Using Varying Levels of Formic Acid to Limit Growth of *Salmonella gallinarum* in Contaminated Broiler Feed

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ABSTRACT : Reported here are the effects of added formic acid on inhibitory effect of *Salmonella gallinarum* in poultry feed. Two experiments were conducted to investigate the viability of *S. gallinarum* and pH of poultry feed using different dietary formic acid levels (0.0, 0.5, 1.0 and 1.5%) on inhibitory effect of *S. gallinarum* in broiler feed. Experiment one was conducted to investigate the viability of *S. gallinarum* and pH of artificially contaminated diet at 0, 1, 3, 5 and 7 days after treatment *in vitro*. Formic acid showed a significant ($p<0.05$) reduction in the viability for all treatments with time after treatment. Various formic acid levels *in vitro* showed a reduction in the pH of the diet depending upon the concentration of treated acid, and the diet remained acidic below the growth range of *S. gallinarum*. This meant that the bacterial cells were exposed to stressful conditions that made them unable to grow. Experiment two was conducted to find out the effect of dietary formic acid levels on *S. gallinarum* colonization and pH in the contents of crop, small intestine, large intestine and ceca and mortality rate of broiler chicks at 7, 14 and 21 days of age when fed artificially contaminated diet with *S. gallinarum*. The numbers of *S. gallinarum* re-isolated from all treated groups except in groups treated with 0.5% formic acid, decreased significantly ($p<0.05$) compared with the control group. The treatment significantly ($p<0.05$) lowered the pH of the crop, small intestine, large intestine and ceca contents in all groups except the groups treated with 0.5% formic acid compared with the control. All treated groups showed a significant ($p<0.05$) reduction in overall mortality rate during the experimental period (3 to 21 days) compared with the control. The results indicate that addition of formic acid in a total concentration of 1.5% to the diet of newly hatched broiler chicks significantly decreases the contamination of diet with *S. gallinarum*. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 390-395)***

Key Words : Salmonella Gallinarum, Broiler Feed, Formic Acid, Contamination, Feed Additive

INTRODUCTION

S. gallinarum is one of the major pathogens of concern to the industry in the early days of poultry production intensification (Barrow, 1993). Contaminated feed is the major source of Salmonella infections in poultry.

Domestic animals have been considered the largest reservoir of Salmonella organisms, and Salmonella contaminated feed has been implicated as a major contributor in maintaining the reservoir.

In a previous report, Westerfeld et al. (1970) suggested that a chemical feed additive may have reduced Salmonella initially present without eliminating them from contaminated feed. *In vitro* sensitivity tests indicated that 0.1% concentration of the additive was effective in inhibiting the growth of Salmonella in concentrations up to 10^7 cells/ml. Williams (1981) considered feed as an important source for transmission of Salmonella to poultry. Presence of Salmonellae in small numbers in feed is undesirable and in many countries renders feed unfit for poultry consumption. Accordingly, different methods for treatment of such feed against Salmonellae were suggested.

Incorporation of formic acid, as well as other organic acids, had a disinfecting effect on contaminated feed and its sufficiently antibacterial effect in the alimentary tract was useful for this purpose (Iba and Junior, 1995). The anti-Salmonella effects of this acid, were thought to be the result of diffusion of undissociated acid into the bacterial cell and the reduction of intracellular pH (Cherrington et al., 1991). The optimum level of formic acid to prevent food borne salmonellosis in broiler chicks was not determined.

This study aimed to determine the effect of adding different levels of formic acid on artificially contaminated feed with *S. gallinarum in vitro*, and to determine the possible antibacterial effect of feeding formic acid on crop, ceca and intestinal pH contents, to reduce the colonization of *S. gallinarum*, and its effect on mortality rates of broiler chicks which are reared on feed that was artificially contaminated with *S. gallinarum*.

MATERIALS AND METHODS

Experiment one

This experiment was conducted to investigate the viability of *S. gallinarum* and pH of artificially-contaminated diet treated with different levels of formic acid at 0, 1, 3, 5 and 7 days after treatment *in vitro*. The

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treated diet was kept in sterile plastic bags of 1 kg at room temperature and contained *S. gallinarum* 1.36×10^6 cfu/kg diet. Five treatments were randomly assigned to five groups with three replicates.

Experiment two

This experiment was conducted to find out the effect of dietary formic acid on *S. gallinarum* colonization and pH in the contents of the crop, small intestine, large intestine and ceca, and mortality rate of broiler chicks at 7, 14 and 21 days of age when fed artificially-contaminated diet with S. *gallinarum* 1.36×10^6 cfu/kg diet at day 3 of age. The diets and the chicks were checked for the absence of Salmonella (Linton et al., 1985).

One hundred and eighty day-old Hubbard broiler chicks were purchased from a commercial hatchery and were randomly assigned to five treatments with three replicates each with 12 chicks. Wood shavings were used as bedding. The treated diet and water were freely available during the study period. Chicks were fed basal diet for the first three days of age and the treated diet was introduced on day 4 of age. The feeders and drinkers were cleaned routinely to avoid reinfection.

The treatments of both experiments were : Basal diet without *S. gallinarum* challenge (negative controls).

S. gallinarum challenge with no formic acid (control).
S. gallinarum challenge with 0.5% formic acid.
S. gallinarum challenge with 1.0% formic acid.
S. gallinarum challenge with 1.5% formic acid.

Incorporation of formic acid into the diet

Formic acid (85%) was obtained from Gainland Chemical Co., Sandycroft, Germany and was incorporated into the diet as percent (volume/weight) (Hinton and Linton, 1988) at the required rate (0.5, 1.0 and 1.5%). The total liquid volume of formic acid and sterile distilled water was 50 ml per kg diet. The diets in both experiments, was prepared by hand and mixed to ensure a complete homogenization of formic acid into the diet.

Bacterial strain

A primary isolate of *S. gallinarum* was obtained from Animal Health Institute, Ministry of Agriculture, Amman, Jordan.

Artificial contamination of diets

The method of contamination in experiment 1 was as described by Iba and Junior (1995). One ml of nutrient broth containing *S. gallinarum* (1.36×10^6 cfu/ml) was mixed into 1 kg diet by hand. The bags were inverted continuously to ensure complete distribution. Formic acid was then added shortly after contamination. In experiment 2, the diet was contaminated by a method described by Hinton (1986) in which an over night nutrient broth culture containing *S. gallinarum* 1.36×10^6 cfu/ml as percent 1/5,000 (volume/weight) was mixed by hand slowly and thoroughly until complete homogenization. Formic acid was incorporated before contamination. The overall liquid volume of nutrient broth and sterile distilled water was 50 ml/kg diet. An equal volume of sterile distilled water was added to the diet that did not receive formic acid or *S. gallinarum*.

Sampling

In experiment 1, three-10 g samples from 3 sites of each bag was collected to be cultured (Williams, 1981). In experiment 2, chicks were randomly selected and were sacrificed by neck dislocation. Three 1 g samples from crop, small intestine, large intestine and cecum were pooled together and considered as one sample of 3 g per section per group (Izat et al., 1990a).

The samples for the pH measurement was prepared in the same manner as discussed above for culture process, but the weight was 0.2 g (for the three sites and for the three chicks) and pooled together (0.6 g) and considered as one sample (Corrier et al., 1990).

Isolation and enumeration of *S. gallinarum*

Each sample was pre enriched in buffered peptone water at 37°C for 24 h. This culture was then transferred to selenite broth at 42°C for 24 h. Subcultures were then made to SS agar at 37°C for 24 h; negative plates were re-incubated for an additional 24 h. Colonies typical for *S. gallinarum* were counted and confirmed biochemically by inoculating in triple sugar iron at 37°C for 24 h (Andrew et al., 1984). Results (average cfu duplicate of each sample) were logarithmically ($\log_{10}$) - transformed.

Measurements of the pH

The pH determination was performed after culture process using the method described by Corrier et al. (1990). Each sample (0.6 g) was suspended in 2.4 ml sterile distilled water. The suspension was shaken vigorously and the pH was determined by pH-meter.

Statistical analysis

The logarithmically-transformed counts and pH values were subjected to Analysis of Variance (ANOVA), where a significant F-statistic was indicated by ANOVA, and a Student's t-test was used for separation of significantly different means, with probability level ($p<0.05$), using the General linear Model (GLM) procedures of Statistical Analysis System (SAS, 1994).

Table 1. Means of viable numbers of *S. gallinarum* (log_{10} cfu/g) in the different levels of formic acid-treated-artificially-contaminated diet with *S. gallinarum* at intervals after contamination

Days	Treatment			
	Control	0.5% FA	1.0% FA	1.5% FA[1]
0	5.32_{a}^{v}	5.05_{b}^{v}	4.34_{c}^{v}	4.22_{d}^{v}
1	5.23_{a}^{v}	4.78_{b}^{w}	4.13_{c}^{w}	3.82_{d}^{w}
3	4.79_{a}^{w}	4.06_{b}^{x}	3.18_{c}^{x}	2.83_{d}^{x}
5	4.33_{a}^{x}	3.74_{b}^{y}	2.85_{c}^{y}	2.33_{d}^{y}
7	4.23_{a}^{x}	3.14_{b}^{z}	2.82_{c}^{y}	1.65_{d}^{z}

Standard error of log_{10} *Salmonella gallinarum*=0.04.

FA[1]: formic acid

[a, b, c, d] Means with different subscripts in the same row are significantly different ($p<0.05$).

[v, w, x, y, z] Means with different superscripts in the same column are significantly different ($p<0.05$).

Table 2. Means of the pH of the different levels of formic acid-treated-artificially-contaminated diet with *S. gallinarum* at intervals after contamination

Day	Treatment				
	Negative control	Control	0.5% FA	1.0% FA	1.5% FA[1]
0	6.17_{a}	6.21_{a}	5.84_{b}	5.36_{c}^{w}	4.81_{d}^{x}
1	6.20_{a}	6.20_{a}	5.83_{b}	5.34_{c}^{w}	4.90_{d}^{wx}
3	6.19_{a}	6.19_{a}	5.85_{b}	5.46_{c}^{v}	4.96_{d}^{w}
5	6.20_{a}	6.21_{a}	5.89_{b}	5.52_{c}^{v}	5.11_{d}^{v}
7	6.18_{a}	6.19_{a}	5.90_{b}	5.53_{c}^{v}	5.09_{d}^{v}

Standard error (pH)=0.03. FA[1]: formic acid.

[a, b, c, d] Means with different subscripts in the same row are significantly different ($p<0.05$).

[v, w, x] Means with different superscripts in the same column are significantly different ($p<0.05$).

RESULTS AND DISCUSSION

Experiment one

Viable numbers of S. gallinarum in the diet : means of viable numbers of *S. gallinarum* in the diets receiving different treatments are presented in Table 1. There was a significant ($p<0.05$) reduction in log_{10} cfu *S. gallinarum* of the diet of all treatments compared with control group. The results also showed a significant ($p<0.05$) reductions in the viability of added Salmonella for all treatments with time after treatment. The result indicates that increased concentrations of formic acid reduces the number of viable *S. gallinarum* and the loss in viability can be explained by the designation of the bacterial cells, as well as the effect of formic acid (Iba and Junior, 1995). This result illustrated the high antibacterial activity of formic acid at the various levels of formic acid at various times after treatment.

pH of the diet : means of the pH values of the diets receiving different treatments are shown in Table 2. There were significant ($p<0.05$) reductions in the pH of the diet of all treatments compared with control group. There was no significant ($p<0.05$) difference between positive and negative controls. There was a significant ($p<0.05$) increase in the pH of the diet at 3 days after treatment of all treated groups. This can be explained by the volatility of formic acid: however. the pH of the diet remained acidic throughout the experimental period.

These results showed that the pH of the diet was decreased depending on the concentration of added acid. Smyser and Snoeyenbos (1979) found that the pH of meat and bone meal was shifted to acidity by the addition of 0.1% formalin and remained acidic for the trial period of 16 days. These results. also. illustrated that the pH values were below the growth range of *S. gallinarum*. (6.5-7.5) which means that the bacterial cells were exposed to stressful conditions that made them unable to either replicate or cope with acidic conditions (Nassar et al.. 1994: Thompson and Hinton, 1996,1997).

Table 3. Means of the colonization numbers of *S. gallinarum* (log_{10} cfu/g) on days 7, 14 and 21 in some alimentary canal contents of broiler chicks fed different levels of formic acid-treated-artificially-contaminated diets

Organ	Day	Treatment			
		Control	0.5% FA	1.0% FA	1.5% FA[1]
Crop	7	5.25_{a}^{v}	4.37_{b}^{v}	4.21_{c}^{v}	4.14_{c}^{v}
	14	5.19_{a}^{v}	3.36_{b}^{w}	3.42_{b}^{w}	3.16_{c}^{w}
	21	4.42_{a}^{w}	3.15_{b}^{x}	3.12_{b}^{x}	2.94_{c}^{x}
Small intestine	7	5.36_{a}^{v}	4.40_{b}^{v}	4.14_{c}^{v}	4.21_{c}^{v}
	14	4.34_{a}^{w}	4.10_{b}^{w}	3.40_{c}^{w}	3.21_{d}^{w}
	21	4.29_{a}^{w}	4.39_{a}^{v}	3.07_{b}^{x}	2.96_{c}^{x}
Large intestine	7	5.36_{a}^{v}	4.40_{b}^{v}	4.14_{c}^{v}	4.21_{c}^{v}
	14	4.34_{a}^{w}	4.10_{b}^{w}	3.40_{c}^{w}	3.21_{d}^{w}
	21	4.29_{a}^{w}	4.39_{a}^{v}	3.07_{b}^{x}	2.96_{c}^{x}
Ceca	7	5.73_{a}^{v}	4.35_{b}	4.11_{b}^{y}	3.42_{c}^{y}
	14	5.24_{a}^{w}	4.20_{b}	3.41_{c}^{w}	3.26_{c}^{v}
	21	4.44_{a}^{x}	4.26_{a}	3.16_{b}^{x}	2.89_{b}^{w}

Standard error : Crop log_{10} *S. gallinarum*=0.03; Small intestine log_{10} *S. gallinarum*=0.04; Large intestine log_{10} *S. gallinarum*=0.03: (Ceacum log_{10} *S. gallinarum*)=0.10.

FA[1]: formic acid.

[a, b, c, d] Means with different subscripts in the same row are significantly different ($p<0.05$).

[v, w, x] Means with different superscripts in the same column are significantly different ($p<0.05$).

Experiment two

The colonization numbers of S. gallinarum in some alimentary canal contents :

i) Crop : results shown in Table 3 indicated that there were significant ($p<0.05$) reductions in log_{10} cfu *S. gallinarum* from crops of all treatments compared with control group at day 7, 14 and 21.

The results of this experiment showed the antibacterial activity of formic acid in a concentration-dependent manner. These results were in agreement with those described by Thompson and Hinton (1997) who found that the Bio-Add™ at 6.8 and 12 g/kg diet reduced the number of S. *enteritidis* from the crops of hens by a factor of 10. and also with those of Hinton and Linton (1988) who showed high bactericidal activity of formic acid at 0.66% in the crop.

Table 4. Means of the pH on days 7, 14 and 21 of some alimentary canal contents of broiler chicks fed different levels of formic acid-treated-artificially-contaminated diet with *S. gallinarum*

Organ	Days	Treatment				
		Negative control	Control	0.5% FA	1.0% FA	1.5% FA[1]
Crop	7	4.58_{a}^{v}	4.52_{a}	4.30_{b}^{w}	4.12_{c}^{w}	4.07_{c}^{w}
	14	4.51_{a}^{w}	4.49_{a}	4.33_{b}^{vw}	4.35_{b}^{v}	4.19_{c}^{v}
	21	4.58_{a}^{v}	4.52_{a}	4.38_{b}^{v}	4.33_{b}^{v}	4.22_{c}^{v}
Small	7	6.30_{a}	6.25_{a}	6.11_{b}^{w}	6.00_{c}^{w}	6.03_{c}^{w}
intestine	14	6.30_{a}	6.26_{a}	6.26_{a}^{v}	6.17_{b}^{v}	6.13_{b}^{v}
	21	6.33_{a}	6.19_{a}	6.19_{b}^{vw}	6.10_{c}^{v}	6.07_{c}^{vw}
Large	7	6.21_{a}	6.23_{a}^{v}	6.15_{b}^{v}	6.17_{ab}^{v}	6.07_{c}
intestine	14	6.16_{a}	6.14_{a}^{w}	6.06_{b}^{w}	6.05_{b}^{w}	6.06_{b}
	21	6.16_{a}	6.15_{a}^{w}	6.05_{b}^{w}	6.05_{b}^{w}	6.03_{b}
Ceca	7	5.78_{ab}	5.82_{a}	5.71_{bc}	5.67_{cd}	5.60_{d}
	14	5.81_{a}	5.76_{a}	5.76_{ab}	5.68_{b}	5.66_{b}
	21	5.77_{a}	5.76_{a}	5.75_{a}	5.68_{ab}	5.63_{b}

Standard error : Crop pH=0.02; Small intestine pH=0.03: Large intestine pH=0.03: Ceacum pH=0.04. FA[1]: formic acid.

[a, b, c, d] Means with different subscripts in the same row are significantly different ($p<0.05$).

[v, w] Means with different superscripts in the same column are significantly different ($p<0.05$).

which inhibited the bacterial cells growth and reproduction after one week. Alshawabkeh and Tabbaa (2002) found that the number of *S. gallinarum* positive culture in crop of chicks, decreased significantly ($p<0.05$) in the groups provided 0.6, 1.2 and 1.8% of propionic acid in the diet at the days 1, 8 and 15 post inoculation.

ii) Small intestine : means of the colonization numbers of *S. galliarum* in the small intestinal contents of broiler chicks receiving different treatments are presented in Table 3.

These results paralleled that of earlier work by Izat et al. (1990b) who found that a buffered propionic acid at 0.8% reduced the number of *S. typhimurium* ($\log_{10}$ cfu/g) from 5.61 to 5.46, and were in agreement with Rouse et al. (1988) who found that the propionic acid-treated feed at 0.5% resulted in a Salmonella- free small intestine in chickens after 7 days. The results illustrated that formic acid reduces the pH in the digestive tract, since the acid molecules inhibited the growth of Salmonella, but did not eliminate them from the intestinal tract at day 21 of age. These results also agree with those of McHan and Shotts (1992) who, found that the effect was higher as the level of formic acid was increased to inhibit Salmonellae.

iii) Large intestine : results in Table 3 showed a significant ($p<0.05$) reduction in $\log_{10}$ cfu *S. gallinarum* in large intestines of all treatments compared with control group at day 7 and 14, while, no significant ($p>0.05$) difference in $\log_{10}$ cfu *S. gallinarum* in large intestine between 0.5% formic acid and the control at day 21. As for 0.5% formic acid treatment, there was no significant ($p>0.05$) difference in $\log_{10}$ cfu *S. gallinarum* in large intestines between day 7 and 21. However, a significant ($p<0.05$) reduction in $\log_{10}$ cfu *S. gallinarum* in large intestines was observed only at day 14 of the study period.

iv) Cecum : means of the colonization numbers of *S. gallinarum* in the cecal contents of broiler chicks receiving different treatments are presented in Table 3. There were significant ($p<0.05$) reduction in $\log_{10}$ cfu *S. gallinarum* in cecum in all treatments compared with control group at day 7 and 14. At day 21 there was no significant ($p>0.05$) difference in $\log_{10}$ cfu *S. gallinarum* in cecum of 0.5% FA treatment compared with control group, however, a significant ($p<0.05$) reduction was observed when 1.5% FA and 1.0% FA treatments were compared with control groups at day 21. As for 1.5% FA treatment, a persistent colonization till day 14, but a significant ($p<0.05$) reduction in $\log_{10}$ cfu *S. gallinarum* in cecum at day 21 was observed.

The results of this experiment, suggested that formic acid is likely to have an antibacterial effect, since it reduced the pH of the digestive tract in a concentration-dependent manner (McHan and Shotts, 1992; AL-Tarazi and Alshawabkeh, 2003). Besides, Chung and Geopfert (1970) observed that the growth of Salmonella in acidic media was more dependent on the nature of acid molecules than on pH. The organism could grow at pH 4.05 when the acid was hydrochloric or citric. However, if the acids were acetic, propionic or formic, the limiting pH values were 5.04, 5.5 and 4.0 respectively.

The pH of some alimentary canal contents :

i) Crop : there was a significant ($p<0.05$) reduction in the pH of crop for all treatments compared with control group at day 7, 14 and 21 of the experiment. Meanwhile, there was a significant ($p<0.05$) reduction in the pH of crop of the negative control group at day 14, but no significant ($p<0.05$) difference observed at day 21 compared with day 7. Furthermore, there was a significant ($p<0.05$) increase in the pH of crop for 0.5% FA treatment during the study period. As for 1.0% FA treatment, it showed a significant ($p<0.05$) increase in the pH of crop at day 14 and persisted till day 21 and this trend was observed in 1.5% FA treatment.

Table 5. Means of mortality rate during days 7, 14 and from (3-21 days) of broiler chicks fed different levels of formic acid-treated-artificially-contaminated diet with *S. gallinarum*

Day	Treatment				
	Negative control	Control	0.5% FA	1.0% FA	1.5% FA[1]
7	2.78	11.11	5.55	2.78	5.55
14	7.41	8.33	7.87	7.87	0.00
21	6.67 $_b$	33.33 $_a$	8.33 $_b$	5.56 $_b$	0.00 $_b$
3-21	11.11 $_b$	27.78 $_a$	13.89 $_b$	11.11 $_b$	5.55 $_b$

Standard error: Mortality rate=4.84; Overall mortality rate=4.97. FA[1]: formic acid.

[a, b] Means with different subscripts in the same row are significantly different ($p<0.05$).

These results illustrated the effect of various levels of formic acid on the pH of the crop. This indicated that the pH decreased as the formic acid level increased, and this lower pH inhibited the growth of *S. gallinarum* in the crop before degradation or absorption occurred in the intestines. Although, the pH of the crop is fairly low this is not the case in the newly hatched chicks (Iba and Junior, 1995; AL-Tarazi and Alshawabkeh, 2003).

ii) Small intestine : means of the pH values of small intestinal contents of broiler chicks receiving different treatments are shown in Table 4. There was a significant ($p<0.05$) reduction in the pH of small intestinal contents of 0.5% FA treatment compared with control group at day 7 and 21, while, no significant ($p>0.05$) differences at day 14. As for 1.0% FA and 1.5% FA treatments, the pH values of small intestine were significantly ($p<0.05$) lower compared with control and 0.5% FA treatments during the study period. As for the 0.5% FA and 1.5% FA treatments there was a significant ($p<0.05$) increase in the pH of small intestine with time. 1.0% FA treatment caused a significant ($p<0.05$) increase in the pH of small intestine at day 14, which persisted till the end of the experiment.

The results of this experiment illustrated that the pH values were decreased in a concentration-dependent manner, although, the pH values remained high and near neutrality, since little formic acid reaches the small intestine due to metabolism and absorption (Hume et al., 1993). However, this little amount is responsible for the slight effect on small intestine pH (Furuse et al., 1991). The reduction in the $\log_{10}$ cfu *S. gallinarum* from small intestine may be due to the small amount of formic acid reaching the small intestines, such that the pH of the digestive canal affected negatively the proportion of undissociated acid molecules. The antibacterial activity of formic acid for gram-negative bacteria, provided that there were sufficient undissociated acid molecules present and that they were in contact with bacteria for enough time, and this undissociated acid diffused into the bacterial cells caused a lower pH inside the cell (Cherrington et al., 1991; Furuse et al., 1991; Thompson and Hinton, 1997).

iv) Large intestine : Results in Table 4 indicated that significant ($p<0.05$) reductions in the pH of large intestine were observed in all treatments compared with control group at day 14 and 21 of the study period, while, a significant ($p<0.05$) decrease in the pH of large intestine of 0.5% FA and 1.0% FA treatments were observed compared with control at day 7. For the negative control and 1.5% FA treatments there were no significant ($p>0.05$) differences in the pH of large intestine among days. However, for 0.5% FA treatment a significant ($p<0.05$) reduction in the pH of large intestine occurred at day 14 and persisted till the end of the experiment and this trend was observed in control group and 1.0% FA treatment.

These results indicated that the pH values for the large intestine, which was closest to neutrality, were affected by all dietary formic acid levels due to the small amount of acid that reached the large intestine because of absorption and metabolism (Hume et al., 1993). However, at these pH values, the $\log_{10}$ cfu *S. gallinarum* was reduced due to the same reasons described in the case of the small intestines as well as the fact that *S. gallinarum* take a long time to colonize the large intestine (Xu et al., 1988; AL-Tarazi and Alshawabkeh, 2003).

v) Cecum : The results in Table 4 indicated that the pH values of cecal contents were less affected by the dietary formic acid levels used as a result of metabolism and absorption. However, a significant decrease in cecal pH was observed. This was accompanied by a decrease in the colonization of *S. gallinarum*, since the colonization needs a long time to be established in the ceca (Xu et al., 1988; AL-Tarazi and Alshawabkeh, 2003). Moreover, the acid nature of the ceca played a major role in the inhibition of *S. gallinarum* growth.

vi) Mortality rate : Data in Table 5 showed a significant ($p<0.05$) reduction in mortality rate of all groups compared with control group at day 21 only, with no significant ($p>0.05$) differences among 1.5% FA, 1.0% FA, 0.5% FA and negative control treatments at the same period. All treatments showed a significant ($p<0.05$) reduction in overall mortality rate during (3-21 days) compared with control group.

These results of mortality rate are in agreement with those described by AL-Tarazi and Alshawabkeh, (2003) who, reported that the addition of formic and propionic acids mixture in a total concentration of 2.0% or more to the diet of newly hatched infected layer chicks with *S. pullorum* reduced the chick mortality rate. Junior and Barrow (1996) reported that the Bio-Add ™ treatment at 0.68% reduced mortality rate from 77% in untreated chicks to 33% in chicks given treated feed.

CONCLUSIONS

Various *in vitro* formic acid levels had an inhibitory

effect on *S. gallinarum,* and caused a reduction in the pH of the diet in a dose-dependent manner. Supplementing the feed with formic acid reduced *S. gallinarum* colonization and lowered the pH in the contents of the crop. small intestine. large intestine. and cecum. Various levels of formic acid decreased mortality rate in all treatments of experimentally infected chicks.

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