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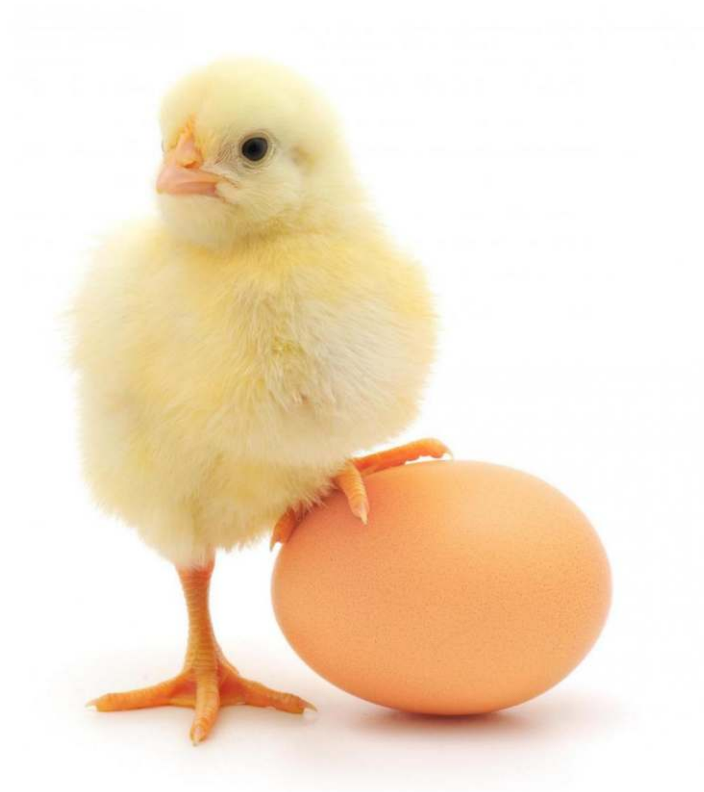

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Reduction of Broiler House Malodor by Direct Feeding of a Lactobacilli Containing Probiotic

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Abstract: Studies were conducted to investigate the direct feeding of a lactobacilli containing probiotic, Ecozyme, on broiler house malodor. Results indicated that feeding a diet containing the probiotic decreased ($P<0.05$) the environmental ammonia level in broiler house as well as pH and moisture content of excreta during the growth period. Gas chromatography/mass spectrometry data indicated that the quantity of volatile malodor compounds was much lower for the house with birds fed a diet containing lactobacilli than those of the controls. Volatile compounds such as 1-propanol, 1-butanol, 3-methyl hexane, 2-methylhoptane, dimethyl benzene, 3-methylheptane, octane, 2, 4-dimethyl hexane and octamethylcydotetrasiloxane were decrease to an undetectable level in the treatment room. Many major malodor indicators such as 2-butanone, hexanal, dimethyl disulfide and dimethyltrisulfide were also reduced by the feeding of lactobacilli containing diet. All 16 sensory panelists detected the odor difference between the treatment and control rooms. The control room has a higher malodor score than those of the treated one.

Key words: Malodor, probiotic, ammonia, volatile, excreta

Introduction

The malodor arising from the animal farms comprises a large proportion of environmental pollution complaints for the industry. Swine and poultry houses are the main sources of such odors (Yasuhara, 1987). Environmental ammonia and other volatile compounds play an important role in the malodor of poultry farms. The malodor of chicken house is caused by many different low boiling point volatile compounds (Yasuhara *et al.*, 1984). These malodor compounds always have very low detectable threshold for human being and even lower than many detectable limit of machine (Shigeta, 1976).

Ammonia and sulfur-containing compounds play an important role in malodor of chicken house (Yasuhara, 1987). Numerous laboratories and field studies have shown how ammonia affects birds' health and performance (Blake, 1998). Suppressing ammonia production can be beneficial for improving animal health and enhancing growth because ammonia produced in the intestinal mucosa can exert significant damage to the surface of cell. Isshiki (1979) reported that feeding of *Lactobacillus casei* suppressed ammonia content in chicken blood. He indicated that feeding of *L. casei* resulted in a decrease of non-protein nitrogen in blood, including uric acid, ammonia and urea. Chiang and Hsieh (1995) reported that feeding probiotics containing *L. acidophilus, S. faecium and B. subtilis* reduced the concentration of ammonia in the excreta and litter of broilers. Recently, Yeo and Kim (1997) reported that feeding broiler chicks a diet containing *L. casei* as a probiotic decreased urease activity in the small intestine content during the first 3 weeks. However, no information concerning the ammonia and other odor compounds of chicken house environment was reported.

There are many types of probiotic preparations on the market. Ecozyme (Ecozyme Biotich Inc., TX) is one of several commercial probiotic products. This product is a mixture of poultry feed and several groups of *Lactobacillus sp.*, including *L. casei, L. brevis, L. buchneri* and *L. plantarum*. This probiotic has been used to improve the farm environment in Taiwan, but scientific data are unavailable to show the effectiveness of malodor reduction level in the chicken house. Therefore, the objective of this research was to investigate the effect of this lactobacilli based probiotic, Ecozyme, on environmental air characteristics of broiler house.

Materials and Methods

Birds, Diets and Rooms: Two experiments were conducted. The log number of lactobacilli in the Ecozyme supplemented feed of the first experiment as represented in Rogosa medium (Difco, Detroit, MI) counts was 6.0 cfu/g and the log number of lactobacilli for the second experiment was 3.0 cfu/g.

In each experiment, 56 day-old male birds (Arbor Acres) were assigned to eight 75x65x35 cm cages with seven birds per case. Four cages were randomly assigned to each treatment of 1) control feed (Clover brand, Starkville, MS) and 2) control feed supplemented with 5% Ecozyme (Ecozyme Biotech Inc., TX). The feed was mixed every 2 days in order to keep constant quality and quantity of lactobacilli cultures. Birds were reared in two separated rooms of the same dimension and equipped with an identical automatic controlled heating and ventilation systems. Each room housed four cages with

Table 1: Volatile organic compounds of broiler house air as affected by a lactobacilli type probiotic, Ecozyme, supplementation in diet

Peak #	Retention Time (Min)	Peak Area		Possible Compound
		Control	Treated	
1	4.9	6,374,200	639,712	ethanol
2	5.0	9,340,486	1,527,456	2-propanone
3	5.2	2,138,888	249,403	2-propanol
4	5.5	1,382,542	782,923	dichloromethane
5	5.8	1,322,293	234,703	2-methyl pentane
6	5.9	1,758,078	ND	1-propanol
7	6.0	1,363,380	167,545	3-methyl pentane
8	6.3	5,884,128	872,717	2-butanone
9	6.6	905,531	108,447	2-butanol
10	7.9	2,341,155	294,506	benzene
11	8.2	894,363	ND	1-butanol
12	8.3	1,232,773	ND	3-methyl hexane
13	9.1	1,609,700	248,807	haptene
14	10.2	409,213	ND	cyclohexane
15	11.1	9,281,490	1,283,033	dimethyl disulfide
16	11.9	324,572	ND	2-methylheptane
17	12.1	1,034,032	ND	dimethyl benzene
18	12.3	260,924	ND	3-methylheptane
19	13.7	425,447	ND	octane
20	13.8	817,381	444,359	hexanal
21	14.4	322,119	204,110	hexamethyl cyclotrisiloxane
22	19.2	189,050	ND	2,4-dimethyl hexane
23	23.5	344,475	82,175	dimethyltrisulfide
24	23.7	383,396	ND	Octamethyl cydotetrasiloxane

Each value represents mean of two observations. ND = not detected

the same treatment within the rooms to ensure no interaction between the two treatments.

Each room had continuous lighting and maintained a temperature of 32.2 °C initially, then reduced to 23.8 °C after 2 weeks and remained at 23.8 °C for the rest of feeding period. Chicks were allowed to have free access to feed and water during the 6-week grow-out period.

Analyses

Environmental Ammonia: Environmental ammonia was determined by using a Kitagawa Toxic Gas Detector (Kitagawa, Matheson, NJ) and ammonia detecting tubes (0-20 ppm capacity, Matheson, NJ). The detecting tubes were broken at both ends by using a tip cutter and inserted into the detector. One hundred milliliter air samples were used for each measurement.

Volatile Organic Compounds: At the end of the fifth week feeding, the volatile organic compounds (VOC) of the broiler room air were sampled by pumping the broiler room air through a Tenax TA steel tube trap (60/80 mesh, Supelco, Bellefonte, PA) for 2 hr at an air flow rate of 60 mL/min. The sampling tubes were then thermally desorbed by a thermal desorption Autosystem (Perkin Elmer ATD400, Norwalk, CT). The trapped VOC were desorbed at 250 °C for 15 min and condensed at –30 °C by liquid nitrogen. The temperature was immediately raised to 300 °C at 40 °C/sec rate and the VOC were transferred in helium at 1.0 mL/min onto a capillary Gas Chromatography (HP5890 Series II GA)/Mass Spectrometry (GC/MS) (HP 5972 mass selective detector) (Hewlett-Packard Co., CA) system with a Supelco SPB-1 fused silica capillary column (30 m, 0.32 mm i.d., 4.0 um thickness; Bellefonte, PA).

Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The temperature was programmed to rise from 220 °C at 3 C/min and finally held at 220 °C for 1 min. The GC-MS condition was set as follows: injector temperature, 180 °C and ionization energy, 70 eV. A computer search of standard mass spectra was conducted on the Wisely Standard Library to identify the compounds. These computer matches were "tentative" since no additional analytical technique was used to verify the identification.

Sensory Evaluation: Fifteen staff members and students were chosen as panelists for evaluating the odor by a sensory test. A paired-comparison test (Larmond, 1977) was used for the evaluation.

Table 2: Odor description of some volatile organic compounds as isolated from a broiler house

Compound	Odor Description and Reference
Ethanol	Alcohol (Angelino, 1991; Grosch and Schieberle, 1991)
2-propanone	Pungent, sweetish taste (Merck Index, 1968)
2-propanol	Mixture of ethanol and acetone odor (Merck Index, 1968)
1-propanol	Alcoholic and slightly stupefying odor (Merck Index, 1968)
2-butanone	Acetone-like odor (Merck Index, 1968)
1-butanol	Weak fusel oil odor (Merck Index, 1968)
3-methyl hexane	Solvent odor (Merck Index, 1968)
Cyclohexane	Solvent odor (Merck Index, 1968)
Dimethyl benzene	Aromatic (Jovanovic and Milovanovic, 1993)
Hexanal	Green grass (Grosch and Schieberle, 1991; Lin, 1993) Cut grass (Overton and Manura, 1998)
Dimethyltrisulfide	Cooked cabbage (Yang, 1997)

pH: The pH of fecal content was determined by using a pH meter (Model LS, Sargent-Welch Co., Springfield, NJ).

Moisture Content: Moisture content of fecal material was determined according to the method as described in AOAC (AOAC, 1990).

Statistical Analyses: Data were analyzed by Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS/STAT, 1990). When there were significant differences ($P<0.05$), least significant difference (LSD) test was used to separate the means (Steel and Torrie, 1980).

Results and Discussion

Volatile Ammonia Production in Broiler Farming: The environmental ammonia concentration increased as the broiler age increased. Adding Ecozyme in broiler feed reduced environmental ammonia concentration in broiler house when compared to those of the controls (Fig. 1). After the end of first 2 weeks of feeding, no apparent difference ($P>0.05$) in the ammonia readings between the two treatments was found. One explanation could be that the chickens were small and the excreta impact on environmental air was correspondingly minimal. The ammonia concentration in the air increased dramatically after 3 weeks of bird age and leveled off after 5 weeks. Feed containing log 3.0 cfu/g (Exp. 2) of lactobacilli performed as well as that of log 6.0 cfu/g (Exp. 1) in volatile ammonia reduction (Fig. 1). Chiang and Hsieh (1995) reported that probiotics containing *L. acidophilus, S. faecium* and *B. subtilis* reduced the concentration of ammonia in the excreta and broilers' litter. However, no data indicated that the reduction of malodor in the chicken house air by probiotics was reported. Our data indicated that volatile ammonia can be reduced on the chicken farm by feed a lactobacilli type probiotic which should be of practical value to the poultry industry.

Reduction of Excreta pH: Feeding broilers with a diet containing Ecozyme reduced the pH of excreta or birds when compared to those of the controls (Fig. 2). This is probably due to the metabolites from the lactic acid bacteria such as lactic acid and acetic acid in the digestive system (Tramer, 1966; Sorrels and Speck, 1970). No differences in the pH of excreta were observed between the two groups from the first week of feeding. The pH were increased dramatically after two weeks and then leveled off at the third week (Fig. 2). The time required for lactobacilli to establish colonies in the chicken gut may be one factor responsible for this phenomenon.

Reduction of Excreta Moisture Content: The moisture content of excreta from the lactobacilli treated group was lower ($P<0.05$) than those of the controls (Fig. 3). The moisture of fecal content increased as the age of birds progressed. Lower moisture content of fecal material would aid in the convenience of clean-up in the broiler house. No published data concerning the effect of probiotic on moisture content of excreta were available. Wet litter is a problem in the broiler breeder industry. Apart from ventilation problems and water spillage, wet litter is directly associated with excessive excretion of watery droppings (Ogunji *et al.*, 1983). Results of this study showed the moisture content can be reduced when broilers are fed a diet with a lactobacilli type probiotic. This may help the broiler breeder industry solve the wet litter problem.

Reduction of Volatile Organic Compounds in Broiler House Atmosphere: The total quantity of volatile organic compounds (VOC) in the control broiler room was much higher than those in the treatment room (Table 1). When direct feed lactobacilli to broilers, many VOC production reduced to an undetectable level, including 1-propanol, 1-butanol, 3-methyl hexane, 2-methylheptane, dimethyl benzene, 3-methylheptane, octane, 2, 4-dimethyl hexane and octamethylcydotetrasiloxane. This may reduce different type odors in the treatment room, such as alcoholic-like odor (1-propanol), fusel oil odor (1-butanol) and solvent odor (3-methyl hexane). Many major malodor indicators such as 2-butanone (acetone-

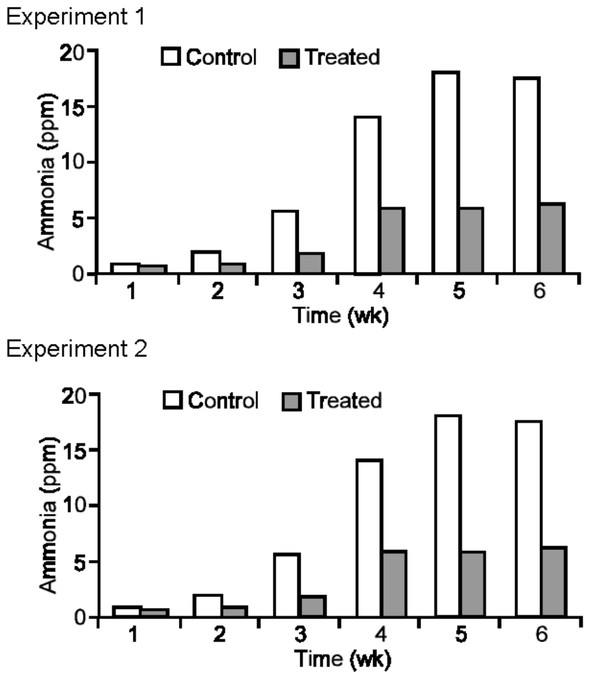

Fig. 1: Environment ammonia concentration of broiler room as affected by Ecozyme diet supplementation. Exp. 1: log 6 lactobacilli cfu/g feed; Exp 2: log 3 lactobacilli cfu/g feed.

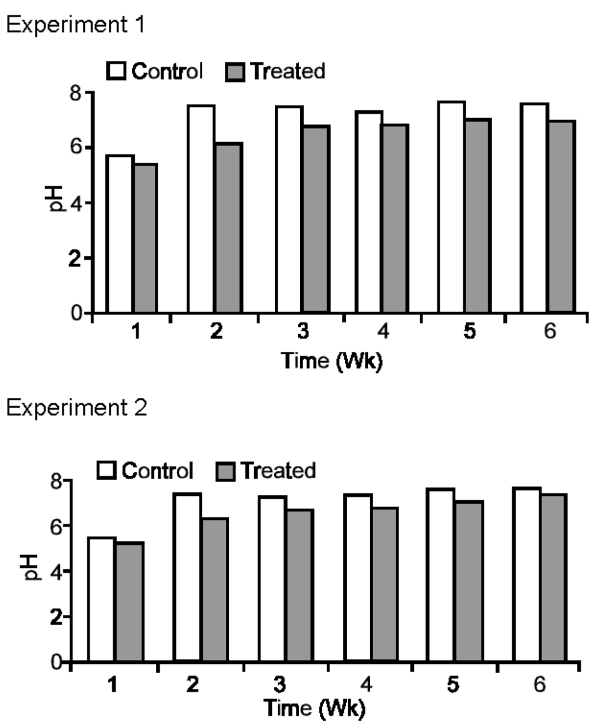

Fig. 2: Broiler fecal pH as affected by Ecozyme diet supplementation. Exp. 1, log 6 lactobacilli cfu/g feed; Exp. 2, log 3 lactobacilli cfu/g feed.

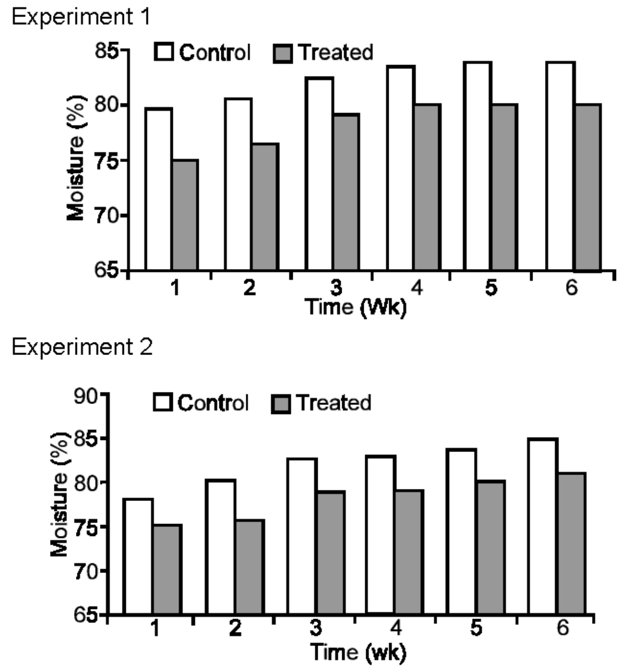

Fig. 3: Broiler fecal moisture content as affected by Ecozyme diet supplementation. Exp. 1, log 6 lactobacilli cfu/g feed; Exp. 2, log 3 lactobacilli cfu/g feed.

like odor), hexanal (green-grass odor), dimethyl disulfide (cooked cabbage odor) and dimethyltrisulfide (cooked cabbage odor) were identified in both broiler rooms; but much lower quantities were recorded in the treatment room(Table 1). The odor descriptions of the malodor compounds are summarized as Table 2.

The alcohol compounds were usually higher in the control room than the treatment room. Ethanol, 1-propanol, 1-butanol and 2-butanol were remarkably abundant in the control room. Only one aldehyde, hexanal, was detected in this experiment and its concentration was much higher in the control room than in the room with Ecozyme supplement in the diet. Two alkanones, 2-propanone and 2-butanone were also detected. These compounds are widespread among animal fecal materials (Yasuhara, 1987) and play an important role in the chicken excreta odor. In addition, two sulfur-containing compounds and many alkanes were also detected in this experiment.

Sensory Evaluation of Malodor Level: All the 16 panelists detected the odor difference between the two rooms through paired comparison odor evaluation after 3 and 5 weeks of broiler age. As expected, the control room has a higher malodor score than those of the treatment room (Table 3). The strong malodor may have included the ammonia and several VOC in the broiler room.

Table 3: Malodor evaluation of broiler house air by sensory subjects

	Experiment 1		Experiment 2	
	Day 15	Day 38	Day 15	Day 38
Total subject	16	16	16	16
No of subject scored:				
Extremely difference	0	12	0	11
Much difference	1	2	0	2
Slight difference	7	2	7	3

Experiment 1: log 6 lactobacilli cfu/g feed. Experiment 2: log 3 lactobacilli cfu/g feed.

In addition to the GC/Mass analysis of malodor compounds for determining the malodor odor, the subjective sensory measure can be used for verifying and comparing the results. Using panelists to determine the level of malodor in chicken house is difficult because of the malodor may make panelists uncomfortable. Therefore, little or no reports determining the level of malodor in the chicken house were published.

Conclusion: Results of this study indicated that supplementing broiler with a lactobacilli type probiotic, Ecozyme, reduced the pH and moisture content of excreta. The environmental volatile ammonia and volatile organic compound levels were also decreased by the lactobacilli supplement. Many VOC in the air were reduced to an undetectable level when diet was supplemented with this lactobacilli type probiotic.

In addition to the potential improvement of bird health and performance, research data provided an alternative method in solving the environmental problem on poultry farming.

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References

Angelino, S. A. G. F., 1991. Beer. In Marrse, H. (Ed), Volatile Compounds in Foods and Beverages. Pages: 581-616. Marcel Dekker, New York, NY.

AOAC, 1990. Official Method of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC.

Blake, J. P., 1998. Many options are available for reducing ammonia in litter. In Poultry Times, Nov. 2nd, 1998.

Chiang, S. H. and W. M. Hsieh, 1995. Effect of direct-fed microorganisms on broiler growth performance and litter ammonia level. Asian-Aust. J. Anim. Sci., 8: 159-162.

Grosch, W. and P. Schieberle, 1991. Bread. In Marrse, H. (Ed), Volatile Compounds in Foods and Beverages. Pages: 41-78. Marcel Dekker, Inc., New York, NY.

Isshiki, Y., 1979. Effect of lactobacilli in the diet on the concentration of nitrogenous compounds and minerals in blood of chickens. Japanese Poult. Sci., 16: 254-258.

Jovanovic, K. P. and Milovanovic, 1993. Analysis of volatile compounds in almond and plum kernel oil. J. Am. Oil Chem. Soc., 70: 1102-1104.

Larmond, E., 1977. Laboratory Method for Sensory Evaluation of Food. Canada Department of Agriculture, Ottawa.

Lin, C. F., 1993. Flavor Chemistry of Fish Oil. In Lipids in the Food Flavor. Pages: 208-232. Ho, C.T. and Hartman, T. G. Ed., American Chemistry Society, Washington, D.C.

Merck Index, 1968. An Encyclopedia of Chemicals and Drugs. 8th Ed., Merck & Co., Inc., Pahway, NJ.

Ogunji, P. A., R. N. Brewer, D. A. Roland, Sr. and D. Caldwell, 1983. Effect of dietary sodium chloride, protein and strain difference upon water consumption and fecal moisture content of broiler breeder males chickens. Poult. Sci., 62: 2497-2500.

Overton, S. V. and J. J. Manura, 1998. Analysis of volatile organic in cooking oils by thermal desorption-gas chromatography-mass spectrometry. J. Agri. Food Chem., 43: 1314-1320.

SAS/STAT, 1990. "User's Guide, Release 6.03", SAS Institute Inc., Cary, NC.

Shigeta, Y., 1976. Odor pollution and deodorization techniques. IPC Kogai Boshi Kenkyukai, Tokyo, 386-424.

Sorrels, K. M. and M. L. Speck, 1970. Inhibition of *Salmonella gallinarum* by culture filtrates of *Leuconostoc citrovorum*. J. Dairy Sci., 59: 338-342.

Steel, D. A. and J. H. Torrie, 1980. Principles and Procedures for Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York, NY.

Tramer, J., 1966. Inhibitory effect of *Lactobacillus acidophilus*. Natural, 211: 204-205.

Yang, S. P., 1997. Identification of character-impact aroma compounds in cooked tail meat of brown shrimp (*Penaeus aztecus*). Thesis, Mississippi State University, Mississippi State, MS.

Yasuhara, A., K. Fuwa and M. Jimbu, 1984. Identification of odors compounds in fresh and rotten swine manure. Agri. Biol. Chem., 48: 3001-3010.

Yasuhara, A., 1987. Identification of volatile compounds in poultry manure by gas chromatography-mass spectrometry. J. Chrom., 387: 371-378.

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.