*Article*

# Investigation of possible synergistic effect of using formic acid and plant essential oil in broiler chicks drinking water on performance and gut microflora

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**Abstract**
The possible synergistic effect of using a combination of formic acid (FA) and plant essential oils (EO) in broiler chick's drinking water was investigated. Performance and gut microflora were assessed from day old to 42 d of age. The experiment was carried out using a completely randomized design with factorial arrangement (2×3). Factors were included formic acid (0, 1000 and 2000 ppm) and EO (0 and 250 ppm) level which were administered through drinking water. Both FA and EO improved performance criteria but their combination failed to create a synergistic effects. Chicks received FA supplemented water had significantly lower numbers of *C. perfringens* and coliforms. Administration of EO also significantly lowered numbers of gut pathogenic bacteria (*C. perfringens* and coliforms) while did not affect lactobacilli population. Results obtained in our study suggest a synergistic effect of using FA and EO simultaneously only in reducing gut pathogenic bacteria.

**Keywords** formic acid; plant essential oil; gut microflora; broiler.

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## 1 Introduction

The gastrointestinal tract plays a vital role in the digestion and absorption of nutrients required for maintenance and growth. The proliferation of pathogens in the intestines often results in inflammatory responses that cause productivity losses, increased mortality, and increased contamination of poultry products. Sub-therapeutic antibiotics have long been used in broiler diets for growth improvement and the control of intestinal pathogens. However, issues regarding the development of antibiotic-resistant bacteria and intensive use of sub-therapeutic antibiotics have led to public demand to limit the use of antibiotics in animal agriculture (McCartney, 2002). Consequently, there is growing demand for natural alternatives to sub-therapeutic antibiotics that can sustain or improve farm performance and safety of broiler products. Probiotic, prebiotic, organic acid and plant essential oils have been considered as antibiotic alternatives.

Although the antibacterial mechanism(s) for organic acids are not fully understood, they are capable of exhibiting bacteriostatic and bactericidal properties. Given the weak acid nature of most of these compounds, pH is considered a primary determinant of effectiveness because it affects the concentration of un-dissociated acid formed (Davidson, 2001). It has been traditionally assumed that un-dissociated forms of organic acids can easily penetrate the lipid membrane of the bacterial cell and once internalized into the neutral pH of the cell cytoplasm dissociate into anions and protons (Eklund, 1983, 1985; Salmond et al., 1984; Cherrington et al., 1990, 1991; Davidson, 2001). Generation of both of these species potentially presents problems for bacteria that must maintain a near neutral pH cytoplasm to sustain functional macromolecules. Export of excess protons requires consumption of cellular adenosine triphosphate (ATP) and may result in depletion of cellular energy (Davidson, 2001).

The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property (Conner, 1993) and chemical structure (Farag et al., 1989) could play a role. It is thought that membrane perforation or binding is the principle mode of action (Shapiro and Guggenheim, 1995; Stiles et al., 1995), leading to an increase of permeability and leakage of vital intracellular constituents (Juven et al., 1994), resulting in impairment of bacterial enzyme systems (Farag et al., 1989).

The objective of this study was to evaluate if a synergistic effects exist between organic acids and plant essential oil in maintaining a profitable microflora in chicken gut.

## 2 Materials and Methods

A total of 300 feather sexed male and female day old Ross 308 broiler chicks were used in this experiment. Chicks were weighed individually and randomly assigned to floor pens (100×120 cm) so that pens had equal sex ratio, initial weight and weight distribution.

The experiment was carried out using a completely randomized design with factorial arrangement (2×3). Factors were included formic acid (0, 1000 and 2000 ppm) and EO (0 and 250 ppm) level which administered through drinking water. Four replicate of 15 chicks per each were received experimental treatments. Experimental treatments were included: 1) control (C) with no additive in drinking water, FA1 group with 1000 ppm formic acid in their drinking water, FA2 group with 2000 ppm formic acid, EO group with 250 ppm plant essential oils (*Origanum Vulgare* and *Thymus vulgaris* ) and FA1-EO with 1000 ppm formic acid and 250 ppm plant essential oil and FA2-EO with 2000 ppm formic acid and 250 ppm plant essential oil. The experiment started at one day old and lasted at 42 d of age. Each pen was equipped with a tube feeder and a bell waterer. Pens were placed inside a commercial farm so that experimental animals were reared with their contemporaries at the farm. The lighting schedule was 23L:1D throughout the experiment, with controlled temperature and humidity. Room temperature was maintained according to industry standards. All the chickens received the same diets throughout the experiment. Diets formulated according to Ross 308 manual (Aviagen, 2009). None of the experimental diets contained antibiotics or coccidiostats, and diets were in mash form. Throughout the experimental period, birds had *ad libitum* access to feed and water. Water was refreshed every day after cleaning the waterer. Feed consumption and BW for each pen were recorded at 21 and 42 d of age to calculate feed conversion. Mortality was recorded daily and when calculating feed conversion, the BW of dead birds was taken into account.

### 2.1 Bacterial enumeration

On d 42, two birds were selected at random from each pen, weighed, and killed by cervical dislocation and their intestinal tracts were removed. Samples of fresh digesta (0.1 to 0.2 g) from the ileum (Meckel's diverticulum to 1 cm proximal to the ileocecal junction) and ceca were collected aseptically in pre weighed 15-mL sterilized plastic tubes containing 1 mL of 0.1% sterile peptone buffer with 5 g/L of Cys hydrochloride

(Sigma Chemical Co., St. Louis, MO). The digesta samples were pooled from 2 birds from each pen. The samples were immediately placed on ice and kept there until plated, within 3 h of collection. The numbers of *C. perfringens,* Coliforms and *lactobacillus* determined using the method described by Dahiya et al. (2007).

### 2.2 Statistical analysis

Treatment means were tested for significance using the General Linear Models (GLM) procedure of SAS software (SAS Institute, 2001). A group of 15 birds in a pen constituted an experimental unit for experimental treatment. Feed intake, weight gain, feed conversion, and $\log_{10}$ transformed bacterial population data were analyzed as a completely randomized design. Differences between treatment means were separated using Duncan`s multiple range test. Significant difference was defined as $P$<0.05.

## 3 Results

### 3.1 Growth performance

Body weight (BW) and feed:gain of chickens during 0-21 d and 22-42 d of age are presented in Table 1. Neither organic acid (FA) nor essential oil (EO) supplementation had no significant effect on BW and feed:gain at 21 d of age. However, at 42 d of age, chicks received FA solely or in combination with EO, had better BW (P<0.001) and feed:gain ratio (P<0.002) in comparison to control group. Different levels of FA (1000 or 2000 ppm) did not affect performance of broiler chicks. Chicks received EO supplemented water had heavier BW (0.05) at 42 d of age in comparison to control group, but feed gain ratio were not affected by EO supplementation. Combination of FA and EO did not result in a synergistic effect to further improve BW or feed:gain ratio.

**Table 1** Effect of drinking water supplementation with formic acid, plant essential oils and their combination on broiler chick's performance

| Treatment | | BW 21 d of age | Feed:gain 21 d of age | BW 42 d of age | Feed:gain 42 d of age |
|---|---|---|---|---|---|
| FA | | | | | |
| 0 | | 809.63 | 1.36 | $2226.00^{b}$ | $2.02^{a}$ |
| 1000 | | 817.63 | 1.33 | $2291.75^{a}$ | $1.96^{b}$ |
| 2000 | | 824.88 | 1.33 | $2320.00^{a}$ | $1.94^{b}$ |
| SEM | | 5.51 | 0.01 | 15.53 | 0.01 |
| EO | | | | | |
| 0 | | $809.58^{b}$ | 1.36 | $2260.58^{b}$ | 1.99 |
| 250 | | $825.17^{a}$ | 1.32 | $2297.92^{a}$ | 1.96 |
| SEM | | 4.50 | 0.01 | 12.68 | 0.01 |
| FA | EO | | | | |
| 0 | 0 | $797.75^{b}$ | $1.39^{a}$ | $2186.25^{b}$ | $2.05^{a}$ |
| 0 | 250 | $821.50^{a}$ | $1.33^{b}$ | $2265.75^{a}$ | $1.99^{b}$ |
| 1000 | 0 | $814.50^{ab}$ | $1.34^{ab}$ | $2270.50^{a}$ | $1.97^{b}$ |
| 1000 | 250 | $820.75^{a}$ | $1.32^{b}$ | $2313.00^{a}$ | $1.95^{b}$ |
| 2000 | 0 | $816.50^{ab}$ | $1.34^{ab}$ | $2325.00^{a}$ | $1.94^{b}$ |
| 2000 | 250 | $833.25^{a}$ | $1.32^{b}$ | $2315.00^{a}$ | $1.94^{b}$ |
| SEM | | 7.79 | 0.02 | 21.96 | 0.02 |

FA:Formic acid, EO:Essential oil; Means with different superscript are significantly different

### 3.2 Ileum

The number of *Lactobacilus,* C. *perfernges* and coliforms in digesta of ileum and cecum are presented in table 2. A significant effect of FA on numbers of *Lactobacilus* was observed, whereas EO did not show any significant effect. Chicks received water supplemented with 1000 or 2000 FA had significantly higher counts of Lactobacillus (Table 2).

Both FA and EO supplementation of water significantly decreased numbers of *C. perfernges* in ileum. Chickens from control group had significantly higher *C. perfringens* count in comparison to other treatment groups. A synergistic effect of combining FA and EO on the numbers of *C. perfernges* were also observed, so that, treatment groups with combination of FA and EO had significantly lower *C. perfernges* compared to those received either FA or EO solely.

Coliforms population in the ileum of chicks received water with FA or EO supplements were significantly lower than those received non-supplemented water. Combining FA and EO resulted in further decrease in coliforms population in comparison to single addition of either FA or EO.

### 3.3 Cecum

Neither FA nor EO did not shows any significant effect on *Lactobacilus* numbers in the cecum. Although adding 2000 ml of FA to drinking water significantly decreased *C. perfergens* number in comparison to control group, but 1000 ml FA failed to create such effects (Table 2).

Addition of FA to drinking water resulted in significant decrease in the number of coliforms compare to control. Furthermore, higher levels of FA (2000) had significantly lower coliforms count compare to those received water with 1000 ml FA. Supplementation of drinking water with EO also decreased numbers of coliforms in the Cecum. Simultaneous addition of both FA and EO to drinking water, resulted in more depression in coliforms count in the Cecum compared to single addition of them.

Addition of 2000 ml FA and also EO supplemented water significantly lowered *C. perfergens* in Cecum. Although combination of FA and EO resulted in lower *C. perfergens* count in Cecum but this synergistic effect was significant only at 1000 ml FA.

## 4 Discussion

Health and performance promoting effects have been demonstrated for organic acids and plant essential oils. Besides improvement in hygiene and a corresponding reduction of pathogen intake, effects on feed digestion and absorption and on stabilization of gut flora eubiosis have been demonstrated in a number of investigations. Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract) and reducing potential proliferation of unfavourable microorganisms. Acidification of gut stimulates enzyme activity and optimises digestion and the absorption of nutrients and minerals. Herbal essential oils assist in colonization of the beneficial microbial population within the gastrointestinal tract to more balanced levels (Zaika, 1988; Jang et al., 2007). Besides their antimicrobial properties (Ultee et al., 2002), they also exhibit antioxidant (Basmacioğlu et al., 2004), antifungal (Bang et al., 2000; Shin and Lim, 2004), digestion-stimulating, and enzymatic (Jamroz et al., 2003, 2005; Hernandez et al., 2004) activities. In the present study we used formic acid and EO solely or in combination to investigate if there could be a synergistic effect. Although reports about the effects of broiler chicks water acidification with formic acid is rare, Elwinger et al. (1993) reported improved BWG and FCR in broiler chicks received dietary formic acid. Garcia et al. (2007) and Runho et al. (1997) reported improved FCR in broiler chicks received dietary formic acid, whereas BWG was not affected by addition of formic. Gurcia et al. (2007) suggested that better FCR was due to improved dry matter and crude protein digestion. Furthermore improved broiler performance by supplementation with single acids was noticed for formic acid (Vogt et al., 1981). Contrarily, Herna´ndez et al. (2006) failed to observe any effect on the performance of chickens when formic acid (5,000 or 10,000 ppm) was added to the feeds. Nevertheless, the experiment was performed under ideal conditions of experimentation, which could explain the lack of effects observed, because the growth-enhancing effects of antimicrobial additives become apparent when chickens are subjected to suboptimal conditions, such as a less digestible diet or a less clean environment. Since we placed experimental pens in industry condition, beneficial effects of organic acid on chicks performance were observed at 42 d of age.

**Table 2** Effect of drinking water supplementation with formic acid, plant essential oils and their combination on broiler chickens gut micro flora.

| Treatment | *C. perfringens* | | Coliforms | | | *Lactobacilus* | |
|---|---|---|---|---|---|---|---|
| Organic acid | | Ileum | Cecum | Ileum | Cecum | Ileum | Cecum |
| 0 | | $4.32^{a}$ | $4.53^{a}$ | $7.42^{a}$ | $7.50^{a}$ | $8.80^{b}$ | 9.32 |
| 1000 | | $3.38^{b}$ | $4.38^{a}$ | $6.76^{b}$ | $6.87^{b}$ | $9.11^{a}$ | 9.15 |
| 2000 | | $3.20^{b}$ | $4.00^{b}$ | $6.48^{b}$ | $6.48^{c}$ | $9.10^{a}$ | 9.16 |
| SEM | | 0.13 | 0.11 | 0.15 | 0.11 | 0.09 | 0.13 |
| Essential Oil | | | | | | | |
| 0 | | $4.06^{a}$ | $4.53^{a}$ | $7.28^{a}$ | $7.35^{a}$ | 8.94 | 8.99 |
| 250 | | $3.20^{b}$ | $4.02^{b}$ | $6.50^{b}$ | $6.55^{b}$ | 9.06 | 9.43 |
| SEM | | 0.07 | 0.09 | 0.12 | 0.09 | 0.07 | 0.11 |
| FA | EO | | | | | | |
| 0 | 0 | $4.70^{a}$ | $4.87^{a}$ | $7.55^{a}$ | $7.87^{a}$ | $9.12^{b}$ | $9.10^{ab}$ |
| 0 | 250 | $3.95^{b}$ | $4.20^{b}$ | $7.30^{a}$ | $7.12^{b}$ | $9.10^{b}$ | $9.55^{a}$ |
| 1000 | 0 | $3.97^{b}$ | $4.72^{a}$ | $7.40^{ab}$ | $7.25^{b}$ | $9.17^{b}$ | $9.07^{ab}$ |
| 1000 | 250 | $2.80^{c}$ | $4.05^{b}$ | $6.12^{c}$ | $6.50^{cd}$ | $8.42^{c}$ | $9.22^{ab}$ |
| 2000 | 0 | $3.52^{b}$ | $4.17^{b}$ | $6.90^{b}$ | $6.95^{bc}$ | $8.52^{c}$ | $8.80^{b}$ |
| 2000 | 250 | $2.87^{c}$ | $3.82^{b}$ | $6.07^{c}$ | $6.02^{d}$ | $9.67^{a}$ | $9.52^{a}$ |
| SEM | | 0.18 | 0.16 | 0.21 | 0.16 | 0.13 | 0.19 |

FA:Formic acid, EO:Essential oil, Means are $\log_{10}$ cfu/g of ileum or Cecum contents, Means with different superscript are significantly different

Antibacterial effects of EO are well established. Active components of *Origanum Vulgare* and *Thymus vulgaris* (thymole and carvacrol) which we used in our experiment have been shown to decrease Cp counts (Briozzo et al., 1988; Dorman and Deans, 2000) in vitro. Furthermore, a field study conducted by Kohler (1997) with a commercial preparation of EO showed a reduction of Cp as compared to positive control diet containing zinc bacitracin. Same to results obtained in our study, Losa and Kohler (2001) and Mitsch et al. (2004) found a reduction of the average number of Cp in broiler chicks intestine when their diet supplemented with EO of *Thymus vulgaris* and *Origanum vulgare.* However, Evans et al. (2001) reported that a mixture of essential oil from colve, peppermint, thyme and lemon did not reduce numbers of Cp in broiler chicks intestine.

In the studies of Mathlouthi et al. (2011), Celiktas et al. (2007) and Qussalah et al. (2006), the oregano essential oil showed antimicrobial effects against *Escherichia coli*. Reduced number of total coliform bacteria in broiler chicks received EO in our study agreed with those reported by Michiels et al. (2009).

Peric et al. (2010) examined a commercial phytogenic product containing essential oil of oregano, anis and citrus and did not observe any effects of EO on lactic acid bacteria. They suggested that plant essential oils are only active against pathogenic bacteria. Similarly, we did not observe any significant effects of EO on *Lactobacillus* number.

Based on results obtained here in our study it could be concluded that although simultaneous addition of FA and EO failed to create a synergistic effect on performance of broiler chicks, their combination may have a synergistic effect in reducing pathogenic bacteria of gut lumen. This may be due to the fact that EO could damage the bacteria cell membrane facilitating the penetration of organic acids into the bacteria cytoplasm.

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