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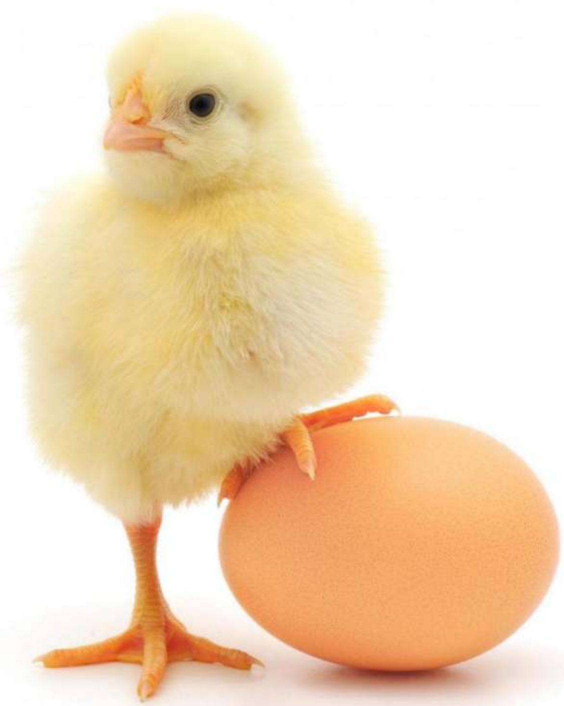

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Effect of *Lactobacillus* Cultures and Oxytetracycline on the Growth Performance and Serum Lipids of Chickens

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Abstract: A feeding experiment was carried out for 42 days to evaluate the effects of a mixture of 12 *Lactobacillus* cultures (LC) or oxytetracycline (OTC) on growth performance and serum lipids of broiler chicks (Hubbard). Two hundred and seventy 1-day-old chicks were assigned randomly to three dietary treatments. The treatments were (I) a basal diet (control) (ii) basal diet+1 g kg^{-1} LC and (iii) basal diet+50 mg kg^{-1} OTC. Each dietary treatment had six replicate cages with 15 chicks per cage. Body weights and feed to gain ratios of broilers were determined at 21 and 42 days of age while serum lipids were determined at 42 days of age. From 1 to 42 days of age, broilers fed LC or OTC had significantly ($p<0.05$) better growth than the control broilers. The feed conversion ratios were improved significantly ($p<0.05$) during the growing (1 to 21 days of age) and finishing (22 to 42 days of age) periods in broilers fed LC or OTC, but the best feed to gain ratio was obtained in the LC-fed broilers. Serum total cholesterol was significantly ($p<0.05$) reduced in broilers supplemented with LC as compared to broilers receiving OTC or control diet. Low density lipoprotein cholesterol and triglycerides were significantly ($p<0.05$) lower in broilers fed LC but significantly ($p<0.05$) higher in broilers fed OTC when compared to the control broilers. The results indicated that LC had a hypocholesterolaemic effect on broilers in contrast to OTC.

Key words: *Lactobacillus*, oxytetracycline, growth performance, serum lipid, chicken

Introduction

For over 50 years, antibiotics have been used as growth promoters in livestock feeds to improve animal performance and health. It has been reported that the addition of antibiotic growth promoters to animal diets increases productivity 72% of the time in 12,153 trials (Rosen, 1996). The effectiveness of antibiotics is at least partially explained by the antimicrobial activity against some pathogenic bacteria. However, in recent years, a growing concern on the possible development of antibiotic-resistant bacteria from continuous feeding of sub-therapeutic levels of antibiotic has resulted in a severe restriction on the use of antibiotics in animal feeds in many countries. Removal of antibiotic growth promoters is likely to increase the variability of broiler performance and this has led to considerable interest to find other ways to achieve the same growth promotion in livestock without the use of antibiotics to ensure that the livestock industry will not be adversely affected. The potential of probiotics (direct-fed microbials) as a substitute for antibiotic growth promoters has been considered. Probiotics are live microorganisms which beneficially affect the host animal by improving their intestinal microflora (Fuller, 1999). As research in the field of probiotic progresses, experiments with more well-characterized probiotic cultures have demonstrated beneficial effects in many cases (Fuller, 1999).

There is increasing evidence that intestinal microflora may play an important role in the lipid metabolism of the host animal. The beneficial effects of probiotics in lowering cholesterol in egg yolk (Abdulrahim *et al*., 1996; Li *et al*., 2006) and serum (Jin *et al*., 1998; Kalavathy *et al*., 2003) have been reported. Diets supplemented with probiotics can also significantly reduce plasma triglycerides in hens (Abdulrahim *et al*., 1996).

Oxytetracycline, a broad-spectrum antibiotics, is no longer used as a growth promoter for livestock in many countries, but is still used by poultry farmers in Malaysia. This study was conducted to compare the effect of a probiotic consisting of a mixture of 12 *Lactobacillus* strains with that of oxytetracycline, on (i) growth performance and (ii) serum blood lipids (total cholesterol, triglycerides and low density lipoprotein cholesterol) of broiler chickens.

Materials and Methods

Animals and diets: A total of 270 1-day-old male broiler chicks (Hubbard), obtained from a commercial hatchery, were randomly assigned to 18 cages of 15 chicks each. The cages, with raised wire netted floors, were kept in an open house under natural conditions. The chicks were brooded with heating lamps of 100 W for 14 days. Chicks were allowed free access to feed and water. The feed was in a mash form and was added to the feeder

Table 1: Composition of basal diet[1]

Ingredient (g kg^{-1} unless otherwise stated)	Starter (1 to 21 days)	Grower (22 to 42 days)
Ground yellow maize	538.9	603.0
Soybean meal	362.0	318.7
Fish meal	30.0	30.0
Palm oil	37.3	24.4
60% choline chloride	2.5	2.0
Vitamin mineral mix[1]	1.0	1.0
Salt (NaCl)	2.0	1.0
DL-methionine	1.80	0.40
Limestone	13.0	13.0
Dicalcium phosphate	11.5	6.5
Total	1000.0	1000.0
Calculated analysis ($g\ kg^{-1}$ except energy)		
Crude protein	220.0	199.9
Crude fat	63.1	52.2
Crude fibre	38.0	36.5
Calcium	10.2	9.0
Phosphorus	4.5	3.5
Metabolizable energy (MJ kg^{-1})	13.06	13.06

[1]Vitamin mineral mix (per kg vitamin mineral mix): iron 100 g; manganese 110 g; copper 20 g; zinc 100 g; iodine 2 g; selenite 0.2 g; cobalt 0.6 g; antioxidant (santoquin) 0.6 g; folic acid 0.33 g, thiamin 0.83 g; pyridoxine 1.33 g; biotin 2% 0.03 g; riboflavin 2 g; cyanocobalamin 0.03 g; D-calcium pantothenate 3.75 g; niacin 23.3 g; retinol 2000 mg; cholecalciferol 25 mg; α-tocopherol 23,000 mg

daily at 0900 h. The chicks were divided into three treatment groups with six replicate cages for each dietary treatment and the cages were randomized with respect to the dietary treatments. The dietary treatments were: (i) basal diet (control) (ii) basal diet+1 g kg^{-1} mixture of 12 *Lactobacillus* strains and (iii) basal diet+50 mg kg^{-1} oxytetracycline (OTC). The 12 *Lactobacillus* strains (LC) and the method of preparation as a feed supplement were the same as those described by Jin *et al.* (1996c, 1998). The basal diet was a corn-soybean diet formulated to meet the nutrient requirements (NRC, 1994) for starter (1 to 21 days) and grower (22 to 42 days) periods. The composition of the basal diet is shown in Table 1. The viable LC cells were mixed daily with the basal diet at feeding time while OTC was incorporated into the basal diet weekly. Prior to feeding, the body weights of individual chicks were obtained at 1, 21 and 42 days. Feed consumed was recorded daily on per cage basis, the uneaten feed was collected once daily before the morning feeding and feed conversion was calculated (total feed: total gain). Mortality was recorded as it occurred and percentage mortality was determined at the end of the study. The experiment was carried out for 42 days under typical tropical conditions (mean environment temperature was about 30°C; relative humidity was between 90-95%). The experiment complied with the guidelines of the Consortium Guide (1988) with respect to animal experimentation and care of animals under study.

Sampling Procedures and Assay for Serum Lipids: At 42 days of age, two chickens were randomly selected from each cage and euthanized by severing the jugular vein. The blood was collected in non-heparanized collection tubes to obtain the serum. Blood samples were centrifuged at 2,000 *g* for 10 min and the serum was transferred using individual pasteur pipettes into vials and stored at-20°C until used. Serum samples were analyzed for total cholesterol, low density lipoprotein cholesterol and triglycerides by enzymatic diagnostic kits[1].

Statistical analysis: Treatment effects were compared using analysis of variance and treatment means were separated using the least significant difference. The computation was done by using the SAS programme (Statistical Analysis System Institute, 1997).

Results and Discussion

The effects of the dietary treatments on the mean body weight, weight gain, feed intake and feed conversion are summarized in Table 2. The results showed that at 21 and 42 days of age, broilers fed LC or OTC had significantly ($p<0.05$) heavier body weights than the control broilers. Body weight gains were also significantly ($p<0.05$) improved by LC or OTC diets during the growing (1 to 21 days) and finishing periods (22 to 42 days) and throughout the experimental period (1 to 42 days). The body weights or weight gains between the LC-and OTC-fed birds were not significantly different.

The effect of LC in the present study on body weight and weight gain is consistent with the results of earlier studies using LC on different broiler strains (Jin *et al.*, 1998; Zulkifli *et al.*, 2000; Kalavathy *et al.*, 2003). Similar significant improvements on growth performance were also observed in broilers fed OTC or LC diet before (1 to 21 days) and during (22 to 42 days) a 3-h episode of 36°C heat stress each day (Zulkifli *et al.*, 2000). The results of the present study indicated that LC has a similar effect as OTC in improving the body weight and weight gain of broiler chicken. Similar findings were also reported for broilers fed *Bacillus coagulans* or virginiamycin (Cavazzoni *et al.*, 1998) and *L. acidophilus* or zinc bacitracin (Abdulrahim *et al.*, 1999) during late growth periods. In contrast, Yeo and Kim (1997) reported that only broilers fed *L. casei* improved the average daily weight gain during the first 3-week period compared to the control or chloroxytetracycline treatments. Mohan *et al.* (1996) also reported that the beneficial effects on body weight gains were observed in broilers fed flavophospholipol or flavophospholipol in combination with Probiolac (a commercial probiotic mixture containing lactic acid bacteria, *Aspergillus oryzae* and *Torulopsis*) but not in the control or Probiolac treatment alone.

Table 2: Effects of *Lactobacillus* cultures or oxytetracycline on body weight, weight gain, feed intake and feed to gain ratio of broiler chickens for 42 days

	Dietary treatment[1]			
	Control	LC	OTC	SEM
Body weight (g)				
day 1	41.30[a]	41.24[a]	42.02[a]	0.26
day 21	632.47[b]	741.83[a]	740.08[a]	10.96
day 42	1700.33[b]	1976.58[a]	1962.42[a]	35.90
Weight gain (g)				
1-21 days	591.17[b]	700.59[a]	698.07[a]	11.02
22-42 days	1067.87[b]	1234.76[a]	1222.34[a]	37.83
1-42 days	1659.04[b]	1935.35[a]	1920.41[a]	35.85
Feed intake (g)				
1-21 days	1048.00[b]	1033.97[b]	1104.01[a]	17.14
22-42 days	2475.89[a]	2422.89[a]	2618.81[a]	70.26
1-42 days	3523.89[ab]	3456.86[b]	3722.82[a]	71.17
Feed:Gain (g:g)				
1-21 days	1.77[a]	1.48[c]	1.58[b]	0.03
22-42 days	2.32[a]	1.97[c]	2.14[b]	0.04
1-42 days	2.12[a]	1.79[c]	1.94[b]	0.03
Mortality (%)				
1-42 days	2.27	1.89	6.53	ND

[a-c]Means within a row with no common superscript differ significantly ($p<0.05$), [1]Control = basal diet; LC = basal diet+1 g kg^{-1} mixture of 12 *Lactobacillus* strains; OTC = basal diet+50 mg kg^{-1} oxytetracycline. ND, no data

Table 3: Serum lipid concentrations in broiler chickens fed *Lactobacillus* cultures or oxytetracycline from 21 to 42 days of age

	TC	TG	LDLC
Dietary treatment[1]	(mg/dl)		
Control	136.62[a]	84.20[b]	43.50[b]
LC	119.27[b]	63.29[c]	38.12[c]
Antibiotic	146.66[a]	105.31[a]	49.98[a]
SEM	4.77	6.68	1.76

[a-c]Means within a column with no common superscript differ significantly ($p<0.05$), [1]Control = basal diet; LC = basal diet+1 g kg^{-1} mixture of 12 *Lactobacillus* strains; OTC= basal diet+50 mg kg^{-1} oxytetracycline, TC = Total cholesterol; TG = Triglycerides; LDLC = Low density lipoprotein cholesterol

From 1 to 21 days of age, broilers given OTC had significantly ($p<0.05$) higher feed intake than those fed LC or control diets. However, from 22 to 42 days of age, there were no significant differences in feed intake of broilers among the treatments and from 1 to 42 days of age, feed intake was significantly ($p<0.05$) higher in broilers given OTC compared to LC but there were no significant differences between control and the other two dietary treatments. Significant differences existed for feed to gain ratios between treatments. The feed to gain ratios were improved significantly ($p<0.05$) in broilers supplemented with LC or OTC compared to the control from 1 to 21, 22 to 42 and 1 to 42 days of age, with the best feed to gain ratio obtained in the LC treatment. From 1 to 42 days of age, the feed to gain ratios of LC-fed broilers were reduced by 0.33 and 0.15 units when compared to the control and OTC-fed broilers, respectively. The mortality was 2.27%, 1.89% and 6.53% in the control, LC-fed and OTC-fed broilers, respectively, from 1-42 days of age (Table 2). In an earlier study using LC, Zulkifli *et al.* (2000) observed that better feed efficiency was achieved in Hubbard and Shaver broilers fed LC diet compared to OTC or control diets from 1 to 21 days of age. However, when the broilers were subjected to cyclic heat stress from 22 to 42 days of age, the improved feed efficiency in LC-fed broilers was not observed, as more feed was consumed by the broilers due to the stress (Zulkifli *et al.*, 2000). Prior to heat stress, broilers receiving the OTC di*et al*so significantly ($p<0.05$) improved the feed efficiency, however, during heat stress, there was no significant difference between OTC and the control diets. Abdulrahim *et al.* (1999) reported that the feed conversion ratio was best in broilers supplemented with *L. acidophilus* in combination with bacitracin and poorest in broilers fed bacitracin alone, while broilers fed *L. acidophilus* alone had feed conversion ratio similar to that of the control. In contrast, Mohan *et al.* (1996), Yeo and Kim (1997) and Cavazzoni *et al.* (1998) did not find any improvement in the feed to gain ratio or feed intake of broilers fed either probiotics or antibiotics.

It is apparent that both antibiotics and probiotics function by modifying the intestinal microflora. It has been proposed that the beneficial effect of probiotics is mainly mediated by competitive exclusion and by antagonistic activity towards pathogenic bacteria (Fuller, 1999). On the other hand, the effectiveness of antibiotics is probably due to the suppression of microbial population in the intestinal tract that compete with the host for nutrients (Bedford, 2000). Various modes of action have been suggested for the different classes of antibiotics. Oxytetracycline is known to inhibit bacterial growth by interfering with protein synthesis to stop cell division of bacteria. Jonsson and Conway (1992) reported that the growth-promoting effect of probiotics in livestock is less consistent than that observed with antibiotic supplementation. The inconsistent results obtained on the effects of commercial probiotics may be attributed to the composition, characteristics, viability and method of administration of the probiotic strains (Fuller, 1999). The beneficial effect of LC in the present study is probably due to the ability of the 12 *Lactobacillus* strains to quickly and strongly attach to the chicken intestine (Jin *et al.*, 1996c), to antagonize and competitively exclude some pathogenic bacteria (Jin *et al.*, 1996a, b) and to increase antibody production (Zulkifli *et al.*, 2000) in chicken.

The serum total cholesterol, Low Density Lipoprotein Cholesterol (LDLC) and triglycerides of broilers fed the control or treatment diets are summarized in Table 3. Broilers fed LC diet had significantly ($p<0.05$) lower serum total cholesterol (by about 13 or 19%, respectively) when compared to those fed control or OTC diet. However, there was no significant difference in serum total cholesterol between control and those fed OTC. The serum LDLC was significantly ($p<0.05$) lower in broilers fed LC and significantly ($p<0.05$) higher in broilers fed OTC when compared to the control birds. In a recent study, Li *et al.* (2006) found a significant decrease in yolk cholesterol concentration of hens supplemented with *Bacillus subtilis*. Similar reduction in cholesterol were observed in eggs and serum of laying hens fed *L. acidophilus* or *L. acidophilus* in combination with bacitracin (Abdulrahim *et al*., 1996) and broilers fed Probiolac or Probiolac in combination with flavophospholipol (Mohan *et al*., 1996), but in both these studies, antibiotics had no effect when administered alone. In contrast, Shaddad *et al.* (1985) found OTC to decrease serum cholesterol in chicken.

The results from the present study indicated that LC possesses a hypocholesterolemic effect on broilers in contrast to OTC. Although there have been numerous reports on the hypocholesterolemic effect of probiotics, little is known on the mechanism (s) involved. Fukushima and Nakano (1995) suggested that probiotics influence the cholesterol blood levels by inhibition of cholesterol synthesis. De Rodas *et al.* (1996) proposed that the cholesterol lowering effect of probiotics is due to the binding of cholesterol to the cellular membrane of bacterial cells and also through deconjugation of bile salts which may interfere with the enterohepatic cycle. Increased deconjugation of bile salts will result in greater fecal excretion of bile acids which in turn reduces cholesterol in the body pool as cholesterol is the precursor of bile acids.

The serum triglyceride level was significantly ($p<0.05$) lower (by 24.8 or 40%, respectively) in broilers fed LC when compared to those fed control or OTC diet (Table 3). Broilers fed OTC had the highest serum triglyceride concentration; it was 20% higher than that of the control broilers. Similar finding was reported by Shaddad *et al.* (1985) who demonstrated that the administration of OTC increased serum triglyceride concentration in chicken. Supplementation of probiotic *L. acidophilus* (Abdulrahim *et al*., 1996) or *B. subtilis* (Santoso *et al*., 1995) to chickens have also been shown to reduce the serum triglycerides. In contrast, Haddadin *et al.* (1996) found that continuous supplementation of *L. acidophilus* to chickens did not reduce the triglyceride levels in plasma or egg yolk. From the results of this study, it is evident that broilers given the antibiotic diet had significantly elevated serum lipids compared to those fed LC diet.

Conclusion: The supplementation of LC as a probiotic for broilers significantly improved growth and feed conversion which were equivalent to that provided by the antibiotic OTC. However, only LC exerted beneficial effects in improving the serum blood lipids. The results of the present study indicate that the potential of probiotics as an alternative to antibiotic growth promoters deserves serious attention.

References

Abdulrahim, S.M., M.S.Y. Haddadin, E.A.R. Hashlamoun and R.K. Robinson, 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. Br. Poult. Sci., 37: 341-346.

Abdulrahim, S.M., M.S.Y. Haddadin, N.H.M. Odetallah and R.K. Robinson, 1999. Effect of *Lactobacillus acidophilus* and zinc bacitracin as dietary additives for broiler chickens. Br. Poult. Sci., 40: 91-94.

Bedford, M., 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. World's Poult. Sci. J., 56: 347-365.

Cavazzoni, V., A. Adami and C. Castrovilli, 1998. Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci., 39: 526-529.

Consortium, 1988. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Division of Agriculture, NASULGC (National Association of State Universities and Land-Grant Colleges) Consortium Publication, Champaign, IL.

De Rodas, B.Z., S.E. Gilliland and C.V. Maxwell, 1996. Hypocholesterolemic action of *Lactobacillus acidophilus* ATCC 43121 and calcium in swine with hypocholesterolemia induced by diet. J. Dairy Sci., 79: 2121-2128.

Fukushima, M. and M. Nakano, 1995. The effect of a probiotic on faecal and liver lipid classes in rats. Br. J. Nutr., 73: 701-710.

Fuller, R., 1999. Probiotics for farm animals. In: Probiotics: A Critical Review (Ed. G.W. Tannock). Horizon Scientific Press, Wymondham, England, pp: 15-22.

Haddadin, M.S.Y., S.M. Abdulrahim, E.A.R. Hashlamoun and R.K. Robinson, 1996. The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. Poult. Sci., 75: 491-494.

Jin, L.Z., Y.W. Ho, A.M. Ali, N. Abdullah and S. Jalaludin, 1996a. Effect of adherent *Lactobacillus* spp. on in vitro adherence on salmonellae to the intestinal epithelial cells of chickens. J. Appl. Bacteriol., 81: 201-206.

Jin, L.Z., Y.W. Ho, N. Abdullah, A.M. Ali and S. Jalaludin, 1996b. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chickens. Lett. Appl. Microbiol., 23: 67-71.

Jin, L.Z., Y.W. Ho, A.M. Ali., N. Abdullah, K.B. Ong and S. Jalaludin, 1996c. Adhesion of *Lactobacillus* isolates to intestinal epithelial cells of chicken. Lett. Appl. Microbiol., 22: 229-232.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers diets containing *Lactobacillus* cultures. Poult. Sci., 77: 1259-1265.

Jonsson, E. and P. Conway, 1992. Probiotics for pigs. In: Probiotics: The Scientific Basis (Ed. R. Fuller). Chapman and Hall, London, pp: 260-316.

Kalavathy, R., N. Abdullah, S. Jalaludin and Y.W. Ho, 2003. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br. Poult. Sci., 44: 139-144.

Li, L., C.L. Xu, C. Ji, Q. Ma, K. Hao, Z.Y. Jin and K. Ki, 2006. Effects of dried *Bacillus subtilis* culture on egg quality. Poult. Sci., 85: 364-368.

Mohan, B., R. Kadirvel, M. Bhaskaran and A. Natarajan, 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. Br. Poult. Sci., 37: 395-401.

National Research Council, 1994. Nutrient Requirements of Poultry, 9th Ed. National Academic Press, Washington, DC.

Rosen, G.D., 1996. The nutritional effects of tetracyclines in broiler feeds. In: Proceedings of World's Poultry Congress Vol II, New Delhi, India, pp: 141-148.

Santoso, U., K. Tanaka and S. Ohtani, 1995. Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. Br. J. Nutr., 74: 523-529.

SAS Institute Inc., 1997. SAS/STAT User's Guide: Statistics: Release 6.12. Ed. SAS Institute Inc., Cary, North Carolina.

Shaddad, S.A., I.A. Wasfi, M.A. Maglad and S.E. Adam, 1985. The effect of oxytetracycline on growth and lipid metabolism in poultry. Comp. Biochem. Physiol., 80C: 375-80.

Yeo, J. and K. Kim, 1997. Effect of feeding diets containing an antibiotic, a probiotic or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci., 76: 381-385.

Zulkifli, I., N. Abdullah, M.N. Azrin and Y.W. Ho, 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci., 41: 593-597.