



**EFFECTS OF ACIDIFIER SUPPLEMENTATION ON
PRODUCTIVE PERFORMANCE, SERUM
LIPOPROTEIN LEVEL AND CARCASS
CHARACTERISTICS OF BROILER**

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Roll No. 0214/07

Registration No. 193

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**A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Animal and Poultry Nutrition**

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DECEMBER 2016

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December 2016

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This is to certify that we have examined the above Master's thesis and have found that the thesis is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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List of Abbreviation

ANOVA	-	Analysis of variance
ARC	-	Agricultural Research Council
BBS	-	Bangladesh Bureau of Statistics
BBC	-	British Broadcasting Corporation
BER	-	Bangladesh economic review
BC	-	Before Christ
BMD	-	Bangladesh Meteorological Department
BCRDV	-	Baby Chick Ranikhet Disease Vaccine
Ca	-	Calcium
CF	-	Crude fibre
CP	-	Crude protein
DM	-	Dry Matter
EE	-	Ether extract
EU	-	European Union
FAO	-	Food and agriculture organization
IBD	-	Infectious Bursal Disease
GIT	-	Gastro intestinal tract
DNA	-	Deoxyribonucleic Acid
DOC	-	Day Old Chick
LW	-	Live weight
Kg	-	Kilogram
FCR	-	Feed conversion ratio
LW	-	Live weight
SEM	-	Standard error of mean
NFE	-	Nitrogen free extract
NS	-	Non significant
P	-	Phosphorus
<	-	Less than
>	-	Greater than
e.g	-	Example
et al.	-	And his associates
etc.	-	Et cetera
gm	-	Gram
%	-	Percentage
i.e.	-	That is
Sig.	-	Significance
Ref.	-	Reference
MS	-	Master of Science
ME	-	Metabolic energy
CVASU	-	Chittagong Veterinary and Animal Sciences University
SGPT	-	Serum Glutamic-Pyruvic Transaminase
SGOT	-	Serum Glutamic Oxaloacetic Transaminase

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Abstract

One hundred Cobb 500™ broiler chicks were used in a 28-day trial at Chittagong Veterinary and Animal Sciences University (CVASU) poultry research shed to study the effects of supplementation of water acidifier on performance parameters, carcass characteristics and serum lipoprotein level in commercial broiler. Birds were divided into five watery treatment groups designated as T₀, T₁, T₂, T₃ and T₄ and each treatment was further divided into two replication having 20 birds per replicate. Acidifier was supplemented at 0%, 0.1%, 0.2%, 0.3% and 0.4% in water for different treatment groups respectively. All birds had free access to ad-libitum feeding and watering. Results indicated that, average daily weight gain was significantly ($p < 0.001$) lower from 49.4 to 44.1 g/day at 2nd week and insignificantly ($p > 0.05$) less from 62.5 to 59.6 g/day at 3rd week, but at the 4th week of age body weight gain increased insignificantly ($p > 0.05$) 85.0 to 93.6 g/day at the level of acidifier supplementation increased from 0% to 0.4% in water compare to control group. The highest daily average weight gain 93.6g was recorded in T₄ group and the lowest daily average weight gain 80.0g was recorded in T₀ group at 4th week. Unlike to weight gain, feed intake differed significantly from 2nd ($p < 0.01$), 3rd ($p < 0.05$) and 4th ($p < 0.01$) weeks of age at the level of acidifier supplementation increased. Feed intake decreased from 148.5g/day to 136.1g/day at 4th week of age at the level of acidifier supplementation increased from 0% to 0.4%. In the same week, the highest daily average feed intake 148.5g was recorded in T₁ group and the lowest daily average feed intake 136.1g was recorded at T₄ group. FCR was differed significantly at 2nd ($p < 0.001$) and 4th ($p < 0.05$) weeks of age at the level of acidifier supplementation increased and also dissimilar ($p > 0.05$) at 3rd week in acidifier treatment group compare to control group. However, best FCR (1.4) was observed in the T₄ group and worst FCR (1.8) was observed in the T₀ group at 4th week of age. In case of water intake, acidifier was cause to decrease water intake significantly ($p < 0.001$) from 2nd and 3rd weeks of age at the level of acidifier supplementation increased, but increased water intake significantly ($p < 0.05$) at 4th week of age. The highest daily average water intake 375.0 ml was recorded at T₄ group and the lowest daily average water intake 359.4 ml was recorded at T₁ group. In addition to performance parameter, acidifier had significantly ($p < 0.001$) decreased abdominal fat weight and significantly ($p < 0.01$) increased gizzard weight. LDL and HDL level of blood serum were significantly ($p < 0.01$) decreased at 4th week in acidifier supplementation group. However, supplementation of acidifier had no influence ($p > 0.05$) on blood serum pH. It could therefore be inferred that, increasing levels of supplemental acidifier substantially improve performance parameter and carcass characteristics at later stage (During 4th week and onward) of commercial broiler with decreasing serum lipoprotein level (LDL and HDL).

Keywords: water acidifier, carcass characteristics, feed intake, feed conversion ratio, serum lipoprotein level, water intake and weight gain.

Chapter I: Introduction

High level of production and efficient feed conversion are the need of modern poultry industry, of which to a certain extent could be achieved by the use of specific feed additives. But this performance may be lessening by different types of pathogenic microorganisms. For preventing from these diseases and improvement of growth performance, antibiotics were used worldwide in poultry industry in the past 60 years. But continuous misuses of antibiotics in livestock production, especially poultry industry resulted many concerns about 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 (Ghahri *et al.*, 2013).

Therefore, animal researchers and animal food producers are looking for suitable feed additives to improve poultry performance. Organic acids have been used as dietary supplements in animal production for the past 50 years, mainly as additives in pig diet. In poultry, their application is relatively recent; it started from the late 1970's and early 1980's. Scientist reported firstly at 1981, improvement of broiler performance was occurred by using formic acid (Luckstadt and Mellor, 2011). In 2006, acidifier supplementation to poultry diets has augmented, when European Union (EU) banded the use of antibiotics as growth promoter (Abdel-Fattah *et al.*, 2008).

In general, acidifiers are functional feed additives made from organic acids which are organic compounds with acidic properties associated with their carboxyl group $-COOH$ and their salts. A number of different organic acids and salts can be used as acidifiers. The most common acids using as acidifiers for poultry are formic acid, acetic acid, propionic acid, butyric acid, lactic acid, citric acid, fumaric acid, malic acid, sorbic acid etc and their salts. Organic acids are found in several fruits and vegetables.

According to modern farming standards, chicken mortality rates must not exceed 4% (Brzoska *et al.*, 2013). In our environmental conditions, tropical conditions play an important role to accelerate bacterial growth with increasing water temperature which impaired productivity in the poultry along with excessive mortality. In this situation, acidifiers have

contributed greatly to the profitability in poultry and also provide people with health and nutritious poultry products.

Several scientific reports demonstrated that acidifiers and organic acids could different functions; the most important effect of the acidifier is its anti-microbial effect. It works in different ways; firstly short chain of organic acids for example formic acid can penetrate the bacterial cell membranes and destroy DNA unlike the inorganic acids which cannot penetrate bacterial cell (Koh *et al.*, 2014), secondly the free hydrogen proton of a dissociated organic acid lowers pH, thereby creating unfavorable conditions for bacterial pathogens, thirdly the undissociated form of organic acids directly penetrates the lipid membrane of Gram-negative bacteria (Ricke, 2003) and cease the bacteria's growth by inhibiting oxidative phosphorylation and causing increased energy expenditure (H⁺-ATPase pump) and finally acidifiers modify the P^H of both the feed and the animal's digestive tract and can disrupt the normal cell function and protein synthesis of various gut microorganisms. Several scientific reports demonstrated that acidifiers and organic acids has capacity to decrease the intra-luminal concentration of coliform bacteria and other acid-intolerant organisms, such as *Campylobacter spp* and *Salmonella spp*, known to be involved in digestive disorders (Thompson and Hinton, 1997) along with other pathogenic microorganisms like- *Clostridium spp*, *Enterococcus spp* etc. and balance bacterial population in poultry (Hedayati *et al.*, 2013).

Furthermore, it can decrease the frequency of coccidiosis in broiler (Dhama *et al.*, 2008). Low-molecular-weight organic acids, particularly propionic acid, also inhibit the disease caused by different species of mold, fungi and yeast (Brzoska *et al.*, 2013).

Beside these antimicrobial effects, it can accelerate immune capacity of bird and remove 'heat stress' which can fall the productive performance of a broiler. In addition, it has been suggested that lowering the pH by organic acids improve nutrient absorption (Biggs and Parsons, 2008). 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).

Acidifier has number of further different important functions; 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.

In view of current researches, it can be concluded that the acidifiers 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.

Possibility of substitution of supplemental antibiotic with acidifier still is subject of research and controversy, especially the efficiency of acidifier addition for the purpose of full substitution of antibiotic in broiler diets.

1.1 Justification of the study

Use of antibiotic in broiler production is now discouraged and EU already banned due to the negative public health effects. In near future the use of antibiotic may be restricted all over the world. The antimicrobial properties of acidifier may decrease the disease frequency in broiler production, in turn may have a positive impact on animal health status and nutrient digestibility. Since the use of in-feed antibiotics will be restricted in the future, there will be growing interest in using acidifier as a bioactive compound for improving gut health. Furthermore, acidifier seems to contribute to a certain extent to a reduced mold, yeast caused diseases. There is also some evidence that acidifier may keep the human health by avoiding antibiotic residual effects and drug resistance effects. Additionally, considerable improvements in performance and carcass quality such as improved carcass quality and lower feed conversion have been reported. In particular, under certain physiological conditions, acidifier has positive effect against coccidiosis and has capacity to increase the utilization of mineral particles and other nutrients along with immune boosting properties, which may lead to the livestock production in a positive manner. Maximum study, used acidifier in feed level which has some controversial effects; to avoid this circumstances, the study is going to know its effects in water level, concentration level of acidifiers in water and its better concentration level of acidifiers on broiler.

1.2 General objective

To measure the effects of water acidifier in broiler production.

1.3 Specific objectives

1.3.1 To observe the effects of acidifier supplementation on feed intake, water intake, weight gain and FCR in commercial broiler.

1.3.2 To identify the effects of acidifier supplementation on carcass characteristics in commercial broiler.

1.3.3 To evaluate the effect of acidifiers supplementations on serum lipoprotein level (LDL and HDL) in commercial broiler.

1.4 Research questions

1.4.1 Is there any effect of acidifier on productive performance of broiler?

1.4.2 Which level of water acidifier improves productive performance of bird?

1.4.3 Have any effect of acidifier on carcass characteristics of broiler?

1.4.4 Does acidifier influence blood serum P^H of broiler?

1.5 Scope of the Study

The purpose of the study was to assess the effectiveness of water acidifier on productive performance, carcass quality, serum lipoprotein level of blood and blood serum pH in broiler. This study involved acidifier supplement, effectiveness of acidifier and verify the level of acidifier.

1.6 Limitations of the study

1.6.1 The sample size was only 100 birds due to resource limitation.

1.6.2 Seasonal variations were not observed due to limited study period.

1.6.3 Temperature could not be controlled due to load shedding and other unexpected circumstances.

1.6.4 Bio-security was not so good due to its location.

Chapter II: Review of Literature

2.1 Acidifier

Acidifier word, originated from the word acidify by adding with (+ -er) in late 18th century. In general, acidifiers as functional feed additives made from organic acids and their salts are included in feeds or water in order to lower the pH of the feed, water, gut and microbial cytoplasm thereby inhibiting the growth of pathogenic intestinal microflora (Paul *et al.*, 2007a). Organic acids are organic carboxylic acids, including fatty acids and amino acids, of the general structure R-COOH and it have been used extensively for more than 25 years in swine production and more recently in poultry (Fuller, 1989).

Antibiotics and acidifiers both feed additives are possessing beneficial effects but antibiotic use in the poultry industry has been intensively controversial because of the development of bacterial resistance and potential consequences on the human health (Cheng *et al.*, 2014).

Although, antibiotics as a growth promoter, has a capacity to fulfill different physiological functions (nutrient absorption and feed intake), nutritional functions (energy and nitrogen retention), metabolic functions (liver protein synthesis) and other functions along with enhance the immunity, but it has some negative effects; it hampers the time of feed transit, gut wall diameter, gut wall length, gut energy and vitamin synthesis along with ammonia production, toxic amine production and fatty acid oxidation. Moreover, Europe reported bacterial resistance to Vancomycin firstly in 1980; CC398- Methicillin-resistant *Staphylococcus aureus* was produced by the use of antibiotic in livestock. Study also released by CSE, India found antibiotic residue in chicken and antibiotic resistant bacteria have been found in Brazillian Cattle. By concerning above fact, EU banded using antibiotics on poultry production in 2006 (Castanon, 2007).

So, the alternatives to antibiotics are researched. Among this two compounds, organic acids are promising alternatives (Gunal *et al.*, 2006). Health of the gut is one of the major factors governing the performance of birds and thus, the economics of poultry production (Islam *et al.*, 2008) and the profile of intestinal microflora play an important role in gut health. Purpose

of giving acidifiers in poultry production is to lower the P^H below 5, inhibit the growth of harmful bacteria directly and indirectly and reduce the buffering capacity of feed and water.

Therefore, it improves feed and water hygiene by reducing P^H and inhibiting microbial growth, gut health by reducing P^H and improving pepsin activity, enzyme secretion and nutrient digestibility. The antibacterial action of organic acids depends on whether the bacteria are pH sensitive or not. Thus, the antibacterial effect of organic acids is by modification of bacterial internal pH, inhibition of bacterial fundamental metabolic functions and accumulation of toxic anions in bacteria and disruption of bacterial cellular membrane.

Poultry industry started to use acidifiers in the mid decade of 1970 to 1980. Many studies showed that, acidifier has many benefits to the broiler industry and specific target of organic acid usage in Poultry are disease control, enhance growth performance along with improving carcass yield and can be used for various metabolic pathways for energy generation. The results also showed that acidifier has many benefits to the broiler industry including; improved carcass yield (Garcia *et al.*, 2007), increased carcass yield and breast percentage as well as decreased abdominal fat (Castellini *et al.*, 2002) and decreased mortality (Patten and Waldroup, 1988).

Other studies have indicated that acidifier can cause improved growth, feed conversion efficiency and breast yield when supplemented in a antibiotic free diet (Islam, 2012), improved performance under heat stress and improved dressing percentage in 42 day old male broilers (Nourmohammadi *et al.*, 2010). Some other benefits include: improved weight gain and feed efficiency (Abdel-Fattah *et al.*, 2008). Acidifiers has growth prompting effects by reducing of P^H , inhibiting bacterial growth, improving gut health, increasing digestion, absorption of the nutrients, increasing the retention of protein, amino acids and minerals, improving gut morphology and reducing the formation of biogenic amines, particularly in high protein and containing added synthetic amino acid. Buffering capacity, composition of diets, qty of fermentable carbohydrates, presence of toxic metabolites such as biogenic amines, type/pka/dose of supplemented acid, colonization and activity resulting in acid production, receptors for bacterial colonization on epithelial villi and immunity level are the factors affecting acidifier's outcome.

Acidifiers has some risk factors; decrease diet palatability (when added at excessive level), lower feed intake (due to the strong odour and flavour) and corrosiveness which may lead to hamper the production performance of broiler (Aclkgoz *et al.*, 2011). To avoid risk factors, evaluate the natural BC of feeds to determine the minimum amount of acid required, use of slow release form of acid and use of organic acid with fatty acid and monoglycerides and diglycerides mixes to form micro granules.

2.2 Chemistry of organic acid

Organic acids are organic carboxylic acids, including fatty acids and amino acids, of the general structure R-COOH (Al-Kassi and Mohssen, 2009). 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 hydroxyl group such as lactic, malic, tartaric and citric acids or " Short chain carboxylic acids containing double bonds like fumaric and sorbic acids. 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.

2.3 Acidifiers and its characteristics

Organic acids are mainly divided into two types, one is short chain fatty acid; formic acid, acetic acid, propionic acid etc. reduce pH & affect directly gram (-) bacteria and fumaric 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.

Table 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

Source: Daa vision, 2014.

2.4 Mode of action 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).

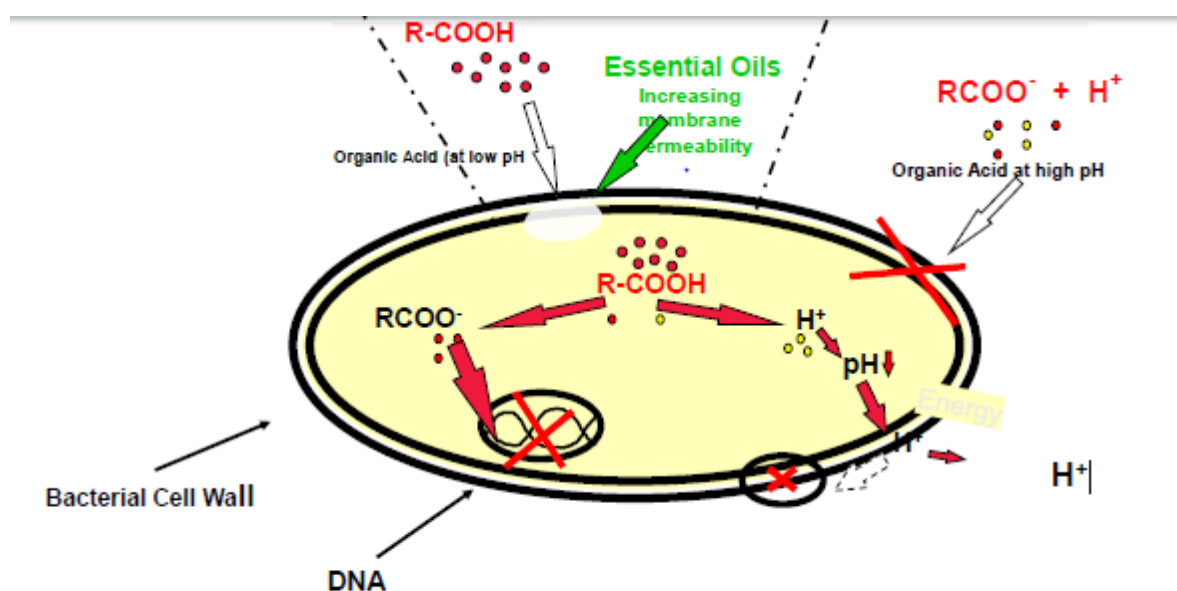


Figure 1. Mode of action of organic acids

Source: Daa vision, 2014.

2.4.1 For pH sensitive bacteria

The mode of action in pH sensitive bacteria is shown in Figure 1. 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 For 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 nondissociated 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 Antimicrobial activity of organic acids

The addition of organic acids in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria. 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 (Gharib Naseri *et al.*, 2012). Currently, drinking water acidification is another implementation in the broiler industry drinking used for improving performance. Subsequent studies indicated that addition of organic acid to the drinking water helps to reduce the level of pathogens in the water and the crop/proventriculus, to regulate gut microflora, to increase the digestion of feed and to improve growth performance (Byrd *et al.*, 2001; Aclkgoz *et al.*, 2011). Byrd *et al.*, (2001) suggested that incorporation of 0.5% organic acids (lactic acid, acetic acid or formic acid) in the drinking water during pre-transport feed withdrawal may reduce Salmonella and Campylobacter contamination of crops and broiler carcasses at processing.

Similarly, 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. Paul *et al.*, (2007b) 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. Fernández-Rubio *et al.*, (2009) found that sodium butyrate (in both partially protected with vegetable fats and unprotected forms) was able to prevent *Salmonella* colonization in the crop and caeca of broilers, whereas only the partially protected source of the butyrate salt reduced internal organ colonization (liver).

Table 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.

Source; Daa vision, 2014.

2.6 Effect of organic acids on immunity

Several studies demonstrated that organic acids could stimulate the natural immune response in poultry as well as broiler. Lohakare *et al.*, (2005) found that the infectious bursal disease (IBD) titers measured post vaccination showed significantly higher IBD titers in the ascorbic acid (0.2%) supplemental group. They explained that the possibility of increasing the antibody to vaccination in ascorbic-acid-supplemented chickens might be due to speeding up of differentiation of lymphoid organs by increasing the activity of the hexose monophosphate pathway, thus increasing the circulating antibody. The significant increase of CD4 and TCR-II lymphocytes by addition of organic acid indicated the increase of lymphocyte to exogenous antigen and provokes immune response quickly. Hassan *et al.*, (2010) found that at 21 days of age of the broiler, dietary addition of organic acids (Sunzen Corporation SdnBhd, Malaysia; 0.15% in a starter diet) resulted in significant increases in antibody titres against Newcastle disease.

Abdel-Fattah *et al.*, (2008) 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 immunity response. Above workers also suggested that the improvement in bird immunity could be related to the inhibitory effects of organic acids on gut system pathogens. Citric acid supplementation (0.5%) enhanced the density of the lymphocytes in the lymphoid organs, enhancing the non-specific immunity (Haque *et al.*, 2010).

2.7 Effect of organic acid on the gastrointestinal tract

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; Rodriguez-Lecompte *et al.*, 2012). Garcia *et al.*, (2007) reported that broilers fed diets containing formic acid had the longest villi compared with control. This trophic effect was demonstrated by Frankel *et al.*, (1994), who found an increase in villus height, crypt depth and surface area in the colon and jejunum of rats fed diets supplemented with butyric acid. Similarly, Leeson *et al.*, (2005) and Panda *et al.*, (2009) reported that butyrate, irrespective of concentrations

(0.2%, 0.4% or 0.6%) in the broiler's diet, improved the villus length and crypt depth in the duodenum. In another study, the highest duodenal, jejunal and ileal villus heights were recorded in the birds fed diets supplemented with 3% butyric acid, 3% fumaric acid and 2% fumaric acid, respectively (Adil *et al.*, 2011). Moreover, the muscularis thickness was decreased in all the segments of small intestines (Teirlynck *et al.*, 2009). This reduction in the muscularis thickness is helpful in improving the digestion and absorption of nutrients as reported by that the thickening of mucous layer on the intestinal mucosa contributes to the reduced digestive efficiency and nutrient absorption.

In some studies, organic acid also significantly improved villus height in the duodenum, jejunum and ileum. Pelicano *et al.*, (2005) reported that, higher villus height in the ileum with the diet based on organic acid. The increase of villus height of different segments of the small intestine may be attributed to the role of the intestinal epithelium as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen (Khan and Iqbal, 2016).

So organic acid salts reduced the growth of many pathogenic intestinal bacteria. Consequently, organic salts reduced intestinal colonization and infectious process, thereby, decreased inflammatory process at the intestinal mucosa, this improved villus height and function of secretion, digestion and absorption of nutrients (Iji and Tivey, 1998).

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. Garcia *et al.*, (2007) 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. Similarly, 2% citric acid in the broiler diet also increased the retention of DM, CP and neutral detergent fiber (Ao *et al.*, 2009).

In one study, the gross energy, CP and EE digestibility at 19 days was found to be lower in the non-supplemented group, as compared with supplemented group. The results were similar at 39 days of lower nutrient digestibility's in the no supplemented group as compared with the ascorbic-acid-added diet (Lohakare *et al.*, 2005).

Organic acid supplementation improved CP and ME digestibilities by reducing microbial competition with the host for nutrients, endogenous nitrogen losses and production of ammonia and organic acids raised gastric proteolysis and improved the digestibility of protein and amino acids. Organic acids lowered the pH of the chyme and thus enhanced the digestibility of protein.

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.

Dietary addition of organic acids can also improve the digestibility of minerals and increase the utilization of the phytate phosphorus (Boling *et al.*, 2000; Park *et al.*, 2009). Supplementation of the mixture of organic acid (propionic acid and sodium bantonite) in the broiler diet caused an increase in digestibility and availability of nutrients (such as Ca and P) due to developing desirable microflora (*Lactobacillus spp.*) of the digestive tract, which in turn results in increasing mineral elements' retention and bone mineralization (Ziaie *et al.*, 2011).

2.9 Effect of organic acid on broilers 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 but superior for FCR. Chicks fed the diet supplemented with organic acids showed a significant 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).

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).

2.10 Other possible effects

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; Esmailipour *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.

Some researchers have also proposed that organic acids may stimulate energy metabolism by providing energy sources for epithelial cells in the GIT. For instance, some organic acids such as fumaric and citric acids are intermediates of the tricarboxylic acid cycle, and butyric acid is the direct energy source for epithelial cells in the GIT (Pryde *et al.*, 2002).

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).

2.11 Factors affecting inconsistent results

The responses of broiler chickens to dietary organic acids have shown considerable inconsistency. There have been many successful demonstrations of positive effects of dietary 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, 2011).

The extent of the effects was also variable among the previous experiments using different inclusion levels and sources of organic acids. The reduction in the feed intake might be due to the strong taste associated with the organic acids which would have decreased the palatability of the feed, thereby reducing feed intake.

Some studies also showed no performance difference, in comparison with the negative control and/or the birds fed antibiotics (Gunal *et al.*, 2006; Kopecky *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. Moreover, most of the studies that used organic acids as additives in broilers diets were conducted in low health challenge environments which could explain the inconsistent results, because the growth-enhancing effects of antimicrobial additives become apparent when chickens are subjected to suboptimal conditions, such as a less digestible diet or a less clean environment. This inconsistency would be related to the source, the amount of organic acids used and the composition of the diets. More research is required to determine the effects of dietary organic acids on feed palatability or feed choice in broiler chickens.

Chapter III: Materials and Methods

3.1 Study area

The experiments were carried out from July 2016 to December 2016, at the Department of Animal Science and Nutrition poultry research shed and research laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

3.2 Study Period

The study was conducted during October 2016 to November 2016. October-November are considered as monsoon and post monsoon seasons respectively (Islam *et al.*, 2014). In October average temperature was 31.5⁰ C; average humidity was 82%; and average precipitation was 184.8 mm. In November average temperature was 29.8⁰ C; humidity was 78% and average precipitation was 67.5 mm (BMD, 2015; Weatherbase, 2013; BBC weather, 2013).

3.3 Experimental birds

The day-old chicks (Cobb 500 strain) of mixed sex (male and female) were purchased from an agent of Nahar Agro Complex Limited, Jhautala Bazar, Khulshi, and Chittagong, Bangladesh. All chicks were examined for any kind of abnormalities and uniform size during purchasing. Average body weight of purchased chicks was about 46.60±0.01gm.



Figure 2. Day old chicks

3.4 Chemical composition of selected acidifier

The commercial name of acidifier used in this experiment is Hameco-P^H of square company and composed from 7 organic acids and some others compound (Table 3).

Table 3. Composition of experimental acidifier (Hameco-P^H, square company limited, Bangladesh).

Acidifiers composition	Percentages of contents
Citric acid	2%
Sorbic acid	2.5%
Formic acid	15%
Acetic acid	14%
Lactic acid	2%
Propionic acid	7%
Amonium formate	24%
Amonium propionate	7%
L-ascorbic acid	1%
Yeast extract	2%
Propylene glycol	5%
water	18.5%
Total	100%

Here, the sum of content of all organic acids and its salts (Acidifier) were 73.5%. So, at the level of 0%, 0.1%, 0.2%, 0.3% and 0.4% of acidifier was equal to 0ml/litter, 1.36ml/litter, 2.72ml/litter, 4.08ml/litter, and 5.44ml/litter of water, respectively on its concentration. And acidifier was supplemented in drinking water at the aforementioned levels in different treatment groups.

3.5 Design of experiment

Birds were assigned to Completely Randomized Design (CRD). A total of 100 birds were equally and randomly allocated and distributed in five dietary treatment groups (T₀, T₁, T₂, T₃ and T₄) with two replications per treatment. These groups were treated with acidifiers at the levels of 0%, 0.1%, 0.2%, 0.3% and 0.4%, respectively in regular drinking water of broilers along with regular homogenous optimum diets (basal diet) for all groups. There were 20 birds per treatment group and 10 birds per replication. The bird rearing period was 4 weeks. The first week was adaptation period and the actual trial with acidified water was started from second week. Layout of experiment is shown in Table 2.

Table 4. Layout of the experiment

Dietary treatments	No. of birds per replicate		No. of birds per treatment
T ₀ (Basal diet + 0 % Acidifier)	R ₁	10	20
	R ₂	10	20
T ₁ (Basal diet + 0.1 % acidifier in water)	R ₁	10	20
	R ₂	10	20
T ₂ (Basal diet + 0.2 % acidifier in water)	R ₁	10	20
	R ₂	10	20
T ₃ (Basal diet + 0.3 % acidifier in water)	R ₁	10	20
	R ₂	10	20
T ₄ (Basal diet + 0.4 % acidifier in water)	R ₁	10	20
	R ₂	10	20
Grand total			100

3.6 Management

3.6.1 Housing

Poultry shed was prepared for broiler rearing by thoroughly washed and cleaned by using tap water treated with caustic soda. For killing microorganism, water diluted phenyl solution 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 disinfection the house was left for one week for drying. All windows were opened for proper ventilation. After one-week, lime was spread on the floor and around the shed for strictly maintaining bio-security. Floor space for each bird was 0.17 sq. ft. in brooding box and 0.57 sq. ft. in the cage. Each replication of 10 birds were placed into the single brooding box during brooding period (up to 14 days) and rest of the period each replication was transferred in a single cage. The replica was randomly distributed in different brooding boxes and cages.



Figure 3. Brooder box



Figure 4. Birds in a cage

3.6.2 Brooding

The brooding boxes were ready for broiler chicks rearing after proper cleaning and drying. Dry and clean newspaper was placed in floor of brooding box as bedding materials and was changed for every 6 hours intervals in whole brooding period.

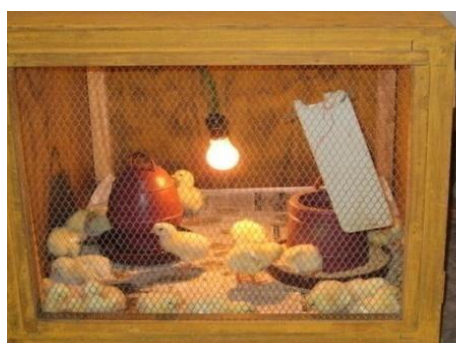


Figure 5 Box brooding of chicks



Figure 6. Placed newspaper in a box

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 weeks respectively.

3.6.3 Temperature and humidity control of experiment

Broiler shed was not environmentally controlled fully, 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 guni bag were used to prohibit fluctuating the room temperature as well as humidity.

3.6.4 Feeding and watering

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. Rations for all treatment groups were iso-energetic and iso-nitrogenous. Feed and water were supplied ad-libitum to all group 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.

3.6.5 Vaccination

All birds were vaccinated properly against Newcastle disease and Infectious Bursal disease along with boosting dose against Newcastle disease at the age of 18th days of old.

Table 5. Vaccination schedule

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

After each vaccination, multivitamin (Rena-WS, Renata) was supplied @ 1g/5 liter of drinking water along with vitamin-C to overcome the stressed effect of vaccination and adversity of weather.



Figure 7: BCRDV and IBD Vaccine



Figure 8: Vaccination of chicks

3.6.6 Sanitation

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

3.7 Laboratory work

3.7.1 P^H measurement of acidified and normal water

The P^H of normal and acidifier drinking water was measured by using digital P^H meter following the standard procedure.



Figure 9. Normal water P^H



Figure 10. Acidified water P^H

3.7.2 Carcass measurement

During this study, bird weight was measured by treatment on a weekly basis. Weekly weight gains were calculated and these figures were used with the weekly consumption to determine feed conversion ratios. On days 28 of the study, twenty birds, randomly selected from each replication then killed by severing of 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.



Figure 11. Separation of heart



Figure 12. Thymus of broiler



Figure 13. Separation of all organs



Figure 14. Weighted of breast muscle

3.7.3 Serum preparation

Blood samples were collected from the jugular vein of 4 birds from each group (2 birds from each replicate) at the end of third week and fourth week using 3 ml sterile syringe and 23-gauge needle. Immediately after collection blood samples were transferred into vacutainers (without anticoagulant). Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and the obtained serum was stored into the ependroff tube. Sera were marked and stored in - 20°C until being analyzed for determining the level of HDL and LDL in blood serum.



Figure 15. Collected blood



Figure 16. Centrifugation



Figure 17. Collection of serum



Figure 18. Collected sera

3.7.4 Estimation of serum lipoprotein level

HDL and LDL of serum were determined by semi-automated method by using Humalyzer-3000 (Wiesbaden, Germany). In both parameters the commercial kit of RANDOX Company (<http://www.randox.com/reagent>) were used and followed the manufacturer's procedure.

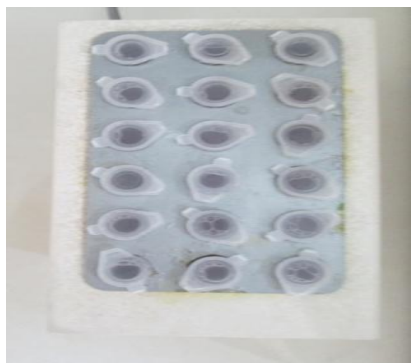


Figure 19. Incubation of sera



Figure 20. Humalyzer machine

3.7.5 Blood serum P^H

Blood serum p^H was determined by P^H paper. Collected sera was reserved at epindroph tube and P^H paper were emerged into sera and waited for a few seconds to observe the color of P^H paper and matched with the color bands of P^H paper box.



Figure 21. P^H measurement



Figure 22. Changed color of P^H paper

3.8 Data collection

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 fourth week. The weekly weight gain was calculated by deducting weight of to corresponding weeks.

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

3.8.2 Feed intake

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

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 following formula.

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

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 by using SPSS 16.0. Means showing significant differences was compared by Dunnet Test. A P value of <0.05 or <0.01 were considered statistically significant.

Chapter IV: Results

The experiment was carried out to find out the effect of acidifier on the performance parameters, carcass characteristics, blood serum P^H and lipoproteins level of Cobb-500 broilers. 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 indicate that, weekly average live weight differed significantly ($p < 0.001$) at 2nd weeks and 3rd weeks ($p < 0.05$) but insignificantly at the end of 4th week of age at the level of acidifier supplementation 0% to 0.4%. Highest weekly average live weight (1612.0 g/bird) was recorded at 0.1% acidifier treatment group and the lowest average live weight (1562.0 g/bird) was recorded at 0.2% acidifier treatment group at 4th week (Table 6).

Table 6. Live weight (g/bird) of the experimental broiler birds supplemented with water acidifiers.

Age of Bird	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	Sig.
Initial	211.6	211.4	211.3	211.7	211.4	0.1	NS
2 nd week	557.5 ^d	553.5 ^c	525.5 ^c	540.0 ^b	520.5 ^a	4.6	***
3 rd week	995.0 ^b	991.5 ^b	951.5 ^a	944.5 ^a	937.0 ^a	8.9	*
4 th week	1590.0	1612.0	1562.0	1578.0	1592.5	9.3	NS

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non- Significant ($p > 0.05$); * = Significant ($p < 0.05$); *** = Significant ($p < 0.001$); Initial = End of 1st week; 2nd, 3rd and 4th weeks = End of 2nd, 3rd and 4th week of age; a, b, c and d = Means having different superscript in the same row differ significantly.

4.2 Weight gain

Weight gain of the experimental birds varied in irregular fashion during the entire experimental period. It was revealed that, weight gain decreased significantly ($p < 0.001$) at 2nd week of age but differed insignificantly at 3rd and 4th weeks of age at the level of acidifier supplementation increased. It was observed that, highest daily average weight gain (49.4 g/bird/day) was recorded at 0% acidifier treatment group and the lowest daily average weight gain (44.1

g/bird/day) was recorded at 0.4% acidifier treatment group at 2nd week at the level of acidifier supplementation increased from 0% to 0.4%. But, highest average daily weight gain (93.6 g/bird/day) was recorded at 0.4% acidifier treatment group and the lowest average daily weight gain (85.0 g/bird/day) was recorded at 0% acidifier treatment group at 4th week (Table 7).

Table 7. Weight gain (g/bird/day) of the experimental broiler birds supplemented with water acidifiers.

Age of Bird	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	Sig.
2 nd week	49.4 ^d	48.9 ^{cd}	48.7 ^c	46.9 ^b	44.1 ^a	0.7	***
3 rd week	62.5	62.6	57	57.8	59.6	0.9	NS
4 th week	85.0	88.7	87.2	90.5	93.6	1.0	NS

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non- Significant (p>0.05); *** = Significant (p<0.001); 2nd, 3rd and 4th weeks = End of 2nd, 3rd and 4th week of age; a, b, c and d = Means having different superscript in the same row differ significantly.

4.3 Feed intake

Similar to weight gain, feed intake differed significantly (p<0.01) at 2nd week within all the water treatment groups. But, at the 3rd and 4th weeks of age feed intake of bird were also differed significantly (p<0.05) and (p<0.01). Highest feed intake (64.5 g/bird/day) was recorded at 0.3% acidifier treatment group and the lowest feed intake (59.8 g/bird/day) was recorded at 0.1% acidifier treatment group at 2nd week of age. And, highest feed intake (150.0 g/bird/day) was recorded at 0.1% acidifier treatment group and the lowest feed intake (136.1 g/bird/day) was recorded at 0.4% acidifier treatment group at 4th week of age (Table 8).

Table 8. Feed intake (g/bird/day) of the experimental broiler bird's water supplemented with acidifier.

Age of Bird	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	Sig.
2 nd week	63.9 ^b	59.8 ^a	60.9 ^a	64.5 ^b	61.3 ^a	0.6	**
3 rd week	93.6 ^b	91.9 ^b	89.1 ^{ab}	89.7 ^{ab}	85.0 ^a	1.1	*
4 th week	148.5 ^a	150.0 ^a	142.2 ^a	141.7 ^a	136.1 ^a	2.25	**

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; * = Significant (p<0.05); ** = Significant (p<0.01); 2nd, 3rd and 4th weeks = End of 2nd, 3rd and 4th week of age; a and b = 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 increased significantly (p<0.001) at 2nd week of age but differed insignificantly at 3rd week of age at the level of acidifier supplementation increased. But at the 4th week of age, it decreased significantly (p<0.05) at the level of acidifier supplementation increased. It was observed that, the worst FCR (1.8) was recorded at 0% acidifier treatment group and the best FCR (1.4) was recorded at 0.4% acidifier treatment group at 4th week of age (Table 9).

Table 9. FCR of the experimental broiler bird's water supplemented with acidifier.

Age of Bird	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	Sig.
2 nd week	1.3 ^b	1.2 ^a	1.3 ^a	1.4 ^c	1.4 ^c	0.02	***
3 rd week	1.5	1.5	1.6	1.6	1.4	0.02	NS
4 th week	1.8 ^c	1.7 ^{bc}	1.6 ^{bc}	1.6 ^{ab}	1.5 ^a	0.03	*
Initial – 4 week	1.6 ^b	1.5 ^b	1.5 ^b	1.5 ^b	1.4 ^a	0.01	*

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non- Significant (p>0.05); * = Significant (p<0.05); *** = Significant (p<0.001); 2nd, 3rd and 4th weeks = End of 2nd, 3rd and 4th week of age; a, b and c = Means having different superscript in the same row differ significantly.

4.5 Water intake

Water intake of the experimental birds was recorded daily basis throughout the whole experimental period but calculated results are done by measuring total water intake on weekly basis with measuring water intake separately on day and night. Results indicate that, average daily water intake at morning was significantly differed at 2nd week ($p<0.01$), 3rd week ($p<0.05$) and 4th week ($p<0.05$) and also varied significantly at 2nd week ($p<0.001$) and 3rd week ($p<0.001$) but insignificantly differed at 4th week ($p>0.05$) week of age at night at the level of acidifier supplementation 0% to 0.4%. By this concern, total average daily water intake was significantly differed at 2nd week ($p<0.001$), 3rd week ($p<0.001$) and 4th week ($p<0.05$). Highest total water intake (375.0 ml/bird/day) was recorded at 0.4% acidifier treatment group and the lowest total water intake (359.4 ml/bird/day) was recorded at 0.1% acidifier treatment group at 4th week (Table 10).

Table 10. Water intake (ml/bird/day) of the experimental broiler bird's water supplemented with acidifier.

Day (7.00 AM to 7.00 PM)							
Age of Bird	T₀	T₁	T₂	T₃	T₄	SEM	Sig.
2 nd week	67.1 ^c	66.2 ^{bc}	64.3 ^{ab}	63.3 ^a	62.4 ^a	0.6	**
3 rd week	117.6 ^b	117.2 ^b	116.6 ^{ab}	115.1 ^a	115.1 ^a	0.4	*
4 th week	185.6 ^a	184.8 ^a	186.7 ^a	193.3 ^b	195.9 ^b	1.6	*
Night (7.01 PM to 6.59 AM)							
2 nd week	56.5 ^c	55.4 ^c	52.8 ^b	51.4 ^b	47.3 ^a	1.1	***
3 rd week	110.4 ^c	109.7 ^c	108.4 ^b	106.7 ^a	105.6 ^a	0.6	***
4 th week	176.5	174.7	175.0	178.2	179.2	0.7	NS
Total water intake / bird							
2 nd week	125.0 ^d	121.5 ^c	117.1 ^b	114.7 ^b	109.7 ^a	1.8	***
3 rd week	228.0 ^a	226.8 ^a	224.9 ^b	221.8 ^a	220.9 ^a	0.9	***
4 th week	362.0 ^a	359.4 ^a	361.6 ^a	371.5 ^b	375.0 ^b	2.2	*

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non- Significant ($p>0.05$); * = Significant ($p<0.05$); ** = Significant ($p<0.01$); *** = Significant ($p<0.001$); 2nd, 3rd and 4th weeks = End of 2nd, 3rd and 4th week of age; a, b and c = Means having different superscript in the same row differ significantly.

4.6 Serum lipoprotein level

Serum lipoprotein (mg/dl) level differed significantly ($p < 0.01$) at 4th week although it was statistically similar ($p > 0.05$) at 3rd week. The highest average value of serum LDL (135.4 mg/dl) was recorded at 0.4% water acidifier treatment group, whereas the lowest value (121.4 mg/dl) was found at 0% water acidifier treatment group at 3rd week during the experimental period. And, the highest average value of serum LDL (164.7 mg/dl) was recorded in at 0.1% water acidifier treatment group, whereas the lowest value (95.7 mg/dl) was found at 0.4% water acidifier treatment group at 4th week during the experimental period. On the other hand, the highest and lowest average value of serum HDL was 96.0 mg/dl at 0.4% water acidifier treatment group and 73.2 mg/dl at 0.3% water acidifier treatment group at the age of 3rd week. And, the highest and lowest average value of serum HDL was 108.9 mg/dl at 0.1% water acidifier treatment group and 55.6 mg/dl at 0.4% water acidifier treatment group at the age of 4th week (Table 11).

Table 11: Serum lipoprotein level of the experimental broiler supplemented with varied level of water acidifier.

Parameter		Water treatment					SEM	Sig.
		T ₀	T ₁	T ₂	T ₃	T ₄		
LDL(mg/dl)	3 rd week	121.4	121.5	122.6	123.7	135.4	2.8	NS
	4 th week	113.0 ^a	164.7 ^b	123.7 ^a	111.1 ^a	95.7 ^a	7.0	**
HDL(mg/dl)	3 rd week	76.2 ^a	84.1 ^{ab}	87.3 ^{ab}	73.2 ^a	96.0 ^b	2.7	*
	4 th week	70.4 ^a	108.9 ^b	98.0 ^b	66.3 ^a	55.6 ^a	5.7	**

N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁ = water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non- Significant ($p > 0.05$); * = Significant ($p < 0.05$); ** = Significant ($p < 0.01$); 3rd and 4th weeks = End of 3rd and 4th week of age; a and b = Means having different superscript in the same row differ significantly.

4.7 Carcass characteristics

Among the different carcass characteristics the abdominal fat was decreased significantly ($p < 0.001$) and gizzard weight increased significantly ($p < 0.05$) at the 4th week of age at the level of water acidifier supplementation increased from 0% to 0.4% However, it did not differ significantly ($p > 0.05$) in other parameters of amongst water acidifier treatments.

Table 12. Carcass characteristics of the experimental birds water supplemented with acidifier.

Parameters(%)	Water treatments					SEM	Sig.
	T ₀	T ₁	T ₂	T ₃	T ₄		
Dressing weight	58.0	58.3	58.8	59.3	60.0	0.6	NS
Drumstick weight	8.5	7.8	8.9	8.5	9.0	0.2	NS
Thigh weight	9.1	10.2	10.1	10.1	9.5	0.2	NS
Breast weight	21.2	22.4	22.4	22.5	23.0	0.3	NS
Neck weight	2.8	4.0	4.0	3.8	3.8	0.2	NS
Back weight	8.6	8.7	8.4	9.0	9.0	0.2	NS
Wing weight	9.0	9.3	9.0	9.3	8.9	0.2	NS
Feet weight	4.3	4.2	4.4	4.3	4.4	0.1	NS
Liver weight	3.1	3.2	3.8	3.4	3.4	0.1	NS
Heart weight	0.4	0.4	0.4	0.3	0.4	0.01	NS
Abdominal fat weight	1.4 ^d	1.4 ^c	1.4 ^{bc}	1.3 ^{ab}	1.3 ^a	0.01	***
Gizzard weight	1.3 ^a	1.3 ^a	1.3 ^a	1.3 ^a	1.4 ^b	0.02	**
Proventriculus weight	0.7	0.7	0.7	0.7	0.8	0.01	NS
Head weight	2.3	2.4	2.5	2.6	2.5	0.07	NS
Spleen weight	0.1	0.1	0.1	0.1	0.1	0.00	NS
Thymus weight	0.4	0.4	0.5	0.4	0.4	0.01	NS

The measurement of carcass parameters are based on live weight of birds; N = Number of birds in a treatment: 20; T₀ = water without acidifier; T₁= water containing 0.1% acidifier; T₂ = water containing 0.2% acidifier; T₃ = water containing 0.3% acidifier; T₄ = water containing 0.4% acidifier; SEM=Standard Error of Mean; NS=Non-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.

Chapter V: Discussion

This study tested the effects of acidifier supplementation on broilers. We hypothesized that acidifier supplementation may have a variety of benefits, both in terms of performance and economic point of view, which could play a key role in future broiler production, although it has some bad effects on broiler in early stage. Different pathogenic microorganism cause different diseases in chickens that present 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 acidifier as water treatment on broilers during a typical production life of 28 days.

5.1 Weight gain

After the completion of experiment, study got insignificant result in body weight of birds among the groups was observed at 4th weeks but significant at 2nd week and 3rd week of age. This results from experiment is comparable with the results of (Pinchasov and Jensen, 1989). In the results Denli *et al.*, (2003) they observed slow increase in weight, using organic acid in the diet. They reported that live weight was not affected significantly by organic acid treatments in broiler chickens, but in our experiment live weight was increased at the 4th week of age, it may be due to failure of consuming acidifier or failure of adaptation to acidifier at very early life of birds (Král *et al.*, 2011).

One the other hand, the 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 (Patterson and Burkholder, 2003; Choudhari *et al.*, 2008), reduction of the growth depressing metabolites produced by microorganism in the gut (Knarreborg *et al.*, 2004), prevention of exponential multiplication of common pathogenic bacteria (*E. coli*, *Salmonella spp.*, *Streptococcus spp.*, etc.), and alteration of the pH in the gut (George *et al.*, 1982; Brennan *et al.*, 2003). The responses of broiler chickens to water acidifier have shown considerable inconsistency.

Although, 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. Some studies also showed no performance difference, in comparison with the negative control and/or the birds fed antibiotics (Gunal *et al.*, 2006;

Kopecky *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

In case of feed intake, the study finds that, all the result of weekly feed intake of all experimental weeks were differed significantly from 2nd week to 4th week. Feed intake was decreasing with increasing concentration of acidifier in water at whole experiment time. The highest feed intake was observed at 0% water acidifier treatment group and lowest feed intake was occurred at 0.4% water acidifier treatment group at 2nd, 3rd and 4th weeks of age of broilers. This study was similar to Stipkovits *et al.*, (1992) and Islam *et al.*, (2008) who reported that acidifier in poultry water decreased feed intake. In tandem with this, on day 28, the feed intake decreased in all levels of acidifier and have shown a significant decrease with more severely on 0.5% level (Hedayati *et al.*, 2013).

Along with this similar results, some showed dissimilar results, they found supplementation of 0.2% or 0.3% acidifier had no effect on feed intake than those without acidifier (Adil *et al.*, 2011). Some researcher also showed positive effect on feed intake (Islam *et al.*, 2008). The reduction in the feed intake might be due to the unfavorable taste associated with the organic acids which would have decreased the palatability, thereby reducing feed intake which cause of significantly decreasing body weights at 21 and 42 days of age (Aclkgoz *et al.*, 2011).

5.3 Feed conversion ratio (FCR)

The weekly feed conversion at different ages of broilers supplemented with water acidifier indicated acidifier improved feed conversion ratio of broiler. The better feed conversion ratio for the groups with acidifiers 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). Acidifiers modified intestinal microflora and helped to improve bird's performance; health statue as well as reduced the microbial use of nutrients (Snyder and Westmann, 1987). The lowering of the pH, optimized the activity of proteases and beneficial bacteria (Nava *et al.*, 2009) and enhanced feed conversion by broiler birds.

Accordingly, Adil *et al.*, (2011) 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; Brzoska *et al.*, 2013). The improvement in FCR possibly due to better utilization of nutrients in the birds fed organic acids in the diet.

However, in contrast to present study, Brzoska *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 (Kral *et al.*, 2011).

5.4 Water intake

Water intake of this result is similar with others, Aclkgoz *et al.*, (2011) who indicated that acidifier supplementation significantly decreased water intake. It may due to reduction of water pH from 7.4 to 4.5 with organic acid supplementation (Vieira *et al.*, 2008; Aclkgoz *et al.*, 2011). Over use of organic acids such as citric and acetic acids might reduce water and feed intake due to the unfavorable taste of water. In addition, acidified water might lead to sub-clinical intestinal problems, in spite of the fact, acidifier suggested to use in water at later stage of broiler production (Oviedo, 2006). Although it decreases water intake at early stage but increase water intake at later stage which cause of improving broiler performance by declining heat stress, cause of respiratory alkalosis, elevated reparation (Al-Tarazi and Alshwabkeh, 2003; Bilgili, 2002) and making balance of several minerals (Sas, 2000) and induced perturbation of bird mineral balance. It has been observed that acidifier supplementation decrease serum Na, Ca, and Mg, concentration (Teeter *et al.*, 1985; Steiner, 2006; Stonerock and Lückstädt, 2007).

5.5 Serum lipoprotein level

These findings of serum lipid profile have 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; 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.

5.6 Carcass characteristics

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 the findings of (Islam *et al.*, 2008). This result is also similar with others, Brzóska *et al.*, (2013) and Hedayati *et al.*, (2013) who indicated that acidifier supplementation significantly increased gizzard weight (Islam *et al.*, 2008). One study reported that, acidifier has capacity to decrease abdominal fat (Castellini *et al.*, 2002) .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. Closely similarity was seen in other studies.

However, were lower than the values reported by Ogunwole *et al.*, (2011) in broiler fed acidified diets. The heart and liver of the various treatment group of this experiment; though varied numerically but did not differ significantly. This finding has agreement with Ogunwole *et al.*, (2011) who also reported no significant difference in liver weight and heart weight of broilers treated with dietary acidifiers. This result also inconsistent with some research article; Islam *et al.*, (2008) stated that dietary acidifier improved carcass yield by approximately 3 -5 % in poultry. Drumstick muscle yield was improved 4-5% by addition of acidifier (Islam *et al.*, 2008).

Chapter VI: Conclusion

The study investigates effect of acidifier supplementation in Cobb 500 broiler under intensive rearing system. The birds were assessed based on performance parameter, serum lipoprotein level (LDL, HDL) and carcass characteristics .It was evident that, there was a positive relationship between acidifier supplementation and performance parameters with decreasing LDL and HDL level in blood serum at later stage. Highest weight gain was recorded in the bird's drinking water containing 0.4% acidifier supplement at 4th week of age. Similar to weight gain FCR were also improved but feed, water intake were decreased in birds drinking water supplemented with acidifier. There were no unusual changes in the serum lipoprotein level and carcass characteristics in comparison to the reference level. Our study suggests acidifier as a potential water supplement with basal diet at inclusion level of 0.4 % at later stage of broilers (During 4th week and onward). However a long term study with larger sample size and multi dimensional temporal pattern is suggested for increasing the sensitivity of the study.

Chapter VII: Recommendation

The use of acidifiers in drinking water is a relatively recent development in poultry production. In tropical production systems, this may play a vital role in providing hygienic drinking water and reducing pathogen load, thus having enormous potential as an integral component of a successful bio-security programme. The author along with other authors has used acidifier under a wide variety of conditions in South and South East Asia. This particular study, carried out in Bangladesh, demonstrates that including water acidification in broiler production has beneficial effects on the performance of broilers at the later stage and may be considered as a low-cost option to improve production parameters in general.

Inclusion level of 0.4 % acidifier are recommended in regular drinking water of broiler at later stage (During 4th 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 financial constraints and technical limitations, some vital blood parameters like Glucose, SGPT, SGOT, White blood cell count (WBC), calcium, phosphorus and other trace minerals both in meat and feed were not analyzed. These parameters could have vital impact on human health. The study will explore new horizon for investigating those parameters as future study.

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