Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using the direct method

M. N. Anwar,[*] V. Ravindran,[*,1] P. C. H. Morel,[*] G. Ravindran,[†] and A. J. Cowieson[‡]

[*]*Institute of Veterinary, Animal and Biomedical Science, Massey University, Palmerston North 4442, New Zealand;* [†]*Institute of Food, Nutrition and Human Health, Massey University, Palmerston North 4442, New Zealand; and* [‡]*Poultry Research Foundation, University of Sydney, Camden, NSW 2570, Australia*

ABSTRACT The objective of the study that is presented herein was to determine the true ileal calcium (Ca) digestibility in meat and bone meal (MBM) for broiler chickens using the direct method. Four MBM samples (coded as MBM-1, MBM-2, MBM-3 and MBM-4) were obtained and analyzed for nutrient composition, particle size distribution and bone to soft tissue ratio. The Ca concentrations of MBM-1, MBM-2, MBM-3 and MBM-4 were determined to be 71, 118, 114 and 81 g/kg, respectively. The corresponding geometric mean particle diameters and bone to soft tissue ratios were 0.866, 0.622, 0.875 and 0.781 mm, and 1:1.49, 1:0.98, 1:0.92 and 1:1.35, respectively. Five experimental diets, including four diets with similar Ca concentration (8.3 g/kg) from each MBM and a Ca and phosphorus-free diet, were developed. Meat and bone meal served as the sole source of Ca in the MBM diets. Titanium dioxide (3 g/kg) was incorporated in all diets as an indigestible marker. Each experimental diet was randomly allotted to six replicate cages (eight birds per cage) and offered from d 28 to 31 post-hatch. Apparent ileal Ca digestibility was calculated by the indicator method and corrected for ileal endogenous Ca losses to determine the true ileal Ca digestibility. Ileal endogenous Ca losses were determined to be 88 mg/kg dry matter intake. True ileal Ca digestibility coefficients of MBM-1, MBM-2, MBM-3 and MBM-4 were determined to be 0.560, 0.446, 0.517 and 0.413, respectively. True Ca digestibility of MBM-1 was higher ($P < 0.05$) than MBM-2 and MBM-4 but similar ($P > 0.05$) to that of MBM-3. True Ca digestibility of MBM-2 was similar ($P > 0.05$) to MBM-3 and MBM-4, while that of MBM-3 was higher ($P < 0.05$) than MBM-4. These results demonstrated that the direct method can be used for the determination of true Ca digestibility in feed ingredients and that Ca in MBM is not highly available as often assumed. The variability in true Ca digestibility of MBM samples could not be attributed to Ca content, percentage bones or particle size.

Key words: calcium digestibility, broiler, meat and bone meal, direct method

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INTRODUCTION

Recent interest in the determination of phosphorus (P) digestibility in feed ingredients (WPSA, 2013) necessitates the measurement of calcium (Ca) digestibility because of the close relationship between P and Ca metabolism. Meat and bone meal (MBM) is an important organic Ca source in poultry diets and contains approximately 100g/kg Ca (NRC, 1994). However, wide variations have been reported in the Ca concentration of MBM from different sources depending on its origin (bovine, porcine, or mixed) and proportion of meat and bones. Calcium concentration of MBM is reported to range from 40 to 150 g/kg (Drewyor and Waldroup, 2000; Sulabo and Stein, 2013). These variations in Ca concentration may cause variations in the digestible Ca content of MBM. A recent study by Sulabo and Stein (2013) reported that the apparent total tract Ca digestibility for pigs was reduced with increasing Ca concentration in MBM.

Currently there is no established method available for the determination of Ca digestibility in poultry. However, three different methods, namely direct, difference, and regression, are used for the determination of amino acid digestibility in feed ingredients, and these can also be deployed for estimation of Ca digestibility (Ravindran and Bryden, 1999; Lemme et al., 2004). Historically, Ca availability has been described in terms of bioavailability relative to calcium carbonate, and it is generally assumed that Ca availability from Ca sources is very high (Blair et al., 1965; Peeler, 1972; Reid and Weber, 1976).

In a previous study (Anwar et al., 2015) the regression method was used for the determination of true ileal Ca digestibility in three MBM sources for broiler

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[1]Corresponding author: v.ravindran@massey.ac.nz

chickens, and it was found that the digestibility coefficients varied from 0.46 to 0.60. These values were lower than the apparent total tract digestibility of Ca (0.53 to 0.81) in MBM for pigs determined by the direct method by Sulabo and Stein (2013). The possible reasons for the observed discrepancy may include differences in animal species, source of MBM, and the methodology used.

When apparent digestibility coefficients are determined by the direct method, they must be corrected for endogenous Ca losses to determine the true Ca digestibility coefficients. The purpose of this study was to determine the true ileal Ca digestibility of four MBM samples in broiler chickens using the direct method. Ileal endogenous Ca losses were also determined following feeding of a Ca-free diet.

MATERIALS AND METHODS

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethic Committee.

Diets

Meat and bone meal samples, of a mixture of bovine and ovine origin, from four commercial rendering sources (coded as MBM-1, MBM-2, MBM-3, and MBM-4) were obtained and representative samples were analyzed in duplicate for dry matter (DM), crude protein (CP), crude fat, ash, Ca, phosphorus (P), particle size distribution and bone and soft tissue fractions (Table 1). A total of five experimental diets were developed, including a Ca- and P-free diet and four diets containing each MBM (Table 2). Meat and bone meal served as the sole source of Ca and P in assay diets. Inclusion level of each MBM was set to obtain 8.3 g Ca/kg diet, which was below the recommended dietary Ca requirement (8.5 g) for broiler finisher diets (Ross, 2007). Titanium dioxide (3g/kg) was added in all diets as an indigestible marker.

Birds

Day-old male broilers (Ross 308) were obtained from a local hatchery and were raised on floor pens in an environmentally controlled room. Temperature was maintained at 31°C on d 1 and gradually reduced to 20°C by 27 d of age, and then maintained till d 31. The birds were fed commercial broiler starter crumbles (9.0 g/kg Ca and 4.5 g/kg P) until d 20. On d 21, birds were moved to colony cages to acclimatize them. Between d 21 and 23, the crumbles were gradually changed to a broiler starter diet in mash form as the experimental diets were in mash form. On d 28, birds were individually weighed and allocated to 30 cages (8 birds per cage) on weight basis so that the average bird weight per cage was similar. The five experimental diets were then randomly allotted to six replicate cages each. The diets, in mash form, were offered ad libitum and the birds had free access to water. Group body weights and feed intake were recorded on d 28 and 31.

Particle Size Distribution

To determine the particle size of calcium sources, a set of sieves (Endocott, London, UK) sized 2, 1, 0.5, 0.212, 0.106, and 0.075 mm and a sieve shaker were used as described by Baker and Herman (2002). Samples in duplicates were passed through the sieve stack on shakers for 10 minutes. The amount of sample retained on each sieve was determined and, the geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated for each sample. These calculations were based on the assumption that the weight distribution of the samples is logarithmically normal (Wilcox et al., 1970).

Bone and Meat Fractions

Bone and meat fractions of MBM samples were determined by the flotation method described by Khajarern and Khajarern (1999). Ten grams of each meat and bone meal sample was weighed in beaker and mixed

Table 1. Analyzed nutrient composition and percentage composition of bone and soft tissue fractions of the four meat and bone meal (MBM) samples (g/kg, as fed basis).[1]

	MBM-1	MBM-2	MBM-3	MBM-4
Dry matter	925	943	956	953
Crude protein	536	488	474	482
Crude fat	114	93	88	128
Ash	237	357	362	251
Calcium	71	118	114	81
Phosphorus	37	60	59	41
Ca:P ratio	1.91	1.96	1.92	1.97
Bone and soft tissue fractions				
Bone	40.2	50.5	52.1	42.6
Soft tissue	59.8	49.5	47.9	57.4
Bone:Soft tissue	1:1.49	1:0.98	1:0.92	1:1.35

[1]Samples were analyzed in duplicate.

Table 2. Ingredient composition and analysis (g/kg as-fed basis) of meat and bone meal (MBM) diets.

	MBM diets				Ca– and P-free diet
	MBM-1	MBM-2	MBM-3	MBM-4	
Corn starch	349.35	370.85	369.85	355.6	451.45
Dextrose	349.35	370.85	369.85	355.6	451.45
Dried egg albumen	100	100	100	100	–
Meat and bone meal	115	70	72	102	–
Cellulose	50	50	50	50	50
Soybean oil	20	20	20	20	20
Potassium bicarbonate	8	10	10	8.5	14.8
Sodium bicarbonate	3	3	3	3	3
Sodium chloride	–	–	–	–	4
Titanium dioxide	3	3	3	3	3
Trace mineral-vitamin premix[1]	2.3	2.3	2.3	2.3	2.3
Calculated analysis					
Metabolizable energy, (kcal/kg)	3504	3573	3570	3524	3726
Crude protein	144.7	117.1	117.1	132.2	1.4
Calcium[2]	8.30	8.30	8.30	8.30	0.01
Total phosphorus[2]	4.44	4.34	4.43	4.33	0.06
Non-phytate phosphorus	4.44	4.34	4.43	4.33	–
Ca: Non-phytate phosphorus	1.86	1.92	1.87	1.91	–
Analyzed values					
Dry matter	906	907	906	907	903
Calcium	9.89	8.03	9.71	8.25	0.14
Total phosphorus	4.34	3.54	4.45	3.64	0.24

[1]Supplied per kilogram of diet: vitamin A, 12,000 IU; cholecalciferol, 4,000 IU; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 10 mg; folic acid, 3 mg; biotin, 0.25 mg; cyanocobalamin, 0.02 mg; dl-α-tocopherol acetate, 80 mg; niacin, 60 mg; Ca-D pantothenate, 15 mg; menadione, 4 mg; choline chloride, 600 mg; Co, 0.25 mg; I, 1.5 mg; Mo, 0.25 mg; Se, 0.26 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; Fe, 60 mg; antioxidant, 100 mg.

[2]Calculated based on analyzed values of MBM samples.

well with 90 mL carbon tetrachloride to dissolve the fat. The samples were then allowed to settle, and the floating meat and submerged bone fractions were separated on separate filter papers (Whatman no.4). Both fractions were dried at 110°C for 10 minutes, allowed to cool and weighed to calculate the proportions of bone and meat fractions.

Sample Collection and Processing

On d 31, all birds were euthanized by intravenous injection (0.5 mL per kg body weight) of sodium pentobarbitone (Provet NZ Pty. Ltd., Auckland, New Zealand) and ileal digesta were collected as described by Ravindran et al. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~ 40 mm proximal to the ileo-cecal junction. The ileum was then divided into two halves and the digesta were collected from the lower half towards the ileo-cecal junction. Digesta from birds within a cage were pooled, frozen immediately and subsequently lyophilized. Lyophilized samples were ground to pass through 0.5-mm sieve and stored in air-tight containers at 4°C till chemical analysis

Chemical Analysis

Representative samples of diets and ileal digesta were analyzed for DM, CP, fat, ash, Ca, P and titanium. Dry matter was determined using the standard procedure (method 930.15; AOAC International, 2005). Nitrogen was determined by combustion (method 968.06; AOAC International, 2005) using as CNS-200 carbon, nitrogen and sulphur analyzer (LECO® Corporation, St. Joseph, MI). Fat was determined by the Soxhlet method (method 991.36; AOAC International, 2005). Ash was determined gravimetrically by standard AOAC procedure (method 942.05; AOAC International, 2005). Titanium dioxide was determined by the procedure of Short et al. (1996). Calcium and P were determined by standard AOAC procedures (method 968.08D; AOAC International, 2005).

Calculations

The true ileal digestibility coefficient of Ca was calculated according to the procedure outlined by Adedokun et al. (2008) for the estimation of amino acid digestibility. Apparent ileal digestibility coefficients of Ca were calculated using titanium ratio in the diets and digesta.

$$\text{AIDC} = 1 - [(\text{Ti}_\text{I}/\text{Ti}_\text{O}) \times (\text{Ca}_\text{O}/\text{Ca}_\text{I})]$$

where AIDC is apparent ileal digestibility coefficient of Ca, Ti_I is the titanium concentration in the diet, Ti_O is the titanium concentration in the ileal digesta, Ca_O is the Ca concentration in the ileal digesta, and Ca_I is the Ca concentration in the diet. All analyzed values were expressed as gram per kilogram of DM.

Ileal endogenous Ca losses (g/kg DM intake) were calculated by the following formula.

$$IECaL = Ca_O \times (Ti_I/Ti_O)$$

where IECaL is ileal endogenous Ca losses, Ti_I is the titanium concentration in the diet, Ti_O is the titanium concentration in the ileal digesta, Ca_O is the Ca concentration in the ileal digesta.

True ileal digestibility coefficients of Ca of the test diets were then calculated as follows:

$$TIDC = AIDC + [IECaL\,(g/kg\ of\ DMI)/Ca_I\,(g/kg\ of\ DM)]$$

where TIDC and AIDC represent the true ileal digestibility and apparent ileal digestibility coefficients of Ca, respectively, while IECaL represents the ileal endogenous Ca losses (g/kg of DMI) and Ca_I represents the Ca concentration in diet (g/kg of DM).

Statistical Analysis

The data were analyzed as a one-way ANOVA using the General Liner Model of SAS (2004). Cage means served as the experimental unit. Differences were considered significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

RESULTS AND DISCUSSION

The nutrient composition of the four MBM samples is shown in Table 1. Analyzed Ca and P concentrations ranged between 71 and 118, and 37 and 59 g/kg, respectively. According to the definition of Association of American Feed Control Officials (AAFCO, 2000), MBM is a rendered product of mammalian tissue and bones, exclusive of any hair, horn, hoof, and blood. It should contain a minimum of 40 g/kg P, maximum of 550 g/kg CP, and the Ca concentration should not be more than 2.2 times the P concentration. If the P concentration is less than 40 g/kg and CP is more than 550 g/kg, then the meal is considered to be meat meal. The CP, Ca, and P concentrations of the samples used in this study were within the range to be considered as MBM. The Ca concentration of the four MBM samples in current study was within the range reported in previous studies (Waldroup 1999; Sulabo and Stein, 2013).

Ash and Ca concentrations of MBM-1, MBM-2, MBM-3 and MBM-4 were 237, 357, 362 and 251 g/kg, and 71, 118, 114 and 81 g/kg, respectively. Similar to the trend reported by Sulabo and Stein (2013), Ca concentration of the MBM samples was observed to be directly related with ash content. Bone and soft tissue proportions of the MBM samples are presented in Table 1. Bone percentage of MBM-1, MBM-2, MBM-3 and MBM-4 was observed to be 40.2, 50.5, 52.1 and 42.6%, respectively. Bone and soft tissue fractions of MBM-2 and MBM-3 were similar and their bone percentage was higher than MBM-1 and MBM-4. The lowest bone to soft tissue ratio was observed for MBM-1 and the highest for MBM-3. In the present study, ash and Ca concentration of MBM samples increased with increase in bone fractions of the samples. A direct relationship between ash concentration and bone fraction of the MBM samples has also been described previously (Dale, 1997; Mendez and Dale, 1998).

Analyzed Ca and P concentrations of the experimental diets are presented in Table 2. Analyzed Ca concentration of two of the four diets differed from calculated. Calcium concentrations of MBM-1 and MBM-3 diets were 1.59 and 1.41 g/kg higher, while those of MBM-2 and MBM-4 diets were close to calculated values. The observed differences may be reflective of the difficulty in obtaining representative samples due to the particle size of Ca-rich bone fraction.

Particle size distribution of the four MBM samples is presented in Figure 1. In the current study, particle size distribution was classified as fine (< 0.5 mm), medium (0.5 to 1.0 mm) and coarse (> 1.0 mm). According to this classification, proportions of fine, medium and coarse particles in MBM-1, MBM-2, MBM-3 and MBM-4 were 5, 41, 5, and 14%, and 63, 34, 60, and 57%, and 32, 25, 35, and 29%, respectively. Geometric mean diameters (GMD) of MBM-1, MBM-2 and MBM-3 were determined to be 0.866, 0.622, 0.875, and 0.781 mm, respectively. The corresponding geometric standard deviations were 1.52, 1.95, 1.51 and 1.61, respectively.

Body weight gain and feed intake of birds fed the experimental diets during the 3-d experimental period are summarized in Table 3. Daily gain of birds fed the MBM-1 diet was higher ($P < 0.05$) than those of other MBM diets. There was no difference in the weight gain of birds fed MBM-3 and MBM-4 diets, while that of birds fed the MBM-2 diet was lower ($P < 0.05$) than those fed the other diets. Feed intake of birds was lower ($P < 0.05$) on the MBM-2 diet compared to those of other diets.

The apparent ileal digestibility coefficients of Ca for MBM-1, MBM-2, MBM-3 and MBM-4 were determined to be 0.552, 0.438, 0.509 and 0.404, respectively (Table 3). A recent study with pigs has also reported wide variations, with apparent total tract Ca digestibility coefficients ranging between 0.53 and 0.81, in eight MBM sources (Sulabo and Stein, 2013). Overall, these data do not support the general assumption that Ca in MBM is highly bioavailable.

Ileal endogenous Ca losses (mean ± SE; n = 6) were determined to be of 88 ± 21 mg/kg of DM intake in birds fed a Ca- and P-free diet, and this value was used to calculate the true Ca digestibility coefficients. To authors' knowledge, no previous published data are available on the ileal endogenous Ca losses in broiler chickens. It can be seen that these endogenous flow estimates are negligible in the context of undigested Ca in the ileal digesta, resulting in differences of less than 0.01 between apparent and true digestibility coefficients

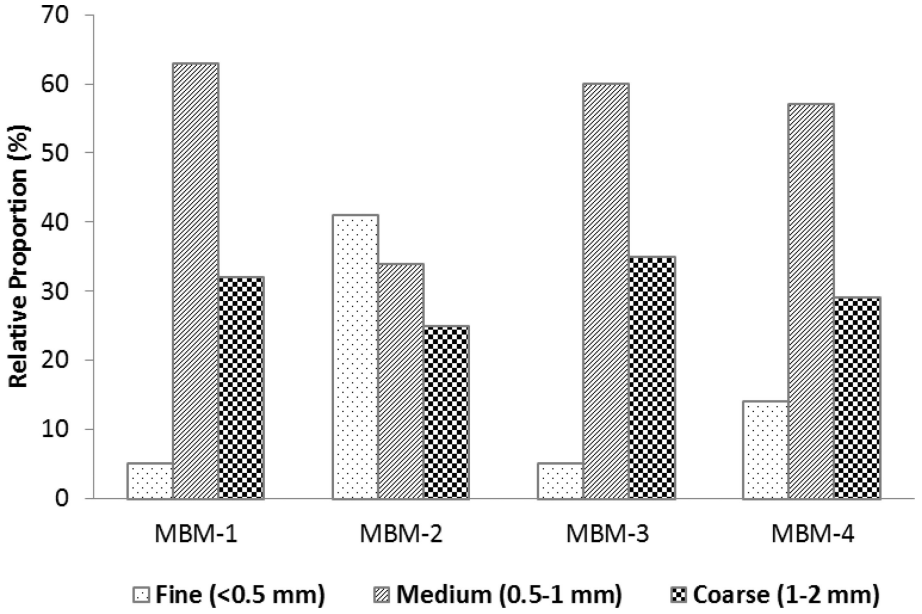

Figure 1. Particle size distribution of the four meat and bone meal samples.

Table 3. Body weight gain (g/bird/d), feed intake (g/bird/d) and apparent and true ileal calcium digestibility coefficients of the four meat and bone meal (MBM) samples.[1,2]

	MBM-1	MBM-2	MBM-3	MBM-4	SEM[3]	Probability P≤
Weight gain	46[a]	28[c]	35[b]	37[b]	1.64	0.05
Feed intake	107[a]	97[b]	105[a]	106[a]	1.68	0.05
Apparent ileal digestibility	0.552[a]	0.438[b,c]	0.509[a,b]	0.404[c]	0.033	0.05
True ileal digestibility[4]	0.560[a]	0.446[b,c]	0.517[a,b]	0.413[c]	0.033	0.05

[a–c]Values with a different superscript in a row differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicates (8 birds per replicate); experimental diets were offered from 28 to 31 d of age.

[2]Feed intake of birds on Ca and P-free diet was 63 g/bird/d and birds lost weight at the rate of 20 g/bird/d.

[3]Pooled standard error of mean.

[4]Corrected for ileal endogenous Ca losses (88 mg/kg dry matter intake) determined following the feeding of a Ca- and P-free diet.

(Table 3). A relevant question in practice, therefore, is whether the correction for endogenous Ca flow is necessary and whether the apparent value can be considered as an acceptable estimate of Ca digestibility in ingredients for poultry. It must be noted; however, that this estimate is indicative of only basal endogenous Ca losses and that specific endogenous Ca losses may be higher in practical diets containing P and phytate. Calcium-deficient diets can lead to low plasma Ca concentration, which increases the release of parathyroid hormone and production of 1,25-dihydroxycholecalciferol, causing increased intestinal Ca absorption (Proszkowiec-Weglarz and Angel, 2013). A portion of endogenous Ca may, therefore, be more efficiently reabsorbed on Ca-free diet as compared to practical diets, and this may result in the underestimation of endogenous Ca losses.

The length of feeding experimental diet to measure the Ca digestibility is critical as Ca in plasma is tightly regulated and, Ca- and P-deficient diets can influence the Ca homeostasis mechanism and can affect the estimation of digestible Ca content of the test ingredient (Proszkowiec-Weglarz and Angel, 2013). In the current work, this issue was addressed by using experimental diets that were formulated to contain recommended dietary Ca and P concentration for broiler finisher and fed for 3 d. The same length of assay has been previously used for determination of P digestibility at our laboratory (Mutucumarana et al., 2015). Clearly, further research is warranted to better understand the effect of assay length in order to establish the time frame for Ca and P digestibility assays.

Although not of direct relevance to the current work, ileal endogenous P losses were also calculated and determined to be 354 ± 39 mg/kg of DM intake. No comparable data are available on ileal endogenous P losses in poultry fed P-free diets. But this estimate is lower than the value of 446 mg/kg DM intake reported by Rutherfurd et al. (2004) using a minimal P diet.

True ileal Ca digestibility coefficients of MBM-1, MBM-2, MBM-3 and MBM-4 were 0.560, 0.446, 0.517 and 0.413, respectively (Table 3). The true Ca digestibility of MBM-1 was higher ($P < 0.05$) than those

Table 4. True ileal calcium digestibility coefficients of three meat and bone meal (MBM) samples as influenced by methodology.[1,2]

	MBM-1	MBM-2	MBM-3	Probability P≤
Direct method	0.560[a]	0.446[b]	0.517[a,b]	0.05
Regression method[3]	0.600[a]	0.463[b]	0.497[a,b]	0.05

[a–c]Values with a different superscript in a row differ significantly ($P < 0.05$).

[1]Data were subjected to two-way analysis of variance.

[2]MBM effect, $P < 0.05$; method effect, $P > 0.05$; MBM x method, $P > 0.05$.

[3]From Anwar et al. (2015). The same MBM samples were evaluated in both studies.

of MBM-2 and MBM-4, but similar ($P > 0.05$) to that of MBM-3. The digestibility of MBM-2 was similar ($P > 0.05$) to those of MBM-3 and MBM-4. The digestibility of MBM-3 was higher ($P < 0.05$) than that of MBM-4. The factors responsible for the observed variability between MBM samples are not clear. However, ash and Ca concentration (Traylor et al., 2005; Sulabo and Stein, 2013) and particle size distribution (Burnell et al., 1989) of MBM have been reported to influence the Ca and P utilization in pigs. Apparent digestibility coefficient of Ca in MBM for pigs has been reported to be negatively correlated to its bone to soft tissue ratio and, concentrations of ash, Ca and P (Sulabo and Stein, 2013). In contrast, the bone to soft tissue ratio and nutrient profile of MBM-1 and MBM-4 in the present study were similar, but their digestibility coefficients differed markedly.

Average particle size (GMD) of MBM samples in this study varied from 0.622 to 0.866. No published data are available on the effect of particle size of MBM on Ca utilization in poultry. In pigs, P from large bone particles has been reported to be absorbed less efficiently than that from finely ground bones (Burnell et al., 1989). In present study, observed differences in Ca digestibility of four MBM samples cannot be explained on the basis of variations in particle size, as the average particle size of MBM-2 was lower than the other three samples used, while its Ca digestibility was lower than those of MBM-1 and MBM-4, but similar to that of MBM-3.

In our previous study (Anwar et al., 2015), the regression method was used to determine the true Ca digestibility of three MBM samples. In the present study, true digestibility was determined for the same three samples by the direct method, thus enabling comparison between the two methods. The digestibility estimates determined by the regression method are automatically corrected for endogenous losses and represent the true digestibility values, while those determined by the direct method are apparent values and need to be corrected for endogenous losses. The advantage of the direct method is that it is less laborious, less expensive, and simple compared with the regression method in part because fewer diets and birds are required. The comparison of true Ca digestibility coefficients of MBM samples determined by the direct and regression methods (Table 4) showed that the ranking and trend in variation in digestibility between samples within each method were similar and that there was no difference ($P > 0.05$) in digestibility of MBM determined by these two methods.

In the present study, the direct method was used for the estimation of true Ca digestibility of MBM for poultry. It must be noted, however, that MBM has a Ca:P ratio of 2:1, indicating a good balance between these interdependent minerals and therefore optimal Ca absorption. Given this balance is critical for the absorption of Ca, the applicability of the direct method to common feed ingredients with much lower Ca:P ratios need to be evaluated in future studies. In the four MBM samples under test, the true ileal Ca digestibility coefficients ranged between 0.413 and 0.560, indicating that the Ca in MBM is not highly available. There is also likelihood that these values can be even lower in practical diets containing phytate (Selle et al., 2009). The observed variability in Ca digestibility cannot be attributed to differences in the contents of ash, Ca and bone or particle size.

REFERENCES

AAFCO. 2000. Official Publication of the Association of American Feed Control Officials. AAFCO, Washington, DC.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535–2548.

Anwar, M. N., V. Ravindran, P. C. H. Morel, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens. Anim. Feed Sci. Technol. 206:100–107.

AOAC International. 2005. Official Methods of Analysis, 18th ed. AOAC Int., Washington, DC.

Baker, S., and T. Herman. 2002. Evaluating particle size. MF-2051 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University, Manhattan, KS.

Blair, R., P. English, and W. Michie. 1965. Effect of calcium source on calcium retention in the young chick. Br. Poult. Sci. 44: 355–356.

Burnell, T. W., G. L. Cromwell, and T. S. Stahly. 1989. Bioavailability of phosphorus in meat and bone meal for pigs. J. Anim. Sci. 67:38 (Abstr.).

Dale, N. 1997. Metabolizable energy of meat and bone meal. J. Appl. Poult. Res. 6:169–173.

Drewyor, M. A., and P. W. Waldroup. 2000. Utilization of high levels of meat and bone meal in broiler diets. J. Appl. Poult. Res. 9: 131–141.

Khajarern, J., and S. Khajarern. 1999. Manual of Feed Microscopy and Quality Control, 3rd ed. Klang Nana Vittaya Company Limited, Khon Kaen, Thailand.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J. 60:423–438.

Mendez, A., and N. Dale. 1998. Rapid assay to estimate calcium and phosphorus in meat and bone meal. J. Appl. Poult. Res. 7: 309–312.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94:1611–1618.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Peeler, H. T. 1972. Biological availability of nutrients in feeds: availability of major mineral ions. J. Anim. Sci. 35:695–712.

Proszkowiec-Weglarz, M., and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility1. J. Appl. Poult. Res. 22:609–627.

Ravindran, V., and W. L. Bryden. 1999. Amino acid availability in poultry - In vitro and in vivo measurements. Aust. J. Agric. Res. 50:889–908.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens. Anim. Sci. 81:85–97.

Reid, B. L., and C. W. Weber. 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. Poult. Sci. 55:600–605.

Ross, . 2007. Ross 308 Broiler: Nutrition Specification. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Rutherfurd, S.M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poult. Sci. 83:61–68.

SAS Institute. 2004. SAS® Qualification Tools User's Guide Version 9.1.2. SAS Inst. Inc., Cary, NC.

Selle, P., A. J. Cowieson, V. Ravindran, and V. 2009. Calcium interactions with phytate and phytase: Consequences for pig and poultry nutrition. Livestock Sci. 124:126–141.

Short, F.J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Sulabo, R., and H. H. Stein. 2013. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285–1294.

Traylor, S. L., G. L. Cromwell, and M. D. Lindemann. 2005. Effect of particle size, ash content, and processing pressure on the availability of phosphorus in meat and bone meal for swine. J. Anim. Sci. 83:2554–2563.

Waldroup, P. W. 1999. Nutritional approaches to reducing phosphorus excretion by poultry. Poult. Sci. 78:683–691.

Wilcox, R. A., Deyoe, C. W. Deyoe, and H. B. Pfost. 1970. A method for determining and expressing the size of feed particles by sieving. Poult. Sci. 49:9–13.

WPSA. 2013. Determination of phosphorus availability in poultry. World's Poult. Sci. J. 69:687–698.