Effects of feeding microbial phytase and *Lactobacillus* spp. on growth performance and mineral utilization of broiler chickens

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Introduction

Phytase enzyme and probiotics are often used as feed supplements in poultry production. The enzyme phytase enhances phytate phosphorous (P) availability. Phosphorous is an essential mineral required in poultry diets for normal growth and development. To meet metabolic demands, broiler chickens require 0.45 – 0.50 g of P per 100 g diet, while laying hens require 0.21 – 0.35 g P per 100 g feed. Poultry feeds contain ingredients primarily of plant origin, and about two thirds of P are present in the form of phytic acids complex. However, due to lack of phytase enzyme, phytate P is essentially unavailable to the birds. Hence inorganic P is usually added to the diet and as a consequence of the inability of bids to utilize phytate-P, considerable amount of organic P is excreted and may create a significant pollution problem when the manure is applied to land. In previous studies, a new bacterial species, *Mitsuokella jalaludinii*, from the rumen of cattle has been shown to be effective as a phytase supplement in broiler chickens (Lan et al., 2002a,b). While a probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Probiotics for farm animals have been shown to have beneficial properties by influencing the indigenous microflora resulting in improve growth performance, better health control and enhancement in feed digestion. Various strains of *Lactobacillus* isolated from the gut of chicken have been successfully used as a probiotic (Jin et al., 1998). Separate studies on the effects of phytase enzyme from *M. jalaludinii* or of *Lactobacillus* spp. as a probiotic on performances and nutritive value of feed in broilers and layers have been conducted. However the effects of using both feed supplements have not been studied. In the present study, the effects of feeding both phytase enzyme and *Lactobacillus* spp. on the performance, mineral utilization and nitrogen retention of broilers were investigated.

Materials and Methods

Mitsuokella jalaludinii as a phytase source and *Lactobacillus* spp. as a probiotic supplement were prepared according to the procedures described by Lan et al. (2002b) and Jin et al. (1998), respectively. Four hundred and forty eight one-day old male broiler chicks (Avian-43) and eight diets were used. The diets were designed to determine the effects of *M. jalaludinii* and *Lactobacillus* spp. on mineral utilization and growth performance of broilers fed either a normal basal diet containing 0.49 % (starter diet) or 0.45% (grower diet) available-P (aP) or low-aP diet containing 0.25 % (starter diet) or 0.23 % (grower diet) aP. The diets were: basal diet + heat treated *M. jalaludinii* (B), basal diet + *M. jalaludinii* (BM), basal diet + *Lactobacillus* cultures + heat treated *M. jalaludinii* (BL), basal diet + *Lactobacillus* cultures + *M. jalaludinii* (BLM), low-aP diet + heat treated *M. jalaludinii* (T), low-aP diet + *M. jalaludinii* (TM), low-aP diet + *Lactobacillus* cultures + heat treated *M. jalaludinii* (TL) and low-aP diet + *Lactobacillus* cultures + *M. jalaludinii* (TLM). The concentration of freeze-dried *Lactobacillus* cultures was 1×10^9 cells/g and they were supplemented at 1g/kg feed. The phytase used was equivalent to 500 U/kg feed for starters and 300 U/kg feed for growers. All diets were formulated to contain similar levels of crude protein, metabolizable energy, lysine and methionine. The experimental period was 6 weeks. Feed consumption was recorded on per cage basis daily. Total faecal collection was conducted on days 18 to 20 from each cage. At the same time 100 g of feed samples were sampled and stored at -20°C. Feed and faecal samples were later used for proximate analyses and minerals (N, Ca, P, Zn, Cu and Mn). Three birds from each cage were randomly selected and sacrificed. Blood samples were collected in heparinized tubes for plasma collection. Left tibia bone from each bird was collected and ashed for mineral analysis. The various organs (crop, proventriculus, gizzard, bursa, ileum and liver) and abdominal fat were dissected and weighed.

Results and Discussion

The body weight (BW) gain and feed conversion ratio (FCR) of broilers fed basal diet supplemented with *Lactobacillus* (BL and BLM) were significantly improved ($P<0.05$), but the feed intake was not affected. Supplementation of *M. jalaludinii* culture to chickens fed basal diet (BM) did not enhance body weigh gain or FCR of the birds. Chickens fed low-aP diet supplemented with *M. jalaludinii* with or without *Lactobacillus* cultures (TLM, TM) showed significantly higher BW gain and feed intake when compared to broilers fed low-aP diet (T). Chickens fed low-aP diet supplemented with *Lactobacillus* cultures (TL) showed lower BW gain and feed intake when compared to chickens fed low-aP diet supplemented with *M. jalaludinii* (TM). This indicates the ability of phytase produced by *M. jalaludinii* in improving the growth performance of chickens fed low-aP diets. The BW gain and FCR of broilers fed low-aP diet supplemented with both *M. jalaludinii* and *Lactobacillus* cultures (TLM) were significantly improved ($P<0.05$) when compared to broilers fed basal (B) or low- aP (T) diets. The best FCR was observed in chickens fed low-aP diet supplemented with both *M. jalaludinii* and *Lactobacillus* cultures. The improved FCR could be the result of a combination of effects like the enhancement in mineral utilization and increase in energy and protein digestion. Supplementation of both *M. jalaludinii* and *Lactobacillus* cultures significantly reduced ($P<0.05$) the abdominal fat deposition in broilers as compared to chickens fed basal diet. Broilers fed diets supplemented with *Lactobacillus* cultures had lower ($P<0.05$) serum cholesterol level. *Mitsuokella jalaludinii* supplementation to chickens fed low-aP diet (TM) significantly increased ($P<0.05$) the digestibility of dry matter (DM), P, Ca, Zn and N. *Mitsuokella jalaludinii* supplementation to chickens fed low-aP diets (TM, TLM) significantly improved ($P<0.05$) the P retention when compared to the other six dietary treatments. Phosphorous excretion of broilers fed low-aP diet was much lower with *M. jalaludinii* supplementation (TM, TLM) as compared to broilers fed the basal diets. Phosphorous excretion could be reduced by about 48% with *M. jalaludinii* supplementation. Broilers fed low-aP diets showed higher ($P<0.05$) N retention when supplemented with *M. jalaludinii*. This indicates the increase in the availability of N in the feed. It is known that phytic acid in the plant feeds can bind protein to form phytate-protein complex which is less soluble. Hence, the increase in the digestibility of N indicates the ability of the enzyme phytase to hydrolyzse the phytate-protein complex. *Mitsuokella jalaludinii* supplementation also increased ($P<0.05$) Ca retention in chickens fed low-aP diets. The P content of tibia DM was significantly increased ($P<0.05$) in broilers fed TM or TLM diets. The tibia P of broilers fed these diets were comparable to those fed basal diets. However, Ca content in tibia DM was significantly reduced ($P<0.05$). On the other hand, Ahmad et al. (2000) reported a significant increase in Ca of tibia DM of broilers fed corn-soybean diet supplemented with microbial phytase. *Lactobacillus* cultures supplementation to broilers fed low-aP diet showed no improvement in P or Ca content of tibia DM. Although, tibia Ca was significantly($P<0.05$) reduced in broilers fed low-aP diet with *M. jalaludinii* supplementation (TM and TLM), but the values were comparable to those fed basal diets. Dietary *M. jalaludinii* supplementation did not influence the concentrations of Zn, Cu and Mn in tibia ash. Supplementation of *M. jalaludinii* culture to chickens fed low-aP diet significantly increased ($P<0.05$) plasma P and Ca concentrations. No significant differences were found in the weight (% body weight) of liver, spleen, bursa, gizzard, proventriculus and ileum of broilers given the different dietary treatments. During the first three weeks, mortality was low and no death was observed in birds fed B, TM and TL diets. However, during the last three weeks, mortality rate increased (2-10%) in all dietary treatments, except for birds fed BL diet, where no death was recorded. The mortality of broilers fed diet T was significantly higher (13.3 %) than those fed BL or TML diets (3.3 %). This indicates the ability of *Lactobacillus* spp. in improving the health conditions of the broilers.

Conclusions

The results showed that the performance and nutrient utilization of broilers fed a corn-soybean meal diet containing low available P (aP) supplemented with a combination of freeze-dried active *M. jalaludinii* and *Lactobacillus* cultures were significantly improved ($P<0.05$). There were no significant effects on the weight of organs of broilers fed low-aP diets supplemented with both bacterial cultures. The abdominal fat deposition and serum cholesterol level of broilers were also significantly reduced ($P<0.05$) with the supplementation of *M. jalaludinii* and *Lactobacillus* cultures. The supplementation of *M. jalaludinii* to chickens increased P and N retention, hence reducing the potential of P pollution caused by P excretion and increasing the bioavailability of protein, respectively. The use of *Lactobacillus* spp. as probiotics enhances growth performance as well as reduces the mortality rate of the broilers.

References

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Benefits from the study

More than 60% of phosphorous (P) in poultry diets are present as phytate P. Theoretically, the P contents of feeds should be sufficient to meet the requirement of poultry. Unfortunately, phytate P is poorly utilized by poultry because of the lack of the digestive enzyme phytase. *Mitsuokella jalaludinii*, isolated from the rumen of a cattle, is used as a feed supplement to increase the utilization of phytate compounds in chicken. The enzyme phytase dephosphorylates phytate compounds to release the bound P, hence increasing the availability of P. This will reduce the requirement of inorganic P which is normally added to the diet and the amount of P entering the environment. The enzyme also improves Ca and N utilisation. The *Lactobacillus* spp. isolated from the chicken intestine have been successfully used as probiotics. The research findings show the beneficial effects of using both *M. jalaludinii* and the *Lactobacillus* spp. in broilers fed low-aP diets. The *M. jalaludinii* improves mineral utilisation and digestibilities of feed and reduces P excretion in poultry waste, while the *Lactobacillus* spp. improve the growth performance and reduce the mortality rate.

Project Publications in Conference Proceedings

1. Lee HC, Abdullah N, Zulkifli I and Ho YW. 2000. Storage test on *Lactobacillus* cultures used as a feed supplement for poultry. In: Proceedings 23[rd] Symposium Malaysian Society for Microbiology. p 216-218.

2. Lee HC, Abdullah N, Zulkifli I and Ho YW. 2001. Effects of supplemental *Mitsuokella* sp. culture and *Lactobacillus* cultures on the bioavailability of phosphorous and calcium in broiler chickens. In: Proceedings 23[rd] Malaysian Society Animal Production Conference. p 120-122.

3. Lee HC, Abdullah N, Zulkifli I and Ho YW. 2003. Effects of *Mitsuokella Jalaludinii* and *Lactobacillus* spp. supplementation on the growth performance, tibia P and Ca of broiler chickens. In: Proceedings International Conference of Animal Nutrition, in CD (not paginated).

Graduate Research

	Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
	Lee Hooi Ching	Effects of *Mitsuokella jalaludinii* and *Lactobacillus* cultures supplementation on the performance and nutrient utilization of broiler chickens	Animal Nutrition	M.Sc	2002

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