Using Dietary Propionic Acid to Limit *Salmonella gallinarum* Colonization in Broiler Chicks

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ABSTRACT : This experiment was conducted to study the effect of dietary propionic acid on inhibitory effect of *Salmonella gallinarum*. Day-old 144 broiler chicks were randomly distributed into four dietary treatments, three replicates per treatment. The four dietary treatments consisted of 0.0, 0.6, 1.2, and 1.8% dietary propionic acid. Chicks were challenged orally on three days of age with 10^6 cfu *Salmonella gallinarum*. Sample of crop and cecal contents were obtained on Days 1, 8 and 15 post-inoculation. The pH of crop contents decreased significantly (p<0.01) for groups of chicks provided 1.2 and 1.8% propionic acid in the diet as compared with those provided 0.6% propionic acid and control group. The pH of cecal contents decreased significantly (p<0.01) for groups of chicks provided with 0.6, 1.2 and 1.8% of propionic acid as compared with control group. The number of *Salmonella gallinarum* positive culture in the crop and ceca of chicks, decreased significantly (p<0.01) from the groups provided with 0.6, 1.2 and 1.8% of propionic acid in the diet at day 1, 8 and 15 post-inoculation. The addition of 1.8% propionic acid in the diet provided a high level of protection against colonization than did 0.6 and 1.2%. These results indicate that the addition of propionic acid in a concentration of 1.2 or more to the diet of newly hatched chicks significantly decreases crop and cecal colonization by *Salmonella gallinarum*. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 2 : 243-246)***

Key Words : *Salmonella gallinarum*, Broiler Chicks, Propionic Acid, Inoculation, Diet Additive.

INTRODUCTION

Various investigators exposed the feasibility of altering diet as a mean of controlling Salmonella in chickens (Alshawabkeh, 1996; 1997; Cherrington et al., 1990; Corrier et al., 1990; Hinton et al., 1985; Oliveira et al., 2000; Thompson and Hinton, 1997). Later works suggested that short chain fatty acids produced from carbohydrates affect Salmonella colonization (Alshawabkeh, 1996; McHan and Shotts, 1992; Oyofo et al., 1989; Toeless et al., 1993). Propionic acid has been used as a diet additive reportedly to reduce pathogenic bacterial and fungal contamination (Hinton and Linton, 1988; Michael et al., 1993). The anti-salmonellae effects of propionic and other organic acids, such as formic, acetic and lactic acids, were thought to be the result of diffusion of the undissociated organic acids into the bacterial cell and the reduction of intracellular pH (Cherrington et al., 1991; Hinton, 1990). Decreasing pH of the intestine enhances resistance to Salmonella colonization (Alshawabkeh, 1996; Corrier et al., 1992; Toeless et al., 1993). Crop and ceca have been regarded as the principal sites of Salmonella colonization (Brownell et al., 1970). The shedding of *Salmonella enteritidis* into dropping fosters horizontal transmission to other chicks (Turnbull et al., 1974), so that some reduction in the number of *Salmonella enteritidis* cells shed in feces should help control the spread of infection among farms. However, no such studies were conducted using *Salmonella gallinarum*. Therefore, the major objective of this study was to determine the possible antibacterial influence of propionic acid added to the chicken diet on crop and cecal pH to reduce the colonization of *Salmonella gallinarum* in these organs in Jordan.

MATERIAL AND METHODS

Chickens

One hundred forty four, one-day-old Hubbard broiler chicks, Salmonella free, were obtained from a commercial hatchery in Jordan. Chicks were floor-reared and fed a commercial diet, with constant lighting at a mean temperature of 32°C with supplemental heat supply and relative humidity of 45%. Diet and water were cultured for the presence of Salmonella using a standard culture method (Anderw et al., 1978).

Salmonella

Primary poultry isolate of *Salmonella gallinarum* was obtained from the Animal Health Institute, Ministry of Agriculture, Amman, Jordan. This isolate was selected for resistance to novobiocin and nalidixic acid and maintained on nutrient agar. Media used to culture the resistant isolate contained 25 μg /ml novobiocin and 20 μg/ml nalidixic acid, to inhibit the growth of other bacteria. Challenge inoculate was prepared from 24 h selenite F. broth culture incubated at 42°C and serially diluted to 10^6 cfu/0.25 ml in sterile distilled water. The viable cell count of the challenged

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inoculum was confirmed by colony counts on brilliant green agar (BGA) plates.

Experimental protocol

Day-old 144 broiler chicks were randomly allotted to four dietary treatments, three replicates per treatment. Four dietary treatments consisted of 0.0, 0.6, 1.2 and 1.8% dietary propionic acid.

Chicks in all groups were challenged per os on day 3 of the experiment with 10^6 cfu of *Salmonella gallinarum* directly deposited into their crops by a syringe (Barrow et al., 1994). Diet containing propionic acids were prepared and replaced daily for all groups. The required amount of propionic acid was dissolved in 100 ml sterilized distilled water and mixed with 2 kg of diet. These 2 kg were mixed with the total amount of diet. At day 1, 8 and 15 post-inoculation, 4 chicks from each group were killed by cervical dislocation. Sample of 0.2 g crop and cecal contents were collected aseptically. These samples were diluted into 1:10, 1:100, 1:1000 and 1:10000 in sterile distilled water and 0.1 ml sample was streaked on BGA plates containing 2 g Surf per 1000 ml media (Reusse and Meyer, 1972). Plates were incubated at 37°C for 24 h. Salmonella colonies were evaluated from the number of cfu according to Smith and Tucker (1975).

To identify chicks that were positive for Salmonella in crop and cecal culture but not detected at 1:100 plate dilution, one gram samples of cecal and crop contents from each chick were placed separately in a plastic Stomacher bag containing 10 ml of sterile distilled water. Each bag was blended for 30 sec and 90 ml of selenite F. broth was added and incubated for 24 h at 43°C. After incubation, the broth was streaked on BGA plates, incubated for 24 h at 37°C and examined for the presence of typical *Salmonella gallinarum* colonies. Cecal and crop contents that were negative for *Salmonella gallinarum* culture at 1:100 dilution on BGA plates and after selenite F. enrichment and BGA plating were scored as 0 cfu. Cecal and crop contents that were culture negative at 1:100 dilution on BGA plates but positive after selenite F. enrichment and BGA plating were arbitrarily assigned 1.50 Log_{10} Salmonella per g of cecal and crop contents (Nisbet et al., 1993). Typical *Salmonella gallinarum* colonies were confirmed by biochemical tests on triple sugar iron agar and lysine iron agar and serological tests with somatic 0 antiserum 1, 9, 12.

Determination of crop and cecal pH

Crop pH was measured *in situ* by inserting on electrode into an incision in the crop before removing its contents. The pH of ceca were measured by collection of 0.2 g of cecal contents from each chick, suspended in 1.8 ml of sterile glass-distilled water and pH was measured immediately with glass electrode (Nisbet et al., 1994).

Statistical analysis

Data were analyzed by the analysis of variance using the Statistical Analysis System (SAS) for colonization percentage (Luginbake and Schlotzhaver, 1989). For treatments that were pronounced significant, Student's t-test was utilized for separation between means.

RESULTS

Effect of dietary propionic acid on crop pH

Crop contents were examined for pH at day 1, 8 and 15 post-inoculation with *Salmonella gallinarum*. There were no significant differences detected between crop pH in chicks given dietary 0.6% propionic acid and control chicks. However, pH of crop contents decreased significantly ($p<0.01$) for groups of chicks provided 1.2 and 1.8% propionic acid in the diet as compared with those provided 0.0 and 0.6% propionic acid (table 1).

Effect of dietary propionic acid on cecal pH

The pH of the cecal contents decreased significantly ($p<0.01$) for groups of chicks provided with 0.6, 1.2 and 1.8% of propionic acid in diet as compared with the control group. However, there was no significant difference in groups provided 1.2 and 1.8% propionic acid in the diet after post-inoculation (table 2).

Crop and cecal colonization by *Salmonella gallinarum*

Compared with control group, the number of

Table 1. Effect of providing propionic acid in diet on crop pH of broiler chicks challenged with 10^6 cfu *Salmonella gallinarum* at day 1, 8 and 15 post-inoculation

Propionic acid %	Days post-inoculation		
	1	8	15
0.0	5.35 ± 0.22^a	5.31 ± 0.29^a	5.33 ± 0.18^a
0.6	5.00 ± 0.29^a	5.19 ± 0.11^a	5.12 ± 0.24^a
1.2	4.62 ± 0.34^b	4.83 ± 0.53^b	4.73 ± 0.17^b
1.8	4.49 ± 0.16^b	4.51 ± 0.33^b	4.59 ± 0.34^b

[a,b] Means in the same column with different superscripts differs significantly ($p<0.05$).

Table 2. Effect of providing propionic acid in diet on cecal pH of broiler chicks challenged with 10^6 cfu *Salmonella gallinarum* at day 1, 8 and 15 post-inoculation

Propionic acid %	Days post-inoculation		
	1	8	15
0.0	5.72 ± 0.30^a	5.84 ± 0.16^a	4.90 ± 0.14^a
0.6	5.18 ± 0.20^b	5.18 ± 0.80^b	4.67 ± 0.61^a
1.2	4.67 ± 0.50^c	4.68 ± 0.26^c	3.97 ± 0.23^b
1.8	4.51 ± 0.17^c	4.60 ± 0.52^c	3.89 ± 0.29^b

[a,b,c] Means in the same column with different superscript differs significantly ($p<0.05$).

Salmonella gallinarum positive culture in the crop and cecal of chicks decreased significantly ($p<0.01$) from the groups provided with 0.6, 1.2 and 1.8% of propionic acid in the diet at day 1, 8 and 15 post-inoculation (tables 3 and 4).

DISCUSSION

There was a reduction in the pH of the crop contents as the acid concentration in the diet increased and the differences between mean values were statistically significant. Hume et al. (1995) reported that crop pH was already sufficiently low at 5 to cause undissociated propionic acid to increase to a bactericidal level when enough total propionic acid is present. The significant reduction in the number of *Salmonella enteritidis* in the crop and ceca by adding propionic acid in the diet of broiler chicks indicates a strong potential for the use of propionic acid in the diet to reduce infection of *Salmonella enteritidis* in poultry flocks. Matlho et al. (1997) and Vanderwal (1995) already showed that decontamination of diet by acidification reduced carcass contamination during slaughter and also improved weight gain and diet conversion. However, the propionic acid concentration incorporated into the diet should be as low as possible for economic reasons and to avoid detrimental effects on meat quality and palatability.

In this study, addition of propionic acid to the diet of experimentally infected chickens with *Salmonella gallinarum*, gradually inhibited its growth. This means it is preferable to use such chemicals in the diet as reported by Iba and Berchier (1995), who could not isolate Salmonella from any sampling of birds fed diet containing 0.5% or 0.75% of formic acid-propionic acid mixture (Bio-Add^{TM}). However, other investigators reported that the decrease of Salmonellae in crop and cecal contents as compared to the control were the result of the presence in the crop and ceca of bacteria harmful to Salmonella (Narmi and Rantala, 1973), and less likely the result of conditions created by dietary propionic acid. Conditions in the crop and ceca that produce a pH of about 5 much initial lactic acid concentration and high total propionic acid concentration and high levels of undissociated propionic acid have been reported to reduce the number of Salmonella (Hinton et al., 1990, 1991). When dietary propionic acid was increased to 1.2 or 1.8% of the diet, *Salmonella typhimurium* in the crop and ceca was reported to be significantly lower (McHan and Shotts, 1992). This suggests that a formic-propionic acid mixture is active in the alimentary tract (Hinton and Linton, 1988; Iba and Berchier, 1995; Oliveira et al., 2000; Thompson and Hinton, 1997), particularly in the crop, before degeneration or absorption in the intestine occurs. Although the pH of the crop of the adult chickens is fairly low; this is not the case in newly hatched chicks (Smith, 1965). It seems likely, therefore, that undissociated acid, lactate ions and protons would be present in crop. Which of these is more important in the antibacterial effect is still unclear (Cherrington et al., 1990). The results here suggest that the propionic acid is likely to be antibacterial effect on *Salmonella gallinarum* both in the crop as well as the ceca. The addition of 1.8% propionic acid level in the diet provided a high level of protection against colonization than did 0.6 and 1.2% propionic acid levels in the diet.

The results of this study indicate that the addition of propionic acid in a concentration of 1.2 or more to the diet of newly hatched chicks significantly decreases crop and cecal colonization by *Salmonella gallinarum* in countries with conditions like Jordan.

Table 3. Average $\log_{10}$ means of *Salmonella gallinarum* recovered from crop at day 1, 8 and 15 post-inoculation

Propionic acid %	Days post-inoculation		
	1	8	15
0.0	6.32±0.41^{a}	6.22±0.35^{a}	6.15±0.18^{a}
0.6	5.43±0.29^{b}	5.12±0.24^{b}	4.42±0.35^{b}
1.2	5.21±0.39^{b}	4.99±0.49^{b}	4.39±0.10^{b}
1.8	4.53±0.19^{c}	4.23±0.41^{c}	4.00±0.28^{c}

[a,b,c] Mean in the same column with different superscript differs significantly ($p<0.05$).

Table 4. Average $\log_{10}$ means of *Salmonella gallinarum* recovered on cecal culture at day 1, 8 and 15 post-inoculation

Propionic acid %	Days post-inoculation		
	1	8	15
0.0	5.13±1.27^{a}	5.13±1.17^{a}	5.07±1.11^{a}
0.6	4.02±1.31^{b}	4.00±1.01^{b}	3.84±1.23^{b}
1.2	3.91±1.15^{b}	3.83±1.02^{b}	3.35±1.13^{c}
1.8	3.61±1.44^{c}	3.29±1.03^{c}	3.23±1.46^{c}

[a,b,c] Mean in the same column with different superscripts differ significantly ($p<0.05$).

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