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Effect of Ash Content on Protein Quality of Meat and Bone Meal

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ABSTRACT The effect of ash concentration on amino acid (AA) composition, true AA digestibility, and protein efficiency ratio (PER; weight gain per unit of protein intake) of meat and bone meal (MBM) was evaluated. Commercially rendered MBM samples containing 16 to 44% ash were obtained from two sources. Additional samples of MBM varying in ash from 9 to 63% were obtained by chloroform floatation or lab screening of a beef crax sample. Protein quality of selected MBM samples was assessed by determining true AA digestibility using the precision-fed cecectomized rooster assay and by a PER chick growth assay wherein chicks were fed 10% CP diets containing a MBM as the only source of dietary protein from 8 to 18 d of age.

Increases in Ala, Pro, Gly, and Arg as a percentage of CP were observed in all MBM samples as ash percentage increased, with Pro and Gly accounting for most of the increase. In contrast, the levels (% of CP) of all essential AA, other than Arg, decreased as ash level increased. For example, Lys concentrations per unit of CP decreased from 5.7 to 4.0% as ash increased from 9 to 63%. There was little or no effect of ash content on AA digestibility of MBM varying in ash from 9 to 44%. The PER of MBM markedly decreased from 3.34 to 0.72 as ash increased from 16 to 44%, and most of the effects of ash on PER were not due to differences in dietary Ca and P levels.

The results indicate that the reduction in protein quality of MBM as ash content increases is almost entirely due to a decrease in analyzed essential AA per unit of CP, not a decrease in digestibility of AA.

(*Key words*: meat and bone meal, protein quality, ash, poultry)

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Abbreviation Key: AA = amino acid; PER = protein efficiency ratio; MBM = meat and bone meal; NPR = net protein ratio.

INTRODUCTION

Annually, an estimated 1.4 billion kg of animal by-product protein meals are utilized in poultry rations in the U.S. (Muirhead, 1996). The majority of the latter is meat and bone meal (MBM) that is produced from animal offal that is not directly consumed by humans. Animal offal consists of restaurant grease, plate waste, trimmings and bones, viscera, and undigested feed, blood, heads, hooves, hides, and dead livestock, all of which are considered unfit for human consumption. Due to rendering the wide variety of raw materials, differences in the final chemical composition of MBM can occur. One of the raw materials that can affect the composition and protein quality of MBM is bone. For example, increased bone or ash content has been shown by Dale (1997), Mendez and Dale (1998), and Wang and Parsons (1998) to have a negative effect on protein and energy concentrations. Eastoe and Long (1960) estimated that 83% of the protein in bone is collagen. Boomgaardt and Baker (1972) and Berdanier (1998) have shown that collagen and gelatin (refined collagen) are deficient in most essential amino acids (AA) such as Trp, sulfur AA, and Ile, while surfeit in hydroxyproline, Pro, and Gly. Therefore, any increase in bone content of the raw materials may have a negative effect on protein quality due to its high collagen content and poor AA balance.

Although the effects of increased ash on the chemical composition of MBM are well documented, the effects of ash on protein quality are not totally clear. It is expected that some decrease in protein quality with increased ash will occur due to the changes in AA concentrations discussed above. In addition, an increase in ash could further decrease protein quality if digestibility or bioavailability of AA is reduced. The effects of ash content on AA digestibility are basically unknown. In limited previous work from our lab, Johnson and Parsons (1997) and Johnson et al. (1998) showed that protein efficiency ratio (PER; chick weight gain per unit of CP intake) decreased from 1.70 to 1.0 as ash content increased from 24 to 35% in two samples of MBM. In contrast, mean AA digestibilities of the 24 and 35% ash MBM samples were not significantly different ($P > 0.05$) (70.8 and 76.3%, respectively). Thus,

TABLE 1. Description and analysis of meat and bone meals (MBM) varying in ash content[1]

Description[2]	Ash	DM	CP	Ca	P	Gross energy
			(%)			(kcal/kg)
Low ash air classified—Company 1	16.5	95.5	52.4	4.5	1.9	5,060
Original MBM—Company 1	35.2	95.2	43.9	12.2	4.7	3,528
Low ash air classified—Company 2	20.7	96.0	54.4	4.3	1.9	4,719
Original MBM—Company 2	26.0	96.2	53.1	6.4	2.8	4,290
High bone meal—Company 2	44.4	93.8	42.3	14.1	6.4	2,687
Beef crax—Company 3	34.0	93.1	45.0	10.8	5.0	3,705
Screened	26.9	92.1	48.2	8.5	4.0	4,157
Screened	39.3	91.9	44.7	13.3	6.3	3,096
Screened	60.6	91.3	27.9	20.6	9.6	1,677
Chloroform flotation	8.8	94.2	70.4	2.8	1.1	4,687
Chloroform flotation	62.8	94.1	30.6	23.0	10.0	1,578

[1]Values are on an as-fed or air-dry basis.

[2]The low ash, fraction air classified, original MBM, high bone meal, and beef crax samples were obtained from commercial rendering plants and the lab screened, and chloroform filtration samples were prepared from laboratory processing of the beef crax sample. The two low ash MBM were obtained from air classification of the original MBM listed directly below them.

increased bone ash had no negative effect on AA digestibility in the two MBM samples evaluated in the previous studies.

The objective of the current study was to better define the effect of ash content on protein quality, particularly AA digestibility, in many MBM samples varying greatly in ash.

MATERIALS AND METHODS

Meat and Bone Meals

A description of the 11 MBM samples that were evaluated is shown in Table 1. The first six MBM samples were obtained from two different commercial rendering companies. The first four samples were a low-ash MBM from air classification and the original MBM from which the low-ash fraction was obtained from each of the two companies. Air classification is a process whereby air is blown through MBM to separate meat from bone on the basis of particle size and density. In addition, we obtained a high-bone MBM from the same commercial plant as the low ash and original MBM from the second company and an unground beef crax sample from a third company. The beef crax sample was acquired for use in the screening separation process described later. Beef crax is the MBM that exits the cooker before it has been finely ground; thus, it contains large particles of bone that can be easily separated. The ash content (Association of Official Analytical Chemists, 1980) of the six commercial samples varied from 16.5 to 44.4%. To produce MBM samples with even greater variation in ash, the beef crax sample was separated into low and high ash fractions using screening or chloroform floatation. In the screening method, bone shards were initially hand picked, washed, and ground to produce an all-bone or very high ash fraction. The remaining crax material was then separated into three additional fractions, by sifting through screens ranging from 850 μm to 6.3 mm in diameter, and ground. The ash content of the latter three samples varied from 26.9 to 60.6%. To obtain a lower ash MBM, we used the chloroform floatation method of Mendez and Dale (1998). Approximately 20-g samples of the ground beef crax sample were added to 500 mL of chloroform, agitated with a glass rod, and allowed to settle for 30 s. The top or floating material (mostly meat) was then decanted with the chloroform and filtered through Whatman® filter paper (#541) under vacuum. The material that sank to the bottom (mostly bone) of the beaker was similarly collected. The residual chloroform in the two fractions was evaporated under a fume hood, and the samples were then further ground. The ash content of the low and high ash fractions was 8.8 and 62.8%, respectively.

Ingredient Analysis

In addition to ash, all MBM samples were analyzed for DM, CP (N × 6.25), gross energy, Ca, and P using procedures of the AOAC (1980). All AA concentrations, except Trp, were analyzed following hydrolysis of samples in 6 *N* HCl for 24 h at 110 C by ion-exchange chromatography[3] (Spackman et al., 1958). Analyses of Met and Cys were conducted using a modified procedure of Moore (1963), in which the samples were oxidized with performic acid, diluted with deionized water, lyophilized, acid hydrolyzed as described above, and analyzed separately using ion-exchange chromatography. Selected MBM samples were also analyzed for Trp by ion-exchange chromatography following hydrolysis in LiOH.[4]

Bioassays to Determine Protein Quality

Three experiments were conducted, and all surgical and animal care procedures were approved by the University of Illinois Laboratory Animal Care Advisory Com-

[3]Amino acid analysis performed with a Beckman 6300 Analyzer, Beckman Instruments Corp., Palo Alto, CA 94302.

[4]Performed by Degussa Corp., Allendale, NJ 07401.

mittee. In Experiments 1 and 2, true digestibility of AA in selected MBM samples varying in ash were determined using the precision-fed rooster assay of Sibbald (1986). The ages of the Single Comb White Leghorn roosters were 61 and 65 wk in Experiments 1 and 2, respectively. The roosters were cecectomized at 25 wk of age using the procedure of Parsons (1985). Roosters were housed in individual cages with raised-wire floors in an environmentally controlled room with a daily photoperiod of 16 h light and 8 h darkness and had access to water and feed ad libitum. Prior to each experiment, roosters were feed deprived for 24 h, and then four roosters were crop intubated with 30 g of a MBM sample followed by 48-h collection of excreta. Feed deprived, cecectomized roosters were used to correct for endogenous AA excretion. All excreta samples were lyophilized, weighed, ground to pass through a 60-mesh screen, and analyzed for AA content as described earlier.

In Experiment 1, eight MBM samples were evaluated. The first four samples were the original MBM and the low ash fractions from air classification from the two commercial sources. The fifth sample was the high bone sample (44.4% ash). The last three samples were the 26.9, 39.3, and 60.6% ash MBM samples from the screening method. In Experiment 2, four samples were evaluated. The first three samples were the ground beef crax sample and the low and high ash samples from the chloroform floatation procedure. In the fourth sample, Ca and P (limestone and dicalcium phosphate) were added to the latter low ash sample to equal the Ca and P contents of the high ash sample. This treatment evaluated whether the high Ca and P contents of the high ash sample had any effect on AA digestibility.

The protein efficiency ratio (PER) and net protein ratio (NPR) of selected MBM samples were determined in Experiment 3. One-week-old New Hampshire x Columbian Plymouth Rock female chicks were fed a 23% CP corn-soybean meal diet for the first 7 d posthatching. By using the procedures of Sasse and Baker (1973), chicks were feed deprived overnight, weighed, and wing-banded, and four groups of five female chicks were allotted to each treatment. The chicks were housed in thermostatically controlled starter batteries with raised-wire floors. The starter batteries were kept in an environmentally controlled room with 24 h of light provided daily. Chicks were given access to feed and water ad libitum throughout the experiment.

Ten dietary treatments were evaluated. The first treatment was a N-free diet (Table 2). In the other treatments, the N-free diet was supplemented with a MBM sample as the only source of dietary protein to supply 10% CP. The MBM replaced cornstarch:dextrose on a 2:1 (wt:wt) basis. We evaluated the MBM samples that contained 16.5, 35.2, 20.6, 26.9, and 44.4% ash. Four additional treatments were evaluated in which the Ca and P contents of the low ash MBM diets (16.5 and 20.6% ash) were equalized to the levels in their original MBM counterpart or the 44.4% ash MBM. Thus, the Ca and P contents of the 16.5% ash MBM diet were increased to those of the 35.2 and 44.4% ash MBM diets, and the Ca and P contents of the 20.6% ash MBM diet were increased to those of the 26.9 and the 44.4% ash MBM diets. The increases in Ca and P were achieved by adding limestone and dicalcium phosphate in place of cornstarch and dextrose. The objective of the latter treatments was to determine if any variation in PER or NPR among the MBM samples was due to differences in dietary Ca and P levels rather than protein quality per se. The PER was calculated as body weight gain (g)/CP intake (g), and NPR was calculated as [body weight gain (g) – body weight gain (g) of chicks fed N-free diet]/CP intake (g).

Statistical Analysis

Data from the precision-fed cecectomized rooster assays and PER chick assay were subjected to ANOVA procedures using SAS® software (SAS Institute, 1985) for a completely randomized design. Statistical significance of differences among individual treatment means was then determined using the least significant difference test after the ANOVA indicated a significant ($P < 0.05$) F-value (Carmer and Walker, 1985).

RESULTS AND DISCUSSION

As expected, the CP and gross energy content of the MBM samples decreased as ash concentration increased, whereas the Ca and P contents increased as ash content increased (Table 1). These findings are in agreement with previous reports by Dale (1997), Mendez and Dale (1998), and Wang and Parsons (1998).

The AA concentrations (weight basis) in the MBM samples selected for nutritional evaluation are shown in Table 3. As expected, the concentrations of most AA decreased as ash increased. To better illustrate the effect of ash on the AA composition among the MBM samples, AA were

TABLE 2. Composition of the N-free basal diet used in the chick growth assay; Experiment 3[1]

Ingredient	Amount (%)
Cornstarch/dextrose (2:1)	89.04
Soybean oil	5.00
$MgSO_4 \cdot 7H_2O$	0.35
K_2CO_3	0.90
NaCl	0.50
Trace mineral mix[2]	0.15
Vitamin mix[3]	0.20
Choline·Cl (99%)	0.20
α-tochopheryl acetate	0.002
Ethoxyquin	0.0125

[1]Diet calculated to contain 1.0% Ca and 0.45% available P.

[2]Trace mineral mix provides the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 75 mg; iron ($FeSO_4 \cdot 7H_2O$), 75 mg; zinc (ZnO), 75 mg; copper ($CuSO_4 \cdot 5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; selenium (Na_2SeO_3), 0.1 mg.

[3]Vitamin mix provided the following (per kg of diet): thiamin·HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine·HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione, 2 mg; ascorbic acid, 250 mg; cholecalciferol, 15 μg; retinyl acetate, 1,789 μg.

TABLE 3. Amino acid concentrations in meat and bone meals (MBM) differing in ash[1]

	Percentage ash content[2]										
Amino acid	16.5	35.2	20.7	26.0	44.4	34.0	26.9	39.3	60.6	8.8	62.8
	(%)										
Asp	4.08	3.23	4.20	4.10	2.87	3.39	3.55	3.02	1.77	5.50	1.92
Thr	1.88	1.33	1.99	1.85	1.11	1.44	1.58	1.25	0.58	2.53	0.62
Ser	2.05	1.59	2.20	2.04	1.42	1.67	1.75	1.68	0.91	2.82	0.89
Glu	6.55	5.26	6.84	6.77	4.64	5.49	5.73	4.97	2.97	8.81	3.14
Pro	4.09	4.27	3.92	4.26	4.35	4.23	3.88	4.15	3.28	5.78	3.42
Gly	5.20	5.82	4.93	5.74	6.61	6.09	5.74	6.48	5.60	7.96	5.86
Ala	3.50	3.28	3.60	3.86	3.60	3.44	3.50	3.56	2.53	5.02	2.55
Val	2.46	1.78	2.65	2.55	1.71	1.92	2.13	1.66	0.76	3.32	0.85
Ile	1.74	1.21	1.91	1.81	1.00	1.25	1.39	1.08	0.47	2.21	0.51
Leu	3.55	2.48	3.82	3.64	2.25	2.68	3.08	2.38	1.08	4.70	1.14
Tyr	1.27	0.84	1.40	1.30	0.68	0.90	1.05	0.86	0.30	1.63	0.31
Phe	1.94	1.41	2.05	1.95	1.29	1.49	1.66	1.34	0.67	2.58	0.68
His	1.47	1.17	1.50	1.46	0.99	0.90	1.19	0.89	0.52	1.54	0.41
Lys	3.00	2.08	3.13	3.08	2.02	2.37	2.68	2.06	1.13	3.99	1.22
Arg	3.38	3.05	3.51	3.66	3.13	3.25	3.30	3.32	2.30	4.88	2.28
Met	0.77	0.55	0.82	0.76	0.46	0.61	0.64	0.50	0.25	1.05	0.23
Cys	0.66	0.41	0.62	0.60	0.27	0.37	0.43	0.38	0.08	0.74	0.10
Trp	0.40	0.25	0.44	0.39	0.17	NM[3]	0.33	0.21	0.06	NM	NM

[1]Values are on an as-fed or air-dry basis.

[2]The 16.5 and 35.2 and the 20.7 and 26.0% ash samples are the low ash fraction from air classification and the original MBM from two commercial sources, respectively; the 44% ash sample is a commercial high bone sample; the 34.0% ash sample is beef crax MBM; the 26.9, 39.3, and 60.6% ash samples are from lab screening of the beef crax sample; the 8.8 and 62.8% ash samples are from chloroform separation of the beef crax sample.

[3]NM = Not measured.

calculated on a per unit of CP basis (Table 4). An increase in ash content resulted in a decrease in the concentration of all essential AA per unit CP, except Arg, and increased concentrations of Pro, Gly, Ala, and Arg. For example, as ash increased from 26.9 to 60.6% in the lab screened samples, Lys was reduced from 5.6 to 4.0% of CP, and Met + Cys was reduced from 2.2 to 1.2% of CP. Conversely, as ash increased among the same MBM samples, Pro increased from 8.1 to 11.8% of CP and Gly increased from 11.9 to 20.1% of CP. The reduction in most essential AA and increases in several nonessential AA as ash increased is in agreement with earlier studies (Johnson et al., 1998).

TABLE 4. Amino acid concentrations as percentage of CP in meat and bone meals (MBM) differing in ash[1]

	Percentage ash content[2]										
Amino acid	16.5	35.2	20.7	26.0	44.4	34.0	26.9	39.3	60.6	8.8	62.8
	(%)										
Asp	7.79	7.35	7.73	7.72	6.78	7.53	7.38	6.75	6.35	7.81	6.28
Thr	3.59	3.04	3.66	3.49	2.64	3.20	3.28	2.81	2.06	3.60	2.03
Ser	3.91	3.62	4.04	3.84	3.36	3.71	3.64	3.75	3.25	4.01	2.90
Glu	12.5	12.00	12.57	12.76	10.96	12.20	11.89	11.12	10.64	12.51	10.26
Pro	7.80	9.72	7.21	8.04	10.29	9.40	8.06	9.28	11.75	8.21	11.18
Gly	9.92	13.26	9.06	10.81	15.63	13.53	11.90	14.50	20.07	11.31	19.15
Ala	6.68	7.47	6.61	7.27	8.52	7.64	7.26	7.95	9.07	7.13	8.33
Val	4.69	4.07	4.86	4.81	4.04	4.27	4.43	3.72	2.71	4.72	2.77
Ile	3.32	2.75	3.51	3.41	2.37	2.78	2.89	2.42	1.68	3.14	1.65
Leu	6.77	5.65	7.02	6.85	5.31	5.96	6.41	5.31	3.88	6.68	3.73
Tyr	2.42	1.92	2.58	2.45	1.61	2.00	2.17	1.92	1.07	2.32	1.02
Phe	3.70	3.21	3.78	3.67	3.06	3.31	3.46	3.00	2.41	3.66	2.23
His	2.80	2.66	2.75	2.75	2.33	2.00	2.47	1.99	1.87	2.19	1.33
Lys	5.73	4.74	5.76	5.80	4.78	5.27	5.57	4.61	4.05	5.67	4.00
Arg	6.45	6.96	6.45	6.89	7.40	7.22	6.86	7.42	8.24	6.93	7.44
Met	1.47	1.24	1.50	1.44	1.08	1.36	1.34	1.11	0.89	1.49	0.75
Cys	1.26	0.94	1.14	1.13	0.63	0.82	0.88	0.85	0.27	1.05	0.31
Trp	0.76	0.57	0.81	0.74	0.40	NM[3]	0.68	0.47	0.22	NM	NM

[1]Values are on an as-fed or air-dry basis.

[2]The 16.5 and 35.2 and the 20.7 and 26.0% ash samples are the low ash fraction from air classification and the original MBM from two commercial sources, respectively; the 44% ash sample is a commercial high bone sample; the 34.0% ash sample is beef crax MBM; the 26.9, 39.3, and 60.6% ash samples are from lab screening of the beef crax sample; the 8.8 and 62.8% ash samples are from chloroform separation of the beef crax sample.

[3]NM = Not measured.

TABLE 5. True amino acid digestibility coefficients for meat and bone meals (MBM) differing in ash; Experiment 1[1]

	Percentage ash content[2]								
Amino acid	16.5	35.2	20.7	26.0	44.4	26.9	39.3	60.6	Pooled SEM
	(%)								
Asp	82.4^{a}	75.7^{b}	61.0^{d}	61.3^{d}	77.0^{ab}	80.1^{ab}	75.5^{bc}	69.0^{c}	2.25
Thr	85.9^{a}	80.8^{ab}	80.9^{ab}	80.7^{ab}	84.1^{a}	81.3^{ab}	82.2^{ab}	77.6^{b}	1.99
Ser	83.1	77.9	79.8	78.9	82.6	79.8	83.3	77.2	2.83
Glu	87.5^{a}	82.3^{ab}	79.4^{b}	80.4^{b}	80.8^{b}	84.1^{ab}	83.0^{ab}	72.0^{c}	2.00
Pro	86.4^{a}	80.8^{b}	80.5^{b}	83.9^{ab}	79.7^{b}	89.3^{a}	86.1^{ab}	71.3^{c}	2.60
Ala	87.7^{a}	82.5^{abc}	80.1^{bc}	81.9^{abc}	79.1^{c}	85.9^{ab}	85.1^{abc}	70.5^{d}	2.10
Val	85.7^{a}	81.3^{a}	80.9^{a}	81.4^{a}	83.1^{a}	80.9^{a}	81.2^{a}	70.2^{b}	2.05
Ile	86.5^{a}	82.0^{ab}	82.4^{ab}	83.2^{ab}	82.9^{ab}	78.7^{b}	81.3^{ab}	71.2^{c}	2.26
Leu	89.3^{a}	84.8^{ab}	83.3^{b}	84.1^{ab}	85.8^{ab}	84.0^{ab}	84.7^{ab}	74.7^{c}	1.96
Tyr	81.7^{a}	74.2^{a}	80.3^{a}	78.1^{a}	75.9^{a}	70.6^{a}	73.8^{a}	56.8^{b}	3.85
Phe	96.1	93.9	90.5	90.7	93.9	90.8	94.0	93.7	2.25
His	85.3^{a}	83.8^{a}	74.5^{b}	82.8^{ab}	91.0^{a}	88.8^{a}	85.0^{a}	88.2^{a}	3.08
Lys	86.3^{a}	82.0^{ab}	77.4^{b}	78.5^{b}	85.0^{a}	86.1^{a}	81.0^{ab}	77.6^{b}	2.00
Arg	91.7^{a}	86.0^{ab}	87.0^{ab}	88.3^{ab}	82.2^{b}	89.2^{a}	88.3^{ab}	72.8^{c}	2.22
Met	86.6^{a}	82.1^{a}	80.0^{ab}	80.5^{ab}	81.6^{ab}	75.3^{bc}	72.8^{c}	68.9^{c}	2.32
Cys	65.3^{bc}	53.4^{c}	66.6^{bc}	68.1^{abc}	90.7^{a}	49.2^{c}	63.3^{c}	87.9^{ab}	8.13
Trp[3]	81.7	74.9	79.7	75.0	76.2	76.2	75.3	68.5	. . .

$^{a-d}$Means within a row with no common superscripts are significantly different ($P \leq 0.05$).

[1]Mean values of four cecectomized roosters per sample.

[2]The 16.5 and 35.2 and the 20.7 and 26.0% ash samples are the low ash fraction from air classification and the original MBM from two commercial sources, respectively; the 44% ash sample is a commercial high bone sample; the 26.9, 39.3, and 60.6% ash samples are from lab screening of a beef crax sample.

[3]Trp values are calculated from one pooled excreta samples from four cecectomized roosters.

Because the protein in bone is approximately 83% collagen (Eastoe and Long, 1960) and collagen is deficient in most essential AA, a high bone concentration will negatively affect the AA profile or AA balance in MBM.

In Experiment 1, there was little or no significant effect of increased ash content on digestibility of AA for the original and low ash, air classified MBM samples from the two commercial sources (Table 5; 16.5 vs. 35.2% ash and 20.7 vs. 26.0% ash). Moreover, digestibilities of most AA in the 44.4% ash high bone MBM were generally similar and not significantly different ($P > 0.05$) from the 20.7 and 26.0% ash MBM that came from the same company and processing plant. Comparison of the screened samples (26.9, 39.3 and 60.6% ash) revealed that AA digestibility was not significantly affected as ash increased from 26.9 to 39.3%; however, as the concentration of ash increased to 60.6%, the digestibility of most AA, except Cys, was reduced. For example, as ash increased from 26.9 to 60.6%, Lys digestibility decreased from 86 to 78%, whereas Cys digestibility increased from 49 to 88%, respectively. When reviewing the results for the commercial MBM and lab screened samples, the lack of an effect of ash content on AA digestibility for MBM varying in ash from 16 to 44% is in agreement with an earlier study from our laboratory (Johnson et al., 1998) that found no significant difference in AA digestibility for two MBM samples containing 24 or 35% ash.

In Experiment 2, there were no significant differences ($P > 0.05$) in AA digestibility between the 34.0% ash beef crax sample and the 8.8% ash sample from chloroform separation (Table 6). In contrast, digestibilities of all AA except Cys in the 62.8% ash sample were lower than those in the 34 and 8.8% ash samples ($P \leq 0.05$). The latter observation generally agrees with the results of Experiment 1 for the 60.6% ash MBM sample obtained from screening. Adding limestone and dicalcium phosphate to the 8.8% ash sample, to equalize its Ca and P to the 62.8% ash sample, had no effect ($P > 0.05$) on AA digestibility. These results indicate that the lower AA digestibility of the 62.8% ash sample was not due to its high Ca and P contents.

The combined results of Experiments 1 and 2 clearly indicate that ash levels in the range of 8.8 to 44.4% have little or no effect on AA digestibility. There does seem to be some reduction in AA digestibility for samples containing an excess of 60% ash. The latter samples were essentially 100% bone. The reason for the reduced AA digestibility in the 61 to 63% ash samples is unknown. The reduced AA digestibility in the latter samples is of little practical importance because the ash content of commercial MBM samples is seldom less than 16% or more than 35%.

Results of the chick growth assay (Experiment 3) showed that increased ash content in MBM samples had a definite negative effect on PER and NPR values (Table 7). Comparison of the first two commercial MBM samples (Treatments 2 and 3) revealed that the PER and NPR values of the 35.2% ash MBM were significantly lower ($P < 0.05$) than those of the 16.5% ash MBM. When the two MBM samples from the other company (Treatments 6 and 7) were compared, the PER and NPR values of the 26.9% ash sample were numerically lower than those of the 20.6% ash MBM sample. The lack of a significant difference between the PER and NPR of the two latter samples was probably largely due to only a 6% difference

TABLE 6. True amino acid digestibility coefficients for meat and bone meals (MBM) differing in ash, calcium and phosphorus; Experiment 2[1]

Amino acid	Percentage ash content[2]				
	34.0	8.8	8.8 + Ca/P[3]	62.8	Pooled SEM
	(%)				
Asp	74.0[a]	75.7[a]	75.4[a]	58.6[b]	5.25
Thr	88.3[a]	89.5[a]	86.7[a]	64.8[b]	5.04
Ser	86.5[a]	88.1[a]	87.9[a]	58.8[b]	5.57
Glu	85.3[a]	87.9[a]	88.2[a]	62.6[b]	4.75
Pro	84.0[a]	86.6[a]	88.1[a]	61.1[b]	5.40
Ala	84.3[a]	88.4[a]	88.0[a]	60.3[b]	5.00
Val	87.0[a]	88.8[a]	86.5[a]	62.2[b]	4.63
Ile	88.0[a]	89.3[a]	88.7[a]	63.0[b]	4.47
Leu	89.4[a]	91.0[a]	90.6[a]	63.8[b]	4.48
Tyr	90.3[a]	90.8[a]	85.6[a]	30.9[b]	3.77
Phe	93.6[a]	95.2[a]	100.8[a]	76.8[b]	4.76
His	78.2[a]	80.5[a]	72.2[a]	49.6[b]	5.70
Lys	86.4[a]	89.4[a]	89.0[a]	71.9[b]	3.62
Arg	88.7[a]	92.4[a]	91.6[a]	62.4[b]	4.80
Met	86.0[a]	88.0[a]	88.0[a]	52.6[b]	4.90
Cys	82.0	79.0	81.3	68.2	9.47

[a-b]Means within a row with no common superscripts are significantly different ($P \leq 0.05$).

[1]Mean values of four cecectomized roosters per sample.

[2]The 34.0% ash is beef crax MBM; the 8.8 and 62.8% ash are from the chloroform-separated beef crax MBM.

[3]Calcium and phosphorus were added using dicalcium phosphate and limestone to equalize the Ca and P to that of the 62.8% ash MBM.

in ash concentration. Moreover, the PER and NPR values of the 44.4% ash MBM were much lower than those of the 20.6 and 26.9% ash MBM from the same company and the 16.5 and the 35.2% ash MBM samples from the other commercial source.

Increasing the Ca and P contents of the 16.5% ash MBM diet to equal the 35.2 and 44.4% ash MBM diets resulted in a significant reductions ($P < 0.05$) in PER and NPR values (Treatment 2 vs. 4 and 5). Likewise, increasing the Ca and P contents of the 20.6% ash MBM diet to equal the 44.4% ash MBM diet also resulted in a significant reduction ($P < 0.05$) in PER and NPR values (Treatment 6 vs. 9). However, the PER and NPR values of the 16.5% ash MBM diets containing increased Ca and P were still much higher than those of the 35.2 and 44.4% ash MBM diets. Similarly, the PER and NPR values of the 20.6% ash diet with increased Ca and P were much higher than those for the 44.4% ash MBM diet. These results indicate that the lower PER and NPR values for the highest ash MBM diets were primarily due to a decrease in protein quality and not high dietary Ca and P levels. These results agree with limited earlier work from our lab (Johnson and Parsons, 1997) in which increasing the Ca and P content of a 24% ash MBM diet to that of a 35% ash

TABLE 7. Effect of varying levels of ash, Ca, and P on the protein quality of meat and bone meals (MBM); Experiment 3[1]

Dietary treatment[2]	Dietary Ca	Dietary P	Weight gain	Gain:feed	PER[3]	NPR[4]
	(%)		(g)	(g:g)	(%)	
1) N-free diet	1.00	0.45	−12.3[g]	−0.121[g]	...	...
2) 10% CP from 16.5% ash MBM	1.00	0.45	69.9[a]	0.334[a]	3.34[a]	3.93[a]
3) 10% CP from 35.2% ash MBM	2.78	1.07	34.1[e]	0.209[e]	2.09[f]	2.85[f]
4) As 2 + Ca + P equal to Diet 3	2.78	1.07	63.9[ab]	0.302[b]	3.02[b]	3.61[b]
5) As 2 + Ca + P equal to Diet 10	3.43	1.52	59.1[b]	0.291[b]	2.91[bc]	3.52[bc]
6) 10% CP from 20.6% ash MBM	1.00	0.45	48.3[cd]	0.253[cd]	2.53[de]	3.18[de]
7) 10% CP from 26.9% ash MBM	1.24	0.50	42.0[de]	0.234[de]	2.34[ef]	3.03[ef]
8) As 6 + Ca + P equal to Diet 7	1.24	0.50	54.4[bc]	0.272[bc]	2.72[cd]	3.34[cd]
9) As 6 + Ca + P equal to Diet 10	3.43	1.52	39.0[de]	0.216[e]	2.16[f]	2.84[f]
10) 10% CP from 44.4% ash MBM	3.43	1.52	9.6[f]	0.072[f]	0.72[g]	1.68[g]
Pooled SEM			3.6	0.011	0.10	0.08

[a-g]Means within a column with no common superscripts are significantly different ($P \leq 0.05$).

[1]Means of four groups of five female chicks each from 8 to 18 days posthatching; average initial weight = 98.4 g.

[2]The 16.5 and 32.5% ash MBM and the 20.6 and 26.9% ash MBM are the low ash fraction from air classification and the original MBM from two commercial sources, respectively; the 44% ash sample is a commercial high bone sample.

[3]PER = protein efficiency ratio = weight gain (g) ÷ protein intake (g).

[4]NPR = net protein ratio = [weight gain (g) − weight gain (g) of chicks fed N-free diet] ÷ protein intake (g).

MBM diet did not significantly affect ($P < 0.05$) PER and NPR values.

Protein quality, as defined by Boorman (1992), is the ability of a feedstuff to supply essential AA relative to an animal's metabolic needs. Protein quality includes the total AA balance or profile and the bioavailability of the total AA. The three experiments in this study have shown that AA digestibility of MBM is not affected by levels of ash within a range that is normally encountered commercially. The overall protein quality of MBM, however, is negatively affected by increased ash content. The latter effect is almost entirely due to negative effects on AA balance or profile of the MBM, not reduced AA digestibility.

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