Digestive and Bacterial Enzyme Activities in Broilers Fed Diets Supplemented with Lactobacillus Cultures

L. Z. Jin,* Y. W. Ho,†,[1] N. Abdullah,† and S. Jalaludin‡

*Department of Animal Science, McGill University, Quebec, Canada; †Institute of Bioscience, and ‡Department of Animal Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT A study was carried out to investigate the effects of adherent *Lactobacillus* cultures on 1) amylolytic, lipolytic, and proteolytic enzyme activities in the contents of the small intestine (from the distal end of the duodenum to the ileocecal junction) and 2) bacterial β-glucuronidase and β-glucosidase activities in the intestinal contents and feces of broiler chickens. Three dietary treatments were randomly assigned to three groups of chicks, i.e., basal diet only (control group), basal diet + 0.1% dried culture of *Lactobacillus acidophilus*, and basal diet + 0.1% dried culture of a mixture of 12 *Lactobacillus* strains. The results showed that supplementation of the adherent *Lactobacillus* cultures to chickens, either as a single strain of *L. acidophilus* or as a mixture of 12 *Lactobacillus* strains, increased significantly ($P < 0.05$) the levels of amylase in the small intestine. However, the proteolytic and lipolytic activities in the small intestine were not affected by addition of either of the adherent *Lactobacillus* cultures. Addition of either *L. acidophilus* or a mixture of 12 *Lactobacillus* strains was also found to reduce significantly ($P < 0.05$) the intestinal and fecal β-glucuronidase and fecal β-glucosidase but not the intestinal β-glucosidase at 40 d of feeding.

(*Key words*: *Lactobacillus*, broiler, probiotics, digestive enzymes, bacterial enzymes)

2000 Poultry Science 79:886–891

INTRODUCTION

Direct-fed microbials (probiotics) have been utilized to improve animal performance by maintaining "normal" microflora of host animals. The modes of action of probiotics still remain unclear, although the efficiency of these products to enhance animal production has been discussed extensively (Fuller, 1989; Sissons, 1989; Jin et al., 1997). Several modes of action of probiotics involving the changes of intestinal microflora have been examined (Fuller, 1989; Jin et al., 1997).

The intestinal microflora population is a complex ecosystem composed of a large variety of bacteria. The metabolic capacity of microflora is extremely diverse and can produce positive and negative effects on gut physiology (Macfarlane and Cummings, 1991). There is, therefore, a great deal of interest in the possibility of altering the intestinal microbiota in a beneficial way with the aim of improving the health of the host. Lactic acid bacteria have been considered potentially useful in this respect (Sanders, 1993). Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract. These microorganisms have been reputed to modify the intestinal milieu and to deliver enzymes and other beneficial substances into the intestines (Marteau and Rambaud, 1993).

Bacterial enzymes, such as β-glucosidase (EC 3.2.1.21) and β-glucuronidase (EC 3.2.1.31), are the major microbial glycosidases in the intestinal tract. These enzymes release noxious metabolites from nontoxic glycosides and prolong the lifetime of toxicants in the body. Results from studies on the effects of *Lactobacillus* culture on bacterial enzymes are often contradictory. Goldin and Gorbach (1977) found that feeding a *Lactobacillus acidophilus* culture to rats fed a grain diet had no effect on fecal β-glucuronidase, but there was a significant decrease in β-glucuronidase activity in rats fed a meat diet. Later, Goldin et al. (1980) reported that the addition of a viable *L. acidophilus* supplement to the diet of humans significantly decreased fecal bacterial β-glucuronidase; the level returned to normal 30 d after withdrawal of the supplement. β-Glucuronidase reduction was also reported by feeding yogurt to pigs (Cole et al., 1987). In contrast, Lessard and Brisson (1987) reported that fecal β-glucuronidase and β-glucosidase activities of weaned pigs were not affected by addition of *Lactobacillus* fermentation product to their diets. In addition, very few reports have focused on the effect of *Lactobacillus* cultures on the digestive enzymes in chicken intestine, although the effect of intestinal microflora on digestive enzymes has been demonstrated in several host species by comparing the activity in conventional and germ-free animals (Szabo, 1979; Ratcliffe, 1985).

Received for publication September 21, 1999.
Accepted for publication February 23, 2000.
[1]To whom correspondence should be addressed: ywho@ibs.upm.edu.my.

TABLE 1. Composition of the basal diet fed to broilers

Ingredient and composition	0 to 21 d	22 to 40 d
	(%)	
Corn	64.55	66.55
Soybean meal (44.6%)	22.50	23.50
Fishmeal	11.00	7.00
Limestone powder	0.55	1.20
Potassium dihydrogen phosphate	0.00	0.50
Mineral[1] and vitamin[2] premix	0.50	0.50
Sodium chloride	0.35	0.35
Choline-Cl	0.35	0.35
DL-methionine	0.20	0.05
Calculated analysis		
CP (N × 6.25)	21.35	19.97
ME, kcal/kg	3,100	3,100
Lysine	1.20	1.05
Methionine + cystine	0.95	0.74
Calcium	0.92	0.91
Phosphorus	0.76	0.72

[1]Provided per kilogram of complete feed: iron ($FeSO_4 \cdot 7H_2O$), 80 mg; zinc (ZnO), 40 mg; manganese ($MnSO_4 \cdot H_2O$), 60 mg; iodine (Iodized NaCl), 0.8 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; selenium (Na_2SeO_3), 0.2 mg; and cobalt ($CoCl_2$), 0.4 mg.

[2]Provided per kilogram of complete feed: vitamin A, 4,500 IU (retinyl acetate); cholecalciferol, 1,000 IU; vitamin E, 25 IU (dl-α-tocopheryl acetate); menadione, 1.5 mg; vitamin B_{12}, 0.02 mg; riboflavin, 3 mg; pantothenic acid, 5 mg; niacin, 20 mg; folic acid, 0.5 mg; thiamine, 1.5 mg; biotin, 0.5 mg; and pyridoxine, 2.5 mg.

Therefore, an experiment was carried out to investigate the effects of adherent *Lactobacillus* cultures on 1) amylolytic, lipolytic, and proteolytic enzyme activities in the contents of small intestine and 2) bacterial β-glucuronidase and β-glucosidase activities in the intestinal contents and feces of broiler chicken.

MATERIALS AND METHODS

Animals and Diets

One hundred eighty 1-d-old Arbor Acres broiler chicks were randomly divided into three groups of 60 chicks each. Three dietary treatments were randomly assigned to the three groups of chicks. Chicks in Group 1 were fed basal diet only (control group), Group 2 was fed basal diet + 0.1% dried culture of *L. acidophilus*, and Group 3 was fed basal diet + 0.1% dried culture of a mixture of 12 *Lactobacillus* strains. Compositions of the basal diet and calculated nutrient levels for the experiment are presented in Table 1. The diets were formulated to meet nutrient requirements (NRC, 1984) and were provided ad libitum in mash form throughout the experimental period. The chicks were raised in wooden cages (90 × 120 × 50 cm, length × width × height) in an open house under natural conditions. Each cage was provided with a self-feeder and a waterer. There were 12 chicks per cage, and five cages were used for each dietary treatment. Heating lamps of 100 W were used to supply brooding heat in the first 2 wk. Chicks were weighed individually at 1 and 40 d of age to determine weight gain. Feed consumed on cage basis was recorded daily, the uneaten discarded and feed efficiencies were 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 40 d under typical tropical conditions (24 to 34 C, 70 to 100% relative humidity). All animal management and sampling procedures were in accordance with the guidelines of the Consortium Guide (1988).

Bacterial Culture Preparation

The 12 strains of *Lactobacillus* (2 strains of *L. acidophilus*, 3 strains of *L. fermentum*, 1 strain of *L. crispatus*, and 6 strains of *L. brevis*), isolated from chicken intestine, were the same as those described by Jin et al. (1996d). A single strain of *L. acidophilus* I 26 (the most adherent of the 12 *Lactobacillus* strains, Jin et al., 1996d) or a mixture of 12 strains of *Lactobacillus* was inoculated into MRS broth[2] and incubated at 37 C for 24 h. The bacterial cells were harvested by centrifugation at 2,000 × *g* for 20 min at 4 C, and the bacterial pellets were lyophilized and stored at –20 C until used. To obtain a concentration of 1 to 2 × 10^9 cells per gram, the *Lactobacillus* cultures were diluted with cornstarch and skimmed-milk powder, based on their original colony-forming units per gram determined on MRS agar.[2] These products, containing either *L. acidophilus* I 26 or a mixture of *Lactobacillus* strains, were stored at 4 C and mixed into the feed each day. The viability of the *Lactobacillus* cultures was checked biweekly to ensure that the viability of the cultures remained at 1 to 2 × 10^9 viable cells per gram.

Sampling Procedure

Fecal samples and small intestinal contents (digesta) were collected after 40 d of feeding. Five chickens (one per cage) were randomly selected from each treatment. The chickens were euthanatized by severing the jugular vein, and the contents taken from the small intestine were digesta from the distal end of the duodenum to the ileo-caecal junction. To ease the sampling process, the mesentery was cut to uncoil the tract. A homogenous intestinal digesta sample was collected by massaging the tract from both ends. Care was taken to remove blood and excess tissue from each digesta sample to minimize interference with the enzyme assay. The digesta samples were stored immediately at –70 C until used.

Feces were collected immediately after they were dropped on a paper sheet that was laid on a plastic tray placed directly below the cage. The fecal samples were put into bottles filled with CO_2 and were stored at –70 C until used.

The small intestinal digesta samples were diluted 10×, based on the sample weight, with ice-cold PBS (pH 7.0), homogenized for 60 s, and sonicated for 1 min with three

[2]Oxoid Ltd., Basingstoke, Hampshire, RG24 8PW, UK.

cycles at 30-s intervals. The sample was then centrifuged at 18,000 × *g* for 20 min at 4 C.

Fecal samples were lyophilized and ground to pass through a 1-mm sieve, and 1 g was suspended in 10 mL of ice-cold 0.1 *M* potassium phosphate buffer (pH 7.0). The suspension was homogenized in a prechilled homogenizer for 1 min, and the homogenate was disrupted by sonication at 4 C for 1 min, three times, at 30-s intervals. It was then centrifuged at 18,000 × *g* for 15 min.

To prevent possible enzyme degradation, the intestinal digesta samples, fecal samples, and supernatants were kept on ice throughout the preparation. The supernatants from both intestinal and fecal samples were divided into small portions and stored at –70 C for enzyme assays.

Digestive Enzyme Assay

Amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1.) activity was determined using the method of Somogyi (1960). Amylase activity unit (One Somogyi 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 per milligram of intestinal digesta protein. The substrate used in the analysis was cornstarch. All chemicals were purchased in a kit (No. 700) from Sigma Chemical Company.[3] The analytical procedure was in accordance with Sigma instructions. When amylase activity of the sample was too high, 0.5% NaCl solution was added to dilute the sample.

Lipase (LPS, triacylglycerol lipase, EC 3.1.1.3.) activity was assayed using the method described by Tietz and Fiereck (1966). Lipase activity unit (Sigma-Tietz Units) was equal to the volume (mL) of 0.05 *M* NaOH required to neutralize the fatty acid liberated during 6 h incubation with 3 mL of lipase substrate at 37 C per milligram of intestinal digesta protein. Olive oil was used as the substrate in this assay. All chemicals were purchased in a kit (No. 800).[3]

Protease activity was analyzed using the modified method of Lynn and Clevette-Radford (1984). The protease activity unit was defined as milligrams of azocasein degraded during 2 h incubation at 38 C per milligram of intestinal digesta protein. Azocasein was used as the substrate.

Bacterial Enzyme Assay

β-Glucosidase (EC 3.2.1.21) activity was analyzed under anaerobic conditions by using the method of Goldin and Gorbach (1976). β-Glucosidase activity unit was expressed as micromolar of nitrophenol released per hour per milligram of fecal protein or intestinal digesta protein. The amount of nitrophenol released was determined by comparison with a standard nitrophenol curve.

β-Glucuronidase (EC 3. 2. 1. 31) activity was measured under anaerobic conditions using a modified method of Fishman et al. (1967). One β-glucuronidase activity unit (One Modified Sigma Unit) will liberate 1 μg of phenolphthalein from phenolphthalein glucuronic acid per hour at 56 C per milligram of fecal protein or intestinal digesta protein. All the chemicals were acquired in a kit (No. 325)[3]. The amount of phenolphthalein released was determined by comparison with a standard phenolphthalein curve.

Protein Determination

The intestinal digesta and fecal protein concentrations were determined by the method of Lowry et al. (1951) that was modified by Ohnishi and Barr (1978). Ovine serum albumin was used as a standard. All chemicals were purchased in a kit (No. 690)[3].

Statistical Analysis

Mean values between treatments were compared using analysis of variance followed by least significant difference. The computation was done by using the general linear models procedures of the SAS program (SAS Institute, 1988).

RESULTS AND DISCUSSION

Addition of either a single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains to the basal diet significantly increased ($P < 0.05$) the body weight of broilers after 40 d of feeding (Table 2). The feed to gain ratios were decreased by 0.11 ($P < 0.05$) or 0.16 unit ($P < 0.05$) for the birds fed diets with *L. acidophilus* or a mixture of *Lactobacillus*, respectively (Table 2). The present results are in accordance with our previous reports (Jin et al., 1998a,b). Similar results on the beneficial effects of *Lactobacillus* cultures on the growth of chicken have also been reported by several workers (Tortuero, 1973; Dilworth and Day, 1978; Watkins et al., 1982; Jin et al., 1996b; Mohan et al., 1996; Yeo and Kim, 1997).

The results on the effects of adherent *Lactobacillus* cultures on the small intestinal digestive enzymes are shown in Table 3. Supplementation of *L. acidophilus* or a mixture of *Lactobacillus* cultures to chickens significantly increased ($P < 0.05$) the levels of amylase after 40 d of feeding. This result is similar to the finding of Collington et al. (1990), who reported that inclusion of a probiotic (a mixture of multiple strains of *Lactobacillus* spp. and *Streptococcus faecium*) resulted in significantly higher carbohydrase enzyme activities in the small intestine of piglets. The lactobacilli colonizing the intestine may secrete the enzyme, thus increasing the intestinal amylase activity (Duke, 1977; Sissons, 1989). The adherent *Lactobacillus* spp. used in the present experiment had been found to be able to produce amylase extracellularly and intracellularly in vitro (L. Z. Jin, unpublished data). The present result that showed that supplementation of *Lactobacillus* cultures to chicken increased the amylolytic activity in the intestine supports this finding.

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.

TABLE 2. Effect of *Lactobacillus* cultures on body weight gain and feed efficiency of broilers

	Body weight[1] (g)				
Treatment	Initial weight	Final weight	Gain	Feed to gain ratio[2]	Mortality[1] (%)
Control	50.2	1,632.0[b]	1,581.8[b]	2.14[a]	7.4
L. acidophilus	50.4	1,705.2[a]	1,654.8[a]	2.03[b]	7.0
Mixture of *Lactobacillus*	50.2	1,679.5[a]	1,629.3[a]	1.98[b]	3.9
SEM	1.45	6.67	5.84	0.085	
Source of variation	(*P*)				
Diet	NS	0.008	0.0001	0.031	

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$).
[1]n = 60.
[2]n = 5.

In contrast to the intestinal amylase activity, the intestinal lipolytic and proteolytic activities of broilers did not increase significantly after 40 d of feeding diets supplemented with either a single strain of *L. acidophilus* or a mixture of *Lactobacillus*. This result is not surprising as extracellular protease and lipase of the *Lactobacillus* spp. were not detected by an in vitro test (L. Z. Jin, unpublished data). There has been no previous report on the effect of probiotic on the intestinal lipolytic activity. However, the result on the intestinal proteolytic activity was similar to those reported by earlier workers. Salter and Fulford (1974) found that the presence of bacteria had no effect on the digestion of dietary protein in chickens, and Lepkovsky et al. (1964) reported that the activities of intestinal protease in germfree chickens were similar to those of conventional ones. In piglets, Collington et al. (1990) observed that feeding a probiotic had no effect on the intestinal depeptidase activity before or after weaning.

Table 4 shows the effect of feeding adherent *Lactobacillus* cultures on the intestinal and fecal β-glucuronidase and β-glucosidase activities of chicken after 40 d of feeding. Intestinal β-glucuronidase level was significantly ($P < 0.05$) reduced by the addition of either *L. acidophilus* or a mixture of *Lactobacillus*, but fecal β-glucuronidase activity was significantly ($P < 0.05$) reduced by only *L. acidophilus* and not by a mixture of *Lactobacillus* (Table 4). Similar results on intestinal β-glucuronidase activity was observed by Cole et al. (1984), who reported that young birds supplemented with yogurt showed a marked reduction of β-glucuronidase activity when compared with birds without yogurt supplement. Several reports on rats and humans also showed that supplementing *Lactobacillus* to the hosts decreased the fecal β-glucuronidase (Ayebo et al., 1980; Goldin et al., 1980; Goldin and Gorbach, 1984; Ling et al., 1994; Djouzi et al., 1997).

TABLE 3. Effect of *Lactobacillus* cultures on the amylolytic, proteolytic, and lipolytic activities in the small intestinal contents of broilers

	Enzyme activity		
Treatment	Amylolytic (Somogyi Unit)	Proteolytic (Unit)	Lipolytic (Sigma-Tietz Unit)
Control	7.41[b]	73.98	18.27
L. acidophilus	10.54[a]	78.73	18.73
Mixture of *Lactobacillus*	10.44[a]	74.60	22.82
SEM	0.88	3.64	3.52
Source of variation	(*P*)		
Diet	0.021	NS	NS

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$). Each mean represents five replicates.

β-Glucuronidase is believed to be largely responsible for the hydrolysis of glucuronides in the lumen of the gut. This reaction is potentially important in the generation of toxic and carcinogenic substances as many compounds are detoxified by glucuronide formation in the liver and subsequently enter the bowel via the bile. In this manner, toxic aglycones can be regenerated in the bowel by bacterial β-glucuronidase (Gorbach, 1986). Although these potential harmful metabolites may not cause disease to chickens, they may hinder the performance or reduce feed utilization.

The results in the present study demonstrated that fecal β-glucosidase activity was significantly ($P < 0.05$) lower in birds fed diets with either *L. acidophilus* or a mixture of *Lactobacillus* but that intestinal β-glucosidase levels were not affected (Table 4). There is a paucity of information on the effect of *Lactobacillus* culture on β-glucosidase activity in poultry, but there have been some reports on human and rats. Ayebo et al. (1980) reported that ingestion of *L. acidophilus* culture at 2×10^6 cells/ml reduced the fecal β-glucosidase levels in humans. β-Glucosidase is involved in the carcinogenicity of the naturally occurring substance. It has been postulated that amygdalin (a major cyanogenic glucoside) is hydrolyzed in the gut by bacterial β-glucosidase to yield mandelonitrile, which is unstable and is readily hydrolyzed to release toxic cyanide (Goldin and Gorbach, 1976, 1984). Recently, Wollowski et al. (1999) found that *Lactobacillus* inactivated carcinogens and prevented DNA damage in the colon of rats.

The reduction of β-glucuronidase and β-glucosidase activities in chickens fed *Lactobacillus* cultures may be

TABLE 4. Effect of *Lactobacillus* cultures on β-glucuronidase and β-glucosidase activities in the small intestinal contents and feces of broilers

	β-Glucuronidase activity (μM/h per mg protein)		β-Glucosidase activity (μg/h per mg protein)	
Treatment	Intestinal content	Feces	Intestinal content	Feces
Control	3.42[a]	1.30[a]	6.50	5.86[a]
L. acidophilus	1.95[b]	0.67[b]	6.59	1.43[b]
Mixture of *Lactobacillus*	1.55[b]	1.00[ab]	5.44	1.66[b]
SEM	0.41	0.17	0.51	0.33
Source of variation	(*P*)			
Diet	0.034	0.042	NS	0.001

[a,b]Means within columns with no common superscript differ significantly ($P < 0.05$). Each mean represents five replicates.

attributed to the partial replacement of the intestinal microflora with *Lactobacillus* strains. The *Lactobacillus* cultures may reduce β-glucuronidase and β-glucosidase activities by attaching themselves along the chicken intestine, thus preventing colonization of other bacteria, especially *Escherichia coli*. The *Lactobacillus* strains used in the present study have been shown to possess a strong ability to adhere to the intestinal epithelium of chicken (Jin et al., 1996d). They are also able to antagonize and competitively exclude some pathogenic bacteria in vitro and to inhibit the growth of several serotypes of *E. coli* in vitro (Jin et al., 1996a,c,d, 1998a,b). In a survey of gut bacteria, Hawksworth et al. (1971) found that *E. coli* produced 15.4 times more β-glucuronidase per strain than any other genera tested. Furthermore, over 90% of *E. coli* strains are able to produce β-glucosidase, whereas only less than 40% of *Lactobacillus* strains show an ability to produce glucosidase (Drasar and Hill, 1974).

ACKNOWLEDGMENTS

The authors thank R. R. Marquardt, Department of Animal Science, The University of Manitoba (Winnipeg, Canada) for his helpful comments and suggestions. The fund provided by the Ministry of Science, Technology and Environment of Malaysia under the Intensification of Research Priority Areas (IRPA) Program (Project Code 01-02-04-0094) is acknowledged.

REFERENCES

Ayebo, A. D., I. A. Angelo, and K. M. Shahani, 1980. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. Milchwissenschaft 35:73–84.

Cole, C. B., P. H. Anderson, S. M. Philips, R. Fuller, and D. Hewitt, 1984. The effect of yogurt on the growth, lactose-utilizing gut organisms and β-glucuronidase activity of cecal contents of a lactose-fed, lactase-deficient animal. Food Microbiol. 1:217–222.

Cole, C. B., R. Fuller, and M. J. Newport, 1987. The effect of diluted yogurt on the gut microbiology and growth of piglets. Food Microbiol. 4:83–85.

Collington, G. K., D. S. Parker, and D. G. Armstrong, 1990. The influence of inclusion of either an antibiotic or a probiotic in the diet on the development of digestive enzyme activity in the pig. Br. J. Nutr. 64:59–70.

Consortium, 1988. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Division of Agriculture, NASUL6C, Washington, DC.

Dilworth, B. C., and E. J. Day, 1978. *Lactobacillus* cultures in broiler diets. Poultry Sci. 57:1101.

Djouzi, Z., C. Andrieux, M.-C. Degrvry, C. Bouley, and O. Szylit, 1997. The association of yogurt starters with *Lactobacillus casei* DN 114.001 in fermented milk alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats. J. Nutr. 127:2260–2266.

Drasar, B. S., and M. J. Hill, 1974. Human Intestinal Flora. Academic Press, London, UK.

Duke, G. E., 1977. Avian digestion. Pages 313–320 *in*: Physiology of Domestic Animals 9th ed. G. E. Duke, ed. Cornell University Press, Ithaca, NY.

Fishman, W. H., K. Kato, C. L. Anstis, and S. Green, 1967. Human serum β-glucuronidase: Its measurement and some of its properties. Clin. Chem. Acta. 15:435–438.

Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365–378.

Goldin, B. R., and S. L. Gorbach, 1976. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. J. Natl. Cancer Inst. 57:371–377.

Goldin, B. R., and S. L. Gorbach, 1977. Alterations in fecal microflora enzymes related to diet, age, *Lactobacillus* supplements, and dimethylhydrazine. Cancer 40:2421–2426.

Goldin, B. R., and S. L. Gorbach, 1984. Alterations of the intestinal microflora by diet, oral antibiotic, and *Lactobacillus*: decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. J. Natl. Cancer Inst. 73:689–695.

Goldin, B. R., L. Swenson, J. Dwyer, M. Sexton, and S. L. Gorbach, 1980. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. J. Natl. Cancer Inst. 64:255–261.

Gorbach, S. L., 1986. Function of the normal human microflora. Scand. J. Infect. Dis. 49:17–30.

Hawksworth, G. M., B. S. Drasar, and M. J. Hill, 1971. Intestinal bacteria and the hydrolysis of glycosidic bonds. J. Med. Microbiol. 4:451–459.

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

Jin, L. Z., Y. W. Ho, N. Abdullah, A. M. Ali, and S. Jalaludin, 1998a. Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. Anim. Feed Sci. Technol. 70:197–209.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin, 1996b. Influence of dried *Bacillus subtilis* and Lactobacilli cultures on

intestinal microflora and performance in broilers. Asian-Australasian J. Anim. Sci. 9:397–404.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin, 1997. Probiotics in poultry: Modes of action. World's Poult. Sci. J. 53:351–368.

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

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

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

Lepkovsky, S., M. Wagner, F. Furuta, K. Ozine, and T. Koike, 1964. The proteases, amylase and lipase of the pancreas and intestinal contents of germfree and conventional chicken. Poultry Sci. 43:722–731.

Lessard, M., and G. J. Brisson, 1987. Effect of a *Lactobacillus* fermentation product on growth, immune response and fecal enzyme activity in weaned pigs. Can. J. Anim. Sci. 67:509–516.

Ling, W. H., R. Korpela, H. Mykkanen, S. Salminen, and O. Hanninen, 1994. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J. Nutr. 124:18–23.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

Lynn, K. R., and N. A. Clevette-Radford, 1984. Purification and characterization of hevin, a serin protease from *Hevea braziliensis*. Biochemistry 23:963–964.

Macfarlane, G. T., and J. H. Cummings, 1991. The colonic flora, fermentation and large bowel digestive function. Pages 51–92 *in*: The Large Intestine: Physiology, Pathophysiology and Disease. S. F. Phillips, J. H. Pemberton, and R. G. Shorter, ed. Raven Press, New York, NY.

Marteau, P., and J. C. Rambaud, 1993. Potential of using lactic acid bacteria for therapy and immunomodulation in man. FEMS Microbiol. Rev. 12:207–220.

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

National Research Council, 1984. Nutrient Requirements of Poultry. 8th rev. ed. National Academic Press, Washington, DC.

Ohnishi, S. T., and J. K. Barr, 1978. A simplified method of quantitating proteins using the burette and phenol reagents. Anal. Biochem. 86:193–198.

Ratcliffe, B., 1985. The influence of the gut microflora on the digestive processes. Pages 245–267 *in*: Proceedings of the 3rd International Seminar on Digestive Physiology of Pig. National Institute of Animal Science, Copenhagen, Denmark.

Salter, D. N., and R. J. Fulford, 1974. The influence of the gut microflora on the digestion of dietary and endogenous proteins: Studies on the amino acid composition of the excreta of germ-free and conventional chicks. Br. J. Nutr. 32:625–636.

Sanders, M. E., 1993. Summary of conclusions from a panel of experts on health attributes of lactic cultures: Significance to fluid milk products containing cultures. J. Dairy Sci. 76:1819–1828.

SAS Institute, 1988. SAS/STAT User's Guide, Release 6.03 Edition, SAS Institute Inc., Cary, NC.

Sissons, J. W., 1989. Potential of probiotic organisms to prevent diarrhea and promote digestion in farm animals: A review. J. Sci. Food Agric. 49:1–13.

Somogyi, M., 1960. Modification of two methods for the assay of amylase. Clin. Chem. 6:23–27.

Szabo, J., 1979. Protein, carbohydrate and fat degrading enzymes in the intestine of germfree and conventional piglets. Pages 125–128 *in*: Clinical and Experimental Gnotobiotics. Proceedings of the VIth International Symposium on Gnotobiology. T. M. Fliedner, H. Heit, D. Niethammer, and H. Pflieger, ed. Gustav Fischer Verlag, Stuttgart, Germany.

Tietz, N. W., and E. A. Fiereck, 1966. A specific method for serum lipase determination. Clin. Chem. Acta 13:352–355.

Tortuero, F., 1973. Influence of implantation of *Lactobacillus acidophilus* in chicks on the growth, feed conversion, malabsorption of fats syndrome and intestinal flora. Poultry Sci. 52:197–203.

Watkins, B. A., B. F. Miller, and D. H. Neil, 1982. In vivo effects of *Lactobacillus acidophilus* against pathogenic *Escherichia coli* in gnotobiotic chicks. Poultry Sci. 61:1298–1308.

Wollowski, I., S.-T. Ji, A. T. Bakalinsky, C. Neudecker, and B. L. Pool-Zobel, 1999. Bacteria used for the production of yogurt inactivate carcinogens and prevent DNA damage in the colon of rats. J. Nutr. 129:77–82.

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. Poultry Sci. 76:381–385.