EFFECTS OF FEEDING MINT LEAF (*Mentha pulegium L.*) AS AN ALTERNATIVE TO ANTIBIOTICS ON PERFORMANCE OF BROILER

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CERTIFICATE

*This is to certify that the thesis entitled, "**EFFECTS OF FEEDING MINT LEAF (Mentha pulegium L.) AS AN ALTERNATIVE TO ANTIBIOTICS ON PERFORMANCE 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 bonafide research work carried out by **KHANDAKAR REZAUL ISLAM,** Registration No. **20-11141,** Semester: **July-December/2021** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by her.

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Dedicated

To

My Beloved Parents

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The Author

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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
mg	=	Mille Gram
BCR	=	Benefit Cost Ratio

EFFECTS OF FEEDING MINT LEAF AS AN ALTERNATIVE TO ANTIBIOTICS ON PERFORMANCE OF BROILER

ABSTRACT

The research was conducted to determine the effects of feeding mint leaf powder on the performance of broiler chicken. A total of 225 day-old Lohman meat broiler chicks were allocated randomly to five treatment groups with three replications having 15 birds per replication. There were five groups. e. g. T_0 (Basal feed), T_1 (Basal feed+Antibiotic), T_2 (Basal feed+1.0% mint leaf powder), T_3 (Basal feed+1.50% mint leaf powder) and T_4 (Basal feed+2.0% mint leaf powder). Result demonstrated that the body weight (g) was insignificantly (P>0.05) higher in T_2 (1965) and lowest in T_1 (1838.66). The body weight gain (g) was insignificantly (P>0.05) higher in T_2 (1923) than other groups including control group. Feed consumption (g) was significantly (P<0.05) higher in T_3 (2489) than other groups including control group. The better feed conversion ratio (FCR) was significantly (P<0.05) observed in T_2 (1.20) group compared to other groups. Dressing percentage was insignificantly (P<0.05) higher in T_3 (70.25) and lower in T_0 (67.95). The weight (g) of liver, heart, spleen and gizzard in T_2 was significantly higher (P<0.05). The weight (g) of thigh, drumstick and back in T_2 were insignificantly (P>0.05) higher. Insignificantly (P>0.05) survivability rate (%) was better in all groups except T_4. BCR is insignificantly higher (P>0.05) in treatment group T_2 (1.91) than control group (T_0). Average counts of *E. coli* and *Salmonella sp.* bacteria were insignificantly (P>0.05) lowest in T_3 (6.18) and T_4 (4.56) respectively. Significantly (P<0.05) more WBC was found in T_0 (14.60) and less in T_4 (1.20). But, T_2 (12.23) group was better result than others group. Significantly (P<0.05) the highest lymphocyte was found in T_4 (3.23) and lowest in T_3 (2.220). But, T_2 (2.68) treatment group has better result than other groups including control group. Significantly (P<0.05) the highest granulocyte was found in T_0 (11.73) and lowest in T_1 (3.55). But, T_2 (9.69) treatment group has better result than others group. Better result was found in T_2 (basal feed+1.0% mint leaf powder) group than others including control group (T_0).

CHAPTER I

INTRODUCTION

Poultry is the most important and advanced segment of the livestock sector in Bangladesh. Over the years, the demand for poultry products in Bangladesh has grown significantly; per capita consumption per year increased to 8.5 kg poultry meat and 5.1 kg (104 pieces) eggs (DLS, 2019). However, to meet the growing domestic demand, the productivity of the Bangladesh poultry sector needs to increase significantly.

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 arisen 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 (Wray and Davies, 2000). The banning of the use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in poultry production. As one of the alternatives, herbal extracts have been already used as feed supplements to improve growth performance under the intensive management systems (William and Losa, 2001). Antibiotics have been used for many years ensure a healthy growth for animals and increase yield and quality. However, since microorganisms develop resistance to antibiotics and leave residues in edible tissues, it has been revealed that these substances pose a risk for human health (Gümüş and Imik, 2016; Ölmez *et al*., 2021). After the prohibition of antibiotics as growth promoters in livestock and poultry, the search for alternative feed additives has focused on medicinal and aromatic plants (MAP) (Westendarp, 2005; Şahin *et al*., 2020). Medicinal and aromatic plants (MAPs) and their extracts have affected on digestibility and blood serum parameters in ruminant animals when added as feed ingredients. It has been determined that it regulates insulin metabolism by affecting serum glucose levels in sheep and calves and has positively affect total protein, calcium (Ca), and phosphorus (P) levels in sheep (Aslani *et al*., 2007; Mahgoub *et al*., 2008). It has also been stated that MAPs and their extracts show antioxidant activity (Gümüş et al., 2017). Pennyroyal (*Mentha pulegium* L.), which belongs to the Mentha genus of the Lamiaceae family, is an aromatic plant used worldwide in food, cosmetics, and health found free in nature. Pennyroyal leaves are rich in polyphenols, such as linalool, menthone, menthan, pulegon, piperitenone and other flavonoids. It has been determined that pennyroyal has antioxidant and cytotoxic properties besides these effects (Goodarzi and Nanekarani, 2014; Ölmez and Yörük, 2021).

Herbal plants *Mentha sp.* initiatives broiler performance. Mentha, the genus in Labiatae family, includes 20 species that can be found all over the world. *Mentha pulegium L.* is one of the *Mentha* species commonly known as pennyroyal. It is native to Europe, North Africa, Minor Asia and the near East (Chalchat *et al*., 2000). The flowering aerial parts of pennyroyal have been traditionally used for its antimicrobial properties in the treatment of cold, sinusitis, cholera, food poisonings, bronchitis and tuberculosis (Zargari, 1990). In addition, it is also used as an antiflatulent, carminative, expectorant, diuretic, antitussive and menstruation agent (Newall, 1996). *Mentha pulegium L.* and other species of the genus *Mentha* pose antimicrobial (Mahboubi and Haghi, 2008), antioxidant (El- Ghorab, 2006), cytotoxic (Shirazi *et al*., 2004) and abortifacient (Soares and *et al*, 2005) properties. Based on the reports of Geran *et al*, (2010), of 0.10, 0.20 and 0.30% supplementation of pennyroyal essential oils did not significantly affect feed intake, weight gain and feed conversion in broilers. Ghalamkari *et al*, (2012) did not show significant effects on broiler performance by using 0.5 and 1.0% pennyroyal. Modiry *et al*. (2010) reported that the use of 1.50% of different mixtures of *Urtica dioica*, *M. pulegium* and *T. vulgaris* medicinal plants in broiler diets improved their performance and carcass quality. An application of 0.75% of mixtures of *Urtica dioica*, *M. pulagum* and *T. vulgaris* medicinal plants in the growing period had positive effects on the performance and carcass traits of broilers (Nobakht *et al*., 2010). Since few studies on the use ofmedicinal plants especially *M. pulegium L.* in broilers are present, this study was conducted to evaluate the effects of using different levels of *M. pulegium L.* on performance and carcass traits of broilers. Mentha is used as herbs which are an ancient source of medicine, flavoring, beverages, dyeing, fragrances and cosmetics uses that have attracted biotechnology, cosmetics, pharmaceutical and food industries. Mentha is a genus of widely distributed aromatic perennial herbs that grows in the temperate regions of Eurasia, Australia and South Africa. The mint species possesses both medicinal and commercial importance. The leaves, stems and flowers of Mentha species are used in various foods to offer aroma and flavor and is also used in herbal teas. It has also been used as a folk remedy for treatment of fevers, headaches, digestive disorders, bronchitis, ulcerative colitis, liver complaints etc. Spearmint (*Mentha* *spicata*) and Peppermint (*Mentha piperita*) are among the important members of the Lamiaceae family (Zaidi and Dahiya, 2015). Spearmint is an aromatic herbal plant used widely in cosmetic, confectionary, chewing gum, food, tooth paste, pharmaceutical industries and for essential oil productions. It is an important herb used fresh and dried for folk medicine such as stimulant and carminative. The essential oil is extracted from freshly harvested mint leaves or from dried leaves via distillation process. The essential oil obtained has been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Singh and Aggarwal, 2013). The essential oil contains significant amounts of limonene, dihydrocarvone, and 1, 8- cineol (Hussain *et al*., 2010). The distinctive smell of spearmint oil is because of its most abundant compound carvone. Moreover, Al kassie (2010) found that there is an important in performance traits (weekly body weight, feed conversion ratio and dressing percentage) for broiler chicks fed diet supplemented with 0.25, 0.50, 1.0 and 1.50% peppermint compared with the control group and there was no significant effect was noticed on the addition of the peppermint to the diet on blood traits (Packed cell volume%, Red blood cell count, Hemoglobin concentration% and White blood cell count). This results in agreement with the observations made by Ocak *et al*. (2008), Cross *et al*. (2007) and Bampidis *et al*. (2005).

Throughout the world, the use of antibiotics is considered as dietary growth promoter, today their use as growth promoters in animal nutrition has become undesirable due to the appearance of residues and resistant strains of bacteria; (Yoshimura *et al*., 2000). The phasing out of Antibiotic Growth Promoters (AGP) will affect the poultry and animal industry widely. To minimize the loss in growth, there is a need to find alternative to AGP. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immune stimulant and other management practices (Banerjee, 1998). Compared with synthetic antibiotics or inorganic chemicals, these plants and their derived products have reported to be less toxic, residue free and thus considered as ideal feed additives in animal production (Hashemi and Davoodi, 2010). These herbal plants exert positive effects on growth and health of animals probably by their immune stimulatory properties (Guo *et al*., 2004).

From the above consideration, objectives of the study are as follows-

1. To investigate the effect of feeding mint leaf powder on growth performance broiler chicken
2. To investigate the immune parameter and caecal microbial count of broiler chicken fed mint leaf powder
3. To find out an alternative to antibiotic for growth promotion of broiler chicken

CHAPTER II

REVIEW AND LITERATURE

In recent years, there has been a steep rise in poultry production throughout the world. As a result, it is gradually becoming a major thrust in the world economy especially in the livestock sector. Rising prices of feeds certainly have reduced the profitable nature of broiler farming. For better utilization of feed and to improve feed efficiency, growth promoting feed additives viz. probiotics, prebiotics and herbal bio-enhancers are added to poultry ration. Most of the information available in the scientific literature pertaining to use of herbal supplements have been found in various species of poultry and therefore work on colored chicken has been reviewed based on the other poultry species. In this section, studies on mint (*Mentha pulegium L*) in poultry have been described.

2.1 Mint leaf

Mentha (also known as mint, from Greek míntha, Linear B mi-ta) is a genus of the plant belonging to family Lamiaecae. Peppermint (Mentha) is one of the oldest medicinal plants in the world which is a cross between two species namely *M. aquatic* and *M. spicata* and distributed mostly in temperate and sub-temperate regions of the world. It is estimated that 13 to 18 species exist, and the exact distinction between species is still unclear. Hybridization between some of the species occurs naturally. The genus has a sub-cosmopolitan distribution across Europe, Africa, Asia, Australia, and North America.

Taxonomy of *Mentha pulegium L.*

Kingdom: Plantae

Subkingdom: Tracheobionta – Vascular plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae

Order: Lamiales

Family: Lamiaceae

Genus: Mentha

Table 1: Composition of mint leaf

Elements	Percentage %
Menthol	35–60
Menthone	2–44
Menthyl acetate	0.7–23
1,8-cineole	1–13
Menthofuran	0.3–14
Iso-menthone	3–4
Neomenthol	3–4
Limonene	0.1–6
β-caryophyllene	1.6–1.8

(**Source**: https://www.intechopen.com)

2.2 Properties of mint leaf

Mentha is an herbal plant, which contains various types of essential oils, such as menthol, mentone, cavone, methyl acetate and piperitone. These essential oils promote biological effects such as antimicrobial growth, anti-oxidation and stimulation of bile acid secretion, growth improvement and ammonia reduction in broiler. It was also found that the supplementation of dried peppermint in diets can improve the growth performance of broilers (Khempaka *et al*., 2013). Peppermint oil is one of the most popular and widely used essential oils, mostly because of its main components- menthol and menthone. Peppermint oil is used for flavouring pharmaceuticals and oral preparations. Mint is the richest source of natural menthol (Sharma and Tyagi, 1991; Shasany *et al*., 2000). It is also used as a flavouring agent in cough drops, chewing gums, confectionery and alcoholic liqueurs. It is used in medicines for internal use. Its pleasant taste makes it an excellent gastric stimulant (Budavari *et al*., 1989, Gupta, 1991). Plant-derived natural products are extensively used as biologically active compounds. Among them, essential oils were the first preservatives used by man (Thompson, 1989). Many of these crude mixtures have been found to have antifungal, antimicrobial, cytostatic and insecticidal activities (Sivropoulu *et al*., 1995). The essential oil of both these aromatic plants showed strong insecticidal activity. Active constituents in mint (Akbari and Torki, 2014) reported different species of mint are of particular importance because of their abundant essential oils that include menthol, carvone, limo-nene, beta pinene, Mentone, alpha-pinene and geraniol, and effective pharmacological compounds. However, the main flavonoid component of mint is menthol. Studies show that medicinal plants due to its antioxidant and flavonoid compounds could play an important role in improving cardiovascular system. Ameri *et al*. (2016) reported that peppermint, in addition to the essential oils, also contains tannins, glycosides, saponins and other bioactive components.

Table 2. Mint species and its commercial and medicinal uses (Wiersema and Leon, 1999)

Species	Usage
M. pulegium L. (European pennyroyal) Medicine	Ornamental, oil or medicinal
M. arvensis L. (corn mint; field mint)	Weed
M. x gracilis (Sole)	Essential oil
Ginger mint, Red mint, Scotch mint, Scotch spearmint	Essential oil
M. longifolia L. Huds. (horsemint)	Poison, medicinal
M. x piperita L. nothosubsp. piperita (peppermint)	Flavoring and essential oil
M. aquatica L. (water mint)	Flavoring
M. requienii Benth. (Corsican mint)	Ornamental
M. suaveolens Ehrh. syn: M. rotundifolia auct (apple mint)	Ornamental

2.3 Beneficial effects of mint alternative to antibiotics

The unfavourable effect of chemical products especially 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 phytogenics as feed additives is gaining importance due to their antimicrobial and stimulatory effects on digestive system (Jamroz *et al*., 2003; Jang et al., 2004). They include herbs, spices or plants that are used to keep the gut microflora of poultry normal, which is a prerequisite for cost efficient and eco-friendly poultry production (Windisch and Kroismayr, 2006). It has been estimated that there are 250,000-500,000 species of plants on earth (Borris, 1996). Relatively, a small percentage (1-10%) of these is used as food by both humans and other animal species (Cowan, 1999). Compared with synthetic antibiotics or inorganic chemicals, these plants and their derived products have reported to be less toxic, residue free and thus considered as ideal feed additives in animal production (Hashemi and Davoodi, 2010). These herbal plants exert positive effects on growth and health of animals probably by their immune stimulatory properties (Guo *et al*., 2004). Kashmir often referred to as paradise on earth is located at the north western tip of Himalayan biodiversity hotspot (Hussian, 2001). The region has a number of phytobiotics which may have the potential to promote production performance in chicken; and one amongst them being mint (*Mentha piperita*), locally known as Pudina. Mint is a member of the Labiatae family and is widely used in herbal medicine and believed to be beneficial in as immunity enhancer (Nanekarani *et al*., 2012). Mint is mostly consumed after a meal because of its ability to reduce indigestion and intestinal spasms by reducing the gastrocholic reflux (Spirling and Daniels, 2001). The main action of its leaves and flowers is due to the presence of abundant menthol which is the main phenolic component having antibacterial activities (Schuhmacher *et al*., 2003). Mint also contains polyphenolic compounds and hence could possess strong antioxidant properties (Dorman *et al*., 2003). Further, the supplementation of enzyme in poultry diets has been reported to improve the performance (Yousuf *et al*., 2012) by degrading non-starchy polysaccharides and improving their digestion; having beneficial effect on gut morphology and thus improving absorption of nutrients (Tufarelli *et al*., 2007; Yousuf *et al*., 2011; Qureshi *et al*., 2016a). In view of such beneficial effects of mint and enzyme, a study was conducted to evaluate the efficiency of mint leaves, with or without enzyme supplementation on blood biochemistry, carcass characteristics and sensory attributes of broiler chicken.

2.4 Beneficial effect of mint in humans

Shah and Mello (2004) reported peppermint oil vapour is used as inhalant for respiratory congestion. Peppermint tea is used to treat cough, bronchitis, inflammation of oral mucosa and throat. It is used in infant to treat colic, diarrhea, indigestion and nausea. Lamiaceae family is one of the families of flowering plants. Genus Mentha, is an important member of this family. Spearmint oil has anti-fungal, anti-micro bial, anti-inflammatory, anti-tumor and antioxidant activity. Various beneficial medicinal effects of spearmint have been found, such as preventing chemotherapy-induced nausea and vomiting (CINV), treatment of respiratory and digestive system disorders, hypertension, anxiety and even for relieving menstrual pain (Nozhat *et al*., 2014).

2.5 Antibacterial activity

Al-Sum and Al-Arfaj (2013) reported that mentha extract at different concentrations (1:1, 1:5, 1:10, and 1:20) was active against all tested bacteria except for *S. aureus* and the highest inhibitory effect was observed against *S. mutans* using the well diffusion method. Antibacterial activity of aqueous extracts of selected commonly used mint were screened against multi drug resistant bacteria and so was concluded that their extracts can be used against multi drug resistance bacteria capable of causing both nosocomial and community acquired infections.

2.6 Gastrointestinal effects

Shams *et al*. (2015) reported that peppermint oil has been clinically studied for symptomatic treatment of certain gastrointestinal conditions, including Irritable Bowel Syndrome. The improvement in feed intake with the addition of peppermint leaf could be attributed to the carbohydrates and their main component which stimulated the appetizing and digestive process in birds (Steiner, 2009). Peppermint leaf significantly ($P<0.05$) affected feed conversion ratio during the 42 days of age. This is related to the development of the broiler chicks' gut morphological changes of gastrointestinal tissues can be induced by differences in gut load of microbial content including their metabolites (Xu *et al*, 2003).

2.7 Effect on central nervous system

Hazati *et al*. (2014) reported that wild mint (*Mentha longifolia*) known as horse or habek mint is often used in the domestic herbal remedy, being valued especially for its antimicrobial, antiseptic, antispasmodic, choleretic, carminative and central nervous system stimulant properties. Peppermint oil use as a sedative has expanded in the tropics. It has been applied on the affected area to reduce pain and to improve blood flow (Schuhmacher *et al*. 2003; Jamroz *et al*. 2003). Peppermint oil or peppermint tea is often used to treat gas and indigestion, and it may also increase the flow of bile from the gallbladder (Mimica Dukic *et al*. 2003; Forster, 1996). Also Mentha species can be used in reducing gas and cramping, alcoholic beverages, rheumatism and toothache (Shah and Mello, 2004).

2.8 Immunomodulatory activity

Banerjee (1998) noted that phasing out of Antibiotic Growth Promoters (AGP) will affect the poultry and animal industry widely. To minimize the loss in growth, there is a need to find alternative to AGP. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immune stimulant and other management practices. Lavinia *et al*. (2009) reported that essential oils extracted from mint are immunological stimulators. Galib and AI-Kassie (2010) studied the performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita L*.). A total of 200 (Hubbard) broiler chicks were studied, 5 levels of whole peppermint, 0.00%, 0.25%, 0.50%, 1.0% and 1.50% were incorporated into the basal diet for six weeks. The feeding period for all groups lasted for 42 days. It was concluded that there was an improvement in performance traits for all treated groups compared with the control group. Al Ankari *et al*. (2004) reported that the use of herbal mint (*Mentha longifolia*) in broiler chicken diets increased antibody titers against NDV. This suggests that the plant’s essential oil may stimulate the immune system.

2.9 Anti-stress activity

Ameri *et al*. (2016) reported the effect of different levels of mint (*Mentha piperita*) plant powder on immune system of broilers under heat stress condition, birds were fed on 1.0 and 2.0% mint leaf powder and basal diet supplemented with 300 mg/kg vitamin E. Heat stress was created by setting room temperature at 34 ˚C for 8 hours per day from the 35th to the 42nd day of experiment. Results showed significant differences ($P<0.05$) for feed conversion ratio (FCR) at 21 days and body weight at 42 days of the experiment. Birds treated by 2.0% peppermint powder and 1.0% peppermint powder showed higher and lower body weight gain, respectively, at 21 days of age, when compared with birds fed basal diet and vitamin E. A significantly higher level of total Ig, IgM and IgG was found for peppermint powder than other treatment groups at 35 days and 42 days of age. Significant interactions were observed between diet and sex on IgG at 35 days of the experiment ($P<0.05$). There were significant ($P<0.05$) differences among the treatments for total white blood cells, lymphocytes, heterophils, heterophils to lymphocytes ratio at 42 days of experiment and 2.0% peppermint powder increased total white blood cells values compared to basal diet and vitamin E. The peppermint powder significantly ($P<0.05$) higher in 1.0% peppermint powder as compare to basal diet groups respectively. In general, results indicated that supplementation of peppermint powder in the diet did not improve bursa of fabricius and spleen weight of broiler chicken, but had an antioxidative potential to improve oxidative stability and immune response.

2.10 Effect of mint in poultry growth

Amasaib *et al*. (2013) reported that birds fed 1.0% spearmint in the second and third week did grow better than those fed higher levels of spearmint (1.50%, and 2.0%). The body weight gain was not significantly affected by addition of spearmint. Arab *et al*. (2016) studied different levels of peppermint (*Mentha piperita*) plant powder usage on feed conversion ratio (FCR), body weight (BW), feed intake (FI), body temperature, carcass parts (breast and thigh) and internal organs (liver, heart, gizzard) weights in broiler chicken. A total of 192 broiler chicken were randomly divided into 4 experimental treatments with 4 replicates (12 birds per replicate) arranged in a completely randomized design. Experimental diets consisted of: (1) basal diet (control); (2) basal diet + 1.0% peppermint powder; (3) basal diet + 2.0% peppermint powder and (4) basal diet + 300 mg of vitamin E per kilogram. Heat stress was performed by setting room temperature on 34 ˚C for 8 hours/day from 35 to 42 days of age. Results showed that peppermint powder supplemented in all levels, significantly affected the FCR at 21 days of age and BW at 42 days of age ($P<0.05$). Birds treated with basal diet plus vitamins E showed better then control FCR at 21days of age. Body weight and feed consumption were significantly reduced in birds in the heat stress group. Peppermint powder supplementation at the level of 1.0% significantly ($P<0.05$) reduced body temperature compared with the control group during heat stress period. Significant differences ($P<0.05$) were observed between dietary treatments for the relative weights of carcass, breast and thigh at 35 days of age and breast, gizzard and liver relative weights at 42 days of age. Birds fed basal diet plus vitamin E had higher carcass weight than the control groups on 35 days. In general, the results revealed that peppermint powder as a natural anti-oxidant had beneficial effects on chicken growth performance, body temperature regulation and carcass and internal organ weights. Seyed *et al*. (2013) conducted a study in broilers to evaluate the effect of dietary supplementation of four medicinal plants on the performance, blood lipids and micro-flora population in the ileum. Three hundred and thirty-six-day old Ross broiler chicks were used in a completely randomized study with 6 treatments and 4 replicates each. The diets were iso-caloric and iso-nitrogenous and contained 15, 3, 2 and 2 g/kg of dried cumin, peppermint, yarrow (*Achillea millefolium*) and polly herbs, respectively. Two dietary treatments were considered the negative (containing no medicinal plant or antibiotic) and positive (containing flavomycin at 0.4 g/kg) control groups. Flavomycin and peppermint supplementation to the diet increased the FI and body weight gain (BWG) of the broiler chickens compared to the control (P<0.01) Dietary flavomycin significantly increased body weight gain (BWG) in contrast to the other dietary treatments (P<0.05). Peppermint and cumin supplementation to the diet increased the BWG of the broiler chickens, whereas dietary polly herb and yarrow significantly reduced the BWG and increased feed conversion ratio (FCR) when compared with broilers fed the negative control diet (P<0.05). Dietary flavomycin and peppermint increased the concentration of triglycerides (TG), low density lipoprotein (LDL) and total cholesterol in serum (P<0.05). Addition of flavomycin or peppermint to the diet significantly (P<0.05) reduced the ileal bifid bacteria and clostridia. So, it was concluded that peppermint improved growth performance and by adding it to the diet could be an alternative to the use of antibiotics as growth promoters in poultry production.

2.10.1 Body weight of poultry

Chickens fed diet supplemented with 2.0% spearmint (T_4) had significantly lower body weight and total body weight gain compared with the control group and the other treatments (Asadi e*t al*., 2017). Amasaib *et al*. (2013) who reported significant improvement in body weight in broiler chickens supplemented with spearmint (*Mentha spicata*). Data on body weight changes indicated that during 6th week, T_2 had significantly higher (P<0.05) body weight than T_5, T_6 and T_7 and comparatively higher (P<0.05) body weight than T_1, T_3 and T_4 respectively. However, during 7th week, T_4 had significantly higher body weight than T_3, T_5 and T_6 and comparatively higher body weight than T_1, T_2 and T_7 respectively. Further, T_4 had significantly higher (P<0.05) body weight than T_3, T_5 and T_6 and comparatively higher body weight than T_1, T_2 and T_7 at 8^{th} weeks of age. The significantly higher body weight in 0.75% mint leaf meal supplemented group in our study is in line with the observations of Amasaib *et al*. (2013) who also reported birds fed 1.0% spearmint had higher body weight than those fed higher levels of spearmint (1.50%, 2.0%). In addition, Isha *et al*. (2018) noted that chickens fed diet supplemented with 2.0% spearmint had significantly lower body weight compared to control or fed spearmint at lower concentrations. Further, in our study, it was found that there was no significant difference in the weekly body weight among the different treatment groups up to 5^{th} week of age. Similar results have also been obtained in our studies (Galib *et al*., 2010, Demir *et al*., 2008).

2.10.2 Body weight gain of poultry

Ocak *et al*. (2008) reported that feeding broilers with peppermint resulted in significant improvements in daily weight gain in grower and finisher periods. Asadi *et al*. (2017) reported that birds fed peppermint power @4.5 g/kg basal feed, peppermint powder had significantly better ($P<0.05$) daily weight gain compared to the control group. Amasaib *et al*. (2013) formulated experimental diets with four levels of spearmint (*Mentha spicata*) of 0, 1.0, 1.50 and 2.0%. However, the body weight gains for the treatments were 1481.63, 1512.81, 1519.57 and 1519.63. The results indicated that supplementation of different levels of spearmint to the diets of broilers improved body weight gain.

Mehranpoor (1995) reported the positive effect of different levels of peppermint i.e. increasing average daily weight gain was due to its decreasing effect on gastrointestinal disorders, thus strengthening the digestive system and improving feed efficiency. Ocak *et al*. (2008) reported that during the starter period, there was no significant difference in the daily weight gain of birds receiving peppermint and the control diet, although control treatment had the least average daily weight gain among other treatments. Al Ankari *et al*. (2004) reported that body weight gain was also increased with increasing level of spearmint in the basal diet with the ranking found to be as follows, diet C recorded higher value for body weight gain when compared to diet A and diet B. It was concluded that birds fed diet (1.50% spearmint) were observed to have best performance in terms of total body weight gain, total feed intake and economic value.

These may be attributed to the effect of some antimicrobial components which may act as growth promoters.

2.10.3 Feed consumption

Deyoe *et al*. (1962) reported that feeding graded levels of spearmint (*Mentha spicata*) had no significant effect ($P<0.05$) on feed intake. However, the highest feed intake was obtained by the birds fed 1.0% spearmint during second and third week. The increment in feed intake may be due to the flavor effect of spearmint. Amasaib *et al*. (2013) formulated experimental diets with four levels of spearmint (*Mentha spicata*) of 0, 1.0, 1.50 and 2.0%. Average feed intake obtained from the experiment were 2680.20, 2679.11, 2708.55 and 2692.57 for diets 0, 1.0, 1.50% and 2.0%, respectively the results indicated that the supplementation of different levels of spearmint to the diets of broiler improved feed intake.

2.10.4 Feed Conversion ratio

Aida *et al*. (2018) reported significant improvement in FCR of chicks fed on the diet supplemented with spearmint than control. Results obtained indicated that the control group has significantly better weekly FCR and phase wise FCR compared to the other treatment groups during the initial phase. Though the weekly feed consumption did not indicate any clear trend from 6th week onwards, but during 4-8 weeks of age, FCR was significantly better ($P<0.01$) in 0.75% mint leaf meal supplemented group compared to 0.50%, 1.0%, 1.25% and comparatively better than control group. Amasaib *et al*. (2013) reported that effect of spearmint on feed FCR which was found to be insignificant in the first five weeks of age, but FCR was significantly affected by addition of spearmint in the sixth week ($P<0.05$) with 1.50% yielding the best results. However, in the present study, during 0-8 weeks of age, FCR was significantly better ($P<0.01$) in T_1 than T_3, T_5, T_6, T_7. Further, FCR was comparatively better in T_1 than T_2 and T_4 during 0-8 weeks of age. Contrary to our results, Isha *et al*. (2018) reported that supplementation of different levels of spearmint to the diets of broiler chicks improved feed conversion ratio. However, Ocak *et al*. (2008) reported that there was no significant difference in FCR of broilers fed diets supplemented with 0.20% dry peppermint leaves compared to the control group. After feeding supplemental ME for eight weeks, FCR was improved ($P<0.05$) for those birds in 100 ME and 200 ME relative to the control diet. It has been claimed herbal supplements do not affect FCR in laying hens (Christaki *et al*., 2012;

Ghasemi *et al*., 2010;). However, other studies indicated beneficial effects on FCR from feeding herbal products to laying hens (Bolukbasi & Erhan 2007; Lippens *et al*., 2005; Rahimi *et al*., 2011; Sharma *et al*., 2020).

2.11 Haemato-biochemical parameters

Akbari and Torki (2014) studied the effect of dietary chromium picolinate (Cr pic), peppermint essential oil, or their combination on growth performance and blood biochemical parameters of female broiler chicks raised under heat stress conditions (HS, 23.9 to 38 °C cycling). Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were obtained from 1 to 42 days of age. Furthermore, at the end of the experiment (day 42), birds were bled to determine some blood biochemical parameters and weighed for final body weight (BW). ADFI, ADG, and BW were not influenced significantly by dietary Cr pic and peppermint. A significant (P=0.012) interaction between dietary Cr pic and peppermint on FCR was detected. FCR was significantly better in chicks fed the diet including both Cr pic and peppermint compared with the Cr pic group. Significant interaction (P<0.01) between dietary peppermint and Cr pic on serum concentrations of triglycerides, glucose and albumin were observed, but the other measured blood biochemical parameters were not statistically affected by dietary treatments (P>0.05) The serum concentrations of glucose, triglycerides were significantly decreased (P<0.05) in broilers fed the diet including both Cr pic and peppermint. Plasma chromium content increased significantly (P<0.05) in birds fed the Cr pic-included diet compared with the control group. It was concluded that dietary supplementation with combined peppermint and Cr pic had beneficial effects on some blood biochemical parameters of female chicks reared under heat stress conditions. Ayman *et al*. (2016) reported that, albumin, globulin ratio, total cholesterol (TCH), creatinine and ALT of chick plasma at 35 days old are dietary AGP and the two levels of peppermint oil tended to increase concentrations of total protein and globulin while no significant differences were found for albumin values dietary supplementation with peppermint leaves 1.50, peppermint oil 125, PO250 and AGP, resulted in lower A: G ratio than control (4.14) and PL3.0 (2.05) and ranged between 1.14 and 1.20. On the other hand, total cholesterol in plasma for both control and AGP groups was significantly lower compared to all peppermint treatments (P<0.05).

A/G ratio didn't show significant difference between treatments. Khurshid *et al*. (2017) observed that broiler chicken diets supplemented with raw mint leaves @ 10g/ kg and 20 g/ kg did not result in any significant difference in serum glucose, total protein, cholesterol, SGPT and SGOT levels. Devi *et al*. (2017) conducted an experiment supplementing pudina leaf powder @ 0, 5.0, 7.50 and 10.0 g/kg in the diet of laying hens. It was observed that 12-week supplementation of pudina leaf powder at different levels resulted in significant increase in serum total protein, albumin, globulin, HDL-cholesterol and LDL cholesterol ratio and a significant decrease in serum glucose, total cholesterol, LDL cholesterol, VLDL-cholesterol, triglycerdies, uric acids, AST and ALT levels.

2.12 Effect of mint leaf in carcass quality of poultry

Adibnezhad *et al*. (2014) evaluated the effects of combination of two herbs peppermint and thyme powder on broiler performance and carcass characteristics. This experiment was done in a completely randomized design using 300 one day-old broilers (Ross) with five treatments and three replicates. Treatments included control (without any additives; T_1), 1.0% peppermint and 0.50% of thyme (T_2), 1.0% peppermint and 1.0% thyme (T_3), 2.0% peppermint and 0.50% thyme (T_4) and 2.0% peppermint and 1.0% of thyme (T_5). Results showed that feed intake in whole period (42 days) was not affected by treatments ($P>0.05$), although in the period of 1 to 21 days, there was significant increase ($P<0.05$) in T_2 in terms of daily feed intake. Average daily gain in 1-21 days, 22-42 days and in the entire period was not affected, although numerically the largest weight gain was found in T_5. Best feed conversion ratio (FCR) was observed in T_3 in total period although FCR was not affected significantly in all three experimental periods. The results showed that carcass weight, thigh weight, wing weight, chest weight, neck weight and liver weight at the end of the period was significantly high in T_5. However, the back weight, gizzard weight, intestinal length and abdominal fat were not affected by treatments. The results showed that the supplemented of 2.0% peppermint and 1.0% of thyme in poultry rations has a good impact on performance and carcass characteristics. The dressing percentage was not significantly affected in broiler chicks by addition of spearmint (Amasaib *et al*., 2013).

Al-Ankari *et al*. (2004) reported protein and fat contents in breast and thigh muscles were not influenced by habek including 150 g/kg habek into broiler diet. Further, there was a significant improvement in the mean body weight, daily average gain, feed intake and food conversion ratio. Peppermint powder had an effect on the weight of heart, liver, gizzard, and abdominal fat in broilers. Data showed that there was significant difference ($P<0.05$) on liver weight between birds fed 3.0 g/kg, 4.50 g/kg and 6.0 g/kg peppermint powder compared to the control group (Asadi *et al*., 2017).

2.13 Other activities of mint leaf

Galib and Al-Kassie (2010) reported that mentha as aromatic plant has been traditionally used as medicine. It extends the shelf life of food, inhibit bacteria and fungi growth. Hernandez *et al*. (2004) reported supplementation of poultry diets with aromatic plants have a stimulating effects on digestive system of the animals through the increasing the production of digestive enzymes and by improving the utilization of digestive products through enhanced liver function. Peppermint oil or peppermint tea is often used to treat gas and indigestion, and it may also increase the flow of bile from the gallbladder (Dukic *et al*., 2003).

CHAPTER III

MATERIALS AND METHOD

3.1 Statement of the experiment

The research was conducted at Sher-e-Bangla Agricultural University poultry farm, Dhaka, with 225 one-day-old commercial Lohmann meat (Indian river) broiler chicks for a period of 28 days from 27th August to 25th September, 2021 to assess the individual and effect of feeding mint leaf as an alternative to antibiotics on the performance of broilers.

3.2 Collection of experimental birds

A total of 225 one-day-old Lohmann meat broiler chicks (Indian river) were collected from Kazi hatchery distribution point, Savar, Dhaka. Average weight of day-old chick was 42.0 g of the study.

3.3 Experimental materials

The chicks were collected from Kazi hatchery and carried to the university poultry farm early in the morning. Then the chickens were kept in the electric brooders for 7 days by maintaining standard brooding protocol. During brooding time only control treatment was given. After successful brooding the chicks were distributed randomly in five (5) treatments. Each treatment had three (3) replications like R_1, R_2 and R_3 where each replication contains 15 birds. The total number of treatments were five (5) and their replications were fifteen (15).

3.4 Experimental treatments

The mint leaf was mixed properly with commercial feed at three different level. The experimental treatments were following:

T_0: Basal feed

T_1: Basal feed+Antibiotic (Amoxicillin, dose-1 g/3 kg feed)

T_2: Basal feed+1.0% mint leaf powder

T_3: Basal feed+1.50 % mint leaf powder

T_4: Basal feed+2.0% mint leaf powder

Table 3: Experimental layout

Distribution of treatments and birds			No. of birds
T_1R_3 (15)	T_2R_2 (15)	T_0R_1 (15)	45
T_0R_2 (15)	T_1R_1 (15)	T_2R_3 (15)	45
T_2R_1 (15)	T_0R_3 (15)	T_1R_2 (15)	45
T_4R_1 (15)	T_3R_2 (15)	T_4R_3 (15)	45
T_3R_3 (15)	T_4R_2 (15)	T_3R_1(15)	45
Total birds			**= 225**

3.5 Collection of mint leaf and preparation of mint leaf powder

For preparing of mint leaf powder, went to kawran bazar, Dhaka and bought 41 kg mint leaf and took the leaf & soft stem part of the mint tree and properly dried on the sunlight to decrease moisture up to 15-20%. After that used hot air oven to keep the moisture below 15%. Grinder machine is used to make fine powder. Then, mixed the mint leaf powder with feed into each treatment with the given proportion in a bowl without control and antibiotic treatment every week and served that mixed feed to the broiler chicken.

Processing of experimental mint leaf powder

- Purchase of fresh peppermint herbs from local market
- Washed and dried under sun for 4-5 days
- Collection of the dried mint leaves
- Dried leaves ground to powder mint leaf powder packaged for use

3.6 Preparation of experimental house

The experimental house was properly cleaned and washed by using tap water. Ceiling, walls, floor, feeder and waterer were thoroughly cleaned and disinfected by spraying diluted disinfectant solution. The house was divided into 15 pens of equal size using wood materials after proper drying. A group of 15 birds were randomly shifted to each pen of the 5 treatments. One feeder and one waterer were distributed each pen. The stocking density was 1 m^2/10 birds.

3.7 Experimental diets

Starter and grower commercial kazi broiler feed were purchased from the market. Starter diet was enriched with following elements:

Table 4: Nutrient contents in starter and grower broiler ration

Name of the elements	Starter broiler ration %	Grower broiler ration %
Protein	21.0	19.0
Fat	6.0	6.0
Fiber	5.0	5.0
Ash	8.0	8.0
Lysine	1.20	1.10
Methionine	0.49	0.47
Cysteine	0.40	0.39
Tryptophan	0.19	0.18
Threonine	0.79	0.75
Arginine	1.26	1.18

(**Source:** Kazi Feed, 50 kg feed packet)

The feeding program was divided into two phases including starter and grower diets that were fed from 0 to 14 days and 15 to 28 days respectively.

3.8 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.

3.8.1 Brooding of baby chicks and lighting program

The experiment was conducted on 27th August, 2021. The highest temperature was 39^{0} C and lowest 26^{0} C. The highest relative humidity was 99% and lowest 51% in the poultry house. Brooding will be done for first 2 weeks, in first day temperature will be set 35^0 C and then lowered stepwise to ambient temperature. For the first 4 days, lighting program will be 24 hrs. of light and then stepwise lowered to 21 hrs. of light and 3 hrs. of dark. The birds were grouped in five treatments.

3.8.2 Room temperature and relative humidity

Daily maximum and minimum room temperature and humidity were recorded with the digital hygrometer. Average room temperature and percentage of the relative humidity for the experimental period was recorded and collected in a fixed time every day.

3.8.3 Litter management

Rice husk was used as litter at a depth of 6 cm. Every day remove ammonia gas along with harmful gasses to running exhaust fan of the tunnel ventilation and to reduce parasite infestation. After 3 weeks of age droppings on the upper layer of the litter were cleaned and fresh litter was added.

3.8.4 Feeding and watering

Feed and fresh clean water were given to the bird *ad-libitum*. One feeder and one drinker were provided in each pen for one group of birds. Everyday feeders were cleaned and drinkers were washed daily morning.

3.8.5 Biosecurity measures

Recommended vaccination, sanitation program was performed in the farm and which help to prevent the disease from the farm. All chicks were provided Vitamin-A, D, E, K, Vitamin-C, Vitamin-B Complex, Ca and electrolytes.

3.8.6 Vaccination

Vaccines were collected from medicine shop (HIPRA Company) and provided to the birds according to product card of Ceva Sante Animale marketed in Bangladesh by ACI animal health limited, Tejgaon, Dhaka.

Table 5: The vaccination schedule

Age of birds	Name of the disease	Name of vaccine	Route of administration
2 days	IB+ND	CEVAC BI L Vaccine	One drop in one eye
9 days	Gumboro	CEVAC IBD L Vaccine	One drop in one eye
16 days	Gumboro	CEVAC IBD L Vaccine	Drinking water
19 days	ND	CEVAC NEW L Vaccine	Drinking water

3.8.7 Medication

Medicine were collected from medicine shop and offered to the birds according to the schedule. The medication schedule was given in Table 6.

Table 6: The medication schedule

Medicine	Dose	Time period (Days)
Glucose	50 g /1 L Water	1
Vitamin C	5 ml/1-2 L Water	5
HIPRACHOK AMINO	1 ml/1 L Water	5

3.8.8 Ventilation

The broiler shed was open sided. Due to having short wall, it was very easy to enter fresh air into the farm and remove polluted gas from the farm. Besides ventilation was maintained as per requirement by polythene screen.

3.8.9 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant (Potassium iodide) was used to disinfect the feeders, waterers and house also.

3.8.10 Study parameters

Every day, temperature and relative humidity was measured at 10 a.m. Besides, weekly feed consumption, weekly live weight and death of chicks were recorded to calculate mortality percentage. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter liver, heart, spleen, gizzard, thigh, drumstick, back, wing, and intestine were measured from each broiler chicken. Dressing yield was calculated for each replication to determine the dressing percentage. Blood test was done to examine the immune parameter. Microbial load was also counted in laboratory.

3.9 Live weight

3.9.1 Data collection

The initial live weight of one-day-old chicks and weekly live weight of each replication was kept to find out the final live weight record per bird. The expected weight of broiler chicken depends on the age of the broiler, sex, and other factors that have to be kept in order to attain the expected weight.

3.9.2 Feed consumption

Feed consumption is a variable phenomenon and is influenced by several factors such as strain of the bird, energy content of the diet, ambient temperature, density of birds in the shed, hygienic conditions and rearing environment. Daily feed consumption was recorded of each replication to get weekly and total feed consumption.

3.9.3 Mortality of chicks

The number is calculated by total deaths, divided by the number of chickens present in the house on that day, multiplied by 100. You can use this spreadsheet to calculate cumulative daily mortality rates. Daily death record for each replication was counted till 28 days to calculate the mortality.

3.9.4 Dressing yield

Dressing yield was calculated by using the following formula-

Dressing yield = Live weight – (blood + feathers + shank + head + liver + heart + digestive system)

3.9.5 Determination of Dressing percentage of broiler chicken

Three birds were taken randomly from each replication at the 28th days of age and slaughtered to calculate dressing percentage of broiler chicken. All birds were slaughtered by halal method with knife. All the live birds were weighed before slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and prefer to complete bleed out at least for 2 minutes. Outer skin of the broiler chicken was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose feathers and other foreign materials from the carcass. Then the carcass was eviscerated and dissected according to the methods by Jones (1982). Liver and heart were removed from the remaining viscera and then the gall bladder was removed from the liver. Then the gizzard was removed. Lastly dressing yield was calculated by subtracting feathers, blood, head, shank, heart, liver and digestive system from the weight.

3.10 Calculation

3.10.1 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

3.10.2 Feed intake

Individual feed intake of each bird is the sum of feed intake for maintenance and growth. Feed intake of the dead bird in the cage should be used to correct cage feed intake and hence feed efficiency during the final data analysis. Feed intake was calculated dividing the total feed consumption in the replication by number of the birds in each replication.

$$\text{Feed intake(g/bird)} = \frac{\text{Feed intake in a replication (gm)}}{\text{Number of birds per replication}}$$

3.10.3 Growth performance and feed conversion ratio

Birds of each replication pen were weighed by digital balance at the end of every week to calculate average weight gain (AWG) weekly. The average weekly feed intake (AWFI) was calculated by considering the difference of given and unconsumed feed at the end of each week. The feed efficiency or FCR was calculated in every week. Mortality of the birds was recorded daily to calculate and adjust the feed intake and feed efficiency. Feed Conversion Ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

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

3.11 Immune parameters

Blood of broiler chicken was collected with syringe and preserved in refrigerator. Then Lab analysis was done at Euro-Bangla Heart Hospital and Diagnostic center, Dhanmondi-15 and they had provided of different blood immune parameter of broiler chicken. Immune parameters Include-Hemoglobin, RBC, WBC, Platelet, Neutrophil, Lymphocyte, Monocyte, Eosinophil, PCV etc. (Calder. P. C., 2007).

3.12 Caecal microbial count

For caecal microbial counts, caecal samples were collected from all 15 replications pens each treatment at day 28. Fresh caecal samples were collected within 2 hrs from each replicate pen per treatment and transferred into clean plastic containers. The caecal samples were immediately transferred to the laboratory in an ice box for the enumeration of *Salmonella sp.* and *E. coli*. The viable counts of bacteria in the caecal samples were then determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) in respective media. The selective medium used for isolation of Salmonella was *Salmonella Shigella* agar (HiMedia, India) and for *E. coli*, Eosin-methylene blue (EMB) agar (HiMedia, India). Eosin-methylene blue (EMB) agar and *Salmonella Shigella* agar plates were incubated for 24 h at 37 °C. The colony counts were then enumerated and results are presented as log10-transformed data (Don, *et. al*., 2009).

3.12.1 Preparation of dilution

At the end of the experiment, caecal samples were collected from broiler chicken. Sterilized test tubes with 9 ml of distilled water were used. One gm of sample content from each sample was mixed in 9 ml of sterilized distilled water in a test tube and shacked well, its ratio was 1:10 and dilution factor was 10^1. Then 1 ml liquid was collected from 1:10 ratio in test tube and mixed in 9 ml of sterilized distilled water in a test tube. Its ratio was 1:100 and dilution factor were 10^2. Finally, 1:1000 and 1:10000 ration was made in same way and their dilution factor was 10^3 and 10^4 respectively.

3.12.2 Preparation of agar medium

36 grams EMB and SS agar powder was mixed in 1000 ml distilled water. Mixed until suspension was uniform. It was heated to dissolve the medium completely. Dispensed and sterilized by autoclaving at 15 lbs. pressure and temperature 121°C for 15 minutes. Then it was poured into the petri dish. It was cooled to 50°C and shacked in order to oxidize the methylene blue (i.e., to restore its blue colour) and to suspend the flocculent precipitate. One ml of liquid of 1:10000 ratio test tube was collected for each sample and poured to petri dish which was partially filled with EMB medium.

3.12.3 Incubation

Petri dishes were sent to bacterial growth chamber for 24 hr. at 37 °C.

3.12.4 Bacterial colony count

After 24 hours *E. coli* and Salmonella colonies were counted by colony counter and following formula was used to estimate *E. coli* and Salmonella population-

$$\text{CFU/g} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Volume of inoculated}}$$

3.13 Benefit Cost Ratio

Benefit cost ratio(BCR) was calculated as the total income of the study divided by total cost of production (Cost Inclusive Evaluation, by-Nadini Persuad).

$$\text{BCR} = \frac{\text{Total income (Tk.)}}{\text{Total cost of production (Tk.)}}$$

3.14 Statistical analysis

Total data were complied, tabulated and analyzed in according to the objectives of the study. Excel program was practiced for the preliminary data calculation. The collected data was subjected to the statistical analysis by applying one-way ANOVA using Statistical Package for the Social Sciences (SPSS version 25.0) in according to the principles of completely randomized design (CRD). Differences between means were tested using the Duncan's multiple comparison test, and significance was set at $P<0.05$.

Some photographic view of the study

Collection of mint leaf

Dried mint leaf

Powder of mint leaf

Plate 1: Collection and preparation of mint leaf

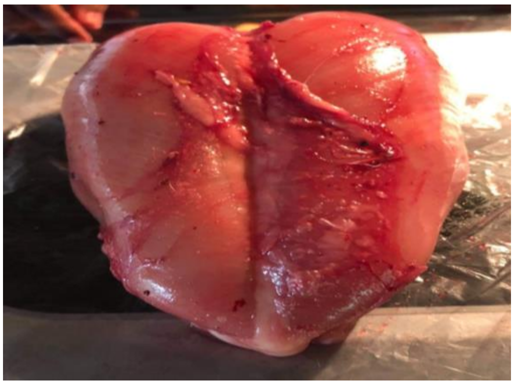
Supervision of supervisor

Data recording

Processing of organ

Blood collection

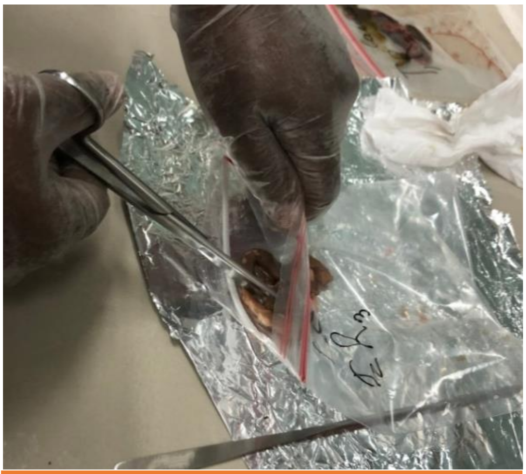
Plate 2: Supervision of supervisor and activities during the experiment



Breast

Different internal organ

Plate 3: Different organs of broiler



Sample collection

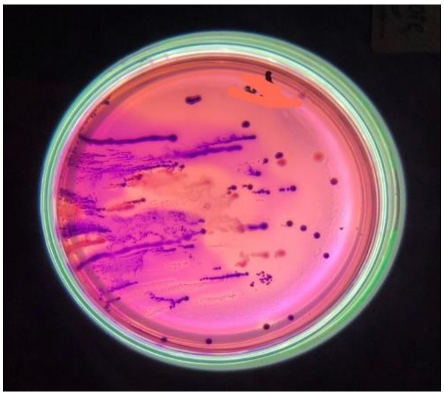
Vortex mixing

Dilution of sample

Agar making

Agar in petri dish

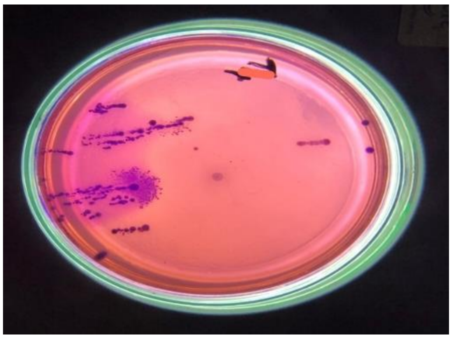
Plate 3: Agar making and colony counting



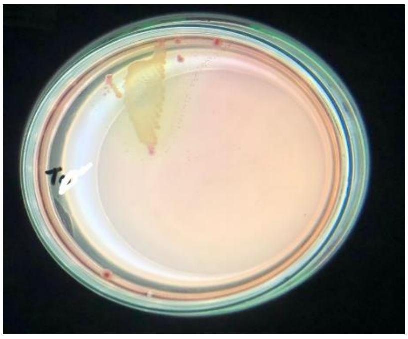
Colony of *E. coli*

Colony of Salmonella sp.

Plate 4: Colony counts of *E. coli in EMB agar and* Salmonella sp. on SS agar of T_0 group

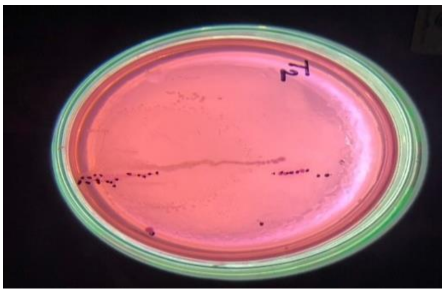


Colony of *E. coli*

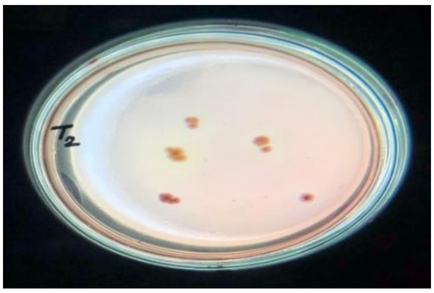


Colony of Salmonella

Plate 5: Colony counts of *E. coli in EMB agar and* Salmonella sp. on SS agar of T_1 group



Colony of *E. coli*



Colony of Salmonella sp.

Plate 6: Colony counts of *E. coli in EMB agar and* Salmonella sp. on SS agar of T_2 group

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 mint leaf on broiler production. The data are given in different tables and figures. The results have been discussed and possible interpretations of the research are given under the following headings.

4.1 Production performances

In this chapter, discussed the effect of mint leaf on broiler production that helps the body growth of broiler chicken. The chicks were randomly divided into five experimental treatment groups. The five groups were T_0 (Basal feed), T_1 (Basal feed+Antibiotic), T_2 (Basal feed+1.0% mint leaf powder), T_3 (Basal feed+1.50% mint leaf powder) and T_4 (Basal feed+2.0% mint leaf powder). The performance traits *viz*. body weight, body weight gain, feed consumption, FCR, weekly feed consumption, weekly body weight gain, weekly FCR, dressing percentage, different dressed organ weight, survivability rate, benefit cost ratio and immune parameter were discussed in this chapter.

4.1.1 Body weight

Table 7 showed that the effect of different level of mint leaf on body weight was discussed. The relative body weight (g) of broiler chickens in the different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1897.00±99.74, 1838.66±92.86, 1965.00±159.55, 1872.00±62.28 and 1866.66±65.21, respectively. The highest body weight was found in T_2 and lowest in T_1. The higher body weight in T_2 group might be due to treatment with basal feed+1.0% mint leaf powder. Chickens fed diet supplemented with 2.0% mint had significantly lower body weight and total body weight gain compared with the control group and the other treatments (Asadhi *et al*., 2017). Amasaib *et al*. (2013) who reported significant improvement in body weight in broiler chickens supplemented with mint.

4.1.2 Body weight gain

Table 7 showed that the effect of different level of mint leaf on body weight gain of broiler was described. 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 1853.00±9.91, 1796.66±25.40, 1923.00±16.22, 1830.67±41.97 and 1824.33±11.93, respectively. The highest body weight gain was found in T_2 and lowest in T_1. The overall body weight gains of different treatment groups showed that there were no significant (P>0.05) effects on body weight gains. The higher body weight in T_2 group might be due to treatment with basal feed+1.0% mint leaf powder. The higher body weight gain observed in broilers fed the peppermint diet may be related to the properties of propernyol (Lovkova *et al*., 2001). Al-Kassie, (2010) found that the difference in body weight gain between the control and peppermint group was not reflected in the body weights of slaughter age.

4.1.3 Feed consumption (FC)

Table 7 showed that the total feed consumption (g) of broiler chicken was discussed. Here, the relative total feed consumption (g) of broiler chicken in different treatment groups were 2262.33±99.73(T_0), 2156.33±67.66 (T_1), 2334.33±108.21 (T_2), 2489.00±61.78 (T_3) and 2288.33±94.93 (T_4), respectively. The highest feed consumption was found in T_3 and lowest in T_2 group. The overall feed consumption of different treatment groups showed that there were significant (P<0.05) effects on feed consumption. Deyoe *et al*. (1962) reported that feeding graded levels of mint (*Mentha spicata*) had no significant effect (P>0.05) on feed intake. However, the highest feed intake was obtained by the birds fed 1.0% spearmint during second and third week. The increment in feed intake may be due to the flavor effect of spearmint. Amasaib *et al*. (2013) formulated experimental diets with four levels of mint (*Mentha spicata*) of 0, 1.0, 1.50 and 2.0%. Average feed intake obtained from the experiment were 2680.20, 2679.11, 2708.55 and 2692.57 for diets 0, 1.0%, 1.50% and 2.0%, respectively the results indicated that the supplementation of different levels of mint leaf to the diets of broiler improved feed intake.

4.1.4 Feed Conversion Ratio (FCR)

Table 7 showed that the FCR of this experimental study was described. The FCR of the different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.22±0.02, 1.20±0.02, 1.21±0.05, 1.36±0.06 and 1.25±0.05, respectively. There were no significant (P>0.05) difference in the different experimental group of the experiment. However, T_1 treatment is better among different treatment groups, might be due to treat with basal feed+antibiotic. The FCR of T_0, T_1, T_2 and T_4 groups were showed better than T_3 group. Because, number of flock size was very small; management was very good, better effect of mint leaf was better and 100% survivability rate in the group. Aida *et al*. (2018) reported significant improvement in FCR of chicks fed on the diet supplemented with mint leaf than control. There was significant effect of supplemented of mint levels 0.50, 1.0 and 2.0% compared with the control group on feed conversion ratio and the results showed improvement in FCR of chicks that fed on the diets supplemented with mint.

Table 7: Effect of Mint leaf on body weight (BW), body weight gain (BWG), total FC and FCR

Treatments	Body weight (g/bird) (Mean±SE)	Body weight gain (g/bird) (Mean±SE)	Total FC (g/bird) (Mean±SE)	FCR±SE (Mean±SE)
T_0	1897.00±99.74	1853.00±19.91	2262.33^{ab}±99.73	1.22^{b}±0.02
T_1	1838.66±92.86	1796.66±25.40	2156.33^{b}±67.66	1.20^{b}±0.02
T_2	1965.00±159.55	1923.00±16.22	2334.33^{ab}±108.21	1.21^{ab}±0.05
T_3	1872.00±55.50	1830.67±41.97	2489.00^{a}±61.78	1.36^{b}±0.06
T_4	1866.66±65.21	1824.33±11.93	2288.33^{ab}±94.93	1.25^{b}±0.05
Level of significance	**NS**	**NS**	*	*

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; Values: Mean±SE (n=45); 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
- FC= Feed Consumption
- FCR= Feed Conversion Ratio

4.1.5 Weekly Feed Consumption

Figure 1 showed that the weekly feed consumption (g) of broiler chicken was discussed. At first week, the highest feed consumption (g) was found in T_1 (257.33) and lowest in T_4 (256). In case of 2nd week, the highest feed consumption (g) was found in T_3 (471.67) and lowest in T_2 (451.33); In case of 3rd week, the highest feed consumption (g) was found in T_3 (635) and lowest in T_2 (599) and in case of 4th week, the highest feed consumption (g) was found in T_0 (983.67) and lowest in T_3 (793.33).

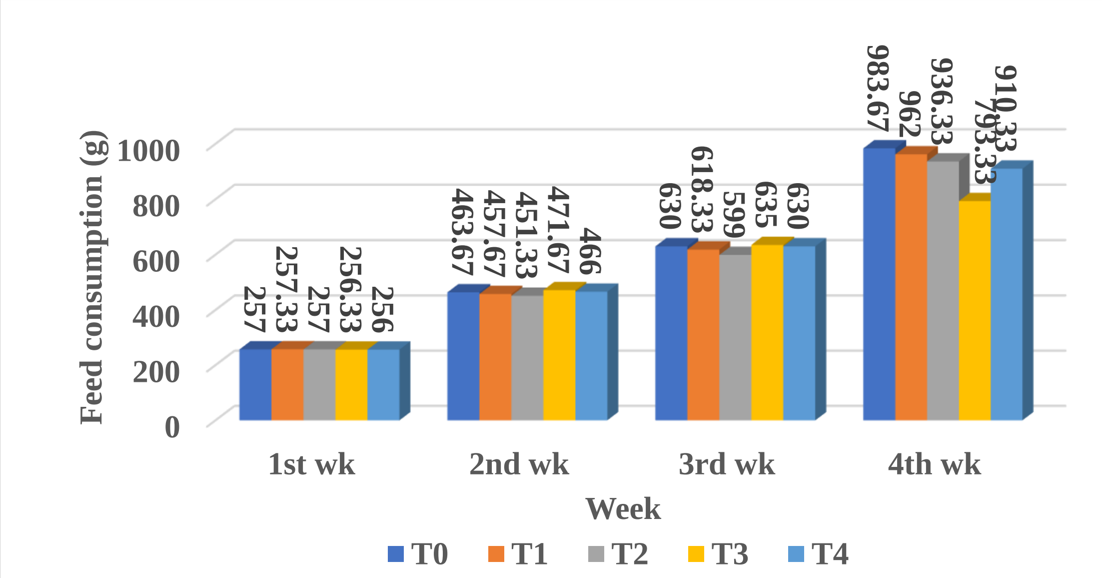


Figure 1: Weekly Feed Consumption

4.1.6 Weekly Body Weight Gain

Figure 2 showed that the weekly body weight gain (g) of broiler chicken was discussed. At first week, the highest body weight gain (g) was found in T_0 (232.87) and lowest in T_2 and T_4 (228.33). In case of 2nd week, the highest body weight gain (g) was found in T_3 (372.27) and lowest in T_2 (335.13); In case of 3rd week, the highest body weight gain (g) was found in T_2 (583.53) and lowest in T_4 (507.47) and in case of 4th week, the highest body weight gain (g) was found in T_0 (755.67) and lowest in T_1 (691.33).

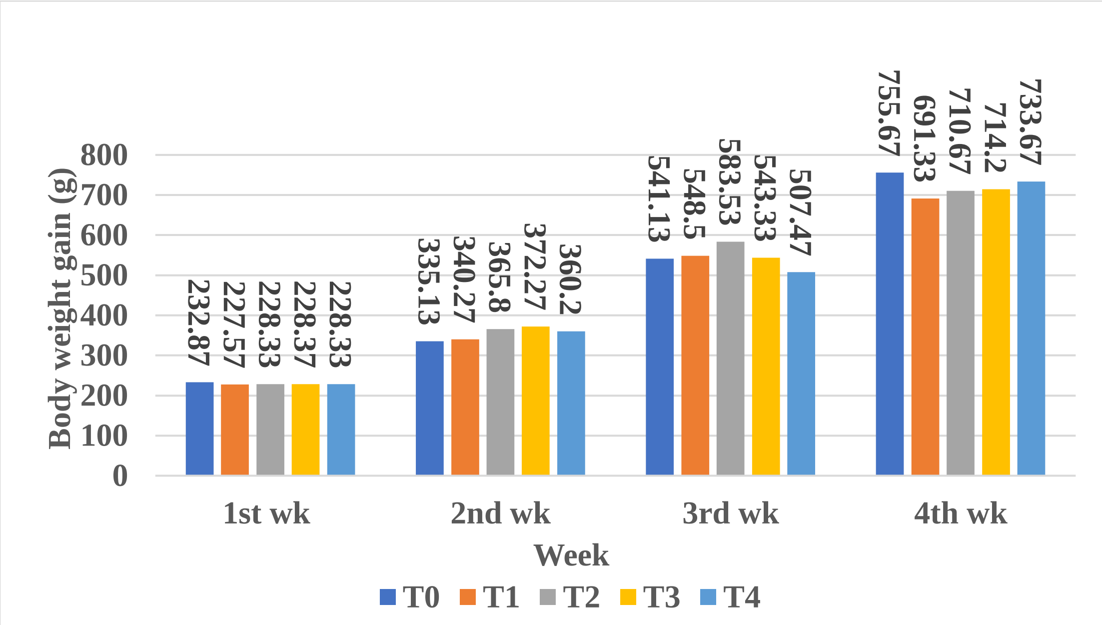


Figure 2: Weekly Body Weight Gain

4.1.7 Weekly Feed Conversion Ratio

Figure 3 showed that the weekly feed conversion ratio of broiler chicken was discussed. At first week, better feed conversion ratio was found in T_0 (1.10) and worse in T_2 (1.14). In case of 2nd week, better feed conversion ratio was found in T_2 (1.23) and worse in T_0 (1.38); In case of 3rd week, better feed conversion ratio was found in T_2 (1.05) and worse in T_4 (1.24) and in case of 4th week, better feed conversion ratio was found in T_3 (1.17) and worse T_1 (1.39).

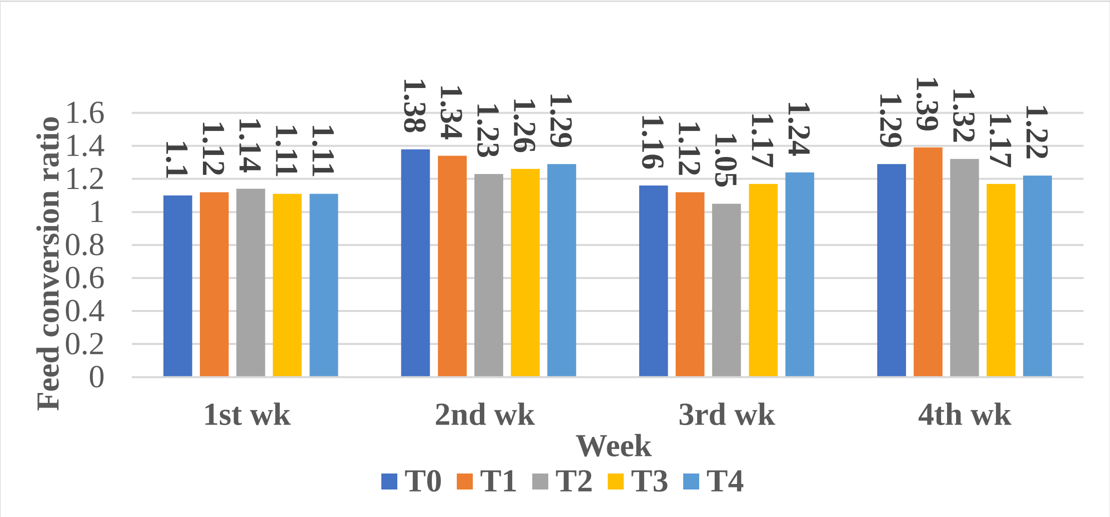


Figure 3: Weekly Feed Conversion Ratio

4.2 Dressing percentage of broiler chicken

Table 8 showed that the live weight (g), dressing yield (g) and dressing percentage of the different treatment groups were discussed. Dressing percentage of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 67.95, 68.68, 70.01, 70.25 and 68.63, respectively. There was no significant difference (P>0.05) in the dressing percentage in this research. However, dressing percentage in basal feed+1.50% mint leaf powder treated group T_3 was the highest than other groups. This is might be due to the effect of Basal feed+1.50% mint leaf powder compared with control group. The results showed that the supplemented of 2.0% peppermint and 1.0% of thyme in poultry rations has a good impact on performance and carcass characteristics. The dressing percentage was not significantly affected in broiler chicks by addition of mint (Amasaib *et al*., 2013).

Table 8: Effect of Mint leaf on dressing percentage of broiler chicken

Treatments	Live Weight (g/bird) Mean±SE	Dressing Yield (g/bird) Mean±SE	Dressing percentage Mean±SE
T_0	2071.33±63.58	1408.33±51.32	67.95±0.39
T_1	2145.33±170.27	1474.00±121.49	68.68±0.61
T_2	2234.66±31.20	1545.33±32.12	70.01±1.13
T_3	2108.33±73.56	1479.66±30.11	70.25±1.07
T_4	2118.66±84.66	1455.33±73.40	68.63±1.01
Level of Significance	**NS**	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error

4.3 Carcass characteristics

4.3.1 Effect of mint leaf on thigh, drumstick, back and wing weight (g) of broiler chicken

Table 9 showed the effect of different level of mint leaf 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 206.00±2.88, 215.67±30.22, 227.00±15.53, 185.00±6.65 and 196.33±3.71, respectively. The relative weight (g) of drumstick in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 184.67±7.31, 182.00±18.33, 193.33±7.62, 178.33±5.20, and 188.00±1.15, respectively; the relative weight (g) of back in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 234.00±2.30, 254.33±23.58, 258.66±11.89, 245.66±7.31 and 248.66±2.02, respectively; the relative weight (g) of breast in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 642.33±7.87, 598.33±11.43, 582.00±9.24, 560.33±5.62 and 564.00±7.98, respectively; and 248.66±2.02, respectively; the relative weight (g) of wing in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 118.33±1.45, 111.00±10.21, 115.66±4.05, 99.66±5.78 and 114.66±1.45, respectively. The weight (g) of thigh, drumstick and back in T_2 were non significantly ($P>0.05$) higher than the other groups including control group. But the weight (g) of wing in T_0 was significantly (P>0.05) higher than the other treatment groups. The better result in T_2 group might be due to the positive effect of basal feed+1.0% mint leaf powder compared with control group (T_0). Al-Ankari *et al*. (2004) reported that contents in thigh muscles were not influenced by wild mint feeding in broiler diet.

Table 9: Effect of mint leaf on thigh, drumstick, back and wing weight (g) of broiler chicken

Treatments	Thigh (g/bird) (Mean±SE)	Drumstick (g/bird) (Mean±SE)	Back (g/bird) (Mean±SE)	Breast (g/bird) (Mean±SE)	Wing (g/bird) (Mean±SE)
T_0	206.00±2.88	184.67±7.31	234.00±2.30	642.33±7.87	118.33^{a}±1.45
T_1	215.67±30.22	182.00±18.33	254.33±23.58	598.33±11.43	111.00^{ab}±10.21
T_2	227.00±15.53	193.33±7.62	258.66±11.89	582.00±9.24	115.66^{ab}±4.05
T_3	185.00±6.65	178.33±5.20	245.66±7.31	560.33±5.62	99.66^{b}±5.78
T_4	196.33±3.71	188.00±1.15	248.66±2.02	564.00±7.98	114.66^{ab}±1.45
Level of Significance	**NS**	**NS**	**NS**	**NS**	*****

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; 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

4.3.2 Effect of mint leaf on Liver, heart, spleen and gizzard weight (gm) of broiler chicken

Data presented in Table 10 showed 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 49.33, 52.66, 60.16, 49.33 and 49.33, respectively; the relative weight (g) of heart in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 8.16, 8.80, 9.50, 7.50 and 9.06, respectively; the relative weight (g) of spleen in different treatment groups

T_0, T_1, T_2, T_3 and T_4 were 3.00, 6.33, 6.67, 4.67, and 5.00, respectively; the relative weight (g) of gizzard in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 22, 24.67, 32.67, 20.00 and 21.67, respectively. The weight (g) of liver, heart, spleen and gizzard in T_2 was significantly higher ($P<0.05$) than the other groups including control group (T_0). Amal (2012) found that the addition of peppermint was no significant effect of the supplementation of different levels of peppermint in chicken's diets on carcass weight percentage, gizzard, liver, heart, edible parts and carcass yield. Meanwhile, the abdominal fat percent did not significantly affect by addition of peppermint on chicken diets compared to the control group. Lee *et al*. (2003) determined an increase in relative liver weight for birds given thyme, but this was seen only at the age of 21 days and not at 40 days that led increases of body weight. Also Galib and Al-Kassie (2010) showed the effect of peppermint on liver weight. They also reported that liver weight of control group was higher than those of the other groups. Ameri *et al*. (2016) reported that chicks fed diet supplemented with 1.0% peppermint powder had the highest gizzard weight compared to control and other treatments at 42 days of age. Khaligh *et al*. (2011) observed no significant differences in liver weight of chicks fed with herb mixtures. Guo *et al*. (2000) reported that the use of medicinal plants has led to the increased weight of the lymphoid organs such as thymus and spleen in broiler chickens, this difference may be due to different levels of medicinal plants.

Table 10: Effect of mint leaf on liver, heart, spleen and gizzard weight (g) of broiler chicken

Treatments	Liver (g/bird) (Mean±SE)	Heart (g/bird) (Mean±SE)	Spleen (g/bird) (Mean±SE)	Gizzard (g/bird) (Mean±SE)
T_0	$49.33^{b}\pm0.88$	$8.16^{bc}\pm0.16$	$3.00^{b}\pm0.22$	$22.00^{b}\pm0.57$
T_1	$52.66^{b}\pm3.17$	$8.80^{ab}\pm0.41$	$6.33^{ab}\pm1.2$	$24.67^{b}\pm2.96$
T_2	$60.16^{a}\pm1.42$	$9.50^{a}\pm0.28$	$6.67^{a}\pm1.2$	$32.67^{a}\pm1.20$
T_3	$49.33^{b}\pm1.20$	$7.50^{c}\pm0.81$	$4.67^{ab}\pm1.45$	$20.00^{b}\pm0.57$
T_4	$49.33^{b}\pm1.76$	$9.06^{ab}\pm0.23$	$5.00^{ab}\pm0.57$	$21.67^{b}\pm0.88$
Level of Significance	*	*	*	*

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; 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

4.4 Survivability rate (%) of broiler chicken

Data presented in figure 5 showed that the survivability rate (%) of the experimental study was described. The relative survivability rate (%) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 100, 100, 100, 100 and 97.77, respectively. Survivability rate (%) was higher in treated group T_1 and T_2 than control group T_0. There was no significant difference ($P>0.05$) in Survivability rate (%). The better result was found in all groups except T_4. But, better result also found in T_2 (100) due to effect of basal feed+1.0% mint leaf powder.

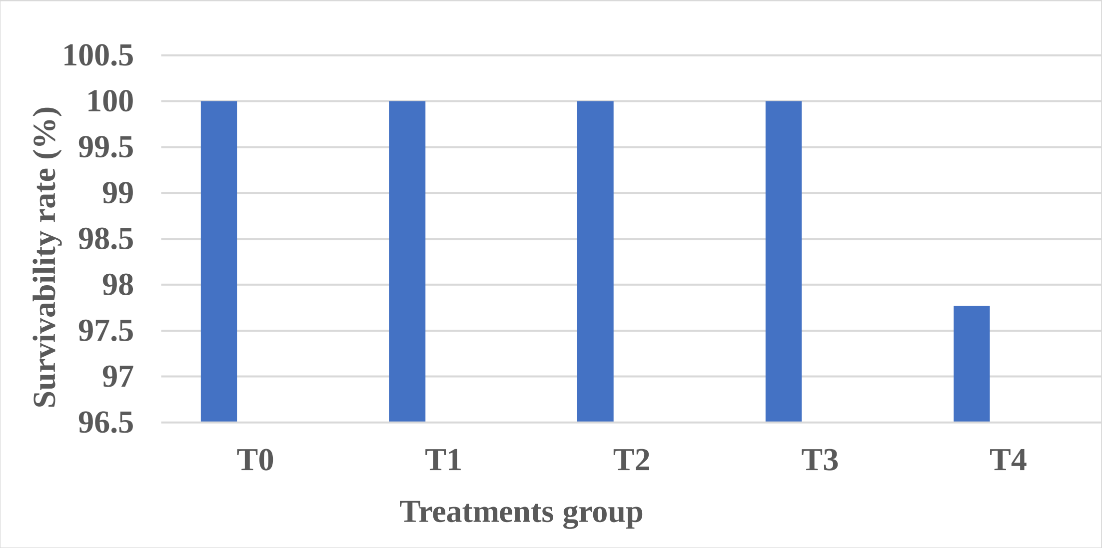


Figure 5: Effect of mint leaf on survivability rate

4.5 Immune parameters

Table 11 showed that immune parameters of broiler chicken was discussed. WBC ($x10^9$/L) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 14.60, 5.83, 12.23, 6.43 and 1.20, respectively. More WBC ($x10^9$/L) was found in T_0 and less in T_4. But, T_2 treatment group has better result than others group. This is might be due to the effect of basal feed+1.0% mint leaf powder. Lymphocyte ($x10^9$/L) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 2.69, 2.54, 2.68, 2.22 and 3.23, respectively. The highest lymphocyte ($x10^9$/L) was found in

T_4 and lowest in T_3. But, T_2 treatment group has better result than others group including control group. This is might be due to the effect of basal feed+1.0% mint leaf powder. Granulocyte ($x10^9$/L) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 11.73, 3.55, 9.69, 4.10 and 6.78, respectively. The highest granulocyte ($x10^9$/L) was found in T_0 and lowest in T_1. But, T_2 treatment group has better result than others group. This is might be due to the effect of basal feed+1.0% mint leaf powder. All parameters were significantly different ($P<0.05$) in this table 13. Lavinia *et al*. (2009) reported that essential oils extracted from mint are immunological stimulators. Compared with synthetic antibiotics or inorganic chemicals, mint plants and their derived products have reported to be less toxic, residue free and thus considered as ideal feed additives in animal production (Hashemi and Davoodi, 2010). These herbal plants exert positive effects on growth and health of broilers probably by their immune stimulatory properties (Guo *et al*., 2004)

Table 11: Effect of mint leaf on immune parameters of broiler chicken

Treatment	WBC ($x10^9$/L)	Lymphocyte ($x10^9$/L)	Granulocyte ($x10^9$/L)
T_0	$14.60^{a}\pm0.07$	$2.69^{cd}\pm0.07$	$11.73^{a}\pm0.06$
T_1	$5.83^{ab}\pm0.05$	$2.54^{cd}\pm0.13$	$3.55^{b}\pm0.11$
T_2	$12.23^{bc}\pm0.09$	$2.68^{c}\pm0.11$	$9.69^{c}\pm0.06$
T_3	$6.43^{bd}\pm0.06$	$2.22^{b}\pm0.01$	$4.10^{d}\pm0.05$
T_4	$1.20^{bc}\pm0.03$	$3.23^{a}\pm0.06$	$6.78^{e}\pm0.04$
Level of Significance	*	*	*

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; Values: Mean±SE (n=45); 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

4.6 Caecal microbial count

The effect of different level of mint leaf on caecal microbial count on broiler chickens showed in the Table 14. Average counts of *E. coli* bacteria (log 10 value) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 7.21, 6.94, 6.63, 6.18 and 6.47, respectively. The highest average counts of *E. coli* bacteria were found in T_0 and lowest in T_3. This is might be due to the effect of basal feed+1.50% mint leaf powder. Average counts of *Salmonella sp.* bacteria of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 7.39, 6.93, 6.17, 5.87 and 4.56, respectively. The highest average counts of *Salmonella sp.* bacteria were found in T_0 and lowest in T_4. This is might be due to the effect of basal feed+2.0% mint leaf powder. Average counts of *E. coli* bacteria and average counts of *Salmonella sp.* bacteria (log 10 value) was non significantly lower (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 peppermint in gut load of microbial content including their metabolites (Xu *et al*, 2003).

Table 12: Effect of mint leaf on microflora (log10 CFU/g) in the caecum of broiler chicken

Treatments	No. of *E. coli* colony (CFU/g) (Mean±SE)	No. of *Salmonella sp.* colony (CFU/g) (Mean±SE)
T_0	7.21	7.39
T_1	6.94	6.93
T_2	6.63	6.17
T_3	6.18	5.87
T_4	6.47	4.56
Level of significance	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error

4.7 Cost benefit ratio analysis

Cost benefit ratio analysis was discussed 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 1.85, 1.91, 1.39, 1.22 and 1.26, respectively. BCR is significantly higher ($P<0.05$) in treatment group T_1 than control group (T_0). This is might be due to the effect of basal feed+antibiotic (amoxicillin).

Table 13: Effect of mint leaf on cost benefit ratio analysis of different treatment groups

Treatments	Total cost (Tk./bird) (Mean±SE)	Sell price (Tk./bird) (Mean±SE)	Profit (Tk./bird) (Mean±SE)	BCR Mean±SE
T_0	157.06±3.53	290.77±3.12	133.71±2.59	1.85±0.03
T_1	152.89±3.41	292.74±4.13	139.85±0.75	1.91±0.01
T_2	203.90±5.45	284.53±0.785	80.62±5.92	1.39±0.03
T_3	232.65±3.12	285.44±6.56	52.78±8.91	1.22±0.04
T_4	236.48±10.09	297.70±1.94	61.22±10.05	1.26±0.05
Level of Significance	**NS**	**NS**	**NS**	**NS**

Here, T_0= Basal feed, T_1= Basal feed+Antibiotic, T_2= Basal feed+1.0% mint leaf powder, T_3= Basal feed+1.50% mint leaf powder, T_4=Basal feed+2.0% mint leaf powder; Values: Mean±SE (n=15); Applying: One-way ANOVA (SPSS, Duncan's method)

- SE= Standard Error

CHAPTER V

SUMMARY AND CONCLUSION

The present study was conducted at Sher-e-Bangla Agricultural University (SAU), Dhaka Poultry Farm for a period of four weeks using different level of dietary mint leaf powder in feed. The specific objective of this study was under taken to determine the effect of different level of dietary mint leaf powder to assess alternative to antibiotics & production performance of broiler. A total of 225 day-old Lohman meat broiler chicks were purchased from Kazi hatchery, Savar, Dhaka. The experimental broilers were allocated randomly to five treatment groups with three replications having 15 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+Antibiotic), T_2 (Basal feed+1.0% mint leaf powder), T_3 (Basal feed+1.50% mint leaf powder) and T_4 (Basal feed+2.0% mint leaf powder). 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 non significantly ($P>0.05$) higher in T_2 (1965±159.55) and lowest in T_1 (1838.66±92.86). The body weight gain (g) was non significantly ($P>0.05$) higher in T_2 (1923±16.22) than other groups including control group. Feed consumption (g) was significantly ($P<0.05$) higher in T_3 (2489±61.78) than other groups including control group. There was 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 significantly ($P<0.05$) observed in T_2 (1.20±0.02) than other groups including control group. Dressing percentage non significantly ($P<0.05$) higher in T_3 (70.25) and lower in T_0 (67.95). The weight (g) of liver, heart, spleen and gizzard in T_2 was significantly higher ($P<0.05$) than the other groups including control group (T_0). The weight (g) of thigh, drumstick and back in T_2 were non significantly ($P>0.05$) higher than the other groups including control group. But the weight (g) of wing in T_0 was significantly ($P>0.05$) higher than the other treatment groups. There was no significant difference ($P>0.05$) in survivability rate (%). Survivability rate (%) was better in all groups except T_4. BCR is non significantly higher ($P>0.05$) in treatment group T_2 (1.91±0.01) than control group (T_0). The colony count of *E. coli* bacteria were non significantly lower ($P>0.05$) in treatment group T_3 (6.18) than control group T_0 (7.21); the colony counts of *Salmonella sp.* bacteria were non significantly lower (P>0.05) in treatment group T_4 (4.56) than control group T_0 (7.39). Significantly (P<0.05) more WBC ($x10^9$/L) was found in T_0 (14.60) and less in T_4 (1.20). But, T_2 (12.23) treatment group has better result than others group. Significantly (P<0.05) the highest lymphocyte ($x10^9$/L) was found in T_4 (3.23) and lowest in T_3 (2.220). But, T_2 (2.68) treatment group has better result than others group including control group. Significantly (P<0.05) the highest granulocyte ($x10^9$/L) was found in T_0 (11.73) and lowest in T_1 (3.55). But, T_2 (9.69) treatment group has better result than others group. The above research was found that body weight (g), body weight gain (g), dressing percentage, weight (g) of liver, heart, spleen, gizzard, thigh, drumstick and back were better result in T_2 than other groups including control group. FCR and BCR were higher in T_1 (basal feed+antibiotic) but better in T_2 (basal feed+1.0% mint leaf powder) group than other groups including control group. Dressing percentage was increased in T_3 (Basal feed+1.50 % mint leaf powder) but also increased in T_2 (basal feed+1.0% mint leaf powder) group than other groups. The count of *E. coli* and *Salmonella sp.* bacteria were insignificantly (P>0.05) decreased in treatment group T_2 than control group (T_0) and T_1 group also. It is concluded that better result was found in T_2 (basal feed+1.0% mint leaf powder) group than control group. Therefore, the research recommended that broiler rearing with 1.0% mint leaf powder could be used on used as an alternative to antibiotics for growth of broiler chicken.

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix I: Effect of mint leaf on body weight (BW) (g/bird) of broiler chicken

Treatments	Replications	1st week (g/bird)	2nd week (g/bird)	3rd week (g/bird)	4th week (g/bird)
T_0	R_1	275.0	599.0	1151.0	1865.0
T_1	R_1	275.7	614.50	1188.0	1870.0
T_2	R_1	266.0	633.4	1164.0	1891.0
T_3	R_1	268.8	638.6	1194.8	1901.0
T_4	R_1	270.0	576.0	1081.0	1873.0
T_0	R_2	275.3	611.6	1129.6	1907.6
T_1	R_2	266.0	617.0	1249.0	1845.0
T_2	R_2	268.0	611.4	1208.0	1892.0
T_3	R_2	274.3	648.3	1165.0	1896.0
T_4	R_2	275.0	622.0	1115.0	1822.0
T_0	R_3	275.3	609.0	1153.0	1915.0
T_1	R_3	263.0	602.0	1147.0	1862.0
T_2	R_3	262.0	625.6	1229	1928.0
T_3	R_3	269.6	631.6	1176.6	1821.0
T_4	R_3	266.0	642.6	1155.0	1886.0

Appendix II: Effect of mint leaf on body weight gain (BWG) (g/bird) of broiler chicken

Treatments	Replications	1st week (g/bird)	2nd week (g/bird)	3rd week (g/bird)	4th week (g/bird)	Total BWG (g/bird)
T_0	R_1	232.0	327.0	556	717.0	1832.0
T_1	R_1	232.7	335.8	570.5	677	1816.0
T_2	R_1	224.0	374.4	536.6	735.0	1870.0
T_3	R_1	225.8	376.8	560.2	712.2	1875.0
T_4	R_1	228.0	351.0	509	753.0	1841.0
T_0	R_2	234.3	341.7	521	783.0	1880.0
T_1	R_2	226.0	348.0	528	686.00	1788.0
T_2	R_2	229.0	353.4	604.6	690.0	1877.0
T_3	R_2	233.3	376.0	519.7	736.0	1865.0
T_4	R_2	232.0	351.0	498	714.0	1795.0
T_0	R_3	232.3	336.7	547	767.0	1883.0
T_1	R_3	224.0	337.0	543	711	1815.0
T_2	R_3	219.0	369.6	609.4	707	1905.0
T_3	R_3	226.6	364.0	551	649.4	1791.0
T_4	R_3	225.0	378.6	515.4	734.0	1852.0

Appendix III: Effect of mint leaf on feed consumption (FC) (g/bird) of broiler chicken

Treatments	Replications	1st week FC (g/bird)	2nd week FC (g/bird)	3rd week FC (g/bird)	4th week FC (g/bird)	Total FC (g/bird)
T_0	R_1	258.0	458.0	635.0	835	2186.0
T_1	R_1	259.0	458.0	627.0	924.0	2268.0
T_2	R_1	257.0	431.0	531.0	893.0	2112.0
T_3	R_1	255.0	473.0	640.0	856.0	2224.0
T_4	R_1	255.0	460.0	637.0	1013.0	2365.0
T_0	R_2	257.0	469.0	616.0	1203.0	2545.0
T_1	R_2	257.0	452.0	601.0	1011.00	2321.0
T_2	R_2	259.0	457.0	623.0	989.0	2328.0
T_3	R_2	256.0	476.0	631.0	861.0	2224.0
T_4	R_2	257.0	465.0	624.0	777.0	2123.0
T_0	R_3	256.0	464.0	639.0	913.0	2272.0
T_1	R_3	257.0	463.0	627.0	951.0	2298.0
T_2	R_3	255.0	466.0	643.0	927.0	2291.0
T_3	R_3	258.0	466.0	634.0	663.0	2021.0
T_4	R_3	256.0	473.0	629.0	941.0	2299.0

Appendix IV: Effect of mint leaf on feed conversion ratio (FCR) of broiler chicken

Treatments	Replications	1st week	2nd week	3rd week	4th week	Final FCR
T_0	R_1	1.11	1.40	1.14	1.16	1.19
T_1	R_1	1.11	1.36	1.09	1.36	1.24
T_2	R_1	1.14	1.15	0.98	1.21	1.12
T_3	R_1	1.12	1.25	1.14	1.20	1.18
T_4	R_1	1.11	1.31	1.25	1.34	1.28
T_0	R_2	1.09	1.37	1.18	1.53	1.35
T_1	R_2	1.13	1.29	1.13	1.47	1.29
T_2	R_2	1.13	1.29	1.03	1.43	1.24
T_3	R_2	1.09	1.26	1.21	1.16	1.19
T_4	R_2	1.10	1.32	1.25	1.05	1.18
T_0	R_3	1.10	1.37	1.16	1.19	1.20
T_1	R_3	1.14	1.37	1.15	1.33	1.26
T_2	R_3	1.16	1.26	1.05	1.31	1.20
T_3	R_3	1.13	1.28	1.15	1.02	1.12
T_4	R_3	1.13	1.24	1.22	1.28	1.24

Appendix V: Effect of mint leaf on dressing percentage of broiler chicken

Treatments	Replications	Average live weight (g/bird)	Eviscerated weight (g/bird)	Dressing %/bird
T_0	R_1	2165	1483	68.45
T_1	R_1	2090	1410	67.46
T_2	R_1	2178	1531	70.29
T_3	R_1	2045	1467	71.73
T_4	R_1	2216	1510	68.14
T_0	R_2	1950	1310	67.18
T_1	R_2	2464	1709	69.36
T_2	R_2	2296	1626	70.81
T_3	R_2	2025	1435	70.86
T_4	R_2	1950	1310	67.18
T_0	R_3	2099	1432	68.22
T_1	R_3	1882	1303	69.23
T_2	R_3	2214	1546	69.83
T_3	R_3	2255	1537	68.16
T_4	R_3	2190	1546	70.59

Appendix VI: Effect of mint leaf on thigh, drumstick, back, breast and wing weight (g/bird) of broiler chicken

Treatments	Replic ations	Thigh (g/bird)	Drumstick (g/bird)	Back (g/bird)	Breast (g/bird)	Wing (g/bird)
T_0	R_1	206	180	234	573.0	118
T_1	R_1	196	170	237	698.0	114
T_2	R_1	205	179	243	624.0	109
T_3	R_1	194	187	248	564.0	99
T_4	R_1	201	190	252	476.0	117
T_0	R_2	201	175	230	598.0	116
T_1	R_2	275	218	301	607.0	127
T_2	R_2	257	205	282	576.0	123
T_3	R_2	172	169	232	585.0	90
T_4	R_2	189	186	245	544.0	112
T_0	R_3	211	199	238	756.0	121
T_1	R_3	176	158	225	490.0	92
T_2	R_3	219	196	251	546.0	115
T_3	R_3	189	179	257	532.0	110
T_4	R_3	199	188	249	672.0	115

Appendix VII: Effect of mint leaf on liver, heart, spleen and gizzard weight (g) of broiler chickens

Treatments	Replications	Liver (g/bird)	Heart (g/bird)	Gizzard (g/bird)	Spleen (g/bird)
T_0	R_1	49	8	22	2
T_1	R_1	53	9	26	4
T_2	R_1	59	9	31	9
T_3	R_1	51	8	21	2
T_4	R_1	46	9	20	4
T_0	R_2	48	8	21	4
T_1	R_2	58	8	29	7
T_2	R_2	63	10	35	5
T_3	R_2	47	7	19	5
T_4	R_2	50	8.7	23	6
T_0	R_3	51	8.5	23	3
T_1	R_3	47	9.4	19	8
T_2	R_3	58.5	9.5	32	6
T_3	R_3	50	7.5	20	7
T_4	R_3	52	9.5	22	5

Appendix VIII: Effect of mint leaf on survivability rate of broiler chicken

Treatments	Replications	Survivability rate (%)
T_0	R_1	100
T_1	R_1	100
T_2	R_1	100
T_3	R_1	100
T_4	R_1	100
T_0	R_2	100
T_1	R_2	100
T_2	R_2	100
T_3	R_2	100
T_4	R_2	93.33
T_0	R_3	100
T_1	R_3	100
T_2	R_3	100
T_3	R_3	100
T_4	R_3	100

Appendix XIII: Effect of mint leaf on immune parameters of broiler chicken

Treatments	Replications	WBC ($X 10^9/L$)	Lymphocyte ($X 10^9/L$)	Granulocyte ($X 10^9/L$)
T_0	R_1	14.8	2.81	11.83
T_1	R_1	5.9	2.36	3.43
T_2	R_1	12.3	2.58	9.47
T_3	R_1	6.4	2.24	4.1
T_4	R_1	10.3	3.29	6.79
T_0	R_2	14.3	2.68	11.87
T_1	R_2	5.7	2.5	3.45
T_2	R_2	11.9	2.59	9.85
T_3	R_2	6.6	2.21	4.0
T_4	R_2	10.1	3.3	6.78
T_0	R_3	14.7	2.59	11.5
T_1	R_3	5.9	2.75	3.78
T_2	R_3	12.5	2.88	9.75
T_3	R_3	6.3	2.22	4.2
T_4	R_3	10.2	3.1	6.77

Appendix X: Effect of mint leaf on microflora (log10 cfu/g) in the caecum of broiler chicken

Treatments	Replications	Colony counts of *E. coli* bacteria (log 10 cfu/g)	Colony counts of Salmonella bacteria (log 10 cfu/g)
T_0	R_1	7.21	7.41
T_1	R_1	6.93	6.85
T_2	R_1	6.62	6.34
T_3	R_1	6.15	5.86
T_4	R_1	4.90	3.69
T_0	R_2	7.21	7.39
T_1	R_2	7.20	7.39
T_2	R_2	6.63	5.30
T_3	R_2	6.18	5.87
T_4	R_2	5.91	4.72
T_0	R_3	7.21	7.38
T_1	R_3	6.94	6.56
T_2	R_3	6.64	6.32
T_3	R_3	6.20	5.87
T_4	R_3	6.91	3.72

Appendix IX: Effect of mint leaf on benefit cost ratio (BCR) of broiler chicken

Treatments	Replications	Total cost (Tk./bird)	Receipt per bird (Tk./bird)	Profit (Tk./ bird)	Benefit Cost Ratio (BCR)
T_0	R_1	189.36	256.08	66.72	1.35
T_1	R_1	203.49	267.0	64.49	1.31
T_2	R_1	200.85	289.52	88.67	1.44
T_3	R_1	226.49	292.32	65.82	1.29
T_4	R_1	236.59	289.32	52.72	1.22
T_0	R_2	214.87	271.68	56.80	1.24
T_1	R_2	211.11	285.68	67.57	1.35
T_2	R_2	207.93	285.84	77.90	1.37
T_3	R_2	218.39	291.60	73.20	1.32
T_4	R_2	208.31	292.28	83.97	1.40
T_0	R_3	186.31	276.48	90.17	1.50
T_1	R_3	198.06	264.43	66.37	1.32
T_2	R_3	193.43	282.80	89.37	1.46
T_3	R_3	197.21	279.64	82.43	1.41
T_4	R_3	206.54	279.88	73.34	1.35

Appendix XI: Broiler house temperature (^0C)

Days	Maximum	Minimum
01	35.20	30.10
02	34.60	29.40
03	33.30	28.80
04	33.70	28.70
05	31.30	28.30
06	33.40	28.20
07	33.60	28.60
08	34.60	28.50
09	34.50	28.10
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	27.30
28	28.40	28.80

Appendix XII: Relative humidity (%)

Days	Maximum	Minimum
01	85	56
02	89	57
03	93	65
04	97	72
05	84	75
06	91	63
07	95	56
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	94	55
23	94	73
24	92	70
25	95	60
26	94	55
27	86	64
28	95	54