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Effect of graded levels of formic acid on gut microflora count, serum biochemical parameters, performance and carcass yield of broiler chickens

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ABSTRACT

The influence of formic acid (FA) supplementation in diets on the performance of broiler chicken was evaluated. Control starter (0–3 wk) and finisher (4–6 wk) diets were formulated to contain 2900 kcal ME/kg with 22% CP and 3000 kcal ME/kg with 20% CP, respectively. In addition, 6 experimental diets were constituted with the inclusion of 50 g antibiotic (furazolidone)/ quintal or FA at 0.2, 0.4, 0.6, 0.8 or 1.0% to the control diet. Each diet was offered *ad lib.* in cages to 6 replicates of 6 chicks each from day-old to 6 wk of age. Formic acid at 0.6% of the diet significantly improved the body weight gain and feed conversion ratio, which was as par with the antibiotic supplemented group. Increasing the concentration of FA from 0.6 to 0.8% further improved both the above parameters with no subsequent improvement thereafter. The digestibility of crude protein and calcium and their concentrations in serum, and dressed yield were significantly higher and abdominal fat content was lower in the dietary groups that contained 0.6 to 1% FA. Dietary addition of FA at 0.6% and above reduced the *Escherchia coli* counts in crop but not in the small intestine and caecum. Higher concentration of FA (0.8% and 1%) was more beneficial in reducing the *Escherichia coli* counts in small intestine (SI) and caecum and was as par with the antibiotic group. From this study, it is concluded that 0.8% formic acid in the diet of broiler chickens elicited optimum performance and provided better results than antibiotic (furazolidone).

Key words: Broiler chickens, Carcass yield, Formic acid, Gut microflora count, Serum biochemical parameters

The microbial balance of the gut is of importance in maintaining gut health (Gabriel *et al*. 2006). For achieving high levels of economic efficiency, poultry are raised under intensive production systems which inflict considerable stress altering the gut environment, creating an imbalance in the gut microflora, ultimately affecting the bird's performance. The use of antibiotics as routine feed additives has been banned worldwide because of public concern over antibiotic residual effects and the development of drug resistance bacteria. In the absence of this option, use of organic acids in the diet is one such promising strategy (Dibner and Butin 2002). Amongst organic acids, short chain fatty acids are considered as potential alternatives to antibiotic growth promoters (Leeson *et al*. 2005, Ao *et al.* 2009). Several investigations were conducted to determine the effects of formic acid (FA) on the performance and health of broilers (Hinton and Linton 1988, Patten and Waldroup 1988), however, conflicting information is available. Therefore, the present study was conducted to evaluate the effect of graded levels of formic acid on gut pH and microflora count, serum biochemical parameters, performance and carcass yield of broiler chickens.

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MATERIALS AND METHODS

Day-old commercial broiler chicks (252) were wing banded and randomly distributed into 42 raised-floored stainless steel battery brooder pens with 6 chicks per pen. Uniform management and vaccination schedules were followed for all the birds. Control starter (0–3 wk) and finisher (4–6 wk) diets were formulated to contain 2900 kcal ME/kg and 22% CP and 3000 kcal ME/kg and 20% CP, respectively (Table 1). Subsequently 6 other experimental diets were formulated adding 50 g antibiotic (furazolidone)/ quintal or organic acid at 0.2, 0.4, 0.6, 0.8 or 1% to the control diet. Organic acids and antibiotic was added to the basal diet at the expense of maize. The organic acid used was formic acid. Each diet was fed at random to 6 replicates of 6 chicks each throughout the experimental period of 6 weeks.

Body weight of chicks (individual) and feed intake (replicate-wise) were recorded at weekly interval. Feed conversion ratio was calculated as the ratio between feed consumed and weight gained. On 14th day, 6 birds from each dietary treatment were sacrificed and crops, small intestine

Table 1. Composition of control diet (%)

	Starter (0–3 wk)	Finisher (4–6wk)
Ingredient		
Maize	56.12	58.24
Soybean meal	38.60	35.80
Dicalcium phosphate	1.88	1.62
Shell grit	0.72	0.68
Salt	0.40	0.40
DL- methionine	0.21	0.20
Choline chloride (50%)	0.10	0.06
Vegetable oil	1.62	2.66
[1]Vitamin premix	0.05	0.04
[2] Mineral premix	0.10	0.10
Toxin binder	0.20	0.20
Total	100.00	100.00
Nutrient composition		
ME (kcal/kg)	2900	3000
Protein (%)	22	20
Lysine (%)	1.21	1.07
Methionine (%)	0.53	0.50
Av. phosphorus (%)	0.45	0.40
Calcium (%)	0.90	0.85

[1]Supplies per kg diet: vitamin A, 16,500 IU; vitamin D_3, 3200 ICU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin B_1, 1.2 mg; vitamin B_2, 10 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 12 mg; niacin, 18 mg; pantothenic acid, 12 mg; [2]Mn, 90 mg; Zn, 72 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.

and caecum were removed quickly and stored at 4°C for *Escherchia coli* count. The crop was incised to expose inner lining, which was thoroughly washed with normal physiological saline to make the volume to 10 ml. The *E. coli* counts were made in aliquots drawn from the saline extract. Intestinal contents (dudeno-jejunal junction) and caecal contents, approximately 1 g from each bird were collected and suspended in 9 ml of nutrient broth. Serial dilution of each sample were made in nutrient broth and *E. coli* counts were made on MacConkey's agar and EMB agar by surface spread method.

A 3-day (22–24) metabolic trial using all the birds from 3 replicates of each group involving total collection of faeces was conducted to determine the digestibility of dry matter (DM), nitrogen (N), Ca and P. Each replicate was treated as a single unit. The daily feed intake and faeces voided were recorded. Representative samples of feed offered, residue left and faeces voided were analyzed for N, Ca and TP concentrations (AOAC 1990). On 22nd day, 3 ml blood was collected from 6 birds from each treatment group and concentration of calcium (Ca), phosphorus (P), protein and alkaline phosphatase (ALP) activity in the serum was estimated by using diagnostic kits. For carcass yield, 1 bird representing the mean body weight of each replicate (6 birds /treatment) was selected and sacrificed by cervical dislocation on 43 day of age. The data on weight of edible carcass, giblet (liver, heart and gizzard), small intestine, abdominal fat were recorded and all the data were expressed as percentage of the pre-slaughter weight. Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran 1989). The means of different treatments were compared with Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Dietary addition of formic acid at 0.6% of the diet significantly improved body weight gain (BWG) and feed conversion ratio (FCR) which was at par with the antibiotic supplemented group (Table 2). Increasing the concentration of FA from 0.6 to 0.8% further improved both the above parameters with no subsequent improvement thereafter (1.0%). Formic acid at 0.8% of the diet produced significantly higher BWG and better FCR than control or antibiotic supplemented group. Thus, our findings suggested that FA supplementation in the diet of broiler chickens could be beneficial and can spare antibiotic in diet to improve performance. Dietary supplementation of formic acid (0.5–1.0%) in broilers up to 7 weeks of age had beneficial effect on body weight and feed utilization and inclusion of FA even at 1% level had no adverse effects on body weight, feed utilization or livability (lzat *et al*. 1990), a similar finding observed in the present study. In another study, Eidelsburger and Kirchgessner (1994) reported that calcium formate at 1.5% improved feed utilization in broilers. However, in this study the same benefit could be observed at a lower level (0.8%).

Dietary addition of formic acid did not influence digestibility of dry matter (DM) or phosphorus (P). However, CP and Ca digestibilities were significantly influenced by the FA (Table 3). Formic acid up to 0.4% did not affect digestibility of CP and Ca, but increasing the concentration to 0.6% significantly increased the digestibility of CP and

Table 2. Effect of dietary addition of formic acid on body weight gain and feed conversion ratio in broiler chickens

Treatments	0–3 wk		0–6 wk	
	Body weight gain (g)	Feed conversion ratio	Body weight gain (g)	Feed conversion ratio
Formic acid (0.2%)	585^{c}	1.56^{a}	1272^{c}	1.83^{a}
Formic acid (0.4%)	597^{c}	1.54^{a}	1289^{c}	1.80^{bc}
Formic acid (0.6%)	619^{b}	1.48^{b}	1311^{b}	1.78^{bc}
Formic acid (0.8%)	637^{a}	1.47^{bc}	1361^{a}	1.75^{c}
Formic acid (1.0%)	635^{a}	1.46^{c}	1354^{a}	1.77^{c}
Control	584^{c}	1.54^{a}	1268^{c}	1.84^{a}
Control + antibiotic	620^{b}	1.55^{a}	1318^{b}	1.83^{a}
SEM	4.18	0.006	6.48	0.008

Means with different superscripts in a column differ significantly ($P < 0.05$).

Table 3. Effect of dietary inclusion of organic acid on the apparent digestibility of DM, CP, Ca and P in broilers during metabolic trial (22–25 d)

Treatments	Apparent digestibility (%)			
	DM	CP	Ca	P
Formic acid (0.2%)	73.76	39.55^{b}	36.96^{b}	59.89
Formic acid (0.4%)	73.05	3.9.40^{b}	36.51^{b}	59.90
Formic acid (0.6%)	72.37	41.27^{a}	38.74^{a}	59.71
Formic acid (0.8%)	73.45	41.20^{a}	38.94^{a}	59.48
Formic acid (1.0%)	73.57	41.20^{a}	3.8.78^{a}	58.76
Control	73.59	38.91^{b}	36.92^{b}	57.92
Control + antibiotic	72.61	38.44^{b}	36.78^{b}	58.90
SEM	0.55	0.22	0.16	0.28

Means with different superscripts in a column differ significantly ($P < 0.05$).

Table 4. Effect of dietary addition of formic acid on serum biochemical parameters of broiler chickens

Treatments	Protein (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (IU/L)
Formic acid (0.2%)	3.17^{c}	9.28^{c}	5.79	48.24^{a}
Formic acid (0.4%)	3.12^{c}	9.40^{c}	5.84	47.20^{a}
Formic acid (0.6%)	3.71^{b}	9.72^{b}	5.82	38.24^{b}
Formic acid (0.8%)	3.94^{a}	9.90^{a}	5.92	36.08^{b}
Formic acid (1.0%)	3.83^{ab}	9.88^{a}	5.89	36.14^{b}
Control	3.24^{c}	9.36^{b}	5.86	47.68^{a}
Control + antibiotic	3.30^{c}	9.38^{b}	5.78	37.54^{a}
SEM	0.06	0.05	0.06	1.02

Means with different superscripts in a column differ significantly ($P < 0.05$).

Ca. Further increasing the concentration of FA from 0.6 to 1.0% in the diet had no extra advantage on the above 2 parameters. Morz (2000) reported significant improvement in apparent digestibility of protein with the addition of formic, fumaric and butyric acids in broiler chickens. Combination of lactic acid and formic acids also improved digestibility of protein, Ca and P in pigs (Jongboled *et al*. 2000). However, in this study no influence of organic acid was observed on P digestibility. One of the hypotheses for increased digestibility of protein was the reduction of microbial deamination of amino acids by addition of organic acids (Eckel *et al*. 1992). In the present study, the digestibility of Ca also improved significantly due to dietary inclusion of FA. Ca digestion and absorption is better under acidic condition. It could be hypothesized that organic acid addition possibly reduced the pH of intestinal tract of birds leading to better digestibility and subsequent absorption of Ca (Jongboled *et al*. 2000).

The concentration of protein and calcium (Ca) increased and alkaline phosphatase (ALP) activity decreased significantly in serum by dietary addition of 0.6% FA, however, phosphorus concentration in serum was not influenced (Table 4). Amongst the levels of FA in the diet, 0.8% FA proved to be best, which resulted in higher levels of protein and Ca in serum compared to other treatment groups. In the present study, the higher serum protein and Ca levels was observed in groups fed organic acid could be due to better digestibility of nutrients. It has been reported that organic acid supplementation in the diet of broilers reduce stress. The findings of the present study further supports the above report as ALP activity (more the ALP activity higher is the stress) reduced significantly due to dietary supplementation of FA (0.6% and above).

Dietary addition of FA at 0.6% and above reduced the *Escherchia coli* (*E. coli*) count in crop but not in the small intestine and caecum (Fig. 1). Higher concentration of FA (0.8% and 1%) was more beneficial in reducing the *E. coli* counts in SI and caecum and was at par with the antibiotic supplemented group. Our findings further suggested that organic acid can replace antibiotic in eliminating harmful micro-organisms like *E. coli* from the gut. Concomitant to

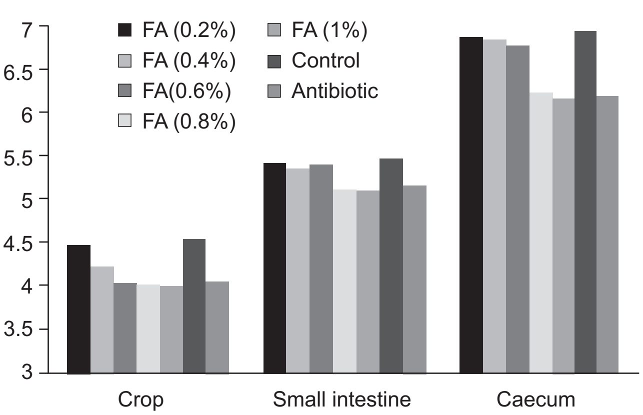

Fig 1. Effect of dietary addition of formic acid on *Escherchia coli* counts (log 2) in the gut of broiler chickens.

Table 5. Effect of dietary inclusion of formic acid on dressed yield, giblet, abdominal fat content and small intestine weights of broiler chickens

Treatments	% live weight			
	Dressed weight	Giblet	Abdominal fat	Small intestine
Formic acid (0.2%)	71.37^{c}	5.46	1.58^{a}	3.68
Formic acid (0.4%)	71.34^{c}	5.38	1.61^{a}	3.89
Formic acid (0.6%)	72.38^{b}	5.39	1.23^{b}	3.46
Formic acid (0.8%)	72.82^{a}	5.48	1.05^{c}	3.92
Formic acid (1.0%)	72.80^{a}	5.54	1.17^{bc}	3.64
Control	71.28^{c}	5.52	1.56^{a}	3.44
Control + antibiotic	72.42^{c}	5.44	1.55^{a}	3.48
SEM	0.22	0.06	0.04	0.12

Means with different superscripts in a column differ significantly ($P < 0.05$).

the findings of this study, Hinton and Linton (1988) observed reduced infection rate of *Salmonella* in broiler by dietary addition of 0.6% FA. The adhesion index of *Salmonella* increased with increase in pH and decreased at low pH due to the antibacterial effect of the dietary addition of organic acids (formic, propionic and lactic acids) as reported by Kamala *et al*. (2001).

Improvement in dressing yield and reduction of abdominal fat were found in birds fed 0.6% FA compared to the control, antibiotic or 0.2–0.4% FA in diets (Table 5). Formic acid at 0.8% further enhanced the dressed yield and lowered the abdominal fat content. A similar finding was observed in one of our earlier study with organic acid (butyric acid–0.6%) in broiler chickens (Panda *et al*. 2008). From this study, it is concluded that 0.8% formic acid in the diet of broiler chickens elicited optimum performance and provided better results than furazolidone.

REFERENCES

AOAC. 1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists. Arlington, Virginia, USA.

Ao T, Canton, A H, Pescatore, A J and Ford M J. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poultry Science* **88**: 111–17.

Dibner J J and Putin R J. 2002. Use of organic acids as a model to study the impact of Gut microflora in nutrition and metabolism. *Journal of Applied Poultry Research* **11**: 453–63.

Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42.

Eckel B F, Roth F × and Kemme P A. 1992. Influence of formic acid on daily weight gain, fed intake, feed conversion rate and digestibility: 1. Nutritive value of organic acids in the rearing of piglets. *Journal of Animal Physiology and Animal Nutrition* **67**: 198–205.

Eidelsburger U and Kirchgessner M. 1994. Effect of organic acids and salts in the feed on fattening performance of broilers. *Archives Geflugelkonde* **58**: 268–77.

Gabriel I, Lessire M, Mallet S and Gullot J F. 2006. Microflora of the digestive tract: Critical factors and consequences for poultry. *Worlds Poultry Science Journal* **62**: 499–511.

Hinton M and Linton A H. 1988. Control of Salmonella infections in broiler chickens by the acid treatment of their feed. *Veterinary Record* **123**: 416–18.

Izat A L, Tidwell N M, Thomas R A, Reiber M A, Adams M H, Colberg M and Waldroup P W. 1990. Effects of a buffered propionic acid in details on the performance of broiler chickens and on microflora of the intestine and carcass. *Poultry Science* **69**: 818–26.

Jongbeld A W, Morz R and Kemme P A. 2000. The effect of microbial phytase, organic acids and their interaction in diets for growing pigs. *Livestock Production Science* **67**: 113–22.

Kamala K, Vijayasarahi S K, Sreenivasagowda, R N and Rao S. 2001. Effect of organic acid on in vitro colonization of Salmonella gallinarum to caudal ileal and caecal epithelial cells of chickens. *Indian Journal of Animal Sciences* **71**: 1038–40.

Leeson S, Namkung H, Antongiovanni M and Lee H. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. *Poultry Science* **84**: 1418–22.

Morz Z. 2000. Supplementary organic acids and their effects with microbial phytase in diets for pigs and poultry. *Proceedings of Annual Conference on Phytase in Animal Nutrition*. Lublin, Poland.

Panda A K, Rama Rao S V, Raju M V L N and Shyam Sunder G. 2008. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. *Asian Australian Journal of Animal Sciences* (*in Press*)

Patten J D and Waldroup P W. 1988. Use of organic acids in broiler diets. *Poultry Science* **67**: 1178–82.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 7th edn. Oxford and IBH. Publishing Company Pvt. Ltd., New Delhi.