Efficacy of Supplementation of a Phytase-Producing Bacterial Culture on the Performance and Nutrient Use of Broiler Chickens Fed Corn-Soybean Meal Diets

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ABSTRACT We evaluated the efficacy of supplementation of active *Mitsuokella jalaludinii* culture (AMJC) on the growth performance, nutrient use, and mineral concentrations in tibia bone and plasma of broiler chickens fed corn-soybean meal diets. Dietary treatments included low-nonphytate P (NPP) feed (containing 0.24% and 0.232% NPP for chicks from 1 to 21 and 22 to 42 d of age, respectively), low-NPP feed added with different levels of AMJC (equivalent to 250, 500, 750, and 1,000 U phytase/kg of feed), and normal-NPP feed (containing 0.46 and 0.354% NPP for chicks from 1 to 21 and 22 to 42 d of age, respectively). Supplementation of AMJC to low-NPP feed increased ($P < 0.05$) weight gain and feed intake and decreased ($P < 0.05$) feed:gain ratio of chickens during the whole experiment (Days 1 to 42). Supplementation of AMJC increased ($P < 0.05$) the AME value, digestibility of DM and CP, and retention of P, Ca, and Cu. Mn retention in broilers was only increased ($P < 0.05$) by AMJC supplementation from 18 to 20 d of age, and Zn retention was improved ($P < 0.05$) only at a high level of AMJC (equivalent to 1,000 U phytase/kg of feed) 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 significantly ($P < 0.05$) 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 (equivalent to 1,000 U phytase/kg of feed) was used. In conclusion, AMJC supplementation to low-NPP feed improved growth performance; AME value; digestibility of CP and DM; use of Ca, P, and Cu; and bone mineralization.

(*Key words*: phytase-producing bacteria, broiler, nutrient use)

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INTRODUCTION

About two-thirds of the total 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). A large proportion of phytate in the feed consumed by chickens is passed through the digestive tract and excreted in the manure. Hence, in poultry production, producers have to supplement expensive phosphates in feeds to meet an animal's dietary requirements. Furthermore, phytate is considered as an antinutritive factor: it can ionically chelate with nutritionally important minerals, such as Ca^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+}, thus reducing their bioavailability (Erdman, 1979). Phytate can also bind with protein at a broad range of pH to form a phytate-protein complex, which is less soluble, resulting in decreased protein digestibility (Carnovale et al., 1988). Phytate has also been found to inhibit trypsin and pepsin activities (Singh and Kirkorian, 1982).

It has been well demonstrated that phytase supplementation improves P use in broilers and layers (Simons et al., 1990; Schöner et al., 1991; Broz et al., 1994; Sebastian et al., 1996a,b; Um et al., 1999). Phytase supplementation also has a positive effect on the bioavailability of other minerals such as Ca (Schöner et al., 1991; Broz et al., 1994; Sebastian et al., 1996a), Zn (Thiel and Weigand, 1992; Sebastian et al., 1996a; Yi et al., 1996b), and Cu (Sebastian et al., 1996a) in broiler chickens. It has been reported that phytase supplementation improves N digestibility in pigs (Yi et al., 1994) and N retention in broiler chickens (Ravindran et al., 1999; Kies et al., 2001) and laying hens (Van der Klis and Versteegh, 1991).

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Abbreviation Key: AMJC = active *Mitsuokella jalaludinii* culture; AP = available P; NPP = nonphytate P.

All of the phytases used in these studies originated from *Aspergillus niger*. Very little research has been conducted on microbial phytase from other sources, such as ruminants. Ruminants, in contrast to monogastrics, are able to use phytate-P efficiently (Nelson et al., 1976). The special ability of ruminants to utilize phytate is attributed to phytase-producing bacteria in the rumen (Raun et al., 1956). Rumen bacteria are one of the major microorganisms in the rumen ecosystem, and they produce many kinds of enzymes, including phytase, which have potential for industrial application (Cheng et al., 1999). Recently, in the process of large-scale screening for phytase-producing bacteria from rumens, a new phytase-producing bacterial species, *Mitsuokella jalaludinii*, which can hydrolyze phytate in the feed of chickens effectively in vitro has been isolated from rumens of cattle in Malaysia (Lan et al., 2002). The objective of this study was to determine the efficacy of supplementation of active *M. jalaludinii* culture broth on the performance and use of nutrients in broiler chickens fed a corn-soybean meal diet with low available P (AP).

MATERIALS AND METHODS

Preparation of M. jalaludinii Culture Broth as a Phytase Supplement

Mitsuokella jalaludinii was cultured in reduced rice bran-soybean milk (RB-SM) medium containing 15% rice bran, 20% soybean milk, and 0.15% L-cysteine[3] and was incubated anaerobically at 39 C for 12 h. After 12 h of incubation, about 1.0 g of the fermented broth was diluted to 20 mL by adding 0.1 *M* acetate buffer (pH 5.5), and 5 mL was centrifuged at 8,000 × *g* for 20 min at 4 C. The pellet was washed twice with 5 mL of acetate buffer. The washed pellet was mixed with 5 mL of acetate buffer containing 5.1 m*M* sodium phytate[3] (assay mixture, pH 5.5) and incubated at 37 C in a shaking water bath for 15 min. At the end of incubation period, the reaction was terminated by adding 5 mL 5% trichloracetic acid.[4]

The released P was determined by the method of Heinonen and Lathi (1981). For determining the native P in the enzyme and substrate, the enzyme and substrate in the assay mixture were replaced with acetate buffer, and the P concentration was determined as described above. After subtracting the native P in the enzyme and substrate, the released P from the assay mixture was used to calculate the phytase activity. A unit of phytase activity is defined as the amount of enzyme that liberates 1 μmol P/min under the given assay conditions. The activity of phytase was found to be 7.90 to 8.05 phytase U per min per g of fermented broth at pH 5.5 and 37 C. After measuring the phytase activity, the fresh *M. jalaludinii* culture broth was used as a phytase supplement.

For determination of amylase and protease activities in the *M. jalaludinii* pellet, the washed pellet was suspended in 10 mL ice-cold 0.1 *M* potassium phosphate buffer (pH 6.8) and then disrupted by sonication at 4 C for 1 min, eight times, at 1-min intervals. The solution was centrifuged at 18,000 × *g* for 15 min. The supernatant was used to determine the enzyme activity. The amylase (α-1,4-glucan 4-glucanohydrolase, E.C. 3.2.1.1.) and protease activities of the supernatant were determined using the method of Somogyi (1960) and Lynn and Clevette-Redford (1984), respectively. An amylase activity unit was defined as the amount of amylase that will cause formation of reducing power equivalent to 1 mg of glucose in 30 min at 40 C. The protease activity unit was defined as milligrams of azocasein degraded during 2 h of incubation at 38 C. The activities of α-amylase and protease were found to be 3.6 to 7.4 and 1.9 to 9.3 units per gram of fermented broth, respectively.

Birds and Feeding Treatments

Three hundred thirty-six 1-d-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. The wire-floored cages (0.9-m length × 0.6-m width × 0.5-m height) were kept in an open house. Heat was provided with a heating lamp during the first 2 wk. The feed was provided ad libitum in a mash form and added to the feeder twice daily at 0900 and 1800 h. Four cages were used for each dietary treatment. Six dietary treatments were used: low nonphytate P (low-NPP) feed (Diet T1), low-NPP feed + 250 U phytase/kg of feed (Diet T2), low-NPP feed + 500 U phytase/kg of feed (Diet T3), low-NPP feed + 750 U phytase/kg of feed (Diet T4), low-NPP feed + 1,000 U phytase/kg of feed (Diet T5), and normal-NPP feed (Diet T6). The low-NPP feed for chickens from 1 to 21 and 22 to 42 d of age contained 0.24% and 0.232% NNP, respectively. The normal-NPP feed for chickens from 1 to 21 d and 22 to 42 d of age contained 0.460 and 0.354% NPP, respectively.

The composition of the basal diets is shown in Table 1. The normal-NPP diet contained similar concentration of Ca as the low-NPP diet, whereas the concentration of NPP was formulated to be similar as the total P content of the low-NPP diet (for chicks from 1 to 21 d of age). As such, the low-NPP diets would have similar nutrient content and Ca:NPP ratio as normal-NPP diet, provided the total dephosphorylation of phytate in the low-NPP diets takes place in vivo.

To ensure that all experimental diets were isonitrogenous and isocaloric, all diets were incorporated with the same percentage of *M. jalaludinii* culture broth. In Treatments 1 and 6, the feeds were incorporated with thermally inactivated *M. jalaludinii* culture broth, in Treatments 2 to 5, the feeds were incorporated with different weights of fresh active *M. jalaludinii* culture (AMJC) broth mixed with different amounts of thermally inactivated culture to obtain varying levels of phytase activity in the feeds. The thermally inactivated culture was prepared by boil-

[3]Sigma Chemical Co., St. Louis, MO.
[4]Fluka Chemie AG, Buchs, Switzerland.

TABLE 1. Composition of basal diets[1]

Ingredient (g/kg unless otherwise stated)	1 to 21 d		22 to 42 d	
	Normal NPP[2]	Low NPP	Normal NPP	Low NPP
Corn	541.0	545.67	600.10	601.50
Soybean meal	362.00	362.00	318.62	318.62
Fish meal	30.00	30.00	30.00	30.00
Palm oil	37.40	37.30	27.40	27.00
60% choline chloride	2.50	2.50	2.00	2.00
Vitamin premix[3]	1.00	1.00	1.00	1.00
Salt (NaCl)	2.00	2.00	1.00	1.00
DL-Methionine	1.68	1.68	0.33	0.33
Limestone	10.37	17.80	13.00	18.50
Dicalcium phosphate	12.00	0.00	6.50	0.00
Sulfamonomethoxine sodium[4]	0.05	0.05	0.05	0.05
Calculated nutrient content				
Ca	9.46	9.45	8.90	8.85
NPP	4.60	2.40	3.54	2.32
Total P	6.86	4.62	5.73	4.52
CP	225.0	225.0	210.9	211.0
ME (kcal/kg)	3,005	3,020	3,027	3,035
Analyzed composition				
CP	227	224	197.4	197.9
Ca	10.06	10.12	9.14	9.06
Total P	6.93	4.77	6.20	4.71
Zn (ppm)	111.1	114.1	108.2	113.4
Mn (ppm)	131.2	126.3	128.0	139.0
Cu (ppm)	32.8	33.2	26.1	25.7

[1]The phytase activities of the fresh active *Mitsuokella jalaludinii* culture broth (AMJC) and thermally inactivated *Mitsuokella jalaludinii* culture broth were determined before the cultures were mixed with the feed at feeding time (see Materials and Methods). The rates of AMJC incorporated into the feed to obtain phytase activities equivalent to 250, 500, 750 and 1,000 U phytase/kg feed were 3.11 to 3.17, 6.21 to 6.33, 9.32 to 9.49, and 12.42 to 12.66%, respectively.

[2]NPP = nonphytate phosphorus.

[3]Supplied per kilogram of diet: Fe, 100 mg; Mn, 110 mg; Cu, 20 mg; Zn, 100 mg; I, 2.0 mg; Se, 0.2 mg; Co, 0.6 mg; santoquin, 0.6 mg; vitamin A, 6,667 IU; vitamin D, 1,000 IU; vitamin E, 23 IU; vitamin K_3, 1.33 mg; cobalamin, 0.03 mg; thiamin, 0.83 mg; riboflavin, 2.0 mg; folic acid, 0.33 mg; biotin, 0.03 mg; pantothenic acid, 3.75 mg; niacin, 23.3 mg; pyridoxine, 1.33 mg.

[4]For prevention of coccidiosis.

ing AMJC at 100 C for 2 min, and its phytase activity was found to be inactivated by measuring the phytase activity by using the method described earlier.

The phytase activities of the AMJC and thermally inactivated broth were determined before the cultures were mixed daily with the feed at feeding time. The rates of AMJC incorporated into the feed to obtain phytase activities equivalent to 250, 500, 750, and 1,000 U phytase/kg feed were 3.11 to 3.17%, 6.21 to 6.33%, 9.32 to 9.49%, and 12.42 to 12.66%, respectively.

Feed consumption was recorded daily on a per-cage basis. The residual feed was collected once daily before the morning feeding. Body weight and body weight gain of birds were measured individually on weekly basis. Mortality was recorded daily and was used to adjust the total number of birds by the end of each week to determine the feed intake. All animal management and sampling procedures were in accordance with the guidelines of the Consortium Guide (1988). The experimental period was 42 d.

Sampling Procedures

Feed intake was recorded and total excreta were collected for 3 consecutive d (for exactly 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 was taken daily from each treatment for 3 consecutive d. The excreta from each cage were collected at 24-h intervals. The feed samples and excreta were stored separately at –20 C until used for analysis.

Analyses of Feeds and Excreta

The feed samples and feces were dried at 65 C to a constant weight and then ground through a 1-mm mesh screen. Feeds and excreta were analyzed for proximate components according to the AOAC procedures (1984). For analysis of minerals, samples of feeds and excreta were wet-ashed with HNO_3-H_2SO_4-$HClO_4$ (3:1:1, vol:vol:vol). Ca, Mn, Zn, and Cu concentrations were determined using an atomic absorption spectrophotometer,[5] whereas P was determined by the method of Pearson (1976). All samples were assayed in duplicate.

[5]Model SpectrAA 300/400, Varian, Inc., Palo Alto, CA.

TABLE 2. Effects of *Mitsuokella jalaludinii* supplementation on feed intake, body weight, and feed to gain ratio of broiler chickens fed a corn-soybean meal diet for 42 d[1]

	Dietary treatment[2]						
	T1	T2	T3	T4	T5	T6	SEM
Body weight (g)							
Day 1	43[a]	43[a]	41[a]	42[a]	42[a]	42[a]	0.6
Day 21	639[b]	716[a]	727[a]	731[a]	742[a]	738[a]	8.9
Day 42	1,744[b]	2,055[a]	1,996[a]	2,044[a]	2,041[a]	1,984[a]	27.1
Weight gain (g)							
1–21d	596[b]	674[a]	685[a]	689[a]	699[a]	696[a]	8.7
22–42 d	1,105[c]	1,338[a]	1,270[ab]	1,313[ab]	1,300[ab]	1,246[b]	27.3
1–42 d	1,701[b]	2,012[a]	1,955[a]	2,002[a]	1,999[a]	1,942[a]	27.0
Feed intake (g)							
1–21d	901[b]	1,012[a]	975[a]	1,002[a]	1,009[a]	1,011[a]	14.1
22–42 d	2,337[b]	2,570[a]	2,488[a]	2,529[a]	2,521[a]	2,524[a]	43.2
1–42 d	3,238[b]	3,582[a]	3,463[a]	3,531[a]	3,530[a]	3,535[a]	49.0
Feed:gain (g:g)							
1–21d	1.51[a]	1.50[a]	1.42[b]	1.45[ab]	1.44[ab]	1.45[ab]	0.022
22–42 d	2.12[a]	1.92[b]	1.96[b]	1.93[b]	1.94[b]	2.03[ab]	0.033
1–42 d	1.90[a]	1.78[b]	1.77[b]	1.76[b]	1.77[b]	1.82[b]	0.020

[a-c]Means in the same row without a common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of 14 chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

Plasma and Tibia Analyses

At the end of the third week (Day 21), five chicks were randomly selected from each cage, weighed, and euthanized by severing the jugular vein. The heparinized blood sample was collected from each bird, and the plasma was separated by centrifugation at 3,000 × *g* for 15 min at 4 C. The plasma samples (4 mL each) of three birds from each cage were digested with HNO_3 until colorless and then used for mineral determination.

The left tibia was removed from each bird. After removing all the soft tissues, the tibia was dried at 105 C until constant weight and then ignited at 600 C for 8 h. The ash was dissolved with 20 mL concentrated HNO_3, and the solution was boiled until colorless. Concentrations of Ca, Mn, Zn, Cu, and P in tibia ash and plasma were determined using the methods described above.

Statistical Analyses

Data obtained were analyzed by the general linear models procedures for analysis of variance (SAS Institute, 1997). Cage means were used to evaluate broiler performance, nutrient digestibility, bone ash, and bone and plasma minerals. Significant differences among treatment means were separated using the multiple range test with a 5% probability.

RESULTS

Body Weight, Weight Gain, Feed Intake, and Feed to Gain Ratio

The effects of supplementing AMJC on the performance of broiler chicks are summarized in Table 2. There were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio. Chicks fed a low-NPP diet without AMJC supplementation (Diet T1) had significantly ($P < 0.05$) lower body weight at Days 21 and 42 compared to those fed other dietary treatments. Supplementation of AMJC to the low-NPP diet (equivalent to 250 to 1,000 U phytase/kg of feed, Diets T2 to T5) significantly increased the body weight gain by 14.9 to 18.3% during the whole experimental period (Days 1 to 42). However, the body weights at 42 d of age were not significantly different among the birds fed diets supplemented with different levels of AMJC. No difference was observed for body weights at Day 42 between chicks consuming the normal-NPP diet (Diet T6) and those consuming the low-NPP diet with different levels of added AMJC (Diets T2 to T5).

Chicks fed a low-NPP diet without AMJC supplementation (Diet T1) consumed significantly less feed than those fed other dietary treatments. There was no difference in feed intake measured at the different experimental periods between chickens fed the normal-NPP diet and the low-NPP diet supplemented with different levels of AMJC.

Feed conversion ratio of broilers from 1 to 21 d of age was significantly improved by the supplementation of a medium level of AMJC (equivalent to 500 U phytase/kg of feed) to the low-NPP diet. From 22 to 42 d or 1 to 42 d of age, feed conversion ratio was significantly improved by the supplementation with all levels of AMJC to the low-NPP diet. However, the feed conversion ratio was similar between the normal-NPP and low-NPP diets supplemented with different levels of AMJC from 1 to 21 d, 22 to 42 d, and 1 to 42 d of age.

TABLE 3. Effects of *Mitsuokella jalaludinii* supplementation on the AME of diets and apparent digestibility of CP and DM in broiler chickens fed a corn-soybean meal diet[1]

	11 to 13 d			18 to 20 d		
Dietary treatment[2]	AME (kcal/kg DM)	CP (%)	DM (%)	AME (kcal/kg DM)	CP (%)	DM (%)
T1	3,056.3^{c}	60.2^{c}	67.0^{c}	3,137.4^{c}	59.3^{c}	71.4^{c}
T2	3,346.4^{a}	67.5^{ab}	72.8^{a}	3,313.5^{b}	60.8^{bc}	72.1^{bc}
T3	3,338.3^{a}	67.8^{a}	72.8^{a}	3,350.6^{b}	61.6^{ab}	72.8^{b}
T4	3,202.3^{b}	65.4^{b}	70.3^{b}	3,330.6^{b}	60.7^{bc}	72.4^{b}
T5	3,311.1^{a}	67.3^{ab}	72.4^{a}	3,411.4^{a}	63.2^{a}	73.9^{a}
T6	3,078.8^{c}	57.5^{d}	66.7^{c}	3,177.9^{c}	53.9^{d}	68.2^{d}
SEM	26.0	0.79	0.51	13.80	0.65	0.26

[a-d]Means in the same column without a common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of 14 chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

AME and Apparent Digestibility of CP and DM of Diets

The AME and digestibility of CP and DM of diets in broilers are shown in Table 3. From 11 to 13 d of age, AMJC supplementation (Diets T2 to T5) significantly increased the AME of the low-NPP diet as compared to the normal-NPP (Diet T6) or the low-NPP diet without AMJC supplementation (Diet T1). Among the treatments with AMJC supplementation, the AME values were not affected by increasing levels of AMJC supplementation except for the diet supplemented with AMJC equivalent to 750 U phytase/kg, which had significantly ($P < 0.05$) lower AME value. From 18 to 20 d of age, addition of AMJC to the low-NPP diet significantly increased the AME of diets. Diet supplemented with a high level of AMJC (equivalent to 1,000 U phytase/kg of feed) had significantly higher AME value as compared to diets supplemented with lower levels of AMJC. The AME value of the normal-NPP diet was similar to the low-NPP diet without AMJC supplementation but significantly lower than the low-NPP diets with added AMJC.

The apparent digestibility of CP followed a pattern similar to that of AME. From 11 to 13 d of age, AMJC added at various levels to the low-NPP diet increased the apparent digestibility of CP when compared to the low-NPP diet without AMJC supplementation or the normal-NPP diet. From 18 to 20 d of age, supplementation of AMJC to the low-NPP diet with equivalents of 250 to 1,000 U phytase/kg of feed significantly increased the apparent digestibility of CP when compared to the normal-NPP diet, but compared to the low-NPP diet without AMJC supplementation, only supplementations equivalent to 500 and 1,000 U phytase/kg of feed significantly improved CP digestibility.

The apparent digestibility of DM was also significantly ($P < 0.05$) influenced by the dietary treatments. From 11 to 13 d of age, the apparent digestibility of DM was significantly higher in dietary treatments with AMJC supplementation than in other dietary treatments. From 18 to 20 d of age, DM digestibility was significantly improved by supplementation to medium to high levels of AMJC (equivalent to 500 to 1,000 U phytase/kg of feed), but it was not influenced by supplementation of a low level of AMJC (equivalent to 250 U phytase/kg of feed).

Apparent Bioavailability of Minerals

The effects of AMJC supplementation on the retention of minerals in chickens are summarized in Table 4. The lowest P retention (53.1% from 11 to 13 d of age and 48.7% from 18 to 20 d of age) was found in birds consuming the low-NPP diet without AMJC supplementation (Diet T1). Supplementation of AMJC to the low-NPP diet significantly increased ($P < 0.05$) P retention in chickens by 17.9 to 20.0 and 16.6 to 19.0 percentage units from 11 to 13 and 18 to 20 d of age, respectively. The P retention in chicks fed the normal-NPP diet was 60.3 and 51.2% from 11 to 13 and 18 to 20 d of age, respectively, which were 10.7 to 12.8 (11 to 13 d of age) and 14.1 to 16.5 (18 to 20 d of age) percentage units lower ($P < 0.05$) than those in chickens fed the low-NPP diet added with AMJC. The retention of P was similar among the treatments of AMJC supplementation, except for the treatment with AMJC supplementation at a low level (equivalent to 250 U phytase/kg of feed) from 18 to 20 d of age, which was significantly lower.

Supplementation of AMJC to the low-NPP diet significantly increased the Ca retention in chickens by 9.1 to 10.6 and 9.7 to 16.6 percentage units from 11 to 13 and 18 to 20 d of age, respectively. Chicks receiving AMJC supplementation retained 2.7 to 4.2 and 2.5 to 9.4 percentage units more Ca ($P < 0.05$) from 11 to 13 and 18 to 20 d of age, respectively, as compared to those consuming a normal-NPP diet. It was observed that the improvements of Ca retention were not as pronounced as those of P retention.

Addition of AMJC to the low-NPP diet numerically improved the Mn retention in chickens from 11 to 13 d of age, but the improvement was not significant. From 18 to 20 d of age, supplementation of medium to high

TABLE 4. Effects of active *Mitsuokella jalaludinii* culture (AMJC) supplementation on the retention rates of P, Ca, Mn, Zn, and Cu[1]

	11 to 13 d					18 to 20 d				
Dietary treatment[2]	P (%)	Ca (%)	Mn (%)	Zn (%)	Cu (%)	P (%)	Ca (%)	Mn (%)	Zn (%)	Cu (%)
T1	53.1^{c}	44.0^{c}	17.4^{ab}	32.7^{b}	58.3^{b}	48.7^{d}	39.9^{e}	25.4^{b}	30.0^{b}	41.8^{b}
T2	71.5^{a}	53.1^{a}	24.8^{a}	29.6^{b}	65.3^{a}	65.3^{b}	49.6^{c}	26.6^{b}	27.1^{bc}	54.3^{a}
T3	71.1^{a}	53.4^{a}	23.5^{a}	36.8^{ab}	64.7^{a}	67.1^{a}	53.8^{b}	30.4^{a}	31.0^{b}	50.9^{a}
T4	71.0^{a}	53.8^{a}	21.3^{a}	33.2^{ab}	63.7^{a}	67.7^{a}	56.5^{a}	32.1^{a}	30.5^{b}	54.7^{a}
T5	73.1^{a}	54.7^{a}	22.1^{a}	43.9^{a}	65.1^{a}	67.2^{a}	53.2^{b}	31.1^{a}	38.9^{a}	50.2^{a}
T6	60.3^{b}	50.4^{b}	14.1^{b}	14.1^{c}	54.3^{b}	51.2^{c}	47.1^{d}	17.8^{c}	21.9^{c}	33.5^{c}
SEM	0.95	0.61	2.30	3.48	1.88	0.53	0.55	1.15	2.37	1.88

[a-e]Means in the same column without a common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of 14 chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

levels of AMJC (equivalent to 500, 750, and 1,000 U phytase/kg of feed) to the low-NPP diet significantly increased the retention of Mn in chickens. From 11 to 13 and 18 to 20 d of age, Mn retention was significantly higher in chickens fed the low-NPP diet containing different levels of AMJC than in those fed the normal-NPP diet.

Significant improvement of Zn retention in chickens was observed only when a high level of AMJC (equivalent to 1,000 U phytase) was incorporated into the low-NPP diet (11 to 13 and 18 to 20 d of age). Generally, the retention of Zn in chickens fed the normal-NPP diet was significantly lower than in those fed the low-NPP diet with or without AMJC addition from 11 to 13 and 18 to 20 d of age. Zinc retention tended to increase as the level of AMJC supplementation increased.

Supplementation of AMJC to the low-NPP diet significantly increased the Cu retention in chickens by 5.4 to 7.0 and 8.5 to 13.0 percentage units from 11 to 13 and 18 to 20 d of age, respectively. Chickens fed the normal-NPP diet retained significantly less Cu than those fed the low-NPP diet with AMJC supplementation from 11 to 13 and 18 to 20 d of age.

Intake of P was significantly higher in birds fed the normal-NPP or low-NPP diet added with AMJC when compared to those fed the low-NPP diet without AMJC (Table 5). Supplementation with AMJC to the low-NPP diet also significantly decreased the amount of P excreted by each bird per day from 11 to 13 and 18 to 20 d of age (Table 5). Most P excreted (mg/bird/d) was from chickens fed the normal-NPP diet.

Tibia Ash and Mineral Contents

The effects of AMJC supplementation on mineral concentrations in the ash and DM of tibia are shown in Table 6. Supplementation of AMJC to the low-NPP diet significantly increased tibia ash percentage. The tibia ash percentage in chickens fed the low-NPP diet supplemented with AJMC was comparable to that in chickens fed the normal-NPP diet. The Ca concentration in tibia ash was significantly increased when medium to high levels of AMJC (equivalent to 500, 750, and 1,000 U phytase/kg of feed) were supplemented to the low-NPP diet. Supplementing medium to high levels of AMJC (equivalent to 500, 750, and 1,000 U phytase/kg of feed) to the low-NPP diet increased the Ca concentration in tibia ash to a level comparable to that of chickens fed the normal-NPP diet.

The P and Zn concentrations in tibia ash were not affected by the dietary treatments. Generally, supplementation of AMJC to the low-NPP diet significantly decreased ($P < 0.05$) Mn and Cu concentrations in tibia ash.

Chickens fed the low-NPP diet without AMJC supplementation had significantly lower Ca and P concentrations in the DM of tibia than those fed other dietary treatments. The Ca content in tibia DM of chicks fed the low-NPP diet supplemented with medium to high levels of AMJC (equivalent to 500 to 1,000 U phytase/kg of feed) was similar to that of chicks fed the normal-NPP diet. Supplementing AMJC to the low-NPP diet significantly increased the P content in tibia DM to a level comparable to that of chickens fed the normal-NPP diet. On the other hand, the low-NPP diet supplemented with AMJC significantly decreased Mn content in tibia DM to a level similar to that obtained with the normal-NPP diet. The supplementation of AMJC at medium (equivalent to 500 U phytase /kg of feed) or high level (equivalent to 1,000 U phytase /kg of feed) significantly decreased the Cu content in DM of tibia. The concentration of Zn in DM of tibia was not affected by dietary treatments.

Plasma Minerals

The effects of AJMC supplementation on plasma mineral levels are shown in Table 7. Plasma Ca and Mn concentrations were not influenced by the dietary treatments. Chickens fed the normal diet or a low-NPP diet with different levels of AJMC had significantly higher plasma P concentrations than those fed the low-NPP diet without AMJC supplementation. Supplementing AJMC to the low-NPP diet at low (250 U phytase/kg of feed) or medium (500 U phytase/kg of feed) level increased the plasma P to a level comparable with that obtained

TABLE 5. Effects of active *Mitsuokella jalaludinii* (AMJC) supplementation on intake and excretion of P in broiler chickens fed a corn-soybean diet[1]

	11 to 13 d			18 to 20 d		
Dietary treatment[2]	P intake (mg/bird/day)	P excreted (mg/bird/day)	P excretion (%)	P intake (mg/bird/day)	P excreted (mg/bird/day)	P excretion (%)
T1	222[c]	104[b]	46.9[a]	416[c]	213[b]	51.3[a]
T2	262[b]	75[c]	28.5[c]	481[b]	167[c]	34.7[c]
T3	253[b]	73[c]	28.9[c]	480[b]	158[c]	32.9[c]
T4	255[b]	74[c]	28.9[c]	469[b]	151[c]	32.3[c]
T5	250[b]	67[c]	26.9[c]	477[b]	157[c]	32.9[c]
T6	428[a]	169[a]	39.8[b]	791[a]	386[a]	48.8[b]
SEM	7.51	3.99	0.94	9.99	5.51	0.54

[a-c]Means in the same column without a common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of 14 chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

with the normal-NPP diet. As AMJC supplementation level increased (equivalent to 750 U or 1,000 U phytase/ kg of feed), the plasma P concentrations increased to levels that were significantly greater than that obtained with the normal-NPP diet. The concentration of Zn in plasma was significantly increased by supplementation of a high level (1,000 U phytase/kg of feed) of AMJC, but it was not influenced by other dietary treatments.

DISCUSSION

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. These results are similar to those reported previously by Sebastian et al. (1996 a,b). In the present study, the low-NPP diet was formulated to contain 0.24% NPP, which was much lower than the NRC-recommended (1994) AP requirement (0.45% AP) for growing broilers (NRC, 1994). 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. The result indicates that AMJC supplementation could effectively improve the poorer growth and lower feed intake caused by the low NPP content in the diet.

Our results are consistent with those reported in earlier studies on microbial phytase by several researchers (Simons et al., 1990; Broz et al., 1994; Sebastian et al., 1996a,b). The improvements on growth rate and feed intake by AJMC supplementation were evidently related to the phytase activities of AJMC. Supplementation of AMJC also significantly improved feed efficiency over the whole experiment (Days 1 to 42) when compared to

TABLE 6. Effects of supplemental active *Mitsuokella jalaludinii* culture (AMJC) on tibia bone ash, P, Ca, Mn, Zn, and Cu contents in the ash and DM of tibias of 21-d-old broiler chickens[1]

	Dietary treatment[2]						
	T1	T2	T3	T4	T5	T6	SEM
Ash (% DM)	44.6[b]	49.7[a]	53.4[a]	50.5[a]	52.4[a]	50.5[a]	1.21
Minerals in ash							
Ca (% ash)	25.1[c]	26.2[bc]	27.0[ab]	27.4[a]	26.4[ab]	27.4[a]	0.33
P (% ash)	19.2[a]	19.1[a]	19.3[a]	19.3[a]	20.0[a]	19.3[a]	0.37
Mn (ppm in ash)	35.3[a]	27.7[b]	24.7[b]	24.8[b]	24.0[b]	26.0[b]	1.55
Zn (ppm in ash)	798.9[a]	729.7[a]	796.3[a]	734.9[a]	645.4[a]	627.6[a]	66.12
Cu (ppm in ash)	21.0[a]	15.4[ab]	7.1[c]	13.3[bc]	7.8[c]	11.6[bc]	1.96
Minerals in DM							
Ca (% DM)	11.4[c]	12.9[b]	14.3[a]	13.9[a]	13.8[a]	13.9[a]	0.29
P (% DM)	8.6[c]	9.5[b]	10.3[ab]	9.9[ab]	10.5[a]	9.7[ab]	0.26
Mn (ppm in DM)	15.8[a]	13.7[b]	13.1[b]	12.6[b]	12.6[b]	13.1[b]	0.64
Zn (ppm in DM)	356.0[a]	407.0[a]	429.3[a]	377.2[a]	339.0[a]	316.2[a]	41.20
Cu (ppm in DM)	9.1[a]	7.5[ab]	3.8[c]	6.6[abc]	4.1[c]	5.8[bc]	0.97

[a-c]Means in the same row with no common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of 5 chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

TABLE 7. Effects of active *Mitsuokella jalaludinii* culture (AMJC) supplementation on the concentrations of plasma total phosphorus (P), calcium (Ca), manganese (Mn) and zinc (Zn) in 21-d-old broiler chickens[1]

Dietary treatment[2]	Ca (mg/dL)	P (mg/dL)	Mn (μg/dL)	Zn (μg/dL)
T1	6.67[a]	11.6[c]	160.5[a]	204.7[b]
T2	6.43[a]	20.0[ab]	181.8[a]	194.0[b]
T3	6.54[a]	18.7[ab]	185.1[a]	228.5[b]
T4	6.81[a]	21.1[a]	173.1[a]	240.9[b]
T5	6.15[a]	20.8[a]	190.3[a]	352.5[a]
T6	5.98[a]	16.0[b]	189.3[a]	217.5[b]
SEM	0.61	1.80	45.44	17.80

[a-c]Means in the same column with no common superscript differ significantly ($P < 0.05$).

[1]Data represent mean values of four replicate cages of three chicks each.

[2]Experimental diets: T1= low-nonphytate P (NPP) diet (without *M. jalaludinii* phytase supplementation); T2 = low-NPP diet + 250 U phytase of *M. jalaludinii*; T3 = low-NPP diet + 500 U phytase of *M. jalaludinii*; T4 = low-NPP diet + 750 U phytase of *M. jalaludinii*; T5 = low-NPP diet + 1,000 U phytase of *M. jalaludinii*; T6 = normal-NPP diet.

the low-NPP diet without AMJC supplementation. Compared to chickens fed the normal-NPP diet, chickens consuming the low-NPP diet added with AMJC also tended to have a lower feed:gain ratio. The improvement in feed efficiency may not be related only to the phytase activity but also to other enzymes, such as the protease and α-amylase activities, in AMJC. Several researchers (Simons et al., 1990; Broz et al., 1994; Sebastian et al., 1996a) have reported that microbial phytase supplementation has no significant effect on feed conversion ratio in broiler chickens. However, Zyla et al. (1996) demonstrated that supplementation of a novel *Aspergillus niger* mycelium into corn-soybean meal-based feed with low AP content significantly improved the feed efficiency of growing turkeys.

Recently, Zyla et al. (2000) also found that feed efficiency was significantly improved in broilers when phytase, acid phosphatase, and pectinase were supplemented together to a low-NPP wheat-based feed. In the present study, no significant differences in body weight gain or feed:gain ratio were noted among chickens receiving different levels of AMJC over the whole experimental period (Days 1 to 42). This result indicates that AMJC incorporated into a low-NPP diet in the form of fresh broth at an equivalent to 250 U phytase/kg feed 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).

Supplementation of AJMC significantly increased DM and CP digestibilities and the AME value of the low-NPP diet. Recently, Namkung and Leeson (1999) reported that supplementation of phytase to broiler diets significantly increases the N-corrected AME_n. Yi et al. (1996c) found that adding Natuphos phytase to a corn-soybean diet with low AP content linearly increases the apparent digestibility of DM. Several researchers have reported that CP digestibility was significantly increased by microbial phytase supplementation in broiler chickens (Kies et al., 2001), turkeys (Yi et al., 1996a), and laying hens (Van der Klis and Versteegh, 1991).

Supplementation of AMJC to the low-NPP diet increased the P and Ca retentions in broiler chickens. These findings are comparable with the results of earlier studies on microbial phytase supplementation in broiler chicken diets (Simons et al., 1990; Schöner et al., 1991; Broz et al., 1994, Yi et al., 1996c; 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 (Ahmad et al., 2000). Supplementation of AMJC (at 500, 750, and 1,000 U phytase/kg of feed) to the low-NPP diet significantly increased the Mn retention by 5.04 to 6.7 percentage units in birds at 18 to 20 d of age. Windisch and Kirchgessner (1996) reported similar findings. They found that phytase supplementation increased Mn retention by 3.8 percentage units in broiler chickens and 3.0 percentage units in piglets. In contrast, Mohanna and Nys (1999) could not find any improvement in Mn retention by microbial phytase supplementation in broiler chickens. From 18 to 20 d of age, the Zn retention in chickens was significantly improved when a high level of AMJC (equivalent to 1,000 U phytase/kg of feed) was supplemented to a low-NPP diet. The higher Zn retention, which resulted from AMJC supplementation, might be due to improvement of Zn use from the phytate-mineral complex or the phytate-mineral-protein complex.

Studies to investigate the relationship between phytase supplementation and Zn bioavailability have been conducted in poultry (Thiel and Weigand, 1992; Roberson and Edwards, 1994; Biehl et al., 1995; Sebastian et al., 1996a; Yi et al., 1996b; Lou et al., 1997), rats (Rimbach et al., 1997), and swine (Windisch and Kirchgessner, 1996), but the results are contradictory. Thiel and Weigand, (1992), Biehl et al. (1995), and Yi et al. (1996b) reported that Zn retention in broiler chickens was significantly increased when the birds were fed a Zn-deficient diet supplemented with microbial phytase.

Supplementation of phytase to a diet with high Zn content (70.9 ppm/kg) was also found to increase the use of Zn in broiler chickens (Sebastian et al., 1996a). In contrast, Roberson and Edwards (1994) demonstrated that Zn retention in broiler chickens was not affected by adding phytase to a diet containing 35 to 45 ppm Zn. Lou et al. (1997) also could not find any significant effect of phytase supplementation on Zn retention rate, although they found that the Zn content of tibia was increased by the phytase supplementation in broiler chickens. Supplementation of AMJC to the low-NPP diet increased the Cu retention in chickens. Sebastian et al. (1996a) also reported similar results; they found a significant improvement in Cu retention in broiler chickens by using microbial phytase supplementation.

Reduction of P excretion is particularly important in the reduction of P pollution by poultry manure. In the present study, the increase of P retention resulted in a significant decrease in P excretion (mg P/bird per d).

Compared with the normal-NPP diet, P excretion (mg P/bird per d) was reduced by 56.8% (11 to 13 d of age) and 59.1% (18 to 20 d of age) by supplementing AMJC (equivalent to 500 U phytase per kg feed). Yi et al. (1996c) found that P excretion by broiler chickens linearly decreased by 30 to 60% as 350 to 1,050 U phytase/kg of feed was added to a diet containing 0.27% AP. Earlier, Simons et al. (1990) reported that supplemental phytase could lower P excretion by more than 40% by increasing P availability. Um et al. (1999) reported similar results with hens.

The ash percentage of tibia was significantly increased by AMJC supplementation. This result is in agreement with the findings of previous studies on microbial phytase in broiler chickens (Broz et al., 1994; Sebastian et al., 1996a; Mohanna and Nys, 1999; Ahmad et al., 2000). Zyla et al. (1996) also reported that the tibia ash percentage is significantly improved in turkeys fed a low-AP diet (0.17% AP) with added phytase (1,000 U/kg), and this improvement is further increased when the turkeys are fed a low-AP diet with an added enzyme cocktail (1,000 U phytase/kg, 100,000 U acid phosphatase/kg, 42,000 U acid protease/kg, and 2.4% pectinase) or a fungal mycelium. Similar results were also reported in a recent study on broiler chickens (Zyla et al., 2000).

Increased bone ash by AMJC supplementation in the present study suggested an improvement in bone mineralization due to the increase in Ca and P availability. This result also indicated that inorganic P and Ca were liberated from phytate in the feed by *M. jalaludinii* phytase and were effectively incorporated into the bone.

Supplementation of AMJC to the low-NPP diets did not affect the concentration of P in tibia ash but increased ($P < 0.05$) the Ca concentration in tibia ash. On the other hand, AMJC supplementation significantly increased the Ca and P concentrations in the DM of tibia. Broz et al. (1994) found that phytase supplementation to a corn-soybean diet did not affect the P and Ca concentrations in the tibia ash of chickens. Sebastian et al. (1996a) also found that phytase supplementation did not influence the P, Ca, Zn, or Cu concentration in the tibia ash of chickens but significantly increased the P and Ca concentrations in the DM of tibia heads.

Recently, Ahmad et al. (2000) reported that phytase supplementation had no effect on the P or Ca concentration in the whole tibia ash but significantly increased the Ca and P concentrations in tibia DM of chickens. Earlier, Young et al. (1993) found that phytase supplementation increased bone weight, ash percentage, weight of ash, and weights of Ca and P in the fat-free third metatarsal bone of swine, but the concentration of P in the ash was not affected by phytase supplementation.

The Zn concentration in the whole tibia ash was not significantly influenced by AMJC supplementation. This result is supported by the results of Sebastian et al. (1996a) in which microbial phytase supplementation did not affect Zn concentration in the ash of tibia shaft of broiler chickens even though the relative retention rate of Zn was improved. The lack of effect of AMJC supplementation on tibia Zn concentration may be attributed to the high concentration of dietary Zn (>100 ppm) used in the present study. Mohanna and Nys (1999) reported that dietary addition of microbial phytase increased Zn deposition in bone tissue when the dietary Zn was less than 60 ppm but had no effect on tibia Zn concentration when the dietary Zn was greater than 60 ppm.

Supplementation of AMJC to the low-NPP diet significantly reduced the tibia Mn concentration of chickens to a level that was similar to that of chicks fed a normal-NPP diet. This finding is in contrast to that reported by Mohanna and Nys (1999), who found that addition of microbial phytase tended to increase Mn concentration in tibia of chickens fed a basal diet containing 31to 41 mg Mn/kg of feed. The reason is not known for the reduction in the tibia Mn concentration of chickens when AMJC was supplemented. It may be that the higher deposition rate of Ca and P in chickens receiving the normal-NPP diet or low-NPP diet added with AMJC had negative effects on the Mn deposition in the bone. The supplementation of AMJC to the low-NPP diet significantly reduced the Cu content in tibia ash. This result concurs with the finding of Um et al. (1999) in which supplementation of 250 U phytase/kg of feed reduced the Cu content of tibia ash in 48-wk-old hens, but it is in contrast to the result of Sebastian et al. (1996a), who found no significant effect of phytase supplementation on the Cu concentration in tibia ash of broiler chickens.

Supplementation of AMJC to the low-NPP diet only increased plasma P but did not significantly affect the plasma Ca concentration. This result is in agreement with the reports by Edwards (1993) and Roberson and Edwards (1994). However, some of the previous studies indicate that supplementation of microbial phytase increases plasma P but reduces plasma Ca concentration. The different results may be due to the difference in concentrations of NPP, Ca, and other components in the experimental diets used.

Plasma Mn was not influenced by any dietary treatments, although it was found that AMJC supplementation could increase Mn retention in the chickens. This finding could be due to an enterohepatic mechanism that controls any excess of Mn over a wide range of dietary intakes (Hall and Symonds, 1981). Plasma Zn was not significantly affected by low to medium levels of AMJC supplementation (equivalent to 250, 500, and 750 U phytase/kg of feed) but was significantly increased by a high level of AMJC supplementation (equivalent to 1,000 U phytase/kg of feed). The result implies that the plasma Zn concentration may be affected by the level of phytase supplemented in the diet.

Mohanna and Nys (1999) reported that at low dietary Zn, incorporation of microbial phytase into the corn-soybean meal significantly increases plasma Zn by 20 to 30%, but the effect of microbial phytase on plasma Zn concentration is limited when the dietary Zn level is greater than 70 ppm. Sebastian et al. (1996a) also found that there is no effect of microbial phytase supplementation (600 U

phytase/kg) on the plasma Zn level in chicks fed a low-AP diet containing a high level of Zn (70 ppm).

The results from the present study demonstrated that supplementation of AMJC to a corn-soybean diet containing low NPP significantly ($P < 0.05$) improved the performance of broiler chickens. Supplementation of AMJC significantly increased ($P < 0.05$) the AME value, digestibility of DM and CP, and retention of P, Ca, and Cu but significantly decreased ($P < 0.05$) excretion of P by broiler chickens. Thus, the inorganic P supplemented to a normal-NPP diet of chickens could be effectively replaced by AMJC without any adverse effect on the performance and nutrient use of broilers.

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