Egypt.Poult.Sci.Vol (38)(I): (223-239)(2018) **(1793)**

Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

EFFECT OF ORGANIC ACIDS SUPPLEMENTATION ON NUTRIENTS DIGESTIBILITY, GUT MICROBIOTA AND IMMUNE RESPONSE OF BROILER CHICKS

Faten A. M. Attia[1*]; Haiam S. Abd EL-Haliem[1]; I. H. Hermes[1]; and Manal M. A. Mahmoud[2]

[1]Anim. Prod. Dep., Fac. of Agric.,[2] Nut. and Clinic. Nut. Dep., Fac. of Veter. Med., Suez Canal Uni., Ismailia 41522, Egypt.

*__Corresponding author:__ email: Faten Attia; E-mail: famattia@yahoo.com

Received: 12/02/2018 Accepted: 27/02/2018

ABSTRACT: This study aimed to investigate the effect of citric (CA) and fumaric (FA) acids (0, 15 and 30 g/kg diet for each) on nutrients digestibility, the population of bacteria inhabiting the gut, histopathological changes in the intestine and lymphoid organs and meat physical characteristics of broilers. One–hundred twenty-one-day old unsexed "Cobb" chicks were randomly distributed into five treatments of three replicates eight chicks each. Control (no additives), 15 g CA, 30 g CA, 15 g FA and 30 g FA were evaluated from 0 to 6 weeks of age. Dry matter, crude protein (CP), ash, calcium (Ca), and phosphorus (P) apparent digestibility were significantly improved by 30 g FA/kg diet compared to the control. Fumaric acid at 30 g/kg diet showed higher ether extract, and nitrogen free extract apparent digestibility compared to the control. A significant increment in P and a non-significant higher CP, ash, and Ca apparent digestibility were noticed in 30 g CA/kg diet compared to the control. A significant reduction in total bacterial and total *Enterobacteriaceae* counts in all acid treatments compared to the control, with the least count in 30 g FA/kg diet group. The ileum of organic acids treated birds expressed a healthy intestinal structure with an abundance of goblet cell proliferation. Lymphoid organs showed mild to moderate hyperplasia of their lymphoid follicles indicating immune response improvements. It could be concluded that acids supplementation improved gut microbiota, immune response and apparent nutrients digestibility.

Keywords: Broilers –Citric - Fumaric acid - Nutrient digestibility- Meat characteristics.

INTRODUCTION

Feed additives have beneficial effects on controlling pathogenic and enhancing the growth of beneficial microorganisms. Antibiotics possess these beneficial effects but their use in the poultry industry banned due to fear of antibiotic resistance and its effect on human health. Research for antibiotics alternatives is carried on and some of these promising alternatives are organic acids. Health of the gastrointestinal tract (GIT) is an important factor controlling performance of birds and economics of poultry production. Dietary organic acids (OA) are able to inhibit microbial growth in the food, preserve the microbial balance in the GIT, modify the intestinal pH, and improve the solubility of feed ingredients, digestion and absorption of nutrients (Islam et al, 2008). Data illustrated that OA have antimicrobial activity, depending on acid concentration and the bacterial species exposed to it (Khan and Iqbal, 2016). This study conducted to investigate the effect of citric and fumaric acids supplementation (at two different concentrations) on nutrient digestibility, population of GIT bacteria, histopathological changes in the intestine and lymphoid organs and meat physical characteristics of broilers.

MATERIALS AND METHODS

Management and treatments

This study was approved by Animal department, College of Agriculture, Suez Canal University committees, Egypt and was performed in compliance with relevant laws and institutional guidelines. One hundred twenty-one-day old unsexed "Cobb" broiler chicks obtained from a commercial hatchery, were used. Chicks were weighed, wing-banded and randomly distributed into five treatments × three replicates of eight chicks per replicate. Chicks raised in brooder batteries with wire mesh floors. Feed and water supplied *ad-libitum* during the 6 weeks experimental period. Artificial light provided for 23 hours per day. Chicks were vaccinated at the 5th and the 18th days of age with Hitchner and Lasota**,** respectively while the vaccination at the 14th and the 24th days of age was by Gumboro. Two types of OA (citric, CA and fumaric, FA) at two levels each (15 and 30 g/kg diet) plus control were used. Chicks fed three types of diets (starter, grower and finisher) in mash form (Table 1). Diets formulated according to (NRC, 1994) and chemical analysis was done according to (A.O.A.C, 1990). At the end of the experimental period (6 weeks of age), the following measurements were taken.

Nutrients Digestibility

A digestion trial was performed to determine the total apparent digestibility of nutrients. Four birds from each treatment, close to the average body weight, were used. Birds housed in individual metabolic cages and fed the corresponding finishing diet for seven days for adaptation and another three days for collection. Each cage was prepared with a feeder, water cup and an excreta collection tray. Feed intake and excreta were accurately determined. Daily excreta from each bird was separately collected, pooled for each bird and stored at -20° C until further analysis. Both excreta and feed were dried at 70° C for 24 hours; then weighed, grind and placed in glass jars until chemical analyses. Dry matter, crude protein, ether extract, ash, phosphorus and calcium were determined in the experimental diets and excreta according to the (A.O.A.C, 1990).

Bacteriological analysis of caecal content

At the end of the experimental period, two birds were chosen from each replicate (six birds per treatment) and slaughtered for bacteriological analysis. The contents of one caecum per bird collected aseptically in a sterile test tube. One gram of the content was suspended in 9 ml sterile buffered peptone water; then shaken thoroughly by an electric touch mixer. Viable total bacterial counts were obtained by using ten-fold serial dilutions of samples that plated on nutrient MacConkey agar for total *Enterobacteriaceae* species (Foods, 1998; Guban et al., 2006). All plates incubated for 24 hours at 37°C and the counts were determined by using the dilution that contains between 30 and 300 CFU per plate.

Histopathological examination

Specimens from duodenum, ileum and lymphoid organs (bursa, spleen and thymus) were collected in 10% buffered formalin for histopathological examination (Bancroft et al., 1990).

Meat physical characteristics

Parameters measured were color, tenderness, water holding capacity and pH of fresh meat samples. Color was evaluated using Minolta colorimeter (Minolta Co. Ltd., Osaka, Japan). Color attributes of meat; lightness 'L', redness 'a' and yellowness 'b' were evaluated (Rørå and Einen, 2003). Tenderness (Kg/cm^2) and water holding capacity (WHC, cm^2) were measured (Volovinskaia and Kelman, 1962). Method of (Aitken et al., 1962) was used for measuring pH.

Statistical analysis

Data were subjected to analysis for significance by a one-way ANOVA model (as a completely randomized design) using the General Linear Models (GLM) procedures of SPSS (IBM SPSS statistics, version 22, USA). Treatments differences were considered significant at $P \leq 0.05$ for all measurements except for the bacteriological analysis of caecal content, which was considered significant at $P \leq 0.01$. Means comparisons were performed using Duncan's multiple range tests (Duncan, 1955).

RESULTS

Organic acids (30 g/kg diet) improved the apparent digestibility of all nutrients. Dry matter, CP, ash, Ca and P apparent digestibility were significantly ($P \leq 0.05$) improved by 30 g FA/kg diet compared to the control. Fumaric acid at 30 g/kg diet showed a higher ether extract (EE) and nitrogen free extract (NFE) apparent digestibility compared to the control. A significant increment in P and numerically higher CP, ash, and Ca apparent digestibility in 30 g CA/kg diet compared to the control. Numerical improvements in CP and P apparent digestibility were noticed by 15 g CA or FA supplementation (Table 2).

Gut microbiota was positively altered by OA supplementation ($P \leq 0.01$). A significant reduction in the total bacterial count (TBC) and total *Enterobacteriaceae* count were detected in all acid treated groups compared to the control, with the least count noticed in the 30 g FA/kg diet group (Table 2).

Water holding capacity was significantly reduced by all acids supplementation compared to the control with the lowest values observed for 30 g CA/kg diet group. Also, OA supplemented groups had lower pH with the lowest ($P \leq 0.05$) values noticed for 15 g FA/kg diet. Color and tenderness were numerically affected by OA supplementation (Table 3).

Histopathological examination of the duodenum showed normal integrity of epithelial cell lining as well as duodenal glands. The birds treated with both types of OA had taller villi with prominent goblet cells (Figure1). A healthy intestinal structure with an abundance of goblet cell proliferation that was stained by Alcian blue stain and PAS reaction in the ileum of examined birds. No differences between high and low doses of both OA were seen (Figure 2 and 3). All lymphoid organs (bursa, spleen and thymus) showed mild to moderate hyperplasia of their lymphoid follicles indicating increased immune-response, hyperplasia of the lymphoid follicle and reticuloendothelial cells for all doses of OA, mainly for 15 g CA followed by 15 g FA/kg diet (Figure 4).

DISCUSSION

Results presented in Table 2 reveal improvement in apparent digestibility in 30 g OA treated groups. These results agree with those reported by Ghazalah et al. (2011) who indicated that 5 g fumaric or formic acid and 7.5 g acetic or 20 g CA/kg diet improved digestibility of CP, EE, CF and NFE of broiler diets. Also, 20 g CA/kg diet improved the retention of DM, CP and neutral detergent fiber in broiler diet (Ao et al., 2009). Gross energy, EE, and CP digestibility were significantly lower in the non-treated group compared to ascorbic acid supplemented group (Lohakare et al., 2005).

Positive effects of OA on digestibility were related to the slower feed passage in the intestinal tract and the nutrients absorption improvement (Van Der Sluis, 2002). In addition, these positive effects might be due to reducing the competition of microorganisms with the host nutrients, reducing the endogenous nitrogen losses and ammonia production (Omogbenigun et al., 2003). Moreover, OA improve gastric proteolysis and enhance the digestibility of protein and amino acids (Samanta et al., 2010). Also, it was reported that lowering the pH of chime increase the pepsin activity, which leads to enhancing the protein digestibility (Afsharmanesh and Pourreza, 2005). Furthermore, OA increase production of pancreatic juice that might lead to protein digestion improvement as a result of the presence of high concentration of trypsinogen, chymotrypsinogen A, chymotrypsinogen B, pro-carboxy peptidase A and pro-carboxy peptidase B (Adil et al., 2010).

Supplementation of OA may enhance the digestibility of minerals and increase utilization of phytate phosphorus (Boling et al., 2000; Park et al., 2009). Organic acids supplemented in broiler diet caused enhanced digestibility and improved nutrients availability (Ca and P). This might be due to developing desirable microflora (Lactobacillus spp.) in GIT, which in turn increase elements retention and bone mineralization (Ziaie et al., 2011). The acidic anion forms a complex with Ca, P, magnesium and zinc**,** and results in minerals digestibility improvement (Edwards and Baker, 1999).

The present research indicated a significant reduction in the total bacterial and *Enterobacteriaceae* counts in OA treated groups. This is in harmony with Gunal et al. (2006) who showed that the use of OA mixture significantly decreased the total bacterial and gram-negative bacteria counts in broiler chicken. Józefiak et al. (2007 found a bacterial growth depression in broilers fed OA and concluded that the changes in microbial populations are in the lower parts of the gut (i.e. caeca). Also, Pirgozliev et al., (2008) reported a significant reduction in total Coliform numbers in the ileum and caecum of broiler treated with OA. These acids can penetrate the bacterial cell wall and disrupt the normal physiology of the 'pH sensitive' ones that cannot tolerate a wide internal and external pH gradient. Besides, OA might have a direct effect on poultry GIT bacterial population, resulting in a reduction of some pathogenic bacteria levels, which compete with birds for nutrients (Khan and Iqbal 2016).

Meat pH, WHC, tenderness and color are important characteristics to evaluate meat quality that affects consumer opinion of palatability and products cost. The pH value reflects the meat acid content and affects the meat texture, drip loss and color. In the present study, OA treatment significantly lowered WHC with numerical pH and color reduction. Similarly, supplementing broiler diets with 0.25, 0.5 and 0.75 g microencapsulated blends of organic acids and essential oils (250 g CA, 167 g sorbic acid, 17 g thymol, 10 g vanillin and 556 g starch as a carrier) had no significant effects on neither the color nor the pH values of breast meat (Mohammadi Gheisar et al., 2015). On the other side, dietary 1.0, 2.0 and 4.0 g phenyl lactic acid/kg diet (Kim et al., 2014) or 2.0 and 4.0 g/kg diet (Wang et al., 2010) had no effect on the pH, L, or a values of broilers' breast meat; although, the WHC and shear force values were increased and b value was decreased significantly. Zhang et al. (2008) showed that diets treated with 2.5, 5.0 and 7.5 ginosinic acid/kg diet had no effect on the color of breast meat or L and a values of thigh meat, but the b value of thigh meat was decreased when birds fed 7.5 g inosinic acid. The contradictory between the data reported here and the former findings might be due to the type of acids used concentrations and diet composition.

Intestinal health is an important key for efficient nutrient absorption that leads to improving feed efficiency and growth of broilers. The current data revealed that duodenum of birds treated with OA had taller villi with prominent goblet cells. In addition, ileum of examined birds expressed a healthy intestinal structure with the abundance of goblet cell proliferation. Same effects were reported by Abdelrazek et al. (2016); Kum et al. (2010) and Panda et al. (2009). They found that OA supplementation significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler. Adil et al (2010) observed the highest duodenal, jejunal and ileal villus heights in birds fed diets treated with 3.0g butyric acid, 30g FA and 20g FA/kg of diet, respectively. Crypt depth of duodenum, jejunum and ileum were not affected by different treatments. In addition, muscularis thickness was decreased in all segments of small intestines. Reduction in muscularis thickness enhanced digestion and absorption of nutrients. As indicated by the results reported here, OA reduced the growth of many pathogenic intestinal bacteria. Therefore, improves villus height and function of secretion, digestion and absorption of nutrients which resulted from reducing intestinal colonization, infections, and inflammation of the intestinal mucosa.

The major constituents of the avian immune system are the lymphoid organs. In this study, the lymphoid organs (bursa, spleen and thymus) had mild to moderate hyperplasia of their lymphoid follicles, which indicate that the immune response was increased. Previous studies showed that OA might stimulate a natural immune response in poultry. Abdel-Fattah et al. (2008) and Ghazalah et al (2011) showed that birds fed diet supplemented with OA had heavier immune organs (bursa of fabricius and the thymus) and increased level of globulin in their serum. They suggested that the improvement in immunity might be due to the inhibitory effects of these OA on gut pathogens. In addition, OA supplementation caused hyperthyroidism and peripheral conversion of T4–T3, which indicate that these birds had better immune competence and bursa growth (Abde-Fattah et al., 2008). Citric acid at 5.0 g/kg diet enhanced the density of lymphocytes in lymphoid organs, improving non-specific immunity (Haque et al., 2010). In another study, post vaccination significantly increased infectious bursal disease antibody titers in birds fed on a diet supplemented with ascorbic acid. This could be due to increasing the activity of the hexose monophosphate pathway that leads to speeding up of differentiation of lymphoid organs, thus increasing the circulating antibody (Lohakare et al., 2005).

CONCLUSION

This study indicated that OA supplementation, irrespective of type and level, improved gut microbiota of broilers. The birds supplemented with both types of OA had taller villi with prominent goblet cells of duodenum and ileum. In addition, OA improved nutrient total apparent digestibility. For economic purposes, we recommend the use of lower level of OA (15 g/kg diet). Further research is required under the practical commercial production conditions.

ACKNOWLEDGEMENT

The authors wish to express their deepest gratitude for Dr. Dalia Mansour, Poultry and Rabbit Disease Department, for the bacteriological analysis, and Dr. Amina Dessouki, Department of Pathology, Faculty of Veterinary Medicine for the interpretation of histopathological samples.

Table (1): Composition and chemical analysis of the experimental diets.*

Ingredients g/kg diet	Starter (0-3rd wk.)			Grower (4-5th wk.)			Finisher (6th wk.)		
Yellow corn	521.3	500.0	500.0	603.9	586.0	562.1	650.0	630.0	609.8
Soybean meal (44%)	294.4	290.5	239.7	260.0	238.1	234.9	263.8	248.8	233.5
Corn gluten meal 60%	96.5	100.0	137.0	60.0	80.0	85.0	15.0	30.0	45.0
Vegetable oil	47.0	53.5	51.0	40.0	44.0	51.0	40.0	45.0	50.0
Ground limestone	14.1	14.0	14.1	14.4	15.0	15.0	13.0	13.1	13.2
Di-Ca phosphate	16.8	17.0	17.5	12.2	12.3	12.3	10.0	10.0	10.0
Vit.& min. premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
DL- Methionine	1.0	1.0	0.6	0.3	0.1	0.1	0.2	0.1	0.1
L-Lysine	0.9	1.0	2.1	1.2	1.5	1.6	0.0	0.0	0.4
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Organic acid level[2]	0.0	15.0	30.0	0.0	15.0	30.0	0.0	15.0	30.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated values g/kg diet									
ME (Kcal/Kg diet)	3193	3184	3190	3192	3195	3190	3182	3182	3183
Crude protein	235.1	232.8	235.2	204.2	205.6	205.4	180.7	181.7	182.9
Calcium	10.01	10.00	10.01	9.02	9.22	9.20	8.04	8.03	8.02
Inorganic Phosphorus	4.50	4.50	4.51	3.55	3.52	3.50	3.12	3.08	3.05
Lysine	11.08	11.04	11.05	10.06	10.09	10.10	8.94	8.64	8.69
Methionine	5.18	5.17	5.02	3.89	3.82	3.83	3.23	3.22	3.31
Methionine & Cysteine	9.11	9.07	9.00	7.33	7.33	7.33	6.30	6.33	6.46
Determined values g/kg diet									
Moisture	81.4	81.4	84.7	80.5	80.5	84.4	77.2	80.2	80.1
Crude protein	229.0	227.5	227.8	198.5	199.2	198.3	178.9	179.2	178.7
Crude fiber	35.0	35.1	35.3	38.4	36.9	37.4	37.5	35.3	35.5
Ether extract	37.4	38.2	37.8	34.9	39.2	36.3	35.4	34.4	35.7
Ash	71.2	72.5	75.0	75.6	76.1	78.0	61.2	59.7	59.0
Nitrogen free extract	546.0	545.3	539.4	572.1	568.1	565.6	609.8	611.2	611.0

*Diets formulation according to (NRC, 1994) and chemical analysis according to (AOAC, 1990).

[1]Supplied per kg diet: vitamin A (retinol acetate), 3600 µg; vitamin D_3 (cholecalciferol), 50µg; vitamin E (α-tocopheryl acetate), 10 mg; vitamin K_3 (Menadione), 2.0 mg; Thiamine, 1.0 mg; Riboflavin, 5.0 mg; Pyridoxine, 1.5 mg; Cyanocobalamin, 0.01 mg; Pantothenic acid, 10 mg; Nicotinic acid, 30 mg, Folic acid, 1.0 mg; Biotin, 0.05 mg; Choline chloride, 250 mg; Cu, 10 mg; I, 1.0 mg; Fe, 50 mg; Zn, 50 mg; Mn, 60 mg; Co, 0.1 mg; and Se, 0.1 mg.

[2]Citric or fumaric acids**.**

Table (2): Effect of organic acids supplementation in broiler diets on apparent nutrients digestibility and bacteriological count (Mean± SE)

Parameters	Treatment (g/kg diet)				
	Control	**CA15**	**CA30**	**FA15**	**FA30**
*Apparent digestibility coefficient (g/g)					
DM	0.780^{b}±0.011	0.760^{b} ±0.011	0.786^{b} ±0.015	0.769^{b} ±0.002	0.823^{a}±0.016
CP	0.611^{b} ±0.020	0.625^{b} ±0.016	0.665^{ab} ±0.021	0.628^{b} ±0.002	0.728^{a} ±0.025
EE	0.694^{ab}±0.019	0.604^{c}±0.030	0.648^{bc} ±0.027	0.651^{bc}±0.003	73.31^{a} ±0.029
Ash	0.346^{b} ±0.031	0.339^{b} ±0.036	0.371^{b} ±0.022	0.326^{b} ±0.009	0.523^{a} ±0.036
NFE	0.902^{ab} ±0.003	0.876^{c} ±0.006	0.894^{ab} ±0.008	0.891^{bc} 0.002	0.912^{a} ±0.007
Ca	0.734^{b} ±0.027	0.723^{b} ±0.015	0.747^{ab} ±0.028	0.727^{b} ±0.004	0.798^{a} ±0.013
P	0.227^{c} ±0.034	0.241^{c} ±0.028	0.355^{b} ±0.015	0.244^{c} ±0.010	0.488^{a} ±0.022
^{1}Bacteriological count (mean ×10^{5}cfu/g of sample)					
T. bacterial count	88.33^{a} ±4.41	33.33^{b} ±4.41	20.00^{c} ±5.77	10.33^{cd} ±2.60	3.67^{d} ±0.88
T. Enterobacteriaceae	70.00^{a} ±5.77	14.00^{c} ±2.31	30.00^{b} ±5.77	15.00^{c} ±2.89	8.00^{c} ±1.16

*Means with different superscripts in the same row are significantly different ($P \leq 0.05$).

[1]Means with different superscripts in the same row are significantly different ($P \leq 0.01$).

CA = citric acid (15 or 30 g)   FA = fumaric acid (15 or 30 g)

Table (3): Effect of organic acids supplementation in broiler diets on meat physical characteristics (Mean± SE)

Parameters	**Treatments (g/kg diet)**				
	Control	**CA15**	**CA30**	**FA15**	**FA30**
Color attributes of meat:					
Lightness 'L'	52.47±0.70	50.61^{a} ±0.31	51.19^{a} ±0.83	50.63^{a} ±0.43	50.66^{a} ±0.68
Redness 'a'	2.81±0.20	2.85^{a} ±0.22	2.56^{a} ±0.17	2.52^{a} ±0.13	2.96^{a} ±0.28
Yellowness 'b'	11.02 ±0.32	10.67^{a} ±0.37	10.10^{a} ±0.29	10.28^{a} ±0.24	10.43^{a} ±0.41
Tenderness(Kg/cm^2)	3.46±0.08	3.66^{a} ±0.16	3.60^{a} ±0.13	3.50^{a} ±0.06	3.39^{a} ±0.009
Water holding capacity ($cm^{2)}$	3.10 ±0.05	2.43^{bc}±0.11	2.28^{c}±0.09	2.65^{b} ±0.12	2.71^{b}±0.09
pH	6.03±0.13	5.91^{ab}±0.06	5.73^{ab} ±0.10	5.54^{b}±0.12	5.66^{ab} ±0.17

Means with different superscripts in the same row are significantly different ($P \le 0.05$).

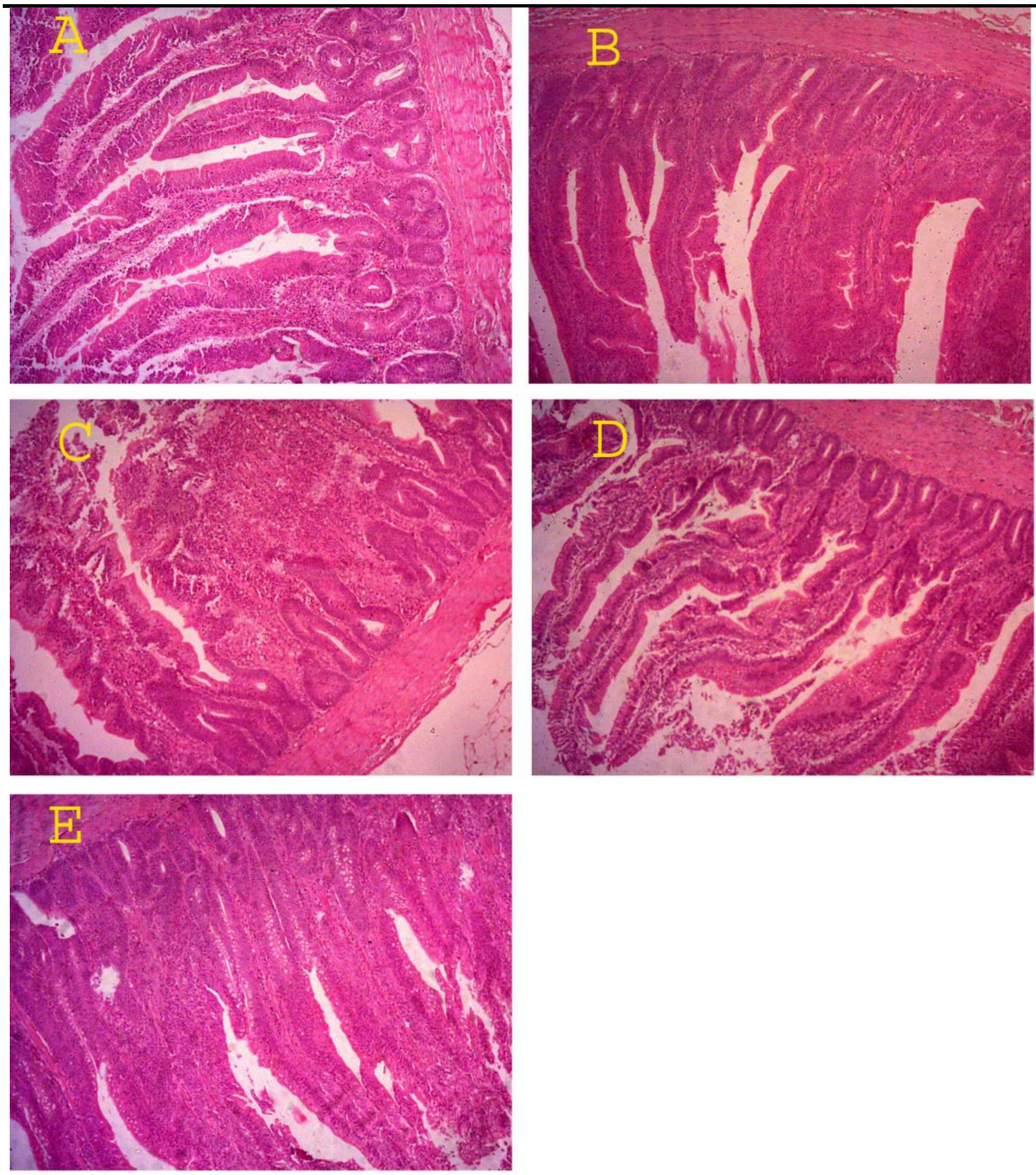


Figure (1): Duodenum, (A) Control showing normal histological structure. (B) Citric acid (15 g/kg diet) showing a mild increase in the number of goblet cells in epithelial lining the villi. (C) Citric acid (30 g/kg diet) showing mild to moderate sloughing and desquamation of epithelial lining the villi. (D) Fumaric acid (15 g/kg diet) showing normal histological integrity of epithelial cell lining the villi. (E) Fumaric acid (30 g/kg diet) showing hyperplasia of epithelial lining the villi; H&E. X 200.

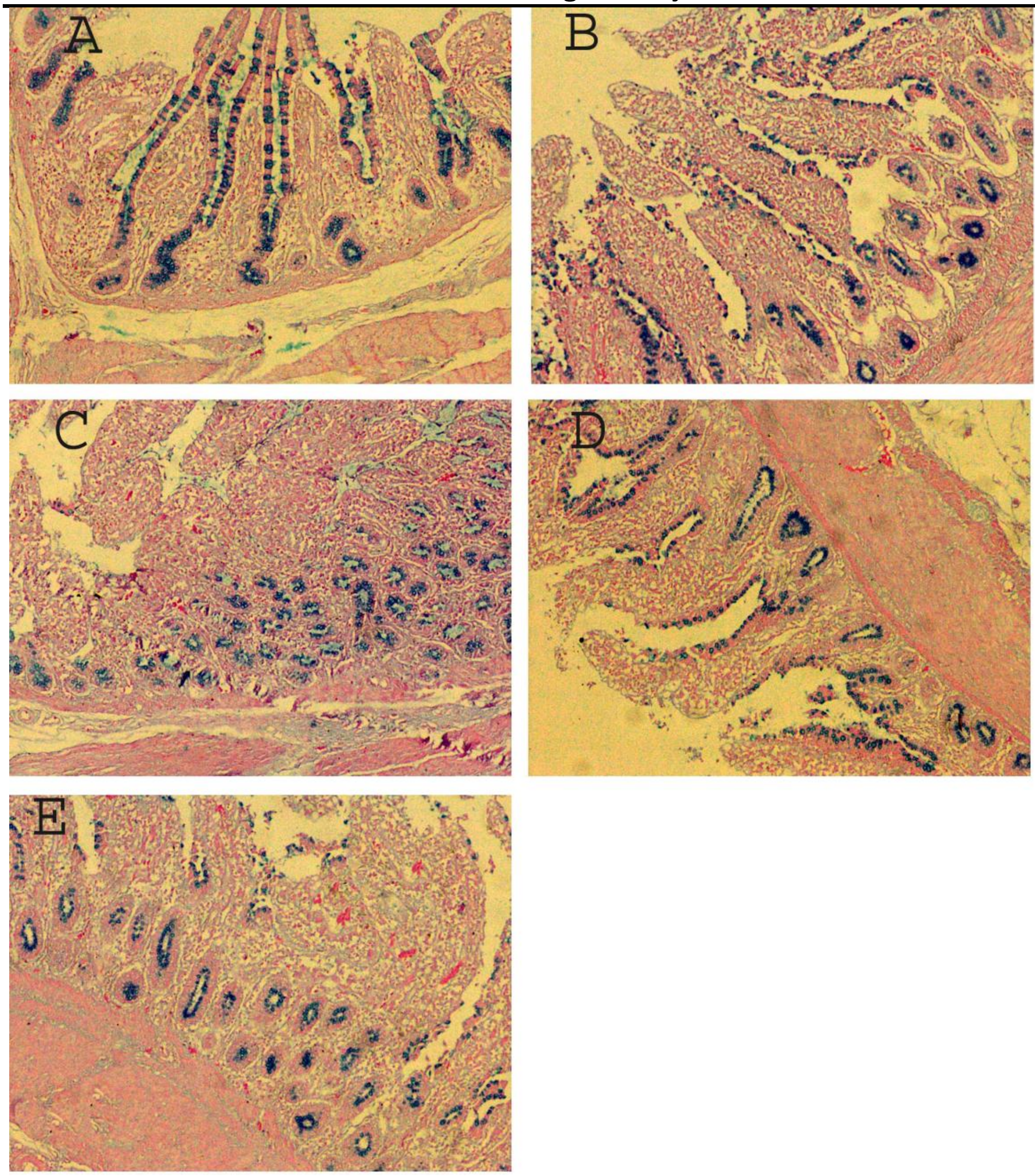


Figure (2): Ileum,(A) Control showing no pathological changes**. (**B) Citric acids (15 g/kg diet) showing basophilic granules represent goblet cells activity**. (**C) Citric acid (30 g/kg diet) showing slight increase number of mucous secreting cells. (D) Fumaric acid (15 g/kg diet) showing an increase in the number of goblet cells especially in intestinal crypt (basophilic granules). (E) Fumaric acid (30 g/kg diet) showing marked increase in the number of goblet cells in intestinal crypts; Alcian blue. X 200.

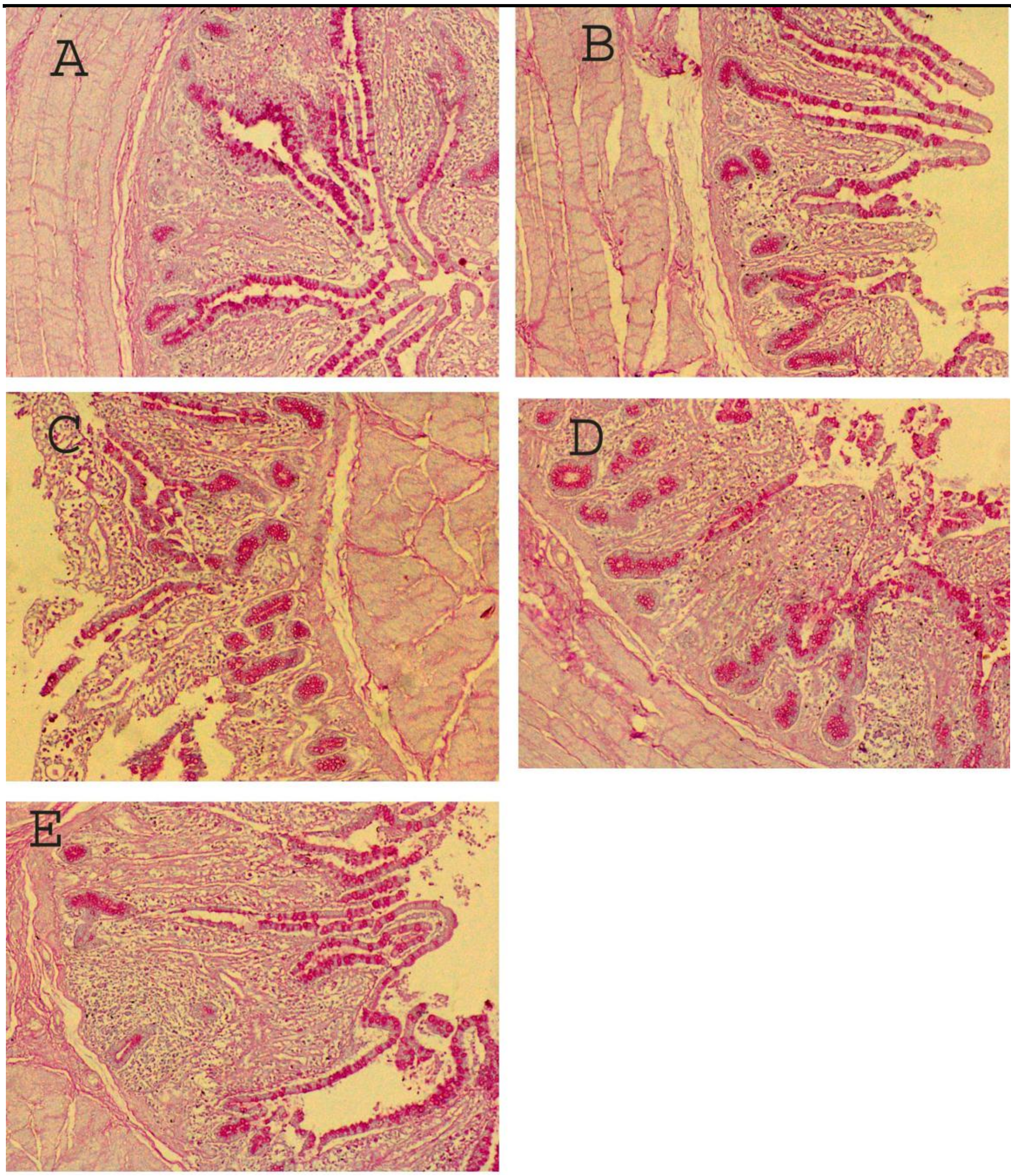
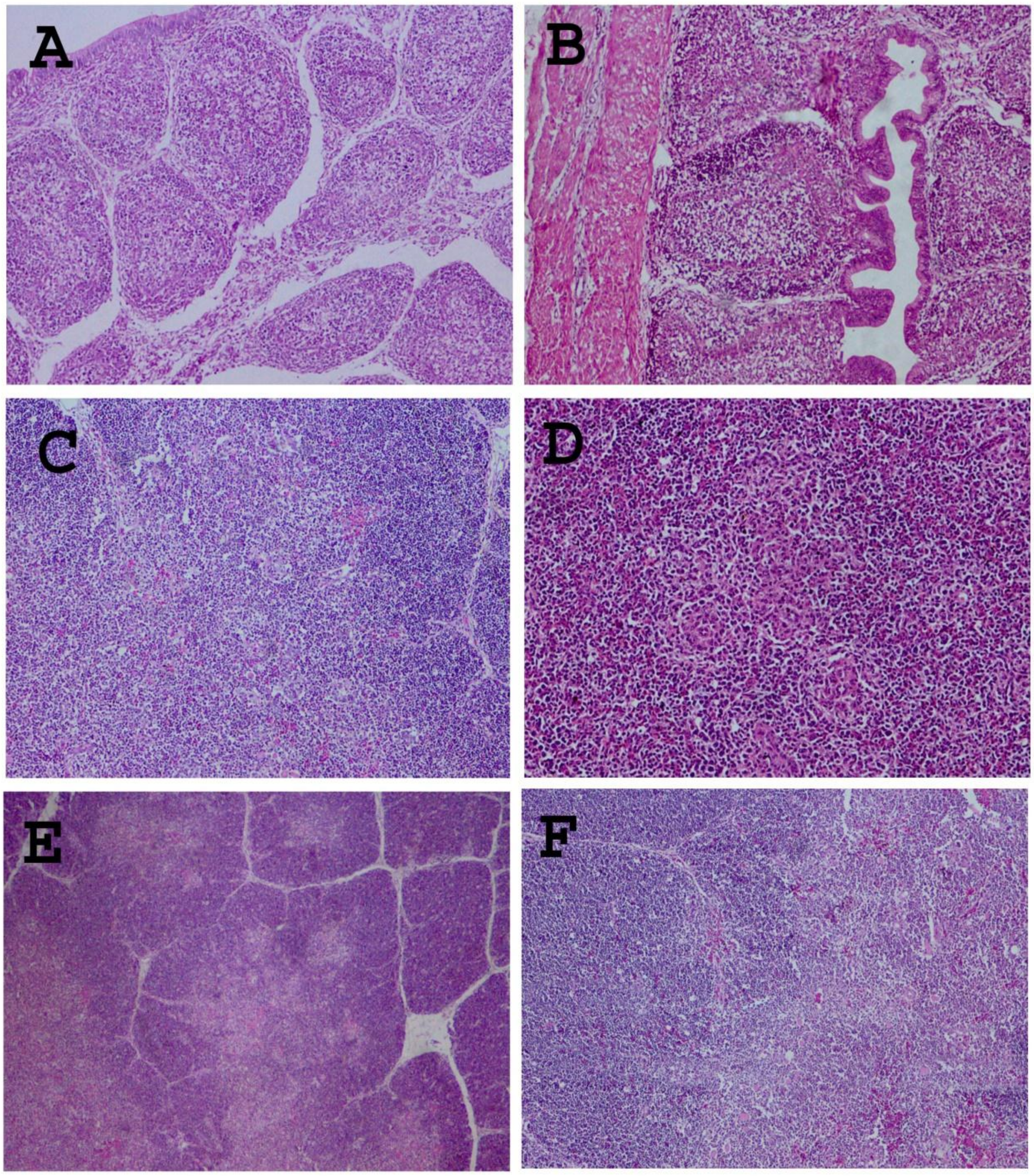


Figure (3): Ileum, (A) Control showing a moderate increase in the number of goblet cells that appeared by PAS as eosinophilic granules. (B and C) Citric acid (15 and 30 g/kg diet) showing increased the number of mucous secreting cells. (D) Fumaric acid (15 g/kg diet) showing marked increase number of mucous secreting as eosinophilic granules. **(**E) Fumaric acid (30 g/kg diet) showing a moderate increase in the number of mucous secreting cells; PAS Reaction. X 200.



Figure(4): Supplementation of citric or fumaric acids (15 g/kg diet) showing the following: (A and B) Bursa of normal to mildly hyperplastic lymphoid follicles; (C and D) Spleen of moderate hyperplasia of white pulp and reticuloendothelial cells;(E and F) Thymus of hyperplasia of lymphocytes forming the cortex and medulla of the thymus; H&E. X 200.

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Broilers –Citric - Fumaric acid - Nutrient digestibility- Meat characteristics.

الملخص العربى

تأثير إضافة الاحماض العضوية على هضم المركبات الغذائية وبكتريا القناة الهضمية والاستجابة المناعية فى كتاكيت اللحم

[1]فاتن عبد العزيز محمود عطية ، هيام سيد عبد الحليم ، إسماعيل حافظ هرمس ، [2]منال محمود

[1]قسم الانتاج الحيوانى – كلية الزراعة - [2]قسم التغذية والتغذية الأكلينكية – كلية الطب البيطرى – الاسماعلية ،45122 – جامعة قناة السويس – جمهورية مصر العربية

هدفت هذه الدراسة الي التعرف علي تأثير كلا من حمض الستريك وحمض الفيوماريك (15، 30 جم / كجم عليقة) علي هضم المركبات الغذائية ، العشائر البكتيرية للقناة الهضمية ، التغيرات الهستوباثولوجية للأمعاء والأعضاء الليمفاوية والخصائص الطبيعية للحوم دجاج التسمين. تم توزيع 120 كتكوت عمر يوم من سلالة ال Cobb الغير مجنسة توزيعا عشوائيا علي خمس معاملات، كل منها تضم ثلاث مكررات، بكل مكررة ثمانية كتاكيت. نتج تحسن ملحوظ في معاملات الهضم الظاهري لكل من المادة الجافة ، البروتين الخام ، الرماد ، الكالسيوم والفوسفور وذلك عندإضافة الأحماض العضوية بمستوي 30 جم/ كجم عليقة مقارنة بمجموعة الكنترول. أظهر حمض الفيوماريك (30 جم / كجم عليقة) أعلي معامل هضم ظاهري لكل من مستخلص الإثير والمستخلص الخالي من الأزوت مقارنة بمجموعة الكنترول. لوحظ زيادة معنوية في معامل الهضم الظاهري للفوسفور وغير معنوية بالنسبة لمعاملات هضم البروتين الخام ، والرماد والكالسيوم بالنسبة لمجموعة ال 30 جم حمض ستريك /كجم عليقة مقارنة بمجموعة الكنترول. كذلك لوحظ إنخفاض معنوي في العدد الكلي للبكتيريا وكذلك العدد الكلي للبكتيريا المعوية المعدية (انتيروبكترياسى) بالنسبة لكل المجاميع المضاف لعلائقها الأحماض العضوية مقارنة بمجموعة الكنترول. واتضح ان أنسجة اللفائفي للطيور المعاملة بالأحماض العضوية كانت سليمة وبها زيادة في عدد الخلايا الكأسية (خلايا جوبلت). أظهرت الأعضاء الليمفاوية تضخم خفيف إلى متوسط في الحويصلات الليمفاوية والتي تشير الي تحسن الإستجابة المناعية.

يمكن الإستنتاج أن إضافة الأحماض العضوية حسنت كل من العشائر البكتيرية في القناة الهضمية والإستجابة المناعية ومعاملات الهضم الظاهري للمركبات الغذائية.