EFFECTS OF PROBIOTICS AND METHANOIC ACID ON THE PERFORMANCE OF BROILER CHICKEN

A THESIS

BY

G-M-KABIRUL HOQUE

Registration No. 1705034

Semester: July-December, 2018

MASTER OF SCIENCE (M.S.)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

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Approved as to style and content by

....

Assistant Prof. Dr. Kamruzzaman **Supervisor**

Prof. Dr. Tahera Yeasmin **Co-supervisor**

....

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

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

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DECEMBER, 2018

Dedicated to
My
Beloved Parents

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The author

ABSTRACT

The experiment was conducted at Ekram Poltry farm, Domar, Nilphamari to determine the Effects of probiotics and methanoic acid on the performance of broiler. A total of 120 day-old broiler chicks (Cobb 500) fed diets T_0, T_1, T_2 and T_3 having three replications in each. The diet was then divided into 4 equal parts for treatment groups. Supplementation of control diet, supplementation of 0.75% probiotics in control diet, supplementation of 0.75% of methanoic acid in water supplementation, and o.5% probiotics in control diets combined with 0.5% of methanoic acid in water were made available for treatment $T_{0,}$ T_1, T_2 and T_3 respectively. The birds were reared in floor management system. Body weight gains, feed conversion ratio (FCR), mortality and meat yield traits were recorded. The present study revealed that there was no significant (P>0.05) variation of initial body weight (g/broiler) among the dietary groups and final body weight (g/broiler) and body weight gain. The highest body weight was found in T_1 (1732gm), followed by T_3 (1725gm), T_2 (1715.66gm), and T_0 (1695gm) respectively. The lowest FCR was in dietary group T_1 (1.55) and highest in dietary group T_0 (1.62) at 28th day (4 weeks) of age and the intermediates are in T_2 (1.58) and T_3 (1.57) respectively. It was found that there was no significant (P<0.05) difference among the dietary groups in case of carcass weight (gm), live weight (gm), thigh weight (gm), breast weight (gm) and there was almost similar (P>0.05) among the dietary groups for heart weight (gm), gizzard weight(gm), liver weight(gm) and heart weight (gm). Carcass weight in T_1 (1313gm) and live weight T_1 (1763gm) were non significantly (P<0.05) different compared to control T_0 (1117gm) and T_0 (1620gm) respectively. No mortality was found among the dietary groups during experimental period.

Keyword: Probiotics and Methanoic acid, Broiler performance

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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
GIT	: Gastro Intestinal Tract
FCR	: Feed Conversion Ratio

CHAPTER-I
INTRODUCTION

Broiler production is one of the most important and promising sector in poultry industry in terms of advantage of quick return that plays a vital role in the economic growth of Bangladesh. It has been proven that the genetic potentiality of the fast growing commercial broilers is achieved in the shortest possible time by the application of modern nutri-biotechnology. However, the optimum growths of broilers are seriously hampered by the invasion of pathogenic microorganisms. In order to cope with the challanges of growth-inhibiting microorganisms, some antibiotics like bacitracin, virginiamycin, flavomycin, avilamycin, tiamulin, colistin sulphate, oxytetracycline, aureomycin, chlortetracycline, neomycin sulphate, erythromycin and enrofloxacin have been used for several decades in broiler feed at a sub-therapeutic level. Antibiotics are double edge weapon. Antibiotics that are used as AGP in broiler feed have been shown to increase meat yield and improve feed efficiency with substantial reduction in pathogenic bacteria in the host gut (Gaskins *et al.,* 2002). They are also widely used in veterinary field for reducing the incidence of diseases. However, indiscriminate use of antibiotics in broiler production leads to the development of antibiotic resistant pathogenic bacteria, thereby causing resistance to medicines, persistence of infections and treatment failure. Several studies provided evidence that inappropriate and excessive use of antibiotics has led to the accumulation of their residues in edible broiler carcass which poses a major threat and potential risk to public health (Donoghue, 2003; El-Kahky and Allam, 2005; Nisha, 2008; Shareef *et al.,* 2009; Jallailudeen *et al.,* 2015). In Bangladesh, a recent study has shown that high levels of residues of major antibiotics like tetracycline, ciprofloxacin, enrofloxacin and amoxicillin were found mostly in liver, kidney, thigh meat and breast meat of broilers (Sattar *et al.,* 2014). The European Union has reported that about 25,000 patients died each year from infections caused by drug resistant bacteria, which is equivalent to €1.5 billion of medical healthcare costs (Ziggers, 2011). For this reason, most of the poultry meat consumer groups are avoiding meat from birds fed on diets containing antibiotics. In consequence, the European Union has banned the use of antibiotics in animal production since 2006 and other developed countries have limited the antibiotic use in poultry production. However, the ban of AGP demands the search for more suitable and safer alternatives to antibiotics that would promote growth, feed utilization and gut health without having any residual effect on poultry products.

Recently, many feed additives referred to as natural growth promoters or non-antibiotic growth promoters have been evaluated which include probiotics, prebiotics, synbiotics, acidifiers, phytobiotics, etc. (Ricke, 2003; Alavi *et al.*, 2012).

Feed additive antibiotics have been used as growth promoters for more then 50 years in the feed industry all over the world. The mode of action of antibiotics is the suppressing of the detrimental effect of pathogenic bacteria in the gut. Since the proposed total ban on sub-therapeutic feed antibiotics, products such as prebiotics, organic acids and probiotics are receiving considerable attention in animal nutrition because of their non-residual and non-resistant properties (Mellor, 2000; Gill, 2001; Hertrampf, 2001; Kocher, 2005; Plail, 2006). The beneficial effects on protein and energy digestibility and also on immune stimulation of these additives have been demonstrated in detail in previous studies. Probiotics (Vanbella *et al.*, 1990; Jin *et al.*, 1998) and prebiotics (Shane, 2001; Ferket, 2004) act as growth promoters feed savers, nutritional bio-regulators, immune stimulators and help in improving performance and health.

Feed organic acids suppress the growth of certain species of bacteria, particularly acid-intolerant species such as E. coli, Salmonella spp. and Campylobacter ssp. (Ricke, 2003; Dibner, 2004; Luckstadt, 2005). Their principal role is to lower and stabilize the pH in the stomach and intestines so that the gut environment is too acidic for normal bacterial growth. Additionally, they improve protein digestion in young animals by stimulating pancreatic enzyme secretion. Thus, dietary organic acids suppress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that the digestive enzymes function at maximal capacity (Broek, 2000; Mellor, 2000; Dibner & Winter, 2002; Ricke, 2003; Best, 2004; Dibner, 2004).

Prebiotics are defined as non-digestive feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the digestive tract. The prebiotic, mannan oligosaccharide (MOS), is a carbohydrate, derived from yeast cell walls, and can block pathogenic bacterial proliferation and stimulate the non-specific immune system; thus tending to improve the health and growth performance of birds (Hertampf, 2001; Iji *et al.*, 2001; Shane, 2001; Ferket, 2004; Kocher, 2005). Probiotics are pure cultures of one or more live microorganisms given orally. They proliferate in the gastrointestinal tract (GI) of the host and ensure that the bird maintains a beneficial microbial population in the GI tract by limiting the damage caused by pathogenic bacteria, reinforcing intestinal mucosal integrity and creating a positive balance of digestive microflora. Improved epithelial cell integrity, increased immune response, well balanced gut microflora, better utilisation and digestion of diet are also additive beneficial effects of dietary probiotics (Vanbella *et al*., 1990; Jin *et al*., 2000; Wenk, 2000; Panda *et al*., 2001; Linge, 2005).

Antibiotics have been used to protect the animal health and to improve growth for many years. Aim of using antibiotics in feed sector was to get advantage from feeding and increasing protection against some diseases, toxins, and making better the absorption of nutrients in intestines. Confidence has diminished about antibiotics used for improving performance and reducing stress factors because of the risk development of bioresistance against bacteria in human. Consumers are being sensible about this matter. New regulation therefore taking into account public health restricts to use of antibiotics as a feed and foodstuff supplement Turkey, especially after EU decisions has restricted the use of antibiotics in feed-foodstuff supplement though regulation from the Ministry Agricultural since 30 sept. 1999. (Nir and Senkoylu 2000, Sayg1c1 and Gunal 2004). Currently, probiotics and the organic acids seem the most interesting alternative supplement in regard of minimizing economic loses.

Objective of the study

- To determine the effects of Probiotics and Methanoic acid on the performance of broiler.
- To observe the carcass traits by supplementation of Probiotics and Methanic acid.

CHAPTER-II

REVIEW OF LITERATURE

Review of related literature is necessity in the sense that it provides scope for reviewing the stock of knowledge and information relevant to the proposed research. Despite the fact that a few numbers of works have been done in Bangladesh related to this research, there are some published reports and related activities. However, the limited numbers of works so far published are mentioned here along with other related works. A short description on the available literature relevant to the present investigation has been presented below:

Youcef Mehdi *et al.* 2018. Antibiotics are used to fight bacterial infections. However, a selective pressure gave rise to bacteria resistant to antibiotics. This leaves scientists worried about the danger to human and animal health. Some strategies can be borrowed to reduce the use of antibiotics in chicken farms. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytoncides, nanoparticles and essential oils.

Kumarasamy Deepa1 *et al.* 2018. In modern commercial broiler chicken production, the birds are inevitably exposed to various stress due to rapid growth, intensive poultry rearing, high stock density resulting in diminishing immune competence, gut health etc. This paves way to greater susceptibility of the birds to illness, infection and mortality. To overcome these losses, mostly antibiotics are being incorporated in feed. These antibiotics have possible lead to the emergence and dissemination of multiple antibiotic resistant pathogens and reduction in response to human and animal infections. The ban of antibiotic growth promoters in many countries necessitates to find an alternative to suppress microbial load particularly the gut. Probiotics, prebiotics or organic acids have being included to replace antibiotics. Of which, prebiotics are costlier affecting economics in poultry production, while probiotics have different degrees of survivability in feed and in the gut environment. Organic acids could be the possible choice as alternative to antibiotics. In poultry production, organic acids have not gained as much attention as in swine production. Generally, short chain fatty acids (formic acid, acetic acid, butyric acid) are preferred acidifiers, among which, butyric acid (BA) is considered as the prime enterocyte energy source, necessary for development of Gut Associated Lymphoid Tissue (GALT) and has the highest bactericidal efficacy against the acid-intolerant species such as Escherichia coli and Salmonella sp. with selective stimulation of beneficial gut bacteria.

Saleem *et al.* 2016. This study was conducted to investigate the comparative efficacy of three different concentrations of acetic acid on broiler chickens experimentally challenged with Salmonella enterica serovar Pullorum (S. Pullorum). A total of 360 birds were divided into five groups (A–E). Group A served as the unchallenged, untreated control. All the birds in groups B–E were challenged with 4 × 104 cfu/mL of S. Pullorum. Birds in groups C, D, and E were respectively treated with three different concentrations, 0.5%, 1%, and 1.5%, of acetic acid. Pathological examination revealed congested and hemorrhagic liver, hepatitis and necrotic areas in the liver, hydropericardium, focal necrosis in the spleen, epithelial desquamation of the intestinal mucosa, and congested lungs and hazy appearance of air sacs in birds challenged with S. Pullorum. Acetic acid supplementation (1%) helped to reduce the number and severity of these gross and histopathological changes. Counts of S. Pullorum in cecal digesta were significantly reduced with increasing concentrations of acetic acid ($P < 0.05$). However, acetic acid supplementation at a higher rate (1.5%) also showed adverse effects in terms of higher percentage of diarrhea and bad growth performance in birds challenged with S. Pullorum. Among the three different concentrations, 1% acetic acid supplementation showed partially protective effects by showing better growth performance, lower feed conversion ratios ($P < 0.05$), and lower rates of gross and histopathological changes.

Chen G. Olnood *et al.* 2016. A total of 294 one-day-old Cobb broiler chickens were used to investigate the effects of four Lactobacillus strains on gut microbial profile and production performance. The six dietary treatments, each with 7 replicates were: 1) basal diet (negative control), 2) one of four strains of Lactobacillus (tentatively identified as Lactobacillus johnsonii, Lactobacillus crispatus, Lactobacillus salivarius and an unidentified Lactobacillus sp.) and 3) basal diet with added zinc-bacitracin (ZnB, 50 mg/kg). Results showed that the addition of probiotic Lactobacillus spp. to the feed did not significantly improve weight gain, feed intake and feed conversion rate (FCR) of broiler chickens raised in cages during the 6-week experimental period, but tended to increase the number of total anaerobic bacteria in the ileum and caeca, and the number of lactic acid bacteria and lactobacilli in the caeca; and to significantly increase the small intestinal weight (jejunum and ileum). Furthermore, all 4 probiotics tended to reduce the number of Enterobacteria in the ileum, compared with the control treatments. The probiotics did not affect the pH and the concentrations of short chain fatty acids (SCFA) and lactic acid in both the ileum and caeca.

Abdullah-Al-Masud *et al.* 2016. The effect of feeding probiotic (Bio-Top; Bacillus subtilis and Bacillus licheniformis), acidifier (Sal-Stop), antibiotic growth promoter (AGP) or probiotic plus acidifier was investigated in commercial broiler. A total of four hundred Cobb 500 day-old straight run chicks were randomly distributed to 5 different dietary groups having 4 replications each. The number of birds in each replication was 20. The five dietary groups were as control (basal diet; BD), BD containing AGP at a level of 20g/100kg, BD containing probiotic at a level of 200g/100kg, BD containing acidifier at a level of 200g/100kg; and BD containing an equal amount of probiotic plus acidifier (200g/100kg). Broilers that received either probiotic, acidifier or a mixture of probiotic and acidifier (1:1) exhibited higher body weight gain, lower feed conversion ratio (FCR) and higher costeffectiveness compared with the broilers fed on control diet ($P<0.05$). However, feeding of diet containing both probiotic and acidifier resulted in the highest growth rate and net profit in all dietary regimens. Broilers fed on probiotic and acidifier in a mixture had FCR similar to other treatment groups. This study indicated that the diet containing probiotic-acidifier mixture seems to be more cost-effective in promoting growth performance of broilers, as an alternative to the AGP, as compared to the use of probiotic or acidifier alone in the diet.

Naela M. Ragaa *et al*. 2016. An experiment was conducted to evaluate the effects of thyme, formic acid (FA) and thyme plus formic acid in broiler ration on performance, carcass traits, blood biochemical parameters, intestinal microbial load, and histological picture of intestine as well as immunity parameters of broilers. A number of 480-day old broiler chicks were divided into 4 groups with three replicates of 40 chicks each. Experimental groups included T_1, control group with no thyme or formic acid supplementation, T_2 group which was fed on basal diet supplemented with thyme (1g/kg diet), T_3 group received the basal diet supplemented with formic acid (5g/kg) and T_4 group was fed on basal diet supplemented with thyme (1g/kg) plus formic acid (5g /kg). The results showed that the use of thyme or formic acid or formic plus thyme had significant effects on growth performance and carcass traits of broilers ($P<0.05$). The highest % of breast and thigh was observed in group T_4, while an improvement in villus height was observed in all supplemented groups compared to control group but the highest was observed in T_4. It is concluded that using of thyme or formic acid in broiler feeds have significant effects on performance and immunity parameters.

Agboola AF *et al.* 2015. The effects of organic acid, probiotic and a combination of the two on performance and gut morphology in broiler chickens were investigated. Two hundred and forty one-day-old Arbor Acre broiler chicks were randomly assigned to five dietary treatments with six replicates, each with eight birds. The treatments were basal diet (negative control, NC), basal diet + antibiotic (positive control (PC)), NC + 0.4% organic acid (OA), NC + 0.3% probiotic (PB) and NC + 0.4% OA and 0.3% PB. Reduced body weight gain (BWG) was recorded for birds on the NC diet at the starter phase and over the total period. The addition of OA significantly increased BWG compared to values obtained in birds on the NC and other diets. Diet had no effect on BWG at the grower phase or on feed intake, dry matter intake and feed conversion ratio in any growth phase. Gain: feed ratio was lowered in the NC diet, but improved significantly by OA and PB in the starter phase. Organic acid supplementation reduced the weight of the bursa of Fabricius. The weight of pancreas, height of villi and crypt depth were reduced in birds on the NC diet compared with those on OA, PB and a combination of these. Diet had no effects on the weights of the lungs, heart, spleen, kidneys, liver, villus width, villus height crypt depth ratio, lactic acid bacteria, coliform bacteria and total bacterial count in any gut section. In conclusion, supplementation of broiler diets with OA and PB could improve their growth and gut morphology better than antibiotics would, with a greater positive effect in the starter phase.

Jong Woong Kim *et al.* 2015. The objective was to summarize and describe the possible mode of action of dietary organic acids and their effects on growth performance of broiler chickens. Previous experiments have suggested that dietary organic acids decrease pH in diets and subsequently reduce pH in the proximal and distal intestine, increase nutrient utilization, and inhibit pathogenic bacterial growth in the gastrointestinal tract (GIT). The degree of pH reduction is usually greater in the upper part of the GIT (crop, proventriculus, and gizzard) than in the lower part of the GIT (duodenum, jejunum, ileum, and cecum). Bactericidal effects of dietary organic acids have been observed for pathogenic bacteria and even for beneficial bacteria to some extent. However, few significant results regarding bacterial modulation in the GIT have been reported. Dietary organic acids can improve dry matter and protein utilization in some experiments, but the extent of improvement in nutrient utilization is smaller than has been anticipated. Growth performance is likely improved, but results have been inconsistent due to variations in sources and inclusion levels of dietary organic acids. Differences in other dietary components and experimental environments among previous experiments likely contribute to the variable results. This review suggests that the effects of dietary organic acids on broiler chickens are not fully understood. Further experiments are required to reliably demonstrate the mode of action of dietary organic acids and their growth-promoting effects on broiler chickens.

Franciszek Brzoska *et al.* 2013. An experiment with 608 broiler chickens was conducted to investigate the effect of dietary acidifier level on body weight, feed consumption and conversion, mortality, dressing percentage, postmortem carcass traits, tissue composition of breast and leg muscles, and plasma chemical parameters. Feeding the acidifier to chickens at 3, 6 and 9 g/kg of the diet reduced the pH of starter and grower diets from 6.90 to 5.89, and from 6.28 to 5.73, respectively. Compared to the control group, dietary acidification significantly increased body weight of chickens by 6.2, 8.2 and 8.2% at 21 days of age, and by 2.7, 3.6 and 3.7% at 42 days of age, respectively ($P<0.01$). Mortality decreased from 2.58% in the control group to 0.00–0.59% in the experimental groups ($P<0.01$). Acidification of the diets increased EEI-index from 327 (control group) to 348 points in the experimental group supplemented with 9% (9 g/kg) acidifier, but had no significant effect on feed consumption and feed conversion ratio among treatments. The relative weight of breast and leg muscles, gizzard, liver and carcass depot fat was not affected by dietary treatments. Breast muscles represented 27.7% (control group) and 27.9% (experimental groups) of the carcass weight. Leg muscles made up 21.5% and 20.7% of the carcass weight, respectively. There were no significant differences in chemical composition of breast and leg muscles, including dry matter, protein and fat content. No significant differences between the control and experimental chickens were noted for determined blood plasma constituents, glucose, total protein, triglycerides, total cholesterol and high density lipoprotein. The results suggested that organic acid acidifier used in this experiment at the rates of 3 to 9 g/kg diet has a growth enhancing and mortality reducing effect in broiler chickens, with no significant influence on carcass yield, proportion of individual carcass parts and blood plasma constituents. It seems that the amount of 6 g of the applied acidifier per kilogram of feed may be recommended as the optimum dietary level if protein in the diet does not exceed 200–230 g crude protein per kilogram of diet.

Behrouz Rezanezhad Dizaji1 *et al.* 2012. This experimental trial was conducted to investigate the effects of dietary supplementations of prebiotic, probiotic, synbiotic and acidifier on broiler performance and organ's weights of broiler chickens. One hundred and sixty 1-dold Ross 308 broiler chickens were randomly assigned to one of five dietary treatments for six week. The dietary treatments were 1- Control, 2- Basal diets supplemented with prebiotic (1kg of ActiveMOS/ton) 3- Basal diets supplemented with probiotic (150/100/50gr of Protexin/ton of the starter, grower and final diets respectively) 4- Basal diets supplemented with synbiotic (1kg of Amax4x/ton) 5- Basal diets supplemented with acidifier (2 liter Globacid/ton). The highest body weight observed in synbiotic group, which was significantly (P0.05) but higher than control group (P0.05). Daily weight gain was significantly (P0.05) difference between experimental groups. Feed conversion ratio decreased significantly (P0.05) differences in feed conversion ratio of broiler chickens in prebiotic and probiotic groups compared with control group. The weight of proventriculus, Gizzard, liver, and Bursa did not differ ($P>0.05$) between groups. Additionally, the weight of Spleen increased significantly ($P<0.05$) in probiotic group compared with control group.

K. Gharib Naseri *et al.* 2012. Campylobacter is known to be one of the most common causes of human intestinal disorders. Since poultry are known to be the main reservoirs for this pathogen, decreasing this bacterium in intestinal tract could be beneficial in reducing contamination of poultry products. The effects of probiotic (PrimaLac®), medicinal plant (Sangrovit®) and organic acid (Selko-pH®) as broiler feed additives on cecal colonization, and fecal excretion of broilers were studied. Other parameters such as performance, immune response and intestinal morphology were also determined. A total of 300 broiler chicks (Cobb 500) were divided into 5 groups. Groups consisted of unsupplemented feed (negative and positive controls), probiotic, medicinal plant and drinking water containing organic acid mixture. Except for the negative control group, all chickens were orally challenged with (109 cfu mL-1) Campylobacter jejuni at day 21. Cecal and fecal samples were collected for Campylobacter count. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were determined weekly and cumulatively. BW and FI in the probiotic treated group were higher (P< 0.05) than the positive control group. On day 49 all supplemented treatments showed a reduction of Campylobacter colonization in cecal contents (P< 0.05). Fecal samples showed reductions (P< 0.05) on day 35 and 42. Villi height of duodenum and jejunum in the probiotic and medicinal plant treated groups were improved (P< 0.05). Immune response was significantly higher in these two groups (P< 0.05). These effects could be due to the antibacterial effects of the used feed supplements. Our results indicate that these feed additives could be potential treatments for reducing Campylobacter in the intestine of broilers. Probiotic and medicinal plant improve growth performance of these birds.

Paula Fajardo *et al.* 2012. The aim of this study was to evaluate the potential of two probiotic preparations, containing live lactic acid bacteria (Lactococcus lactis CECT 539 and Lactobacillus casei CECT 4043) and their products of fermentation (organic acids and bacteriocins), as a replacement for antibiotics in stimulating health and growth of broiler chickens. The effects of the supplementation of both preparations (with proven probiotic effect in weaned piglets) and an antibiotic (avilamycin) on body weight gain (BWG), feed intake (FI), feed consumption efficiency (FCE), relative intestinal weight, and intestinal microbiota counts were studied in 1-day posthatch chickens. The experiments were conducted with medium-growth Sasso X44 chickens housed in cages and with nutritional stressed Ross 308 broiler distributed in pens. Consumption of the different diets did not affect significantly the final coliform counts in Sasso X44 chickens. However, counts of lactic acid bacteria and mesophilic microorganisms were higher in the animals receiving the two probiotic preparations ($P < 0.05$). In the second experiment, although no differences in BWG were observed between treatments, Ross 308 broilers receiving the probiotic Lactobacillus preparation exhibited the lowest FCE values and were considered the most efficient at converting feed into live weight.

Martin Kral *et al.* 2011. Probiotics and organic acids are widely accepted as an alternative to in-feed antibiotics in poultry production. We carried the experiment with broiler chickens. In experiment we research effect of probiotic and acetic acids on the performance of broiler chickens. A total number of 200 one day old broiler chickens were distributed to two dietary groups. Broiler chickens in control group were fed with standard feed mixture and experimental group 1% vinegar contained 5% acetic acid used in drinking water and probiotics mixed with feed mixture. Body weight, FCR and GIT pH were recorded. The performance showed no statistically significant increase in body weight (P>0.05) in the weeks 1, 2, 3 and 4 of age. The body weight of broiler chickens was significant increase (P>0.05) in weeks 5, and 6 of age. In different segments of the GIT was not statistically significant (P>0.05) difference of pH between the control and experimental groups.

Sheikh Adil *et al.* 2010. The aim of the study was to determine the effect of dietary supplementation of organic acids on the performance, intestinal histomorphology, and blood biochemistry of broiler chicken. The birds in the control (T1) group were fed the basal diet whereas in other treatment groups basal diet was supplemented with 2% butyric acid (T2), 3% butyric acid (T4), 2% fumaric acid (T4), 3% fumaric acid (T5), 2% lactic acid (T6), and 3% lactic acid (T7). Broiler chicken fed diets supplemented with organic acids had significantly (*P* <. 0 5) improved body weight gains and feed conversion ratio. No effect (*P* <. 0 5) on cumulative feed consumption was observed. The addition of organic increased villus height in the small intestines but the differences were not significant (*P* <. 0 5) in case of the ileum. Serum calcium and phosphorus concentrations were increased (*P* <. 0 5) but no effect (*P* <. 0 5) on the concentration of serum glucose and cholesterol, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetate transaminase (SGOT) was observed. The results indicated that the organic acid supplementation, irrespective of type and level of acid used, had a beneficial effect on the performance of broiler chicken.

Hassan *et al.* 2010. A grower broiler experiment (from 14 to 35 days of age) was conducted to study the effect of using two commercial mixtures of organic acids (Galliacid and Biacid) to substitute antibiotic growth promoter (Eneramycin) on performance, carcass characteristics and intestinal microflora. 400 (Ross 308) broiler chicks were used. A basal corn-soybean meal diet were formulated and served as a control treatment. The control diet was supplemented with either 0.06% Galliacid, 0.1% Biacid or 0.02% Eneramycin. Birds fed the Galliacid-supplemented diet had 16% (p<0.001) more gain than the control, while those fed the Biacid- or Enramycinsupplemented diets recorded 3 and 5.5% more gain, respectively. Organic acids mixtures and Enramycin supplementation significantly (p<0.001) improved feed conversion ratio. These results indicated that birds fed either organic acid mixtures or Enramycinsupplemented diets utilized feed more efficiently than those fed the control diet. Galliacid significantly ($p<0.01$) increased dressing percentage and bursa weight (% body weight). No significant differences were detected on liver, spleen and thymus (% body weight) among treatments. Galliacid or Biacid significantly ($p<0.001$) decreased intestinal *Escherichia coli* and *Salmonella* compared to the control and Enramycin-supplemented diets. Dietary Enramycin significantly ($p<0.001$) decreased *Escherichia coli,* but had no effect on *Salmonella* counts. In conclusion, organic acid mixtures are more efficient than antibiotic growth promoter (Enramycin) in improving broiler performance and decreasing intestinal *Escherichia coli* and *Salmonella* spp., and could be successfully used to substitute antibiotic growth promoters in broiler diets. However, not all of the organic acid mixtures gave the same effect either on performance or intestinal bacterial counts.

Bozkurt *et al.* 2009. A study was conducted to investigate the effect of dietary supplementation of an organic acid, a probiotic or a prebiotic alone or the prebiotic combined with the organic acid or the probiotic on the performance and slaughter characteristics of broiler chickens fed a maize-soya based diet. The six dietary treatments were: a basal diet (negative control) and diets containing 0.5 g mannan oligosaccharide/kg (prebiotic), or 1.0 g formic acid/kg (organic acid), or a probiotic at 0.5 g/kg, or 0.5 g prebiotic/kg + 1.0 g organic acid/kg, or 0.5 g prebiotic/kg + 0.5 g probiotic/kg feed. Each treatment consisted of eight pens with 50 birds per pen (25 male + 25 female). All dietary supplements, alone and in combination improved live weight significantly at both 21 and 42 days of age compared with the control. However, combinations of the prebiotic with either the organic acid or the probiotic had no additive benefit at 21 and 42 days of age in comparison with the prebiotic alone. The feed intake of the birds was significantly increased with prebiotic supplementation at day 21, but not at day 42. Organic acid significantly improved feed conversion ratio at day 21. The combination of prebiotic and probiotic significantly improved the feed conversion ratio at both 21 and 42 days in comparison with the control. At days 21 and 42 bird mortality was significantly higher in the treatments containing organic acid and organic acid with the prebiotic. In the female birds no slaughter traits were affected by dietary treatments. However, liver weight as a percentage of live weight in the male birds was significantly lowered with prebiotic and probiotic supplementation. Prebiotic supplementation with organic acid resulted in a significantly lower weight of the small intestines compared with the control. In general, the different feed additive regimens that include the prebiotic, probiotic, organic acid, prebiotic with organic acid and prebiotic with probiotic improved the growth rate of the birds significantly compared to the control treatment. The significant improvement in feed conversion ratio when the prebiotic and probiotic were supplemented together suggests a synergism between them.

Irshad Ahmad 2006. A probiotic is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance. It has been used as a substitute of antibiotics that is being used in considerable amounts as growth promoters in broilers production and is, associated with incalculable risks for human health resulting from the use of particular feed additives. This article reviews the scientific data showing that probiotics may positively affect various physiologic functions in ways that will permit them now or in the future to be classified as functional foods for which health claims (of enhanced production or reduction in disease risk) will be authorized. The article has been prepared under various subheadings including introduction into probiotics, mode of action including immune enhancement, growth stimulation, feed conversion ratio, competition for adhesion receptors, digestion and absorption and health management of diseased animals. The authors own results have been reviewed including: i) poultry growth is promoted with the increasing doses of probiotics up to a certain limit. The growth pattern increased relative to the control, up to 1.0 gram per 10 kg feed but beyond that the pattern was reversed; ii) no difference could be detected in feed conversion ratio of broilers as compared to control; iii) crypt cells proliferation of small intestine increased with the use of probiotics as compared to control. Present/future aspect of probiotics, is the last component of the article including; discovery of more probiotic organisms through genetic engineering.

Celik1 *et al.* 2007. This experiment was conducted to study the effects of probiotic (P) and organic acid (OA) on performance and some organs in broiler chicks. Seventy two 1-d-old (male and females) broiler chicks (Ross PM) consisted of 4 groups (Control, 02 % Probiotic (Saccharomyces cerevisiae), 04 % OA and 02 % P + 04 % OA, each of which had 25 chicks. The analysis of the data indicated that no significant effect between the groups was found in body weight, feed intake, hot carcass, gizzard, liver and large intestine weight, but a significant difference in small intestine weight was observed

(P<0.001). According to the results of this study, it was found that the provision of mentioned probiotic or organic acids to the diet of broiler throughout 42 days had no effect on performance.

Gunal *et al.* 2006. Effects of an antibiotic growth promoter (flavomycin), a probiotic mixture (protexin) or a mixture of organic acids including plant extract and mineral salts (genex) on performance, intestinal microbial flora and tissue morphology have been examined in 160 day-old Ross 308 broiler chicks. Commercial corn-soybean- based broiler starter and grower diets were formulated as basal diets for control treatment Basal diets were supplemented with a probiotic (0.1% protexin), an antibiotic growth promoter (0.1% flavomycin), an organic acids mixture (0.2% geriex) or a combinaiion of a probiotic with an organic acids mixture (0.1% protexin+0.2% genex). In total, five dietary treatments were employed in the trial. Live weight gain, feed intake, feed conversion ratio and mortality were not affected by dietary treatments throughout the experiment. However, relative weight of the small intestine of antibiotic treatment had significantly less than that of the basal diet. Intestinal microbial flora and tissue were determined at 2lt and 42' days. In both periods, antibiotic or organic acids mixture treatments significantly decreased total bacteria counts. In addition to that all treatments significantly decreased gram negative bacteria counts compared to the basal diet. Probiotic treatment significantly increased ileum and jejunum villus height, whereas antibiotic treatment significantly decreased muscularis thickness compared to the basal diet.

Alcicek *et al.* 2004. The aim of the present study was to investigate the effect of dietary supplementation with an essential oil mixture, a commercially available organic acid and a probiotic on growth performance and carcass yield of broilers. One thousand two hundred and fifty sexed one day-old broiler chicks were randomly divided into five treatment groups of 250 birds each (negative control, organic acid, probiotic and essential oil mixture (EOM) at two levels). Each treatment group was further sub-divided into five replicates of 50 birds (25 male and 25 female) per replicate. The oil in the EOM was extracted from different herbs growing in Turkey. An organic acid at 2.5 g/kg diet, a probiotic at 1 g/kg diet and the EOM at 36 mg and 48 mg/kg diet were added to the basal diet of the birds. There were significant effects of dietary treatments on body weight gain, feed intake, carcass yield and intestinal weight of the broiler at 42 days of age. At day 42, birds fed the diet containing 36 mg EOM/kg showed the highest body weight gain. This was followed by chicks on the diet containing 48 mg EOM/kg, the probiotic, the organic acid and the negative control, in descending order. The addition of the essential oil mixture to the diet improved the feed conversion ratio significantly as compared to the negative control and the organic acid treatment. The feed intakes at days 21 and 42 were significantly different between the treatments. The addition of 48 mg EOM/kg increased carcass yield significantly above the other treatments, while the addition of EOM and the organic acid reduced the intestinal weight significantly. It was concluded that the supplementation of the herbal essential oil mixture to broiler diet had beneficial effects on body weight gain, feed conversion ratio and carcass yield

Muzaffer Denli1 *et al.* 2003. The specific aim of this study was to determine the effects of the supplementation of separate probiotic (protexin), including organic acid combination, plant extracts, mineral salts (genex) and antibiotic (flavomycin) to broiler diets on performance, abdominal fat weight, abdominal fat percentage, liver weight, intestinal weight, intestinal length, intestinal pH, carcass weight, carcass yield of broiler chicks. In this study. 84 one-day old male broiler chicks were used and divided equally into 6 groups. When the control group was fed a dietwithoutsupplemented diet probiotic(0.1% protexin), organic acid (0.2% genex), probiotic + organic acid (0.1% protexin + 0.2% genex), antibiotic (0.15% flavomycin) and antibiotic + organic acid (0.15% flavomycin +0.2% genex) were added to the diets of the experimental groups respectively. The experimental period was 42 days. The results obtained in the experiment showed that the group receiving 0.15% flavomycin + 0.2% genex supplemented in the basal diet was exhibited higher body weight gain, teed intake and carcass weight and better feed efficiency respectively than the control and other groups ($P<0.05$). However liver weight, intestinal pH, and abdominal fat weight were not affected significantly by probiotic, antibiotic and organic acid treatments ($P>0.05$).

CHAPTER-III
MATERIALS AND METHODS

3.1 Statement of the research work

The experiment was conducted at Ekram Poultry farm, Domar, Nilphamari to determine the Effects of probiotics and methanoic acid on the performance of broiler. (Cobb 500) during the period from 27 August to 23 September, 2018.

3.2 Experimental birds

A total of 120 day-old broiler chicks (Cobb 500) were purchased from Provita Hateries Limited, Domar, Nilphamari, Bangladesh.

3.3 Layout of the experiment

The day-old chicks were reared at brooder house to adjust with the environmental condition up to 7 days. After 7 days, chicks were randomly allocated in four dietary treatment groups having three (3) replications in each and 10 birds per replications. The layout of the experiment is shown in Table 1

Table: 1. Layout showing the distribution of experimental broilers

Dietary groups		Number of broilers in each replication			Total
		R_1	**R_2**	**R_3**	
Control (without probiotics or methanoic acid)	**T_0**	10	10	10	30
Control+0.75% probiotics	**T_1**	10	10	10	30
Control+0.75%methanoic acid	**T_2**	10	10	10	30
Control+0.5%methanoic acid+0.5%probiotics	**T_3**	10	10	10	30
Total No. of broilers		40	40	40	120

3.4 Collection of feed ingredients

Feed ingredients used in the experiment were purchased from a feed shop of Dinajpur town.

3.5 Preparation of the experimental diet

Ready feed was used for the experimental study. At first required amount of ready feed ingredients were weighed by digital weighing balance. The diet was then divided into 4 equal parts for treatment groups of diet, supplementation of 0.75% probiotics in control diet, supplementation of 0.75% of methanoic acid in water supplementation, and o.5% probiotics in control diets combined with 0.5% of methanoic acid in water were made available for treatment $T_{0,}$ T_1, T_2 and T_3 respectively. During the time of mixing cross mixing was applied. Mixing was done manually. The experimental period were divided into two phases (broiler-starter and broiler-grower). The broiler chicks were fed broiler starter for 0 and 14 days and broiler grower for 15 to 28 days of age.

3.6 Calculated composition of experimental diets.

Nutrients	**Amount(kg/100kg feed)**	
	Starter (1-14 days)	**Grower (15-28 days)**
Crude protein (%)	21.50	21
Crude fiber (%)	4.5	4.5
Crude fat (%)	4.5	5
Ash	6.0	6
Lysine (%)	1.27	1.18
Methionine (%)	0.50	0.52
Calcium (%)	1.05	1
Phosphorus(%)	0.50	0.5
Moisture (%)	11	11
Metabolizable Energy, ME (kCal/kg)	2950	3100

3.7 Management of the experimental birds

Similar care and management in all treatment groups throughout the experimental period was practiced. At the initiation of the experiment, chicks were individually weighed recorded as initial body weight. The following management practices were followed during the whole experimental period and these management practices were identical for all dietary groups.

3.8 Managemental practices

3.8.1 Housing and equipment

The experimental house contained 20 cages each had a floor space .of l20 cmx76cm among those 12 cages were considered for this trial. The cages were properly cleaned, washed and disinfected with bleaching powder. The experimental house was properly cleaned and washed by forced water using a hose-pipe. Then, the room was disinfected by bleaching powder solution. After 15 days, the room was disinfected with Virkon solution (50 ppm). At the same time, all feeders, plastic buckets, waterers and other necessary equipment's were also properly cleaned, washed and disinfected with bleaching powder solution, subsequently dried and left them empty for a week before the arrival of chicks.

3.8.2 Litter management

During the experiment period for the first 7 days litter was covered by clean newspaper and newspaper was removed when it becomes dirty. After that period the birds were reared on rice husk littered floor having a depth of 4 cm. Before use of litter calcium carbonate was spread on the floor. After first week, upper part of the litter with droppings were removed regularly and stirred three times a week up to the end of the experiment. The litter was disinfected with Virocid® solution in every other day. Litter materials, when found damp for any reason, were removed to prevent accumulation of ammonia and other harmful gases. At the end of each week, litter was stirred to break its compactness and maintain proper moisture. At the end of 2nd and 3rd weeks of age, dropping were cleaned from the surface of litter.

3.8.3 Brooding

Additional heat was provided to brood the chicks when it was necessary. Brooding temperature was kept at 34^{o} C in the first 1 week of age and decreased gradually until they were adjusted to normal environmental temperature of the house and final temperature was 28^{o} C at the end of experiment. Additional heat was provided by fitting 100-watt electric bulb at the center of the pen about 12 inches above the floor from the 7-day old. The height of the bulbs was increased by raising the bulb gradually as per need of temperature. Paper was used on two sides of the house and in ventilators to protect cold and stormy wind. These sheets were removed partly or completely particularly at the later stage of finishing period when room temperature was found favorable. Daily room temperature (^{o}C) was recorded every six hours with a thermometer.

Fig.1. Brooding of Young Chicks

3.8.4 Lighting

All birds were exposed to continuous lighting of 23 hours and one hour dark period per day throughout the experimental period. The dark period was practiced to make the broilers familiar with the possible darkness due to electricity failure. Supplementary light at night was provided by electric bulb by hanging at a height of 2.8 meters to provide necessary lighting.

3.8.5 Temperature

The house temperature was maintained at 34°C for the first week. In the course of the trial period the temperature was gradually reduced from 34°C to 32°C during first week, 32°C to 30°C in the second week, 32°C to 28°C in the third week and there after remain almost constant until the end of the trial.

3.8.6 Floor, feeder and water space

An area of 10 sq. feet was allotted for 10 birds in each pen; therefore floor space for each bird was 1 sq. feet. One round feeder and one round waterer were provided in each pen for 10 birds; required feeding and drinking space was providing according to the number of birds in each replication.

3.8.7 Feed and water management

At the first week Feeds were supplied to the chicks on clean newspapers at three hours interval for the first 3 days. Linear feeder and round plastic drinker were used during brooding period. After that linear feeder was replaced by round plastic feeder. Feed and fresh water were supplied to the treatment T_0. Feeds were supplied with probiotics thrice daily (once at morning, at noon and again at night) in treatment T_1 and T_3 and water was supplied with formic acid thrice daily (once at morning, at noon and again at night) in treatment T_2 and T_3. Feeders were cleaned at the end of each week and drinkers were washed daily.

Fig.2. Feeding of broiler

Fig.3. Watering of broiler

3.8.8 Sanitation

Adequate sanitary measures were taken during the experimental period. The entrance point and veranda were kept clean and solution of bleaching powder and potassium permanganate ($KMnO_4$) was kept in foot bath alternatively

3.8.9 Immunization

All birds were vaccinated against Baby chick Ranikhet Disease Infectious bronchitis at day one by the company. The birds were vaccinated against Ranikhet and Infectious Bursal (Gumboro) diseases by following schedule at the evening-

3.8.10 Applied vaccination program

Diseases	Day	Vaccine	Route	Time
Ranikhet	5	BCRDV	Eye	Evening
Gumboro	11	Gumborovac	drinking water	Evening
Gumboro	17	Gumborovac	drinking water	Evening
Ranikhet	22	RDV	drinking water	Evening

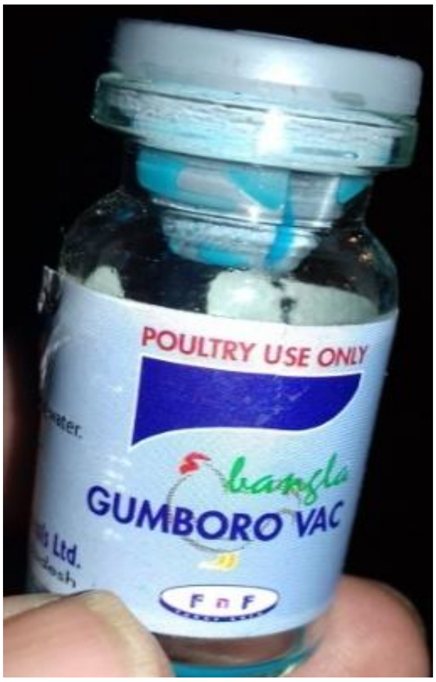

Figure: 4. Vaccination

3.8.11 Clinical observation

The birds were critically observed twice a day for clinical sign if any (slow movement, infrequent sitting, lack of appetite, significant changes of feathering, paralysis etc.) and for monitoring other activities.

3.8.12 Medication

Immediately after unloading from the chick boxes the chicks were given Glucose and Vitamin-C to prevent the stress occurring during transport. Water soluble vitamin and normal saline were also provided for the first 3 days of brooding. During the course of experimental period, electrolytes and vitamin-C were added with the drinking water to combat stress due to high environmental temperature (33 to 35^{o}C).

3.9 Data collection and record keeping

The following records were kept during 28 days of rearing period:

i. Live weight.
ii. Feed consumption.
iii. Feed conversion ratio.
iv. Mortality
v. Temperature: Five times daily during the experimental period.
vi. Dressing yield: At the end of the experiment one broiler was slaughtered from each replication to estimate dressing yield.

3.9. 1 Live weight gain

Birds were weighed in a group at the beginning of the trial and then every week at the age of 7, 14, 21, 28 days. The weighing was done using pan balance.

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

$$\text{Body weight gain} = \text{Final weight} - \text{Initial weight.}$$

3.9.2 Feed intake

Feed offered daily and refusal at the end of each week was recorded. Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

$$\text{Feed Intake (g/bird)} = \frac{\text{Feed intake per replication}}{\text{No.of birds per replication}}$$

3.9.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

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

3.9.4 Temperature and relative humidity

During the experimental period the temperature and the relative humidity (RH) of the experimental house and pens at chick level were recorded two times a day (8AM and at 5 PM) with the help of an automatic thermo hygrometer.

3.9.5 Mortality

Mortality was recorded daily treatment wise when occurred

3.9.6 Processing of broilers

After termination of the experiment, one bird weighing average of pen weight from each replication was selected randomly. Feed was withdrawn from the pens 24 hours prior to slaughter but water was available to facilitate proper bleeding. Birds were slaughtered according to halal method. Following slaughter, broilers were allowed to bleed for about 2 minutes. Then the birds were scalded in hot water (55-65^{o} C) for about 120 seconds in order to loosen the feather of the carcasses and weighed again. Breast meat, thigh meat, drumstic meat were separated from the carcass. Finally, processing was performed by removing head, shank, viscera, oil gland, kidney and giblets. As soon as these were removed the gall bladder was removed from the liver and pericardial sac and arteries were cut from the heart. Cutting it loose in front of the proventriculus and then cutting with both incoming and outgoing tracts removed the gizzard. Then, it was split open with knife, emptied and washed and the lining removed by hand.

3.9.7 Dressing yield

Dressing yield is based on the relationship between the dressed carcass weight and live bird weight after things like the skin and internal organs have been removed. Dressing yield can be calculated by taking weight of the carcass divided by weight of live bird.

$$\text{Dressing yield} = \frac{\text{Weight of the carcass}}{\text{Weight of live bird}} \times 100$$

Fig.5.Carcass & different parts of broiler

3.10 Economy of broiler production

The cost of broiler production for each treatment group was calculated based on the market price of feed ingredients, cost of chicks, citric acid and acetic acid and management cost (labor, medicine, electricity and litter depreciation) to produce per kg of live broiler at the time of trial. The income from per kg of live broiler in different treatment groups was calculated by the selling price of per kg live broiler.

3.11 Statistical analysis

Data on different variables were subjected to analysis of variance (ANOVA) in a Complete Randomized Design (CRD). The significant differences between the treatment means were calculated from analysis of variance (ANOVA) table. All analyses were performed by using “IBM SPSS statistics 22” Program.

CHAPTER-IV

RESULTS AND DISCUSSION

4.1 Performance of broiler of experimental birds

This experiment was conducted to study on feed consumption, feed conversion ratio, live weight gain and mortality of birds and carcass traits were used as criteria of response of broiler to different dietary levels of citric acid, acetic acid and their combination are presented in different tables and discussed under the following subheadings.

4.2 Body weight

The effect of probiotics and methanoic acid on highest body weight gain is shown in table 4. The present study revealed that there was no significant ($P>0.05$) variation of initial body weight (g/broiler) among the dietary groups but final body weight (g/broiler) and body weight gain were significantly ($P<0.05$) different among the dietary groups. The initial body weight (g/broiler) in T_0, T_1, T_2 and T_3 group was (38.00±0.03), (39.07±0.04), (41.00±0.09), (37.00±0.05). At 7 days of age, the body weight was almost similar in different dietary groups. Significant different($p<0.05$) were found at 14 days, 21 days and 28 days of age on body weight gain. The highest body weight was found in T_1 (1732gm), followed by T_3 (1725gm), T_2 (1715.66gm), and T_0 (1695gm) respectively.

Birds on dietary group T_2 showed the lowest ($P<0.05$) weight gain and dietary group T_1 showed the highest ($P<0.05$) weight gain between T_0, T_2 and T_3 dietary groups. Dietary groups T_3 showed improved growth when administration of both probiotics in diets and methanoic acid in water was done. The lower growth rate of water administration containing methanoic acid was evident in dietary groups T_2 throughout the trial and confirmed at the end of the trial. The growth reduction in treatment T_2 seemed to be a consequence of a depressed water intake induced by application of methanoic acid in water. The result is in agreement, who found lower weight gain. The chicks belonged to treatment T_1 showed highest weight gain which was significantly ($P<0.05$) higher compared to treatment T_0, T_2 and T_3 respectively. The results obtained in the study agreed with previous findings (Shen-HuiFang *et al.*, 2005; Denil *et al.* 2003) where improved weight gain was observed with administration of citric acid in diets at 0.3, 0.5 and 0.7%, respectively. The results contradict with the findings of previous researchers Pinchasov *et* *al.*, (2000) where depressed weight gain was observed with application of acetic acids in diets.

Table 4.1 Body weight gain and mortality in different dietary groups at different ages of birds

Age in days /Parameters	Dietary groups				Level of Significance
	T_0	T_1	T_2	T_3	
Initial body weight	38.00±0.03	39.07±0.04	41.00±0.09	37.00±0.5	NS
7th	220.00±7.42	219.00±8.38	221.33±7.42	222.33±4.46	NS
14th	594.33 ±14.54	617.00±16.03	600.33±10.54	607.66±16.15	NS
21th	1310.00±24.38	1330.00±16.20	1316.00±18.38	1320.00±24.72	NS
28th	1712.66±38.72	1732.00±46.10	1714.00±30.72	1725.00±11.77	NS
+Mortality (%)	00.00	00.00	00.00	00.00	NS

T_0= Control diet

T_1= Control diet + 0.75% probiotics

T_2= Control diet + 0.75% methanoic acid

T_3= Control diet + 0.5% probiotics + 0.5% methanoic acid

±= Standard error

^{abc}means having different superscript in the same row differed significantly (P<0.05)

NS= Non significant

4.3 Feed intake

The feed intake of birds fed different diets are shown in Table 4.2. Feed intake (g/broiler) was almost similar among the dietary groups. The feed intake (g/broiler) in T_0 (2691.66gm), T_1 (2705gm) T_2 (2695gm) and T_3 (2700gm) respectively. Feed intake was lowest in dietary group T_0 (2691.66gm) and the highest in dietary group T_1 (2705gm).

Table 4.2 Feed intakes (g) in different dietary groups at different ages of birds

Age in days /Parameters	Dietary groups				Level of Signifi-cance
	T_0	T_1	T_2	T_3	
7th	250.33±2.60	251.66±3.75	245.66±1.20	255.00±2.88	NS
14th	604.66.37±2.60	609.00±7.09	593.33±8.81	605.66±3.48	NS
21th	1584.66±2.60	1600.00±5.77	1591.66±4.40	1603.33±6.00	NS
28th	2691.66±7.26	2705.00±10.40	2695.00±2.88	2700.00±5.77	NS

T_0= Control diet

T_1= Control diet + 0.75% probiotics

T_2= Control diet + 0.75% methanoic acid

T_3= Control diet + 0.5% probiotics + 0.5% methanoic acid

±= Standard error

^{abc}means having different superscript in the same row differed significantly ($P<0.05$)

NS= Non significant

4.4 Feed conversion ratio

The feed conversion ratio (FCR) of the experimental birds is shown in Table 4.3.The lowest FCR was in dietary group T_1 (1.51) and highest in dietary group T_0 (1.62) at 28th day (4 weeks) of age. From the table it is found that probiotics treated group (T_1) showed better FCR and control diet treated group (T_0) showed higher FCR but administration of methanoic acid treated group T_3 (1.55) showed better FCR than treatment T_2 (1.58). Administration of probiotics showed best feed conversion ratio as compared to control group. The results are in well agreement with the findings of (Afsharma Pesh *et al.*, 2005) where FCR was found with administration of citric acid in poultry diet.

Table 4.3 Feed conversion ratio (wt gain/feed intake) of different birds of different dietary groups.

Age in days	Dietary groups				Level of Significance
	T_0	T_1	T_2	T_3	
7^{th}	1.10±0.002	1.05±0.006	1.08±0.16	1.07±0.02	NS
14^{th}	1.49±0.08	1.35±0.02	1.46±0.02	1.41±0.01	NS
21^{th}	1.57±0.07	1.44±0.02	1.53±0.008	1.49±0.009	NS
28^{th}	1.62±0.00	1.51±0.08	1.58±0.01	1.55±0.01	NS

T_0= Control diet

T_1= Control diet + 0.75% probiotics

T_2= Control diet + 0.75% methanoic acid

T_3= Control diet + 0.5% probiotics + 0.5% methanoic acid

±= Standard error

^{abc}means having different superscript in the same row differed significantly (P<0.05)

*= 5% level of significance

NS= Non significant

4.5 Dressing Parameters

It is found from the Table 4.4 that highest live weight (1763gm) in group T_1 and lowest live weight (1667gm) in group T_0 similarly from group T_2 and T_3 live weight (1710gm) and (1733gm) respectively which are significant. Carcass weights were significant and highest weight (1313.33gm) found from dietary groups T_1 and lowest weight in dietary groups T_0 (1117.00gm).

Table 4.4 Meat yield traits of broilers of different dietary groups (gm)

Parameter (gm)	Dietary groups				Level of Significance
	T_0	T_1	T_2	T_3	
Live weight	1667.00±22.26	1763.00±12.02	1710±40.33	1733±22.17	NS
Carcass weight	1117.00±87.22	1313.33±52.06	1171.00±50.20	1283.66±50.20	NS
Breast weight	521.66±24.55	585.00±20.81	527.00±18.26	540.00±33.83	NS
Thigh weight	153.33±6.00	170.00±12.14	163.00±12.99	169.00±8.00	NS
Gizzard weight	49.00±2.08	55.00±4.04	50.00±0.57	54.00±5.89	NS
Liver weight	46.00±3.51	55.33±4.04	46.00±1.52	52.00±4.73	NS
Heart weight	10.00±2.08	14.00±1.52	10.00±1.00	13.66±2.33	NS
^{abc}means having different superscript in the same row differed significantly ($P<0.05$) NS= Non significant					

T_0= Control diet

T_1= Control diet + 0.75% probiotics

T_2= Control diet + 0.75% methanoic acid

T_3= Control diet + 0.5% probiotics + 0.5% methanoic acid

±= Standard error

It is also observed from the Table 4.4 that weight of shank in all treatments did not differ significantly ($P>0.05$) among different groups. Head weight, gizzard weight and liver weight did not significantly ($P>0.05$) among different groups. Heart, spleen and intestine weight also did not differ significantly ($P>0.05$).

CHAPTER-V

SUMMARY AND CONCLUSIONS

The study was carried out 120 day old Cobb 500 broiler chicks to evaluate the effect of supplementation of probiotics and methanoic acid on growth, feed intake, feed consumption and carcass characteristics of broilers. The experimental birds were distributed randomly to 4 dietary groups T_0 (Basal diet), T_1 (Basal diet + 0.75% probiotics), T_2 (Basal diet + 0.75% methanoic acid), T_3 (Basal diet + 0.5% probiotics + 0.5% methanoic acid) each with 3 replications each having 10 broilers. Diets and fresh drinking water were provided to the chicks adlibitum during experimental period. Body weight, feed consumption, FCR, mortality and meat yield traits of broiler on different dietary groups were recorded and calculated and analyzed by using SPSS version 22 software. The highest body weight of broilers in dietary groups T_0 (1712.00 gm), T_1 (1732.00 gm), T_2 (1714.00 gm) and T_3 (1725.00 gm) respectively at 28 days. Body weight gain was affected significantly ($P<0.05$) by using 0.75% probiotics and 0.75% methanoic acid in the diet of broiler.

Feed conversion ratio (FCR) among the birds received 0.75% probiotics and 0.75% methanoic acids affected significantly ($P<0.05$). Addition of 0.75% probiotics was the most effective and efficient followed by dietary groups T_0, T_2 and T_3 respectively. No mortality was found in all dietary groups. Carcass weight (gm) was the highest in dietary groups T_1 (1313.33gm) and the lowest in T_0 (1117gm). Carcass weight was affected significantly ($P>0.05$) by using 0.75% probiotics and 0.75% methanoic acid during the experimental period. Therefore, addition of probiotics up to the level of 0.75% in broiler diet enhances productivity and feed conversion ratio (FCR).

Considering the above facts it may be concluded that supplementation of 0.75% probiotics in the diet had positive significant effect on live weight, feed intake and feed conversion ratio (FCR) with no detrimental effect on meat yield traits. Therefore, 0.75% probiotics can successfully be used in broiler diet.

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