EFFICACY OF YOGURT AND PANAMIN® LIQUID IN BROILER PRODUCTION

MS THESIS

BY

MD. ASADULLAH AL-GALIB

Registration No. 1505258

Session: 2015-2016

MASTER OF SCIENCE (MS)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2017

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Submitted to the department of Physiology and Pharmacology,

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December, 2017

Dedicated to

My

Beloved Parents

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The Author *December, 2017*

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LIST OF ABBREVIATIONS

B.wt.	:	Body weight
Conc.	:	Concentration
cu mm	:	Cubic millimeter
ESR	:	Erythrocytes Sedimentation Rate
et al.	:	Associates
Fig.	:	Figure
FCR	:	Feed Conversion Ratio
gm	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
j.	:	Journal
kg	:	Kilogram
lit	:	Litre
Ltd.	:	Limited
mg	:	Milligram
ml	:	Milliliter
mm^3	:	Cubic millimeter
No	:	Number
PBS	:	Phosphate Buffer Solution
PCV	:	Packed Cell Volume
SD	:	Standard Deviation
SEM	:	Standard Error Mean
SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
±	:	Plus minus

°C : Degree centigrade

ABSTRACT

This study was conducted to investigate the effect of yogurt (as probiotic) and commercially available Panamin® (as amino acid) on the overall performance of broiler chicks, measuring the body weight gain, feed intake, feed conversion ratio and blood parameter during the period from 25th February 2017 to 24th March, 2017at the area Hatimara, Kashimpur, Gazipur under the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Thirty day old broilers chicks were randomly distributed into 3 main groups T_0, T_1 and T_2 comprising of 15 birds each group. Commercial ration was fed *ad libitum* to all the three groups. The yogurt (as probiotic) and Panamin® (as amino acids) were given at the rate of 5 gm/L of water and 1 ml/L of water to group T_1 and T_2 respectively while group T_0 was kept as control. Group T_2 supplemented with Panamin® showed higher live weight at day 20th and 30th which was significantly ($p \leq 0.05$) different from control group. The FCR value of T_0, T_1 and T_2 was 1.62, 1.56 and 1.49 respectively. FCR value of T_2 group was slightly better than other group and economics of production were analyzed and found that net profit per broiler was Tk. 0.15, Tk. 6.18 and Tk. 13.86 respectively. Birds of each group and subsequent hematological analysis were done. TEC and Hb value are significantly increased ($p \leq 0.05$) in T_2 group than control group. It was found that birds supplemented with Panamin® possess positive effect on growth performance and blood parameter in broiler.

Key words: Broiler, Yoghut, Panamin®, FCR

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Feed is a major component, affecting net return from the poultry business, because 60 to 75% of the total input, in terms of money is spent on feed purchase (Asghar *et al.,* 2000; Farooq *et al.,* 2001). To ensure more net return and to minimize high expenses on feed, are the main challenges, for which many research strategies have been practiced such as introducing feed supplements and feed additives etc. (Parvez, 1992). Different feed additives are used either in feed or water to improve growth and production performance of poultry birds. Probiotics is one of the effective feed additives. Probiotic is derived from Greek word probiosmeaning for life. A probiotic is a live microbial feed supplement, which quickly establishes in the gut to suppress colonization and growth of harmful bacteria and improving its intestinal microbial balance (Fuller, 1992). There are different types of bacteria that are found to have appositive key role on the performance of poultry birds.

The poultry industry has developed in several areas such as nutrition, genetics, management to maximizing the efficiency of growth performance and meat yield. However, the poultry industry has focused more attention towards addressing public concern for environmental and food safety. Animals including poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli, Salmonella ssp., Clostridium perfringens* and *Campylobacter sputorum*. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al.,* 2000). This leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies (Truscott and AI-Sheikhly, 1977; Miles *et al.,* 1984; Waldroup *et al.,* 1985).

However, the antibiotic growth promoters have been under scrutiny for many years and have been removed from the market in many countries (Ratcliff, 2000). Their usefulness has seldom been contested, it is their relatedness with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that causes concerns (Philips, 1999). In light of that situation, the feed manufacturers and the animal growers have been actively looking to an efficacious alternative to antibiotic growth promoters. Probiotics and organic acids are the most promising alternative to antibiotics. Probiotics are viable microbial additives which assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes (Green and Sainsbury, 2001). It was reported that probiotics benefit the host animal by stimulating synthesis vitamins of B-groups, improving immunity stimulation, preventing harmful microorganisms, providing digestive enzymes and increasing of production of volatile fatty acids (Fuller, 1989; Rolfe, 2000; Coates and Fuller, 1977). However, acidification with various weak organic acids to diets such as formic, fumaric, propionic, lactic and sorbic have been reported to decrease colonization of pathogen and production of toxic metabolites, improve digestibility of protein and of Ca, P, Mg and Zn and serve as substrates in the intermediary metabolism (Kirchgessner and Roth, 1988). Several studies demonstrated that the supplementation of organic acid or probiotic to broiler diets increased the growth performance, reduced diseases and management problems (VIademirova and Sourdjiyska, 1996; Jin *et al.,* 1998; Vogt *et al.,* 1981; Runho *et al.,* 1997).

Yogurt a dairy product can be used effectively as probiotic. Yogurt contains mainly lactobacilli and other beneficial bacteria that have strong positive health effects. Yogurt could aid digestion and inhibit the development of pathogens by improving the balance of microbes living in the digestive tract (Metchnikoff, 1998). Lactobacillus one of many friendly species of intestinal microflorais considered as beneficial bacteria in its ability to aid in the breakdown of proteins, carbohydrate and fats in food and help absorption of necessary elements and nutrients such as minerals, amino acids and vitamins by the host. They lower the pH of the gut by converting sugar to lactic acid, which inhibits the growth of enter pathogens. Lactobacillus sp. can get quickly colonized in the gut epithelium to deprive sites for attachments of pathogens. They have immunoregulatory actions by increasing macrophage activity and also by enhancing the production of irnmnnoglobulins.

Panamin is the product of amino acid and trace element mixture, which has recently been introduced in market. Incompatibles with all feed compounds and additives for animal nutrition. Optimizes the nutritional value of other feed proteins. Minimizes quality variations of the flock. Maximizes production and production yields. It confers maximum performance to poor diets or when feed intake is reduced. However, there is paucity of information regarding the efficacy and beneficial effects of Panaminin poultry. Without any tangible evidence from empirically derived data, the adoption of this innovation in the poultry industry cannot be guaranteed.

In poultry ration, along with the vitamins and minerals, proteins have been playing critical role in development of musculature. One of the most important areas is amino acid nutrition. Amino acids are the basic constituents of living matter because they are building blocks of proteins and their proper balance in the diet is required to maintain protein quality, feed consumption, growth rate and carcass composition and as a result receiving considerable attention in industry (Baker, 2009). Methionine, choline and lysine are universally recognized as the most limiting amino acids in broiler diets based on corn and soybean meal (Baker, 1997; Han and Baker, 1991). The supplementation of broiler feeds with these amino acids is very common in the poultry industry. Synthetic methionine and choline are listed among the prohibited synthetic substances and its usage has been questioned in organic farming practices (Fanatico *et al.,* 2009). The concept of organic farming recently came up with various objectives in general and to avoid residual effects of synthetic drugs in particular. Nearly 80% of world's population relies upon herbal medicine for basic health care needs. Many herbs are rich source of these essential amino acids and also mimic the activity like that of methionine, choline or biotin and may be supplemented along with ration to replace synthetic in feed.

Objectives:

The specific objective of this study is to determine

1. To observe the effect of yogurt and panamin® on growth performance in broilers.
2. To know the alteration of hematological parameters in broiler after supplementation of yogurt and panamin®.
3. To evaluate the compare of cost effectiveness between yogurt and panamin®.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

2.1 Ideal amino acid ratios for broiler chicks

Poultry meat and eggs are poised to play a greater role than present, in global food security in the coming years, as the preferred and primary protein source. Efficient production requires precision, of which dietary amino acids are central. As primary poultry breeders continue to provide broiler strains that have improved annual growth, health, and muscle accretion with lower feed intake, nutritionists should continue to assess digestible Lysine needs and subsequent ratios of essential amino acids to digestible Lysine.

Amino acid requirements ratio to lysine have become a popular means of expressing requirements, particularly since factors such as protein accretion potential (i.e., bird strain) and dietary energy density have little effect on the ideal ratios. However, period of growth (i.e., age), gender, and criterion of response can have important effects on particular ratio. Extensive studies with lysine, for example, have shown that males have higher requirements than females, and maximal feed efficiency (i.e., gain: feed ratio) requires a higher dietary level of lysine than maximal body weight gain (Dozier III *et al.,* 2008; Garcia *et al.,* 2006; Han and Baker, 1994; Mack *et al.,* 1999). The Met requirement for maximal feed conversion efficiency may also be higher than the need for maximal weight gain (Mack *et al.,* 1999). One would think that if sex and criterion of response affect lysine and SAA requirements, all other indispensable amino acids would be similarly affected. However, this appears not to be the case. Baker *et al.* (2002) in studying lysine, tryptophan, threonine, isoleucine, and valine requirements of young male chicks found that only lysine was required in higher concentration for maximal gain: feed than for maximal weight gain. Earlier work with threonine and tryptophan also showed that the requirement of broiler chicks was similar for both maximal weight gain and maximal feed efficiency (Rosa *et al.,* 2001a, Bashan *et al.,* 2003). Moreover, in the threonine and tryptophan studies of Rosa *et al.* (2001a, b) the requirement for males and females was similar. That the lysine requirement is affected by gender but other amino acids are not affected adds a complicating factor to use of ideal rations for broiler chicks. Thus, for separate-sex feeding, female chicks having a 10% lower lysine requirement than male chicks mean that females would need to have ratios (to lysine) for all other indispensable amino acids adjusted upwards by approximately 10%. The simplest solution to this gender difference in ratios is to use the male (gain: feed) requirement for lysine together with the male ideal ratios, i.e., for both sexes. This suggests also, that there is no practical or economic advantage to separate-sex feeding.

Because protein gain as a percentage of total bodyweight gain decreases as growing chicks advance in both age and weight, requirements (% of diet) for all amino acids decrease with age and weight. The NRC (1994) model shows these decreasing requirements in three growth periods: starter phase (0–3 weeks), grower phase (3–6weeks) and finisher phase (6–8 weeks). Emmett's laboratory at the University of Arkansas used the ideal ratio concept to propose phase-feeding programs in which two or more diets could be blended multiple times during the 6to 8-week feeding period of broiler chicks (Pope and Emmert, 2001, 2002; Pope *et al.,* 2002, 2004; Warren and Emmert, 2000). Due to maintenance: proteinaccretionconsiderations, ideal ratios for certain amino acids (SAA, threonine, and tryptophan) may increase, but only slightly, as birds advance in age and weight.

Bregendahl *et al.* (2008) conducted an extensive study on true digestible amino acid requirements of laying hens, and concluded that with a lysine requirement (for maximal egg mass) of 538 mg/day the ideal ratios (% of lysine) would be 94% for SAA, 79% for isoleucine, 93% for valine, 77% for threonine, and 22% for tryptophan. These ratios, particularly that for tryptophan: lysine are higher than ratios used for growth of broilers. The Bregendahl *et al.* (2008) study represents a ‘‘first attempt’’ to find ideal amino acid ratios for laying hens. Undoubtedly, these estimated ratios will be revised and refined in the years ahead.

Work from the University of Alberta has focused on determining amino acid requirements, and ultimately ideal amino acid ratios (i.e., requirements rationed to lysine), fore-lay broiler-breeder pullets that average 20 weeks of age and weigh 3 kg (Coleman *et al.,* 2003; Tabiri *et al.,* 2002). Indirect L-[1-(14C)] phenylalanine indirect oxidation methodology has been used to estimate requirements, and thus far an estimate of 0.49% of the diet (366 mg/day) for lysine has been made. Requirements of broiler-breeder pullets have been unknown up to this point, so the work being done in the

Kroger/Ball laboratory at the University of Alberta will greatly aid our understanding of protein–amino acid needs in this class of poultry**.**

2.2 Effect of lysine on body composition

Several authors have recently shown that lysine supplementation, at levels above the requirement for maximal growth rate, results in specific and significant effects on body composition. That is the case for breast meat (BM) yield (Hickling *et al.,* 1990; Moran and Bilgili, 1990). Similarly, lysine can modify fat deposition, this was very well demonstrated by Grisoni (1991) by using eight levels of lysine supplementation. From this experiment it can be concluded, using a monomolecular model, that the total lysine requirement (0.99 asymptote) for gain, feed conversion ratio (FCR), and AF were, respectively, 9.10, 10.07, and 14.3 g/kg. Unfortunately, BM yield was not measured in this experiment.

Using seven levels of lysine supplementation Han and Baker (1994) studied the effect of lysine on gain, FCR, BM yield, and AF percentage between 3 to 6 weeks of age. By recalculating the digestible lysine requirement from their data on male boilers and using the monomolecular model, requirements for reaching the 0.99 asymptote were 10.14 g/kg for gain and 10.6 g/kg for AF percentage. More recently, Tesseraud *et al.* (1996) reinvestigated the effects of lysine level on the growth of different muscles and protein renewal. It is clearly shown that a lysine deficiency specifically reduces Pectoralis major growth; these muscles having Type IIb fibers. In contrast, proportions of Sartorius and Anterior latissimusdorsi, which contain both Type I and IIb fibers, were less influenced by lysine (10.1 g/kg vs 7.7 g/kg); this observation was made at 2, 3, and 4 wk. of age. Renewal of muscle proteins was studied in the same experiment. Lysine deficiency increased both relative protein synthesis and degradation (percentage per day) in the three muscles studied.

The specific effect of lysine intake on body composition was recently reinvestigated in a large collaborative research program, involving TNO-ILOB-Holland (Dr. Schutte), the Rijksstationvoor Kleinveeteelt-Belgium (Dr. De Groote), INRA-Station de Recherches Avicoles (Dr. Leclercq), and Degussa and Euro lysine companies. This study dealt with requirements of male broiler chickens for lysine, SAA, threonine, arginine, valine, isoleucine, and tryptophan during the finishing period (20 to 40 d of age). Details of this experiment are still to be published. This paper will give a summary of the lysine, threonine, and valine results observed in that study. Comparison between these three amino acids is particularly powerful because six levels of each amino acid were compared and all three amino acids were studied in the same experiment (same time, same poultry house, same genotype of broiler, same basal diet, same dissection procedure).

2.3 Effect of lysine on feed conversion

As the lysine deficiency decreases growth rate, FCR increases. However, there seems to be an additional effect that is not accounted for the effect on gain. It is likely that the effect on body composition leads to a better FCR, because lipid gain is reduced by high levels of lysine.

This effect on FCR has been observed by a number of authors. The experiment of Grisoni (1991) shows that the requirement for FCR, using the monomolecular model and the performance of 1.01 asymptote, was 10.07 g total lysine/kg feed, whereas the requirement for gain was 9.10 g/kg. In the experiment from Han and Baker (1994) the requirement for FCR (1.01 asymptote) was 11.55 g digestible lysine/kg of feed, which was very close to the requirement for gain (10.14 g/kg). The requirement for FCR was always higher than that for gain, whatever the mathematical model used. The “broken line” model, resulted in 9.24 and 10.10 g total lysine/kg feed, as requirements for gain and FCR respectively. Requirements needed to reach the 0.99 asymptote were 9.69 and11.84 total lysine/kg feed for gain and FCR, respectively. It appears, therefore, that the lysine requirement for FCR is higher than the requirement for gain.

2.4 Threonine research in broiler

The threonine requirement for young broilers has been studied extensively in the past decade. During this time, estimates of threonine requirements in young male and female broilers ranged from 0.68 to 0.79% and 0.58 to 0.75% of the diet, respectively. In addition to varying requirements, nutrient levels that induce threonine imbalances and crude protein levels that affect threonine requirements are poorly understood. Commercial diets composed of corn, soybean meal, bakery meal, and poultry meals contain sufficient amounts of threonine for broiler production. Dietary inclusions of milo, wheat, wheat minds, and meat meals in broiler rations may cause threonine to be a pressure point in linear programming. Nevertheless, wide variations in published threonine requirements of broilers render the use of crystalline L-threonine problematic because they leave the optimal threonine level for broiler performance subject to conjecture.

Thomas *et al.* (1986) conducted two experiments with male broilers from 7 to 21 days of age fed graded levels of threonine which were added to a peanut meal, soybean meal, and corn-based diet supplemented with synthetic amino acids to meet current Maryland standards. In both experiments, eight levels of threonine and two levels of coccidiostats (salinomycin and stenoral) were employed. Regardless of coccidiostat, the optimum level of threonine for weight gain and feed efficiency ranged from 0.73% to 0.7%. The second experiment employed a lower level of threonine in the basal diet (OS%), which was achieved by substituting peanut meal for soybean meal. The threonine requirement for feed efficiency as determined by a regression equation was 0.73%. Thus, the threonine requirement for body weight gain and feed efficiency was reported to be between 0.73 and 0.77% of the diet. Previous findings by these authors Thomas *et al.* (1979) demonstrated that the threonine requirement for broilers aged 14 to 21 days was between 0.80 and 0.85% of diet. Thus, estimates of the threonine requirement for broilers vary considerably within the same laboratory. However, Thomas *et al.* (1987) further evaluated the threonine requirement for both male and female broilers from 7 to 21 days of age. Synthetic amino acids were added to a corxdpeanut meal basal diet (0.55% threonine) so that all amino acids except threonine were at a minimum of 104% of the Maryland standards. The threonine requirements for males and females were estimated at 0.72% and 0.67%, respectively. This estimated threonine requirement for males was similar to results previously Thomas *et al.* (1986).

Uzu (1986) fed European experimental diets varying in threonine to broilers from 1 to 42 days of age. The four experiments utilized a variety of basal diets (corn/soy vs. corn/ soyl peanut me a 1; wheat/soy vs wheat/soy/peanut meal). The threonine requirement for growth and feed efficiency averaged across all experiments was 0.73- 0.75% (1 to 21 days of age) and 0.68% (21 to 42 days of age). In addition, the threonine requirement varied little with respect to whether wheat or corn was used in the basal diets. This study indicated that threonine is not a limiting amino acid in typical or low protein corn-soybean meal diets.

Robbins (1987) found the threonine requirement of 1Cday-old female Peterson crossbred broiler chicks to be 3.7% of dietary crude protein. In these experiments, broilers received diets ranging from 10.98 to 18.3% crude protein, which were supplemented with graded levels of L-threonine. The threonine requirement was determined by estimating the inflection point of the best-fit line Robbins (1986). Analysis of the threonine requirement as a percentage of diet ranged from 0.58 to 0.78%. Hence, the threonine requirement of chicks fed the high protein diet (20% crude protein) was 29% higher than that of chicks fed the low protein diet (15% crude protein). However, when the threonine requirement was expressed as a percentage of crude protein it ranged only from 3.77 to 3.87%, indicating that the threonine requirement increases as dietary crude protein increases. Threonine requirements expressed as a percentage of dietary crude protein, however, did not vary with the dietary crude protein content of the diet. If threonine is expressed as a percentage of dietary crude protein and the estimation of 3.7% is correct, the NRC (1994) value of 0.80% threonine in a 23% crude protein diet for broilers from 1 to 21 days of age is too low. However, a previous section of this review discussed threonine's sparing effect on glycine. Glycine provides two carbons and one nitrogen in the uric acid molecule. Indeed, a higher threonine requirement in high crude protein diets may indicate excess nitrogen excretion.

The threonine requirement up to 3 wk. of age for male Vantress x Arbor Acre broilers was determined by feeding threonine deficient (0.59%) milo-soybean meal diets (Smith *et al.,* 1988). Broilers were fed a 15% crude protein diet from intact protein sources and supplemented with crystalline amino acids to obtain a diet which provided a minimum of 110% of the suggested amino acid recommendations (NRC, 1984). Broilers in these experiments received dietary treatments from 7 to 18 or 21 days of age. The threonine requirements for weight gain and feed efficiency were 0.68% and 0.79%, respectively. The threonine requirement for feed efficiency conforms to the NRC (1994) requirement of 0.80% of diet from 1 to 21 days of age.

The 21 to 42-day threonine requirements for body weight gain and feed efficiency were estimated to be 0.66 and 0.68% of the diet, respectively (Penz *et al.,* 1991). The basal diet of wheat, corn gluten meal, soybean meal, and meat and bone meal contained 0.64% threonine and 20% crude protein. Abdominal fat was evaluated in addition to performance and was not affected by dietary threonine. This experiment suggests that the NRC (1994) 21 to 42-day requirement of 0.74% of diet is too high.

Rangel-Lugo *et al.* (1994) evaluated the threonine requirements for weight gain and feed efficiency up to 14 days of age in chicks fed wheat-peanut meal-based diets of 20 and 25% crude protein. This study utilized Peterson x Hubbard broilers reared in environmentally controlled Petersime battery brooders. The threonine requirements for maximal weight gain in the 20 and 25% crude protein diets were 0.67 and 0.77%, respectively. These results are similar to Robbins (1987) in that as dietary crude protein increases the threonine requirement increases, presumably for excess nitrogen excretion. In the 25% crude protein diets, the threonine requirements for weight gain were 0.82 and 0.86%. The threonine requirement (as a percentage of crude protein) was 3.44 in the 25% crude protein diet. Thus chicks receiving the 25% crude protein diet had a higher threonine requirement than those receiving the 20% crude protein diet. In addition to these requirements, the threonine requirement in broilers aged 16 to 28 days receiving a cord peanut meal diet containing 20% crude protein was determined to be 0.63% of diet for weight gain and 0.69% of diet for feed efficiency. The threonine requirement for feed efficiency is higher than that of weight gain, and the threonine requirement increases as crude protein increases.

Holsheimer *et al.* (1994) used typical European diets to test the threonine requirement of Hybrid male and female broiler chicks up to 28 days of age. In two experiments they evaluated eight graded levels of threonine and two crude protein levels of either 16 and 20% or 16 and 22%. Their design employed floor pens (22 chick pen) with six replication treatment. In Experiment 1, female chicks were fed threonine-supplemented diets from 10 to 28 days of age and the threonine requirement was 0.63%. In Experiment 2, male and female chicks were fed a threonine supplemented diet from 7 to 21 days of age and the threonine requirement was 0.73%. These studies were conducted to demonstrate a minimum value for the threonine requirement. More importantly, when chicks received a 16% crude protein diet supplemented with 0.25% threonine (total dietary threonine, OM%), they grew as well as chicks receiving a 22% crude protein diet containing 0.85% threonine from corn and soybean meal. Furthermore, chicks fed the low protein threonine-supplemented diet had superior feed conversion compared to those fed the 22% crude protein cord soybean meal diet. These authors stated that nutritionists who implement low protein diets must make extrapolations from these results as to how much threonine can be supplemented for maximal profit.

Thomas *et al.* (1995) evaluated the threonine requirement in Ross x Avian male and female broilers aged 35 to 47 days. Peanut meal was added to the basal diet, which contained 18.4% crude protein and 0.56% threonine. L-threonine was added in 0.02% increments up to 0.68%. They suggested that the threonine requirement for finishing broilers does not exceed 0.56%. This requirement is much lower than that recommended by the NRC (1994) of 0.68% for the 42 to 56-day period.

Kharlakian *et al.* (1996) evaluated the threonine requirements for weight gain and feed conversion in male and female Avian x Avian broilers aged 5 to 7 wk. Threonine-deficient diets consisted of corn and peanut meal and were adequate in all amino acids except threonine. Threonine requirements were estimated using broken line methodology. The estimated threonine requirement for weight gain and feed conversion was 0.57% in males. In females, the estimated threonine requirement was 0.52% for weight gain and 0.55% for feed conversion. Although these estimated requirements are below NRC (1994) suggested recommendations, performance of male and female broilers receiving the corn-peanut meal diets was below that of male and female broilers receiving the corn-soybean meal control diet (containing 0.70% threonine).

Kidd *et al.* (1996) conducted two studies evaluating threonine responses in low crude protein diets utilizing threonine limiting ingredients. Experiment 1 evaluated graded levels of threonine (92 to 112% of the NRC) in milo, soybean meal, wheat, and meat and bone meal based diets from 1 to 56 days of age. A linear improvement in feed conversion was observed from 1 to 42 days of age. This suggests that threonine may become more important in older birds, possibly because of a higher maintenance requirement. A second experiment was conducted in broilers aged 21 to 42 days, evaluating additional threonine in a low crude protein diet with adequate methionine and lysine or a low crude protein diet balanced with all essential amino acids except threonine. Weight gain was optimized in low crude protein diets containing 0.78% threonine, but was statistically the same as in low crude protein diets containing 0.66% threonine and the high crude protein diet containing0.78% threonine. However, the low crude protein diet with adequate essential amino acids and a threonine content of 0.78% had the lowest weight gain. Feed conversion was optimized in low crude protein diets containing 0.78% dietary threonine. In addition, efficiency of protein utilization for daily body weight gain was lowest in the high crude protein diet, but abdominal fat was lowest in the high crude protein diet. This study indicates that dietary crude protein in broilers 21 to 42 days old may be reduced from 20.0% to 16.8% provided that synthetic methionine, L-lysine, and L-threonine are added to the 16.8% crude protein diet.

2.5 Valine and isoleucine requirement in the literature

To better understand the amino acid requirements of broilers, knowledge of their carcass and feather amino acid contents is helpful. Stilborn *et al.* (1997) estimated the feather amino acid contents from broilers. The authors found a Val:Lys ratio of 198% at day 14. This ratio increased to 338 % day 28and up to 378% at day 112. The corresponding Illy's ratios were 145%, 234% and 283%. It shows the impact of both amino acids for synthesis of feather protein. In the carcass (without feather) an average Valles and Illy's ratios of 79% and 60%, respectively, can be measured (GfE, 1999). An exhaustive literature review show that there is a huge variability in published requirements with Val:Lys = 81% ± 19.7 and Ile: Lys = 70% ± 17.

2.6 The valine requirement of broilers as a ratio to lysine

Due to the wide variation in published requirements, it is very difficult to interpret and reach conclusions about the Valine and Isoleucine requirements of broilers. In order to investigate this further, a database was built in which the available data on the Valine and Isoleucine responses and requirements of broiler were compiled. It could be found in world literature 28 valine and 66 isoleucine trials (articles or abstracts from scientific journals). The expression of the requirement as Val: Lys and Iso: Lys needs to test if TD Lysine level is sub limiting. This was done by graphical comparison to the TD Lysine requirement at different ages of modern broiler genotypes (adapted from Ross 308 (2007) and Cobb 700 (2008) management guides). In a second step, the TD amino acid profiles of basal diets were compared to Baker and Han (1994) in order to determine if any amino acids other than Valine or Isoleucine were limiting in the diet. In addition, because a 2-level study does not allow the determination of a requirement, such studies were eliminated from the compilation. At the end only ten published studies could be selected for the evaluation of Val: Lys ratio. The table 1 gives the experimental design of these studies.

In order to estimate the TD Val: Lys ratio which optimizes ADG and G: F ratio of broilers, the performance in the ten selected trials was represented in a curvilinear plateau model. The meta-analytical model took into account a trial effect by estimating a plateau for each trial separately. There is a clear response in ADG and G: F ratio to increased TD Val: Lys ratios, which validates the selection procedure. The TD Val: Lys ratio of 80% appears as a minimum to optimize broiler growth and feed efficiency.

The compilation of the data for carcass parameter shows a strong effect of TD Val: Lys ratio on weight of carcass and breast meat (Corzo *et al.,* 2004; 2007). However, carcass yield (Thornton *et al.,* 2006, Corzo *et al.,* 2004; 2007) and breast meat yield (Leclerq, 1998, Thornton *et al.,* 2006, Corzo *et al.,* 2004; 2007) were not affected. As carcass weight and breast meat weight increased together with increased Val: Lys ratios, the ratio between breast meat and carcass weight did not change. However, meeting the bird's requirement for Valine is of key importance in ensuring the optimal usage of Lysine which is well known to increase breast meat yield (Berri *et al.,* 2008).

2.7 The isoleucine requirement of broilers as a ratio to lysine

As result, from 66 published trials only 6 trials fulfilled the conditions in order to evaluate the TD Ile: Lys ratio. The mean characteristics of the trials selected. The 6 trials differ in the broilers' ages, sex, genetics and nutritional values of the feed. This explains the differences in ADG, ADFI and G: F ratio between the trials. In order to estimate the TD Ile: Lys ratio that optimizes ADG and G: F ratio in broilers, growth performance of the six trials was compiled using the same method as for Valine. There is a clear response in ADG and G: F ratio to increased TD Ile: Lys ratios, which validates the selection procedure. A minimum TD Ile: Lys ratio of 67 appears to be necessary to optimizes broiler performance.

However, it is important to notice that in four trials (Hale *et al.,* 2004, Kidd *et al.,* 2004) the authors used blood cells (animal product). Blood have a specific imbalance in their amino acid profiles, which is characterized by a relative high Valine and Leucine content and particularly poor level of Isoleucine. This raw material is often used in Isoleucine dose-response trials because it easily creates an Ile deficiency. It is well documented that the imbalance between these three amino acids (all branched-chain amino acids) is a factor that impacts animal response in broilers (D'Mello and Lewis, 1970 a, b; Burnham *et al.,* 1992) and piglets (Wiltafsky *et al.,* 2010). D'Mello and Lewis (1970a) observed in growing broilers (7-21 days) that circulating levels of Isoleucine and Valine were lowered by an excess of Leucine. In addition, Valine and Isoleucine requirement increased with Leucine supply (D'Mello and Lewis, 1970b). Burham *et al.* (1992)

observed that dietary Leucine set at 1.76 times the requirement level depressed chick growth. However, an excess of Valine does not seem to impact bird's response to Isoleucine (D'Mello and Lewis, 1970a). Therefore, further trials in order to estimate the optimal Ile:Lysratio in broiler diets without blood cells or high Leucine level is needed.

2.8 Effect of methionine on broiler performance

Panda *et al.* (2007), was conducted an experiment to know the effect of dietary concentration of lysine (Lys) and methionine (Met) on performance of Vanaraja breeder (female parent line) chicks during juvenile stage. Nine diets were formulated to contain 3 different concentrations of Lys (0.9, 1.0 and 1.1%) and Met (0.4, 0.5, and0.6%) each in factorial manner. Each experimental diet was fed adlibitum to eight replicates of six chicks each during 0-6 weeks of age. The interaction between Lysand Met for body weight gain, feed conversion ratio, antibody titre to SRBC, cell mediated immune response in response to PHA-P inoculation, slaughter traits (dressing weight, giblet and abdominal fat content) and weight of immune organs (spleen, bursa and thymus) were found to be significant. However, bodyweight gain was influenced independently by the main factor Lys and Met. Birds fed diet containing 1.0% Lys gained significantly ($P<0.05$) higher weight as compared to that of 0.9 or 1.1% Lys. Neither Lys nor Met levels in diet influenced feed conversion ratio, humeral, and cell mediated immune response, slaughter traits and weight of immune organs like bursa and spleen. The findings of the present study suggested that Vanaraja breeder (female parent line) chicks require 1% lysine and 0.4% methionine in the diet for realizing optimum performance during 0 to 6 weeks of age.

Effect of dietary methionine (0.1 to 0.9% supplementary methionine) on the hepatic microsomal mixed-function oxidase (MFO) system were studied by Takahashi and Horiguchi (1991) in 3 experiments with male broiler chickens. Results showed that dietary methionine concentration giving highest growth rates also produced highest MFO activity. Dietary methionine deficiency and excess amount in diets both reduced MFO activity although the effect was not constant in methionine deficiency.

Another experiment was conducted by Halder, *et al.* (2007) to find out the effect of sodium arsenate with or without extra supplementation of methionine or methioninebetaine combination on reproductive performance and immune status of layer chicken. Rhode Island Red (RIR) pullets (120; 16 weeks old) randomly distributed in four equal groups (each having 3 replicates with 10 birds each (9hens, 1 cock) were offered either control (C), or C supplemented with either 1/20th LD50 arsenic (LD50 As) through water (T_1), 1/20th LD50 As through water with 50% more methionine (i.e. 50gmethionine/100kg of feed) than requirement through feed (T_2), 1/20th LD50 As through water with 25% more methionine than requirement and betaine (substitute of 25% extra methionine) through feed (T3). Fertility, hatchability rate of eggs with reduced embryo mortality and congenital defects than T_1 group. Chicks weight after hatching showed significant ($p<0.05$) variation among the groups. Immune status of different groups of bird showed significant ($p<0.01$) difference.

2.9 Trace minerals in poultry nutrition

Micronutrients as trace minerals play a vital role in various metabolic, enzymatic and biochemical reactions ultimately leading to better growth rate, egg production and feed efficiency. Trace minerals have a very important role to play in the mechanism of nutrient circulation in the animal organism. Deficiency or imbalance of any of these vital micronutrients results in deficiency disease, metabolic disorders, poor growth rate, low egg production, low hatchability and low feed efficiency.

2.10 Absorption of trace minerals in the intestine

Trace Minerals are absorbed in the intestine in ionic forms. They are usually supplemented in inorganic salts, which get dissociated after entering the system. The free metal ion first gets attached to an organic molecule or 'escort'. Due to multiple antagonistic reactions of ingredients in the animal digestive system and limited ligand /escort availability whatever minerals are able to find escorts are absorbed and balance is excreted. The absorption process of mineral is well expressed in the fig.1

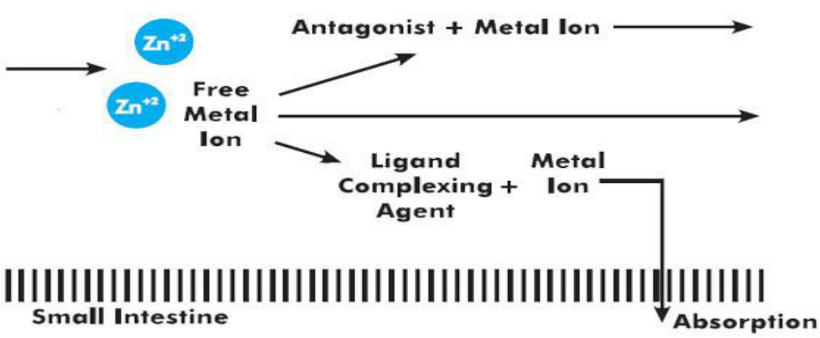

Fig 1: Trace Mineral absorption in the gut

2.11 Role of trace minerals

Trace elements function as part of larger organic molecules. Iron is a part of hemoglobin and cytochrome, and iodine is a part of thyroxin. Copper, manganese, selenium, and zinc function as essential accessory factors to enzymes. If any of these minerals is deficient, the functional activity of the organic moiety requiring the presence of the mineral will be decreased. The physiological activity and its deficiency symptoms are tabulated in Table 1.

Table 1: Role of trace minerals in animal body

TRACE MINERALS	BIOLOGICAL FUNCTION	DIETARY SOURCES
Iron	Iron is essential for the production of haemoglobin, myoglobin, cytochromes and many other enzyme systems. Iron is one of the primary metals involved in lipid oxidation.	Rich dietary sources of iron include; blood meal, kelp meal, coconut meal, meat and bone meal, sunflower seed meal, dried distillers soluble alfalfa meal, crab meal **etc. Recommended Doses:70 mg/ kg of feed**
Zinc	Metabolism of lipid, protein, and carbohydrate. Actives in the synthesis and metabolism of nucleic acids (RNA) and proteins. Action of hormones and in wound healing. Reduced viral penetration inhabits proteases involved in viral capsid formation and increases antibody production.	chick hatchery meal ,dried Candida yeast, dehydrated fish soluble, dried distillers grains with soluble etc Recommended Doses: 90 **mg/kg of feed**
Manganese	Manganese functions as an enzyme activator; The manganese is essential for bone formation, regeneration of red blood cells, carbohydrate metabolism, and the reproductive cycle. It repair and maintenance of epithelial tissues, bone formation, in urea synthesis, amino acid metabolism and glucose oxidation.	kelp meal, rice bran, dehydrated poultry manure, palm kernel meal, crab meal, wheat bran etc. Recommended **Doses:45** mg/kg of feed
Copper	Copper participates in hematopoiesis, copper dependent metalloenzymes responsible for oxidation reduction and in the absorption and metabolism of iron. formation of the pigment melanin and skin pigmentation, bone formation, nerve fiver	fish soluble, corn distillers dried soluble, dehydrated sugar cane molasses corn gluten meal, linseed meal, soybean meal, dried brewers grains, wheat mill run, millet, etc Recommended Doses:9 mg/kg of feed
Cobalt	Red blood cell formation and the maintenance of nerve tissue, an activating agent for various enzyme systems. synthesis of vitamin B12	Copra meal, linseed meal, dried brewer's yeast, fish meal, meat meal, cottonseed meal, and soybean meal etc. **Recommended Doses:0.9 mg/kg** of feed
Iodine	odine is an essential component of thyroid hormones important in regulating the metabolic rate of all body processes. It has roles in thermoregulation, intermediary metabolism, reproduction, growth and development, hematopoiesis, and circulation and neuromuscular functioning	All food stuffs of marine origin, and in particular seaweed meal, marine fish and crustacean meals etc. Recommended **Doses: 4.5 mg/kg of** feed
Selenium	Protects cells from deleterious effects of peroxides. Selenium acts along with vitamin E to function as a biological antioxidant to protect polyunsaturated phospholipids in cellular and subcellular membranes from peroxidative damage. Zinc functions as a cofactor in several enzyme, make stress free	Dehydrated fish soluble, fish meal ,dried brewer's yeast, corn gluten meal, dried torula yeast, rapeseed meal,etc **Recommended Doses:0.19 mg/kg of** feed
Chromium	Chromium is associated with the glucose tolerance factor, an organometallic molecule that potentiates the action of insulin, important in carbohydrate metabolism.	Chick shell meal , shrimp tail meat, Artemia salina, dried brewer's yeast, shellfish, liver, etc. **Recommended Doses:0.7mg/kg of feed**

2.12 Different class of poultry require different levels of trace minerals

Broilers and Layers are the two categories in poultry. The requirement of micronutrients differs according to their activities. Broilers are expected to grow fast in a very short duration, whereas layers are slow growing birds with a productive lifespan of 72 weeks. Broilers are expected to put on weight rapidly whereas Layers are expected to remain lean to produce eggs. There is no definite ratio between different trace mineral requirements for these two categories. If we assume the example of Iron and Copper, it is found that requirement of iron in layers is high than that of broilers, whereas requirement of copper is just opposite, i.e. requirement of copper is low in layers as compared to broilers. The above considerations are of significance whilst formulating Trace Mineral formulations. In the Indian scenario it is observed that, the trace minerals do not get due respect whilst formulating the feed. Many of the trace mineral manufacturers formulate trace minerals in general and do not consider the different requirements of broilers and layers. Moreover, in a study conducted by Prasad *et al.* (2002) observed that certain mineral supplements available in the Indian market do not conform to quality standards. Many manufacturers, in an attempt to bring the cost lower, use cheaper and locally available ingredients; hence the supplies show enough of compositional variability. Prasad *et al.* (2002) analyzed samples of mineral mixtures marketed by 10 different firms and found that none of them matched the BIS specifications. All the samples were either deficient or in excess for one or more essential trace minerals.

2.13 Probiotic

The term ‘probiotic’ is derived from Greek and means pro: for and bios: life (for life) in contradiction to antibiotic which means: against life. The term probiotic was first introduced by Lilly and Stillwell (1965) to describe growth-promoting factors produced by microorganisms. Parker (1974) first specified designation ‘probiotic’. He defined probiotics as microorganisms or substances, which contribute to the balance of the intestinal microflora. Crawford (1979) defined probiotics as a culture of specific living microorganisms, primarily *Lactobacillus spp.* that are implanted in the organism and ensure the rapid and effective establishment of a beneficial intestinal population. Fuller (1989) discussed the definition given by Parker (1974) and considered it too broad, as cultures, cells, and metabolites are also included in antibiotic preparations. He redefined ‘probiotic’ as a live microbial feed additive, which beneficially affects the animal by improving its microbial balance. Havenaar *et al.* (1992) pointed out that the definition of ‘probiotic’ made by Fuller (1989) was restricted to feed supplements, animals, and their intestinal tract. Therefore, they generalized Fuller’s definition of ‘probiotic’ as a mono or mixed culture of living microorganisms, which beneficially affect the host by improving the properties of the indigenous micro-flora.

Through 1989, United States Department of Agriculture (USDA) advised manufactures to use the term: ‘direct-fed microbial’ (DFM) instead of ‘probiotic’ (Miles and Bootwalla, 1991). The USFDA defined DFM as a source of live naturally occurring microorganisms, including bacteria, fungi, and yeast. Vanbelle *et al.* (1990) pointed out that most researchers considered ‘probiotic’ for selected and concentrated viable counts of lactic acid bacteria. Koh *et al.* (1992) pointed out that as a biological product for newly hatched chicks a bacterial culture producing acetic acid could be used. Such a culture might be supplied to the chicks either through their drinking water or by the feed. For controlling the biological balance in the chicken’s intestinal tract different probiotics may be used.

2.14 Yoghut in broiler performance

Endens *et al.* (2003) reported that yoghut improved digestion, absorption and availability of nutrition accompanying with a positive effect on intestine activity and increasing digestive enzymes. Jin *et al.* (1998) reported that in low levels of *Lactobacillus* culture (0.05, 0.01%), feed intake rate has been increased, while Timmerman *et al. (*2006) found inconsistent results, maybe because of type of diet ingredients which can affects probiotic’s growth or their metabolites.

Sultan *et al.* (2006) in 2009 reported that yogurt successfully improved the overall performance and gross return of the experimental birds the same as this study. The main probiotics in yogurt are Lactic Acid bacteria, which can elevate the immune system of host. Suitable function, nonexistence of residue in poultry productions and environmental protection. Also, it has been reported, that *L. acidophilus* can absorb cholesterol from in vitro system, and this phenomenon can decrease the cholesterol level of medium (Gilliland *et al.,* 1985). In addition, *Lactobacillus* bacteria can increase the protein digestibility and availability of minerals for its host like Cu, Mn, Ca, Fe, P and etcby Sultan *et al.* (2006).

Mansoub NH (2011) conducted a research showed that the serum total cholesterol and Triglycerides concentration were significantly reduced by dietary with Yogurt compared to the control group. These results supported with the work of Afsharmanesh *et al.* (2003) reported that Yogurt decreased the cholesterol level numerically too, like what happened in his study.

The cholesterol level of serum significantly decreased in groups supplemented with probiotics in compared to control group. There are many reports that are in agreement with Mansoub NH (2011). *L. acidophilus* is capable to deconjucate glycocholic and taurocholic acids under anaerobic condition. Deconjucation of gallbladder acids in small intestine can affects control of serum cholesterol, while deconjucated acids are not capable to solve and absorb fatty acids as conjugated acids. As a consequence, they prevent from absorption of cholesterol. Also free gallbladder acids attach to bacteria and fibers and this can increase the excretion of them. Moharrery *et al.* (2006) reported that fat digestion rate is linked to rate of gallbladder acids in digestion latex, and subsequently the lipid concentration. *L. acidophilus* and *L. casei* in diet or water cause a decrease in gallbladder acids in digestion latex and this resulted in a reduction in ability of fat digestion and therefore decreasing lipid level of blood.

Aftahi *et al.* (2006) conducted a study on the influence of yoghurt and protein boost on broiler growth, feed intake, feed conversion ratio, livability and profitability production was studied from 1 to 35 days of age. A total of 120 day-old Arbor Acres commercial broiler chicks were distributed randomly into five dietary treatments. Each treatment had 3 replications each of 8 birds. The experimental treatments T_1 considered as control and T_2, T_3, T_4 received 3.0g, 4.0g, 5.0g sour yoghurt, respectively per liter of drinking water and T5 received 1.0g protein boost per 10 liter of drinking water throughout the experimental period. Improvement was observed in body weight gain and feed conversion ratio of broiler of T5 group at 35 days of age compared to other groups. Satisfactory improvement was observed in birds of T4 treatment group. Diet of different treatments had no significant ($p>0.05$) effect on livability of broiler. It was concluded that yoghurt and protein boost could show beneficial effect on broiler performance at the level tested and the inclusion of yoghurt at a level of 5 g per liter of drinking water could be economized broiler production.

Sultan *et al.* (2006) was conducted to investigate the comparative effect of yogurt as probiotic with the commercially available probiotic protein on the overall performance of broiler chicks, measured in terms of gain in body weight, feed intake, feed efficiency and economics. One hundred and twenty-day old broiler chicks were randomly distributed into 3 main groups A, B and C that were further divided into 4 sub-groups comprising of 10 birds each. Commercial ration was fed ad libitrnn to all the three groups. The probiotics, yogurt and protein were given at the rate of 5 rnL -l of water and 1 g L -l of water to group B and A, respectively while group C was kept as control. The data were statistically analyzed, using Completely Randomized Design. The yogurt resulted in significantly ($p<0.05$) higher weight gain, feed efficiency, dressing percentage and gross return both in starter as well as finisher phases and Protein did not affect ($p>0.05$). Mean FCR value in starter phase was 1.86, 1.69 and 1.84 for groups A, B and C, respectively. Group B showed significantly ($p<0.05$) improved feed efficiency than other two groups. Mean weight gain in starter phase for group A, B and C was 592.5, 633.8 and 610.0 g, respectively. Group B had significantly higher body weight gain ($p<0.05$). Feed intake in starter phase was 1104.5, 1076.2 and 1115.7 grams for group A, Band C, respectively, there was no significant difference ($p>0.05$) in all the three groups. Mean FCR in finisher phase were 2.55, 1.98 and 2.51 for group A, B and C, respectively. Group B showed significantly improved feed efficiency ($p<0.05$) as compared to other groups. No significant ($p>0.05$) difference was found in feed intake in finisher phase for groups A, Band C. Feed intake was 3281.075, 3136.37 and 3227.25 g, respectively. Mean weight gain in finisher phase for group A, Band C was 1282.3, 1579.5 and 1280.6 g, respectively. Group B had significantly ($p<0.05$) higher weight gain than group A and C. The dressing percentage of group B was found highly significant ($p<0.05$), that was 62.95, 67.82 and 62.89% for group A, B and C, respectively. Mortality was recorded during experimental period. Percent Mortality was 5, 0 and 5% for group A, B and C, respectively. Economics was calculated for feed cost and gross return. Mean feed cost per bird was 70.08, 55.76 and 56.45 rupees for group A, Band C, respectively. Group A, showed significantly higher ($p<0.05$) feed cost as compared with other groups. Gross between calculated were 93.74, 110.7 and 94.53 rupees for group A, B and C, respectively. Group B had significantly higher gross return ($p<0.05$) as compared with other groups A and C. It was found that yogurt successfully improved the overall performance and gross return of the experimental birds.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Research area and research period

The experiment was conducted at Hatimara, Kashimpur, Gazipur, Bangladesh under the department of physiology and pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, during the period from 25th February 2017 to 24th March, 2017.

Layout of Experiment

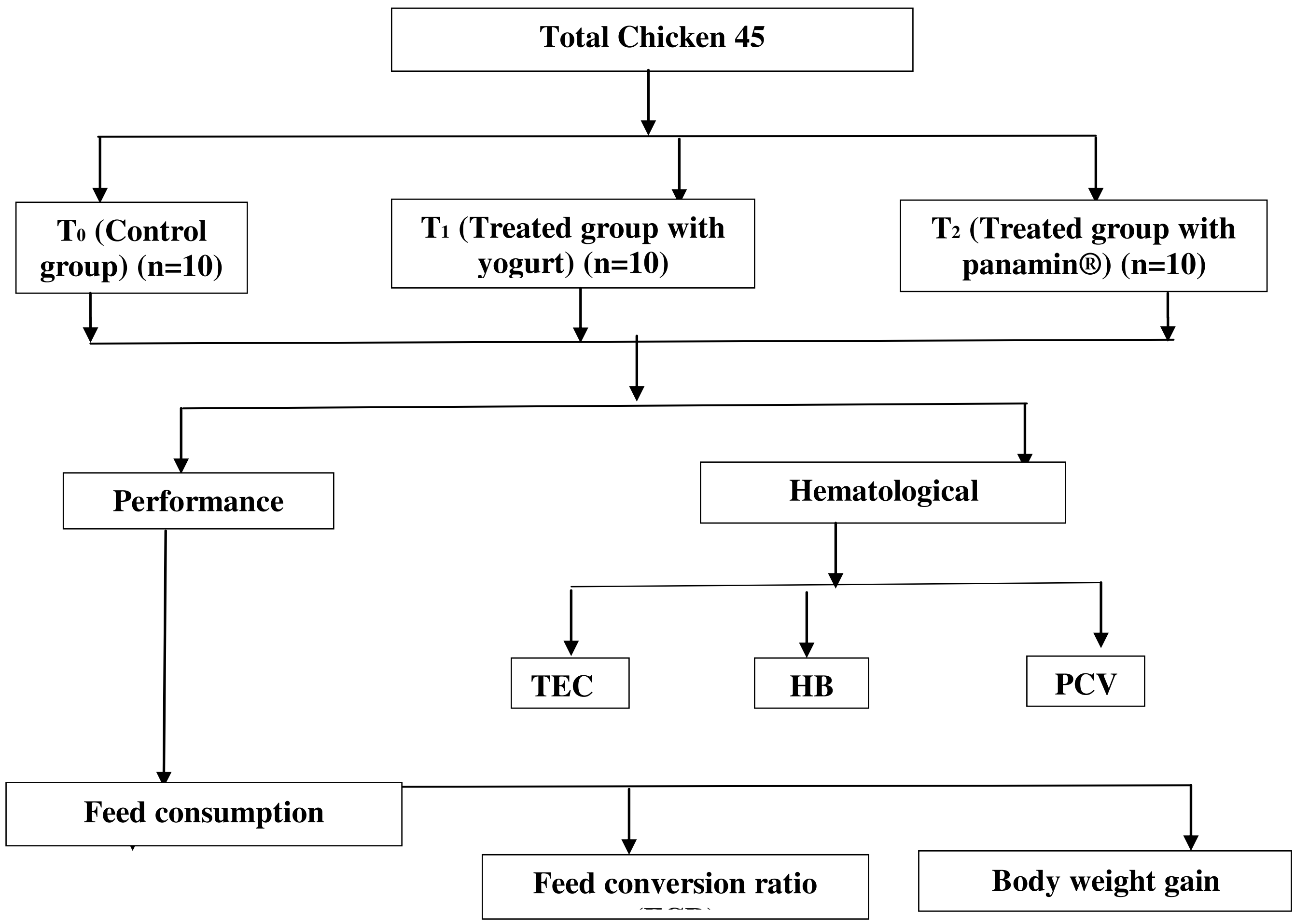

Fig 2: Layout of study design

3.2 Research design

An experimental study was undertaken to assess the comparative efficacy of yogurt and panamin® in broiler production.

Fourty five (45) days Cob-500 broiler chicks were randomly divided into 3 equal groups every groups 15 birds (T_0, T_1 and T_2) for assessing the efficacy of yogurt and panamin® as growth promoter on broilers.

* Group 'T_0': were kept as control.

* Group 'T_1': were treated with yogurt.

* Group 'T_2': were treated with panamin.

3.3 Selection of chicks as study population

A total number of fourty five (45) day-old chicks "A" grade healthy chicks (Cobb-500) were purchased from Kazi Farms Group, Gazipur as pre-contract basis for this study and were divided randomly into three groups T_0, T_1 and T_2 for treatment Control, Yogurt and panamin®. The birds were provided with feeder drinker and bulb.

3.4 Management of chicks

Chicks were placed in a chick guard (brooding house) and during brooding period, temperature was maintained as 95°F and humidity was 65% for the 1st week and for the next week, temperature was 88-90°F and humidity was 70%. Commercially available starter feeds (Grambangla Poultry and Fish Feed Limited) were provided for 13 days (2950 Kcal/Kg ME; 22.7% CP). From 14 to 26 days, they were given Grower Feeds (Grambangla Poultry and Fish Feed Limited) (3080Kcal/Kg ME and 21% CP) and after 26 days, again provided the readymade pellet feed (Grambangla Poultry and Fish Feed Limited) which containing 3050 Kcal/Kg ME and 22% CP. Appropriate vaccination schedule was maintained against different viral diseases such as Marek's vaccine at Day-0, Baby Chick Ranikhet Disease Vaccine (BCRDV) at Day-3 and Day-22 and Gumboro vaccine at Day-11. Everyday *ad libitum* water mixed with various vitamins, minerals, electrolytes, enzymes, phytozyme, probiotics, extract of *Yucca schidigera*, glucose and saline were provided.

Fig. 3: Panamin®

Fig. 4: Yogurt

Fig. 5: Broiler in experimental shed

3.4.1 General monitoring task

On the brooding period, chicks were monitored every two hours interval, checked the temperature and humidity of chick guard. The floor of chick guard was maintained by rice husk and paper materials.

The papers were replaced with new paper every twelve hours interval for first 5 days of brooding and only rice husk was used as bedding material and changed every 24 hours interval until Day-11. Proper ventilation was maintained for ensuring the comfort of the birds.

3.4.2 Supplementation with yogurt and panamin®

Yogurt used 5gm/Liter in water and panamin® liquid 1ml/Litre in morning. Group T_1 was supplemented with yogurt and group T_2 was supplemented with panamin® in drinking water and group T_0 was kept as control.

The yogurt is collected from a super store and the yogurt is manufactured by arong Dairy, Mirpur, Dhaka.

Panamin® is a composition of multi amino acids, it's marketed by square pharmaceuticals, Mohakhali, Dhaka, Bangladesh.

Table 2: Composition of panamin®

Composition of panamin® in liquid per litre	
DL-Methionine	1.35mg
L-Lysine HCL	4500mg
Histidine	2000mg
Phenylalanine	3400mg
Serine	3800mg
Therionine	2900mg
Valine	3500mg
Arginine	5200mg
Cystine	1050mg
Isoleucine	3275mg
Leucine	5700mg
Tryphtophane	1200mg
Tyrosine	3200mg
Papain	1020mg
Glycine	3150mg

Source: Panamin® is a composition of multi amino acids, it's marketed by square pharmaceuticals, Mohakhali, Dhaka, Bangladesh.

Table 3: Composition of yogurt

Composition of yogurt	
kilo calories	149
Total carbohydrates	12 g
Total fat	8.5 g
Cholesterol	32 mg
Protein	9 g
Calcium	296 mg
Phosphorus	233 mg
Potassium	380 mg
Sodium	113 mg
Vitamin A	243 IU
Vitamin C	1.2 mg
Vitamin D	~
Vitamin E	0.1 mg
Vitamin K	0.5 µg
Thiamine	0.1 mg
Riboflavin	0.3 mg
Niacin	0.2 mg
Vitamin B6	0.1 mg
Folate	17.2 µg
Vitamin B12	0.9 µg
Choline	37.2 mg
Betaine	~
Water	215 g
Ash	1.8 g

3.5 Data collection

The experiment was conducted according to the completely randomized designing and data about 10thdays interval body wt., feed consumptions and mortality were recorded during the experimental period

3.5.1 Collection of blood

Blood was collected at Day-30 from jugular vein of three randomly selected chicks in each group and 1.5ml blood was placed into both vacutainer containing anticoagulant and without anticoagulant with identity number and group name in each vacutainer.

3.6 Measurement recording of body weight

The measuring of body weight by weight scale randomly at 10^{th} day, 20^{th} day and 30^{th} day.

3.7 Determination of TEC

Thomas red blood cell pipette was used and pipette was filled up to 0.5 marks with blood and diluting fluid (Hayem's solution). The dilution of the contents was 1:200. The counting chamber was filled with the contents and was placed under microscope. Counting of cells and calculation of TEC was performed as per method indicated by Coffin (1955).

3.8 Determination of hemoglobin

The Hb estimation was performed by the acid Hematin method with the Shali's Hemometer. The result was read as per method described by Coffin (1955).

3.9 Determination of Packed Cell Volume (PCV)

The haematocrit tube was filled up with well-mixed blood by special loading pipette. Then the tube was centrifuged at 3000rpm for half an hour and reading was taken. The PCV was determined as per method described by Coffin (1955).

3.10 Calculation of FCR

Initially, every chick was weighed to measure appropriate growth and then randomly 10 chicks (10%) were weighed at 10 days' interval. Feed Efficiency or Feed Conversion Ratio (FCR) was calculated according to the following formula-

$$\text{Feed efficiency or FCR} = \frac{\text{Unit of feed consumed}}{\text{Unit of weight gain}}$$

3.11 Evaluation of hematological parameters

EDTA containing blood samples were used to determine the hematological parameters such as ESR, PCV, Hb, TEC and DLC at day 30.

(a) Total Erythrocyte Count (TEC)

(b) Hemoglobin estimation (Hb)

(c) Packed Cell Volume (PCV)

(d) Erythrocyte Sedimentation Rate (ESR)

3.12 Statistical analysis

The data thus collected regarding weight gain, feed consumption, feed conversion ratio and different hematological parameters of control and treated groups were analyzed statistically by the well-known student's test ('t' test): Statistical Package for the Social Sciences (SPSS version 20) (Wellman, 1998).

CHAPTER IV

RESULTS

CHAPTER-IV

RESULTS

This experiment was conducted to evaluate the effect of yogurt (as probiotic) and panamin (as amino acids) on the basis of growth performance and hematological alterations in broiler.

4.1 Effect of yogurt and panamin® supplementation on growth performance of broilers

In this study, the birds supplemented with yogurt in T_1 group and panamin in T_2 group and their weight gained or growth performance was recorded in Table 4.

Table 4 showed that, in control group (Group T_0) initial average live weight on 10th day was 282.60 ± 0.84 g, on 20th day was 850.45± 3.67 g and final live weight on 30th day was 1608.20 ± 7.87 g, and feed conversion ratio (FCR) was 1.62. In Group T_1initial average live weight on 10th day was 287.60 ± 0.79 g, on 20th day live weight 900.85 ± 19.22 g and final live weight gain 1710.25 ± 17.89 g and FCR 1.56. In Group T_2 initial average live weight on 10th day was 280.30 ± 0.73gm, on 20th day live weight 920.40 ± 23.56 g and on day 30th final live weight gain 1752.10 ± 13.78 g and FCR 1.49.

The average body weight of bird in T_2 group (panamin®) is comparatively higher than other two group at day 10th, 20th and 30th.The birds of 20th days supplemented with yogurt and panamin® has higher live weight and statistically significantly (at 5% level) than control group. On day 30, the average live weight of bird was increased significantly than control group (Table 4).

Table 4. Growth performance of broiler on day 10th(a), day 20th(b) and day 30th(c) through body weight

Variables	Treatments	Average weight (Mean ± SEM)	P value	Significance level
Live weight (g) on 10th day	T_0	282.60 ± 0.84a	0.10	NS
	T_1	287.60 ± 0.79c		
	T_2	280.30 ± 0.73a		
Live weight (g) on 20th day	T_0	850.45 ± 3.67b	0.02	*
	T_1	900.85 ± 19.22c		
	T_2	920.40 ± 23.56a		
Live weight (g) on 30th day	T_0	1608.20±7.87b	0.03	*
	T_1	1710.25±17.89b		
	T_2	1752.10±13.78c		
Weight gain (g) on 30th day	T_0	1412.60 ± 8.45c	0.09	NS
	T_1	1480.65 ± 21.65b		
	T_2	1525.95 ± 18.09a		
Feed consumption (g)	T_0	2820 ± 45.87a	0.12	NS
	T_1	2802 ± 36.34a		
	T_2	2810 ± 49.86c		
FCR	T_0	1.62		
	T_1	1.56		
	T_2	1.49		

We know,

* = Significant at 5% level of probability,

** = Significant at 1% level of probability,

NS = No Significant

Here,

** Significantly increased (P<0.01) at 1% level of probability.

* Significantly increased (P<0.05) at 5% level of probability.

SEM= Standard Error of Mean

4.2 Effect of Yogurt and panamin® on Hematological Parameters of Broiler:

Observation of hematological parameter (RBC, Hb, PCV, ESR) on 30th day between the control and yogurt and panamin® treated group (Table 5).

The effect of Yogurt and panamin® on blood parameter are shown in the following table-

Table 5: Hematological Parameters of Broiler

Days	Blood parameters	Treatments	Average Blood Parameters Value (Mean ± SEM)	P Value	Significance Level
30th Days	TEC	T_0	2.2 ± 3.28a	0.01	**
		T_1	2.5 ± 1.75b		
		T_2	2.8 ± 1.31a		
	Hb	T_0	8.35 ± 2.74c	0.02	*
		T_1	8.12 ± 1.90c		
		T_2	9.37 ± 3.13b		
	PCV	T_0	21.24 ± 2.13a	0.9	NS
		T_1	19.91 ± 1.25b		
		T_2	20.02 ± 1.21b		
	ESR	T_0	1.9 ± 0.29c	1.2	NS
		T_1	2.2 ± 0.45a		
		T_2	2.1 ± 0.42b		

We know,

* = Significant at 5% level of probability,

** = Significant at 1% level of probability,

NS = No Significant

Here,

** Significantly increased (P<0.01) at 1% level of probability.

* Significantly increased (P<0.05) at 5% level of probability.

SEM= Standard Error of Mean

Table 6: Cost of different groups

Description	Group-T_0	Group-T_1	Group-T_2
Cost/chick (Taka)	26	26	26
Average feed consumed (Kg)/chicks	2.82	2.8	2.79
Feed price/kg (Taka)	40.1	40.1	40.1
Feed cost (Taka)	113.08	112.28	111.87
Yoghut/ panamin®/Broiler	0	2.25	1.8
Miscellaneous (Taka)	25	25	25
Total cost/broiler (Taka)	164.08	163.28	162.87
Average live weight (Kg)	1.493	1.561	1.623
Sale price/Kg live wt. (Taka)	110	110	110
Sale price/broiler (Taka)	164.23	171.71	178.53
Net profit/broiler (Taka)	0.15	6.18	13.86

CHAPTER V

DISCUSSION

CHAPTER-V
DISCUSSION

The effects of yogurt and panamin® supplementation were tested on growth performance and blood parameters, according to recommended amounts during broilers treatments. The study was carried out as dietary additives on broilers during a typical production period of 30th days.

In our study, the effect of yogurt and panamin® on performance of broiler chickens has been proven that the average live body weight were higher in yogurt and panamin® supplemented group than control group at day 10th, 20th and 30th. Birds supplemented with panamin® showed higher body weight gain than yogurt supplemented group. Table 4 showed that, in control group (Group T_0) initial average live weight on 10th day was 282.60 ± 0.84 g, on 20th day was 850.45± 3.67 g and final live weight on 30th day was 1608.20 ± 7.87 g, and feed conversion ratio (FCR) was 1.62. In Group T_1initial average live weight on 10th day was 287.60 ± 0.79 g, on 20th day live weight 900.85 ± 19.22 g and final live weight gain 1710.25 ± 17.89 g and FCR 1.56. In Group T_2 initial average live weight on 10th day was 280.30 ± 0.73gm, on 20th day live weight 920.40 ± 23.56 g and on day 30th final live weight gain 1752.10 ± 13.78 g and FCR 1.49. The average body weight of bird in T_2 group (panamin®) is comparatively higher than other two group at day 10th, 20th and 30th.The birds of 20th days supplemented with yogurt and panamin® has higher live weight and statistically significantly (at 5% level) than control group. On day 30, the average live weight of bird was increased significantly than control group (Table 4). This finding was supported by Thakur *et al.,* (2014). In agreement with previous studies (Denbow *et al.,* 1995; Kornegay *et al.,* 1996; Sebastian *et al.,* 1996), amino acids supplementation increased body weight gain and feed intake in broiler. Based on the results, it is suggested that supplementation of growth promoter and multivitamin-mineral premix in broilers even after feeding of commercial feed has positive effect on the growth of broilers.

Proteins have a critical role in poultry ration, along with vitamins and minerals to the development of musculature. One of the most important areas is amino acid nutrition. Amino acids are the basic constituents of living matter as building blocks of proteins and their proper balance in the diet is required to maintain protein quality, feed consumption, growth rate and carcass composition and as a result receiving considerable attention in industry. Methionine, choline and lysine are universally recognized as the most limiting amino acids in broiler diets based on corn and soybean meal (Baker, 1997).

Broilers have high dietary protein requirements, for either maximizing broiler performance or profit, requires more knowledge related to the requirements of protein and amino acids and their effects on the growth performance and development of broiler chicken (Beski *et al.,* 2015).

In our study, Yogurt has a positive role for the growth performance on broiler chicken and it was supported by the similar findings of Mansoub (2011). Yogurt contains mainly lactobacilli and other beneficial bacteria that have strong positive health effects. Yogurt could aid digestion and inhibit the development of pathogens by improving the balance of microbes living in the digestive tract. *Lactobacillus* is one of the most friendly species of intestinal micro flora is considered as beneficial bacteria due to its ability to aid in the breakdown of proteins, carbohydrate and fats in food and help absorption of necessary elements and nutrients such as minerals, amino acids and vitamins by the host (Sultan *et al.,* 2006).

Drinking water supplemented with yogurt which resulted in better absorption of the nutrients present in the gut and finally leads to the improvement in feed conversion ratio (Boostani *et al.,* 2013).

The main probioticts in yogurt are Lactic Acid bacteria, which can elevate the immune system of host. In addition, Lactobacillus bacteria can increase the protein digestibility and availability of minerals for its host like Cu, Mn, Ca, Fe, P etc (Mansoub, 2011).

Growth promoters are getting popularity as feed additives due to their beneficial effect on gut health and immunity, and growth performance (Panda *et al.,* 2009). Though, their mechanism of action varies, positive effect can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality and regulation of the intestinal microflora (Peric *et al.,* 2009).

In our study, we found that Hb, PCV, TEC, ESR has been increased in the treatment groups than control group. The effect of Yogurt and panamin® on blood parameter are tested on 30th day. TEC of T_0 was 2.2 ± 3.28, T_1 was 2.5 ± 1.75 and T_2 was 2.8 ± 1.31 on

30th day. Hb of T_0 was 8.35 ± 2.74, T_1 was 8.12 ± 1.90 and T_2 was 9.37 ± 3.13 on 30th day. PCV of T_0 was 21.24 ± 2.13, T_1 was 19.91 ± 1.25 and T_2 was 20.02 ± 1.21 on 30th day. ESR of T_0 was 1.9 ± 0.29, T_1 was 2.2 ± 0.45 and T_2 was 2.1 ± 0.42 on 30th day (Table 5). Our findings were supported by Rahman *et al.,* (2013) who found that Hb, TEC, PCV increased in the treatment group groups supplemented with probiotics. Panamin supplemented group showed the increased level of Hb, PCV, TEC and ESR in our study. This result was agreed with Paul *et al.,* (2010) who got significant increased level of Hb, PCV, TEC and ESR after providing amino acid and mineral supplementation.

In our study, total cost/broiler of control group was 164.08 taka whereas T_1 (Yogurt) and T_2 (Panamin) group revealed the total cost/broiler 163.28 taka and 162.87 taka. Average live weight was more in T_2 (Panamin) group (1.623 kg) than T_1 (Yogurt) (1.561 kg) and control group (1.493 kg). Net profit/broiler was higher in T_2 (Panamin) group (13.86 taka/bird) than T_1 (Yogurt) (6.18 taka/bird) and control group (0.15 taka/bird).

CHAPTER VI

CONCLUSION

CHAPTER-VI
CONCLUSION

The study was carried out to investigate the effect of yogurt (as probiotic) and commercially available panamin® (as amino acid) on growth performance of broiler chicks. The yogurt (as probiotic) and panamin® (as amino acids) were given at the rate of 5 gm/L of water and 1 ml/L of water to group T_1 and T_2 respectively while group T_0 was kept as control. Group T_2 supplemented with panamin® showed higher live weight at day 20th and 30th which is significantly differ than control group. The FCR value of T_0, T_1 and T_2 was 1.62, 1.56 and 1.49 respectively. TEC and Hb value are significantly increased in T_2 group than control group. It was found that the broiler supplemented with panamin® dictated the positive effect on growth performance in broiler.

From the findings it could be suggested that supplementation of Yogurt and Panamin are essential for improving the growth performance of broilers, to increase the hematological parameters and cost effectiveness. However, elaborate studies are necessary to observe the effects of Yogurt and Panamin with more biochemical parameters before making any comment.

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