EFFECT OF DRINKING WATER pH ON PRODUCTION PERFORMANCE AND BACTERIAL LOAD OF SONALI CHICKEN

A Thesis

By

DR. MD. ATIQUR RAHMAN

Registration No. 1605476

Semester: January – June, 2018

MASTER OF SCIENCE (M.S.)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

MAY, 2018

EFFECT OF DRINKING WATER pH ON PRODUCTION PERFORMANCE AND BACTERIAL LOAD OF SONALI CHICKEN

A Thesis

By

DR. MD. ATIQUR RAHMAN

Registration No. 1605476

Semester: January – June, 2018

Submitted to

The Department of Dairy and Poultry Science

Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

MAY, 2018

EFFECT OF DRINKING WATER pH ON PRODUCTION PERFORMANCE AND BACTERIAL LOAD OF SONALI CHICKEN

A Thesis

By

DR. MD. ATIQUR RAHMAN

Registration No. 1605476

Semester: January – June, 2018

Approved as to style and content by

…….....	**……...……**
Prof. Dr. Mst. Afroza Khatun	**Assistant Prof. Dr. Md. Kamruzzaman**
Supervisor	**Co-supervisor**

……

(Prof. Dr. Tahera Yeasmin)
Chairman, Examination Committee
and
Chairman of the Department

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

MAY, 2018

Dedicated to
My
Beloved Parents

ACKNOWLEDGEMENT

The author express all praise due to the "Almighty Allah" who has created us to explore the hidden fact of the nature for the benefit of mankind and enabling the author to pursue his higher study and to submit his thesis for the degree of Master of Science in Poultry Science.

The author express his sincere appreciation, deep heartfelt gratitude and indebtedness to his reverend supervisor, Professor Dr. Mst. Afroza khatun, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for his scholarly guidance, supervision, inspiration, instruction, constructive criticism, valuable advice and untiring assistance in all phases of the research work, and as well as successful completion of the thesis.

The author deeply indebted and sincerely grateful to his research co-supervisor Dr. Md. Kamruzzaman, Assistant Professor, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for his constructive advices, encouragement, fruitful criticisms and scholastic supervision throughout the entire period of research work.

Finally, the author has much pleasure to express his heartfelt indebtedness and gratitude to his beloved parents, sisters specially his elder brother who opened the gate and paved the way for his higher study and relatives who had always sacrificed their causes of happiness for his constant inspiration throughout his academic life.

The author would also express his sincere thanks to all the lab staffs, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for their sincere help and cooperation throughout the study period.

The author

ABSTRACT

This study was conducted to evaluate the efficacy of drinking water pH on production performance, dressing yield parameter and microbial load of sonali cross breed chicken. For this purpose 120 day old chicks were randomly assigned into four treatment groups namely (T_0, T_1, T_2, and T_3) having three replication in each treatment group with 10 birds in each replication. Experimental birds in T_1, T_2 and T_3 were provided drinking water @ 5.5, 6.5 and 7.5 pH level. Final live weight gain and feed efficiency of birds were not found insignificantly different among the treatment groups ($p>0.05$). Highest body weight gain was found (863.60±25.21g) in T_2 group that was received @ 6.5 pH in water compared to control group T_0 and the lowest body weight gain was found (794.60±27.02g) in T_3 group that was received @ 7.5 pH in water statistically similar to the control group T_0. Meat yield parameters did not show significant differences among the treatment groups compared to control group. This study is also indicated that microbial load was significantly ($p<0.01$) reduced in the acidic groups when compared to the control alkaline group. Highest *E. coli* count was found (233.33±12.01) in T_3 groups and lowest *E. coli* count was found (170.00±11.54) in T_1 groups. Highest *salmonella* count was found (225.00±14.43) in T_3 groups and lowest was count (145.00±8.66) in T_2 groups. Total production cost for per kilogram weight was lowest in T_2 (142.00±1.12TK.) group and highest in T_1 (142.80±1.36Tk.) group. The net profit from per kilogram sonali chicken was statistically similar ($p>0.05$). The highest profit was found in T_2 (18.00±1.2Tk.) group and lowest was found in T_3 (13.8±1.18Tk.) group. Based on the result it could be concluded that controlling pH in the drinking water at the effective level has a statistical similar in production performance and potential effect on bacterial load of sonali chicken.

Keywords: Hameco-pH, Sonali chicken, production performance, and Microbial load.

CONTENTS

LIST OF TABLE

LIST OF FIGURE

LIST OF ABBREVIATION AND SYMBOLS

ANOVA	: Analysis of variance
CRD	: Completely Randomized design
DM	: Dry Matter
Dr.	: Doctor
et al.	: Associates
Fig.	: Figure
g	: Gram
HSTU	: Hajee Mohammad Danesh Science and Technology University
kcal	: kilo-calorie
Ltd.	: Limited
ME	: Metaboilizable energy
ML	: Mille Litter
^{o}C	: Degree Celsius
Prof.	: Professor
SEM	: Standard Error of Means
Sl.	: Serial Number
Tk.	: Taka
%	: Percentage
&	: and
/	: Per/or
@	: At the rate of
+	: Plus/and
<	: Less than
>	: Greater than
±	: Plus-minus
µl	: Micro Liter
BBS	: Bangladesh Bureau of Statistics
pH	: Power of Hydrogen
ME	: Metabolizable energy
GIT	: Gastro Intestinal Tract
FCR	: Feed conservation Ratio

CHAPTER-I
INTRODUCTION

Bangladesh is considered as one of the most appropriate countries in the world for rearing poultry. The poultry industry plays a crucial role in economic growth and simultaneously, creates numerous employment opportunities (Shamsuddoha, *et al.*2003). Regardless of religion and age almost all people are fond of chicken meat. People of any age can take poultry meat without hesitation for less content of fat compared to other meats. We have to increase the animal protein production to make our people sound and healthy. Protein intake is recommended to be in the range of 0.8 to 1.6g/d per kg body weight for human requires minimum 20.44kg protein per person (average 70kg body weight) per year. It indicates that it is a crying need to increase the meat production according to the requirements. In Bangladeshi food culture, people always try to find the indigenous (*Desi*) cock for its tenderness and good taste. One of the reasons is that poultry meat is still compact to heat, but in sonali meat some portions are separated from the bone, that's why this is not suitable for making roast. Majority people like cockerels weighing about 650-700g, so that they can economically make maximum four roasts of 120-130g. Practically, it is seen in the market that for a festival or usual consumption people have been buying most of the cockerels. The demand of cockerels is bigger than that of production. Local chicks could not meet the demand of the people in an overpopulated country where about 142 million people living in an area of 143,999 square kilometers (BBS March, 2001). For the necessity of time Fayomi and *Sonali* (Rhode Island Red × Fayomi) growing straight run chicks have been taking their place beside the indigenous chicks for their adaptability and acceptability under the climatic conditions of Bangladesh (Anisuzzaman and Wahid, 1988). Moreover, they have tenderness and good taste as liked as *Desi* chicks. On another observation, *sonali* crossbred stated as more suitable chicks for meat and egg production to rear in rural areas for higher adaptability and disease resistance (Ali and Wahid, 1989). Crossbred progenies were superior to purebred in growth rate, meat quality, body weight and feed conversion compared to that of respective purebreds (Dubrynia, 1958; Masic and Khalifah, 1965). Crossbred *Sonali* might have a higher growth rate, viability and meat yield because a certain level of hybrid vigor could be expected as their parents Rhode Island Red (RIR) and Fayomi are 2 different breeds. Rhode Island Red is bigger sized chicks than that of Fayomi. The breed that will show better performance could be recommended besides the native cockerels to

partially fulfill the demand of meat. In northern region of Bangladesh, *sonali* is more popular for meat production. Highlighted concern over antibiotic resistance, natural alternatives; probiotic (Fuller, *et al.* 1989) and some organic acid (Cheveerach *et al.*2004) or combination of them are used in the diets assume to be their positive effect on health and growth of broiler. There are several types of organic acids; citric acid, acetic acid, lactic acid, fumeric acid, malic acid, ascorbic acid etc. and also their different combinations are used in poultry diets (Callsen, 1999). They have a specific antibacterial effect at a low pH and may help to reduce overall bacterial numbers or modify bacterial species distribution in the gut and increase nutritive value to the diet and thus improved their health. Health of the gut is one of the major factors governing the performance of poultry.

Organic acid treatments composed of individual acids and blends of several acids have been found to perform antimicrobial activities similar to those of antibiotics (Wang *et al.*2009). The European Union allowed the use of organic acids and their salts in poultry production because these are generally considered safe (Adil *et al.*2010). Organic acids have been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are particularly effective (Luckstadt, 2014). In the European Union, these two organic acids and several others (lactic, citric, fumaric and sorbic acids) and their salts (e.g. calcium formate, calcium propionate) are used under the classification 'feed preservative' (Luckstadt & Mellor, 2011). In the poultry industry, the use of water with adequate physical, chemical and microbiological quality it is of fundamental importance. Since many birds have access to the same water source, quality problems will affect a great number of animals. The drinking water plays an important role in the transmission of some bacterial, viral and protozoan diseases that are among the most common poultry diseases. Important factors to prevent waterborne diseases in poultry the protection of supply sources, water disinfection and the quality control of microbiological, chemical and physical characteristics. Water is an essential nutrient for birds and therefore quality preservation is fundamental for good herd performance. Effective Salmonella control on the farm is based on preventing Salmonella from entering and spreading in a farm. Salmonella is a cause of bacterial food-borne disease in humans, and can often be attributed to contaminated food products. It is estimated that around 2.6%, 10.6% and 17.0% of human *salmonellosis* cases are attributable to turkeys, sonali chicken and laying hens, respectively. Some researchers are suggesting the use of organic

acids as a cheaper and safe alternative to antibiotics, but limited studies have been done in Bangladesh to compare the effects of using organic acids and antibiotics on the growth, meat yield and economic feasibility of rearing popular dual purposes *sonali* chicks.

Keeping above information in mind, present research was aimed with the following objectives:

- To know the effect of different pH level on production performance of Sonali Chicken.
- To evaluated the bacterial load (*E. coli* and *salmonella)* on faeces of Sonali Chicken.

CHAPTER-II
REVIEW OF LITERATURE

Organic Acid is an organic compound with acidic properties associated with their Carboxyl group –COOH group. In General Organic acids are considered to be any carboxylic acid including fatty acid & amino acid. Organic Acids are weak Acid & do not disassociate completely in water. Prevention of diseases and enhancement of growth are critical factors in modern poultry production. Keeping these thinking in mind, poultry farmers are indiscriminately using different antibiotics. But continuous and unnecessary use of antibiotics cause antibiotic resistance and residual existence (Waldroup *et al.*2003) in poultry products, is the major health concern now a day. The short-chain acids (C1–C7) are found to be associated with antimicrobial activity. They are either simple mono-carboxylic 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). Generally organic acids with antimicrobial activities have a pKa value in the range of 3 and 5. Organic acid treatments composed of individual acids and blends of several acids have been found to perform antimicrobial activities similar to those of antibiotics (Wang *et al.* 2009). The European Union allowed the use of organic acids and their salts in poultry production because these are generally considered safe (Adil *et al.*2010). Organic acids have been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are particularly effective (Luckstadt, 2014). In the European Union, these two organic acids and several others (lactic, citric, fumaric and sorbic acids) and their salts (e.g. calcium format, calcium propionate) are used under the classification ‘feed preservative’ (Luckstadt & Mellor, 2011).

2.1 Application ways of organic acids

Sprayed as a liquid directly in to feedstuff & compound feed. Powder forms are added directly or via premix. Liquid form via drinking water.

2.1.1 Mode of action

Un-dissociated form of acid RCOOH has the ability to penetrate the bacterial cell wall. 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 (Luckstadt, 2014). This results into destruction of the cytoplasm, and further growth of the bacteria is inhibited. Molecule of organic acid also attacks the DNA of bacteria results in its death (Tripathi, 2017).

2.1.2 Organic acid usage in Poultry to improve Birds Performance

Organic acids have growth promoting properties and can be used as alternatives to antibiotics (Fascina *et al.*2012) and they also reported that the use of an organic acids mixture (comprising 30.0% lactic acid, 25.5% benzoic acid, 7% formic acid, 8% citric acid and 6.5% acetic acid) in broiler diets improved its performance as compared to the control diet at 42 days of age and organic acids provided better carcass characteristics. Supplementation of organic acids in feed is found to improve the production parameters like body weight and feed conversion ratio (FCR) in broiler. The improvement may be attributed to better utilization of nutrients resulting in increased body weight gain in the birds supplemented with organic acids in the feed.

2.1.3 Use of organic acid to improve Nutrient Digestibility

Organic acids have been considered to be suitable alternatives for improving nutrient digestibility. Organic acids are supposed to lower the pH of the chyme and thereby enhanced the protein digestibility. Addition of organic acids may also improve the digestibility of minerals. Supplementation of the mixture of organic acid in the broiler birds diet may lead to an increase in overall digestibility and availability of nutrients (such as Ca and P) due to developing beneficial microflora (*Lactobacillus* spp.) of the digestive tract. The low ME of a soybean meal for poultry is due mainly to the very poor digestibility of the carbohydrate fraction. The galacto-oligosaccharides in the soyabean meal cannot be digested in the small intestine of poultry because of the absence of the endogenous α-(1, 6)-galactosidase enzyme (Lee *et al.*2015). Ao (2005), 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.

2.1.4 Organic acid usage in Poultry to improve GIT

Having a sound intestinal health of poultry is one of the key aspects to achieve best growth rate, production performance and feed efficiency. It has been found that organic acid supplementation significantly increases the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks. Garcia *et al.*(2007), reported that poultry 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 villous height and crypt depth.

2.1.5 Organic acid usage in Poultry as Antimicrobial agent

In general, potential bacterial targets of biocidal com-pounds include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and function (Denyer, *et al*. 1998; Davidson, 2001). Although the antibacterial mechanism(s) for organic acids are not fully understood, they are capable of exhibiting bacteriostatic and bactericidal properties depending on the physiological status of the organism and the physicochemical characteristics of the external environment. Given the weak acid nature of most of these compounds, pH is considered a primary determinant of effectiveness because it affects the concentration of undissociated acid formed (Davidson, 20011). It has been traditionally assumed that undissociated forms of organic acids can easily penetrate the lipidmembrane of the bacterial cell and once internalized intothe neutral pH of the cell cytoplasm dissociate into anionsand protons (Eklund, 1983, 1985; Salmond *et al.*1984; Cherrington *et al.*1990, 1991; Davidson, 2001). Generation of both of these species potentially presents problems for bacteria that must maintain a near neutral pH cytoplasm to sustain functional macromolecules. Export of excess protons requires consumption of cellular adenosine triphosphate (ATP) and may result in depletion of cellular energy (Davidson, 2001).

2.2 Water acidification

Salmonella can persist and grow in water given the right conditions. The diversity and concentration of Salmonella increases as temperatures rise. For better Salmonella control, the microbiological test of water is needed, especially if the source of water is a well or river (Tripathi, 2017).

Water acidification can help prevent Salmonella. The supplementation of acids in drinking water reduces the pH level and bacterial counts. A very important feature of water acidification is the pH level and corrosive properties of the acidifier. Very often, farmers apply acids without knowing the pH level of water. If the acidification is too strong, the pH level of the water goes below 4 and this has a negative impact on the equipment and water intake of animals (Luckstadt and Theobald, 2011).

2.2.1 The use of acidifiers in controlling Salmonella

Salmonella control is key to preventing the introduction of Salmonella on the farm. Correct farm management, bio-security measures, targeting small groups of animals and preventing the return of sick animals to the main production unit all contribute to the prevention of Salmonella spread. Acidification of feed and water minimizes Salmonella infection and promotes good gut health, thereby enhancing the performance of animals. By Natalia Roth, Product manager acidifiers, Biomin Holding, Austria (Tripathi, 2017).

Effective Salmonella control on the farm is based on preventing Salmonella from entering and spreading in a farm. Salmonella is a cause of bacterial food-borne disease in humans, and can often be attributed to contaminated food products. It is estimated that around 2.6%, 10.6% and 17.0% of human salmonellosis cases are attributable to turkeys, broiler and laying hens, respectively (Jones, 2011).

Salmonella is a common component of the gut micro flora of animals and can be found in the faeces of affected animals. Faecal pollution is the main culprit for the contamination of feed and water. Poultry can also become infected and act as reservoirs of Salmonella (Zimmerman, 1998). In order to ensure a high level of poultry performance, farmers should pay close attention to farm management and Salmonella prevention. Regular testing and observing the critical points of the production chain are necessary for preventing Salmonella occurrences and contamination.

There must be adequate Salmonella monitoring and control at the hatchery and breeder farms. Control starts with getting healthy young chicks to the farm. On arrival, the chicks should be Salmonella free. Samples from transport equipment and faeces should be taken to determine the Salmonella status.

2.2.2 Disease Control: *E. Coli, Salmonella, Clostridia, Entrococcus* etc.

The performance of poultry is enhanced by the addition of organic acids in diet as these organic acids decrease the pathogenic bacteria from feed. Most commonly the bacteria that affect the gut health of poultry include *Salmonella, Clostridia, Entrococcus, Campylobacter* and *Escherichia coli* (Tripathi, 2017). These can be checked by inclusion of an organic acid in the diet. The most important basic principle on which these acidifiers work is that the non-dissociated organic acids can penetrate the bacteria cell wall and destroy the normal physiological functions of pH sensitive bacteria (meaning that they cannot tolerate a wide internal and external pH gradient) (Luckstadt and Theobald, 2011) . Adding to that, the organic acids in poultry seem to have a direct effect on the bacterial population of gastrointestinal tract (GIT). Individual acid has its own range of microbial activity in terms of pH range, membrane structure, physiology etc. Generally the mixture or combination of acids shows different pKa values and have a broad spectrum activity.

2.3 pH

The acidity or alkalinity of water is measured by pH. A pH of 7 indicates that the water is neutral, a pH less than 7 indicates acidity, and a pH greater than 7 indicates alkalinity. Low pH water can be unpalatable, corrosive to equipment, and may have a negative impact on performance. High pH water is also unacceptable since it reflects high levels of calcium and magnesium, which can clog watering systems. Poultry accept water on the acid side better than they accept water on the alkaline side (Zoetis, 2013).

2.3.1 Composition of Hameco-pH

Chemical Composition	Amount (%)
Acetic acid	14.00
Ascorbic acid	1.00
Citric acid	100
Lactic acid	2.00
Formic acid	15.00
Propionic acid	7.00
Sorbic acid	2.5o
Yeast extract	2.00
Ammonium format	24.00
Ammonium propionate	7.00
Propylene glycol	5.00
Water	18.50

2.3.2 Maintain Optimum Drinking Water pH for Healthier birds

Providing quality water in adequate amounts is vital for poultry performance. Birds consume nearly twice as much water as they do feed. Anything that reduces their water intake will have an adverse effect on their feed intake. The water supply is an important source of nutrition, but it also can be an entryway for diseases, leaving birds vulnerable and the entire flock exposed. Water lines can harbor pathogens, especially from biofilm buildup. Key water quality factors affecting water intake on poultry farms include pH, hardness and total dissolved solids (Zoetis, 2013).The pH of water is a measure of its acidity or alkalinity. A numeric scale for measuring pH runs from 1 to 14. Neutral water (neither acid nor alkaline) has a pH of 7. Acidic water has a pH lower than 7; if pH is greater than 7, water is alkaline or basic. Measuring pH with a test kit generally is

inexpensive. Research shows that pH is a major factor in determining the amount of drinking water that birds consume. Along with pH, the chemicals used to control pH affect water's palatability and the amount of water that birds drink. Chemicals used to modify water pH also affect efficacy of antimicrobials and disinfectants, as well as vaccines, mineral buildup in water lines and mineral transfer to the gut (Tripathi, 2017).

2.3.3 Function of Acids and pH Control

Alkaline water can have a bitter taste that is undesirable to birds. Current research recommends that poultry water be maintained within a pH range of 6 to 6.5, but it's been shown that birds are tolerant of pH 4 to 8 on a continuous basis. Birds also are tolerant of pH 2 to 3 for short periods. Maintaining water pH at 6.5 to 7 will keep minerals suspended in water. Dropping pH below 6.5 will begin to dissolve scale from drinkers and pipes. A pH of 5 or lower can corrode metal. At pH levels below 7, chlorine as hypochlorous acid is effective and fast-acting as a disinfectant. The recommended range of free chlorine in poultry water is 3 to 5 parts per million. For good residual effect, pH should be below 4.5 for acids used as disinfectants in water lines. Bird performance can be improved by maintaining water pH within an optimum range. The optimum water pH also improves the efficacy of vaccines, antibiotics and antimicrobials administered through the water system. Controlling pH can help reduce scale and biofilm buildup in the water system. Lowering pH also can help lower bacteria populations, including *Salmonella*, in the water system and in birds. Effective water acidification products are available to help maintain poultry water at optimum pH levels for better bird health and feed conversion (Zoetis, 2013).

2.3.4 pH/Acid Tolerance and Temperature

The pH of mayonnaise plays an important role in its structure and stability. Mayonnaise is an emulsion stabilized by denatured proteins forming a network that can be impacted by the isoelectric pH of the egg yolk protein. When the charge on the proteins is minimized, the viscoelasticity and stability of the mayonnaise is at its highest. Food safety guidelines published online by the Government of New South Wales (NSW), Australia, suggest that a pH at or below 4.2 has shown to be effective in controlling Salmonella in raw-egg products, however, there are numerous factors that influence the bactericidal efficiency such as the type of acid used, temperature water activity, garlic, ginger, and pepper. Many bacterial species induce responses to environmental stress. When Salmonella spp. are

exposed to a stress this can produce cross-tolerance to many or various stresses. Gruzdev *et al*, 2011. reported that following carbon starvation, Salmonella spp. demonstrated greater tolerance to low pH, hyperosmolarity, heat, polymyxin B, and peroxides. Another study conducted by Leyer and Johnson demonstrated that exposure of Salmonella to mild acids (pH 5.8) could induce adaptation to lower pH, heat, NaCl (2.5 M), crystal violet and polymyxin B. Additionally, subjecting S. enteric cells to an initial acid shock or pH 5.8 or 4.5 before inoculating mayonnaise (pH 4.2–4.5) increased the survival rate and persistence of the organism at 4 _C. Salmonella can also achieve pH homeostasis, which is when the intracellular pH is maintained compared with the environmental pH . Homeostasis is facilitated by cellular proton pumps and potassium/proton and sodium/proton antiport systems (Zoetis, 2013). The ability of Salmonella to decrease proton extrusion and membrane proton conductance enables the cell to be protected against acid stress. Additionally, S. typhimurium has a regulated response to further protect from acid stress, which is called the acid tolerance response (ATR). The ATR protects Salmonella spp. at pH levels of 3.0–4.0, but is activated when environmental pH values are between 6.0 and 5.5 and when pH homeostasis fails. These pH conditions are referred to as the postshock stage and the preshock stage, respectively. During the postshock stage, stimulation of 43 acid shock proteins takes place in order to prevent and repair the damage done to macro molecules by the acids. In contrast, studies conducted by Alvarez-Ordonez *et al.(*2012) and Samelis *et al.*,(2003) suggested that S. typhimurium vulnerability to acid stress is dependent on growth temperature. S. typhimurium growth was observed in the temperature range of 25–37^{0}C at pH 4.5. Alali *et al.*2012. Proposed that lowering the pH of the mayonnaise-based homemade salads decreased the rate of survival of Salmonella regardless of the temperature. According to a study conducted by Koutsoumanis *et al.*2004. the minimum pH value that permitted the growth of S. typhimurium was 3.94 within the temperature range 25–35 ^{0}C (Keerthirathne, 2016).

2.4 Organic acids on growth performance

2.4.1 Organic acids and live weight

Denli *et al.*(2003) showed 1.25% dietary FA had higher p<0.05) weight gain. Higher body weight gain was obtained for the supplementation organic acid has been reported. Christian *et al.*2004. observed that organic acid blend (3kg inclusion rate per ton of feed) increased the growth of broiler under controlled conditions. The body weight of broiler at 6 week of age was higher (p<0.05) in the groups fed diet containing organic acid at

1kg/ton or 1 .5kg/ton (Thirumeignanam *et al.*2006). Paul *et al.*(2007) reported that ammonium format or calcium propionate at the level of 3g/kg feed increased the live weight at 21 day in sonali. Nezhad *et al.*(2007) reported increased growth of broiler for supplementation of citric acid (0.0, 2.5% and 5.0%) with microbial phytase. Moghadam *et al.*(2006) administrated dietary citric acid (0.0, 1.5% and 3.0%) and phosphorus (0.3, 0.035 and 0.4%) in broiler for a period of 2 weeks (from 8 to 21 days) and observed increased live weight. Shen Huifang *et al.*(2005) used 0.3, 0.5 and 0.7% dietary citric acid in yellow chicken and 0.3% citric acid gave highest growth. Live weight was increased ($p<0.05$) by supplementation of citric acid in Ross x Ross and Hampshire x Columbian chicks (Rafacz-Livingston *et al.*2005). The effects of supplemental organic acids and chromium (Cr) were studied to ascertain on production and carcass traits of broiler (Sarnanta, 2008). They concluded that, instead of individual supplementation, a combination of Cr and organic acids may improve the production of broiler. Zhang *et al.*(2005) used citric acid, fumeric and malic acid and found the mixture to support maximum live weight broiler. Formic acid (5,000ppm and 10,000ppm in the diet of chicken improved ($p<0.05$) growth (Garcia *et al.*2007). Ivanov (2005) recorded increased live weight of broiler using lactic acid bacteria (3%), citric acid (0.7%) and baker's yeast (1%). Addition of citric acid and ascorbic acids in broiler increased live weight (Afsharmanesh and Pourreza, 2005). They also reported higher live weight by 18% with citric acid along with ascorbic acid, phytase and vitamin Chitra *et al.*,(2004) without specifying dose reported ascorbic acid with probiotic in broiler increased live weight. Maiorka *et al.*(2004) used citric acid along with fumenc, lactic and ascorbic acids and found increased growth performance of broiler. Andrys *et al.*(2003) documented highest live weight in Ross 208 male and female broiler received the acidifier FA-30 (citric acid and phosphoric acid). Nudiens (2002) supplementing acidifier (10 kg/ton feed) in broiler found increased live weight. Kahraman, *et al.*(1997) without specifying dose used Acid Lac Dry; citric, lactic, fumeric, propionic and fumeric acid and/or zinc bacitracin in broiler. They reported that Acid Dry with zinc bacitracin ($p<0.05$) increased live weight at 3 weeks of age. Garcia *et al.*(2007) supplemented diet apramycin (100 ppm) and organic acidmixture (50% formic acid+50% propionic acid) at 0.0, 0.1 and 0.2% level and found that supplementation of apramycin and organic acids (0.1%) alone increased live weight but combined did not result in a cumulative effect. Patten and Waldroup (1988) reported that addition of 0.5 or 1.0% fumaric acid improved ($p<0.0l$) body weights of broiler. Skinner *et al.*(1991) reported that addition of 0.125% fumaric acid ($p<0.05$)

improved 49-day body weight of females and average weight gain of both sexes. Kassim and Norziha (1995) reported that additive of acetic acid to diet (400 or 600 mg/kg) increased live weight. Vieira *et al.*(2008) reported improved, body weight on diets supplemented with a blend of organic acids (40% lactic, 7% acetic, 5% phosphoric and 1% butyric). Owens *et al.*(2008) reported 12 % increase in total live weight gain and about 9 % improvement in gain feed ratio with diets supplemented with dietary organic acids. Improvement in live body weight by organic acid supplementation (containing acetic acid, citric acid and lactic acid, each at 1.5 and 3.0 % in the diet) was also observed by Abdel-Fattah *et al.*(2008). Mazanowski *et al.*(1981) noted that the addition of citric acid solution for sonali checken decreased live weight. Pinchasov and Elmalich (2000) used dietary propionic and acetic acid and observed decreased live weight with the inoculation of the acids.

2.4.2 Organic acids and feed conversion

Islam *et al.*(2008) showed 1.25% FA group had better ($p<0.05$) feed conversion (FC) than that of groups received 5.0 and 7.5% fatty acid. Higher feed intake for orgauic acid supplementation has been reported (Denli *et al.*2003). The better feed conversion noticed in the group containing organic acid at 1 kg/ton (Thirumeignanam *et al.*2006). Formic acid (5,000ppm and 10,000ppm in the diet of chicken improved ($p<0.05$) feed conversion (Garcia *et al.*2007).Vieira *et al.* (2008) reported improved feed conversion with diets supplemented with a blend of organic acids (40% lactic, 7% acetic, 5% phosphoric and 1% butyric). Improvement in feed conversion by organic acid supplementation (containing acetic acid, citric acid and lactic acid, each at 1.5 and 3.0 % in the diet) was also observed by Abdel Fattah *et al.(*2008). Paul *et al.* (2007) reported that ammonium formate or calcium propionate at the level of 3g/kg feed increased feed conversion at day 21 in broiler. Afsharmanesh and Pourreza (2005) showed citric acid and ascorbic acid, phytase, low-P (3.1 5g/kg), low-Ca (7.9g/kg) and vitamin D3 increased feed conversion in broiler. Nezhad *et al.*(2007) used citric acid (0.0, 2.5 and 5.0%) and microbial phytase and observed increased feed conversion in sonali. Zhang *et al.* (2005) used citric acid and fumeric and malic acid without specifying dose and reported the mixture to support higher feed conversion in sonali. Arefin (2002) without specifying dose mentioned nutria in chicken to improve feed conversion. Chitra *et al.*(2004) without specifying dose reported higher feed conversion for ascorbic acid with probiotic in broiler. Mazanowski *et al.* (1981) reported that the addition of citric acid solution to the starting and fmishing

feeds of sonali increased feed conversion. Kahraman and Bostan (1998) without specifying dose used Acid Lac Dry; citric, lactic, fumeric, propionic and fumeric acid and/or zinc bacitracin in broilers. They reported that best feed conversion obtained in group fed organic acid combination + zinc bacitracin. Nudiens (2002) supplementing acidifier (10kg/ton feed) in broiler found increased feed conversion. Shen-HuiFang *et al.*(2005) using 0.3, 0.5 and 0.7% dietary citric acid in Yellow chicken observed increased feed conversion. Celik *et al.*(2003) found increased feed conversion in sonali chicken by supplementing propionic acid, fumeric acid, citric acid and sorbic acid containing acidifier (0.5kg/ton). Izat, (1989) without specifying dose reported buffered propionic acid as an alternative to antibiotics improved feed conversion of sonali Chicken. Reported that additive of acetic acid to diet (400 or 600mg/kg) increased feed conversion. Waldroup, *et al.* (1967) reported that addition of 0.5 or 1.0% fumaric acid did not ($p>0.05$) influence feed conversion. Garcia *et al.* (2000) supplemented apramycin (l00ppm) and organic acid mixture (50% formic acid+50% propionic acid) at 0.0, 0.1 and 0.2% levels and found decreased feed conversion. Skinner *et al.*(1991) reported that addition of 0.125% fumaric acid had no effect on feed conversion. Citric acid (0.0. 0.5, 3.0%) had no significant effects on feed conversion in sonali s (Moghadam *et al.*2006). Atapattu and Nelligaswatta (2005) reported feed conversion was not affected by the inclusion of 2 levels (1 and 2%) of dietary citric acid. Andrys *et al.*(2003) reported that broiler ROSS 308 treated with acidifier FA30 (citric acid and phosphoric acid) did not affect feed conversion.

2.4.3 Organic acids and survivability

Shen-HuiFang *et al.*(2005) using 0.3, 0.5 and 0.7% dietary citric acid in Yellow chicken observed highest survivability in group fed 0.3% citric acid. Zhang *et al.*(2005) used citric acid and fumeric and malic acid without specifying dose and found increased survivability in broiler. Nudiens (2002) supplementing acidifier (10kg/ton feed) in sonali s found increased survivability. Chitra *et al.*(2004) without specifying dose reported higher survivability for ascorbic acid with probiotic in broilers. Arefin (2002) without specifying dose mentioned nutrilac in chicken to increase survivability.

2.5 Drinking Water as a Risk Factor to Poultry Health

2.5.1 Water as a vehicle of infection for poultry

Water is the most abundant and widely distributed chemical compound in the world. In the natural state, water is one of the purest compounds known; nevertheless, it is currently difficult to find a freshwater source that has not been altered by man. This fact is related to characteristics of countries in development, such as Brazil, where wastewaters from agriculture and urban areas, which might contain high levels of pathogenic microorganisms, are disposed of on the soil or into aquatic environment. The residues are then carried to the superficial and underground waters by the rain. The use of consumption water with high physical, chemical and microbiological qualities is of fundamental importance in animal production because many animals have access to the same water source and a problem in the water quality would affect a great number of animals. This is particularly relevant in poultry production, where one single water source serves thousands of animals. Therefore, control measures must be considered as priority, in order to prevent the occurrence of diseases that are spread through water, and would certainly result in great economical losses. Although water does not provide ideal conditions for pathogenic microorganism to multiply, they will generally survive for enough time to allow waterborne transmission. Water is, therefore, an excellent transmission route of agents responsible for human and animal diseases, mainly those in which fecaloral transmission occurs, since contamination of water supplies is still gradually increasing as a result of urban and rural activities. Preventive measures and also solutions to problems that already exist must be the aim of every person by Amaral LA do, (2004).

The scenario is not so different in the rural area, where many factors increase the risk of occurrence of waterborne diseases. Examples of such factors are the inadequate disposal of organic and inorganic residues from agriculture and livestock productions; the lack of concern regarding the quality control of the drinking water given to animals, resulting in the animals drinking water of very low quality; and finally the general belief that any water sources in the rural area have good quality and can be used as drinking water for both humans and animals, no matter if they have been submitted to adequate water treatment or not. The use of potable water in animal rearing is a preventive approach that is expected from farmers, mainly from poultry farmers, who are unique in many aspects in Brazil. The intensive methods of rearing poultry have as consequence the more

preventive consciousness regarding diseases. Disease dissemination through water can result in great losses to the producer, besides the hazards of carrying zoonosis pathogens to the herd, which would reflect in a Public Health problem. Diseases that can be transmitted to the bird flock though the drinking water may originate from water contamination by feces and secretions of sick birds, or by the utilization of water already contaminated by pathogenic organisms that originate from other animal species and the man, such as in the case of salmonella and Escherichia coli, respectively. Diseases caused by bacteria, virus and protozoa are among the most common diseases in the poultry industry in which drinking water plays an important role.

2.5.2 Poultry diseases potentially transmitted by water

2.5.2.1 Bacterial diseases

Chronic Respiratory Disease (CRD)

Etiologic Agent: *Mycoplasma gallisepticum*. Thedisease might be complicated by the presence of Escherichia coli.

Main clinical signs: respiratory distress, weight loss, respiratory rales, decreased egg production, poor flock uniformity and feed conversion, increased carcass condemnation. The etiological agent may contaminate water by the expectorations of the birds and Escherichia coli maybe present by fecal contamination of the drinking water.

2.5.2.2 Colibacillosis

Etiological Agent: *Escherichia coli.*

Main signs: exacerbation of respiratory symptoms, which are complicated by septicemia, occurring after stressing situations. The pathogen may be present due to fecal pollution of the water.

2.5.2.3 Avian Cholera

Etiological agent: *Pasteurella multocida.*

Main signs: appetite loss, prostration, decreased egg production, cyanotic combs, high mortality, and respiratory signs. The pathogen may be present as a result from fecal pollution of the water.

2.5.2.4 Fowl Typhoid

Etiological agent: *Salmonella Gallinarum.*

Main signs: prostration, green diarrhea, mortality, and decreased production. The agent may be present in the water as a result of fecal contamination.

2.5.2.5 Diseases caused by virus

Newcastle Disease Etiological agent: *Paramyxovirus.*
Main signs: respiratory, neural or digestive signs, decreased egg production, high mortality. The etiological agent may be present in the water due to pollution by feces and discharges from the respiratory tract of infected birds.

2.5.2.6 Infectious bronchitis

Etiological agent: *Coronavirus.*
Main signs: respiratory impairment, decreased egg production. The etiological agent may contaminate water by fecal pollution or by discharges from the respiratory tract of infected birds

2.5.2.7 Marek's disease

Etiological agent: *Herpesvirus.*
Main signs: weight loss, paralysis, and mortality. The etiological agent may be present in the water due to epithelial desquamation of infected birds.

2.5.2.8 Avian encephalomyelitis

Etiological agent: *Picornavirus.*
Main signs: ataxia, tremor of head, neck, and limbs. The agent may be present in water due to fecal contamination.

2.5. 2.9 Gumboro disease

Etiological agent: *Birnavirus.*
Main signs: paleness, prostration, and low resistance. The etiological agent may be present in water due to fecal contamination.

2.5.2.10 Protozoan Diseases

Histomoniasis Etiological agent: *Histomonas meleagradis.*
Main signs: prostration, ruffled feathers, and yellowish diarrhea. The etiological agent may be present in the water by fecal pollution.

2.5.2.11 Coccidiosis

Etiological agent: *Eimeria sp.*

Main signs: dark feces with blood, drooping wings, ruffled feathers, loss of pigmentation in the shanks and combs, and flock yield lower than expected. The etiological agent may be present in the water by fecal pollution.

2.6 Water management

2.6.1 Conduct water tests

Each farm should have its well water tested. Water quality can change during periods of heavy rain or drought, and additional water tests during these periods will ensure that water lines continue to deliver adequate water volume for both the birds and the cooling systems.

2.6.2 Change filters regularly

Sediment and other particulates can cause leaky water nipples that can have negative effects on litter quality. Clogged filters restrict water flow to the drinker and cooling systems. In some cases, simple cartridge filters may not be adequate, such as for water with high iron. In those cases, consider other water treatments.

2.6.3 Flush water lines regularly

Perform a high pressure flush on water lines between each flock and after adding supplements through the medicator (i.e., vaccine, medications, vitamins, electrolytes, etc.).

2.6.4 Plan ahead before treating water

Before implementing water treatment or sanitation programs, consult your county agent to be sure contaminants in your water will not react negatively and cause the water system to become clogged.

2.7 Microbiological Control of the Drinking Water for Birds

The control of the microbiological quality of the water used in the poultry industry is of fundamental importance. The knowledge of water microbiological characteristics is therefore necessary. It should be noted that the classification of the interior waters in Brazil advises that waters up to class 3 can be used as drinking water for animals. In other words, they should have values of total and fecal coli forms of 20,000/100ml and 4,000/100 ml, respectively. The observation of such values in the drinking water of larger

animals may not result in health damage (Brasil, 1986). Concerning poultry production, these limits may represent sanitary problems to the flock. The birds are smaller and precocious animals, and their lower resistance may cause them to be more susceptible to infections, mainly caused by pathogens of intestinal origin that might be present in water with the fecal pollution index mentioned above. Therefore, Macari (1997) and Englert (1998) recommend that waters with portability levels similar to levels applicable to humans should be also used for birds. Corroborating these considerations, (Nemedi 1984, cited by Geldreich, 1998) verified that when the levels of fecal coliforms in the water were 106, 105, 104, 103, 102 and 10, the percentages of Salmonella isolation were 100%, 99%, 66%, 33%, 21% and 11%, respectively. Schwartz and Waggoner *et al.*(1984) (cited by Carter & Sneed, 1996) and Reddy *et al.*1995).considered that the number of microorganisms in the drinking water of birds should be 100 CFU/ml for total bacteria and 50 CFU/ml for coliforms. The mean levels of Escherichia coli in the water of a sonali chicken farm that used bell-type drinkers were 104 microorganisms/ml in the first week of life (Barros *et al.*2001), a concerning finding since this is a high fecal contamination associated to young age of the birds. Meza (1989) states that there should be a better bacteriological control of the water provided to the birds during the initial phase, since there is a fast bacterial growth and the health risk is increased for the for birds from 1 to 21 days of age. It must be pointed out that the water that is supplied to the birds in many farms is contaminated in the water sources. It has been reported that the samples from the water sources and reservoirs were contaminated by Escherichia coli in 10 sonali chicken and laying hen farms, evidencing fecal pollution of the samples (Amaral *et al.*1999; Amaral *et al.*2001). Burcham *et al.*(1992) assessed water samples from 105 wells of 65 flocks in the United States and reported that fecal coliforms were present in 45% of the samples whereas Salmonella was present in 7.6%, what evidences that well water may pose a risk to bird health. Drinkers are important factors to the microbiological quality of the water provided to the birds. Open water supplies, such as troughs and bell drinkers, may present high contamination levels of 107 and 104 per ml for mesophiles and fecal coliforms (Carr *et al.*1988). In the closed system (nipple), the quality of the water offered to the birds is better protected and there are no deleterious effects on bird performance compared to the open systems (Carpenter *et al.*1992). Amaral *et al.*(1999) and Amaral *et al.*(2001) observed significant differences in Escherichia coli numbers when open and closed drinkers were compared in sonali chicken and laying hen farms.

The risk of contamination with salmonellas was 6 to 7 times higher when the water given to birds was exposed to the environment (Renwick *et al.*1992). Besides, more water samples were positive to salmonellas in a sonali chicken facility when water was provided in troughs and therefore water was considered an important means of re-infection in birds (Morgan-Jones, 1980). Salmonellas were isolated from 21.6% of the sonali chicken farms and from 12.3% of the water samples examined in Canada by Poppe *et al*, (1991). The use of open drinkers in the majority of the farms was favorable to contamination and the presence of salmonellas in the litter was considered an important contamination route of the water provided to the birds. Microorganisms from the genus Campylobacter are also important for the poultry industry and may be transmitted though water. Kapperud *et al.*(1993) reported a risk 3.5 higher of birds being infected by such microorganisms when the drinking water was not disinfected with chloride. Furthermore, Campylobacter jejuni was isolated from the biofilm present in the nipple supplying pipes when the birds were infected, whereas no microorganism was isolated when the birds were not colonized (Zimmer *et al.*2003).

2.8 Natural feed additives

It is known that dietary supplementation with natural growth promoters (NGP) can assist in Salmonella prevention (Jones, 2011). In a trial 84 day-old sonali chicks (Ross) from the same origin were randomly divided into three treatment groups. The control group received no feed additives, whereas two trial groups received dietary supplementation with two different natural growth promoters.

Trial group I received a diet supplementation with an NGP consisting of a blend of formic and propionic acids at inclusion levels of 3.0 kg/t feed. Trial group II received a diet supplemented with an NGP consisting of a blend of organic acids, a phytochemical and a permeabilising substance at an inclusion level of 1 kg/t feed. A permeabilising substance was identified to weaken the outer membrane of Gram-negative bacteria and facilitate the entry of organic acids and phytochemicals in the cell disturbing its vital functions. At three days of age all chicks were orally inoculated, the challenged dose was 104 cfu/bird of Salmonella enteritidis (Tripathi, 2017).

At 7 and 14 days post infection (dpi), the caecal content from 12 birds was taken and analysed quantitatively and qualitatively for Salmonella. At 11dpi, faecal samples were taken and analysed qualitatively for Salmonella. The results of this study showed that in the groups supplemented with the natural growth promoters, Salmonella was neither detected in the quantitative and qualitative culture in caecal samples at 14dpi nor in the qualitative culture in faecal samples at 11dpi.The present trial results are in accordance with scientific literature which has shown that organic acid blends are effective in preventing the caecal colonisation of newly-hatched chicks by Salmonella enterica serovars Typhimurium, Enteritidis, Agona and Infantis (Iba & Berchieri Jr, 1995). This is consistent with previous findings by Hinton & Linton (1988), who reported that while a blend of organic acids did not completely eliminate Salmonella from treated feed, there was no caecal colonisation by Salmonella when this feed was given to the birds.

2.9 Farm hygiene and biosecurity

Contamination of the resident environment of animal housing can be a source of Salmonella infection. Keeping buildings clean and disinfecting farm equipment helps to minimize the danger of infection. Improving farm personnel hygiene and the control of visitors are important factors for reducing the risk of Salmonella (Jones, 2011). Washing hands and disinfection as well as the cleaning of overalls and disinfection of boots before entering the stable are associated with decreased Salmonella prevalence. The relatively small cost incurred may be offset by decreased transfer of other performance impairing pathogens.

Since all vertebrates are susceptible to the Salmonella infection, contact with other species may pose an infection risk to other animals. Pests (rodents, wild birds, and other wildlife species) have often been implicated as potential sources of Salmonella (Tripathi, 2017). It has been recognized that flies and beetles also serve as a potential reservoir and carriers for Salmonella. It is therefore important to ensure proper vermin and pest control on a farm.

CHAPTER-III
MATERIALS AND METHODS

3.1 Location of the study

The experiment was conducted at the Poultry farm under the Department of Dairy and Poultry science of HSTU, Dinajpur. 5200 during the period from 31 October 2017 to 1 January 2018. Commercial sonali chick was used in this study for a period of 9 weeks to find out the effects of different pH levels on the performance of Sonali chicken.

3.2 Experimental birds

One hundred twenty vigorous day- old Sonali chicks were procured from Rafid hatchery limited, Joypurhat.

3.3 Layout of the experiment

The experiment was conduct in complete randomized design (CRD)**.** The chicks were randomly distributed to four dietary treatment groups (T_0, T_1, T_2, and T_3,) having three replications in each treatment. The chicks were reared in separated pens according to treatments and replications, each treatment group contain of 10 birds. The layout of the experiment is shown in the following table:

Table 1: Layout of the experiment

Dietary treatment	**No. of chicks in each replication**			**Total number of chicks in each treatment**
	R_1	**R_2**	**R_3**	
T_0	10	10	10	30
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
Total				120

Where,

T_0: control (Natural water)

T_1: 5.5 pH level

T_2: 6.5 pH level

T_3: 7.5 pH level

3.4 Preparation of the experimental house

HSTU poultry farm was used for rearing experimental birds to evaluate the efficacy of Organic Acid on growth performance and antibacterial effect. Experimental shed was constructed with compartment for housing for ten birds. Each compartment was dimensions 54x42 inch for length and breadth, respectively. The shed was constructed by iron net and wooden materials. At first the experimental house was properly washed and cleaned by using tap water. Ceiling, walls, and floor are thoroughly cleaned and subsequently disinfected with bleaching powder, then the room was left vacant for two weeks. Later the house was again disinfected with virocid solution 1ml per 3 liter water, at the same time, all federals, watarers and other necessary equipment were also properly cleaned, washed and disinfectant with bleaching powder. After drying the house was used for this study.

3.5 Adjustment of different pH level

At first plane water was taken in a jar. Which contains near about 7 pH value Measured by pH meter. To get 7.5 pH value added sodium carbonate drop by drop in the water for increasing pH level. After few minutes, check the reading by pH meter. Finally reached the 7.5 pH value. To get 6.5 pH and 5.5 pH value added Hemko pH drop by dropin the water for decreasing pH value. After few minutes check the reading by pH meter and finally reached 6.5 pH and then similarly 5.5 pH.

3.6 Experimental diet

The experimental diet was provided into two phages (Sonali-starter and Sonali-grower), starter was provided 0 to 30 days and grower was days 31 to 63 days of experiment. The experimental diets were purchased from local market in Dinajpur, namely company (Naris Poultry and Hatchery Limited). Organic Acid was collected from local market in Dinajpur.

3.7 Routine Management

The birds were reared to similar care and management in all treatment groups throughout the experimental period. The following management practices were followed whole experimental period.

3.7.1 Litter Management

Fresh and dried rice husk was used as litter at a depth 2-3 inch. After 5 weeks old litter was totally removed and new litter was provided as same depth. The litter was stirred one time per day from four weeks to up to the last day of experimental period.

3.7.2 Floor Space

Each pen 4.5×3.5 sq. ft. was allocated for feeding, watering, and housing for 10 experimental birds.

3.7.3 Brooding Management

Brooding is the first management of day old chick. In brooding period electric brooder was used to provide suitable heat in chick for maintaining their body temperature. The brooder was hanged just above the bird level at the center of chick guard. Before entry day old chick fresh dried litter provide at depth 3 inch then covered by newspaper. Pre-heating the brooding space and temperature adjust at 33±2 $^{\circ}$C. After entry day old chick provided vitamin C and glucose, one-hour latter feed was provided. At first day temperature maintain 33± 2°C then gradually decrease 1°C per day. Temperature and humidity recoded by using clinical thermometer and hygrometer.

Figure: 1 Brooding Management

3.7.4 Lighting Management

The birds were exposed to 23 hours of lighting and 1-hour dark period throughout the experimental period.

3.7.5 Feeding and drinking

Provide *ad libitum* feed and water through the experimental period.

Figure: 2. Feeding and drinking

3.7.6 Vaccination

Name of Vaccine	Name of diseases	Age(days)	Route of administration
IB + ND	Infectious Bronchitis & Newcastle	5^{th}	One drop in one eye
IBD	Gumboro	10^{th}	One drop in one eye
IBD	Gumboro	17^{th}	Through drinking water
ND	Newcastle	22th	Through drinking water
ND	Newcastle	42th	Through drinking water

3.7.7 Sanitation

Drinkers were washed daily in the morning and feeders were cleaned weekly before being used. Strict sanitary measures were followed during the experimental period.

3.8 Temperature and relative Humidity measure

Temperature (^{0}C) was recorded by clinical thermometer and relative humidity (%) was recorded by digital hygrometer three time daily.

3.9 Slaughtering of the Birds

Prior to slaughtering the birds were fasted for 8 hours, but water was provided *ad libitum*. Two birds were randomly selected in each replication for slaughtering. The live weight of birds was taken individually before slaughtering. At the time of slaughtering the birds were secured by holding both shanks with one hand and both wings with other hand by the help of an assistant to prevent struggling. Slaughtering was done by Halal Method with sharp knife. Complete bleeding was accomplished by raising the bird approximately 45^o so that the caudal part will be higher than the head. After complete bleeding was done then removal of shank, head and skin. Finally evisceration was done manually to separate liver, spleen, heart, gizzard, and meat yield.

Figure: 3. Slaughtering of the Birds

Figure: 4. Weighting of the meat

3.10 Collection of feces

For bacteriological analysis two birds were randomly selected in per replication. Feces was collected from cloaca.

3.11 Storage and Transport of fecal sample

After collection of feces it was kept air tight polythine bagthen store at 4^0C.Then the feces sample was send in Microbiology Laboratory of Microbiology Department in VAS faculty for analysis. Eosin Methylene Blue (EMB) agar medium was prepared by suspending 36.0 g in 1 litre of distilled water and Salmonella Shigela agar media was prepared by suspending 50g in 1 liter distilled water. This was brought to boil to dissolve completely and then sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 55 ºC, it was poured into the petri dish and checked for sterility by overnight incubation. The next day, the freshly collected faecal sample i.e 1 gram of faeces from the experimental birds at random from each group in three replicates was suspended in 9 ml of sterile normal saline and serially diluted from test tub. Form the last dilution a loopful of inoculums was streaked on the media and then incubated at 37 ºC for 24 hours to screen for the presence of E. coli as per the standard method.

3.12 Data collection and record keeping

The following records were kept during the experimental period: Initial DOCs weight and after brooding weight of chicks. Weekly Body weight gain and feed intake was recorded replication wise in each treatment group at last day of week. Mortality was recorded daily if death occurred. The different meat yield parameters like, carcass, thigh, breast meat, head, heart, liver, spleen, gizzard and meat yield parameter for individual birds were recorded after slaughtering. Temperature and relative humidity was recorded three times daily (8 hour interval).

3.13 Calculation

1. Total weight gain in (kg). This was computed as a group by subtracting the initial weight from the final weight.

   Total gain in weight = final weight – initial weight

2. Dressing percentage: The dressing percentage of sonali chicken was calculated as follows:

Dressing (%) = (Dressed Weight÷ Body Weight) x 100

3. Total feed consumption (kg).The amount of feeds consumed by the birds from the start until the end of the experiment (63 days). This was computed by adding the total feeds offered after the total left- over have been subtracted.

Total feed consumption = total feed offered – total left-over

4. Feed efficiency. This was obtained per treatment by dividing the total feed consumed by the total gain in weight. Feed efficiency is computed for the whole duration of the experiment (63 days).

Feed efficiency = total feed consumed / total gain in weight

5. Total cost of the total feed consumed (PhP). This was obtained by multiplying the cost of feed per kilogram to the total feed consumed.

Cost of the total feed consumed = cost of feed per kilogram × total feed consumed

6. Feed cost per kg gain of sonali chicken (PhP). The feed cost per kilogram of gain in weight and this was computed as the price of feeds per kilogram multiplied by the total gain in weight.

Feed cost per kilogram gain (PhP) = price of feeds per kg × total gain in weight

7. Mortality rate (%) = no. of dead chickens / total no. of birds as a group × 100

8. Cost of production (PhP). This includes the cost of stocks, feeds, commercial antibiotics and vitamins, electricity, and materials used.

9. Gross income (PhP). This was obtained as a group by multiplying the sum of the final weight of the birds by the price per kilogram of live weight.

Gross Income = total weight of the birds (as a group) × price per kilogram

10. Net income (PhP). This was obtained by subtracting the cost of production from the gross income.

Net income = gross income – cost of production

3.14 Statistical analysis

The data of feed consumption, growth performance, carcass characteristics and bacterial count were recorded and analyzed by SPSS version-20 software by using one way ANOVA accordance with the principles of Complete Randomized Design (CRD). All values were expressed as Mean±SEM and significance was determined when P is <0.05. Mean was compared among the treatment groups by using DMRT (Ducans multiple rang test).

CHEPTER-IV
RESULTS AND DISCUSSION

This experiment was conducted to evaluate the efficacy of different pH levels on production performance in terms of weekly body weight gain, final live weight gain, feed intake, feed efficiency, dressing percentage, meat yield parameters and microbial count such as E. coli and Salmonella on Sonali chicken.

4.1 Weekly Body weight gain

Table 1. showed that after 7 days of brooding, initial body weight of chicks in different dietary treatment was similar. The live weight of birds in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, $6^{th,}$$7^{th}$$8^{th}$ and 9^{th} weeks did not significantly ($P>0.05$) vary among the treatment groups. The efficacy of different pH levels in different water such as @ 5.5, 6.5 and 7.5 in drinking water up to 9^{th} weeks increase live weight gain day by day. In 9^{th} weeks the highest body weight gain was found (863.60±25.21g) in T_2 group that was received @ 6.5 pH in water and the lowest body weight gain was found (794.60±27.02g) in T_3 group that was received @ 7.5 pH in water ($p>0.05$). Within the treatment group @ 5.5, 6.5 and 7.5 pH in drinking water live weight was found (800.66±22.69g), (863.60±25.21 g) and (794.6±27.02 g). The result of this study showed that increase pH @ 7.5 decrease live weight gain although no significant difference was found ($p>0.05$). However, the highest result was found @ 6.5 pH level in drinking water as similar to the plain water. Inclusion level of pH 6.5 in drinking water was showed maximum live weight (863.60±25.21 g) and minimum live weight was showed (794.60±27.02g) in pH 7.5 treatment group T_3 at the terminal stage of experiment.

4.2 Body weight gain

Initial body weight of sonali chicks fed on different dietary treatments was similar ($p>0.05$). Final live weight gain was not statistically significant ($p>0.05$) among the different treatment group. The highest body weight gain was attained in birds that received pH 6.5 in drinking water. However, treatment groupT_2 was highest body weight gain compared to control group $T_{0,}$ and treatment groupT_1 and T_3. Highest body weight gain (834.60±15.22) was found in T_2 group and lowest body weight gain (765.60±14.80) was found in T_3 group ($p>0.05$).

Table 1. Effect of supplementation of Organic acid on weekly body weight, and body weight gain of sonali chicken.

Parameters	T_0 Normal water	T_1 5.5 p^H	T_2 6.5p^H	T_3 7.5 p^H	Level of Sign.
Initial live wt.(g)	29.00±0.00	29.00±0.00	29.00±0.00	29.00±0.00	NS
1st week	84.5±3.4	85.33±4	87.5±3.6	84.50±3	NS
2nd week	171.06±3.21	181.00±4.60	176.60±3.73	176.66±3.97	NS
3rd week	219.73±5.97	221.33±5.34	227.00±5.45	213.66±5.31	NS
4th week	306.33±7.50	307.26±13.87	307.40±11.15	302.53±7.15	NS
5th week	412.40±12.50	401.81±16.08	433.46±5.99	412.80±9.14	NS
6th week	494.86±6.15	493.93±14.25	517.13±12.66	486.53±12.45	NS
7th week	607.00±13.89	598.00±13.83	622.53±17.91	585.53±13.33	NS
8th week	727.33±21.09	726.66±18.66	752.00±15.31	700.93±17.82	NS
9th week	804.33±20.07	800.66±22.69	863.60±25.21	794.60±27.02	NS
Final body wt. gain	775.33±13.40	771.66±18.30	834.60±15.22	765.60±14.80	NS

The mean values with different superscript (a to c) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean

NS=Non significant, * statistically significant ($P<0.05$)

4.3 Feed intake

The cumulative feed intake of sonali chicken in different dietary treatment during experimental periods was almost statistically similar and the differences were insignificant ($p>0.05$). However, the lowest feed intake (2097.12±25.34 g) was found T_1 group. The birds of T_2 group took containing 6.5 pH value showed higher feed intake (2210.10±28.12 g) due to the normal pH value. In (Table 2) we found that normal water group and T_2 group showed highest feed intake.

4.4 Feed efficiency

At the experimental period feed efficiency of different treatment groups statistically insignificant ($P>0.05$).The birds of T_2 groups took containing 6.5 pH value converted feed to meat most efficiently. The feed efficiency of T_2 treatment groups was statistically insignificant ($P>0.05$) with T_0, T_1 and T_3 treatment group. From (Table 2) feed efficiency was increased with 6.5 and normal pH value in drinking water.

Table 2. Effect of pH value on feed intake, feed efficiency, mortality, and mortality percentage of sonali chicken.

Parameter	T_0 Normal water	T_1 5.5 p^H	T_2 6.5p^H	T_3 7.5 p^H	Level of sig
FCR	2.75±0.01	2.73±0.05	2.65±0.04	2.78±0.03	NS
Feed intake (g)	2131.25±18.23	2097.12±25.34	2210.10±28.12	2126.70±18.21	NS
Mortality %	0	0	0	0	

The mean values with different superscript (a to c) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean
NS=Non significant, * statistically significant ($P<0.05$).

4.5 Dressing percentage

After slaughtering and eviscerating, remove all edible and non edible by-product, dressing percentage of different treatment group showed in (Table 3). The Table indicated that, there were no significant differences among the treatment group ($p>0.05$). The dressing percentages were observed in T_2 (51.96±0.36%), T_1 (50.33±0.88%), T_3 (50.44±1.28%), and T_0 (50.86±0.59%) respectively. The highest dressing percentage was found (51.96±0.36%) in T_2 treatment group and lowest was found (50.33±0.88%) in T_1 treatment group ($p>0.05$).

4.6 Breast meat

Breast meat obtained (Table 3) was statistically insignificant ($P>0.05$) among the different treatment group. Supplementation of 6.5 pH in drinking water was higher breast weight compare to control group and T_3 treatment group. However, highest weight was found (120.66±9.8g) that receive 6.5 pH in drinking water and lowest was found (98.66±16.17g) in T_3 treatment group.

4.7 Thigh meat

Data obtained from (Table 3) thigh meat of sonali chicken was statistically insignificant ($p>0.05$) among the different treatment group. Best result was observed in supplementation of 6.5 pH treated group T_2 (146.66±4.05g) whereas lowest was found in T_1 group (138.66±5.20g).

4.8 Heart, Liver and Gizzard weight

Heart, gizzard and liver weight of sonali chicken in different dietary treatment groups was statistically insignificant ($p>0.05$). From (Table 3) it was seen that liver and gizzard weight maximum in T_2 treatment group and minimum in T_1 treatment group. Heart weight was similar in control and treatment group.

Table 3. Effects of pH on meat yield parameters of sonali chicken

Parameter	**T_0 0**	**T_1 5.5 p^H**	**T_2 6.5 p^H**	**T_3 7.5 p^H**	**Level of sig**
Final Live wt. (g)	804.33±20.07	800.66±22.69	863.60±25.21	794.60±27.02	NS
Dressing (%)	50.86±0.59	50.33±0.88	51.96±0.36	50.44±1.28	NS
Breast meat wt. (g)	108.00±4.00	117.33±2.90	120.66±9.8	98.66±16.17	NS
Thigh meat wt.(g)	142.00±2.00	138.66±5.20	146.66±4.05	142.00±16.04	NS
Heart (g)	5.00±0.57	5.66±0.33	5.33±0.66	5.33±0.66	NS
Gizzard (g)	32.66±1.33	27.33±2.40	30.00±2.30	38.66±4.05	NS
Liver (gm)	22.00±1.15	20.66±1.33	26.00±1.15	23.33±1.33	NS

The mean values with different superscript (a to b) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean
NS=Non significant, * Statistically significant ($P<0.05$)

4.9 Faecal total bacterial count

The effect of pH on the faecal total bacterial count is presented in the Table 4. The E. coli and Salmonella bacterial count was significantly ($p<0.01$) reduced in the treatment groups when compared to the control groups. The E. coli and Salmonella bacterial load was increased in the control and T_3 group which was provided only the normal drinking water and pH 7.5 as against the T_1, T_2 groups. Highest E. coli count was found (233.33±12.01) in T_3 groups and lowest E. coli count was found (160.00±11.54) in T_1 groups. Highest salmonella count was found (225.00±14.43) in T_3 groups and lowest was count (130.00±5.77) in T_1 group. However one log reduction was noticed in the group T_2.

Table 4 Effect of pH on E. coli and Salmonella count on sonali chicken

Parameters	T_0 Natural water ml/L	T_1 5.5 $\mathbf{p^H}$	T_2 6.5 $\mathbf{p^H}$	T_3 7.5 $\mathbf{p^H}$	Level of sign.
Salmonella	216.66±12.01	130.00±5.77	145.00±8.66	225.00±14.43	*
E.coli	203.33±8.81	160.00±11.54	173.33±17.63	233.33±12.01	*

The mean values with different superscript (a to b) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean
NS=Non significant, * Statistically significant ($P<0.05$)

4.10 Economic efficiency of production

Production cost of sonali chicks in this study are presented in (Table 5). Spending on feed, chick, vaccine, medicine, litter, hemico pH, miscellaneous (labour, electricity, transport cost) were constituted cost/chick and cost/kg live weight. Total production cost per kilogram weight gain lowest was (142.00±1.12TK.) found in T_2 group and highest was found (142.80±1.36Tk.) in T_1 group. The net profit from per kilogram sonali chicken was statistically similar ($p>0.05$). The highest profit (18.00±1.2Tk.) was found T_2 group and lowest (13.8±1.18Tk.) was found in T_3 group.

Table 5: Cost benefit analysis of different dietary treatment on sonali chicken production

Parameters (Tk.)	**T_0 0 ml/L**	**T_1 5.5 p^H**	**T_2 6.5 p^H**	**T_3 7.5 p^H**	**Level of .**
Chick cost/chick	15	15	15	15	NS
Litter cost/chick	4	4	4	4	NS
Vaccine + medicine	10	10	10	10	NS
organic acid cost/ chick	0	2	4	6	NS
Feed cost/ kg production	110.00±1.3	108.80±1.36	106.00±1.12	111.2±1.18	NS
Miscellaneous cost/ chick	3	3	3	3	NS
Total cost Tk./kg production	142.50±1.3	142.80±1.36	142.00±1.12	146.20±1.1	NS
Selling price Tk./kg	160	160	160	160	NS
Net profit Tk./kg	17.5±1.30	17.2±1.36	18.00±1.12	13.8±1.18	NS

The mean values with different superscript (a to b) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean
NS=Non significant, * Statistically significant ($P<0.05$)

CHEPTER-V

SUMMARY AND CONCLUSION

The experiment was conducted to evaluate the efficacy of different pH levels on production performance, dressing yield and microbial load of sonali chicken at Hajee Mohammad Danesh Science and Technology University poultry farm, Dinajpur from 31 October 2017 to 1 January 2018. For this purpose 120 day old chicks were purchas from Rafid Hatchery Ltd. After 7 days of brooding the chicks were randomly distributed to four dietary treatment groups (T_0, T_1, T_2, and T_3,) having three replications in each treatment. The chicks were reared in separated pens according to treatments and replications, each dietary treatment group contain of 10 birds.

The result of this study clearly showed that increase pH @ 7.5 decrease live weight gain. However the highst result was found @ 6.5 pH level in drinking warer as similar to the plain water. Inclusion level of pH 6.5 in drinking water was showed maximum live weight (863.60±25.21 g) and minimum live weight was showed (794.60±27.02g) in pH 7.5 treatment group T_3 at the terminal stage of experiment. Control group showed as similar result compared with T_1 group. Highest weight gain was found in T_2 group.

The highest body weight gain was attained in birds that received pH 6.5 in drinking water. However, treatment group T_2 was highest body weight gain compared to control group $T_{0,}$and treatment group T_1 and $T_{3.}$ The result of this study was indicated that pH value 6.5 is better for weekly live weight gain as compared to 5.5 and 7.5 pH value. Highest body weight gain (834.60±15.22) was found in T_2 group and lowest body weight gain (765.60±14.80) was found in T_3 group.

Spending on feed, chick, vaccine, medicine, litter, hemico pH, miscellaneous (labour, electricity, transport cost) were constituted cost/chick and cost/kg live weight. Total production cost per kilogram weight gain lowest was (142.00±1.12TK.) found in T_2 group and highest was found (142.80±1.36Tk.) in T_1 group. The net profit from per kilogram sonali was statistically similar ($p>0.05$). The highest profit (18.00±1.2Tk.) was found T_2 group and lowest (13.8±1.18Tk.) was found in T_3 group.

Data obtained from meat of sonali chicken was statistically insignificant ($p>0.05$) among the different treatment group. Best result was observed in supplementation of 6.5 pH treated group T_2 (146.66±4.05g) whereas lowest was found in T_1 group (138.66±5.20g).

The result of this study suggest that control of pH level in the drinking water can be effective on production performance in sonali chicken. Therefore, more studies are required to determine the effective pH level in the drinking water.

REFERENCES

Abdel-Fattah, S. A., El-Sanhoury, M. H., El-Mednay, N. M. and Abdel-Azeem, F., 2008. Thyroid activity, some blood constituents, organs morphology and perfocnnce of broiler chicks fed supplemental organic acids. International kuiia1 of Poultry Science, 7: 215-222.

Adil S. Tufail B. Gulam A.B., Masood S. and Manzoor R., 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet Med Int. 2010:1–7. Article ID 479485.[PubMed], [Google Scholar]

Afsharmanesh, M. and Pourreza, J., 2005. Effect of calcium, citric acod, ascorbic acid Vitamin D3 on the efficiency of microbial phytase in broiler staters fed wheat-based diets. 1, performance, bone mineralization and ideal digestibility. International Journal of Poultry Science, 4: 418-424.

Alali, W.Q., Mann, D.A. and Beuchat, L.R.. 2012. Viability of salmonella and listeria monocytogenes in delicatessen salads and hummus as affected by sodium content and storage temperature. J. Food Prot., 75, 1043–1056. [CrossRef] [PubMed]

Ali, I. and Wahid, M. A., 1989. Reproductive performance of Fayoumi, Rhode Island Red and Fayoumi × RIR crossbred hens and subsequent growth rate of their offspring up to sexual maturity. M.Sc. Thesis. Submitted to the Department of Poultry Science, Bangladesh Agricultural University, Mymensingh.

Alvarez-Ordonez, A. *et al.*2012. The acid tolerance response of Salmonella spp: An adaptative strategy to survive in stressful environments prevailing in foods and the host. Food Research International, v.45, p.482–492,. Available from: . Accessed: Apr. 29, 2015. doi: 10.1016/j.foodres.2011.04.002.

Amaral L.A., Nader Filho A., Isa H., Barros LSS., 2001. Qualidade higiênicosanit ária e demanda de cloro da água de dessedentação de galinhas de postura coletadas em bebedouros tipo nipple e taça. Revista Brasileira de Ciência Avícola; 3(3):249-255.

Amaral L.A., Rossi Junior O.D. and Cardoso V. 1999. Qualidade higienicosanit ária da água de bebedouros pendular e nipple utilizados na criação de frangos de corte. Revista Brasileira de Ciêcia Avícola; 1(2):145-148.

Amaral LA do, 2004. Drinking water as a risk factor to poultry health. Amaral LA do. Departamento de Medicina Veterinária Preventiva e Reprodução Animal - FCAV/Unesp. Correspondence.

Andrys, R., Klecker, D., Zeman, L. and Marecek, E., 2003. The effect of changed pF values of Feed in isophosphoric diets on chicken broiler performance. Czech-Journal of Animal Science, 48: 197-206.

Anisuzzaman, M. and Wahid, M. A., 1988. A study on growth rate, feed efficiency and livability of Fayoumi chicken under Bangladesh condition. M.Sc. Thesis submitted to the Department of Poultry Science, BAU., Mymensingh, Bangladesh.

Ao T., 2005. Exogenous enzymes and organic acids in the nutrition of broiler chicks: effects on growth performance and in vitro and in vivo digestion. PhD Thesis, University of Kentucky.

Arefin, H., 2002. Result of E coil infection to chickens immunopotentiality with nutniac I. G. A. Product refresher's course. Novertis (Bangladesh) Ltd.

Atapattu, N. S. B. M. and Nelligaswatta, C., 2005. Effect of citric acid on the performance and utilization of phosphorus and Crude protein in broiler chickens fed rice by products based diets. International Journal of Poultry Science, 4: 990-993.

Bangladesh Bureau of Statistics (BBS)., 2001. Statistical Year books., Statistics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh.

Barros LSS, Amaral LA. and Rossi Junior OD., 2001. Aspectos microbiológicose demanda de cloro de amostras de água de dessedentação de frangos de corte coletadas em bebedouros pendulares. Revista Brasileira de Ciência Avícola; 3(2):193-198.

Brasil. Ministerio do interior, 1986. Secretaria Especial do Meio ambinte. a agua que voce bebe. 2ª ed. Brasilla; poltry Science; 74:457-462.

Burcham *et al.1992*. It has been reported that the samples from the water sources and reservoirs were contaminated by Escherichia coli in 10 broiler and laying hen farms, evidencing fecal pollution of the samples.

Callsen, J., 1999. Commercial feed products and feed mixes for piglets. Lansuvalget for Svin, report 409, January.

Carpenter GHRA, Peterson WT, Jones KR, Hypes WA, 1992. Effects of two nipple drinker types with different flow rates on the productive performance of broiler chickens during summerlike conditions. Poultry Science; 71:1450.1456.

Carr LE, Murphy DW. and Wabeck, CJ., 1988. Livestock Environment In: III Proceedings of the International Livestock Environment Symposium; p.279-285.

Carter T.A. and Sneed R.E. 1996. Drinking water quality for poultry. Published by: North Carolina Cooperative Extension Service; [Acessed dec. 14, 2004]. Available at: http://www.bae.ncsu.edu/programs/extension/evans/ps&t-42.html.

Celik, K, Ersoy, Uzatici, E. A. and Erturk, M., 2003. The using of organic acids in California turkey chicks and its effect on performance before pasturing. International Journal of Poultry Science, 2: 446-448.

Cherrington, C. A., M. Hinton and I. Chopra., 1990. Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. J. Bacteriol. 68:69–74.

Cherrington, C. A., M. Hinton, G. C. Mead, and I. Chopra., 1991. Organic acids: Chemistry, antibacterial activity and practical applications. Adv. Microb. Physiol. 32:87–108.

Cheveerach, P., Keuzenkamp, D. A., Lipman, L. J. A. F. and Van Knapen., 2004. Effect of organic acids in drinking water for young broilers on Campylobacter infection, volatile fatty acid production, gut microflora and histological changes. Poultry Science, 83: 330-334.

Chitra, P., Mohan, B. and Viswanathan, K., 2004. Effect of probiotic with ascorbic acid on the carcass characteristics and economics of broiler production in summer season. Department of Poultry Science, Veterinary College and Research Institute, Namakkal-637001, Cheiron, India. 33: 29-33.

Christian, L., Nizamettin, S., Hasan, A. and Aylin, A., 2004. Acidifier — a modern alternative for anti-biotic free feeding in livestock production, with special focus on broiler production. Veterinaria Zootechnika, 27: 91. [Cited from International Veterinary Journal. Vol.6, No.1. Article 76.]

Cited by Carter & Sneed., 1996. *considered* that the *number* of *microorganisms* in the *drinking water* of *birds should* be *100 CFU*/*mL* for *total bacteria* and *50 CFU*/*mL* for *coliforms*. The *mean levels* of Escherichia coli in the water of a broiler farm that used.

Davidson, P. M., 2001. Chemical preservatives and natural antimicrobial compounds. Chap. 29. Pages 593–627 in Food.

Denli, M., Okan, F. and Celik, K., 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pakistan Journal of Nutrition, 2: 89-9 1.

Denyer, S. P. and G. S. A. B. Stewart.,1998. Mechanisms of action of disinfectants. Int. Biodeterior. Biodegradation 41:261–268.

Dubrynia, Ja. A., 1958. Reciprocal crossing with Moscow and Leghorn fowls. Animal Breeding Abstract. British Journal of Poultry Science, 28: 325.

Eklund, T., 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriol. 54:383–389.

Eklund, T., 1985. Inhibition of microbial growth at different pH levels by benzoic and propionic acids and esters of *p*-hydroxybenzoic acid. Int. J. Food Microbiol. 2:159–167.

Englert S., 1998. Produção de frangos de corte. In: Englert, S. Avicultura: tudo sobre raças, manejo e alimentação. 7a ed. Guaíba: Agropecuária;. p.94-151.

Fascina V.B, Sartori J.R, Gonzales E, Barros De Carvalho F, Pereira De Souza IMG, Polycarpo G.V, Stradiotti A.C and Pelícia VC. 2012. Phytogenic additives and organic acids in broiler chicken diets. Revista Brasileira de Zootecnia. 41(10):2189–2197. doi: 10.1590/S1516-35982012001000008[Crossref], [Web of Science ®], [Google Scholar]

Fuller, R., 1989. Probiotics in man and animals. A review. Journal of Biotechnology, 66: 365-378.

G. Avila-Quezada., 2010. In this study, the large concentration of *bacteria* found in plants grown in sterile soil inoculated with *Salmonella* typhimurium, even after 45 days of *growth*, could be due to a lack of competition. In the same study, more apical leaves had significantly higher. Gama M.S.Q. Água, que cura, que nutre, que mata. Aves & Ovos., 1995; p.30-33.

Garcia *et al.*2007. Document reference. Garcia, A. R. ; Batal, A. B. ; Dale, N. M., 2007. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci., 86 (1): 94-101.

Garcia, V. Catala-Gregori, P. Hernandez, F., Megias, M. D. and Madrid., 2007. Effect of Formic Acid and Plant Extracts on Growth, Nutrient Digestibility, Intestine Mucosa Morphology, and Meat Yield of Broilers. Journal of Applied Poultry Research, 16: 555—562.

Geldreich E.E., 1998. The bacteriology of water. In: Colier L, Balaws A, Sussman M. Microbiology and microbial infections. 9th ed. London: Arnold Pub.

Gruzdev, N., Pinto, R. and Sela, S., 2011. Effect of desiccation on tolerance of salmonella enterica to multiple stresses. Appl. Environ. Microbiol., 77, 1667–1673. [CrossRef] [PubMed]

Hinton and Linton., 1988. Deleterious effects of organic and nonorganic acids on bacteria are well documented Levine and Fellers, 1940;Eklund.

Iba, A. M. and Jr. A. Berchieri., 1995. Studies on the use of a formic acid-propionic acid mixture (Bio-Add TM) to control experimental Salmonella infecti on in broiler chickens. Avian Path. 24:304-311.

Islam, M.Z., khandaker, Z.H., Chowdhury, S.D. and Islam, K.M.S., 2008. Effect of citric acid and acetic acid on the performance of broilers. J. Banglariuan Agric. Univ., 6: 315–320.

Ivanov, I., 2005. Laboratory study to determine die effect of a probiotic mixture on broilers. Biotechnology in Animal Husbandry, 21: 107-123.

Izat, A., 1989. Feed additives reduce Salmonella on processed broilers. Asian- Australian Journal of Animal Science, 16:12-30.

Jones, F., 2011. A review of practical Salmonella control measures in animal feed. J. Appl. Poultry Res. 20, 102-113.

Kahraman, R., Abaş, İ., Bostan, K., Tanör, M. A and Kocabağlı, N., Alp, M., 1997. Organik asit ve mayaların broylerlerin performansı, ileum pH'sı ile Enterobacteriacaea populasyonuna etkisi. Pendik Veteriner Mikrobiyol. Derg.,; 28 (2): 171-180.

Kapperud G, Skjerve E, Vik L, Hauge K, Lysaker A, Aalmen I, Ostroff SM and Potter M., 1993. Epidemiological investigation of risk factors for Campylobacter colonization in Norwergian broiler flocks. Epidemiology and Infection; 111(2):245-255.

Kassim, H., and I. Norziha., 1995. Effects of ascorbic acid (vitamin C) supplementation in layer and broiler diets in the tropics. Asian-australas. J. Anim. Sci. 8:607–610

Keerthirathne., 2016. Additionally, S. typhimurium has a regulated response to further protect from *acid* stress, which is called the *acid tolerance* response (ATR) [47]. The ATR protects Salmonella spp. at *pH* levels of 3.0–4.0, but is activated when environmental *pH* values.

Koutsoumanis, K.P. Kendall, P.A. and Sofos, J.N., 2004. Modeling the boundaries of growth of salmonella typhimurium in broth as a function of temperature, water activity, and ph. J. Food Prot., 67, 53–59. [PubMed]

Lee J. Inkyung P. JaIesoon C., 2015. Production of extracellular α-galactosidase by Bacillus sp. LX-1 in solid state fermentation for application as a potential feed additive. Revista Colombiana de Ciencias Pecuarias. 27:194–201.[Web of Science ®], [Google Scholar]

Luckstadt C. Mellor S., 2011. The use of organic acids in animal nutrition, with special focus on dietary potassium diformate under European and Austral-Asian conditions. Recent Adv Animal Nutr Aus. 18:123– 130. [Google Scholar]

Luckstadt C., 2014. Effects of dietary potassium diformate on growth and gastrointestinal health in weaned piglets in Vietnam. Conference on International Research on Food Security, Natural Resource Management and Rural Development organized by the Czech University of Life Sciences Prague, 2014 Sept 17–19.

Luckstadt, C. and Theobald, P., 2011. Dose dependent effects of diformates on broiler performance. In: Standard for Acidifiers. Nottingham University Press, Nottingham, UK. pp. 83- 87.

Macari M. 1997. Qualidade da água e bebouros para frangos de corte: tipos, vantagens e desvantagens. In: Conferência Apinco.97 de Ciência e Tecnologia Avícolas, São Paulo. Brasil. Campinas: Facta; p.121-143.

Maiorka, A., Santin, E., Sugeta, S. M., Almeida, J. G. and Macan, M. 2004. Utilization of prebiotics, probiotics, or symbiotics in broiler chicken diets. Brazilian Journal of Poultry Science, 3: 75-82.

Masic B. and Khalifah M., 1965. The conformation of chick of various breeds and crosses for broiler production. Animal Breeding Abstract, 34: 114. [Cited by Azharul, M. I., Ranvig, H. and Howlider, M. A. R. Department of Poultry Science, Bangaldesh Agricultural University, Mymensing.]

Mazanowski, A., Doruchowski, W. and Konteca, H., 1981. Effect of supplement of citric acid in the feed on growth of broiler chicken. Journal of Biological Science, 29: 129-135.

Meza H., 1989. Controle de qualidade na produção de frangos de corte. Avicultura & Suinocultura Industrial; 80:38-44

Moghadam, A. N., Pourreza, J. and Samile, A. H., 2006. Effect of different levels of citric acid on calcium and phosphorus efficiencies in broiler chicks. Pakistan Journal of Biological Science, 9: 1250-1256.

Morgan-Jones S.C., 1980. The occurrence of Salmonellae during the rearing of broiler birds. British Poultry Science,; 21(6):463-470.

Nemedi., 1984, cited by Geldreich., 1998. Potability *levels* similar to *levels* applicable to humans should be also used for birds. Corroborating these considerations.

Nezhad, Y. E., Shivazad, M., Nazeeradi, M., and Babak, M. M. S., 2007. Influence of citric acid and microbial phytase on performance and phytase utilization in broiler chicks fed a corn-soyabean meal diet. Journal of the faculty of Veterinary Medicine, University of Tehran, 61: 407-413.

Nudiens. J., 2002. Opportunity of genetic potential cross Hybro-G broiler chicks using differently enriched feed. Veterinary Journal of Zootechnology, 19: 82-86.

Owens, B., Tucker, L., Collins, M. A. and McCracken, K., 2008. Effects of different feed additives alone or in combination on broiler performance, gut micro flora and ileal histology. British Journal of Poultry Science, 49: 202-12.

Patten. D. and Waidroup P. W., 1988. Use of organic acids in broiler diets. Poultry Science, 67: 1178-4182.

Paul, S. K., Halder, G., Monthl, M. K. and Samanta, G., 2007. Effect of Organic Acid Salt on the Performance and Gut Health of Broiler Chicken. The Journal of Poultry Science, 44: 389-395.

Pinchasov, Y. and Elmalich, S., 2000. Broiler chick responses to anorectic agent: dietary acetic and propionic acids and the digestive system. Faculty of Agriculture, Hervew University of Jerusalem, Rehovot, Israel. (http:// www.ncbi.nlm.nih.gov/sites/entrez).

Poppe C, Irwin R. J, Messier S, Finley GG, Oggel J., 1991. The prevalence of Salmonella enteritidis and other Salmonella spp. among Canadian registered commercial chicken broiler flocks. Epidemiology and Infection; 107:201-211.

Rafacz-Livingston, K. A., Parsons, C. M. and Jungk, R. A., 2005. The effect of various organic acids on phytate phosphorus utilization in chicks. Poultry Science, 84: 1356-1362.

Reddy M.R, Raju M.V.L.N., Chawak M.M and Rama Rao S.V., 1995. Importance of water in poultry health. Poultry Adviser; 28(6):31-37.

Reddy., 1995. considered *that the* number *of* microorganisms *in the* drinking water *of* birds should *be* 100 CFU/mL *for* total bacteria *and* 50 CFU/mL *for* coliforms. The mean levels of Escherichia coli in the water of a broiler farm that used.

Renwick A.S., Irwin R.J., Clarke R.C., Mcnab W.B and Poppe C., 1992. Epidemiological association between characteristics of registered broiler chicken flocks in Canada and the Salmonella culture status of floor litter and drinking water. Canadian Veterinary Journal; 33:449-458.

Salmond, C. V., R. G. Kroll, and I. R. Booth., 1984. The effect of food preservatives on pH homeostasis in *Escherichia coli*. J. Gen. Microbiol. 130:2845–2850.

Samelis, J., Ikeda, J., Sofos, J., 2003. Evaluation of the ph-dependent, stationary-phase acid tolerance in listeria monocytogenes and salmonella typhimurium dt104 induced by culturing in media with 1% glucose: A comparative study with Escherichia coli o157: H7. J. Appl. Microbiol., 95, 563–575. [CrossRef] [PubMed]

Sarnanta, M. A., 2008. Effect of dietary organic acidsupplementation on egg production, egg quality, and some blood serum parameters in laying hens. International Journal of Poultry Science, 7: 613-62 1.

Schwartz and Waggoner., 1984. considered that the number of microorganisms in the drinking water of birds should be 100 CFU/mL for total bacteria and 50 CFU/mL for coliforms. The mean levels.

Shahidi S., Maziar Y., and Delaram N.Z. 2014. Influence of dietary organic acids supplementation on reproductive performance of freshwater Angelfish (Pterophyllum scalare). Global Vet. 13:373–377. [Google Scholar]

Shamsuddoha, Mohammad Sohel and Mir Hossain. 2003. Problems and Prospects of Poultry Industry in Bangladesh: A Study on Some Selected Areas. The Chittagong University Journal of Business Administration, 19: 200.

Shen-HuiFang, Han-ChuiWang and Du-Bing Wang. 2005. Effect of citric acid on production performance of Three Yellow chicken. China-Poultry, 27: 34-37.

Skinner, J. T., Izat, A. L. and Waldroup, P. W. 1991. Fumaric acid enhances performance of broiler chickens. Poultry Science, 70: 1444-1447.

Thirumeignanam, D., Swain, R. K., Mohanty, S. P. and Pati, P. K. 2006. Effects of dietary supplementation of organic acids on performance of broiler chicken. Indian journal of animal nutrition, 23: 34-40.

Tripathi. S, 2017. Organic Acid is an organic compound with acidic properties associated with their Carboxyl group–COOH group. In General Organic acids are considered to be any carboxylic acid including fatty acid & amino acid. Organic Acids are weak Acid & do not disassociate

Vieira, S. L., Oyarzabal, 0. A., Freitas, D. M., Berres, J., Pena, J. E. M., Torres, C. A. and Coneglian, J. L. B. 2008. Performance of Broilers Fed Diets Supplemented with Sanguinarine-Like Alkaloids and Organic Acids. Journal of Applied Poultry Research, 17: 128-133.

Waldroup, A., Kaniawati, S. and Mauromoustakos, A., 1995. Performance characteristics and microbiological aspects of broiler fed diets supplemented with organic acids. Journal of Food Protection, 58: 482-489.

Waldroup, P. W., D. R. Landes, R. D. Kealy, D. E. Green, and E. C. Stephenson. 1967. Comparison on the growth stimulatory properties of Vigofac and streptomycin in broiler diets with and without fish meal. Poult. Sci. 46:974–976.

Waldroup, P. W., E. O. Oviedo-Rondon and C. A. Fritts. 2003. Comparison of Mos and antibiotic feeding programs in broiler diets containing copper sulfate. Int. J. Poult. Sci. 2:28-31.

Wang J.P, Yoo J.S, Lee J.H, Zhou T.X, Jang H.D, Kim H.J, and Kim I.H. 2009. Effects of phenyllactic acid on production performance, egg quality parameters, and blood characteristics in laying hens. J Appl Poultry Res. 18:203– 209. doi: 10.3382/japr.2008-00071[Crossref], [Web of Science ®], [Google Scholar]

Zealand., 2010. This review presents information on disease outbreaks in human populations linked to Salmonella spp. and Escherichia coli O157: H7 associated with the consumption of fresh produce. It focuses on the processes of bacterial internalization and survival in non-host.

Zhang Vintini, E., Alvarez, S., Medina, M. and Medini, M. 2005. Gut mucosa linmunistimulation by lactic acid bacteria. Journal of Biocell, 24: 223-232.

Zimmer M., Barnhart H., Idris U. and Lee M.D. 2003. Detection of Campylobacterjejuni strains in the waterlines of a commercial broiler and their relationship to the strains that colonized the chickens. Avian Diseases; 47(1):101-107.

Zimmermann, N.G., 1998. Relationship of drinking water quality and broiler performance on Delmarva. Proceedings of the Maryland nutrition conference for feed manufacturers. University of Maryland. pp. 66-76.

Zoetis., 2013. Providing quality water in adequate amounts is vital for poultry performance. Birds consume nearly twice as much water as they do feed. Anything that reduces their water intake will.

APPENDIX

APPENDIX I: Daily temperature (^{0}C) was recorded by clinical thermometer at 7 AM, 2 PM and 7 PM

SL NO	Date	7 AM	2 PM	7 PM
1	7-11-2017	22	26	23
2	8-11-2017	22	27	24
3	9-11-2017	21	26	23
4	10-11-2017	21	26	23
5	11-11-2017	21	26	24
6	12-11-2017	21	27	24
7	13-11-2017	22	28	24
8	14-11-2017	21	27	24
9	15-11-2017	19	25	23
10	16-11-2017	20	25	23
11	17-11-2017	21	25	24
12	18-11-2017	22	28	25
13	19-11-2017	21	27	24
14	20-11-2017	22	27	24
15	21-11-2017	19	26	22
16	22-11-2017	17	24	20
17	23-11-2017	16	23	19
18	24-11-2017	17	23	20
19	25-11-2017	17	23	21
20	26-11-2017	18	23	21
21	27-11-2017	17	23	20
22	28-11-2017	17	23	20
23	29-11-2017	17	24	21
24	30-11-2017	17	24	21
25	1-12-2017	17	23	20
26	2-12-2017	16	23	21
27	3-12-2017	17	24	21
28	4-12-2017	17	24	21
29	5-12-2017	17	24	21
30	6-12-2017	16	23	20
31	7-12-2017	16	23	20
32	8-12-2017	17	22	20
33	9-12-2017	18	24	21

34	10-12-2017	20	25	21
35	11-12-2017	20	25	22
36	12-12-2017	19	24	22
37	13-12-2017	18	24	20
38	14-12-2017	17	23	20
39	15-12-2017	16	23	20
40	16-12-2017	16	23	20
41	17-12-2017	16	23	20
42	18-12-2017	15	20	18
43	19-12-2017	15	20	18
44	20-12-2017	16	22	20
45	21-12-2017	16	22	19
46	22-12-2017	16	23	20
47	23-12-2017	17	23	20
48	24-12-2017	17	23	20
49	25-12-2017	17	23	19
50	26-12-2017	16	20	18
51	27-12-2017	16	20	18
52	28-12-2017	15	20	17
53	29-12-2017	15	20	18
54	30-12-2017	16	20	17
55	31-12-2017	15	20	17
56	01-01-2018	16	20	18

The mean values with different superscript (a to c) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean

NS=Non significant, * statistically significant ($P<0.05$).