Optimization of carbon and nitrogen sources for phytase production by *Mitsuokella jalaludinii* and the effects of supplementation of *Mitsuokella jalaludinii* culture and Natuphos® phytase on the performance and nutrient utilization of broiler chickens

Lan, GQ, Abdullah, N, Jalaludin, S and Ho, YW

Institute of Bioscience
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

Telephone Number of Corresponding Author: 03-89466710
E-mail of Corresponding Author: norhani@fsas.upm.edu.my

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Introduction

Phytate is the major form of phosphorus (P) containing compounds in plants and cereal grains. The nutrient availability of this form of P is poor in the monogastric animals because of the low phytase activity in their digestive tracts. The low digestibility of phytate in monogastric results in the need to supplement inorganic P in the diets, the excretion of indigestible P in the feces which pollutes the environment and the reduction of nutritional value of the feed. Phytate also chelates other minerals like Ca^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+}, thus reducing their bioavailability (Carnovale *et al.*, 1988). The supplement of microbial phytase to monogastrics has been found to significantly improved the phytate-P utilization by the animals (Simons *et al.*, 1990). Microbial phytase supplementation also has a positive effect on the bioavailability of other minerals like Ca, Zn and Cu in broiler chickens (Sebastian *et al.*, 1996 and Yi *et al.*, 1996).

In contrast to monogastric animals, ruminants are able to utilise phytate P readily. The unique capacity of ruminants to utilize phytate is attributed to the phytase-producing bacteria residing in the rumen. In a recent screening for phytase-producing bacteria, a new bacterial species, *Mitsuokella jalaludinii*, was isolated from the rumen of cattle in Malaysia, and it was found to hydrolyze phytate actively *in vitro* (Lan *et al.*, 2002). However, there is no information on the influence of medium components on its phytase production. Thus, the objectives of the present study were to evaluate the effects of different carbon and nitrogen sources on phytase production in batch fermentation and to optimise the carbon and nitrogen concentrations for phytase production. The study on the effects of *M. jalaludinii* culture supplementation on the performance and nutrient utilization of broilers compared to that of a commercial phytase (Natuphos® phytase) was also conducted.

Materials and Methods

Medium MF1 was used to study the effects of carbon (C) and nitrogen (N) sources on phytase production. The C sources (glucose, cellobiose and soluble starch, designated as GCS) in MF1 were replaced with rice bran (RB) or palm kernel cake (PKC) or molasses (ML), and the N sources (trypticase peptone and yeast extracts, designated as TPY), were substituted with soybean milk (SM) or enzymatic digested SM (EDSM) (Lan *et al.*, 2002). To study the optimum conditions of C and N concentrations for phytase production, media at varying concentrations of RB and SM were used. All media were prepared anaerobically. The *M. jalaludinii* cultured in MF1 (12 h, 39°C) was used as the inoculum (5% v/v). Growth was determined by viable counts after 12 h incubation at 39°C. The RB-SM medium was used to study the effects of pH, temperature and glucose on phytase activity. For the feeding trial, *M. jalaludinii* was cultured in RB-SM broth medium (15% RB and 20% SM) for 12 h. After centrifugation (8000×g, 15 min, 4 °C), the bacterial pellet was washed twice with 0.1 M sodium acetate buffer (pH 5.0). The pellet was mixed with sucrose (final concentration 8%, w/w) and freeze-dried to obtain freeze-dried active *M. jalaludinii* culture (FD-AMJC) or dried (65°C, 72 h) to inactivate the bacteria (IMJC). The dried pellet was ground (1mm sieve) and phytase activity was determined. A unit of phytase is defined as the quantity of enzyme which liberates 1µmol inorganic orthophosphate min^{-1} from 5.1mM sodium phytate at pH 5.5 and 37°C. The commercial phytase used was Natuphos® phytase (BASF, Germany). A total of 360 one-day old chicks (Avian-43) were fed *ad libitum* low available P (aP) diet (0.21% aP) supplemented with either FD-AMJC, IMJC or Natuphos® phytase for 21 days. Each chick was weighed and assigned randomly to 24 cages of 15 chicks each. Four diets used werelow-aP feed+2.0% IMJC; low-aP feed+2% FD-AMJC (500U phytase/kg of feed); low-aP feed+2% IMJC+500U of Natuphos® phytase/kg of feed and normal-aP (0.44% aP) feed+2% IMJC. The supplements were mixed daily with diets at feeding time. Parameters measured include growth performance, nutrient utilization, ash content and mineral concentration of tibia and plasma.

Results and Discussion

Viable cell counts among all media were similar, except for the media containing ML as the C source, which had significantly lower counts. However, phytase production by *M. jalaludinii* was significantly ($P<0.05$) increased when the GCS (C source) and TPY (N source) in MF1 medium were replaced with RB or PKC and SM or EDSM, respectively. GCS produced much lower phytase as compared to RB or PKC. In the medium containing GCS, *M. jalaludinii* may utilize glucose and cellobiose more rapidly, resulting in an accumulation of organic acids, such as acetic acid which not only reduces the pH of medium but

is also toxic to the bacterial cells, thus suppressing the enzyme synthesis. SM and EDSM were better N sources for phytase production of *M. jalaludinii* than TPY. The high levels of amino acids in TPY might have repressed phytase production. The best media for phytase production were those containing RB+SM or RB+EDSM. The optimal concentrations of RB and SM in the medium were 15% RB and 20% SM, 20% RB and 10% SM or 20% RB and 20 % SM and the phytase activities in the media were 12.5, 12.9 and 12.7 U g^{-1} culture broth, respectively. Sunitha *et al.*, (1999) reported that by optimising peptone, yeast extract and NaCl concentrations in Luria Bertani (LB) medium, phytase production by *E. coli* increased by 1.2 times. Similarly, by optimizing RB and SM concentrations, the phytase production by *M. jalaludinii* increased by 2.6 times. It was also observed that phytase production by *M. jalaludinii* was induced by supplementing 0.5 % sodium phytate, but the effect was lesser than that obtained from RB supplementation. Inclusion of glucose into RB-SM medium inhibited phytase production, but did not affect viable cell counts, indicating phytase production was not related to number of viable cells. The optimum pH and temperature for phytase production were 7.2 and 39°C, respectively. The addition of 8% sucrose to *M. jalaludinii*, significantly improved the stability of phytase during freeze drying and storage. After 6 weeks of storage at 4°C, 92 % activity still remained.

The feeding trial showed that supplementation of FD-AMJC or Natuphos® phytase to a low-aP diet significantly increased the body weight gain and feed intake of chickens. This indicates that FD-AMJC has a similar effect as Natuphos® phytase in improving the poorer growth caused by the low-aP content in the diet. Although supplementation of FD-AMJC or Natuphos® phytase to low-aP diet significantly improved the feed conversion ratio, the improvement was significantly better in chicks fed FD-AMJC. This may be attributed to not only the effect of phytase but also the effects of other tissue degrading enzymes such as protease and amylase existing in FD-AMJC. Supplementation of FD-AMJC significantly increased the AME value, and DM and CP digestibilities of low-aP diet. The increased AME value and DM and CP digestibilities showed that FD-AMJC supplementation could improve the dietary nutrient utilisation by broiler chickens. Natuphos® phytase supplementation to low-aP diet also increased the apparent digestibility of DM but it had no effect on the AME value and CP digestibility. Supplementation of FD-AMJC or Natuphos® phytase to low-aP diet significantly increased the P retention (11.3 – 25.7 and 6.0 – 25.8 percentage units, respectively) in chickens. These findings are comparable with the results of earlier studies on microbial phytase supplementation in broiler chicken diets (Sebastian *et al.*, 1996). Supplementation of FD-AMJC or Natuphos® phytase to low-aP diet significantly improved the relative retention of Ca. The improvement of Ca availability may be due to the liberation of Ca from the Ca-phytate complex by phytase. Supplementation of FD-AMJC to low-aP diet significantly increased Mn retention by 4.4 and 5.6 percentage units, respectively, in birds at 18 – 20 d of age. At 18 – 20 d of age, the Zn retention in chickens was significantly improved by 18.4 percentage units with FD-AMJC supplementation but not by Natuphos® phytase supplementation. Only FD-AMJC supplementation increased the Cu retention. Supplementation of FD-AMJC or Natuphos® to low-aP feed significantly increased the ash content of tibia; increased the Ca, decreased the Mn but had no effect on the P and Zn concentrations in tibia ash. On the other hand, FD-AMJC and Natuphos® phytase supplementations significantly increased the Ca and P concentrations in the DM of tibia. Higher plasma P and lower plasma Ca concentrations were observed in chickens fed low-aP diet with FD-AMJC or Natuphos® phytase supplements.

Conclusions

Replacement of C and N sources in MF1 medium by RB and SM markedly increased phytase production. The optimal concentrations of RB and SM in the medium for phytase production were 15% RB and 20% SM or 20% RB and 10% SM or 20% RB and 20% SM. Optimal pH and temperature for phytase production were 7.0 and 39°C, respectively. Addition of 8% sucrose improved the stability of phytase activity during the freeze-drying process and storage. Dietary supplementation of FD-AMJC or Natuphos® phytase to low-aP diet could improve the feed intake, feed conversion rate, body weight gain, retention of P, Ca and Mn, ash content of the tibia and the concentrations of plasma P and Zn of broiler chickens. FD-AMJC supplementation to low-aP improved the AME value of the diet, retention of Cu and Zn, and the digestibility of N and DM, but Natuphos® phytase supplementation only improved the digestibility of DM. The results showed that FD-AMJC was more efficient in improving the dietary nutrient utilization in chickens than Natuphos® phytase supplementation.

Benefits from the study

There has been a great deal of interest on the study of microbial phytase production and the optimization of media and conditions for maximum production of the enzyme with the aim to increase yields to make it economical as a commercial product. In this study, *M. jalaludinii* was found to produce high phytase enzyme when grown in a medium containing rice bran and soybean milk. Chickens fed low available P (aP) supplemented with *M. jalaludinii* showed similar growth performance as those fed normal-aP diet. This indicates the effectiveness of the product in improving phytate P utilization, hence decreasing the amount of organic P excreted and reducing the requirement of inorganic P in the diet. The activity of the phytase enzyme produced is comparable to that of Natuphos® phytase, but the presence of other enzymes in the bacterium help further the digestive processes of the birds. Hence, *M. jalaludinii* supplementation was more efficient in improving the dietary nutrient utilization in broiler chickens than Natuphos® phytase. The new bacterial species has the potential to be used commercially as a feed supplement to improve not only P utilization but other minerals as well.

References

Carnovale, E., E. Lugaro, and Lombardi-Boccia, G. (1988). Cereal Chem. 65: 114-117.
Lan, G.Q., Ho, Y.W. and Abdullah, N. (2002). Int. J. Syst. Evol. Microbiol. 52: 713-718.
Sebastian, S. Touchburn, S.P., Chavez, E.R and Lague, P.C. (1996). *Poultry Science*, 75: 729-736.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W, Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R.F. and Verschoor, G.J. (1990). *British journal of nutrition,* 64: 525-540.
Sunitha, K., Lee, J.K. and Oh, T.K. (1999). Bioprocess Engineering, 21: 447-481
Yi, Z., Kornegay, E.T., Ravindran, V. and Denbow, D.M. (1996). *Poultry Science,* 75: 240-249.

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.

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. 1999. In vitro dephosphorylation of phytate in corn-soybean meal for broiler chicken by cell bound phytase of *Mitsuokella* sp. In: Proceedings 3rd UNESCO National Workshop on Promotion of Microbiology in Malaysia. p. 33.
2. Lan GQ, Ho YW, Abdullah N and Jalaludin S. 1999. The efficacy of supplemental *Mitsuokella* sp. culture on the performance of broiler chicken fed corn-soybean diet. In: Proceedings National Congress Animal Health & Production. p. 387-389.
3. 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 23rd Symposium Malaysian Society for Microbiology. p 178 - 179.
4. 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 22nd Malaysian Society of Animal Production Annual Conference. p 169- 170.
5. 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 24th Malaysian Society of Animal Production Annual Conference. p 64-65.

Graduate Research

	Name of Graduate	Research Topic	Field of Expertise	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

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