

## Effects of Supplementing Rumen Bacterial Phytase on the Performance and Nutrient Utilization of Broiler Chickens

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**Key words:** phytase, *Mitsuokella jalaludinii*, poultry, digestion, phosphorous

### Introduction

About two-thirds of the total phosphorus (P) in cereal grains and oil seed meals exist in a phytate-bound form. The availability of phytate P from plant-derived feedstuffs is low in monogastric animals, such as chickens, because of very low or no phytase activity in their digestive tracts (Common, 1989). Hence, in poultry production, expensive phosphates are added in feeds to meet the animal dietary requirement. Phytate is also considered as an anti-nutritive factor as it can chelate important minerals such as  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$ , thus reducing their bioavailability; and it can bind with protein to form a phytate-protein complex which is less soluble, resulting in decreased protein digestibility (Carnovale *et al.*, 1988).

It has been reported that the supplementation of microbial phytase improves the phytate P utilization and the bioavailability of Ca in broiler chickens (Sebastian *et al.*, 1996a,b). All the phytases used in these studies originated from *Aspergillus niger*. Very little research has been conducted on microbial phytase from ruminants. Ruminants are able to utilize phytate P efficiently as they have phytase-producing bacteria in the rumen. Recently, a new phytase-producing bacterial species, *Mitsuokella jalaludinii*, which can hydrolyze phytate in the feed of chickens effectively *in vitro* has been isolated from the rumen of cattle in Malaysia (Lan *et al.*, 2002a). Hence, the objective of this study was to determine the efficacy of supplementation of active *M. jalaludinii* culture on the performance and utilization of nutrients in broiler chickens fed a corn-soybean meal diet with low available P.

### Materials and Methods

Active *M. jalaludinii* culture (AMJC) was prepared according to the procedure described by Lan *et al.* (2002b). The activity of phytase was found to be 7.90 to 8.05 phytase units (U) per min per gram of fermented broth at pH 5.5 and 37°C. Three hundred and thirty six one-day old male broiler chicks (Avian-43) were used. The chicks were weighed individually, wing-banded and assigned randomly to 24 cages of 14 chicks each. Heat was provided with a heating lamp during the first 2 weeks. The feed was provided *ad libitum* in a mash form at 0900 and 1800 h. Four cages were used for each dietary treatment. The six dietary treatments were: (T1) low non-phytate P (low-NPP) feed, (T2) low-NPP feed + 250 U phytase/kg of feed, (T3) low-NPP feed + 500 U phytase/kg of feed, (T4) low-NPP feed + 750 U phytase/kg of feed, (T5) low-NPP feed + 1,000 U phytase/kg of feed, and (T6) normal-NPP. The low-NPP feed for chickens from 1 to 21 and 22 to 42 d of age contained 0.240% and 0.232% NPP, respectively. The normal-NPP feed for chickens from 1 to 21 and 22 to 42 d of age contained 0.460% and 0.354% NPP, respectively. The composition of the basal diets is as reported by Lan *et al.* (2002b). Feed consumption was recorded daily on per cage basis. Body weight and body weight gain of birds were measured individually on a weekly basis. The experimental period was 42 d. Total excreta were collected for 3 consecutive days (exactly for 72 h) from the birds in each cage during Days 11 to 13 and Days 18 to 20. At the same time, 100 g of feed sample were taken daily from each treatment. The feed samples and excreta were stored separately at -20°C until used for analysis. Feeds and excreta were analyzed for proximate components and minerals (P, Ca, Mn and Cu). At the end of third week, five chicks were euthanized and blood samples were collected. The left tibia was removed and ashed. Plasma and tibia ash were analyzed for Ca, Mn, Zn, Cu and P.

### Results and Discussion

Chicks fed low-NPP diet without AMJC supplementation had significantly ( $P < 0.05$ ) lower body weight at Days 21 and 42 compared to those fed other dietary treatments. Supplementation of AMJC to low-NPP diet (250-1,000 U phytase/kg of feed) increased significantly ( $P < 0.05$ ) the body weight gain during the whole experimental period. Feed conversion ratio of broilers from 1 to 42 d of age was significantly ( $P < 0.05$ ) improved by AMJC (250-1,000 U phytase/kg of feed) supplementation to low-NPP diet. The results indicate that AMJC supplementation could effectively improve the poorer growth and lower feed intake caused by the low NPP content in the diet. The results are consistent with those reported in earlier studies on microbial phytase (Broz *et al.*, 1994; Sebastian *et al.*, 1996a,b). The addition of AMJC (250 U phytase/kg feed) into low-NPP diet is adequate to optimize the performance of chickens. This level is lower than that of commercial *A. niger* phytase recommended for broiler chicken diets (BASF, 1996).

From 11-13 and 18-20 d of age, AMJC supplementation significantly ( $P < 0.05$ ) increased the apparent metabolizable energy (AME) of low-NPP diet as compared to normal-NPP or low-NPP diet without AMJC supplementation. The apparent digestibility of CP and DM followed a pattern similar to that of AME. From 11 to 13 d of age, AMJC addition at various

levels to low-NPP diet increased the apparent digestibility of CP when compared to low-NPP diet without AMJC supplementation or normal-NPP diet. From 18 to 20 d of age, supplementation of AMJC (250-1,000 U phytase per kg of feed) to low-NPP diet significantly ( $P<0.05$ ) increased the apparent digestibility of CP when compared to normal-NPP diet. However, the DM digestibility was significantly ( $P<0.05$ ) improved by supplementation only at medium to high levels of AMJC (500-1,000 U phytase/kg of feed) from 18 to 20 d of age.

The lowest P retention was observed in birds fed low-NPP diet without AMJC supplementation. The P retention in chicks fed normal-NPP diet was lower ( $P<0.05$ ) than those in chickens fed low-NPP diet added with AMJC. The increase of P retention resulted in significant decrease of P excretion. The amount of P excreted by chickens supplemented with AMJC (500 U phytase/kg feed) was reduced by 56–59 %. Reduction of P excretion is particularly important in the reduction of P pollution by poultry manure.

Supplementation of AMJC to low-NPP diet increased significantly ( $P<0.05$ ) the Ca retention in chickens. These findings are comparable with the results of earlier studies on microbial phytase supplementation in broiler chicken diets (Sebastian *et al.*, 1996a; Ahmad *et al.*, 2000). The improvement in Ca availability is expected because phytase liberates Ca from the Ca-phytate complex and as the availability of P increases, the availability of Ca also increases.

Supplementation of medium to high levels of AMJC (500-1,000 U phytase/kg of feed) to low-NPP diet significantly ( $P<0.05$ ) increased the retention of Mn in chickens from 18 to 20 d of age. Mn retention was also significantly ( $P<0.05$ ) higher in chickens fed low-NPP diet containing different levels of AMJC when compared to those fed normal-NPP diet. Similar finding was reported by Windisch and Kirchgessner (1996) who found that phytase supplementation increased the Mn retention in broiler chickens and piglets. Significant ( $P<0.05$ ) improvement of Zn retention in chickens was observed only when a high level of AMJC (1,000 U phytase/kg of feed) was incorporated into low-NPP diet.

Cu retention in chickens fed low-NPP diet was significantly ( $P<0.05$ ) increased when supplemented with AMJC from 11 to 13 and 18 to 20 d of age. Chickens fed normal-NPP diet retained significantly ( $P<0.05$ ) less Cu than those fed low-NPP diet with AMJC supplementation. Similar result was also reported by Sebastian *et al.* (1996a) who found a significant improvement in Cu retention in broiler chickens by microbial phytase supplementation.

Chicks fed low-NPP feed added with AMJC had similar tibia ash percentages as those fed the normal-NPP diet. Generally, supplementing AMJC to low-NPP feed increased ( $P<0.05$ ) Ca, decreased ( $P<0.05$ ) Mn and Cu, but did not affect Zn and P concentrations in tibia ash. Supplementing AMJC also increased ( $P<0.05$ ) plasma P but had no effect on plasma Ca or Mn. Plasma Zn concentration was increased only when a high level of AMJC (1,000 U phytase/kg of feed) was used.

## Conclusions

Feeding trials conducted in the present study showed that chicks consuming the low-NPP diet without AMJC supplementation showed slower growth rate, lower retention of P, reduced bone ash of tibia and lower plasma P concentration. However, supplementation with different levels of AMJC (equivalent to 250, 500, 750 and 1,000 U of phytase/kg of feed) to the low-NPP diet increased the body weight gain and feed intake of chickens to a level comparable to that obtained from chickens fed the normal-NPP diet. Supplementation of AMJC to the low-NPP diet also increased the P, Ca, Mn, Zn and Cu retentions in broiler chickens. Increase in P utilization is particularly important in the reduction of the amount of organic P excreted and reducing the requirement of inorganic P in the diet. This will result in a decrease of P in poultry waste entering the environment. The results also indicate that AMJC incorporated into a low NPP diet equivalent to 250 U phytase/kg feed is adequate to optimize the performance of chickens.

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## Benefits from the study

Poultry diet is mostly composed of cereals and their by-products and more than 60% of P is present as phytate P. Theoretically, the P contents of feeds in the diet should be sufficient to meet the requirement of poultry. Unfortunately, phytate P is poorly utilized because of the lack of the digestive enzyme, phytase, to hydrolyze phytate into inorganic P. Phytate also chelates important minerals such as Ca, Zn, Cu, Mn and Fe. The limited ability of poultry to utilize phytate P poses two problems, i.e., the need to supplement inorganic P in the feed and the excretion of large amounts of organic P in manure which pollutes the environment. In the present study, *Mitsuokella jalaludinii* which was used as a feed supplement was isolated from a natural environment, and its supplementation to chicks fed low NPP diet significantly improved the growth performance and mineral utilization of the chicks. Thus, the inorganic P supplemented to a normal NPP diet could be

effectively replaced by the freeze-dried active bacterium without any adverse effect on the performance and nutrient use of the birds.

#### Project Publications in Refereed Journals

1. Lan GQ, Ho YW, and Abdullah, N. 2002. *Mitsuokella jalaludinii* sp. nov., from the rumens of cattle in Malaysia. *Int. J. Syst. Evol. Microbiol.* 52:713-718.
2. Lan GQ, Abdullah N, Jalaludin S and Ho YW. 2002. Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chicken fed corn-soybean meal diets. *Poultry Sci.* 81: 1522-1532
3. Lan GQ, Abdullah N, Jalaludin, S and Ho YW. 2002. Culture conditions influencing phytase production of *Mitsuokella jalaludinii*, a new bacterial species from the rumen of cattle. *J. Appl. Microbiol.* 93: 668-674.
4. Lan GQ, Abdullah N, Jalaludin S and Ho YW. 2002. Optimization of carbon and nitrogen sources for phytase production by *Mitsuokella jalaludinii*, a new rumen bacterial species. *Lett. Appl. Microbiol.* 35: 157-161.

#### Project Publications in Conference Proceedings

1. Lan GQ, Ho YW, Abdullah N and Jalaludin S. 2000. Effects of carbon and nitrogen sources on phytase production from *Mitsuokella* sp. In: Proceedings of the 23<sup>rd</sup> Symposium Malaysian Society for Microbiology. p 178 - 179.
2. Lan GQ, Ho YW, Abdullah N and Jalaludin S. 2000. Efficacy of supplemental *Mitsuokella* sp. culture and Natuphos® phytase on the performance of broiler chickens. In: Proceedings of the 22<sup>nd</sup> Malaysian Society of Animal Production Annual Conference. p 169- 170.
3. Lan GQ, Ho YW, Abdullah N and Jalaludin S. 2001. Efficacy of supplementation of *Mitsuokella* sp. culture on mineral utilization in broiler chickens fed corn-soybean meal diets. In: Proceedings 23<sup>rd</sup> Malaysian Society of Animal Production Annual Conference. p 110-111.
4. Lan GQ, Abdullah N, Jalaludin S and Ho YW Ho. 2002. Efficacy of supplemental freeze dried culture of a new species of *Mitsuokella* and Natuphos® phytase on nutrient utilization of broiler chickens. In: Proceedings 24<sup>th</sup> Malaysian Society of Animal Production Annual Conference. p 64-65.

#### Graduate Research

	Name of Graduate	Research Topic	Field Expertise of	Degree Awarded	Graduation Year
	Lan Ganqiu	Studies on the characterization and utilization of a new phytase-producing bacterium isolated from the rumen of cattle	Animal Nutrition	Ph.D	2001

IRPA Project number: 01-02-04-0365)

UPM Research Cluster:AFF

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