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Original Research Article

Protein digestive dynamics of meat and bone meals in broiler chickens

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ABSTRACT

This study determined the variations in protein digestibilities and digestion rates in broiler chickens offered diets containing 7 different meat and bone meals (MBM). A total of 252 male Ross 308 broiler chickens were offered 7 atypical diets largely based on maize and MBM from 24 to 28 d post-hatch. Each experimental diet was offered to 6 replicates with 6 birds per replicate cage. Excreta were collected in their entirety from 25 to 27 d post-hatch and on 28 d post-hatch. Digesta samples were collected from the proximal jejunum, distal jejunum, proximal ileum and distal ileum. Apparent digestibilities of protein were determined in each segment and apparent digestibilities of amino acids were measured in the distal ileum. There were significant differences in apparent protein digestibility coefficients in the proximal jejunum ($P = 0.006$), where broiler chickens offered the high ash beef meal (diet 7) generated the lowest protein digestibility in the proximal jejunum (0.318). Similarly, there were significant differences in apparent digestibility coefficients in the distal jejunum ($P < 0.001$) and distal ileum ($P < 0.001$) but not in the proximal ileum. More pronounced differences were found in the disappearance rate of protein and there were significant differences in all 4 segments of the small intestine ($P < 0.001$). Broiler chickens offered the high ash beef meal had the lowest protein disappearance rate ($P < 0.001$). No difference was observed in the predicted protein digestion rate ($P = 0.486$) but chickens offered the high ash beef meal had the lowest potential digestible protein (0.662, $P = 0.034$) whereas the highest potential digestible protein (0.739) was detected in diet 5 (containing a beef meal). This study contributed to the establishment of a preliminary database to include digestion rates of starch and protein into practical diet formulation.

1. Introduction

Growth performance and feed conversion efficiency may be determined by both the extent and rate of starch and protein digestion (Liu and Selle, 2015). Glucose provides energy and amino acids provide the building blocks for muscle protein deposition (Pelley and Goljan, 2011). Therefore, the synchrony of starch and protein digestion, glucose and amino acid absorption may be important for optimal protein utilisation and feed conversion efficiency. The importance of the kinetics of carbohydrate and protein digestion in the rumen has long been recognized and considered in practical feed formulation. The relevance of starch and protein digestive dynamics in broiler chickens has been confirmed by Liu et al. (2014, 2013, 2016a, 2016b). Therefore, the present study was conducted to determine the digestion rate of protein in broiler chickens offered different sources of meat and bone meal (MBM). This study belongs the series of 6 experiments to quantify digestion rates of starch and protein in cereal grains, plant-based and animal source protein meals (Liu et al., 2020, 2019).

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MBM is used as an alternative source of protein for broilers in Australia and overseas (Ravindran et al., 2002); however, the inconsistency of its quality as a feedstuff has restricted its inclusion in animal diets. MBM contains 30% to 60% of crude protein with amino acid profiles and digestibility varying depending on their source, origin and rendering method (Adedokun et al., 2007; Ravindran et al., 2002; Wang and Parsons, 1998). Indeed, Ravindran et al. (2002) determined chemical composition, apparent protein and amino acid digestibilities of 19 MBM and reported notable variations in histidine, methionine and cysteine concentrations. Moreover, MBM which undergoes severe treatment and contains hair, skin and bone often results in poor protein digestibility (Kim et al., 2012). Additionally, the digestibility of protein and amino acids of complete diets may be also inconsistent and suboptimal in broiler chickens offered diets containing MBM. Ravindran et al. (2002) suggested MBM was a good source of non-essential amino acids (Glu, Gly, Asp, Pro and Ala) compared to the other protein meals. Nevertheless, Bolarinwa et al. (2012) reported high inclusion rate of MBM impaired protein digestibility in broilers and may have depressed growth performance in broiler chickens.

Therefore, the objective of the present study was to determine the variations in protein digestion rates of MBM in broiler chickens. This was coupled with the determination of protein digestion rates in commonly used protein meals and cereal grains from 3 other studies (unpublished), in order to allow commercial nutritionists to formulate complete diets based on starch and protein digestion rates and assess the merits of this strategy. Given the inconsistency of protein quality in MBM, the hypothesis was that broiler chickens offered various MBM sources would generate different protein digestion rates.

2. Materials and methods

2.1. Experimental design

All of the experimental procedures involving animals were approved by the Animal Ethics Committee of the University of Sydney (Project number 2016/1016). The study comprised of 7 dietary treatments containing different meat bone meals sourced from the Australian industry. Six replicates were included for each treatment and 6 birds were accommodated in each cage. Hence, a total of 252 male Ross 308 broilers were randomly allocated into 42 cages and they were offered experimental diets from 24 to 28 d post-hatch. The origin and label of tested meat bone meals are reported in Table 1. The analysed amino acid and mineral profiles of the test MBM are shown in Table 2. Table 3 reports the digestible amino acids, crude fat and ash concentrations as determined by near infrared spectroscopy (NIR).

2.2. Diet preparation

Seven atypical experimental diets were formulated to be iso-energetic (12.75 MJ/kg) and iso-nitrogenous (195 g/kg CP) as shown in Table 4. Diets were formulated without synthetic amino acid inclusion to avoid any manipulation of protein digestion rates and they are similar to diets evaluated in Ravindran et al. (2005). To evenly mix and pellet experimental diets, maize was used as the base of the diet; however, maize and the 7 MBM were analysed for chemical composition prior to finalization of feed formulation. Maize was included in the diet formulation to facilitate feed intake and prevent weight loss which may be the challenge of measuring digestibilities in MBM. The digestive dynamics of the maize utilised in this study was reported in Moss et al. (2019). The maize was hammer-milled through 4.0-mm screen before mixing with other ingredients. All the diets were cold-pelleted. A dietary marker (Celite World Minerals, Lompoc, CA, USA) was included at 20 g/kg in diets as an inert acid insoluble ash (AIA) marker in order to determine nutrient digestibility coefficients in 4 small intestinal sites. A commercial starter diet based on wheat with 12.13 MJ/kg energy and 220 g/kg CP, was offered to broiler chickens from 0 to 23 d post-hatch.

Table 1
The origin and label of tested meat bone meals (MBM).

Diet	Code	Origin	Label
1	MBM1	Manildra, New South Wales	Ovine meal
2	MBM2	Manildra, New South Wales	Low ash 21% ovine meal
3	MBM3	Laverton, Victoria	Bovine/ovine meal 25% ash
4	MBM4	Dinmore, Queensland	Mixed bovine/ovine meal
5	MBM5	New South Wales	SWI beef meal
6	MBM6	Laverton, Victoria	Mixed bovine/ovine meal
7	MBM7	Dinmore, Queensland	High ash beef meal

2.3. Bird management

At 24 d post-hatch, a total of 252 male Ross 308 broilers were individually identified (wing-tags), weighed and allocated into bioassay cages (6 birds per cage), on the basis of body weights. Bird allocation was such that cage means and variations were almost identical. Thereafter, birds were offered the experimental diets until 28 d post-hatch. Birds had unlimited access to feed and water under an '18-h-on-6-h-off' lighting regime in an environmentally controlled facility. An initial room temperature of 32 ± 1 °C was maintained for the first week, which was gradually decreased to 22 ± 1 °C by the end of the third week and maintained at this temperature for the duration of the feeding study.

2.4. Sample collection and chemical analysis

Amino acid concentrations in MBM and digesta were analysed by the following procedures described in Cohen (2001) and (Truong et al., 2015). For elemental analysis, minerals were analysed on an ICP Emission Spectrometer (iCAP6000 Series) according to manufacturer's instructions (Thermo Electron Corporation, Waltham, M.A., USA).

Initial and final body weights of the birds were determined at 24 and 28 d post-hatch, respectively. Feed intake was measured for the experimental period. Dead and weak birds were identified daily and culled after recording their body weights to correct the feed conversion ratio (FCR). During 25 to 27 d post-hatch, feed intake and excreta output were recorded to determine dry matter basis apparent metabolizable energy (AME), N corrected apparent metabolizable energy (AMEn), N retention, and excreta moisture. Moreover, water intake and excreta dry matter were also monitored during 25 to 27 d post-hatch. Water was offered in separate feeders and the volume was recorded at the beginning and end of the excreta collection period.

Following total excreta collection, excreta was oven dried (air-forced) for 24 h until no further loss of moisture under 80 °C. The gross energy of diets and excreta were determined via bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL, USA). The AME was calculated by the following equation:

$$AME_{diet} = [(\text{Feed intake} \times \text{Gross energy}_{diet}) - (\text{Excreta output} \times \text{Gross energy}_{excreta})]/\text{Feed intake}\ .$$

AMEn values were calculated by correcting to zero N retention, using the factor of 36.54 kJ/g (Hill and Anderson, 1958).

Table 2
The analysed amino acid and mineral profile in test meat bone meals (MBM) (as-is, g/kg).

Item	MBM1	MBM2	MBM3	MBM4	MBM5	MBM6	MBM7	Mean	SD	CV, %
Analysed proximate nutrition composition										
Dry matter	962	961	940	960	951	938	945	951	10.2	1
Crude protein	588	607	505	500	549	509	454	530	53.8	10
Crude fat	100	105	128	127	65	118	84	104	23.2	22
Ash	270	305	318	315	285	270	255	288	24.7	9
Analysed amino acid concentration										
His	8.8	9.7	7.4	9.5	10.6	7.2	4.7	8.3	2.00	24
Ser	19.9	21.4	18.3	17.2	17.4	17.7	11.9	17.7	2.97	17
Arg	36.8	38.7	31.2	29.7	30.8	30.7	28.0	32.3	3.93	12
Gly	54.6	54.6	47.6	49.2	53.5	48.0	56.7	52.0	3.68	7
Asp	34.9	37.5	30.0	31.6	32.4	30.3	22.9	31.4	4.58	15
Glu	61.6	65.7	52.1	48.2	48.0	52.1	39.9	52.5	8.71	17
Thr	17.6	19.2	14.7	14.6	14.6	14.5	9.0	14.9	3.19	21
Ala	30.9	31.8	26.7	28.0	30.0	26.8	25.8	28.6	2.33	8
Pro	37.4	38.1	32.6	33.1	35.2	33.4	35.5	35.0	2.14	6
Lys	25.4	27.4	22.5	23.7	25.2	22.5	16.9	23.4	3.35	14
Tyr	11.8	13.1	9.9	8.9	8.7	9.4	5.4	9.6	2.45	26
Met	7.1	7.8	5.3	5.1	5.8	5.3	4.4	5.8	1.20	21
Val	21.4	22.9	18.7	19.5	20.3	18.3	11.5	18.9	3.65	19
Ile	15.1	16.4	12.8	10.5	9.5	12.6	7.7	12.1	3.08	25
Leu	32.7	35.3	27.8	29.5	30.3	27.0	16.8	28.5	5.88	21
Phe	18.0	19.3	15.2	16.8	17.7	15.0	10.2	16.0	2.99	19
Analysed mineral concentration										
Ca	93.6	69.9	73.3	99.4	125.9	75.4	187.9	103.6	41.97	41
P	46.2	37.2	37.1	46.8	59.3	38.4	87.9	50.4	18.30	36
Na	6.47	6.08	6.17	4.49	4.83	6.22	5.55	5.69	0.76	13
K	5.61	6.07	4.83	1.45	1.31	4.82	1.03	3.59	2.22	62
Cu	0.01	0.01	0.01	0.01	0.00	0.02	0.00	0.01	0.006	64
Fe	0.31	0.37	0.85	0.74	0.44	0.62	0.04	0.48	0.277	57
Mg	2.16	1.80	1.73	1.89	2.32	1.80	3.35	2.15	0.572	27
Mn	0.03	0.03	0.03	0.02	0.01	0.03	0.00	0.02	0.012	57
Sr	0.18	0.13	0.10	0.15	0.16	0.12	0.30	0.16	0.065	40
Zn	0.09	0.09	0.09	0.08	0.07	0.09	0.08	0.08	0.006	8

SD = standard deviation; CV = coefficients of variation.

Table 3
Digestible amino acids, crude fat and ash concentrations in meat bone meals (MBM) and corn analysed by near infrared spectroscopy (g/kg).

Item	MBM1	MBM2	MBM3	MBM4	MBM5	MBM6	MBM7	Corn
Met	5.77	6.15	5.15	4.34	4.67	5.24	3.45	1.37
Cys	1.41	1.48	1.23	1.41	1.20	1.23	0.58	1.44
Met + Cys	7.65	8.10	6.83	6.41	6.22	6.89	4.08	2.87
Lys	19.58	20.61	18.45	17.85	20.44	18.59	14.54	2.05
Thr	11.08	11.60	10.18	10.00	10.50	10.14	6.75	2.26
Trp	2.08	2.25	1.91	1.88	1.94	1.90	0.87	0.47
Arg	31.34	32.29	25.99	24.76	27.49	26.37	25.37	2.91
Ile	11.00	11.67	9.90	8.22	8.34	9.92	6.32	2.35
Leu	23.60	24.80	21.97	23.43	25.09	21.82	14.97	7.64
Val	16.05	16.81	14.87	16.04	16.91	14.74	10.80	3.17
His	7.26	7.67	6.62	7.40	8.29	6.63	4.53	1.95
Phe	13.03	13.63	12.03	13.29	13.92	11.96	8.97	3.15
Crude fat	100	105	128	127	65	118	Outlier	35
Ash	269	245	259	281	316	260	Outlier	11

The N retention was calculated by the following equation:

$$\text{N retention (\%)} = 100 \times [(\text{Feed intake} \times \text{N}_{\text{diet}}) - (\text{Excreta output} \times \text{N}_{\text{excreta}})]/(\text{Feed intake} \times \text{N}_{\text{diet}}).$$

At 28 d post-hatch, birds were euthanized by an intravenous injection of sodium pentobarbitone 3 h after the chicken house was illuminated. Feed intake for 24 h prior to the sampling was recorded to determine the mean retention time (MRT). Abdominal incisions were made to separate 4 sections in the small intestine for instance, proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI). They were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction and their midpoints. Afterward, digesta in each section was collected separately and pooled by cages. Digesta samples were homogenized and freeze dried to determine the MRT and apparent digestibility of protein and amino acids.

$$\text{MRT (min)} = (1{,}440 \times \text{AIA}_{\text{digesta}} \times W)/(\text{Feed intake}_{24\text{ h}} \times \text{AIA}_{\text{feed}}),$$

where $\text{AIA}_{\text{digesta}}$ is the AIA concentration in the digesta (mg/g), W is the weight of dry gut content (g), $\text{Feed intake}_{24\text{ h}}$ is the feed intake over 24 h before sampling (g), and AIA_{feed} is the AIA concentration in the feed (mg/g).

The N and AIA concentrations in both feed and digesta were analysed using method described by Siriwan et al. (1993). Apparent digestibility coefficients of starch and N were calculated by the following equation:

$$\text{Digestibility coefficient} = [(\text{Nutrient}/\text{AIA})_{\text{diet}} - (\text{Nutrient}/(\text{AIA})_{\text{digesta}}]/(\text{Nutrient}/\text{AIA})_{\text{diet}}.$$

Protein (N) disappearance rates (g/bird per d) were deduced from the following equation:

$$\text{Disappearance rate (g/d per bird)} = \text{Average daily feed intake from 24 to 28 d post-hatch (g/bird)} \times \text{Dietary nutrient (g/kg)} \times \text{Nutrient digestibility (apparent digestibility coefficient)}.$$

The pattern of fractional digestibility coefficients was calculated as previously described in Liu et al. (2013). Briefly, it is derived by relating the digestion coefficient at each site with the digestion time (t), which was calculated from the sum of the MRT determined in each intestinal segment. The curve of digestion is often described

Table 4
Diet compositions, calculated nutrient specifications and analysed amino acid concentrations in experimental diets (g/kg).

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Ingredients							
Corn	733	736	670	670	691	683	628
Test MBM	244	236	293	296	267	289	332
Soybean oil	0.2	5	13.9	10.4	18.2	4.9	16.4
Salt	0	0.05	0	0.35	0.44	0	0
Choline chloride 60%	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Celite	20	20	20	20	20	20	20
Calculated nutrient specifications							
AMEn, MJ/kg	12.75	12.75	12.75	12.75	12.75	12.75	12.75
Crude protein	195	195	195	195	195	195	195
Dig. Lys	6.24	6.33	6.74	6.62	6.84	6.74	6.08
Dig. Met + Cys	3.85	3.91	3.82	3.72	3.54	3.84	3.06
Dig. Thr	4.25	4.29	4.39	4.37	4.26	4.37	3.57
Dig. Val	6.02	6.08	6.28	6.67	6.50	6.22	5.39
Dig. Ile	4.22	4.29	4.30	3.84	3.67	4.30	3.41
Dig. Arg	9.80	9.78	9.58	9.30	9.37	9.63	10.27
Total P	12.8	10.3	12.3	15.3	17.3	12.5	30.5
Available P	10.6	8.3	10.1	12.8	14.6	10.3	26.6
Ca	23.3	16.9	21.9	29.8	34.0	22.2	62.7
Ash	86	92	113	113	96	98	104
Na	1.72	1.60	1.94	1.60	1.60	1.93	1.96
K	4.67	4.74	4.43	3.44	3.46	4.47	3.17
Cl	2.14	2.04	2.30	2.08	2.11	2.30	2.30
Crude fat	50	55	75	71	59	63	66
Analysed amino acid concentration[2]							
His	3.21	3.52	3.36	3.88	3.92	3.22	2.77
Ser	6.16	6.53	6.67	6.55	6.18	6.43	5.59
Arg	9.14	9.53	9.53	9.39	9.23	9.26	10.10
Gly	13.50	13.10	14.20	15.20	15.70	13.90	19.90
Asp	10.40	11.20	11.10	11.30	10.90	10.90	9.90
Glu	21.10	22.40	21.50	20.50	19.80	21.60	19.90
Thr	5.14	5.61	5.37	5.32	5.02	5.19	4.22
Ala	9.47	9.72	9.94	10.45	10.43	9.66	10.95
Pro	11.75	11.94	12.43	12.64	12.82	12.33	15.05
Lys	6.45	6.97	7.07	7.37	7.26	6.78	6.26
Tyr	2.57	3.01	2.93	2.80	2.70	2.93	2.26
Met	1.97	2.09	1.98	1.88	1.99	2.03	1.83
Val	6.32	6.79	6.87	7.20	6.93	6.59	5.43
Ile	4.52	4.96	4.81	4.21	3.82	4.71	3.80
Leu	11.82	12.72	12.23	12.74	12.30	11.91	9.98
Phe	5.75	6.19	6.07	6.50	6.33	5.90	5.16
Crude protein	182	193	199	190	182	186	200

MBM = meat bone meal; AMEn = N corrected apparent metabolizable energy; Dig. = digestible.
[1] Vitamin-trace mineral premix supplies per kilogram of diet: retinol 12 MIU, cholecalciferol 5 MIU, tocopherol 50 mg, menadione 3 mg, thiamine 3 mg, riboflavin 9 mg, pyridoxine 5 mg, cobalamin 0.025 mg, niacin 50 mg, pantothenate 18 mg, folate 2 mg, biotin 0.2 mg, copper 20 mg, iron 40 mg, manganese 110 mg, cobalt 0.25 mg, iodine 1 mg, molybdenum 2 mg, zinc 90 mg, selenium 0.3 mg.
[2] Analyses were conducted in duplicates.

by the exponential model developed by Orskov and McDonald (1979):

$$D_t = D_\infty \left(1 - \mathrm{e}^{-kt}\right),$$

where D_t (g/g N) is the protein (N) digested at time t (min), the fraction D_∞ is the amount of potential digestible protein (N) (asymptote) (g/g), k (per unit time, per min) is defined as digestion rate constant. This mathematical model is applied with the assumptions that amino acid absorption does not take place proximal to the small intestine.

2.5. Statistical analysis

Assay data were analysed by one-way ANOVA to determine the significance effect of treatments on responses, Pearson correlation was performed to determine the correlation coefficients between the responses, and Fisher's least significant difference (LSD) was used to compare the mean values of responses. All data were analysed by using JMP Pro 14 (SAS 2016 Institute Inc, JMP Software, Cary, NC, USA).

3. Results

The results of the broilers offered diets containing different MBM on growth performance and nutrient utilisations are presented in Table 5. Broilers offered the diet with MBM2 had the highest ($P < 0.001$) weight gain (301 g/bird) and feed intake (698 g/bird). Broilers offered the diet with MBM 7 generated the lowest weight gain (77 g/bird) and feed intake (496 g/bird). Broilers offered MBM 7 generated the worst FCR whereas those offered MBM2 showed the best FCR (6.569 versus 2.327) compared to those fed other MBM diets ($P < 0.001$). There were significant differences in AME, AME:GE and AMEn in broiler chickens offered diets containing different MBM. Broilers offered MBM 7 had inferior AME (13.11 MJ/kg) and AMEn (12.23 MJ/kg) in comparison to the other experimental diets. There were considerably lower water intake and excreta moisture obtained from the broilers fed diets with MBM 7 ($P < 0.001$).

Table 5
The influence of different meat bone meals (MBM) on growth performance of broilers from 24 to 28 d post-hatch and nutrient utilization of broilers from 25 to 27 d post-hatch.

Item	Weight gain, g/bird	Feed intake, g/bird	FCR, g/g	AME, MJ/kg	AME:GE, MJ/MJ	N retention, %	AMEn	Water intake, mL/d per bird	Water: Feed intake ratio	Excreta moisture, %
Diet 1	256^{bc}	676^{a}	2.655^{bc}	15.11^{b}	0.850^{b}	63.9	13.73^{a}	114^{bc}	1.166	54.8^{b}
Diet 2	301^{a}	698^{a}	2.327^{c}	15.41^{a}	0.848^{bc}	63.8	13.91^{ab}	125^{abc}	1.242	60.5^{ab}
Diet 3	279^{ab}	683^{a}	2.450^{c}	15.39^{a}	0.839^{cd}	66.9	13.75^{ab}	135^{a}	1.341	61.6^{a}
Diet 4	252^{c}	640^{b}	2.552^{c}	15.11^{b}	0.835^{d}	55.7	13.89^{ab}	127^{abc}	1.321	54.9^{b}
Diet 5	184^{d}	551^{c}	3.000^{b}	14.80^{c}	0.864^{a}	62.3	13.67^{b}	109^{c}	1.322	61.7^{a}
Diet 6	275^{bc}	668^{ab}	2.460^{c}	15.25^{ab}	0.837^{cd}	53.6	14.05^{a}	129^{ab}	1.314	61.5^{a}
Diet 7	77^{e}	496^{d}	6.569^{a}	13.11^{d}	0.848^{bc}	54.4	12.23^{c}	82^{d}	1.176	46.7^{c}
SEM	9.2	11.7	0.1528	0.072	0.004	3.99	0.125	6.3	0.0659	2.09
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.123	<0.001	<0.001	0.300	<0.001

FCR = feed conversion ratio; AME = apparent metabolizable energy; GE = gross energy; AMEn = N corrected apparent metabolizable energy; SEM = pooled standard error of mean.
[a, b, c, d, e] Within a column, mean values with unlike superscript letters were significantly different ($P < 0.05$).

The influence of dietary treatment on apparent digestibility of protein (N) and apparent disappearance rate in 4 sites of the small intestine is shown in Table 6. There were significant differences in apparent digestibility coefficients of protein (N) in the proximal jejunum, distal jejunum and distal ileum of the broiler chickens. Broiler chickens offered MBM 7 generated the lowest protein digestibility in the proximal jejunum (0.318, $P = 0.006$). However, the apparent digestibility coefficient of protein in broiler chickens offered MBM 7 compensated and there was no statistical differences between MBM 1, 2 and 7 on apparent protein digestibility in the distal jejunum. The highest apparent protein digestibility in the distal jejunum was generated by chickens offered diets containing MBM 5 ($P < 0.001$). Similarly, the highest distal ileal protein digestibility (0.771) and potential digestible protein (0.739 g/g) were observed in broiler chickens offered diets containing MBM 5 ($P < 0.001$). The disappearance rates of protein (N) in the 4 sites of the small intestine are shown in Table 6. There were significant differences on apparent disappearance rates in all 4 segments of the small intestine. Broiler chickens offered MBM 3 obtained the highest protein disappearance rate, whereas birds offered MBM 7 generated the lowest protein disappearance rate ($P < 0.001$) across all the 4 segments of the small intestine.

The influence different MBM had on apparent amino acid digestibility coefficient in the distal ileum is documented in Table 7. Broilers offered diet 5 generated the highest ($P < 0.001$) coefficient values for all the analysed amino acids. Overall, methionine presented the highest digestible coefficient but aspartic acid showed lowest digestible coefficient values of 0.841 and 0.632, respectively in distal ileum of broilers. Moreover, essential amino acids obtained higher digestible coefficients compared to the digestible coefficients of non-essential amino acids in broilers (0.767 vs. 0.718).

4. Discussion

4.1. Variation in protein digestive dynamics

Digestive dynamics of starch and protein play important roles in broiler performance and protein digestion rate tended to have greater impact than starch digestion rate (Liu and Selle, 2015). The physical and chemical properties of different protein sources in broiler diets determine the site and rate of the absorption of amino acids along the small intestine. Therefore, it is important to consider digestive dynamics of different types of protein (rapid, slow, medium) in diets to obtain optimum growth performance while ensuring the amino acid requirements in broilers.

Eighty-three percent of the total protein in the bone is collagen (Eastoe and Long, 1960) and collagen is poorly digestible (Ravindran et al., 2002). Since Ca and P in MBM are mainly derived from the bone, high ash or Ca and P contents often means high collagen content. In the present study, dietary Ca and P concentrations were negatively correlated with the digestibility coefficient of N in the proximal jejunum ($r = -0.553$ and $r = -0.557$, $P < 0.001$) which may suggest MBM used in the high Ca and P diets may contain higher collagen content which is harder to be digested. Interestingly, the high ash beef meal (diet 7) had the lowest proximal jejunum protein digestibility (0.318) but the second highest distal ileal protein digestibility. The 136% increase in apparent protein digestibility from the proximal jejunum (0.318) to the distal ileum (0.751) has led to the numerically highest protein digestion rate (0.331 per min, $P = 0.486$) observed in broiler chickens offered diet 7. In the present study, there was no significant differences in predicted protein digestion rate in the small intestine ($P = 0.486$).

Table 6
The influence of different meat bone meals (MBM) on apparent digestibility of protein (N) and apparent disappearance rate in the proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI), potential digestible protein (PDP) and protein digestion rate (PDR) in the small intestine of broiler chickens at 28 d post-hatch.

Item	Digestibility coefficients				Disappearance rate, g/d per bird				PDR, per min	PDP, g/g
	PJ	DJ	PI	DI	PJ	DJ	PI	DI		
Diet 1	0.484^{a}	0.598^{cd}	0.689	0.682^{d}	9.821^{cd}	12.127^{cd}	13.958^{bc}	13.861^{c}	0.055	0.664^{c}
Diet 2	0.575^{a}	0.581^{d}	0.674	0.682^{d}	12.649^{ab}	12.834^{bc}	14.892^{ab}	15.069^{b}	0.168	0.669^{bc}
Diet 3	0.584^{a}	0.637^{bc}	0.734	0.735^{bc}	13.163^{a}	14.328^{a}	16.517^{a}	16.526^{a}	0.073	0.711^{abc}
Diet 4	0.575^{a}	0.668^{ab}	0.698	0.742^{ab}	11.404^{abc}	13.211^{b}	13.827^{bc}	14.666^{bc}	0.087	0.717^{ab}
Diet 5	0.465^{a}	0.685^{a}	0.733	0.771^{a}	7.706^{d}	11.327^{d}	12.170^{c}	12.758^{d}	0.174	0.739^{a}
Diet 6	0.512^{a}	0.627^{bc}	0.707	0.703^{cd}	10.506^{bc}	12.852^{bc}	14.509^{b}	14.408^{bc}	0.152	0.696^{abc}
Diet 7	0.318^{b}	0.618^{cd}	0.646	0.751^{ab}	5.050^{e}	9.703^{e}	10.150^{d}	11.816^{e}	0.331	0.662^{bc}
SEM	0.0490	0.0150	0.0320	0.0110	0.8780	0.2990	0.6542	0.2923	0.0710	0.0171
P-value	0.006	<0.001	0.478	<0.001	<0.001	<0.001	<0.001	<0.001	0.486	0.034

SEM = pooled standard error of mean.
[a, b, c, d, e] Within a column, mean values with unlike superscript letters were significantly different ($P < 0.05$).

Table 7
The influence of different meat bone meals (MBM) on apparent digestibility coefficients of amino acids in distal ileum in broiler chickens at 28 d post-hatch.

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	SEM	*P*-value
His	0.727^{c}	0.729^{c}	0.781^{b}	0.793^{b}	0.835^{a}	0.770^{b}	0.778^{b}	0.0102	<0.001
Ser	0.643^{ef}	0.635^{f}	0.696^{cd}	0.732^{b}	0.776^{a}	0.669^{de}	0.711^{bc}	0.0102	<0.001
Arg	0.775^{c}	0.767^{c}	0.819^{ab}	0.824^{ab}	0.840^{a}	0.805^{b}	0.777^{c}	0.0086	<0.001
Gly	0.667^{c}	0.691^{bc}	0.712^{b}	0.760^{a}	0.779^{a}	0.693^{bc}	0.691^{bc}	0.0119	<0.001
Asp	0.528^{e}	0.549^{de}	0.592^{c}	0.705^{b}	0.773^{a}	0.67^{cd}	0.706^{b}	0.0117	<0.001
Glu	0.725^{d}	0.724^{d}	0.779^{bc}	0.796^{b}	0.839^{a}	0.767^{c}	0.780^{bc}	0.0101	<0.001
Thr	0.627^{d}	0.629^{d}	0.700^{bc}	0.719^{b}	0.761^{a}	0.671^{c}	0.695^{bc}	0.0114	<0.001
Ala	0.750^{d}	0.762^{cd}	0.804^{b}	0.814^{ab}	0.837^{a}	0.787^{bc}	0.766^{cd}	0.0099	<0.001
Pro	0.675^{d}	0.696^{cd}	0.721^{bc}	0.736^{ab}	0.753^{a}	0.708^{bc}	0.674^{d}	0.0110	<0.001
Lys	0.673^{d}	0.667^{d}	0.770^{bc}	0.797^{b}	0.831^{a}	0.752^{c}	0.769^{c}	0.0097	<0.001
Tyr	0.643^{c}	0.661^{c}	0.740^{ab}	0.735^{b}	0.789^{a}	0.762^{ab}	0.722^{b}	0.0175	<0.001
Met	0.803^{b}	0.812^{b}	0.864^{a}	0.852^{a}	0.878^{a}	0.860^{a}	0.815^{b}	0.0095	<0.001
Val	0.706^{de}	0.698^{e}	0.759^{bc}	0.774^{b}	0.817^{a}	0.739^{cd}	0.739^{bc}	0.0117	<0.001
Ile	0.698^{c}	0.693^{c}	0.771^{ab}	0.762^{ab}	0.797^{a}	0.749^{b}	0.762^{b}	0.0122	<0.001
Leu	0.768^{c}	0.763^{c}	0.817^{b}	0.814^{b}	0.856^{a}	0.802^{b}	0.822^{b}	0.0104	<0.001
Phe	0.761^{cd}	0.758^{d}	0.817^{ab}	0.808^{b}	0.845^{a}	0.802^{b}	0.788^{bc}	0.0103	<0.001

SEM = pooled standard error of mean.
$^{a, b, c, d, e, f}$ Within a column, mean values with unlike superscript letters were significantly different ($P < 0.05$).

Protein digestion rate was predicted by a fitting exponential model to describe the relationship between apparent digestibility coefficients and their corresponding mean retention time in various segments of the small intestine. The 41% differences between the highest and lowest feed intake may have confounded the rate of digestion by influencing passage rate, retention time and apparent digestibility coefficients. Apparent disappearance rate takes consideration of feed intake and significant differences were observed in all 4 segments of the small intestine. For example, broiler chickens offered diet 7 generated the highest protein digestion rate due to the dramatic increase in apparent protein digestibility from 0.318 in the proximal jejunum to 0.751 in the distal ileum. However, they had the lowest disappearance rate of protein in all segments of the small intestine ($P < 0.001$). This has been reflected on their poorest weight gain and feed conversion efficiency. Therefore, more pronounced variations were observed in apparent disappearance rates than digestibility coefficients and predicted protein digestion rates.

4.2. Variations in amino acid and mineral content

Analysed results of the 7 MBM sources showed that there was a substantial difference in crude protein (454 to 607 g/kg), crude fat (65 to 128 g/kg), ash (255 to 318 g/kg), Ca (73.3 to 187.9 g/kg) and P (37.1 to 87.9 g/kg) concentrations with coefficients of variation (CV) of 10%, 22%, 9%, 41% and 36%, respectively (Table 2). Among the 16 amino acids determined in the present study, isoleucine showed the highest variation with CV of 25.5% (7.7 to 16.4 g/kg), whereas proline showed the lowest CV of 6.12% (33.1 to 38.1 g/kg). As expected, MBM 7, which was labelled as the high ash beef meal, contained the highest Ca (187.9 g/kg) and P (87.9 g/kg). Sodium concentrations are sometimes overlooked in commercial feed formulation; however, high dietary sodium concentration may depress 'extra-phosphoric' responses to phytase in broiler chickens (Ravindran et al., 2008). Therefore, it is important to determine Na concentrations in feed ingredients.

Analysed amino acid contents in the diets and MBM samples were strongly correlated ($r = 0.940$, $P < 0.01$) which was independent to the maize and MBM inclusion levels in each diet. Moreover, dietary amino acids derived from MBM are highly correlated with the total amino acid contents in the diet ($r = 0.962$, $P < 0.01$) which means the small variations in MBM and maize inclusions of experimental diets should not confound the comparison between different MBM.

There are a few studies that have reported the inconsistent compositions (ash, crude protein, crude fat and amino acids composition) of MBM due to the differences in sources, origins, and rendering conditions (Ravindran et al., 2002; Wang and Parsons, 1998). Ravindran et al. (2002) indicated that variations of tissues, such as tendons, ligaments, skeletal muscles, lungs, intestinal tissues and bones in MBM is the main reason for variations of nutrient compositions in MBM. It was also reported that the increasing level of ash might lead to decreased crude protein, crude fat and gross energy in different MBM (Dale, 1997; Ravindran et al., 2002). In contrast, the correlation between ash content and crude protein and crude fat was not significant in MBM tested in the present study. The gross content of amino acids in MBM reflected that higher levels of non-essential amino acids compared to essential amino acids. Hendriks et al. (2002) reported that non-essential amino acids content ranged between 42 and 74 g/kg in MBM which is slightly higher than the range (31.4 to 52.5 g/kg) tested from the present study. In the present study, glycine and glutamic acid were more prominent in MBM. Previous studies showed the largest variation in methionine content of MBM (Hendriks et al., 2002; Ravindran et al., 2002) and the smallest variation in proline concentrations; however, the present study detected the largest variation in isoleucine and the smallest variation in glycine.

4.3. Variation in growth performance and nutrient utilisation

The results of the overall growth performance in the present study was inferior to the Ross 308 performance objective (Aviagen, 2019) in weight gain (633 vs 232 g/bird), feed intake (977 vs 630 g/bird) and FCR (1.54 vs 3.12) from 24 to 28 d post-hatch. This is not surprising as atypical diets largely based on maize and MBM were offered to broiler chickens. The primary objective of the present study was to determine the digestive dynamics of protein and amino acids in broiler chickens offered diets containing different MBM in order to generate preliminary database for least-cost feed formulation based on digestion rates. In the literature, growth performance is often not recorded or reported in digestibility studies; however, they are important indicators of how animals compromised growth in response to atypical diets. It is possible sub-optimal growth performance may have confounded digestibilities reported in this study and in the literature. Moreover, synthetic amino acids are considered instantly digestible and may confound the protein digestion rate; therefore, synthetic amino acids, including lysine,

methionine and threonine were not included in the experimental diets. Strong negative correlations ($P < 0.001$) were detected between the weight gain and dietary concentrations of certain non-essential amino acids including glycine ($r = -0.928$), proline ($r = -0.894$) and alanine ($r = -0.805$). Addition of animal by-products may cause amino acid imbalance in broilers due to their high levels of non-essential amino acids. Moreover, atypical diets were formulated to be iso-energetic without balancing mineral content. Consequently, this may have contributed to the sub-optimal growth performance and feed conversion efficiency in the present study.

Indeed, AME was negatively correlated with Ca ($r = -0.963$, $P < 0.01$) and P ($r = -0.968$, $P < 0.01$) concentrations in the diet. Similarly, the apparent disappearance rate of protein in the distal ileum was negatively correlated with Ca ($r = -0.721$, $P < 0.01$) and P ($r = -0.716$, $P < 0.01$) concentrations in the diet. High inclusion rate of MBM has depressed AME in broiler chickens as reported by Olson et al. (1961). Bolarinwa et al. (2012) reported that higher bone/ash level in MBM has negative impact on AME in broilers. Similarly, Ravindran et al. (2002) found negative correlation between the gross energy and ash content in the diet. In contrast, there is no correlation between the dietary ash content and AME in the present study. Instead, Ca and P was negatively correlated with AME, whereas K and Na was positively correlated with AME. Therefore, despite the initial purpose of the study was to compare MBM quality in broiler chickens and diets were not balanced with Ca, P and Na in order to maximise the comparison, future studies investigating MBM quality need to consider protein and mineral qualities separately. Moreover, in the present study, weight gain was negatively correlated with apparent distal ileal digestibility of histidine ($r = -0.378$, $P < 0.05$), glutamic acid ($r = -0.512$, $P < 0.01$), threonine ($r = -0.409$, $P < 0.01$), lysine ($r = -0.426$, $P < 0.01$), valine ($r = -0.416$, $P < 0.001$), isoleucine ($r = -0.397$, $P < 0.001$) and leucine ($r = -0.467$, $P < 0.001$). FCR was positively correlated with apparent distal ileal digestibility of aspartic acid ($r = 0.418$, $P < 0.01$). Again, this suggested atypical diets used in the present study which were formulated to be iso-energetic without balancing amino acid profile may have led to the sub-optimal growth performance and feed conversion efficiency in the present study.

Francesch and Brufau (2004) suggested that excessive dietary protein may lead to high water intake in broilers and the excessive protein may have to be catabolised and excreted as uric acid. In the present study, imbalanced protein and mineral profiles in experimental diets might have caused high water intake and excreta moisture in broilers offered diet 7.

5. Conclusions

The amino acid and mineral composition of different MBM obtained from various sources in Australia were inconsistent. Consequently, digestive dynamics of protein and distal ileal amino acid digestibility were modified in broiler chickens offered diets containing different MBM. This study is one of the series to quantify protein digestion rate in order to apply the concept of synchronised digestive dynamics into the least-cost feed formulation. Despite the differences in chemical composition and apparent digestibilities of protein and amino acids, the predicted protein digestion rate along the small intestine was not significant in broiler chickens offered diets containing different MBM. Future studies will be conducted to quantify protein digestive dynamics in other common protein meals used in Australia and the relevance of the preliminary database will be verified in the complete diet.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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