EFFECT OF NON-ANTIBIOTIC GROWTH PROMOTER IN BROILER PRODUCTION

A

Thesis

By

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MASTER OF ANIMAL NUTRITION

DEPARTMENT OF ANIMAL NUTRITION, GENETICS AND BREEDING

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CERTIFICATE

This is to certify that the thesis entitled "EFFECT OF NON-ANTIBIOTIC GROWTH PROMOTER IN BROILER PRODUCTION" submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, as partial fulfillment for the requirements of the degree of Master of Science(MS) in Animal Nutrition, embodies the result of a piece of bona fide research work carried out by Nayem Akhter Palash, Registration No.: 19-10117, Semester: Jan-June/2020 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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The Author

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION	FULL WORD
ADG	Average Daily Gain
AGPs	Antibiotic Growth Promoter
ANOVA	Analysis of Variance
Avg	Average
BWG	Body Weight Gain
CEO	Cinnamon Essential Oil
CFU	Colony Forming Unit
Cm^2	Square Centimeter
CP	Crude Protein
DOC	Day Old Chick
DP	Dressing Percentage
e.g.	For Example
EO	Essential Oil
et al	And others/Associates
EU	European Union
FAO	Food and Agricultural Organization
FC	Feed Consumption
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
FI	Feed Intake
g	Gram
GIT	Gastro Intestinal Tract
i.e.	That is
IB	Infectious Bronchitis
Kcal	Kilo Calorie
Kg	Kilogram
L	Liter
M.S.	Master of Science
ME	Metabolizable Energy
ml	Milliliter

LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION	FULL WORD
mm	Millimeter
MT	Metric ton
NAGP	Non- Antibiotic Growth Promoter
ND	Newcastle Disease
No	Number
NS	Non-Significance
OEO	Oregano Essential Oil
RH	Relative Humidity
SAU	Sher-e-Bangla Agricultural University
SE	Statistical Error
SPSS	Statistical Package for Social Science
TM	Trade Mark
Viz	Such as
Vs.	Versus
WBWG	Weekly Body Weight Gain
WHO	World Health Organization
Wks	Weeks

LIST OF SYMBOLS

SYMBOLS	FULL MEANING
^{0}C	Degree Celsius
^{0}F	Degree Fahrenheit
@	At the rate of
:	Ratio
<	Less than
>	Greater than
*	5% level of significance
&	And
/	Per
±	Plus-minus
%	Percentage

EFFECT OF NON-ANTIBIOTIC GROWTH PROMOTER IN BROILER PRODUCTION

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ABSTRACT

This study was conducted to evaluate the effect of non-antibiotic growth promoters in broiler performance. Day-old of "Indian River-Lohmann Meat" broiler chicks (n=150) were randomly allocated into five treatments. Each dietary treatment consisted of 3 replicates having 10 broilers in each of the replication. The dietary treatment without growth promoter considered as control (T_0) and the other four treatments were T_1 (100g multi secondary plant compounds & essential oils/ton feed), T_2 (500g lysozyme enzyme/ton feed), T_3 (500g single secondary plant compound & essential oil/ton feed) and T_4 (500g antibiotic/ton feed). During the experimental periods of 4 weeks, feed intake, body weight gain, feed conversion ratio (FCR), flock uniformity values were calculated. Growth performance parameters were significantly ($P<0.05$) affected by experimental diets. Birds fed 100g multi secondary plant compounds & essential oils/ton feed (T_1) gained superior body weights (1710.33±15.30g) compared to T_0-control (1584.67±12.91g), T_4- antibiotic (1593.33±14.53g) and other dietary treatments. The mean body weight gains (g) at the 1st, 3rd and 4th week of different treatment groups were significantly higher ($P<0.05$) than the control group. The groups fed diets containing 500g single secondary plant compound & essential oil/ton feed (T_3) and 100g multi secondary plant compounds & essential oils/ton feed (T_1) had lower (best) FCR (1.35±0.00) & (1.36±0.00) respectively compared to T_0 control (1.43±0.00) and T_4 antibiotic (1.40±0.01). The inclusion of different dietary treatments had a significant ($P<0.05$) difference in flock uniformity. However, the control (T_0) group had the superior uniformity (73.33±3.66%) and the group treated with antibiotic had the lowest uniformity (56.67±3.33%). It is concluded that non-antibiotic growth promoter can be included in the broiler diet instead of antibiotic growth promoter for better performance.

CHAPTER I

INTRODUCTION

1.1 General Background

Poultry is an important sub-sector of agriculture, which contributes significantly to the economy of Bangladesh. It is one of the promising and emerging agribusiness which started practically during 1980s. Now-a-days broiler industries have become a rapidly developing enterprise among all other sectors of poultry production and a large number of farms are being established in whole country. This profitable business is responsible for employment of rural masses particularly small and marginal farmers. According to recent statistics, total poultry population in our country is 347.03 million from where about 289.28 million chicken and 57.75 million duck in number (DLS, 2018-19).

During 1970-80, the poultry population growth rate was 0.7% which increased to 4% per year during 1990-2005 (Begum, 2008). According to Department of Livestock Service (DLS), 81425 poultry farms got registered until August 2019. Of them, 54411 were broiler farms, 18954 layer farms, 7829 duck farms and 231 were other types of farms.

In Bangladesh, the per capita requirements of meat and eggs are 120 g/day and 104 eggs/ year, respectively however the average per capita availability of meat and eggs are 124.99 g/day and 103.89 eggs/ year (DLS, 2018-19). The demand of meat consumption per head able to fulfill the requirement but egg consumption still lack behind. Poultry can play a pivotal role to retain in meat production level and to achieve the expected egg production.

Chicken meat is an important source of dietary protein, and the industry has developed high grade because of intensive farming techniques, comprehensive and balanced feeding, automation equipment, and other new technologies. Total output from poultry is coming from broiler sector because of its commercialization and also rapid return to the farmers. However, diseases in production are problematic especially with the development of antibiotic-resistant bacteria. Lowered immunity arises the chance of disease occurrence into the farm. Therefore, exploring safe, green and efficient additive that increase immunity in broilers has become a research priority.

1.2 State of the Problems

Poultry are the cheapest source of animal protein, contributing significantly to supply the growing demand for animal food products around the world (Farrell, 2013). The biggest challenge of commercial poultry production is the availability of quality feed on sustainable basis at stable prices.

In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1991) and imbalance of normal microflora (Andremont, 2000).

Antibiotic resistance (AR) which is defined as the ability of an organism to resist the killing effects of an antibiotic to which it was normally susceptible (Madigan *et al*., 2014). This microbial resistance is not a new phenomenon since all microorganisms have an inherent capacity to resist some antibiotics (Hugo & Russel, 1998). However, the rapid surge in the development and spread of AR is the main cause for concern (Marshall & Levy, 2011). In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a contributing factor to the overall burden of AR has emerged (Laxminarayan *et al*., 2015). The extent of usage is expected to increase markedly over coming years due to intensification of farming practices in most of the developing countries (Mathew *et al.,* 2009). The main reasons for the use of antibiotics in food-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals (Castanon 2007).

Antibiotic usage as growth promoters leaves residues in poultry products (meat and eggs) which have deleterious effect on humans as the consumer and also shown to cause bacteria resistance (Donoghue, 2003). Consequently, this steered to the prohibition of sub-therapeutic use of antibiotics as growth promoters.

1.3 Justification of the study

The phytobiotic bioactive substances have some bioactivities such as increase amylase and protease activity, affect the production and activity of digestive enzymes (Jang *et al.*, 2007), improve the poultry growth performance by promoting the proliferation and growth of absorptive cells in the gut so that deeper crypt and higher villus are obtained (Jamroz *et al*., 2006). Phytobiotics are generally known as one of the potential alternative feed additives to AGP in the poultry production process (Windisch *et al.*, 2008). The advantages of phytobiotics or other plant-derived products compared to antibiotics or inorganic chemicals compounds are safer, no residue, not for medical or veterinary purposes and have a favorable effect on livestock production (Hashemi *et al.*, 2008). Herbs, spices, essential oils, and oleoresins are the classification of several kinds of common phytobiotic compounds based on origin and processing (Windisch *et al*., 2008).

Studies have been reported about the antimicrobial activities of several essential oils (Burt, 2004), that make plant origin essential oils potentially to be induced in poultry nutrition. Essential oils can promote the intestinal functions by stimulating the bile secretion, digestive enzymes, and mucus (Platel and Srinivasan, 2004). Essential oils are potentially used in poultry nutrition mainly because of their antimicrobial and antioxidant activity. Those activities can modulate the gastrointestinal ecosystem, stimulate the digestion process and extend to animal metabolism (Lee *et al.*, 2004).

If we implement this study successfully we hope every people in our country will able to meet the requirement of broiler meat by increasing its production.

1.4 Objectives

From the above consideration, the present study was under taken to determine the efficacy of different non antibiotic growth promoter with the following specific objectives:

- ✓ To find out the effective natural alternative of antibiotic growth promoters in broiler nutrition
- ✓ To mitigate the risk of antibiotic residue in broiler meat
- ✓ To evaluate the production performance of broiler.

CHAPTER II

REVIEW OF LITERATURE

Review of literature is advantageous and important for performing any type of survey or experiment which are linked to the proposed study for the amelioration of research work. During the last decade, different studies have been attempted to find nutrition-based health approaches and natural feed additives to improve performance and immunity of poultry, and strongly recommended the use of phytogenic additives, enzymes, probiotics, prebiotics, or organic acids. As a result of residual side effects of antibiotics on human health among these feed supplement phytogenic additives individually have drawn much great attention. Nowadays, there has been growing interest among researchers and the feed industry to prepare a phytogenic additives feed supplement at a low cost that have beneficial effects on broiler growth performance, health status, and product quality of poultry. The literature reviewed here have been limited to these which are considered compatible and related to the objectives of the present study.

2.1 Antibiotics impacts

2.1.1 Impact on chicken growth, digestive tract and immune systems

In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1991) and imbalance of normal microflora (Andremont, 2000).

Animals including poultry are vulnerable to potentially pathogenic microorganism such as *Escherichia coli*, *salmonella ssp.* and *Clostridium perfringens*. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al.*, 2000). According to Truscott and Al-sheikhly (1977), this leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies.

Dono (2014), entitled that the commercially available antibiotics have been used in poultry feed to provide supplementary support to fight against harmful exogenous pathogens. These antibiotics helps to overcome with the morbidity and mortality issues with poultry farming, however can affect the public health by developing drug resistant micro flora. Because of giving concern over the transmission and proliferation of resistant bacteria via the food chain, the European Union in 2006 banned antibiotic growth promoters to be used as additives in poultry (Castanon 2007).

The poultry industry uses antibiotics to improve meat production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth (Barcelo, 2007; Chattopadhyay 2014; Engberg *et a*l., 2000; Harms *et al*., 1986; Emami *et al.,* 2012; Rosen, 1996) and protect the health of birds by modifying the immune status of broiler chickens (Lee *et al*., 2012). This is mainly due to the control of gastrointestinal infections and microbiota modification in the intestine (Dibner and Richards, 2005; Singh *et al.,* 2013; Torok *et al.,* 2011). In addition, the mechanism remains unclear, but antibiotics are likely to act by remodeling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005).

Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of *Lactobacillus* species in broiler duodenal loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota (Dumonceaux *et al.,* 2006). In addition, populations of *Lactobacillus* spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than those in chickens receiving no tylosin (Lin *et al.,* 2013). This decrease in *Lactobacilli* species following the use of antibiotics has been demonstrated in other studies (Danzeisen *et al*., 2011; Lee *et al.*, 2012; Zhou *et al.,* 2007). For reminder, *Lactobacillus* are the primary commensal bacteria for the production of bile hydrolase salt. The decrease in the *Lactobacillus* population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain (Lin *et al.,* 2013).

Lee *et al.* (2012), reported that a change in the intestinal microbiota of chickens can influence their immunity and their health. However, changes in the intestinal microbiota of chickens can be influenced by several factors. These factors include housing

conditions, exposure to pathogens, diet composition and the presence of antibiotics in feed.

2.1.2 Impact on bacterial resistance and public health concern

Antibiotic resistance (AR) which is defined as the ability of an organism to resist the killing effects of an antibiotic to which it was normally susceptible (Madigan *et al*., 2014). This microbial resistance is not a new phenomenon since all microorganisms have an inherent capacity to resist some antibiotics (Hugo & Russel, 1998). However, the rapid surge in the development and spread of AR is the main cause for concern (Marshall & Levy, 2011). In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a contributing factor to the overall burden of AR has emerged (Laxminarayan *et al*., 2015). The extent of usage is expected to increase markedly over coming years due to intensification of farming practices in most of the developing countries (Mathew *et al*., 2009). The main reasons for the use of antibiotics in food-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals (Castanon 2007).

Poultry is one of the most widespread food industries worldwide. Chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (Landers *et al.,* 2012). A large diversity of antimicrobials, are used to raise poultry in most countries (Sahoo *et al.,* 2010; Boamah *et al*., 2016, A large number of such antimicrobials are considered to be essential in human medicine (Aalipour *et al.,* 2013; World Health Statistics, 2017). The indiscriminate use of such essential antimicrobials in animal production is likely to accelerate the development of AR in pathogens, as well as in commensal organisms. This would result in treatment failures, economic losses and could act as source of gene pool for transmission to humans. In addition, there are also human health concerns about the presence of antimicrobial residues in meat (Darwish *et al.,* 2013; Goetting *et al*., 2011) eggs (Addo *et al*., 2011) and other animal products (Mehdizadeh *et al*., 2010; Laxminarayan *et al*., 2013)

Generally, when an antibiotic is used in any setting, it eliminates the susceptible bacterial strains leaving behind those with traits that can resist the drug. These resistant bacteria then multiply and become the dominating population and as such, are able to transfer (both horizontally and vertically) the genes responsible for their resistance to

other bacteria (Madigan *et al*., 2014; Van & Stobberingh, 2000). Resistant bacteria can be transferred from poultry products to humans via consuming or handling meat contaminated with pathogens (Leverstein *et al.,* 2011). Once these pathogens are in the human system, they could colonize the intestines and the resistant genes could be shared or transferred to the endogenous intestinal flora, jeopardizing future treatments of infections caused by such organisms (Laxminarayan *et al*., 2015; Jakobsen *et al*., 2010; de Leenar *et al*., 2005, Ogle, 2013).

2.2 Alternatives to antibiotic as a growth promoter

Antibiotic growth promoters (AGP) are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of AGP resulted in common problems such as development of drug-resistant bacteria, drug residues in the body of birds, and imbalance of normal microflora. As a consequence, it has become necessary to develop alternatives using either beneficial microorganisms or nondigestible ingredients that enhance growth (Awad *et al.,* 2009). Consumers' pressure and worries towards harmful effects of antibiotic use and the ban of antibiotics in EU have prompted researchers to think about alternatives to antibiotics (Diarra and Malouin, 2014).

There is increasing interest in finding alternatives to antibiotics for poultry production. Because of the general problem of increased resistance of bacteria and the decreasing acceptance of the consumers for antibacterial growth promoters (AGPs), different substances, referred as natural growth promoters (NGPs), have been identified as effective and safe alternatives to AGPs. At present, there is a large number of NGPs available in the market, including phytogenic essential oils & enzymes.

The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. There are indeed a number of nontherapeutic alternatives that can substitute antibiotics use. Among these, the most popular are phytogenic feed additives, phytocides, nanoparticles and essential oils, enzymes, organic acids, probiotics, prebiotics, immune stimulants, bacteriocins bacteriophages.

2.2.1 Phytogenic feed additives

Phytogenic feed additives (PFA) derived from plants, herbs and spices are used to improve animal performance. They have been very successful because of their positive effects on growth, improved immune system and reduced stress response. Recent results showed that PFA were good alternatives to antibiotics (Frankic *et al.*, 2009; Ghasemi *et al.*, 2014; Toghyani *et al.*, 2011) and promoted broiler chicken growth (Ghasemi *et al*., 2014; Lei *et al.*, 2015; Toghyani *et al.*, 2011). For example, inclusion of cinnamon 2 g/kg of the diet had a positive effect on growth performance at 28 days of age (974 vs. 850 g) and at 42 days of age (2,111 vs. 1,931 g) (Toghyani_*et al.*, 2011). Also, inclusion of *Lippia javanica* at 5 g/kg in broiler feed had beneficial effects on ADG in the grower period (67 vs. 30 g), slaughter weight (2,213 vs. 1,967 g) and fatty acid profiles of broiler chicken meat (Mpofu *et al*., 2016). According to Mpofu *et al.* (2016), phytogenic extracts in *L. javanica* leaf meal can stimulate glycolysis and increase utilization of energy production and ultimately growth. In addition, a mixture of garlic (5 g/kg) and black pepper (1 g/kg) powder had positive effects on weight gain and broiler chicken consumption index (Kirubakaran *et al.*, 2016).

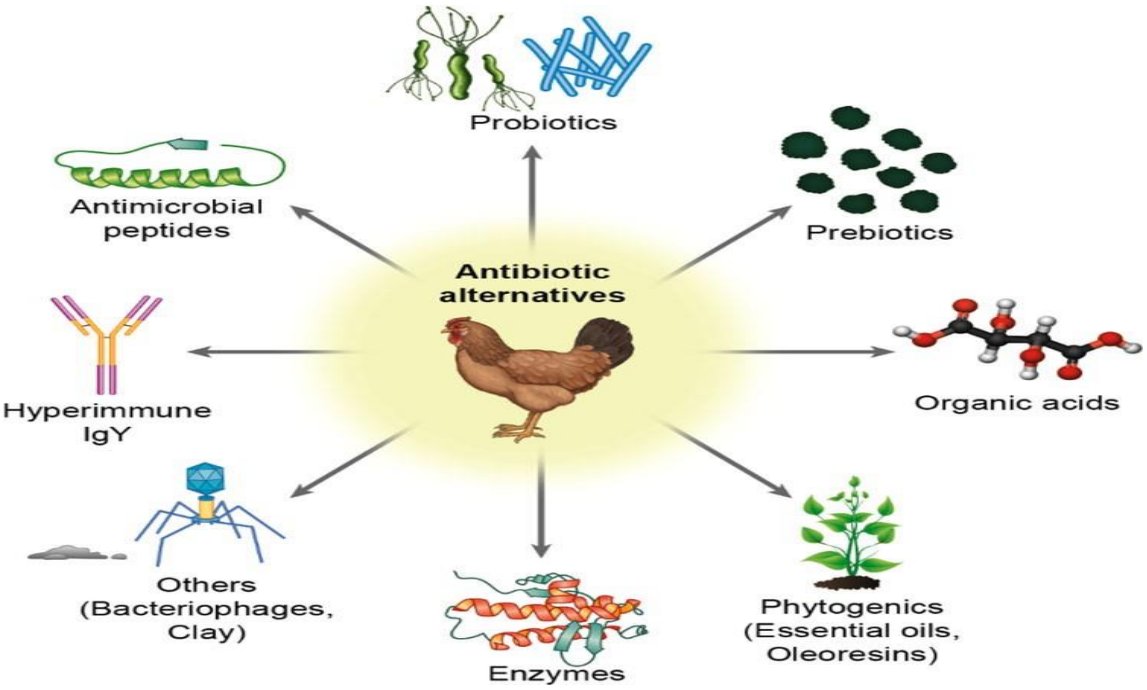
2.2.2 Amino acids and enzymes

According to Cowieson *et al.*, (2006), the feed additive enzymes are produced through fungi and bacteria fermentations. They are used to maximize feed conversion. Enzymes facilitate components degradation such as proteins, phytates and glucans. For example, endo-b-1-4-xylanases and b-1-3, 1-4-glucanases have been used in wheat and barley diets of broilers to improve their digestion. Also, phytase enzyme can increase villus width and decrease crypt depth which can improve ADG (Mohammadagheri *et al.*, 2016). Lysins are bacteriophage endolysins representing an innovative alternative therapeutic option of antibacterial. Lysins are phage-encoded peptidoglycan hydrolases which bring about the bacterial cell lysis when applied exogenously to Gram-positive bacteria (Fenton *et al.*, 2010; Rios *et al.*, 2016). Volozhantsev *et al.* (2011), demonstrated that administration of a combination of a group of lysins containing peptidases, amidases and lysozymes produces an antimicrobial effect against *C. perfringens* in poultry. For example, Ply3626 lysine is an enzyme which has been shown lytic activity against several strains of *C. perfringens*, which is an important cause of food poisoning and leads to economic losses in poultry production (Fenton *et al.*, 2010).

2.2.3 Organic acids

The antimicrobial action of organic acids is due to the fact that non-dissociated acids can diffuse through lipophilic bacteria membrane and disrupt enzymatic reactions and transport system (Cherrington *et al*., 1991). In addition, some studies (Hassan *et al*., 2010; Nava *et al*., 2009) showed that organic acids addition to broiler feed promotes growth, feed conversion rate and feed utilization. Adding organic acids in drinking water gives young chicks a protective efficacy against *Campylobacter* infection (Chaveerach *et a*l., 2004). These acids also have a protective action against *E. coli* (Izat *et al*., 1990). Thus, it has been shown (Mohammadagheri *et al.*, 2016) that supplementation with citric acid (2%) can improve cell proliferation epithelial and villi height of gastrointestinal tract.

Organic acid blend, formic and propionic acid supplementation (0.0525% in drinking water) generates more homogeneous and distinct populations in the intestinal microbiota and increases the colonization of *Lactobacillus* spp. in ileum of chicken (Nava *et al.*, 2009). These changes in the intestinal microbiota and the increase in *Lactobacillus* populations show that organic acid can be used as an alternative to antibiotics to reduce pathogenic bacteria in the gastrointestinal tract (Nava *et al.*, 2009).



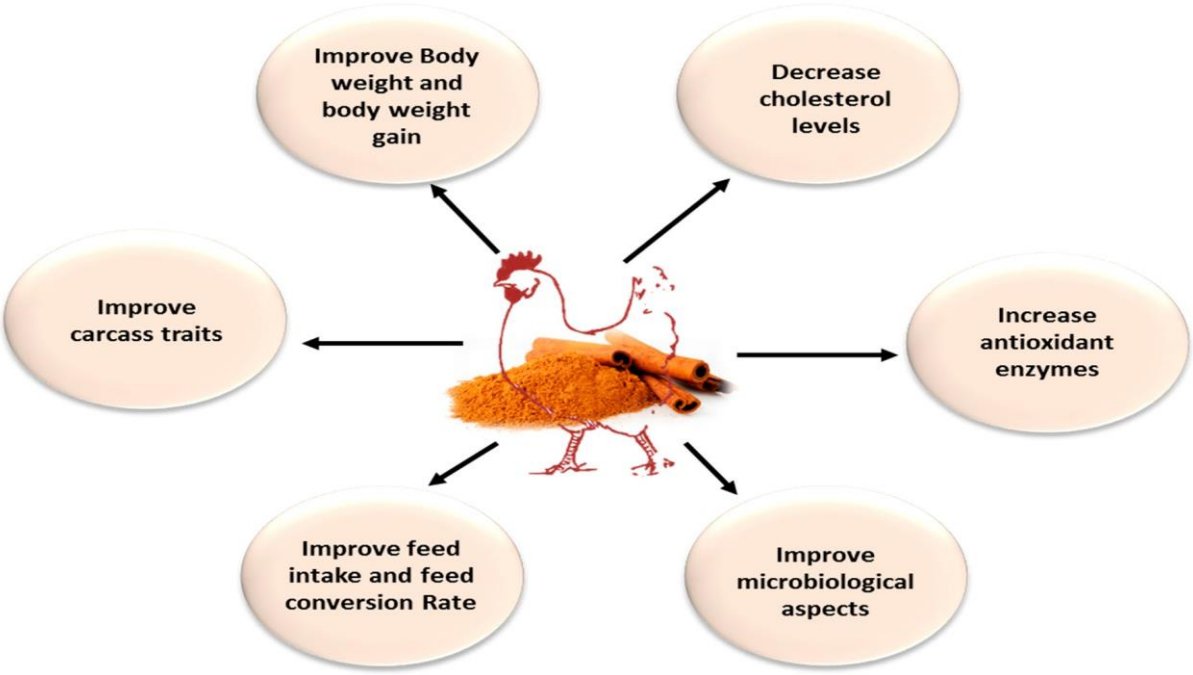
(Source: Gadde *et al*., 2017)

Figure 1. Various classes of antibiotic alternatives are available for use in poultry production.

2.3 Phytogenic feed additives and their essential oils in poultry production

Several plant origin essential oils have been known to prevent the emergence of enteric diseases and pathogens in poultry (Micciche *et al.,* 2019). Feed conversion significantly increased the chicken growth with essential oils blend supplementation in feed consisted of anise, citrus, sage, oregano, and bay leaf due to the high nutrient's availability by modulating in the intestinal ecosystem (Cabuk *et al*., 2006). According to Peng *et al.*, (2016), the inclusion of 300 and 600 mg/kg oregano essential oil (*Origanum genus*) in broiler chicken feed increased the birds average daily gain (ADG) which is to be due to an increase in both villus height and crypt depth of the jejunum. Recent studies (Khattak *et al*., 2014; Pirgozliev *et al.,* 2015; Peng *et al*., 2016) reported that several essential oils have good potential as an alternative to AGP for improving the poultry productivity. Essential oils are also possible to play a preventive and curative of necrotic enteritis diseases in poultry production (Jerzsele *et al*., 2012).

The decrease of the pathogenic bacteria affects positively to increase the nutrient availability for animal utilization due to decrease the nutrient competition and prevent several intestinal diseases (Yitbarek, 2015).



(Source: El-Hack *et al*., 2020)

Figure 2. Advantages of dietary supplementation of cinnamon oil in poultry diet.

Studies have been reported about the antimicrobial activities of several essential oils (Burt, 2004), that make plant origin essential oils potentially to be induced in poultry

nutrition. Essential oils can promote the intestinal functions by stimulating the bile secretion, digestive enzymes, and mucus (Platel and Srinivasan, 2004). Essential oils are potentially used in poultry nutrition mainly because of their antimicrobial and antioxidant activity. Those activities can modulate the gastrointestinal ecosystem, stimulate the digestion process and extend to animal metabolism (Lee *et al*., 2004). Furthermore, by modulating the gastrointestinal ecosystem or having antimicrobial activities, essential oil affects the digestibility of starch, protein (Hernández *et al*., 2004) and fat (Lee *et al*., 2004). The phytobiotics especially essential oils have been known to have a positive effect on the performance and feed intake of poultry by improving flavor and palatability of feed (Grashorn, 2010).

Dietary inclusion of plant origin trans-cinnamaldehyde and eugenol are effectively reduced the pathogenic bacteria (*Salmonella enteritidis*) in 20-d-old broiler chickens (Kollanoor-Johny *et al*., 2012). Moreover, Mitsch *et al*. (2004) reported that dietary inclusion a mixture of several essential oils such as curcumin, carvacrol, piperin, thymol, and eugenol has a positive effect at reducing the colonization and proliferation of *Clostridium perfringens* in the chicken gastrointestinal tract. According to Windisch *et al*., (2008), essential oils have potential activity against *C. perfringens* and *E. coli*. Herbs origin essential oils can be used to decrease *E. coli* (Jang *et al*., 2007) and *Campylobacter* spp. (Kelly *et al.*, 2017) that inhabits the digesta of broiler chickens. Comparing to the inclusion of antibiotic-containing ciprofloxacin, *Oreganum aetheroleum* essential oil can help the chicken against *E. coli* infections by enhancing the cell-mediated and humoral immune responses, thus becoming more effective for the treatment of *E. coli* infection in the broiler chicken (El-Ghany and Ismail, 2013). Oregano and thyme essential oil effectively counter a wide range of pathogenic bacteria such as *Salmonella* strains that inhabited in the gastrointestinal tract of the chicken (Koščová *et al.*, 2006). Some essential oils such as thyme, oregano, rosemary, clove, and cinnamon are used to protect the intestinal wall from damage due to the effects of coccidial multiplication and hence can be used as growth promoters (Hashemi *et al*., 2008). In addition, a positive effect on the activity of trypsin and amylase enzyme has been shown by providing essential oils to chickens (Jamroz *et al*., 2005). One gram per kilogram of thyme essential oil supplementation gives a significant increment in body weight (BW) gain of broiler chickens. Different result is achieved when 10 g/kg of the thyme herb was included in the diet. This observation noted that thyme essential oil has

a better result compared to the herb (Cross *et al*., 2007). Compared to the control group, the mixture of essential oils consist of oregano, anise, and clove shows significantly increment by approximately 16% for the BW gain and after 5 weeks of trial and the inclusion of 200 mg/kg from the mixture of the essential oil gave the best result (Ertas *et al*., 2005).

The phytobiotic bioactive substances have some bioactivities such as increase amylase and protease activity, affect the production and activity of digestive enzymes (Jang *et al*., 2007), improve the poultry growth performance by promoting the proliferation and growth of absorptive cells in the gut so that deeper crypt and higher villus are obtained (Jamroz *et al*., 2006). Phytobiotics are generally known as one of the potential alternative feed additives to AGP in the poultry production process (Windisch *et al*., 2008). The advantages of phytobiotics or other plant-derived products compared to antibiotics or inorganic chemicals compounds are safer, no residue, not for medical or veterinary purposes and have a favorable effect on livestock production (Hashemi *et al*., 2008). Herbs, spices, essential oils, and oleoresins are the classification of several kinds of common phytobiotic compounds based on origin and processing (Windisch *et al*., 2008).

Essential oil is defined as natural, volatile and aromatic substances, oily liquids which can be extracted from several parts of the plants (Bakkali *et al*., 2008). In addition, essential oils are known as plants secondary metabolites which highly contain a lot of isoprenoid compounds (Brewer, 2011).

The hydrophobicity of essential oils or their components become an important characteristic that makes essential oils able to penetrate the lipid-containing bacterial cell membrane and provide the antimicrobial activity (Smith-Palmer *et al*., 2004). In addition, the exposure of essential oil increases the membrane permeability, leading to cell lysis due to leakage of the cell contents (Carson *et al*., 2002). Essential oil constituents have high hydrophobicity properties because of their short carbon chain extension, allowing for tight interactions with lipid cell membranes. The interaction of volatile terpene groups with lipid-containing bacterial cell membrane provides inhibitory activity on cell function and its lipophilic properties which lead to the death of pathogenic bacteria. That inhibitory activity can affect and disrupt the fluidity of cell membrane and mitochondrial membranes (Calo *et al*., 2015). Moreover, crossing the cell membrane and bind to specific proteins also can be done by the oil components as

another inhibitory activity (São Pedro *et al*., 2013). The antimicrobial activity of essential oil is attributable to more than one specific mechanism because of plant-derived compounds mostly contain several chemical groups (Carson *et al*., 2002; Burt, 2004; Smith-Palmer *et al*., 2004). As an illustration, essential oils are generally contained up to more than 100 single constituents (Bilia *et al*., 2014; Calo *et al*., 2015). In general, the chemical contents of essential oil are terpene compounds (mono-, sesqui- and diterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfide which can be divided into terpene compounds and aroma compounds (Bakkali *et al*., 2008). Hence, using essential oils as an antimicrobial agent is hypothesized to reduce the potential for bacteria to develop resistance and spread it out (Smith-Palmer *et al*., 2004).

The limiting factor of essential oils as an alternative to antibiotic is due to most of the constituents have high volatility, thermolabile, photolabile, and less stable (Yitbarek, 2015). The characteristic of essential oil is easy to oxidize when directly exposed to heat, air, light, and humidity because of the high volatility of their constituents (Bilia *et al*., 2014).

In addition, essential oils have become very susceptible to oxidation by light or heat due to their main constituents are unsaturated carbon chains. The oxidation of essential oils produces terpenes that have been known to have high allergenic activity, and other plant metabolites especially oxidized sesquiterpenes with lacone rings and terpenoids (Vigan, 2010).

Therefore, the characteristics of essential oil such as high volatility, unstable substances, and poorly water-soluble limit their possible routes of administration. The consequences of the low solubility of essential oils in biological fluids inhibit their absorption and lead to the very low bioavailability (São Pedro *et al*., 2013; Natrajan *et al*., 2015). Thus, a solution with a new approach is needed to overcome the limiting factor for the application of essential oils to improve their bioavailability.

Table 1. Research on nano emulsions essential oils and their antimicrobial activity

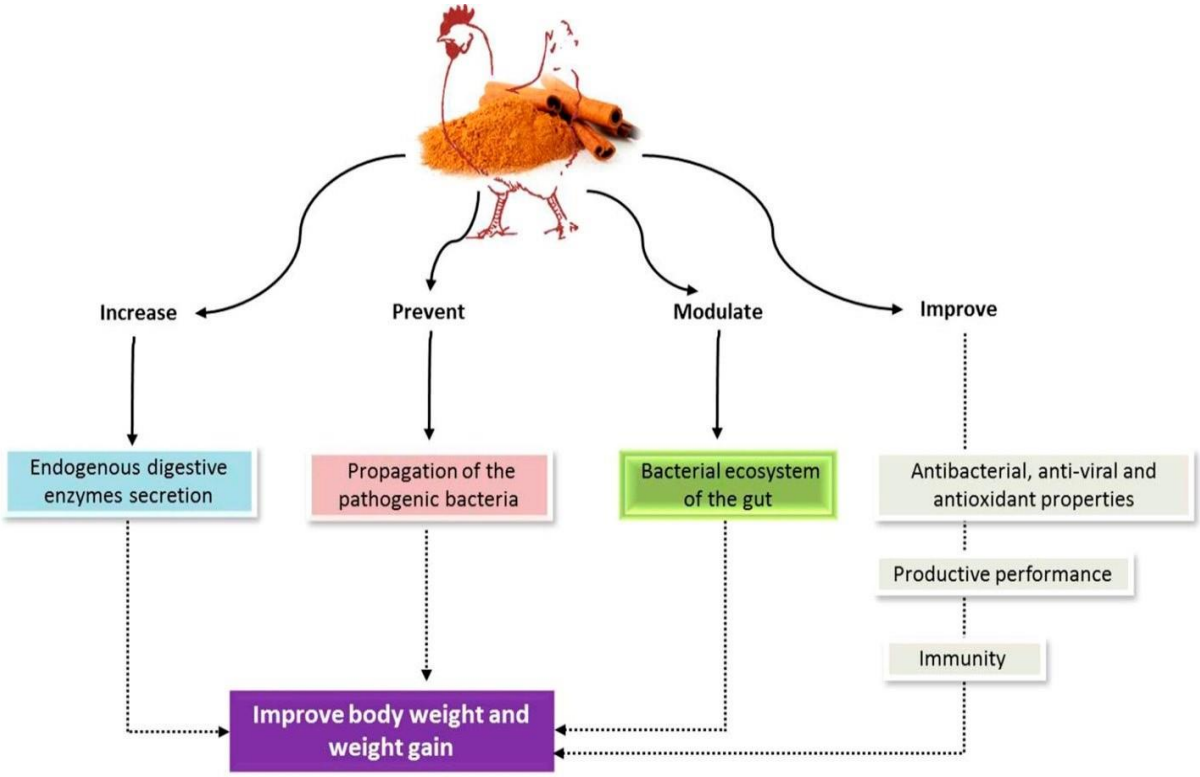
Oil	Targeted	Reference
Cinnamon oil	*Bacillus cereus*	(Ghosh *et al*. 2013)
Oregano, thyme, lemongrass or mandarin oils	*Escherichia coli* and *Listeria innocua*	(Guerra-Rosas *et al.* 2017)
Cinnamon bark oil	*Salmonella enteritidis*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*	(Hilbig *et al*. 2016)
Thymus	*Escherichia coli*	(Moghimi *et al.* 2016)
Oregano oil	*Staphylococcus aureus* ATCC 25923 *and E. coli* ATCC 25922	(Moraes-Lovison *et al.* 2017)
Eucalyptus oil	*Staphylococcus aureus*	(Sugumar *et al*. 2014)
Blended cloves/cinnamon oil	*Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and *Staphylococcus aureus*	(Zhang *et al*. 2017)

2.3.1 Effects on growth performance

2.3.1.1 Body weight and body weight gain

Many trials were carried out to assess the impacts of dietary supplementation with cinnamon (powder and oil) and CEO components as growth promoter agents. Al-Kassie (2009), showed that broilers feeding on diet additives with CEO had a body weight gain significantly higher than the control (without CEO). In addition, Sarica *et al*. (2009), illustrated that supplementation of CEO in quail diets had the same effects of antibiotic, probiotic, enzymes, mannanoligosaccharide, oregano essential oil (OEO), OEO plus CEO on body weight gain (BWG) of quail during the growth period of 0–35 days. Toghyani *et al.* (2011), displayed that the addition of cinnamon to broiler diets by about 2 g/kg significantly improved the final body weight of the chicks. On the other hand, Mehdipour *et al*. (2013), indicated that dietary supplementation with cinnamon oil (200 mg/kg) significantly increased the BWG of quails at 21–35 days old as a comparison with cinnamon powder, antibiotic (virginiamycin) and symbiotic. Shirzadegan (2014), observed a significant increase in the final body weight of broiler chickens feeding on diets supplemented with different concentrations of cinnamon powder (especially at a level of 0.5%). Moreover, Devi *et al*. (2018), showed that supplementation with a combination of CEO and ajwain essential oil in broiler diets significantly increased body weight at age 42 days. However, Lee *et al*. (2003), stated that cinnamaldehyde supplementation in feed had no significant effect on female broilers' weight gain, but water intake was decreased significantly. Muhl and Liebert (2007), reported that the performance of broiler chicks was not significantly affected as a result of using commercial phytogenic feed additives that contain carvacol, capsicum oleoresin, cinnamaldehyde, chelerythrin and alkaloids sanguinarin. Koochaksaraie *et al*. (2011), showed that the inclusion of cinnamon (0.5 to 2 g/kg diet) had no influence on the growth in broiler chickens. Moreover, Tonbak and Çiftçi (2012), concluded that supplementation of cinnamon oil (*Cinnamomum zeylanicum* L.) in the diets at concentrations of 250 and 500 mg/kg diet had no significant influence on the live weight and live weight gain of the quail. Symeon *et al*. (2014) added cinnamon oil to broiler diets at 0.5 or 1.0 mL per kg and summarized that cinnamon oil supplementation did not significantly affect broiler body weight at marketing age. In alternative strategies, emphasis was placed on preventing the spread of pathogenic bacteria and modifying the bacterial ecosystem of the intestine to improve the overall health and immune status,

thereby improving productive performance. The enhancing effect of EO on the feed efficiency and growth performance was due to improving the immune system, regulating the gut micro flora, increasing endogenous digestive enzymes secretion and eliciting antioxidant, antibacterial and anti-viral properties (Kishawy *et al.*, 2019; Saeed *et al.*, 2018; Mahgoub *et al*., 2019). The effect of cinnamon oil on body weight and weight gain is illustrated in Figure 3.



(Source: El-Hack *et al.*, 2020)

Figure 3. The effect of cinnamon oil on body weight and weight gain.

2.3.1.2 Feed intake and feed conversion rate

Studies related to the impact of cinnamon oil on feed intake (FI) and feed conversion rate (FCR) were contradictory, while many researchers (Mehdipour *et al*., 2013; ŞİMŞEK *et al.*, 2015; Torki *et al.*, 2015) concluded that cinnamon oil has beneficial effects on FI and FCR. Al-Kassie (2009), clarified that the chicks fed on diets containing 200 ppm EO resulting from a combination of thyme and cinnamon achieved significant increases in feed efficiency and FI compared to the control. Similarly, Ciftci *et al.* (2009), suggested that broilers receiving a diet supplemented with 500 ppm cinnamon oil showed the best feed conversion efficiency in comparison with avilamycin (antibiotic) groups and the control. In addition, Mehdipour *et al.* (2013), found that

quails' diet supplemented with cinnamon oil (200 mg/kg) significantly improved FCR compared to the control group (0–35 days), while FI was not affected. Moreover, ŞİMŞEK *et al.* (2015), reported that the addition of cinnamon oil to the diets significantly reduced FCR. Torki *et al*. (2015), indicated that FCR was significantly lessened in laying hens housed under cold stress conditions (8.8 ± 3 ˚C) and fed on the diets including Zn and CEO (combined or single) compared with those fed on the control diet. In addition, Mehdipour and Afsharmanesh (2018), showed that the supplementation of cinnamon oil or virginiamycin to quail diets at a level of 200 mg/kg had the same significant beneficial effects on FCR compared to the control group at day 35; however, feed intake did not differ among the groups. In another study, Pathak *et al*. (2017), demonstrated that enramycin supplementation (125 mg/kg feed), or a combination of calcium formate and cinnamaldehyde (500 mg/kg diet), to broilers orally challenged with *E. coli* (10^8 bacteria/bird) on day 14, significantly improved FCR compared with the control group, and concluded that antibiotics can be replaced with EO and organic acid. Contradictory studies were reported by Symeon *et al.* (2014); Sarica *et al*. (2009) and Lee *et al.* (2003), showing that cinnamon oil or powder did not significantly affect the FI or FCR of birds. Lee *et al.* (2003), pointed out that cinnamaldehyde supplementation in feed had no significant influences on the FI and FCR of female broilers, however, water intake was significantly decreased. In addition, Hernandez *et al.* (2004), indicated that broilers feeding on diets treated with 200 ppm essential oil extract (EOE) from cinnamon, pepper and oregano had no significant alterations in FI or FCR at 14 and 21 days of age. Moreover, Tonbak and Çiftçi (2012), reported that supplementation of cinnamon oil (*Cinnamomum zeylanicum* L.) to the diets at concentrations of 250 and 500 mg/kg had no significant impacts on FCR of quail.

Table 2. Some effects of cinnamon essential oil on poultry:

Level	Bird Type	Age (days)	Results	References
200 mg/kg	Broiler chicks	42	Improved body weight gain (BWG), feed conversion rate (FCR) and dressing% Decreased Abdominal fat% Decreased Cholesterol Improved blood haematology	Al-Kassie, 2009
500 mg/kg	Broiler chicks	38	Increased glutathione peroxidase activity in the kidney and liver Reduced plasma malondialdehyde level and ALT activity Increased the phagocytic activity	Faix *et al.*, 2009
500 mg/kg	Broiler chicks	35	Improved BWG and FCR No effects on carcass traits	Ciftci *et al.*, 2009
1g/kg	Japanese quail	35	Decreased lipid profile	Sarica *et al.*, 2009
1g/kg	Broiler chicks	35	Increased concentration of glutathione peroxidase and catalase Decreased breast and thigh meat	Ciftci *et al.*, 2010
200 mg/kg	Japanese quail	35	Improved BWG and FCR Increase water holding capacity of meat	Mehdipour *et al.*, 2013
300 mg/kg	Broiler chicks	35	Improved the performance indices (BWG, FCR and performance index) Increased the carcass yield (dressed weight, drawn weight and eviscerated weight).	Gawande, 2015
3 and 4 g/kg	Broiler chicks	42	Improved BWG and FCR No effects on carcass traits	Devi *et al.*, 2018
200 mg/kg	Broiler chicks	35	Improved FCR Increased Lactobacillus and decreased Coliforms count in the intestine	Mehdipour and Afsharmanesh, 2018
250 mg/kg	Broiler chicks	35	Decreased meat cholesterol No effect on carcass characteristics, meat quality	Gomathi *et al.*, 2018
400 mg/kg	Broiler chicks	42	Improved the immunity Decreased cecal *E. coli*	Yang *et al.*, 2019

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at Poultry Farm of Sher-E-Bangla Agricultural University, Dhaka-1207, with 150-day-old chick for a period of 28 days from 14th September to 12th October, 2020 to assess the probability of using non-antibiotic growth promoter (Mixed or single secondary plant compounds & essential oils, lysozyme enzyme) in commercial broiler diet on growth performance.

3.2 Collection of experimental broilers

A total of 150-day old chicks of "Indian River-Lohmann Meat" strain weighing 49±0.2g average body weight were obtained from Kazi farm limited hatchery, Gazipur, Dhaka.

3.3 Experimental procedures

A total of 150-day-old Indian River strains were collected from Kazi Hatchery. At day seven (7) broiler chicks were randomly divided into five (5) experimental group having three (3) replicates each with ten (10) chicks per replicate. Birds were housed in 3ft x 2ft floor pens on fresh rice husk litter with a 24-h lighting plan. The height of litter was 3 cm. Before being used in the experiment, birds were adapted for 7 days in order to acclimatize in the environment. The experimental diets were prepared by supplementing the control diet with different treatment product. The control diet was formulated without supplementation of any antibiotic or non-antibiotic growth promoter. The required amount of treatment product weighed and initially mixed with small amount of feed and then mixed with bulk quantity of feed in each treatment separately. The collected birds have neither developmental disorders, detectable genital diseases nor other diseases that may cause any problem in the experiment or affect the result of the experiment.

3.4 Experimental treatments

The experimental treatments were followings:

T_0 = No growth promoter (Neither antibiotic nor non-antibiotic)

T_1=100g blend of multiple secondary plant compounds, essential oils & organic acids/metric ton of the feed

T_2 = 500g lysozyme enzyme/metric ton of the feed

T_3 = 500g single secondary plant compound, essential oil/metric ton of the feed

T_4 = 500g bacitracin/metric ton of the feed

Table 3. Layout of the experiment

Treatment groups	No. of replications			Total
	R_1	R_2	R_3	
T_0	10	10	10	30
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_4	10	10	10	30
Total	50	50	50	150

3.5 Preparation of experimental house

The experimental room was properly cleaned and washed by using tap water. Ceiling walls and floor were thoroughly cleaned and disinfected by spraying diluted Iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 15 pens of equal size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen. The stocking density was $1m^2$/10 birds. Different replications of different treatment were allocated randomly in different segment of the farm with significant marking.

3.6 Experimental diets

Starter and grower commercial Kazi broiler feed were purchased from the market. Feeds were supplied 4 times daily by following IR Manual and *ad libitum* drinking water 2 times daily.

Table 4. Name and minimum percentage of nutrients present in Starter ration

Name of ingredients in Grower ration	Minimum present
Protein	21.0 %
ME (kcal/kg)	3050
Fiber	5.0%
Ash	8.0%
Lysine	1.20%
Methionine	0.49%
Cystine	0.40%
Tryptophan	0.19%
Threonine	0.79%

Table 5. Name and minimum percentage of nutrients present in Grower ration

Name of ingredients in Grower ration	Minimum present
Protein	19.0 %
ME (kcal/kg)	3150
Fat	6.0%
Lysine	1.10%
Methionine	0.47%
Cystine	0.39%
Tryptophan	0.18%
Threonine	0.75%
Arginine	1.18%

3.6.1 Collection of treatment products & composition

1. Blend of multiple (Cinnamon, Capsaicin, Carvacrol & Cineole) secondary plant compounds, essential oils & organic acids- **Activo** from Nature care manufacturing industry limited company.

2. Lysozyme enzyme- **Lyovo** from Nature care manufacturing industry limited company.

3. Single (Cinnamon) secondary plant compound, essential oil & organic acid- **Amaril** from Nature care manufacturing industry limited company.

4. Antibiotic (Bacitracin)- **BMD** from Zoetis company.

3.7 Management procedures

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

3.7.1 Brooding of baby chicks

The experiment was conducted during 14^{th} September to 12^{th} October, 2020. The average temperature was 32.5^{0}C in the poultry house. Common brooding was done for one week. After one week the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was $1m^2$. Due to hot climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35^{0}C) with house temperature. So, when the environmental temperature was above the recommendation, then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat and drink. Electric fans were used as per necessity to save the birds from the heat stress.

3.7.2 Room temperature and relative humidity

Daily room temperature (^{0}C) and humidity % were recorded every six hours with a thermometer and a wet and dry bulb thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded.

Table 6. Temperature in the poultry house

Week	Date	Temperature (℃)	
		Avg. Maximum	Avg. Minimum
1st	21.02.19 -27.02.19	37.64	29.64
2nd	28.02.19 -06.03.19	33.57	28.20
3rd	07.03.19-13.03.19	35.28	27.62
4th	14.03.19-20.03.19	36.14	27.92

3.7.3 Litter management

Rice husk was used as litter at a depth of 3 cm. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation. At 3 weeks of age, droppings on the upper layer of the litter were cleaned and for necessity fresh litter was added.

3.7.4 Feeding and watering

Feed and clean fresh water was offered to the birds *ad libitum.* One feeder and one round drinker were provided in each pen for 5 birds. Feeders were cleaned at the end of each week and drinkers were washed daily. All mash dry feed was fed to all birds *ad libitum* throughout the experimental period.

3.7.5 Lighting

At night there was provision of light in the broiler farm to stimulate feed intake and body growth. For first 2 weeks 24 hours light was used. Thereafter 22 hours light and 2 hours dark were scheduled up to 28 days.

3.7.6 Biosecurity measures

To keep disease away from the broiler farm recommended vaccination, sanitation program was undertaken in the farm and its premises.

3.7.7 Vaccination

The vaccines collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule.

Table 7. Vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	IB + ND	CEVAC IB L	One drop in each eye
9 days	Gumboro	CEVAC IBDL	Drinking Water
17days	Gumboro	CEVAC IBDL	Drinking Water
21 days	IB + ND	CEVAC IB L	Drinking Water

3.7.8 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm. Besides ventilation was regulated as per requirement by folding polythene screen.

3.7.9 Sanitation

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

3.8 Study Parameters

3.8.1 Recorded parameters

Weekly lives weight, weekly feed intake and death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed intake per bird in each replication. After slaughter gizzard, liver, spleen, intestine, hear and bursa were measured from each broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage. Blood sample was analysis from each replication to measure.

3.9 Data collection

3.9.1 Live weight

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

3.9.2 Feed intake

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

3.9.3 Mortality of chicks

Daily death record for each replication was counted up to 28 days of age to calculate mortality.

3.10 Calculations

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

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

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

3.10.3 Feed conversion ratio

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

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

3.11 Statistical analysis

The data was subjected to statistical analysis by applying one-way ANOVA (Duncan method-1955) using statistical package for social sciences (SPSS) version 16. Experiment was laid out in Randomized Complete Block Design (RCBD)

Some photograph of chick management and experimental procedure are presented in plate 1-15 below:

Plate 1. Washing of floor with detergent and disinfectant (a & b)

Plate 2. Collection of feed and litter (a & b)

Plate 3. Preparation of chick brooder guard (a & b)

Plate 4. Arrival of day-old chick (DOC) (a & b)

Plate 5. Measuring of temperature and humidity (a & b)

Plate 6. Preparation of experimental room (a & b)

Plate 7. Distribution of chick under different treatments (a & b)

Plate 8. Drinking of chicks (a & b)

Plate 9. Feeding of chicks (a & b)

Plate 10. Vaccination vial of IBD

Plate 11. Vaccination vial of ND and IB

Plate 12. Vaccination of chick (a & b)

Plate 13. Weighing and mixing of treatment additives within the feed (a & b)

Plate 14. Monitoring of research activities by the supervisor sir (a, b & c)

Plate 15. Medication used thorough the experimental period

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

Supplementation of live yeast and oligosaccharide to broiler diets improves growth performance in terms of feed intake, body weight gain and feed conversion ratio (FCR). The chicks were randomly divided into five experimental treatment groups. The five groups were T_0 (control), T_1 (100g multi essential oil/ton feed), T_2 (500g lysozyme enzyme/ton feed), T_3 (500g single essential oil/ton feed) and T_4 (500g antibiotic/ton feed). The performance traits viz. final live weight, body weight gain, feed intake, FCR, flock uniformity, were discussed here.

4.1.1 Final live weight

The relative final live weight (g) of broiler chickens in the different groups T_0, T_1, T_2, T_3 and T_4 presented in (Table 8 & Figure 4) were 1584.67^{b}±12.91g, 1710.33^{a}±15.30g, 1671.33^{a}±12.44g, 1696.33^{a}±10.17g, 1593.33^{b}±14.53g respectively. The highest result was found in T_1 (1710.33^{a}±15.30g) and lowest result was in T_0 (1584.67^{b}±12.91g) control and that was statistically significant ($P<0.05$). Results also demonstrated that the body weights also varied among the treatment groups having statistical significance ($P<0.05$). All the treated groups had higher live weight than control group and moreover all the NAGP (T_1, T_2, T_3) had higher live weight than AGP (T_4). The higher body weight in T_1, T_2 and T_3 group might be due to the diversified (digestive, anti-microbial, anti-oxidative) mode of action of treatment products.

These results are in agreement with those of previous researchers Toghyani *et al.*, (2011), reported that inclusion of cinnamon 2 g/kg of the diet had a significant positive effect on growth performance at 28 days of age (974 vs. 850 g) and at 42 days of age (2,111 vs. 1,931 g). Shirzadegan (2014), observed a significant increase in the final body weight of broiler chickens feeding on diets supplemented with different concentrations of cinnamon powder (especially at a level of 0.5%). Moreover, Devi *et al.*, (2018), showed that supplementation with a combination of CEO and ajwain essential oil in broiler diets significantly increased body weight at age 42 days.

On the other hand, Sarica *et al*., (2009), illustrated that supplementation of CEO in quail diets had the same effects of antibiotic, probiotic, enzymes, mannanoligosaccharide,

oregano essential oil (OEO), OEO plus CEO on body weight gain (BWG) of quail during the growth period of 0–35 days.

4.1.2 Weekly body weight gains (WBWG)

The mean body weight gains (g) at the 1st, 3rd and 4th week of different treatment groups were significantly higher (P<0.05) than control and antibiotic treated group. The mean body weight gains (g) of broiler chicks at 4th week in different groups were T_0 (531.00^{b}±6.55g), T_1 (573.33^{a}±10.10g), T_2 (576.33^{a}±7.21g), T_3 (578.33^{a}±2.40g) and T_4 (510.00^{b}±8.50g) respectively. At the 4th week the highest result was found in T_3 (578.33^{b}±2.40g) and lowest result was in T_4 (510.00^{a}±8.50g) group and that was statistically significant (P<0.05). The data of weekly body weight gains of broiler chicks presented in (Table 9 & Figure 5). Also, the mean of total body weight gains (g) of different treatment groups were significantly higher (P<0.05) than control and antibiotic treated group.

These results are in agreement with those of previous researchers Al-Kassie (2009), showed that broilers feeding on diet additives with CEO had a body weight gain significantly higher than the control (without CEO). Moreover, Tonbak and Çiftçi (2012), concluded that supplementation of cinnamon oil (*Cinnamomum zeylanicum* L.) in the diets at concentrations of 250 and 500 mg/kg diet had no significant influence on the live weight and live weight gain of the quail.

However, Lee *et al*., (2003), stated that cinnamaldehyde supplementation in feed had no significant effect on female broilers' weight gain, but water intake was decreased significantly.

4.1.3 Total feed intake (FI)

Total feed intake of different treated groups and control group have been presented in Table 8. T_1 consumed higher amount of feed (2265.83^{a}±11.84g) and T_4 consumed lower amount of feed (2173.67^{c}±31.85g), whereas T_0, T_2, and T_3 consumed 2202.00^{bc}±13.01g, 2243.93^{ab}±17.37g and 2243.17^{ab}±9.98g feed respectively. Result in total feed intake demonstrated that treatment groups showed significant (P<0.05). The higher feed intake in T_1 group might be due to the digestion enhancing effect of essential oils.

Al-Kassie (2009), clarified that the chicks fed on diets containing 200 ppm EO resulting from a combination of thyme and cinnamon achieved significant increases in feed efficiency and FI compared to the control.

Contradictory studies were reported by Symeon *et al.*, (2014); Sarica *et al*., (2009) and Lee *et al*., (2003), showing that cinnamon oil or powder did not significantly affect the FI or FCR of birds. Lee *et al.*, (2003), pointed out that cinnamaldehyde supplementation in feed had no significant influences on the FI and FCR of female broilers, however, water intake was significantly decreased.

4.1.4 Flock uniformity

Flock uniformity of broiler chicken were presented in Table 12. The higher flock uniformity (73.33^{a}±3.66%) was found in T_0. The lower flock uniformity (56.67^{b}±3.33%) was found in T_4 group. Flock uniformity of different treatment groups were statistically significant ($P<0.05$).

Average flock uniformity of T_1, T_2 & T_3 were (70.00^{ab}±5.77%), (66.67^{ab}±3.33%) & (70.00^{ab}±5.77%) respectively.

4.1.5 Feed conversion ratio (FCR)

The result of feed conversion ratio (FCR) of broilers under different treatment groups have been shown in Table 8. The lowest (best) feed conversion ratio (FCR) 1.35^{d}±0.00 and 1.36^{d}±0.00 were significantly ($P<0.05$) found in T_3 and T_1 group respectively than control birds (1.43^{a}±0.00). However, feed conversion ratio (FCR) was significantly ($P<0.05$) higher in T_0 (1.43^{a}±0.00) and T_4 (1.40^{b}±0.01) groups compared to NAGP treated group. The lowest (best) FCR in T_1 and T_3 group might be due to the diversified (digestive, anti-microbial, anti-oxidative) mode of action of essential oils.

Similarly, Ciftci *et al*. (2009), suggested that broilers receiving a diet supplemented with 500 ppm cinnamon oil showed the best feed conversion efficiency in comparison with avilamycin (antibiotic) groups and the control. In addition, Mehdipour *et al.* (2013), found that quails' diet supplemented with cinnamon oil (200 mg/kg) significantly improved FCR compared to the control group (0–35 days), while FI was not affected. Moreover, ŞİMŞEK *et al*. (2015), reported that the addition of cinnamon oil to the diets significantly reduced FCR. Torki *et al*. (2015), indicated that FCR was significantly lessened in laying hens housed under cold stress conditions (8.8 ± 3 ˚C) and fed on the diets including Zn and CEO (combined or single) compared with those fed on the control diet.

Contradictory studies were reported by Hernandez *et al.* (2004), indicated that broilers feeding on diets treated with 200 ppm essential oil extract (EOE) from cinnamon, pepper and oregano had no significant alterations in FI or FCR at 14 and 21 days of age. Moreover, Tonbak and Çiftçi (2012), reported that supplementation of cinnamon oil (*Cinnamomum zeylanicum* L.) to the diets at concentrations of 250 and 500 mg/kg had no significant impacts on FCR of quail.

Table 8. Effects of NAGP on production performances of broiler chicken

Treatment	Final Live weight (g/bird)	Average BWG (g/bird)	Total FI (g/bird)	Final FCR	Uniformity (%)
T_0	1584.67^{b}±12.91	1538.67^{b}±12.91	2202.00^{bc}±13.01	1.43^{a}±0.00	73.33^{a}±3.66
T_1	1710.33^{a}±15.30	1664.33^{a}±15.30	2265.83^{a}±11.84	1.36^{d}±0.00	70.00^{ab}±5.77
T_2	1671.33^{a}±12.44	1625.33^{a}±30.06	2243.93^{ab}±17.37	1.38^{c}±0.00	66.67^{ab}±3.33
T_3	1696.33^{a}±10.17	1650.33^{a}±12.44	2243.17^{ab}±9.98	1.35^{d}±0.00	70.00^{ab}±5.77
T_4	1593.33^{b}±14.53	1547.33^{b}±14.53	2173.67^{c} ±31.85	1.40^{b}±0.01	56.67^{b}±3.33
Mean ± SE	1651.20±14.85	1605.20±14.85	2225.72±11.32	1.38±0.01	67.33±2.29

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed). Values are Mean ± SE (n=15), one-way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different ($P<0.05$)
- Mean within same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error
- NAGP= Non-antibiotic Growth Promoter

Table 9. Effects of NAGP on body weight gain (BWG) (g/bird) of broiler chickens in different weeks

Treatment	1st Week BWG	2nd Week BWG	3rd Week BWG	4th Week BWG
T_0	204.33^{c}±4.05	300.67±1.33	502.67^{d}±4.37	531.00^{b}±6.55
T_1	233.67^{a}±5.54	296.67±2.33	560.67^{c}±2.33	573.33^{a}±10.10
T_2	220.33^{ab}±2.90	295.67±5.23	533.00^{b}±3.78	576.33^{a}±7.21
T_3	229.33^{a}±5.60	292.33±3.18	550.33^{b}±1.85	578.33^{a}±2.40
T4	213.67^{bc}±3.48	298.33±4.25	525.33^{c}±2.72	510.00^{b}±8.50
Mean ± SE	220.27±3.29	296.73±1.53	534.40±5.52	553.80±8.00

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed). Values are Mean ± SE (n=15), one-way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- NAGP= Non-antibiotic Growth Promoter

Table 10. Effects of NAGP on feed intake (g/bird) of broiler chickens in different weeks

Treatment	1st Week FI	2nd Week FI	3rd Week FI	4th Week FI
T_0	229.33^{b}±4.05	404.33^{a}±3.84	678.33^{c}±3.84	890.00^{a}±7.23
T_1	247.00^{a}±4.72	389.33^{ab}±1.45	727.17^{a}±3.08	902.33^{a}±8.64
T_2	241.00^{ab}±1.73	395.23^{ab}±4.62	708.70^{b}±1.17	899.00^{a}±12.22
T_3	248.00^{a}±4.93	388.00^{b}±6.65	696.33^{b}±2.33	910.83^{a}±3.98
T_4	236.00^{ab}±2.64	398.67^{ab}±4.84	701.67^{b}±7.68	837.33^{b}±21.98
Mean ± SE	240.27±2.35	395.11±2.37	702.44±4.55	887.90±8.42

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed). Values are Mean ± SE (n=15), one-way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different ($P<0.05$)
- Mean within same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error
- NAGP= Non-antibiotic Growth Promoter

Table 11. Effects of NAGP on feed conversion ratio (FCR) of broiler chickens in different weeks

Treatment	1st Week FCR	2nd Week FCR	3rd Week FCR	4th Week FCR
T_0	1.12^{a}±.00	1.34^{a}±.01	1.34^{a}±.00	1.67^{a}±.01
T_1	1.05^{d}±.00	1.31^{b}±.01	1.29^{b}±.00	1.57^{c}±.01
T_2	1.09^{bc}±.01	1.33^{ab}±.01	1.32^{a}±.01	1.55^{c}±.00
T_3	1.08^{c}±.01	1.32^{ab}±.01	1.26^{c}±.00	1.57^{c}±.00
T_4	1.10^{ab}±.01	1.33^{ab}±.00	1.33^{a}±.01	1.64^{b}±.01
Mean ± SE	1.09±.01	1.33±.00	1.31±.01	1.60±.01

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed). Values are Mean ± SE (n=15), one-way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different ($P<0.05$)
- Mean within same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error
- NAGP= Non-antibiotic Growth Promoter

Table 12. Flock uniformity of broiler chickens under different treatments

Treatment	Uniformity
T_0	73.33^{a}±3.66
T_1	70.00^{ab}±5.77
T_2	66.67^{ab}±3.33
T_3	70.00^{ab}±5.77
T_4	56.67^{b}±3.33
Mean ± SE	67.33±2.29

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed). Values are Mean ± SE (n=15), one-way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different ($P<0.05$)
- Mean within same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error
- NAGP= Non-antibiotic Growth Promoter

Average Live Weight Under Different Treatment Group

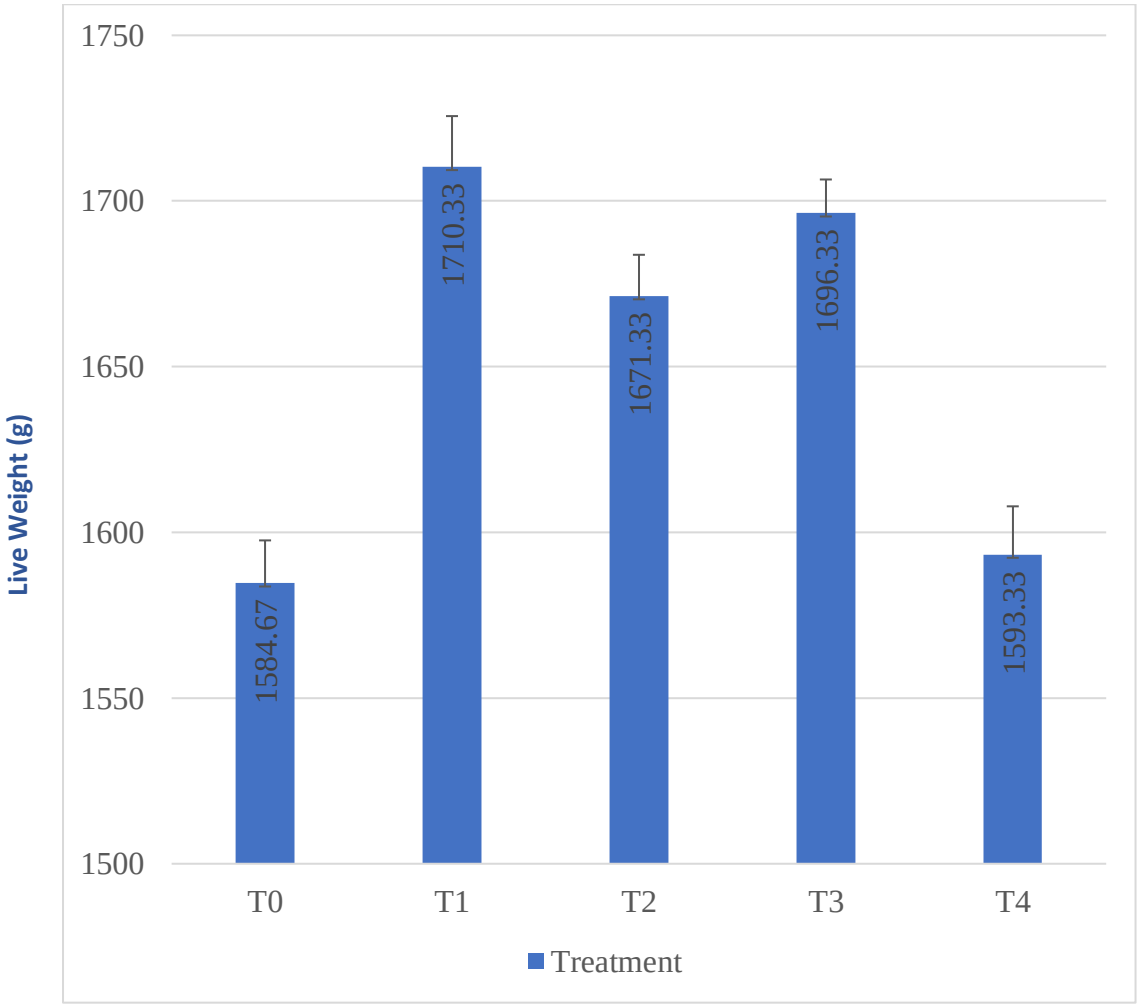


Figure 4. Effects of NAGP on live weight of broiler chickens

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed).

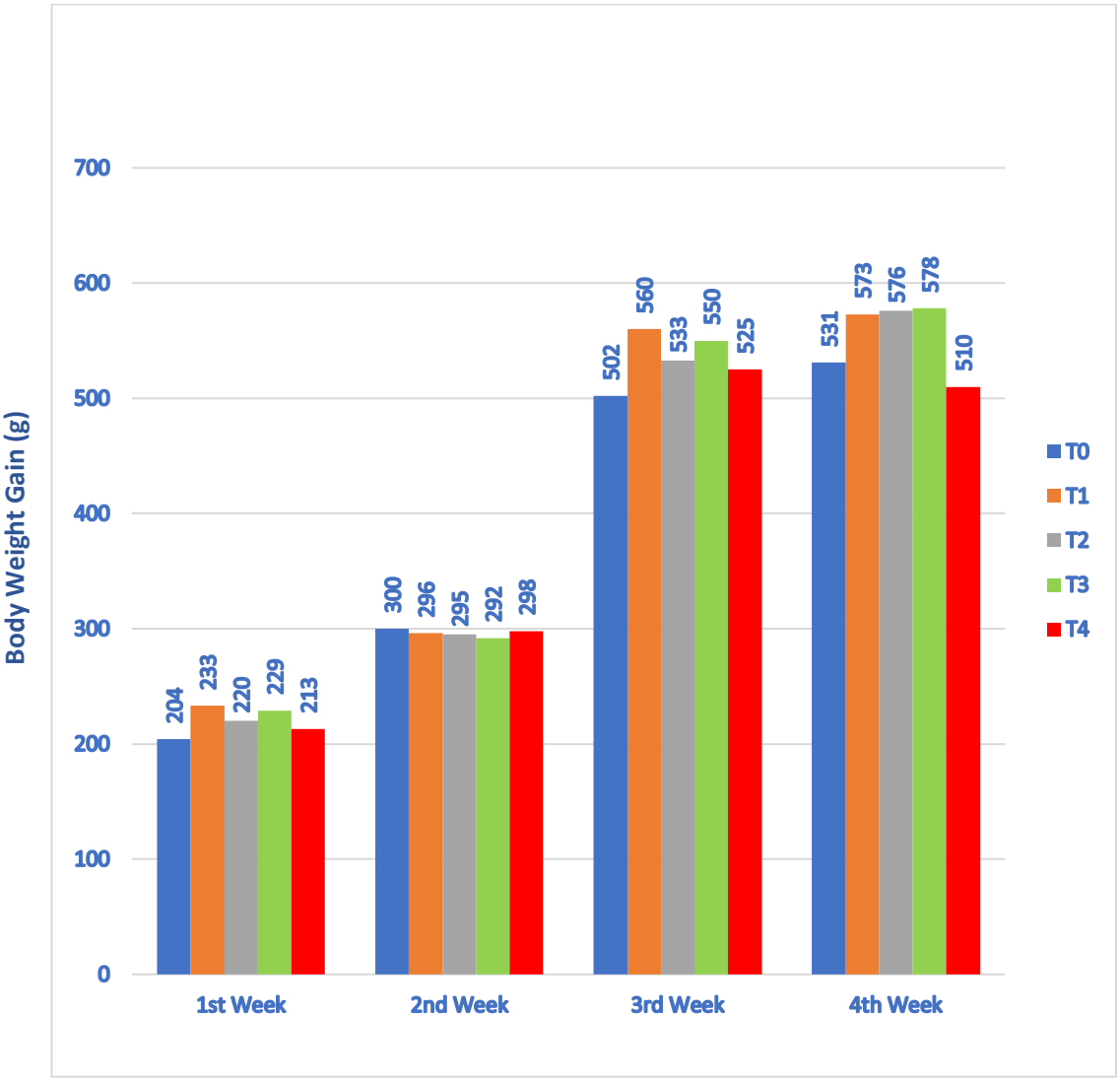


Figure 5. Effects of NAGP on body weight gain of broiler chickens in different weeks

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed).

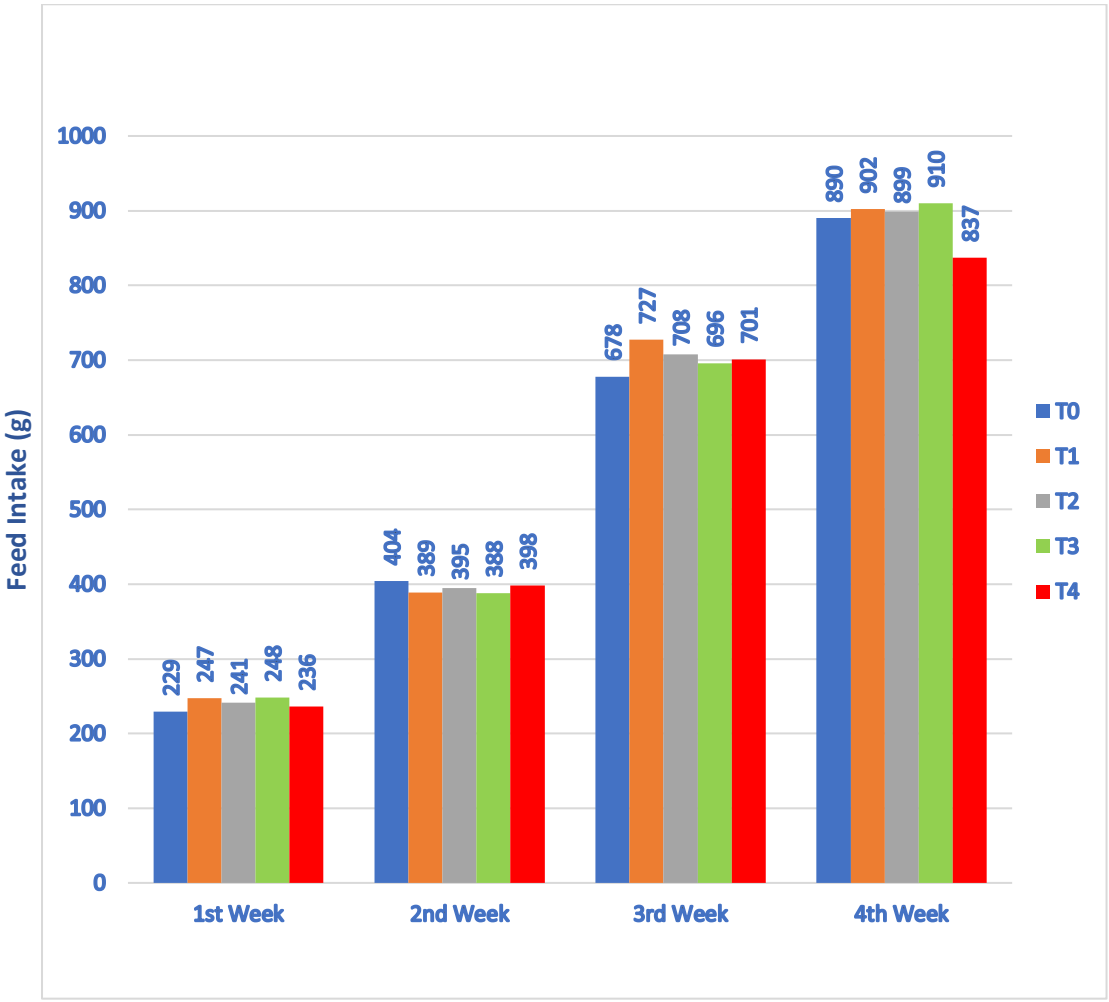


Figure 6. Effects of NAGP on feed intake of broiler chickens in different weeks

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed).

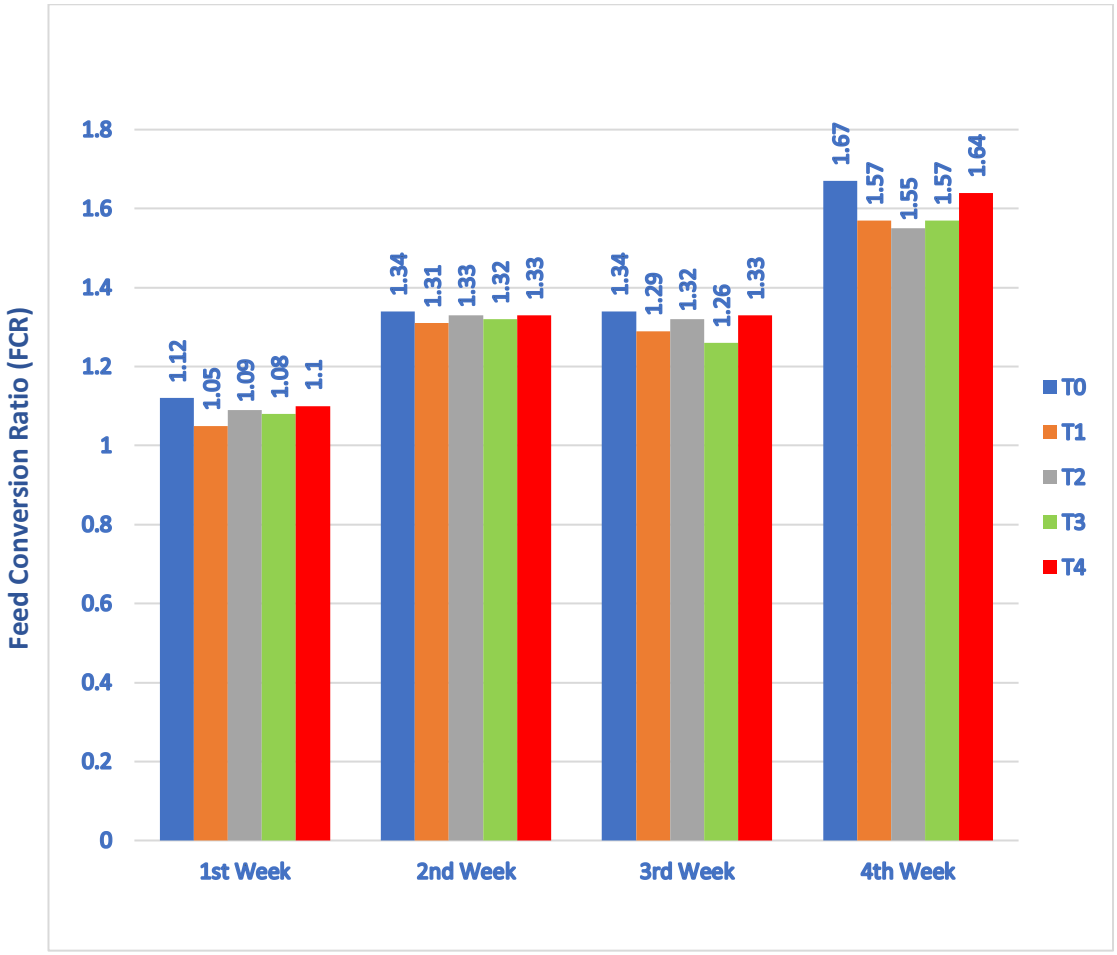


Figure 7. Effects of NAGP on FCR of broiler chickens at different weeks

Here, $T_{0=}$ (control), $T_{1=}$ (100g multi secondary plant compounds & essential oils/ton feed), $T_{2=}$ (500g lysozyme enzyme/ton feed), $T_{3=}$ (500g single secondary plant compound & essential oil/ton feed) and $T_{4=}$ (500g antibiotic/ton feed).

CHAPTER V

SUMMARY AND CONCLUSION

A total of 150-day old chicks of "Indian River-Lohmann Meat" were reared at Sher-E-Bangla Agricultural University Poultry Farm for a period of four weeks using different growth promoters both antibiotic and non-antibiotic. Chicks were divided randomly into 5 experimental groups of 3 replicates (10 chicks were allocated in each treatment group). One of the 5 experimental group was fed diet without any growth promoter were considered as control while, the remaining four groups were fed diet with 100g multi secondary plant compounds & essential oils/ton feed (T_1), 500g lysozyme enzyme/ton feed (T_2), 500g single secondary plant compound & essential oil/ton feed (T_3) and 500g antibiotic/ton feed (T_4). The specific objectives of this experiment were i) To find effective natural alternative of growth promoters in broiler nutrition. ii) To mitigate the risk of antibiotic residue in broiler meat. iii) To evaluate the production performance of broiler. The performance traits *viz.* body weight, weight gain, feed intake, FCR, flock uniformity of broiler on different replication of the treatments were recorded and compared in each group.

A statistically significant difference ($P<0.05$) was noted on final live weight, feed intake, BWG, FCR value of the birds treated with different dietary treatment. Birds fed 100g multi essential oil/ton feed (T_1) gained superior body weights (1710.33±15.30g) compared to T_0- control (1584.67±12.91g), T_4- antibiotic (1593.33±14.53g). The mean body weight gains (g) at the 1st, 3rd and 4th week of different treatment groups were significantly higher ($P<0.05$) than control group. The groups fed diets containing 500g single secondary plant compound & essential oil/ton feed (T_3) & 100g multi secondary plant compounds & essential oils/ton feed (T_1) had lower FCR (1.35±0.00) & (1.36±0.00) respectively compared to T_0 control (1.43±0.00) and T_4 antibiotic (1.40±0.01). The inclusion of different dietary treatments had significant ($P<0.05$) difference on flock uniformity. However, control (T_0) group had the superior uniformity (73.33±3.66%) and the group treated with antibiotic (T_4) had the lowest uniformity (56.67±3.33%).

Analyzing the above research findings, 100g multi secondary plant compounds & essential oils/ton feed (T_1) showed better results than control (T_0) and other treatment groups in terms of improved growth performance with superior body weight gain and

FCR. Among different dietary treatment groups 100g multi secondary plant compounds & essential oils/ton feed (T_1) & 500g single essential oil/ton feed (T_3) showed better result than group 500g lysozyme enzyme/ton feed (T_2), 500g antibiotic/ton feed (T_4) & Control (T_0).

So, NAGP could be used as an alternative of antibiotics on broiler ration especially secondary plant compound based NAGP's and therefore the study recommends for further trial on commercial poultry farm to fix up inclusion level perfectly used in broiler rearing for higher economical return without any adversity.

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APPENDICES

Appendix 1. Feed intake (g/bird) of 1st, 2nd, 3rd and 4th week under different treatment groups

Treatment	Replication	1st Week FI	2nd Week FI	3rd Week FI	4th Week FI	Total FI
T_0	R_1	236	410	680	902	2228
	R_2	222	406	671	891	2190
	R_3	230	397	684	877	2188
T1	R_1	240	387	730.5	890	2247.5
	R_2	245	389	730	898	2262
	R_3	256	392	721	919	2288
T_2	R_1	241	395.7	707.1	907	2250.8
	R_2	238	387	711	875	2211
	R_3	244	403	708	915	2270
T_3	R_1	239	379	692	913.5	2223.5
	R_2	256	384	700	916	2256
	R_3	249	401	697	903	2250
T_4	R_1	240	403	713	881	2237
	R_2	237	404	687	820	2148
	R_3	231	389	705	811	2136

Appendix 2. Body weight gain (BWG) (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments

Treatment	Replication	1st Week	2nd Week	3rd Week	4th Week	Total BWG
T_o	R_1	211	302	506	544	1563
	R_2	197	302	494	526	1519
	R_3	205	298	508	523	1534
T_1	R_1	225	292	565	556	1638
	R_2	232	299	560	573	1664
	R_3	244	299	557	591	1691
T_2	R_1	221	297	527	582	1627
	R_2	215	286	540	562	1603
	R_3	225	304	532	585	1646
T_3	R_1	221	287	549	577	1634
	R_2	240	292	554	583	1669
	R_3	227	298	548	575	1648
T_4	R_1	220	301	527	526	1574
	R_2	213	304	520	507	1544
	R_3	208	290	529	497	1524

Appendix 3. Feed conversion ratio (FCR) of birds under different treatments

Treatment	Replication	1st Week	2nd Week	3rd Week	4th Week
T_0	R_1	1.11	1.35	1.34	1.65
	R_2	1.12	1.34	1.35	1.69
	R_3	1.12	1.33	1.34	1.67
T_1	R_1	1.06	1.32	1.29	1.60
	R_2	1.05	1.30	1.30	1.56
	R_3	1.04	1.31	1.29	1.55
T_2	R_1	1.09	1.33	1.34	1.55
	R_2	1.10	1.35	1.31	1.55
	R_3	1.08	1.32	1.33	1.56
T_3	R_1	1.08	1.32	1.26	1.58
	R_2	1.06	1.31	1.26	1.57
	R_3	1.09	1.34	1.27	1.57
T_4	R_1	1.09	1.33	1.35	1.67
	R_2	1.11	1.32	1.32	1.61
	R_3	1.11	1.34	1.33	1.63

Appendix 4. Production performance of broiler chicken under different treatments

Treatment	Replication	Final Live weight (g/bird)	Total FI (g/bird)	Total BWG (g/bird)	Final FCR
T_0	R_1	1609	2228	1563	1.42
	R_2	1565	2190	1519	1.44
	R_3	1580	2188	1534	1.42
T_1	R_1	1684	2247.5	1638	1.37
	R_2	1710	2262	1664	1.35
	R_3	1737	2288	1691	1.35
T_2	R_1	1673	2250.8	1627	1.38
	R_2	1649	2211	1603	1.37
	R_3	1692	2270	1646	1.37
T3	R_1	1680	2223.5	1634	1.36
	R_2	1715	2256	1669	1.35
	R_3	1694	2250	1648	1.36
T_4	R_1	1620	2237	1574	1.42
	R_2	1590	2148	1544	1.39
	R_3	1570	2136	1524	1.40

Appendix 5. Flock uniformity of broiler chickens under different treatment

Treatment	Replication	Uniformity (%)	Average Uniformity (%)
T_0	R_1	77	
	R_2	66	73.33
	R_3	77	
T_1	R_1	80	
	R_2	70	70
	R_3	60	
T_2	R_1	70	
	R_2	60	66.66
	R_3	70	
T_3	R_1	70	
	R_2	60	70
	R_3	80	
T_4	R_1	50	
	R_2	60	56.66
	R_3	60	