Research Notes

Effect of Feeding Diets Containing an Antibiotic, a Probiotic, or Yucca Extract on Growth and Intestinal Urease Activity in Broiler Chicks

JINMO YEO[1] and KYU-IL KIM[2]

Department of Animal Science, Cheju National University, Cheju 690-756, Republic of Korea

ABSTRACT A 6-wk study was conducted to determine the effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on daily gain, feed conversion ratio, and urease activity and ammonia production in intestinal contents of broiler chicks. Four replicates of 10 broiler chicks (average body weight, 48 g) each were assigned to a control or diets containing 0.1% chloroxytetracycline (antibiotic), 0.1% *Lactobacillus casei* (probiotic), or 0.2% yucca extract. Feeding a diet containing the probiotic significantly ($P < 0.05$) increased average daily gain during the first 3-wk period compared to the control (30.7 vs 28.7 g). This increase was partly accounted for by increased feed intake. During the first 3 wk, feeding the diet containing probiotic significantly ($P < 0.05$) decreased urease activity (per gram of collected contents) in small intestinal contents but not in large intestinal contents, compared with the control. Urease activity determined at 6 wk of age was not significantly affected by diet. Our studies indicate that dietary probiotic decreases urease activity in the small intestinal contents of young chicks and thus may be beneficial for improving animal health and growth, especially during early life.

(*Key words*: urease activity, ammonia production, antibiotic, probiotic, yucca extract)

1997 Poultry Science 76:381–385

INTRODUCTION

Ammonia (including the ammonium ion) produced from amino acid degradation in the body is converted to urea in the mammalian liver or uric acid in the chicken liver. A significant amount of these compounds (e.g., 20 to 25% of urea produced in the mammalian liver) is excreted into the gastrointestinal (GI) tract, and hydrolyzed into ammonia by microbial urease (Wrong, 1981). This ammonia, together with that produced from other nitrogenous substrates, may be used for microbial protein synthesis or may enter the blood stream. Ammonia is one of the microbial products that is known to be toxic to animals (Visek, 1978).

There are many types of bacteria in the ceca that exhibit hydrolytic activity of uric acid (a precursor of urea) in the chicken intestine and of urea (Stutz and Metrokotsas, 1972; Barnes and Impey, 1974), and deaminating activity (Fujita, 1968). Karasawa (1989) found that 70% of uric acid injected into a cecal sac disappeared within 1 h, with a concomitant increase in ammonia concentration.

Some species of the urease-producing bacteria were known to decrease the growth of chickens (Lev *et al.*, 1957), and the lack of growth was counteracted by addition of 45 mg of penicillin/kg diet (Coates *et al.*, 1963). Antimicrobial agents (Lev and Forbes, 1959; Gedek, 1984) and probiotics (Fuller, 1989) have been significant in reducing costs of animal production and have led to new insights into the influence of intestinal flora on the host. Feeding a diet supplemented with antimicrobial agents reduced the number of ureolytic organisms (Varel *et al.*, 1987) and urea hydrolysis in rats (Visek *et al.*, 1959) and chickens (Karasawa *et al.*, 1994). However, no such effects of probiotics were examined in the chicken.

The antibacterial mechanism of probiotics, although not completely known, may include low pH, low redox potential, miscellaneous inhibitory substances (e.g., H_2S, bacteriocins, fatty acids, and deconjugated bile acids), and competition for nutrients and adhesion to receptor sites (Nurmi and Rantala, 1973; Barrow, 1992). The growth-promoting effects of subtherapeutic-level antibiotics (Visek, 1978) or probiotics (Kim and Kim, 1992) used in animal feeds as growth promotants have also been ascribed to suppression of urea hydrolysis and subsequently reduced ammonia production in the GI tract.

Yucca extract has also been used as a feed supplement to reduce ammonia concentration in poultry barns (Johnston *et al.*, 1981). However, whether or not this yucca effect is attained through reduced urease activity has not been clearly demonstrated. The present study was conducted to further evaluate the effect of dietary

Received for publication April 3, 1996.
Accepted for publication September 28, 1996.
[1]Present address: Sam Yang Sa, Co., Andong, Korea.
[2]To whom correspondence should be addressed.

TABLE 1. Composition of diets[1]

Ingredients and composition	Starter 0 to 3 wk	Finisher 4 to 6 wk
	(% as-fed)	
Corn	39.37	54.26
Wheat	30.0	20.6
Soybean meal	16.7	12.4
Rapeseed meal	4.0	4.0
Corn gluten meal	4.3	4.0
Tallow	1.1	0.72
Calcium phosphate	1.6	1.4
Limestone	0.7	0.9
Salt	0.07	0.05
Methionine	0.46	0.50
Lysine	0.43	0.51
Choline chloride	0.24	0.25
$NaHCO_3$	0.90	0.25
Mineral mix[2]	0.10	0.10
Vitamin mix[3]	0.10	0.10
Ethoxyquin	0.03	0.03
Xanthophyll		0.01
Calculated composition		
Crude protein	19.5	17.5
ME, Mcal/kg	3,035	3,107

[1]For antibiotic, probiotic, and yucca extract, 0.1% chloroxytetracycline (Pfizer, Agriculture Division, New York, NY 10017), 0.2% *Lactobacillus casei* (1.2×10^7 cfu/g, Yuhan Yang-haeng, Seoul, Korea) and 0.2% yucca extract (Midori Pharmaceutical Co., Seoul, Korea) was added at the cost of corn, respectively.

[2]Provided in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5.0; I, 1.2; Co, 0.2; Se, 0.1.

[3]Contents per kilogram of diet: vitamin A, 5,000 IU; cholecalciferol, 1,100 IU; tocopheryl acetate, 11 IU; menadione, 1.1 mg; thiamine·HCl, 2.2 mg; riboflavin, 4.4 mg; pyridoxine·HCl, 2.2 mg; cyanocobalamin, 0.66 meq; niacin, 44 mg; Ca pantothenate, 12 mg; choline chloride, 220 mg; folic acid, 0.55 mg; D-biotin, 0.11 mg.

antibiotic, probiotic, or yucca extract on urease activity in the intestinal contents of broiler chicks.

MATERIALS AND METHODS

Animals and Diets

Four replicate groups, each consisting of 10 2-d-old chicks (Arbor Acres), were randomly assigned to a control or diets containing an antibiotic (0.1% chloroxytetracycline), a probiotic (0.1% *Lactobacillus casei*), or a yucca extract (0.2%). Chicks were allowed to have free access to a starter diet during the first 3 wk and then to a finisher diet during the second 3 wk (Table 1). They also had free access to water.

The temperature of the room with continuous lighting was maintained at 34 C initially, and then reduced by 3 C/wk until it reached 21 C, at which temperature the room was maintained for the rest of the feeding period. Body weight was recorded daily, and average daily gain was calculated during the first and second 3-wk periods. Feed intake was monitored and feed conversion ratio (feed:gain) calculated. All animal management and sampling procedures were in accord with the guidelines of the Consortium Guide (1988).

Incubation of Intestinal Contents

One chick from each replicate group, which had the approximate average weight of the group, was killed by cervical dislocation at 3 and 6 wk of age, respectively. The small and large intestine was removed into a plastic container, which was filled with nitrogen gas before being capped tightly. This sample was frozen in dry ice and stored at -20 C until used for incubation. This protocol did not significantly reduce urease activity, when compared to that in fresh contents. Before incubation, the small intestine was slit and homogenized with a Potter-Elvehjem tissue grinder[3] in 2 vol of 0.2 mol/L phosphate buffer in ice. Samples for incubation were taken from the homogenate and the weight of tough serosal residue was subtracted from the total weight of the small intestine when total urease activity in small intestinal contents was calculated. The mucosal layer of small intestine was included to recover microbes attached to microvilli. The contents of the large intestine (ceca + colon) were collected in 50-mL centrifuge tubes, weighed, and diluted 1:2 (wt/vol) with 0.2 mol/L phosphate buffer (pH 6.5).

Two 3-mL samples of diluted contents were transferred into 15-mL centrifuge tubes and 1 mL of 0.4 mol/L urea containing 3.7 kBq of [^{14}C]urea (74 MBq/mmol)[4] was added to one sample. To the other sample was added 1 mL of 0.4 mol/L urea containing no [^{14}C]urea, the mixture was inactivated with 0.4 mL of 3 mol/L H_2SO_4 before incubation, and served as blank. Because the volume of large intestinal contents from chicks killed at 3 wk was not sufficient, the contents of the last 10-cm segment of the small intestine were included in the large intestinal contents. This combined sample was used for incubation. Samples that were not inactivated were incubated in a shaking water bath at 37 C while being flushed with N_2 for the first 2 min and then each unit was clamped sealed.

At the end of the 30-min incubation, an air stream was pulled through the reaction chamber and CO_2 trap (5 mL of 1:2, by volume, mixture of ethanolamine and ethylene glycol monomethyl ether), and 0.4 mL of 3 mol/L H_2SO_4 was added to the inlet tube of the reaction chamber to stop the reaction and release CO_2. Over a 20-min period, CO_2 released was trapped by use of a gas dispersion tube. This technique allowed more than 98% recovery of radioactivity of $Na_2{}^{14}CO_3$, which was added into the reaction chamber and acidified. Preliminary studies showed that CO_2 release from urea was linear over the 30-min incubation when checked over 15- and 30-min incubations. There was no $^{14}CO_2$ contamination in [^{14}C]urea or no chemical ureolysis, confirmed by measuring radioactivity recovered in CO_2 from [^{14}C]urea when it was added to acid-inactivated samples and the mixtures were

[3]Wheaton, Millville, NJ 08332.
[4]Du Pont NEN Research Products, Boston, MA 02118.

incubated for 30 min. The inactivated samples (blanks and incubated samples) were centrifuged at 5,000 × *g* for 10 min and the supernatants were collected into plastic vials and stored at 4 C for later analysis.

Determination of Urease (EC 3.5.1.5) Activity

The radioactivity in 1 mL (taken from 5 mL) CO_2 trap was determined in 15 mL of Bio-Safe II[TM5] using a Liquid Scintillation Counter.[6] Total radioactivity in the CO_2 trap was calculated. Urease activity (micromoles per 30 min per gram of collected contents) was calculated by dividing radioactivity in Bequerels recovered in CO_2 during the 30-min incubation by specific radioactivity of urea (Bequerels per micromole) added to samples, assuming that no significant amounts of urea from the contents contributed to the incubation (Combe *et al.*, 1965). This value was divided by 0.9 to correct for the CO_2 unrecovered (about 10% on average ranging from 8 to 13%) after the 30-min incubation. The amount of CO_2 unrecovered (10%) was observed in a preliminary experiment when $Na_2{}^{14}CO_3$ was added to the large intestinal content sample and incubated for 30 min. This unrecovered CO_2 was considered to be incorporated into bacterial cells.

Determination of Ammonia Production

Net ammonia production during the 30-min incubation was calculated from the difference between the amounts of ammonia in the blank and the incubated sample. Ammonia (including ammonium ion) concentration in the 5,000 × *g* supernatants of both blanks and incubated samples were determined by using a modified method of Weatherburn (1967).

Statistical Analysis

Data were analyzed by one-way analysis of variance. Individual means were compared by the Newman-Keuls test (Snedecor and Cochran 1980), only if the *F* test for the treatment effect was significant ($P < 0.05$).

RESULTS

Average daily gain (30.7 vs 28.7 g) was increased ($P < 0.05$) by feeding the diet containing probiotic during the first 3-wk period as compared to the control, but average feed intake was not. No difference was found between the control and chicks fed diets containing either antibiotic or yucca extract (Table 2). During the second 3-wk period, daily gain was not significantly ($P > 0.05$) different among the diet groups.

Urease activity in the small intestinal contents of chicks fed the diet supplemented with probiotic for 3 wk and then killed was much lower ($P < 0.05$) than that for birds fed the control diet (0.12 vs 0.39 μmol urea hydrolyzed/g collected contents per 30 min). This difference disappeared when urease activity was determined at 6 wk of age due to high variability of response. Neither urease activity nor net ammonia production in large intestinal contents was significantly different among the treatment groups at either 3 or 6 wk of age (Table 2).

DISCUSSION

Microflora inhabiting the GI tract of animals interact with host animals and their populations vary with animal species, site along the GI tract, age, diet, and environment. Healthy animals generally maintain a balanced microbial population that plays an important role in the growth and health of animals. For example, intestinal bacteria metabolize nutrients in the contents, produce short chain fatty acids and lactic acid, and synthesize some vitamins. Some of these activities can be beneficial to the host animals.

Results (Table 2) showed that dietary probiotic suppressed the growth of bacteria that produce urease. This effect along with increased feed intake may be responsible for the increased weight gain during the first 3 wk of feeding in chicks fed the diet supplemented with probiotic. A similar effect was found when broiler chicks were fed a diet supplemented with *Lactobacillus acidophilus* (Tortuero 1973; Francis *et al.*, 1978).

Suppressing urease activity and ammonia production can be beneficial for improving animal health and enhancing growth, as shown in the present study, because ammonia locally produced by ureolysis in the intestinal mucosa can exert a significant damage to the surface cells. Urease has been known to play an essential role in pathogenesis of gastritis induced by *Helicobacter pylori*, and a urease-negative strain did not cause gastritis in gnotobiotic piglets (Smoot *et al.*, 1990; Eaton *et al.*, 1991). Similarly, the generation of ammonia in the rat stomach following instillation of urea in the presence of urease resulted in a deleterious influence on the rat gastric mucosa including stasis of microcirculation, disruption of the surface epithelial cells and necrosis of the mucosa (Murakami *et al.*, 1990).

Reports on the effect of yucca extract on ammonia production have been controversial (Johnston *et al.*, 1981, 1982). The depressed net ammonia production in large intestinal contents by dietary yucca extract shown in Table 2 might have been due to absorption of ammonia by yucca extract and thus reduction in detectable ammonia concentration (Headon and Dawson, 1990), although this result has not been confirmed by others. In addition to ureolysis, deamination, and use of ammonia as a substrate for microbial protein synthesis influence net ammonia production, suggesting a complicated system for a balance between production and utilization of ammonia by intestinal bacteria. Studies of growth

[5]Research Products International Corp, Mount Prospect, IL 60056.
[6]Model 1220, Quantulus, Wallac Oy., Turku, Finland.

TABLE 2. Effect of dietary antibiotic, probiotic, or yucca extract on daily gain, feed intake, feed to gain, and urease activity and ammonia production in intestinal contents of broiler chicks

Variable	Control	Antibiotic[1]	Probiotic[2]	Yucca extract[3]
0 to 3 wk[4]				
ADG, g	28.7 ± 0.5[a]	29.5 ± 0.6[a]	30.7 ± 0.5[b]	29.0 ± 0.4[a]
Feed intake, g/d	45.9 ± 0.7	46.5 ± 0.6	47.6 ± 0.6	45.3 ± 0.5
Feed:gain, g:g	1.6 ± 0.01	1.6 ± 0.03	1.5 ± 0.04	1.5 ± 0.02
4 to 6 wk[4]				
ADG, g	62.7 ± 1.6	67.5 ± 2.6	69.9 ± 2.8	62.6 ± 5.0
Feed intake, g/d	120.4 ± 3.1	128.8 ± 1.1	125.1 ± 1.5	123.9 ± 1.5
Feed:gain, g:g	1.9 ± 0.03	1.9 ± 0.07	1.8 ± 0.05	2.0 ± 0.10
Urease activity[5,6] at 3 wk				
SI[7]	0.39 ± 0.05[a]	0.31 ± 0.12[a]	0.12 ± 0.05[b]	0.17 ± 0.07[ab]
LI[8]	11.8 ± 2.8	11.7 ± 2.4	8.8 ± 3.7	4.4 ± 3.8
Urease activity[5,6] at 6 wk				
SI[7]	0.48 ± 0.18	1.01 ± 0.63	2.64 ± 1.57	2.06 ± 1.18
LI[8]	5.28 ± 1.22	6.76 ± 1.95	5.86 ± 1.70	6.96 ± 2.79
Net ammonia production[6,9]				
At 3 wk	14.2 ± 7.2	11.0 ± 5.7	7.9 ± 1.4	6.9 ± 2.0
At 6 wk	9.2 ± 7.2	12.6 ± 11.0	7.2 ± 3.3	7.4 ± 7.9

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

[1]Antibiotics = chloroxytetracycline (0.1%).

[2]Probiotics = *Lactobacillus casei* (1.2×10^7/g, 0.1%).

[3]Yucca extract 0.2%.

[4]Means ± SEM of 40 (0 to 3 wk of age) or 36 chicks (4 to 6 wk).

[5]Micromoles of urea hydrolyzed/30 min per gram of collected contents at 37 C.

[6]Means ± SEM of 4 chicks.

[7]Small intestine.

[8]Large intestine, at 3 wk, also includes contents of last 10 cm of SI.

[9]Micromoles of ammonia/g collected contents of the large intestine; net production = (after incubation − before incubation or in blank); and ammonia concentrations in blanks (μmol/g collected contents) = 3.85 ± 2.53, 3.12 ± 0.98, 2.68 ± 1.16, or 3.32 ± 1.14 at 3 wk, and 1.15 ± 0.75, 1.38 ± 0.60, 0.91 ± 0.53, or 0.89 ± 0.54 at 6 wk for animals fed the control, or diets containing antibiotic, probiotic, or yucca extract, respectively.

responses to antibiotics have been inconsistent. A positive effect was observed in the case of animals raised in deliberately infected hen houses because these animals had undergone a depression of growth due to microbial infection (Forbes and Park, 1959). However, in the same study no effect was observed in germ-free animals or animals grown in new hen houses. Different responses to antibiotic feeding among studies can be attributable to differences in species or age of animals, diet, and environment. Generally, more significant effects have been reported in younger than older animals, and in on-farm compared to university trials (Zimmerman, 1986).

In conclusion, our findings together with others' (e.g., Karasawa *et al.*, 1994) suggest that urease activity in the GI tract of broiler chicks, especially young chicks can be reduced by dietary probiotics. The urease activity may be used as a measure of a health-promoting potential of dietary growth promotants that act on animals as well as humans through the modification of intestinal microflora.

ACKNOWLEDGMENTS

We thank Tae-Wook Kim, Hak-Moon Park, Yong-Ho Yang, Wang-Sik Lee, and Joon-Ik Song for their technical help.

REFERENCES

Barnes E. M., and C. S. Impey, 1974. The occurrence and properties of uric acid decomposing anaerobic bacteria in the avian cecum. J. Appl. Bacteriol. 37:393–409.

Barrow, P. A., 1992. Probiotics for chickens. Pages 225–257 *in*: Probiotics. R. Fuller, ed. Chapman and Hall, London, UK.

Coates, M. E., R. Fuller, G. F. Harrison, M. Lev, and S. F. Suffolk, 1963. Comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17:141–153.

Combe, E., E. Penot, H. Charlier and E. Sacquet, 1965. Metabolisme du rat "germ-free". Ann. Biol. Anim. Biophys. 5:189–206.

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

Eaton, K. A., C. L. Brooks, D. R. Morgan, and S. Krakowka, 1991. Essential role of urease in pathogenesis of gastritis induced *Helicobacter pylori* in gnotobiotic piglets. Infect. Immun. 59:2470–2475.

Forbes M., and J. T. Park, 1959. Growth of germ-free and conventional chicks: Effect of diet, dietary penicillin and bacterial environment. J. Nutr. 67:69–84.

Francis, C., D. M. Janky, A. S. Arafa, and R. H. Harms, 1978. Interrelationship of lactobacillus and zinc bacitracin in the diets of turkey poults. Poultry Sci. 57:1687–1689.

Fujita, H., 1968. The production of ammonia by microorganisms in alimentary canal of chicks. I. Production of ammonia from L-arginine. Jpn. Poult. Sci. 5:136–141.

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

Gedek, B., 1984 Contemporary concepts of growth promoter use. Pages 315–328 *in*: Proceedings of 4th International Symposium on Antibiotics in Agriculture: Benefit and Malefits. Butterworths, London, UK.

Headon, D. R., and K. A. Dawson, 1990. Yucca extract controls atmospheric ammonia levels. Feedstuffs 62(29):15.

Johnston, N. L., C. L. Quarles, D. J. Fagerberg, and D. D. Caveny, 1981. Evaluation of yucca saponin on broiler performance and ammonia suppression. Poultry Sci. 60: 2289–2292.

Johnston, N. L., C. L. Quarles, D. J. Fagerberg, and D. D. Caveny, 1982. Broiler performance with DSS40 yucca saponin in combination with monensin. Poultry Sci. 61: 1052–1054.

Karasawa, Y., 1989. Ammonia production from uric acid, urea, and amino acids and its absorption from the ceca of the cockerel. J. Exp. Zool. 3(Suppl.):75–80.

Karasawa, Y., T. Ono, and K. Koh, 1994. Inhibitory effect of penicillin on cecal urease activity in chickens fed on a low protein diet plus urea. Br. Poult. Sci. 35:157–160.

Kim, T. W., and K. I. Kim, 1992. Effects of feeding diets containing probiotics or antimicrobial agent on urease activity and ammonia production in the intestinal contents of rats. Korean J. Anim. Sci. 34:167–173.

Lev, M., C.A.E. Briggs, and M. E. Coates, 1957. The gut flora of the chick. 3. Differences in cecal flora between 'infected', uninfected and penicillin-fed chicks. J. Anim. Sci. 16: 364–372.

Lev, M., and M. Forbes, 1959. Growth response to dietary penicillin of germ-free chicks and chicks with a defined intestinal flora. Br J. Nutr. 13:78–84

Murakami, M., J. K. Yoo, S. Teramura, K. Yamamoto, H. Saita, K. Matuo, T. Asada, and T. Kita, 1990. Generation of ammonia and mucosal cell lesion formation following hydrolysis of urea by urease in the rat stomach. J. Clin. Gastroenterol. 12:S104–S109.

Nurmi, E., and M. Rantala, 1973. New aspects of *Salmonella* infection in broiler production. Nature 241:210–211.

Smoot, D. T., H. L. T. Mobley, G. R. Chippendale, J. F. Lewison, and J. H. Resau, 1990. *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. Infect. Immun. 58:1992–1994.

Snedecor, G. W., and W. G. Cochran, 1980. Statistical Methods. Iowa State University Press, Ames, IA.

Stutz, M. W., and M. J. Metrokotsas, 1972. Urease activity in the digestive tract of the chick and metabolism of urea. Poultry Sci. 51:1876. (Abstr.)

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.

Varel, V. H., I. M. Robinson, and W. G. Pond, 1987. Effect of dietary copper sulfate, aureo SP250, or clinoptilolite on ureolytic bacteria found in the pig large intestine. Appl. Environ. Microbiol. 53:2009–2012.

Visek, W. J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447–1469.

Visek, W. J., J. M. Baron, D. M. Switz, 1959. Urea metabolism and intestinal ureolytic activity of rats fed antimicrobial agents. J. Pharmacol. Exp. Therap. 126:359–365.

Weatherburn, M. W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971–974.

Wrong, O. M., 1981. Nitrogen compounds. Pages 133–211 *in*: The Large Intestine: Its Role in Mammalian Nutrition and Homeostasis. O. M. Wrong, C. J. Edmonds, and V. S. Chadwick, ed. John Wiley and Sons, New York, NY.

Zimmerman, D., 1986. Role of subtherapeutic levels of antimicrobials in pig production. J. Anim. Sci. 62(Suppl. 3): 6–17.