**EFFECTS OF DIETARY ESSENTIAL OILS (*Eucalyptus globulus*) AS ALTERNATIVE TO ANTIBIOTICS IN BROILER**

**Md. Ariful Hasan**

**Roll No: 0117/18**

**Registration No: 00438**

**Session: 2017-2018**

**A thesis submitted in the partial fulfillment of the requirements for the**

**Degree of Master of Science in Applied Human Nutrition & Dietetics**



**Department of Applied Food Science and Nutrition**

**Faculty of Food Science and Technology**

**Chattogram Veterinary and Animal Sciences University**

**Chattogram-4225, Bangladesh**

**June 2019**

# AUTHORIZATION

I, Md. Ariful Hasan assure that I have performed all works furnished here in this report. The Information has been collected from books, national and international journals, websites and other references. All references have been acknowledged duly.

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**The Author**

June 2019

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This is to certify that we have examined the above Master’s thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

**--------------------------------------------------------**

(**Professor Dr. Md. Manirul Islam**)

Supervisor

**--------------------------------------------------------**

(**Md. Altaf Hossain**)

Chairman of the Examination Committee

**Department of Applied Food Science and Nutrition**

**Faculty of Food Science and Technology**

**Chattogram Veterinary and Animal Sciences University**

**Chattogram-4225, Bangladesh**

**June 2019**

***Dedication***

***DEDICATED TO MY BELOVED PARENTS***

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**The Author**

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# List of Abbreviations

|  |  |
| --- | --- |
| **Abbreviation** | **Elaboration** |
| **GRAS** | Generally Recognized As Safe |
| ADG | Average Daily Gain |
| g/b | Gram/bird |
| g/b/d | Gram/bird/day |
| **FDA** | the Food and Drug Administration |
| WHO | World Health Organization |
| **%** | Percentage |
| **°C** | Degree centigrade |
| °F | Degree Farenhite |
| v/v | Volume/ Volume |
| w/v | Weight/ Volume |
| **EO** | essential oil |
| BDT | Bangladeshi taka |
| FCR | Feed Conversion Ratio |
| LDL | Low density Lipoprotein |
| HDL | High Density Lipoprotein |
| SE | Standard Error |
| DM | dry matter |
| CP | crude protein |
| CF | crude fiber |
| EE | ether extracts |
| TA | and total ash |
| Fig | Figure |
| FAO | Food and Agriculture Organization |

**Abstracts**

The study was conducted to investigate the effects of different levels of Dietary Essential Oils (*Eucalyptus globulus*) on growth performance, carcass characteristics, meat quality and blood parameters in broiler. A total of 96 day old Ross 308 unsexed broiler chicks were randomly distributed into four dietary treatment groups: T0= Control (basal diet); T1= Antibiotics (basal diet+ 0.1% amoxiciline in drinking water); T2 = Essential oil (basal diet+ 0.4ml/L eucalyptus oil, DM basis ); T3= Essential oil (basal diet+ 0.8ml/L eucalyptus oil, DM basis). Each treatment group consist of 3 replications having 6 birds in a completely randomized design for 28 days trial period. Results indicated that, in 1st week T1 treatment group has the highest average daily gain (ADG). During 2nd ,3rd and 4th week T1 treament group has highest Average daily gain (ADG) while T0 stands for lowest in every week (P< 0.05).

But in terms of FCR T3 has the highest feed conversion ratio while T1 has the lowest. A significant increase in blood HDL level while decreased LDL and total cholesterol in all treatments compared to control (P< 0.05). Considering meat quality, crude protein (CP), ether extract (EE) and ashes were decreased singnificantly in all treatment groups compared to control. In case of mortality T3 treatment group has shown the best result compared to control group.In conclusion, dietary supplementation of *Eucalyptus globulius* essential oil decreased weekly weight gain, better FCR, decreased mortality, CP, EE, total ashes and blood HDL level. On the other hand, it reduced blood LDL and total cholesterol level.

**Keywords:** Broiler, *Eucalyptus globulius,* essential oil, growth performannce, carcass characteristics.

**Chapter-I**

**Introduction**

* 1. **General Feature**

Poultry is considered as a cheap and readily available protein source for human consumption worldwide. In the third world country like Bangladesh poultry sector has proven its promise in eliminating protein deficiency especially from the lower middle class people. But with the rapid growth of poultry sector a major human health concern is noticed relating to the excessive and uncontrolled abuse of antibiotics which is leading to antibiotic resistant bacteria, a major threat to human race. This increasing use of antibiotics may lead to the spread of antibiotic-resistant bacteria in both pigs and humans, which is identified as a signiﬁcant public health threat (Yang et al., 2015). Antibiotics used at sub therapeutic doses also the use of antimicrobial growth promoters have been found beneficial for rapid growth performance in animal production level and prevention of disease occurrence (Barton et al., 2000; Snel et al., 2002). Since 2006 European Union banned the use of Antibiotic Growth Promoters (AGP) in food animal production (Bengtsson and Wierup, 2006). The U.S. Food and Drug Administration placed restrictions on antibiotic use in animals in December 2016 (Zhao et al., 2007). For this reason, it is crying need to develop alternatives to antibiotics to prevent diseases and improve bird performance for the use of organic poultry producers. Beneficial effects of plants and plant extracts that have traditional use are evaluated in many studies. Most common beneficial effects of those plants and plant extracts are stimulating endogenous digestive enzymes and antioxidants (Lee et al., 2004).

Plant-derived products (phytobiotics) are natural, less toxic than antibiotics, and typically residue free in comparison with synthetic antibiotics or inorganic chemicals. Many are certified as Generally Recognized as Safe **(GRAS**) by the Food and Drug Administration (**FDA)** and therefore, make them acceptable to use as feed additives in organic poultry production (Wang et al., 1998).

Several growth and health promoting properties have been attributed to phytobiotics usage in poultry. These benefits are derived by improving gut health including increasing digestibility (Mitsch et al., 2004; Kroismayr et al., 2008), modifying digestive secretions, and sustaining and improving gut histology (Williams and Losa, 2001; Kreydiyyeh et al., 2003; Jamroz et al., 2003). Furthermore, some phytobiotics stabilize the microbiome, which reduces microbial toxins (Steiner, 2006; Windisch et al., 2008; Perič et al., 2010). This, in turn, reduces inflammation and; therefore, protein production can be allocated to growth as opposed to production of immune modulators (Kroismayr et al., 2008; Steiner, 2006).

Scientist have found organic acids (Eckel et al., 1992; De Lange et al., 2010), probiotics (Heo et al., 2004, 2013; Musa and Seri, 2009), enzymes (Bedford and Cowieson, 2012; Kiarie et al., 2013), medium chain fatty acids (Boyen et al., 2008), antimicrobial peptides (Choi et al., 2013) and essential oils (Windisch et al., 2008; Randrianarivelo et al., 2010; Gong et al., 2013) as effective alternatives to antibiotics in feeds.

Among phytobiotics, essential oils have gained more interest due to their antimicrobial and growth promoter properties. Essential oils are compounds obtained by distillation or solvent extraction from aromatic plants, herbs, or spices (Yang et al., 2009). Many EOs contain multiple active components and these components are primarily used to protect the plants from damage caused by insects and bacteria. Each component may have a different mechanism of action and these components can work synergistically (Senatore, 1996; Russo et al., 1998).

The leaves of the eucalyptus tree have been used in aboriginal medicinal remedies for thousands of years. Nowadays they are also used in modern medicine to treat various common ailments. Eucalyptus oil has antibacterial effects on pathogenic bacteria in the respiratory tract. The Inhaled eucalyptus oil vapors are a decongestant and treatment for bronchitis. Cineole controls airway mucus hyper secretion and asthma via anti-inflammatory cytokine inhibition. Eucalyptus oil also stimulates immune system response by effects on the phagocytes ability of human monocyte derived macrophages (Priyanka et al., 2017). Eucalyptus and peppermint oils proved to be able to implement innate-cell mediated, humoral immune response and have a potent immune modulatory effect in chickens (Awaad et al., 2010).

Essential oils have been widely used as traditional medicines to improve health or cure diseases in humans as it has the ability to possess antimicrobial, anti-inﬂammatory and antioxidative activities (Kim et al., 2008; Brenes and Roura, 2010). The bioactive components in essential oils have been identiﬁed and some progress have been made to elucidate the mechanisms underlying the functions of these compounds in animals, leading to increased research efforts to use essential oils to replace antibiotics in animal feeds (Li et al., 2012). However, the application of essential oils in the feed has been mainly based on the antimicrobial effects. Moreover, the minimum inhibitory concentration (MIC) of most essential oils are much higher than the acceptable levels in animal industry in terms of cost-effectiveness and feed palatability (Yang et al., 2015).

Previous researches had been conducted by mixing Eucalyptus oil directly in feed. As Eucalyptus oil is volatile so it is possible to loss mostly by evaporation. Therefore, the present research has been designed by dissolving eucalyptus essential oil into water might be a new approach and it will reduce evaporation.

**Research questions:**

Will present research be able to provide a better result?

Will we get a better alternative to antibiotics?

Will eucalyptus oil positively affect the growth performance, blood parameter and meat quality?

Will it be cost effective for poultry farmers?

* 1. **Objectives**
     1. **General Objective**

This research aims to find an alternative to commercially used antibiotics by using eucalyptus essential oil.

* + 1. **Specific Objectives**
* To find an effective alternative to antibiotics.
* To investigate the growth performance of broiler in supplementation of essential oil.
* To assess the effect of essential oil on meat quality in broiler.
* To determine the blood parameters in supplementation of essential oil.

Chapter-II

Review of literature

**2.1 Essential oils**

Aromatic, volatile and oily liquids extracted from plant materials such as seeds, ﬂowers, leaves, buds, twigs, herbs, bark, wood, fruits and roots are named as essential oils (Brenes and Roura, 2010). Among 3,000 known essential oils, 300 of which have commercially importance and used in pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries as effective alternatives or complements to synthetic compounds (Bakkali et al., 2008). In recent years the food industry and animal producers have increased their interest in the use of Essential Oil (EO), not only for their anti-oxidative and anti-inflammatory properties, but also for their antimicrobial, coccidiostatic, antihelmintic and anti-viral effects (Wei and Shibamoto, 2007; Rhodes et al., 2006; Burt, 2004; Nakatani, 2000; Craig, 1999; Cuppett and Hall, 1998; Hirasa and Takemasa, 1998; Halliwell et al., 1995;). Multiple oils, including carvacrol, thymol , obtained from oregano (Origanum glandulosum) or eugenol (**EUG**) obtained from the oil cloves (Eugenia caryophillis), are reported to inhibit many pathogenic bacteria (Applegate et al., 2010; Kollanoor et al., 2010; Si et al., 2006; Dorman and Deans, 2000)

EOs are gaining more interest in conventional and organic poultry nutrition, primarily focusing on the improvement of gut functions. Stabilizing the microflora in the digestive tract is one of the positive effects of EO, which improves nutrient utilization and absorption. EOs improve nutrient utilization and absorption by increasing the activity of digestive enzymes including trypsin and amylase (Jang et al., 2004; Lee et al., 2003). Additionally, active components increase intestinal secretions of mucus, which prevents the adhesion of pathogens (Jamroz et al., 2006).

Essential oils are more effective against gram-positive than gram-negative bacteria because the gram-negative bacteria have an effective permeability barrier against external noxious agents (Vaara, 1992). Essential oils have been proven as an alternative to antibiotics by many researchers because they have antimicrobial, anti-inﬂammatory, antioxidative, and coccidiostatic properties. They enhance digestibility (Chitprasert and Sutaphanit, 2014) and immunity (Brenes and Roura, 2010), promote gut health by minimizing the effect of the pathogenic bacteria (Chitprasert and Sutaphanit, 2014), and control odor and ammonia emission (Varel, 2002).

Some researchers recorded that the supplementation of the mixture of herbs (thyme, bay, sage, myrtle leaf, fennel and citrus essential oil) to the broiler´s diets, significantly improved live weight, feed efficiency and carcass characteristics of the broilers ([Alçiçek et al., 2004, 2003](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B1)).

It is reported that herbal extracts or active substances may increase feed intake and may improve secretion of endogenous digestive enzymes ([Jamroz et al., 2003](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B24); [Craig, 1999](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B14)).Besides that, it has been reported that essential oils have antibacterial effects in chickens ([Skoufos et al., 2016](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B37); [Giannenas et al., 2014](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B22); Bölükbaşi et al ., 2008, 2009; [Faleiro et al., 2003](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B21); [Cowan, 1999](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2017000500015#B16)).

**2.1.1 Effect of essential oils on feed palatability and digestibility and nutrient metabolism**

Flavor and odor of feed promotes with the addition of essential oils for this reason feed palatability and intake increase (Kroismayr et al., 2006). It is believed that the increased feed palatability associated with the supplementation of essential oils could also be due to their antioxidative properties that can preserve the qualities of diets and prevent the release of unfavorable odors from the diets (Sola-Oriol et al., 2011; Franz et al., 2010). So it might be possible to replace chemical antioxidants (e.g., ethoxyquin and butylated hydroxytoluene) which are frequently used in the animal diet with enough amounts of essential oils (natural antioxidants), particularly when chemical antioxidants are prohibited (Yang et al., 2015).

**2.2 *Eucalyptus***

*Eucalyptus* (family Myrtaceae) is a genus of tall, evergreen tree which is an Australian native, that have around 700 species. It is a magnificent tree cultivated worldwide for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one which have extensive use in perfumery, food and pharmaceutical industry. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal (Batish et al., 2008).

**2.2.1 Scientific Classification**

Kingdom : Plantae

Class : Magnoliapsida

Order : Myrtales

Family : Myrtaceae

Genus : *Eucalyptus*

Species : *Globulus*

**2.2.2 Botanical Information**

Species used : The family includes more than 500 species such as

E. biostatic : Southern Blue Gum, Eurabbie, Victorian Blue Gum

E. globules : Tasmanian Blue Gum

E. maiden : Maiden's Gum

Synonyms : Southern blue gum, Tasmanian blue gum, Maiden” gum

Category : Strong nervine and anxiolytic

Plants parts used : Leaf and Barks



**Figure 1:** *Eucalyptus* tree

**2.2.3 Morphological Description**

The *Eucalyptus globulus* bark sheds often, peeling in large strips. The broad juvenile leaves are borne in opposite pairs on square stems. They are about 6 to 15 cm long and covered with a blue-grey, waxy bloom, which is the origin of the common name "blue gum". The mature leaves are narrow, sickle-shaped and dark shining green. They are arranged alternately on rounded stems and range from 15–35 cm (5.9–13.8 in) in length. The buds are top-shaped, ribbed and warty and have a flattened operculum (cap on the flower bud) bearing a central knob. The cream-colored flowers are borne singly in the leaf axils and produce copious nectar that yields a strongly flavored honey. The fruits are woody and range from 1.5–2.5 cm (0.59–0.98 in) in diameter (Pereira et al., 2014).

**2.2.4 Geographical Distribution**

*Eucalyptus globulus* is naturally distributed in Tasmania and south-eastern Australia, but is now widely planted and naturalized in subtropical regions around the world. In tropical Africa it is found in cool highland regions, especially in Ethiopia, where it was introduced around 1890. The introduction of *Eucalyptus globulus* to Ethiopia is said to have played a major role in the development of the country (Gobel et al., 2002). In recent years *Eucalyptus globulus* is seen to be cultivated in Bangladesh and India mostly for timber.

**2.3 *Eucalyptus* essential oil**

Eucalyptus oils in the trade are categorized into three broad types according to their composition and main end-use: medicinal, perfumery and industrial (Vigo et al., 2004). The most prevalent is the standard cineole-based "oil of eucalyptus", a colourless mobile liquid with a penetrating, camphoraceous, woody-sweet scent. China produces about 75% of the world trade, but most of this is derived from camphor oil fractions rather than being true eucalyptus oil. Significant producers of true eucalyptus oil include South Africa, Portugal, Spain, Brazil, Australia, Chile and Swaziland ( Salari et al., 2006). Global production is dominated by *Eucalyptus globulus*. However, *Eucalyptus kochii* and *Eucalyptus polybractea* have the highest cineole content, ranging from 80-95%. The British Pharmacopoeia states that the oil must have a minimum cineole content of 70% if it is pharmaceutical grade. Rectification is used to bring lower grade oils up to the high cineole standard required. Global annual production of eucalyptus oil is estimated at 3,000 tonnes. The *eucalyptus* genus also produces non-cineole oils, includes piperitone, phellandrene, citral, methyl cinnamate and geranyl acetate. Eucalyptus oil should not be confused with the term "eucalyptol", another name for cineole.

According to (Tyagi et al., 2011) the major constituents of E. globulus essential oil were 1,8-cineole (45.4%), limo-nene (17.8%), p-cymene (9.5%), c-terpinene (8.8%), a-pinene (4.2%) and a-terpineol (3.6%).

Chemical constitutes of *E. globulus* essential oil vapor and oil found by (Tyagi et al., 2011) is shown Table 1 and Table 2.

**Table 1.** Chemical constitutes of *E. globulus* essential oil vapor

|  |  |  |
| --- | --- | --- |
| RT (min) | Compound | Percentage |
| 8.5 | a-Pinene | 4.0 |
| 12.5 | a-Phellandrene | 2.4 |
| 14.0 | Limonene | 29.9 |
| 14.3 | 1,8-Cineole | 34.6 |
| 14.8 | b-Ocimene | 0.5 |
| 15.2 | c-Terpinene | 7.4 |
| 16.1 | p-Cymene | 10.5 |
| 17.6 | Epoxylinalol | 0.1 |
| 22.4 | Linalool oxide | 0.1 |
| 24.7 | Linalool | 0.2 |
| 25.9 | Fenchol | 0.1 |
| 26.5 | Terpinen-4-ol | 0.5 |
| 27.6 | Menthol | 0.2 |
| 28.1 | Pinocarveol | 0.2 |
| 29.3 | a-terpineol | 0.8 |
|  | Monoterpene hydrocarbon | 54.7 |
|  | Oxygenated monoterpenes | 36.8 |
|  | Total identified compound | 91.5% |

Retention Indices on AB-Innowax column, Relative area percentage without using the FID response correction factor, RT: Retention Time (min), (Results are based on GC-MS; MS acquisition started after 4 min).

**Table 2.** Chemical constitutes of *E. globulus* essential oil.

|  |  |  |  |
| --- | --- | --- | --- |
| RT (min) | Compound | Percentage | RI |
| 6.4 | a-Pinene | 4.2 | 1036 |
| 9.0 | b-Myrcene | 1.5 | 1156 |
| 9.1 | a-Phellandrene | 1.3 | 1173 |
| 9.5 | a-Terpinene | 0.4 | 1188 |
| 10.1 | Limonene | 17.8 | 1206 |
| 10.4 | 1,8-Cineole | 45.4 | 1233 |
| 10.8 | b-Ocimene | 1.0 | 1238 |
| 11.2 | c-Terpinene | 8.8 | 1247 |
| 11.9 | p-Cymene | 9.5 | 1269 |
| 13.3 | Epoxylinalol | 0.1 | – |
| 17.7 | Linalool oxide | 0.2 | 1423 |
| 19.8 | Linalool | 0.5 | 1506 |
| 21.1 | Fenchol | 0.1 | 1574 |
| 21.7 | Terpinen-4-ol | 1.4 | 1601 |
| 22.0 | a-Humulene | 0.1 | 1607 |
| 22.7 | Menthol | 0.1 | 1612 |
| 23.3 | Pinocarveol | 0.4 | – |
| 24.4 | a-Terpineol | 3.6 | 1731 |
| 24.5 | Borneol | 0.1 | 1735 |
| 28.4 | Geraniol | 0.1 | 1797 |
|  | Monoterpene hydrocarbons | 44.5 |  |
|  | Oxygenated monoterpenes | 52.0 |  |
|  | Total of identified compound | 96.6% |  |

Retention Indices on AB-Innowax column, Relative area percentage without using the FID response correction factor, RT: Retention Time (min), (Results are based on GC-FID; MS acquisition started after 4 min).

**2.3.1 Use of Eucalyptus essential oil**

A number of studies have demonstrated the antimicrobial properties of Eucalyptus essential oils against a wide range of microorganisms*. E. citriodora* oil has been shown to have a wide spectrum of antifungal activity. In addition, *Eucalyptus camaldulensis* and *Eucalyptus urophylla* are also well known for their antibacterial ([Cimanga et al., 2002](#page7)) and antifungal ([Su YC et al.,](#page8) [2006](#page8)) activities. Several studies have focused on the anti-fungal properties of Eucalyptus essential oils (Somda I et al., 2007; [Ramezani H](#page7) et al., [2002](#page7)). Research carried out by (Han et al., 2011) shows that the performance of pig fed eucalyptus MCFA blend was the same as that of antibiotics.

Traditionally, many Eucalyptus species are used in Chinese folk medicine. For example, hot water extracts of dried leaves of *Eucalyptus citriodora* (lemon-scented Eucalyptus) are used as anti-inflammatory, analgesic and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion ([Silva et al., 2003](#page7)). The essential oils produced by *E. citriodora* are used for medicinal and pharmaceutical purposes ([Ghisalberti, 1996; Leung et al.,](#page7) [1996](#page7)).

In Japan ‘‘Eucalyptus leaf extract’’ has been approved as a natural food additive, and is also included as one of the antioxidants in the ‘‘List of Existing Food Additives in Japan’’ ([Amakura et al., 2002](#page7)). Recent studies focus on the functional aspects of the Eucalyptus extracts/ essential oil. Eucalyptus extracts exhibit various biological effects, such as antibacterial, antifungal, antihyperglycemic and antioxidant activities ([Takahashi T et al., 2004](#page8)).

Chapter-III

Materials and methods

## 3.1 Trail area

The study was conducted in experimental poultry shed of Department of the Animal Science and Nutrition, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh.

## 3.2 Study Period

The overall research work was designed and performed from July 2018 to March 2019. July was considered as a representation of monsoon seasons (Islam et al., 2006). In July, average maximum temperature records cited as 29 to 32°C and humidity was 78 to 82% (BMD, 2017).

## 3.3 *Eucalyptus* Essential oil extraction

### 3.3.1 Collection of *Eucalyptus* leaves

Fresh *Eucalyptus* leaves were collected from several locality of Chattogram region from1st September to 15th October, 2018.



**Fig 2:** Leaf preparation

**3.3.2 Cleaning of leaves**

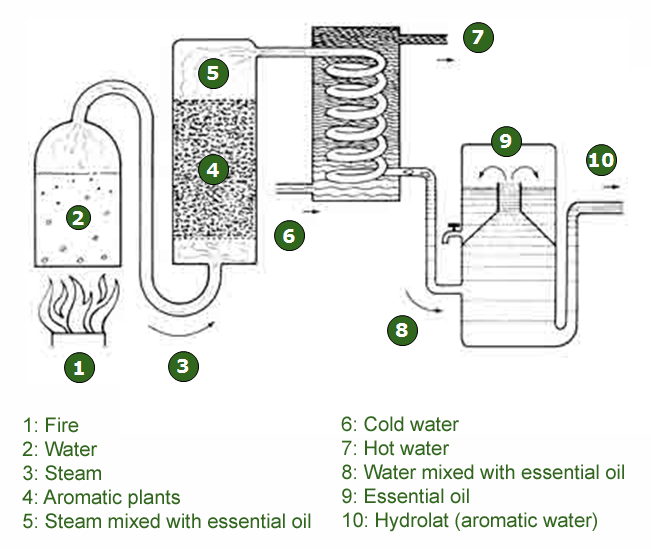
Freshly collected leaves are separated from stem. All kinds of foreign particles are removed from leaves then leaves are taken in a basket. After that leaves are washed with tape water to remove any kinds of dirt.

**3.3.3 Distillation of oil**

Fresh washed leaves are kept in distillation pot then distilled water added to the pot after that lid is tightly fitted to pot, condenser is carefully connected as though no steam can be leaked. After arranging all properly gentle heat is applied from gas stove. Cold water is used to condense the steam.



**Fig 3:** Essential oil distillation



**Figure 4:** Steam distillation process

**3.4 Design of the experiment**

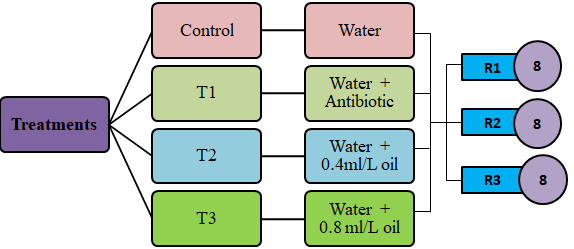
Ninety six chicks were divided randomly into four dietary treatment groups consisting

3 replications having 6 birds in a completely randomized design for 28 days.



**Figure`5:** Dividing the chicks into different dietary treatment groups

T0 = Control (Normal water), T1: Antibiotic (Normal water + Renaquine), T2: Eucalyptus essential oil (Water + 0.4 ml oil/L) and T3: Eucalyptus essential oil (Water + 0.8ml oil/L)



**Figure 6:** Design of the experiment

**3.5 Animals and housing**

Ninety six day old unsexed broiler chicks of Ross 308® were purchased from Progressive Hatcheries Limited, address Chattogram, Bangladesh. All the chicks were examined for abnormalities and uniform size. Average body weight of the chicks was 40.74±0.26 g. The experimental shed was brick cemented with corrugated metal wiring. Floor space for each bird was 0.17 square feet in brooding box and 0.75 square feet in the cage. The cages were further divided into 12 pens. The pens were selected in an unbiased way for uniform distribution of chicks. The chicks were brooded for two weeks. Each pen was allocated for 6 birds. Dry and clean newspaper was placed in the brooding box and changed for every 6 hours. Room temperature and humidity was maintained using 40 watt incandescent lamps and ceiling fans. The birds were exposed to continuous lighting. During brooding period, chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Temperatures were measured by using thermometer.



Figure 7: Temperature and humidity maintain during Brooding period

3.6 Cleaning and sanitation

The shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding boxes, rearing cages and pens were cleaned in the same manner. After cleaning and disinfection, the house was left one week for proper drying. After drying, all doors and windows were closed. The room was fumigated (Adding 35 ml of formalin to 10 g potassium permanganate per cubic meter) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders were cleaned and washed with Temsen® solution (0.3% v/v) weekly before being used further. Drinkers were washed with potassium permanganate (1% w/v) and dried up daily in the morning.

## 3.7 Diets

Feed ingredients were purchased from Pahartali market, Chattogram, Bangladesh. During purchase, cleanliness and date of expiry were checked. Five different types of rations were formulated. Each ration had two different types i.e., starter (0 to 14 days) and finisher (15 to 28 days). All rations were iso-caloric and iso-nitrogenous. The composition of different feed ingredients and nutritive value of starter and grower rations are given in Table 3 and Table 4

**Table 3.** Ingredients names with their chemical composition of the broiler starter ration (1-14 days)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients**  (as percent feed basis) | **T0** | **T1** | **T2** | **T3** | **T4** |
| Maize | 59.50 | 59.25 | 59.00 | 59.25 | 59.00 |
| Rice polish | 1.50 | 1.25 | 1.00 | 1.25 | 1.00 |
| Soya bean meal | 33.20 | 33.20 | 33.20 | 33.20 | 33.20 |
| Vegetable oil | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 |
| Supplement | 0.00 | 0.50 | 1.00 | 0.50 | 1.00 |
| Molasses | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Limestone | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Vit-min premix1 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DCP2 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| DL-Methionine3 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| L-Lysine4 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Toxin binder5 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.0 |
| **Estimated chemical composition** | | | | | |
| Met. Energy6(kcal/kg) | 2965.82 | 2965.75 | 2965.13 | 2966.23 | 2966.30 |
| Crude protein (%) | 20.65 | 20.63 | 20.63 | 20.68 | 20.70 |
| Crude fiber (%) | 3.90 | 4.10 | 4.50 | 4.00 | 4.40 |
| Calcium (%) | 0.94 | 0.98 | 1.00 | 1.10 | 1.30 |
| Phosphorus (%) | 0.75 | 0.65 | 0.60 | 0.80 | 0.84 |
| Lysine (%) | 1.20 | 1.14 | 1.17 | 1.17 | 1.60 |
| Methionine (%) | 0.54 | 0.57 | 0.60 | 0.65 | 0.67 |
| Cysteine and Methionine (%) | 0.74 | 0.75 | 0.73 | 0.70 | 0.67 |
| Tryptophan(%) | 0.25 | 0.23 | 0.22 | 0.32 | 0.29 |

1Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D3 1000 IU, K 1.6 mg, B1 1 mg, B2 2 mg, B3 16 mg, B6 1.6 mg, B9 320 μg, B12 4.8 μg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 μg); 2DCP (18% P, 23% Ca); 3DL-Methionine (Purity 99.0%); 4L-Lysine (Purity 99.0%); 5Toxin Binder (Purity 98%, all imported from Poland); 6Metabolizable energy (kcal/kg).

**Table 4.** Ingredients names with chemical composition of the broiler grower ration (15-28 days)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients (**as percent feed basis**)** | **T0** | **T1** | **T2** | **T3** | **T4** |
| Maize | 60.10 | 59.95 | 59.60 | 59.95 | 59.60 |
| Rice polish | 1.85 | 1.60 | 1.35 | 1.60 | 1.35 |
| Soya bean meal | 31.00 | 31.00 | 31.00 | 31.00 | 31.00 |
| Vegetable oil | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 |
| Supplement | 0.00 | 0.50 | 1.00 | 0.50 | 1.00 |
| Molasses | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Limestone | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Vit-min premix1 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DCP2 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| DL-Methionine3 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| L-Lysine4 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Toxin binder5 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Total | 100 | 100 | 100 | 100 | 100 |
| **Estimated chemical composition** | | | | | |
| Met. Energy6(kcal/kg) | 3057.9 | 3057 | 30.57.5 | 3057.75 | 3058 |
| Crude protein (%) | 19.75 | 19.59 | 19.45 | 19.74 | 19.78 |
| Crude fiber (%) | 6.17 | 6.22 | 6.25 | 6.21 | 6.23 |
| Calcium (%) | 0.94 | 0.92 | 0.9 | 0.97 | 0.99 |
| Phosphorus ( %) | 0.73 | 0.7 | 0.68 | 0.8 | 0.89 |
| Lysine (%) | 1.05 | 1.04 | 1.02 | 1.25 | 1.35 |
| Methionine (%) | 0.53 | 0.52 | 0.51 | 0.6 | 0.68 |
| Cysteine and Methionine (%) | 0.74 | 0.73 | 0.72 | 0.67 | 0.64 |
| Tryptophan (%) | 0.23 | 0.22 | 0.21 | 0.22 | 0.25 |

1Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D3 1000 IU, K 1.6 mg, B1 1 mg, B2 2 mg, B3 16 mg, B6 1.6 mg, B9 320 μg, B12 4.8 μg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 μg); 2DCP (18% P, 23% Ca); 3DL-Methionine (Purity 99.0%); 4L-Lysine (Purity 99.0%); 5Toxin Binder (Purity 98%, all imported from Poland); 6Metabolizable energy (kcal/kg).

**3.8 Experimental Water Preparation**

Normal tape water is provided in all the treatment groups. Iso propeyl alcohol is added as an emulsifier to dissolve Eucalyptus essential oil into water where T0= Normal tape water, T1= Tape water + Antibiotic, T2= Tape water + 0.4% Eucalyptus essential oil, T3= Tape water + 0.8% Eucalyptus essential oil

## 3.9 Feeding of birds

Feed was prepared manually and supplied ad-libitum to the birds on round small feeder and waterer for 0-7 days. After 7th day, small round feeders and waterers were replaced by medium linear feeders (2.21 ft X 0.25 ft.) and round waterers. At 15th day, large linear feeder (3.5 ft. X 0.38 Ft.) and round waterers (3-liter capacity) were provided for feeding and drinking of the birds.

**3.10 Medications**

All birds were vaccinated against Newcastle disease (BCRDV live) and Infectious Bursal Disease on the 4th day followed by a booster dose on 14th day. After each vaccination, multivitamin (Rena-WS, Renata; 1g/ 5liter of drinking water) was supplied along with Vitamin-C to overcome the effect of stress due to vaccination and cold shock.

****

**Figure 8:** Vaccination of poultry

## 3.11 Carcass measurement

At the end of the 28 day trail, one bird was randomly selected from each replicate and killed by severing the jugular vein and carotid artery. Once a bird was adequately bleed out, it was scalded and feather was removed. After de-feathering, the birds were eviscerated and the head and feet were removed as per technique described by Jones (1984). During evisceration process, abdominal fat, liver, spleen and Versa were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.



**Figure 9:** Collection of internal organs

**3.12 Analysis of meat**

After slaughter, 120 g of meat was collected in the air tight bag from each carcass for estimation of the chemical composition of meat. Meat samples were kept at 5°C in air tight bag. After that, chemical analyses of the meat samples were carried out in triplicate for dry matter (DM), crude protein (CP), crude fiber (CF), ether extracts (EE) and total ash (TA) in the animal nutrition laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram as per AOAC (2006).

**Figure 10:** Meat sample analysis

## 3.13 Hematological analysis

Blood samples were collected from the brachial vein of four birds from each group (Two birds from each replicate) using a 3 ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant, ethylene diamine tetra acetic acid and 5ml blood was kept without anticoagulant. Blood was collected without anticoagulant from a total of four birds from each group at 28th days of age. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the ependroff tube by micropipette. Sera samples were marked and stored in -20°C until being analyzed for LDL, HDL, total cholesterol and triglyceride. Randox® veterinary reagent kits were used for determination of the blood parameter of interest. Serum sample was mixed with the respective reagents in an ependroff tube. The serum with reagent was aspired by spectrophoto-metric method which measured the target parameter and immediately the printed result was recorded.



**Figure 11:** Hematological analysis

## 3.14 Data collection

Weight gain, feed intake and FCR were recorded at weekly intervals. Carcass characteristics, hematological and biochemical parameters were recorded at 4th week. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total amounts of feed supplied to the birds. FCR was calculated dividing feed intake by the weight gain.



**Fig 12:** Weight recording

**3.15 Data analysis**

Data were compiled in MS Excel. Raw data related to weight gain, feed intake, FCR, carcass characteristics, hematological and biochemical parameters were tested for normality by using normal probability plot and analyzed for ANOVA by using **SAS (2009).** Means showing significant differences were compared by Duncan’s Multiple Range Test **(Duncan, 1955).** Statistical significance was accepted at p<0.05 for F-tests.

**Chapter-IV**

**Results**

The objective of the conducted study was to observe and interpret effects of Eucalyptus essential oil by observing growth performance, carcass characteristics and biochemical parameters of Ross-308 broilers.

**4.1 Growth performance**

Growth performance of the experimental broiler which include average daily gain (ADG), average daily feed intake (ADFI) and FCR are shown in the table 6.

## 4.1.1 Average daily gain (ADG)

No significant variation was observed in ADG for T2 and T3 during 1st, 2nd and 3rd week (P>0.05) compared to control. A significant increase in ADG was found during 2nd and 4th week (P<0.05) compared to control and treatment groups. Highest ADG was found in antibiotic supplemented group during 2nd, 3rd and 4th week of age. At fourth week, significant difference (P<0.05) observed between the treatment groups where T1 showed the best performance while T3 is the worst (Table 6).

**4.1.2 Average daily feed intake (ADFI)**

Tables 6 represent the average daily feed intake for different treatment groups of this experiment. Average daily feed intake significantly differed among the treatment, antibiotic and control group (P<0.05). During 1st and 2nd week, T1 has the highest ADFI while T2 and T3  has the lowest (P<0.05). In 3rd week, T0 and T1 have the higest feed intake while T2 and T3 have the lowest (P<0.05). During 4th week, T1 treatment group have the highest feed intake and T3 treatment group stand for the lowest (P<0.05).

**4.1.3 Feed conversion ratio (FCR)**

The feed conversion ratio during 1st and 2nd week reduced significantly (P<0.05) in T2 and T3 treatment groups while it was found higher for T1 group. At 3rd and 4th week, T1 showed the highest FCR value while T3 group has the lowest (P<0.05).

**Table 6.** Effects of eucalyptus essential oil on growth performance in broiler

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Age of birds | Parameters | Treatments | | | | P-Value |
| T0  (Mean± SE) | T1  (Mean± SE) | T2  (Mean± SE) | T3  (Mean± SE) |
| 1st week | Initial wt (g/b) | 55.06 ± 0.06 | 55.06 ± 0.06 | 55.00± 0 | 55.11±0.06 | 0.49 |
| Final wt (g/b) | 233.89± 0.56 | 233.33± 0.96 | 231.11± 1.11 | 230.56± 0.56 | 0.0565 |
| ADG(g/b/d) | 25.55± 0.08 | 25.47± 0.13 | 25.16± 0.16 | 25.07± 0.09 | 0.06 |
| ADFI(g/b/d) | 33.5 5b ± 0.38 | 35.32a± 0.33 | 30.867c± 0.50 | 30.41c± 0.23 | <0.0001 |
| FCR | 1.31b± 0.01 | 1.39a± 0.01 | 1.23c± 0.01 | 1.21c± 0.01 | <0.0001 |
| 2nd week | Initial wt (g/b) | 233.89± 0.56 | 233.33± 0.96 | 231.11± 1.11 | 230.56± 0.56 | 0.06 |
| Final wt (g/b) | 598.89a± 8.41 | 606.67ab±11.55 | 572.78bc± 7.22 | 569.44c± 5.30 | 0.03 |
| ADG(g/b/d) | 52.14ab± 1.22 | 53.33a ±1.58 | 48.81b± 0.96 | 48.41b± 0.76 | 0.04 |
| ADFI(g/b/d) | 70.43b± 2.42 | 76.42a± 1.81 | 61.33c± 1.24 | 59.87c± 0.96 | 0.0004 |
| FCR | 1.35b± 0.02 | 1.43a± 0.01 | 1.26c ± 0.01 | 1.24c ± 0.01 | <0.0001 |
| 3rd week | Initial wt (g/b) | 598.89ab ± 8.41 | 606.67a ± 11.55 | 572.78bc ± 7.22 | 569.44c ± 5.30 | 0.03 |
| Final wt (g/b) | 917.22± 22.22 | 919.45± 4.01 | 876.11± 8.41 | 875.00± 0.96 | 0.05 |
| ADG(g/b/d) | 45.48 ± 2.05 | 44.68 ±1.11 | 43.34± 0.36 | 43.65 ± 0.84 | 0.6261 |
| ADFI(g/b/d) | 63.54± 3.18 | 64.67± 2.25 | 54.02± 0.23 | 52.66± 0.83 | 0.005 |
| FCR | 1.40b ± 0.01 | 1.45a ± 0.01 | 1.25c ± 0.01 | 1.21d ± 0.01 | <0.0001 |
| 4th week | Initial wt (g/b) | 917.22± 22.22 | 919.44± 4.01 | 876.11± 8.41 | 875.00± 0.96 | 0.05 |
| Final wt (g/b) | 1297.22a ± 6.96 | 1330.55a ± 12.99 | 1223.33b ± 15.12 | 1176.66c ± 10.13 | <.0001 |
| ADG(g/b/d) | 54.28ab ± 2.74 | 58.73a ± 1.79 | 49.60b ± 0.96 | 43.10c ± 1.39 | 0.002 |
| ADFI(g/b/d) | 76.33b ± 3.53 | 87.88a ± 2.43 | 62.83c ± 1.09 | 52.88d ± 2.01 | <.0001 |
| FCR | 1.40b ± 0.01 | 1.50a ± 0.01 | 1.27c± 0.003 | 1.23d ± 0.01 | <.0001 |

abc Different superscipt in the same row indicate significant variation among treatments (P<0.05).

Each data indicates means of three replications consisting of six birds per replication (n=18)

T0= Control (basal diet);T1=Antibiotics (basal diet+ 0.1% amoxiciline in drinking water);T2= Essential oil (basal diet+ 0.4ml/L eucalyptus oil in drinking water);T3=Essential oil (basal diet+ 0.8ml/L eucalyptus oil in drinking water).

SE= Standard error

## 4.2 Blood serum parameters

Different serum parameters estimated have been presented in table 7. A significant reduction (P<0.05) of LDL value was found in treatment T3 group while it was statistically non-significant for other treatment groups (P>0.05). HDL value was found higher in all three treatment groups except control (P<0.05). In case of total cholesterol T2 group shows the highest value, whereas T1 group showed the highest triglyceride level among treatment groups (P<0.05).

**Table 7**. Effects of eucalyptus essential oil on blood serum parameters in broiler.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Treatments | | | | P- Value |
| T0  (Mean± SE) | T1  (Mean± SE) | T2  (Mean± SE) | T3  (Mean± SE) |
| LDL(mg/dl) | 78.20a ± 1.85 | 71.20a ± 0.72 | 79.43a ± 2.65 | 45.50b ± 6.16 | 0.0004 |
| HDL(mg/dl) | 44.77b ± 0.91 | 57.87a ± 5.19 | 65.00a ± 2.12 | 70.10a ± 4.53 | 0.0058 |
| Total cholesterol(mg/dl) | 123.03b ± 2.64 | 129.07b ± 5.64 | 144.43a ± 4.77 | 115.60b ±1.85 | 0.0057 |
| Triglyceride(mg/dl) | 26.77b ± 2.92 | 82.33a ± 17.93 | 62.83a ± 1.01 | 17.10b ± 1.32 | 0.0031 |

ab Different superscipt in the same row indicate significant variation among treatments (P<0.05).

Each data indicates means of three replications consisting of two birds per replication (n=6)

T0= Control (basal diet);T1=Antibiotics (basal diet+ 0.1% amoxiciline in drinking water);T2= Essential oil (basal diet+ 0.4ml/L eucalyptus oil in drinking water);T3=Essential oil (basal diet+ 0.8ml/L eucalyptus oil in drinking water).

SE= Standard error

## 4.3 Chemical Composition of meat

Value of moisture, ether extract (EE), crude protein (CP) and ashes have been estimated from the lab tests of carcass of different treatment groups. The biochemical lab tests indicated that T3 treatment group showed the lower moisture content (P<0.05). While higher CP was obtained from T1 and T0 (P<0.05). An increased EE and ash value were obtained in T0  treatment group (P<0.05).

**Table 8**. Effects of eucalyptus essential oil on meat proximate components in broiler

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Treatments | | | | P- Value |
| T0  (Mean± SE) | T1  (Mean± SE) | T2  (Mean± SE) | T3  (Mean± SE) |
| DM (%) | 24.93a ± 1.33 | 24.66ab ± 0.34 | 23.77c ± 0.067 | 24.07bc ± 0.15 | 0.0113 |
| Moisture (%) | 75.07ab ± 0.13 | 75.33a ± 0.34 | 76.23a ± 0.067 | 72.57b ± 1.57 | 0.5808 |
| CP (%) | 26.19a ± 0.16 | 26.66a ± 0.12 | 24.74b ± 0.21 | 24.27b ± 0.21 | < 0.0001 |
| EE (%) | 2.20a ± 0.15 | 1.67ab ±0.34 | 1.44b ± 0.23 | 0.66c ± 0.08 | 0.0078 |
| Ash (%) | 1.38a ± 0.03 | 1.28b ± .03 | 1.34ab ± 0.02 | 1.25b ± 0.03 | 0.0664 |

abc Different superscipt in the same row indicate significant variation among treatments (P<0.05).

Each data indicates means of three replications consisting of two birds per replication (n=6)

T0= Control (basal diet);T1=Antibiotics (basal diet+ 0.1% amoxiciline in drinking water);T2= Essential oil (basal diet+ 0.4ml/L eucalyptus oil in drinking water);T3=Essential oil (basal diet+ 0.8ml/L eucalyptus oil in drinking water).

SE= Standard error

## 4.4 Carcass Characteristics

Different carcass characteristics have been presented in table 9. Comparative weights of different treatment groups among the carcass component (meat) and weights of different organs have been sequentially described. There is no significant difference in live weights, liver, bursa and abdominal fat. Though T3 showed the best dressed weight while T0 have found the lowest (P>0.05). In case of spleen weight T0 showed the higher value (P>0.05).

**Table 9**. Effects of eucalyptus essential oil on carcass characteristics and relative organs weight in broiler

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Treatments | | | | P- Value |
| T0  (Mean± SE) | T1  (Mean± SE) | T2  (Mean± SE) | T3  (Mean± SE) |
| Live Wt (%) | 1303.33 ± 82.12 | 1350 ± 70.00 | 1330 ± 10.00 | 1156.67 ± 94.93 | 0.2914 |
| Dress (%) | 55.03c ± 0.43 | 58.56b ± 0.51 | 58.89b ± 0.49 | 61.20a ± 0.33 | 0.2281 |
| Liver (%) | 2.56 ± 0.29 | 2.74 ± 0.17 | 2.61 ± 0.15 | 2.78 ± 0.26 | 0.8895 |
| Spleen (%) | 0.17a ± 0.03 | 0.12ab ± 0.01 | 0.10b ± 0.01 | 0.10b ± 0.01 | 0.0698 |
| Bursa (%) | 0.19± 0.04 | 0.24 ± 0.02 | 0.19 ± 0.05 | 0.14 ± 0.003 | 0.2585 |
| Abdominal fat (%) | 1.78 ± 0.11 | 1.36 ± 0.31 | 1.72 ± 0.17 | 1.29 ± 0.08 | 0.2414 |

ab Different superscipt in the same row indicate significant variation among treatments (P<0.05).

Each data indicates means of three replications consisting of six birds per replication (n=18)

T0= Control (basal diet); T1=Antibiotics (basal diet+ 0.1% amoxiciline in drinking water); T2= Essential oil (basal diet+ 0.4ml/L eucalyptus oil in drinking water); T3=Essential oil (basal diet+ 0.8ml/L eucalyptus oil in drinking water).

SE= Standard error

**4.5 Mortality**

Mortality of bird are shown in the following bar diagrum. From the diagrum it is clear that mortality of T0  treatment group is higher then any other group . lowest mortality is observed in the T3 treatment group where T3 is treated with 0.8ml/L eucalyptus oil in drinking water**.**

**Figure 13:** Mortality of bird

**Table 10.** Effects of eucalyptus essential oil on Cost-benefit analysis in broilers (BDT=Bangladeshi taka)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter/bird | Treatments | | | |
|  | T0 | T1 | T2 | T3 |
| Live weight (kg) | 1.27 | 1.33 | 1.22 | 1.17 |
| Selling price (BDT) | 165.00 | 172.00 | 160.00 | 150.00 |
| Total feed intake (kg) | 1.70 | 1.80 | 1.50 | 1.40 |
| Total Feed cost (BDT) | 71.50 | 75.60 | 63.00 | 58.80 |
| Dug cost (antibiotic) | 0.00 | 13.8 | 0.00 | 0.00 |
| EO cost (BDT) | 0.00 | 0.00 | 5.00 | 10.00 |
| Other cost (BDT)1 | 50.00 | 50.00 | 50.00 | 50.00 |
| Total cost (BDT) | 121.50 | 139.40 | 118.00 | 118.00 |
| Net profit (BDT) | 43.50 | 32.60 | 42.00 | 32.00 |
| Net profit /kg (BDT) | 34.25 | 24.06 | 34.42 | 27.35 |

1Other costs including chick cost, vaccination and transportation cost.

**Chapter-V**

**Discussion**

Effects on performance of different probiotics and dry leaves treated chicks have been discussed during these 28 days of feed trial under this chapter. Weight gain, feed intake, feed conversion ratio, serological parameters, biochemical changes and carcass characteristics has been given more emphasis.

5.1 Average daily gain (ADG)

Current study has revealed that treatment with *E. globulus* EO decreases Average daily gain (ADG) comparing the control group. Current study partially agrees with the research conducted by Farhadi et al. (2016) where dietary supplementation of eucalyptus leaf powder decreased BWG during 7-28 days of age, but dietary addition of Eucalyptus EO had no effect on BWG.

But this is directly opposite to the result found by Ibrahim et al. (2018). A study conducted by Ibrahim et al. (2018) showed that weight gain increases with the increase of Eucalyptus oil concentration actually they have found best result when they treated with 600 mg/kg of feed compared to control group but unfortunately this result does not agree with the result of current study. May be direct consumption of eucalyptus oil with water have negative effect on weight gain. Lower weight gain may be due to lower feed intake in the treatment group. During the study T1 treatment group which is treated with antibiotic have the highest weight gain because antibiotics used at sub therapeutic doses also the use of antimicrobial growth promoters have been found beneficial for rapid growth performance in animal production level and prevention of disease occurrence (Barton et al., 2000; Snel et al.,2002).

5.2 Average daily feed intake (ADFI)

In present study, gradual decrease in feed intake has been observed actually T3 treatment group have the lowest figure compared to control group. After 4th week T1 treatment group has the highest feed intake unfortunately this result does not fully agree with Farhadi et al. (2016). Farhadi et al. (2016) found no effect on feed intake due to Eucalyptus EO supplementation but broilers receiving 3,000 mg/kg ELP in their diet had lower FI during 7-28 days of age.

Antibiotic treated group showed highest feed intake, this may because antibiotics used at sub therapeutic doses also the use of antimicrobial growth promoters have been found beneficial for rapid growth performance in animal production level and prevention of disease occurrence (Barton et al., 2000; Snel et al., 2002).

## 5.3 Feed conversion ratio (FCR)

In the research conducted by Farhadi et al. (2016) where dietary supplementation of eucalyptus leaf powder and Eucalyptus EO have no effect on FCR during 7-28 days of age but in our research we found that dietary addition of Eucalyptus EO had better impact on FCR.

T3 treatment group which is treated with Eucalyptus essential oil (Water + 0.8ml oil/L) showed the best performance in FCR this is because dietary essential oils can improve digestion (Anonymous, 1997; Mellor, 2000 a,b). It might be reasoned that spices and herbs, from which essential oils are derived, have been shown to positively affect food digestion (Pradeep et al., 1991; Pradeep and Geervani, 1994). A number of studies have reported the effect of spices or their active components on bile salt secretion (Bhat and Chandrasekhara, 1987; Bhat et al., 1984, 1985; Sambaiah and Srinivasan, 1991).

**5.4 Blood serum parameters**

Estimation of serum parameters in the current study shows that there are significant changes in serum parameters among the various treatment groups. HDL concentration is found higher in Water + 0.8ml oil/L treated groups while found lower value in control group. In case, LDL, it’s higher in control groups and found lower in Water + 0.8ml oil/L treated group. Total cholesterol is also found higher in control groups and lowest value was found in Water + 0.8ml oil/L treated group. It might be due to synergistic effects of phytochemicals. Decreased level of LDL and Total cholesterol components in treatment groups makes the efficacy of essential oil treated groups while the increased level of HDL components also validate the efficacy of same treatment groups on serological components (Yang et al., 2009) .

**5.5 Chemical Composition of meat**

Chemical estimation of the meat composition shows that moisture components is less in Water + 0.8ml oil/L treated group that of the other treatment groups while Crude protein percentage is higher in T0 and T1 while EE and Ashes are both higher in the T0 group.

**5.6 Carcass Characteristics**

Carcass characteristics show that, weight percentage has no significant difference among the treatment groups but dressing percentage is higher in T3 treatment group. No significant weight difference is found in internal organ which is similar to the result found by (Ibrahim et al., 2018).

**5.7 Mortality**

Mortality rate shows that mortality of bird is higher in T0 treatment group where no antibiotic or essential oil were added. In case of antibiotic it stands 2nd in position in terms of mortality. Best result was found in T3 treatment group where it was treated with eucalyptus essential oil **(**0.8ml oil/L) but (Farhadi et al., 2016**)** concluded that there is no influence on mortality by the dietary supplementation of Eucalyptus EO.

Mortality may be reduced because essential oils have the ability to possess antimicrobial, anti-inﬂammatory and antioxidative activities, (Kim et al., 2008; Brenes and Roura, 2010).

Chapter-VI

Conclusion

The study investigated the effects of Eucalyptus essential oil supplementation on the performance parameters, carcass characteristics and blood parameters in commercial broiler under intensive rearing system. Highest weight gain was observed in birds fed with antibiotic supplement. Optimum feed intake and better FCR were observed in birds fed with Water + 0.8ml oil/L supplement. There were drastically changes in the serum parameters in comparison with the reference level especially in LDL, HDL, Total cholesterol. There is no significant change in weight gain but dressing percentage is higher in birds fed with Water + 0.8ml oil/L supplement. But a significant decrease in mortality was observed. However, a long term investigation with larger sample size and multi-dimensional temporal pattern is suggested for increasing sensitivity and validity of the study under field condition.

Chapter-VII

Recommendations

*Eucalyptus globulus* essential oil has shown negative effect on growth performance of the broiler so right now *Eucalyptus globulus* essential oil cannot be recommended as a diet supplement of broiler. More research is required to use it as an effective supplement for broiler. But if we want to produce low fat meat *Eucalyptus globulus* essential oil can be used but the long term effect of supplementation on productive performance of broilers should be investigated in future.

Due to some unavoidable constraints and technical limitations, some vital blood parameters like glucose, calcium, phosphorus and other trace minerals both in meat and blood were not analyzed. These parameters could have vital impact on human health and will explore new horizon for investigating those parameters as future study.

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**Appendices**

**Appendix A:** Carcass Characteristics

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | |  |  |
| Treatment | Live weight in kg | Kill weight in kg | Dressed weight in g | Liver weight in g | Spleen weight in g | Bursa weight in g | Abdominal fat weight in g |
| T0R1 | 1.14 | 1.11 | 700 | 35.8 | 2.07 | 2.61 | 18.81 |
| T0R2 | 1.37 | 1.33 | 710 | 32.44 | 3.01 | 3.15 | 23.2 |
| T0R3 | 1.4 | 1.35 | 740 | 30.41 | 1.66 | 1.52 | 27.84 |
| T1R1 | 1.37 | 1.34 | 810 | 39.61 | 1.65 | 2.71 | 25.64 |
| T1R2 | 1.22 | 1.2 | 720 | 35.75 | 1.81 | 3.02 | 9.94 |
| T1R3 | 1.46 | 1.42 | 840 | 35.01 | 1.45 | 4 | 20.58 |
| T2R1 | 1.31 | 1.28 | 760 | 30.07 | 1.49 | 3.79 | 20.91 |
| T2R2 | 1.34 | 1.3 | 800 | 37.24 | 1.5 | 1.83 | 27.65 |
| T2R3 | 1.34 | 1.29 | 790 | 36.79 | 0.91 | 2.04 | 20.01 |
| T3R1 | 1.22 | 1.2 | 780 | 40.13 | 0.96 | 1.59 | 17.53 |
| T3R2 | 0.97 | 0.93 | 600 | 24.01 | 1.1 | 1.4 | 11.54 |
| T3R3 | 1.28 | 1.23 | 740 | 32.71 | 1.52 | 1.79 | 15.75 |

**Appendix B:** Serum parameters

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | LDL | HDL | Total Cholesterol | Triglyceride |
| T0R1 | 74.5 | 43.4 | 117.9 | 30.5 |
| T0R2 | 79.9 | 44.4 | 124.5 | 21 |
| T0R3 | 80.2 | 46.5 | 126.7 | 28.8 |
| T1R1 | 72.5 | 67 | 139.5 | 114.4 |
| T1R2 | 71.1 | 49 | 120.1 | 52.4 |
| T1R3 | 70 | 57.6 | 127.6 | 80.2 |
| T2R1 | 77.9 | 63.4 | 141.3 | 64.6 |
| T2R2 | 84.6 | 69.2 | 153.8 | 62.8 |
| T2R3 | 75.8 | 62.4 | 138.2 | 61.1 |
| T3R1 | 57.1 | 62.2 | 119.3 | 19.7 |
| T3R2 | 36.1 | 77.9 | 114 | 15.4 |
| T3R3 | 43.3 | 70.2 | 113.5 | 16.2 |

**Appendix C:** Weekly weight record in kg

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Initial | 1st week | 2nd week | 3rd week | 4th week |
| T0R1 | 0.33 | 1.41 | 3.57 | 5.37 | 7.7 |
| T0R2 | 0.33 | 1.4 | 3.69 | 5.77 | 7.83 |
| T0R3 | 0.331 | 1.4 | 3.52 | 5.37 | 7.82 |
| T1R1 | 0.331 | 1.41 | 3.76 | 5.55 | 8.12 |
| T1R2 | 0.33 | 1.39 | 3.64 | 5.53 | 7.85 |
| T1R3 | 0.33 | 1.4 | 3.52 | 5.47 | 7.98 |
| T2R1 | 0.33 | 1.38 | 3.48 | 5.28 | 7.37 |
| T2R2 | 0.33 | 1.4 | 3.48 | 5.33 | 7.48 |
| T2R3 | 0.33 | 1.38 | 3.35 | 5.16 | 7.17 |
| T3R1 | 0.33 | 1.39 | 3.42 | 5.24 | 7.07 |
| T3R2 | 0.331 | 1.38 | 3.36 | 5.26 | 7.16 |
| T3R3 | 0.331 | 1.38 | 3.47 | 5.25 | 6.95 |

**Appendix D:** weekly weight gain in kg

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | 1st week | 2nd week | 3rd week | 4th week |
| T0R1 | 1.08 | 2.16 | 1.8 | 2.33 |
| T0R2 | 1.07 | 2.29 | 2.08 | 2.06 |
| T0R3 | 1.069 | 2.12 | 1.85 | 2.45 |
| T1R1 | 1.079 | 2.35 | 1.79 | 2.57 |
| T1R2 | 1.06 | 2.25 | 1.89 | 2.32 |
| T1R3 | 1.07 | 2.12 | 1.95 | 2.51 |
| T2R1 | 1.05 | 2.1 | 1.8 | 2.09 |
| T2R2 | 1.07 | 2.08 | 1.85 | 2.15 |
| T2R3 | 1.05 | 1.97 | 1.81 | 2.01 |
| T3R1 | 1.06 | 2.03 | 1.82 | 1.83 |
| T3R2 | 1.049 | 1.98 | 1.9 | 1.9 |
| T3R3 | 1.049 | 2.09 | 1.78 | 1.7 |

**Appendix E:** Weekly feed intake in kg

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | 1st week | 2nd week | 3rd week | 4th week |
| T0R1 | 1.4148 | 2.8728 | 2.502 | 3.262 |
| T0R2 | 1.4338 | 3.1602 | 2.9328 | 2.9252 |
| T0R3 | 1.37901 | 2.8408 | 2.5715 | 3.43 |
| T1R1 | 1.5106 | 3.337 | 2.5418 | 3.855 |
| T1R2 | 1.4734 | 3.2175 | 2.7405 | 3.5032 |
| T1R3 | 1.4659 | 3.074 | 2.8665 | 3.7148 |
| T2R1 | 1.281 | 2.583 | 2.25 | 2.6543 |
| T2R2 | 1.3375 | 2.6624 | 2.2755 | 2.709 |
| T2R3 | 1.2705 | 2.4822 | 2.2806 | 2.5527 |
| T3R1 | 1.2826 | 2.4969 | 2.1658 | 2.2692 |
| T3R2 | 1.29027 | 2.4552 | 2.28 | 2.337 |
| T3R3 | 1.2588 | 2.5916 | 2.1894 | 2.057 |

**Appendix F:** Feed conversion ratio

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | 1st week | 2nd week | 3rd week | 4th week |
| T0R1 | 1.31 | 1.33 | 1.39 | 1.4 |
| T0R2 | 1.34 | 1.38 | 1.41 | 1.42 |
| T0R3 | 1.29 | 1.34 | 1.39 | 1.4 |
| T1R1 | 1.4 | 1.42 | 1.42 | 1.5 |
| T1R2 | 1.39 | 1.43 | 1.45 | 1.51 |
| T1R3 | 1.37 | 1.45 | 1.47 | 1.48 |
| T2R1 | 1.22 | 1.23 | 1.25 | 1.27 |
| T2R2 | 1.25 | 1.28 | 1.23 | 1.26 |
| T2R3 | 1.21 | 1.26 | 1.26 | 1.27 |
| T3R1 | 1.21 | 1.23 | 1.19 | 1.24 |
| T3R2 | 1.23 | 1.24 | 1.2 | 1.23 |
| T3R3 | 1.2 | 1.24 | 1.23 | 1.21 |

**Appendix G:** Meat quality analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | moisture (Sample 10g) | | | | Protein (Sample 0.5g) | |
| Treatments | Wt of petridish | Final wt | Moisture % | dry matter | Titration value | Cp% |
| T0R1 | 39.9 | 42.38 | 24.8 | 75.2 | 15.1 | 26.425 |
| T0R2 | 35.95 | 38.47 | 25.2 | 74.8 | 15 | 26.25 |
| T0R3 | 37.7 | 40.18 | 24.8 | 75.2 | 14.8 | 25.9 |
| T1R1 | 34.05 | 36.56 | 25.1 | 74.9 | 15.3 | 26.775 |
| T1R2 | 38.59 | 41.08 | 24.9 | 75.1 | 15.3 | 26.775 |
| T1R3 | 36.4 | 38.8 | 24 | 76 | 15.1 | 26.425 |
| T2R1 | 36.66 | 39.05 | 23.9 | 76.1 | 14.2 | 24.85 |
| T2R2 | 36.07 | 38.44 | 23.7 | 76.3 | 13.9 | 24.325 |
| T2R3 | 36.06 | 38.43 | 23.7 | 76.3 | 14.3 | 24.75 |
| T3R1 | 36.02 | 38.45 | 24.3 | 75.7 | 15 | 26.25 |
| T3R2 | 34.2 | 37.1 | 29 | 71 | 15.2 | 26.763 |
| T3R3 | 34.1 | 37 | 29 | 71 | 13.8 | 24.102 |

**Brief Biography**

Md. Ariful Hasan completed B.Sc. (Hon’s) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh with CGPA 3.52 (out of 4.00). Now, he is a candidate for the degree of MS in Human Nutrition and Dietetics under the Department of Applied Food Science & Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). He has immense interest to work in public health perspective like production of antibiotic residue free egg, meat, meat products and milk. He has also interest in Natural preservatives, Bio preservation of food, functional food product development, phytobiotics, essential oils and their use in human welfare.

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