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Original Research Article

Studying the effect of formic acid and potassium diformate on performance, immunity and gut health of broiler chickens

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ABSTRACT

Our trial was conducted to study the effects of formic acid (FA) and potassium di-formate (KDF) in broiler ration on performance, carcass traits, blood biochemical, intestinal microbial load, histological picture of intestine and immune parameters of broilers. In this study 360 one-day-old broiler chicks were divided to 3 groups with 3 replicates of 40 chicks each. The trial continued for 35 days. The control group was fed only basal diet (G1). Group 2 (G2) were fed basal diet supplemented with FA (5 g/kg diet), and group 3 (G3) received basal diet supplemented with KDF (5 g/kg diet). The results showed that both FA and KDF significantly increased body weight gain (BWG), dressing percentage of broilers and significantly decreased feed conversion ratio (FCR) ($P < 0.05$). The highest percent of breast and thigh was observed in G3. The improvement in villus height was observed in G2 and G3 compared with the control one, and the highest was in G3. The results evidence that the using of FA or KDF in broiler feeds have significant effects on performance, immune parameters, and gut health without having any significant effects on blood biochemical. However, KDF is more effective than FA as little amount of FA reaches the small intestine due to metabolism and absorption, whereas KDF permits a proportion of FA to pass through the fore-gut intact and enter the small intestinal tract. In addition, FA has a strong odor and corrosiveness to gastrointestinal tract which limits its use.

1. Introduction

In-feed antibiotics have been used for many years in the poultry industry as antibiotic growth promoters (AGP) to enhance both animal health status and productive performance (Bedford, 2000). The mechanisms by which AGP improve growth performance are poorly understood, it is thought that the antibiotics may induce modifications of the gut micro flora which are beneficial to the host (Hassan et al., 2010). Banning in-feed antibiotics for the mono-gastric animals, as in the European Union due to its huge problem for environmental conditions and human health, has put more pressure on animal nutritionists to innovate new alternatives to fill the gap left by removing AGP from the feed industry.

Acidifiers such as pure organic acids have been used as feed preservatives for protecting feed from microbial and fungal destruction (Christian, 2015). Thus, acidifiers could be used as powerful tool in maintaining the health of gastrointestinal tract of poultry, resulting in improving their performance. Acidifiers act as performance promoters by suppressing the growth of acid intolerance bacteria such as *E-coli*, *salmonella* spp., and *Clostridium perfringens* (Naseri et al., 2012). Moreover, organic acids reduce pH in the stomach, which enhance pepsin activity, and increases the digestibility of nitrogen, phosphorus and minerals Christian and Mellor (2011).

Numerous studies have demonstrated that FA is effective against pathogenic bacteria and enhancing growth performance, but strong odor and corrosiveness to gastrointestinal tract limit its use (Øverland et al., 1999). Another important limitation is that the

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organic acids are rapidly metabolized in the fore-gut (crop to gizzard) of birds which reduces their impact on growth performance (Christian and Mellor, 2011). Gut acidifiers are organic acid salts, such as potassium di-formate (KDF), that have received attention as an alternative to formic acid (FA) due to its easiness to handle, little or no corrosive effect and also effective against pathogenic bacteria along whole gastrointestinal tract (Huyghebaert et al., 2011). Potassium diformate is a crystalline powder, where the carbonyl group of FA links with hydroxyl group of potassium formate via a hydrogen bond, which dissociates to FA and potassium formate in the gut, thus FA enters the small intestine intact (Selle et al., 2004).

The purpose of this study to investigate the effect of FA and its salt, KDF, on performance, protein digestibility and gut health (pH, tissue morphology, cecal microbial content) of broiler chickens.

2. Material and methods

2.1. Experimental design

Our trial was conducted at the poultry research farm of the faculty of veterinary medicine – Cairo University. A total of 360 one-day-old broiler chicks (Cobb 500) were obtained from Pyramid Poultry Company in Giza. Chicks were randomly divided into 3 treatment groups, each in 3 replicates (40 chicks/replicate). Birds were housed in an open house system bedded by a layer of wood shaving with a constant lighting program during the whole experimental period (5 weeks). Birds were provided continuously with clean drinking water. All birds were kept under standard hygienic conditions and subjected to prophylactic vaccination program against viral diseases.

The birds were fed a basal diet formulated according to the breed producer requirements. Birds fed corn-soybean meal basal diets (starter, grower and finisher) without any supplementation served as control group (G1). Birds were fed basal diets supplemented with FA at 0.5% (G2) (Ghazalah et al., 2011), and were fed basal diets supplemented with KDF at 0.5% (G3) (Mikkelsen et al., 2009).

Calculations and chemical analysis of different diets were performed according to AOAC (1990). Diet composition and chemical analysis are shown Table 1. Birds in different experimental groups were weighted initially then weekly till the end of the experimental period. Body weight development, body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) were calculated.

2.2. Incorporation of FA into the diet

Formic acid (85%) was obtained from Gainland Chemical Co. (Sandycroft, Germany) and was incorporated into the diet as a percent (vol/wt) (Hinton and Linton, 1988) at the required rate of (0.5%). The total liquid volume of FA and sterile distilled water was 50 mL per kg diet. Then the liquid was mixed to the diet by hand to ensure a complete homogenization of FA into the diet (Al-Natour and Alshawabkeh, 2005).

2.3. Incorporation of Formi into the diet

Formi, the KDF product of ADDCON, NordicAS, Porsgrunn, Norway, contains 35% free FA, 35% formate, and 30% potassium. It was incorporated into the diet as percent (wt/wt) at the required rate (5 g/kg).

Table 1
Diet composition and chemical analysis (as fed basis).

Item	Diet composition along the experimental period		
	Starter	Grower	Finisher
Feed ingredient, %			
Yellow corn	55.44	60.63	62.83
Soybean meal (45.5%)	33.30	27.80	24.35
Corn gluten meal	3.00	3.20	4.20
DL-Met	0.24	0.24	0.20
L-Lys	0.18	0.24	0.16
Soy oil	3.66	3.83	4.33
Mono calcium phosphate	1.64	1.58	1.49
Broiler premix[1]	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10
Lime stone	1.66	1.61	1.59
Sodium chloride	0.35	0.30	0.30
Sodium bicarbonat	0.08	0.12	0.10
Anticoocidial drug	0.05	0.05	0.05
Total	100	100	100
Calculated analysis, %			
ME, kcal/kg	3,033	3,108	3,180
CP	21.50	19.50	18.70
EE	2.65	2.70	2.77
CF	3.02	2.94	2.80
Lysine	1.30	1.20	1.05
Methionine	0.61	0.59	0.55
Threonine	0.85	0.78	0.75
Ca	1.00	1.00	1.00
Total P	0.75	0.72	0.69
Av.P	0.50	0.48	0.45
Na	0.17	0.16	0.16
Cl	0.19	0.17	0.17
Chemical analysis, %			
CP	21.4	19.6	18.9
EE	2.85	2.50	2.90
Ca	1.10	1.05	1.03
Total P	0.73	0.71	0.68

[1] Broiler premix (per kg of diet): vitamin A 15,000 IU, vitamin D_3 1,500 IU, vitamin E 20 mg, vitamin K_3 5 mg, vitamin B_1 3 mg, vitamin B_2 6 mg, niacin 25 mg, vitamin B_6 5 mg, vitamin B_{12} 0.03 mg, folic acid 1 mg, D-biotin 0.05 mg, Ca-Dpantothenate 12 mg, carophyll-yellow 25 mg, and choline chloride 400 mg, Mn 80 mg, Fe 60 mg, Zn 60 mg, Cu 5 mg, Co 0.2 mg, I 1 mg, and Se 0.15 mg.

2.4. Determination of crude protein digestibility coefficient

From 28 to 35 days of age, 3 birds from each treatment were randomly taken and housed in individual cages to determine the crude protein digestibility coefficients. During this period the birds were fed the experimental diet mixed with an indicator (TiO_2 5 g/kg diet). The fresh dropping were collected daily from 30 to 35 days of age in air-tight plastic bags between 08:00 to 09:00 and frozen (−18 °C) for subsequent analysis. The crude protein of feed and dried excreta was done according to AOAC (1990). The TiO_2 was determined according to Short et al. (1996).

2.5. Blood samples and carcass traits

At the end of the experimental period, 3 birds per replicate of experimental groups were randomly taken and slaughtered, scalded at 55 to 65 °C, defeathered, eviscerated and washed with tap water. the breast meat was cut from the remaining upper back and rib cage of the carcass, washed, cooled in ice water tank for 2 h, dried for 10 min. The dressing yield (%), breast muscle yield (BMY %) were recorded according to El-Banna et al. (2008).

Blood samples were taken during slaughter. Non-haemolyzed sera were separated by centrifugation at 1,500 × *g* for 15 min at 4 °C, stored in deep freezer at −20 °C untill analysis to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid and creatinine using commercial kits.

2.6. Measurement of gastro-intestinal pH and cecal microbial content

The pH determination was performed using the method described by Al-Natour and Alshawabkeh (2005). The gastrointestinal tracts were removed. The crop and full stomach were opened, and the respective pH was determined directly using a digital pH meter HANNA HI 2210 bench-top pH meter supplied with HI 1131B glass body pH electrode, HI7662 temperature electrode. The intestinal tract was divided into duodenum, jejunum, ileum, cecum and colon. Each sample (0.6 g) was suspended in 2.4-mL sterile distilled water. The suspension was shaken vigorously and the pH was determined using a pH-meter.

The cecal contents were immediately collected into sterile tubes in ice and transferred to the laboratory of Animal Health Research Institute, Dokki, Gizza, Egypt, for cecal microbial count of total *Clostridia*, *E. coli* and *Salmonella* according to Quinn et al. (1999).

2.7. Histomorphological examination

Tissue specimens from small intestine (ileum) and spleen were collected and fixed in 10% neutral buffered formalin. The fixed tissue specimens were processed and embedded in Paraffin wax, sectioned at 4 μm and then stained with hematoxylin and eosin (Bancroft and Gamble, 2008).

Histoquantitative studies were performed by measuring the villus height, crypt depth and villus:crypt ratio using an Olympus light microscope (Olympus, Japan) and image analysis software as described by Iji et al. (2001). Histoquantitative studies were also performed by counting the number of splenic lymphocytes according to morphometric method of Biljana et al. (2008), the test areas of spleen were 3 random fields under light microscope, lymphocytes in these fields were counted and then the mean values were calculated for each sample.

2.8. Statistical analyses

The obtained data were calculated and statistically analyzed according to Wayne (1998) using SPSS software version 14 for Windows. The differences between groups were determined with variance analysis (one-way ANOVA) using the probability level of 0.05 for the rejection of the null hypothesis. Significant differences among means were determined by the Student-Newman-kuel test. Data were expressed as means ± SEM.

3. Results

3.1. Performance and carcass characteristics

The results of dietary supplementation of FA (G2) and KDF (G3) on growth performance and carcass characteristics of broiler chickens are summarized in Tables 2 and 3; data manufisted that birds of G2 and G3 had a significant ($P < 0.05$) increase in body weight gain (BWG) and a numerical decrease in feed intake (FI) compared with the control group. The results of weight gain and feed intake reflected on feed conversion ratio (FCR) which was significantly ($P < 0.05$) improved in FA and KDF supplemented groups compared with control one. The highest dressing percentage and BMY percentage were recorded in G3 followed by G2 and then G1.

3.2. Crude protein digestibility coefficient

The results of dietary supplementation of FA (G2) and KDF (G3) on crude protein digestibility coefficient of broiler chickens were summarized in Table 2; data revealed that the birds in G2 and G3 had a significant ($P < 0.05$) increase in protein digestibility coefficient compared with the control one.

3.3. Gastro-intestinal pH and cecal microbial content

The gastro-intestinal pH of the treated chicken groups is clarified at Table 4. The results showed that a dietary inclusion of FA and KDF (G2 and G3) resulted in a significant ($P < 0.05$) reduction in the pH of crop, gizzard, duodenum, jejunum, ileum compared with the control one (G1). The results revealed a numerical reduction in the pH of other gut portions (cecum and colon) in KDF (G3), which was less than FA (G2) and the control group.

The results of cecal microbial content in different experimental groups are illustrated in Table 5. The results revealed that there was a significant decrease in total clostridia and *salmonella* spp. isolated from the cecum of the groups supplemented with FA and KDF (G2 and G3) compared with the control group. The results represented a numerical decrease of *E. coli* isolated from the cecum of KDF (G3) compared with that of the control one.

3.4. Histomorphological examination

Data regarding histomorphological parameters at the end of the experiment for the different replicates of experimental groups are presented in Table 6. Results revealed that there was a significant ($P < 0.05$) increase in villus height of ileum in G2 and G3 compared with the control group. Villus/crypt ratio showed a significant ($P < 0.05$) improvement in G2 and G3 compared with control group.

3.5. Immune response

The immune system of birds is complex and is composed of several cells and soluble factors that must work together to produce a protective immune response. The lymphoid organs are the major constituents of the avian immune system (Khan and Iqbal, 2016).

Table 2
Growth performance parameters.

Item	Initial BW, g	Final BW, g	Total weight gain, g	Total feed intake per chick, g	FCR	CP digestibility, %
Control[1]	36.03 ± 3.72[a]	1,601.94 ± 160.5[a]	1,565.91 ± 156.8[a]	3.60 ± 0.38[a]	2.30 ± 0.25[a]	81.43 ± 8.32[a]
FA[2]	36.03 ± 3.65[a]	1,678.94 ± 168.2[b]	1,642.91 ± 164.3[b]	3.24 ± 0.33[a]	1.97 ± 0.21[b]	82.95 ± 8.41[b]
KDF[3]	36.02 ± 3.62[a]	1,688.13 ± 169.1[b]	1,652.11 ± 165.4[b]	3.23 ± 0.34[a]	1.95 ± 0.20[b]	83.21 ± 8.54[b]
P-value	0.761	0.004	0.004	0.065	0.008	0.003

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a,b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

Table 3
Carcass traits in different experimental groups at the end of experimental period.

Item	Live weight, g	Dressing weight, g	Dressing, %	Breast weight, g	Breast muscle yield, %	Thigh weight, g	Thigh muscle yield, %
Control[1]	1,606.45 ± 160.8[a]	1,183.75 ± 118.6[a]	73.69	298.75 ± 29.9[a]	25.24	553.75 ± 55.4[a]	46.78
FA[2]	1,674.21 ± 167.5[b]	1,238.75 ± 124.2[b]	73.99	313.75 ± 31.7[ab]	25.33	596.50 ± 59.6[a]	48.15
KDF[3]	1,689.12 ± 169.1[b]	1,257.50 ± 125.8[b]	74.45	339.50 ± 34.2[b]	26.99	608.75 ± 61.1[a]	48.41
P-value	0.003	0.010		0.055		0.378	

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

Table 4
The pH of different parts of gastrointestinal tract in different experimental groups at the end of the experiment.

Item	Crop	Gizzzard	Dudenum	Jujenum	Ileum	Cecum	Colon
Control[1]	4.54 ± 0.46[a]	3.66 ± 0.38[a]	5.71 ± 0.58[a]	5.86 ± 0.59[a]	5.55 ± 0.57[a]	6.17 ± 0.62[a]	6.09 ± 0.61[a]
FA[2]	4.37 ± 0.44[b]	3.16 ± 0.34[b]	5.17 ± 0.53[b]	5.34 ± 0.54[b]	5.02 ± 0.51[b]	6.16 ± 0.63[a]	5.59 ± 0.58[a]
KDF[3]	4.49 ± 0.46[ab]	3.27 ± 0.33[b]	5.18 ± 0.52[b]	5.18 ± 0.53[b]	5.01 ± 0.50[b]	5.74 ± 0.58[a]	5.54 ± 0.57[a]
P-value	0.043	0.002	0.028	0.042	0.014	0.072	0.248

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

Table 5
Caecal microbial load in different experimental groups.

Item	*E. coli*, $\log_{10}$ cfu/g	Total *Clostridium* count, $\log_{10}$ cfu/g	*Salmonella* (positive sample/total sample)
Control[1]	4.35[a]	330.53[a]	3/9
FA[2]	4.04[a]	236.64[b]	0/9
KDF[3]	4.02[a]	235.23[c]	0/9
P-value	0.156	0.004	

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b, c] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

Table 6
Villi height, crypt depth, and villus:crept ratio of ileum in different experimental groups at the end of the experiment.

Item	Villus height, μm	Crypt depth, μm	Villus:crept ratio
Control[1]	635.30 ± 63.8[a]	252.94 ± 25.4[a]	2.52 ± 0.27[a]
FA[2]	872.17 ± 87.5[b]	212.10 ± 21.6[a]	4.12 ± 0.44[b]
KDF[3]	874.10 ± 87.6[b]	190.50 ± 19.3[a]	4.71 ± 0.49[b]
P-value	0.001	0.074	0.008

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

Table 7
Spleen lymphocyte count in different experimental groups at the end of the experiment.

Item	Number of lymphocyte
Control[1]	1,943.33 ± 194.4[a]
FA[2]	2,973.33 ± 297.5[b]
KDF[3]	2,960.00 ± 296.02[b]
P-value	0.002

FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

The results of our experiment revealed that there was a significant increase in spleen lymphocyte count in G2 and G3 compared with G1 ($P < 0.05$) as clarified in Table 7.

3.6. Serum parameters

Results of serum parameters at the end of the experiment for the different replicates of experimental groups are clarified in Table 8. The results showed that FA and KDF supplementation had no significant effect on serum AST, ALT, uric acid, and creatinine.

4. Discussion

4.1. Performance and carcass characteristics

The reasons that organic acids or their salts improved performance in the broilers may be attributed to that the organic acids

Table 8
Serum parameters in different experimental groups at the end of the experiment.

Item	ALT, U/L	AST, U/L	Uric acid, mg/dL	Creatinine, mg/dL
Control[1]	4.64 ± 0.45	18.32 ± 1.9	9.73 ± 0.80	1.33 ± 0.09
FA[2]	4.65 ± 0.49	18.25 ± 2.0	9.62 ± 0.89	1.31 ± 0.13
KDF[3]	4.74 ± 0.51	17.99 ± 1.8	9.70 ± 0.91	1.43 ± 0.15
P-value	0.066	0.064	0.057	0.071

ALT = alanine aminotransferase; AST = aspartate aminotransferase; FA = formic acid (Gainland Chemical Co., Sandycroft, Germany); KDF = potassium di-formate, which is formi (ADDCON, NordicAS, Porsgrunn, Norway).
[a, b] Within a column, values with different superscripts are significantly different ($P \leq 0.05$).
[1] Group 1 was with 0% FA and 0% KDF.
[2] Group 2 was with 0.5% FA.
[3] Group 3 was with 0.5% KDF.

and their salts improve protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, lowering the incidence of sub-clinical infections and secretion of immune mediators, reducing the production of ammonia and other growth suppressing microbial metabolites (Dibner and Buttin, 2002; Chowdhury et al., 2008). Moreover, gut acidification stimulates pancreatic enzyme secretion and activity and thus optimizes nutrient digestion and absorption in young animal, making amino acids more available for protein deposition within the body so improves carcass leanness (dressing %) (Mellor, 2000).

Our results of FA on BWG and FCR of broiler chickens are compatible with Adil et al. (2010, 2011) who found that chicks fed the diet supplemented with organic acids showed a significant improvement in the FCR as against the chicks fed the control diet. Several investigators reported that FA (0.1% to 1.0%) had positive effect on growth performance of broiler (Garciá et al., 2007; Bozkurt et al., 2009). Ghazalah et al. (2011) recorded that dietary supplementation of FA at (0.25%, 0.5% and 1%) increase BWG, improve FCR. However, 0.5% FA supplemented group recorded the heaviest BWG and the best FCR. Brzóska et al. (2013) reported that organic acid (0.3% to 0.9%) had a growth enhancing effect and mortality-reducing effect in broiler chickens. Contrary to our findings, Hernadez et al. (2006) reported that FA (0.5% to 1%) did not affect BW, BWG and FCR of broiler.

The improvement in BWG and FCR by KDF supplementation was discussed by Christian and Mellor (2011) who concluded that double salts of organic acids, such as KDF and sodium diformate (NaDF), which reach the small intestine, have been shown to have a significant impact on nutrient utilization. The same was recorded by Selle et al. (2004) who found that dietary supplementation of KDF at 6% significantly increased BWG and FI from 16 to 35 days post–hatch. Similarly, Helen and Christian (2010) who reported that the addition of diformate at (0.1%, 0.3% and 0.5%) was found to enhance individual live weight and FCR with increasing dosage, the best results in respect of these parameters were obtained for a dosage of 0.3% diformate. Tohru et al. (2011) recorded that dietary KDF supplementation at 1% significantly increased the body weight of broiler chickens. Christian (2014) studied the results of 17 trials with NaDF inclusion, which ranged from 0.1% to 0.6% and concluded that the dietary NaDF could improve broiler production worldwide. These results did not comply with Samuel et al. (2009) who found that adding KDF to diet at 0, 0.3%, 0.6%, 0.9% and 1.2% linearly reduced feed intake and weight gain.

Concerning to carcass traits, our results are consistent with the result of Tohru et al. (2011) who found that dietary supplementation of KDF at 1% significantly increased breast muscle, thighs and wings. On the other hand, Denli et al. (2003) reported that organic acid mixture of propionic and FA had no effect on the carcass yield at the end of the experiment compared with control. Similarly, Garciá et al. (2007) recorded that FA supplementation at 0.5% or 1.0% did not affect carcass, right breast and right thigh yields of broilers at 49 days of age. Brzóska et al. (2013) reported that organic acid (0.3% to 0.9%) had no significant influence on carcass yield or proportion of individual carcass parts.

4.2. Crude protein digestibility coefficient

The increase in crude protein digestibility coefficient may be attributed to that organic acids raised gastric proteolysis and improved protein and amino acids digestibility as reported by Samanta et al. (2010). It was thought that the organic acids supplementation lowers the pH of the chime which might increase the pepsin activity and thus enhance the digestibility of protein (Afsharmanesh and Porreza, 2005). Proteolysis of proteins by pepsin produced peptides which activated the release of hormones including gastrin and cholecystokinin. The pancreatic secretion increased by organic acids led to better digestion of proteins due to the high concentration of trypsinogen, chymotrypsinogen A, chymotrypsinogen B, procarboxypeptidase A and procarboxypeptidase B (Adil et al., 2010). According to Van Der Sluis (2002), the positive effect of organic acids on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings.

Our results agree with that of Hernández et al. (2006) and Garciá et al. (2007) who recorded that supplementation of FA (0.5% or 1.0%) in broiler finisher diet was found to improve apparent ileal digestibility of dry matter (DM) and CP as compared with control. Similarly, Ao et al. (2009) observed that 2% citric acid in the broiler diet also increased the retention of DM, CP and neutral detergent fiber. Also, Ghazala et al. (2011) reported that 0.5% dietary supplementation of either fumaric or FA improved both ME and nutrient digestibility, like, crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE) of broiler diets.

Concerning the effect of KDF on crude protein digestibility, it is speculated that KDF supplementation improved epithelial cell proliferation in the gastrointestinal tract which might increase in N retention and CP digestibility coefficient. Selle et al. (2004) observed that dietary KDF supplementation at 12 g/kg increased N retention by 5.6%. Also, Christian (2015) stated that 0.3% sodium diformate supplementation to broiler diet numerically increased protein digestibility.

4.3. Gastro-intestinal pH and cecal microbial content

Our results agree with that of Alshawabkeh and Kanan (2005) who reported that 0.5% to 1.5% FA supplementation to broiler diet reduced significantly the intestinal pH. The same was recorded by Ghazalah et al. (2011) who concluded that dietary supplementation of FA (0.25%, 0.5% and 1.0%) significantly reduced pH values of different gastrointestinal tract segments crop, gizzard, duodenum, jejunum and ileum compared with control group.

Regarding the antimicrobial effect of organic acid, it is suggested that the un-dissociated form of organic acids is the basic form by which they could exert their antimicrobial effect (Ostling and Lindgren, 1993). The Organic acids are lipid soluble in the un-dissociated form, in which they are able to passively diffuse through the microbial cell wall and disrupt the normal physiology of certain types of bacteria that we call ‘pH sensitive’ meaning that they cannot tolerate a wide internal and external pH gradient (Van Immerseel et al., 2006). Once in the cell, the acid releases the proton in the more alkaline environment, resulting in a decrease of intracellular pH leading to inhibition of the action of important

microbial enzymes and nutrient transport systems, which inhibits the ability of the bacteria to multiply (Huyghebaert et al., 2011). Furthermore, the $RCOO^-$ anions produced from the acid can disrupt DNA and protein synthesis, putting the organism under stress, so that it is unable to replicate rapidly (Russell and Diez-Gonzalez, 1998).

Our results are in harmony with that of Van Immerseel et al. (2006); Naseri et al. (2012) who concluded that the organic acid supplementation in poultry diet have a beneficial effect in controlling intestinal bacterial infection by *Salmonella*, *Campylobacter* and *E. coli*. Similarly, Hassan et al. (2010) found that dietary supplementation of organic acid mixtures (fumaric acid, calcium format, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate and hydrogenated vegetable oil) is more efficient than the antibiotic growth promoter (Enramycin) in decreasing intestinal *E. coli* and *Salmonella* spp. Different studies by (Byrd et al., 2001; Açıkgöz et al., 2011; Hamed and Hassan, 2013) indicated that addition of organic acid to the drinking water helps to reduce the level of pathogens in the water and the crop/proventriculus, to regulate gut micro flora. It is believed that the organic acid administration in feed or water mainly metabolized and absorbed in the upper gastro-intestinal segments of poultry as recorded by (Thompson and Hinton, 1997; Van Immerseel et al., 2006; Hassan et al., 2010). Furthermore, the dissociation kinetics of organic acid salts such as KDF permits a proportion of FA to pass through the fore-gut intact and enter the small intestinal tract. So that, the KDF able to reduce *C. perfringens* and control necrotic enteritis in broiler flocks at (0.45%) Mikkelsen et al. (2009). Fernández et al. (2009) found that sodium butyrate (in both partially protected with vegetable fats and unprotected forms) was able to prevent *Salmonella* colonization in the crop and cecum of broilers. Christian et al. (2012) had tested 2 different dosages of sodium diformate (NaDF) (0.1% and 0.3%) in a commercial broiler diet and recorded a significant reduction in faecal levels of *E. coli* in both treated groups. Counteractive to our results, Paul et al. (2007) who found that organic acid salt (ammonium formate or calcium propionate; 3 g/kg diet) supplementation lowered *E. coli* count in the gut, but the *clostridial* count was unaffected. Tohruet al. (2011) observed that KDF supplementation to broiler diet at 1% did not affect the count of *Enterococcus faecalis,* coliforms, and lactic acid bacteria in the cecum.

4.4. Histomorphological examination

The increase of villus height may be attributed to the antimicrobial action of organic acidifier (FA and KDF) which reduces the growth and colonization of many pathogenic bacteria, therefore reduces the infectious and inflammatory process at the intestinal mucosa, leading to increased villus height and function of secretion (Loddi et al., 2004; Pelicano et al., 2005).

Coincide with our results, Garcıá et al. (2007) who observed that the groups fed diets containing FA at (0.5% and 1%) had the longest villi compared with control. Similarly, Panda et al. (2009) recorded that butyrate supplementation irrespective of concentrations 0.2%, 0.4% or 0.6% in the broiler's diet, improved the villus length and crypt depth in the duodenum. Subsequent study by Adil et al. (2010) recorded the increase in duodenal, jejuna and ileal villus heights in the birds fed diets supplemented with 3% butyric acid, 3% fumaric acid and 2% fumaric acid. Kum et al. (2010) and Rodríguez-Lecompte et al. (2012) reported that (1.0% sorbic acid and 0.2% citric acid) supplementation significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks at 14 days of age.

Our results of KDF on the villus height of ileum are compatible to those of Franco et al. (2005) who found a beneficial effect with the use of KDF on the intestinal mucosa of broilers. Also, Paul et al. (2007) who found that organic acid salt (ammonium formate, calcium propionate and calcium lactate) supplementation to the broiler diet increased the villus height of different segments of the small intestine than the control group. Christian (2015) tested the effect of sodium diformate (0.3%) inclusion in a typical corn-soy diet of broiler chicken, and observed a significant increase in villi height of jejunum and ileum at d 39.

4.5. Immune response

Several studies elucidated that organic acids could stimulate the natural immune response in poultry. The improvement in bird immunity could be related to the inhibitory effects of organic acids on gut system pathogens (Abdel-Fattah et al., 2008; Ghazala et al., 2011). Chowdhury et al. (2008) reported a higher density of lymphocytes in the cecal tonsils and ileum in citric acid-fed broiler chickens. Moreover, Haque et al. (2010) found an increase in the density of the lymphocytes in the lymphoid organs by 0.5% citric acid supplementation. These findings raise the possibility that dietary organic acids improve both humeral and cellular immunity.

4.6. Serum parameters

The results of serum parameters indicates that both FA and KDF supplementation had no effect on liver and kidney functions. The same were recorded by Hernadez et al. (2006) who stated that using FA at 0.5%, 1.0% in broiler diets had no effect in blood metabolites compared with control.

5. Conclusion

From the above mentioned results, it could be concluded that FA supplementation, irrespective of the form, had a beneficial effect on performance, and immunity of broiler chicken without having any significantly effects on blood biochemical parameters. Moreover, FA is effective against acid intolerant species such as *E. coli*, *Salmonella* and Clostridium count in ceacum. However, KDF is more effective than FA as little amount of FA reaches small intestine due to metabolism and absorption. While KDF permits a proportion of FA to pass through the fore-gut intact and enter the small intestinal tract.

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