GROWTH PERFORMANCE AND LIPID PROFILE OF BROILER CHICKENS FED ON HONEYWEED (*Leonurus sibiricus*) SUPPLEMENTED DIET

A THESIS

BY

SAIYED TAUFIQUR RAHMAN

Registration No.: 1705249

Session: 2017-2018

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Submitted to the Department of Biochemistry and Molecular Biology,
Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200
In partial fulfillment of the requirements for the degree of

MASTERS OF SCIENCE

IN

BIOCHEMISTRY AND MOLECULAR BIOLOGY

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DINAJPUR-5200

DECEMBER, 2018

Dedicated
To
My Beloved Parents
&
Wife

ACKNOWLEDGEMENTS

At the inception, I bow to the grace and mercy of "Almighty Allah" without whose grace I could not have pursued higher studies and completed the thesis work leading to the degree of Master of Science (M.S.) in Biochemistry.

*Allah Almighty had been so helpful in His blessings by giving the author a prospect to toil under the esteem supervision of Associate Professor and chairman **Dr. Md. Abu Sayed**, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The author have no words to express gratitude for his keen inspiration, scholastic and dynamic guidance, invaluable advice, scrupulous support, constructive criticism and encouragement throughout the course of the study.*

*It's a privilege to express my profound sense of gratitude and indebtedness to my co-supervisor **Dr. Md. Kamruzzaman (Mitoo)**, Assistant Professor, Department of Dairy and Poultry Science, HSTU, Dinajpur, for his valuable assistance, timely supervision of the research work, technical guidance, exclusive suggestions, helpful criticism and meticulous review of the manuscript for the improvement of this dissertation.*

*I am extremely happy expressing my recognition and gratefulness to **Dr. Mst. Nur-E-Nazmun Nahar, Shukla Rani Das, Dr. Md. Yeasin Prodhan, Shefali Akter, Dr. Md. Azizul Haque** of the Department of Biochemistry and Molecular Biology, HSTU, Dinajpur, for their nice cooperation and inspiration during the period of study.*

Thanks are extended to all staff of the Department of Biochemistry and Molecular Biology HSTU, Dinajpur for their cooperation towards the completion of the research work.

I feel much pleasure to convey the profound thanks to my friends and well-wishers for their cooperation, encouragement and help during the ongoing of the research.

Last but not least I express my deepest sense of gratitude and heartfelt thanks to my beloved parents, wife, sister and other relatives for their blessings, understandings, encouragement, patience and dedicated efforts to educate the author to this level.

December, 2018 ***The author***

ABSTRACT

Large amount of feed additives including antibiotics have been used in poultry feeds. Researchers have found out the adverse effect of antibiotic used in animal feed. Honeyweed (*Leonurus sibiricus*) is a herbaceous plant found in many countries in Asia, America and Sub-Saharan Africa region used as medicinal purpose by folk practitioners to control some non-communicable diseases. Buckwheat seed has also been reported to decrease serum lipid profile. This study was aimed to investigate the effects of combined effect of honeyweed and buckwheat seed powder in poultry feed on serum cholesterol, triglycerides, high density lipoprotein (HDL), examine mortality rate and growth performances of broiler to formulate a low cost and eco-friendly diet. Total 150 one day old chicks were taken and divided into five groups having five replications each consisting of six birds. Along with commercial control (T1) and own formulated feed (T2) there were OC (Own control) with 5% HW (Honeyweed) (T3), OC with 10% HW (T4) and OC with 15 % HW (T5). Results revealed that newly formulated supplemented feed has significant effects on feed intake and body weight gain of the birds. However, supplementation of honeyweed with buckwheat significantly ($p<0.05$) decreased serum triglycerides but elevated the HDL contents in broiler. Interestingly, honeyweed supplemented feed decreased mortality rate of the broilers to 3.32%. Our results suggest that this newly formulated feed with honeyweed and buckwheat could be considered as an alternative natural feed additive instead of hazardous synthetic antibiotics for safe poultry meat production.

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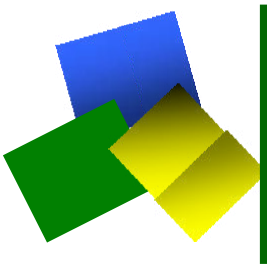
BCS	=	Black cumin seed
BMB	=	Biochemistry and Molecular Biology
BV	=	Biological value
BWS	=	Buckwheat seed
dl	=	Deciliter
et al.	=	And others
EU	=	European Union
FCR	=	Feed conversion ratio
g	=	Gram
HDL	=	High density lipoproteins
i.e.	=	That is
LDL	=	Low density lipoproteins
mL	=	Mililitre



CHAPTER I

INTRODUCTION

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INTRODUCTION

Meat and other animal products can play a significant role in alleviating the nutritional status of the people. Meat is an excellent source of high quality and readily digestible protein. They are also good sources of micronutrients (Bender, 1992). A variable but moderate energy content, highly digestible proteins (with low levels of collagen) of good nutritional quality, unsaturated lipids (mainly found in the skin and easily removed),vitamin B-complex (mainly thiamin, vitamin B6 and pantothenic acid), and minerals (such as iron, zinc, and copper) make poultry meat a valuable food. Epidemiological studies performed across the world, in highly diverse populations with different food preferences and nutritional habits; provide solid information on the association between poultry consumption, within a balanced diet, and good health. A recent survey of several countries found that 34 percent of the people surveyed in South Asia and 59 percent in Sub-Saharan Africa were suffering from severe energy deficiency (Smith and Wiseman, 2007). Consumption of poultry meat, as part of a vegetable-rich diet, is associated with a risk reduction of developing overweight and obesity, cardiovascular diseases, and type 2 diabetes mellitus. Also, white meat (and poultry in particular) is considered moderately protective or neutral on cancer risk. Moreover, poultry meat consumption also contributes to the overall quality of the diet in specific ages and conditions (prior to conception, during pregnancy up to the end of breastfeeding, during growth, and in the geriatric age) and is suitable for those who have an increased need for calorie and protein compared to the general population (Marangoni *et al.,* 2015). In developing countries, the diet of people living in cities usually contains more animal protein than that of rural people, mainly because urban people are more prosperous, but also because they generally have access to a wider variety of foods at local markets. In low-income countries, commercially produced chicken meat is well placed to

satisfy the demands of a rapidly increasing affluent, middle class who can afford to pay for broiler chickens. Facilities and infrastructure for producing broiler chickens can be established quickly and soon start generating. Not only is chicken meat seen as a healthy meat, but it is also the cheapest of all livestock meats.

According to World Health Organization, cardiovascular diseases include coronary heart disease (heart attacks), cerebrovascular disease, raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. It is known that hypercholesterolemia is a risk factor for cardiovascular diseases (CVD) such as atherosclerosis and myocardial infarction, which is a common cause of mortality and morbidity (Wald *et al.,* 1995). However, presence of cholesterol in broiler meat is a main factor discouraging the consumption of such foods (Krieger *et al.,* 1998). Now a day, people are more concern about the nutritional quality and related possible health hazards of dietary components. Most of the people restrict eating broiler meat due to the fear of having high cholesterol content in it (Abeywardena *et al*., 2003). This situation has led to missing of an excellent source of nutrients in the diet thus causing protein malnutrition in Bangladesh. Therefore, it is a timely need to reveal measures to produce broiler meat with low cholesterol level as it will make broiler meat more attractive to the people and hence broiler meat consumption could be increased. It then will have a significant effect to reduce the protein malnutrition problem as well as to reduce the health hazards due to consumption of broiler meat. One way of lowering broiler meat cholesterol is to incorporate cholesterol reducing factors into the diets of broiler. Although several factors, such as life style, a diet rich in cholesterol, age and hypertension have been reported to cause heart failure (Schaefer *et al,.* 1995). High levels of cholesterol, particularly LDL cholesterol, are mainly responsible for hypercholesterolemia (Krieger *et al.,* 1998). Increased generation of oxidized LDL is a major factor in the vascular damage associated with high

cholesterol levels (Pritchard *et al*., 1995). Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the anti-oxidative functions of various medicinal plants (Gang *et al.,* 2009).

Feed additives improve the quantity of food production. Despite of using antibiotics as additive, use of natural resources plays a critical role to meet the escalating nutritional needs of a growing world human population. Good results are obtained with these substances. Due to the risk of the development of direct antibiotic resistance of pathogens in the species receiving the feed, as well as indirect resistance to similar antibiotics used in human medicine led to the ban of all sub-therapeutic levels of growth-promoting antibiotics by the European Union (Council of the European Union, 1970) and many other countries, including Bangladesh. As this may negatively affect the profitability of poultry farming, alternative substances and strategies for growth promotion and disease prevention are being investigated, among which phytogenic and herbal products have received increased attention, since they have acquired acceptability among consumers as natural additives (Toghyani *et al.,* 2010). In previous studies it is found that phytogenic feed additives of plant origin normally proved safe, healthy, eco-friendly, cost effective, not directly associated with problems and less hazardous than synthetic feed additives in poultry production (Islam *et al.,* 2011).

Phytogenic feed additives of plants origin are generally believed to be safe, healthier, less subject to hazards and not accompanied by problems than synthetic feed additives**.** Natural growth promoters, aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects (Wenk *et al.*, 2000) and their stimulating effect on animal digestive systems (Jang *et al*., 2007). Under the intensive management systems, natural growth promoters are already being used as feed supplements to improve growth performance. These extracts when supplemented to animals

diets can play a significant role in supporting both performance and health status of the animal (Manzanilla *et al*., 2004).

Leonurus sibiricus is a ubiquitous herbaceous plant grown in crop fields in many countries in Asia and South America (Sayed *et al*., 2016). *L. sibiricus* acts works as an effective therapeutic against diabetes, menstrual irregularities, and bronchitis. Studies show that it has medicinal effects on endometritis, myocardial cells and diabetes. Studies show that this plant has cytotoxic activity, antimicrobial activity, analgesic, anti-inflammatory, anti-oxidant, antiatherogenic and anti-hemorrhagic activity.

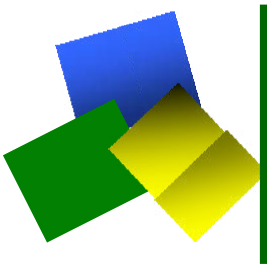
Another plant of nutritional and medicinal importance is Buckwheat (*Fagopyrum esculentum* Moench.); an ancient dicotyledonous crop belonging to the family Polygonaceae (Edwardson, 1996). It is cultivated and adapted to the marginal lands with harsh environments. This non-glutinous pseudo cereal is consumed as functional food mainly in China, Japan, and Eastern Europe (Zhang *et al*., 2012). Buckwheat seed (BWS) contains: vitamins such as B_1, B_2 and a factor facilitating the absorption of vitamin B; minerals including K, Mg, Ca, Fe, Se, Zn; and other compounds, such as protein with balanced amino acid composition, phytosterols, soluble carbohydrates, D-chiro-inositol, flavonoids, phenolic acids, tocopherols, inositol phosphates, rutin. Its starch composition is similar to cereals, but has higher amounts of amino acids such as lysine, methionine, and cystine, which are more typical of legumes (Zheng, *et al*., 1998). The literature suggests that buckwheat has reasonable feed value, roughly comparable to oats. Additionally buckwheat is rich in unsaturated fatty acids. Therefore, consumption of products made from buckwheat is considered to be helpful in preventing human disorders such a cardiovascular diseases, hypercholesterolemia or hypertension (Karamac, 2010). The addition of protein products of buckwheat to diets significantly lowers the levels of cholesterol in serum, liver, and gallbladder of hamsters and suppresses the formation of gallstones by altering cholesterol metabolism whereas protein

extracts are more efficient in lowering the blood cholesterol level, particularly that of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) (Tomotake *et al*., 2006). It was shown through numerous studies on buckwheat that, this plant plays an antioxidant, anti-inflammatory and anti-hypertensive role. Its medicinal property such as high antioxidant activity is due the presence of bioactive compounds such as flavones, flavonoids, phytosterols and myo-inositol (Zhang *et al*., 2012). The antimicrobial effect of buckwheat has also been reported (Świątecka *et al*., 2013). In our previous study, we found that supplementation of buckwheat seed powder in broiler feed significantly improve growth performance, decrease mortality rate, increase serum high density lipoproteins (HDL-cholesterol) and reduce serum triglycerides (Sayed *et al*., 2016).

Combined effect of honeyweed and buckwheat supplemented diets may improve broilers' productive performance such as weight gain, feed intake, feed conversion ratio (FCR) and survivability, and at the same time suppressed harmful intestinal bacteria. Because buckwheat seed-supplemented diets in broiler feed significantly improved growth performance, increased serum high-density lipoproteins (HDL-cholesterol) and reduced serum total cholesterol, LDL cholesterol and triglycerides concentrations (Siddiqui *et al.,* 2015). Although several reports (Świątecka *et al., 2013*) have focused on the effects of honeyweed extract can regulate lipid profile as well as control microbial growth (Sayed *et al*., 2015). Considering the medicinal advantages of buckwheat seed and honeyweed, the current study was designed to evaluate the combined effect of these two medicinal plants on growth performance and serum biochemical metabolites in broiler chicks.

Therefore, the objectives of this study were to evaluate the combined effects of honeyweed and buckwheat seed on-

1. Growth performances of broiler chickens
2. Lipid profile of Broiler chickens.



CHAPTER II

REVIEW OF LITERATURE

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Supplementation of poultry feed with pharmacological products, either for preventive purposes, as prevention of certain diseases or as growth stimulators is a common phenomenon, primarily for young chicks. However, currently there is considerable controversy regarding the use of antibiotics as growth promoters in poultry production that has led to restriction or even a complete ban of these substances in some countries. The removal of antibiotic growth promoters might have a negative economic impact. Therefore, search for alternative additives has been incentivized among the possibilities in natural products from plant origin (Scheuermann *et al*., 2009).

It was estimated that there are 250000- 500000 species of plants on earth (Hashemi and Davoodi, 2010). Many scientists have searched for alternatives to antibiotics through utilization of the extracts from some of these plants (Alcicek *et al*., 2003). There are several non-pharmacological products from the group prebiotics, probiotics, organic acids and other essential oils, medicinal plants or parts of plants which are alternatives to antibiotics as growth stimulators (Simon *et al*, 2005).

Many traditional plants products are in use owing to their therapeutic potential. In diet-based therapies, research investigations confirmed the importance of various plants including garlic, green tea, ginger, mulberry, thyme etc. nonetheless, several avenues are yet to be explored. Many developing countries possess a diversity of indigenous plants and many of them hold growth and antimicrobial promoting benefits. The present research plan was an effort in this direction; accordingly honeyweed (*Leonurus sibiricus*) its nutraceutical worth.

2.1 The effect of synthetic growth promoters in poultry feed

Growth promoters (chemical products, antibiotics, enzymes. etc) play an active role in the experimental and commercial production of large and small animals as well as poultry. Supplementation of several growth promoters from different sources to poultry feed as feed additives have been used for years to improve the profitability of poultry production. A wide variety of antibiotics is routinely added to animal feed in sub-therapeutic doses for growth promotion of animals produced for human consumption (Martin *et al*., 1995). These substances improve the profitability of poultry production by controlling pathogenic bacteria in the gut mucosa and improving the utilization of nutrients (Dorgham *et al*., 1994) thereby improving weight gain, feed conversion ratio and uniformity.

Although good results were obtained with these substances, their use might have unfavorable effects. Antibacterial substances those are used as growth promoters in animal husbandry carry an incalculable risk for human health (Witte *et al*., 2000). The indiscriminate use of antibiotics as feed additives could lead to an increased number of antimicrobial-resistant bacteria, and ultimately compromise the treatment of bacterial infections in humans (McDermott *et al*., 2001). Antibiotics may accumulate in the tissues of animals and be ingested by consumers whose own resident microflora may become antibiotic-resistant (Ranger *et al*., 1996). This antibiotic resistance may lead to problems with antibiotic therapy in humans and other animals (Kolawole and Shittu, 1997). Therefore, major changes occurred in the use of antimicrobial agents for growth promotion in different countries. In 1986, the Swedish Government banned the use of antimicrobial growth promoters (Wierup, 2001). Denmark banned the use of avoparcin in 1995 and virginiamycin in 1998. The glycopeptide-resistant *E. faecium* in broilers was decreased after the ban of avoparcin from 72.7% in 1995 to 5.8% in 2000 (Aarestrup and Jensen, 2001).

However, removal of antibiotics from the diet may negatively affect profitability of the poultry industry. But, it is indispensable to minimize these components, and deals with replacers without any adverse effect on production. Therefore, there is a great demand in developing natural alternatives to antibiotic growth promoters in order to maintain both bird's performance and health (Abdel-Malak *et al*., 1995).

2.2 The effect of natural feed additives in poultry feed

Most of the antibiotic growth promoters have been banned due to multiple resistances and some unwanted results. Nowadays, antibiotic resistant strains of bacteria have increased the concern about the potential public health problems and food safety is more seriously considered than before. On the other hand, the economy of the poultry industry and food production is also factors which may not be overlooked. Therefore, poultry nutritionists are being challenged to develop an alternative for antibiotic growth promoters and the search for alternative feed supplements has been stepped up. Considerable attention has been paid to phytogenic and herbal products as replacements for antibiotic growth promoters (Toghyani *et al*., 2010). Herbs or products including plant extracts, essential oils or the main components of the essential oil are among the alternative growth promoters that are already being used (Ocak *et al*., 2008).

There is evidence suggesting that herbs, spices, and various plant extracts have appetizing, digestion-stimulating and antimicrobial properties. Therefore, possible alternatives to antibiotics may be represented by plant products. Indeed, plant products have been used for centuries as food and medicines. Natural medicinal products made with herbs and spices have also been used as feed additives for poultry. An example of natural feed additive is black cumin seeds (BCS). They have been used for centuries in Asia, Northern Africa, Middle and Far East for the treatment of asthma in the presence of the

antiasthmatic compound nigellone as digestive and appetite stimulant (Gilani *et al*., 2001), hepatoprotective and antitumor agent (Abuharfeil *et al*., 2001).

The positive effects of herbal plants on broilers have been reported by many studies. Their antibacterial potential, hypocholestrolemic effects, growth promoting effects and availability are the most beneficial part of herbs, which have drawn the scientists attention themselves (Mansoub, 2011). Various dietary herbs, plant extracts, especially essential oils, have been studied for their antimicrobial and growth promoter abilities (Fritz *et al*. 1993) reported that the addition of 1.5-3% herb mixture containing 30% *Chamomile recutita* L. in broiler diets improved feed conversion and increased hemoglobin content and leukocyte in blood (Abdel- Malak *et al*. 1995) have reported that the addition of biotonic (herbal mixture) as a feed additive in broiler rations significantly improved live body weight and feed conversion.

Recently, a number of scientific studies have concentrated on the bactericidal and bacteriostatic effects of various herbs and plant extracts (Tucker, 2002). In 1943, Osborn reported more than 60 genera of plants that exhibit inhibitory properties toward the growth of either *E. coli* or *Staphylococcus aureus* or both. Demir *et al*., 2005 reported that replacing antibiotic growth promoter (Zinc Bacitracin) by Rhubarb (*Rheum rhaponticum* Wild.) as a herb did not significantly affect body weight, body weight gain, feed intake, feed efficiency and dry matter content of excreta.

In some reports, the results demonstrated that broilers fed the XT (a blend of capsicum, cinnamaldehyde and carvacrol) diets had significantly increase live weight, better weight gains and feed efficiency than broilers fed a control diet with avilamycin. In addition, XT broilers showed lower caecal counts for *E. coli* and *C. perfringens* (Alipour *et al.,* 2015*,* Jamroz and Kamel, 2002, Shan *et al*. 2002) showed that dietary fructo-oligosaccharides (FOS) significantly increased caecal *bifidobacterium* number, but there was no effect of FOS on caecal *E. coli* number as compared to the control.

2.3 Nutritional profile of honeyweed

A number of studies show that *L. sibiricus* contains many different pharmacologically important chemical compounds. Other studies show the beneficiary effects of *L. sibiricus* as a treatment for cancers (Nagasawa *et al.,* 1990) and cardiovascular disease (Lin *et al.,* 2013). Compounds like LS-1, LS-2, leonotinin, leonotin, dubiin and nepetaefuran isolated from *L. sibiricus* show a considerable cytotoxic effect against leukemia cells in vitro (Satoh *et al.,* 2003). Interestingly, dry leaves of *L. sibiricus* at low dose (0.5 g/kg body weight) in rats and rabbits did not show any toxic effects while high doses (5.0 and 25 g/kg body weight) showed harmful effects (Chua *et al.,* 2006). Furthermore, different extraction methods of leaves and roots show cytotoxic effects on brine shrimp (Bouzada *et al.,* 2009). These results reveal the potential use of both leaf and root extracts of *L. sibiricus* as wormicide for human. Study shows that labdane type compounds are mainly responsible for the cytotoxicity (Chinou *et al.,* 2005). A preliminary report shows that transformed root extract of *L. sibiricus* induces the apoptosis in glioma cells by upregulating Bax/Bcl-2–p53 signaling axis (Sitarek *et al.,* 2016).

Different chemical extracts of *L. sibiricus* show potential antimicrobial activity. Methanol extract inhibits the growth of Bacillus subtilis (de Souza GC *et al*., 2004). The effects of *L. sibiricus* alcoholic extract are notable on Gram-negative and Gram-positive bacteria (Sober´on *et al.,* 2007). Intriguingly, carbon tetrachloride and chloroform extracts show a broad spectrum antibacterial activity than acetone and methanol extracts. Carbon tetrachloride and chloroform extracts inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Escherichia coli*, *Vibrio cholera*, *Shigella dysenteriae* and *Shigella boydii*. The alcohol extracts do not show any inhibition of the growth of *Escherichia coli*. On the contrary, carbon tetrachloride and chloroform extracts significantly inhibit bacterial growth. Reports show that diterpenes of labdane type compounds isolated from the

plants of different family including Lamiaceae show a moderate to strong inhibitory activity on microorganism (Chinou *et al.,* 2005).

Methanol extracts of *L. sibiricus* show a significant analgesic effect on mice (dose: 250 and 500 mg/kg body weight). The anti-inflammatory activity is also demonstrated in rats at the dose of 200 and 400 mg/kg body weight (Islam *et al.,* 2005). Anti-inflammatory effect of *L. sibiricus* is reported in secretion of inflammatory cytokine like tumor necrosis factor-a and interleukin-6 and interleukin-8 in human mast cell line HMC-1 (Shin *et al*., 2009). The aqueous extracts of *L. sibiricus* show more efficient antioxidant activity than ethanol extracts in three different bioassay systems (Chua *et al.,* 2013*)*. The extracts of *L. sibiricus* show not only the antioxidant activity, but also reduce the intracellular reactive oxygen species (ROS) in Chinese hamster ovary cells exposed to hydrogen peroxide. Methanol extract (80% v/v) increases the expression of intracellular antioxidants like superoxide dismutase, catalase and glutathione peroxidase.

Liquid chromatography–mass spectrometry/mass spectrometry and high-performance liquid chromatography analyses reveal that the phenolic compounds and flavonoids modulate the antioxidant genes and repair the oxidative DNA damage. The aerial part of *L. sibiricus* is found to be used for the treatment of menstrual irregularities, amenorrhea, malaria, hypertension and myocardial ischemia. *L. sibiricus* has also clinical implications to reduce the postpartum hemorrhage. In connection with current reports, literature shows the effects of *L. sibiricus* extract for reducing the uterine bleeding in RU486-induced abortion mice. Stachydrine hydrochloride is one of the constituents of *L. sibiricus* which has a function to reduce the uterine bleeding in RU486- induced abortion mice by regulating Th1/Th2/Th17/Treg paradigm. Stachydrine hydrochloride upregulates the mRNA expression of T-bet and RORgt while inhibiting the mRNA expression of GATA-3 and Foxp3 shows the effects on lowering the uterine bleeding in RU486-induced mice. The

isolated leonurine from the methanol extract of *L. sibiricus* shows anti-platelet activity in rabbit (Lin *et al.,* 2013).

2.4 The effect of honeyweed on lipid profile

Cardiovascular disease is one of the leading causes of death in the world. Cholesterol, triglycerides and low density lipoprotein (LDL) are generally considered as the risk factors for cardiovascular diseases. Oxidized-LDL is the major risk factor for atherosclerosis which may promote ROS production.

Lectin-like oxidized LDL receptor-1, the receptor of LDL, functions to promote the vascular dysfunction connecting to endothelial nitric oxide synthase (eNOS) production in the vessel tissues of human and animals. The suppression of eNOS production in human umbilical endothelial cells (HUVEC) is linked to cardiovascular diseases, hypercholesterolemia and atherosclerosis followed by preeclampsia in pregnant women. Nitric oxide (NO) can be synthesized by the activity of eNOS in vascular endothelial and HUVEC cells, which can act as signaling molecule to prevent the aggregation of blood cells. The aqueous extract of *L. sibiricus* along with recombinant interferon-g increase the NO production and tumor necrosis factor-a in mouse peritoneal macrophages. The previous study suggests that *L. sibiricus* has potential effects for reducing atherosclerosis. Furthermore, the ethanol extracts of *L. sibiricus* show the effects in controlling the level of cholesterol, adhesion molecules and ROS in vivo as well as lectin-like oxidized LDL receptor-1 in vitro.

2.5 Nutritional profile of buckwheat

Buckwheat is introduced into the diet as an alternative crop of renewed interest due to its nutritive and health-promoting value. Experiments with animal models have demonstrated that buckwheat flour may alleviate diabetes, obesity, hypertension, and hypercholesterolemia. A number of nutraceutical compounds exist in buckwheat grains and other tissues. These are a rich source

of starch, proteins, antioxidants, and dietary fibre as well as trace elements. The biological value (BV) of buckwheat proteins is comparable to BV of other protein sources. Besides high-quality proteins, buckwheat grains contain some components with prophylactic value: flavonoids, fagopyrins, or thiamin-binding proteins. For the food industry, buckwheat grains are a valuable raw material to be used for the production of functional foods. Buckwheat flour may be a valuable and important ingredient in diets or food products, taking into consideration its nutritive value and potential promotion of human health (Steadman *et al*., 2001).

Starch is the major storage component of buckwheat grains. It is accumulated in the endosperm as an energetic material necessary for the plant growth. In the whole grain of buckwheat, starch content varies from 59% to 70% of the dry mass, demonstrating fluctuations under variable climatic and cultivation conditions. However, current results of starch analysis in buckwheat grains of three polish varieties have shown that the starch content lies in a narrow range, i.e. from 63% to 66% of dry mass. The composition of starch isolated from buckwheat grains differs from that of cereal starches. It may contain higher amounts of proteins, ash, and phosphorus (Soral-Śmietana *et al*., 1984). The content of bound lipids is two times higher than that of free lipids. Amylose content of buckwheat starch granules fluctuates between 15% and 52% (Campbell, 1997).

From the nutritional point of view, there exist three fractions of starch: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Resistant starch is not absorbed in the small intestine and is partly or completely available for fermentation by microflora in the large intestine. It could show similarity to dietary fibre. In uncooked buckwheat grains, RS constitutes 33–38% of total starch, but after cooking only 7–10%. The factors influencing starch availability include its botanical origin and physical properties, the ratio of amylose to amylopectin, and starch interactions with

other constituents. Starch is not only a significant source of energy for human; it is also reported to interact with the gut microflora (Bird *et al*., 2000; Wronkowska *et al*., 2006).

Soluble carbohydrates, including fagopyritols, are concentrated mainly in the embryo, their concentration is low in endosperm whereas their total contents ranges from 1% to 6% (Steadman *et al*., 2001). Fagopyritol A_1 and Fagopyritol B_1 are the most remarkable of all the fagopyritols accumulated. Fagopyritol A_1 is an active substance that may be used in the treatment of diabetes and polycystic ovarian syndrome (PCOS) (Christa *et al*., 2008).

According to the current definition, dietary fibre is the edible part of a plant or analogous carbohydrates that is resistant to digestion and absorption in the human small intestine but is partly or completely fermented by microflora in the large intestine. Dietary fibre consists also of oligosaccharides, polysaccharides, and other hydrophilic derivatives (Gibson *et al*., 2002). Non-starch polysaccharides such as cellulose, hemicelluloses, pectins, gums, and non-cellulosic polysaccharides are the main components of dietary fibre. They are concentrated in tissues with thicker cell walls, aleurone, seed coat and hulls. Total dietary fibre (TDF) is classified in view of its affinity to water as either insoluble dietary fibre (IDF) or soluble dietary fibre (SDF). In general, IDF includes cellulose, lignins, and certain non-cellulosic polysaccharides, while SDF includes pectins and some associated non-cellulosic polysaccharides. The whole grains contain 7% TDF, while bran with hull fragments has 40% TDF (Steadman *et al*., 2001).

Buckwheat is one of the best sources of high quality, easily digestible protein in the plant kingdom. In literature, the protein content of buckwheat grains has been reported to range from 12% to 18.9% (Steadman *et al*., 2001; Liu *et al*., 2001; Wei *et al*., 2003). Bran milling fractions of buckwheat have been shown to be characterized by a high concentration of proteins, whereas the protein concentration in the hull is low, about 4%, however, in the embryo it reaches

55.9%. Buckwheat flour contains from 8.5% to near 19% of proteins depending on the variety, pesticides used, and fertilization that are likely to affect the total concentration of buckwheat proteins.

The major protein fractions of the grains are water-soluble and salt-soluble albumins and globulins representing almost one-half of all buckwheat proteins. Globulins consist of 12-13 subunits with molecular weights from 16 kDa to 66 kDa. The main storage protein of buckwheat grains is 13S globulin (Zhang, 2001). The average albumin content is 21%, whereas the highest one reaches 30-33% (Bharali and Chrungoo, 2003). Buckwheat prolamins have a different characteristic in comparison to wheat, barley, and rye prolamins, which enables buckwheat grains application in the prophylactic of gastrointestinal tract diseases, mainly celiac disease (Kreft *et al*., 2006). Grains may constitute a valuable source of dietary proteins with a high content of essential amino acids, which is important for people who do not tolerate glutenproteins or with proteins deficiency in the diet. Buckwheat proteins are rich in arginine and lysine, the primary amino acids limiting the content of proteins in cereals, whereas the contents of methionine and threonine in buckwheat proteins are low (Wei *et al*., 2003; Tomotake *et al*., 2006).

Buckwheat grains contain from 1.5% to 4% of total lipids (Steadman *et al*., 2001), but the content of raw fat in buckwheat flour exceeds 3% (Soral-Śmietana, 1984). Free lipids isolated from buckwheat grains constitute 2.5% of dry mass, whereas bound lipids about 1.3% dry mass. The highest concentration of lipids was found in the embryo (7-14%), whereas the lowest in the hull (0.4-0.9%). Triacylglycerides are the main components of the neutral fraction of lipids containing fatty acids from C_{12} to C_{22}, with a predominating contribution of: oleic (42%), linolic (32%) and palmitic acids (16%) (Soral-Śmietana *et al.*, 1984).

Generally, the content of minerals in buckwheat grains and their morphological fractions (dry base) reaches: 2-2.5% in whole grains, 1.8-2.0% in kernel, 2.2-

3.5% in dehulled grains, about 0.9% in flour, and 3.4-4.2% in hulls (Li and Zhang, 2001). Buckwheat is rich in potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). P, K, and Mg are most concentrated in bran, particularly in the bran from which the hulls were removed before milling the grains. Buckwheat may be an important nutritional source of microelements such as iron (Fe), manganese (Mn), and zinc (Zn) (Wei *et al*., 1995). Trace elements, e.g. chromium (Cr) or selenium (Se), are occasionally detected at very low levels. Foliar fertilization makes buckwheat grains a rich source of dietary Se and a useful raw material for enriched food products (Steadman *et al*., 2001; Wei *et al.*, 2003;). Buckwheat grains were also demonstrated to contain vitamins: B_1, B_2, B_6. These are concentrated in the peripheral parts of endosperm and embryo, hence the highest quantity of B vitamins is found in the bran.

Buckwheat contains many flavonoid compounds, known for their effectiveness in reducing the blood cholesterol, keeping capillaries and arteries strong and flexible, and assisting in prevention of high blood pressure (Santos *et al*., 1999). Rutin, the main buckwheat flavonoid, is a flavonol glucoside. The flavonoids content and composition in buckwheat seeds is affected by species, growing phase and growing conditions. Flavonoids content in seeds of the wild buckwheat (*Fagopyrum tataricum*) is about 40 mg/g, while in the common buckwheat (*Fagopyrum esculentum*) around 10 mg/g. Many different flavonoids have been isolated and identified in buckwheat grain. Rutin, orientin, vitexin, isovitexin, quercetrin and isoorientin are all present in the hull, while groats contain only rutin and small amounts of isovitexin.

2.6 The effect of buckwheat on lipid profile

Buckwheat (*Fagopyrum* spp.) is an important food in some areas of the world. Recently, it is becoming a common ingredient of functional food products because of its properties (Ahmed *et al.,* 2014). Buckwheat seeds contains: vitamins such as B_1, B_2 and a factor facilitating the absorption of vitamin B;

minerals including K, Mg, Ca, Fe, Se, Zn; and other compounds, such as protein with balanced amino acid composition, phytosterols, soluble carbohydrates, D-chiro-inositol, flavonoids, phenolic acids, tocopherols, inositol phosphates, rutin. Additionally buckwheat is rich in unsaturated fatty acids. Therefore, consumption of products made from buckwheat is considered to be helpful in preventing human disorders such a cardiovascular diseases, hypercholesterolemia or hypertension. (Karamac *et al.,* 2010).

Buckwheat proteins may show a strong supplemental effect with other vegetable proteins due to the well balanced amino acid composition (Li and Zhang, 2001). The Lysine/Arginine and Methionine/Arginine ratios in buckwheat proteins are lower than those in most plant proteins. This indicates that buckwheat should be characterized by the properties capable of lowering blood cholesterol level.

The addition of protein products of buckwheat to diets significantly lowers the levels of cholesterol in serum, liver, and gallbladder of hamsters and suppresses the formation of gallstones by altering cholesterol metabolism (Tomotake *et al*., 2001), whereas protein extracts are more efficient in lowering the blood cholesterol level, particularly that of LDL and VLDL (Tomotake *et al*., 2006). The hypocholesterolemic effect in humans is linked with a lower digestibility of buckwheat proteins and the presence of fibre-like substances, which is indicated by an increase in the contents of neutral and acid sterols in rat faeces observed upon the administration of a diet rich in buckwheat protein products (Tomotake *et al*., 2001).

Buckwheat proteins products (BWP) are acknowledged as preventive nutrients (Liu *et al*., 2001). They are also associated with the suppression of colon carcinogenesis by reducing cell proliferation and with the suppression of mammary carcinogenesis by lowering serum estradiol. They can suppress gallstone formation better than can soy protein isolates (Tomotake *et al*., 2001). Numerous experiments have proved that buckwheat proteins extract

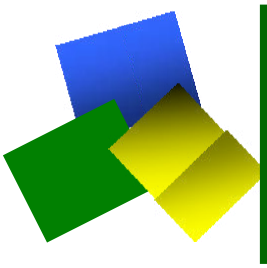
may be used as a potential functional food additive to treat hypertension, obesity, alcoholism, as well as constipation. He concluded that the only epidemiological indication of a beneficial effect of buckwheat comes from a study in Yi people, an ethnic minority in south- west China. Higher individual buckwheat consumption in this population was related to lower serum total cholesterol and low-density lipoprotein, and higher ratio of high-density lipoprotein to total cholesterol.

Wieslander and Norback (2001) used tartary buckwheat to alleviate diabetes mellitus, hypertension, hypercholesterolemia, and gallstones. Animal experimental studies support the view that buckwheat protein could have beneficial effects for various diseases, including hyperlipemia and that buckwheat leaf may have antioxidative properties. The mechanisms remains unclear, but could be related to the low digestibility of buckwheat protein, good nutritional value of buckwheat, or specific effects of phytochemicals in buckwheat, e.g. rutin. Moreover, it was reported that fagopyrins found in buckwheat can be utilized in the treatment of type II diabetes (Li and Zhang 2001; Bonafaccia *et al*., 2003). Epidemiological research is needed to evaluate long term health effects of buckwheat consumption.

As stated by Wieslander *et al.* (2001), intake of Tartary buckwheat cookies reduced the serum level of myeloperoxidase (MPO) by a factor 0.84 ($p = 0.02$). When grouping the two types of buckwheat cookies together, there was a reduction of total serum cholesterol ($p < 0.001$) and HDL-cholesterol ($p < 0.001$) during the study period, with improved lung vital capacity ($p < 0.001$).

In one study, 60 patients with senile hyperlipemia got a daily supplement of 40g tartary buckwheat during eight weeks. No control group was used. They reported a decrease of triglycerides, cholesterol, and low density lipoprotein (LDL) and an increase of the beneficial high density lipoprotein (HDL). They could also note a reduction of systolic and diastolic blood pressure, and a reduction of weight.

In another study, hyperlipemia patients got lower serum triglycerides and cholesterol, after starting eating tartary buckwheat flour and stopping taking medicine against hyperlipemia. Lower fastening blood sugar levels were observed in diabetic patients after daily consumption of 100-150 grams of tartary buckwheat flour during one month (Lin *et al*., 1998).



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

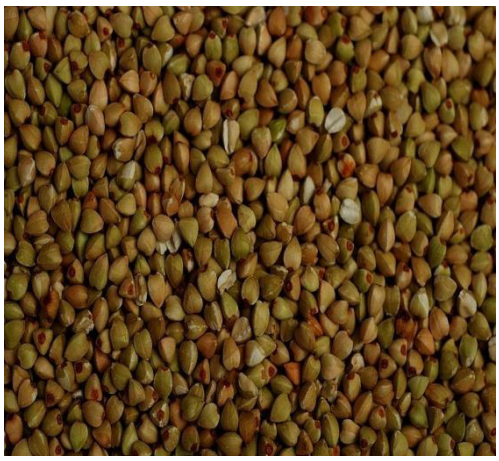
MATERIALS AND METHODS

The present study was conducted in a commercial poultry farm (Joynal Agro and Feed) Pakerhat, Khansama, Dinajpur. Biochemical parameters were measured in the Laboratory, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.1 Materials

3.1.1 Buckwheat and Honeyweed

Buckwheat seed (BWS) was purchased from the local market of Panchagar and honeyweed was collected from Santhia, Pabna, Bangladesh. The Buckwheat seeds were coarsely powdered by a mechanical grinder and then directly mixed with manually prepared diets in appropriate doses The honeyweed leaves were washed with running tab water and dried at room temperature. Dried leaves were kept in the oven at 60°C for 72 hours. Subsequently, oven dried leaves were powdered by grinder. Powders were fed by making solutions with water.



(a) **(b)**

Figure 3.1: (a) Buckwheat seeds (b) Honeyweed

3.1.2 Other feed materials

Sundried and grinded corns, meat and bone meal, rice polish, soybean meal, soybean oil and other feed items were purchased from local market of Dinajpur, Bangladesh and then directly mixed with manually prepared diets in appropriate doses (Table 3.1). Vitamin premix was purchased from Renata Animal Health Ltd.

3.1.3 Experimental birds

A total 150 one-day old broiler chicks (Loman) was purchased from a local hatchery (Jaynal Hatchery, Bangladesh).

3.1.4 Materials required for biochemical study

3.1.5 Chemicals and reagents

Kits for the estimation of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were purchased from CRESCENT diagnostic, Jeddah, K.S.A.

3.1.6 Instruments and appliances

Instruments and appliances used for biochemical study were as follows.

a. Micro grinder (Tekmark, West German)
b. Vortex mixture (Branson 1210, USA)
c. Spectrophotometer (Spectronic®Genesys™, USA)
d. SYSMEX Automated Haematology Analyzer (Model: XN-350)High pressure steam sterilizer (ES-315, Tomy)
e. Centrifuge machine
f. Syringe and needle
g. Pipettes and micropipettes
h. Refrigerator, electric balance, conical flasks, beakers, test tubes, test tube stands and other conventional laboratory instruments.

3.2 Methods

The whole experimental design is presented below:

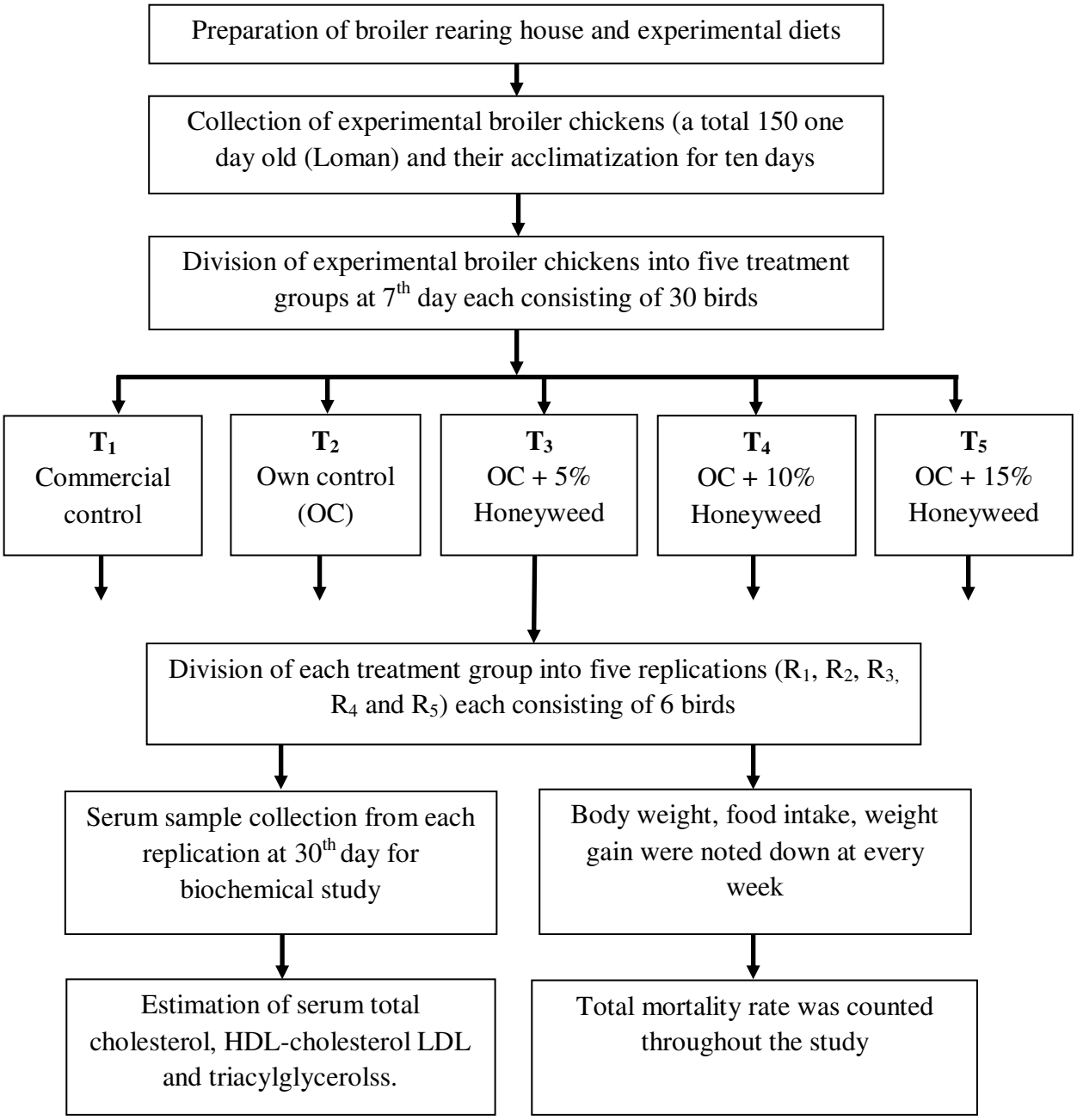


Figure 3.2: Flow chart of the experimental design

3.2.1 Preparation of experimental diets and treatments

The experimental diets in mash form and drinking water were provided *ad libitum.* All diets were formulated manually to meet the nutrient requirements of broiler (NRC, 1994). The chicks were fed starter diet from 1 to 15 days and a finisher diet from 15 to 30 days old broiler. Compositions of the starter and grower rations are presented in table 3.1.

Table 3.1: Composition (%) of experimental diet in different rearing periods

Diet Composition	**1-15days (Starter)**	**15-30days (Grower)**
Maize	40.5	43.4
Soyabean meal	24.36	21.57
Rice Polish	10	10
Meat and Bone meal	4	4
Protein Concentrate	5.5	5.5
Soyabean oil	3	3
Lime stone	0.5	0.5
DCP	1	1
Salt	0.3	0.3
Methionine	0.12	0.1
Broiler Premix	0.31	0.25
Toxin binder	0.3	0.3
Coccidiostates	0.05	0.02
Lysine	0.01	0.01
Enzyme	0.05	0.05
Buckwheat	10	10
Chemical composition of calculated nutrient (%)		
Metaboisable energy (kcal/kg)	2890	3000
Crude Protein	21.30	19.20
Calcium	1.01	0.86
Available phosphorus	0.48	0.40
Sodium	0.16	0.18
Arginine	1.41	1.23
Lysine	1.38	1.15

The treatments of the feed supplementation experiment were as follows -
T_1: Commercial control, (Quality feed ltd.), **T_2:** Own control feed, **T_3:** Own control feed + 5% honeyweed, **T_4:** Own control feed + 10% honeyweed, **T_5:** Own control feed + 15% honeyweed

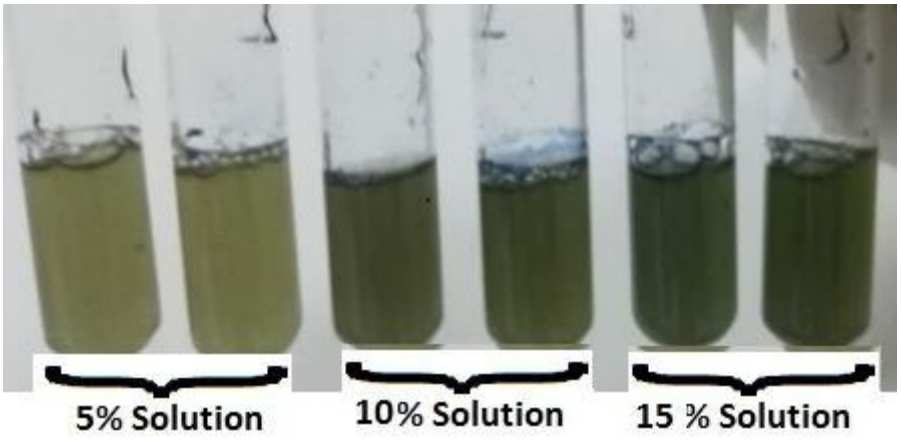


Figure 3.3: Formulated solution of honeyweed leaf powder

Figure 3.4: Own control feed with 10% Buckwheet

3.2.2 Rearing of birds and experimental design

Birds were reared at brooding house to adjust with the environmental condition up to 7 days. After 7 days, they were randomly divided into five dietary treatment groups of 30 chicks each; each treatment was composed of five replicates with six birds in each in a complete randomized design. The birds were housed on floor and routinely managed.

3.2.3 Observation of birds

All the birds were examined twice daily for abnormal clinical signs (restlessness, lordosis, abnormal gait, vices and depression) as well as feed

intake throughout the experiment. They were vaccinated against Newcastle and Gumboro diseases.

3.2.4 The performance trial

During the 30 days of experimental period, growth performances of the birds were recorded. Before giving dietary supplementation treatment, body weight was recorded for each group of birds. Then body weight and feed consumption were recorded daily and body weight gain and feed conversion were then calculated. Mortality was recorded throughout the study.

3.2.5 Feed consumption

Feed consumption is the amount of feed consumed every day. It was calculated for each treatment at daily basis. At the end of the week, the residual amount of feed was weight and subtracted from the known weight of feed at the beginning of week. The product was divided by the total number of birds.

3.2.6 Body weight gain and feed conversion ratio

Body weight was measured for all birds at the beginning of the experiment, and it was repeated at the beginning of the week at the same time. Live weight gain was calculated by subtraction the live weight at the beginning of the week from the live body weight of the next week.

Feed conversion ratio (FCR) was calculated every week at the amount of feed consumption per unit of body weight gain {average weekly feed consumption (g)/ average weekly gain (g)}.

Food Conversion ratio= $\frac{\text{Feed Consumed}}{\text{Weight gain}}$

Figure 3.5: Experimental birds at brooding stage

Figure 3.6: Experimental birds with different treatments

3.2.7 Preparation and storage of serum

The blood was collected from experimental and control birds at 30th day by using a sterilized disposable syringe and needle by wing vein puncture without using any anticoagulant. Each of the syringes with blood sample was kept at normal temperature in an inclined position. After 20 minutes, the serum was collected and centrifuged for 15 minutes at 2500 rpm. After centrifugation, the supernatant were carefully collected by a micropipette and preserved in eppendorf vials. The collected serum samples were stored at -15ºC until used for determination of total cholesterol, HDL-cholesterol, LDL and triglycerides.

3.2.8 Estimation of serum total cholesterol

3.2.8.1 Test principle:

A commercial kit (CRESCENT diagnostic, Jeddah, K.S.A) was used for estimation of serum total cholesterol of birds. Cholesterol esterase (CHE) catalyses the hydrolysis of cholesterol esters to produce cholesterol which is oxidized by cholesterol oxidase (CHO) to yield hydrogen peroxide (H_2O_2). In a coupled reaction catalyzed by peroxidase (POD), quinoneimine dye (red) is formed from (H_2O_2), 4-aminophenazone and phenol.

1. Cholesterol ester + $H_2O \xrightarrow{CHE}$ Cholesterol + fatty acid

2. Cholesterol + $O_2 \xrightarrow{CHO}$ Cholesterol-3-one + H_2O_2

3. 2 H_2O_2 + 4-aminophenazone + phenol $\xrightarrow{POD}$ Quinoneimine + 4 H_2O

3.2.8.2 Reagent Composition:

Reagent A: It consisted of phosphate buffer 30 mmol/L, 4-amino-phenazone 0.3 mmol/L, phenol 5 mmol/L, peroxidase>5 KU/L, cholesterol esterase>150 U/L, cholesterol oxidase>100 U/L and sodiumazide0.05% having the pH 6.5.

Reagent S: Cholesterol standard: It consisted of cholesterol200 mg/dL. It was aqueous primary standard solution of cholesterol.

3.2.8.3 Procedure:

1. The reagents and the standards were brought to room temperature.

2. Pipetting was performed into labelled test tubes as follows.

Item	Blank	Standard	Sample
Serum sample(µL)	-	-	10
Cholesterol standard, S (µL)	-	10	-
Distilled Water (µL)		-	-
Reagent, A (µL)	1000	1000	1000

3. After mixing thoroughly, the tubes were incubated for 10 minutes at room temperature.

4. Absorbance (A) of the standard and the sample was measured by the spectrophotometer at 500 nm against the blank.

3.2.8.4 Calculations:

The cholesterol concentrations in the samples were calculated using the following general formula:

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{A sample}}{\text{A standard}} \times \text{C standard}$$

Here 'A' means absorbance and 'C' for concentration.

The cholesterol standard provided in the kit box had been used to calibrate as follows.

$$\frac{\text{A sample}}{\text{A standard}} \times 200 = \text{mg/dL cholesterol}$$

3.2.9 Estimation of serum HDL-Cholesterol

3.2.9.1 Test principle:

A commercial kit (Human, Germany) was used for estimation of serum HDL-Cholesterol of birds. Phosphotungstic acid and magnesium ions specifically precipitate low and very low density lipoproteins (LDL and VLDL). After centrifugation the cholesterol content of the high density lipoproteins (HDL) in the supernatant can be determined using HUMAN Cholesterolliquicolortest kit.

3.2.9.2 Reagent Composition:

It consisted of phosphotungstic acid 0.55 mmol/L and magnesium chloride 25.00 mmol/L

3.2.9.3 Reagent preparation:

The reagent was prediluted with distilled water before use (80 mL of reagent and 20 mL of water). HUMAN Cholesterolliquicolortest kitis also required for HDL-cholesterol estimation.

3.2.9.4 Procedure:

1. The reagents and the standards were brought to room temperature.
2. 200 µL serum samples was thoroughly mixed with 500 µL diluted reagent.
3. The mixture was allowed to stand for 10 minutes. Then it was centrifuged for 10 minutes at 4000 rpm**.**
4. After centrifugation, clear HDL supernatant was separated from the precipitate within one hour and HDL-cholesterol concentration was then determined using HUMAN Cholesterol liquid colour test kit**.**
5. Pipetting was performed intolabelled test tubes as follows.

Item	Reagent blank	Standard	Sample
Distilled water (µL)	100	-	-
Standard (µL)	-	100	-
HDL supernatant (µL)	-		100
Cholesterol reagent (µL)	1000	1000	1000

6. After mixing thoroughly, the tubes were incubated for 10 minutes at room temperature.
7. Absorbance (A) of the standard and the sample was measured by the spectrophotometer at 500 nm against the blank.

3.2.9.5 Calculations:

The HDL-cholesterol concentrations in the samples were calculated using the following general formula:

$$\text{HDL-cholesterol concentration (mg/dL)} = \frac{\text{A sample}}{\text{A standard}} \times \text{C standard}$$

Here 'A' means absorbance and 'C' for concentration.

The HDL-cholesterol standard provided in the kit box had been used to calibrate as follows.

$$\frac{\text{A sample}}{\text{A standard}} \times 175 = \text{mg/dL HDL-cholesterol}$$

3.2.10 Estimation of serum triglycerides

3.2.10.1 Test principle:

A commercial kit (Human, Germany) was used for estimation of serum triglycerides of birds. Lipases catalyze the hydrolysis of triglycerides to yield glycerol and free fatty acids. The glycerol content is determined enzymetically with the Trinder reaction using glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO) and peroxidase (POD). The end product is a quinoneimine dye the concentration of which at 546 nm is directly proportional to the concentration of triglycerides in the sample.

1. Triglycerides $\xrightarrow{\text{Lipases}}$ Glycerol + free fatty acids

2. Glycerol + ATP $\xrightarrow{\text{GK}}$ Glycerol-3-phosphate + ADP

3. Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetonephosphate + H_2O_2

4. H_2O_2 + 4-aminoantipyrine $\xrightarrow{\text{POD}}$ Quinoneimine + HCl + H_2O + 4-chlorophenol

3.2.10.2 Reagent composition:

Reagent A: It consisted of pipes buffer 50 mmol/L, lipases 150 KU/L, glycerol kinase (GK) >0.4 KU/L, glycerol-3-phosphate oxidase (GPO) >1.5 KU/L, peroxidase (POD) >0.5 KU/L, magnesium 5.0 mmol/L, adenosine triphosphate

(ATP) 1.0 mmol/L chlorophenol 5.0 mmol/L, aminoantipyrine 0.4 mmol/L having the pH 7.5.

Reagent S: Triglycerides standard: It consisted of glycerol equivalent to 200 mg/dL.

3.2.10.3 Procedure:

1. The reagents and the standards were brought to room temperature.

2. Pipetting was performed into labeled test tubes as follows.

Item	Blank	Standard	Sample
Sample (µL)	-	-	10
Triglycerides standard, S (µL)	-	10	-
Reagent, A (µL)	1000	1000	1000

3. After mixing thoroughly, the tubes were incubated for 10 minutes at room temperature.

4. Absorbance (A) of the standard and the sample was measured by the spectrophotometer at 500 nm against the blank.

3.2.10.4 Calculations:

The triglycerides concentrations in the samples were calculated using the following general formula:

$$\text{Triglycerides concentration (mg/dL)} = \frac{\text{A sample}}{\text{A standard}} \times \text{C standard}$$

Here ‘A’ means absorbance and ‘C’ for concentration.

The triglycerides standard provided in the kit box had been used to calibrate as follows.

$$\frac{\text{A sample}}{\text{A standard}} \times 200 = \text{mg/dL triglycerides}$$

3.2.11 Estimation of serum LDL

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

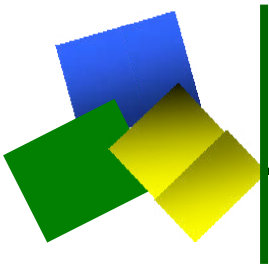
[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

3.2.12 Statistical analysis

The data were analyzed by using the statistical package SPSS statistics 17.0 (One way ANOVA). Differences among treatments, when significant, were ordered using Duncan's multiple range tests. Statements of statistical significance were based on $p \leq 0.05$ or $p \leq 0.01$.



CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

This study was conducted to evaluate the combined effects of doses of buckwheat and honeyweed supplemented diets on growth rate, mortality, serum lipid profile and FCR (Feed Conversion ratio) on the broiler chicks. The formulated diets were supplemented with T1 (Commercial control), T2 (Own control), T3 (Own control + 5% Honeyweed), T4 (Own control + 10% Honeyweed), T5 (Own control+ 15% Honeyweed). The birds were fed diets for a period of 30 days. Some physical parameters were recorded weekly and the chemical parameters were measured also at the end of the feeding trial. All results are expressed as mean ± standard error. The one way analyses of variance of some values were done followed by to Duncan's T-test to evaluate the differences among the mean values.

4.1 Growth performances of broilers feed with different diets

The effect of each formulated diet on growth performances of broiler chicks (Loman) are shown in table 2. We took the body weight of each broiler chick at the 10^{th} day just after completing the brooding period. Initial body weights of birds were (table 2) T1 (272.09 gm), T2 (272.03 gm), T3 (272.12 gm), T4 (273.26 gm) and T5 (272.18 gm) which were statistically non-significant ($p<0.05$). Final body weight of birds with different supplemented diets were T1 (1876.0 gm), T2 (1628.0 gm), T3 (1700.0 gm), T4 (1632.0 gm) and T5 (1650 gm). The highest body weight was found in commercial (T1) diet. Even though, T1, T2 and T3 are statistically non-significant whereas T5 and T5 are statistically significant ($p<0.05$).

Bodyweight gain of broilers of different dietary treatments during experimental period were found highest in T1 (1603.91 gm) i.e. commercial feed followed by T3 (1427.88 gm), T5 (1377.81 gm), T4 (1358.74 gm) and the lowest body

weight gain was found with T2 (1355.97 gm) treatment. The results obtained indicate that body weights of all groups are increased. The highest body weight gain was found in T1 i.e. broiler chicks fed with commercially available feed. However the value of T1, T2 and T3 were statistically non significant. Except commercial feed, T3 (5% HW) showed the highest body weight gain. The value of T3 was statistically significant from that of T2, T4 and T5.

Data are presented on total feed intake basis in order to observe the trend of feed intake among the birds of different dietary treatments in Table 2. Lowest feed intake was found in T3 (2619.20 gm) in comparison with T1 (2706.80 gm), T2 (2688.60 gm), T4 (2692.40 gm) and T5 (2676.20 gm). Feed intake was not found significantly different among the honeyweed supplemented diet.

Feed Conversion Ratio (FCR) was found lowest in T1 (1.69) and highest in T2 (2.07) treatments followed by T3 (1.85), T5 (1.96) and T4 (1.97). Though the FCR is found lowest in commercial feed supplemented treatment, the values of other treatments are statistically non-significant ($p<0.05$).

Table 4.1 Growth Performances of broilers chickens feed with different experimental diets for a period of 30 days

Parameters	Treatments				
	Commercial Control (T1)	OC (T2)	OC + 5% HW (T3)	OC + 10% HW (T4)	OC + 15% HW (T5)
Initial body weight at 10th day (g)	272.09±0.04[a]	272.03 ±0.08[a]	272.12 ±0.03[a]	273.26 ±0.71[a]	272.18±0.56[a]
Final body weight at 30th day (g)	1876.0±53.25[b]	1628.0±123.67[b]	1700.0±60.08[b]	1632.0±51.23[ab]	1650.0±21.21[a]
*Body weight gain (g)	1603.91±53.25[b]	1355.97±123.68[b]	1427.88±60.09[b]	1358.74±50.55[ab]	1377.81±21.10[a]
Total feed intake (g)	2706.80±13.37[b]	2688.60±17.06[a]	2619.20±12.77[a]	2692.40±16.09[a]	2676.20±8.71[a]
**FCR	1.69±.06[a]	2.07±.25[a]	1.85±.09[a]	1.97±0.08[a]	1.96±.03[a]
***Mortality (%)	6.64±4.06[ab]	19.92±6.21[a]	9.96±4.06[ab]	9.96±4.06[ab]	3.32 ± 3.32[b]

Values are expressed as mean standard error of at least five replications each of which contains six birds. Different letter within a row differ significantly (P<0.05). Same letters within a row are statistically non-significant. (P<0.05)

OC=Own Control.

HW=Honeyweed.

*Body Weight gain (g) = Final body weight at 30th day –Initial body weight at 10th day.

** FCR (Feed Conversion Ratio) = Total feed intake (g)/Body weight gain (g).

*** Mortality (%) =Number of dead birds/Total number of birds× 100%.

4.2 Serum biochemical parameters of broilers fed with different diets

The effects of formulated diets on some serum biochemical parameters like total cholesterol, HDL-cholesterol, LDL -cholesterol, triglycerides of broilers are shown in figures (4.1 to 4.4). Serum total cholesterol, HDL-cholesterol, LDL -cholesterol, triglycerides of broiler chicks in different dietary treatments during experimental periods were almost statistically significant in 30^{th} day ($P \leq 0.05$). The results clearly showed that HW supplemented diets had significant effects on serum total cholesterol, HDL-cholesterol, LDL -cholesterol, triglycerides of broilers.

It was observed that the total cholesterol level in T1, T2, T3, T4 and T5 were 131.31, 125.23, 117.20, 113.52 and 111.54 mg/dl, respectively at the end of the experiment (30^{th} day). The lowest value (111.54 mg/dl) was recorded in birds fed with 15% HW and highest value (131.37 mg/dl) was recorded fed with commercial diet (Figure 4.1).

The highest HDL-cholesterol content was found in T5 (66.58 mg/dL) followed by T4 (61.21 mg/dl), T3 (61.79 mg/dl), T2 (45.42 mg/dl) and T1 (36.08 mg/dl) at 30^{th} day (Figure 4.2). In statistical analysis T1 and T2 were significant compare with T2, T3 and T4 HDL-cholesterol is called as good cholesterol, was found significantly ($p \leq 0.05$) highest for HW supplemented diet (Figure 4.2).

Our study showed that LDL- cholesterol level in T1, T2, T3, T4 and T5 were 68.12, 62.23, 55.45, 50.40 and 46.21 mg/dl, respectively. The lowest value (46.21 mg/dl) was recorded in birds fed T5 (Own control +15% honeyweed and highest value (68.12 mg/dl) was recorded in T1 (Figure 4.3).

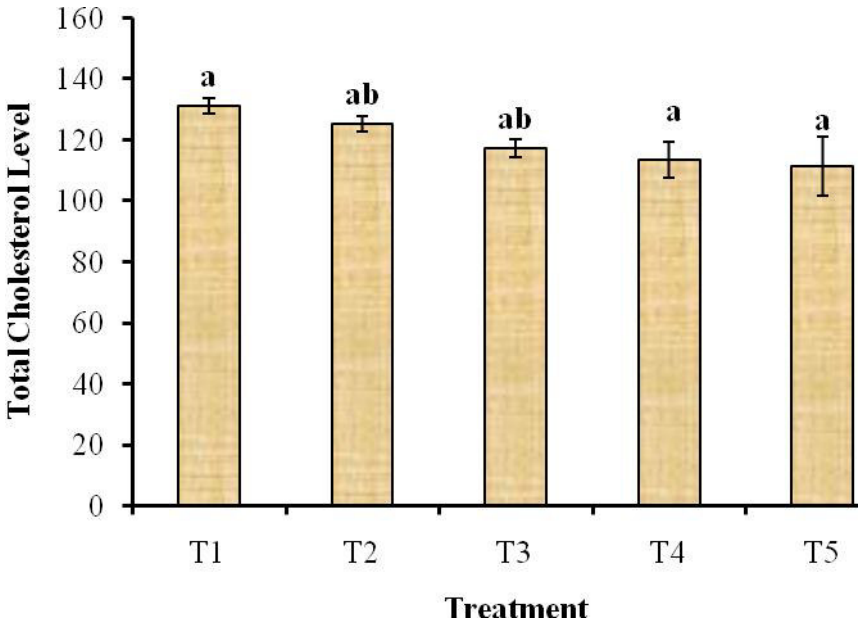


Figure 4.1: Serum total cholesterol of broiler chicks fed with commercial control (T1), Own control (T2), Own control +5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds. Data point bearing different letters are significantly different at $p \leq 0.05$.

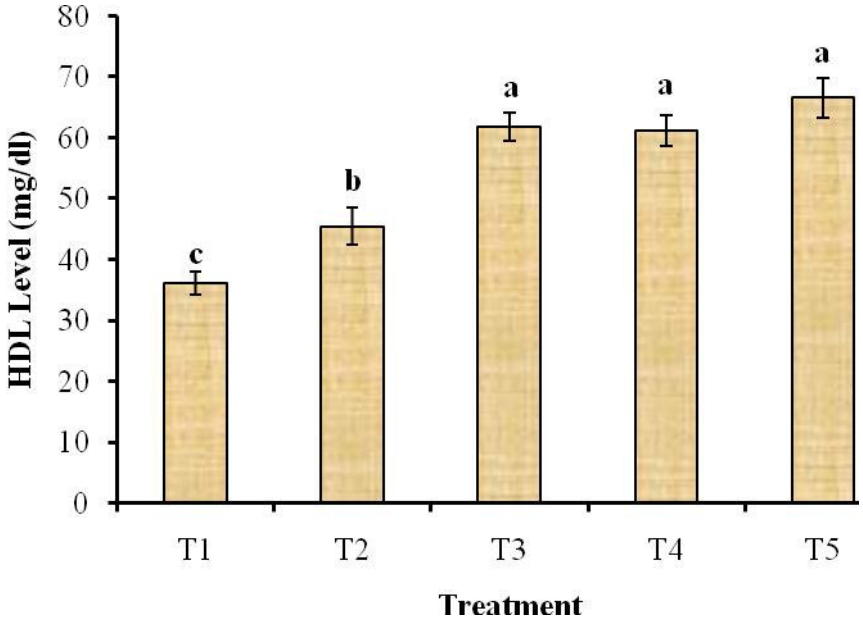


Figure 4.2: HDL level of broiler chicks fed with commercial control (T1), Own control (T2), Own control +5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds. Data point bearing different letters are significantly different at $p \leq 0.05$.

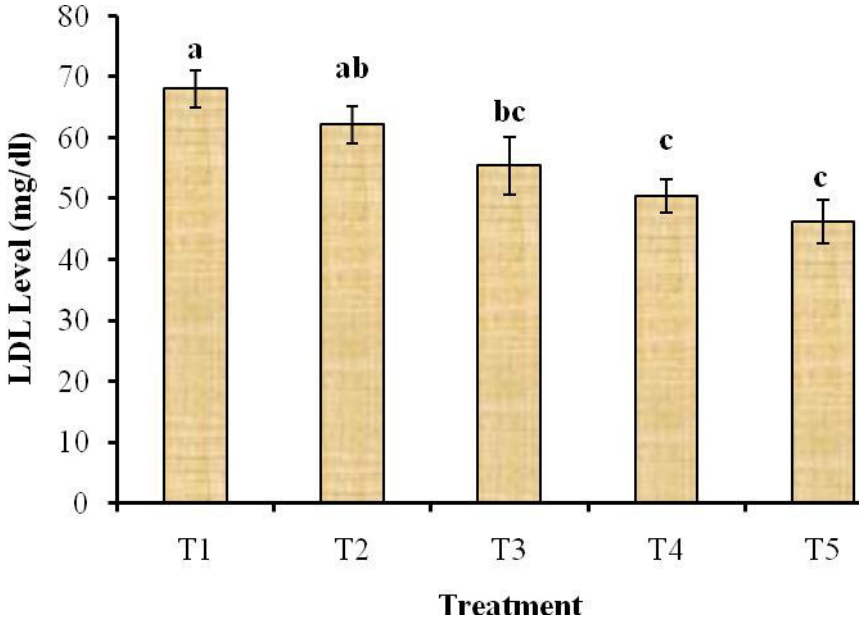


Figure 4.3: LDL level of broiler chicks fed with commercial control (T1), Own control (T2), Own control +5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds. Data point bearing different letters are significantly different at p≤ 0.05.

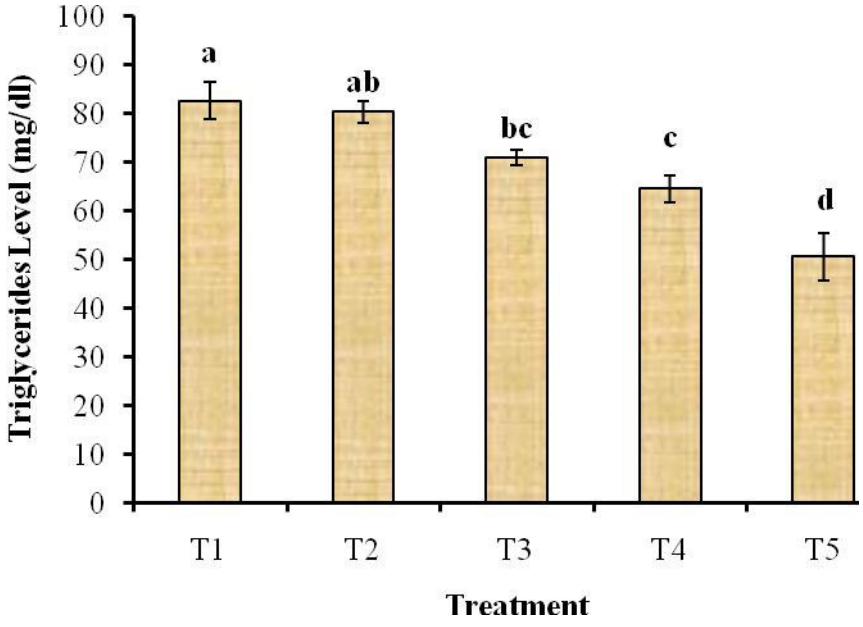


Figure 4.4: Triglycerides level of broiler chicks fed with commercial control (T1), Own control (T2), Own control +5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds. Data point bearing different letters are significantly different at $p \leq 0.05$.

Honeyweed supplemented diets decreased serum triglycerides level. At 30th day, the lowest serum triglycerides content was found in T5 (50.61mg/dl), T4 (64.64 mg/dl), T3 (70.89 mg/dl) compared to control treatments T2 (80.35 mg/dl) and T1 (82.62 mg/dl), respectively (Figure 4.4). All treatments showed statistically significant level with each other.

4.3 Body weight calculation of broiler fed with different diets

Honeyweed supplemented diets increased the body weight gain to a certain level. During the experiment period of 30 day, the highest body weight gain is found in commercial feed supplemented birds. But among the honeyweed added feed supplemented birds, T3 (Own feed + 5% honeyweed) showed the maximum body weight gain. (Figure 4.5)

4.4 Food conversion ratio calculation of broiler fed with different diets

During the experiment period of 30 day, the lowest FCR is found in commercial feed supplemented birds. But among the honeyweed supplemented treatments supplemented birds, T3 (Own feed + 5% honeyweed) showed the lowest FCR value which is more or less similar to the commercial control. (Figure 4.6)

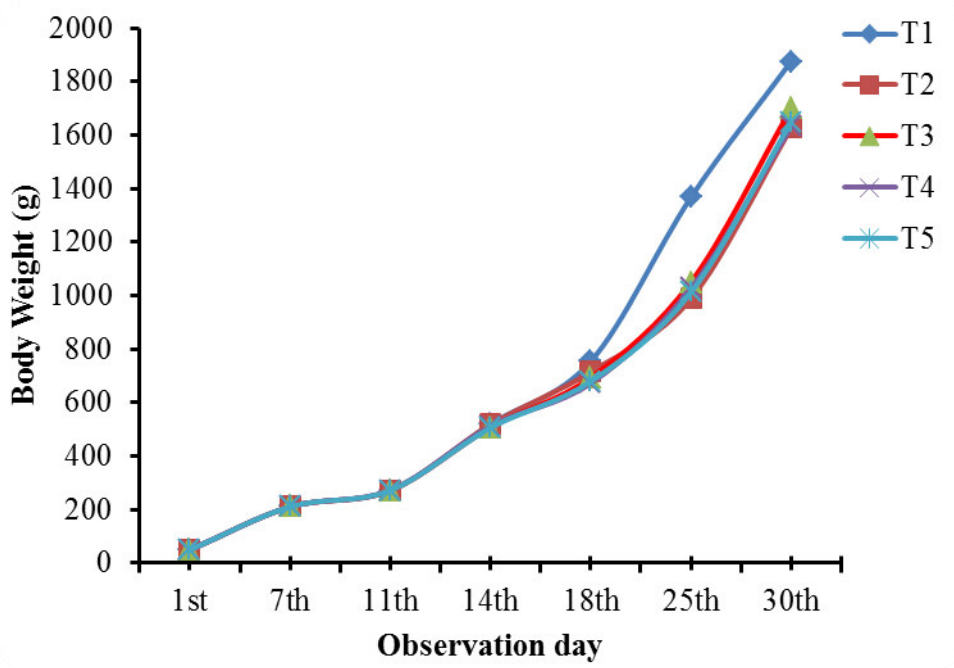


Figure 4.5: Body weight gain of broiler chicks fed with commercial control (T1), Own control (T2), Own control + 5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds.

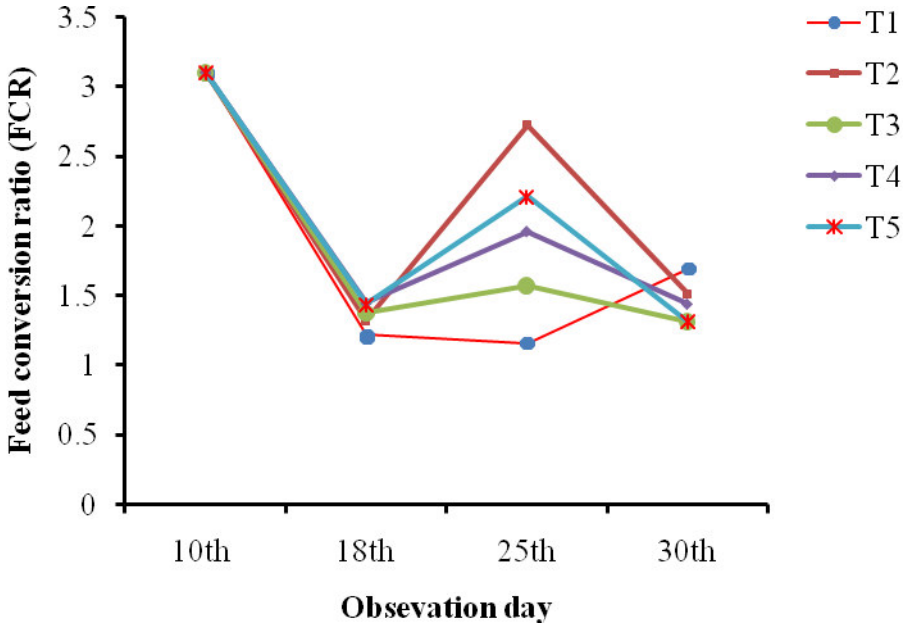
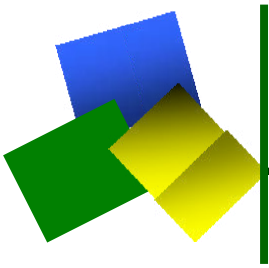


Figure 4.6: Feed Conversion Ratio (FCR) of broiler chicks fed with Commercial control (T1), Own control (T2), Own control + 5% Honeyweed (T3), Own control +10% Honeyweed (T4), Own control +15% Honeyweed at 30th day of treatment. The results are expressed as mean± SD of six birds.



CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

This study was conducted to investigate the effect of different doses of honeyweed supplemented diet on growth performances, serum lipid profile, FCR (Feed Conversion ratio) and mortality rate of broiler. The experimental birds were classified into five treatments differentiated by feeding with commercial control feed (T1), own control feed (T2), own formulated feed supplemented with 5% honeyweed (T3), own formulated feed supplemented with 10% honeyweed (T4), own formulated feed supplemented with 15% honeyweed (T5) for a period of 30 days. Each treatment contained six birds. Some physical parameters were recorded daily and the chemical parameters were measured at 30^{th} of the feeding trial. All results are expressed as mean ± standard deviation. The one way analysis of variance of some values was done followed by Duncan's multiple range tests to evaluate the differences among the mean values.

In this study, the growth performance of broiler chickens was studied in terms of body weight gain (BWG), total feed intake, feed conversion ratio (FCR) and mortality rate. Lipid profile was analyzed by measuring the total cholesterol, HDL, LDL and Triglycerides level.

5.1 Effects of formulated diets on growth performances of experimental birds

Growth performance of various treatments of broilers (Loman) varied widely and is shown in table 4.1. Final body weight of birds with different supplemented diets were T1 (1876.0 gm), T2 (1628.0 gm), T3 (1700.0 gm), T4 (1632.0 gm) and T5 (1650 gm). T1, T2 and T3 are statistically non-significant whereas T5 and T5 are statistically significant.

Bodyweight gain of broilers of different dietary treatments during experimental period were found highest in T1 (1603.91 gm) i.e. commercial feed followed by T3 (1427.88 gm), T5 (1377.81 gm), T4 (1358.74 gm) and the lowest body weight gain was found with T2 (1355.97 gm) treatment.

Similar results were found in chicken when fed diet supplemented with 30% buck wheat seeds (Gupta *et al*., 2002). The grain of this underutilized, non-conventional crop can be fed to chicken to the level of 30% of the diet without any adverse effect on growth and development of the chickens. The research conducted by Jacob and Carter (2008) indicated that up to 60% buckwheat can be included in broiler diets with no significant effect on body gain. Honeyweed can be included in organic broiler diets with significant effect on overall BW gain. Feed conversion increased as the level of honeyweed in the diet increases. As the difference between the price of organic corn and buckwheat widens, it will become more economical to use buckwheat despite the decreased feed efficiency. Studies have shown that the use of growth stimulants have a positive impact on the growth of broiler chickens (Denli, Oka, and Celik 2003). Yang *et al*., (2009) reported that adding antibiotics in broiler chicken diets improves bodyweight gain, feed intake and feed conversion ratio. Bedford, (2000) found that the antibiotics as growth promoters are in direct contact with intestinal micro flora, because these compounds had no effect on the sterile animals. Intestinal microflora by interaction with nutrient digestion may cause a significant effect on the host animal nutrition, health and performance of their growth (Barrow *et al*., 1992). When pathogens are attached to the intestinal mucosa, intestinal functions are strongly influenced (Droleskey *et al*., 1994) and the immune system is threatened (Neish *et al*., 2002). Chickens that were grown in germ-free condition rather than normal chicks that grew to bacteria and viruses exposure had15% higher growth rate (Klasing *et al*., 2001). However, in order to prevent antibiotic resistance in humans against pathogenic bacteria and also remove

residual antibiotics in poultry products, the abuse of antibiotics in poultry production was prohibiting. There is many evidence showing that the use of probiotics in poultry diets improves immune function, improved body weight, diarrhea decrease and feed conversion ratio (Reid and Friendship, 2002; Patterson and Burkholder, 2003).

5.2 Effects of formulated diets on FCR and Mortality rate

A significant result was found in total feed intake and non-significant FCR among different dietary treatments. FCR is lowest in T1 (1.69). But this treatment was supplied with commercial feed that is combined with antibiotics. Bedford, (2000) found that the antibiotics as growth promoters are in direct contact with intestinal micro flora, because these compounds had no effect on the Sterile Animals. Intestinal micro flora by interaction with nutrient digestion may cause a significant effect on the host animal nutrition, health and performance of their growth (Barrow *et al*., 1992). Among other treatments supplied with honeyweed leaves T3 showed the lowest value (1.85). Similar results were found when buckwheat supplemented diets fed to poultry (Sayed *et al*., 2015, Chowdhury and Koh, 2018).

The variations in FCR that occurred among the different fed groups compared to control may be depended on feed intake. Similar results were found when moringa leaf supplemented diets fed to poultry (Nkukwana *et al*. 2014). Thus, contrasting results may also be caused by variations in chemical composition, cultivated regions, environmental and rearing conditions or different ages of birds used in the studies.

Mortality rate is lowest in T5 (3.32 %) followed by T1 (6.64%), T3 (9.96%), T4 (9.96%), T2 (19.92%).The results obtain showed that mortality rate decreased from 19.92% to 3.32% by supplementation of broiler feed ration with honeyweed. It has been reported to improve immunity due to the presence of pharmacologically active constituents. Several studies have

observed that HW has potential as an alternative to antibiotics and vaccination to improve immunity and to reduce mortality in rat owing to the presence of pharmacologically active constituents such as Quercetin, Leonotinin, Rutin, Leonotinin (Sayed *et al*. 2016). Similarly, another medicinal herb, black cumin supplementation in broiler diets has been reported to strengthen the immune system by preventing lipid peroxidation and liver damage (Sogut *et al*., 2012). Our results predicted the similar mechanism with black cumin, even though, we did not conduct the experiment to find out the insight molecular mechanism. Rutin is the most influential pharmacological compound in honeyweed, and is reported to improve immunity, lipid profile, antioxidant activities, cytotoxic actrivity to cancer cell (Sayed *et al*., 2016). The poor performance of chickens fed higher levels of buckwheat was probably due to the characteristics of hardness and springiness of the buckwheat flour and high crude fibre content. Birds have limited ability to digest crude fibre owing to an insufficient amount of the cellulase enzyme in their digestive systems (Sundu, Kumar, and Dingle 2002; Choct 2015). Buckwheat hulls are also rich in antioxidants comprising tocopherols, rutin, quercetin derivatives, and other phenolic substances. The hulls are more abundant in total phenolics and flavonoids in comparison to other parts of buckwheat grain. The antimicrobial activity of phenolic substances has been widely reported (Cushnie and Lamb, 2005).

5.3 Effects of formulated diets on Serum lipid profile

In previous studies, the authors observed that supplementation of BWS and BCS powder in diets significantly decreased serum total cholesterol, triglycerides and LDL-cholesterol concentrations, and the population of *E. coli* in broilers and layers (Islam *et al.*, 2011; Sayed *et al*., 2015; Siddiqui *et al*., 2015). The findings of our current research are more or less in accordance to the findings of some earlier researchers (Guler *et al*., 2006; Abu-Dieyeh *et al*., 2008). The current findings and earlier results (Islam *et al.*, 2011; Sayed *et al*.,

2015; Siddiqui *et al*., 2015) suggest that herbal feed additives might be an effective alternative to synthetic antibiotics for the promotion of health and performance of poultry.

The effects of formulated diets on serum lipids like total cholesterol, HDL-cholesterol, and triglycerides of broilers shown in figures 4.1-4.4. Total cholesterol of broiler chicks in different dietary treatments during experimental periods were almost statistically similar and the differences were not significant ($P<0.05$) from 0 to 30 days. So the results clearly showed that honeyweed supplemented diets up to 15% dietary level had non-significant effects on total cholesterol. HDL level of different diets were significantly changed but among the honeyweed supplemented diet treatments ranged from 5-15% concentration, HDL level changed non-significantly. It is interesting that LDL cholesterol level decreased in case of 5-15% honeyweed supplemented diet throughout the experimental period. Our results are similar to many other findings. There are some phyto-chemicals have already been identified (Sayed *et al*., 2016) which can be played a significant role to regulate the lipid profile of broiler chicks.

According to the results on the lipid profile of broilers in this study it could be deduced that buckwheat seeds and honewyweed have favourable effects on serum metabolites. Both black cumin seeds (BCS) and buckwheat seed (BWS) have been reported to possess hypolipidemic and hypocholesteremic properties in animal studies (El-Bagir *et al*., 2006; Sayed *et al*., 2015). In the current study it was observed that buckwheat seeds and honewyweed supplementation in broiler diets significantly decreased serum total cholesterol, LDL and triglycerides levels, but increased HDL cholesterol compared with the control treatment (Figure 4.2). Similar effects of BCS and BWS on serum lipid profile had been obtained by earlier investigators (Tomotake *et al*., 2006; Islam *et al*., 2011; Sayed *et al*., 2015; Siddiqui *et al*., 2015; Siddiqui and Sayed, 2015). Al-Beitawi and El-Ghousein (2008) reported that feeding broiler chicks with BCS

supplemented diets reduce plasma cholesterol and triglycerides compared with broiler chicks fed control diet. The high weight gain reported in the group fed fenugreek confirmed the results obtained (Abbas *et al*., 2012) who reported that supplementing Fenugreek to broiler diet resulted in an increased body weight. Reduced serum triglycerides and total cholesterol levels were observed, while the serum HDL cholesterol level increased owing to supplementation of layer rations with black cumin seeds (Akhtar *et al*., 2003; Siddiqui and Sayed, 2015). Furthermore, Sayed *et al.* (2015) observed similar results with BWS supplementation with chitosan in broiler diets.

Although the mechanism of lipid profile improvement is not clearly understood from the current study, this may be because of possible cholesterol-lowering mechanisms of Isoquercitrin, Rutin, Leonurineetc, such as inhibition of cholesterol oxidation (Yan *et al*. 2006) and reduced HMG-CoA-reductase activity (Ha *et al*. 2005).Furthermore cholesterol-lowering effect of buckwheat is linked with lower digestibility of buckwheat and the presence of fibre-like substances, which is indicated by an increase in the levels of neutral and acid sterols in broiler faeces observed when a diet rich in buckwheat protein products (Tomotake *et al*., 2001) is administered.

This report, for the first time, has demonstrated that feeding buckwheat (15%) along with (5% to 15%) honeyweed. In addition, several studies suggested that buckwheat has a reasonable feed value, high antioxidant activity and contains lysine with other essential amino acids and bioorganic compounds which reduce mortality, and blood serum cholesterol, triglycerides, LDL and HDL concentrations (Meyers and Meinke 1994; Holasova *et al*., 2002; Zhang *et al*., 2012). Although the mechanisms of the positive influence of a higher level of buckwheat on the lipid profiles of the birds are difficult to explain from the current data, they might be associated with the effects of bioactive compounds in the seeds of buckwheat that affected the lipid profile of the birds (Holasova, *et al*., 2002; Zhang *et al*., 2012). Antioxidant activity and lipid profile-

changing activities of buckwheat were both found in earlier studies (Wieslander, Lin *et al*., 1998; Gang *et al.,* 2012; Zhang *et al.,* 2012). For example, Wieslander observed that the inclusion of buckwheat in human diets significantly decreased serum concentrations of triglycerides, total cholesterol and low-density lipoprotein (LDL), and increased the concentration of beneficial high-density lipoprotein (HDL). In another study, hyperlipemia patients showed lower serum triglycerides and cholesterol concentrations after they began to eat tartary buckwheat flour and stopped taking medicine against hyperlipemia (Lin *et al*., 1998). Buckwheat protein product showed strong hypocholesterolemic activity in rats fed a cholesterol-enriched diet (Kayshita *et al*., 1997; Zhang *et al*., 2012). The mechanism of these effects has still to be clarified, but could be related to the low digestibility of buckwheat protein along with honeyweed, its good nutritional value, or specific effects of phytochemicals in the seeds of buckwheat such as rutin (Halosova *et al*., 2002). As the price of organic maize continues to increase, the lower price for buckwheat may make it an attractive economical replacement in organic broiler diets (Jacob and Carter, 2008). In addition honeweed is a medicianl plant generally grown in road side or any other fallow high land, can be collected free of cost. Therefore, honeyweed and buckwheat could be regarded as an alternative to artificial antibiotics which could include beneficial side effects for sustainable feed production (Jacob and Carter 2008; Leiber *et al*., 2009). Although positive effects have been reported on poultry and rat consuming buckwheat alone (Hirano *et al*., 1990; Razdan and Pettersson, 1994; Morris, 1980; Tanaka *et al*., 1997), this is the first report on positive lipid profile-changing effects of buckwheat with trace amounts of honeyweed. Results in the present study found no detrimental effects of honeyweed.

It was observed that, at 30^{th} day the values of total cholesterol level in T1, T2, T3, T4 and T5 were 131.31, 125.23, 117.20, 113.52 and 111.54 mg/dl respectively. The lowest value (111.54 mg/dl) was recorded in birds fed

Treatment 5 (Own control +15% honeyweed and highest value (131.37 mg/dl) was recorded in Treatment-1 (Commercial control) (Figure 4.1). T1, T4 and T5 treatments are statistically non-significant and T2, T3 are also non-significant to each other.

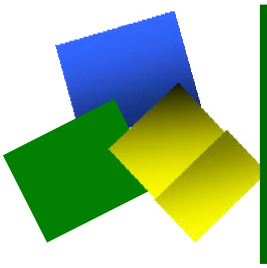
The highest HDL-cholesterol content was found in T5 (66.58 mg/dL) followed by T4 (61.21 mg/dl), T3 (61.79 mg/dl), T2 (45.42 mg/dl) and T1 (36.08 mg/dl) at 30^{th} day (Figure 4.2). In statistical analysis T1 and T2 were significant whereas T2, T3 and T4 were non-significant.

It was observed that, at 30^{th} day the values of LDL- cholesterol level in T1, T2, T3, T4 and T5 were 68.12, 62.23, 55.45, 50.40 and 46.21 mg/dl respectively. The lowest value (46.21 mg/dl) was recorded in birds fed T5 (Own control +15% honeyweed and highest value (68.12 mg/dl) was recorded in T1 (Figure 4.3). Rather than T4 and T5, T1, T2 and T3 were statistically significant.

Honeyweed supplemented diets decreased serum triglycerides level. At 30^{th} day, the lowest serum triglycerides content was found in T5 (50.61mg/dl), T4 (64.64 mg/dl), T3 (70.89 mg/dl) compared to control treatments T2 (80.35 mg/dl) and T1 (82.62 mg/dl) respectively (Figure 4.4). Every treatment showed statistically significant value.

Honeyweed have been reported to possess hypolipidemic and hypocholesteremic properties in animal studies. This study showed honeyweed diets at different levels caused a significant reduction ($p \leq 0.05$) in the levels of serum total cholesterol and triglycerides compared to control treatments. At the same time, honeyweed supplemented diets increased serum HDL level. Previous study (Świątecka, Markiewicz, and Wróblewska 2013) demonstrated the antioxidant activity of honeyweed and provides further evidence of the antinociceptive and anti-inflammatory activities of its extract, which have also been reported by others (Islam *et al*., 2005, Shin *et al*., 2009). In addition, possible mechanisms to attenuate the inflammatory response (proinflammatory

cytokine and oxidative stress) were explored. The phytochemical analysis showed honeyweed contains both phenolic compounds, such as chlorogenic acid, caffeic acid, p-coumarin and ferulic acid, and flavonoids, such as quercetin. With the exception of quercetin, these secondary metabolites are hydroxy derivatives of cinnamic acid (El-Seedi *et al*., 2012). The detection of these compounds in honeyweed agrees with previous studies (Pitschmann *et al*., 2016, Sitarek *et al*., 2016; Sayed *et al*., 2016).



CHAPTER VI

SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSIONS

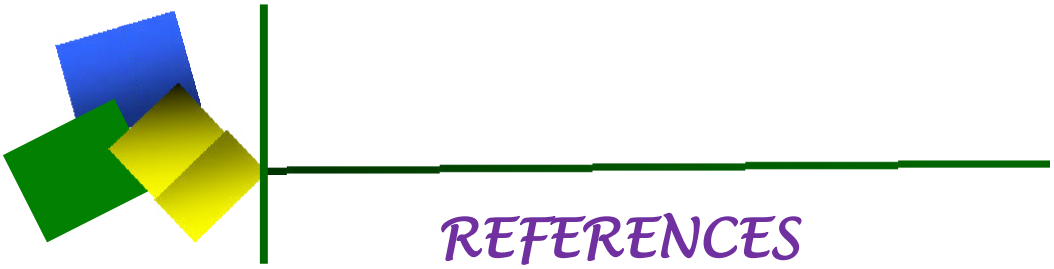
Phytogenic feed additives and plant-derived products are being used in animal feed to improve the performance of agricultural livestock. This trend has recently gained increasing interest, especially for use in swine and poultry. This appears to be strongly driven by the ban on most of the antibiotic feed additives within the EU in 1999, a complete ban enforced in 2006, and ongoing discussions to restrict their use outside the EU because of speculated risk for generating antibiotic resistance in pathogenic microbes. In this context, phytogenic feed additives are discussed possibly to add to the set of non-antibiotic growth promoters, such as organic acids and probiotics, which are already well established in animal nutrition. Phytogenics, however, are a relatively new class of feed additives and our knowledge is still rather limited regarding their modes of action and aspects of their application. Therefore, this study was conducted to investigate the effect of honeyweed supplemented diets on growth performances, serum lipid profile, body weight gain and FCR of broiler chickens.

In relation to growth performance of chickens, highest body weight gain (1603.91gm) and feed intake (2706.80 g) was found in commercially supplemented diet (T1) compared to other treatments. But as we know commercial controlled feed contains antibiotic and this aid the promotion of growth, so T3 that is fed with 5% honeyweed along with own controlled feed showed the best results without the application of antibiotics. Mortality rate was decreased from 19.92% (in T1) to 3.32% (in T5) with the increase of honeyweed supplementation in diets. However, FCR was highest to T2 (1.9). The poor performance of chickens fed higher levels of honeyweed was probably due to the characteristics of off smell of the honeyweed leaf powder.

A significant variation in serum lipid profile (total cholesterol, triglycerides, LDL and HDL-cholesterol) was found among the experimental diet groups. Serum total cholesterol content of broiler chicks at 30 days experimental period varies significantly. All treatments other than controls showed better performance. Honeyweed supplementation in chicken diets decreased serum total cholesterol, LDL and triglycerides while HDL-cholesterol was increased. The lowest serum total cholesterol was found in T5 (111.54 mg/dL) and highest value (131.31 mg/dL) was recorded in Treatment 1.

T5 diet (Own feed+15% honeyweed) had the highest HDL-cholesterol concentration (66.58 mg/dl) and Treatment-1(Commercial controlled feed) diet had the lowest HDL-cholesterol concentration (36.08 mg/dl). Broilers receiving Treatment-1 diet (Commercial control) had the highest LDL-cholesterol concentration (68.12 mg/dl) and Treatment-5 (Own feed+15% honeyweed) diet had the lowest LDL-cholesterol concentration (46.21 mg/dl). Broilers receiving Treatment-5 (Own feed+15% honeyweed) diet had the lowest triglycerides concentration (46.57 mg/dL) and highest triglycerides concentration in Treatment 1(82.47 mg/dL) (Commercial control).

Based on the findings it may be concluded that honeyweed supplemented diets have positive impact on broiler growth rate. Diet supplemented with 5% honeyweed (T5) may be used in poultry industry for increasing the rate of poultry as it showed lowest FCR rate of mortality rate gain compared to control treatments. Moreover the T3 treatment was found to have good impact on lowering the health hazardous serum total cholesterol, LDL and triglycerides along with elevation of serum HDL-cholesterol level with highest body weight gain without the use of any antibiotic. Thus, poultry industries can produce safe and low cholesterol poultry meat as there is a positive correlation between serum cholesterol content and meat cholesterol. However, further in-depth research is highly recommended for assessing the depth mechanisms of lipid profile lowering effect of honeyweed in broiler chickens.



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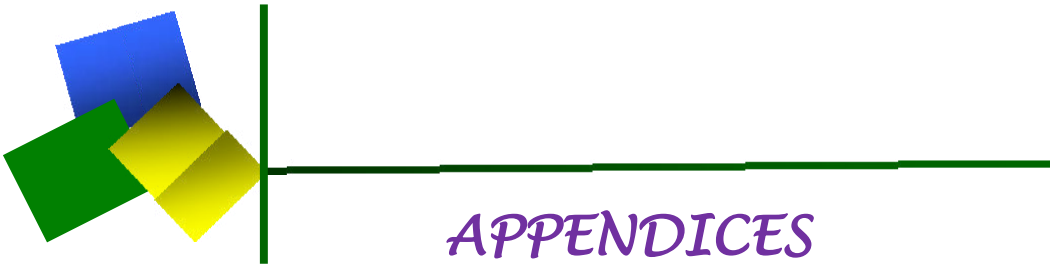

APPENDICES

APPENDICES

Appendix I: Analysis of Variance (ANOVA) table of growth performance

ANOVA table for Initial Body Weight (IBW)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	5.397	4	1.349	1.608	.211
	Within Groups	16.783	20	0.839		
	Total	22.180	24			

ANOVA table for Final Body Weight (FBW)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	216224.00	4	54056.00	2.178	.108
	Within Groups	496280.00	20	24814.00		
	Total	712504.00	24			

ANOVA table for Body Weight Gain (BWG)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	217000.397	4	54250.099	2.193	.107
	Within Groups	494867.743	20	24743.387		
	Total	711868.140	24			

ANOVA table for Feed Intake (FI)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	23032.00	4	5758.00	5.119	.005
	Within Groups	22498.00	20	1124.900		
	Total	45530	24			

ANOVA table for Feed Conversion Ratio (FCR)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	.417	4	.104	1.276	.312
	Within Groups	1.635	20	.082		
	Total	2.053	24			

ANOVA table for Mortality						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	771.568	4	192.892	1.944	.142
	Within Groups	1984.032	20	99.202		
	Total	2755.600	24			

Appendix II: Serum Lipid profile of broiler chickens

Parameters	Treatments				
	Commercial Control (T1)	OC (T2)	OC + 5% HW (T3)	OC + 10% HW (T4)	OC + 15% HW (T5)
Total Cholesterol	131.31±2.56[a]	125.23 ±2.57[ab]	117.20 ±2.99[ab]	113.52 ±5.77[a]	111.54±9.68[a]
*HDL	36.08±1.89[c]	45.42±3.06[b]	61.79±2.30[a]	61.21±2.58[a]	66.58±3.24[a]
**LDL	68.12±3.04[a]	62.23±3.08[ab]	55.45±4.71[bc]	50.40±2.77[c]	46.21±3.56[c]
***Triglycerides	82.62±3.85[a]	80.35±2.29[ab]	70.89±1.56[bc]	64.61±2.72[c]	50.61±4.96[d]

Appendix III: Analysis of variance (ANOVA) table of biochemical parameters

ANOVA table for total cholesterol (1-30 days)						
		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	1501.700	4	375.425	2.519	.074
	Within Groups	2980.607	20	149.030		
	Total	4482.307	24			

ANOVA table for HDL (1-30 days)						
		Sum of Squares	df	Mean Square	F	Sig.
HDL	Between Groups	3327.110	4	831.778	23.519	.000
	Within Groups	707.323	20	35.366		
	Total	4034.434	24			

ANOVA table for LDL (1-30 days)						
		Sum of Squares	df	Mean Square	F	Sig.
LDL	Between Groups	1561.308	4	390.327	6.367	.002
	Within Groups	1226.030	20	61.301		
	Total	2787.338	24			

ANOVA table for triglycerides (1-30 days)						
		Sum of Squares	df	Mean Square	F	Sig.
Triglycerides	Between Groups	3361.381	4	840.345	15.444	.000
	Within Groups	1088.224	20	54.411		
	Total	4449.605	24			