EFFECT OF HUMIC ACID, PROBIOTICS AND THEIR COMBINATION ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS OF BROILER

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CERTIFICATE

*This is to certify that the thesis entitled, " **EFFECT OF HUMIC ACID, PROBIOTICS AND THEIR COMBINATION ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS OF BROILER"** Submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS)** in **Animal Nutrition** embodies the result of a piece of bona fide research work carried out by **MD.Khalaquzzman**, Registration No. **20-11104**,Semester: **JANUARY-JUNE/2022** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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Dedicated

To

My Beloved Parents

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION		FULL MEANING
ANOVA	=	Analysis of Variance
Avg.	=	Average
BWG	=	Body Weight Gain
DP	=	Dressing Percentage
e.g.	=	For Example
et al.	=	And Others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
g	=	Gram
i.e.	=	That Is
L	=	Liter
MS	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
SE	=	Standard Error
SPSS	=	Statistical Package for Social Sciences
Viz.	=	Such As
hrs.	=	Hours
°C	=	Degree Celsius
/	=	Per
%	=	Percentage
±	=	Plus-Minus
:	=	Ratio
m/s	=	Meter Per Second
mg	=	Mille Gram
Cu. Ft.	=	Cubic Feet
BCR	=	Benefit Cost Ratio

EFFECT OF HUMIC ACID, PROBIOTICS AND THEIR COMBINATION ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS OF BROILER

ABSTRACT

The present study was conducted at the National Hatchery Research and development farm Dighulia, Tangail, The Poultry Farm for a period of four weeks using different level of dietary humic acid and probiotics in feed. The specific objectives of this study were under taken to determine the effect of different level of dietary humic acid and probiotics to assess alternative to antibiotics & production performance of broiler. A total of 195 day-old Lohmann meat broiler chicks were allocated randomly to five treatment groups with three replications having 13 broilers per replication. The experiment lasted for 4 weeks and the treatment of various groups consisted of group T_0 (Basal feed), T_1 (Basal feed+0.05% HA), T_2 (Basal feed+0.10%HA), T_3 (Basal feed+0.02% Probiotics) and T_4 (Basal feed+0.05% HA+0.02% Probiotics) respectively. The parameters evaluated in this study were body weight, body weight gain, feed consumption, FCR, flock uniformity, survivability, carcass characteristics, caecal microbial count, immune parameter and BCR of broiler. Result demonstrated that the body weight (g) was not significantly different ($P>0.05$) higher in T_4 (1648.67±91.5) and lower in T_0 (1590.67 ±48.32). The body weight gain (g) was not significantly different ($P>0.05$), however the higher value was found in T4 (1597.33±96.88) and lower value was in T_0 (1542.33±46.30) group. The higher feed consumption was found in T_4 and lower in T_2. The overall feed consumption of different treatment groups showed that there was non significant ($P>0.05$) effects on feed consumption. There was no significant ($P>0.05$) differences in FCR among treatments T_0, T_1, T_2, T_3 and T_4. However the better feed conversion ratio (FCR) was not significantly ($P>0.05$) observed in T_4 (1.32±0.00). Dressing percentage significantly ($P<0.05$) higher in T_4 (69.12 ±2.19) and lower in T_0 (59.57±2.96). The weight (g) of thigh, wing and back in T_4 were significantly ($P<0.05$) higher than the other groups. But the weight (g) of drumstick in T_3 was significantly ($P<0.05$) higher than the other treatment groups. The weight (g) of Liver was significantly higher ($P<0.05$) and Heart, Spleen and Gizzard in T_4 was not significantly higher ($P>0.05$) than the other groups. Flock uniformity (%) was higher in treated group T_4 (82.67±67) than others group including control group T_0. There was no significant difference ($P>0.05$) in flock uniformity (%). There was no significant difference ($P>0.05$) in survivability rate (%). BCR is not significantly higher ($P>0.05$) in

treatment group T_4 (1.29±0.02) than others groups. No. of *E. coli* colony (cfu/g) and no. of *Salmonella sp.* colony (cfu/g) were significantly lower ($P<0.05$) in treatment group T_4 than other groups. The above research was found that body weight (g), body weight gain (g), dressing percentage, weight (g) of liver, heart, spleen, gizzard, thigh, drumstick, back and survivability rate were better result in T_4 than other groups. FCR and BCR were also found better in T_4 (basal feed+0.05%HA+0.02% probiotic) group along with better performance was in humic acid and probiotics treated group. It was concluded that better result was found in T_4 group than others groups. Therefore, the research recommended that broiler rearing with basal feed+0.05%HA+0.02%probiotics along with different level of humic acid and probiotics could be used on broiler production for better performance and profitability.

CHAPTER I

INTRODUCTION

Antimicrobials have been used as feed supplement for more than 50 years in poultry feed to enhance the growth performance and to prevent diseases in poultry. However, in recent year great concern has a risen from the use of antibiotics as a supplement at the sub-therapeutic level in poultry feed due to the emergence of multiple drug resistant bacteria. The banning of the use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in animal production. As the alternatives of humic acid and probiotics have been used as feed supplements to improve growth performance under the intensive management systems.

In veterinary practice, humic substances (HS) have been used as antidiarrheal, analgesic, immune stimulatory and antimicrobial agents in Europe. The main substances that make up the HS are the humic acids (HA), fulvic acids (FA), and humins, and hence, they are a complex mixture of aliphatic chains or aromatic rings with specific content of functional groups, but the concentration of these substances differs according to the raw materials that they originate from. Linarite and lignites, two non-renewable resources, are the primary commercial sources of HS, while compost and worm compost, especially those produced with animal manure, are two environmentally friendly sources of HS. Specifically, HA are defined as naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains.

Probiotic is defined as a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). Probiotics stimulates the growth of beneficial microorganisms and reduces the amount of pathogens thus improving the intestinal microbial balance of the host (Fuller, 1989). Intake of Probiotic lowers the risk of gastro-intestinal diseases by stimulating the growth of beneficial microorganisms (Fuller, 1989). Supplementation of probiotics alleviates the problem of lactose intolerance, the enhancement of nutrients bioavailability, and prevention or reduction of allergies in susceptible individuals. Probiotics are reported to have alsoant mutagenic, ant carcinogenic, hypo cholesterol emic, antihypertensive, anti-osteoporosis, and immune modulatory effects.

To achieve the best profitability in poultry production, gut health is critical for accomplishing the optimum growth, digestion, and nutrient absorption and utilization. It's also essential for immune function and effective post-absorption metabolism. The healthy gut- acts as a barrier against pathogens, protects the bird against toxins and harmful metabolites, and supports the mucosal immune response. Challenges in the poultry industry as enteric disease outbreaks can cause poor feed efficiency, lower weight gain, and an overall increase in mortality, resulting in major economic impacts (Porter, 1998; Dosoky *et al*., 2022). Necrotic enteritis caused by *Clostridium perfringens* is considered one of the most important enteric diseases reported by poultry producers worldwide (Paiva and McElroy, 2014). It negatively affects the intestinal mucosa resulting in decreased digestion and absorption, reduced weight gain and increased feed conversion ratio, and significant economic losses (Elwinger *et al*., 1992; Kaldhusdal *et al*., 2001). Nonantibiotics (probiotics) growth promoters have been used in poultry farms for decades to keep birds healthy and help them grow faster (Huyghebaert *et al.*, 2011). There is a worldwide endeavor to decrease antibiotic use in poultry production, since a high level of microbial resistance to antibiotics, and residues of antibiotics in poultry products can be harmful to humans. Since 2006, the European Union has banned the use of antibiotics as feed additives in animal diets (Saleh *et al.* 2020). scientific evidence suggests that the enormous use of antibiotics can promote bacterial resistance in treated animals (Furtula *et. al*., 2010; Forgetta *et. al.*, 2012) A variety of different supplements, as the alternatives to antimicrobial growth promoters, have been explored to maintain growth performance of broilers (Dickens *et al*., 2000; Ghadban, 2002; Biggs and Parsons, 2008; Chowdhury *et al.*, 2009). Several studies showed that dietary supplementation of lactic acid bacteria (e.g., *Lactobacillus*) improved the performance of broilers in the starter phase (Yeo and Kim, 1997; Zulkifli *et al.*, 2000). Feeding *Saccharomyces cerevisiae* products was shown to improve growth performance of broilers after 21 d of age (Stanley *et al.*, 2004; *Gao et al*., 2009). Although the manner by which probiotics act remains to be clarified, they are thought to function by maintaining the presence of beneficial microorganisms, and competitive exclusion of pathogenic bacteria adherence in the intestine of broilers (Reid and Friendship, 2002; Callaway *et al*., 2008). In addition, (Gao *et al*., 2008and Higgins *et al*., 2008) reported that intestinal immunity was increased in chickens fed diets supplemented with yeast product and *Lactobacillus*-based probiotic culture, respectively. Our previous study on probiotics products incorporating *Lactobacillus fermentum* and *Saccharomyces cerevisiae* indicated that they improved the intestinal balance of the diverse micro flora species in the rectum of broiler chickens (Lei *et al.*, 2009). However, Klasing (1998) reviewed that probiotics enhanced the intestinal microbial balance and intestinal immunity in chickens, which might result in decreased growth performance. (Tugnoli *et al*., 2020). Once they diffuse through the cell membrane of the bacteria cell, the weak organic acid dissociates due to the higher pH of the bacterial cytoplasm, causing the cytoplasm pH to drop rapidly, resulting in the death of the bacteria (Dibner and Buttin, 2002). Additionally, organic acids have other biological activities besides antimicrobial activity, such as improved intestinal health for efficient nutrient consumption and absorption, thus enhancing the overall health and efficiency of broilers (Khan *et al.* 2022). Humic acid (HA) is a naturally occurring component of drinking water, soil, and lignite which is formed by the decomposition of organic matter, especially plants. Positive effects of HA addition in feed have been reported as improved broiler growth by increasing protein digestibility and trace element utilization,(Islam *et al.* 2005; Bahadori*et al*. 2017), improved gut health, allowing for better nutrient utilization and improved health status, influence digestion, immune response and general performance of broilers (Furtula*et al.* 2010). Humic acids have recently been used in the feed and water of poultry to promote their development (Rath *et al*., 2006; Abd El-Hack 2016; Fouda *et al.*, 2021). Salah *et al.* (2015) indicated that HA supplementation significantly increased broiler body weight gain and FCR. To the best of our knowledge, no more research has been done to evaluate the impact of HA with probiotic on broiler performance and other physiological functions when exposed to bacterial infection. Further research into the growth performance and other physiological traits of meat quality and fatty acids contents of commercial broilers fed diets supplement with humic acid and probiotics under clostridium infection appears to be needed in light of these observations. The purpose of the present study was to evaluate the effects of dietary supplementation of humic acid and probiotic single or combined on growth, internal organs, blood biochemistry, and gut morphology.

Objectives

From the above consideration, the present study was under taken to determine the effect of dietary feed additives of humic acid and probiotics with the following specific objectives:

1. To determine the growth performance and carcass characteristics by addition of humic acid and probiotics in broiler feed.
2. To determine the immune response of humic acid and probiotics in broiler production.
3. To identify the alternative of antibiotics in broiler diet.

CHAPTER II

REVIEW AND LITERATURE

It is very important to review the past research works which are related to the proposed study.In recent years the use of antibiotics at sub-therapeutic level as growth promoter caused resistant in bacteria which reside in the gut (Phillips *et al.* 2004, Hernandez *et al.,*2006). Due to this adverse situation, the European Union (EU) and Bangladesh also banned the antibiotics at sub therapeutic level as growth promoters in poultry diet in 2006, since then sub-therapeutic use of antibiotics is not practiced (Castanon 2007, Yang *et al.* 2009). Therefore, the poultry nutritionists are trying to substitute the antibiotics with different natural feed additives, such as organic acids and probiotics that can be beneficial similar in control of infectious diseases and ultimately improve feed efficiency (Zhang *et al*. 2005, Alamo *et al*. 2007, Abd. El-Hack *et al*. 2015, Alagawany *et al.* 2015 a, b, c). By using different feed additives growth and feed conversion ratio will be improved and also result in disease prevention. Organic acids have been practiced for some decades as feed preservatives for protecting the destruction of feed from microbes and fungus (Paul *et al.* 2007).

2.1 Properties and composition of Humic Acid

Humic acid (HA) is an organic substance which is acquired by decomposition of organic matter and having long chain molecular formula with high molecular weight. It is insoluble in strong acids having pH below than 2 and soluble in alkaline media (Islam *et al*., 2005). These substances have medium molecular size and their molecular weight is around 5,000 to 100,000 Dalton. Oxygen represents 33–36 %, and nitrogen represents 4 % in this substance. Because of their medium molecular size, sufficient negative surplus charge on their surfaces for peptizing the macromolecules will be present only in a more alkaline medium with a pH over 8. So their mobility in the soil is very limited in neutral acidic-alkaline condition. HA has been considered for quite a long substantial period of time as an alternative to antibiotic growth promoter in poultry production. Although many experimental studies have shown HA to be largely nontoxic and nonteratogenic, there are reports which showed that inclusion of HA in diets of broiler chickens at the rate of 0.5% might significantly reduce body weight and negatively affect feed conversion ratio (Rath *et al.,* 2006, Kocabagli *et al.,*2002) reported an improvement in feed conversion in birds that were given 0.25% HA either from 0 to 42 d or during grow-out periods only, between d 21 to 42, although the birds did not show improvement in body weight gain. With this background, the present study was designed with the primary objective of ascertaining the responses of broiler chickens fed with diets containing graded levels of HA varying from 0.05% (0.05 g/kg diet) to 0.3% (3 g/kg diet)

2.2 Properties of probiotics

Probiotics are one of the approaches that have a potential to reduce chances of infections in poultry and subsequent contamination of poultry products (Ahmad, 2006). Probiotics are defined as live bacteria-yeast cultures or biological products that are added to drinking water or feed to regulate the ecological balance of micro flora in the digestive tract of animals. These substances prevent the harmful effects of potentially pathogenic microorganisms and allow animals to derive increased benefits from the feed (Dibner and Richards, 2005). The selection of bacteria such as Lactobacillus, Pediococcus, Bacteroides, Bifidobacterium, Bacillus and Streptococcus, for use as probiotics is based on assessment of their metabolic products and their potential to colonize specific sites (Lima *et al.*, 2007). Several studies have shown that addition of probiotics to the diet of broiler and turkey leads to improve the performance (Vicente *et al*., 2007).

2.3 Beneficial effects of HA and Probiotics alternative to antibiotics

The unfavorable effect of chemical products especially antibacterial/antibiotics led to the use of natural products like phytogenics to improve the efficiency of feed utilization and growth performance of poultry (Adil *et al*., 2015). The use of antibiotics at sub-therapeutic level as growth promoter caused resistant in bacteria which reside in the gut (Phillips *et al*. 2004, Hernandez *et al.* 2006). Humic acid was used in poultry as growth promoter (Bailey *et al.* 1996, Parks 1998, Shermer *et al.*, 1998, Eren *et al.*, 2000). Humic acid reduced stress (Enviromate 2002) and enhanced immunity of birds (Enviromate 2002, Humin Tech 2004). Humic acid improved the protein digestibility of animals especially in broilers (Kreutz and Schlikekewey 1992, Huang *et al.*, 1994, Seffner *et al*., 1995, Yang *et al.*, 1996). Use of HA on daily basis showed positive effect on broilers growth performance (Bailey *et al.* 1996, Parks 1998, Shermer *et al*., 1998, Eren *et al.*, 2000, Kocabaðli *et al.*, 2002, Humin Tech 2004). Humic acid supplementation in broiler could improve body weight and feed efficiency and also enhance fat deposition on thigh muscles (Ozturk *et al*., 2010). Humic acid possessed potential to reduce bacterial and mold growth, by inhibiting the mold growth, toxin level can be reduced (Humin Tech 2004). Limited information is available of humic acid in poultry. Therefore, the following study was planned to investigate the impact of different levels of dietary humic acid supplementation on growth performance and carcass traits of broiler chicks.

Broiler chicken feeding a probiotic product improved growth performance in the early stage, but there was no dose response. These results agreed with our previous finding that the growth performance of broilers was increased by feeding probiotics (*Lactobacillus fermentum* and *Saccharomyces cerevisiae*) at levels ranging from 0.1 to 0.3% during 1 to 21 d. We therefore recommended supplementing 0.1% probiotic product in diets, as an alternative to antimicrobial growth promoters, for better performance of broilers during the starter phase. Similarly, Yeo and Kim (1997) and Zulkifli *et al.* (2000) demonstrated that a 0.1% *Lactobacillus*- supplemented diet improved ADG and feed efficiency from 1 to 21 d of age, but not from 22 to 42 d of age. Li *et al.*(2008) found that a commercial probiotic mixture of yeasts and other microbes improved growth performance in the starter phase, and there was no significant difference among different levels (0.2 to 0.6%).

2.4 Gastrointestinal effects

Probiotics can also improve the quality of meat produced and ensuring its safety to human health compared to the conventional production with the use of antibiotics (Dibner and Buttin 2002). More recently, interest in the use of natural and safe growth promoters such as organic acids has increased due to their effectiveness in improving the productive performance of poultry by inhibiting harmful actions of intestinal bacteria providing healthy environment for the birds (Hinton 1997). Humic acid is one of the most complex organic acids formed naturally from the decomposition of organic matter and coal (Alhameed and Jaloob 2016). It contains multiple carboxylic groups and aromatic binds (Mac Carthy 2001) and improves productive performance of the broiler chicks by boosting the immune system and inhibiting pathogenic bacteria and molds in addition to its ability to form a thin protective layer covering the epithelial layer of the gastrointestinal tract that useful against pathogen*s* (Chen *et al* 2001)

2.5 Immune modulatory activity

Humic substances are used in both human and veterinary medicine for their detoxication, antibacterial and antiviral effects, but more and more studies confirm their immune modulatory potential (Gomez-Rosales & Angeles, 2015; Joone & van Rensburg, 2004). Their immune stimulatory effect on infectious diseases is known, and their anti-inflammatory effect and suppression of an excessive immune response, for example in hypersensitivity reactions, have been reported (Islam *et al.,*2005). The effect of humic substances on the immune system is related to the properties of these substances. They form relatively solid complexes with carbohydrates. Subsequently, these complexes allow the formation of glycoproteincharacterized by the ability to bind to NK cells and T lymphocytes. It means that they behave as modulators, and enable subsequent communication between these cells. The ability of humic substances to affect the immunesystem, therefore, lies in the regulation of immune activity (Riede, Zeck-Kapp, Freudenberg, Keller & Seubert, 1991). For poultry husbandry, HS could be a powerful tool in maintaining the gastrointestinal health, thus improving body weight, feed conversion, and ash content of the tibia (Ceylan, Ciftci, & Ilhan2003; Kocabagli, 2002; Taklimi and Isakan2012. The effect of HS, as a binding agent for aflatoxin molecules in the gastrointestinal tract and prevention their absorption decreases the risk of aflatoxin toxicity in poultry. In addition, in laying hens and partridges benefits involving feed intake and egg production have been observed (Dobrzański, Erener, 2009). There is extensive information on the immune system (Schat and Myers, 1991).The immunological function of chick gut-associated lymphoid tissue (GALT) is critical for reducing the incidence of poultry enteric disorders (Reid and Friendship, 2002; Callaway *et al*., 2008) because GALT is exposed to the micro flora from concomitant feed and the environment (Bar-Shira *et al.*, 2003). The immune biotic lactic acid bacteria (Sato *et al*., 2009) and yeast product (Gao *et al.*, 2009) could stimulate the GALT immune system in chicks. Moreover, evidence is accumulating that suggests probiotics might augment Toll-like receptor (TLR) signaling, regulate local mucosal cell-mediated immune responses, enhance dendritic cell-induced T cellhypo responsiveness, and promote epithelial barrier integrity in avian and mammalian species (Jin *et al.*, 1998b; Gao *et al*., 2008; Ng *et al.*, 2009).

2.6 Effect of HA and probiotics on growth performance of broiler

(Khan, 2005) Humic acid was used in poultry as growth promoter (Bailey *et al.* 1996, Parks 1998, Shermer *et al.* 1998, Eren *et al.* 2000). Humic acid reduced stress (Enviromate 2002) and enhanced immunity of birds (Enviromate 2002, Humin Tech 2004). Humic acid improved the protein digestibility of animals especially in broilers (Kreutz and Schlikekewey 1992, Yang *et al*. 1996). Use of HA on daily basis showed positive effect on broilers growth performance (Bailey *et al.* 1996, Parks 1998, Shermer *et al.*, 1998, Eren *et al.*, 2000, Kocabaðli *et al.*, 2002, Humin Tech 2004). Humic acid supplementation in broiler could improve body weight and feed efficiency and also enhance fat deposition on thigh muscles

(Ozturk *et al*., 2010). Humic acid possessed potential to reduce bacterial and mold growth, by inhibiting the mold growth, toxin level can be reduced (Humin Tech 2004).The feed supplemented with probiotics alone or combined with the humic acid were not very promising giving less positive results. This may be explained that the probiotics was negatively affected by the presence of humic acid, indicating that humic acid may inhibited the beneficial microorganisms of the probiotics. Manhob *et al* (2016) also observed that the probiotics Bios B-Gold did not improve boiler's weight gain and feed conversion ratio. Taklimi *et al.* (2012) also observed significant improvement in weight gain and feed conversion ratio by adding the humic acid to the broiler's feed at 0.3%. The addition of 100 ppm humic acid to the drinking water of broiler chicks resulted in significant increase in total weight gain and improvement in feed conversion ratio (Mirnawati *et al*., 2013)

2.7 Body Weight

The body weight of the individual broiler chicks was measured at weekly intervals and the feed consumption was recorded each day. The feed conversion ratio was calculated on the basis of feed intake to body weight gain. After the completion of the fattening period (day 35), the animals were weighed, stunned, killed by cervical dislocation and bled Effect of HA as it helps in the stabilization of gut micro flora which results in improved nutrient absorption and WG (Sherm *et al.,* 1998; Saleh 2016). This improvement in FCR may be caused by the effects of the decrease in the total bacterial count, Salmonella, E. Coli, and Proteus by using HA. On the other hand, the improvement in the FCR with HA supplementation could be possibly due to better utilization of nutrients resulting in increased BW (Lala *et al.,* 2016) which was supported in our study by the improved digestibility of nutrients (crude fibre digestibility and crude protein retention). Similarly, Son *et a*l. (2002) demonstrated that using organic acids in broiler diets decreases feed passage rate resulting in increased digestion time and therefore improved utilization of feed nutrients. Changes in size and structure of internal organs can be indicative of the effect of diet and its components on the development and function of the organs. Dietary supplementation of HA in broilers exposed to clostridium infection did not affect the carcass, breast, thigh, and abdominal fat weights. However, liver, gizzard, heart, and spleen were significantly increased in groups treated with HA and probiotics of each supplement. Our results are in agreement with Avci *et al.,* (2007) who reported that the breast weight of broilers was not affected by supplementation of HA and humates. Likewise, Kocabağli *et al.* (2002) found that HA supplementing did not affect carcass yield or abdominal fat pad percentages in broilers. On the contrary, Aksu and Bozkurt

(2009) and Arif *et al.* (2018) found that breast and thigh weights were increased with HA inclusion in broiler diets. The observed increase in weights of gizzards and spleen could be attributed to the trophic effect of humic acid and probiotics**.** The feed consumption and weight gain differed among treatments and the breeding periods. In 0-3 week G4 chicks had the lowest feed conversion ratio among all the diet groups, groups G1, G2 and G3 did not significantly differ. The G3 chicks had the best feed conversion ratio among all the diet groups for the two periods 4-5 and 0-5 weeks. Mortality rate and economic figure showed that both the probiotic and humic acid significantly affected mortality rate and the economic figure. No mortality was recorded in any of the chicks groups. The economic figure significantly improved in the G3 chicks compared to G1 and G4 groups but not in G2 group.G1, G4 and G2 differ from each other. The addition of humic acid to broiler diets was effective giving best results for most studied traits. The humic acid chicks group had the highest average body weight.

2.8 Body weight gain

It was concluded from the present study that supplementation of humic acid to broiler diet had some beneficial effects in terms of body weight and feed conversion ratio although the effects were not dose dependent and better results were found when holmic acid was supplemented at the rate of 1 g/kg diet. Humic acid at the rate of 1g/kg improved vaccine titer against Newcastle disease and infectious bursal disease. This was corroborated from a higher weight of the thymus in the treated groups. Humic acid also sequestered the pro inflammatory responses as was revealed from a significantly lower inflammatory marker activity (alpha-1-acid-glycopreotein) in circulation. This observation supported the marginally higher villus height observed in the groups supplemented with humic acid. It was finally concluded that supplementation of humic acid in diets of broiler chickens at the rate of 1 g/kg may be beneficial in terms of improving body weight, FCR and vaccine titers though increasing the beyond this level may not be beneficial at least under standard conditions of management where enteric challenges are minimum.

2.9 Feed consumption

Total feed consumption was not affected by addition of probiotic and humate. We noticed the lowest feed consumption for probiotic (1.89 kg), followed by the treatments probiotic+ humic acids (1.90 kg) and humate (1.92 kg). The highest feed consumption was detected in control (1.94 kg). The results obtained from this research are in agreement with Yu *et al.* (2007) who reported that probiotic inclusion did not significantly affects feed consumption. In contrast, some researchers found a positive effect of dietary probiotic supplementation (Samli *et al.*, 2007; Baurhoo *et al*., 2009).

2.10 Feed Conversion ratio

Feed conversion ratio is improved by humic acid supplementation and HA3 diet was the best. There results herein are consistent with the findings of Bailey *et al.* (1996), Avci *et al*. (2007) who postulated the FCR was significantly improved with supplementing varying levels of humates and humic acid in broiler diets. Also, Ceylan *et al.* (2003) performed an experiment to observe the influence of antibiotic growth promoter, probiotics, prebiotics and humic acid fed in corn-soya based diet on growth performance and gut micro flora of broiler chicks; improved feed conversion ratio was observed in a group supplemented with probiotics, prebiotics and humic acid based mixture as compared with control group. In another study using Farmagülatör DRYTM Humates @ 2.5 g/kg, live performance showed better FCR (Kocabaðli*et al*., 2002). Feed conversion energy to the host. The organic acids namely, acetic ratio as affected by probiotics is the subject of acid, butyric acid, propionic acid and others, which controversy. Some studies show that probiotics cause reduction in pH which in turn reduces the activity supplementation in feed of chickens improves the feed of enzymes in the small intestine which is not desired. conversion ratio Alvarez *et al*., Some of the bacteria are useful for the production of 1994; Hamid *et al.*, 1994; Silva *et al*., 2000) while others vitamins i.e. vitamin A & K of the deficient diet in vitamins suggest no such effect on feed conversion ratio (Fuller, 1997). Probiotics have effect on the main physiological functions of the gastrointestinal tract, 1999; Ergun *et al*., 2000; which are digestion, absorption and propulsion 2000). Ahmad (2004) could not detect any difference in (Fioramonti *et al.*, 2003). Ahmad (2004) reported a feed conversion ratio of broilers as compared to controlled. Although many experimental studies have shown HA to be largely nontoxic and nonteratogenic, there are reports which showed that inclusion of HA in diets of broiler chickens at the rate of 0.5% might significantly reduce body weight and negatively affect feed conversion ratio (Rath *et al*., 2006, Kocabagli *et al*., 2002) reported an improvement in feed conversion in birds that were given 0.25% HA either from 0 to 42 d or during grow-out periods only, between d 21 to 42, although the birds did not show improvement in body weight gain. With this background, the present study was designed with the primary objective of ascertaining the responses of broiler chickens fed with diets containing graded levels of HA varying from 0.05% (0.05 g/kg diet) to 0.3% (3 g/kg diet. Moreover, Arafat *et al.* (2015)

claimed that feed conversion of laying hens was improved carcass weight, breast weight, thigh weight and neck weight with the findings of Karaoglu *et al.* (2004) and Avci *et al.* (2007) who reported that carcass yield, abdominal fat, neck weight, breast and thigh weight were not significantly improved by supplementation of humic acid and humates in broilers diet. Similar findings were observed by other researchers (Kemal *et al.,* 2008, Santos *et al.,* 2005, Kaya *et al.,* 2009) who used humates, humic acid and several other feed additives (antibiotic, fumaric acid, probiotic, mannan oligosaccharides, mushroom extract and fructooligosaccharide) in broiler diet. Taklimi *et al.* (2012) also observed significant improvement in weight gain and feed conversion ratio by adding the humic acid to the broiler's feed at 0.3%. The addition of 100 ppm humic acid to the drinking water of broiler chicks resulted in significant increase in total weight gain and improvement in feed conversion ratio (Mirnawati *et al.*, 2013).

2.11 Haemato-biochemical parameters

The humic acid affected serum chemistry values at high concentration. Except for cholesterol, triglyceride, creatinine and lactate dehydrogenase, there was a trend for decrease in protein, albumin, glucose, creatine kinase, blood ureanitrogen, alkaline phosphatase, alanine, aminotransferase,Ca, Fe, and P concentrations. Although the decreasedvalues were statistically different than controls, they didnot reflect any trend that would suggest any toxic effect of HA on muscle, kidney, heart, or liver. The blood chemistry results were concordant with relative organ weight results, which showed no dystrophic enlargement or atrophy as it could happen under maladaptive conditions. At 2.5% level of HA, the reduction in the serum concentrations of Ca, Mg, Fe andP may be due to a metal chelating effects of HA, which is affected by large number of carboxylic acid side chains (Klocking, 1994). The HA increased crop pH but had no effect on the duodenal pH. Hinton *et al.* (2000) showed a positive correlation between the adhesion of pathogenic bacteria, Salmonella and Enterobacteriaceae, to crop epithelium and an increase in crop pH. However, it is not known whether the reverse effect increasing crop pH, would favor the colonization of the crop by those bacteria. If an increase in pathogen colonization had occurred, that was not reflected by birds being sick. On the contrary, the antimicrobial effects of HA have been described in the literature (Klocking, 1994; EMEA 1999,). In both trials humic acid did not have any effect on white blood cell, red blood cell, monocyte and lymphocyte counts, or hematocrit values, but at 4 wk trial HA reduced the blood heterophil counts, causing a decrease in heterophil, lymphocyte . A similar numerical, but not statistically significant, trend was seen in the second trial (Figure 1). The differential effect of HA on blood neutrophils is not understood, but in vitrostudies show the ability of HA to activate blood neutrophils and increase their adhesibility (Riede *et al.*, 1991; Chen *et al.*, 2002). It is possible that HA may cause nonspecific margination of neutrophils leading to their decreasein the blood. An elevated heterophil to lymphocyte ratio is considered an indicator of stress (Gross and Siegel,1983); therefore, the results do not reflect that the chickens experienced stress, which was also evident from their overall health and general activity that appeared no different than control birds. Whether HA beneficial to overall immunity of birds is is not known, but several studies have shown HA having immune-stimulatory, anti-inflammatory, and antiviral effects (Klocking 1994; EMEA 1999; Klocking *et al*., 2002; Joone *et al*., 2003; Joone and van Rensburg, 2004).In conclusion, our studies show that HA produces neither skeletal aberrations nor toxicity in chickens. However, at high concentrations it decreases BW without causing adverse health effects, a property that may be useful in controlling certain production problems related to excess BW gain in breeder males.

2.12 Effect of humic acid and probiotics in carcass quality of broiler chicken

The carcasses were plucked, eviscerated (removal of lungs and the gastrointestinal tract), and weighed. The carcass yield was determined as a proportion of the body weight before slaughter and after evisceration. The yields of breast meat (without bones) and thighs (with bones) were calculated as a proportion of meat weight and carcass weight. The results were expressed as a percentage of the body parts to the entire carcass M., Malíková L. (2010).

2.13 Other activities

The feed supplemented with probiotics alone or combined with the humic acid were not very promising giving less positive results. This may be explained that the probiotics was negatively affected by the presence of humic acid, indicating that humic acid may inhibited the beneficial microorganisms of the probiotics. Manhob *et al.* (2016) also observed that the probiotics Bios B-Gold did not improve boiler's weight gain and feed conversion ratio. Taklimi *et a*l (2012) also observed significant improvement in weight gain and feed conversion ratio by adding the humic acid to the broiler's feed at 0.3%. The addition of 100 ppm humic acid to the drinking water of broiler chicks resulted in significant increase in total weight gain and improvement in feed conversion ratio (Mirnawati *et al.*, 2013).

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at National Research and Development (RND)Poultry Farm under National Hatchery Ltd., Dighulia, Tangail, with 195-day-old chick for a period of 28 days from 2ndJanuaray to 30thJanuarary, 2023.The experiment was performed by applying different concentration levels of humic acid and probiotics to assess the immune response, growth performance and carcass characteristics of broiler chickens.

3.2 Collection of experimental broilers

A total of 195-day old chicks of "Lohmann Meat (Indian River)" strain having 40±0.5g average body weight were collected from Kazi farm limited hatchery, Nayanpur, Gazipur.

3.3 Experimental materials

The collected chicks were carried to National research and development poultry farm. They were kept in electric brooders equally for 7 days by maintaining standard brooding protocol. The chicks are brooding in 5 different brooder each brooder contain 39 chicks to use 5 different diet was given for experiment. After seven days for proper handling and data collection, the chicks of each treatment group were divided into three replications and in each replication of dietary treatment, there were 13 birds. After 28 days of nursing and feeding, data were collected for the following parameters: feed intake, live weight, body weight gain, feed conversion ratio, carcass characteristics, total blood count, profit per bird and benefit-cost ratio.

3.4 Experimental treatments

The feed additives of humic acid and probiotics were mixed properly with commercial broiler feed at different level. The experimental treatments were following:

T_0: Basal feed

T_1: Basal feed+ 0.05%HA

T_2: Basal feed+0.10% HA

T_3: Basal feed+0.02%Probiotics

T_4: Basal feed+0.05%HA+0.02%Probiotics

Table 1: Lay out of the experiment

Treatment	Replication			Total
	R1	**R2**	**R3**	
T_0	13	13	13	39
T_2	13	13	12	39
T_2	13	13	13	39
T_3	13	13	13	39
T_4	13	13	13	39
Total	65	65	65	195

3.5 Collection of experimental feed

For research of the dietary supplementation of humic acid and probiotics mixed and basal feed are collected from National feed Mill Gazipur. In Feed Mill this treated feed production was possible due to I am employer of National Feed Mill Ltd., Maize and soybean meal based diet.

3.6 Collection of Humic Acid and Probiotics

For research purpose humic acid is collected from Hovers Agro Vet Ltd. brand name Minarva, manufacture by Pacta, Italy. Probiotics collected from KEMIN, brand name Enterosure..

Table 2: Description of Humic Acid

Brand Name	Minerva
Manufacture by	Pacta, Italy
Marketing by	Hoovers Agro vet Ltd.Bangladesh
Humic acid	Humic acids (HA), fulvic acids (FA), and humans complex mixture

Table 3: Description of Probiotics

Brand Name	Enterosure
Manufacture by	KeminAmerica
Marketing by	KeminIndia
Composition	*Bacillus subtilis,Bacillus licheniformis*

3.7 Preparation of broiler house

The broiler shed was an open sided natural house. It was a tin shed house with concrete floor. The experimental room was properly cleaned and washed by using tap water. All the equipment of the broiler house was cleaned and disinfected. There was 6 inch. side wall around the shed with no ceiling. The floor was above 1ft. from the ground and the top of the roof was above 12ft. from the floor. The house was disinfected by n-alkyl dimethyl benzyl ammonium chloride (TimsenTM) solution before starting the experiment. After proper drying, the house was divided into pens as per lay-out of the experiment by plastic net.Before enter the chicks in house was fumigated by formalin and potassium permanganate @ 500 ml formalin and 250 g potassium permanganate (i.e. 2:1) for 35 m^3 experimental area. Rice husk was used as a litter material to keep free the floor from moisture.

3.8 Experimental diets

Starter and grower commercial National broiler feed were purchased from the National Feed Mill. Starter diet was enriched with minimum 4 times daily by following Lohmann Meat (Indian River) Manual and *ad libitum* drinking water 2 times daily. Detail composition of feed are presented in table 4 & 5.

Table 4. Name and minimum percentage of ingredients present in starter ration

Name of ingredients in Starter ration	Minimum percentage Present
Protein	22.0
Fat	6.0
Fiber	4.0

Name of ingredients in Starter ration	Minimum percentage Present
Ash	6.0
Lysine	1.30
Methionine	0.55
Cysteine	0.40
Tryptophan	0.19
Threonine	0.79

(**Source**: National starter feed 50 kg packet)

Table 5: Name and minimum percentage of ingredients present in grower ration

Name of ingredients in Grower ration	Minimum percentage Present
Protein	21.0
Fat	6.0
Fiber	4.0
Ash	6.0
Lysine	1.25
Methionine	0.49
Cysteine	0.39
Tryptophan	0.18
Threonine	0.75
Arginine	1.18

(Source: National grower feed 50 kg packet)

3.9 Management procedures

Feed intake and body weight were recorded every week. Survivability was recorded for each replication up to 28 days of age. The following management procedures were followed during the whole experiment period

3.9.1 Care of day old chicks

Just after arrival of day old chicks to the poultry house the initial weight of the chicks were recorded by a digital electronic balance, vaccination was done and distributed them under the hover for brooding. The chicks were supplied glucose water with Vitamin-C to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers to start feeding for the first 24 hours.

3.9.2 Brooding of baby chicks

Electric brooder was used to brood chicks. Partitioning brooding was done due to different experimental treatment. Each brooder had one hover, three of 100W bulb and a round chick guard to protect for 39 chicks and make 5 Brooder for 195 birds. Thereafter 7 days baby chicks were randomly distributed to the pen according to the design of the experiment. The recommended brooding temperature was 35-21^{0}C from 1st to 4th weeks of age. Due to winter season environmental temperature was below 25^{0}C. So, at that all time there was need of extra heat to brood the baby chicks. After one week a 200-watt electric bulb was hanged in every pen up to the market age of birds. Moreover, at that time the wall polythene sheet spread over the net-wire to protect the broiler chicks from cold and wind.

3.9.3 Room temperature and relative humidity

Daily room temperature (℃) and humidity were recorded with a thermometer and a wet and dry bulb thermometer respectively. Daily of room temperature and percent relative humidity for the experimental period were recorded and presented. Average of room temperature and percent relative humidity for the experimental period was recorded and presented in Table 8

Table 6. Average temperature and humidity

Week	Date	Temperature (℃)		Humidity (%)	
		Average Maximum	Average minimum	Average Maximum	Average minimum
1st	02.01.23- 09.01.23	34.04	30.92	92.42	61.14
2nd	10. 01.23-16.01.23	33.34	29.30	93.28	69.71
3rd	17.01.23-23.01.23	31.20	28.05	92.28	70.85

Week	Date	Temperature (°C)		Humidity (%)	
		Average Maximum	Average minimum	Average Maximum	Average minimum
4th	24.01.23-30.07.21	29.42	27.97	93.71	62.14

3.9.4 Feeding and drinking

Crumble feed was used as starter (0-2 wks.) and pellet feed for grower (3-4 wks.) ration. *Ad libitum* feeding was allowed for rapid growth of broiler chicks up to the end of the four weeks. Fresh clean drinking water was also supplied *Ad libitum*. Feeds were supplied 3 times: morning, noon and night. Water was supplied two times daily: morning and evening. Left over feeds and water were recorded to calculate actual intake. Digital electronic balance and measuring plastic cylinder was used to take record of feed and water. Weekly feed consumption (gm)/bird were calculated to find out weekly and total consumption of feed. All feeders and drinkers were washed and sun-dried before starting the trial. One plastic made round feeder and one drinker were kept in the experimental pen. Feeder and drinker size were changed according to the age of the birds. Feeders were washed at the end of the week and drinkers once daily.

3.9.5 Lighting

At night there was provision of light in the broiler house to stimulate feed intake and rapid body growth. A 200watt incandescent bulb lights (1000 lumen) were provided to ensure 24 hours' light for first 2 weeks. Thereafter 23 hours' light and one-hour dark were scheduled up to marketable age. At night one-hour dark was provided in two times by half an hour.

3.9.6 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation was easy to remove polluted gases from the farm. Besides, on the basis of necessity ventilation was regulated by folding polythene screen. The open space around the farm was favorable for cross ventilation.

3.9.7 Bio-security measures

Biosecurity is a set of management practices that reduce the potential for introduction and spread of diseases causing organisms. To keep disease away from the broiler, farm the following vaccination, medication and sanitation program was undertaken. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-A, D, E, K and Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

3.9.8 Vaccination

The vaccines were collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule. One ampoule vaccine was diluted with distilled water according to the recommendation of the manufacturer. The cool chain of vaccine was maintained strictly up to vaccination. The vaccination schedule of broiler is shown in Table 7.

Table7. Vaccination schedule

Age	Name of Disease	Name of Vaccine	Route of vaccination
Day 3	Infectious Bronchitis + Newcastle Disease (IB+ND)	CEVAC BI L	One drop in eye
Day 11	Gumboro (IBD)	CEVAC IBDL	One drop in eye
Day 18	Gumboro (IBD)	CEVAC IBDL	Drinking water
Day 21	IB+ND	CEVAC IBDL	Drinking water

3.9.9 Medication

Vitamin-B complex, vitamin-A, D3, and E were used against deficiency diseases. Electromin and Vitamin-C also used to save the birds from heat stress. The medication program is presented in the Table 5

Table 8. Medication programmed

Medicine	Composition	Dose	Period
Liva -Vit	Liver-extract Vitamin B-complex	1-2ml/1L water	3-5days (all groups)
Vita AD_3E	AD_3E	1g/3L water	6-9days(all groups)

3.9.10 Sanitation

Proper hygienic measures were maintained throughout the experimental period. Cleaning and washing of broiler shed and its premises were under a routine sanitation work. Flies and insects were controlled by spraying Phenol and Lysol to the surroundings of the broiler shed. The attendants used farm dress and shoe. There was a provision of Foot Bath at the entry gate of the broiler shed to prevent any probable contamination of diseases. Strict sanitary measures were followed during the experimental period.

3.9.11 Recorded parameters

Daily temperature, Feed and water was calculated. Weekly lives weight, weekly feed consumption and death of chicks to calculate mortality percent were taken during the study. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter carcass weight and gizzard, liver, spleen and heart were measured from each broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage.

3.10 Data collection

3.10.1 Live weight

The initial day-old live weight and weekly live weight of each replication was kept to get final live weight record per bird.

3.10.2 Dressing yield

Dressing yield of bird was obtained from live weight subtracting blood, feathers, head, shank and inedible viscera.

3.10.3Feed consumption

Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird

3.10.4 Survivability of chicks

Daily death record for each replication was counted up to 28 days of age to calculate the mortality, if occurred that indicated the survivability of the bird.

3.10.5 Dressing procedures of broiler chicken

Three birds were picked up at random from each replicate at the 28th day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by halal method or overnight (12 hours) but drinking water was provided *ad-libitum* during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Heart and liver were removed from the remaining viscera by cutting them loose and then the gall bladder was removed from the liver. Cutting it loose in front of the proventiculus and then cutting with both incoming and outgoing tracts removed the gizzard. Giblet were collected after removing the gall bladder. All the carcasses were washed with cold water inside and out to remove traces blood, loosely attached tissue or any foreign materials. Then the eviscerated weight of carcasses was recorded. Thereafter the weight of carcass cuts such as breast, thigh (both), drumstick (both), back, neck, wing (both), heart, liver and gizzard was taken. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight. Liver, heart, gizzard and neck were considered as giblet. Percent of breast, thigh, drumstick, back, wing, giblet and abdominal fat were found as DP.

3.10.6 Immune parameter:

At the end of the experiment blood sample was collected randomly from each replication of every treatment. 2mL blood was collected from wing vein with syringe in a vacationer. Vacationer contains EDTA solution which prevent blood coagulants. Few hours after collection the blood sample was tested by Auto Blood Analyzer in Janata diagnostic and consultation center, Tangail.

3.11 Calculation

3.11.1 Live weight gain

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

Body weight gain = Final weight – Initial weight

3.11.2 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

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

3.11.3 Feed conversion ratio (FCR)

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

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

3.11.4 Dressing percentage

Dressing yield was found by subtracting blood, feathers, head, shank and digestive system from live weight. Liver, heart, gizzard and neck were considered as giblet. Dressing percentage of bird was calculated by the following formula-

$$DP = \frac{Dressing\ yield\ (g)}{Live weight\ (g)} X 100$$

Dressing yield = Breast, thigh, drumstick, back, wing, giblet, abdominal fat weight.

Statistical analysis

Total data were complied, tabulated and analyzed in accordance with the objectives of the study. Excel Program was practiced for preliminary data calculation. The collected data was subjected to statistical analysis by applying one-way ANOVA using Statistical Package for Social Sciences (SPSS version 16.0, 2008). Differences between means were tested using Duncan's multiple comparison test, LSD and significance was set at $P<0.05$.

Some photographic view during the experimental period

Plate 1: Preparation of brooder **Plate 2: weighing of day old chick**

Plate3. Weighing and mixing of additives

Plate 5: feed brand (NFL Plate 6 :Humic acid Plate 7: Probiotics

Plate 8. IB and IBD vaccination **Plate 9: Medicine**

Plate10 **:Vaccine indicator Plate 11: Weighing of feed** **Plate 12 : Watering**

Plate 13 : Feeding

Plate 14: Weighing after 3w Plate 14: Weighing after 4w Plate 15: Blood collection

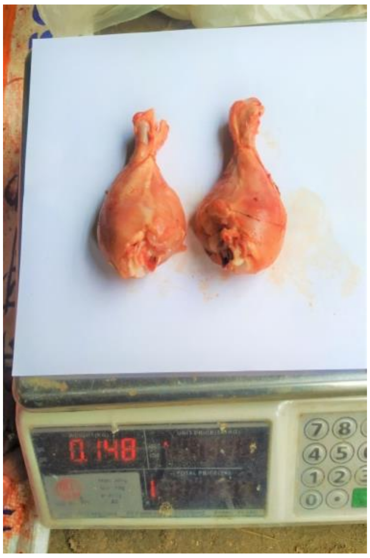

Plate 16 : Weighing of slaughtering Plate 17 : Weight of thigh Plate 18 : weight of drumstick

Plate 19 : Weight of breast Plate 20: Weight of wing Plate 21: Weight of back

Plate 22 : Weight of liver **Plate 22 : Weight of heart** **Plate 22 : Weight of spleen**

Plate 23: Weight of gizzard **Plate 24: Weight of head** **Plate 25: Weight of neck**

CHAPTER IV

RESULTS AND DISCUSSION

Results obtained from the present study have been presented and discussed in this chapter with a view to study the effect of humic acid and probiotics onbroiler production. The data are given in different tables. The results have been discussed and possible interpretations of the research are given under the following headings.

4.1 Production performances

In this chapter, the effect of humic acid and probiotics on broiler production are discussed that helps the body growth of broiler chicken. The chicks were randomly divided into five experimental treatment groups. The five groups were T_0 (Control), T_1 (Basal feed+0.05%HA), T_2 (Basal feed+0.10%HA), T_3 (Basal feed+0.02% Probiotics) and T_4 (Basal feed+0.05% HA+ 0.02% Probiotics). The performance traits *viz*. body weight, body weight gain, feed consumption, FCR, dressing percentage, different dressed organ weight, survivability rate, flock uniformity, benefit cost ratio, caecal microbial count and immune parameter were discussed in this chapter.

4.1.1 Body weight

Table 9 showed the effect of different level of HA and Probiotics on body weight of broiler. The relative body weight (g) of the broiler chickens in the different treatment groups T_0, T_1, T_2,$T_3$and T_4were 1590.67 ±48.32, 1616.67±72.99, 1610.00±25.23, 1595.67±73.65 and 1648.67±91.51 respectively. The highest body weight was found in T_4 and lowest in T_1,The overall body weight of different treatment groups showed that there was no significant (P>0.05) effects on body weight. The higher body weight in T_4 group might be due to treatment with basal feed+0.05%HA+0.02% probiotics. O. C. Turgut L. 2004 reported that significant improvement was found in body weight in broiler chickens supplemented with humic acid and humic acid+ probiotics..

4.1.2 Body weight gain

Table 9 showed the effect of different level of HA and Probiotics on body weight gain of broiler. The relative body weight gain (g) of broiler chickens in the different treatment groups T_0, T_1, T_2,T_3 and T_4 were 1542.33±46.30, 1574.00±75.88, 1552.33±17.63, 1545.00±76.37 and 1597.33±96.88 respectively. The highest body weight gain was found in T_4 and lowest in

T_0.The overall body weight gains of different treatment groups showed that there was no significant (P>0.05) effects on body weight gains. The higher body weight gain in T_4 group might be due to treatment with basal feed+0.05%HA+0.02%probiotics.This results are in agreement with those obtained by Bujko J. (2014).

4.1.3 Feed consumption (FC)

Table 9 showed the effect of different level of HA and probiotics on total feed consumption (g) of broiler chicken. Here, the relative total feed consumption (g) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 2153.33±15.25, 2127.67±17.14, 2151.00±25.74, 2160.00±6.81 and 2165.00±5.86 respectively. The highest feed consumption was found in T_4 and lower in T_2. The overall feed consumption of different treatment groups showed that there was not significant (P>0.05) effects on feed consumption. Deyoe *et al*. (1962) reported that feeding graded levels of HA, probiotics and HA+probiotics had no significant effect (P>0.05) on feed intake. The increment in feed intake may be due to the effect of humic acid and probiotics. The results indicated that the supplementation of different levels of humic acid and probiotics to the diets of broiler to improved feed intake (Samanta, M. and P. Biswas, 1997).

4.1.4 Feed Conversion Ratio (FCR)

Table 9 showed the effect of different level of HA and probiotics on FCR of this experimental study. The FCR of the different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.35±0.05, 1.32±0.06, 1.33±0.01, 1.39±0.06 and 1.32±0.07 respectively. There was no significant (P>0.05) difference in the FCR of the research. However, better FCR were found in T_1 and T_4 treatment group from others different treatment groups, might be treated with HA and probiotics. Cmiljanic *et al.* (2001) reported significant improvement in FCR of chicks fed on the diet supplemented with HA and HA+ probiotics than control group.

Table 9: Effect of HA and Probiotics on body weight (BW), total FC and FCR

Treatments	Body weight±SE (g/bird)	Body weight gain±SE(g/bird)	Total FC±SE (g/bird)	FCR±SE
T_0	1590.67±48.32	1542.33±46.30	2153.33±15.25	1.35±0.05
T_1	1616.67±72.99	1574.00±75.88	2127.67±17.14	1.32±0.06
T_2	1610.00±25.23	1552.33±17.63	2151.00±25.74	1.33±0.01
T_3	1595.67±73.65	1545.00±76.37	2160.00±6.81	1.39±0.06

T_4	1648.67±91.51	1597.33±96.88	2165.00±5.86	1.32±0.07
Level of Significance	**NS**	**NS**	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+0.05%HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02% Probiotics, T_4=Basal feed+0.05% HA+0.02% Probiotics; Values: Mean±SE (n=15); Applying: One-way ANOVA with Duncan's method for post-hoc test (SPSS v.26)

- Mean with the different superscripts are significantly different (P<0.05)
- Mean with the same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.2 Dressing percentage

Table 10 showed the effect of different level of HA and Probiotics on live weight (g), dressing yield (g) and dressing percentage of the different treatment groups of broiler chickens. Dressing percentage of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 59.57^a±2.96, 61.71^a±2.71, 65.08^a±1.88, 63.42^a±3.81 and 69.12 ^{a}±2.19 respectively. There was no significant difference (P>0.05) in the dressing percentage in this research. However, dressing percentage was higher in T_4 than other groups including control group. This is might be due to the effect of basal feed+0.05%HA +0.02% probiotics compared with others groups including control group. The results showed that the supplemented of 0.05%HA and 0.02% probiotics in poultry rations has a good impact on performance and carcass characteristics. However Ozturk E. *et al.*, 2010 indicated that the dressing percentage was not significantly affected in broiler chicks by addition of humic acid and probiotics.

Table 10: Effect of HA and Probiotics on dressing percentage

Treatments	Live weight±SE (g/bird)	Dressing yield±SE (g/bird)	Dressing percentage±SE
T_0	1590.67±48.32	945.33 ^{c}±31.86	59.57±2.96
T_1	1616.67±72.99	994.00^{bc}±15.94	61.71±2.71
T_2	1610.00±25.23	1047.00^b±14.17	65.08±1.88
T_3	1550.67±73.65	978.33^{bc}±22.99	63.42±3.81
T_4	1648.67±91.51	1135.67^a±26.56	69.12±2.19
Level of Significance	**NS**	**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+0.05%HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02% Probiotics, T_4=Basal feed+0.05% HA+0.02% Probiotics; Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.3 Carcass characteristics

4.3.1 Thigh, drumstick, back and wing weight (gm) of broiler chicken

Table 10 showed the effect of different level of HA and Probiotics on thigh, drumstick, back and wing weight (g) of the different treatment groups of broiler chicken. The relative weight (g) of thigh in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 125.67±2.33, 134.33±2.33, 135.67±7.31, 149.00±2.31 and 173.00±4.91 respectively. The relative weight (g) of drumstick in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 116.67±2.72, 129.67±3.84, 146.67±2.33, 151.67±11.02 and 150.00±3.21 respectively; The relative weight (g) of back in different treatment groups T_0, T_1, T_2,$T_3$and T_4 were 166.0±5.85, 164.7±2.60, 188.3±6.38, 175.3±2.91 and 190.3±7.13 respectively. The relative weight (g) of wing in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 58.67±1.20, 64.33±2.33, 74.00±.57, 62.00±1.52 and 87.33±4.09 respectively. The weight (g) of thigh, back and wing in T_4 and drumstick in T_3 were significantly higher than the other groups including control group. The better result in T_4 group might be due to the positive effect of basal feed+0.05% HA+0.02% probiotics were compared with control group (T_0). Erener G. (2010):

Table 11: Effect of HA and Probiotics on thigh, drumstick, back and wing weight (g) of broiler chicken

Treatments	Thigh±SE (g)	Drumstick±SE (g)	Back±SE (g)	Wing±SE (g)
T_0	125.67^{c}±2.33	116.67^{c}±2.72	166.0^{b}±5.85	58.67^{c}±1.20
T_1	134.33^{c}±2.33	129.67^{bc}±3.84	164.7^{b}±2.60	64.33^{c}±2.33
T_2	135.67^{bc}±7.31	146.67^{ab}±2.33	188.3^{a}±6.38	74.00^{b}±.57
T_3	149.00^{b}±2.31	151.67^{a}±11.02	175.3^{ab}±2.91	62.00^{c}±1.52
T_4	173.00^{a}±4.91	150.00^{a}±3.21	190.3^{a}±7.13	87.33^{a}±4.09
Level of Significance	**	**	*	***

Here, T_0= Basal feed, T_1= Basal feed+0.05% HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02%Probiotics, T_4=Basal feed+0.05%HA+0.02% Probiotics Values: Mean±SE (n=13); Applying: One-way ANOVA (SPSS, Duncan's method)

- Mean with the different superscripts are significantly different (P<0.05)

- Mean with the same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.3.2 Liver, heart, spleen and gizzard weight (gm) of broiler chicken

Data presented in table 11showed the effect of different level of mint leaf on liver, heart, spleen and gizzard weight (g) of broiler chickens in different treatment groups of broiler chickens. The relative weight (g) of liver in different treatment groups T_0, T_1, T_2,T_3 and T_4 were 42.33±0.88, 42.67±.88, 44.67±1.45, 41.67±1.20 and 48.33±1.85 respectively; the relative weight (g) of heart in different treatment groups T_0, T_1, T_2,T_3 and T_4 were 8.33±0.33, 9.33±0.33, 9.67±0.66, 9.33±0.33 and 9.33±0.33 respectively; the relative weight (g) of spleen in different treatment groups T_1, T_2, T_3,T_4 and T_5 were 2.33±0.33, 3.00±.00, 3.33±.33, 3.33±.33 and 5.00±0.57 respectively; the relative weight (g) of gizzard in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 23.00±1.15, 28.00±.57, 26.33±2.40, 26.67±1.67and 29.00±2.08 respectively. The weight (g) of liver, spleen and gizzard in T_4 and heart in T_2 was significantly higher (P<0.05) than the other groups including control group. The better result in T_4 group might be due to the effect of basal feed+0.05%HA + .02% probiotics compared with control group (T_0). It has been stated that supplementation of probiotics has no effect on the performance of broiler chicks (ZuAnon *et al.,* 1998; system (Fuller, 1989; Ergun *et al.,* 2000). But Baidya *et al.* (1993) stated that probiotics were the most effective growth promoter. Probiotics fed chickens had more weight than other groups Mohan *et al.,* 1996; Zulkifli *et al*., 2000; Lan *et al.*, 2003). Recently, it has been reported that poultry growth is promoted with the increasing doses of probiotic (Protexin, Hilton, Pharma, Karachi Pakistan) from 0.5 to 1.5 grams per 10 kg feed. In our laboratory the growth pattern of treated birds showed an increase in weight gain relative to the control, up to 1.0 gram per 10 kg feed but beyond that the pattern was reversed (Ahmad, 2004).

Table 12: Effect of HA and Probiotics on liver, heart, spleen and gizzard weight (g) of broiler chickens

Treatments	Liver±SE (g)	Heart±SE (g)	Spleen±SE (g)	Gizzard±SE (g)
T_0	42.33^{b}±0.88	8.33^{a}±0.33	2.33^{a}±0.33	23.00^{b}±1.15
T_1	42.67^{b}±0.88	9.33^{a}±0.33	3.00^{a}±0.00	28.00^{ab}±0.57
T_2	44.67^{ab}±1.45	9.67^{a}±0.66	3.33^{a}±0.33	26.33^{ab}±2.40
T_3	41.67^{b}±1.20	9.33^{a}±0.33	3.33^{a}±0.33	26.67^{ab}±1.67
T_4	48.33^{a}±1.85	9.33^{a}±0.33	5.00^{a}±0.57	29.00^{a}±2.08

Level of Significance	**	NS	NS	*

Here, T_0= Basal feed, T_1= Basal feed+0.05% HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+.02%Probiotics, T_4=Basal feed+0.05%HA+0.02% Probiotics Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- Mean with the different superscripts are significantly different ($P<0.05$)
- Mean with the same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error
- 'NS' = Not significant, ($p>0.05$) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.4 Flock uniformity (%) and survivability rate (%)

4.4.1 Flock uniformity (%)

Data presented in table13 showed the effect of different level of HA and Probiotics on flock uniformity (%) of broiler chickens. The relative flock uniformity (%) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 77.67±9.59, 79.00±3.78, 64.33±4.33, 69.67±8.41 and 82.67±3.33 respectively. Flock uniformity (%) was higher in treated group T_1 and T_4 than control group T_0. There was no significant difference ($P>0.05$) in flock uniformity (%). The better result in T_4 group might be due to the effect of basal feed+0.05%HA + 0.02% probiotics compared with control group (T_0).

4.4.2 Survivability rate (%)

Data presented in table 12 showed the effect of different level of HA and probiotics on survivability rate (%) of the experimental study. The relative survivability rate (%) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 97.43±2.56,100±0.00, 100±0.00, 100±0.00 and100±0.00 respectively. Survivability rate (%) was lower in treated group T_0 than other group. There was no significant difference ($P>0.05$) in survivability rate (%).

Table 13:Effect of HA and Probiotics on survivability rate (%) and flock uniformity (%)

Treatments	Flock Uniformity (%)	Survivability Rate (%)
T_0	77.67±9.59	97.43±2.56
T_1	79.00±3.78	100±0.00
T_2	64.33±4.33	100±0.00
T_3	69.67±8.41	100±0.00

T_4	82.67±3.33	100±0.00
Level of Significance	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+0.05% HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02%Probiotics, T_4=Basal feed+0.05%HA+.02% Probiotics .Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.5 Cost benefit ratio analysis

The effect of different level of HA and probiotics benefit ratio analysis showed in the Table 13. Benefit cost ratio (BCR) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.28±0.04, 1.31±.06, 1.29±0.01, 1.24±0.05 and 1.32±0.07 respectively. BCR is not significantly higher (P>0.05) in treatment group T_4 than others groups including control group (T_0). This is might be due to the effect of basal feed+0.05%HA+0.02% probiotics.

Table 14: Effect of HA and Probiotics on cost benefit ratio analysis of different treatment groups

Treatments	Total cost±SE (Tk./Bird)	Sell price±SE (Tk./Bird)	Profit±SE (Tk./Bird)	BCR±SE
T_0	179.27±.92	230.64±7.00	51.36±7.93	1.28±0.04
T_1	177.71±1.04	234.41±10.58	56.69±11.60	1.31±.06
T_2	179.13±1,56	233.44±3.65	54.31±2.34	1.29±0.01
T_3	179.68±.41	224.84±10.67	45.15±10.51	1.24±0.05
T_4	179.98±0.35	239.05±13.26	59.06±13.47	1.32±0.07
Level of Significance	**NS**	**NS**	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+0.05% HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02%Probiotics, T_4=Basal feed+0.05%HA+0.02% Probiotics. Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

4.6 Caecal microbial count

The effect of different level of HA and Probiotics on caecal microbial count on broiler chickens showed in the Table 14. No. of *E. Coli* colony (cfu/g)of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 16300000±57735.03, 8966666±272845.09, 4300000±57735.02, 1500000±57735.02 and 81000±577.35 respectively. The highest No. of *E. coli* colony (cfu/g) was found in T_0 and lowest in T_4. This is might be due to the effect of basal feed+0.05HA+0.02% probiotics. Number of Salmonella colony (cfu/g)of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 25000000±577350.26, 3200000±0.00, 2100000±57735.03, 746666±3333.33 and 51666.66±881.92 respectively. The highest no. of Salmonella colony (cfu/g) was found in T_0 and lowest in T_4. This is might be due to the effect of basal feed+ HA+Probiotics. Number of *E. Coli* colony (cfu/g) and number of Salmonella colony (cfu/g) were significantly ($P<0.05$) in treatment group T_3 and T_4 respectively than control group (T_0).Morphological changes of gastrointestinal tissues can be induced by different level of humic acid, probiotics and combined in gut load of microbial content including their metabolites (Olnood C. G., *et al.* 2015).

Table 15: Effect of HA and Probiotics on no. of *E. coli* colony (cfu/g) and no. of salmonella colony (cfu/g) of broiler production

Treatments	No. of *E. coli* colony (cfu/g)±SE	No. of *Salmonella sp.* colony (cfu/g)±SE
T_0	16300000^{a}±57735.03	25000000^{a}±577350.26
T_1	8966666^{b}±272845.09	3200000^{b}±0.00
T_2	4300000^{c}±57735.02	2100000^{c}±57735.03
T_3	1500000^{d}±57735.02	746666^{d}±3333.33
T_4	81000^{e}±577.35	51666.66^{d}±881.92
Level of significance	***	***

Here, T_0= Basal feed, T_1= Basal feed+0.05% HA, T_2= Basal feed+0.10% HA, T_3=Basal feed+0.02%Probiotics, T_4=Basal feed+0.05%HA+0.02% Probiotics. Values: Mean±SE (n=13); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error
- 'NS' = Not significant, (p>0.05) '*'= p-value< 0.05, '**'= p-value < 0.01; '***'=p-value < 0.001

Table 16: Effect of Humic Acid and Probiotics on immune parameter

Treatment	Hemoglobin (g/dl)	WBC(/cumm)	RBC(/cumm)	Platelet(cumm)	Neutrophil (%)	Lymphocyte(%)	Monocyte (%)	Eosinophil (%)	PCV(%)
T_0	$9.64^{a}\pm0.96$	$13900^{a}\pm950.43$	$3.8^{a}\pm0.83$	$225000^{a}\pm48045.12$	$74.00^{ab}\pm3.21$	$19.67^{ab}\pm3.18$	$2.33^{a}\pm0.88$	$4.0^{a}\pm1.0$	$30.54^{ab}\pm5.78$
T_1	$9.69^{a}\pm0.48$	$15926.67^{a}\pm2026.67$	$4.38^{a}\pm0.98$	$271000^{a}\pm57657.03$	$75.67^{ab}\pm2.03$	$20.33^{ab}\pm1.45$	$2.67^{a}\pm0.33$	$3.67^{a}\pm0.88$	$38.59^{b}\pm3.17$
T_2	$9.55^{a}\pm0.91$	$13520^{a}\pm1540.69$	$3.25^{a}\pm.49$	$360333^{a}\pm62317.29$	$81.67^{a}\pm1.2$	$15.33^{b}\pm0.33$	$1.67^{a}\pm0.66$	$4.67^{a}\pm1.20$	$28.24^{ab}\pm2.21$
T_3	$8.37^{a}\pm0.46$	$10866.67^{ab}\pm2085.13$	$2.79^{a}\pm.57$	$239333^{a}\pm75235.93$	$70.33^{b}\pm4.09$	$24.00^{a}\pm3.21$	$2.00^{a}\pm0.57$	$3.0^{a}\pm0.0$	$25.74^{b}\pm1.94$
T_4	$8.46^{a}\pm0.52$	$7620^{b}\pm520$	$2.59^{a}\pm0.49$	$234666.7^{a}\pm73076.06$	$69.67^{b}\pm1.45$	$27.67^{a}\pm2.40$	$2.00^{a}\pm0.57$	$2.67^{a}\pm0.33$	$27.16^{b}\pm2.15$
Level of Significance	**NS**	*** ($p<0.05$)**	**NS**	**NS**	**NS**	*** ($p<0.05$)**	**NS**	**NS**	**NS**

- Here, T_1= Basal feed, T_2= Basal feed+0.05% HA, T_3= Basal feed+0.10% HA, T_4=Basal feed+0.02%Probiotics, T_5=Basal feed+0.05%HA+0.02% Probiotics. Values: Mean±SE(n=13); Applying: One-way ANOVA (SPSS, Duncan's method);

CHAPTER V

CONCLUSION AND RECOMMENDATION

The present study was conducted at the National Hatchery Research and Development (RND) Farm Dighulia, Tangail for a period of four weeks using different level of dietary humic acid and probiotics in feed. The specific objectives of this study was under taken to determine the effect of different level of dietary humic acid and probiotics to assess alternative to antibiotics & production performance of broiler. A total of 195 day-old Lohmann meat broiler chicks were purchased from Kazi Hatchery, Nayonpur, Gazipur. The experimental broilers were allocated randomly to five treatment groups with three replications having 13 broilers per replication. The experiment lasted for 4 weeks and the treatment of various groups consisted of group T_0 (Basal feed), T_1 (Basal feed+0.05% HA), T_2(Basal feed+0.10%HA), T_3(Basalfeed+0.02%Probiotics) and T_4(Basal feed+0.05%HA+0.02%Probiotics). The parameters evaluated in this study were the bird's performance like body weight, body weight gain, feed consumption, FCR, flock uniformity, survivability, carcass characteristics, caecal microbial count, immune parameter and BCR on broiler rearing. Result demonstrated that the body weight (g) was not significantly (P>0.05) higher in T_4 (1648.67^a±91.5) and lowest in T_0 (1590.67^a ±48.32). The body weight gain (g) was also not significantly (P>0.05) difference, the highest result was found in T4 (1597.33^a±96.88) and lowest result was in T_0 (1542.33^a±46.30) group. The highest feed consumption was found in T_4 and lowest in T_1. The overall feed consumption of different treatment groups showed that there was not significant (P>0.05) effects on feed consumption There was no significant (P>0.05) difference in FCR among T_0, $T_{1,}$ T_2, T_3 and T_4. The better feed conversion ratio (FCR) was observed in T_4 (1.32^a±0.0)than other groups including control group. Dressing percentage was (P<0.05) higher in T_4 (69.12 ^{a}±2.19) and lower in T_0 (59.57^a±2.96). The weight (g) of thigh, wing and back in T_4 were significantly (P<0.05) higher than the other groups including control group. However the weight (g) of drumstick in T_3 was significantly (P<0.05) higher than the other treatment groups. The weight (g) of liver was significantly higher (P<0.05) and heart, spleen and gizzard in T_4 was not significantly higher (P>0.05) than the other groups including control group (T_0). Flock uniformity (%) was higher in treated group T_4 (82.67±67) than others group including control group T_0. There was no significant difference (P>0.05) in flock uniformity. There was no significant difference (P>0.05) in survivability rate (%). BCR is not significantly higher

(P>0.05) in treatment group T_4 (1.29±0.02) than control group (T_0). Numberof *E. coli* colony (cfu/g) and number of *Salmonella sp.* colony (cfu/g) were significantly lower (P<0.05) in treatment group T_4 than control group (T_0). The above research indicated that body weight (g), body weight gain (g), dressing percentage, weight (g) of liver, heart, spleen, gizzard, thigh, drumstick, back and survivability rate were better in T_4 than other groups including control group. FCR and BCR were also found better in T_4group. Number of *E. coli* colony (cfu/g) and number of *Salmonella sp.* colony (cfu/g) were significantly lower (P<0.05) in treatment group T_4 than control group (T_0) and others groups also. It was concluded that better result was found in humic acid and probiotics treated groups than control group. Therefore, the research recommended that broiler rearing with basal feed+0.05%HA+0.02%probiotics could be used on broiler production for better performance and profitability.

The results revealed that using probiotic and humic acid had significant effects on final body weight and feed consumption of broiler. Supplementation of probiotic and humic acid are favorable to the consumers as broilers have less abdominal fat content. In conclusion, it can be stated that probiotic and humic acid might be promising alternatives for antibiotics eliminate in broiler chicken production.

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix I: Effect of HA and Probiotics on body weight (BW)

Treatments	Replications	1st week	2nd week	3rd week	4th week
T_0	R_1	180	379	963	1500
T_1	R_1	163	389	993	1532
T_2	R_1	175	446	943	1560
T_3	R_1	168	374	923	1695
T_4	R_1	183	456	1016	1603
T_0	R_2	184	417	1014	1607
T_1	R_2	180	468	950	1762
T_2	R_2	159	434	1007	1641
T_3	R_2	151	399	920	1453
T_4	R_2	185	456	1066	1518
T_0	R_3	177	389	933	1665
T_1	R_3	178	458	981	1556
T_2	R_3	167	470	996	1629
T_3	R_3	162	470	896	1504
T_4	R_3	180	471	1150	1825

Appendix II: Effect of HA and probiotics on body weight gain (BWG)

Treatments	Replications	1st week	2nd week	3rd week	4th week	Total BWG
T_0	R_1	139	199	584	537	1459
T_1	R_1	122	226	604	597	1479
T_2	R_1	134	291	497	610	1519
T_3	R_1	127	206	549	634	1659
T_4	R_1	142	273	560	594	1549
T_0	R_2	143	233	597	597	1549
T_1	R_2	139	288	482	749	1724
T_2	R_2	118	275	573	613	1579
T_3	R_2	110	248	521	517	1409
T_4	R_2	144	271	610	604	1459
T_0	R_3	136	212	544	697	1619
T_1	R_3	135	280	523	546	1519
T_2	R_3	126	303	526	680	1559
T_3	R_3	121	269	465	521	1459
T_4	R_3	139	291	495	859	1784

Appendix III: Effect of HA and probiotics on feed consumption (FC)

Treatments	Replications	1st week FC	2nd week FC	3rd week FC	4th week FC	Total FC
T_0	R_1	174	350	653	1005	2182
T_1	R_1	175	333	646	999	2153
T_2	R_1	160	375	669	904	2108
T_3	R_1	175	340	623	1025	2163
T_4	R_1	178	374	600	1024	2176
T_0	R_2	170	353	638	987	2148
T_1	R_2	167	333	630	965	2095
T_2	R_2	176	350	661	997	2176
T_3	R_2	169	363	653	962	2147
T_4	R_2	170	374	605	1014	2163
T_0	R_3	172	353	592	1013	2130
T_1	R_3	174	335	623	985	2117
T_2	R_3	180	350	607	1011	2148
T_3	R_3	172	340	668	990	2170
T_4	R_3	168	374	615	999	2156

Appendix IV: Effect of HA and probiotics on feed conversion ratio (FCR)

Treatments	Replications	1st week	2nd week	3rd week	4th week	Final FCR
T_0	R_1	0.96	1.75	1.12	1.87	1.45
T_1	R_1	1.07	1.47	1.07	1.67	1.4
T_2	R_1	0.91	1.28	1.34	1.48	1.35
T_3	R_1	1,04	1.65	1.13	1.61	1.27
T_4	R_1	0.97	1.37	1.07	1.72	1.35
T_0	R_2	0.92	1.52	1.06	1.65	1.33
T_1	R_2	0.92	1.57	1.30	1.28	1.18
T_2	R_2	1.1	1.27	1.15	1.62	1.33
T_3	R_2	1.11	1.46	1.25	1.86	1.47
T_4	R_2	0.91	1.38	.98	1.67	1.42
T_0	R_3	0.97	1.67	1.08	1.45	1.27
T_1	R_3	0.91	1.19	1.19	1.80	1.37
T_2	R_3	1.07	1.16	1.15	1.48	1.31
T_3	R_3	1.06	1.26	1.43	1.90	1.44
T_4	R_3	0.93	1.29	1.24	1.16	1.18

Appendix V: Effect of HA and probiotics on dressing percentage

Treatments	Replications	Average live weight (g)	Eviscerated wt. (g)	Dressing %
T_0	R_1	1500	940	62.67
T_1	R_1	1532	978	63.89
T_2	R_1	1560	1009	64.69
T_3	R_1	1453	926	63.75
T_4	R_1	1603	1036	64.67
T_0	R_2	1607	1033	64.29
T_1	R_2	1762	1132	64.29
T_2	R_2	1641	1054	64.25
T_3	R_2	1504	960	63.87
T_4	R_2	1518	973	64.11
T_0	R_3	1665	1025	61.58
T_1	R_3	1629	1047	64.31
T_2	R_3	1695	1090	64.52
T_3	R_3	1518	955	62.93
T_4	R_3	1825	1181	64.76

Appendix VI: Effect of HA and probiotics on thigh, drumstick, back and wing weight (g) of broiler chicken

Treatments	Replications	Thigh (g)	Drumstick (g)	Back (g)	Wing (g)
T_0	R_1	125	115	168	61
T_1	R_1	138	134	169	68
T_2	R_1	149	149	187	74
T_3	R_1	150	159	176	61
T_4	R_1	163	145	187	81
T_0	R_2	130	122	175	58
T_1	R_2	135	133	165	65
T_2	R_2	153	142	200	73
T_3	R_2	131	130	180	65
T_4	R_2	173	149	180	86
T_0	R_3	122	113	155	57
T_1	R_3	135	122	160	60
T_2	R_3	145	149	178	75
T_3	R_3	126	116	170	60
T_4	R_3	183	157	204	95

Appendix VII: Effect of HA and probiotics liver, heart, spleen and gizzard weight (g) of broiler chickens

Treatments	Replications	Liver (g)	Heart (g)	Gizzard (g)	Spleen (g)
T_0	R_1	42	9	25	2
T_1	R_1	41	9	28	3
T_2	R_1	45	9	25	3
T_3	R_1	40	10	25	3
T_4	R_1	47	9	30	3
T_0	R_2	44	8	22	3
T_1	R_2	43	10	27	3
T_2	R_2	47	11	23	4
T_3	R_2	41	9	30	4
T_4	R_2	52	9	25	3
T_0	R_3	41	8	21	2
T_1	R_3	44	9	29	3
T_2	R_3	42	9	31	3
T_3	R_3	44	9	25	3
T_4	R_3	46	10	32	4

Appendix VIII: Effect of HA and probiotics on survivability% and uniformity%

Treatments	Replications	Survivability%	Uniformity%
T_0	R_1	92.3	80
T_1	R_1	100	93
T_2	R_1	100	60
T_3	R_1	100	73
T_4	R_1	100	78
T_0	R_2	100	86
T_1	R_2	100	73
T_2	R_2	100	60
T_3	R_2	100	60
T_4	R_2	100	76
T_0	R_3	100	53
T_1	R_3	100	80
T_2	R_3	100	86
T_3	R_3	100	76
T_4	R_3	100	86

Appendix IX: Effect of HA and probiotics on benefit cost ratio (BCR)

Treatments	Replications	Total Cost (Tk./Bird)	Receipt per Bird (Tk./Bird)	Profit (Tk./ Bird)	Benefit Cost Ratio (BCR)
T_0	R_1	181.02	217.5	36.47	1.2
T_1	R_1	179.25	222.14	42.88	1.23
T_2	R_1	176.51	226.2	49.68	1.28
T_3	R_1	179.86	245.77	65.9	1.36
T_4	R_1	180.65	232.43	51.77	1.28
T_0	R_2	178.05	233.02	54.06	1.3
T_1	R_2	175.72	255.49	79.76	1.45
T_2	R_2	181.93	237.94	56.01	1.3
T_3	R_2	178.89	210.68	31.79	1.17
T_4	R_2	179.86	220.11	40.24	1.22
T_0	R_3	177.85	241.42	63.56	1.35
T_1	R_3	178.16	225.62	47.45	1.26
T_2	R_3	178.95	236.2	57.25	1.31
T_3	R_3	180.29	218.08	37.78	1.2
T_4	R_3	179.44	264.64	85.18	1.47

Appendix X: Effect of HA and probiotics on number of *E. coli* colony (cfu/g) and number of Salmonella colony (cfu/g) of broiler production

Treatments	Replications	No. of *E.Coli* colony (cfu/g)	No. of Salmonella colony (cfu/g)
T_0	R_1	16300000	25000000
T_1	R_1	8700000	3200000
T_2	R_1	4100000	2200000
T_3	R_1	1300000	740000
T_4	R_1	810000	50000
T_0	R_2	16300000	25000000
T_1	R_2	16300000	25000000
T_2	R_2	4300000	200000
T_3	R_2	1700000	750000
T_4	R_2	800000	53000
T_0	R_3	16200000	24000000
T_1	R_3	8800000	3600000
T_2	R_3	4500000	2100000
T_3	R_3	1500000	750000
T_4	R_3	8200000	52000

Appendix XI: Broiler house temperature (^0C)

Days	Maximum	Minimum
01	34.75	30.10
02	34.60	29.43
03	33.30	29.80
04	33.70	29.70
05	32.20	28.30
06	33.40	29.20
07	33.60	28.60
08	34.60	28.50
09	34.50	28.65
10	34.10	28.50
11	33.60	28.40
12	32.30	27.10
13	31.60	27.50
14	32.70	26.10
15	28.60	26.20
16	29.30	27.20
17	30.40	27.30
18	31.40	28.50
19	33.90	28.90
20	33.10	29.40
21	31.90	28.90
22	30.40	27.00
23	29.90	28.30
24	30.20	28.10
25	28.90	27.80
26	29.70	28.50
27	28.50	28.30
28	28.40	27.80

Appendix XII: Relative humidity (%)

Days	Maximum	Minimum
01	90	58
02	92	61
03	93	65
04	97	72
05	89	75
06	91	68
07	95	59
08	99	60
09	92	61
10	93	68
11	95	66
12	87	74
13	93	80
14	94	79
15	91	82
16	86	73
17	94	76
18	96	61
19	95	68
20	96	73
21	88	63
22	95	55
23	94	74
24	92	70
25	95	60
26	94	55
27	90	65
28	96	56

Appendix XIII: Effect of HA and probiotics on immune parameters of broiler chicken

Treatment	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5
Replication	R_1	R_1	R_1	R_1	R_1	R_2	R_2	R_2	R_2	R_2	R_3	R_3	R_3	R_3	R_3
Hemoglobin(g/dl)	10.8	10.6	11.0	7.5	8.0	7.73	9.5	9.79	8.5	7.9	10.4	8.98	7.86	9.1	9.5
WBC(/cumm)	13000	11980	12060	6700	7660	15800	17100	16600	13100	6700	12900	18700	11900	12800	8500
RBC(/cumm)	2.44	2.42	2.37	1.64	1.64	3.65	5.41	4.09	3.40	2.9	5.31	5.32	3.29	3.35	3.25
Platelet	130000	163000	236000	90000	90000	260000	290000	415000	330000	325000	285000	360000	430000	298000	289000
Neutrophils(%)	68	72	80	64	70	75	76	81	69	67	79	72	84	78	72
Lymphocytes(%)	25	23	15	30	23	20	18	16	19	29	14	20	15	23	31
Monocytes(%)	4.0	3.0	3.0	3.0	3.0	2.0	2.0	1.0	2.0	2.0	1.0	3.0	1.0	1.0	1.0
Eosinophil(%)	3.0	2.0	3.0	3.0	3.0	3.0	4.0	7.0	3.0	2.0	6.0	5.0	4.0	3.0	3.0
PCV(%)	32.0	33.0	31.0	22.0	23.0	19.88	43.98	29.87	28.53	28.3	39.76	38.79	23.87	26.69	30.2