CHAPTER I: INTRODUCTION

Bangladesh is a country with a very high population density. Agricultural development involving allocatiFon of additional land which is not possible at all, hence, emphasize should be given to other sectors in agriculture like broiler rearing. This sub-sector has proved as an attractive economic benefit, thereby, indicating its` importance for the entire economy. The sector accounts for 14% of the total value of livestock output and is growing rapidly (Raihan, 2008). Thereby, broiler farming seems to be a considerable part of meat production and consumption in the country.

Similarly there is need of high levels of production and efficient feed conversion in modern poultry industry which can be achieved by the use of specific feed additives. But different types of microorganism are deteriorating the performance of these additives by causing different disease. For a long time antibiotic feed additives have been supplemented to poultry feed to stabilize this type of intestinal microbial flora, also to improve the general performances and to prevent some specific intestinal pathology (Hassan *et al*., 2010). But continuous misuses of antibiotics in livestock production, especially in poultry industry has resulted many problems like development of drug-resistant bacteria (Dizaji *et al*., 2012), drug residues in the body of the birds (Yamauchi *et al*., 2006) and imbalance of normal microflora in the gut (Ghahrib *et al*., 2012). Considering this health issues, the European Commission (EC) decided to phase out, and ultimately ban (on 1 January 2006) the marketing and use of antibiotics as growth promoters in feed (Annonymous, 2006). But removal of antibiotics has created different problems in poultry performance like increasing feed conversion ratio and incidence of certain animal diseases, such as (subclinical) necrotic enteritis (Dibner and Richards, 2005). In such situation researchers were compelled to find out the effect of other non-therapeutic alternatives like organic acids, enzymes, probiotics, prebiotics, herbs, essential oils and immune stimulants as feed additives in poultry production and were looking for suitable feed additives to improve poultry performance.

Organic acids have been used as dietary supplements in animal production for the last 50 years, mainly as feed additives in pig diet (Cole *et al*., 1968). In poultry diet as

additive, their application is relatively recent, started from the late 1970's and early 1980's. Scientist noticed firstly at 1981, enhancement of broiler performance was occurred by using formic acid (Lückstädt and Mellor, 2011). In 2006, acidifier supplementation to poultry diets has illumined, when European Union (EU) banned the use of antibiotics as growth promoter (Abdel-Fattah *et al*., 2008). Individual or blends of several organic acids have been found to perform antimicrobial activities similar to antibiotics (Wang *et al*., 2009). European Union now allowed the use of organic acids and their salts in poultry production as these are considered safe (Adil *et al*., 2010). Moreover, as a group of chemicals, organic acids are considered to be any organic carboxylic acid of the general structure R-COOH (including fatty acids and amino acids). The short chain acids (C1–C7) are associated with antimicrobial activity. They are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids or carboxylic acids with the hydroxyl group such as lactic, malic, tartaric and citric acids or short-chain carboxylic acids containing double bonds like fumaric and sorbic acids (Shahidi *et al*., 2014). Organic acids are weak acids and are only partly dissociated. Most organic acids with antimicrobial activity have a pKa between 3 and 5. A wide range of organic acids with variable physical and chemical properties exists, of which many are used as drinking water supplements or as feed additives (acidifiers). Many are also available as sodium, potassium or calcium salts (and/or partially esterified) and has been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are particularly effective (Lückstädt, 2014). Studies demonstrated that supplementation of organic acids to broiler diets increased growth performance, reduced diseases and management problems (Vlademirova and Sourdjiyska, 1996; Runho *et al*, 1997; Jin *et al*, 1998; Gunal *et al*, 2006; Islam *et al*, 2008; Ao *et al*, 2009). Furthermore, the use of organic acids has been reported to protect the young chicks by competitive exclusion (Mansoub *et al*., 2011), enhancement of nutrient utilization, and growth and feed conversion efficiency (Lückstädt and Mellor, 2011). It enhance growth performance of broiler with lowering the FCR by enhancing various metabolic pathways for energy generation (Nourmohammadi *et al*., 2010) and several studies support the statement that dietary inclusions of acidifiers have improved growth performance in broiler chickens along with carcass characteristics (Lakshmi and Sunder, 2015). It has been suggested that lowering the pH by organic acids improve nutrient absorption (Biggs and Parsons, 2008) by poultry. The results also notify that acidifiers affect production performances

of broiler effectively by improving digestibility of protein, Ca, P, Mg, Zn and serving as a substrate in the intermediary metabolism (Fallah and Rezaei, 2013;). Besides, organic acids have number of further different important functions like addition of organic acids in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria such as Salmonella, Campylobacter and Escherichia coli which can be controlled by supplementation of an organic acid in diet (VanImmerseel *et al*., 2006; Naseri *et al*., 2012), which indicate the antimicrobial activity of organic acid. Again several studies demonstrated that organic acids could stimulate the natural immune response in poultry (Lohakare *et al*., 2005, Houshmand *et al*., 2012; Abbas *et al*., 2013). Considering all those effect it can be concluded that organic acid enhance the animal's immune system, inhibit the proliferation of pathogenic organisms and increase the capacity to utilize nutrients which help to improve the broiler performance along with its carcass characteristics. The possibility to substitute antibiotic with organic acid is still a subject of research and controversy, especially the efficiency of organic acid addition for the purpose of full substitution of antibiotic in broiler diets.

However, discrete use of antibiotic is not encouraged and European Union has already banned the use of antibiotic considering its harmful effect on human health. In some countries, such as the USA, consumer pressure is pushing the poultry industry to rear birds without antibiotics (Castanon 2007). As a result in future use of in-feed antibiotic can be restricted in all over the world. Since the use of in-feed antibiotics will be restricted in the future, there will be growing interest in using organic acid as a bioactive compound for improving gut health. The antimicrobial activity of organic acid may decrease the incidence of disease caused by different microorganism or mold & yeast in broiler. Use of organic acid may also alleviate the fear of antibiotic residue and effect of antibiotic resistance. Moreover, considerable improvements in performance and carcass quality such as improved carcass quality and lower feed conversion have been reported. Moreover their capacity to increase nutrient digestibility by improving utilization of different mineral particles may lead to the livestock production in a positive manner. In most of the study organic acid was used in feed level which has some controversial effect. To avoid this circumstances this study is going to know its effect in water level with the following objectives.

Objectives of this study

1. To observe the effect of organic acid and on growth performance in broilers.
2. To observe the effect of organic acid on carcass quality in broiler.
3. To observe the effect of organic acid on blood profile in broiler.

Scope of the study

The purpose of the study was to assess the comparative effectiveness of three different organic acids with antibiotic on productive performance, carcass quality, blood parameter in broiler. This study involved organic acid supplement, effectiveness of organic acid and verify the level of organic acid.

CHAPTER II: REVIEW OF LITERATURE

2.1 Organic acid

The term 'organic acids' refers to all those acids built on a carbon skeleton, known as carboxylic acids, which can alter the physiology of bacteria, causing metabolic disorders that prevent proliferation and cause death. Organic acids are organic compound with acidic properties. They are weak acids, including fatty acids and amino acids, of the general structure R-COOH (Al-Kassi and Mohssen, 2009). Their acidity depends on their carboxyl group –COOH. The short chain acids (C1-C7) are associated with antimicrobial activity. Organic acids are weak acids and are only partly dissociated. Most organic acids with antimicrobial activity have a pKa-the pH at which the acid is half dissociated between 3 and 5.

2.2 Classification of Organic acid

Depending on the nature of the carbohydrate radical they can be classified into 3 groups, which are Aliphatic; lauric, myristic, palmitic etc(saturated) & oleic, linoleic, linolenic, arachidonic etc (unsaturated), Aromatic; benzoic, salicylic, gallic, phthalic etc. Alycyclic; quinic, shikimic etc. Again depending on the chain length organic acid can be divided into two types, one is short chain fatty acid; formic acid, acetic acid, propionic acid etc. reduce pH & affect directly gram (-) bacteria and fumeric acid, citric acid, malic acid, lactic acid etc. have indirect effect on the bacterial population by pH reduction, acting mainly on stomach and rest one is multi chain fatty acid; capric acid, caprylic acid, lauric acid which have direct and strong antimicrobial effect on gram(+) and gram(-) bacteria.

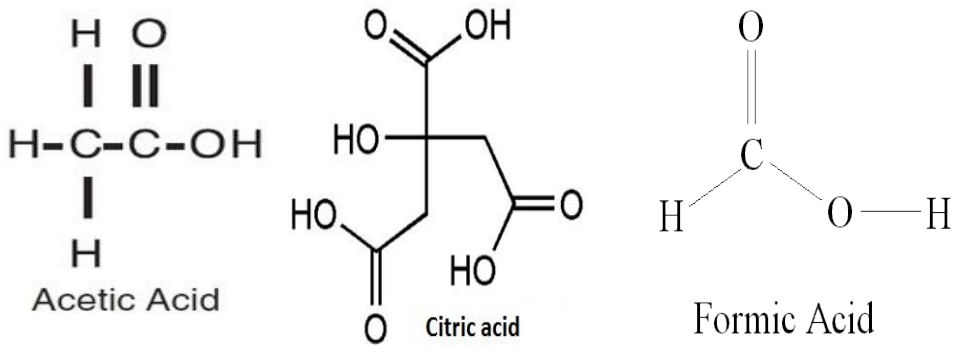

Fig 2.1: Chemical structure of acetic, citric and formic acid.

2.3 Characteristics of organic acids

Table 2.1. Different types of organic acid and their characteristics

Organic acid	pk_a	solubility	P^H lowering	Taste	Corrosivity
Formic acid	3.75	+++	+++	----	---
Acetic acid	4.76	+++	++	++	--
Propionic acid	4.88	+++	+	±	-
Butyric acid	4.82	+++			
Lactic acid	3.83	++	++++	++	+
Sorbic acid	4.76				
Benzoic acid	4.17	-			
Fumaric acid	3.02	-	++	±	±
	4.38				
Malic acid	3.40	++			
	5.10				
Tartaric acid	2.93	++			
	4.23				
Citric acid	3.13	++			
	4.76				
	6.40				
Phosphoric acid	2.15	+++			
	7.10,12				
	.32				

+= Positive; ++= Moderate; +++= Highly; ++++= Extremely; -=Negative

2.4 General Mode of action (MoA) of organic acid

At low pH un-dissociated acid are lipophilic and can diffuse across cell membranes including bacteria & molds. Once in the bacterial cell, the higher pH of cytoplasm cause dissociation of the acids and the resulting reduction in pH due to the release of H+ disrupt the enzymatic reactions & nutrient transport system. Molecule of organic acid also attacks the DNA of bacteria that turns into to death. Mode of action of acidifiers depends on its sensitivity to P^H. Only Certain types of bacteria are sensitive to pH (ex.: E. coli, Salmonella sp., L. monocytogenes, C. perfringens etc) while other types of bacteria are not sensitive (Bifidobacterium sps., Lactobacillus sps etc) (Dibner and Buttin, 2002).

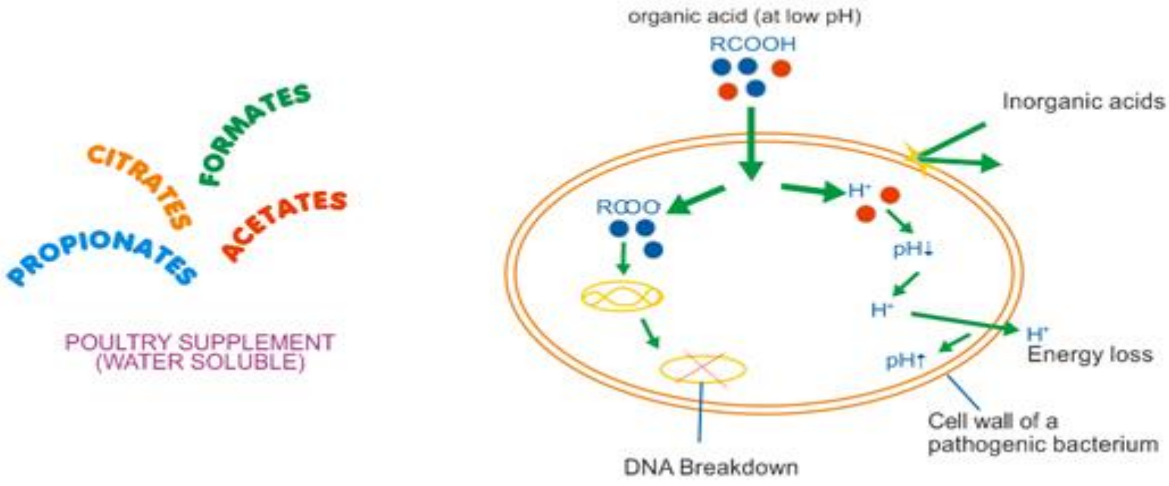

Fig 2.2 : Mode of action of organic acid

2.4.1 In case of pH sensitive bacteria

The mode of action in pH sensitive bacteria is shown in Fig 2.2. Organic acids in undissociated (non-ionized, more lipophilic) state penetrate the semi permeable membrane of bacteria cell wall and enter cytoplasm. At the internal pH of bacteria (~7.0), the undissociated organic acids dissociate, releasing H+ and anions (A-). The internal pH of bacteria decreases. The pH sensitive bacteria are unable to tolerate a large spread between the internal and the external pH. A specific H+ -ATPase pump acts to bring the pH inside the bacteria to a normal level. This phenomenon consumes energy and eventually can stop the growth of the bacteria or even kill it. The lowering of pH

also suppresses the enzymes (e.g. decarboxylases and catalyses), inhibit glycolysis, and prevent active transduction. The anionic (A-) part of the acid trapped inside the bacteria (it can diffuse freely through the cell wall only in its non-dissociated form), becomes toxic involving anionic imbalance leading to internal osmotic problems for the bacteria.

2.4.2. In case of non-pH sensitive bacteria

The non-pH sensitive bacteria tolerate a larger differential between internal and external pH. At a low internal pH, an organic acid re-appear in a non-dissociated form and exits the bacteria. Equilibrium is created and the bacteria do not suffer. Dietary organic acids and their salts are able to inhibit microbial growth in the food and consequently to preserve the microbial balance in the gastrointestinal tract. In addition, by modifying intestinal pH, organic acids also improve the solubility of the feed ingredients, digestion and absorption of the nutrients (Khan and Iqbal, 2016). Acidifiers effect beyond; improve digestive enzyme activity, growth of gastrointestinal mucosa, microbial phytase activity and increased pancreatic secretion.

2.5 Antibacterial activity of organic acids

Organic acids have an antibacterial effect because they diffuse through the bacterial cell membrane, and then dissociate into anions and protons thus disturbing the electron-balance inside the cell (Philipsen, 2006). Most common bacteria that affect the intestinal health of broiler are Salmonella, Campylobacter and Escherichia coli which can be controlled by supplementation of an organic acid in diet (Van Immerseel *et al*., 2006; GharibNaseri *et al*., 2012). Salmonella is a human pathogen that is commonly found in poultry products. From a public health point of view, it is necessary to control this biological hazard. Scientist investigated the efficacy of each 1.0% of formic acid and different blends of formic acid, propionic acid and sodium formate in different feed materials (Koyuncu *et al*., 2013). Organic acid mixtures (fumaric acid, calcium format, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate and hydrogenated vegetable oil) were found to be more efficient than the antibiotic growth promoter (Enramycin) in decreasing intestinal E. coli and Salmonella spp. (Hassan *et al*., 2010). Furthermore, the organic acids in poultry might have a direct effect on the gastrointestinal tract (GIT) bacteria population, reducing the level of some pathogenic

bacteria and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients.

Table 2.2. Antimicrobial activity of different types of organic acids.

Name of organic acid	Yeasts	Fungi	Gram (-) Bacteria	Gram (+) Bacteria	Stafylo-/ streptococcus spp
Formic acid	+++	0	++++	0	0
Acetic acid	+	-	+++	0	0
Propionic acid	++	++++	0	0	0
Sorbic acid	++++	+++	+++++	0	0
Benzoic acid	+++	+++	+++++	0	0
Lactic acid	-	-	+++	0	0
Caprylic-and caprinic acid	++	++	+++	+++++	++++
Lauric acid-GML90	+++	++	++	++++	++++++

+= Sensitive; ++= Moderate sensitive; +++= Highly sensitive; ++++= Extremely sensitive;+++++= Super sensitive; -= Not sensitive; 0= Not known.

(Paul *et al*., 2007) found that organic acid salt (ammonium formate or calcium propionate; 3 gm/kg diet) reduced coliform count in broiler feed compared to control, whereas the clostridium count was unaffected. Mohyla *et al*., (2007) observed that Salmonella load was significantly reduced in the upper digestive tract but not in the lower digestive tract when acidified sodium cholorite (produced by the combination of sodium chlorite with citric acid or sodium acid sulphate) was added to the drinking water at a level of 0.06% for the last 24 hours or 5 days.

2.6 Effect of organic acids on immune system

The immune system of birds is complex and is composed of many cells and soluble factors that must work together to produce a protective immune response. Major

element of the avian immune system are the lymphoid organs (Abdel-Fattah *et al*., 2008) and Ghazalah *et al*., (2011) reported that birds fed an organic-acid-supplemented diet had heavier immune organs (bursa of Fabricius and the thymus) and also a higher level of globulin in their serum. Concentration of globulin is used as an indicator for measuring immune response. Above workers also suggested that the development in bird immunity could be related to the inhibitory effects of organic acids on gut system pathogens. Citric acid supplementation (0.5%) increased the density of the lymphocytes in the lymphoid organs, increasing the non-specific immunity (Haque *et al*., 2010). Several studies demonstrated that organic acids could stimulate the natural immune response in poultry as well as broiler. Rodríguez-Lecompte *et al*., (2012) reported that supplementation of combined probiotics and organic acids (sorbic and citric acid) to broiler diets resulted in better responses of gut morphology and their effects were more apparent in the duodenum and ileum when the gut was fully developed.

2.7 Effect of organic acid on gut health

Good intestinal health in the poultry industry is of great importance to achieve target growth rates and feed efficiency. Organic acid (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 (Kum *et al*., 2010; Rodríguez-Lecompte *et al*., 2012). Garcia *et al*., (2007) reported that broilers fed diets containing formic acid had the longest villi (1273 and 1250 μm for 0.5 and 1.0% formic acid, respectively) compared with control (1088 μm). Similarly, crypts of jejunum were deeper in birds fed the formic acid diet (1.0%) than birds fed the antibiotic diets (266 vs. 186 μm, respectively; $P < .05$) in the same experiment. Thus, formic acid supplementation increased both the villus height and crypt depth. Short-chain fatty acids have been demonstrated to stimulate the proliferation of normal crypt cells, enhancing healthy tissue turn over and maintenance. Pelicano *et al*., (2005) reported that, higher villus height in the ileum with the diet based on organic acid salts compared with diet fed without mannan oligosaccharide + Organic acid salt. Paul *et al*., (2007) found that the histology of intestinal parts revealed that organic acid salt (ammonium formate and calcium propionate) supplementation increased the villus height of different segments of the small intestine than the control group possibly by reducing intestinal colonization of pathogenic and nonpathogenic bacteria.. Consequently, there

is a decrease in the villus height, increase in the cell turnover and decrease in the digestive and absorptive capacities (Pelicano et al., 2005). So organic acid salts reduced the growth of many pathogenic intestinal bacteria.

2.8 Effect of organic acid on nutrient digestibility

Organic acids normally used as an acidifier in poultry feeds have been considered to be attractive alternatives for improving nutrient digestibility. Ghazalah *et al*., (2011) reported that dietary 0.5% of either fumaric or formic acid and 0.75% of acetic or 2% citric acid improved both ME and nutrient digestibility, that is, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) of broiler diets. Moreover, Ghazalah *et al*., (2011) and Garcia *et al*., (2007) reported that supplementation of formic acid (0.5% or 1.0%) in broiler finisher diet was found to improve apparent ileal digestibility (AID) of dry matter (DM) (67.8% or 68.8%, respectively) and CP (72.5% or 73.5%, respectively) as compared with control (56.4% DM and 60.7% CP). Similarly, 2% citric acid in the broiler diet also increased the retention of DM, CP and neutral detergent fiber (Ao *et al*., 2009). Other research added 2% citric acid to the soyabean meal as substrates in the in vitro trial. The result indicated that addition of citric acid increased the activity of α-galactosidase resulting in decreased the crop pH. He reported that citric acid decreased the crop pH and enhanced the activity of α-galactosidase in the crop in vivo trial. When citric acid (3% or 4%) was supplemented to chicks, it improved amino acid digestibility (AAD) at 4 days (3% units), but this effect did not carry through to 21 days. The results for AAD indicated that gluconic acid and citric acid had no consistent effects. Organic acids lowered the pH of the chyme and thus enhanced the digestibility of protein. It is thought that the lower pH of the digesta due to the organic acid supplementation might increase the pepsin activity (Afsharmanesh and Pourreza, 2005). According to Diogo *et al*., (2015), 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. Centeno *et al*., (2007) found that the AID of CP and dispensable and indispensable amino acids were not affected by the addition of citric acid and the microbial phytase enzyme in the broiler diet. Dietary addition of organic acids can also improve the digestibility of minerals and increase the utilization of the phytate phosphorus (P) (Boling *et al*., 2000 ; Park *et al*., 2009).

2.9 Effect of organic acid on broiler performance

In poultry production, organic acids have not gained as much attention as in pig production (Langhout, 2000). Organic acids have growth-promoting properties and can be used as alternatives to antibiotics (Khan and Iqbal, 2016). Dietary supplementation of organic acids increased the body weight and feed conversion ratio (FCR) in broiler chicken. Panda *et al*., (2009) reported that 0.4% butyrate in the broiler diet was similar to antibiotics in maintaining body weight gain (646 and 642 g, respectively) but superior for FCR. No added advantage on these parameters was obtained by enhancing the concentration of butyrate from 0.4% to 0.6% in the diet. Contrary to the findings of the above study, Leeson *et al*., (2005) and Antongiovanni *et al*., (2007) suggested a lower level (0.2%) of butyrate to maintain the performance of broiler chickens. Adil *et al*., (2010) found that the highest weight gains were achieved in the birds fed 3% fumaric acid as compared to the group fed diet supplemented with 3% lactic acid. Chicks fed the diet supplemented with organic acids showed a significant ($P < .05$) improvement in the FCR as against the chicks fed the control diet. The improvement in the FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain in the birds fed organic acids in the diet. The above workers also conducted another trial, in which broilers were given basal diet supplemented with 2–3% each of butyric acid, fumaric acid and lactic acid (Adil *et al*., 2011). Chicks fed the diets supplemented with organic acids showed a significant improvement in the FCR as against the chicks fed the control diet. The improvement in FCR could be possibly due to lesser feed intake resulting in increased body weight gain because of better utilization of nutrients in the birds fed organic acids in the diet. Recently, Brzóska *et al*., (2013) reported that organic acid (0.3–0.9%) had a growth enhancing and mortality-reducing effect in broiler chickens, with no significant influence on carcass yield or proportion of individual carcass parts. The organic acid mixtures might be more efficient than some antibiotic growth promoter in improving broiler performance. Such a positive impact of dietary acidifiers on growth performance might be attributed to a reduction of pH values in the feed and digestive tract, serving as a barrier against pathogenic organisms which are sensitive to low pH; the direct antimicrobial effect; the reduction in buffering capacity in conjunction with improving nutrient digestibility (Ghazalah *et al*., 2011). Improvements in broiler performance in response to organic acids are often reported

2.10 Effects of organic acids with drinking water

In recent years, addition of organic acids in drinking water is another implementation in the broiler farms for improving growth performance (Açıkgöz *et al*., 2011; Alzawqari *et al*., 2013; Chaveerach *et al*., 2004). Studies indicated that addition of organic acid in drinking water helps to reduce the level of pathogens in the water and the crop/proventriculus, to regulate gut microflora, to increase the digestion of feed and to improve growth performance (Byrd *et al*., 2001). Acidifier added to the diet promotes machine corrosion, moisture absorption and acid volatilization during the process of granulating or storing (Zhu *et al*., 2014). Therefore, it is hypothesized that addition of organic acids via drinking water can avoid these problems. It is well known that drinking water is the most important factor for the spread of bacterial infection on the farm. 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 microflora, to increase the digestion of feed and to improve growth performance (Philipsen, 2006). Most studies have concentrated mainly on the effects of water acidification on Campylobacter and Salmonella contaminations in broilers where they reported decreased numbers of Campylobacter in the cecal contents of birds which consumed acidified water (Byrd *et al*., 2001; Chaveerach *et al*., 2004). Several studies have also reported that both dietary formic and propionic acids reduce Salmonella and E.coli in the small intestinal, cecal, and fecal contents of chickens (Al-Tarazi and Alshawabkeh, 2003). Moharrery and Mahzonieh, (2005) observed that the addition of 0.1% malic acid to water significantly reduced E. coli counts in the small intestine of layer chickens. Byrd *et al*., (2001) suggested that the lactic acid provided in the drinking water reduces the pH of the crop and might be provided as a temporary carbon source for beneficial bacteria normally present in the crop. Moreover, the use of formic acid in the drinking water did not significantly affect the number of Salmonella-positive intestines. Desai *et al*., (2007) indicated that inclusion of a combination of formic and propionic acids in the drinking water increased weight gain and improved feed conversion ratio in broilers, which they considered was the cause of higher nitrogen retention. Feed is usually withdrawn for several hours before slaughter in order to reduce the potential for carcass contamination from the crop and intestinal contents (Bilgili, 2002). However, feed withdrawal may cause a decrease in lactic acid concentration of the crop which may

also be accompanied by an increase in the crop pH and in Salmonella crop contamination (Corrier *et al*., 1999a). Nevertheless, the incidence of Salmonella crop contamination might be increased up to fivefold during feed withdrawal and is probably caused by coprophagy (Corrier *et al*., 1999b). Byrd *et al*., (2001) and De Avila *et al*., (2003) suggested that incorporation of some organic acids in the drinking water during the pre-slaughter feed withdrawal period significantly reduced Salmonella and Campylobacter contamination of crops and broiler carcasses at processing. High ambient temperature causes significant economic losses in the broiler industry owing to decreased body weight, poor feed conversion ratio and increasing mortality. Heat-stress leads to panting, decreases the partial pressure of $C0_2$ in blood and causes respiratory alkalosis (Bottje and Harrison, 1985; Teeter *et al*., 1985). Therefore, acidifiers have been used to alleviate negative effects of heat stress and to improve broiler performance by altering acid-base balance. However, data on the effects of acidified water on other species of intestinal bacteria in broiler chickens are limited.

2.11 Other effects of organic acid

Previous experiments have reported that dietary organic acids can influence phosphorus utilization in corn-soybean meal diets fed to broiler chickens (Boling *et al*., 2000 ; Esmaeilipour *et al*., 2011). Phosphorus utilization may be increased due to the chelating properties of organic acids with calcium, which can result in increased phytate-phosphorus solubility, increasing their ability to be hydrolyzed (Centeno *et al*., 2007). Some researchers have also proposed that organic acids may stimulate energy metabolism by providing energy sources for epithelial cells in the GIT; (Partanen and Mroz, 1999). For instance, some organic acids such as citric acid is intermediates of the tricarboxylic acid cycle, and butyric acid is the direct energy source for epithelial cells in the GIT (Partanen and Mroz, 1999 ; Pryde *et al*., 2002). However, no data have elucidated the cellular roles of organic acids in the energy metabolism of broiler chickens. Furthermore, acidified water is expected to be more effective than dietary acidification, since organic acid intake is decreased depending on the reduction in feed consumption during heat stress (Abbas *et al*., 2013).

CHAPTER III: MATERIALS AND METHODS

3.1 Study Area of Experiment

The experiments were carried out at the poultry research shed of the Department of Animal Science and Nutrition and research laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

3.2 Study Period

The overall research work was conducted from July to December 2017 where the actual feeding trial on broiler was carried out in between 30 June to 27 July 2017 where July was considered as monsoon seasons (Islam and Uyeda, 2006). In July average maximum temperature was 29 °C and humidity was 78% (BMD, 2017).

3.3 Experimental birds

The day-old chicks (Cobb 500 strain) of mixed sex (male and female) were purchased from an agent of the Nahar Agro Complex Limited, Jhautala Bazar, Khulshi, Chittagong, Bangladesh. Before purchasing, all chicks were examined for uniform size and any kind of abnormalities. The average body weight of purchasing chicks was about 46.48±0.01gm.

Figure 3.1: Day old chicks

3.4 Selected organic acid and antibiotic for experiment

The name of the organic acids selected for this experiment were acetic acid, citric acid , formic acid which had been brought in liquid form from Taj Scientific Store (a local shop for experimental solutions), Chittagong, Bangladesh. On the other hand the commercial name of the antibiotic used for this experiment was Renamycin®

3.5 Design of experiment

A total of 100 birds were equally and randomly allocated and distributed in five treatment groups (T_0, T_1, T_2, T_3 and T_4) followed by Completely Randomized Design (CRD) with two replications per treatment. Group T_0 was the control group where the water pH was 7 and group T_4 was treated with antibiotic @ 1.5ml/litre as per the recommended commercial dose. Group T_1, T_2, T_3 were treated with citric, formic, and acetic acid at the level of 1.25 ml/L, 0.5ml/L, 2ml/L respectively maintaining the pH 4.5 in regular drinking water. All the birds were provided regular homogenous optimum diets (Standard diet) for all groups. There were 20 birds per treatment group and 10 birds per replication. Layout of the experiment is shown in **Table 3.1**

Table 3.1: Layout of the experiment

Water treatments	No. of birds per replicate		No. of birds per treatment
T_0 (Basal diet+ Normal water)	R_1	10	20
	R_2	10	
T_1 (Basal diet + citric acid in water)	R_1	10	20
	R_2	10	
T_2 (Basal diet + formic acid in water)	R_1	10	20
	R_2	10	
T_3 (Basal diet + acetic acid in water)	R_1	10	20
	R_2	10	
T_4 (Basal diet + antibiotic in water)	R_1	10	20
	R_2	10	
Grand total			100

3.6 Management procedure of the experiment

3.6.1 Housing: At first, poultry shed was selected and prepared for broiler rearing. The broiler shed was thoroughly washed and cleaned by using tap water with caustic soda. For killing microorganism, phenyl solution (according to the manufacturer guideline) was also spread on the floor, corners and ceiling. Following this, brushing was done by

using steel brush and clean water. Brooding boxes and broiler cages were also cleaned by using tap water, caustic soda and phenyl solution in the same manner. After cleaning and disinfecting the house was left for one week for drying. All windows were opened for proper ventilation. After one-week, the lime was spread on the floor and around the shed for strictly maintaining bio-security. Arrangement for rearing broilers was made according to treatments and replications. The compartments were selected in an unbiased way, according to treatments and replications for uniform distribution of chicks.

Fig 3.2 : Cleaning of floor, cage, tray, feeder, waterer and disinfecting the brooder box.

3.6.2 Brooder and cage space: Each box brooder having 2.38 ft. × 2.08 ft. was allocated for 30 birds. After 14 days later broiler birds were transferred to cage having 3.5 ft. × 1.63 ft. for 10 birds. Therefore, floor space for each bird in the brooding box was 0.17 sq. ft. and cage was 0.57 sq. ft. respectively.

3.6.3 Brooding of chicks: The brooding boxes were ready for broiler chicks rearing after proper cleaning and drying. Dry and clean newspaper were placed on the floor of the brooding box as bedding materials and was changed for every 6 hours intervals in whole brooding period. Brooding temperature was maintained by using 100, 50 and 25 watt incandescent lamps in each brooding box. The broilers were exposed to continuous lighting. During the brooding period chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th week respectively.

Fig 3.3: Placing paper and brooding cage with chicks

3.6.4 Temperature and humidity control: Broiler shed was not environmentally controlled, 200 watt incandescent lamps were used to keep the optimum temperature and electric fans were used to distribute the room temperature. In adverse condition, the system had been changed; in cold weather gunny bag were used to prohibit fluctuating the room temperature as well as humidity.

3.6.5 Feeding and watering of birds: Readymade feed of CP Company limited, Bangladesh was supplied to the birds in two different growth stages i.e. starter and grower. Starter ration (crude protein: 22%) was offered from day 0 to 14 days and grower ration (crude protein: 21%) was offered from day 15 to 28.

Fig 3.4: Feeding and watering of birds in cage and brooder

Rations for all treatment groups were iso-energetic and iso-nitrogenous. Feed and water were supplied ad-libitum to all groups of birds in three different times in a day (7.00, 14.00 and 22.00 h) to the birds throughout the experimental period. Feed and water was

given to birds on small feeder and small waterer in the early stage of brooding. In each brooding box, feeding was done by using one round feeder and watering was performed with one round waterer having a capacity of 1.5 liter. The feeders and drinker were fixed in such a way so that the birds could eat and drink conveniently. During the period of cage rearing large liner feeder (3.5 ft. X 0.38 ft.) and large round waterer with a capacity of three liters were used.

3.6.6 Vaccination followed during experiment: All birds were vaccinated properly against Newcastle disease on the 6th days and Infectious Bursal disease on 12th days. After each vaccination, Nutrilac was supplied @ 1g/5 liter of drinking water as per the recommended dose to overcome the stressed effect of vaccination and cold weather.

Table 3.2: Vaccination Schedule

Age of birds	**Name of diseases**	**Name of vaccine**	**Route of administration**
6th days	New Castle Disease	BCRDV (Live)	One drop in one eye
12th days	Infectious Bursal Disease	IBD	One drop in one eye

Fig 3.5: IBD vaccine, BCRDV vaccine, Vaccine diluting and Vaccination

3.6.7 Sanitation procedure followed: Bio-security was maintained strictly during the whole experimental period. Footbath containing potassium permanganate was kept at the entrance of the poultry shed and was changed daily. Feeders were cleaned and washed with detergent and clean water, weekly before being used further. Drinkers were washed with potassium-per-magnate and dried up daily in the morning

3.7 Laboratory work during experiment

3.7.1 Carcass measurement: On days 28 of the study, twenty birds, randomly selected from each replication, and then killed by severing the jugular vein and carotid artery. Once a bird had been allowed to adequately bleed out, the skin with feather was removed using knife and hand force. After defeathering, the birds were eviscerated and the head and feet were removed. During the evisceration process, abdominal fat and liver were excised and weighed. Dressed birds were weighed to obtain a dressed carcass weight. Carcasses were cut into different cuts like- breast, back, thigh, drumstick etc. to measure individual cuts weight. The weights of visceral organs also measured.

Fig 3.6: Cutting of body parts and weighing

3.7.2 Meat Quality Test: Chemical analyses of the meat samples were carried out for Dry Matter (DM), Crude Protein (CP), Ether Extracts (EE) and total ash (TA) in the Animal Nutrition laboratory, CVASU, Chittagong as per AOAC (2006). From each treatment adequate amount of meat sample was taken and preserved in an air tight bag to carry them in the laboratory for analysis during the experimental period. After slaughtering the bird, 120 g of meat was collected in the air tight bag from each carcass for the estimation of chemical composition of meat. Then drying of the sample was performed in oven at 80° C. After drying chemical analysis was done for DM, CP, EE and TA as per AOAC (2006).

Fig 3.7: Estimation of dry matter and meat sample in air tight bag.

Fig 3.8: Estimation of crude protein, ether extract and ash.

3.7.3 Hematological analysis: Blood samples were collected from the brachial vein of two birds from each group (one birds from each replicate) using a 3 ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant EDTA.

Fig 3.9: Collected blood sample and serum collection from blood.

The total red blood cell (Erythrocyte) counts were performed in a 1:200 dilution of blood in Hayem's solution. The differential leukocyte counts were determined by preparation of blood smears stained with Wright's stain. The Hb concentration was evaluated by matching acid hematin solution against a standard colored solution found

in Sahl's hemoglobinometer. Packed cell volume (PCV) was measured by standard manual technique after centrifugation of a small amount of blood using micro-hematocrit capillary tubes (Coles, 1986)

3.7.4 Biochemical analysis: Blood was collected without anticoagulant from a total of two birds from each group at 21th and 28th days of age of broilers. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the ependroff tube by micropipette.

Fig 3.10: Serum sample and some reagent for test of SGOT, SGPT, Total protein & Creatine.

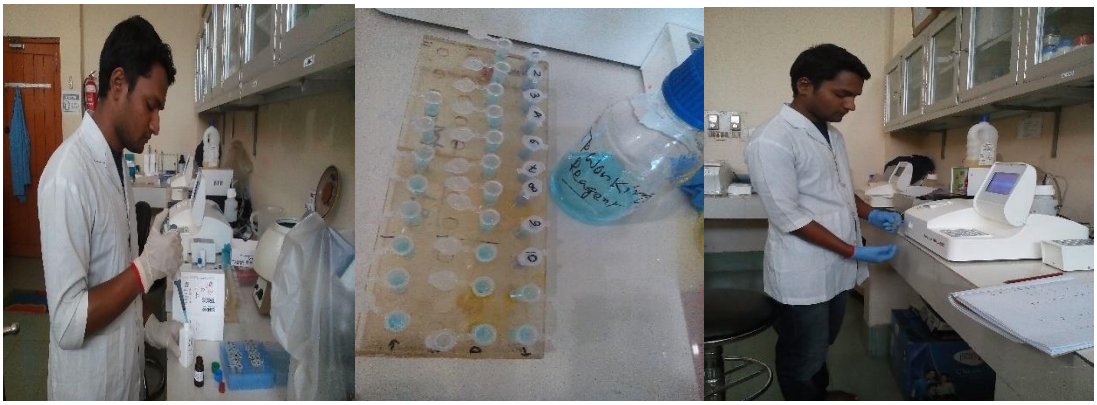

Fig 3.11: Preparing serum for test and Estimating biochemical parameter by Humalyzer.

Sera were marked and stored in -20°C until analyzed for total protein, tryglyceride, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), serum cholesterol, serum creatinine, by Humalyzer 3000 (Wisbaden, Germany). It was semi-automatic machine, microprocessor-controlled photometer with large graphic LCD screen. Randox® veterinary reagent kits were used for determination of the blood parameter of interest. Serum sample was mixed with the respective reagents with a specified time (as per manual) in an ependroff tube. Then the serum with reagent was aspired by spectrophotometric method which measured the target parameter and immediately the printed result was recorded in the blood parameter sheet.

3.8 Data collection

Data regarding the experimental work were collected and recorded according to the set objectives. Following parameters were recorded throughout the experimental period.

3.8.1 Weight gain: Weight of the chicks was recorded at the end of first week, second week, third week and forth week. The weekly weight gain was calculated by deducting weight of two corresponding weeks.

Weight gain = (Final body weight-Initial body weight)

3.8.2 Feed intake: Feed intake was calculated by deducting the left over feeds from the total amounts of supplied feed to the broilers. Feed intake was calculated as gm/bird/day.

3.8.3 Feed Conversion Ratio (FCR): The amount of feed intake per unit of weight gain is the feed conversion (FC). This was calculated by using the following formula.

$$FCR = \frac{Feed\ intake\ (kg)}{Weight\ gain\ (kg)}$$

3.9 Statistical analysis

All the data of performance, carcass characteristics and blood parameters were entered into MS excel (Microsoft office excel-2007, USA). Data management and data analysis were done by one way ANOVA using SPSS 16.0. Means showing significant differences was compared by DMRT Test (Duncan, 1955). The P value of <0.05, <0.01 or <0.001 was considered statistically significant.

CHAPTER IV: RESULTS

The experiment was carried out to find out the effect of organic acid on the performance parameters, carcass characteristics, blood parameter of Cobb-500 broilers to substitute antibiotic. The results obtained from the study have been described in this chapter.

4.1 Live weight

Live weights of the experimental birds were recorded weekly basis throughout the whole experimental period. Results indicated that, weekly average live weight differed highly significant ($p<0.001$) at 3rd and significant ($p<0.01$) at 4th weeks but insignificant at 1st & 2nd week of age. Highest weekly average live weight (1666.50 gm/bird/week) was recorded in T_1 (citric acid) treatment group and the lowest average live weight 1497.00 gm/bird was recorded in T_2 (formic acid) treatment group at 4th week.

Table 4.1. Live weight (gm/bird/week) of the experimental broiler birds supplemented with organic acid & antibiotic.

Age of birds	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
1st week	197.7	201	202	200.75	201.25	0.89	NS
2nd week	522	542.15	511.05	541.9	515.5	6.56	NS
3rd week	982.5^{bc}	978.9^{bc}	810.7^{a}	950^{b}	1024.35^{c}	36.62	***
4th week	1581.5^{b}	1666.5^{c}	1497^{a}	1619^{bc}	1632^{bc}	28.94	**

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$). a, b and c = Means having different superscript in the same row differ significantly.*

4.2 Weight gain

The weight gain of the experimental birds revealed that a significant level of variations were found during the 1st, 3rd and 4th week (**Table 4.2**). Considering the data on 2nd week, live weight gains were differed insignificantly ($p>0.05$) among the treatment groups. In term of weight gain, T_1 (Citric acid) group was performed better than other

groups and finally highest average daily weight gain (687.8 gm/bird/week) was found in T_1 (citric acid) group. It was observed that weight gain of T_2 group (Formic acid) was lowest at 3rd week of age.

Table 4.2. Weight gain (gm/bird/week) of the experimental broiler birds supplemented with organic acid & antibiotic.

Age of birds	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
1st week	149.55^{a}	153.9^{bc}	154.75^{b}	153.9^{bc}	153.45^{bc}	0.91	*
2nd week	324.5	341.15	309.05	341.8	314.25	6.7	NS
3rd week	459.95^{bc}	437.1^{b}	299.15^{a}	409.85^{b}	508.85^{c}	34.96	**
4th week	604.25^{a}	687.6^{b}	686.3^{b}	669^{b}	587.55^{a}	21.25	**

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$). a, b and c = Means having different superscript in the same row differ significantly.*

4.3 Feed intake

Similar to weight gain, feed intake differed significant ($p<0.01$) at 3rd week within all the water treatment groups. At the 4th weeks of age feed intake of bird were also significant ($p<0.05$). Highest feed intake (1086.95 gm/bird/week) was recorded at T_2 (formic acid) group and the lowest feed intake (1013 gm/bird/week) was recorded T_4 (antibiotic treatment) group at 4th week of age.

Table 4.3. Feed Intake (gm/bird/week) of the experimental broiler birds supplemented with organic acid & antibiotic.

Age of birds	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
1st week	`175.7	175.1	175.2	174.6	178.5	0.89	NS
2nd week	448.7	430.45	418.6	426.3	439	6.33	NS
3rd week	809^{cd}	687.05^{bc}	549.95^{a}	650.05^{ab}	839.45^{d}	52.88	**
4th week	1071.05^{b}	1075.7^{b}	1086.95^{b}	1077.3^{b}	1013.05^{a}	14.55	*

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$); a, b, c and d = Means having different superscript in the same row differ significantly.*

4.4 Feed Conversion Ratio (FCR)

FCR of the experimental birds varied in irregular fashion during the entire experimental period. It was revealed that, FCR differed significantly ($p<0.05$) at 2nd week of age within the treatment group.

Table 4.4. Feed Conversion Ratio (FCR) of the experimental broiler birds supplemented with organic acid & antibiotic

Age of birds	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
1st week	1.174^b	1.137^a	1.132^a	1.134^a	1.163^b	0.01	**
2nd week	1.29^a	1.3^a	1.35^b	1.311^a	1.39^b	0.01	*
3rd week	1.75^b	1.59^a	1.83^b	1.58^a	1.64^a	0.04	**
4th week	1.77^b	1.59^a	1.58^a	1.61^a	1.72^b	0.04	*
0-4th week	1.5^b	1.4^a	1.47^b	1.4^a	1.47^b	0.02	**

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$);a, b and c = Means having different superscript in the same row differ significantly.*

FCR increased gradually at 3rd & 4th week of age and varied significantly ($p<0.01$) & ($p<0.05$) at 3rd and 4th week respectively. It was observed that, the highest FCR (1.5) was recorded T_0 (control) treatment group and the lowest FCR (1.4) was recorded in T_1 (citric acid) & T_3 (acetic acid) treatment group considering the whole 28 days trial period.

4.5 Hematological analysis

The blood samples were collected from the brachial vein of two birds from each group (one birds from each replicate). The blood hematological parameters of experimental birds have been presented in the **Table 4.5**.

4.5.1. Packed Cell Volume (PCV) value: The packed cell volume (%) did not differ ($p>0.05$) within all water treatment groups at 3rd and 4th week. The maximum average value of PCV (31.5) was observed in T_0 group at 3rd week and the minimum average value (26.5) was observed in the T_3 at the same week.

Table 4.5. Blood hematological parameters of the experimental broiler birds fed water supplemented with organic acid at 3rd and 4th week of age.

Parameter (%)	Week	Water Treatment					SEM	Level of Sig.
		T_0	T_1	T_2	T_3	T_4		
PCV	3rd	31.5	28.5	27.5	26.5	30.5	0.93	NS
	4th	31	28.5	29.5	30.5	30.5	0.45	NS
ESR	3rd	2.5^{ab}	2^{a}	2^{a}	3.5^{b}	2^{a}	0.29	*
	4th	1.5^{bc}	1.75^{bc}	0^{a}	0.5^{ab}	2^{b}	0.38	*
TEC	3rd	2.6	2.725	2.08	2.605	2.765	0.12	NS
	4th	2.08	2.035	2.305	2.82	2.48	0.14	NS
Haemoglobin	3rd	6.7	7.4	7.2	7.1	7.9	0.19	NS
	4th	6.5	6.5	6.9	7.3	6.6	0.15	NS
Lymphocyte	3rd	67	74	67	72.5	67	1.55	NS
	4th	64.5	63	59.5	62.5	61	0.86	NS
Heterophil	3rd	21.5	21	22.5	19.5	18.5	0.71	NS
	4th	26.5	27.5	31.5	28	27.5	0.86	NS
Eosinophil	3rd	6.5	4.5	5	6	5	0.37	NS
	4th	3	4	4.5	3.5	4	0.25	NS
Monocyte	3rd	3.5	6	5	6.5	4.5	0.53	NS
	4th	5.5	4.5	4	6	4	0.41	NS
Basophil	3rd	1.5	1.5	0.5	0.5	1	0.22	NS
	4th	0.5	1	0.5	1	0.5	0.12	NS

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$);); a, b and c = Means having different superscript in the same row differ significantly.*

4.5.2. Erythrocyte Sedimentation Rate (ESR) Value: Erythrocyte sedimentation rate (%) differed significantly ($p<0.05$) at 3rd week and 4th week (**Table 4.5**) due to supplementation of organic acid and antibiotic. The maximum average value of ESR (3.5) was observed in T_3 group at 3rd week and the minimum average value (0.0) was observed in the T_2 at 4th week

4.5.3. Total Erythrocyte Count (TEC) Value: Total erythrocyte count (%) remained unchanged ($p>0.05$) at 3^{rd} and 4^{th} weeks (**Table4.5**) of age among water treatments groups. The maximum average value of total erythrocyte count (2.82) was observed in T_3 group at 4^{th} week and the minimum average value (2.035) was observed in the T_1 group in the same week.

4.5.4. Haemoglobin Value: Supplementation of organic acid had no marked influence ($p>0.05$) on haemogloblin (%) in the experimental birds. The highest average value (7.9) was found in the T_4 group at 3^{rd} week and the lowest average value of haemoglobulin (6.5) was found in the T_0 & T_1 group at 4^{th} week.

4.5.5 Lymphocyte Value: The lymphocyte (%) did not differ ($p>0.05$) within treatment groups at 3^{rd} and 4^{th} weeks of age. Highest average value (74) was observed in T_1 group at 3^{rd} week and the lowest average value (61) was observed in the T_4 group at 4^{th} week.

4.5.6. Heterophil Value: The heterophil (%) did not differ ($p>0.05$) within treatment groups at 3^{rd} and 4^{th} week of age.. The highest average value of heterophils (31) was found in the T_2 at 4^{th} week and lowest average value (18.5) was found in the T_4 group at 3^{rd} week.

4.5.7. Eosinophil Value: The blood eosinophil (%) did not exhibit marked changes (**Table 4.5**) within experimental groups. The maximum average value of eosinophil (6.5) was observed in T_0 group at 3^{rd} week and minimum average value (3.0) was observed in the T_0 group at 4^{th} week.

4.5.8. Monocyte Value: The monocyte (%) remained constant ($p>0.05$) both at 3^{rd} and 4^{th} weeks. The highest average value (6.5) was recorded in the T_3 group at 3^{rd} week. In contrast, the lowest average value (2.0) was found in the T_0 group at the same week.

4.5.9. Basophil Value: Supplementation of organic acid and antibiotic had no significant changes on basophil (%) at 3^{rd} and 4^{th} week of the experimental birds. Highest average value (1.5) was found in the T_0 & T_1 group at 3^{rd} week. In contrast, the lowest average value of (0.5) was recorded in T_2 & T_3 group at 3^{rd} week of age and in T_0, T_2, T_4 at 4^{th} week of age.

4.6 Biochemical Analysis:

The blood serum biochemical parameter of experimental birds have been presented in the **Table 4.6**

4.6.1. Serum Glutamic Oxaloacetic Transaminase (SGOT) Value: SGOT of the collected sample did not differ ($p>0.05$) at 3rd and 4th week in the treatment group. At the end of the experimental period, highest serum SGOT value (114) was found in T_4 group at 3rd week whereas the lowest value (78.4) found in T_0 group at 4th week.

Table 4.6. Serum parameters of the experimental broiler birds fed water supplemented with organic acid at 3rd and 4th week of age.

Parameter	Week	Water Treatment					SEM	Level of Sig.
		To	T1	T2	T3	T4		
GOT (μ/L)	3rd	103	110.9	110.9	106.6	114.3	1.96	NS
	4th	78.4	92.8	112	104.05	134	9.33	NS
GPT (μ/L)	3rd	7	6.8	6.4	5.95	6.95	0.19	NS
	4th	14.4	37.6	24.1	7.4	41.1	6.48	NS
Cholesterol (mg/dl)	3rd	97.95	99.5	109.15	117.55	121.8	4.73	NS
	4th	102.35	71.1	65.1	71.75	107.7	8.86	NS
LDL (mg/dl)	3rd	73.8^{a}	71.35^{a}	97.35^{b}	102.9^{b}	107.45^{b}	7.52	**
	4th	85	55.25	53.15	38.8	88.95	9.77	NS
HDL (mg/dl)	3rd	57	69.7	92.76	91.17	80.77	6.73	NS
	4th	45.5	37.75	55.65	81.5	29.6	8.97	NS
Creatinine (mg/dl)	3rd	0.5^{b}	0.5^{b}	0.1^{a}	0.45^{b}	0.45^{b}	0.07	**
	4th	0.15	0.2	0.2	0.05	0.15	0.02	NS
TP(mg/dl)	3rd	44.65	52.85	54.3	54.7	53.75	1.87	NS
	4th	42.3	76.3	43	49.5	78.3	8.12	NS
TG(mg/dl)	3rd	171.95	138.35	228.05	214.85	131.8	19.49	NS
	4th	56	66.8	71.55	78.85	72.85	3.82	NS

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$);); a, b and c = Means having different superscript in the same row differ significantly.*

4.6.2. Serum Glutamate Pyruvate Transaminase (SGPT) Value: The SGPT level of the birds did not differ significantly ($p>0.05$) at 3rd and 4th week of age (**Table 4.6**). The

maximum average of SGPT level (41.1) was found in T_4 group at 4th week; whereas the minimum level (5.95) was found in T_3 at 3rd week of age.

4.6.3. Serum Cholesterol Value: Cholesterol level (mg/dl) did not differ significantly ($p>0.05$) at 3rd & 4th week of age. The highest average value of serum cholesterol (121.8) was recorded in T_4 group at 3rd week whereas the lowest value (65.1) was found in the T_2 group at 4th week during the experimental period.

4.6.4. Serum Low Density lipoprotein (LDL) Value: Lipoprotein level (mg/dl) differed significantly ($p<0.01$) at 3rd week although it was statistically similar ($p>0.05$) at 4th week. The highest average value of serum LDL (107.45 mg/dl) in T_4 group at 3rd week of age whereas the lowest LDL value (38.8 mg/dl) in T_3 at 4th week of age during the experimental period.

4.6.5 Serum High Density Lipoprotein (HDL) Value: Serum HDL level (mg/dl) did not differed significantly ($p>0.05$) at 3rd & 4th week of age. The highest average value of serum HDL (92.76) was recorded in T_2 group at 3rd week whereas the lowest value (29.45) was found in the T_4 group at 4th week during the experimental period.

4.6.6. Serum Creatinine Value: Creatinine level (mg/dl) level differed significantly ($p<0.01$) at 3rd week although it was statistically similar ($p>0.05$) at 4th week. The highest average value of serum creatinine (0.5 mg/dl) in T_0 & T_1 group at 3rd week of age whereas the lowest LDL value (0.05 mg/dl) in T_3 at 4th week of age during the experimental period.

4.6.7. Serum Total Protein (TP) Value: Total protein (mg/dl) did not differ ($P>0.05$) at 3rd and 4th week (**Table 4.6**). Maximum average value (78.3) was observed in T_4 group at 4th week and the minimum average value (42.3) was observed in the T_0 group at the same week.

4.6.8 Serum Triglyceride (TG) Value: Triglyceride level did not differ significantly ($p>0.05$) at 3rd and 4th week of age (**Table 4.6**). The maximum average of TG level (228.05) was found in T_2 group at 3rd week; whereas the minimum level of TG level (56) was found in T_0 group at 4th week.

4.7 Meat Quality Test of Experimental Birds

The meat composition of the birds changed significantly in terms of dry matter and protein in different treatment group. But no significant changes($p>0.05$) were observed in Ether extract and ash percentage in different treatment group.

Table 4.7. Meat quality test of the experimental broiler birds supplemented with organic acid.

Parameter(%)	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
Dry Matter	28.38^{b}	25.98^{a}	27.41^{ab}	25.68^{a}	25.49^{a}	0.56	*
Crude Protein	71.51^{ab}	74.37^{bc}	70.26^{a}	81.81^{d}	77.61^{c}	2.09	**
Ether Extract	10.22	14.97	18.16	8.58	14.106	1.71	NS
Ash	4.69	4.68	4.33	4.66	5.39	0.17	NS

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$); a, b, c and d = Means having different superscript in the same row differ significantly.*

4.8 Carcass Charateristics

The carcass parameters significantly differed ($p<0.05$) in terms of dressing percentage, thigh and abdominal fat weight at 28 days. However, though other parameter differed numerically but it did not differ significantly ($p>0.05$) amongst dietary treatments. Other carcass parameters were statistically similar ($p>0.05$) throughout the entire experimental period.

Table 4.8. Carcass characteristics of the experimental birds fed water supplemented with organic acid at 4th week of age.

Parameter(%)	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
Dressing	57.5^{ab}	59.5^{b}	56.5^{ab}	58^{ab}	56^{a}	0.61	*
Drumstick	8.34	9.98	9.74	10.97	10.26	0.43	NS
Thigh	8.42^{a}	11.15^{ab}	9.23^{ab}	12.04^{b}	12.15^{b}	0.75	*
Wing	4.76	4.94	5.21	4.78	5.55	0.14	NS
Breast wt	21.05	24.73	22.50	23.05	21.96	0.61	NS
Neck	2.23	2.56	2.35	2.27	2.00	0.09	NS
Back wt	10.38	11.08	9.78	9.12	10.03	0.32	NS
Head	2.23	2.88	2.54	2.59	2.06	0.14	NS
Abdominal fat	2.03^{b}	1.24^{a}	1.05^{a}	1.74^{a}	2.05^{b}	0.20	**
Heart	0.42	1.65	0.61	0.46	0.47	0.23	NS
Liver	1.81	2.10	2.83	2.16	2.37	0.16	NS
Gizzard	1.38	1.56	1.36	1.04	1.28	0.08	NS
Proventriculus	0.62	0.54	0.62	0.46	0.49	0.03	NS

*N = Number of birds in a treatment: 20; T_0 = water without organic acid; T_1= water containing citric acid; T_2 = water containing formic acid; T_3 = water containing acetic acid; T_4 = water containing antibiotic; SEM=Standard Error of Mean; * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$);); a, b and c = Means having different superscript in the same row differ significantly.*

4.9 Cost-benefit analysis

The cost benefit analysis of the total experiment was done which shows us that the net profit differed significant ($p<0.001$). Highest net profit of taka 19.13 per broiler was found in T_1 (citric acid) group and lowest net profit of taka 4.339 was found in T_2 (formic acid) group.

Table 4.9. Cost-benefit analysis of broiler fed water supplemented with organic acid and antibiotic.

Parameter	Water Treatment					SEM	Level of Sig.
	T_0	T_1	T_2	T_3	T_4		
Live weight (kg)	1.58	1.66	1.49	1.62	1.63	0.02	**
FCR (0-4 week)	1.5	1.4	1.47	1.4	1.47	0.02	**
Feed intake (kg)/broiler	2.37	2.324	2.19	2.268	2.391	0.03	**
Feed cost per kg	43	43	43	43	43	0	NS
Feed cost/broiler	101.96	100.84	95.3	98.27	104.19	1.58	*
Chick cost	45	45	45	45	45	0	NS
Medication cost/broiler	5	5	5	5	5	0	NS
Labor cost/broiler	15	15	15	15	15	0	NS
Acid+Antibiotic cost	0	5	5	5	5	1	NS
Overhead costs/broiler*	10	10	10	10	10	0	NS
Total cost/broiler	176.91	180.84	175.3	178.27	184.19	1.49	*
Market price/kg broiler	120	120	120	120	120	0	NS
Market price/broiler	189.6^{b}	199.2^{c}	179.4^{a}	$194.4b^{c}$	195.6^{bc}	3.53	**
Net profit/broiler	12.81^{bc}	19.13^{d}	4.39^{a}	16.05^{cd}	11.643^{b}	2.49	***

*T_0=Water without organic acid; T_1=Water containing citric acid; T_2=Water containing formic acid; T_3=Water containing acetic acid; T_4=Water containing antibiotic; SEM=Standard Error of Mean; *=Overhead costs: costs for housing, feeder, waterer, sanitation equipments, disinfectants, extra labor, electricity and depreciation cost of the building;); a, b, c and d = Means having different superscript in the same row differ significantly.*

CHAPTER V: DISCUSSION

This study tested the effects of organic acid supplementation on broilers. We hypothesized that organic acid supplementation may have a variety of benefits, both in terms of performance and economic, which could play a major role in future broiler production, although it has some bad effects on broiler in early stage. Different pathogenic microorganism cause different diseases in chickens which create challenges from both a performance side as well as animal survivability. Water acidification has been shown to aid by showing its antimicrobial effect. This study investigated the effect of organic acid applied through water on broilers during a typical production life of 28 days.

5.1 Weight gain

Regarding the effect of organic acid supplementation on productive traits during the experimental period, it was evident that live weight and weight gain were significantly increased by citric and acetic acid supplementation as compared with control group. The obtained results confirmed the previous findings of several researcher (Shen-HuiFang *et al*., 2005: Denli *et al*., 2003: Stipkovits *et al*., 1992) who found that addition of citric acid to poultry diet significantly improved body weight. The results contradict with the findings of previous researcher Pinchasov et al. (1994) where depressed weight gain was observed with application of acetic acids in diets. But reduction of water pH from 7 to 4.5 with formic acid supplementation significantly decreased body weights as compared to control. This results is in agreement with the observations of Vieira *et al*. (2005) that acid mixture supplementation at different levels to water led to significantly reduced body weight gain but did not affect feed conversion ratio and mortality in broilers. However, our results similar with the findings of Pesti *et al*. (2004) indicating that acidified drinking water increased body weight in comparison to normal drinking water (2,146g vs. 2,117 g). In addition, Watkins *et al*. (2004) and Cornellison *et al*. (2005) found that water acidification did not affect the performance of turkeys and broilers. The difference in those results were possibly consequences of differences in the type and concentration of organic acid used in the studies. We observe that live weight and weight gain in antibiotic treated group increased comparing to

control group but decreased comparing to citric acid treated group. This findings is similar to the findings of Açıkgöz *et al*. (2011). This results also supports the findings of Hassan *et al*., (2010) where superiority of Galliacid compared to Biacid or Enramycin was indicated where Galliacid and Biacid were commercial organic acid and Enramycin was commercial antibiotic fed as growth promoter. In addition, Gauthier, (2005) reported that, contrary to antibiotics, organic acids have other properties like; lowering of the chyme pH consequently, enhancing of protein digestion. Here we observe that there was no significant variation in live weight and weight gain during first two week but statistically significant result was found during 3rd and 4th week. In a study conducted by Daskiran *et al*. (2004), it was stated that early exposure to dietary acidifier might cause an adaptation to acidifier in birds and reduce the subsequent therapeutic activity of acidifier. Therefore, they proposed to use the acidifiers in the grower phase rather than in the starter phase in order to reduce economic losses from heat stress. In the results Denli *et al*. (2003) observed slow increase in weight, using organic acid in the diet. The significant positive effect at later stage of the acidifier group was because of the stimulating role on enzymatic secretion; mainly on synthesis of gastric and pancreatic lipase (Tellez *et al*., 2012; Patterson and Burkholder., 2003; Choudhari *et al*., 2008) due to the reduction of the growth depressing metabolites produced by microorganism in the gut (Feighner and Dashkevicz, 1987; Knarreborg *et al*., 2004), due to the prevention of exponential multiplication of common pathogenic bacteria (E. coli, Salmonella spp, Streptococcus spp, etc.) and due to the alteration of the pH in the gut (Brennan *et al*., 2003). There have been many successful demonstrations of positive effects of organic acids on growth performance, whereas other studies were unable to find beneficial effects or even reported negative effects on growth performance due to its rapidly metabolized capacity in the foregut the crop to the gizzard (Lückstädt, 2014). Some studies also showed no performance difference, in comparison with the negative control and/or the birds fed antibiotics (Gunal *et al*., 2006; Vieira *et al*., 2005; Kopecký *et al*., 2012). There are conflicting results regarding the use of acidifiers in poultry and, according to (Hernandez *et al*., 2006), these effects depend on the chemical form of the acid, pKa values, bacterial species, animal species and the site of action of acids.

5.2 Feed intake

From our finding it is evident that average feed intake was lower in organic acid treated group comparing to control and antibiotic group and differed statistically ($P<0.05$) only at 3rd and 4th week of age. These results agree with the finding of previous researchers (Darko et al., 1991; Frigg et al., 1983 and Stipkovits et al., 1992) where depressed feed intake was observed. Some others research also showed some dissimilar result, they found supplementation of 0.2% or 0.3% acidifier had no effect on feed intake than those without acidifier (Adil *et al*., 2010) and has also positive effect on feed intake (Islam *et al*., 2008).

At 4th week the highest feed intake was in citric acid treated group which is similar to the results of Islam *et al*. (2008). Among the organic acid there was lower feed intake in formic acid treated group which was accompanied by retarded growth to be the consequence of depressed water intake by the application of formic acid in water. The reduction in the feed intake might be due to the unfavorable taste associated with the formic acid which would have decreased the palatability of the feed, thereby reducing feed intake which cause of significantly decreasing body weights at 14 and 21 days of age (Vieira *et al*., 2008 : Acikgoz *et al*., 2011).

5.3 Feed conversion ratio (FCR)

The weekly feed conversion at different ages of broilers supplemented with organic acid in water indicated that acidification of water improved feed conversion ratio of broiler. Our result shows that feed conversion or nutrient utilization was lowest in control group but much better in organic acid and antibiotic treatment group. Here organic acid and antibiotic treated group gave almost same FCR which is similar to the findings of Hassan *et al*. (2010). The better feed conversion ratio in organic acid treated groups was might be due to the lowering of the pH of the digestive organ which led to better digestion, absorption and utilization of nutrients (Dhama *et al*., 2011). The lowering of the pH, optimized the activity of proteases and beneficial bacteria (Partanen and Mroz, 1999; Nava *et al*., 2009; Overland *et al*., 2000) and enhanced feed conversion by broiler birds. According to Adil *et al*. (2010) showed that, in slow growth type chickens, supplementation of 0.3% acidifier improved weight gain and feed conversion. In a recent study, the addition of 0.1% acidifier to water improved feed

efficiency of broiler (Hedayati *et al*., 2013 and Brzóska *et al*., 2013). The improvement in FCR could be possibly due to lesser feed intake resulting in increased body weight gain because of better utilization of nutrients in the birds fed organic acids in the diet. However, in contrast to present study, Brzóska *et al*. (2013) did not find any effect of acidifier on feed conversion in broilers. One other study demonstrated that addition of acidifier in water for broilers improved feed conversion ratio at later stage (Král *et al*., 2011).

5.4 Carcass characteristics

It is evident from the **Table 4.8** that in citric acid treated group the dressing yield was improved by about 2% when compared with the control group. This result did not agree with previous findings of Garcia *et al*. (2000) and Kahraman *et al*. (1997) where no significant effect was observed. But this result partially agreed with Sapra and Mehta., (1990), who found increased edible meat yield with increasing body weight. The increased dressing yield on citric acid treated group might be due to increasing live weight. Moreover the **Table 4.8** of carcass yield represented a significant change in abdominal fat weight of all treatments group and other parameters were not significantly changed. It is evident from the **Table 4.8** that all the organic acid treated bird has lower abdominal fat which is similar to Castellini *et al*. (2002) where it is reported that, acidifier has the capacity to decrease abdominal fat. This similarity was also seen in Garcia *et al*. (2007) who reported that the abdominal fat of the acidifier supplemented chicks was less than that of the control group. In case of heart and liver of the various treatment group of this experiment; though varied numerically but did not differ significantly. This is in agreement with Ogunwole *et al*. (2011) who also reported no significant difference in liver weight and heart weight of broilers treated with dietary acidifiers. acidifier supplemented chicks was less than that of the control group.

Other parameters of all the treatment groups were not significantly changed. These results indicate that there were no statistically significant differences in carcass quality between the control and trial groups in other parameters which is similar to Islam *et al*. (2008).

5.5 Hematological Changes

Here we observe that the number of Heterophil, eosinophil basophil, monocyte and lymphocyte in maximum cases increased comparing to antibiotic. It seems that the microbial interaction and effect on local immune stimulation results increase of white blood cells and immunity. These results are in accordance with findings of Zareshahneh *et al*. (2007) and EFSA, (2010). Moreover no significant difference were found in blood haemoglobin level in all treatment which resemble to the results of Al-Mayah and Al-Ahmed (2005). Again highest lymphocyte value of 74 at 3rd week in citric acid treated group supports the result of Haque *et al*. (2010) where it was reported that citric acid supplementation (0.5%) enhanced the density of the lymphocytes in the lymphoid organs, enhancing the non-specific immunity.

5.6 Biochemical changes

Supplementation of organic acids showed no significant($P>0.05$) difference in the concentration of serum GOT, GPT and creatinine(4th week) level among all the treatment groups including the control group confirming the earlier findings of Abdel-Fattah *et al*. (2008) who concluded that dietary supplementation of organic acids could be done up to the level of 3% in the diet of broiler chicken without causing any adverse effect on the kidney and liver functions. Though there was found significant difference in serum creatinine level at 3rd week of age but no signigicant difference in 4th week was observed. Again the data of serum cholesterol, LDL (low density lipoproteins) and HDL (high density lipoproteins) in **Table 4.6** revealed that, broilers watered with acidified drinking water were exhibited a lower level of serum LDL and higher level of HDL(except in citric acid treated group) compared with with control group and antibiotic group at 4th week of age but it is dissimilar to 3rd week of age. This findings of serum lipid profile are in agreement with Fallah and Rezaei., (2013), who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. A significant decrease was observed in serum lipoprotein level in acidifier treatment (SAS, 2000; Kamal and Ragaa, 2009; Abdel-Fattah *et al*., 2008). The role of organic acids in decreasing blood fat may explain via their effect on decreasing intracellular microbes by prevention of microbial enzymes activity and forcing cellular bacteria for using energy in order to release protons which cause forming of mass intracellular

anions. Data presented in Table 4.8 showed that, dietary supplementation of organic acids exhibited relatively noticeable increase, although insignificant in the serum concentration of total protein at 4th week compared with non- supplemented control. The present result coincides with Abdo *et al*. (2004) who obtained simiral result in broiler chicks due to acetic acid inclusion.

5.7 Chemical composition of meat

It was observed that there was significant difference in dry matter and protein percentage of meat which is in contrast with Brzóska *et al*. (2013) where the author got no signficant difference among dry matter, crude protein, ether extract and ash of breast and leg muscle of broiler birds treated with dietary acidifier which is in the same line of our findings of getting no significant difference in ether extract and ash percentage of broiler. The sigificant difference in dry matter and protein percentage may be due to the sample containing mixed meat of different body parts.

CHAPTER VI: CONCLUSION

From the overall experimentation, it was found that weekly average live weight difference was highly significant ($p<0.001$) at 3rd week and significant ($p<0.01$) at 4th week and highest weekly average live weight was recorded in citric acid (T_1) treatment group. Results of weight gain differed significantly ($P<0.01$) during 3rd and 4th week of age and increased weight gain observed on citric acid (T_1) treated group when compared with the control and antibiotic group during 4th week of age. Similar to weight gain, feed intake differed significant ($p<0.01$) at 3rd week within all the treatment groups. At the 4th weeks of age feed intake of bird were also significant ($p<0.05$. It was revealed that, FCR differed significantly ($p<0.05$) at 2nd week of age within the treatment group. It increased gradually at 3rd & 4th week of age and varied significantly ($p<0.01$) & ($p<0.05$) at 3rd and 4th week respectively. It was observed that, the highest FCR was recorded control (T_0) treatment group and the lowest FCR was recorded in citric acid (T_1) & acetic acid (T_3) treatment group considering the whole 28 days trial period. In addition to performance parameter, organic acid had significant effect on dressing percentage, thigh weight and abdominal fat weight. The meat composition of the birds changed significantly in terms of dry matter and protein in different treatment group but no significant changes($p>0.05$) were observed in ether extract and ash percentage in different treatment group. Similar to performance parameter ESR differed significantly ($p<0.05$) at 3rd and 4th week of age. Interestingly, Blood Lymphocyte, Monocyte, Eosinophil, Basophil, Heterophil, Hb, TEC, and PCV remained unchanged ($p>0.05$) throughout the whole experimental period irrespective of organic acid and antibiotic supplementation. Serum LDL and creatinine level differed significantly ($p<0.01$) at 3rd week of age. However, supplementation of organic acid had no influence ($p>0.05$) on Serum Glutamic Pyruvic Transaminase, Serum Glutamic Pyruvic oxaloacetate, Cholesterol, High density lipoprotein, Total Protein and Triglyceride. Maximum net profit per broiler was obtained from birds fed water supplemented with citric acid. It could therefore be inferred that, supplementation of citric acid improve performance parameter, carcass characteristics and net profit without interfering blood and serum parameters in commercial broiler. Therefore this study suggests citric acid as potential growth promoter to substitute antibiotic growth promoter for commercial broiler farming.

CHAPTER VII: RECOMMENDATION

The use of organic acid in drinking water is a relatively recent development in poultry production. In tropical production systems, this may play a pivotal role in providing hygienic drinking water and reducing pathogen load, thus having enormous potential as a sole component of a successful bio-security programme. However, supplementation of citric acid at 4.5 pH level is recommended in regular drinking water of broiler at later stage (During $3^{rd}/4^{th}$ week and onward) for better growth but the long term effect of acidifier supplementation on productive performance of broilers should be investigated in future.

Due to some unavoidable constraints and technical limitations, some vital blood parameters like Glucose, calcium, phosphorus and other trace minerals both in meat and feed were not analyzed. These parameters could have vital impact on human health and will explore new horizon for investigating those parameters as future study.

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BRIEF BIOGRAPHY

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Appendix A

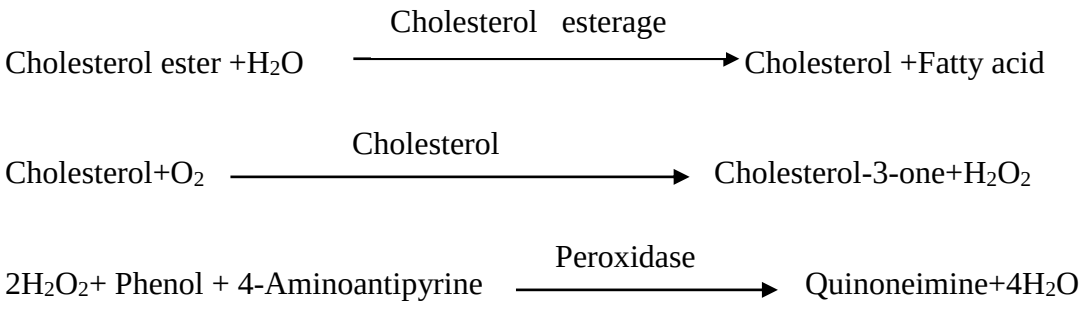
Methods of estimating different biochemical parameters (according to manufactures instruction)

Cholesterol assay

Principle

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

Reactions



Materials and reagents

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was an enzmatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tube was taken. Then 10µl of cholesterol standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. 1000µl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

Triglyceride assay

Principle

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenezone and 4-Chlorophenol under the catalytic influences of peroxidase.

Materials and reagent

1. Serum sample
2. TG conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

The sterile eppendorf tubes were taken. Then 1000µl TG standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

LDL assay

Principle

The principles outcome of LDL is based on the principle of competitive bindings between LDL and LDL reagent. Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (PH-5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant. The absorbance of this complex is proportional to the LDL concentration in the sample.

Materials and reagents

1. Serum sample
2. LDL conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

The sterile eppendorf tubes were taken. Then 100µl of LDL standards was taken in an eppendorf tube and 100µl of sample serums were taken in each eppendorf tube. 1000µl of LDL conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. The LDL concentration of the supernatant was determined within 1 hour after centrifugation. LDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with LDL conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

HDL assay

Principle

Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitavily by the addition of phosphotangstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density Lipoprotein) fraction, which remains in the supernatant, is determined.

Materials and reagents

1. Serum sample
2. HDL conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol.

Procedure

The sterile eppendorf tubes were taken. Then 400µl of HDL standards was taken in an eppendorf tube and 200µl of sample serums were taken in each eppendorf tube. 100µl of distilled water was then added to each eppendorf tube. The eppendorf tube was kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. Then 50 µl HDL concentration of the supernatant was taken and 1000 µl Cholesterol reagent added determined within 1 hour after centrifugation. HDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with HDL conjugate reagent was examined by automated humalyzer and the r reading was taken. The standard value was used as a compared tool, absorbent paper or paper towel or cotton and gloves.

Total protein assay

Principle

The principle outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

Materials and reagents

1. Serum sample
2. Total protein conjugate reagent
3. Precision pipettes: 20µl and 1.0ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20µl of total protein standards was taken in an eppendorf tube and 20µl of sample serums were taken in each 24 eppendorf tube. 1000µl of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Total protein standards with conjugate.