

## Effect of Dietary Formic Acid Level on Artificially Contaminated Broiler Feed with *Salmonella Gallinarum*

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### ABSTRACT

Reported here are the effects of additive formic acid on inhibitory effect of *Salmonella gallinarum* in poultry feed. Two experiments were conducted to investigate the viability of *S. g.* and pH of poultry feed using different dietary formic acid level (0.0, 0.5, 1.0 and 1.5%) on inhibitory effect of *S. g.* in broiler feed.

Experiment one was conducted to investigate the viability of *S. g.* 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 depend upon the concentration of treated acid and the diet remains acidic below the growth range of *S. g.*, which means that the bacterial cells were exposed to stressful conditions that make 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.g.* The numbers of *S.g.* re-isolated from all treated groups except groups treated with 0.5% formic acid, decreased significantly ( $p < 0.05$ ) compared with the control groups. 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.

The mortality rate of all treated groups showed a significant ( $p < 0.05$ ) reduction in overall mortality rate during (3-21 days) compared with the control.

These results indicate that the addition of formic acid in a total concentration of 1.5% to the diet of newly hatched broiler chicks decreases significantly the contamination diet with *S.g.*

**KEYWORDS:** *Salmonella gallinarum*, formic acid ,colonization , feed.

### 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 single reservoir of salmonella organisms, and salmonella contaminated feed has been implicated as a major contributor in maintaining the reservoir.

A previous report by Westerfeid et al. (1970) suggesting that a chemical feed additive may have reduced salmonella initially present without eliminating them from contaminated feed and *in vitro* sensitivity tests indicating a 0.1% concentration of the additive was effective in inhibiting the growth of salmonella in concentrations up to  $10^7$  cells /ml. In nutrient, broth suggested the need for further studies with the additive using poultry feed and feed ingredient.

Williams (1981) considered feed as an important source for transmission of salmonella to poultry. Presence of salmonella in small numbers in these feed is undesirable and in many countries render them unfit for poultry consumption. Accordingly, different methods for

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treatment of such feed against salmonella were suggested.

Formic acid as well as other organic acids incorporation had a disinfecting effect on contaminated feed and its sufficiently antibacterial effect in the alimentary tract to be useful for this purpose (Iba and Junior, 1995). The anti salmonella effects of this acid, were thought to be the result of diffusion of these undissociated acid into the bacterial cell and the reduction of intracellular pH (Cherrington et al., 1991). Unfortunately, they did not ascertain the optimum levels of formic acid to protect food borne salmonellosis, in addition, it is effective in preventing intestinal colonization of *Salmonella* organisms from naturally or artificially contaminated feed.

Therefore, the objectives of this investigation were to study the effect of adding different levels of formic acid on artificially contaminated feed with *Salmonella gallinarum in vitro*, and to determine the possible antibacterial effect of feeding formic acid on crop, ceca and intestinal content pH, to reduce the colonization of *S.gallinarum*, and their 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 treated diet was kept in sterile plastic bags of 1 kg at room temperature and contained *S. gallinarum*  $1.36 \times 10^6$  cfu/kg diet. Five treatments were randomly assigned to five groups (diet bags) 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*  $1.36 \times 10^6$  cfu/ kg diet at 3 days of age . The diets were checked for the absence of *Salmonella* and the chicks too (Linton et al., 1985).

One hundred and eighty day - old Hubbard broiler chicks were purchased from a commercial hatchery and were randomly assigned to 5 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 3 of age, (and after 24 hrs of contamination) the feeders and drinkers were cleaned routinely to avoid reinfection.

The treatments of both experiments were:

- 1) Basal diet without *S. gallinarum* challenge (negative controls).
- 2) *S. gallinarum* challenge with no formic acid (control).
- 3) *S. gallinarum* challenge with 0.5% formic acid,
- 4) *S. gallinarum* challenge with 1.0% formic acid, and
- 5) *S. gallinarum* challenge with 1.5% formic acid.

**Incorporation of formic acid into the diet:** formic acid (85%) was obtained from a commercial company (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 to be in the diet was 50 ml per kg diet. The diet in the two experiments, was incorporated by hands and electrical mixer to insure 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 described by Iba and Junior (1995), in which, 1 ml of nutrient broth containing *S. gallinarum* ( $1.36 \times 10^6$  cfu/ml) was mixed into 1 kg diet by hands and by converting the bags continuously to insure complete distribution, formic acid was added shortly after contamination. In experiment 2 the diet was contaminated by a method described by Hinton (1986) by which an overnight nutrient broth culture containing *S.gallinarum*  $1.36 \times 10^6$  cfu/ml as percent 1/5000, (volume/weight) was mixed by hands slowly and thoroughly until complete homogenization. Formic acid was incorporated before contamination because feed mills refuse to mix formic acid to *Salmonella* - contaminated diet. 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 from 3 sites of each bag was collected to be cultured (Williams, 1981). In experiment 2, chicks were randomly selected and were

killed by neck dislocation, and 3, 1g sample from (crop, small intestine, large intestine and cecum) were pooled together and considered as one sample of 3g per section per group (Izat *et al.*, 1990a) if there were 2 chicks: a 1.5 g sample from each section/chick, and if there was 1 chick: a 3g from each section/ chicks were taken to be collected.

The samples for the pH measurement were prepared in the same manner discussed above for culture process, but the weight was 0.2g (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 hrs. This culture was then transferred to selenite broth at 42 °C for 24 hrs. Subcultures were then made to SS agar at 37 °C for 24 hrs; negative plates were re-incubated for an additional 24 hrs. Colonies typical for *S. gallinarum* were counted and confirmed biochemically by inoculating in triple sugar iron at 37 °C for 24 hrs (Andrew *et al.*, 1984). Results (average cfu duplicate of each sample) were logarithmically ( $\log_{10}$ ) - transformed per gram sample.

**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 the insertion of the glass electrode of pH-meter.

**Statistical analysis:** the logarithmically-transformed counts and pH value 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).

## Results and Discussions

### Experiment One:

**Viable numbers of *S. gallinarum* in the diet:** means of the viable numbers of *S. gallinarum* in the diet 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 the control group. The results showed a significant ( $P < 0.05$ ) reduction in the viability for all treatments with

time after treatment, the result indicated that the increase concentrate of formic acid reduces the number of viability *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 diet 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 the control group. Furthermore, for the positive control, negative control, and 0.5% formic acid groups there were no significant ( $p < 0.05$ ) differences in the pH of the diet among time of testing during the observation period. Although, there was a significant ( $p < 0.05$ ) increase in the pH of the diet 3 days after treatment of 1.0% FA treatment, this can be explained by the volatileness property 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 upon the concentration of the treated acid when increased the concentrate acid the pH diet will decrease. Smyser and Snoeyenbos (1979) found that the pH of meat and bone meal were 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 make them unable to neither replicate nor cope with this acidic condition, and thus, they were greatly affected by the acidic condition (Nassar *et al.*, 1994, and Thompson and Hinton, 1996 and 1997).

### Experiment Two:

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

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

The results of this experiment showed the antibacterial activity of formic acid in a concentration-dependent manner and these results were in agreement with those described by Thompson and Hinton (1997) who found that the Bio-Add<sup>TM</sup> at 6.8 and 12 g/kg diet

reduced the number of *S. enteritidis* from the crop of hens by a factor of 10, and agree with Hinton and Linton (1988) who showed high bactericidal activity of formic acid at 0.66% in the crop which inhibit the bacterial cells to grow and reproduce after one week, as well as, the acidic nature of the crop has high negative impact on bacterial cell. Alshwabkeh and Tabbaa (2002) found the number of *S. gallinarum* positive culture in the crop of chicks, decreased significantly ( $p < 0.05$ ) from the groups provided 0.6, 1.2 and 1.8 % propionic acid in the diet on the days 1, 8 and 15 post inoculation.

**Small intestine:** means of the colonization numbers of *S. galliarum* in the Small Intestinal (SI) 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* - negative small intestine of chickens after 7 days. The results illustrated that formic acid reduces the pH in the digestive tract, since the acid molecules inhibit the growth of *Salmonella*, but did not eliminate them from the intestinal tract at day 21 of age and these results agree with McHan and Shotts (1992), found that the effect was higher as the level of formic acid increased to inhibit *Salmonellae*.

**Large intestine:** results in Table (3) showed that a significant ( $P < 0.05$ ) reduction in  $\log_{10}$  cfu *S. gallinarum* in large intestines of all treatments compared with the control group on 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 control on 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 on day 14 of the study period.

**Cecum:** means of the colonization numbers of *S. gallinarum* in the cecal (Ce) contents of broiler chicks receiving different treatments are presented in Table (3). There was a significant ( $P < 0.05$ ) reduction in  $\log_{10}$  cfu *S. gallinarum* in Ce in all treatments compared with control group on days 7 and 14. On day 21 there was no significant ( $P > 0.05$ ) difference in  $\log_{10}$  cfu *S. gallinarum* in Ce 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 on day 21. Meanwhile, no significant ( $P > 0.05$ ) differences in  $\log_{10}$  cfu *S. gallinarum* in Ce of 0.5% FA treatment. 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 Ce on day 21 was observed.

The results of this experiment, suggested that formic acid is likely to be antibacterial since formic acid reduced the pH of the digestive tract in a concentration-dependent manner (McHan and Shotts, 1992). Besides, Chung and Geopfert (1970) observed that the growth of *Salmonella* in acidic media was more dependent on the 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:

**Crop:** there was a significant ( $P < 0.05$ ) reduction in the pH of Cr for all treatments compared with the control group on days 7, 14 and 21 of the experiment. Meanwhile, there was a significant ( $P < 0.05$ ) reduction in the pH of Cr of the negative control group on day 14, but no significant ( $P < 0.05$ ) difference observed on day 21 compared with day 7. Furthermore, there was a significant ( $P < 0.05$ ) increase in the pH of Cr 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 Cr on day 14 and persisted till day 21 and this trend was observed in 1.5% FA treatment.

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).

**Small intestine:** means of the pH values of Small Intestinal (SI) contents of broiler chicks receiving different treatments are shown in Table (4). There was a significant ( $P < 0.05$ ) reduction in the pH of SI of 0.5% FA treatment compared with the control group on day 7 and 21, while, no significant ( $P > 0.05$ ) differences on day 14. As for 1.0% FA and 1.5% FA treatments, the pH values of SI 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 SI with time. 1.0% FA treatment caused a significant ( $P < 0.05$ ) increase in the pH of SI on 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 (SI) may be due to the little amount of formic acid reached the small intestines, such that the pH of the digestive canal affect negatively the proportion of undissociated acid molecules. The antibacterial activity of formic acid for gram-negative bacteria, provided that there were sufficient amounts of undissociated acid molecules present and that they are 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).

**Large intestine:** Results in Table (4) indicated that significant ( $P < 0.05$ ) reductions in the pH of LI 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 LI of 0.5% FA and 1.0% FA treatments compared with control on day 7. Anyhow, for the negative control and 1.5% FA treatments, there were no significant ( $P > 0.05$ ) differences in the pH of LI among days. However, for 0.5% FA treatment a significant ( $P < 0.05$ ) reduction in the pH of LI occurred on day 14 and persisted till the end of the experiment and this trend was observed in the 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 little amount of formic 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 *S. gallinarum* took a long time to colonize the large intestine (Xu *et al.*, 1988).

#### **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). Moreover, the acid nature of the ceca played a major role in the inhibition of *S. gallinarum* growth.

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

These results of mortality rate were in agreement with Junior and Barrow (1996) who found that the Bio-Add™ treatment at 0.68% reduced mortality rate from 77% in untreated chicks to 33% in chicks given treated feed.

## **CONCLUSIONS**

1. Various formic acid levels *in vitro* had an inhibitory effect on *S. gallinarum*. Also, showed a reduction in the pH of the diet in a dose - dependent manner.
2. Supplementing the diet with formic acid gave a decrease in the colonization numbers of *S. gallinarum* and pH in the contents of the crop, small intestine, large intestine and cecum.
3. Various levels of formic acid decreased mortality rate in all treatments of experimentally infected chicks.

**Table (1): Means of the viable number 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.**

Treatment Day	Control	0.5% F A	1.0% F A	1.5% F A
0	5.32 <sub>a</sub> <sup>v</sup>	5.05 <sub>b</sub> <sup>v</sup>	4.34 <sub>c</sub> <sup>v</sup>	4.22 <sub>d</sub> <sup>v</sup>
1	5.23 <sub>a</sub> <sup>v</sup>	4.78 <sub>b</sub> <sup>w</sup>	4.13 <sub>c</sub> <sup>w</sup>	3.82 <sub>d</sub> <sup>w</sup>
3	4.79 <sub>a</sub> <sup>w</sup>	4.06 <sub>b</sub> <sup>x</sup>	3.18 <sub>c</sub> <sup>x</sup>	2.83 <sub>d</sub> <sup>x</sup>
5	4.33 <sub>a</sub> <sup>x</sup>	3.74 <sub>b</sub> <sup>y</sup>	2.85 <sub>c</sub> <sup>y</sup>	2.33 <sub>d</sub> <sup>y</sup>
7	4.23 <sub>a</sub> <sup>x</sup>	3.14 <sub>b</sub> <sup>z</sup>	2.82 <sub>c</sub> <sup>y</sup>	1.65 <sub>d</sub> <sup>z</sup>

\*. Standard error (SE) of  $\log_{10}$  Sg= 0.04.

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.**

Treatment Day	Negative Control	Control	0.5% FA	1.0% FA	1.5% FA
0	6.17 <sub>a</sub>	6.21 <sub>a</sub>	5.84 <sub>b</sub>	5.36 <sub>c</sub> <sup>w</sup>	4.81 <sub>d</sub> <sup>x</sup>
1	6.20 <sub>a</sub>	6.20 <sub>a</sub>	5.83 <sub>b</sub>	5.34 <sub>c</sub> <sup>w</sup>	4.90 <sub>d</sub> <sup>wx</sup>
3	6.19 <sub>a</sub>	6.19 <sub>a</sub>	5.85 <sub>b</sub>	5.46 <sub>c</sub> <sup>v</sup>	4.96 <sub>d</sub> <sup>w</sup>
5	6.20 <sub>a</sub>	6.21 <sub>a</sub>	5.89 <sub>b</sub>	5.52 <sub>c</sub> <sup>v</sup>	5.11 <sub>d</sub> <sup>v</sup>
7	6.18 <sub>a</sub>	6.19 <sub>a</sub>	5.90 <sub>b</sub>	5.53 <sub>c</sub> <sup>v</sup>	5.09 <sub>d</sub> <sup>v</sup>

\*. SE (pH)= 0.03.

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 ( $P < 0.05$ ).

**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	Treatment Day	Control	0.5% F A	1.0% F A	1.5% F A
Crop	7	5.25 <sub>a</sub> <sup>v</sup>	4.37 <sub>b</sub> <sup>v</sup>	4.21 <sub>c</sub> <sup>v</sup>	4.14 <sub>c</sub> <sup>v</sup>
	14	5.19 <sub>a</sub> <sup>v</sup>	3.36 <sub>b</sub> <sup>w</sup>	3.42 <sub>b</sub> <sup>w</sup>	3.16 <sub>c</sub> <sup>w</sup>
	21	4.42 <sub>a</sub> <sup>w</sup>	3.15 <sub>b</sub> <sup>x</sup>	3.12 <sub>b</sub> <sup>x</sup>	2.94 <sub>c</sub> <sup>x</sup>
Small Intestine	7	5.36 <sub>a</sub> <sup>v</sup>	4.40 <sub>b</sub> <sup>v</sup>	4.14 <sub>c</sub> <sup>v</sup>	4.21 <sub>c</sub> <sup>v</sup>
	14	4.34 <sub>a</sub> <sup>w</sup>	4.10 <sub>b</sub> <sup>w</sup>	3.40 <sub>c</sub> <sup>w</sup>	3.21 <sub>d</sub> <sup>w</sup>
	21	4.29 <sub>a</sub> <sup>w</sup>	4.39 <sub>a</sub> <sup>v</sup>	3.07 <sub>b</sub> <sup>x</sup>	2.96 <sub>c</sub> <sup>x</sup>
Large Intestine	7	5.36 <sub>a</sub> <sup>v</sup>	4.40 <sub>b</sub> <sup>v</sup>	4.14 <sub>c</sub> <sup>v</sup>	4.21 <sub>c</sub> <sup>v</sup>
	14	4.34 <sub>a</sub> <sup>w</sup>	4.10 <sub>b</sub> <sup>w</sup>	3.40 <sub>c</sub> <sup>w</sup>	3.21 <sub>d</sub> <sup>w</sup>
	21	4.29 <sub>a</sub> <sup>w</sup>	4.39 <sub>a</sub> <sup>v</sup>	3.07 <sub>b</sub> <sup>x</sup>	2.96 <sub>c</sub> <sup>x</sup>
Ceca	7	5.73 <sub>a</sub> <sup>v</sup>	4.35 <sub>b</sub>	4.11 <sub>b</sub> <sup>v</sup>	3.42 <sub>c</sub> <sup>v</sup>
	14	5.24 <sub>a</sub> <sup>w</sup>	4.20 <sub>b</sub>	3.41 <sub>c</sub> <sup>w</sup>	3.26 <sub>c</sub> <sup>v</sup>
	21	4.44 <sub>a</sub> <sup>x</sup>	4.26 <sub>a</sub>	3.16 <sub>b</sub> <sup>x</sup>	2.89 <sub>b</sub> <sup>w</sup>

\*. SE (Cr  $\log_{10}$  Sg)= 0.03. \*. SE (SI  $\log_{10}$  Sg)= 0.04. \*. SE (LI  $\log_{10}$  Sg)= 0.03. \*. SE (Ce  $\log_{10}$  Sg)= 0.10.

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 ( $P < 0.05$ ).

**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	Treatment Day	Negative Control	Control	0.5% FA	1.0% FA	1.5% FA
Crop	7	4.58 <sup>a</sup> <sub>v</sub>	4.52 <sub>a</sub>	4.30 <sup>b</sup> <sub>w</sub>	4.12 <sup>c</sup> <sub>w</sub>	4.07 <sup>c</sup> <sub>w</sub>
	14	4.51 <sup>a</sup> <sub>w</sub>	4.49 <sub>a</sub>	4.33 <sup>b</sup> <sub>vw</sub>	4.35 <sup>b</sup> <sub>v</sub>	4.19 <sup>c</sup> <sub>v</sub>
	21	4.58 <sup>a</sup> <sub>v</sub>	4.52 <sub>a</sub>	4.38 <sup>b</sup> <sub>v</sub>	4.33 <sup>b</sup> <sub>v</sub>	4.22 <sup>c</sup> <sub>v</sub>
Small Intestine	7	6.30 <sub>a</sub>	6.25 <sub>a</sub>	6.11 <sup>b</sup> <sub>w</sub>	6.00 <sup>c</sup> <sub>w</sub>	6.03 <sup>c</sup> <sub>w</sub>
	14	6.30 <sub>a</sub>	6.26 <sub>a</sub>	6.26 <sup>a</sup> <sub>v</sub>	6.17 <sup>b</sup> <sub>v</sub>	6.13 <sup>b</sup> <sub>v</sub>
	21	6.33 <sub>a</sub>	6.19 <sub>a</sub>	6.19 <sup>b</sup> <sub>vw</sub>	6.10 <sup>c</sup> <sub>v</sub>	6.07 <sup>c</sup> <sub>vw</sub>
Large Intestine	7	6.21 <sub>a</sub>	6.23 <sup>a</sup> <sub>v</sub>	6.15 <sup>b</sup> <sub>v</sub>	6.17 <sup>ab</sup> <sub>v</sub>	6.07 <sub>c</sub>
	14	6.16 <sub>a</sub>	6.14 <sup>a</sup> <sub>w</sub>	6.06 <sup>b</sup> <sub>w</sub>	6.05 <sup>b</sup> <sub>w</sub>	6.06 <sub>b</sub>
	21	6.16 <sub>a</sub>	6.15 <sup>a</sup> <sub>w</sub>	6.05 <sup>b</sup> <sub>w</sub>	6.05 <sup>b</sup> <sub>w</sub>	6.03 <sub>b</sub>
Ceca	7	5.78 <sub>ab</sub>	5.82 <sub>a</sub>	5.71 <sub>bc</sub>	5.67 <sub>cd</sub>	5.60 <sub>d</sub>
	14	5.81 <sub>a</sub>	5.76 <sub>a</sub>	5.76 <sub>ab</sub>	5.68 <sub>b</sub>	5.66 <sub>b</sub>
	21	5.77 <sub>a</sub>	5.76 <sub>a</sub>	5.75 <sub>a</sub>	5.68 <sub>ab</sub>	5.63 <sub>b</sub>

\*. SE ( Cr pH)= 0.02. \*. SE ( SI pH)= 0.03. \*. SE ( LI pH)= 0.03. \*. SE (Ce pH)= 0.04.

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).

**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*.**

Treatment Day	Negative Control	Control	0.5% FA	1.0% FA	1.5% FA
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 <sub>b</sub>	33.33 <sub>a</sub>	8.33 <sub>b</sub>	5.56 <sub>b</sub>	0.00 <sub>b</sub>
3-21	11.11 <sub>b</sub>	27.78 <sub>a</sub>	13.89 <sub>b</sub>	11.11 <sub>b</sub>	5.55 <sub>b</sub>

\*. SE (MR)= 4.84.

\*.SE (Overall MR)= 4.97.

a, b. Means with different subscripts in the same row are significantly different (P < 0.05).

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(7, 5, 3, 1, 0.0)

(p<0.05)

( )

( 0.0, 0.5, 1.0 and 1.5% )

(p

0.5%

(21, 14, 7)

<0.05)

1.5%

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.2004/10/5

2003/11/21

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