Vet.Med.J.,Giza. Vol.45,No.3.(1997):287-293 .

EFFECT OF SELECTED SUGARS ON SALMONELLA ENTERITIDIS COLONIZATION IN BROILER CHICKS

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SUMMARY

Effect of selected sugars on body weight, mortality and the incidence and level of Salmonella enteritidis in cecal contents of broiler chicks was studied. One hundred forty four, day old broiler chicks were divided into 6 groups each had 2 replicates of 12 chicks. Groups 1 and 2 were provided water only. Groups 3, 4, 5 and 6 were provided in drinking water 5% matose, lactose, sucrose and dextrose, respectively starting on day 1. Groups 2-6 were challenged orally with 10^6 cfu Salmonella enteritidis at day 3 of age. At days 10, 20 and 30 after challenge inoculation, body weight, mortality and cecal contents of 4 chicks in each group were evaluated for the incidence of Salmonella enteritidis. The differences in body weight between groups provided sugars and control groups were significantly higher at ($P<0.05$). Body weight in birds provided lactose was heavier than the birds provided other sugars. There was no significant effect between groups provided sugars and control group in mortality rates. Salmonella enteritidis colonization of the cecal contents was significantly decreased ($P<0.05$) in chicks provided sugars. Lactose was the most effective sugar regarding lowering colonization . Results show that providing sugars in the drinking water of broiler chicks improvs body weight and inhibit cecal colonization of Salmonella enteritidis.

INTRODUCTION

Salmonella enteritidis (Se) in poultry has recently emerged as a major public health concern (Anon, 1994a) . Chickens are widely reservoirs and chicken products have been cited as a source of human infection (Anon, 1994b). The number of reported ourtbreaks of (Se) in chickens increased dramatically in Jordan (Annual report, 1995, Alshawabkeh and Yamani, 1996). The infeciton in poultry may lead to contamination of broiler carcasses during processing and increased when Salmonella are introduced into the processing facility in the intestinal tract and feces contamination (Holmberg and Potter, 1984, Houston, 1987). Reduction of Salmonella colonies in the intestines of broiler chicks may greatly reduce contamination of meat products and carcasses during processing . Recent reports indicated that the addition of carbohydrates inhibited the adherence and prevented colonization of bacteria to intestinal epithelial cells. (Swanson, 1973, Ofek et al., 1975, Jones and Freter, 1976, Oyofo et al., 1989 McHan et al., 1989 McHan et al., 1991). In addition, (Oyofo et

al., 1989) suggested that during the first 10 days of age of broiler chicks, the addition of carbohydrates which are poorly digested by fowls, to the drinking water inhibits colonization of Salmonella typhimurium in the intestines. The purpose of the present study was to determine the selective sugars to be provided in the drinking water to reduce or inhibit intestinal colonization by (Se) in broilers.

MATERIALS AND METHODS

Experimental Chicks: One hundred forty four, one day-old Hubbard Salmonella free broiler chicks were obtained from a commercial hatchery in Jordan, and placed in electrically heated battery brooders and umedicated feed and water were provided *ad libitum*. Before use, the feed and water were cultured for the presence of Salmonella using a standard culture method (Andrew et al., 1978.

Bacteria: Salmonella enteritidis isolate was obtained form Animal Health Institute, Ministry of Agriculture, Amman, Jordan. It was selected for resistance to nalidixic acid at 20 ug/ml and novobiocin at 25 ug/ml to inhibit the growth of other bacteria. Challenge inocula were prepared from selenite F broth cultures incubated at 37°C for 24 hours (Old and Duguid, 1970). The cultures were serially diluted to 10^6 cfu/0.25 ml in sterile distilled water. The viable cell concentration of the challenge inoculum was confirmed by colony counts on brilliant green agar (BGA) plates.

Expermiental protocol:

The chicks were randomly allotted into six equal treatment groups of 12 chicks each, with three replicates. Groups were treated as folows. Group 1, unchallenged control. water only; Group 2 challenged control, water only; Group 3 challenged , maltose in water; Group 4 challenged, lactose in water; Group 5, challenged sucrose in water, Group 6, challenged , dextrose in water, Group 1 and 2 received tap water, and the rest of the groups received 5% (w/v) of their particular sugar in tap water. Chicks in groups 2-6 were challenged per os on day 3 of the experiment with 10^6 cfu of (Se) directly into the crop by syringe. Drinking water containing sugars was prepared and replaced daily.

Reduction or Inhibition test:

At 10, 20 and 30 days after challenge inoculation, 4 chicks in each group were killed by cervical dislocation, and a portion of each chicks cecal contents (0.2g) was collected aseptically. The collected cecal contents were diluted 50-fold in sterile distilled water, and a 0.1ml sample was streaked onto (BGA) plates containing 2 g Surf per 1000 ml media (Reusse and Meyer, 1972). Plates were incubated at 37°C for 24 hours, and resulting Salmonella colonies evaluated for number of colony-forming units according to the method of (Smith and Tucker, 1975). Typical Salmonella colonies were confirmed by biochemical tests on triple sugar iron agar and lysine iron agar and serological tests with somatic (O) antigens (1, 9, 12) and Flagellar (H) antigens phase 1 (gm) and phase 2 (1, 7) (Cooper and

Thoms, 1996). (Se) colony plate counts were expressed as log. Plates were incubated at 37°C for 24 hours, and the resulting Salmonella colonies per gram of cecal contents were calculated (Corrier et al., 1991).

Weight determination

Body weights of chicks in all groups were measured on day 1 and days 10, 20 and 30 after challenge inoculation to ascertain if the addition of sugars to the drinking water had any effect on growth.

Statistical analysis: Data were analyzed by Chi-Square analysis using the SAS program (Luginbake and Schlotzhaver, 1987). Students t-test was used for separation of significantly different means. Chi-square analysis was used for percentage of colonization.

Results and discussions: There were no significant differences between replicates in all groups. Out of 24 broiler chicks per group, (Se) colonized 22 chicks (91.7%) in the maltose groups , 21 chicks (87.5%) in the sucrose group, and 23 chicks (5.8%) in the dextrose group (Table) . The lactose group showed the least colonization in 16 chicks (66.7%). The (Se) challenged control groups showed 100% colonization, and the unchallenged control group showed no colonization. Differences in percentages of colonization were significantly reduced at ($P<0.05$) (Table 1). Sugars are not hydrolyzed or absorbed in the intestinal tract of chickens and as much as 50% of ingested sugars in poultry diets may be excreted unchanged. Because of its slow digestion and absorption, sugars pass into the lower portions of the intestine and ceca and are subsequently available for fermentation (Alkinson et al., 1957, Pjescak, 1970, Morishita et al., 1982, Hume et al., 1992). Decreasing (Se) colonization in the broiler chicks provided sugars to the drinking water was associated with the reduction in cecal pH, which may have resulted form sugars fermentation in the ceca. These data are similar to those reported previously (Oyofo et al., 1989, DeLoach et al., 1990. Corrier et al., 1991, Hume et al., 1992, Tellez et al., 1993, Alshawabkeh, 1996, 1997). There were no significant interactions of selected sugars influencing body weight at 10 days after challenge of broiler chicks that were given sugars and challenged after 3 days of age (Table 1), when compared with broiler chicks unchallenged (control). At 20 days after challenge, birds that received lactose were heavier than the birds receiving maltose, sucrose, dextrose, challenged control and unchallenged control groups. There was no significant effect of groups that received maltose, sucrose, dextrose and unchallenged control but significant from those groups challenged control (Table 1). At 30 days after challenge birds that received lactose and maltose were heavier than the other groups. The differences in body weight were significant at ($p<0.05$) (Table 1). There was no significant effect of groups provided sugars and control group. The differences in mortality rates between the different sugars were not significant (Table 1). This is in contrast to the report of (Ammerman et al., 1980 a, b, 1989), while (Waldroup et al., 1993) reportd that the addition of fructooligosaccharides to nutritionally complete broiler diets at 0.37% had little consistent effect

on growth rate, feed utilization, mortality and incidence or severity of salmonella contamination of processed broiler carcasses. The results of the effect of selected sugars on cecal colonization are shown in Table 2 . Compared with the controls, the mean number of (log10) (Se) recovered per gram of cecal contents decreased in the chicks treated with sugars. At day 10 after challenge, the mean number (log10) of Salmonella enteritidis cells recovered from the cecal contents of control groups was 6.2±0.6 whereas 5.87±0.8, 3.9±1.4, 5.95±0.57 and 5.93±1.2 for maltose, lactose, sucreose and dextrose treated chicks, respectively. At 20 and 30 days after challenge, there was a highly significant (P values given in Table 2) decrease in the number log10. (Se) recovered from cecal contents of treated chicks that received lactose, maltose, sucrose and dextrose. The results support the conclusions of other researchers that addition of sugars increases colonization resistance against variety of Salmonella serotypes (Corrier et al., 1990a, Corrier et al., 1990b, DeLoach et al., 1990, Oyofo et al., 1989, Corrier et al., 1991, Mchan et al., 1991, Alshawabkeh, 1996, 1997). Results of the present study indicate that adding sugars to the drinking water of broiler chicks significantly decreases the number of Salmonella cecal culture positive chicks and cecal colonization by Salmonella enteritidis.

Table 1: Effect of selected sugars on Salmonella enteritidis colonization, body weight and mortality in chicks.

Groups	No. of infected chicks /total	Colonization* %	Body Weight days / g.			Mortality / total	
			10	20	30	No	%
Unchallenged (control)	0 / 24	0.0	142.0 [a]	335 a	557 a	0.00/24 a	0.00
Challenged (control)	24/24	100 [a]	132.5 [a]	306 b	551 b	4.00/24 b	17.0
Challenged (maltose)	22/24	91.7 [a]	147.5 [a]	352 a	613 c	0.00/24 a	0.00
Challenged (lactose)	16/24	66.7 b	132.5 [a]	378 c	626 c	2.0/24 ab	9.00
Challenged (sucrose)	21/24	87.5 [a]	132.5 [a]	334 [a]	566 a	1.00/24 a	4.00
Challenged (dextrose)	23/24	95.8 a	143.9 a	330 a	547 a	1.00/24 a	4.00

*** Number of chicks colonization by Salmonella enteritidis divided by the number of chicks infected x 100.**
Values in column followed by different letters differ significantly at (p<0.05).

Table 2: Effect of selected sugars on Salmonella enteritidis colonization, of ceca of broiler chicks.

Groups	Treatment	Average Log means of S. enteritidis at days post inoculation		
		10	20	30
1	Unchallenged	0.00± 0.0	0.0± 0.0	0.0±0.0
2	Challenged	6.20±0.6 [a]	6.01±0.5 [a]	5.94±0.41 [a]
3	Challenge(Maltose)	5.87±0.8 [a]	5.30±0.6 [b]	4.91±0.32 [b]
4	Challenged(lactose)	3.90±1.4 [b]	3.1±0.35 [c]	2.86±0.43 [c]
5	Challenged(Sucrose)	5.95±0.57 [a]	5.13±0.22 [b]	4.53±0.51 [b]
6	Challenged(Dextrose)	5.93±1.20 [a]	5.01±0.35 [b]	4.41±0.48 [b]

Values the mean ± standard error of the mean of 24 broiler in each treatment Values followed by different lower-case letters are significantly different (P<0.01)

nowledgement:

s research was supported by a grant from the nship of Scientific Research at the University ordan.

FERENCES

nosn, R. L., F. H. Kratzer, and G. F. Stewart (1957): actose in animal and human feeding A review. J. Dairy ci 40: 140-142.

Alshawabkeh, K. (1996): The effect of whey supplementation in feed of broiler chicks challenged with S. gallinarum. Dirasat 23: 8-13.

Alshawabkeh, K. (1996): Effects of dietary lactose and the cecal anaerobic microflora on the colonization of S. enterica serovar gallinarum biovar gallinarum in broiler Chicks. Dirasal 24: 9-15.

Alshawabkeh, K. and M. Yamani (1996): Prevalence of salmonella in poultry farms in jordan. Dirasat. 23: 67-72.

Ammerman, E. C. Quarles, and P. V. Twining Jr. (1980a): Effect of dietary fructooligosaccharides on feed efficiency in floor-pen reared male broilers. Poult. Sci. 1988a 67 (suppl. L): 1. (Abstr).

Ammerman, E. C. Quarles, and P. V. Twining. J. (1980b): Broiler response to the addition of dietary fructooloigosaccharides. Poultry Sci. 1988 b. 67: (suppl. 1): 46. (Abstr)

Ammerman, E., C. Quarles, and P. V. Twining, Jr (1989): Evaluation of fructooligosaccharides on perfomance and carcass yield of male broilers. Poultry Sci, 1989 68 (suppl. 1) : 167. (Abstr.)

Andrew, W. H. O., P. L. Poeline, C. R. Wilson and A. Romero (1978): Isolation and identificantion of Salmonella. In. Bacteriological Analytical Manual, chapter 6,5th ed Association of Official Analytical chemists, Washington, B. C. 1-29.

Annual Reports of Animal Helath Department (1993): Ministry of Agriculture, Amman, Jordan.

Annual Reports of Animal Health Department (1995): Ministry of Agriculture, Amman. Jordan.

Anon, 1(994a): Communicable Disease Reports, Annual Reports of the Public Helath Laboratory Service London.

Anon (1994b): Animal Salmonellosis. Annual Reports of the Ministry of Agriculture, Fisheries and Food, London

Cooper, G. L. and C. L. Thorns (1996): Evaluation of SEF14 Fimbiral Dot Blot and Falgellar Western Blot Tests as Indicators of S. enteritidis Infection in Chickens The Vet. Rec. 17: 149-153.

Corrier, D. E. Hinton, Jr., R. L. Ziprin, R. C. Beier, and J. R. DeLoach, (1990a): Effect of Dietary lactose on cecal pH. Bacteriostatic Volatile Fatty Acid. And S. typhimurium colonization of Broiler Chicks. Avian Dis 34: 617-625.

Corrier, D. E., A. Hinton, Jr., R. L. Ziprin and J. R. DeLoach (1990b): Effect of dietary lactose on S. colonization of market age broilers. Avian Dis, 34 668--676.

Corrier, D. B. Hargis, A. Hinton, Jr., D. Lind Caldwell, J. Manning and J. DeLoach (1991): E anaerobic cecal microflora and dietary lact colonization resistance of layer chicks toi Salmonella enteritidis. Avian Dis, 35: 337-343

DeLoach, J. R., B. A. Oyofo, D. E. Corrier, L. F. K R. L. Ziprin and J. O. Norman. (1990): Reductio typhimurinum concentration in broiler chicks by n whey. Avian Dis, 34: 389-392.

Holmberg. S. C., and M. E. Potter (1984): Current sta human salmonellosis prospects for control. Proc. Annual Meeting of the U. S. Animal Helath Associ 513-519.

Houston, D. L. (1987): USDA and Salmonella Control 91st. Annual Meeting of the U. S. Animal He Association, 445-450.

Hume, M. E., L. F. Kubena, R. C. Beier, A. Hinton, JR. E. Corrier, and J. R. DeLoack, (1992): Fermentatio lactose in broiler chicks by cecal anaerobes. Poult. 71: 1464-1470.

Jones, G. W., and R. Freter, (1976): Adhesive properties Vibro cholera. Nature of the interaction with isolat rabbit bursh border membranes and human erythrocyt Infect. Immune 14: 240-245.

Luginbake , R. C., and S. D. Schlotzhaver, SAS/STA (1987): Guide for Personal Computers, 6th Ed. SA Insitiute Gray, N. C. 555-573.

McHan, F, E. B. Shotts and J. Brown (1991): Effect feeding selected carbohydrates on the in vivo attachme of Salmonella typhimurium in chick ceca Avian Dis. 35 328- 331.

McHan F, N. A. Cox, L. C. Blankenship, and J. S. Bailery (1989): In vitro attachment of Salmonella typhimurium to the ceca exposed to selected carbohydrates. Avian Dis 33: 340-344.

Morishita, Y. R. Fuller, and M.. E. Coates (1982): Influence of dietary lactose on the gut flora of chicks. Br. Poult. Sci 23: 349-359.

Nisbet, D. J., D. E. Corrier, C. M. Scanlan, A. G. Hollister, R. C., Beier and J. R.DeLoach (1994): Effect of dietary lactose and cell concentration on the ability of a continuous-flow-derived bacterial culture to control Salmonella cecal colonization in broiler chickens. Poult. Sci 73: 56-62.

Ofek, I., E. H. Beachey, W. Jefferson and G. L. Campbell (1975): Cell membrane-binding properties of Group A stroptococcal lipoteichoic acid J. Exp. Med. 141: 990-1003.

Old, D. C., and J. P. Duguid (1970): Selective outgrowth of fimbriate bacteria in static liquid medium. J. Bacteriol 103: 447-456.

Oyofo, B. A. J. R. DeLoach, D. E. Corrier, J. O. Norman, R. L. Ziprin and H. H. Molenhauer, (1989): Effect of carbohydrates on Salmonella typhimurium colonization in broiler chickens . Avian Dis 33: 531-534.

Pjescak, M. (1970): On biological effects of lactose in layers. Acta Zootech 21: 122-130.

Reusse, U. U. and A. Meyer (1972): Der "pril-Mannit-Agar" in der Salmonella-Diagnostik, Zbl. Bakt. 1. Abt. Orig, 219 555-557.

Smith, H. W., and J. F. Tucker (1975): The effect of antiblotic therapy on the fecal excretion of S. typhimurium by experimentaly infected chickens J. Hyg. 75 275-292.

Swanson, J. (1975): Studies on Gonococcus infection IV. Pili: Their role in attachment of Gonococci to tissue culture cells J. Exp Med. 137: 571-589.

Tellez, G. C. E. Dean, D. E. Corrier, J. R. DeLoach, L. Jaeger and B. M. Hargis (1993): Effect of dietary lactose on cecal morphology, pH, organic acid and S. enteritidis organ invasion in Leghorn chicks Poult Sci. 72: 636-642.

Waldroup, A. L., J. T. Skinner, R. E. Hierholzer, and P. W. Waldroup (1993): An evaluation of fructooligosacchiaride in diets for broiler chickens and effects on Salmonella contamination of carcasses. Poultry Sci. 72: 643-650.

Zibrin, R. L. D. E. Corrier, A. Hinton, Jr., R. C. Beier, G. E. Spates, J. R. DeLoach, and M. H. Elissalde (1990): Intracloacal S. typhimurium infection of broiler chickens Reduction of colonization with anaerobic organisms and dietary lactose Avian Dis, 34: 749-753.