EFFECTS OF PROBIOTIC, YOGHURT AND ACIDIFIERS ON THE PERFORMANCE OF COMMERCIAL BROILERS

A THESIS

BY

MD. RASHIDUL ISLAM

Registration No. 1605480

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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DEPARTMENT OF DAIRY AND POULTRY SCIENCE

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Dedicated to

My

Beloved Parents

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'The Author

December, 2018

ABSTRACT

An experiment was carried out with 120 Lohman meat broiler chicks (Day old) to evaluate the effect of supplementation of Probiotic (Synlac), yoghurt and acidifier (FraAC34) on the performance of broiler were procured from Aman Poultry and Hatchery limited. The Synlac, yoghurt and FraAC34 were purchased from local market. The treatment Groups were Group A (1g Synlac/2L), Group B (5g yoghurt/1L) , Group C (1ml Fra AC34/1L) and group D (control). At 35 days of age the body weight gain at different dietary treatments were 1981.67g, 1951.67g, 1946.34g and 1906.67g in Group A, B, C and D respectively and the difference was non-significant. Feed consumption was higher in Group A but there was no significant difference among the different treatments. Feed consumption during the whole period (0-35 days) were 3186.67g, 3138.33g, 3106.67g and 3104g in Group A, B, C and D respectively. FCR during the experimental period (0-35 days) were 1.58, 1.58, 1.56 and 1.59 in Group A, B, C and D respectively. Not any bird died during the experimental period, so livability was 100%. The production performance was higher in synlac, yoghurt and FraAC34 than control but no significant difference was found.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	: Analysis of variance
BBS	: Bangladesh Bureau of Statistics
CRD	: Completely Randomized design
DM	: Dry Matter
Dr.	: Doctor
et al.	: Associates
FCR	: Feed conservation Ratio
Fig.	: Figure
G	: Gram
GIT	: Gastro Intestinal Tract
HSTU	: Hajee Mohammad Danesh Science and Technology University
kcal	: kilo-calorie
Ltd.	: Limited
ME	: Metaboilizable energy
ME	: Metabolizable energy
ML	: Mille Litter
^oC	: Degree Celsius
pH	: Power of Hydrogen
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

CHAPTER-I

INTRODUCTION

The poultry sector of Bangladesh has become a giant contributor for fulfilling the protein demand of the nation. In this sector broiler industry is playing a vital role by supplying cheapest meat to the nation. The main aim of broiler production is attaining maximum weight of the birds in a minimum period of time. The feed accounts 70% of the total cost of broiler production.So it is essential to utilize the feed most efficiently to have minimum production cost. A number of feed additives like antibiotics, growth promoters etc have been used to enhance the production performance of broiler. But excessive dependency on medication threatens the mankind for antibiotic resistance. Now adays antibiotics are the most important things that must be handled with proper care. Global awareness is being raised for minimizing the use of antibiotics in food producing animals. So use of others alternatives are now essential to save the world as well as enhance the production of commercial broilers.

The term probiotic is etymologically derived from the Latin preposition pro ("for" or "in support of") and the Greek word (biotic), which literally means "for life". There is a huge body of evidence that support the significant positive impacts of probiotics and bioactive compounds on poultry performance and health. Probiotic bacteria improve the economic indexes and resistance to pathogens of laying or meat-type chickens (Hippenstiel *et al.*, 2011; Aazami *et al.*, 2014; Cean *et al.*, 2015; Mountzouris *et al.*, 2007). Lactobacilli and Enterococci have been widely used as probiotics in the poultry industry (Kabir *et al.*, 2004; Awad *et al.*, 2009; Aazami *et al.*, 2014; Mountzouris *et al.*, 2007). The main postulated health benefits associated with probiotics include improving the gut microflora balance, stimulating the immune reaction, producing different antimicrobial substances, modulating the immune response, producing digestive enzymes, and reducing cholesterol levels (Ramirez- Chavarin *et al.*, 2013; Smug *et al.*, 2014). Recent studies have confirmed that the addition of probiotic, symbiotic, and medicinal plant additives to feeds enhance nutrient bioavailability, health and immune status, and carcass yield and quality of Japanese quails (Yalçın *et al.*, 2000; Siriken *et al.*, 2003; Chimote *et al.*, 2009; Sharifi *et al.*, 2011; Kasmani *et al.*, 2012; Babazadeh *et al.*, 2011; Kheiri *et al.*, 2015). The main factors that affect the general probiotic effects are probiotic species, strain origin, and application levels (Mountzouris *et al.*, 2007; Amerah *et al.*, 2013).

Probiotic, unlike antibiotic, imply the use of live microorganism rather than specific products of their metabolism. Probiotics can be classified into two major types viable microbial cultures and microbial fermentation products. The mode of action have been suggested as beneficial change in gut flora with reduction in population of (*E. Coli*; b) lactate production with subsequent change in intestinal PH, production of antibiotic-like substances, reduction of toxin release (suppression of *E. coli*) (Leeson, S. and summers, J D, 2005). Probiotics beneficially affect the host animal by improving its intestinal balance and create gut conditions that suppress harmful microorganisms and favor beneficial ones (Line EJ *et al.,* 1998, Mead GC, 2000). They have been shown to maintain health by reducing risk diseases, possibly through a reduction in proliferation of pathogenic species, maintaining micro biota balance in the gut enhancing immune system and increasing resistance to infection (Mead GC, 2000, Mountzouris KC *et al.,* 2007). They have been also shown to improve the growth performance of poultry (Kabir SML, Rahman MM and Rahman MB, 2004).

Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii, Bulgaricus sps*. Although fermented milk products such as yogurts were originally developed simply as a means of preserving the nutrients in milk. Yogurt contains mainly lactobacilli and other beneficial bacteria's that have strong positive health effect. Yogurt could aid digestion and inhibit the development of pathogens by improving the balance of microbial living in the digestive tract. Due to its probiotical potential, yogurt can be used instead of commercial antibiotic (Panda AK *et al.,* 2003). The objective of this study was to investigate the effects of supplementation of probiotic and dry yogurt powder on the productive performance and intestinal microbial micro flora of broiler chickens under commercial conditions.

Yogurt can be used as an effective probiotic. It contains lactobacilli and other beneficial bacteria that have strong positive health effects, aid digestion and inhibit the pathogens by improving the balance of microbes in the digestive tract (Metchnikoff, 1998). Antibody production against Newcastle disease virus (NDV) in a group of broiler chicks treated with probiotic has been reported to be significantly higher 10 days post immunization as compared to untreated group (Khaksefidi and Ghoorchi, 2006). Use of probiotic PrimaLac (Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium and Bifidobacterium bifidium) results in a significantly ($P<0.05$) enhanced broiler performance by improving body weight, decreasing the feed conversion ratio and improvement in antibody responses to Newcastle disease (Talebi *et al.*, 2008).

The acid that is originated naturally is called organic acid. There are several types of organic acids like acetic acid, lactic acid, fumeric acid, malic acid, ascorbic acid, propionic acid, butyric acid etc. Various organic acids and their derivatives are used as acidifiers in the drinking water of poultry. Various types of acidifiers under several brand names are available in the market, FraAC34 is one of them marketed by ACI Animal Health Ltd, Bangladesh. It contains Monoglyceride of Propionic acid (monopropionin), Monoglyceride of Butyric acid (monobutyrin) and encapsulated essential oils. It is claimed to be a good alternative of antibiotic growth promoter. It balances GUT's microflora and controls digestive upsets and increases productivity. Since probiotics, yoghurt and acidifiers in water are claimed to improve the performance of birds, so it would be interesting to conduct an experiment with these products to investigate their beneficial effects. Keeping this view in mind, this research work was undertaken with the objectives of evaluating the effect of Probiotic (Synlac), yoghurt and acidifiers (FraAC34) on the performance of broiler.

Objectives:

i) To determine the effects of dietary supplementation of probiotic, yogurt and acidifier on the performance of commercial broilers.

ii) To estimate the effects of dietary supplementation of probiotic, yogurt and acidifiers on body weight gain.

iii) To evaluate the effects of dietary supplementation of probiotic, yogurt and acidifiers on the performance of feed consumption of broiler.

CHAPTER-II

REVIEW OF LITERATURE

The scientific interest on the effect of feed additives of different origin has been studied for the better growth of the broiler. The most common additives are probiotic, organic acid or acidifiers, yoghurt and others. Although a number of scientists have studied on these additive most of the researchers have worked with probiotic, yoghurt and little number of them have worked with organic acid or acidifiers.

2.1 Some of the research reports are reviewed

2.1.1 What is probiotics?

Probiotics are feed supplement of live microbial origin which has beneficial effect on the host animal by improving the intestinal microbial balance. Probiotics are viable microbial and microbial fermentation products which exert their beneficial effects by decreasing the undesirable micro-flora population in the gastro-intestinal tract (Chiang and Hseih, 1995) and build-up resistance against diseases by stimulating the immune system (Cheeke, 1991). Probiotics have also been an approach that has been reported to have the potential to reduce enteric disease in poultry and subsequent contamination of poultry product (Patterson and Burkholder, 2003). (Guilot, 2000) observed that there has been a renewed interest in the incorporation of probiotics as a result of reduction in the use of antibiotics as feed additive in animals. Many probiotics are isolated from gastro intestinal tract of healthy animals and hence natural which makes them devoid of unhealthy side effects to the animal and subsequently to the consumer (WU *et al.,* 2008). (Bonsu *et al.* 2012) found that the inclusion of a probiotic product, RE-3, in the diets of layers and broilers resulted in considerable reduction of body fat and serum cholesterol content (up to 16%) in broilers, a 15% decrease in cholesterol level of eggs, improved egg weight and reduced mortality in both broilers and layers. (Dei *et al.,* 2010) also indicated that the addition of RE-3 to the diets of grower birds significantly reduced mortality compared to birds on a control diet containing no probiotic. Probiotic (RE-3) has also been found to have a positive influence on average daily gain in pigs (Okai *et al*., 2010).

Numerous studies showed that addition of probiotics have positive effects on growth rate, feed utilization, feed efficiency and mortality rate (Sen *et al.,* 2012; Manal, 2012). However, the efficacy of probiotics depends upon the selection of more efficient strains, gene manipulation, combination of several strains and the combination of probiotics and synergistically acting components. The use of multi-strain probiotics seems to be the best way of potentiating the efficacy of probiotics as it beneficially affects the host by improving the growth-promoting bacteria with competitive antagonism of pathogenic bacteria in the gastrointestinal tract. Hence, keeping in view multi-strain probiotics was used to evaluate the effect of probiotics supplementation on growth performance, feed consumption, feed conversion ratio, carcass characteristics, mortality and economics of feeding in broiler chicks.

2.2 Composition of probiotic

Synlac is one kind of water soluble multi-strain commercial probiotics, which is marketed by ACI Animal Health (Bangladesh) Ltd. Synlac contains a unique mixture of microorganisms. Synlac is composed of 4 strains of beneficial bacteria.

Table 2.1 Composition of Synlac (Water soluble multi-strain probiotics)

Bacteria	**Colony Forming Unit**
Lactobacillus acidophilus and Lactobacillus plantarum	7×10
Bacillus subtilis	3×10
Enterococcus faecium	1×10^2

Source: ACI Animal Health, Bangladesh Ltd.

2.3 Function of microbes present in probiotics

Function of microbes present in Synlac is as follows:

***Lactobacillus acidophilus*:** Stimulates immunity, suppresses microbial enzymes activity involved in production of carcinogens, promotes growth of farm animals and in chicks antagonizes *Salmonella typhimurium, Staphyllococcus. aureus and E. coli.*

***Lactobacillus plantarum*:** Characteristic lumen organisms, ferments wide range of carbohydrates, acid tolerant, production of organic acids and bacteriocins.

***Enterococcus faecium*:** Prevents *E. coli* diarrhoea, increases cellulytic activity in caecum of chicken, and antagonizes *Salmonella typhimurium* in mice.

Bacillus subtilis: A healthy probiotic strain that supports digestion, enzyme production, immune and digestive system health.

2.4 Probiotic and the performance of broiler

There are many types probiotic preparation in the market.

Table 2.2 List of different types of probiotics

Product	Company	Target Animal	Total CFU Count	Bacterial Strain	Yeast Strain
Synlac	ACI Animal Health Ltd.	Poultry	Lactobacillus acidophilus and Lactobacillus plantarum 7×10 cfu, Bacillus subtilis 3×10 cfu, Enterococcus faecium 1×10^2 cfu	Lactobacillus acidophilus and Lactobacillus plantarum, Bacillus subtilis, Enterococcus faecium	-
Protexin	NOVARTIS	Poultry	2×10^9	S. thermophilus	Aspergillus oryzae
Boost	(Bangladesh Ltd.)			Lactobacillus casei L. plantarum L. bulgaricus L. acidophilus B.bifidum	
Provilac	Vetcare	Poultry	2×10^9	L. acidophilus L. sporogens S. faecium	Saccharomyces cerevisiae
Yeasacc	All tech	Poulty, pig	1.3×10^7	L. acidophilus S. faecium	Saccharomyces cerevisiae
EM	Integrated Nature Farming	pig, poultry, cattle, fish	6.9×10^5 (Bacteria) 480/ml (Yeast)	Lactobacillus spp. Rhodopseudomonas spp	Saccharomyces spp
Toycerin	Toy Jazo	Poultry	10×10^{10}	Bacillus toyoi	-

Source: Product profile of different company leaflets.

Many studies have been conducted to list the efficacy of such preparations on broiler performance like feed intake, body weight gain, feed conversion, mortality etc. The literatures reviewed are given in the following subheadings:

2.5 Effects of probiotic on feed intake and feed conversion

Abudabos *et al*. (2015) reported that broiler birds supplemented with antimicrobial growth promoter (Neoxyval), prebiotic (TechnoMos) and probiotic (GalliPro) gained more body weight and good feed efficiency than control and symbiotic (TechnoMos and GalliPro) from 0 to 42 days of age. Marshed and Abudabos (2015) reported that the commercial broiler body weight gain was higher when supplemented with antibiotic growth promoter and probiotic compared with control (273.2 g) at the age of 14^{th} day. Li Y B *et al*. (2014) observed that the broilers weighed 18.4% and 10.1% more at the age of 42 days, supplemented with *Bacillus subtilis* and *Lactobacillus acidophilus*. Hammady *et al*. (2014) reported that the broilers received 1g or 1.5g probiotic /kg diets had significantly higher body weight gain than the antibiotic (Neomycin : 200mg/kg diet) and control . Hammady *et al*. (2014) reported that the broiler birds fed antibiotic Neomycin (200mg/kg diet) or probiotic diets (1g and 1.5g/kg) had significantly better FCR values than those of birds fed the control diets.

Sarmah Sankar *et al.* (2014) observed that the diet supplemented with antibiotic and Probiotic @ 50g/100 kg of feed and a combination of antibiotic and probiotic @ 20 and 25g each/100 kg of feed. Showed no effect on feed intake and feed conversion efficiency in the broilers. Roozbeh Shabani *et al.*, (2012). After the FDA ban on fluoroquinolones from being using in poultry over concerns that it was a driving force behind antibiotic-resistant bacteria, the use of probiotic bacteria has become increasingly popular for improved nutrition. The aim of this study was to assess the effects on growth performance by introducing three kinds of commercial probiotics, to the diet of broiler chickens, commercial strain, 308 vertexes in Iran. For this purpose, chickens were divided into four groups include: (a) control group, without probiotics, (b) experimental group containing Protexin, (c) experimental group containing Primalac, (d) experimental group containing Calciparine. The effects of probiotics on growth performance were measured and results shows that feeding broilers with probiotics have significant effects ($P < 0.05$) on average daily gain (ADG) and feed conversion ratio (FCR), while it appeared insignificant on daily feed intake (DFI). However, the results of this research reveal that that feeding chicken broilers with these probiotics have positive effects on growth performance of chicken broilers. Thus, the use of these probiotics is highly recommended.

Roozbeh Shabani *et al.* (2012) observed that the feeding broilers with probiotics (Protexin, Primalac and Calciparine) significantly (P < 0.05) improved average daily gain and feed conversion ratio, however the feed intake was non effected (P > 0.05). Bai et al. (2012) fed the diets supplemented with an antibiotic (100 mg of chlortetracycline/kg of diet, 0.1%, or 0.2% probiotic 1 × 107 cfu/g of Lactobacillus fermentum and 2 × 106 cfu/g of Saccharomyces cerevisiae). The average daily gain and feed efficiency were improved (P< 0.05) in broilers fed the probiotic diet during starter phase. Chae et al. (2012) observed that the supplementation of Lactobacillus acidophilus, Bacillus subtilis (BS) and Saccharomyces cerevisiae at 0.30% to broilers diets improved (P<0.05) body weight gain. Bansal et al. (2011) observed that the inclusion of probiotics showed increased growth rate at 2nd, 3rd and 5th week of age in commercial broilers. Ignatova et al. (2009) observed significant increase in body weight significantly (P<0.001) compared with control when supplemented with probiotic containing Lactobacillus and Bifidobacterium strains in the chicken.

Mountzouris *et al*. (2009) evaluated response of broilers fed with 108, 109, 1010 cfu probiotic/kg of diet and 2.5 mg of avilamycin/kg of diet. The results revealed that the overall feed conversion ratio values were similar and significantly (P<0.05) better for probiotic and antibiotic groups compared with control. Awad *et al.* (2008) showed a slight improvement in performance traits in broilers fed the probiotic *Lacto-bacillus sps* at 1kg/ton of feed. M. Midilli, *et al.* (2008). This study was conducted to investigate the effects of probiotic and/or prebiotic supplementation ongrowth performance and serum IgG concentrations in broilers. One thousand two hundred one-day old Ross-308 broiler chicks of mixed sex were randomly divided into four treatment groups of 300 birds each. The treatments were: Starter diets: 1) Unsupplemented control diet; 2) Probiotic (Bio-Plus 2B® 0.05%); 3) Prebiotic (Bio-Mos® 0.2%); 4) Probiotic and Prebiotic mixture (Bio-Plus 2B® 0.05% and Bio-Mos® 0.2%). The grower diets were: 1) Control with no supplements; 2) Probiotic (Bio-Plus 2B® 0.05%); 3) Prebiotic (Bio-Mos® 0.1%); 4) Probiotic and Prebiotic mixture (Bio-Plus 2B® 0.05% and Bio-Mos® 0.1%). Each treatment group was further sub-divided into five replicates of 60 birds per replicate. The chicks were fed the broiler starter diet for the first 21 d and the broiler grower diet between days 22 and 42. Dietary probiotic and/or prebiotic supplementation did not significantly affect body weight, body weight gain, feed intake, carcass weight, carcass yield or concentration of immunoglobulin (IgG) in the serum. However, feedconversion ratio was improved significantly in the supplemented treatments compared to the unsupplemented control. Probiotic and/or prebiotic supplementation did not significantly affect any of the examined parameters except for an improved feed conversion ratio.

Khaksefidi and Groorchi (2006) observed positive effect on production performance of broiler chicks supplemented with probiotic (*Bacillus subtilis*) at 50 mg/kg during 1-42 days of age. Anjum *et al*. (2005) observed a significant ($P<0.05$) improvement in feed conversion ratio of broilers fed on protexin-supplemented diet at 100g/t in starter and 50g/t in finisher diets compared to control diets. Khaksefidia *et al.*, (2005) carried out an experiment with 320 broilers to assess the influence of supplementation of probiotic on growth, microbial status and carcass quality and found that feed conversion of probiotic fed groups were superior ($P<0.05$) to control group at the 4^{th}, 5^{th}, and 6^{th} weeks.

Yu *et al.* (2004) fed probiotics in commercial broilers and reported that chickens fed diet containing 0.2% probiotics had higher weights and feed conversion ratio. Priyankarage *et al.*, (2003) reported that feeding of commercial probiotic preparation (protexin) had no significant effect on feed conversion of the broilers. Veeramani *et al.* (2003) carried out an experiment with 200 day old broilers providing with 0.5% and 1 % lactic acid, 0.2% and 0.4 % NaCl and observed that broilers provided with 0.5% lactic acid in drinking water had significantly ($P<0.05$) lower feed consumption and the best feed efficiency compared to other groups.

Hamid *et al.* (2001) found that the addition of probiotic (protexin) at the rate of 1 g/liter drinking water of broiler improved feed conversion. Ladukar *et al.* (2001) found that average feed intake during the experiment did not differ significantly among different treatments and also the feed conversion was not influenced by the supplementation of probiotic. Zulkifli *et al.*, (2000) observed that broilers fed a diet containing Lactabacillus culture less feed and had better feed efficiency ratios during the growing period (1 to 21 days), but superior feed efficiency did not extended to the finishing period (22 to 42 days), where the broilers were subjected to 3 hours episodes of heat stress ($36 \pm 1^{o}C$) each day, Supplementation of probiotic (protexin TM) with or without antibiotic, to the rations had no significant effect on feed conversion of broiler as reported by Ergun *et al.*, (2000).

2.6 Effects of probiotic on mortality of birds

Youcef Mehdi *et al.* (2018) reported that probiotics maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. C.M. Pender *et al.* (2016) conducted a research to investigate the effects of *in ovo* administration of probiotics on hatchability, performance, immune organ weights, and lesion scores in broiler chicks during a mixed *Eimeria* infection. No differences were seen among groups for hatchability as well as for body weight (BW), BW gain (BWG), or immune organ weights prior to the *Eimeria* challenge. On day 9, the non-challenged birds with probiotic supplementation had higher BW and BWG than the non-supplemented controls while no differences were seen among the challenged groups. On day 15, probiotic supplemented birds had improved BW compared to the non-supplemented birds as well as increased BWG from day 9 to 15. Bursa weight was not affected by treatment at any time point while spleen weight was greater in supplemented birds on day 15. Birds receiving the probiotic had significantly lower mortality than non-treated birds. Additionally, gross lesion severity was reduced due to probiotic supplementation in all intestinal segments evaluated. These results suggest that *in ovo* supplementation of probiotics may improve early performance and provide protection against a mixed *Eimeria* infection.

Gnikpo A.F *et al.* (2016) evaluated the efficiency of a feed ingredient with probiotic properties (FIPP) on growth performances of Giant White Bouscat red eye rabbits. The experiment was carried out during 56 days on 150 weaned rabbits in Cuniglo farm in Southern of Benin. Rabbit had 35 days-old and weighted on average 669.94 ± 69.55 g. The experimental design was a randomized complete block with five dietary treatments (TF0, T0, T1.5, T3 and T4.5) and six replicates per treatment. TF0 rabbits were fed with the control diet and received antibiotics, T0 rabbits were fed only with the control diet; T1.5, T3 and T4.5 rabbits were fed with the control diet supplemented with 1.5%, 3% and 4.5% of FIPP respectively. The results showed that the rabbits fed TF0, T1.5 and T3 had the best weight performances compare to those fed T0 and T4.5% dietary treatments. Hematological, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) (p=0.058) and lymphocyte (LYM) are improved with the supplementation of FIPP. The lowest feed cost was in T3 (1815 FCFA/kg weight gain) followed by TF0 (1812 FCFA/kg weight gain); T1.5 (1860 FCFA/kg weight gain); T0 (2144 FCFA/kg weight gain) and T4.5 (2859 FCFA / kg weight gain). The economic feed efficiency had the same trends as feed costs. We can conclude that FIPP at concentrations of 1.5% and 3% had an effect on growth performances and on blood characteristics of rabbits.

D. Harrington *et al.* (2016) shown that direct-fed microbials (DFMs) based on spore-forming bacteriasupplemented in poultry feed are able to improve broiler performance. In the present study, theinteraction of feed supplemented withBacillus subtilisand different metabolizable energy (ME) levels in feed on Cobb broiler performance were evaluated. Broilers were fed diets containingan average ME of 3,117 (100% ME), 3,054 (98% ME), 2,991 (96% ME), or 2,930 (94% ME) kcal/kg either with or withoutB.Subtilis (8×105cfu/g feed) in a 4×2 factorial design. Birdswere fed equal amounts of starter and grower feed to approximate for equal amino acid intakeand finisher feed ad libitum. Birds fed diets supplemented withB. Subtilis were able to achievehigher final BWs and lower FCRs than their respective controls, irrespective of the level ofthe energy reduction. Whe birds were fedB. Subtilis and diets containing 3,054 kcal/kg ME(98% ME), they were able to achieve equivalent performance to control birds fed non-Bacillus–supplemented rations containing 3,117 kcal/kg (100% ME). Regression analysis indicatedB.subtilishad a ME contribution of+62 kcal/kg feed. This improved the performance of birdsfed B. subtilis, and a 2% reduced ME (98% ME) diet resulted in a significantly lower feed costper kilogram weight gain ($0.399/kg weight gain) compared to control birds fed a standard100% ME diet ($0.417/kg weight gain) and a potential cost saving of $0.018/kg weight gain. Inaddition, overall mortality was 2.51% lower inB. Subtilis supplemented groups. Consequently, in addition to confirming the performance benefits associated with B.subtilis , it is suggestedthat supplementation ofB. Subtilis in feed confers economic savings as well.

Jason M. Neal-McKinney *et al.* (2012) reported that *Lactobacillus* can be used to reduce the colonization of pathogenic bacteria in food animals, and therefore reduce the risk of foodborne illness to consumers. As a model system, we examined the mechanism of protection conferred by *Lactobacillus* species to inhibit *C. jejuni* growth *in vitro* and reduce colonization in broiler chickens. Possible mechanisms for the reduction of pathogens by lactobacilli include: 1) stimulation of adaptive immunity; 2) alteration of the cecal microbiome; and, 3) production of inhibitory metabolites, such as organic acids. The *Lactobacillus* species produced lactic acid at concentrations sufficient to kill *C. jejuni in vitro*. We determined that lactic acid produced by *Lactobacillus* disrupted the membrane of *C. jejuni*, as judged by biophotonics. The spectral features obtained using

Fourier-transform infrared (FT-IR) and Raman spectroscopy techniques were used to accurately predict bacterial viability and differentiate *C. jejuni* samples according to lactic acid treatment. FT-IR spectral features of *C. jejuni* and *Lactobacillus* grown in co-culture revealed that the metabolism was dominated by *Lactobacillus* prior to the killing of *C. jejuni*. Based on our results, the development of future competitive exclusion strategies should include the evaluation of organic acid production.

Timmerman HM *et al.* (2006) developed a multispecies (MSPB) and a chicken-specific (CSPB) probiotic preparation in fluid form. The MSPB contained different probiotic species of human origin, whereas the CSPB consisted of 7 Lactobacillus species isolated from the digestive tract of chickens. In a field trial with broilers, MSPB treatment resulted in a slight increase (by 1.84%) in broiler productivity based on an index taking into account daily weight gain, feed efficiency, and mortality. The CSPB treatment reduced mortality in 2 subsequent field trials and raised productivity by 2.94 and 8.70%. In a controlled trial with broilers showing a high index of productivity, probiotic treatment further raised productivity by 3.72%. Based on the present 4 studies in combination with 9 studies published earlier, it is suggested that with higher productivity rates of the broilers the effect of probiotics becomes smaller.

A. Khaksefidi and Sh. Rahimi, (2005) was conducted a study with three hundred and twenty broiler chickens to evaluate the influence of supplementation of probiotic on growth, microbiological status and carcass quality of chickens. The probiotic contained similarproportions of six strains of variable organisms namely Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faecium and Torulopsis sps and was fed at 100 mg/kg diet. The body weight and feed conversion of probiotic fed groups were superior ($p<0.05$) compared to the control group in the 4th, 5th and 6thweeks. The chickens fed the diet with probiotic had lower ($p<0.05$) numbers of coliforms and Campylobacter than chickens fed the control diet. All chickens' carcasses on the control diet were positive for Salmonella while only 16 of the 40 carcasses were positive from chickens fed diets containing probiotic. The leg and breast meat of probiotic fed chickens were higher ($p<0.05$) in moisture, protein and ash, and lower in fat as compared to the leg and breast meat of control chickens. Hamid *et al.*, (2001) reported that the probiotic (protexin @ of 1 g/liter drinking water) had the lowest mortality than control group

2.7 Yoghurt and its uses in commercial broiler industry

Traditional yoghurt is also considered as probiotics and it is the most popular fermented milk product in the world. Generally, yoghurt is two types sweet and sour. Sweet yoghurt is generally prepared from mixed culture of *Streptococcus lactis, Streptococcus thermophilus, Streptococcus citrophilus and Lactobacillus plantarum.* Sour yoghurt is prepared by seeding milk with a combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Usually, the starter culture containing *Streptococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* used for manufacture of yoghurt. In fresh yoghurt the amount of these microorganisms (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) together are in concentration of 10^8 cells/ml.

2.7.1 Function for yoghurt

1. Yoghurt increases the bioavailability of many essential nutrients such as Ca, Mg and Zn ions.
2. Yoghurt could inhibit growth of intestinal carcinoma through increased activity of Ig A, T cells and macrophages.
3. Yoghurt allows the absorption of lactose in hydrolyzed form.

2.7.2 Yoghurt and broiler performance

M.S.M. Nafees and M. Pagthinathan (2017) was conducted a experiment to study the effect of dietary supplementation of lactic acid cultures (5×1010 cfu of Streptococcus thermophilus and Lactobacillus bulgaricus per gram) on growth parameters of Lohmann Indian River broiler chicks. Fifty-two unsexed day-old chicks were randomly divided into two groups. Each group was subdivided into two replicates and housed in 108×108 cm pens and reared with a deep litter system. The birds were fed with commercial broiler starter ration for the first 21 days and from 22 to 40 days, they were randomly allocated to one of the two dietary treatments: broiler finisher (control) or broiler finisher supplemented with 1% lactic acid cultures (experimental diet). The study showed that treatment effects on feed intake, live-weight, dressing percentage and weight of the internal organs of broilers were not significant (P>0.05). Total feed intake was 3 786.3 g on control diet and 3 785.3 g on experimental diet. The values for the respective growth parameters on control diet were: live-weight, 2 562.5 g; live-weight gain, 85.1 g/d and relative growth rate, 83.1 g kg-1 d-1. The values for the respective growth parameters on experimental diet were: live-weight, 2 371.4 g; live-weight gain, 77.4 g/d and relative growth rate, 82.0 g kg-1 d-1. Dressed weight (1 828.6 g) was higher (P<0.05) on control diet. There was no difference in FCR. The findings could be due to combined effects of insufficient bacterial count, and genotype and growth stage of broilers

S. Seif *et al.* (2013) reported that a total of 150 one day old broiler chickens (Cobb 500) divided into 5 equal groups: control (T1), 3.0 g prebiotic per kilogram basal diet (T2), 5.0 g probiotic yoghurt per liter of water and 3.0 g prebiotic per kilogram basal diet (T3), 10.0 g probiotic yoghurt per liter of water and 3.0 g prebiotic per kilogram basal diet (T4) and 20.0 g probiotic yoghurt per liter of water and 3.0 g prebiotic per kilogram basal diet (T5). Performance was assessed by measuring the weekly feed consumption and weight gain. The food conversion ratio (FCR) was also calculated for each group. All chickens were bled at the end of experiment. The body weight gain signi±cantly (P< 0.05) improved in T5 treatment group than other groups. There was no signi±cant difference in weight gainamong the treatment groups during the period from 1-14 days of age (P< 0.05). During 15 to 42 days of age the highest weight gain was in T5 followed by T4, T3, T2 and T1 and differences were signi±cant between the treat-ments. There was no signi±cant difference in feed intake among the groups. At the end of experiment the FCR in T5 group differed signi±cantly (P< 0.05) from other groups. The amounts of RBC, WBC, and PCV are lower in control group than other groups, but not signi± cantly (P> 0.05). The ratio of heterophile to lymphocyte was higher in control group than other groups, signi±cantly (P< 0.05).

Boostani *et al.* (2013) evaluated the effect of the probiotic thepax and yogurt (as probiotic) on the growth response and intestinal microflora results of broiler chickens. Two hundred forty day-old Ross 308 broilers were equally distributed into 12 floor pens and reared for 42 day. The treatments consisted of yogurt (10, 5 and 2.5% during starter, grower and finisher periods in the drinking water, respectively) and thepax (1000, 500, 250 g/ton^{-1} in the starter, grower and finisher diets, respectively), resulting three experimental diets and a control group. Each dietary treatment was fed *ad-libitum* to four replicate groups of 20 birds at the beginning of rearing period. Birds and feed were weighed on days 21 and 42. The results of experiment indicate that diets containing feed additives improved broiler performance. The body weight gain and feed conversion ratio improved significantly more ($p \leq 0.05$) with the thepax treatment compared with the control broilers during the total rearing period. The highest ($p \leq 0.05$) carcass and thigh values were recorded for broilers fed the diet supplemented with thepax and yogurt, respectively. The lowest abdominal fat pad value was obtained in broilers fed the diet supplemented with thepax. On d 21, thepax and yogurt significantly reduced ($p \leq 0.05$) cecal *Escherichia coli* and *Clostridium perfringens* populations compared with the control group. In conclusion, thepax and yogurt improved broilers growth response and conferred intestinal health benefits to chickens by improving their microbial ecology.

Seif *et al.* (2013) evaluated that the effects of probiotic yoghurt and prebiotic utilization on performance and some blood parameters of broiler chickens. Materials, Methods & Results: A total of 150 one day old broiler chickens (Cobb 500) divided into 5 equal groups: control (T1), 3.0 g prebiotic per kilogram basal diet (T2), 5.0 g probiotic yoghurt per liter of water and 3.0 g prebiotic per kilogram basal diet (T3), 10.0 g probiotic yoghurt per liter of water and 3.0 g prebiotic per kilogram basal diet (T4) and 20.0 g probiotic yoghurt per liter of water. Sohail Hassan Khan *et al.* (2011) conducted a research to evaluate three different probiotics, using drinking water supplemented with protexin, biovet and yoghurt. The day old broiler chicks were randomly divided into 12 separate floor pens each comprising 25 birds and three pens (replicates) per treatment group following completely randomised design. At 28 and 39 days of age body weight (BW) and feed to gain ratio (FCR) were determined. At the end of experiment, nine birds per treatment were sacrificed to evaluate carcass characteristics, abdominal fat contents and the internal organs. Blood haemato-biochemical parameters were determined. Haemagglutination inhibition antibody titres against Newcastle disease virus and lymphoid organs weight/body weight ratio were also determined. The BW of birds given probiotics was significantly greater than control (without probiotics) at both 28 and 39 days of age. Similarly, better FCR was observed in birds those given drinking water with probiotics. There was less mortality recorded with probiotics treatments. Differences in carcass characteristics, organs weight, meat composition and haematological values among all the treatments were non-significant. However, abdominal fat contents reduced significantly in probiotics supplemented groups as compared to control and cholesterol contents were reduced significantly supplemented groups as compared to control at both 21 and 39 days of age. Feeding of probiotics did positively affect the immune system within the parameters measured. It may be concluded that performance, blood chemistry, immunity against disease and economic efficiency in broilers could be maintained when supplementing any probiotic incorporated in broiler's drinking water.

Sohail Hassan Khan *et al.* (2011) conducted a experiment to evaluate three different probiotics, using drinking water supplemented with protexin, biovet and yoghurt. The day old broiler chicks were randomly divided into 12 separate floor pens each comprising 25 birds and three pens (replicates) per treatment group following completely randomised design. At 28 and 39 days of age body weight (BW) and feed to gain ratio (FCR) were determined. At the end of experiment, nine birds per treatment were sacrificed to evaluate carcass characteristics, abdominal fat contents and the internal organs. Blood haemato-biochemical parameters were determined. Haemagglutination inhibition antibody titres against Newcastle disease virus and lymphoid organs weight/body weight ratio were also determined. The BW of birds given probiotics was significantly greater than control (without probiotics) at both 28 and 39 days of age. Similarly, better FCR was observed in birds those given drinking water with probiotics. There was less mortality recorded with probiotics treatments. Differences in carcass characteristics, organs weight, meat composition and haematological values among all the treatments were non-significant. However, abdominal fat contents reduced significantly in probiotics supplemented groups as compared to control and cholesterol contents were reduced significantly supplemented groups as compared to control at both 21 and 39 days of age. Feeding of probiotics did positively affect the immune system within the parameters measured.

G L Bohoua (2008) studied on one hundred and forty (140) Red Island chicks. The gained bodyweight and feeding efficiency index were calculated were taken as performance parameters. To conduct this experiment, four batches of 35 chicks were used. The following feeding supplementations were added to the basic feed. The first batch (STA) received the only the basic feeding. Batches 2 (YEA), 3 (YOG), 4 (YEA + YOG) received respectively 3% palm wine settling, 3% yogurt powder, 1.5% palm wine settling and 1.5% yogurt powder. Data were collected every week and the performance was measured. The best results in terms of body weight and the feeding efficiency index were obtained with batch 3 (YOG). This batch was followed by batch 2 (YEA), batch 4 (YEA + YOG) and batch 1 (STA). For the ratio used, palm wine yeast and yogurt probiotics combined had a detrimental effect on the weight and the feeding efficiency index.

Aftahi *et al.* (2006) studied the indulgence of yoghurt and protexin boost on broiler growth, feed intake, feed conversion ratio, livability and profitability production from 1 to 35 days of age of broiler chicks. She concluded that yoghurt (5g/liter of drinking water) and protein boost (1g/liter of drinking water) could economize broiler production.

Hossain (2004) revealed that yoghurt and protein boost could not show any beneficial effect on broiler performance at the level tested but was effective in redacting abdominal fat pad, total viable count (TVC) and total coliform count (TCC) while increased bursa weight and length of small intestine. Bhatt *et al.,* (1995) fed 4 strains of *Lactobacillus bulgaricus* (6.8×10^{10} cells/kg feed) in diet of broiler up to 6 weeks of age and observed that only one strain improved survivability during finishing period.

2.8 Organic acid or acidifiers

The acid that originates naturally is called organic acid. Organic acid and their derivatives act as non-therapeutic additives. They are commonly used as acidifiers. Acidifiers played a dual role within agriculture. Acids and their salts have long been used for to preserve food and feedstuffs. There are several types of organic acids like butyric acid, propionic acid, acetic acid, lactic acid, fumeric acid, malic acid and ascorbic acid etc. FraAC34 the trade name of organic acidifiers , it contains monoglycerides of fatty acids and has strong antimicrobial properties, enhances bird's immunity, balances gut's micro flora, controls diarrhoea and increases productivity & decreases mortality.

2.8.1 The advantages of acidifiers

a) It is an effective preservative
b) Its antimicrobial efficiency is generally improved with increasing chain length and degree of instauration.
c) It lowers the pH

2.8.2 Functions of acidifiers

- Maintain an optimum pH in the stomach, allowing correct activation and function proteolytic enzymes.
- Optimize protein digestion in the stomach
- Stimulate feed consumption by improving palatability of feed.
- Inhibit the growth of pathogenic bacteria, yeasts and moulds
- Improve protein and energy digestibility by reducing microbial competition with the host of nutrients, as well as endogenous nitrogen losses
- Lower the incidence of sub clinical infections

2.8.3 The special properties of FraAC34

a) It contains monoglyceride of Propionic acid (Monopropionin), monoglyceride of Butyric acid (Monobutyrin) and encapsulated essential oils.
b) It has no pungent smell and stable molecule up to temperature of 200 degree Celsius.
c) It can resist all feed processing without loss activity.
d) It is stable at different pH's.
e) It has effect to control Salmonella.
f) It has polar molecule and can be administered via drinking water.
g) It has strong antibacterial properties against pathogens like *E.coli, Clostridia, Salmonella, Streptococcus* etc. These monoglycerides are taken up in the bloodstream to protect the animals against negative effect of the pathogens.
h) It is a good alternative to replace antibiotic growth promoters.
i) Monoglycerides of fatty acids have much stronger antibacterial properties than the pure acid.

2.8.4 Organic acid (acidifiers) and broiler performance

Hresko Samudovska, *et al.* (2017) determined the effect of acidifier added to drinking water on growth rate, performance index, flock uniformity, weight of edible giblets and immune organs, fermentation process in the caecum and excretion of dry matter and crude protein in broiler chicks. They found acidification of drinking water had positive effect on growth rate during finisher phase ($P<0.05$) and reduction of crude protein in faeces ($P<0.001$). Although not statistically significant, water acidification increased flock body weight uniformity. No significant effect of water acidification were observed on performance index, weight of edible giblets and immune organs, pH and concentration of short chain fatty acids in caecum content as well as content of dry matter in faeces.

Vinus *et al.* (2017) observed that organic acids and their salts are able to inhibit microbial growth in the food and consequently to preserve the microbial balance in the gastrointestinal tract. Modifications to the gastrointestinal microflora which reduce pathogen attachment may have a profound effect on the structure of the intestinal wall. Butyric acid is one such SCFA, which has higher bactericidal activity when the acid is undissociated. Butyrate also appears to play a role in development of the intestinal epithelium. Bakheit M. Dousa, *et al.* (2016) was carried out an experiment to study the effect of probiotics, acidifiers and their combination on broiler growth performance and blood chemistry. The results showed that statistically there was no significant increase in body weight gain at weeks 1, 3, 4, 5 and 6 of age. The body weight of broiler chickens was significantly increased at weeks 2 of age when adding 0.05% bacillus subtilis. It concluded that inclusion of probiotic and probiotic plus acidifier to broiler chickens diets improved live body weight, blood serum parameters were not affected except blood calcium and glucose.

Kim JW, Kim JH, Kil DY (2015) observed the 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). Bactericidal effects of dietary organic acids have been observed for pathogenic bacteria and even for beneficial bacteria to some extent. 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. Vitor Barbosa Fascina et al. (2012) evaluated the influence of isolated or associated phytogenic additives (PA) and organic acids (OA) on nutrient digestibility, performance and carcass characteristics of broiler chickens. The phytogenic additives and organic acids, isolated or associated, improve the nutrient digestibility of the diet and replace the growth promoting antibiotics. The use of organic acids isolated or associated with phytogenic additives in broiler diets improves broiler performance in comparison with free antibiotic performance enhancer at 42 days of age.

A. Galib Al-Kassi and M. Aqeel Mohssen (2009) conducted an experiment to compare the effects of single and synergistic organic acids (formic and propionic acids) on broiler performance. Three hundred one day-old mixed sexes broiler (Arbor-Acres) were divided into five groups of 60 birds each and randomly assigned to five treatment diets. A control group is considered where no added acids. Group 2 and group 3 are formed with 0.1 % formic acid and 0.2% propionic acid respectively. The forth group is formed with 0.3% organic acids (formic and propionic acids). Group 5 is formed by the addition of 0.3% biotronic acid. The results indicated that group 2, 3, 4 and 5 showed significantly higher ($p<0.05$) in average live weight, average daily gain, average daily feed consumption and mortality rate compared with the control group. Nevertheless at the same time treatment 4 showed significant decrease in the feed conversion ratio compared with other treatments.

Nezhad *et al.* (2007) studied the effect of citric acid and phytase on performance and utilization of dietary nutrient in broilers fed a corn-soybean meal diet with 252 one day old broilers were fed with different levels of citric acid and found that live weight gain and feed conversion efficiency improved on diets supplemented with citric acid ($P<0.05$). No significant effect was observed on feed intake, ash, and carcass yield, With regards to the effect of the integration between citric acid and microbial phytase, significant improvement on live weight gain and feed conversion efficiency were also observed.

Zhang *et al.* (2005) carried out an experiment by feeding fumeric acid, citric acid and malice acid together. They reported that the organic mixture had better body weight gain, Shen *et al.* (2005) studied with One hundred and twenty-three Yellow chickens (aged 1 day) were randomly divided into 4 groups (groups I, II, III and IV). The control group was fed on the basal ration, and those in the group II, III and IV on the basal ration supplemented with citric acid at 0.3 0, 0.5 and 0.7% respectively. The production performance was investigated at 42 days. The highest average daily gain, feed conversion rate and survival rate were recorded at 0.3% citric acid. Chitra et al., (2004) obsedved the effect of probiotic with ascorbic acid on the carcass characteristics and economics of broiler production in summer season. They found better body weights, feed conversion efficiency, livability and dressing percentage due to probiotic and Ascorbic Acid Supplementation. feed consumption, FCR and Survivability.

Arefin (2002) conducted an experiment to find the immunopotentiality of nutrilac in chicken. He reported to vaccination, reduces gut pH and make balance gut micro flora resulting improved FCR, body weight and reduced early chick mortality. Samanta *et al.,* (1995) studies with 120 unsexed day old commercial broilers to assess the effect of feeding probiotic and pure lactic acid (0.25% in drinking water) on the performance of broiler. They reported that treatments had no significant effect on the growth performance of broilers. Izat *et al.,* (1998) suggested that feed conversion and carcass quality of broiler can be enhanced by dietary organic acid as an alternative of antibiotics. The addition of organic acid to the diet of broiler chickens changes the pH within intestine (Kahraman *et al.,* 1999). Muzaffer *et al.,* (2003) studied the effect of organic acid along with probiotic. They reported that organic acid group showed better weight gain, feed efficiency FCR and good carcass quality than control and others.

An experiment was conducted by Kahraman *et al.* (1998) to determine the effects of an organic acid combination (Acid Lac Dry: lactic, fumaric, propionic, citric and formic acid) and/or zine bacitracin on the body weight gain, feed efficiency, microflora and pH in the ileum content of broilers. They reported that organic acids with zine bacitracin significantly ($P<0.05$) increased body weight at 3 weeks of age but not at 6 weeks of age when compared with control and organic acid combination (Dry supplemented). The best feed to gain ratio was obtained in group fed organic acid combination+ Zinc bacitracin supplemented diet during this experiment. Carcass weight and dressing percentage were not affected by any treatment. Mean ileal pH was significantly ($P<0.05$) higher in group fed the additives in combination than the animals treated with cither organic acid (Lac Dry) or zinc bacitracin. However, the combination had the lowest number of Enterobacteriaceae in the intestinal material. In Malaysia, Kassim *et al.*, (1995) reported that additive of acetic acid to diet (400 or 600 mg/kg) improved body weight and feed efficiency.

Research Gaps and the Present Study

Synlac is one of the most widely used probiotics marketed in Bangladesh by ACI Animal Health (Bangladesh) Limited. A large number of works with probiotics has been conducted in abroad since 1970's. Some researchers have claimed beneficial effects of probiotics on the performance of broilers while others found no response. So, the present study was undertaken to evaluate the effect of Synlac, yoghurt and FraAC34 on the broiler performance.

CHAPTER-III
MATERIALS AND METHODS

3.1 Statement of Research Work

The experimental work was conducted at a poultry farm in Dhupchachia, Bogura to investigate the effect of probiotics (Synlac), yoghurt and acidifiers (Fra AC34) on the performance of broiler chicks (Lohman meat). The trial was conducted from 1st october to 5th November, 2018.

3.2 Preparation of the experimental house and equipment

The experimental house was divided into 12 small pens of equal size (6 ft × 2.5 ft= 15 sq. ft) for 10 birds in every pen. As a result the floor space for each bird was 1.5 sq. ft. In commercial broiler farming in Bangladesh each bird is given 1.1-1.3 sq. ft space according to season. But for small confined pens in this experiment we provided more space for each bird. The experimental house was properly cleaned and washed by forced water using a hose-pipe. After two weeks the room was disinfected with Timken disinfectant. At the same time all feeders, plastic buckets, waterers and other necessary equipments were also properly cleaned ,washed and disinfected with bleaching powder solution and Timken solution , subsequently dried and left them empty for two weeks before the arrival of chicks.

3.3 Collection of probiotic, yoghurt and acidifiers

A commercial probiotic “Synlac” and Acidifier “Fra AC 34” were purchased from local market which was marketed by ACI Animal Health Ltd. Yoghurt was also purchased from local market.

3.4 Collection of the birds

One hundred twenty Lohman broiler chicks (Day old) were procured from Aman Poultry and Hatchery limited.

3.5 Source of feed

The experimental broiler chicks were supplied Broiler starter feed from day 1 to day 15 and broiler grower feed from day 16 to last day of the experiment. Both the starter and grower feed was collected from Aman feed limited. The starter feed was in crumble and grower feed was in pellet form. The nutritional value of the Aman Broiler Feed was collected from the company profile that is presented in Table 3.1

Table 3.1 Nutritional value of Aman Broiler Feed

Nutritional Ingredients	**Starter feed**	**Grower feed**
Moisture % (maximum)	11	11
Crude protein %(minimum)	22.5	21
Metabolic Energy (minimum)Kilocal/kg	3000	3100
Crude Fiber% (maximum)	3	3
Fat% (minimum)	4-5	5-6
Calcium%(minimum)	1	0.96
Phosphorus%(minimum)	0.50	0.48
Methionine%(minimum)	0.50	0.48
Lysine%(minimum)	1.3	1.25
Methionine+Cystine(minimum)	0.89	0.84
Sodium%(minimum)	0.20	0.17
Chloride%(minimum)	0.2	0.2
Vitamins and minerals	Standard level	Standard level

3.6 Layout of the experiment

120 chicks were randomly distributed into 4 treatment groups A, B, C and D with three replications in each treatment. The number of birds in each replication was 10. Group A, B and C were given 1g Synlac/2L, 5g yoghurt/1L and 1ml Fra AC34/1L drinking water respectively, and group D was kept as control. The layout of experiment is shown in Table 3.2

Table 3.2 Layout showing the distribution of experimental birds

Treatments	**Number of birds in each replication**			**Total**
	R_1	R_2	R_3	
Group A (1g Synlac/2L water)	10	10	10	30
Group B (5g yoghurt/1L water)	10	10	10	30
Group C (1ml FraAC34/1L water)	10	10	10	30
Group D (Control)	10	10	10	30
Total number of birds	40	40	40	120

3.7 Management of experimental birds

The care and management practices were followed according to following description throughout the experimental period. All the management practices were identical to all treatment groups during the experiment.

3.7.1 Feed and water management

The experimental birds were given Aman broiler starter feed from day 1 to day 15 and Aman broiler grower feed from day 16 to day 35. During the first three days chicks were given feed on newspaper at three hours interval and then on tray feeders up to 9 days old. From day 10, every small pen was provided with a round feeder. Feed was given four times from day 4 to day 14. From day 15 feed was given thrice a day. Water was served *ad libitum* four times a day with a round drinker in each replication. Feeders and waterers were set up in such a way that the birds were able to reach them conveniently. Feeders were cleaned at the end of each week and waterers were cleaned twice daily.

3.7.2 Litter management

Fresh, clean and dried rice husk was used as litter materials at a depth of 2 inch. The litter of each pen was covered with clean newspaper up to day 10. At day 15 the upper part of the litter with droppings were removed and regularly stirred. The litter material was disinfected with Virocid spray (3 ml/L water) in every alternate day. Litter materials, when found wet for any reason, were removed to prevent dampness that could accelerate ammonia and other harmful gases**.**

3.7.3 Brooding management

During brooding period the chicks were provided heat by electric bulbs. A 100 watt bulb was hung in each replication. The temperature was maintained at 34 ^{0}C during the first week and then gradually decreased. The electric bulbs were hanged just above the bird's level and were moved up and down to adjust the heat. The room temperature and humidity were measured by an automatic digital thermo-hygrometer. For maintaining room temperature the house was covered by cloth curtain leaving 6 inch to 1 feet gap at the top for ventilation purpose.Before placing the birds into brooder the temperature was adjusted at optimum level by preheating the brooder about 3 hours earlier of the chick's arrival.

3.7.4 Lighting

The house was provided a light period of 24 hours during first three weeks. After day 21 the light period was 23 hours per day.

3.7.5 Immunization

The experimental birds were vaccinated according to the following schedule of table 3.3

Table: 3.3 Vaccination programs of the experimental birds.

Age of the bird (Day)	Name of vaccine	For specific disease	Route of administration
3	Cevac BI L	Bronchitis and Newcastle Disease	Eye drop
10	Cevac IBD L	Gumboro	Eye drop
18	Cevac IBD L	Gumboro	Eye drop
21	Cevac NEW L	Newcastle Disease	Eye drop

Vaccines were procured from ACI Animal Health Ltd.

3.7.6 Medication

Synlac, yoghurt and FraAC34 were supplied to the respective treatment groups. Besides these in drinking water no other antibiotics or growth promoter was used during the experiment. The day old chicks were supplied glucose and vitamin C for 6 hours at a dose of 50 gm per litre drinking water as they were stressed due to journey. The adult birds were supplied saline and vitamin C to reduce stress whenever the mid day temperature was too high.

3.7.7 Sanitation

Adequate hygiene and sanitation were maintained during the experimental period. The entrance point and surroundings of the farm were kept clean and Virocid solution were sprayed on daily basis.

3.7.8 Bio-security

Strict biosecurity measures were taken during the experiment. Equipments were cleaned and disinfected regularly. Entrances of people were restricted except relevant personnel's. Before entrance hands were washed with soap and separate shoes were used. Virocid spray was used for disinfection. Adequate precautions were taken in case of vaccination. Dead birds were buried away from the farm and sick birds were isolated immediately to a separate place from the experimental pens. The farm was kept free of rats, cats, dogs and wild animals.

3.8 Processing of broilers

At the end of the experiment the weight of the birds was taken and average body weight was calculated. Two birds from each pen were randomly selected for determining meat yields. All birds from each treatment were kept without feed for 12 hours prior to slaughter. But water was supplied *ad libitum* to facilitate bleeding. After complete bleeding birds were immersed in hot water (about 55 ^{0}C) for two minutes for proper defeathering. The feathers were removed by hand and again individual weight of the birds was taken. After that processing was performed by removing head, shank, viscera, oil gland, kidneys and lungs with the help of knife. Heart and gizzard were removed from the remaining parts by cutting them loose. The gall bladder was cut off from the liver and pericardial sac and arteries were cut by knife from the heart. After removal of gizzard by cutting it in front of the proventriculus, it was split open with knife and the fecal materials were removed. The gizzard was washed with clean water and the lining was removed by hand. Then the thymus glands were separated from both sides between head and neck region. The Bursa of Fabricious which is located close to the vent was separated out with the tip of the knife.

3.9 Data collection and record keeping

The following data was recorded throughout the experimental period.

3.9.1 Body weight

The chicks of each replication were weighed at beginning of the experiment. After that birds of all replications were weighed every week in the morning at 7 AM prior to feeding and finally weight was taken at day 35. Replication-wise weekly average body weight was recorded.

3.9.2 Body weight gain

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

3.9.3 Feed consumption

The amount of feed consumed by the birds of different replications were calculated from the amount of supplied feed at each week and the amounts that were retained at the end of the week. Feed intake was adjusted for the birds which died during the experiment by necessary calculation.

3.9.4 Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated by dividing the cumulative feed consumption by average body weight up to certain period of production.

3.9.5 Temperature and relative humidity

The temperature and relative humidity of the experimental house and respective pens were recorded four times a day during the whole experimental period at 6 am, 12 pm, 6pm and 12 am with the help of an automatic digital thermo-hygrometer.

3.10 Statistical analysis

Data were analyzed by Analysis of Variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). The significance differences between the treatment means were calculated by the Duncan's Multiple Range Test (Duncan, 1955). All analysis were performed by SPSS Program. The level of significance was set > 0.05.

Picture Gallary

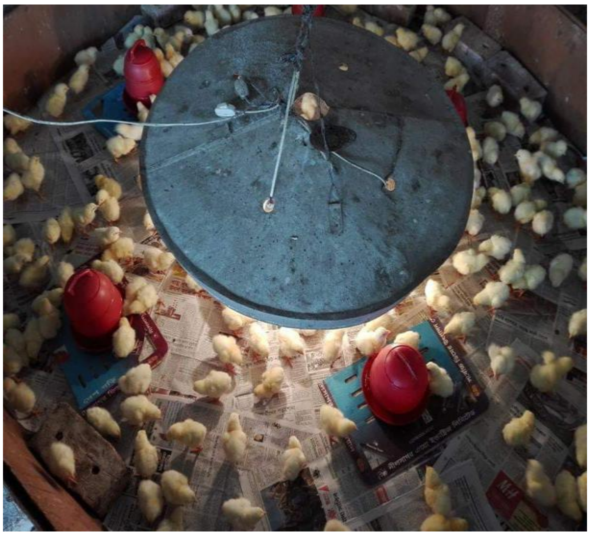


Fig. 1: Brooding management

Fig. 2: Examination of Day old chick

Fig. 3: Different groups of broiler

Fig.4: Feed supply in different groups

Fig. 5: Litter management

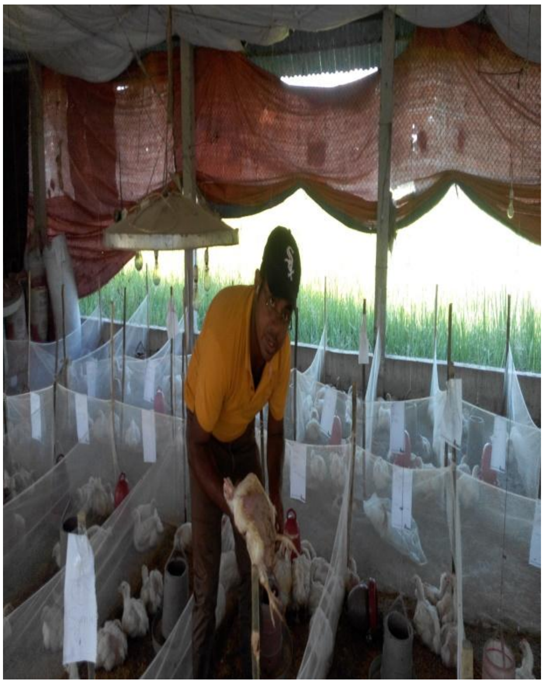


Fig. 6: Health examination

Fig.7: 35 day’s broiler and selling of broiler

CHAPTER-IV
RESULTS AND DISCUSSION

4.1 Performance of broiler

The results of productive performance in term of body weight gain, feed consumption, and feed conversion ratio of birds supplied Synlac, yoghurt and FraAC34 in drinking water are presented and discussed in the following sections.

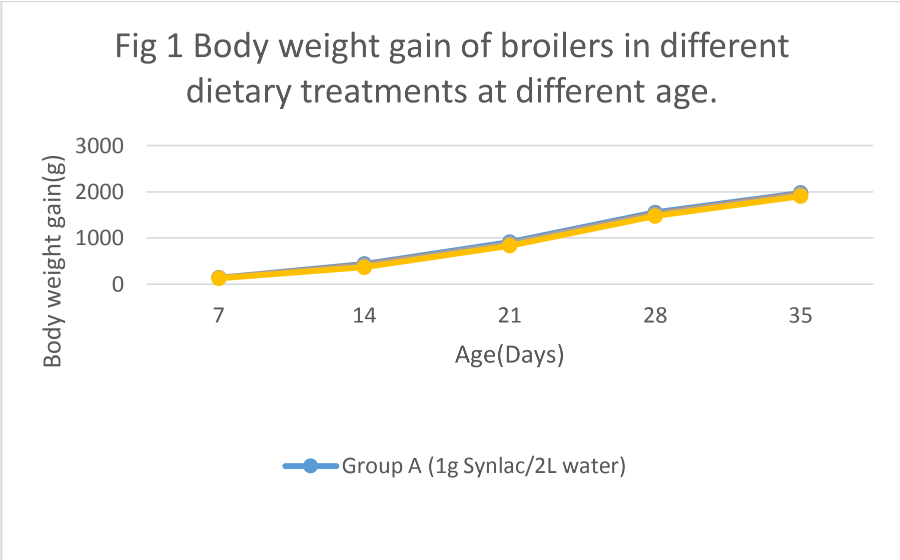
Table 4.1 Productive performance of broilers receiving Synlac, yoghurt and FraAC34 in drinking water (0-35 days)

Variables	Group A (1g Synlac/2L water)	Group B (5g yoghurt/1L water)	Group C (1ml FraAC34/1L water)	Group D (Control)	Sig.
Initial body weight (g/bird)	40 [a] ±1.00	40 [a] ±1.00	40.33 [a] ±0.58	40 [a] ±1.00	NS
Final body weight (g/bird)	2021.67 [b] ±36.86	1991.67 [a b] ±20.82	1986.67 [a b] ±2.89	1946.67 [a] ±37.86	NS
Body weight gain (g/bird)	1981.67 [b] ±37.81	1951.67 [a b] ±6.66	1946.33 [a b] ±2.31	1906.67 [a] ±37.74	NS
Feed consumption (g/bird)	3186.67 [a] ±32.15	3138.33 [a] ±68.25	3106.67 [a] ±50.33	3104 [a] ±90.27	NS
FCR	1.58 [a] ±0.036	1.58 [a] ±0.032	1.56 [a] ±0.025	1.59 [a] ±0.045	NS

Different variables under different programs indicate average ±SD, NS= Non-significant.

4.1.1 Body weight gain

Table 4.1 shows the initial body weight and final body weight and the weight gain differences among all treatment groups. There is no significant differences among the results (P>0.05). Initial body weight of day old chicks were almost similar. The final body weight and weight gain were highest in the Group A (Synlac), then Group B, C and lowest in the group D (control). Body weight gain was 1981.67 ±37.81g, 1951.67 ±6.66g, 1946.33 ±2.31g and 1906.67 ±37.74g in Group A, B, C and D respectively. Figure 1 shows the body weight gain of broilers in different treatment groups at different age. These results are agreed Kritas *et al.* (2008); Falcao-e-Cunha *et al*. (2007); Mourao JL. (2007); Copeland *et al.* (2009); Mateos GG, *et al.* (2010); Combes *et al.* (2012), Rotolo L *et al*. (2014).They also found non significant improvement of live weight gain through probiotics.



4.1.2 Feed consumption

Cumulative feed consumption (g/bird) in Group A, B, C and D were 3186.67±32.15g, 3138.33 ±68.25g, 3106.67 ±50.33g and 3104±90.27g respectively (table 4.1). It is clear that synlac, yoghurt and fraAC34 did not significantly affect feed consumption but slightly improved it in broiler than control. Higher feed consumption in Probiotic supplemented group was in agreement with the results of Mateos *et al.* (2010); Combes *et al.* (2012); Marounek *et al.* (2007) and Rotolo L, *et al.* (2014). In the current study tendency of better feed consumption was observed in synlac, yoghurt and FraAC34 than control. The results are partially consistent with the observation of Nezhad *et al.*, (2007) who found non significant improvement in feed intake by feeding organic acid. 4.1.3 Feed Conversion Ratio (FCR) FCR of Group A, B, C and D were 1.58±0.036, 1.58±0.032, 1.56±0.025 and 1.59 ±0.045 respectively at the end of the trial which are shown in table 4.1. The result is non significant though the other groups had slightly better FCR than control. This was in close agreement with the observation of previous researchers Mohan et al.(1996); Yeo and Kim (1997); Lima *et al.* 2002 and Priyankarage *et al.*, (2003); Ergun *et al.* (2000). The effect of organic acid on better FCR was observed by Arefin (2002); Muzaffer *et al.*, (2003); and Zhang *et al*., (2005).

4.1.4 Mortality

The mortality in all treatment groups were zero. The livability was 100%. The results agreed with Zhang *et al.*, (2005); Aftahi *et al.* (2006) and Watkins *et al.*, (1982). This might be due to strict biosecurity and strong flock management, proper vaccination which enhanced the immunity.

CHAPTER-V

SUMMARY AND CONCLUSION

An experiment was carried out to evaluate the effect of supplementation of Synlac, yoghurt and acidifier (FraAC34) on the performance of broiler. One hundred twenty Lohman meat broiler chicks (Day old) were procured from Aman Poultry and Hatchery limited. The Synlac, yoghurt and FraAC34 were purchased from local market. The chicks were randomly distributed to four treatment groups A, B, C and D, having 3 replications each. Group A, B and C were given 1g Synlac/2L, 5g yoghurt/1L and 1ml Fra AC34/1L drinking water respectively, and group D was kept as control. The experimental broiler chicks were supplied Broiler starter feed from day 1 to day 15 and broiler grower feed from day 16 to last day of the experiment. Both the starter and grower feed was collected from Aman feed limited. Feed and water were served adlibitum to the chicks throughout the experiment period. Identical care and management were followed for all treatment groups.

At 35 days the body weight gain at different dietary treatments were 1981.67g, 1951.67g, 1946.34g and 1906.67g in Group A, B, C and D respectively and the difference was non-significant. Feed consumption was higher in Group A but there was no significant difference among the different treatments. Feed consumption during the whole period (0-35 days) were 3186.67g, 3138.33g, 3106.67g and 3104g in Group A, B, C and D respectively. FCR during the experimental period (0-35 days) were 1.58, 1.58, 1.56 and 1.59 in Group A, B, C and D respectively. Not any bird died during the experimental period, so livability was 100%. The production performance was higher in synlac, yoghurt and FraAC34 than control but no significant difference was found.

So it may be concluded that, Probiotic (Synlac), yoghurt and acidifier (FraAC34) improved the production performance slightly but did not have any significant improvement in the performance of commercial broilers.

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APPENDICES

Appendix 1: Body weight (g/bird) of broilers at different treatments

Treatments	Replication	Age(days)					
		D-1	**D-7**	**D-14**	**D-21**	**D-28**	**D-35**
Group A (1g Synlac/2L water)	R_1	41	187	482	950	1600	1980
	R_2	39	183	480	960	1590	2050
	R_3	40	180	485	955	1595	2035
Mean		40	183.33	482.33	955	1595	2021.67
Group B (5g yoghurt/1L water)	R_1	41	180	452	910	1565	2000
	R_2	39	182	450	925	1555	1985
	R_3	40	185	455	920	1560	1990
Mean		40	182.33	452.33	918.33	1560	1991.67
Group C (1ml FraAC34/1L water)	R_1	41	178	450	910	1560	1990
	R_2	40	180	450	920	1550	1985
	R_3	40	182	445	915	1555	1985
Mean		40.33	180	448.33	915	1555	1986.67
Group D (Control)	R_1	40	170	410	870	1520	1990
	R_2	41	167	400	880	1510	1930
	R_3	39	165	405	875	1515	1920
Mean		40	167.33	405	875	1515	1946.67

Appendix 2: Body weight gain (g/bird) of broilers of different treatments

Treatments	Replication	Age(days)				
		0-7	0-14	0-21	0-28	0-35
Group A (1g Synlac/2L water)	R_1	146	441	909	1559	1939
	R_2	144	441	921	1551	2011
	R_3	140	445	915	1555	1995
Mean		143.33	442.33	915	1555	1981.67
Group B (5g yoghurt/1L water)	R_1	139	411	869	1524	1959
	R_2	143	411	886	1516	1946
	R_3	145	415	880	1520	1950
Mean		142.33	412.33	878.33	1520	1951.67
Group C (1ml FraAC34/1L water)	R_1	137	409	869	1519	1949
	R_2	140	410	880	1510	1945
	R_3	142	405	875	1515	1945
Mean		139.67	408	874.67	1514.67	1946.34
Group D (Control)	R_1	130	370	830	1480	1950
	R_2	126	359	839	1469	1889
	R_3	126	366	836	1476	1881
Mean		127.33	365	835	1475	1906.67

Appendix 3: Cumulative feed consumption (g/bird) of broilers of different treatments

Treatments	Replication	Age(days)				
		0-7	0-14	0-21	0-28	0-35
Group A (1g Synlac/2L water)	R_1	140	460	1200	2245	3200
	R_2	144	469	1230	2255	3210
	R_3	142	465	1250	2190	3150
Mean		142	464.67	1226.67	2230	3186.67
Group B (5g yoghurt/1L water)	R_1	145	460	1250	2240	3200
	R_2	140	465	1210	2210	3150
	R_3	140	470	1205	2200	3065
Mean		141.67	465	1221.67	2216.67	3138.33
Group C (1ml FraAC34/1L water)	R_1	143	455	1207	2116	3060
	R_2	140	460	1200	2227	3160
	R_3	141	465	1190	2150	3100
Mean		141.33	460	1199	2164.33	3106.67
Group D (Control)	R_1	140	460	1205	2115	3162
	R_2	139	465	1200	2222	3000
	R_3	140	460	1185	2145	3150
Mean		139.67	461.67	1196.67	2160.67	3104

Appendix 4: Cumulative feed conversion ratio of broilers at different treatments

Treatments	Replication	Age(days)				
		0-7	0-14	0-21	0-28	0-35
Group A (1g Synlac/2L water)	R_1	0.75	0.95	1.26	1.40	1.62
	R_2	0.79	0.98	1.28	1.42	1.57
	R_3	0.79	0.96	1.31	1.37	1.55
Mean		0.77	0.96	1.28	1.40	1.58
Group B (5g yoghurt/1L water)	R_1	0.81	1.02	1.37	1.43	1.60
	R_2	0.77	1.03	1.31	1.42	1.59
	R_3	0.76	1.03	1.31	1.41	1.54
Mean		0.78	1.03	1.33	1.42	1.58
Group C (1ml FraAC34/1L water)	R_1	0.80	1.01	1.33	1.36	1.54
	R_2	0.78	1.02	1.30	1.44	1.59
	R_3	0.77	1.04	1.30	1.38	1.56
Mean		0.79	1.03	1.31	1.39	1.56
Group D (Control)	R_1	0.82	1.12	1.39	1.39	1.59
	R_2	0.83	1.16	1.36	1.47	1.55
	R_3	0.85	1.14	1.35	1.42	1.64
Mean		0.83	1.14	1.37	1.43	1.59