DIETARY EFFECTS OF GREEN TEA EXTRACT AN ALTERNATIVE OF ANTIBIOTIC ON BROILER PRODUCTION

A THESIS

BY

Registration No. 1505267
Session: 2015-2016
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MASTER OF SCIENCE (MS)

IN

ANIMAL NUTRITION

DEPARTMENT OF GENERAL ANIMAL SCIENCE AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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[Submitted to the Department of General Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree]

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DEPARTMENT OF GENERAL ANIMAL SCIENC AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

December, 2017

Dedicated to

My parents

For their undefined encouragements

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The Author
December, 2017

ABSTRACT

This study was conducted to evaluate the effect of green tea extract as alternative of antibiotic on broiler production. Experiment was conducted for a period of 35 days with a number of 100 day old cob-500 broiler chicks. Birds were divided into five dietary treatment groups with 4 replications each having 5 birds per replication. Green tea extract (GTE) was supplemented in the diets as a natural growth promoter at three different levels (0.5%, 1% and 2%).Broilers fed on the GTE diets were compared to those fed on the un-supplemented (control) and antibiotic (oxytetracyline). Significant differences were observed in body weight, feed intake and feed efficiency among the GTE supplemented group. Also significantly higher breast muscle weight than other treatments ($p<0.05$). Blood serum cholesterol were significantly reduced ($p<0.05$) by GTE feeding. In conclusion, the GTE supplementation at 0.5% may be suitable for growth performance and improve feed efficiency for broiler production.

Key words: Broiler, Green tea extract, Antibiotic

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Abbreviation and Acronyms

FE	Feed efficiency
FI	Feed intake
GTE	Green Tea Extract
gm	Gram
HSTU	Hajee Mohammad Danesh Science and Technology University
TC	Total Cholesterol
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
kg	Kilogram
mg	Milligram
OCT	Oxytetracycline
SEM	Standard error mean
SPSS	Statistical Package for the Social Sciences
wk	week

CHAPTER I
INTRODUCTION

Poultry production has developed in a large number of developing countries around the world as an important source of earning for the rural people. The recognition of small – scale commercial poultry production helps to accelerate the pace of poverty reduction riding in new height in Bangladesh. The poultry industry has been successfully becoming a leading industry of the country. The sector is also growing rapidly for last two decades though it started farming during mid sixties in this country. It has already capable to rise at an annual growth of around 20 per cent during last two decades (Mahboob & Moulude, 2012).

Poultry production is a specialized one and concentrating more on the high performance birds. The successful Poultry production is high genetic potential, balanced nutrition and health maintenance. There is a major demand to produce high quality poultry meat and egg at low price without use antibiotic and other medicines in poultry feed and water (Shivappa *et al*., 2012).

Antibiotic growth promoters (AGP) are used in poultry production for improving feed utilization, increasing general health of chickens and subsequently improving their productive performance through different modes of action (Nasir and Grashorn, 2008; Attia, *et al.,* 2011), although, excessive use of anti-biotic had negative effects on environment and human health (Al-harithi, 2002; Nasir and Grashorn, 2008). Usage of antibiotics as growth promoter in poultry diet has been banned due to concerns about their residues in animal tissues.(Roe & Pillai, 2003 and Saleha *et al.*, 2009) and the inclusion of antibiotics to animal diet was banned in some parts of the world (Simon, 2006).

Antimicrobial properties as well as growth promoting effects of various plants and plant extracts (Hanczakowska and Urbanczyk, 2002; Cross *et al.,* 2007; Ocak *et al.*, 2008; Erener *et al.*, 2010; Sarker *et al.*, 2010; Toghyani *et al.*, 2010).

Phytogenic and herbal feed additives might improve the growth performance of poultry has not been confirmed yet and thus, a systematic approach on the efficacy and safety of compounds used as feed additives. The assumption is that differences in results are

consequences of numerous factors, such as the type and part of plant used and their physical properties, the time of harvest, the preparation method of phytogenic additive and compatibility with other food components (Yang *et al.,* 2009).

Green tea extract is obtained from a nonoxidized and unfermented leaves of the evergreen plant *Camellia sinensis* that grows mainly in tropical and sub-tropical climates. The most abundant constituent of green tea extracts (GTE) is catechins which has antibacterial activities (Cao *et al.,* 2005; Hara-Kudo, 2005), as well as antitumorigenic, anti-inflammatory, antiproliferative, antiviral, anti-parasitic and antioxidative properties (Crespy and Williamson, 2004; Fujiki, 2005; Jang *et al.,* 2007). The GTE is a rich source of polyphenolic compounds and could possess strong antioxidant properties (Crespy and Williamson, 2004; Fujiki, 2005).

In view of these, the present work has been undertaken with the following objectives-

a) To determine the effects of Green tea extract as a substitute for an antibiotic in broiler diets on productivity and carcass characteristics.
b) To evaluate the effect of Green tea extract on lipid profile of blood plasma of broiler chicken.

CHAPTER II

REVIEW OF LITERATURE

Green tea extract (*Camellia sinensis)* contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols (GTPs) are flavonols, commonly known as catechins. Products derived from green tea are mainly extracts of green tea in liquid or powder form that vary in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%). The major flavonoids of green tea are various catechins, which are found in greater amounts in green tea than in black or Oolong tea (Vinson JA, 2000). There are four kinds of catechins mainly find in green tea: epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG (Sano *et al.,* 2001). The preparation methods influence the catechins both quantitatively and qualitatively; the amount of catechins also varies in the original tea leaves due to differences in variety, origin, and growing conditions (khokhar *et al.,* 2002). The preparation of fresh green tea cannot totally extract catechins from the leaves; therefore the concentration found differs from the absolute values determined through the complete extraction of leaves (Fernandez *et al.,* 2000).

Camellia sinensis

Camellia sinensis foliage

2.1 Zoological classification of green tea extract

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Ericales

Family: Theaceae

Genus: *Camellia*

Species: ***C. sinensis***

Source: https://en.wikipedia.org/wiki/Camellia_sinensis

2.2 Chemical composition of green tea extract

The chemical composition of green tea extact is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-*N*-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and α-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons). Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves (Belitz and Grosch, 1997). Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophylline. In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as the anine present (Vinson, 1992).

Compound	Green Tea extract*	Black tea*
Protein	15	15
Amino acids	4	4
Fiber	26	26
Others carbohydrates	7	7
Lipids	7	7
Pigments	2	2
Minerals	5	5
Phenolic compounds‡	30	5
Oxidized phenolic compounds§	0	25

Table 1: Composition (%) of green tea extract and black tea

Source: https://en.wikipedia.org/wiki/Camellia_sinensis

2.3Antimicrobial effects of green tea extract

2.3.1 Anticoccidial effects

Eimeria infection causes extensive destruction of the intestinal epithelium that results in reduced feed efficiency, body weight gain and a temporary reduction in egg production (Dalloul & Lillehoj, 2005).

Anticoccidial effect of green tea extract on Eimeria parasites has been published (Jang *et al.*, 2007). The results showed that green tea extract (0.5% and 2.0%) fed to five-week-old chickens for two weeks prior to infection with Eimeria maxima (E. maxima) (10000 sporulated oocysts per bird) significantly ($p < 0.05$) reduced shedding of oocysts in faeces by 38.5% and 51.5%, respectively. The green tea-based diet, however, did not improve body weight loss due to infection with E. maxima.

The higher concentration of green tea extract supplementation showed a greater protective effect and reduction in faecal oocyst shedding. Green tea extract components have shown anti-parasitic activities in vitro by inhibiting egg hatching and larval development and inactivating the infective larvae of Teladorsagia circumcincta and Trichostrongylus colubriformis (Molan *et al.*, 2003, 2004).

2.3.2 Antiviral effects

The structure-activity data of green tea catechin derivatives may applying as alternative anti-viral agents. The potential anti-viral activity of a unique nutrient mixture (NM) (containing green tea extract, lysine, proline, ascorbic acid, N-acetyl cysteine and selenium, amongst other micronutrients) and its components on A/H5N1 at viral dosages of 1.0, 0.1 and 0.01 TCID50. Antiviral activity was studied in cultured cell lines PK, BHK-21, and Vero-E6. Virus lysing activity was determined by co-incubation of virus A/H 5 N 1 with NM for 0 minutes to 60 minutes, followed by residual virulence titration in cultured SPEV or BHK-21 cells. The NM demonstrated high antiviral activity evident even at prolonged periods after infection. Its antiviral properties were comparable to those of conventional drugs (amantadine and oseltamivir); however, NM had the advantage of affecting viral replication at the late stages of the infection process (Deryabin *et al.*, 2008).

2.3.3 Effects on intestinal flora

The effects of green tea extract on the caecal flora in eight 24-day-old and eight 56-day-old chickens that had consumed a basal diet or a diet supplemented with green tea extract (2 ml/kg diet) for periods of up to 56 days. On day 24 of the experiment, the number of total bacteria and bacteroidaceae significantly decreased, but staphylococci increased. The frequency of the occurrences of pseudomonads and yeasts significantly increased, but that of moulds decreased (Terada *et al.*, 1993).

The effects of GTE supplement on counts of caecal microflora in female broiler chickens fed on semi-purified diets from 28 days to 42 days of age. They found that the counts of bifidobacteria, bacteroidaceae, Peptococcaceae, lactobacilli, Eubacteria and lecithinase-positive bacteria (clostridia, streptococci, staphylococci and bacilli) in the GTP group were lower than those of the control group. They reported that green tea and its chemical components show antibiotic-like effects of non-selectively decreasing total counts of all microflora (Cao *et al.*, 2005).

Polyphenols extracted from green tea have been shown to have inhibitory effects on Gram-positive bacteria as well as Gram-negative bacteria (Gadang *et al.*, 2008).

The efficacy of green tea extract against various bacterial strains can be related to differences in cell membranes (Ikigai *et al.*, 1993).

The antibacterial activities of EGCG on various strains of Staphylococcus (Gram positive cocci) and Gram negative rods including Escherichia coli, Klebsiella pneumoniae and Salmonella They found that 50μg/mL – 100 μg/mL was required to inhibit growth of Staphylococcus and concentrations higher than 800 μg/mL were required to inhibit Gram-negative rods (Yoda *et al.*, 2004).

2.4 Effect of green tea extract on broilers performance

Diets supplemented with high level of GTE reduced BWG, feed and water intakes although feed conversion ratio (FCR) was not significantly influenced while abdominal fat and dressing percentage were significantly decreased (Kaneko *et al.*, 2000).

Added four levels of green tea powder (0.50%, 0.75%, 1.00% and 1.50%) to broiler starter and finisher diets. They observed decrease feed intake and body weight gain at a higher dose, but tended to improve FCR. Dressing percentage was not affected by green tea, although proportions of some parts of the carcass were influenced. The proportion of thigh meat was increased by the 1.50% level feed while that of wing meat was decreased in all treatment groups. The quantity and percentage of abdominal fat were decreased significantly with supplementation (Biswas and Wakita, 2001).

1.00%, 2.50% and 5.00% of green tea in broiler diets linearly reduced body weight gain of the chicks (Kaneko *et al.*, 2001).

1.00% to 1.50% green tea supplement in broiler diet had the effect of reducing body weight gain of the chicks (Uuganbayar, 2004).

The optimum level of green tea by-product (0.50%, 1.00% and 2.00%) in diets. They observed non-significant differences in feed intake and feed efficiency amongst treatments (Yang *et al.*, 2003).

Body weight gain, feed intake and feed efficiency from 28 days to 42 days of age were not improved; however, mortality was significantly reduced by supplementation with green tea by-products (Cao *et al.*, 2005).

A liquid hydro alcoholic extract of fresh green tea (0.1 g/kg or 0.2 g/kg) in broiler diets. The dietary green tea extract increased the body weight, feed efficiency, carcass weight and dressing percentage. The broilers in green tea supplemented groups consumed more feed than the control birds throughout the entire experimental period. The dietary green tea extract increased redness and yellowness values of the breast meat. Thus, the green tea extract appeared to have a measurable impact on CIE colour values of the breast meat in broilers (Guray *et al.,* 2011).

The addition of green tea at 5 g/kg of broiler feed had no significant effect on the chemical composition of plasma total lipids, cholesterol, plasma aspartate aminotransferase and alanine aminotransferase activities (El-Deek and Al-Harthi, 2004).

reported that the addition of green tea by-product to diets tended to decrease blood low-density lipoprotein (LDL) cholesterol content compared to the control group, although there were no significant differences amongst treatments (Yang *et al.,* 2003).

Liver cholesterol, fat and serum cholesterol were signifi-cantly reduced ($P > 0.05$) by feeding GTE supplemented-diet (Biswas and Wakita, 2001).

GT flowers powder improved FCR efficiently and interfered with micelles solubilization of cholesterol in the gut and decreased cholesterol absorption (Fujiki and Suganuma, 2002)

2.5 Effect of green tea extract on poultry production

0.67% Japanese green tea extracts mixed with drinking water slightly reduced the egg weight of the hens (Yamane *et al.,* 1999).

Supplementation of layer diets with 0.50% extract of green tea and 1.50% powder of green tea had no significant effects on feed intake, egg production and egg weight (Ariana *et al.,* 2011).

200 mg or 400 mg of EGCG exerted antioxidant effects and linearly improved feed intake from 29.6 g/day to 30.9 g/day and egg production from 84.30 %/day to 90.10%/day in heat-stressed quails (Sahin *et al.,* 2010).

The effect of adding green tea leaves (1.00% to 5.00%) and its aqueous extract (0.5 L/100 kg to 2.5 L/100 kg of ration) to laying-hen diets. The results revealed that the

improvements in egg production, egg mass and feed conversion values due to 1.00% green tea leaves compared to the control were 5.59%, 6.79% and 7.84%, respectively. The corresponding level (0.5 L/100 kg diet) of hot water green tea extract resulted in improvements of 6.78%, 7.46% and 8.65%, respectively (Abdo *et al.,* 2010).

2.6 Effects on absorption of metal ions

Catechins can affect iron absorption, particularly iron deficiency (Samman *et al.,* 2010; Nelson *et al.,* 2004). Green tea ingestion over a long period does not affect the apparent absorption of copper, whereas it decreases that of zinc and increases that of manganese (Deng *et al.,* 1998). catechin intake does not affect the plasma concentration of these ions (Mclnerney and Dreosti, 1996). Green tea catechins have the potential to affect absorption and metabolism of ions because flavonoids interact with a variety of metal ions (Mira *et al.,* 2002).

2.7 Effects on drug-metabolizing enzymes

Long-term ingestion of green tea increases UDP-glucuronosyl transferase activity in rats(Samman *et al.,* 2010; Maliakal pp *et al.,* 2001; Sohn OS *et al.,* 1994), and after being absorbed, catechins are metabolized by drug-metabolizing enzymes in various organs (Donovan JL *et al.,* 2001; Okushio K *et al.,* 1999).The increased glucuronidation through UDP-glucuronosyl transferase induction is postulated to contribute to the anticarcinogenic effect of green tea by facilitating the metabolism of chemical carcinogens into inactive products that are readily excreted. The interaction between 2-amino-3-methylimidazol (4, 5-f) quinoline (IQ) and green tea catechin metabolism was examined (Embola *et al.,* 2001). IQ is a precarcinogen that was originally detected in an extract of fried meat. The major route of IQ biotransformation in rats is cytochrome P450 in the first step, followed by conjugation to a sulfate and a glucuronide conjugate. Green tea modifies IQ metabolism in rats, increasing the formation of IQ glucuronides, which are then excreted in the urine. Moreover, protection against cancers induced by polycyclic aromatic hydrocarbons by green tea catechins may be due to the inhibition of their cytochrome P450 metabolism, but the effect of green tea on cytochrome P450 enzymes depends on the particular form. The long-term consumption of green tea increases cytochrome P450 1A1 and 1A2 activities, but not 2B1 and 2E1 activities, in

normal rats. However, it is difficult to draw conclusions about a beneficial effect of green tea against carcinogens involving only modulation of this metabolic pathway.

2.8 Effects on antioxidant markers and oxidative stress

Green tea is a popular neutraceutical as an antioxidant. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Halliwell B *et al.,* 1985). Catechins are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e. vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system. (Abdel-Rahman, 2009).

In vivo studies showed that green tea catechins increase total plasma antioxidant activity. (Yokozawa *et al.,* 2002; Skrydlewska *et al.,* 2002). Intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta; these enzymes are implicated in cellular protection against reactive oxygen species (Skrydlewska *et al.,* 2002; Negishi *et al.,* 2004). This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration. Malondialdehyde, a marker of oxidative stress, also decreases after green tea intake (Yokozawa *et al.,* 2002, 2004) These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect. Since catechins can act as antioxidants *in vitro,* they might prevent the oxidation of other antioxidants, such as vitamin E. However, ingestion of green tea catechins does not modify the plasma status of vitamins E and C *in vivo* (Skrydlewska *et al.,* 2002; Tijburg *et al.,* 1999; Alesion *et al.,* 2003). Nevertheless, one study reported that catechins increase vitamin E concentration in low-density lipoprotein and in this way could protect low-density lipoprotein against peroxidation (Tijburg *et al.,* 1999).

2.9 Effects on carbohydrate metabolism

Catechins reduced plasma triglyceride levels in an oral glucose-tolerance test in normal rats (Wu LY *et al.,* 2004). Green tea extract intake reduced these values in both Zucker rats and rats fed a sucrose-rich diet (Hasegawa et al., 2003; Yang *et al.,* 2001). Several

human- and animal-based studies suggested that green tea and its flavonoids have antidiabetic effects (Wu LY *et al.,* 2004; Iso *et al.,* 2006). Green tea flavonoids were also shown to have insulin-like activities as well as insulin-enhancing activity (Anderson *et al.,* 2002).

Green tea extract promoted glucose metabolism in healthy animal's volunteers at 1.5 g/kg as shown in oral glucose-tolerance tests. Green tea extract also lowered blood glucose levels in diabetic db+/db+ mice and streptozotocin-diabetic mice two to six hours after administration at 300 mg/kg without affecting serum insulin level, whereas no effect was observed in control mice (+m/+m and normal ddY mice)

2.10 Effect of EGCG on diabetes

Green tea and green tea extracts were demonstrated to modify glucose metabolism beneficially in experimental models of type II diabetes mellitus (Wu LY *et al.,* 2004; Tsuneki *et al.,* 2004). In addition, EGCG ameliorates cytokine-induced β cell damage *in vitro* and prevents the decrease of islet mass induced by treatment with multiple low doses of streptozotocin *in vivo*.

Intragastric administration of EGCG at a dose of 75 mg/kg resulted in a Cmax of 128 mg/l total plasma EGCG and a terminal half-life of 83 minutes (Lambert *et al.,* 2010). Furthermore, in humans an oral intake of EGCG at a dose of 50 mg (0.7 mg/kg) resulted in a Cmax of 130 mg/l total plasma EGCG and a terminal half-life of 112 minutes (Ullmann U. *et al.,* 2003). These results indicate that rodents must be orally administered 100- to 600-fold more EGCG (depending on whether they are administered by gavage or by feed admixture) to achieve similar plasma concentrations as those found in humans. Total plasma EGCG concentrations shown to be efficacious in mice and rats can be reached by an intake of low to moderate doses of EGCG in humans.

2.11 Effect on obesity

African black tea extract has been shown to suppress the elevation of blood glucose during food intake and reduce the body weight in KK-A(y)/TaJcl diabetic mice (Shoji *et al.,* 2006). Although few epidemiological and clinical studies have shown the health benefits of EGCG on obesity and diabetes, the mechanisms of its actions are emerging

based on various laboratory data. These mechanisms may be related to certain pathways, such as through the modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, and redox status (Yang *et al.*, 2001).

A double-blind, placebo-controlled, cross-over design study showed that consumption of a beverage containing green tea catechins, caffeine, and calcium increases 24-h energy expenditure by 4.6%, but the contribution of the individual ingredients could not be distinguished. It was suggested that such modifications were sufficient to prevent weight gain. It has been reported that the body weights of rats and their plasma triglyceride, cholesterol, and low-density lipoprotein cholesterol were significantly reduced by feedings of Oolong, black, and green tea leaves to the animals. In addition, the inhibition of growth and suppression of lipogenesis in MCF-7 breast cancer cells may be through down-regulation of fatty acid synthase gene expression in the nucleus and stimulation of cell energy expenditure in the mitochondria. When fed to mice, EGCG purified from green tea decreased diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. The increased and prolonged sympathetic stimulation of thermo genesis by the interaction between polyphenols and caffeine could be of value in assisting the management of obesity (Dulloo *et al.*, 2000).

CHAPTER III

MATERIALS AND METHODS

3.1 Location of research work

The experiment was conduct to study the effect of green tea extract in the diets of broiler chicks, at Kornai local private farm, near to the HSTU campus, Basherhat, Dinajpur. The laboratory works were done at operation theater, Faculty of Veterinary Medicine, HSTU, Dinajpur. Hematological test were performed private diagnostic center, Dinajpur.

3.2 Collection, processing and storage of green tea extract (GTE)

I collect fresh green leaves from Green care tea garden, Tetulia, Panchagarh. The green leaves were cleaned and sun-dried on a hygienic polyethylene paper. The leaves were ground properly by a grinder machine.

Photo 1: Preparation of green tea extract

Dried green tea powder (300 g), extracted with 500 ml distill water, sonicated for 3 h, filtered, and extracted .The filtrates were transferred to 50 ml tubes for use.

3.3 Preparation of the experimental house and equipment

A gable type open sided house was used for experimental purpose. The room was divided into 5 separate pens of equal size by using wire net and bamboo materials. The experimental room was thoroughly brushed and swiped. After that the room walls and wire nets were disinfected by spraying oxycol solution (Manufactured by EWABO chemikalien Gmbh &Co.). Feeders, waterers, buckets and all other necessary equipments were also properly, washed and disinfected by povisep solution.

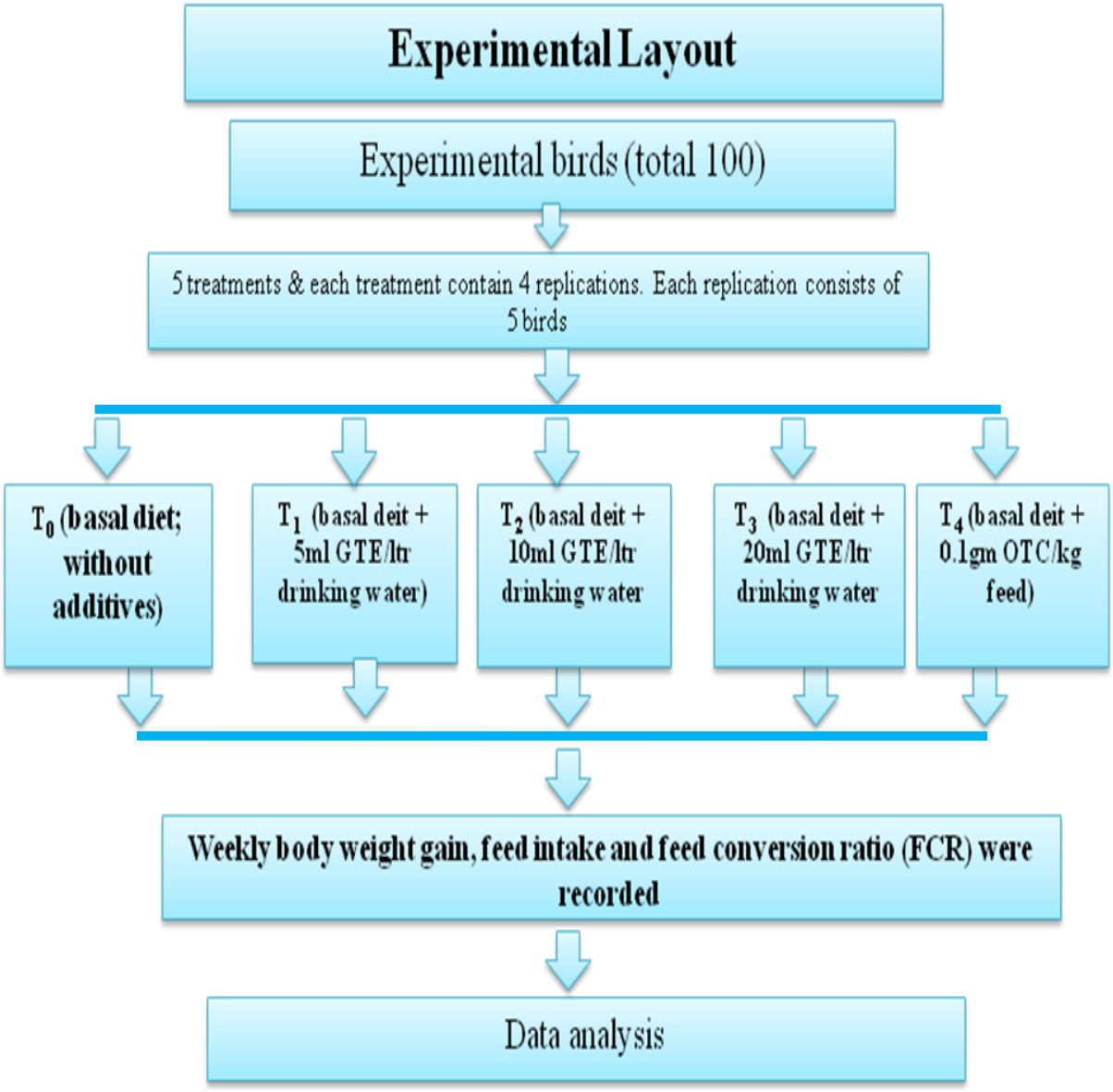
3.4 Collection of the experimental broiler

DOC Cobb 500 commercial broiler chicks were used for this experiment. The chicks were collected from Nourish Poultry and Hatchery.

3.5 Experimental design

Broilers were randomly divided into five (5) equal groups (5×20) and each group is divided into 4 replication each having 5 birds per replication i.e. 4×5, then the group was numbered as group T_0, T_1, T_2, and T_4. The experimental diets were designed as T_0: control, T_1: 0.5% GTE, T_2: 1% GTE, T_3: 2% GTE and T_4: 0.1 gm of Oxytetracyclin.

3.6 Experimental layout



3.7 Formulation of broiler ration

At 1st weeks broiler was feed with broiler pre starter. The 2nd weeks broiler was feed with broiler mash starter. 3rd weeks to 5th weeks broiler was feed with Mash finisher. The nutrient requirements (ME, CP, CF, EE, Ca, P, Lysine and Methionine) were satisfied as per requirement as recommended for Cobb-500 broiler strain diet and also same for all treatment groups.

Table 2: Ingredient composition of broiler pre-starter ration (100kg feed)

Ingredients	**Broiler pre-starter**
Maize	60.00 kg
Rice polish	5.00 kg
Soybean	25.00 kg
Fish meal	7.00 kg
Oyster shell	1.00 kg
Salt	300 g
Methionine	100 g
Lysine	100 g
Vitamin Premix (broiler)	250
Feed zyme	50 g
Soybean oil	.5 Litter
DCP	250 g
Choline chloride	50 g
Toxin Binder	200g
Salmonellar killer	150g
Coccidiostate	50g
Total	100.00 kg

Source**:** Nourish Poultry and Hatchery Ltd.®, Bangladesh

Table 3: Ingredient composition of broiler Mash starter ration

Ingredients	**Broiler Mash starter**
Maize	60.00 kg
Rice polish	5.00 kg
Soybean	25.00 kg
Fish meal	7.00 kg
Oyster shell	1.00 kg
Salt	300 g
Methionine	100 g
Lysine	100 g
Vitamin Premix (broiler)	250
Feed zyme	50 g
Soybean oil	.5 Litter
DCP	250 g
Choline chloride	100 g
Toxin Binder	250g
Salmonellar killer	250g
Coccidiostate	50g
Total	100.00 kg

Source**:** Nourish Poultry and Hatchery Ltd.®, Bangladesh

Table 4: Ingredient composition of broiler mash finisher ration

Maize	58.00 kg
Rice polish	7.00 kg
Soybean	25.00 kg
Fish meal	7.00 kg
Oyster shell	1.00 kg
Salt	300 g
Methionine	100 g
Lysine	100 g
Vitamin Premix (broiler)	250
Feed zyme	50 g
Soybean oil	.5 Litter
DCP	250 g
Choline chloride	100 g
Toxin Binder	250g
Salmonellar killer	250g
Coccidiostate	50g
Total	100.00 kg

Source**:** Nourish Poultry and Hatchery Ltd.®, Bangladesh

Table 5: The vaccination schedule

Age of experimental birds (day)	Name and type of vaccine	Preparation of dilution	Dose and route of administration
5	Cevac New-L	1 ampoule was diluted with 10 ml of distilled water	One drop in each eye
10	Cevace IBD-L	1 ampoule was diluted with 10 ml of distilled water	One drop in each eye
15	Cevace IBD-L	Diluted with Drinking water 2itter	Drinking water
21	Cevac New-L	Diluted with Drinking water 3 litter	Drinking water

Source: Manufactured by Ceva Sante Animale and marketed by ACI Animal Health

Photo 2: Vaccination of birds

3.8. Sanitation

The experimental period adequate hyiegene and sanitation were maintained. The entrance and the outside of the shed should be kept clean and sprinkle bleaching power alternative day.

3.9 Bio-security

The experimental period proper bio-security measures taken. Entrance of personnel was restricted except workers, researchers. At the time of visiting farm the following procedure should be maintain:

- Feet were dipped in a footbath containing disinfectant solution.
- Hands should be dipped in hand dippe and shoes were changed.

3.10 Record Keeping

3.10.1 Body weight:

The broiler chicks were weighted as a group at the beginning of the experiment and then every seven days interval.

Photo 3: Measurement of body weight

3.10.2 Feed intake

The amount of feed consumed by the experimental birds was different groups was recorded weekly basis.

3.10.3 Feed conversation ratio

Feed conversation ration was calculated by using the following formula

$$\text{Feed conversation ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{live weight gain (g)}}$$

3.10.4 Mortality

It was calculated on the basis of total number of birds housed and number of birds died during the experimental period.

3.11 Collection of Blood Serum

Sample blood was collected at age 35 day and with a syringe and needle directly through wing vein puncture without using any anticoagulant. By the end of each treatment all the birds were sacrificed.

Photo 3: Blood collection

Photo 4: Thigh Muscle weight measurement

Photo 5: Breast Muscle weight measurement

3.12 Blood profile measurements

Total plasma cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were determined in plasma using biochemical analysis. The very low density lipoprotein (VLDL) was calculated using the following formula where results were expressed in mg/dL.

VLDL Cholesterol = Plasma triglycerides/5

However, the low density lipoprotein (LDL) was estimated as the following:

LDL Chol = Total Chol – (VLDL Chol + HDL Chol) (Adler and Holub 1997)

3.13 Statistical analysis

- The Experiment was performed under CRD (Complete Randomized Design)
- One way ANOVA following the SPSS (Version 20.00) was used for analysis.
- All data were expressed as mean ± SEM. Differences were considered signification at $P \leq 0.05$

CHAPTER-IV

RESULTS AND DISCUSSION

Dietary effects of green tea extract an alternative of antibiotic on broiler production are presented in Table 6-10.

4.1 Effect of green tea extract on body weight, Feed intake and Feed conversion of broilers

Table 6: Weekly Live weight (g/bird) of broiler in different treatment groups

Age (wk)	T_0	T_1	T_2	T_3	T_4	Level of significance
Day old	42.17±.07	42.28 ±.071	42.06 ±.09	42.30 ±.15	42.12 ±.05	NS
1st week	156.1 ±2.45	152.2 ±2.16	156.4 ±2.04	154.7 ±2.48	157.4 ±2.55	NS
2nd week	405.3 ±2.63	409.7 ±3.49	404.5 ±2.48	402.3 ±2.04	398.8 ±3.87	NS
3rd week	790.2 ± 4.56[a]	861.3 ± 4.30[d]	840.2 ± 3.89[c]	818.0 ±6.38[b]	810.7 ±3.49[b]	*
4th week	1217.0 ±6.16[a]	1403.0 ± 4.55[e]	1321.0± 5.71[d]	1244.0 ±3.63[b]	1272.0± 2.94[c]	*
5th week	1887.0 ±3.74[a]	2034.0 ± 2.16[e]	1961.0± 1.47[e]	1903.0 ±4.29[b]	1935.0± 1.19[c]	*

NS = None significance

*Correlation is significant at the 0.05 level

Table 7: Weekly feed intake (g/bird) of broiler in different treatment groups

Age (wk)	T_0	T_1	T_2	T_3	T_4	Level of significance
1st week	122.1 ±1.41	121.5 ±.72	120.3 ±1.08	123.5 ±1.55	121.7 ±1.47	NS
2nd week	329.9 ±2.16	339.9 ± 4.71	328.9 ±2.68	319.6 ±2.16	328.5 ±3.24	NS
3rd week	556.0 ± 3.89[a]	609.1 ± 2.86[d]	604.8 ± 2.49[cd]	596.4 ± 2.78[c]	578.7 ± 2.78[b]	*
4th week	898.8 ± 4.09[c]	858.8 ±4.26[b]	871.4 ±4.30[b]	801.6±4.08[a]	887.5 ± 3.19[c]	*
5th week	1288.0± 17.79[c]	1219.0 ±4.02[a]	1227.0 ±2.68[a]	1256.0± 2.16[b]	1256.0 ±2.32[b]	*
Total	3194.0 ±1.87[d]	3148.0 ±3.34[b]	3152.0 ±1.08[b]	3097.0 ±3.34[a]	3173.0 ± 1.08[c]	

NS = None significance

*Correlation is significant at the 0.05 level

Table 8: Weekly feed conversion ratio of broiler in different treatment groups

Age (wk)	T_0	T_1	T_2	T_3	T_4	Level of significance
1st week	0.78 ±.01	0.79 ±.02	0.76 ±.02	0.79 ±.01	0.77 ±.02	NS
2nd week	1.11 ±.01	1.12 [a] ±.01	1.11 ±.01	1.11 ±.01	1.12 ±.01	NS
3rd week	1.27 ±.01	1.24 [a] ±.02	1.25 ±.01	1.26 ±.02	1.26 ±.02	NS
4th week	1.558 ± .02[c]	1.370 c±.01[a]	1.450 ±.02[b]	1.480 ±.02[b]	1.460 ±.02[b]	*
5th week	1.63 ±.02[b]	1.58 a ±.01[a]	1.60 ±.02[b]	1.62 ±.01[b]	1.61 ±.02[b]	*

NS = None significance

*Correlation is significant at the 0.05 level

Green tea extract containing diet at different ages of body weights, feed intake, and feed conversion are presented in table 6-8. Body weight was equal in all groups at 2 wks, though higher body weight was observed in 0.5% GTE feeding group for the following 5 wks. Feed intake measured on a group basis tended to decrease with GTE feeding, in which 0.5% supplementation level seemed most inhibitory. The resultant feed conversion tended to be improved in 0.5% GTE feeding groups.

Kaneko *et al.,* (2001) observed the beneficial effect of green tea on the productive performance and lean meat production of the broilers and ion other study Sarker *et al.,* (2010) reported that the diet containing 0.5% green tea was effective for the growth performance and meat composition of broilers

The feed intake is important factor that determines the rate of growth and body composition achieved by animals throughout their lifecycles (Richards, 2003). The improvement in the body weight and feed efficiency of birds fed with diets containing the GTE shows that the use of these products is a feasible alternative to antimicrobial feed additives used as growth promoters. The discrepancy between the studies may be explained that the differences in total catechin content and its major components, such as epicatechin, epigallocatechin, epicatechin gallette, epigallocatechin gallette of the green tea and green tea extract used in the studies.

The action mechanism of phytogenic and herbal feed additives varies depending on numerous factors (Yang *et al*., 2009), the significant influence of the additive on the final body weight and feed efficiency in our study could be attributed to the composition of the basal diet, origin and polyphenols of the green tea used and the time of harvest, the preparation method of phytogenic additive and/or the environmental conditions. The growth promoting agents may have more impact when the diet used is less digestible and well-nourished, healthy chicks do not respond to antibiotic supplements provided that they are housed under the clean and disinfected conditions (Lee *et al.,* 2003; Hernandez *et al.,* 2004; Cross *et al.,* 2007; Toghyani *et al et al.,* 2010).

4.2 Effect of green tea extract on Carcass Characteristics of broilers

Table 9: Different body parts weight in different treatments groups

Age (wk)	T_0	T_1	T_2	T_3	T_4	Level of significance
Dressed wt (g)	$1057.0^{d} \pm 2.48^{a}$	1139.0 ± 4.71^{e}	1098.0 ± 4.02^{d}	1066.0 ± 3.19^{b}	1084.0 ± 2.48^{c}	*
Thigh muscle (g)	283.0 ± 1.78^{a}	$305.1^{a} \pm 1.87^{d}$	294.6 ± 1.65^{c}	285.5 ± 2.27^{ab}	290.4 ± 1.87^{b}	*
Breast muscle (g)	$320.8^{d} \pm 3.19^{a}$	$345.5^{a} \pm 2.62^{d}$	333.4 ± 1.78^{c}	323.5 ± 1.08^{ab}	329.1 ± 1.87^{b}	*
Intestine (g)	10.94 ±.01	11.80 ±.02	11.37 ±.01	11.04 ±.01	11.23 ±.01	NS
Liver (g)	56.61 ±.02	57.02 ±.01	58.83 ±.01	$56.59 \pm .01^{a}$	58.08 ±.01	NS
Gizzard (g)	75.48 ±.12	79.36 ±.11	78.44 ±.15	76.12 ±.11	$77.44^{b} \pm .07$	NS

NS = None significance

*. Correlation is significant at the 0.05 level

Green tea extracts containing diet on carcass characteristics of broilers are presented in table 10. Intestine, liver and gizzard did not significantly differed ($p>0.05$) among the experimental birds. However dress weight is significantly higher in 0.5% GTE supplemented and antibiotic group compare to control group. Breast and thigh muscle weight were significantly higher in the broiler supplemented with 0.5% GTE and antibiotic compare to the control group.

4.3Effect of green tea extract on blood profile of broilers

Table 10: Blood profile of different treatment groups

Age (wk)	T_0	T_1	T_2	T_3	T_4	Level of significance
Total cholesterol (mg/dl)	156.0 ± 2.55^{d}	115.0 ±2.16 ^{a}	124.4 ±1.87 ^{b}	141.6 ± 1.08^{c}	197.3 ± 1.08^{e}	*
HDL (mg/dl)	16.25 ±.85^{b}	25.40 ± 1.08^{d}	12.24 ±.71^{a}	20.50 ± 1.08^{c}	30.75 ± 1.47^{a}	*
LDL (mg/dl)	119.3 ±1.47	118.7 ±1.19	116.3 ±2.27	115.8 ±2.27	117.2 ± 1.47	NS
Triglyceride (mg/dl)	124.7 ±1.47^{b}	116.3 ±1.47^{a}	117.2 ±1.08^{a}	118.8 ± 1.04^{a}	132.3 ±1.31^{c}	*

NS = None significance

** Correlation is significant at the 0.05 level

Effect of green tea extract on blood profile are presented in table 11. All GTE supplemented group showed significantly lower total cholesterol, triglyceride ($p < 0.05$) when compared to the control group.

1.0 and 2.0% green tea extract with lard/cholesterol diets significantly reduced blood cholesterol level in rats (Muramatsu *et al.*, 1986).

1.0% tea polyphenol in diets decreased significantly the serum cholesterol content in rats (Sano *et al.*, 1991). It was demonstrated in other studies that high catechin contents in green tea may have an inhibitory effect on the intestinal absorption of lipid (Ikeda *et al.*, 1992).

0.5 to 1.5% green tea supplementation in broiler diets and 1.0% to 2.0% green tea powder on layers had effects on reducing the cholesterol content of broiler meat and egg yolk of layers (Uuganbayar *et al.*, 2005, 2006). These trial results are similar to those obtained at inclusion levels from 0.5% in our experiment.

CHAPTER V

SUMMARY AND CONCLUSIONS

A total of 100 DOC Cobb 500 broiler chicks were randomly considered to evaluate the effects of a green tea extract an alternative of antibiotic on the performance to commercial broiler reared up to 5 weeks of age. The chicks were divided into five dietary treatment groups. These were control, green tea extract @ 0.5%, Green tea extract@ 1.0%, Green tea extract@ 2.0% and antibiotic @ 0.1g/kg feed having 4 replications in each group. Feed was supplied ad libitum and fresh drinking water was made available at all times.

The weekly live weight of broiler in different groups showed no significant difference among the various groups from 1st wk to 2 nd wks buts there were significant difference from 3 rd wks to 5th wks. In 5th wks higher live weight was in T_1 that were 2034 g/bird with 0.5% GTE and lower weight was in T_0 that were 1887 g/bird with no additives.

The weekly feed intake of broiler in different groups showed no significant difference among the various groups from 1st wk to 2 nd wks buts there were significant difference from 3 rd wks to 5th wks. In 5th wks higher feed intake was in T_0 that were 1288 g/bird with no additives and lower feed intake was in T_1 that were 1227 g/bird with 0.5% GTE.

The weekly feed conversion of broiler in different groups showed no significant difference among the various groups from 1st wk to 3rd wks buts there were significant difference from 4th wks to 5th wks. In 5th wks higher feed conversion was in T_0 that were 1.63 with no additives and lower feed conversion was in T_1 that were 1.58 with 0.5% GTE.

Carcass characteristics of broiler supplemented with green tea extract and antibiotic in diet is lightly affected. lntestine, liver and gizzard did not significantly differed ($p>0.05$) among the experimental birds. However dress weight is significantly higher in 0.5% GTE supplemented and antibiotic group compare to control group. Breast and high muscle weight were significantly higher in the broiler supplemented with 0.5% GTE and antibiotic compare to the control group.

Green tea extract has an effect on the blood profile. The cholesterol level is significantly ($p>0.05$) vary in different groups. Here the total cholesterol and Triglyceride are lower in the group of broiler supplied 0.5% GTE compare to other group of GTE and antibiotic group**.** The experiment shows that feeding of green tea extract to broiler has no effect on LDL of blood profile.

In conclusion, results from the current trail suggested that the GTE supplementation may be suitable for growth performance and improve feed efficiency for broiler production. The dietary GTE supplementation at 0.5% to broiler chicken diets is favorable to the consumers because it makes broilers with less fat and cholesterol deposition, and less oxidative profile without serious adverse effect on general performance.

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