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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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.
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 MBM 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 mol of inorganic phosphate from 5.0 mM sodium phosphate 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 PR330 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.

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 Siriwian 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 amylogluco-amylase, 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:

\[
\text{Digestibility Coefficient} = \frac{(\text{Nutrient/}AIA)_{\text{diets}} - (\text{Nutrient/}AIA)_{\text{digesta}}}{(\text{Nutrient/}AIA)_{\text{diets}}}
\]

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
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 (1.555 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 ($P < 0.05$) deprived 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 diets without MBM (398 versus 329 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 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.

### 4. Discussion

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 ($P < 0.05$) between MBM and phytase for toe ash where phytase significantly increased toe ash and apparent ileal digestibility 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, $P < 0.0001$).
The influence of dietary treatments on growth performance in broiler chickens.

| Treatments | MBM, g/kg | Phytase, FYT/kg | 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 |
|------------|----------|----------------|---------------------|---------------------|---------|---------------------|---------------------|---------|---------------------|---------------------|---------|
| 1–14 days post-hatch | 15–36 days post-hatch | 1–36 days post-hatch |
| 2 | 0 | 0 | 398<sup>b</sup> | 562<sup>ab</sup> | 1.411 | 2.043 | 3.300 | 1.618<sup>bc</sup> | 2.441 | 3.862 | 1.583<sup>bc</sup> |
| 3 | 0 | 1,000 | 429<sup>a</sup> | 587<sup>a</sup> | 1.372 | 2.132 | 3.310 | 1.554<sup>c</sup> | 2.561 | 3.897 | 1.523<sup>c</sup> |
| 4 | 60 | 0 | 407<sup>ab</sup> | 563<sup>ab</sup> | 1.384 | 2.041 | 3.229 | 1.582<sup>bc</sup> | 2.448 | 3.792 | 1.549<sup>bc</sup> |
| 5 | 60 | 1,000 | 403<sup>b</sup> | 546<sup>b</sup> | 1.357 | 1.956 | 3.279 | 1.684<sup>a</sup> | 2.359 | 3.825 | 1.628<sup>a</sup> |
| 6 | 120 | 0 | 36<sup>1</sup> | 542<sup>b</sup> | 1.500 | 1.808 | 3.457 | 1.913<sup>a</sup> | 2.169 | 4.000 | 1.844<sup>a</sup> |
| 7 | 120 | 1,000 | 353<sup>c</sup> | 507<sup>c</sup> | 1.442 | 1.809 | 3.303 | 1.827<sup>c</sup> | 2.161 | 3.810 | 1.764<sup>c</sup> |
| SEM | 7.911 | 11.140 | 0.0293 | 37.205 | 61.360 | 0.0395 | 40.335 | 65.930 | 0.0327 |

Main effects: MBM, g/kg

| Significance (P-value) | Pooled standard error of mean. |
|------------------------|--------------------------------|
| MBM | <0.0001 | 0.001 | 0.037 | <0.0001 | 0.125 | <0.0001 | 0.125 | <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<sup>1</sup> | 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.

<sup>1</sup> Comparison between treatments 1 and 2.

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 containing MBM.
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 glycineme digi-

with 60 g/kg MBM and phytase supplementation on ileal digestibility coefficients of essential amino acids in broiler chickens at 36 days post-hatch.

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 phytase 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 digi-

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 | 0.731 | 4.42  | 16.03 | 21.61     | 223       | 29.54     | 314b      | 367       |
| 3          | 0         | 1,000          | 31.29 | 2.33 | 0.777 | 3.74  | 16.20 | 20.13     | 180       | 32.90     | 367a      | 371       |
| 4          | 60        | 0              | 32.65 | 2.28 | 0.741 | 3.76  | 16.58 | 19.13     | 182       | 31.97     | 302bc     | 340       |
| 5          | 60        | 1,000          | 31.73 | 2.24 | 0.749 | 3.76  | 16.05 | 18.72     | 168       | 21.13     | 279bc     | 340       |
| 6          | 120       | 0              | 32.07 | 2.30 | 0.743 | 3.66  | 16.45 | 21.69     | 191       | 25.61     | 273c      | 328       |
| 7          | 120       | 1,000          | 32.68 | 2.34 | 0.737 | 3.91  | 16.65 | 20.23     | 186       | 20.67     | 291bc     | 354       |

Main effects: MBM, g/kg

SEM 0.6268 0.0834 0.0195 0.1448 1.245 2.712 12.94 10.22

Phytase, FYT/kg

SEM 0.000 0.001 0.006 0.011 0.016 0.021 0.026 0.031

Significance (P-value)

MBM 0.001 0.006 0.011 0.016 0.021 0.026 0.031

Phytase 0.000 0.006 0.011 0.016 0.021 0.026 0.031

Interaction 0.000 0.006 0.011 0.016 0.021 0.026 0.031

Positive control 0.000 0.006 0.011 0.016 0.021 0.026 0.031

P-value 0.000 0.006 0.011 0.016 0.021 0.026 0.031

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 | 0.843 | 0.878 | 0.945 | 0.854 | 0.815 | 0.809 |
| 3          | 0         | 1,000          | 0.866 | 0.838 | 0.836 | 0.843 | 0.881 | 0.944 | 0.854 | 0.818 | 0.813 |
| 4          | 60        | 0              | 0.852 | 0.821 | 0.828 | 0.829 | 0.887 | 0.940 | 0.845 | 0.812 | 0.801 |
| 5          | 60        | 1,000          | 0.868 | 0.833 | 0.829 | 0.845 | 0.876 | 0.949 | 0.857 | 0.825 | 0.822 |
| 6          | 120       | 0              | 0.830 | 0.799 | 0.809 | 0.815 | 0.842 | 0.927 | 0.841 | 0.783 | 0.775 |
| 7          | 120       | 1,000          | 0.815 | 0.764 | 0.765 | 0.784 | 0.828 | 0.921 | 0.807 | 0.759 | 0.738 |

Main effects: MBM, g/kg

SEM 0.0101 0.0108 0.0099 0.0097 0.0069 0.0053 0.0096 0.0097 0.0111

Phytase, FYT/kg

SEM 0.868 | 0.838 | 0.835 | 0.842 | 0.880 | 0.945 | 0.854 | 0.817 | 0.811 |
| 60        | 0.860 | 0.827 | 0.824 | 0.837 | 0.873 | 0.944 | 0.851 | 0.818 | 0.811 |
| 120       | 0.823 | 0.781 | 0.787 | 0.800 | 0.835 | 0.924 | 0.824 | 0.771 | 0.756 |

Significance (P-value)

MBM <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001

Phytase 0.929 | 0.381 | 0.198 | 0.601 | 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 0.529 | 0.481 | 0.373 | 0.439 | 0.402 | 0.722 | 0.478 | 0.587 | 0.355 |

1 Comparison between treatments 1 and 2.

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.
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 cystine 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 the ‘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.

| 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\(^a\) | 0.822 | 0.868 | 0.842 |
| 4          | 60        | 0              | 0.786 | 0.747 | 0.883 | 0.768\(^a\) | 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\(^a\) | 0.764 | 0.790 | 0.808 |
| 7          | 120       | 1,000          | 0.732 | 0.632 | 0.858 | 0.719\(^a\) | 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

| Phytase, FYT/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**

| Significance (P-value) | MBM | Phytase | Interaction | Positive control | P-value\(^1\) |
|------------------------|-----|---------|-------------|-------------------|------------|
| <0.0001                | <0.0001 | 0.002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 0.025                  | 0.235 | 0.994 | <0.0001 | 0.994 | 0.067 | 0.744 |
| 0.276                  | 0.158 | 0.178 | 0.008 | 0.083 | 0.162 | 0.183 |
| 0.808                  | 0.811 | 0.899 | 0.803 | 0.833 | 0.876 | 0.859 |
| 0.129                  | 0.420 | 0.802 | 0.315 | 0.341 | 0.759 | 0.208 |

**SEM** = 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.
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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