Chapter 1: Introduction

Bangladesh is the most densely populated country in the world, with 158.9 million people living in this land. Though she has maintained an impressive track record of 7.24% economic growth rate over the past decade, in addition to reducing poverty, **(BBS, 2018)** her Population remains little bit far away from adequate protein demand and food security due to loss of arable land, rising sea levels, frequent flooding and extreme weather patterns and climate change **(WFP, 2017).** Protein is a vital macronutrient of every cell in the body which plays an important role to make hair and nails, enzymes, hormones and other body chemicals, building block of bones, muscles, cartilage, skin and blood and repair tissues **(Neil Osterweil, 2005)**. Poultry meat is an excellent source of protein and other nutrients. Due to improve digestibility, broiler meat is now worldwide accepted food for all kind of people. Poultry farming provides not only economic benefits to the poor farmers but also help to improve the health of their family. Approximately 20% of the protein consumed in Bangladesh originates from poultry **(Das et al., 2008)**. To achieve enough protective immunity, rearing system is combined with good hygiene and management, supplementing feed additives (probiotics, enzyme and acidifier), they functions efficiently to maintain growth and production and protect the herds against infectious agents. Probiotic, enzyme and acidifier also can enhance the growth and production performance of flocks without any side effects. Probiotics are feed additives that contain mono or mixed culture living microorganisms includes live bacteria, yeast and their metabolites which promote beneficial effects of favoring the balance of the intestinal microbes, protect toxins produced by pathogenic organisms on the host **(Fuller, 1989; Islam et al., 2004)**. The use of probiotics has become widely accepted as a natural health supplements that are responsible for the production of vitamin B complex and digestive enzymes for stimulation of intestinal immunity to promote health for both humans and animals. Moreover, enzymes are such kind of protein which is biological catalyst composed of amino acids with vitamins and minerals **(Khattak et al., 2006)**. The price fluctuation of feed ingredients has been a major constraint in most of the developing countries, therefore, cheaper nonconventional and commonly available feed ingredients (wheat, maize, rice polish, til oil cake, soybean meal etc.) in Bangladesh have to be used which contain higher percentage of Non-Starch Polysaccharides (NSP) along with starch not easily digested by poultry

(Jin et al., 1997; Morgan et al., 1995). Poultry produce no enzymes for the hydrolysis of NSP which is present in the cell wall of the grains. This adverse effect can be overcome by supplementation of exogenous enzymes which have been shown to lower viscosity of intestinal contents **(Verstegen, 2002; Bedford, 1996)** improve digestibility of starch, protein and fat **(Annison and Choct, 1991; Bedford, 1995)**. Enzymes are the most widely used about 80% as alternative to antibiotics **(Sheppy et al., 2001)**.

Acidifiers are that type of water medication which contains several acids with pH regulation in intestine and antibacterial property that contain acetic acid, propionic acid, phosphoric acid, citric acid, lactic acid, formic acid, fusaric acid and salts of each acid. Acidifiers are also synthetic compounds between acidifiers and their salts **(Mahdavi et al., 2013**). Acidifiers are weak acid & do not split completely in water. Acidifiers increases digestive enzymes of intestine for extending food digestion, pH regulation of intestine and microflora balance, the increase of mineral absorption in optimum pH, palatability of food, increases immunity level **(Bedford, 2000)** enhancing of protein digestion (Gauthier, 2002) increases pepsin activity and the reduction in gastric pH **(Afsharmanesh and Pourreza, 2005; Hersey, 1987)** reduces the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells **(Langhout, 2000)**.

Antibiotic supplementation at the sub-therapeutic level to the poultry diet is common as it reduces the incidence of disease and improves growth rate, feed efficiency and meat quality. Although people enjoy the benefits from antibiotics used in animal production, the extensive use of antibiotics as therapeutics and growth promoters could lead to problems such as antibiotic residues and increased bacterial resistance **(Islam et al., 2014)**. Thus, Probiotic, enzyme & acidifier used as alternative sources of antibiotic with equal efficacy need to be evaluated Therefore, probiotic, enzyme and acidifier helps and repairs and restore the antibiotic causing deficiencies in the gut flora **(Ghosia et al., 2011)** penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria **(Dhawale, 2005).**

Probiotic, enzyme and acidifier supplementation reduces mortality, keep birds healthy, increase feed intake, improve digestion and feed conversion rate and weight gain **(Jin et al., 1999).** However, antibiotic discouraged to use growth promoters because of their residual effect in broiler meat.

1.1 Justification of the Study

Broiler meat is a good source of lean meat for fulfillment of the demand of protein for human. There is no religious taboo regarding consumption of broiler meat. Poultry is one of the fastest growing and most promising industries with the brightest future for our country. But, now-a-days, use of antibiotic drastically, it's becoming a great threat for poultry industry. Because of using antibiotic as growth promoter grows resistance against antibiotic. That's why, difficulties arises in case of preventing and controlling the microbial disease. So, probiotics, enzyme, acidifier are used as natural health supplements in feeds and they are replacing the use of antibiotic growth promoters or chemical supplements.

1.2 Objectives

- To observe the effects of feed additives (probiotics, enzyme and acidifier) on growth performance, meat quality and quantity of broiler meat
- To measure the hematological and biochemical effects in commercial broiler
- To evaluate cost benefit of rearing broilers supplementing feed with probiotics, enzyme and acidifier

1.3 Research questions

- Is there any effect of probiotics, enzyme and acidifier on productive performance and blood parameter of broiler?
- Is there any effect of probiotics, enzyme, acidifier have any effect on carcass characteristics of broiler?

1.4 Scope of the Study

The purpose of the study is to assess the effectiveness of probiotics, enzyme, acidifier on productive performance, carcass quality, hematological and biochemical change maximum productivity and better carcass quality.

1.5 Major limitations of the study

- The sample size was only 100 birds due to resource limitation
- Seasonal variations were not observed due to limited study period
- Comparative meat evaluation based on chemical properties was not done

Chapter 2: Review of Literature

In a highly competitive world with ever increasing productive demands, animals and birds are stressed by various factors. The intensive system of livestock or poultry rearing promotes growing young ones, in the absence of dam, thus depriving them from acquiring enough protective immunity, which enables easier invasion of various infectious pathogens. In this context, combined with good hygiene and management, supplementing probiotic, enzyme and acidifier holds much promise as they functions effectively to maintain growth and production in animal husbandry operations and protect the herds against infectious agents. Moreover, these feed additives have no side effects, when compared to its antibiotic counterparts.

2.1 What is a Probiotic?

Probiotics or direct feed microbials (DFM) are naturally occurring and selected live microorganisms that create a positive impact on the physiological status of the host. This is often accomplished by their ability to alter the intestinal microbial balance in a beneficial manner, which in turn will improve the health and wellbeing of animals, birds or human beings **(Anandakumar and Lakshminarayan, 1997.** Probiotics are live microorganisms that affect the host animal by improving its intestinal balance. Probiotics is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance **(Fuller R., 2001).** Probiotic organisms help to improve the environment of the intestinal tract. It may also be defined as living microorganisms, which is given to animals assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes **(Sinnons and Sainsbury, 2001).** Probiotics are those viable microbes (bacteria and fungi) which have beneficial effect on the host animal (**Ghadban, 2002).** Probiotic containing Lactobacillus species provide resistance to the host against disease causing agents like *E.coli*, Salmonella, Campylobacter and *Eimeria acervulina* **(Dalloul et al., 2003).** Probiotics are green live microorganisms when administered in adequate amounts conferring a health benefit to the host". It neither has any residues in animal products, nor exerts any antibiotic resistance. Probiotics have a good impact on the poultry performance **(Koenen et al., 2004 and Mountzouris et al., 2007)**.

2.2 Criteria for an ideal probiotic

An ideal probiotic should contain sufficient number of viable microorganisms which can withstand the hostile gut environment like pH variations. It should be stable in large numbers and be non-pathogenic, non-toxic and preferably host-specific strain(s) of beneficial microbes. Probiotics include Gram-positive organisms, acid and bile resistant, and must be having a short generation time. These should adhere to intestinal epithelium; have the ability to rapidly and efficiently colonize the intestine and edge out the pathogenic microbes. These should preferably overcome pelleting temperatures and be compatible with most feed additives. Also these should have good sensory properties **(Vegad, 2004; Dhama & Singh, 2010; Hajati & Rezaei, 2010)**.

2.3 Mode of Action of Probiotic

Probiotic reduced the incidence of bacterial infection **(Mulder, 1991)** which competes with pathogen probiotic increases the intestinal dwellers lactobacillus which competes with pathogen. It helps to remove gastrointestinal pathogen with digesta. That's why now-a-days probiotics is widely used in poultry production systems to inhibit the harmful effects and the growth of pathogenic bacteria like Salmonella, *Escherichia coli*, *Clostridium perferinges* and *Campylobacter jejuni*. The antibacterial substances can be bacteriocins, lactocin, lactocidin, acidolin, acidophilin, reuterin, bulgaricin, Acidifier (lactic and acetic acid), lysozyme, lactoferrin, hydrogen peroxide or lactoperoxidase **(Jin et al., 1997)**. Acidifier and volatile fatty acids (lactic, acetic, butyric and propionic acids) produced by probiotic organisms decrease the intestinal pH and inhibit the growth of pathogenic bacteria, there are many substrate in gut environment, the probiotic strains grow well in the gut environment and colonize to efficiently utilize this available substrates. Thus they compete with harmful microbes for use of available nutrients in the intestinal tract **(Nava et al., 2005)**.

2.4 Commonly used microbes as probiotic

Generally, live apathogenic bacterial strains belonging to genus *Lactobacillus*, *Streptococcus*, *Bacillus* or *Enterococcus* and the yeast *Saccharomyces*, are used in livestock and poultry. The strains of lactic acid producing bacteria, which have specificity of adhering to the intestinal epithelium, and *Aspergillus oryzae*, which confers beneficial impact on performance of poultry are frequently used in this

industry. *Lactobacillus* and *Bifidobacterium* species have been used most exhaustively in humans. Since probiotics may also include fungi and yeast, besides bacteria, therefore the use of term "Direct Feed Microbials (DFM)" has been suggested. The most commonly used probiotics contain one or a mixture of harmless microbes. The microbes generally considered for developing probiotic growth promoters are *Lactobacillus acidophilus, L. sporogenes, L. bulgaricus, L. casei, Lactobacillus paracasei, L. plantarum, L. cellobiosus, L. salivarius, L. reuteri, L. animalis, Streptococcus faecium, Streptococcus cristatus, S. thermophilus*; *Bacillus subtilis, Bacillus coagulans, Bifidobacterium bifidum, Saccharomyces cerevisiae, Saccharomyces boulardii, Enterococcus faecium, Pediococcus acidilactici, Pediococcus pentosaceus, Escherichia* spp., *Lactococcus* spp., *Torulopsis* spp., *Aspergillus oryzae* and *Hawaiian spirulina (blue greenalgae)* etc. **(Vegad, 2004; Patterson & Burkholder, 2003; Czerucka et al., 2007; Dhama et al., 2008; Dhama & Singh, 2010; Hajati & Rezaei, 2010)**. Most commonly used among these are *Lactobacillus*-based probiotics. Yeast and *Lactobacillus sporogens* are highly resistant to pelleting temperatures and storage at different environmental conditions.

2.5 Applications of probiotic in poultry

Probiotics maintain the proper balance of useful microbial population in the intestine of bird, which is important for the efficient feed conversion, growth, productivity and stimulation of birds' immune mechanisms to combat pathogens. The mechanism of action of probiotics in poultry production system includes establishing and maintaining healthy gut microflora, improving digestion and utilization of nutrients, competitive exclusion of harmful bacteria/pathogens, decreases pH and releases various antibacterial substances, neutralization of toxins, competition for nutrients with pathogens, reduction in ammonia production and stimulation of the immune system **(Anandakumar & Lakshminarayan, 1997; Patterson & Burkholder, 2003; Boirivant & Strober, 2007).** It has been proved that effective probiotics help to accelerate development of normal microflora in chicks and poults **(Vicente et al., 2007; Bansal et al., 2011)**. Even spray applications of probiotic have been found to be effective against *Salmonella* infection in chicks **(Wolfenden et al., 2007**). As feed additive, probiotics show a good impact on improve weight and size **(Nahashon et al., 1992; Saadia and Soliman, 2010)** and feed consumption and conversion **(Cavit, 2003; Kim et al., 2003)**

2.6 Effects of probiotic

2.6.1 Effects on Performance

Khaksefidi and Rahimi1 (2005) were conducted 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 body weight and feed conversion of probiotic fed groups were superior ($p<0.05$) compared to the control group in the 4th, 5th and 6th weeks. 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. **Mohan et al. (1996)** also stated that the use of probiotic in feed had a beneficial effect on body weight gain of broiler chicks from 4^{th} to 6^{th} week of age. Other studies also report favorable response of inclusion of probiotic in poultry diets **(Jin et al., 1996)** found that inclusion of probiotic (*Lactobacilli* and *Bacillus subtilis*) in diet stimulated favorable microbial balance in gut and consequently improved feed efficiency and growth performance in broilers and broilers fed probiotic supplemented diet had better weight gain and feed efficiency when compared to the broilers fed the unsupplemented diet.

2.6.2 Effects on Immunity:

It reduces the chick mortality by stimulating the immune system **(Koenen et al., 2004**). This immunestimulatory action provide immunoglobulins, stimulate cell mediated immunity, elevate interferon production, increases macrophage, lymphocyte and natural killer cell It increases intra-epithelial lymphocytes of intestinal lymphoid tissue, which responds to microbes by secreting immunoglobulin A (IgA) **(Haghighi et al., 2006)**, **Yurong et al., (2005)** reported increases in the number of Ig producing cells (IgM and IgG) detected in Peyer's patches and the cecal tonsils of chicks by day 7 and 10, respectively, following administration of a probiotic culture in the drinking water. Probiotics have previously been associated with activation of innate immunity through phagocytic cells.

2.6.3 Effect of reducing ammonia production

Probiotic has antagonistic properties to antagonists ammonifying bacteria. They reduce ammonia content in the lumen by retarding microbial breakdown of nutrients. It also decreases urease activity in the small intestine and non-protein nitrogen, uric

acid, ammonia and urea **(Anandakumar and Laksmimarayan, 1997; Fuller, 2001)**. It reduces shedding of epithelial lining which facilitate ammonia gas production.

Enzyme is one of the major nutritional advances in the last fifty years. Indeed, the theory of feed enzymes is simple. Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, due to absence the necessary enzyme to degrade them. Nutritionists can help the animal by identifying these indigestible compounds and feeding a suitable enzyme. These enzymes come from microorganisms that are carefully selected for the task and grown under controlled conditions. The poultry industry readily accepts enzymes as a standard dietary component, especially in wheat, maize, soybean meal etc.

2.7 What is Enzymes?

Enzymes are one of the many types of protein in biological systems which catalyze the rate of a reaction. They are involved in all anabolic and catabolic pathways of digestion and metabolism. Enzymes tend to be very specific catalysts that act on one or, at most, a limited group of compounds known as substrates. Enzymes are not living organisms and are not concerned about viability or cross infection **(Acamovic and Cleary, 1996).**

2.8 Sources of Enzymes

Enzymes were used in the preparation of foods long before there was any awareness of enzymes as such, possibly as long ago as 10,000 years. The industrial exploitation of microbial enzymes in the Western world started 100 years ago with the patenting of a process for the production of alpha-amylase from the fungus *Aspergillus oryzae.* Enzymes are produced in every living organism from the highest developed animals and plants to the simplest unicellular forms of life, as they are essential for metabolic process. **(Khattak, 2006)**. It is possible to produce large amounts of cheap enzyme by continually selecting favorable microbes, growing them in advanced fermentation systems and by streamlining the extraction and purification of the enzyme**.** Microorganisms that generally involved in production of enzymes are; Bacteria (*Bacillus subtilis, Bacillus lentus, Bacillus amyloliquifaciens* and *Bacillus stearothermophils*), Fungus (*Triochodermalongi brachiatum, Asperigillus oryzae* and *Asperigillus niger*) and Yeast (*S. cerevisiae*)

2.9 Types of enzyme available for poultry

Some of the enzymes that have been used over the past several years include cellulase (ß-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases. Enzymes in the feed industry have mostly been used for poultry to neutralize the effects of the viscous, nonstarch polysaccharides in cereals such as barley, wheat, rye, and triticale. These antinutritive carbohydrates are undesirable, as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein. Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in plants but also reduces environmental pollution. Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, ß-galactosidases to neutralize certain antinutritive factors in non-cereal feed stuffs.

2.10 Effect of enzyme

2.10.1 Effect on poultry nutrition

The use of enzymes in animal feed is of great importance. Consistent increase in the price of feed ingredients has been a major constraint in most of the developing countries. As a consequence cheaper and nonconventional feed ingredients have to be used which contain higher percentage of Non-Starch Polysaccharides (soluble and insoluble/crude fibre) along with starch? Non Starch Polysaccharides (NSPs) are polymeric carbohydrates which differ in composition and structure from starch **(Morgan et al., 1995)** and possess chemical cross linking among them therefore, are not well digested by poultry **(Annison, 1993)**. A part of these NSPs is water-soluble which is notorious for forming a gel like viscous consistency in the intestinal tract, thus by reducing gut performance. These pentosans also greatly increase the water intake by birds, which lead to unmanageable litter problems caused by wet and sticky droppings. This deteriorates the hygienic conditions and carcass quality. On the other hand, ß-glucans adversely affects all nutrients, especially protein and starch utilization and are known to give rise highly viscous conditions in the small intestine of the chicks. Poultry do not produce enzymes for the hydrolysis of Non-Starch Polysaccharide present in the cell wall of the grains and they remain unhydrolyzed. This results in low feed efficiency. Enzymes break down the NSPs, decreases

intestinal viscosity and eventually improve the digestibility of nutrients by improving gut performance

2.10.2 Effect of Reduction in digesta viscosity

Enzymes added to poultry diets; especially diets containing cereals rich in NSP such as wheat, barley, and rye, reduced viscosity in the diet and digesta. **Morgan et al., (1995)** and **Muramatsu et al., (1992)** found that that enzyme supplementation of wheat based diets significantly reduced foregut digesta viscosity of birds. The reduction in foregut digesta viscosity was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endoxylanase into smaller compounds and thus reduction in viscous effects of the feed because foregut digesta viscosity is directly proportional to the molecular weight of wheat arabinoxylans (Bedford and Classen, 1993). As a result of endo-xylanase and ß-glucanase supplementation, the long backbones of the arabinoxylans and ß-glucans are cleaved into shorter fragments, thereby reducing their viscosity **(Gruppen et al., 1993)**. Similar findings on digesta viscosity were also reported by **Bedford and Classen (1993); Bhatt et al., (1991) and Dunn (1996)** who inferred that the high viscosity in the gut contents caused by the pentosans led to increased water intake of the birds, which resulted in the wet and sticky droppings.

2.10.3 Effect of improvement in nutrient digestibility

Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley maize **(Saleh et al., 2003)** oats, rye and wheat and to those containing pulses, such as lupins. The effect of enzyme supplementation on Dry Matter Digestibility (DMD) in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 **(Schutte et al., 1995)** to 17% in poultry. The enzymes currently used in monogastric diets are predominantly glycanases, which cleave NSPs into smaller polymers, thereby removing their ability to form viscous digesta and enhancing nutrient digestibility. The effects of glycanases are generally nonspecific, except for their effect on fat (greater effect on saturated fat than on unsaturated fat). Another enzyme used in feed is phytase, which increases the utilization of phytate phosphorus.

2.11 Factors affecting the benefits of enzyme

The degree of improvement obtained by adding enzymes to the diet depends on many factors **(Bedford, 1996),** including the type and amount of cereal in the diet; the level of antinutritive factor in the cereal, which can vary within a given cereal (for example, low- versus high-ß-glucan barley); the spectrum and concentration of enzymes used; the type of animal (poultry tend to be more responsive to enzyme treatment than pigs); and the age of the animal (young animals tend to respond better to enzymes than older animals); type of gut micro flora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the micro flora in their intestines, have a greater capacity to deal with negative viscosity effects **(Allen et al., 1995; Choct et al. 1995; Vukic Vranjes and Wenk, 1993).**

2.12 What is Acidifier?

In general, acidifier as efficient feed additives made from acidifier and their salts are included in feeds or water in order to lower the pH of the feed, water, gut and microbial cytoplasm thereby inhibiting the growth of pathogenic intestinal microflora **(Paul et al., 2007).** Acidifier are organic carboxylic acids including fatty acids and amino acids, of the general structure R-COOH. Antibiotics and acidifier both feed additives are possessing beneficial effects but antibiotic use in the poultry industry has been intensively debated because of the development of bacterial resistance on the human health **(Cheng et al., 2014).**

2.13 Biochemistry of acidifier

Acidifier are organic carboxylic acids, including fatty acids and amino acids, of the general structure R-COOH **(Al-Kassi and Mohssen, 2009)**. There are three classes of acid includes simple monocarboxylic acids, carboxylic acids and short chain carboxylic acids which are vacillated antimicrobial activity. Simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, carboxylic acids with hydroxyl group such as lactic, malic, tartaric and citric acids and Short chain carboxylic acids containing double bonds like fumaric and sorbic acids. Most of the acidifier half dissociated between pH 3 and pH 5.

2.14 Acidifier and its characteristics

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

fumeric acid, citric acid, malic acid, lactic acid etc. have indirect effect on the bacterial population by pH reduction, acting mainly on stomach and rest one is multi chain fatty acid; capric acid, caprylic acid, lauric acid which have direct and strong antimicrobial effects on gram positive and gram negative bacteria.

2.15 Mode of action of Acidifier

Undissociated lipophilic acid can diffuse across cell membranes including bacteria & molds. **(Dibner and Buttin, 2002).** Acidifier can penetrate pH sensitive liphophilic bacterial (ex.: *E. coli, Salmonella sp., L. monocytogenes, C. perfringens* etc) cell wall attacks the DNA of bacteria that turns to death. At the internal pH of bacteria (~7.0), the undissociated acidifier dissociate, releasing H+ and anions (A-). The internal pH of bacteria decreases. The pH sensitive bacteria are unable to tolerate a large spread between the internal and the external pH. Dietary acidifier and their salts are able to inhibit microbial growth in the food and consequently to preserve the microbial balance in the gastrointestinal tract. In addition, by modifying intestinal pH, acidifier also improve the solubility of the feed ingredients, digestion and absorption of the nutrients **(Khan and Iqbal, 2016)** acidifier effect beyond; improve digestive enzyme activity, growth of gastrointestinal mucosa, microbial phytase activity and increased pancreatic secretion.

2.16 Antibacterial activity of acidifier

The addition of acidifier in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria. Most common bacteria that affect the intestinal health of broiler are Salmonella, Campylobacter and Escherichia coli which can be controlled by supplementation of an acidifier in diet **(Van Immerseel et al., 2006; Gharib Naseri et al., 2012).** From a public health point of view, it is necessary to control this biological hazard, to decrease chicken carcass by adding acidifier to the feed or drinking water at appropriate times, which can hinder its multiplication. Salmonella can multiply in the gastrointestinal tract of birds and potentially be excreted in the faeces during growing phase **(Kušar et al., 2010).** Currently, drinking water acidification is another implementation in the broiler industry drinking used for improving performance. Subsequent studies indicated that addition of acidifier to the drinking water helps to reduce the level of pathogens in the water and the crop/ proventriculus, to regulate gut microflora, to increase the digestion of feed and to

improve growth performance **(Aclkgoz et al., 2011).** They suggested that the lactic acid provided in the drinking water reduces the pH of the crop and might be provided as a temporary carbon source for beneficial bacteria normally present in the crop. Moreover, the use of formic acid in the drinking water did not significantly affect the number of Salmonella-positive intestines were found to be more efficient than the antibiotic growth promoter (Enramycin) in decreasing intestinal *E. coli* and *Salmonella spp*. **(Hassan et al., 2010)**. Furthermore, the acidifier in poultry might have a direct effect on the gastrointestinal tract (GIT) bacteria population, reducing the level of some pathogenic bacteria and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients. This may be due to the fact that the propionic acid or propionate possesses mainly anti-mould characteristics **(Zha and Cohen, 2014; Van Immerseel et al., 2009)** reported that acidifier administered in feed and water was not effective further down the intestinal tract. According to some authors, most of the short-chain fatty acids (i.e. propionic, formic) used in diets or water are metabolized and absorbed in the upper gastro-intestinal segments of poultry (Hamed and Hassan, 2013). Thus, their role in modifying host microflora populations in the lower parts is limited **(Józefiak et al., 2010).** Recently, some researchers have suggested transport of short-chain fatty acids further down the gastrointestinal tract by microencapsulation in a lipid shell. The protective lipid matrix used for microencapsulation allows acidifier to have an effect all along the gastro-intestinal tract, since they are slowly released during digestion **(Fernández-Rubio et al., 2009).** It has been reported appear to be much more effective against Salmonella than short-chain fatty acids ($C \leq 4$; formic, acetic, propionic and butyric acids). Moreover, **(Kwon and Ricke, 1998)** found that amongst the short-chain fatty acids, butyrate has the highest bactericidal against the acid-intolerant species such as *E. coli* and *Salmonella spp*.

2.17 Effects of acidifier

2.17.1 Effects on immunity

The immune system of birds is complex and is composed of several cells and soluble factors that must work together to produce a protective immune response. Major constituents of the avian immune system are the lymphoid organs **(Abdel-Fattah et al., 2008)** and **(Ghazalah et al., 2011)** reported that birds fed an organic-acid-

supplemented diet had heavier immune organs (bursa of Fabricius and the thymus) and also a higher level of globulin in their serum. Several studies demonstrated that acidifier could stimulate the natural immune response in poultry as well as broiler. **(Lohakare et al., 2005)** found that the infectious bursal disease (IBD) titers measured post vaccination showed significantly higher IBD titers in the ascorbic acid (0.2%) supplemental group. They explained that the possibility of increasing the antibody to vaccination in ascorbic-acid-supplemented chickens might be increased the activity of the hexose monophosphate pathway, thus increasing the circulating antibody. **(Hassan et al., 2010)** found that at 21 days of age of the broiler, dietary addition of acidifier. However, at 42 days of age, a non-significant difference ($P > 0.05$) was noticed between treatments. Concentration of globulin is used as an indicator for measuring immunity response. Above workers also suggested that the improvement in bird immunity could be related to the inhibitory effects of acidifier on gut system pathogens. Citric acid supplementation (0.5%) enhanced the density of the lymphocytes in the lymphoid organs, enhancing the non-specific immunity **(Haque et al., 2010). Rodríguez-Lecompte et al., (2012)** reported that supplementation of combined probiotics and acidifier (sorbic and citric acid) to broiler diets resulted in better responses of gut morphology and their effects were more apparent in the duodenum and ileum when the gut was fully developed.

2.17.2 Effect on nutrient digestibility

Acidifier normally used as an acidifier in poultry feeds have been considered to be attractive alternatives for improving nutrient digestibility. **Ghazalah et al., (2011)** reported that dietary 0.5% of either fumaric or formic acid and 0.75% of acetic or 2% citric acid improved both ME and nutrient digestibility, that is, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) of broiler diets. Moreover, **Ghazalah et al. (2011**) and **Garcia et al. (2007**) reported that supplementation of formic acid (0.5% or 1.0%) in broiler finisher diet was found to improve apparent ileal digestibility (AID) of dry matter (DM) (67.8% or 68.8%, respectively) and CP (72.5% or 73.5%, respectively) as compared with control (56.4% DM and 60.7% CP). In one study, the gross energy, CP and EE digestibility at 19 days was found to be 76.20%, 72.62% and 67.65% in the non-supplemented group, which was significantly lower as compared with 78.01%, 76.07% and 72.85% in the 2.0% supplemental ascorbic acid group, respectively. The results were similar at 39

days of lower nutrient digestibility in the nonsupplemented group as compared with the ascorbic-acid-added diet **(Lohakare et al. 2005).** The galacto-oligosaccharides in the soyabean meal cannot be digested in the small intestine of poultry because of the absence of the endogenous α-(1, 6)-galactosidase enzyme. Other research added 2% citric acid to the soybean meal as substrates in the in vitro trial. The result indicated that addition of citric acid increased the activity of α-galactosidase resulting in decreased the crop pH. He reported that citric acid decreased the crop pH and enhanced the activity of α-galactosidase in the crop in vivo trial. Acidifier supplementation improved CP and ME digestibility by reducing microbial competition with the host for nutrients, endogenous nitrogen losses and production of ammonia (**Omogbenigun et al., 2003)**. **Samanta et al. (2009)** reported that acidifier raised gastric proteolysis and improved the digestibility of protein and amino acids. Acidifier lowered the pH of the chyme and thus enhanced the digestibility of protein. It is thought that the lower pH of the digesta due to the acidifier supplementation might increase the pepsin activity **(Afsharmanesh and Pourreza, 2005)**. Proteolysis of proteins by pepsin produced peptides which activate the release of hormones including gastrin and cholecystokinin. The pancreatic secretion increased by acidifier to enhance the production of pancreatic juice, which led to better digestion of proteins due to the high concentration of trypsinogen, chymotrypsinogen A, chymotrypsinogen B, procarboxy peptidase and procarboxy peptidase B **(Adil et al., 2010)**. According to **(Diogo et al., 2015)** the positive effect of acidifier on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings. **Centeno et al. (2007)** found that the AID of CP and dispensable and indispensable amino acids were not affected by the addition of citric acid and the microbial phytase enzyme in the broiler diet. They did not observe a synergistic effect of microbial phytase and dietary citric acid on amino acid digestibility. A possible explanation may be that the citric acid complexed with calcium (Ca) and decreased its binding to phytate, increasing the susceptibility of the phytate to hydrolysis by the enzyme. Smulikowska et al. (2009) reported that fat-coated acidifier preparations increased nitrogen (N) retention in comparison with the un-supplemented control diet. The increase in N retention can be connected with greater epithelial cell proliferation in the gastrointestinal tract. Non-protected acidifier added into poultry feed are readily digested **(Sugiharto, 2014),** while the fat-coated preparation prevented dissociation of acidifier in the stomach and helped to address

their bioactivity towards distal parts of the intestine and effectively modulate the intestinal microflora and mucosal morphology in chickens **(Hu and Guo, 2007).** Supplementation of the mixture of acidifier (propionic acid and sodium bantonite) in the broiler diet caused an increase in digestibility and availability of nutrients (such as Ca and P) due to developing desirable microflora (Lactobacillus spp.) of the digestive tract, which in turn results in increasing mineral elements' retention and bone mineralization **(Ziaie et al., 2011).** The acidic anion has been shown to complex with Ca, P, magnesium and zinc, which results in an improved digestibility of these minerals **(Edwards and Baker, 1999).**

2.17.3 Effects on broilers performance

High levels of production and efficient feed conversion are the need of the modern broiler industry which to a certain extent could be achieved by the use of specific feed additives. Acidifier have growth-promoting properties and can be used as alternatives to antibiotics **(Khan and Iqbal, 2016)**. Dietary supplementation of acidifier increased the body weight and feed conversion ratio (FCR) in broiler chicken. Chicks fed the diet supplemented with acidifier showed a significant ($P < 0.05$) improvement in the FCR as against the chicks fed the control diet. The improvement in the FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain in the birds fed acidifier in the diet. The above workers also conducted another trial, in which broilers were given basal diet supplemented with 2–3% each of butyric acid, fumaric acid and lactic acid **(Adil et al., 2011)** Chicks fed the diets supplemented with acidifier showed a significant improvement in the FCR as against the chicks fed the control diet. The improvement in FCR could be possibly due to lesser feed intake resulting in increased body weight gain because of better utilization of nutrients in the birds fed acidifier in the diet. Recently, **Brzóska et al. (2013)** reported that acidifier (0.3–0.9%) had a growth enhancing and mortality-reducing effect in broiler chickens, with no significant influence on carcass yield or proportion of individual carcass parts. The acidifier mixtures might be more efficient than some antibiotic growth promoter in improving broiler performance. Such a positive impact of dietary acidifier on growth performance might be attributed to a reduction of pH values in the feed and digestive tract, serving as a barrier against pathogenic organisms which are sensitive to low pH; the direct antimicrobial effect; the reduction in buffering capacity in conjunction with improving nutrient digestibility **(Ghazalah et al., 2011).**

2.18 Additional probable effects

Previous experiments have reported that dietary acidifier can influence phosphorus utilization in corn-soybean meal diets fed to broiler chickens **(Boling et al., 2000; .** Phosphorus utilization may be increased due to the chelating properties of acidifier with calcium, which can result in increased phytate-phosphorus solubility, increasing their ability to be hydrolyzed **(Centeno et al., 2007).** Some researchers have also proposed that acidifier may stimulate energy metabolism by providing energy sources for epithelial cells in the GIT; **(Partanen and Mroz, 1999).** For instance, some acidifier such as fumaric and citric acids are intermediates of the tricarboxylic acid cycle, and butyric acid is the direct energy source for epithelial cells in the GIT **(Pryde et al., 2002).** However, no data have elucidated the cellular roles of acidifier in the energy metabolism of broiler chickens. Furthermore, acidified water is expected to be more effective than dietary acidification, since acidifier intake is decreased depending on the reduction in feed consumption during heat stress **(Abbas et al., 2013).**

2.19 Effect of Antibiotic compared to probiotic, enzyme and acidifier

Poultry scientists have used many techniques like supplementation of feed additives, natural or synthetic origin in a compound feed to improve weight gain, feed efficiency to meet protein requirements of rapidly increasing population. These additives include antibiotics, prebiotics, probiotics, enzymes and coccidiostats **(Saegusa et al., 2004)**. Poultry diets usually contain antibiotic growth promoters to enhance performance of birds. The addition of antibiotics is not cost effective and also has an issue of bacterial resistance. As an alternative to antibiotic growth promoters, probiotics can be used for competitive exclusion of bacterial pathogens **(Karaoglu and Durdag, 2005)**. Dietary supplementations of probiotics prevent the spread of pathogens and improve growth performance, immune response in poultry birds by modulating native microflora (**Bezkorovainy, 2001**). The under field conditions have generally been gastrointestinal tract in chicks is sterile at inconsistent **(Stavric et al., 1992)**. Results from hatching, and immediately bacteria from the trials conducted with broiler fed various environment or the diet colonize it. After this first probiotics were inconsistent. Some researchers colonization, new bacterial species have more reported positive responses of weight gain and difficulties to establish themselves. The microflora of the gastrointestinal tract is unlikely to be modified by feed enzymes in a

similar manner to that achieved with antibiotic growth promoters (Adams, 2001) The development of resistance to certain antibiotics poses real problems to the animal and public health **(Hofacre et al., 2001)**. Consequently, many additives (probiotics, enzyme and acidifier) raise a particular interest as products of substitution to antibiotics in order to improve the production performances and the health of animals **(Revington, 2002).**

Chapter 3: Materials and Methods

3.1. Study area

The experiments were carried out at Chittagong Veterinary and Animal Sciences University experimental farm and analysis were performed in Department of Animal Science and Nutrition; Department of Physiology, Biochemistry and Pharmacology research laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

3.1. Study Period

The total research period was six months started from January to June 2017 but the actual feeding trial on broiler was carried from June 29 to July 27, 2017. June and July was considered as monsoon seasons where average maximum temperature was (**27-32) °C** and humidity was **78%**.

3.2. Experimental birds

The day-old chicks (Cobb 500™ strain) of mixed sex (male and female) were purchased from an agent of the Nahar Agro Complex Limited, Jhautala Bazar, Khulshi, Chittagong, Bangladesh. Before purchasing, all chicks were examined for uniform size and any kind of abnormalities. The average body weight of purchasing chicks was tried to keep similar (about 47.68±0.01gm).

Figure 3.1 Collection of day old chicks

3.3 Experimental medication

The commercial name of Probiotic, Enzyme & Acidifier used in this experiment were Protexin powder of Novartis, Enyme powder of Popular and Acidifier Vet Liquid of ACI Pharmaceuticals Limited Bangladesh, respectively.

Composition of probiotics preparation

- *Lactobacillus planterum*
- *Lactobacillus bulgaricus*
- *Lactobacillus acidophillus*
- *Lactobacillus ramnosus*
- *Bifodobacterium bifidum*
- *Streptococcus thermophillus*
- *Enterococcus facium*
- *Aspergillous oryza*
- *Candida pintolopesi*

Composition of Enzyme (IU)

- Cellulase 20,000
- Xylanase 2,00,000
- Protease 20
- Amylase 40,000
- Phytase 20
- Pectinase 1,400
- Invertase 400
- Hemicellulase 500
- Lipase 20
- Alpha-galactosidase 100

Composition of Acidifier (%)

- Lactic acid 15
- Citric acid 15
- Formic acid 10
- Phosphoric acid 2
- Acetic acid 5
- Tartaric acid 15
- Propionic acid 10

3.4. Design of experiment

A total of 100 birds were equally and randomly allocated and distributed in five treatment groups (T_0, T_1, T_2, T_3 and T_4) with two replications per treatment followed by a Completely Randomized Design. These groups were treated with Probiotic, Enzyme & Acidifier @ 0 mg/l.5L, 1mg/ l.5L, 1.5 mg/ l.5L l.5ml / l.5L and l.5 mg/ l.5L respectively in regular drinking water of broilers along with regular homogenous optimum diets (Standard diet; NRC, 1994) for a period of 4 weeks. Layout of the experiment is shown in Table 1.

Table 3.1 Layout of the experiment.

Water treatments	**No. of birds per replicate**		**No. of birds per treatment**
T_0 (Basal diet + 0 mg/1.5L)	R_1	10	20
	R_2	10	
T_1 (Basal diet + 1mg/ l.5L) Probiotics	R_1	10	20
	R_2	10	
T_2 (Basal diet + 1.5 mg/ l.5L)Enzyme	R_1	10	20
	R_2	10	
T_3 (Basal diet + l.5 ml/ l.5L)Acidifier	R_1	10	20
	R_2	10	
T_4 (Basal diet + l.5 mg/ l.5L) Antibiotic	R_1	10	20
	R_2	10	
Grand total			100

3.5. Management

Standard management procedure was tried to maintain for the entire experimental periods. However, the overall management system was as follows:

3.5.1. Housing

At first, poultry shed was selected and prepared for broiler rearing. The broiler shed was thoroughly washed and cleaned by using tap water with bleaching powder and caustic soda. For killing microorganisms, phenyl solution (15 ml/5 liters) was also spread on the floor, corners and ceiling followed by brushing by using steel brush and clean water. Brooding boxes and broiler cages were also cleaned by using tap water, caustic soda and phenyl solution in the same manner. After cleaning and disinfecting, the house was left for one week for drying where all windows were kept open for proper ventilation. After a week, the lime was spread on the floor and around the shed

for strictly maintaining bio-security. Arrangement for rearing broilers was made according to treatments and replications. The compartments were selected in an unbiased way, according to treatments and replications for uniform distribution of chicks.

3.5.2. Brooder and cage space

Each brooder box having 2.38 ft. × 2.08 ft. was allocated for 20 birds. After 14 days of age of birds, they were transferred to cage having 3.5 ft. × 1.63 ft. for 10 birds. Therefore, floor space for each bird in the brooder box was 0.17 sq. ft. and cage was 0.57 sq. ft. respectively.

Fig. 3.2 Brooder box with chicks

3.5.3. Brooding

The brooder boxes were ready for broiler chicks rearing after proper cleaning and drying. Dry and clean newspaper were placed on the floor of the brooder box as bedding materials and was changed for every 6 hours intervals in whole brooding period. Brooding temperature was maintained by using 100, 60 watt incandescent lamps in each brooder box. The broilers were exposed to continuous lighting. During the brooding period chicks were brooded at a temperature of 95°F, 90°F, 85°F and 80°F for the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} week respectively.

3.5.4. Temperature and humidity control during experiment

Broiler shed was not environmentally controlled, 200 watt incandescent lamps were used to keep the optimum temperature and electric fans were used to distribute the room temperature. In adverse condition, the system had been changed; in cold weather gunny bag were used to prohibit fluctuating the room temperature as well as humidity.

3.5.5. Feeding and watering

Ready-made feed of C.P. Bangladesh Co., Ltd., Bangladesh was supplied to the birds in two different growth stages i.e. starter and grower. Starter ration was offered from day 0 to 14 days and grower ration was offered from day 15 to 28. Feed and water were supplied ad-libitum to all groups of birds in three different times in a day (7.00,

14.00 and 22.00 h) throughout the experimental period. Feed and water was given to birds on small feeder and small drinkers in the early stage of brooding. In each brooder box, feeding was done by using one round feeder and watering was performed with one round drinker having a capacity of 1.5 liters. The feeders and drinkers were fixed in such a way so that the birds could eat and drink conveniently. During the period of cage rearing, large linear feeder (3.5 ft. × 0.38 ft.) and large round drinker with a capacity of three liters were used. The nutritive value of the diets, provided by the manufacturer is presented in Table 3.2

Table 3.2 Nutritive value of basal diet in broiler feeding.

Specification	Type of a diet/Age of chicken (days)	
	Starter (1 - 14)	Grower (15 - 28)
ME (Kcal.kg^{-1})	3000.00	3100.00
Crude protein (%)	21.50	20.00
Crude fiber (%)	5.00	5.00
Fat (%)	3.50	3.00
Lysine (%)	1.25	1.20
Methionine (%)	0.50	0.45

3.5.6. Vaccination

All birds were vaccinated properly against Newcastle disease on the 4th day and Infectious Bursal disease on 12th day. After each vaccination, Immunue enhancer (Immolyte®, Sqaure) was supplied @ 1ml/2 liters of drinking water along with 1 lemon/ 5 litre water to overcome the stressed effect of vaccination and cold weather.

Table 3.3 Vaccination schedule

Age of birds	Name of diseases	Name of vaccine	Route of administration
4th day	New Castle Disease	BCRDV (Live)	One drop in eye
12th day	Infectious Bursal Disease	IBD	One drop in eye

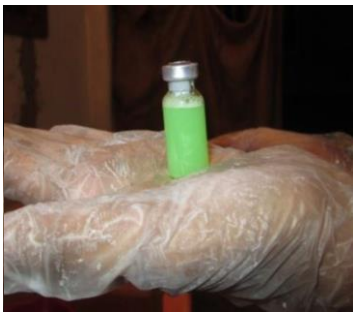


Fig. 3.3 BCRDV and IBD Vaccine

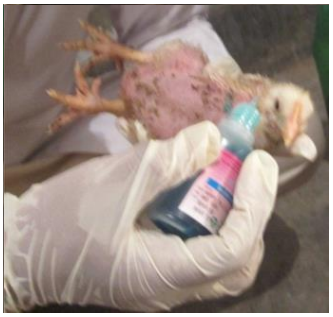


Fig. 3.4 Vaccination of chicks

3.5.7. Sanitation

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

3.6 Laboratory work

The work done in the laboratory discussed as follows:

3.6.1. Carcass characteristics

On days 28 of the study, twenty birds randomly selected from each replication, weighed and then sacrificed by severing of the jugular vein and carotid artery. Once a bird had been allowed to adequately bleed out; the skin with feather was removed using knife and hand force. After defeathering, the birds were eviscerated and the head and feet were removed. During the evisceration process, abdominal fat and liver were excised and weighed. Dressed birds were weighed to obtain a dressed carcass weight. Carcasses were cut into different cuts like- breast, back, thigh, drumstick etc. to measure individual cuts weight. The weights of visceral organs also measured.

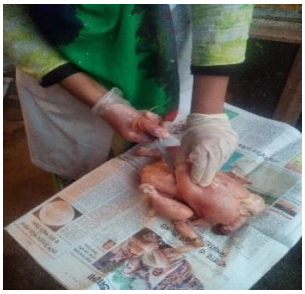


Fig. 3.5 Cutting of body parts

Fig. 3.6 Weighing of the breast meat

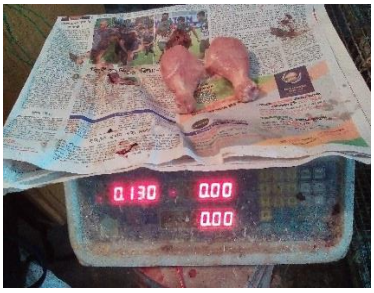


Fig. 3.7 Weighing of the drum stick

3.6.2 Proximate analysis of meat

Chemical analyses of the samples were carried out duplicate for dry matter (DM), crude protein (CP), crude fiber (CF), ether extracts (EE) and total ash (TA) in the Animal Nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong as per AOAC (2006). After slaughtering the bird, 120 g of meat was collected in the air tight bag from the each carcass for the estimation of chemical composition of meat. Then drying of the sample was performed in oven at 80° C. After drying, chemical analysis was done for DM, CP, CF, EE and TA as per AOAC (2006).

Fig. 3.8 Chopping of meat

Fig. 3.9 Digestion of sample

Fig. 3.10 Extracting of ether

Fig. 3.11 Ashing of sample

3.6.3 Hematological analysis

Blood samples were collected from the brachial vein of two birds from each group (one bird from each replicate) using a 3 ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the

anticoagulant, ethylene diamine tetra acetic acid. The total red blood cell (Erythrocyte) counts were performed in a 1:200 dilution of blood in Hayem's solution. The differential leukocyte counts were determined by preparation of blood smears stained with Wright's stain. The Hb concentration was evaluated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobinometer. Packed cell volume (PCV) was measured by standard manual technique after centrifugation of a small amount of blood using micro-hematocrit capillary tubes.

3.6.4 Serum analysis

Blood was collected without anticoagulant from a total of 2 birds from each group at 21^{th} and 28^{th} days of age of broilers. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the ependroff tube by micropipette. Sera were marked and stored in -20°C until analyzed for Cholesterol, Triglyceride, total protein, AST, ALT, LDL, HDL by Humalyzer 3000 (Wisbaden, Germany). It was semi-automatic machine, microprocessor-controlled photometer with large graphic LCD screen. Randox® veterinary reagent kits were used for determination of the blood parameter of interest. Serum sample was mixed with the respective reagents with a specified time (as per manual) in an ependroff tube. Then the serum with reagent was aspired by spectrophotometric method which measured the target parameter and immediately the printed result was recorded in the blood parameter sheet.

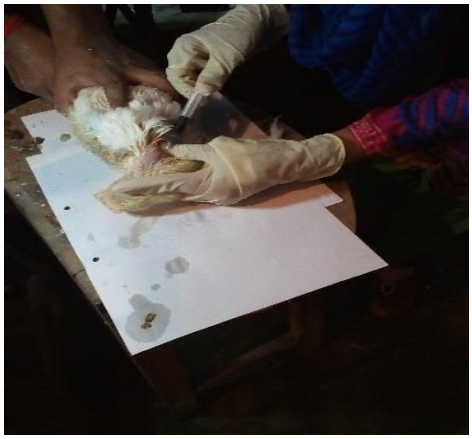


Fig. 3.12 Collected blood sample

Fig 3.13 Mixing of serum

3.7. Data collection

Following parameters were recorded throughout the experimental period.

3.7.1. Weight gain

The weight of chicks was recorded at first day and then weekly intervals. These measures were done along the whole experimental period. The weight gain was calculated by deducting initial body weight from the final body weight of the birds during specific period.

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

3.7.2. Feed intake

Feed intake was calculated by deducting the left over feed from the total amount of supplied feed to the broilers. Feed intake was calculated as g/bird/day.

Feed intake = (Offered feed -Residual feed)

3.7.3. Feed conversion ratio (FCR)

During this study, bird weight was measured by treatment on a weekly basis. Weekly weight gain was calculated and these figures were used to the weekly consumption to determine feed conversion ratio. The amount of feed intake per unit of weight gain is the feed conversion (FC). This was calculated by using the following formula.

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

3.8 Statistical analysis

All the data of growth performance and carcass characteristics and blood parameters were entered into MS excel (Microsoft office excel-2013, USA). Data management and data analysis were done by using ANOVA (Winer *et al.*,, 1991) by using SPSS 16.0. Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was accepted at $P < 0.05$.

Chapter 4: Results

The experiment was carried out to measure the effect of probiotic, enzyme and acidifier on the performance parameter, carcass characteristics and blood parameters of Cobb-500 broilers. The results obtained from the study have been described in this chapter.

4.1 Live weight

Live weight of the experimental broiler birds was recorded weekly throughout the whole experimental period (Table 4.1). Results indicated that, weekly average live weight differed significantly ($p<0.001$) at 4^{th} weeks but insignificantly from 1^{st} to 3^{rd} weeks of age as the level of probiotic, enzyme & acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml / l.5L respectively was added in regular drinking water. Highest average live weight (1693.3g/bird) was recorded in T_2 group followed by T_3, T_4, T_1 and the lowest average live weight (1581.9 g/bird) was recorded in the T_0 group at 4^{th} week.

Table 4.1 Live weight (g/bird) of the experimental broiler birds

Age of bird	Experimental treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
1^{st} week	197.9	206.5	200.1	201.3	201.6	1.40	NS
2^{nd} week	522.1	525.4	523.7	523.1	515.9	1.60	NS
3^{rd} week	958.9	985.4	1019.8	1005.3	982.9	10.4	NS
4^{th} week	1581.9^{a}	1600.5^{a}	1693.3^{b}	1676.8^{b}	1636.0^{ab}	21.3	***

T_0=without probiotic, enzyme, acidifier & antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM=Standard Error of Mean; ***=Significant ($p<0.001$); a,b=Means having different superscript in the same row differ significantly.

4.2 Weight gain

Weight gain of the experimental birds varied in a regular fashion during the entire experimental period (Table 4.2). It was revealed that, weight gain differed significantly at 2^{nd}, 3^{rd} and 4^{th} weeks ($p<0.05$) of age as the level of probiotic, enzyme & acidifier supplementation increased. It was speculated that, as the level of probiotic, enzyme, acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml /

l.5L respectively in regular drinking water weekly average weight gain increased from 88.9 to 100.2g/d in 2nd to 4th week. Among the treatment groups, highest average weight gain (100.2g/d) was observed in T_1 group followed by T_2, T_3, T_4 and the lowest average live weight gain (88.9 g/d) was recorded in the T_0 group at the 4th week.

Table 4.2 Weight gain (g/bird/d) of the experimental broiler birds

Age of bird	Experimental treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
2nd week	46.3^{ab}	45.7^{ab}	47.4^{bc}	45.9^{ab}	44.9^{a}	0.40	*
3rd week	62.45^{a}	65.7^{ab}	70.85^{ab}	68.85^{bc}	66.8^{abc}	1.40	*
4th week	88.9^{a}	100.2^{c}	96.3^{bc}	96.0^{bc}	93.2^{ab}	1.80	*

T_0=without probiotic, enzyme, acidifier & antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$); *=Significant ($p<0.05$); a,b,c =Means having different superscript in the same row differ significantly.

4.3 Feed intake

Results indicated that, Feed intake differed significantly ($p<0.001$) at 2nd weeks, ($p<0.05$) at 4th weeks but insignificant at 3rd weeks of age as the level of probiotic, enzyme & acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml / l.5L respectively in regular drinking water (Table 4.3). Highest average feed intake (159.7 g/bird) was recorded in T_0 group followed by T_1, T_4, T_3 and the lowest average feed intake (154.9 g/bird) was recorded in the T_2 group at 4th week.

Table 4.3 Feed intake (g/bird/d) of the experimental broiler birds

Age of bird	Experimental treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
2nd week	59.9^{d}	55.2^{c}	53.1^{a}	53.6^{b}	55.6^{c}	1.20	***
3rd week	111.1	107.6	102.8	107.1	114.8	2.00	NS
4th week	159.7^{a}	174.0^{b}	154.9^{a}	160.6^{ab}	167.4^{ab}	3.30	*

T_0=without probiotic, enzyme, acidifier & antibiotic; T_1 = Water containing 1mg probiotic/ l.5L water; T_2 = Water containing 1.5 mg enzyme/ l.5L water; T_3 = Water containing l.5ml acidifier/ l.5L water; T_4 = Water containing l.5 mg antibiotic/ l.5L water; SEM=Standard Error of Mean; NS=Non-Significant ($p>0.05$); *=Significant ($p<0.05$); ***=Significant ($p<0.001$); a,b,c,d =Means having different superscript in the same row differ significantly.

4.4 Feed Conversion Ratio (FCR)

FCR did not differ ($p>0.05$) within experimental birds at 3rd week irrespective of the level of Probiotic, Enzyme & Acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml / l.5L respectively in regular drinking water (Table 4.4). However, the difference was highly significant ($p<0.001$) at the 2nd week and significant ($p<0.05$) at 4th weeks, the best FCR (1.4) was observed in the T_2 group followed by T_1 T_3, T_4 at 0-4th week and worst FCR (1.6) was recorded in the T_0 group at 4th week of age.

Table 4.4 FCR of the experimental broiler birds fed

Age of bird	Experimental treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
2nd week	1.3^{d}	1.2^{c}	1.1^{a}	1.2^{b}	1.2^{c}	0.03	***
3rd week	1.8	1.6	1.5	1.6	1.7	0.05	NS
4th week	1.8^{c}	1.7^{bc}	1.6^{a}	1.7^{ab}	1.8^{abc}	0.04	*
0-4 week	1.6^{c}	1.5^{abc}	1.4^{a}	1.5^{ab}	1.6^{bc}	0.04	*

T_0=without probiotic, enzyme, acidifier & antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$); a,b,c,d =Means having different superscript in the same row differ significantly.

4.5 Hematological parameters

The blood parameters of birds were analyzed and the results were presented as follows:-

4.5.1 Packed cell volume (PCV)

The packed cell volume (%) did not differ ($p>0.05$) within all water treatment groups at 3rd and 4th week (Table 4.5). The maximum average value of PCV (33.5) was observed in T_1 group at 4th week and the minimum average value (29.5) was observed in the T_2 group at the same week.

4.5.2 Total erythrocyte count (TEC)

Similar to erythrocyte sedimentation rate, total erythrocyte count (%) remained unchanged ($p>0.05$) at 3rd and 4th weeks (Table 4.5) of age among Water treatments groups. The maximum average value of total erythrocyte count (3.1) was observed in T_3 group at 3rd week and the minimum average value (2.1) was observed in the T_0 group at week.

4.5.3 Lymphocyte

The lymphocyte (%) did not differ ($p>0.05$) within dietary treatment groups at 3rd and 4th weeks of age. Highest average value (67.5) was observed in T_3group at 3rd week and the lowest average value (61.0) was observed in the T_4 group at 4thweek.

4.5.4 Heterophil

The heterophil (%) was similar ($p>0.05$) at 3rd week and 4th week of age as the level of Probiotic, Enzyme & Acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml / l.5L respectively in regular drinking water. The highest average value of heterophil (30.5) was found in the T_1group at 4thweek and lowest average value (21.0) was found in the T_4 group at 3rdweek.

4.5.5 Eosinophil

The blood eosinophil (%) did not exhibit marked changes (Table 4.5) within experimental groups. The maximum average value of eosinophil (6.5) was observed in T_0 at 3rdweek and minimum average value (3.0) was observed in the T_0 group at 4thweek.

4.5.6 Monocyte

The monocyte (%) remained constant ($p>0.05$) both at 3rd and 4th weeks. The highest average value (7.0) was recorded in the T_3 group at 3rd week. In contrast, the lowest average value (2.0) was found in the T_1group at the 3rdweek.

4.5.7 Basophil

Supplementation of water treatment had on no effect ($p>0.05$) on basophil (%) at 3rd and 4th week. Highest average value (2.0) was found in the T_3 group at 3rd week. In contrast, the lowest average value of (0.0) was recorded in T_1 and T_2 group jointly of at the same week.

4.5.8 Hemoglobulin (Hb)

Supplementation of water treatment had no marked influence ($p>0.05$) on hemoglobulin (%) in the experimental birds. The highest average value (7.9) was found in the T_4 group at 3rd week and the lowest average value of hemoglobulin (6.5) was found in the T_0 group at 4th week.

Table 4.5 Hematological parameters of the experimental broiler birds

Parameters (%)	Week	Experimental treatments					SEM	Sig.
		T_0	T_1	T_2	T_3	T_4		
PCV	3rd	31.5	30.0	32.0	30.5	30.5	0.40	NS
	4th	31.0	33.5	29.5	32.0	30.5	0.70	NS
TEC	3rd	2.6	2.2	2.5	3.1	2.8	0.10	NS
	4th	2.1	2.8	2.2	2.4	2.5	0.10	NS
Hemoglobin	3rd	6.7	7.2	7.7	7.8	7.9	0.20	NS
	4th	6.5	7.3	6.5	6.8	6.6	0.10	NS
Lymphocyte	3rd	67.0	66.5	70.5	67.5	67.0	0.70	NS
	4th	64.5	60.0	63.0	65.0	61.0	0.90	NS
Heterophil	3rd	21.5	21.5	21.5	21.5	21.0	0.10	NS
	4th	26.5	30.5	28.0	25.0	27.5	0.90	NS
Eosinophil	3rd	6.5	7.0	3.5	4.5	5.0	0.60	NS
	4th	3.0	5.0	4.0	4.0	4.0	0.30	NS
Monocyte	3rd	3.5	2.0	4.0	7.0	6.0	0.90	NS
	4th	5.5	4.5	5.0	5.0	4.0	0.20	NS
Basophil	3rd	1.5	0.5	0.5	2.0	1.0	0.30	NS
	4th	0.5	0.0	0.0	1.0	0.5	0.20	NS

T_0=without Probiotic, Enzyme, Acidifier & Antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$);); ***=Significant ($p<0.001$); a,b=Means having different superscript in the same row differ significantly.

4.6 Serum parameters

4.6.1 Low density lipoprotein (LDL)

Serum LDL level did not differ ($p>0.05$) 4th week but differ ($p>0.05$) at 3rd week of water treatment groups. The lowest average value (50.7 mg/dl) was found in the T_1 group at 3rd and the highest average value (98.7 mg/dl) was observed in the T_3 groups at 3rd week.

4.6.2 High density lipoprotein (HDL)

Serum HDL (mg/dl) did not differ ($P>0.05$) at 3rd and 4th week (Table 4.6). Maximum average value (72.0 mg/dl) was observed in T_3 group at 3rd week and the minimum average value (41.7 mg/dl) was observed in the T_0 group at 4th week.

4.6.3 Triglyceride (TG)

The serum triglyceride (mg/dl) was statistically differed ($p<0.05$) within dietary treatment groups at 3rd and 4th weeks of age (Table 4.6). The maximum average value (193.3) was observed in T_4 group at 3rd week and the minimum average value (47.3) was observed in the T_1 group at 4thweek.

4.6.4 Total Protein (TP)

Serum total protein (mg/dl) did not differ ($P>0.05$) at 3rd and 4th week (Table 4.6). Maximum average value (7.2) was observed in T_2 group at 3rd week and the minimum average value (4.0) was observed in the T_4 group at 4th week.

4.6.5 Cholesterol

Serum cholesterol (mg/dl) level did not differ ($P>0.05$) at 3rd and 4th week (Table 4.6). The highest average value of serum cholesterol (107.7) was recorded in T_4group at 3rd week whereas the lowest value (52.8) was found in the T_1 group at the same week during the experimental period.

4.6.6 Alanine Transaminase (ALT)

The Alanine Transaminase (ALT) level did not differ significantly ($p>0.05$) at 3rd and 4th week of age (Table 4.6). The maximum average of ALT level (49.5) was found in T_2 group at 4th week; whereas the minimum level (5.9) was found in same group at 3rd week.

4.6.7 Aspartate Transaminase (AST)

The Aspartate Transaminase (AST) statistically does not differed ($p<0.05$) at 3rd and 4th week in the treatment groups. At the end of the experimental period, highest serum AST value (136.1) was found in T_3 group at 4thweek whereas the lowest value (98.1) found in T_1 group at the same week.

Table 4.6 Serum parameters of the experimental broiler birds

Parameters (mg/dl)	Experimental treatments						SEM	Sig.
	Week	T_0	T_1	T_2	T_3	T_4		
LDL	3rd	69.9^{ab}	50.7^{a}	79.0^{bc}	98.7^{c}	62.6^{ab}	8.10	**
	4th	65.4	52.1	66.1	70.9	74.0	3.70	NS
HDL	3rd	59.9	64.9	60.1	72.0	60.2	2.30	NS
	4th	41.7	71.9	39.0	45.6	42.8	6.00	NS
Total protein	3rd	5.5	6.5	7.2	4.9	5.4	0.40	NS
	4th	4.2	5.0	6.1	5.0	4.0	0.30	NS
Triglyceride	3rd	172.0^{ab}	132.5^{a}	189.8^{a}	131.2^{a}	193.3^{b}	13.5	*
	4th	56.3^{a}	47.3^{a}	66.2^{ab}	69.9^{ab}	83.9^{b}	6.20	*
Cholesterol	3rd	98.0	93.9	103.0	98.9	104.8	1.90	NS
	4th	102.4	52.8	105.2	92.4	107.7	10.1	NS
ALT	3rd	7.0	8.1	5.9	8.3	7.0	0.40	NS
	4th	14.4	19.6	49.5	20.7	41.1	6.80	NS
AST	3rd	103.0	126.9	122.1	112.3	114.3	4.10	NS
	4th	108.8	98.1	110.2	136.1	134.0	7.50	NS

T_0=without probiotic, enzyme, acidifier and antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$); **=Significant ($p<0.01$); *=Significant ($p<0.05$); a,b=Means having different superscript in the same row differ significantly

4.7 Carcass characteristics

The carcass parameters significantly differed ($p<0.01$) in terms of dressing weight, neck fat and differed ($p<0.05$) in terms of thigh weight, abdominal fat weight at 28 days. However, it did not differ significantly ($p>0.05$) in other parameters of amongst dietary treatments. Other carcass parameters were statistically similar ($p>0.05$) throughout the entire experimental period.

Table 4.7 Carcass characteristics of the experimental birds

Carcass parameters (%)		Experimental treatments					Sig.
	T_0	T_1	T_2	T_3	T_4	SEM	
Dressing weight	49.2^{a}	55.0^{ab}	66.2^{c}	59.4^{bc}	49.5^{ab}	3.20	*
Drumstick weight	9.6	7.9	9.4	8.0	11.1	0.50	NS
Breast weight	24.6	22.9	26.0	22.3	26.2	0.80	NS
Thigh weight	9.09	10.2	10.09	10.07	9.47	0.21	NS
Wing weight	5.1	5.5	4.9	5.1	4.5	3.50	NS
Head weight	2.8	2.0	2.1	2.3	2.3	0.10	NS
Neck weight	1.5	1.8	1.7	1.6	1.5	0.10	NS
Neck fat weight	2.2^{b}	1.5^{a}	1.3^{a}	1.5^{a}	2.2^{b}	0.05	*
Heart weight	0.3	0.5	0.5	0.5	0.5	0.20	NS
Abdominal fat weight	2.9^{b}	2.0^{a}	2.3^{a}	2.3^{ab}	1.9^{a}	0.04	*
Liver weight	2.1	2.3	2.4	2.2	2.4	0.20	NS
Gizzard weight	1.6	1.4	1.0	1.5	1.7	0.06	NS
Proventiculous weight	0.4	0.9	0.3	0.6	0.4	0.10	NS
Back weight	7.4	7.2	9.9	10.7	10.6	0.10	NS
Feet	3.8	3.9	4.5	4.0	23.7	0.70	NS

T_0= without Probiotic, Enzyme, Acidifier & Antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$); *=Significant ($p<0.05$); **=Significant ($p<0.01$); a,b,c=Means having different superscript in the same row differ significantly.

4.8 Chemical composition of meat

The chemical composition of meat such as Dry matter (DM), crude protein (CP) and ether extract (EE) did not differ significantly ($p>0.05$) irrespective of Probiotic, Enzyme, Acidifier & Antibiotic supplementation but Ash differ significantly ($p<0.01$). There were no marked ($p>0.05$) changes in the chemical composition of meat in terms of Dry matter (DM), crude protein (CP) and ether extract (EE) content in different water treatment group.

Table 4.8 Chemical composition of meat (%) of the experimental birds

Parameter (g/100g)	Experimental treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
DM	28.38	25.80	26.74	26.95	25.50	0.50	NS
CP	71.62	78.58	80.24	78.38	80.85	1.60	NS
EE	4.72	4.67	4.78	4.64	5.31	0.10	NS
Ash	10.22^{a}	11.96^{ab}	10.65^{a}	15.84^{c}	14.09^{bc}	1.10	**

DM= Dry Matter; CP=Crude Protein; EE= Ether Extract;T_0=Diet without Probiotic, Enzyme, Acidifier & Antibiotic; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$);**=Significant ($p<0.01$); abc=Means having different superscript in the same row differ significantly

4.9 Cost-benefit analysis

Table 4.9 Cost-benefit analysis of experimental birds

Parameter	Experimental treatments					SEM	P-value
	T_0	T_1	T_2	T_3	T_4		
Live weight	1.6	1.6	1.7	1.7	1.6	0.02	*
FCR	1.6^{c}	1.5^{abc}	1.4^{a}	1.5^{ab}	1.6^{bc}	0.04	*
Feed Intake (kg)/ broiler	2.6	2.5	2.3	2.4	2.5	0.05	NS
Feed cost/ kg broiler	70.6	66.4	60.6	63.8	68.2	1.70	NS
Feed cost /broiler	113.0	106.3	103.0	108.4	109.1	1.60	NS
Chick cost	45.0	45.0	45.0	45.0	45.0	0.00	NS
Trial treatment cost/ broiler	0.0	2.1	2.4	8.0	1.9	1.30	NS
Medication cost/ broiler	2.3	2.3	2.3	2.3	2.3	0.00	NS
Vaccine Cost	6.0	6.0	6.0	6.0	6.0	0.00	NS
Overhead cost	18.0	18.0	18.0	18.0	18.0	0.00	NS
Total cost	184.3	179.7	176.7	187.7	182.3	1.80	NS
Market price/kg	130.0	130.0	130.0	130.0	130.0	0.00	NS
Market price/broiler	208.0	208.0	221.0	221.0	208.0	3.20	NS
Net profit (TK)/bird	23.7^{a}	28.4^{a}	44.3^{b}	33.3^{a}	25.7^{a}	3.60	**

T_0=Diet without Probiotic, Enzyme, Acidifier and antibiotics; T_1 = Water containing 1mg Probiotic/ l.5L water; T_2 = Water containing 1.5 mg Enzyme/ l.5L water; T_3 = Water containing l.5ml Acidifier/ l.5L water; T_4 = Water containing l.5 mg Antibiotic/ l.5L water; SEM= Standard Error of Mean; NS=Non-Significant ($p>0.05$); *=Significant ($p<0.05$);

=Significant ($p<0.01$); *=Significant ($p<0.001$); abcd=Means having different superscript in the same row differ significantly.*=Overhead costs: costs for housing, feeder, waterer, sanitation equipments, disinfectants, extra labor, electricity and depreciation cost of the building.

From Table 4.9 Cost benefit analysis did not differ ($p>0.05$) within experimental birds irrespective of the level of Probiotic, Enzyme & Acidifier supplementation at the rate of 1mg/ l.5L, 1.5 mg/ l.5L and l.5ml / l.5L respectively in regular drinking water (Table 4.9). However, the differences were significant ($p<0.05$) at live weight, FCR and $p< 0.01$ at net profit though some cost excluded. Highest net profit was observed at T_2 and Lowest was T_0 group.

Chapter 5: Discussion

This study investigates the effects of probiotic, enzyme and acidifier supplementation in water to quantify its effects on productive performance, carcass characteristics and hematological and biochemical parameters in commercial broiler. It was hypothesized that probiotic, enzyme and acidifier when supplemented above ingredients would exhibit a variety of benefit in terms of performance and economic characteristics which would ultimately play a vital role in future broiler production. Antibiotic resistance is present thread for human health as well as animal health perspectives. Adding feed additives have been shown as best alternative to antibiotic. This study measures the effect of probiotic, enzyme and acidifier as water cum feed additives on broiler performance during a typical production period of 28 days.

5.1 Weight gain

The effects of probiotic (T_1), enzyme (T_2) and acidifier (T_3) compared to antibiotic (T_4) supplementation in drinking water on broiler chicken are investigated. Weight gain on 2^{nd} week was almost similar among groups. On 3^{rd} week the highest weight gain was recorded in treated groups lowest in control group T_0. At 4^{th} week higher weight gain was recorded in treated groups than control group T_0. The study showed significantly increases ($p<0.05$) weight gain at 2^{nd}, 3^{rd} and 4^{th} weeks (Table 4.2). The increased weight gain observed in treated groups may be due to an increased feed absorption, utilization, digestion and metabolism of supplied nutrient specially protein essential for weight gain. The effect of addition of enzyme was similar to some of the previous findings **(Meng et al., 2005; Saleh et al., 2005 and Wang et al., 2005)** all of them concluded that improved feed utilization by exogenous enzyme is responsible for an improved weight gain in broilers. **Rosin et al. (2007)** investigated that increasing the growth performance of broiler chickens by supplementing their diets with exogenous enzymes can contribute to positive changes in gut health. **Lazaro et al. (2003)** reported that enzyme supplementation might improve weight gain by improving nutrient digestibility. This mechanism might be induced, at least partially by a reduction of the viscosity. These results, however, not consistent with others **(Rahman et al., 2013; Kabir et al., 2005; Mohan et al., 1996; Panda et al., 1999; Ahmed et al., 2004)** who's indicated that the highest weight gain recorded in combination of probiotic and enzyme supplementation may be due to synergistic effect of combined treatments of probiotics and enzymes. In the study found that T_1 is

more significant than other study due to more feed intake which was supported by **Panda et al. (2006)** who found that effect of dietary supplementation of probiotic Lactobacillus significantly enhanced body weight in broilers. **(Anjum et al., 2005; Mahdavi et al., 2013; Lutful Kabir, 2009)** also reported that, probiotic supplementation is beneficial for growth performance of broiler chicks. Our results disagree with the findings of **Guntal et al. (2006)** and **Mountzouris et al. (2007)** who found that the use of probiotic products in the feed had no significant effect on broiler body weight which may supplementation with feed.

The results from experiment were compared with results of **Denli et al. (2003)** who observed slow increase in weight, using organic acid in the diet. He also reported that live weight was not affected significantly by organic acid treatments in broiler chickens, but in our experiment live weight was increased at the 4^{th} week of age, it may due to failure of consuming acidifier during the starter phase than the grower phase to respond **(Král et al., 2011).**The positive effect at later stage of the acidifier group was because of the stimulating role on enzymatic secretion; mainly on synthesis of gastric and pancreatic lipase **(Tellez et al., 2012; Patterson and Burkholder, 2003; Choudhari et al., 2008),** due to the reduction of the growth depressing metabolites produced by microorganism in the gut **(Knarreborg et al., 2004)** due to the prevention of exponential multiplication of common pathogenic bacteria (E. coli, Salmonella spp, Streptococcus spp etc.) and due to the alteration of the pH in the gut **(Brennan et al., 2 003).** The responses of broiler chickens to water acidifier have shown considerable inconsistency. There have been many successful demonstrations of positive effects of organic acids on growth performance, whereas other studies were unable to find beneficial effects or even reported negative effects on growth performance due to its rapidly metabolized capacity in the foregut the crop to the gizzard **(Lückstädt, 2014)**. Some studies also showed no performance difference, in comparison with the negative control and/or the birds fed antibiotics (**Vieira et al., 2005; Kopecký et al., 2012)**. There are conflicting results regarding the use of acidifiers in poultry and according to **Hernandez et al. (2006)** these effects depend on the chemical form of the acid, pKa values, bacterial species, animal species and the site of action of acids.

5.2 Feed intake

Results indicated that, feed intake differed significantly ($p<0.001$) at 2^{nd} weeks, ($p<0.05$) at 4^{th} weeks but insignificant at 3^{rd} weeks of age (Table 4.2). Comparatively lower feed intake in enzyme supplemented group at 4^{th} week than other group agrees with the findings of **Hajati H, (2010)** who reported that enzyme supplementation significantly decreased feed intake may be due to enzyme supplementation might improve broiler performance by improving nutrient digestibility. These findings disagree with **Ezema Chuka, (2014)** who reported that both enzyme and probiotic have no significant differences ($P>0.05$) in feed consumption among the experimental groups where as our study reported that feed intake differ significantly at 2^{nd} and 4^{th} weeks of rearing among the groups than control group. This finding is in agreement with earlier observations by **Ezema (2007)**. Although, **Craig et al. (2008)** observed that enzyme supplementation increased feed intake.Comparatively in probiotic group increases the feed intake which agree with **Zhang and Kim (2014)** reported an increase body in FI in chicken fed with multistrain probiotics compared with that in control group fed basal diet. **Shareef and Dabbagh (2009)** also reported that probiotic (Saccharomyces cervisiae) supplementation of broilers had significantly increased feed consumption. Probiotics elaborates digestive enzymes which help the host enzymes to increase digestibility and improve efficiency of feed utilization and weight gain. It has also been shown that probiotics breakdown feed into smaller substances making their digestion and absorption by the host animal easier. These findings disagree with **Yousefi and Karkoodi (2007)** who reported that feed consumption was not affected by the dietary probiotic supplementation. Results from a study by **Babazadeh et al. (2011)** indicated that probiotics did not have any significant positive effect on broilers FI.

In case of feed intake, the study finds that, feed intake decreases than control group at 2^{nd} to 4^{th} weeks. This study was similar to **Islam et al. (2008)** who reported that acidifier in poultry water decreased feed intake. In tandem with this, on day 28, the feed intake decreased and has also positive effect on feed intake. The reduction in the feed intake might be due to the unfavorable taste associated with the organic acids which would have decreased the palatability of the feed, thereby reducing feed intake which cause of significantly decreasing body weights at 21 and 42 days of age **(Vieira et al., 2008 and Aclkgoz et al., 2011).**

5.3 Feed conversion ratio (FCR)

The weekly feed conversion at different ages of broilers water supplemented with probiotic, enzyme and acidifier indicated that they improved feed conversion ratio of broiler. FCR did not differ ($p>0.05$) within experimental birds at 3^{rd} weeks irrespective of the level of probiotic, enzyme & acidifier supplementation (Table 4.4). However, the difference was highly significant ($p<0.001$), at the 2^{nd} week and significant ($p<0.05$) at 3^{rd} & 4^{th} weeks. This shows that feed conversion or nutrient utilization was lowest in T_0 and T_4 among treatment groups in which was the control and antibiotic but much better in T_2 group among treatment groups. The better feed conversion ratio for the groups with probiotic, enzyme, acidifier were might be due to the lowering of the pH of the digestive organ which led to better digestion, absorption and utilization of nutrients **(Bengmark, 1998 and Dhama et al., 2011).**

Lawal et al. (2010) observed significant differences ($P<0.05$) among different experimental diets for weight gain, feed intake and feed conversion ratio. The lowest feed consumption and weight gain were observed in control group while the highest were obtained in groups that fed with multi enzyme supplementation. Also, poor feed conversion ratio (FCR) was observed in control group and best FCR (lowest) were in groups that were fed multi enzymes. Enzyme supplementation might improve broiler performance by at least two mechanisms: increasing feed intake and improving nutrient digestibility. Both mechanisms might be induced, at least partially, by a reduction of the viscosity's reduced viscosity decreases retention time of digesta in the gut, allowing more consumption and therefore improving growth and feed conversion ratio **(Lazaro et al., 2003)**. This study partially supported **Hajati (2010)** reported that weight gain, feed intake and feed to gain ratio was decreased by enzyme supplementation from 1-44 days ($p<0.05$). **Dina et al. (2016)** concluded that the use of probiotics improve the performance parameters which including weekly feed consumption, weekly body weight gain, main weekly body weights and FCR.

The study disagree with Joanna **Boirivant et al. (2007)** who reported neither body weight gain nor FCR nor mass of the liver, pancreas and gastrointestinal tract were significantly influenced by supplementing the diet with either additive polish journal of food and nutrition sciences. Acidifiers modified intestinal microflora and helped to improve bird performance, health statue as well as reduced the microbial use of nutrients. The lowering of the pH, optimized the activity of proteases and beneficial bacteria **(Nava et al., 2009)** and enhanced feed conversion by broiler birds. **Azza**

Kamal and Naela Ragaa (2014) stayed that broiler chicken fed diets supplemented with organic acids had significantly ($p < 0.05$) improved feed conversion ratio. The recent study disagree with **Watkins et al. (2004)** found no significant improvement in average weights and feed conversion in broiler chicken drinking acidified water. **Sheikh et al. (2011)** observed that the birds fed diets supplemented with organic acids showed significantly ($p<0.05$) higher body weight gains and feed conversion ratio. The improvement in FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain (Table 4.2) in the birds fed organic acids in the diet. Accordingly **Adil et al. (2010)** showed that, in slow growth type chickens, supplementation of acidifier improved weight gain and feed conversion. The improvement in FCR could be possibly due to lesser feed intake resulting in increased body weight gain because of better utilization of nutrients in the birds fed organic acids in the diet. However, in contrast to present study **Brzóska et al. (2013)** did not find any effect of acidifier on feed conversion in broilers. One other study demonstrated that addition of acidifier in water for broilers improved feed conversion ratio at later stage **(Král et al., 2011).**

5.4 Hematological Parameters

The study revealed no significant difference ($P>0.05$) in the Pack cell volume, red blood cell, hemoglobin, white blood cell, heterophils, lymphocytes, monocytes, basophils etc which agree with **Mansoub (2010)** reported that there was no significant difference ($P>0.05$) in the Pack cell volume, red blood cell count, mean corpuscular hemoglobin, white blood cell count, heterophils, lymphocytes, monocytes and basophils. These results are in disagreement with the earlier findings of **Jin et al. (1997)** who reported that probiotic increased the hematological profile of poultry either due to its direct effects on haemopoetic organs or the indirect effects on the intestinal micro flora. However, hematological parameters are always influenced by environmental changes and nutrition.

5.5 Serum Parameters

Results of blood biochemical parameter represented in Table 4.6 revealed that, broilers watered with probiotic, enzyme & acidifier were exhibited a lowest level of serum low density lipoproteins (LDL) compared with non-supplemented with control group at 3rd week of age but it is dissimilar to 4^{th} week of age which supported **Biggs**

and Parsons (2008) who reported that supplementation of probiotic and organic acid decreased LDL ($p<0.05$) at 4^{th} week. **Kavalati et al. (2003)** also found that supplementation of probiotic (lactobacillus) and decreases LDL. Decreasing of blood LDL values by adding supplementation of probiotic to broiler diet was also observed in **panda et al. (2000)** studies who reported that adding probiotic had significant effect on SRBC antibody. **Haghani et al. (2005)** reported that adding probiotic to broiler diet improved immune reaction especially IgM and IgG. Based on the results of the present study **Rozbeh Fallah and Hasan Rezaei (2013)** at 21 days of age significant differences were observed in LDL and triglycerides levels between treatments ($p>0.05$). **Mansoub (2010)** reported that there is a significant decrease in the serum level of triglycerides between control group and groups treated with *Lactobacillus acidophilus* and *Lactobacillus casei* supplemented in broiler diet in combination with water or alone. **Goli and Aghdam (2015)** adding enzyme significantly increased the concentration of blood LDL at 21($p<0.05$) & decreased triglyceride at 21 ($p<0.05$) and multi enzyme supplementation can improve broiler performance. Our findings disagree with **Daneshyar et al. (2009)** who reported there was not a significant difference between treatments for triglyceride. This findings of serum lipid profile are in agreement with **Fallah and Rezaei, (2013)** who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. A significant decrease was observed in serum lipoprotein level in acidifier treatment **(Sas, 2000; Abdel-Fattah et al., 2008).** The role of organic acids in decreasing blood fat may explain via their effect on decreasing intracellular microbes by prevention of microbial enzymes activity and forcing cellular bacteria for using energy in order to release protons which cause forming of mass intracellular anions. **Amudovská and Demeterová (2010)** stated a significant decrease of AST activity in the blood serum on the 35th day in the treatment group does not show any harmful effect of supplemented acidifier on the health of chickens as increased levels of AST is symptomatic for hepatic damage. Other biochemical parameters (total protein, albumin, uric acid, glucose, total lipids, cholesterol, triglycerides, ALP, Ca, P) were not significantly affected **(Harr, 2006)** but that's fully contradictory from my study. **Abdel-Fattah et al. (2008)** found significantly lower serum concentrations of cholesterol and total lipids and significantly higher concentrations of Ca and P in chicks fed acidified diets (citric acid, acetic acid or lactic acid in 1.5 % or 3 % concentration) in comparison with the control group.

5.6 Carcass characteristics

The carcass characteristics of experimental broiler on 28th day are shown on the (Table 4.7). The table represented a significant decrease in abdominal fat, neck fat and increase dressing weight, at all treatments group and other parameters were not significantly changed. These results indicated that there were no statistically significant differences increases quality between the control and trial groups in other parameters which is similar to **Islam et al. (2008)** and **Lee et al. (2010)** reported that no significant differences in the relative weights of the liver, abdominal fat, right leg or right breast muscle among treatment groups. **Café et al. (2002)** also reported that addition of 1000 mg multi enzyme per kg of diet had no significant effect on breast, thigh and wing components. In this study, however, abdominal fat was affected significantly by enzyme ($p > 0.05$). In Partial similarities to these results, **Islam et al. (2008) and Café et al. (2002)** reported that enzyme had a significantly ($p \leq 0.03$) higher proportion of abdominal fat at 42 days and 49 days. This study disagree with **Ashkan and Nasir (2012)** stayed that Effects of enzyme supplementation on carcass characteristics on 44 days age are shown in Enzyme supplementation increased carcass percentage and thighs + drumsticks percentage significantly ($p<0.05$) **(Biswas et al., 1999).** According to **Goil et al. (2015)** in carcass traits no differences ($P>0.05$) the percentage weight of carcass traits were not affected by dietary treatments except for the percentage of the eviscerated weight that increased in the broilers fed diets containing probiotic ($p<0.05$). One study reported that, acidifier has capacity to decrease abdominal fat **(Castellini et al., 2002).** This similarity was also seen by **Garcia et al. (2007)** who reported that the abdominal fat of the acidifier supplemented chicks was less than that of the control group. Close similarity was seen in other studies. The heart and liver of the various treatment group of this experiment; though varied numerically but did not differ significantly. This is an agreement with **Ogunwole et al. (2011)** who also reported that there was no significant difference in liver weight and heart weight of broilers treated with dietary acidifiers. This result also inconsistent with some research article **(Islam et al., 2008)** stated that dietary acidifier improved carcass yield by approximately 3-5% in poultry. This study disagree with **Denli et al. (2003) and Leeson et al. (2005)** who observed that addition of the acidifier to drinking water did not influence the hot carcass yield and abdominal fat pad.

5.7 Chemical composition of meat

The chemical composition of meat such as Moisture (M), Dry matter (DM), Crude protein (CP) and Ether extract (EE) did not differ significantly ($p>0.05$) irrespective of probiotic, enzyme, acidifier and antibiotic supplementation but total ash differ significantly ($p<0.05$) this study agree **Joy and Samuel (1997)** noted that inclusion of Lactobacillus sporogenes in broiler diets did not influence carcass protein. In contrast, **Khaksefidi and Rahimi (2005)** reported that the leg and breast meat of probiotic fed chickens were higher ($p<0.05$) in moisture, protein and ash as compared to the leg and breast meat of control chickens. This indicates a better retention of minerals especially calcium, phosphorus, nitrogen and improved protein efficiency ratio in probiotic fed birds as compared to control birds. **Pietras (2001)** also reported meat of chickens given probiotic (*Lactobacillus acidophilus* and *streptococcus faecium* bacteria) on the whole rearing period had significantly higher protein content, while crude fat and total cholesterol contents tended to decrease.

Chapter 6: Conclusion

The study investigates the effect of probiotic, enzyme and acidifier supplementation in Cobb 500 broiler under intensive rearing system. After complete discussions of the study it was found that significantly lowest feed intake and best FCR as well as highest net profit was observed in birds containing enzyme supplementation (T_2) was added in regular drinking water. It was also observed that average weight gain increased significantly as the level of probiotic, enzyme & acidifier supplementation was added in regular drinking water. Furthermore, the result showed no unusual changes in the blood and serum parameters in comparison to the standard reference level. Similar to performance parameter and carcass characteristics were improved in terms of dressing percentages, neck fat yield in enzyme supplemented group. Hence, this study suggests that enzyme can be used as a potential water supplement as alternative to antibiotic growth promoter as well as improve performance parameter, carcass characteristics and net profit without interfering blood and serum parameters in commercial broiler. However a long term study with larger sample size and combination of probiotic, enzyme and acidifier are suggested for better result of the study for increasing sensitivity and validity of the study under field condition.

Chapter 7: Recommendation

The use of probiotic, enzyme and acidifier in drinking water is a relatively recent development in poultry production. In tropical production systems, this may play a vital role in providing hygienic drinking water and reducing pathogen load, thus having enormous potential as an integral component of a successful bio-security programme. This particular study, carried out in Bangladesh, demonstrates that including probiotic, enzyme and acidifier in broiler production has beneficial effects on the performance of broilers at the later stage and may be considered as a low-cost option to improve production parameters in general. Therefore, enzyme could be an important and economical solution for profitable broiler production in tropical environment as well as stressful condition. Combination of probiotic, enzyme and acidifier are recommended in regular drinking water of broiler for better growth, best FCR on productive performance of broilers should be investigated in future along with some vital blood parameters like Glucose, Total albumin, White blood cell count (WBC), calcium, phosphorus and other trace minerals both in meat and feed were not analyzed. These parameters could have vital impact on human health. The study will explore new prospect for investigating those parameters as future study.

Chapter 8: References

Abbas G, Khan SH and Rehman H-u. 2013. Effects of formic acid administration in the drinking water on production performance, egg quality and immune system in layers during hot season. Avian Biology Research. 6 (3): 227-232.

Abdel-Fattah S, El-Sanhoury M, El-Mednay N and Abdel-Azeem, F. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7(3): 215-222.

Aclkgoz Z, Bayraktar H and Altan O. 2011. Effects of formic acid administration in the drinking water on performance, intestinal microflora and carcass contamination in male broilers under high ambient temperature. Asian-Australasian Journal of Animal Sciences. 24(1): 96-102.

Adams CA. 2001. Interactions of feed enzymes and antibiotic growth promoters on broiler performance. Brufau J. (ed.). 54: 71-74

Adil S, Banday M, Bhat G, Qureshi S and Wani S. 2011. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Liv. Res. Rur. Dev. pp. 23 (1).

Afsharmanesh M and Pourreza J. 2005. Effects of Calcium, Citric Acid, Ascorbic Acid, Vitamin Dsub3/sub on the Efficacy of Microbial Phytase in Broiler Starters Fed Wheat-Based Diets I. Performance, Bone Mineralization and Ileal Digestibility. International Journal of Poultry Science.

Ahmed K and Taghi G. 2006. Effect of probiotic on performance and immunocompetence in broiler. The Journal of poultry science. 43: 296-300.

Al-Kassi AG and Mohssen MA. 2009. Comparative study between single acidifier effect and synergistic acidifier effect on broiler performance. Pakistan Journal of Nutrition. 8 (6): 896-899.

Amudovská Alena and Demeterová Maria. 2010. Effect of water acidification on performance, carcass characteristic and some variables of intermediary metabolism in chicks. Acta Veterinaria (Beograd). 60(4): 363-370.

Anandakumar K and Lakshminarayan NG. 1997. Probiotics: Importance in poultry production. Poult. Advisor. April Issue. pp: 7-9.

Anjum MI, Khan AG, Azim A and Afzal M. 2005. Effect of dietary supplementation of Multi-Strain Probiotic on broiler growth performance. Pakistan Vet. J. 25: 25-29.

Annison G and Choct M. 1991. Anti-nutritive activities of cereals non-starch polysaccharides in broiler diets and strategies minimizing their effects. Worlds Poultry Science Journal. 47: 232-242.

Ashayerizadeh A, Dabiri N, Mirzadeh KH and Ghorbani MR. 2011. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. Journal of Cell and Animal Biology. 5(8): 152-156.

Ashkan Kavyani, Farid Farivar, Shima Mokhtari Karchegani and Nasir Landy. 2012. Efficiency of A Multi-strain Probiotic (Protexin) on Performance and Carcass Traits in Broiler Chicks. International Conference on Life Science and Engineering. V 45.

Azza M. Kamal and Naela M, Ragaa. 2014. Effect of Dietary Supplementation of Organic Acids on Performance and Serum Biochemistry of Broiler Chicken. Nature and Science. 12(2): 38

Babazadeh D, Vahdatpour T, Nikpiran H, Jafargholipour MA and Vahdatpour S .2011. Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (Coturnix japonica). Indian Journal of Animal Sciences. 81(8): 870–874.

Bangladesh Bureau of Statistics (BBS), 2018.

Bansal GR, Singh VP and Sachan N. 2011. Effect of probiotic supplementation on the performance of broilers. Asian J. Anim. Sci. 5: 277-284.

Bedford M. 2000. Removal of antibiotic growth promoters from poultry diets, implications and strategies to minimize subsequent problems. World 's Poult. Sci. J. 56: 347-365.

Bedford MR, Schulze H. 1998. Exogenous enzymes for pigs and poultry. Nutr Res Rev. 11: 91-114.

Bedford MR. 1996. The effect of enzymes on digestion. J Appl Poultry Res 5: 370-378

Bengmark S .1998. Ecological control of the gastrointestinal tract: The role of probiotic flora Gut. 42(1): 2-7.

Bezkorovainy A. 2001. Probiotics: Determinants of survival and growth in the gut. Am. J. Clin. Nutr. 73: 399-405.

Biggs P and Parsons C. 2008. The effects of several organic acids on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. Poultry science. 87(12): 2581-2589.

Biswas T, Mandal L, Sarker SK. 1999. Studies of enzymes supplementation and herbal preparation at different levels of energy of the performance of broiler. J Inter academia. 3:53–58.

Boirivant M and Strober W. 2007. The mechanism of action of probiotics: antimicrobial effects of probiotics. Curr. Opin. Gastroenterol. 23(6): 679-692.

Boling S, Douglas M, Snow J, Parsons C and Baker D. 2000. Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. Poultry science. 79(9): 1335-1337.

Brzóska F, Śliwiński Band, Michalik-Rutkowska O. 2013. Effect of dietary acidifier on growth, mortality, post-slaughter parameters and meat composition of broiler chickens/Wpływ zakwaszacza diety na masę ciała, śmiertelność, wydajność rzeźną i skład mięsa kurcząt rzeźnych. Annals of Animal Science. 13(1): 85-96.

Café MB, Borges CA, Fritts CA, Waldroup PW. 2002. Avizyme improves performance of broilers fed corn-soybean meal-based diets. J Appl Poult Res. 11:29–33. 10.1093/japr/11.1.29

Castellini C, Mugnai C and Dal Bosco A. 2002. Effect of organic production system on broiler carcass and meat quality. Meat science. 60(3): 219-225.

Cavit A. (2003): Effect of dietary probiotic supplementation on growth performance in the chickens. Turkish J. Vet. Anim. Sci. 28: 887–891.

Centeno C, Arija I, Viveros A and Brenes A. 2007. Effects of citric acid and microbial phytase on amino acid digestibility in broiler chickens. British poultry science. 48(4): 469-479.

Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L and Yuan Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Frontiers in microbiology. 5: 69-83.

Choudhari A, Shinde S and Ramteke B. 2008. Prebiotics and Probiotics as Health promoter. Veterinary world. 1 (2): 59-61.

Craig L, Wyatt T, Mike B. 2008. Mechanism of Action for Supplemental NSP and Phytate Enzymes in Poultry Diet. AB Vista Feed Ingredients Ltd in their paper to the Carolina; Poultry Nutrition Conference.

Dalloul RA, Lillehoj HS, Tamim NM, Shellem TA, Doerr JA. 2005. Induction of local protective immunity to Eimeria acervulina by a Lactobacillus-based probiotic. Comp. Immun. Microbiol. Infect. Dis. 28: 351-361.

Daneshyar M, Kermanshahi H, Golian A. 2009. Changes of biochemical parameters and enzyme activities in broiler chickens with cold-induced ascites. *Poultry Science*. 88: 106–110.

Das SC Chowdhury, Khatun SD, Nishibori MA, Isobe MN and Yoshimura Y. 2008. Poultry production profile and expected future projection in Bangladesh. World's Poultry Science Journal. 64:99.

Denli M, Okan F and Celik K. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pakistan Journal of Nutrition. 2(2): 89-91.

Dhama K and Singh SD. 2010. Probiotics improving poultry health and production: an overview. Poultry Punch. 26(3): 41

Dibner J and Buttin P. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. The Journal of Applied Poultry Research. 11(4): 453-463.

Dina MW, Shibat El-hamd and Hams MA Mohamed. 2016. Effect of Probiotics on Salmonella Enteritidis Infection in Broiler Chickens Egypt. J. Chem. Environ. Health. 2 (2): 298 -314.

Diogo V, Koomen E and Kuhlman T. 2015. An economic theory-based explanatory model of agricultural land-use patterns: The Netherlands as a case study. Agricultural Systems. 139: 1-16.

Edwards H and Baker D. 1999. Effect of dietary citric acid on zinc bioavailability from soy products using an egg white diets with zinc sulfate hepatahydrate as the stander. Poult. Sci. 78 (suppl 1): 576.

Ezema C. 2007. The performance of broilers fed palm kernel cake-based diet supplemented with bioactive yeast. MSc Dissertation. Dept. of Animal Health and Production, University of Nigeria, Nsukka.

Ezema Chuka. 2014. Comparative Study of the Effects of Probiotic and Commercial Enzyme on Growth Rate, Haematology and Serum Biochemistry of Broiler Chicken. Pubmed.

Fallah R and Rezaei H. 2013. Effect of dietary prebiotic and acidifier supplementation on the growth performance, carcass characteristics and serum biochemical parameters of broilers. Journal of Cell and Animal Biology. 7 (3): 21-24.

Fernández-Rubio C, Ordóñez C, Abad-González J, Garcia-Gallego A, Honrubia MP, Mallo JJ and Balaña-Fouce R. 2009. Butyric acid-based feed additives help protect broiler chickens from Salmonella Enteritidis infection. Poultry science. 88 (5): 943-948.

Fuller R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66: 365-378.

Fuller R. 2001. The chicken gut micro flora and probiotic supplements. J. Poult. Sci. 38: 189-196

Garcia V, Catala-Gregori P, Hernandez F, Megias M and Madrid J. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. The Journal of Applied Poultry Research. 16 (4): 555-562.

Gharib Naseri K, Rahimi S and Khaki P. 2012. Comparison of the effects of probiotic, organic acid and medicinal plant on Campylobacter jejuni challenged broiler chickens. Journal of Agricultural Science and Technology. 14: 1485-1496.

Ghazalah A, Atta A, Elkloub K, Moustafa ME and Shata RF. 2011. Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. International Journal of Poultry Science.

Goli S and Aghdam Shahryar H. 2015. Effect of Enzymes Supplementation (Rovabio and Kemin) Me on some Blood Biochemical Parameters, Performance and Carcass Characterizes in Broiler Chickens. Iranian Journal of Applied Animal Science. 5(1): 127-131 127.

Guillot JF. 1998. Les probiotiques en alimentation animale. Cah. Agric. 7:49-54

Gunal M, Yayli G, Karahan O and Sulak O. 2006. The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci. 5: 149-155.

Haghighi HR, Jianhua G, Carlton L, Glyes M, Hayes A, Sanei B, Parvizi P, Gisavi H, James R, Chambers and Shrif SH. 2005. Modulation of antibody-mediated immune Response by probiotics in chickens. Clinical and diagnostic laboratory immunology. 12: 1387-1392.

Hajati H and Rezaei M. 2010. The application of probiotics in poultry production. Inter. J. Poult. Sci. 9: 298-304.

Hajati H. 2010. Effects of Enzyme Supplementation on Performance, Carcass characteristics, Carcass Composition and Some Blood Parameters of Broiler Chicken. American Journal of Animal and Veterinary Sciences 5 (3): 221-227.

Haque M, Islam KM, Akbar M, Chowdhury R, Khatun M, Karim M and Kemppainen B. 2010. Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. Canadian journal of animal science. 90 (1): 57-63.

Harr KE. 2006. Diagnostic value of biochemistry, In: Harrison GJ, Lightfoot TL, editors, Clinical Avian Medicine. V: 2.

Hassan H, Mohamed M, Youssef AW and Hassan ER. 2010. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australasian Journal of Animal Sciences. 23 (10): 1348-1353.

Hassan HM, Amani A, Youssef W, Mohamed MA. 2011. Using commercial enzyme preparations in male and female broilers fed low energy diets. Egypt J Anim Prod. 48:247–259.

Hernandez F, Garcia V, Madrid J, Orengo J, Catalá P and Megias M. 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. British poultry science. 47 (1): 50-56.

Hu Z and Guo Y. 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Animal feed science and technology. 132 (3): 240-249.

Islam M, Khandaker Z, Chowdhury S and Islam K. 2008. Effect of citric acid and acetic acid on the performance of broilers. Journal of the Bangladesh Agricultural University. 6 (2): 315-320.

Islam MM, Ahmed ST, Kim YJ, Mun HS, Kim YJ, Yang CJ. 2014. Effect of Sea Tangle (Laminaria japonica) and Charcoal Supplementation as Alternatives to Antibiotics on Growth Performance and Meat Quality of Ducks. Asian-Australas J Anim Sci. 27(2): 217-24.

Jin LZ, Ho YW, Abdullah N and Jalaludin S. 1997. Probiotics in poultry: Modes of action. World Poultry Sci J 53: 351-368.

Joy AD and Samuel JJ. 1997. Effect of probiotic supplementation on the performance of broiler. J. Vet. Anim. Sci. 28(1): 10-14.

Józefiak D, Kaczmarek and Rutkowski A. 2010. The effects of benzoic acid supplementation on the performance of broiler chickens. Journal of animal physiology and animal nutrition. 94 (1): 29-34.

Kabir SML, Rahman MM, Rahman MB, Rahman MM, Ahmed SU. 2004. The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci. 3: 361-364.

Kalavathy R, Abdullah N, Jalaludin S and Ho YW. 2003. Effect of lactobacillus culthres on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chicken. British. Poultry. Science. 44: 139-144.

Karaoglu M and Durdag H. 2005. The influence of dietary probiotics (Saccharomyces cerevisiae) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. Int. J. Poult. Sci. 4: 319 -316.

Khaksefidi A and Ghoorchi T. 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. J. Poult. Sci. 43: 296-300.

Khan SH and Iqbal J. 2016. Recent advances in the role of organic acids in poultry nutrition. Journal of Applied Animal Research. 44 (1): 359-369.

Khattak FM, Pasha TN, Hayat Z and Mahmud A. 2006. Enzymes in Poultry Nutrition. J. Anim. Pl. Sci. 16(1-2)

Knarreborg, A, Lauridsen, C, Engberg RM and Jensen, SK. 2004. Dietary antibiotic growth promoters enhance the bioavailability of α-tocopheryl acetate in broilers by altering lipid absorption. The Journal of nutrition, 134 (6): 1487-1492.

Koenen ME, Karmer J, Vander Hulst R, Heres L, Jeurissen SH and Boersma WJ. 2004. Immunomodulation by probiotic Lactobacilli in layer and meat-type chickens. British Poultry Science. 45: 355-366.

Král, M, Angelovičová, M, Mrázová, Ľ, Tkáčová, J and Kliment, M. 2011. Probiotic and acetic acid effect on broiler chickens performance. Scientific Papers Animal Science and Biotechnologies. 44 (1): 62-64.

Kušar D, Pate M, Mićunović J, Bole-Hribovšek V and Ocepek M. 2010. Detection of Salmonella in poultry faeces by molecular means in comparison to traditional bacteriological methods. Slovenian Veterinary Research. 47 (2).

Kwon Y and Ricke S. 1998. Induction of acid resistance of Salmonella typhimurium by exposure to short-chain fatty acids. Applied and environmental microbiology. 64 (9): 3458-3463.

Langhout P. 2000. New additives for broiler chickens. pp: 24–27

Lawal TE, Iyayi EA, Adeniyi BA and Adaramoye OA. 2010. Biodegradation of Palm Kernel Cake with Multienzyme Complexes from Fungi and its Feeding Value for Broilers. International Journal of Poultry Science. 9 (7): 695-701.

Lazaro R, Garcia M, Medel P and Mateos GG. 2003. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poult. Sci. 82: 132-140.

Leeson S, Namkung H, Antongiovanni M and Lee E. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry science. 84 (9): 1418-1422.

Lohakare J, Ryu M, Hahn TW, Lee J and Chae B. 2005. Effects of supplemental ascorbic acid on the performance and immunity of commercial broilers. The Journal of Applied Poultry Research. 14 (1): 10-19.

Lückstädt C and Mellor S. 2011. The use of organic acids in animal nutrition, with special focus on dietary potassium diformate under European and Austral-Asian conditions. Recent Adv Anim Nutr Aust. 18: 123-130.

Mahdavi AH, Rahman HR and Pourreza J. 2005. Effect of probiotic supplements on egg quality and laying hen's performance. International Journal of Poultry Science. 4(7): 488–492.

Mahdavi S, Zakeri A, Mehmannavaz Y and Nobakht A. 2013. Comparative Study of Probiotic, Acidifier, Antibiotic Growth Promoters and Prebiotic on Activity of Humoral Immune and Performance Parameters of Broiler Chickens. Iranian Journal of Applied Animal Science. 3(2): 295-299.

Mansoub NH. 2010. Effect of Probiotic Bacteria Utilization on Serum Cholesterol and Triglycerides Contents and Performance of Broiler Chickens. Global Veterinaria. 5(3): 184–186.

Meng X, Slominski BA, Nyachoti CM, Campbell LD. and Guenter W. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poultry

Miles RD, Bootwalla SM. 1991. Direct-fed microbials in animal production. In Direct-Fed Microbials in Animal Production. A Review: National Food Ingredient Association: West Des Monies, Iowa, USA. 117-132

Mohan S, Haker D, Pfeifer A. and Schiffrin EJ. 1996. Probiotics and Immune response. Clinical review and allergy Immunology. 22: 287-886.

Morgan AJ and Bedford MR. 1995. Advances in the development and application of feed enzymes. Australian Poultry Science Symposium. 7: 109–115.

Mountzouris K, Tsirtsikos P, Kalamara E, Nitsch SG, Schatzmayr G and Fegero K. 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult. Sci. 86: 309–317.

Mulder RWAW. 1991. Probiotics as a tool against Salmonella contamination. World Poultry Misset. 7: 6-7.

Nahashon SN; Nakaue HS and Mirosh LW. 1992. Effect of direct-fed microbials on nutrient retention and production parameters of laying pullets. Poult. Sci. 71(1): 111.

Nava GM, Bielke LR, Callaway TR and Castaneda MP. 2005. Probiotic- alternatives to reduce gastrointestinal infections: the poultry experience. Anim. Health Res. Rev. 6(1): 105-118.

Neil Osterweil. 2005. The Benefits of Protein: Beef up your knowledge of protein and good dietary sources of microbial phytase in broiler startrs fed wheat-based diets: perfprmance, bone mineralization and ideal digestibility.

Omogbenigun, F Nyachoti C and Slominski B. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. Journal of animal science. 81 (7): 1806-1813.

Panda A, Rao SR, Raju M and Sunder GS. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australian Journal of Animal Science. 22 (7): 1026-1031.

Panda AK, Reddy MR, Rama Rao SV, Raju Mvln and Paraharajj NK. 2000. Growth, carcass characteristics, immunocomponence and response to Escherchia coli of broiler fed diets with various level of probiotic. Archive fur geflugelkunde. 64: 152-156

Panda SJC, Sahu BJC and Rao. 1995. Urea tolerance in broiler chicken. Indian journal of Poultry Science. 63(5): 54-68.

Partanen KH and Mroz Z. 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews. 12 (1): 117.

Patterson JA and Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82: 627-631.

Paul S, Samanta G, Halder, G and Biswas P. 2007. Effect of a combination of organic acid salts as antibiotic replacer on the performance and gut health of broiler chickens. Livestock Research for rural development. 19 (11): 2007.

Pietras M. 2001. The effect of probiotic on selected blood and meat parameters of broiler chickens. J. Anim. Feed. Sci. 10 (2):297-302.

Rahman MS, Mustari A, Salauddin M and Rahman MM. 2013. Effects of probiotics and enzymes on growth performance and haematobiochemical parameters in broilers. J. Bangladesh Agril. Univ. 11(1): 111–118.

Ramasamy K, Abdullah N, Wong MC, Karuthan C and Ho YW. 2010. Bile salt deconjugation and cholesterol removal from media by *Lactobacillus* strains used as probiotics in chickens. Journal of the Science of Food and Agriculture, 90(1): 65–69.

Revington B. 2002. Feeding poultry in the post-antibiotic era. Multi-State Poultry Meeting. Available from: http://ag.ansc.purdue.edu/poultry/multistate/multi-state.pdf.

Rodríguez-Lecompte J, Yitbarek A, Brady J, Sharif S, Cavanagh M, Crow G, Guenter W, House J and Camelo-Jaimes G. 2012. The effect of microbial-nutrient interaction on the immune system of young chicks after early probiotic and acidifier administration. *Journal of animal science*. 90 (7): 2246-2254.

Rosin EA, Blank G, Slominski BA and Holley RA. 2007. Enzyme supplements in broiler chicken diets: in vitro and vivo effects on bacterial growth; Journal of the Science of Food and Agriculture. 87 (6): 1009-1020.

Saadia MH and Soliman NK. 2010. Effect of probiotic (Saccharomyces cerevisiae) adding to diets on intestinal microflora and performance of hyline layers hens. J. Am. Sci., 6(11):159-169.

Saarela M, Mogensen G, Fondens R, Matto J and Mattila-Sandholm T. 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84, 197–215.

Saegusa SM, Totsuka S, Kaminogawa and Hosoi T. 2004. Candida albicans and Saccharomyces cerevisiae induce interleukin-8 production from intestinal epithelial-like Caco-2 cells in the presence of butyric acid. FEMS Immunal Med. Microbiol. 41: 227–235.

Saleh F, Tahir M, Ohtsuka A and Hayashi K. 2005. A mixture of pure cellulase, hemicellulase and pectinase improves broiler performance. British Poultry Science. 46: 602-6.

Salminen, S, Wright A von, Morelli L, Martean P, Brassart D, Vos WM, Fonden R and. Matills-Sandholm T. 1998. Demonstration of safety of probiotic-A Review. Intl. J. Food Microbiol. 44:93-106

Sas J. 2000. JMP statistics and graphics guide, version 4. SAS Institute, Inc., Cary, North Carolina. Science. 84(1): 37-47.

Shareef AM. 2009. Effect of probiotic (Saccharomyces cerevisiae) on performance of broiler chicks. Iraqi Journal of Veterinary Sciences. 23: 23–29.

Sheikh Adil, Tufail Banday, Gulam Ahmad Bhat, Mir Salahuddin, Mashuq Raquib, Syed Shanaz.2011. Response of broiler chicken to dietary supplementation of organic acids. Journal of Central European Agriculture. 12(3): 498-508

Sheppy C, Bhat M and Hazlewood. Some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids.

Sinnons and Sainsbury. 2001. Potential organisms to prevent diarrhea and promote digestion in farm animals. Journal of Food and Agricultural science. 49: 1-13.

Stavric S. 1992. Experience of the use and Lactobacilli cultures on intestinal microflora of probiotics for the salmonella control in and performance in broilers. Asian-Australasian poultry. Lett. Appl. Microbiol. 14:69-71.

Sugiharto, S. 2014. Role of nutraceuticals in gut health and growth performance of poultry. Journal of the Saudi Society of Agricultural Sciences.

Tellez G. 2007. Effect of Organic Acids and Probiotics on Salmonella enteritidis Infection in Broiler Chickens.

Thomke S and Elwinger K. 1998. Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. Ann. Zootech. 47:245-271

Van Immerseel F, De Zutter L, Houf K, Pasmans F, Haesebrouck F and Ducatelle R. 2009. Strategies to control Salmonella in the broiler production chain. World's Poultry Science Journal. 65 (03): 367-392.

Van Immerseel F, Russell J, Flythe M, Gantois I, Timbermont L, Pasmans F, Haesebrouck F and Ducatelle R. 2006. The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathology. 35 (3): 182-188.

Vegad JL. 2004. Poultry Diseases: A Guide to Farmers and Poultry Professionals. International Book Distributing Co. Lucknow UP. 343-346 & 674-683.

Verstegen MWA, Williams BA. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. AnimBiotechnol. 13: 113-127.

Vicente JL, Avina L, Torres-Rodriguez A, Hargisand B and Tellez G. 2007. Effect of a Lactobacillus spp based probiotic culture product on broiler chicks performance under commercial conditions. Int. J. Poult. Sci. 6: 154-156.

Vieira S, Oyarzabal O, Freitas D, Berres J, Pena J, Torres C and Coneglian J. 2008. Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. The Journal of Applied Poultry Research. 17 (1): 128-133.

Wang ZR, Qiao SY, Lu WQ and Li DF. 2005. Effects of enzyme supplementation on performance, nutrient digestibility of broilers fed wheat-based diets. Poultry Science. 84: 875-81.

Watkins S, Cornelison J, Tillery C, Wilson M and Hubbard R. 2004. Effects of water acidification on broiler performance, Avian Advice. 6: 4-6.

Wolfenden AD, Vicente JL, Higgins JP, Andreatti R, Higgins SE, Hargis BM and Tellez G. 2007. Effect of organic acids and probiotics on Salmonella Enteritidis infection in broiler chickens. Int. J. Poult. Sci., 6: 403-405.

World Food Program Bangladesh Country Overview, 2017. http://www.wfp.org/countries/Bangladesh/overview.

Yousefi M and Karkoodi K. 2007. Effect of Probiotic Thepax and Saccharomyces cerevisiae Supplementation on Performance and Egg Quality of Laying Hens. International Journal of Poultry Science. 6(1): 52-54.

Yurong Y, Ruiping S, Shimin Z and Yibao J. 2005. Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. Arch. Anim. Nutr. 59: 237–246

Zha C and Cohen A. 2014. Effects of anti-fungal compounds on feeding behavior and nutritional ecology of tobacco budworm and painted lady butterfly larvae. Entomology, Ornithology & Herpetology: Current Research. 2014.

Zhang ZF and Kim IH. 2014. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poultry science. 93(2): 364–370.

Brief biography of the author

This is DR. Nasima Akter, a Candidate for the degree of MS in Animal and Poultry Nutrition, Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, CVASU. She passed the Secondary School Certificate Examination from Cox'sBazar Govt. Girls' High school in 2007 with GPA 5.00 followed by Higher Secondary Certificate Examination from Cox'sBazar Govt. College in 2009 with GPA 4.80. She obtained his Doctor of Veterinary Medicine Degree in 2014 with CGPA 3.72 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh.

Appendix A

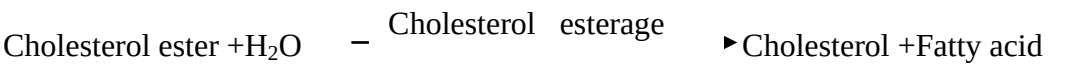
Methods of estimating different biochemical parameters (according to manufactures instruction)

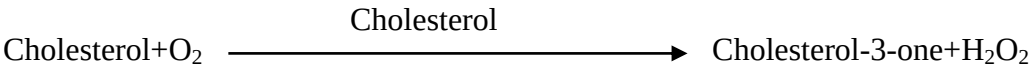
Cholesterol assay

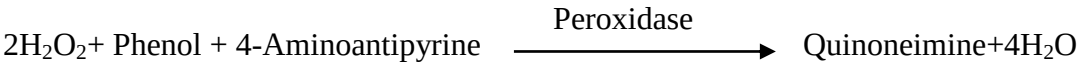
Principle

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

Reactions







Materials and reagents

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was an enzmatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tube was taken. Then 10µl of cholesterol standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. 1000µl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was

examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

Triglyceride assay

Principle

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenezone and 4-Chlorophenol under the catalytic influences of peroxidase.

Materials and reagent

1. Serum sample
2. TG conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

The sterile eppendorf tubes were taken. Then 1000µl TG standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

LDL assay

Principle

The principles outcome of LDL is based on the principle of competitive bindings between LDL and LDL reagent. Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (PH-5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant. The absorbance of this complex is proportional to the LDL concentration in the sample.

Materials and reagents

1. Serum sample
2. LDL conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

The sterile eppendorf tubes were taken. Then 100µl of LDL standards was taken in an eppendorf tube and 100µl of sample serums were taken in each eppendorf tube. 1000µl of LDL conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. The LDL concentration of the supernatant was determined within 1 hour after centrifugation. LDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with LDL conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

HDL assay

Principle

Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitavily by the addition of phosphotangstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density Lipoprotein) fraction, which remains in the supernatant, is determined.

Materials and reagents

1. Serum sample
2. HDL conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips,distilled water, 70% alcohol.

Procedure

The sterile eppendorf tubes were taken. Then 400µl of HDL standards was taken in an eppendorf tube and 200µl of sample serums were taken in each eppendorf tube. 100µl of distilled water was then added to each eppendorf tube. The eppendorf tube was kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. Then 50 µl HDL concentration of the supernatant was taken and 1000 µl Cholesterol reagent added determined within 1 hour after centrifugation. HDL standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with HDL conjugate reagent was examined by automated humalyzer and the r reading was taken. The standard value was used as a compared tool, absorbent paper or paper towel or cotton and gloves.

Total protein assay

Principle

The principle outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

Materials and reagents

1. Serum sample
2. Total protein conjugate reagent
3. Precision pipettes: 20µl and 1.0ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20µl of total protein standards was taken in an eppendorf tube and 20µl of sample serums were taken in each 24 eppendorf tube. 1000µl of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Total protein standards with conjugate.

Estimation of AST

Procedure:

Fresh ddH_2O was aspirated and a new Gain Calibration was performed in flow cell mode. AST in the run test screen was selected and water blank was carried out as instructed. In a test tube 0.05 ml sample and 0.5 ml reagent was taken which was mixed and aspirate dinto the Rx Monza by pipette. In cuvette 0.1 ml sample and 1 ml co-enzyme (α-oxoglutarate) was taken and mixed. The initial absorbance was read after 1 min and again after 1, 2 and 3 min and then calculated. The absorbance change per minute is between 0.11 and 0.16 at 340/ Hg 334 nm or 0.06 and 0.08 at Hg 365 nm.

Estimation of ALT

Reagent Composition

	Contents	Concentration in the text
Rla.	**Buffer/Substrate**	
	Tris buffer	100 mmol/l, pH 7.5
	L-alanine	0.6 mol/l
Rlb.	**Enzyme/Coenzyme/α-oxoglutarate**	
	α-oxoglutarate	15 mmol/l
	LD	≥ 1.2 U/ml
	NADH	0.18 mmol/l

Procedure

Aspirate Fresh ddH_2O was aspirated and a new gain calibration in flow cell mode was performed. Select ALT was selected in the Run Test screen and water blank was carried out as instructed. In a test tube 0.05 ml sample and 0.5 ml reagent was taken which was mixed and aspirate dinto the Rx Monza by pipette. In cuvette 0.1 ml sample and 1 ml co-enzyme (α-oxoglutarate) was taken and mixed. The initial absorbance was read after 1 min and again after 1, 2 and 3 min and then calculated. The use of Saline and Randox Calibration Serum Level 3 is recommended for Calibration. Calibration is recommended with change in reagent lot or as indicated by quality control procedures.

Appendix B

Table 1. Nutritive value of basal diet in broiler feeding. **(Starter)**

Estimated chemical composition (DM basis)	
Metabolizable Energy (Kcal/kg)	3000
Crude Protein (%)	21.50
Crude Fiber (%)	5.00
Fat (%)	3.5
Lysin (%)	1.25
DL Methionine (%)	0.5

Table 2 Nutritive value of basal diet in broiler feeding. **(Grower)**

Estimated chemical composition (DM basis)	
Metabolizable Energy (Kcal/kg)	3100
Crude Protein (%)	20.00
Crude Fiber (%)	5.00
Fat (%)	3.00
Lysin (%)	1.20
DL Methionine (%)	0.45