Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.23 / n.1 / 001-008

http://dx.doi.org/10.1590/1806-9061-2020-1332

Original Article

Mitsuokella Jalaludinii Supplementation Improved Nutrient Utilization of Broilers Fed Low-Available Phosphorus Diet

■Author(s)

Tang HC[I,II] https://orcid.org/0000-0002-8368-9506
Sieo CC[I,III] https://orcid.org/0000-0002-1503-1649
Abdullah N[I,IV] https://orcid.org/0000-0002-7005-4202
Chong CW[V] https://orcid.org/0000-0002-6881-8883
Omar AR[I,VI] https://orcid.org/0000-0002-0495-0869
Mohamad R[III] https://orcid.org/0000-0001-5672-1905
Ho YW[I] https://orcid.org/0000-0003-4461-5456
Yong CY[I,III] https://orcid.org/0000-0002-0842-6386

[I] Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[II] International Medical School, Management and Science University, Seksyen 13, 40100 Shah Alam, Selangor, Malaysia
[III] Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[IV] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[V] School of Pharmacy, Monash University – Malaysia Campus, Bandar Sunway, Selangor, Malaysia.
[VI] Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

■Mail Address

Corresponding author e-mail address
Abdul Rahman Omar
Department of Veterinary Pathology & Microbiology Faculty of Veterinary Medicine – Universiti Putra Malaysia – 43400 Serdang, Selangor, Malaysia.
Phone: +03-89468268
Email: aro@upm.edu.my

■Keywords

Broiler, mineral retention, Mitsuokella jalaludinii, nutrient utilization, phytase.

Submitted: 04/June/2020
Approved: 15/October/2020

ABSTRACT

Phytase enzyme is supplemented to poultry feed to improve phosphorus (P) availability. *Mitsuokella jalaludinii*, bacteria isolated from the rumen of cattle, has been reported as a cheaper alternative source of phytase. As much nutrients are trapped within the phytate complex, we hypothesized that the supplementation of *M. jalaludinii* phytase to poultry feed would enhance nutrient utilization by poultry. In the current study, the efficacy of freeze-dried *M. jalaludinii* cells (Mj) as feed supplement for broilers fed low-available phosphorus (low-aP) diet was evaluated. Day-old male Cobb raised in battery cages were assigned to three treatment groups [normal-available phosphorus diet with heat-deactivated Mj (DMj); low-aP diet with DMj; and low-aP diet with Mj], each consisting of four replicates (10 birds per replicate) for a 3-weeks feeding period. Feed intake was recorded daily from day 1-21, whereas broilers were weighted at day 1, 7, 14, and 21. Total excreta were collected at day 11-13 and 18-20. At day 21, twelve broilers from each treatment group were slaughtered to collect plasma and tibia. The results showed that Mj significantly enhanced broilers' live weight and feed conversion ratio compared to the control groups (p<0.05). Supplementation with Mj have also enhanced the level of P, Ca, Mn, Cu, and Zn in the sera; and Ca and Mn in the tibia at day 18-20 sampling period (p<0.05). As Mj supplementation can enhance nutrient utilization particularly in broilers fed with low-aP diet, it could provide the market with another option in improving broilers' growth rate at a lower cost.

INTRODUCTION

Phosphorus (P) is one of the most important macro-minerals required by poultry as it is essential for the development and maintenance of the skeletal system as well as in numerous biochemical reactions in the body and being a part of many important metabolites involved in metabolic processes, such as phosphates used for energy, and the synthesis of DNA and RNA (Haque & Hossain 2012).

The main ingredients in poultry feed such as cereal grains and plant protein sources have high phosphorus content presence mainly in the form of phytate (up to 80% of total P), which is poorly utilized in monogastric animals including poultry. Phytate (the salt form of phytic acid, inositol-6-phosphate) can bind proteins and minerals as well as interact with endogenous enzymes, thus reducing nutrient utilization (Selle *et al.* 2012). It exerts negative effect on protein digestion either through the direct binding of proteins to phytates, forming binary protein-phytate complexes or through the binding of proteins to the ions attached to phytates, forming ternary protein-phytate complexes. These phytate complexes can be found in plant feedstuffs naturally, and the majority of the complexes may form within the gastrointestinal

tract of the animals due to the presence of many metal cations (Selle *et al.,* 2000).

Phytases are enzymes that break down phytate by hydrolysing its phosphate groups and at the same time, releasing its chelated or bound nutrients such as minerals, proteins and starches (Qvirist *et al.,* 2017). In order for monogastric animals to utilize the bound nutrients, degradation of phytate is necessary by exogenous phytase since monogastric animals do not have enough phytase enzymes in the intestinal tract. The supplementation of exogenous phytase enzyme has become a regular practice in monogastric animal nutrition to overcome the anti-nutritive effects of phytates. As phytase supplementation increases P availability, it reduces the need for addition of inorganic P into poultry feed, thus reduces P pollution which causes eutrophication in land water bodies (Bhavsar & Khire 2014).

A bacterial species, locally isolated from the rumen of cattle, known as *Mitsuokella jalaludinii*, was able to produce phytase with activity comparable to the commercial phytase enzyme Natuphos® (Lan *et al.,* 2012). Not only does the enzyme have wider working pH range, it can also function in the presence of metal chelating agents. In addition, *M. jalaludinii* phytase is a cheaper alternative to conventional phytase, as it is produced through semi-solid state fermentation using agricultural by-products, where the phytase supplementation was added to the feed in form of freeze-dried bacteria (Tang *et al.,* 2018; Tang *et al.,* 2020; Tang *et al.,* 2017). Previously, we have demonstrated that the *M. jalaludinii* phytase supplementation to low-available phosphorus (aP) diet was able to improve broilers' body weight gain and feed conversion ratio (FCR) independent to the caeca microflora (Tang *et al.,* 2020). In the current research, we attempt to study the nutrient retention, particularly on phosphorus (P), calcium (Ca), manganese (Mn), copper (Cu), and zinc (Zn) in broilers fed with low-aP diet supplemented with freeze-dried *M. jalaludinii* cells containing functional phytase activity (Mj). Additionally, the nutrient concentration in the bone and blood of the broilers were also measured to study the effect of *M. jalaludinii* phytase supplementation towards prevention of nutrient leaching.

MATERIALS AND METHODS

Ethics statement

This study was carried out in accordance with the guidelines and approval by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) with the reference number: UPM/IACUC/AUP-R14/2015 with respect to animal welfare ethics and animal management.

Phytase production

Phytase from *M. jalaludinii* was produced under anaerobic condition by semi-solid state fermentation process at 39 °C for 12 h using rice bran and fish meal as substrates, as described in Tang *et al.* (2018). The fermented substrate was filtered and the filtrate was centrifuged at 10,000 × *g* for 20 min. The pellets obtained were washed twice with normal saline (0.85% NaCl) to remove spent substrates. The pellets collected were either freeze-dried or oven dried at 65 °C for 72 h for the preparation of active (Mj) and deactivated (DMj) *M. jalaludinii* phytase, respectively. The absence of phytase activity in DMj was confirmed as described by Yanke *et al.* (1998) and Heinonen & Lahti (1981). The dried *M. jalaludinii* cells were ground through 1 mm sieve. The active Mj enzyme was added to the low-available P (low-aP) diet at the level of 500U/kg feed. Same amount of DMj was added to the normal-available P (normal-aP) and low-aP diet as controls.

Diet preparation

The ingredients and nutrient composition of the experimental diets are summarized in Table 1. The nutrient composition of both normal-aP and low-aP diets was the same, except the aP in low-aP diet was lower than in the normal diet. All diets were maintained iso-nitrogenous and iso-caloric by adding equal amount of freeze-dried and thermally inactivated oven-dried *M. jalaludinii* cells. The three experimental diets were low-aP+Mj, normal-aP+DMj, and low-aP+DMj. The Mj and DMj were mixed daily with the other ingredients manually before the morning feeding.

Broilers management and experimental design

A 3-weeks feeding experiment with completely randomized design was conducted. One hundred and twenty male Cobb (day-old), purchased from a local hatchery were assigned to three dietary treatments with four replicates (10 birds per replicate). The birds were reared in battery cages in a well-ventilated open house.

Sample collection

Daily feed intake was recorded for each replicate and live weight of broilers (1-day, 7-days, 14-days, and 21-days old) was determined weekly for the calculation of feed conversion ratio (FCR). At day 21,

Table 1 – Ingredients and nutrient compositions of normal-available *p* and low-available *p* diets.

Ingredient(g/kg)	Normal-aP	Low-aP
Corn	542.63	551.02
Soybean meal	366.92	366.92
Fish meal	19.99	19.99
Palm oil	39.88	36.98
60% choline chloride	2.00	2.00
Vitamin premix	0.30	0.30
Mineral premix	1.00	1.00
Salt (NaCl)	2.00	2.00
DL- methionine	2.00	2.00
Limestone	10.99	17.79
Dicalcium phosphate	12.29	0.00
Calculated nutrient content		
Available phosphorus (%)	0.4	0.2
Total phosphorus (%)	0.8	0.5
Calcium (%)	1.0	1.0
Crude protein (%)	21.4	21.4
Metabolisable energy (kcal/kg)	3032	3036
Analyzed composition		
Crude protein (%)	20.2	20.5
Carbohydrate (%)	57.8	57.9
Fat (%)	5.4	5.4
Phosphorus (ppm)	5316	3580
Calcium (ppm)	5458	5789
Copper (ppm)	11	12
Manganese (ppm)	106	103
Zinc (ppm)	92	92

Vitamin premix: vitamin A 50.00 MIU; vitamin B1 10.00 g; vitamin B2 30.00g; vitamin B6 20.00g; vitamin B12 0.100g; vitamin D3 10.00 MIU; vitamin E 75.00g; vitamin K3 20.00g; Calcium D-Pantathenate 60.00g, Nicotinic Acid 200.00g; Folic Acid 5.00g; Biotin 235.00g; Antioxidant, Anticaking and Carrier added

Mineral premix: Se 0.200g; Fe 80.00g; Mn 100.00g; Zn 80.00g; Cu 15.00g; KCl 4.00g; MgO 0.60g; $NaCO_3$ 1.50g; I 1.00g; Co 0.25g

aP: Available phosphorous

12 broilers from each treatment group were randomly selected, weighed and sacrificed. Blood samples were collected from the jugular vein into vacutainer plastic blood collection tubes containing lithium heparin (BD-367880). Plasma was collected after centrifugation at 3000 × g for 15 min at 4 °C. The left tibia bone was removed from each sacrificed chicken and cleaned manually by removing all adhering tissues. The feed, excreta, plasma and tibia samples were used for minerals determination.

Chemical analyses

Feed samples were mixed well and kept in a plastic container. The faeces samples were collected at 24 h interval, by wrapping the trays under each compartment with a clean plastic sheet. All contaminants such as feathers and feed residues present on the plastic were removed before the faeces being mixed and kept in sealed plastic bags. The feed and faeces were kept at -20 °C before sending for analysis. Poultry feed and faeces samples were outsourced to Food & Agriculture Analysis Laboratory, Laboratory & Technical Services Centre, Malaysian Agricultural Research and Development Institute (MARDI) for chemical analyses. The minerals (Ca, P, Cu, Mn, Zn) were determined using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The apparent retention of minerals was calculated using the following equation:

Apparent nutrient retention (%) = (DMfi× nutrient DMfi) – (DMe× nutrient DMe) / DMfi × nutrient DMfi

Where DMfi= dry matter of feed intake, g

DMe= dry matter of excreta, g

Statistical analyses

All data collected were analyzed by using statistical software, IBM® SPSS for Microsoft Windows® version 22.0. Data were subjected for analysis of variance (ANOVA), and the means were tested by Duncan multiple range test for significant difference at $p<0.05$. The results were reported as means ± standard error (SE).

RESULTS

Live weight and feed conversion ratio

The live weight and FCR of broilers were significantly improved ($p<0.05$) in broilers fed low-aP+Mj diet compared to the controls at day 14 and day 21 (Table 2). However, live weight of broilers fed low-aP+DMj diet were similar to that fed normal-aP+DMj at both time periods. Unlike day 21, there was no significance difference in the FCR at day 14 for broilers fed low-aP+DMj and normal-aP+DMj. Regardless, the FCR for broilers receiving low-aP+Mj are significantly better than broilers fed normal-aP+DMj diet at both time periods.

Minerals retention

The apparent retentions of P, Ca, Mn, Cu and Zn of broilers fed different dietary treatments are shown in Table 3. At both day 11-13 and day 18-20 sampling periods, *p* retention was significantly higher ($p<0.05$) for broilers fed with low-aP+Mj diet compared to that of control diets. However, Ca, Mn, Cu and Zn retentions did not differ significantly among dietary treatments at both sampling periods, although broilers fed with low-aP+Mj diet are generally higher than that of normal-aP+DMj and low-aP+DMj.

Table 2 – Live weight and feed conversion ratio (FCR) of broilers fed normal-available P and low-available *p* diets.

Parameter	Dietary treatments		
	Normal-aP+DMj	Low-aP+Mj	Low-aP+DMj
Day 14			
Live weight (g/bird)	379.3±6.0[b]	404.3±0.9[a]	380.5±7.9[b]
FCR	1.495±0.023[a]	1.328±0.014[b]	1.505±0.036[a]
Day 21			
Live weight (g/bird)	673.8±4.2[b]	719.3±10.7[a]	677.2±6.4[b]
FCR	1.580±0.012[b]	1.458±0.017[c]	1.663±0.013[a]

Data represent the means ± SE of 4 replicate cages with 10 chickens for each cage.

[a-c] Means in the same row with different superscripts differ significantly (p<0.05).

aP: Available phosphorus

Mj: Freeze-dried active *M. jalaludinii* phytase

DMj: Oven-dried deactivated *M. jalaludinii* phytase

FCR: feed conversion ratio

Table 3 – Apparent retention of phosphorus (P), calcium (Ca), manganese (Mn), copper (Cu) and zinc (Zn) of broilers fed normal-available P and low-available P diets.

Minerals (%)	Dietary treatments		
	Normal-aP+DMj	Low-aP+Mj	Low-aP+DMj
Day 11-13			
P	67.5±1.03[b]	83.5±5.02[a]	76.8±2.07[ab]
Ca	71.7±3.24	79.6±3.01	70.6±2.18
Mn	56.2±2.01	62.3±6.98	59.8±6.63
Cu	34.3±7.34	55.5±5.71	33.7±8.46
Zn	56.5±4.21	66.2±7.90	59.4±5.37
Day 18-20			
P	72.0±3.72[b]	82.9±0.47[a]	75.5±0.82[ab]
Ca	77.7±2.40	82.9±1.46	80.0±5.55
Mn	54.8±5.87	71.1±2.23	52.5±9.67
Cu	38.2±4.90	51.4±2.77	42.3±5.13
Zn	58.6±58.6	67.3±1.94	59.2±9.01

Data represent the means ± SE of 4 replicate cages with 10 broilers for each cage.

[a-b] Means in the same row with different superscripts differ significantly (*p*<0.05).

aP: Available phosphorus

Mj: Freeze-dried active *M. jalaludinii* phytase

DMj: Oven-dried deactivated *M. jalaludinii* phytase

Tibia minerals

The results of Mj supplement on the mineral contents of tibia bones are presented in Table 4. Broilers receiving low-aP+Mj diet had similar P and Zn concentration in the tibia bone as those receiving normal-aP+DMj diet, but broilers fed low-aP+DMj diet had significantly lower concentrations (*p*<0.05). However, Ca was more concentrated (*p*<0.05) in tibia

Table 4 – Mineral contents (P, Ca, Mn, Cu, Zn) in tibia bone of broilers fed normal- available P and low-available P diets.

Minerals (ppm)	Dietary treatments		
	Normal-aP+DMj	Low-aP+Mj	Low-aP+DMj
P	28707±393[a]	29737±493[a]	27071±297[b]
Ca	50861±907[b]	52311±484[a]	51858±317[ab]
Mn	1.57±0.03[b]	1.72±0.04[a]	1.49±0.03[b]
Cu	1.92±0.05	2.00±0.09	1.82±0.06
Zn	60.23±1.57[a]	59.95±1.65[a]	56.75±0.85[b]

Data represent the means ± SE of 4 replicate cages with 10 broilers for each cage.

[a-b] Means in the same row with different superscripts differ significantly (p< 0.05).

aP: Available phosphorus

Mj: Freeze-dried active *M. jalaludinii* phytase

DMj: Oven-dried deactivated *M. jalaludinii* phytase

bone of broilers fed with low-aP+Mj diet than those fed normal-aP+DMj diet, but similar to those fed low-aP+DMj diet. While for Mn: broilers fed low-aP+Mj diet had a higher concentration than the other two dietary treatment groups (p<0.05). However, no significant differences were observed among dietary treatments on tibia Cu content.

Plasma minerals

Table 5 shows the effect of Mj supplement on the mineral contents in blood plasma. The plasma of broilers fed normal-aP+DMj showed higher levels of P and Cu than the other dietary groups (p<0.05). However, Ca content was significantly lower (p<0.05) in the normal-aP+DMj group in comparison to the low-aP+DMj group. There was no significant difference in Mn and Zn concentrations of plasma among all dietary groups.

Table 5 – Mineral contents (P, Ca, Mn, Cu, Zn) in blood plasma of broilers fed normal- available P and low-available P diets.

Minerals (ppm)	Dietary treatments		
	Normal-aP+DMj	Low-aP+Mj	Low-aP+DMj
P	117.7±0.7[a]	111.7±0.7[b]	109.7±2.0[b]
Ca	69.88±1.46[b]	72.38±1.50[ab]	76.02±2.00[a]
Mn	0.08±0.01	0.08±0.01	0.09±0.01
Cu	0.38±0.02[a]	0.29±0.01[b]	0.24±0.01[c]
Zn	4.98±0.04	5.37±0.30	5.33±0.11

Data represent the means ± SE of 4 replicate cages with 10 broilers in each cage.

[a-c] Means in the same row with different superscript differ significantly (p<0.05)

aP: Available phosphorus

Mj: Freeze-dried active *M. jalaludinii* phytase

DMj: Oven-dried deactivated *M. jalaludinii* phytase

DISCUSSION

Since monogastric animals such as poultry lack digestive phytase to hydrolyze phytates in the feed, inorganic P in the form of dicalcium phosphate is often added to their diet to meet the nutrient requirement. However, this practice gave rise to environmental problems, as excessive amount of P is released through the excreta. Potentially, addition of phytase enzyme into poultry feed can eliminate the need to add inorganic P, thereby reducing P pollution (Bhavsar & Khire 2014) while reducing feed cost (PR New Wire, 2015). Moreover, supplementation of phytase is known to reduce the anti-nutritional effects of phytates and improve the digestibility of nutrients by releasing chelated or bound nutrients such as minerals, proteins and starches (Kiarie *et al.,* 2015; Qvirist *et al.,* 2017).

The phytase produced in *M. jalaludinii* has been demonstrated to be active at low pH (2.5 - 7.0) (Lan *et al.*, 2012). This pH range offers an advantage to nutrient digestion, as the upper part of the poultry digestive tract including the crop, proventriculus and gizzard have pH of 5.5, 4.4, and 3.5 respectively (Dersjant-Li *et al.,* 2015). In the present study, an improvement in growth performance was observed in broilers fed low-aP supplemented with freeze-dried *M. jalaludinii* phytase (low-aP+Mj diet). This result indicates that Mj is capable of breaking down protein-phytate complexes, thus facilitates the release of nutrients for digestion and utilization in the broilers.

The present study showed that freeze-dried *M. jalaludinii* enhanced *p* retention significantly (p<0.05) as compared with the broilers fed with normal diet (Table 3). This finding is congruent with the meta-analysis on the effects of phytase supplementation on P retention conducted by Bougouin *et al.,* (2014) which showed the positive effect of phytase supplementation in P retention for both layers and broilers. Ca retention was comparable in all dietary treatments at both sampling periods. This result was similar to that reported by (Ajith *et al.,* 2018), who observed no significant difference in Ca retention for broilers supplemented with laboratory-produced phytase from *Aspergillus foetidus*.

Overall, the inclusion of Mj did not have significant effect in Mn, Cu and Zn although it showed better retention numerically in treatment groups fed with Mj at both sampling periods in comparison to those given normal diet as well as those with DMj diet. Phytase is known to help simple stomach animals such as broilers digest phytate, thus releasing nutrients trapped within the phytate complex, potentially improve mineral retention in these animals (Camden *et al.,* 2016). However, Aoyagi & Baker (1995) did not observe significant Cu retention when microbial phytase was included in the dietary treatment. Roberson & Edwards (1994) also could not find any improvement for Zn retention in broiler chickens fed

with phytase. Discrepancies in the results probably arise due to various factors like feeds, levels of phytase included in the feed, as well as animal factors such as age, breeds of chickens, genetics and sex (Kies *et al.,* 2001). Consequently, the similar live weights between normal-aP+DMj broilers and those fed low-aP+DMj diets (Table 2) suggest that the DMj may have beneficial effect to some extend which supplements low-aP diet, even without active phytase.

The ability of microbial phytase to break down the insoluble phytate-mineral complex is expected to increase the availability of P and other minerals to the broilers, which might allow more minerals to be deposited in the bones and thus increase bone mineralization. In the present study, the effect of Mj supplementation in the low-aP diet was positively reflected by the similar P and Zn concentrations in the tibia bone of both normal-aP+DMj and low-aP+Mj diets. The concentration of tibia Ca on the other hand, increased significantly (p<0.05) in broilers fed with low-aP+Mj diet compared to ones fed with normal-aP+DMj. This is in agreement with Viveros *et al.* (2002), where they have demonstrated the effect of microbial phytase supplementation to corn-soybean meal diet increased the Ca concentrations but not P concentration in tibia. It is also worth mentioning that Broz *et al.* (1994) reported in their findings that there was no increment of both the *p* and Ca concentrations in tibia bone ash of broilers fed low-P, maize-soybean meal diet supplemented with *Aspergillus niger* phytase when compared to basal diet. Mj supplementation also increased the deposition of Mn (p<0.05), but not of Cu. The increase in Mn of tibia was similar to the findings reported by Mohanna & Nys (1999), where microbial phytase supplement enhanced Mn concentration in tibia. Rutherfurd *et al.* (2012) did not observe any improvement in Cu with additional phytase, which is similar to our finding. The effects of phytase supplementation on tibia bone mineralization are rather inconsistent probably due to several nutritional factors, such as the concentration of phytates in poultry feed, types and dosage of microbial phytase used, and grain source which influences phytate degradation rate (Dersjant-Li *et al.,* 2017).

As opposed to tibia mineral retention, Bilal *et al.* (2015) reported that microbial phytase supplementation in broilers fed low-P diet showed no effect on serum Ca, but significantly increased serum P concentration (p<0.05). In the present study, the level of Ca was not influenced by phytase supplement, which is in agreement with the finding reported by B*ilal et al.* (2015). According to Sousa *et al.* (2015), the reason why Ca concentration in blood was not affected by phytase was probably due to Ca homeostasis, which is maintained by the parathyroid hormone (PTH), 1,25 di-hydroxy vitamin D (1,25(OH)2 D3) and calcitonin in the intestines, bone, and kidneys. The Ca flows between these organs and extracellular fluid are regulated by these hormones, thus reducing the variation of Ca concentration in blood level under normal circumstances. While Bilal *et al.* (2015) reported an increment in the serum *p* concentration, Kliment & Aneglovičová (2011) found no change for P content in the blood of broilers (Cobb 500) supplemented with microbial phytase. In this experiment, Mj supplementation did not affect the plasma P concentration in broilers (same as low-aP+DMj), where the concentration was found to be lower than broilers fed with normal-aP diet.

The results presented in Table 5 show P concentrations in blood plasma of all dietary treatments were about 1.5 times higher compared to Ca concentration. Whereas, in tibia bone (Table 4), the Ca concentration was found to be about 1.8 times higher than that of P concentration for all groups. This was due to the homeostasis between P and Ca as the titer of Ca was much higher in bone, where 99% of Ca was actually deposited in the skeleton (Proszkowiec-Weglarz & Angel 2013).

Cu concentration in plasma of broilers fed with low-aP+Mj diet on the other hand was lower than that of normal-aP diet but higher than that of low-aP diet, suggesting a mild positive effect in Cu regulation. Lastly, plasma Mn and Zn concentration were not affected by Mj supplementation, although the overall retention of these minerals was found to be increased in percentage.

CONCLUSIONS

The inclusion of freeze-dried *M. jalaludinii* in low-aP diet was able to enhance live weight and feed conversion ratio of broilers. Overall, supplementation of Mj also improved nutrient retention in broilers compared to the controls. This study further justifies the use of Mj as an alternative source of phytase for poultry feed.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Science, Technology and Innovation of Malaysia under eScience

Fund (06-01-04-SF1376). We would like to express our deep appreciation to our late Associate Professor Dr Sieo Chin Chin for her contribution.

CONFLICT OF INTEREST

All authors certify that there is no conflict of interest to declare.

REFFERENCES

Ajith S, Shet D, Ghosh J, Awachat VB, Bhat KS, Pal D, Elangovan AV. Effect of immobilized fungal phytase on growth performance and bone traits of broilers fed with low dietary calcium and phosphorus. Veterinary World 2018;11758-11764.

Aoyagi S, Baker DH. Effect of microbial phytase and 1,25-dihydroxycholecalciferol on dietary copper utilization in chicks. Poultry Science 1995;74(1):121-126.

Bhavsar K, Khire JM. Current research and future perspectives of phytase bioprocessing. RSC Advances 2014;4(51):26677-26691.

Bilal T, Atis S, Keser O. The effects of microbial phytase on serum calcium and phosphorus levels and alkaline phosphatase activities in broilers fed diets containing different levels of phosphorus. Acta Scientiae Veterinariae 2015;43:1-15.

Bougouin A, Appuhamy JA, Kebreab E, Dijkstra J, Kwakkel RP, France J. Effects of phytase supplementation on phosphorus retention in broilers and layers: a meta-analysis. Poultry Science 2014;93(8):1981-1992.

Broz J, Oldale P, Perrin-Voltz AH, Rychen G, Schulze J, Nunes CS. Effects of supplemental phytase on performance and phosphorus utilisation in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. British Poultry Science 1994;35(2):273-280.

Camden BJ, Morel PCH, Thomas DV, Ravindran V, Bedford MR. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Animal Science 2016;73(2):289-297.

Dersjant-Li Y, Awati A, Schulze H, Partridge G. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. Journal of the Science of Food and Agriculture 2015;95(5):878-896.

Dersjant-Li Y, Wealleans AL, Barnard LP, Lane S. Effect of increasing Buttiauxella phytase dose on nutrient digestibility and performance in weaned piglets fed corn or wheat based diets. Animal Feed Science and Technology 2017;234:101-109.

Haque N, Hossain A. Phytase: their biochemistry, physiology and application in poultry. International Journal of Livestock Research 2012;2(2):30-41.

Heinonen JK, Lahti RJ. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. Analytical Biochemistry 1981;113(2):313-317.

Kiarie E, Woyengo T, Nyachoti CM. Efficacy of new 6-phytase from *Buttiauxella* spp. on growth performance and nutrient retention in broiler chickens fed corn soybean meal-based diets. Asian-Australasian Journal of Animal Sciences 2015;28(10):1479-1487.

Kies AK, Van Hemert KHF, Sauer WC. Effect of phytase on protein and amino acid digestibility and energy utilisation. World's Poultry Science Journal 2001;57(2):109-126.

Kliment M, Aneglovičová M. The effect of microbial phytase on blood performance of broiler chickens. Scientific Papers Animal Science and Biotechnologies 2011;44(1):58-61.

Lan G, Abdullah N, Jalaludin S, Ho YW. Effects of freeze-dried *Mitsuokella jalaludinii* culture and Natuphos ((R)) phytase supplementation on the performance and nutrient utilization of broiler chickens. Journal of the Science of Food and Agriculture 2012;92(2):266-273.

Mohanna C, Nys Y. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. Animal Feed Science and Technology 1999;77(3):241-253.

PR News Wire. Feed enzymes market worth $1,371.03 million by 2020. Available from : https://www.prnewswire.com/news-releases/feed-enzymes-market-worth-137103-million-by-2020-503287641.html. 2015 May [cited 2020 April].

Proszkowiec-Weglarz M, Angel R. Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility. Journal of Applied Poultry Research 2013;22(3):609-627.

Qvirist L, Vorontsov E, Veide Vilg J, Andlid T. Strain improvement of *Pichia kudriavzevii* TY13 for raised phytase production and reduced phosphate repression. Microbial Biotechnology 2017;10(2):341-353.

Roberson KD, Edwards HM. Effects of 1,25-Dihydroxycholecalciferol and Phytase on Zinc Utilization in Broiler Chicks1. Poultry Science 1994;73(8):1312-1326.

Rutherfurd SM, Chung TK, Thomas DV, Zou ML, Moughan PJ. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. Poultry Science 2012;91(5):1118-1127.

Selle PH, Cowieson AJ, Cowieson NP, Ravindran V. Protein–phytate interactions in pig and poultry nutrition: a reappraisal. Nutrition Research Reviews 2012;25(1):1-17.

Selle PH, Ravindran V, Caldwell A, Bryden WL. Phytate and phytase: consequences for protein utilisation. Nutrition Research Reviews 2000;13(2):255-278.

Sousa Jd, Albino L, Vaz R, Rodrigues K, Da Silva G, Renno L, *et al.* The effect of dietary phytase on broiler performance and digestive, bone, and blood biochemistry characteristics. Brazilian Journal of Poultry Science 2015;17(1):69-76.

Tang HC, Chin S, Abdullah N, Mohamad R, Omar A, Chong C, *et al.* Production of phytase by *Mitsuokella jalaludinii* in semi-solid state fermentation of agricultural by-products. Sains Malaysiana 2018;47(2):277-286.

Tang HC, Sieo CC, Abdullah N, Chong CW, Gan HM, Mohd Asrore MS, *et al.* Effects of supplementing freeze-dried *Mitsuokella jalaludinii* phytase on the growth performance and gut microbial diversity of broiler chickens. Journal of Animal Physiology and Animal Nutrition 2020;104(1):116-125.

Tang HC, Sieo CC, Abdullah N, Chong CW, Ho YW. Preservation of phytase enzyme produced by anaerobic rumen bacteria, Mitsuokella jalaludinii. Journal of Biochemistry, Microbiology, and Biotechnology 2017;5(1):13-17.

Viveros A, Brenes A, Arija I, Centeno C. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poultry Science 2002;81(8):1172-1183.

Yanke LJ, Bae HD, Selinger LB, Cheng KJ. Phytase activity of anaerobic ruminal bacteria. Microbiology (Reading) 1998;144(Pt 6):1565-1573.