### **REFERENCES**

- Association of Official Analytical Chemists, 1984. Official Method of Analysis. 14th ed. AOAC, Arlington, Virginia.
- Babinszky, L., J. M. van der Meer, H. Boer, and L. A. den Hartog, 1990. An in vitro method for prediction of the digestible CP content in pig feeds. *J. Sci. Food Agric.* 50:173–178.
- CVB, 1999a. Feed table. Data on chemical composition, digestibility and nutritive value of feedstuffs. Central Bureau for Livestock Feeding, Lelystad, The Netherlands.
- CVB, 1999b. Abbreviated table 1999. Feeding standards for farm livestock and nutritive value of feedstuffs. Central Bureau for Livestock Feeding, Lelystad, The Netherlands.
- Dale, N., 1997. Metabolizable energy of meat and bone meal. *J. Appl. Poult. Res.* 6:169–173.
- Dale, N. M., and H. L. Fuller, 1982. Applicability of the true metabolizable energy system in practical feed formulation. *Poultry Sci.* 61:351–356.
- Dolz, S., and C. De Blas, 1992. Metabolizable energy of meat and bone meal from Spanish rendering plants as influenced by level of substitution and method of determination. *Poultry Sci.* 71:316–322.
- EC Method, 1972. Pages 11–12 *in*: PB no. L123, dated 29 May 1972. Product Board Animal Feed, Den Haag, The Netherlands.
- Esteve-Garcia, E., J. Brufau, and L. Llauredó, 1994. Bioavailability of lysine in meat and bone meal. Effects of level of inclusion and feeding regime. Pages 348–351 *in*: Proceedings 9th European Poultry Conference. WPSA (United Kingdom Branch), Glasgow, UK.
- Feedstuffs, 1999. Feedstuffs ingredient analysis table: 1999 edition. Feedstuffs Reference Issue 71:25–30.
- Johns, D. C., C. K. Low, and K. A. C. James, 1986a. Comparison of amino acid digestibility using the ileal digesta from growing chickens and cannulated adult cockerels. *Br. Poult. Sci.* 27:679–685.
- Johns, D. C., C. K. Low, J. R. Sedcole, M. P. Gurnsey, and K. A. C. James, 1987. Comparison of several in vivo digestibility procedures to determine lysine digestibility in poultry diets containing heat-treated meat and bone meals. *Br. Poult. Sci.* 28:397–406.
- Johns, D. C., C. K. Low, J. R. Sedcole, and K. A. C. James, 1986b. Determination of amino acid digestibility using caecotomized and intact adult cockerels. *Br. Poult. Sci.* 27:451–461.
- Johnson, M. L., and C. M. Parsons, 1997. Effects of raw material source, ash content, and assay length on protein efficiency ratio and net protein values for animal protein meals. *Poultry Sci.* 76:1722–1727.
- Johnson, M. L., C. M. Parsons, G. C. Fahey, Jr., N. R. Merchen, and C. G. Aldrich, 1998. Effect of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *J. Anim. Sci.* 76:1112–1122.
- Just, A., J. A. Fernández, and H. Jorgensen, 1982. The value of meat and bone meal for pigs. *Beret. Statens Husdyrbrugsfors.* 525:52.
- Kadim, I. T., and P. J. Moughan, 1997a. Development of an ileal amino acid digestibility assay for the growing chicken-effect of time after feeding and site of sampling. *Br. Poult. Sci.* 38:89–95.
- Kadim, I. T., and P. J. Moughan, 1997b. Ileal amino acid digestibility assay for the growing meat chicken-effect of the imposition of a fasting period and the nature of the test diet. *Br. Poult. Sci.* 38:285–290.
- Kiiskinen, T., and L. Huida, 1984. Metabolizable energy value and digestibility of some protein sources for poultry. *Ann. Agric. Fenn.* 23:26–38.

- Kling, M. and W. Wöhlbier, 1977. Futtermittel aus Landtieren. Pages 217–219 *in*: Handelsfuttermittel. M. Kling and W. Wöhlbier, ed. Eugen Ulmer GmbH, Stuttgart, Germany.
- Martosiswoyo, A. W., and L. S. Jensen, 1988. Available energy in meat and bone meal as measured by different methods. *Poultry Sci.* 67:280–293.
- Moughan, P. J., J. Schrama, G. A. Skilton, and W. C. Smith, 1989. In vitro determination of nitrogen digestibility and lysine availability in meat and bone meals and comparison with in vivo ileal digestibility estimates. *J. Sci. Food Agric.* 47:281–292.
- National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
- Parsons, C. M., F. Castanon, and Y. Han, 1997. Protein and amino acid quality of meat and bone meal. *Poultry Sci.* 76:361–368.
- Payne, R. W., P. W. Lane, P. G. N. Digby, S. A. Harding, P. K. Leech, G. W. Morgan, A. D. Todd, R. Thompson, G. Tunnicliffe Wilson, S. J. Welham, and R. P. White, 1993. Genstat 5. Reference Manual. Clarendon, Oxford, UK.
- Pesti, G. M., and H. M. Edwards, Jr., 1983. Metabolizable energy nomenclature for poultry feedstuffs. *Poultry Sci.* 62:1275–1280.
- Ravindran, V., and W. L. Bryden, 1999. Amino acid availability in poultry in vitro and in vivo measurements. *Austr. J. Agric. Res.* 50:889–908.
- Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden, 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. *Br. Poult. Sci.* 40:266–274.
- Sato, H., T. Seino, T. Kobayashi, A. Murai and T. Y. Yugari, 1984. Determination of tryptophan content of feed and feedstuffs by ion exchange liquid chromatography. *Agric. Biol. Chem.* 48:2961–2969.
- Schram, E., S. Moore, and E. W. Bigwood, 1954. Chromatographic determination of cystine as cystic acid. *Biochem. J.* 57:35–37.
- Ten Doesschate, R. A. H., C. W. Scheele, V. V. A. Schreurs, and J. D. van der Klis, 1993. Digestibility studies in broiler chickens: influence of genotype, age, sex and method of determination. *Br. Poult. Sci.* 34:131–146.
- Terpstra, K., and A. J. N. Bisalsky, 1976. Analysis of organic matter and gross energy values in urine samples (translated title). Report 131.76. Spelderholt, The Netherlands.
- Van der Klis, J. D., 1993. Physico-chemical chyme conditions and mineral absorption in broilers. PhD Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.
- Wang, X., and C. M. Parsons, 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. *Poultry Sci.* 77:834–841.
- Williams, C. H., D. J. David, and O. Iismaa, 1962. The determination of chromic oxid in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59:381–385.
- Yamazaki, M., and H. Kamata, 1986. Amino acid availability of feed ingredients for poultry. *Jpn. Poult Sci* 23:147–156.
- Yap, K. H., I. T. Kadim, R. D. King, and P. J. Moughan, 1997. An ileal amino acid digestibility assay for the growing meat chicken-effect of feeding method and digesta collection procedures. *Asian Australasian J. Anim. Sci.* 10:671–678.