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Original research article

The influence of meat-and-bone meal and exogenous phytase on growth performance, bone mineralisation and digestibility coefficients of protein (N), amino acids and starch in broiler chickens

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ABSTRACT

The objective of this study was to examine the influence of meat-and-bone meal (MBM) and phytase inclusion on growth performance, bone mineralisation and apparent digestibility coefficients of nutrients in broiler chickens offered wheat-based diets. The feeding study comprised 7 dietary treatments: positive control (PC, 9.0% Ca and 4.5% available phosphorous [AvP] in starter, 7.0% Ca and 3.5% AvP in finisher); negative control (NC, 7.2% Ca and 3.0% AvP in starter, 5.2% Ca and 2.0% AvP in finisher) diets incorporating a 3 × 2 factorial array of 3 MBM inclusions (0, 60, 120 g/kg) and 2 levels of phytase supplementation (0 and 1,000 FYT/kg). Each treatment was allocated to 6 replicated pens with 30 birds per pen in an environmentally-controlled deep litter facility. A total of 1,260 one-day-old male Ross 308 chicks were offered starter diets from 1 to 14 days post-hatch and finisher diets from 15 to 36 days post-hatch. There were significant ($P < 0.05$) interactions between MBM inclusions and phytase supplementation on weight gain and feed intake in starter diets. Phytase significantly increased weight gain in diets without MBM and did not influence weight gain in diets with 60 and 120 g/kg MBM. Collectively, increasing MBM inclusion significantly reduced weight gain in starter diets ($P < 0.0001$). There were dietary interactions ($P < 0.01$) on toe ash where phytase significantly improved toe ash in diet without MBM and did not influence toe ash in the other two groups of negative control diets. There were no dietary treatment interactions on apparent ileal digestibility coefficients of starch and protein except that diets without MBM had significantly ($P < 0.01$) lower ileal starch digestibility and diets with 120 g/kg MBM had significantly ($P < 0.0001$) lower ileal protein digestibility. No dietary influence on ileal fat digestibility was observed. There were dietary interactions on ileal digestibilities of isoleucine, valine and glycine. Phytase significantly increased glycine digestibility in diets with 60 and 120 g/kg MBM but not in diets without MBM. Including 120 g/kg MBM significantly ($P < 0.01$) depressed apparent digestibility coefficients of 13 *ex* 16 amino acids in the distal ileum. This study demonstrated the negative impacts of MBM on amino acid digestibility and growth performance. Also, responses to phytase were more pronounced in diets without MBM, which may have been due to their relatively lower available P and higher phytate concentrations in comparison to diets containing MBM.

1. Introduction

Meat-and-bone meal (MBM) is a potential source of protein, calcium and phosphorus in poultry diets for some parts of the world, including Australia, China and Southeast Asia. However, there are considerable variations in its protein quality and Ca and P concentrations. Ravindran et al. (2002) reported substantial variations in ash (13.0–56.5 g/100 g), crude protein (38.5–67.2 g/100 g), crude fat (4.3–15.3 g/100 g) and apparent ileal digestibility of amino acids in 19 MBM samples from different rendering plants.

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Batterham et al. (1986b) reported that the availability of lysine in 8 MBM ranged from 0.68 to 0.88 for broiler chickens. The variations and poor amino acid digestibilities in MBM may be due to processing damage and the bone to soft tissue ratios (Eastoe and Long, 1960). Nowadays, it is well accepted that the quality of MBM is variable; therefore, MBM is analysed for chemical compositions prior to incorporation into broiler diets. In Australia, the inclusion rate of MBM in broiler diets rarely exceeds 60 g/kg. There are limited reported studies investigating the impact of higher MBM inclusions on growth performance and nutrient utilisation in broiler chickens and the majority of previous studies were based on maize-based diets. One objective of this study was to investigate the influence of nil, standard and high MBM inclusions on growth performance, bone mineralisation, apparent digestibility coefficients and nutrient utilisation in broiler chickens offered wheat-based diets. The hypothesis was that the standard inclusion of 60 g/kg MBM may not depress growth performance and nutrient digestion in broiler chickens whereas the higher inclusion of 120 g/kg MBM may show negative impacts on broiler performance and protein digestion.

Phytate and phytate-bound phosphorus (P) is ubiquitous in plant-sourced feed ingredients and phytate limits P bioavailability and poses ecological problems as excreted P pollutes the environment. Phytate-degrading feed enzymes or phytases have been shown to liberate phytate-bound P and improve protein and energy utilisation (Ravindran, 1995; Selle and Ravindran, 2007). However, the majority of studies in the literature evaluated the beneficial influence of phytase in broiler diets with only plant source feed ingredients such as soybean meal and canola meal. As discussed above, MBM is potentially an important source for Ca and P in poultry diets in some parts of the world and because MBM does not contain phytate, MBM containing diets could be less likely to respond to exogenous phytase. Therefore, it is desirable to investigate the impact of phytase on the performance of broilers offered diets containing MBM. In the present study, exogenous phytase was included in diets with 3 MBM inclusion levels with the hypothesis that phytase responses in diets containing MBM would be less pronounced than control diets not containing any MBM.

2. Materials and methods

This feeding study complied with the specific guidelines of the Animal Ethics Committee of the University of Sydney. The feeding study comprised 7 dietary treatments as tabulated in Table 1. It included positive control (PC) diet with 9.0% Ca and 4.5% available phosphorous (AvP) in starter, 7.0% Ca and 3.5% AvP in finisher and negative control diets with NC, 7.2% Ca and 3.0% AvP in starter, 5.2% Ca and 2.0% AvP in finisher. Negative control diets were supplemented with 60 or 120 g/kg MBM (10.27% Ca, 4.95% P and 0.61% Na), without or with phytase (1,000 FYT/kg, 1 FYT is defined as the activity that releases 1 μmol of inorganic phosphate from 5.0 mM sodium phytate per minute at pH 5.5 and 37 °C, RONOZYME HiPhos). In finisher diets, the analysed Ca and P concentrations were 9.4% and 5.9%, respectively, in the PC diet, and on average, the NC diets contained 6.1% of Ca and 6.1% of total P. Wheat-based diets were formulated to meet the nutritional requirements of broiler chickens as shown in Table 2. In the MBM supplemented diets, the inclusion levels of wheat, soybean and vegetable oil were adjusted accordingly to obtain similar protein and energy concentrations in all 4 dietary treatments. Analysed protein and amino acid in finisher diets are shown in Table 3. The birds were offered starter diets from 1 to 14 days post-hatch and finisher diets from 15 to 36 days post-hatch. The starter diets were fed as mash; whereas, the finisher diets were steam-pelleted through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 80 °C by the automatically controlled introduction of steam into the conditioner with a residence time of 14 s. Finally, the pelleted diets were cooled in a vertical cooler to room temperature and crumbled. Wheat was hammer-milled (3.2 mm screen) prior to dietary incorporation. Acid insoluble ash (Celite World Minerals, Lompoc, CA, USA) was included in finisher diets at 15 g/kg as an inert marker to determine apparent digestibility coefficients of protein, amino acids and starch at the distal ileum.

Table 1
The arrangement of experimental diets for broiler chickens.

Diet	Type	Meat and bone meal, g/kg	Phytase, FYT/kg
1	Positive control	0	0
2	Negative control	0	0
3	Negative control	0	1,000
4	Negative control	60	0
5	Negative control	60	1,000
6	Negative control	120	0
7	Negative control	120	1,000

A total of 1,260 one-day-old male chicks (Ross 308) were placed in 42 pens (6 replicates per treatment and 30 chicks per pen) in the environmentally-controlled deep litter facility. The birds had unlimited access to feed and water under a 24-h lighting regime of 23 light:1 dark for the first three days followed by 16 light:8 dark for the rest of the feeding period. An initial room temperature of 32 ± 1 °C was maintained for the first week, gradually decreased to 22 ± 1 °C by the end of the third week and maintained at the same temperature until the end of the feeding study. Body weight and feed intake were recorded fortnightly from which feed conversion ratios (FCR) were calculated. The incidence of dead or culled birds was recorded daily and their body-weights were used to adjust FCR calculations.

At day 36, five birds close to the average body weight in each pen were selected and euthanised by intravenous injection of sodium pentobarbitone and the small intestine removed. Digesta samples were collected in their entirety from the distal ileum, which were demarcated by the mid-point between Meckel's diverticulum and the ileo-caecal junction. Left middle toes were collected and pooled for determination of ash content and mineral concentrations. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 550 °C for 16 h. Then the ash content was weighed and analysed for mineral concentrations by plasma mass spectrometry. Digesta samples from birds within a pen were pooled, homogenised, freeze-dried and weighed for chemical analyses. Nitrogen concentrations and acid insoluble ash (AIA) concentrations were determined as outlined by Siriwan et al. (1993). Fat concentration was determined in finisher phase by using the automated Soxhlet extraction as described by Luque de Castro and Priego-Capote (2010). Starch concentration in diets and digesta were determined by a procedure based on dimethyl sulphoxide, -amylase and amyloglucosidase, as described by Mahasukhonthachat et al. (2010). Amino acid analyses were completed in duplicates as outlined by Cohen and Michaud (1993) and cysteine and tryptophan were not determined by this extraction method.

Apparent digestibility coefficients of nitrogen were calculated by the following equation:

$$\text{Digestibility Coefficient} = \frac{(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{digesta}}}{(\text{Nutrient/AIA})_{\text{diet}}}$$

Two-way ANOVA was employed to determine the main effects (MBM inclusion and phytase supplementation) and their interactions by a general linear model procedure using JMP 9.0.0 (SAS Institute Inc. JMP Software, Cary, NC). The experimental units were

Table 2
Dietary composition and calculated nutrient specifications in starter (1–14 days post-hatch) and finisher (15–36 days post-hatch) experimental diets.

Item	Starter[1]				Finisher[1]			
	PC	NC1	NC2	NC3	PC	NC1	NC2	NC3
Ingredient, g/kg								
Wheat	637.7	654.7	666.7	678.7	741.6	750.8	762.8	771.1
Soybean meal	281.0	276.2	204.6	133.0	181.1	172.2	100.6	33.1
Meat and bone meal	0.0	0.0	60.0	120.0	0.0	0.0	60.0	120.0
Vegetable oil	41.6	37.1	36.1	35.1	44.1	39.5	38.5	37.5
NaCl	2.3	2.3	1.3	0.5	1.6	1.5	0.7	0.0
Sodium bicarbonate	2.0	2.0	2.0	1.6	1.8	2.0	1.7	1.0
DL-methionine	2.6	2.5	2.7	2.9	2.5	2.5	2.7	2.8
Lysine HCl	2.7	2.8	3.4	3.9	3.4	3.5	4.0	4.6
Threonine	1.1	1.1	1.3	1.6	1.6	1.7	1.9	2.2
Limestone	8.0	8.4	8.5	8.6	6.7	7.2	7.3	7.3
Dicalcium phosphate	15.4	7.3	8.0	8.6	10.0	1.6	2.3	2.9
Choline chloride 60	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin–mineral premix[2]	5.0	5.0	5.0	5.0	5.0	2.0	2.0	2.0
Celite	0.0	0.0	0.0	0.0	15.0	15.0	15.0	15.0
Nutrient level, g/kg								
Metabolisable energy, kcal/kg	3,060.0	3,060.0	3,060.0	3,060.0	3,148.0	3,148.0	3,148.0	3,148.0
Protein	214.0	214.0	214.0	214.0	181.9	181.9	181.9	182.0
Calcium	9.0	7.2	7.2	7.2	7.0	5.2	5.2	5.2
Total phosphorus	7.3	5.6	5.3	5.0	5.8	4.2	3.9	3.6
Phytate-P[3]	2.68	2.69	2.39	2.10	2.45	2.43	2.13	1.85
Lysine	12.5	12.5	12.5	12.5	10.5	10.5	10.5	10.5
Methionine	5.7	5.7	5.8	6.0	5.2	5.2	5.3	5.5
Methionine + cysteine	9.4	9.4	9.4	9.4	8.5	8.5	8.5	8.5
Threonine	8.5	8.5	8.5	8.5	7.6	7.6	7.6	7.6
Sodium	1.8	1.8	1.8	1.8	1.5	1.5	1.5	1.5
Potassium	8.5	8.4	7.3	6.2	6.8	6.7	5.6	4.4
Chloride	2.4	2.4	2.4	2.4	2.1	2.1	2.1	2.2

[1] PC, positive control; NC1, negative control diet without MBM; NC2, negative control diet with 60 g/kg MBM; NC3, negative control diet with 120 g/kg MBM.
[2] The vitamin–mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.
[3] Phytate-P calculation was based on 2.20 g/kg phytate-P in wheat and 4.53 g/kg phytate-P in soybean meal as reported in Selle et al. (2003).

pooled pen means and differences were considered significant at $P < 0.05$ by Students' t-test.

3. Results

The mortality rate during the experimental period of 2.94% was not influenced by dietary treatment. Birds offered PC diet had significantly higher weight gain (2,427 versus 2,311 g/bird, $P < 0.005$) and FCR (1.550 versus 1.585, $P < 0.05$) than Ross 308 performance objective by one sample *t*-test. The influence of dietary treatments on growth performance during the starter and finisher phases are shown in Table 4. Meat and bone meal significantly ($P < 0.05$) compromised FCR in starter phase by 6.5% from the average of 1.381 in diets with 0 and 60 g/kg MBM to 1.471 in diets with 120 g/kg MBM. Inclusion of 60 g/kg MBM significantly ($P < 0.0001$) depressed weight gain by 4.3% in finisher phase (2,088 versus 1,998 g/bird) and by 3.9% during the total feeding period (2,501 versus 2,403 g/bird); whereas inclusion of 120 g/kg MBM further ($P < 0.0001$) reduced weight gain by 8.7% in finisher phase (2,088 versus 1,808 g/bird) and by 13.4% during the total feeding period (2,501 versus 2,165 g/bird). In starter phase, phytase significantly ($P < 0.05$) increased weight gain by 7.8% in diets without MBM (398 versus 429 g/bird) but did not influence weight gain in broiler chickens offered diets with 60 and 120 g/kg MBM. Also, phytase tended ($P < 0.10$) to improve FCR by 2.9% in starter phase (1.431 versus 1.390). There were interactions ($P < 0.05$) between MBM inclusions and phytase supplementation for FCR in the finisher and total feeding phases. In the finisher phase, phytase numerically improved FCR by 4.0% in diets without MBM (1.618 versus 1.554) and by 4.5% in diets with 120 g/kg MBM (1.913 versus 1.827). Similarly, from 1 to 36 days post-hatch, phytase numerically reduced FCR by 3.8% in diets without MBM (1.583 versus 1.523) and by 4.4% in diets with 120 g/kg MBM (1.844 versus 1.764).

Table 3
Analysed protein and amino acid concentrations (g/kg, dry matter basis) in finisher diets.

Item	Diets						
	1	2	3	4	5	6	7
Protein	184.4	183.7	185.4	190.4	184.1	196.2	195.1
Histidine	3.9	3.8	3.8	3.7	3.4	3.8	3.5
Serine	7.4	7.2	7.2	6.9	6.6	7.1	6.8
Arginine	9.4	9.1	9.1	9.3	9.1	9.6	9.4
Glycine	5.8	5.7	5.7	8.6	10.5	8.6	11.0
Aspartic acid	12.5	12.2	12.2	11.4	10.1	11.7	10.4
Glutamic acid	38.1	37.6	37.5	36.3	35.3	37.4	35.6
Threonine	6.7	6.9	6.8	7.1	6.4	7.2	6.9
Alanine	5.6	5.5	5.4	6.5	7.1	6.6	7.3
Proline	11.3	11.1	11.1	12.3	13.3	12.6	13.6
Lysine	9.8	10.0	9.8	10.2	9.3	10.6	10.3
Tyrosine	3.7	3.5	3.4	3.3	3.1	3.4	3.3
Methionine	4.0	4.1	3.9	4.4	4.2	4.5	4.7
Valine	7.2	7.1	7.1	7.1	6.8	7.3	6.9
Isoleucine	6.1	6.0	6.0	5.6	5.1	5.9	5.2
Leucine	10.9	10.9	10.8	10.4	10.0	10.8	10.2
Phenylalanine	7.6	7.4	7.4	7.0	6.5	7.2	6.7

The influence of MBM inclusion and phytase supplementation on toe ash and apparent ileal digestibility of starch, protein and fat are shown in Table 5. Negative control diets had significantly lower toe ash than the PC diet (10.46 versus 12.08%, $P < 0.005$). Phytase did not influence ($P > 0.05$) distal ileal digestibilities of starch, protein and fat. However, 120 g/kg MBM significantly reduced apparent ileal digestibility of protein by 10.2% (0.805 versus 0.723, $P < 0.0001$). There was an interaction ($P < 0.005$) between MBM and phytase for toe ash where phytase significantly increased toe

Table 4
The influence of dietary treatments on growth performance in broiler chickens.

Treatments	MBM, g/kg	Phytase, FYT/kg	1–14 days post-hatch			15–36 days post-hatch			1–36 days post-hatch		
			Weight gain, g/bird	Feed intake, g/bird	FCR, g/g	Weight gain, g/bird	Feed intake, g/bird	FCR, g/g	Weight gain, g/bird	Feed intake, g/bird	FCR, g/g
2	0	0	398^{b}	562^{ab}	1.411	2,043	3,300	1.618^{bc}	2,441	3,862	1.583^{bc}
3	0	1,000	429^{a}	587^{a}	1.372	2,132	3,310	1.554^{c}	2,561	3,897	1.523^{c}
4	60	0	407^{ab}	563^{ab}	1.384	2,041	3,229	1.582^{bc}	2,448	3,792	1.549^{bc}
5	60	1,000	403^{b}	546^{b}	1.357	1,956	3,279	1.684^{b}	2,359	3,825	1.628^{b}
6	120	0	361^{c}	542^{b}	1.500	1,808	3,457	1.913^{a}	2,169	4,000	1.844^{a}
7	120	1,000	353^{c}	507^{c}	1.442	1,809	3,303	1.827^{a}	2,161	3,810	1.764^{a}
SEM			7.911	11.140	0.0293	37.205	61.360	0.0395	40.335	65.930	0.0327
Main effects: MBM, g/kg											
0			414	575	1.391^{b}	2,088^{a}	3,305	1.586	2,501^{a}	3,879	1.553
60			405	555	1.370^{b}	1,998^{b}	3,254	1.633	2,403^{b}	3,809	1.588
120			357	525	1.471^{a}	1,808^{c}	3,380	1.870	2,165^{c}	3,905	1.804
Phytase, FYT/kg											
0			389	556	1.431	1,964	3,329	1.704	2,353	3,884	1.659
1,000			395	547	1.390	1,965	3,297	1.689	2,360	3,844	1.638
Significance (*P*-value)											
MBM			<0.0001	<0.001	0.037	<0.0001	0.125	<0.0001	<0.0001	0.327	<0.0001
Phytase			0.353	0.347	0.097	0.959	0.533	0.633	0.824	0.460	0.445
Interaction			0.034	0.034	0.858	0.092	0.211	0.048	0.056	0.153	0.043
Positive control			397	553	1.391	2,030	3,206	1.581	2,427	3,759	1.550
P-value[1]			0.887	0.314	0.405	0.749	0.031	0.387	0.737	0.023	0.321

MBM = meat and bone meal; SEM = pooled standard error of mean.
[a,b,c] Means within columns not sharing a common suffix are significantly different ($P < 0.05$).
[1] Comparison between treatments 1 and 2.

Table 5
Influence of meat-and-bone meal and phytase supplementation on ileal digestibility of starch, protein (N), fat, and percentage toe ash in broiler chickens at 36 days post-hatch.

Treatments	MBM, g/kg	Phytase, FYT/kg	Starch	Protein (N)	Fat	Toe ash, %
2	0	0	0.765	0.803	0.879	10.46^{d}
3	0	1,000	0.773	0.807	0.887	11.63^{c}
4	60	0	0.855	0.784	0.900	12.34^{ab}
5	60	1,000	0.856	0.816	0.869	12.01^{bc}
6	120	0	0.822	0.730	0.857	12.62^{a}
7	120	1,000	0.818	0.716	0.843	12.86^{a}
SEM			0.0251	0.0122	0.0264	0.2021
Main effects: MBM, g/kg						
0			0.769^{b}	0.805^{a}	0.883	11.04
60			0.855^{a}	0.800^{a}	0.884	12.17
120			0.820^{ab}	0.723^{b}	0.850	12.74
Phytase, FYT/kg						
0			0.814	0.772	0.879	11.80
1,000			0.816	0.780	0.866	12.16
Significance (*P*-value)						
MBM			0.007	<0.0001	0.341	<0.0001
Phytase			0.932	0.463	0.570	0.038
Interaction			0.971	0.182	0.760	0.003
Positive control			0.802	0.813	0.868	12.08
P-value[1]			0.351	0.551	0.682	0.004

MBM = meat and bone meal; SEM = Pooled standard error of mean.
[a,b,c,d] Means within columns not sharing a common suffix are significantly different ($P < 0.05$).
[1] Comparison between treatments 1 and 2.

ash in diets without MBM (10.46 versus 11.63%) but did not influence toe ash in diets with 60 and 120 g/kg MBM.

The influence of dietary treatments on mineral concentrations in toe ash of broiler chickens at 36 days post-hatch is shown in Table 6. There were no dietary influences on Ca, Mg, P and Cu concentrations in toe ash. Negative control diet without MBM had significantly higher K and Na concentrations in toe ash than the other experimental diets ($P < 0.05$). Inclusion of MBM significantly ($P < 0.05$) decreased Zn concentration from 369 mg/kg to the average of 341 mg/kg in the diets with 60 and 120 g/kg MBM. Phytase significantly ($P < 0.05$) increased Sr concentration in diets without MBM but did not have significant impacts in diets containing MBM.

The influence of dietary treatments on apparent digestibility coefficients of essential and non-essential amino acids in the distal ileum at 36 days post-hatch are shown in Tables 7 and 8. There were no significant differences in apparent distal ileal digestibility coefficients of the 16 amino acids between the PC and NC diets. Consistently, inclusion of 120 g/kg MBM significantly ($P < 0.01$) depressed apparent digestibility coefficients of 13 *ex* 16 amino acids, including arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid, serine, proline and tyrosine. Phytase significantly increased digestibility of alanine (0.760 versus 0.784, $P < 0.05$). There was a dietary interaction ($P < 0.01$) for distal ileal glycine digestibility where phytase significantly enhanced glycine digestibility in diets

Table 6
Influence of meat-and-bone meal and phytase supplementation on mineral concentrations in toe ash of broiler chickens at 36 days post-hatch.

Treatments	MBM, g/kg	Phytase, FYT/kg	Ca, %	K, %	Mg, %	Na, %	P, %	Cu, mg/kg	Fe, mg/kg	Mn, mg/kg	Sr, mg/kg	Zn, mg/kg
2	0	0	31.60	2.83[a]	0.731	4.42[a]	16.03	21.61	223	29.54[a]	314[b]	367
3	0	1,000	31.29	2.33[b]	0.777	3.74[b]	16.20	19.13	180	32.90[a]	367[a]	371
4	60	0	32.65	2.28[b]	0.741	3.76[b]	16.58	19.13	182	31.97[a]	302[bc]	340
5	60	1,000	31.73	2.24[b]	0.749	3.76[b]	16.05	18.72	168	21.13[b]	279[bc]	340
6	120	0	32.07	2.30[b]	0.743	3.66[b]	16.45	21.69	191	25.61[ab]	273[c]	328
7	120	1,000	32.68	2.34[b]	0.737	3.91[b]	16.65	20.23	186	20.67[b]	291[bc]	354
SEM			0.6268	0.0834	0.0195	0.1448	0.3868	1.245	10.96	2.712	12.94	10.22
Main effects: MBM, g/kg												
0			31.45	2.58	0.754	4.08	16.11	20.37	202	31.22	340	369[a]
60			32.19	2.26	0.745	3.76	16.32	18.93	175	26.55	290	340[b]
120			32.38	2.32	0.740	3.78	16.55	20.96	189	23.14	282	341[b]
Phytase, FYT/kg												
0			32.11	2.47	0.738	3.94	16.35	20.81	199[a]	29.04	296	345
1,000			31.90	2.30	0.754	3.80	16.30	19.36	178[b]	24.90	312	355
Significance (*P*-value)												
MBM			0.305	0.001	0.769	0.060	0.536	0.259	0.066	0.020	<0.001	0.011
Phytase			0.690	0.020	0.323	0.246	0.863	0.164	0.030	0.071	0.145	0.240
Interaction			0.484	0.006	0.401	0.009	0.577	0.713	0.198	0.044	0.023	0.422
Positive control			31.47	2.40	0.799	3.94	16.22	18.93	169	23.30	322	337
P-value[1]			0.885	0.018	0.027	0.064	0.068	0.029	0.007	0.014	0.724	0.054

MBM = meat and bone meal; SEM = pooled standard error of mean.
[a,b,c] Means within columns not sharing a common suffix are significantly different ($P < 0.05$).
[1] Comparison between treatment 1 and 2.

Table 7
Influence of meat-and-bone meal and phytase supplementation on apparent ileal digestibility coefficients of essential amino acids in broiler chickens at 36 days post-hatch.

Treatments	MBM, g/kg	Phytase, FYT/kg	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
2	0	0	0.870	0.839	0.834[a]	0.843	0.878	0.945	0.854	0.815	0.809[a]
3	0	1,000	0.866	0.838	0.836[a]	0.843	0.881	0.944	0.854	0.818	0.813[a]
4	60	0	0.852	0.821	0.820[a]	0.829	0.870	0.940	0.845	0.812	0.801[ab]
5	60	1,000	0.868	0.833	0.829[a]	0.845	0.876	0.949	0.857	0.825	0.822[a]
6	120	0	0.830	0.799	0.809[a]	0.815	0.842	0.927	0.841	0.783	0.775[b]
7	120	1,000	0.815	0.764	0.765[b]	0.784	0.828	0.921	0.807	0.759	0.738[c]
SEM			0.0101	0.0108	0.0099	0.0097	0.0069	0.0053	0.0096	0.0097	0.0111
Main effects: MBM, g/kg											
0			0.868[a]	0.838[a]	0.835	0.843[a]	0.880[a]	0.945[a]	0.854[a]	0.817[a]	0.811
60			0.860[a]	0.827[a]	0.824	0.837[a]	0.873[a]	0.944[a]	0.851[a]	0.818[a]	0.811
120			0.823[b]	0.781[b]	0.787	0.800[b]	0.835[b]	0.924[b]	0.824[b]	0.771[b]	0.756
Phytase, FYT/kg											
0			0.851	0.819	0.821	0.829	0.863	0.937	0.846	0.803	0.795
1,000			0.850	0.811	0.810	0.824	0.862	0.938	0.839	0.801	0.791
Significance (*P*-value)											
MBM			<0.001	<0.0001	<0.0001	<0.001	<0.0001	<0.001	0.006	<0.0001	<0.0001
Phytase			0.929	0.381	0.190	0.516	0.800	0.918	0.359	0.741	0.627
Interaction			0.318	0.098	0.022	0.061	0.334	0.354	0.061	0.162	0.039
Positive control			0.878	0.849	0.846	0.854	0.886	0.948	0.864	0.823	0.823
P-value[1]			0.529	0.481	0.373	0.439	0.402	0.772	0.478	0.587	0.355

MBM = meat and bone meal; SEM = pooled standard error of mean.
[a,b,c] Means within columns not sharing a common suffix are significantly different ($P < 0.05$).
[1] Comparison between treatments 1 and 2.

with 60 g/kg MBM by 12.7% (0.768 versus 0.843) and with 120 g/kg MBM by 8.90% (0.628 versus 0.719) but did not alter glycine digestibility in the diets without MBM.

4. Discussion

In order to determine the influence of phytase and MBM in broiler diets with nutrient compositions close to industry practice, the average dietary lysine (10.0 g/kg) and protein (188.5 g/kg) concentrations in finisher diets were formulated to be somewhat less than 2,014 Ross 308 nutrient specifications. However, these reductions in the PC diet (Treatment 1) did not compromise bird performance when compared to 2,014 Ross 308 performance objectives. Calcium and P concentrations were further reduced in NC diets (Treatments 2, 4 and 6) to investigate the capacity of phytase to release phytate-bound P. From 1 to 36 days post-hatch, on average, NC diets supported significantly better weight gain but similar FCR in comparison to 2,014 Ross 308 performance objectives. The reductions in Ca and P in NC diets are evidenced by significantly lower percentage toe ash results in NC diets in comparison to the PC diet. It is not straightforward that phytase tended to reduce FCR in diets with 0 and 120 g/kg MBM but tended to increase FCR in broiler chickens offered 60 g/kg MBM diets resulting in an interaction between MBM inclusion and phytase supplementation. One possible reason is that the 60 g/kg MBM phytase supplemented diet (Treatment 5) had the lowest analysed lysine concentration (9.3 g/kg) which represents an 8.8% reduction from the corresponding non-phytase supplemented diet (Treatment 4, 10.2 g/kg). Moreover, feed intake and lysine digestibility were similar in Treatments 4 and 5. This unintended difference may have contributed to the inferiority of FCR in broilers offered the supplemented 60 g/kg MBM diet.

Table 8
Influence of meat-and-bone meal and phytase supplementation on apparent ileal digestibility coefficients of non-essential amino acids in broiler chickens at 36 days post-hatch.

Treatments	MBM, g/kg	Phytase, FYT/kg	Ala	Asp	Glu	Gly	Ser	Pro	Tyr
2	0	0	0.783	0.799	0.896	0.787[b]	0.820	0.871	0.845
3	0	1,000	0.789	0.799	0.895	0.785[b]	0.822	0.868	0.842
4	60	0	0.786	0.747	0.883	0.768[b]	0.800	0.843	0.824
5	60	1,000	0.830	0.751	0.900	0.843[a]	0.824	0.881	0.839
6	120	0	0.712	0.677	0.874	0.628[d]	0.764	0.790	0.808
7	120	1,000	0.732	0.632	0.858	0.719[c]	0.738	0.804	0.787
SEM			0.0120	0.0138	0.0083	0.0147	0.0108	0.0105	0.0095
Main effects: MBM, g/kg									
0			0.786[a]	0.799[a]	0.895[a]	0.786	0.821[a]	0.869[a]	0.843[a]
60			0.808[a]	0.749[b]	0.891[a]	0.805	0.812[a]	0.862[a]	0.831[a]
120			0.722[b]	0.654[c]	0.866[b]	0.674	0.751[b]	0.797[b]	0.797[b]
Phytase, FYT/kg									
0			0.760[a]	0.741	0.884	0.728	0.795	0.835	0.825
1,000			0.784[b]	0.727	0.884	0.782	0.795	0.851	0.823
Significance (*P*-value)									
MBM			<0.0001	<0.0001	0.002	<0.0001	<0.0001	<0.0001	<0.001
Phytase			0.025	0.235	0.994	<0.0001	0.994	0.067	0.744
Interaction			0.276	0.158	0.178	0.008	0.083	0.162	0.183
Positive control			0.808	0.811	0.899	0.803	0.833	0.876	0.859
P-value[1]			0.129	0.420	0.802	0.315	0.341	0.759	0.208

MBM = meat and bone meal; SEM = pooled standard error of mean.
[a,b,c,d] Means within columns not sharing a common suffix are significantly different ($P < 0.05$).
[1] Comparison between treatments 1 and 2.

Inclusion of 120 g/kg MBM significantly depressed apparent digestibility coefficients of 13 *ex* 16 amino acids, including arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid, serine, proline and tyrosine. Also, there were negative linear relationships between MBM inclusions and digestibilities of all 16 amino acids in the distal ileum. Meat and bone meal inclusions also linearly depressed weight gain and FCR from 1 to 36 days post-hatch. The quality of MBM varies as a result of the rendering process and from the amounts of collagen in the raw materials (Batterham et al., 1986a; Ravindran et al., 2002). Skurray (1974) suggested that suboptimal MBM protein utilisation in chickens may be due to low essential amino acid digestibilities and imbalanced amino acid profiles. Meat and bone meal has a high gelatin content because it contains certain amount of skin, cartilage, and connective tissues; moreover, gelatin is deficient in tryptophan and sulphur-amino acids (Skurray, 1974). Ravindran et al. (2005) reported MBM protein digestibility was 0.61 in comparison to 0.82 in soybean meal for broiler chickens and cystine had the lowest digestibility of only 0.34. Earlier, Wang and Parsons (1998) had reported that cystine digestibility ranged from 0.20 to 0.71 among different 31 MBM samples and the high processing temperature depressed average lysine digestibility by 3.6% from 0.84 to 0.81 but reduced average cysteine digestibility by 20.8% from 0.53 to 0.42. The poor digestibility of cystine following heat treatment is almost certainly indicative of disulphide cross-linkage formation, which is amplified by hydrothermal processes, and results in reduced protein solubility. Hendriks et al. (2002) reported significant correlations between protein solubility and amino acid digestibilities ($r = 0.29$, $P < 0.001$) in 94 commercial MBM samples in New Zealand and Selle et al. (2012) found a correlation between disulphide concentrations and protein solubility ($r = -0.518$, $P < 0.001$) in steam-pelleted sorghum-based diets.

It was anticipated that weight gain responses to phytase supplementation in diets without MBM might be more pronounced than those containing 60 and 120 g/kg MBM. Phytase significantly increased weight gain in broiler chickens offered diets without MBM by 7.8% in the starter phase (398 versus 429 g/bird) and did not influence weight gain in MBM diets. Consistently, similar trends were observed during finisher phase. It is axiomatic that diets containing MBM would have lesser inclusions of plant-sourced soy protein in order to formulate iso-nitrogenous diets. Selle et al. (2003) reported that the average phytate-P was 4.53 g/kg in 22 samples of soybean meal and was 2.20 g/kg in 37 samples of wheat. Based on these two values, the calculated phytate-P concentrations in the starter diets used in the present study were 2.69 g/kg in diets without MBM, 2.39 g/kg in diets with 60 g/kg MBM and 2.12 g/kg in diets with 120 g/kg MBM. In the finisher diets, calculated phytate concentrations were 2.43 g/kg in diets without MBM, 2.13 g/kg in diets with 60 g/kg MBM and 1.85 g/kg in diets with 120 g/kg MBM. Therefore, it could be expected that phytase responses would be less pronounced in MBM diets. Also, in non-phytase supplemented diets, MBM increased toe ash concentrations from 10.46% in diets without MBM to 12.34% in diets with 60 g/kg MBM and 12.62% in diets with 120 g/kg MBM. Toe ash concentrations in NC diets containing MBM were similar to that in the PC diet (12.08%). This suggests that there were no Ca and P deficiencies in these diets so that significant phytase responses - in growth performance from the 'phosphoric effect' of the feed enzyme were observed.

It is not straightforward that FCR response to phytase was actually the most pronounced in diets with 120 g/kg MBM. In diets without MBM, phytase reduced FCR by 4.0% (1.618 versus 1.554) from 15 to 36 days post-hatch and by 3.8% (1.583 versus 1.523) from 1 to 36 days post-hatch. However, in diets contained 120 g/kg MBM, phytase reduced FCR by 4.5% (1.913 versus 1.827) from 15 to 36 days post-hatch and by 4.4% (1.844 versus 1.764) from 1 to 36 days post-hatch. As phytase did not influence toe ash concentration in diets contained 120 g/kg MBM, this improvement may be due to 'extra-phosphoric' effects of phytase supplementation (Selle and Ravindran, 2007). Recently, Truong et al. (2015a) suggested that phytase may enhance absorption of glucose and amino acids by increasing Na^+, -K^+, -ATPase pump activity and this was evidenced by the correlation between apparent digestibility of Na and protein in the distal ileum and the increase of Na digestibility by phytase supplementation. It is possible but has yet to prove that the largest improvement of FCR in diets with 120 g/kg MBM was due to better balanced absorption and availability of glucose and amino acids in response to the poorest amino acid digestion in diets with high MBM inclusion.

In the present study, weight gain and FCR in broiler chickens from 15 to 36 days post-hatch were correlated with distal ileal protein digestibility coefficients but not with starch and fat digestibilities. Liu and Selle (2015) suggested that protein availability may be more critical than starch availability for feed efficiency and protein deposition. The reason being that protein digestion in the gastrointestinal tract is usually slower and incomplete than that of starch (Liu et al., 2013). It is noteworthy that diets without MBM had significantly lower starch digestibility and diets with 120 g/kg MBM had significantly lower protein digestibility than the other diets. However, broiler chickens offered diets with 120 g/kg MBM had the poorest weight gain and FCR which proved the relative importance of protein digestion than starch. Therefore, protein digestion is more likely to be the limiting factor for growth performance and feed efficiency in broiler chickens.

Feed intake from 15 to 36 days post-hatch was negatively correlated with apparent distal ileal digestibility coefficients of protein and all 16 amino acids. Clearly, increased feed intakes may lead to shorter retention times along the gastrointestinal tract and reduced opportunities for digestion and absorption. The intake of digestible nutrients on a daily basis is more important than apparent digestibilities of nutrients. Consequently, Truong et al. (2015b) reported significant correlations between energy utilisation and disappearance rate ratio of starch and protein in the distal jejunum emphasising the importance of both the extent and site of nutrient digestion.

The prime outcome of the present study was the pronounced depressions in performance pursuant to MBM inclusions in broiler diets. A decade ago, as discussed by Selle et al. (2006), a surplus of MBM was produced in Australia and it was economically advantageous to include MBM as a source of P and protein in broiler diets. While Australia remains a MBM exporter, its price has escalated due to increasing demands from the pet-food and Aquaculture industries. As a consequence, MBM inclusions in broiler diets are now considerably less than 60 to 80 g/kg, which once was typical, mainly for this and other reasons. However, the major negative impact of high inclusion of MBM was observed in protein and amino acid digestion. Experimental diets in the present study were formulated based on total amino acids; therefore, the findings from the present study do not necessarily set the limits on inclusion rate of MBM if diets were formulated on a digestible amino acid basis.

5. Conclusion

The hypothesis that MBM would depress broiler performance was valid because there were negative linear relationships between MBM inclusion levels and digestibilities of all 16 amino acids in the distal ileum and 120 g/kg MBM significantly depressed apparent digestibility coefficients of 13 *ex* 16 amino acids. Additionally, phytase had the largest influence on weight gain in diets without MBM and the most pronounced impact on FCR was reported in diets with 120 g/kg MBM. The results of toe ash concentration indicated there was not any Ca and P dietary deficiencies in diets contained MBM and the improvement on FCR in high MBM diets may be due to extra-phosphoric effects of phytase. The present study demonstrated that high inclusion levels of MBM in broiler diets are not recommended for broiler performance and protein utilisation if the diets are formulated on total amino acid basis.

Conflict of interest

All authors read and approved the final version of the manuscript. The authors have no financial or personal conflicts of interest to declare.

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