Effect of Acidifiers on Gastrointestinal Tract Integrity, Zootechnical Performance and Colonization of *Clostridium Perfringens* and Aerobic Bacteria in Broiler Chickens

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Abstract: This experiment was to investigate the effect of acidifiers (Protected organic acidifier, CAPacid®, Neovia, France) on gastrointestinal tract (GIT) integrity, zootechnical performance and colonization of *Clostridium perfringens(C. perfringens)* (type C) and aerobic bacteria in broilers from 1 to 42 days of age under commercial conditions. Obtained results clarified that broiler diets supplemented with acidifier could improve chicken performance ($P < 0.05$). Also, it decreased the mortality rate, intestinal and cecal colonization of both *C. perfringens* (naturally present or experimentally induced) and the total aerobic bacteria. The macroscopic and microscopic lesion scores associated with *C. perfringens* infection were also decreased ($P < 0.05$). The current study has shown the interest of using protected organic acidifiers into the feed of broiler chickens submitted to *C. perfringens* infection. In addition, taking in consideration the facts that organic acids do not require withdrawal period, that bird performance are positively affected by their use and that they increase the shelf-life of products, they can make a valuable contribution to flock health and safety of poultry products as food. This may provide a significant tool for the poultry industry in combating the occurrence of intestinal diseases and in reduction of food borne pathogens.
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Key words: Chickens, Acidifier, *Clostridium perfringens*, Aerobic bacteria, gastrointestinal tract integrity.

1. Introduction:

Microbial imbalance has a deteriorating effect on GIT integrity. The later is a major challenge facing poultry industry specially after banning the sub-therapeutic use of antibiotics growth promoters in 2006. Consequently, feed formulators need efficient alternatives to use as commodities. Natural alternatives concepts based on natural ingredients for GIT integrity and antibacterial action are highly commendable. Lückstädt (2003) mentioned under this point of view that acidifiers can be part of the feeding concept to fill the gap from the antibiotics and to replace antibiotic growth promoters. Because of their pH-reducing and antimicrobial effects, acidifiers appear as one of the most feasible and functional alternative to antibiotics growth promoters. Accordingly, experts in the poultry industry have given the use of acidifiers closer scrutiny. The auspicious effect of acidifiers over the organism is due to the better adhesion of the lactic acid bacteria to the intestinal epithelium in comparison with the pathogenic bacteria, and stooping the implementation of those bacteria over the mucus membranes of the intestine.

Among these micro-organisms, *C. perfringens* is an obligate anaerobic bacterium in the intestinal tract of chickens (Johansson and Sarles, 1948; and Shapiro and Sarles, 1949). This organism is relatively innocuous unless cofactors occur such as undigestible dietary ingredients or diet rapid changes, severe stress, coccidiosis, or immunosuppressive affections (Barnes, 1997). The bacterium's pathogenicity is largely derived from its prolific ability to express protein toxins that are active in the GIT (McClane, 2001). Alpha toxin produced by *C. perfringens* types A and C, and beta toxin produced by *C. perfringens* type C, are those believed responsible for necrotic enteritis which is a common problem among rapidly growing broiler strains of chickens that are raised intensively in modern microenvironments (Kohler et al., 1974 and Hofarce, 1998), and that threatens GIT health and livability of many poultry flocks (Craven et al., 2001). *C. perfringens* may cause damage to the intestinal tract,

leading to poor feed efficiency, decreased rate of gain and increased production costs. On the other hand, *C. perfringens* is responsible for severe food borne enteritis in man and its enterotoxin has been shown to be responsible for food poisoning (Brynestad, 2002). The presence of *C. perfringens* in food such as meat or poultry may be unavoidable (Ghadban et al., 1998).

The potential advantage of organic acids in the feed of poultry has been proven and well documented for decades. However, new trials are still necessary to establish performance results under different production conditions. The purpose of this study is to adopt by both subjective and objective criteria a semi-field trial in an attempt to investigate the effect of a protected acidifier compound (CAPacid®, Neovia) in reducing intestinal and cecal colonization with *C. perfringens* type C and total aerobic bacteria as well as its effect on the zootechnical performance of broiler chickens.

2. Material and Methods

Experimental design

Day old Arbor Acres Plus chicks (n=900) were used in this semi-field trial study. These birds were allotted into 4 equal groups (1-4) of 225 birds assigned into 3 replicates of 75 each. Chicks from group 1 were not infected with *C. perfringens* and received CAPacid® in the feed. Chicks from group 2 were not infected with *C. perfringens* and received control feed without CAPacid®. Chicks from group 3 were infected with *C. perfringens* and received CAPacid® in the feed. Chicks from group 4 were infected with *C. perfringens* and received the control feed without treatment. Chicks were floor reared in pens and kept in environmentally controlled rooms. Chicks of all groups were vaccinated against Newcastle disease and infectious bronchitis using Hitchner B1+ H120 vaccines at 7th day of age and against Avian Influenza at 10th day of age using H5N2 vaccine. Revaccination against Newcastle disease using La Sota vaccine and vaccination against infectious bursal disease using 228-E IBDV vaccine were given at 14th day of age. Avian influenza vaccine was given subcutaneously while other vaccines were administered via drinking water. At 21 d of age, 10 chickens from the 3 replicates of groups 3 and 4 (30 birds / each) were subcutaneously inoculated with 0.5 x 10^8 CFU / bird of *C. perfringens* in phosphate buffered saline (PBS) according to Awaad et al. (2005).

Feeding

Chickens were fed commercial starter ration (23% C.P. and 12’552 kJ/kg ME/kg diet) during the first 3 wk of age, and then commercial finisher ration (19% C.P. and 13’388.8 kJ/kg ME/kg diet) for the final 3 w. The diet compositions are indicated in (Table 1). The ration (mash) was given with the acidifier CAPacid® (Neovia) in a dose of 750 g / T and 250 g / T in starter and finisher rations only for the treated broiler chicken groups respectively. Those untreated groups were fed on a plain ration. During the entire experiment, feed and water were provided with *ad libitum* consumption. Diclazuril (Clinacox) was added as a coccidiostate. No antibiotics were added to the ration.

Table 1. *Ingredient percentage and calculated analysis of broiler diets*

	Starter	Finisher
Ingredients (%)		
Yellow corn	52.45	62.85
Soybean meal 44%	33.24	22.11
Corn gluten meal 60%	7.00	6.65
Oil	3.00	4.00
Di-calcium phosphate	1.80	1.80
Lime stone	1.30	1.30
DL-Methionine	0.22	0.23
Lysine hydrochloride	0.29	0.36
Sodium chloride	0.40	0.40
Premix[1]	0.30	0.30
Calculated composition		
Crude protein (%)	23.00	19.00
Metabolizable energy (kJ/kg)	12’552.00	13’388.80

[1]Each gram of mineral mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg..

Measured parameters:

1. Zootechnical performance:

Chicken performance response variables were determined according to Brady (1968), Sainsbury (1984) and North (1984); Body weight (wt.) and wt. gain were measured on all animals. Feed consumption (g / d / bird), feed conversion ratio (FCR) (g feed / g live body wt.) and carcass characteristics (live body wt., carcass wt., dressing (%), front part {wt. (g) and %}, hind part {wt. (g) and %}, liver wt., heart wt., gizzard wt. and intestine (length and diameter) were measured on birds of groups 1 and 2. For body wt. all birds were weighed individually at 1 d and at 6 wk of age. Feed consumption was measured on the same days of birds weighting.

2. Bioassay:

Intestinal and cecal colonization of *C. perfringens* and total aerobic bacteria were evaluated at 0, 3, 7, 14 and 21 d post infection (PI). Four birds were sacrificed at each date in each pen (12 birds per treatment and per date, 60 birds per treatment for the whole trial, and 240 birds for the whole trial). For colonization of *C. perfringens* from each bird, a portion of the intestinal and cecal contents (0.2 g) were serially diluted in sterile PBS to 1:100, 1:1000, or 1:10000 and 0.1 ml of each dilution and were poured on the surface of sheep blood agar plates and tryptose sulfite-cycloserine (TSC) agar (supplemented by D-cycloserine) with egg yolk emulsion. This was overlaid with the same medium but without egg yolk. After anaerobic incubation at 37°C for 24 hours, typical *C. perfringens* colonies (black colonies) on TSC agar or large dom-shaped colonies with a double zone of hemolysis on blood agar plate, were counted and reported as colony-forming units (CFU) per gram and picked and confirmed by criteria of Harmon (1984) and Carrido et al. (2004). For total aerobic bacterial colonization, 10^{-1} to 10^{-8} serial dilutions were made of intestinal and cecal contents (0.2 g) in sterile PBS from each bird and 0.1 ml of each dilution was plated onto blood agar plate and nutrient agar. The plates were incubated aerobically at 37°C for 24 hours and colonies were counted and calculated per gram.

3. Histopathological assay:

Specimens including liver and intestine were randomly collected from sacrificed chickens at 3, 7, 14 and 21 d PI, fixed in 15% buffered formalin. Paraffin-embedded sections were routinely prepared and stained with Hematoxylin and Eosin (Bancroft et al., 1996), and scored for histopathological lesions according to the method described by Rosales et al. (1989).

Statistical analysis:

Weights and body weight gains were subjected to analysis of variance in a complete design with infection and CAPcid treatment as fixed factors. If significant differences were observed, the mean of groups were compared using Duncan mean test comparison. Other data were treated according to Snedecor (1956) and Cochran and Cox (1960).

3. Results and Discussion

Global potential for animal feed acidifiers and other health products for animals are on rise due to the higher need for top quality poultry. Stable demand from developed countries for meat coupled with escalating consumption in the developing world, improving living standards, and swelling of population are expected to propel the worldwide demand for animal feed additives. On the other hand, food safety is probably the biggest issue facing poultry production systems today. Consumer confidence has a direct correlation to the safety and wholesomeness of the product they will purchase. Preventing contamination of poultry products with food borne pathogens remains a considerable challenge for producers and integrations. One of these food borne pathogens is *C. perfringens* which is responsible for the rare but severe food borne necrotic enteritis in man (enteritis necroticans or pig-bel disease) which is fatal specially in young and elderly and its enterotoxin has been shown to be the virulence factor responsible for causing the symptoms of *C. perfringens* type A food poisoning which is the more common in the industrialized world (Ghadban et al., 1998 and Brynestad, 2002). Hence the widespread use of antibiotics as therapeutic agents and growth promoters result in the development of resistant population of bacteria which made their subsequent use for therapy difficult and result in occurrence of antibiotic residues in the poultry products (DuPont and Steele, 1987); the direction towards the use of environmentally friendly alternatives as natural control method has been emerged. To reduce the risk factors associated with enteropathogens, the industry has installed programs to reduce their incidence. One of these programs is addition of feed acidifiers which has contributed immensely to the minimization of the pathogens.

In the present semi-field trial, the zootechnical performance variables in naturally induced *C. perfringins* chickens showed significant improvement in CAPacid® treated group over the non-treated control group. Regardless statistical analysis, FCR was lower in CAPacid® treated group over the control one at all examined times (Table 3). Statistically significant increase was recorded in

Table 3. *Performance of non infected birds (n=200)*

	Body weight (g)						
	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
Non infected Control	43± 2.9	138± 8.8	324± 9.4	636± 112.5	1007± 157.2	1528± 173.5	2009± 196.7
Infected CAPacid	43±1.8	133±17.5	343±53.9	663± 103.6	1072± 163.8	1581± 194.5	2055± 226.9
Significance	*NS*	*< 0.01* **	*< 0.01* **	*< 0.05* *	*< 0.001* ***	*< 0.01* **	*< 0.1*
	Body weight gain (g)						
	0 – 1 wk	1 – 2 wk	2 – 3 wk	3-4 wk	4 -5 wk	5 – 6 wk	0 – 6 wk
Non infected Control	95 ± 18.2	187± 1.9	310± 82.7	376± 120.4	520± 138.0	472± 132.4	1966± 196.8
Infected CAPacid	90 ± 17.7	210± 6.4	319± 82.4	419± 136.8	504± 130.2	471± 134.8	2012± 227.0
Significance	*< 0.01* **	*0.001***	*NS*	*< 0.01* **	*NS*	*NS*	*< 0.05* *
	Feed consumption (g/d/bird)						
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	
Non infected Control	21.49 ^{a}± 0.04	48.46 ^{b}± 0.68	89.26± 1.83	116.54± 3.59	162.94± 9.00	166.42± 1.45	
Infected CAPacid	19.69 ^{b*}± 0.30	53.37 ^{a}± 1.11	87.36± 1.12	119.53± 4.85	162.99± 8.60	170.21± 2.30	
Significance	*< 0.001* ***	*< 0.05**	*NS*	*NS*	*NS*	*NS*	
	Feed conversion ratio (g feed : g live body weight)						
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	Cumulative
Non infected Control	1.577± 0.027	1.840± 0.047	2.017± 0.057	2.207± 0.086	2.275± 0.135	2.538± 0.045	2.218± 0.049
Infected CAPacid	1.556± 0.028	1.793± 0.042	1.925± 0.045	2.149± 0.125	2.191± 0.105	2.556± 0.047	2.152± 0.049
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
	Carcass characteristics						
	Live body weight (g)	Carcass weight (g)	Dressing (%)	Front part weight		Hind part weight	
				(g)	(%)	(g)	(%)
Non infected control	1993.38±25.90	1438.00^{b}±20.07	72.18±0.41	789.50^{b}±11.81	39.62±0.28	647.88^{b}±9.37	32.53±0.25
Infected CAPacid	2058.72±21.02	1502.31^{a}±18.41	72.95±0.46	823.46^{a}±10.47	39.99±0.30	678.85^{a}±9.35	32.95±0.28
Significance	*NS*	*< 0.05* *	*NS*	*< 0.05* *	*NS*	*< 0.05* *	*NS*
	Carcass characteristics							
	Liver weight		Heart weight		Gizzard weight		Intestine (cm)	
	(g)	(%)	(g)	(%)	(g)	(%)	Length	Diameter
Non infected control	64.38^{b}±1.39	3.208±0.061	9.63±0.26	0.487±0.014	51.75±1.45	2.590±0.062	198.25^{b}±1.38	0.634^{b}±0.007
Infected CAPacid	68.21^{a*}±1.27	3.330±0.067	9.87±0.30	0.479±0.014	53.21±1.33	2.591±0.062	206.39^{a}±1.78	0.667^{a}±0.007
Significance	*< 0.05* *	*NS*	*NS*	*NS*	*NS*	*NS*	*< 0.05* *	*< 0.05* *

Means of each trait within age with different superscripts are significantly different (P ≤ 0.05).

CAPacid® treated group since 2 wk of age till end of the trial. Weight gain was significantly improved by CAPacid® during the periods 1 to 2 wk of age and 3 to 4 wk of age, and the global raising period. There was a significant interaction between the infection and the CAPacid® treatment on the final body wt.; CAPacid® tended to have a more positive effect on infected birds (+5.1%) than in non infected birds (+2.3%). The infected control chickens final wt. was significantly lower than that of non infected control birds whereas final wt. of infected birds treated with CAPacid® was not significantly different from the non infected birds final wt. (Tables 2 and 2 Bis). Vogt et al. (1981), Skinner et al. (1991) and Kirchgessner et al. (1991) reported on increase in broiler performance due to the use of single acids. Lückstädt et al. (2004) recorded that the final body wt. of the treated broiler chickens with an acidifier was significantly increased and other performance data showed better results, the average daily wt. gain was higher in the acidifier group, partly significantly and the FCR was slightly reduced, even if this reduction was not significantly. Our obtained results are in accordance with that reported by Versteegh and Jonbloed (1999) who investigated the effect of lactic acid on the performance of broilers.

Non infected CAPacid® treated group showed a cumulative mortality rate reaching 2% as compared with 5% in non infected control group. On the other hand, during 3th and 6th experimental weeks, significant higher mortality rate was recorded in experimentally infected non-treated group over the treated one and the cumulative mortality rate in this acidifier treated group recorded a lower value than that of non-treated one (3% versus 5.5%) (Table4). Lückstädt et al. (2004) mentioned that feed industry and food production sector still suffer from huge losses due to the contamination of feed with pathogenic bacteria and their related impacts on the animal, such as lower wt. gains or even increased mortality. Kim et al. (2005) concluded that dietary acidifiers appear to be a possible alternative to feed antibiotics in order to improve performance of broilers. It is generally known that dietary acidifiers lower gastric pH, resulting in increased activity of proteolytic enzymes, improved protein digestibility and inhibiting the proliferation of pathogenic bacteria in GIT.

Table 2. *Birds weights (n=200)*

	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
Non infected Control	43 ± 2.9	138 ± 18.8	324 ± 59.4	636 ± 112.5	1007 ± 157.2	1528 ± 173.5	2009[a] ± 196.7
Non Infected CAPacid	43 ± 1.8	133 ± 17.5	343 ± 53.9	663 ± 103.6	1072 ± 163.8	1581 ± 194.5	2055[a] ± 226.9
Infected Control	43 ± 2.8	139 ± 20.5	323 ± 59.5	633 ± 127.6	985 ± 181.7	1493 ± 239.3	1924[b] ± 240.4
Infected CAPacid	43 ± 3.7	131 ± 18.4	338 ± 52.4	661 ± 69.5	1011 ± 124.7	1548 ± 164.3	2023[a] ± 183.4
Infection x Ttmt	*NS*	*NS*	*NS*	*NS*	*< 0.1*	*NS*	*< 0.1*
Non infected	43 ± 2.4	135 ± 18.3	333 ± 57.4	649 ± 108.8	1039 ± 163.7	1554 ± 185.8	2032 ± 213.6
Infected	43 ± 3.3	135 ± 19.8	331 ± 56.5	647 ± 104.2	997 ± 157.6	1520 ± 208.2	1970 ± 221.0
Infection	*NS*	*NS*	*NS*	*NS*	*< 0.001* ***	*< 0.05* *	*< 0.001* ***
Control	43 ± 2.9	139 ± 19.6	324 ± 59.4	635 ± 120.3	997 ± 168.4	1513 ± 204.8	1970 ± 221.6
CAPacid	43 ± 2.9	132 ± 17.9	340 ± 53.1	662 ± 88.2	1047 ± 151.7	1567 ± 183.0	2042 ± 210.3
Treatment	*NS*	*<0.001* ***	*<0.001* ***	*< 0.001* ***	*< 0.001* ***	*< 0.001* ***	*< 0.001* ***

NS: Not significant ((P ≥ 0.05).

Table 2 Cont. *Birds weight gains (n=200)*

	0-1 wk	1-2 wk	2-3 wk	3-4 wk	4-5 wk	5-6 wk	0-6 wk
Non infected	95	187	310	376	520	472	1966
Control	± 18.2	± 51.9	± 82.7	± 120.4	± 138.0	± 132.4	± 196.8
Non Infected	90	210	319	419	504	471	2012
CAPacid	± 17.7	± 46.4	± 82.4	± 136.8	± 130.2	± 134.8	± 227.0
Infected	97	184	310	375	508	447	1882
Control	± 20.0	± 50.6	± 90.7	± 131.9	± 159.7	± 131.5	± 240.6
Infected	88	207	320	373	535	463	1979
CAPacid	± 18.5	± 47.1	± 62.8	± 118.3	± 132.8	± 132.1	± 183.2
Infection x Ttmt	*NS*	*NS*	*NS*	*< 0.05 **	*< 0.1*	*NS*	*NS*
Non infected	92 ± 18.1	198 ± 50.6	315 ± 82.5	398 ± 130.6	512 ± 134.2	472 ± 133.4	1989 ± 213.6
Infected	92 ± 19.7	195 ± 50.2	315 ± 78.4	374 ± 125.4	521 ± 147.8	454 ± 131.7	1927 ± 221.0
Infection	*NS*	*NS*	*NS*	*< 0.05 **	*NS*	*NS*	*< 0.001 ****
Control	96 ± 19.1	185 ± 51.2	310 ± 86.8	375 ± 125.5	515 ± 147.8	461 ± 132.4	1927 ± 221.7
CAPacid	89 ± 18.1	209 ± 46.7	320 ± 73.3	400 ± 131.3	517 ± 132.0	468 ± 133.5	1999 ± 210.3
Treatment	*< 0.001 ****	*< 0.001 ****	*NS*	*< 0.05 **	*NS*	*NS*	*< 0.001 ****

NS: Not significant ((P ≥ 0.05).

Table 4. *Mortality rate (%)*

Groups	Age (wk)						
	1	2	3	4	5	6	Cumulative
Non infected	0.50	2.00	0.00 [b]	0.50	1.50	0.50 [ab]	5.00
Control	± 0.50	± 0.82	± 0.00	± 0.50	± 0.96	± 0.50	± 1.29
Non infected	0.00	0.50	0.00 [b]	0.50	0.50	0.50 [ab]	2.00
CAPacid	± 0.00	± 0.50	± 0.00	± 0.50	± 0.50	± 0.50	± 1.41
Infected Control	0.00 ± 0.00	0.50 ± 0.50	0.00 [b] ± 0.00	0.50 ± 0.50	2.50 ± 0.96	2.00 [a] ± 0.82	5.50 ± 2.22
Infected CAPacid	0.50 ± 0.50	1.00 ± 0.58	1.00 [a] ± 0.58	0.00 ± 0.00	0.50 ± 0.50	0.00 [b] ± 0.00	3.00 ± 0.58

Means with different, superscripts, within column, are significantly different (P ≤ 0.05).

They also hypothesized that acidifiers could be related to reduction of gastric emptying rate, energy source in intestine, chelating minerals, stimulation of digestive enzymes and intermediate metabolism. Dhawale (2005) mentioned that the profile of intestinal microflora plays an important role in gut health and in healthy birds, there is a balance between the Gram-positive and Gram-negative populations of microflora at an ideal pH. He added that a disease condition results when there is a shift towards the enteropathogenic population. Thus, maintenance of the ideal pH for microbial balance is essential for keeping the gut healthy. The use of gut acidifiers has been proven to be of immense help in maintaining the microbial balance of the gut. The mode of action of the acidifier in relation to zootechnical performance can be summarized in that they maintain an optimum pH in the stomach, allowing correct activation and function of proteolytic enzymes, optimise protein digestion in stomach, stimulate feed consumption by improving palatability of feed, inhibit the growth of pathogenic bacteria, yeasts and moulds, improve protein and energy digestibilities by reducing microbial competition with the host for nutrients, as well as endogenous nitrogen losses, lower the incidence of sub-clinical infections, reduce the production of ammonia and other growth-depressing microbial metabolites, increase pancreatic secretion and tropic effects on gastrointestinal mucosa and favour mineral absorption by creating an ideal pH in the intestine.

Bacteriological results of *C. perfringens* colonization as well as total aerobic bacteria colonization in CAPacid® treated groups have clearly showed that using acidifiers is considered a novel and effective alternatives to antibiotics that could reduce the severity of *C. perfringens*-associated necrotic enteritis challenge in broilers. As the cecum is also a main site of *C. perfringens* the effect of the studied acidifier was adopted for cecal colonization of *C. perfringens* where reduction in its count significantly occurred during the entire period of the experiment (42d) on using CAPacid®. It seems that the use of this acidifier can greatly assist in the control of both *C. perfringens* and total aerobic bacteria colonization in broilers (Tables 5-8). Results of bacteriological examination could be explained in the view of the report of Dhawale (2005) who mentioned that organic acids in undissociated state (non-ionised) are more lipophilic, penetrate the semi-permeable membrane of the bacterial cell wall and enter the cytoplasm. He also added that at the internal pH of bacteria (around pH 7,0), the organic acids dissociate, releasing hydrogen ions (H^+) and anions (A^-). The internal pH of the microbe decreases, which the bacteria are unable to tolerate. A specific H^+-ATPase pump acts to bring the pH inside the bacteria level. This phenomenon consumes energy and eventually stops the growth of the bacteria or even kills them. The lowering of pH also suppresses the enzymes, e.g. decarboxylases and catalyses, inhibits glycolysis, prevents active transport and interferes with signal transduction. The anionic (A^-) part of the acid is trapped inside the bacteria and becomes toxic by creating anionic osmotic problems for the bacteria. He concluded that the antibacterial effects of organic acids work through: modification of internal pH; inhibition of fundamental metabolic functions; accumulation of toxic anions and disruption of the cellular membrane.

No histopathological changes could be detected in liver or intestine of either treated or non-treated naturally induced *C. perfringens* groups (Fig. 1; Images 6 and 10). In experimentally infected broiler chickens the livers of non-treated group examined sections revealed Kupffer cells activation, vacuolar degeneration of hepatocytes and perivascular leucocytic cells aggregation mainly heterophils (Fig. 1; Image 1). These histopathological alterations were observed at 3 d PI (1st sample), whereas, at 7 d PI (2nd sample), examined liver showed vacuolar degeneration of hepatocytes, hyperplasia and focal desquamation of epithelial lining bile duct (Fig. 1; Image 2) associated with massive leucocytic cells infiltration in the portal triad (Fig. 1; Image 3) and focal areas of hepatic necrosis. Moreover, at 14 and 21 d PI (3rd and 4th samples), liver of chickens from this group revealed vacuolar degeneration of hepatocytes, perivascular infiltration with heterophils (Fig. 1; Image 4) as well as focal hepatic necrosis. Experimentally *C. perfringens* infected treated group showed no histopathological changes all over the experimental period recorded except vacuolar degeneration of hepatocytes (Fig. 1; Image 5).

Examination of intestinal sections of *C. perfringens* infected non-treated group revealed severe histopathological alterations at 14 and 21 d PI, those alterations described as marked caseous necrosis of intestinal mucosa (Fig. 1; Image 7), activation of mucous secreting cells in lamina epithelialis and marked leucocytic cells infiltrations in lamina propria (mainly heterophils) (Fig. 1; Image 8). While, the infected and CAPacid® treated group showed no histopathological changes all over the experimental period except vacuolar degeneration of hepatocytes of liver and slight edema in lamina propria of intestine (Fig. 1; Image 9). At 14 and 21 d PI, examined sections from this group revealed no histopathological changes. Aforementioned results are assuming that CAPacid® could sustain the GIT integrity damaged by *C. perfringins* infection.

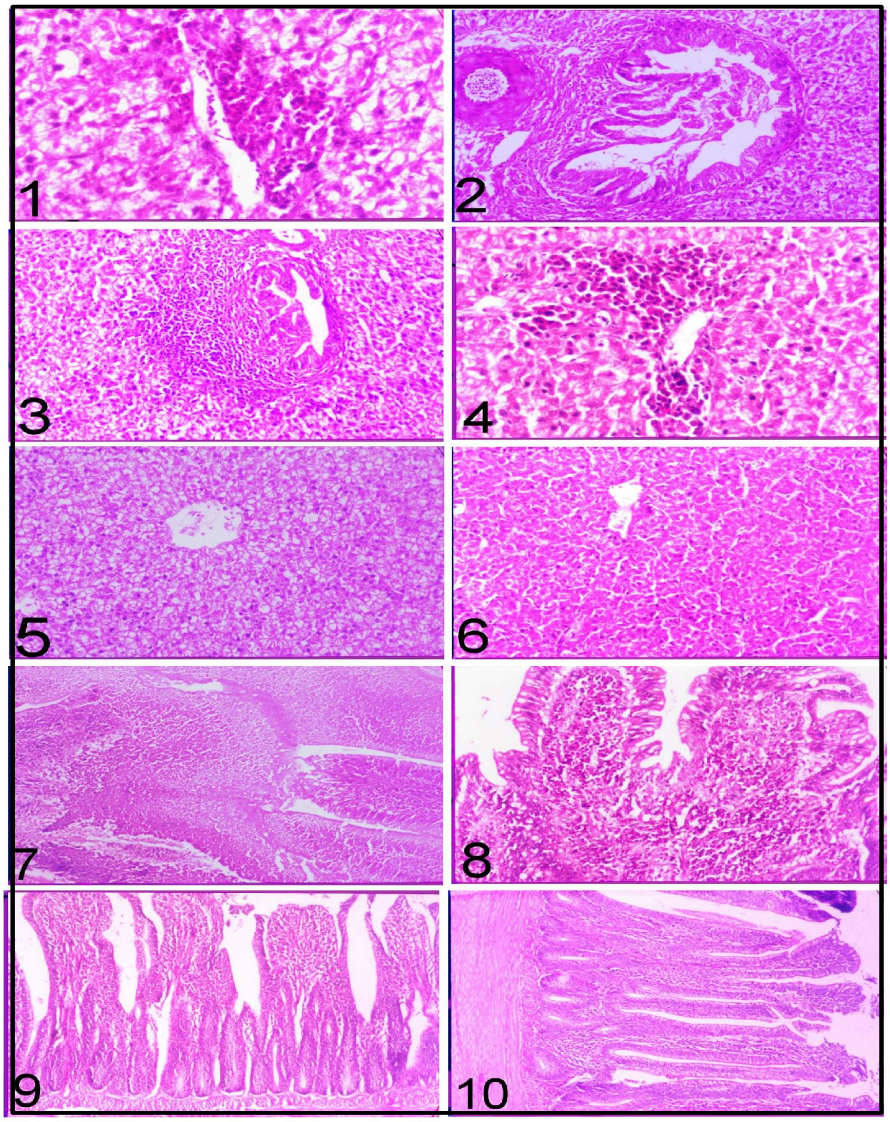

Fig. (1): Image 1: Liver of non-treated broiler chickens 3 d post experimental infection with *C. perfringens* (1st sample PI) showing vacuolar degeneration of hepatocytes and perivascular leucocytic cells aggregation. H & E X 400; Image 2: Liver of non-treated broiler chickens 7 d post experimental infection with *C. perfringens* (2nd sample PI) showing vacuolar degeneration of hepatocytes, hyperplasia and focal desquamation of epithelial lining bile duct. H & E X 200; Image 3: Liver of non-treated broiler chickens 7 d post experimental infection with *C. perfringens* (2nd sample PI) showing vacuolar degeneration of hepatocytes associated with massive leucocytic cells infiltration in the portal triad. H & E X 200; Image 4: Liver of non-treated broiler chickens 14 and 21 d post experimental infection with *C. perfringens* (3rd and 4th samples PI) showing vacuolar degeneration of hepatocytes and perivascular infiltration with heterophils and focal hepatic necrosis. H & E X 400; Image 5: Liver of CAPacid treated broiler chickens 14 d post experimental infection with *C. perfringens* (3rd sample PI) showing vacuolar degeneration of hepatocytes. H & E X 200; Image 6: Liver of CAPacid treated and non-treated non-infected broiler chickens showing no histopathological changes. H & E X 200; Image 7: Intestine of non-treated broiler chickens 21 d post experimental infection with *C. perfringens* (4th sample PI) showing marked caseous necrosis of intestinal mucosa. H & E X 200; Image 8: Intestine of non-treated broiler chickens 14 d post experimental infection with *C. perfringens* (3rd sample PI) showing marked leucocytic cells infiltration in lamina propria. H & E X 200; Image 9: Intestine of CAPacid treated broiler chickens 14 d post experimental infection with *C. perfringens* (3rd sample PI) showing slight edema in lamina propria. H & E X 100; Image 10: Intestine of treated and non-treated non-infected broiler chickens showing no histopathological changes. H & E X 100.

In conclusion the current study has shown the interest of using protected organic acidifiers in broiler diets to struggle *C. perfringens* infection. In addition, taking in consideration the facts that organic acids do not require withdrawal period, that bird performance are positively affected by their use and that they increase the shelf-life of products, they can make a valuable contribution to flock health and safety of poultry products as food. This may provide a significant tool for the poultry industry in combating the occurrence of intestinal diseases and in reduction of food borne pathogens.

Table 5. *Means of C. perfringens colonization in experimentally infected broiler chickens (10^3 CFU / g)*

Time Post Infection	Intestine		Cecum	
	Treated	Non-treated	Treated	Non-treated
0 hr	0.142^{b}±0.045	212.000^{a}± 38.727	0.440^{b}± 0.050	754.000^{a}± 143.149
3 d	3.020^{b}±0.693	2600.000^{a}±339.935	2.982^{b}± 1.107	9000.000^{a}±1173.790
7 d	0.420^{b}±0.049	568.000^{a}± 35.428	0.760^{b}± 0.081	5000.000^{a}±964.019
14d	5.000^{b}±0.471	236.000^{a}± 13.920	8.800^{b}± 0.490	394.000^{a}± 23.247

Means within time and within region of GIT with different, superscripts are significantly different (P≤0.05).

Table 6. *Means of naturally induced C. perfringens (10^3 CFU / g)*

Age	Intestine		Caecum	
	Treated	Non-treated	Treated	Non-treated
21 d	0.142^{b}± 0.045	212.000^{a}±38.727	0.440^{b}± 0.050	754.000^{a}±143.149
24 d	0.360^{b}± 0.069	298.000^{a}±29.469	0.680^{b}± 0.061	1020.000^{a}±106.249
28 d	0.700^{b}±0.037	232.000^{a}±16.918	1.040^{b}±0.027	450.000^{a}±60.663
35 d	1.500^{b}±0.099	34.620^{a}±7.770	2.380^{b}±0.294	66.780^{a}±11.942

Means within time and within region of GIT with different, superscripts are significantly different (P ≤ 0.05).

Table 7. *Means of Total aerobic bacteria colonization in experimentally infected broiler chickens with C. perfringens (10^3 CFU / g)*

Time Post Infection	Intestine		Cecum	
	Treated	Non-treated	Treated	Non-treated
0 h	1.425^{b}±0.354	3420.000^{a}±803.990	4.325^{b}±0.768	1600.000^{a}±266.667
3 d	1.780^{b}±0.191	5760.000^{a}±946.948	12.200^{b}±6.301	10040.000^{a}±1373.010
7 d	4.200^{b}±0.646	1420.000^{a}±121.838	8.800^{b}±0.442	1660.000^{a}± 125.786
14 d	9.000^{b}±1.011	2330.000^{a}±388.387	14.000^{b}±1.265	2326.000^{a}± 570.480

Means within time and within region of GIT with different, superscripts are significantly different (P ≤ 0.05).

Table 8. *Means of Total aerobic bacteria naturally induced colonization (10^3 CFU / g)*

Age	Intestine		Cecum	
	Treated	Non-treated	Treated	Non-treated
21 d	1.425^{b}± 0.354	3420.000^{a}±803.990	4.325^{b}± 0.768	1600.000^{a}±266.667
24 d	0.152^{b}±0.013	5400.000^{a}±805.536	0.244^{b}±0.031	12400.000^{a}±1002.220
28 d	2.780^{b}±0.071	1740.000^{a}±212.498	3.400^{b}±0.137	2780.000^{a}±359.877
35 d	4.140^{b}±0.224	194.000^{a}±16.138	4.580^{b}±0.122	270.000^{a}±25.734

Means within time and within region of GIT with different, superscripts are significantly different ($P \leq 0.05$).

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