Chapter I: Introduction

Bangladesh is a densely populated country where protein is very extravagant for getting formal nourishment to these large populations. Poultry meat can achieve the demand in these circumstances (DLS, 2015). According to the national health strategy, an adult people need 120 g of meat every day and 104 pieces of eggs per year whereas, the availability is only 67.17 and 63.65%; respectively (DLS, 2015). Although meat production has been increasing over time in the country, but the per capita availability is far below the minimum requirement (Begum, 2008). The growth of the range of the poultry commercial enterprise has been developing rapidly than the other food producing animal industries. The ultimate aim of these trade industries is to produce meat and egg in a large volume to fulfill the rapid demand from the population parallel. A lot of commercial poultry enterprise works in the fields through different strategies in Bangladesh where farmers are apprehended to these commercial companies. They face the challenge of getting proper rationed and good quality feed.

Feed is the major component of the entire cost of production in the poultry industry. Broiler and layer feed is formulated with an optimum level of nutrition at reasonable cost for desirable weight gain, production and capability of feed utilization. To make certain more net return and to minimize high cost on feed introducing feed supplement and feed additives has been introduced to commercial feed industry which are the common practical strategy now-a-days (Javed *et al.*, 2009). Mainly feed additives are non nutritive substances used in poultry feed including antibiotics (bacitracin, methylene disalicylate or virginiamycin etc.), enzymes, antioxidants, pellet-binders, antifungal, colored pigments and flavoring agents. Some antibiotics are most effective against gram positive or gram negative or both gram positive and gram negative bacteria. Certain chemotherapeutic agents such as arsenicals and nitrofurans have been found to posses bacteriostatic or bactericidal properties and, at the effective levels, are not toxic to chickens or other host animals (Parks *et al.*, 2000).

The United States food and drug administration approved the use of antibiotics as animal additive without veterinary prescription in 1951 (Jones and Ricke, 2003). Also in the 1950s and 1960s, each European state approved its own national regulations about the

use of antibiotics in animal feed (Castanon, 2007). But many scientific finding suggested that antibacterial used for animal feeding as growth promoters become risky for human and animal health (Sahin *et al*., 2002; Thorns, 2000). That's why World Health Organization (WHO, 1997) has recommended that antibiotic should be phased and replaced by alternatives (Bywater, 2005). The use of the most antibiotics as feed additives has been banned by the EU due to cross-resistance against pathogens and residues in tissues. For this reason, scientists have searched for alternatives to antibiotics. In this view, varieties of substances are used in conjunction with or as alternatives to antibiotics in poultry diets. Herbs and spices, essential oils extracted from aromatic plants enzymes, organic acid, and probiotics all shown promising results for use in organic poultry production (Griggs and Jacob, 2005). Thus, use of antibiotics as a feed additive is no longer acceptable and it is prohibited in developed countries. As a consequence, it has become necessary to develop substitute material and strategies for animal growth advancement and disease prevention. Numerous health benefits have been attributed to the vegetables like onion and garlic, including antibacterial, antiviral, antiparasitic and antifungal properties. In addition, onion and garlic have antihypertensive, hypoglycemic, antithrombotic, antioxidant antihyperlipidemic, and anti inflammatory property (Lampe, 1999). Furthermore, Aji *et al.,* (2011) reported the useful influence of onion bulbs on growth yield of broiler chickens. Goodarzi *et al.,* (2013) reported that the beneficial influence of onion and garlic extract on the growth performance in meat-type broiler chickens. There is a very few works done over the globe regarding the replacement of antibiotic by onion and garlic as a growth promoter, hence, the current study was undertaken with the following objectives.

Objectives of the study

- To evaluate the effects of onion and garlic on the growth performance of broiler.
- To determine the effect of onion and garlic on the carcass characteristics of broiler.
- To observe the effects of onion and garlic on different blood parameters in broiler.

Chapter II: Review of literature

Several compounds such as enzymes, organic acids, probiotics, prebiotics and phytogenics are used to improve the performance. Recently aromatic plants and their associated essential oils or extracts are being concerned as potentially growth promoters. Most essential oils consist of mixtures of compounds such as phenolics and polyphenols, terpenoids, saponines, quinine, esters, flavone, flavonoids, tannins, alkaloids and nonvoltiles residues and their chemical composition and concentration of compounds is variable. These compounds have many effects as antimicrobial, stimulating animal digestive system, antioxidants, anticoccidail increase production of digestive enzymes and improve utilization of digestive products by enhancing liver functions (Ziarlarimi *et al.,* 2011). Plant extracts and spices as single or mixed compounds can be used as a promotion of performance and health condition of the animal (Goodarzi *et al.,* 2014).

2.1. Phytobiotics

Phytochemicals (commonly known as phytobiotics) as the plant derived compounds have wide range of activities in plants, animals and humans. These compounds are the secondary metabolites produced by the plant which possesses characteristic flavor and taste, primarily for its self-protection from being grazed/ eaten by animals and from pest attack.

2.2. Benefits of phytobiotics

Salient benefits of phytobiotics are as follows:

- Favorably alters the microbial population for maintaining the gut health
- Reduces the insult of pathogenic bacteria, virus and parasites in the gut thereby reduces the need for anti-biotic therapy
- Improves the body weight gain and feed efficiency
- Increases the anti-oxidant defense against oxidative stress
- Decreases cholesterol content through inhibiting hepatic enzyme activity
- Stimulates the digestive enzyme secretions and nutrient absorption
- Ameliorate the negative effects of heat stress

- Environmental friendly insecticide and pesticide

Over the years, more than 80, 000 compounds have been identified so far like phenols, flavonoids, tannins, saponins, essential oils, etc. Initially, these compounds were considered as waste, anti-nutritional and health affecting ones. But, now-a-days the approach towards them is changing globally as an antioxidants, digestive enhancer nutraceutical and health promoting substances (Narimani-Rad *et al.,* 2011). Since, the identification of its anti-microbial activity across different groups of organisms (Brut, 2004; Murali *et al.,* 2012) (both gram positive and gram negative organisms). In view of animal production especially in monogastrics (pigs and poultry production) they are mainly used as an alternative antibiotic growth promoter (Khaksar *et al.,* 2012; Karangiya *et al.,* 2016). Although, the exact mechanism of action is not yet known they have been found to favorably alters the gut micro-flora by reducing the number of pathogenic organisms. The probable mechanism of action is the through the alteration in membrane permeability to hydrogen ions (H+). In addition to its antibacterial activities, it also shows antiviral, anti-protozoan and anti-fungal actions. Their anti-fungal actions are getting more importance as these compounds are now being incorporated in to fungicide preparations which are cost effective as well as environmental friendly and also as fly repellent (Mansour *et al.,* 2011).

2.3. Antibiotics use in poultry ration

For several decades, some feed additives such as antibiotics have been vastly used in the poultry rations (Miles *et al.,* 1984; Harms *et al.,* 1986; Eyssen and Desomer, 1963). The antibiotics as growth promoter may produce one or more of the following effect:

- They may favor the growth nutrients-synthesizing microbes or in habit that of nutrient destroying microorganism
- Antibiotics may inhibit the growth of organisms that produced excessive amount of ammonia and other toxic nitrogenous waste products in the intestine ;
- They may improve availability or absorption of certain nutrient ;
- They may improve feed or water consumption or both;
- Antibiotics may instances prevent or cure actual pathological disease which occur either in the intestinal tract or systemically ;

- They may reduce the maintenance cost associated with turnover of the intestinal epithelium (Kahn *et al.,* 2005 and Miles *et al.,* 2006).

2.4. Resistance of antibiotics

The swan committee report (1969) was the first to suggest that the use of sub therapeutic levels of antibiotics for growth promotion and disease prevention could increase the risk of bacteria acquiring resistance to specific antibiotics (Nasir and Grashorn, 2006). The United Kingdom banned the use of penicillin and tetracycline for growth promotion in the 1970s.Sweden and Denmark banned all growth-promoting antibiotics in 1986 and 1999, respectively (FMI, 2006). In 1999, European Union banned four antibiotic growth promoters (virginamycin, spiramycin, tylosin and zinc bacitracin) which are commonly used in feed around the world. The United States banned the use of enrofloxacin in2005, (Colligon, 1999).since 1th January2006 the use of antibiotic growth promoters is prohibited in the European Union (Buchanan *et al.,* 2008). Due to the potential for bacterial resistance and antibiotic residues in animal products (Nasir and Grashorn, 2006) and drug residue in the body of the birds (Burgat, 1999), nowadays, some attempts have been made to replacing these additives with herbs.

2.5. Potential alternatives of antibiotics

Onion and garlic as natural growth promoters can be potential alternatives for common artificial growth promoters like antibiotics. The onion ***(****Allium cepa****)*** belong the Allium genus. Allium is derived from the Greek word for garlic. Onion is abulbous plant greatly tilled for thousands of years in majority countries of the world. It originated in the Near East and Central Asia (Ebesunun *et al.,* 2007).

2.6. Bioactivities of onion as a growth promoter

Onion contains plenty organic sulphur compounds such as Spropylcycteine sulfoxides, S-methyl-cysteine sulfoxide, Trans-S-(1-propenyl) cysteine sulfoxide, and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, b-sitosterol, saponins stigma sterol, sugars and very small amount of volatile oil compounds (Melvin *et al.,* 2009). Numerous health benefits have been attributed to the vegetable, including antibacterial, antiviral, antiparasitic and antifungal properties. In addition, onions have

antihypertensive, hypoglycemic, antithrombotic, antioxidant antihyperlipidemic, and anti inflammatory property (Lampe, 1999).Furthermore, Aji *et al.*, (2011) reported the useful influence of onion bulbs on growth yield of broiler chickens. The serum cholesterol was significantly decreased by dietary dehydrated onion in experimentally hypercholesterolemic rats (Vidyavati *et al.*, 2010). Goodarzi *et al.,* (2013) reported that the beneficial influence of onion extract on the growth performance in meat-type broiler chickens.

2.7. Bioactivities of garlic as a growth promoter

2.7. 1. Actives compounds of garlic

Garlic supplement to broiler chicks has been recognized for its strong stimulating effect on the immune system in addition to its positive effects on digestion in birds due to the very rich aromatic essential content of it (Demir *et al*., 2005). These functions were attributed to the bioactive compounds present in garlic such as alliin, daillyl sulphide and allicin (Amagase and Milner, 1993), which possess antimicrobial activity (Tsao and Yin, 2001) that could be responsible for the growth promoting effect of garlic. Garlic contains an active ingredient called alliin, which, when garlic is crushed in aerobic conditions, is converted by the enzyme allinase into allicin (Lanzotti *et al.,* 2006).The intermediate compound is alkyl sulphonic acid, which has the capacity to acidify the digesta of animals, and the sulphides released from allicin exert strong antibacterial and antioxidant activity (Sallam *et al.,* 2004; Lanzotti, 2006; Bozin *et al.,* 2008). Allyl sulphides exert multiple, antifungal, anti-inflammatory and immune enhancing activity and can regenerate liver tissue (Amagase *et al.,* 2001; Tatara *et al.,* 2005; Kandil *et al.,* 1987). Allicin, the bio-active component of garlic is reported to have the ability to infiltrate pathogen's cellular membranes and subsequent binding to key enzymes that results in blockage of cellular activities. Comprehensive knowledge about the single active compound or their possible synergistic or negative effects is required for the solution oriented developments in herbal treatment (Heinzl & Borchardt, 2015).

Previous studies have already shown that human nutrient supplements and feed additives derived from garlic possess antibacterial properties (Lanzotti, 2006; Toghyani *et al.*, 2011).It exerts health-promoting effects by preventing the development of bacteria, such

as *Escherichia coli*, *Enterobacteria* Spp., and *Salmonella typhimurium* (Kumar and Berwal, 1998; Ross *et al.,* 2001). Medical research on humans and experiments with rats and poultry have confirmed that garlic lowers blood levels of LDL cholesterol and reduces the rate of cholesterol oxidation (Lau, 2001), displays antioxidant and anti-cancer activity (Borek, 2001; Yang *et al.,* 2001), and enhances the immune resistance of living organisms (Kyo *et al.,* 2001). In animal production, garlic is usually used in the form of crushed bulbs, powder, garlic oil, extracts, and in mixtures with other herbs, mainly thyme (Puvača *et al.,* 2013). It increases phagocytic activity, production of interferon, interleukin and tumor necrosis factor α (Hanieh *et al.,* 2010).

2.7.2. Effect of garlic on reduction of cholesterol

There are evidence that garlic has cholesterol lowering effect in humans and animals due to the presence of sulphur-containing bioactive compounds in its homogenates (Chowdury *et al.,* 2002; *Niel et al.,* 1996; Shoetan *et al.,* 1984). Garlic clove has well over 33 sulphur compounds, several enzymes, 17 amino acids and minerals especially selenium (Jennifer, 2002). These phytochemicals which are responsible for garlic sharp flavor are produced when the plant cells are damaged either by chopping, chewing or crushing. As a result of these activities, enzymes stored in cell vacuoles trigger the breakdown of several sulphur containing compounds stored in the cell fluids. The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Allicin (diacyl disulphonate or diallyl sulphides) which is one of the most biological active compounds in garlic does not exist until it is crushed or cut. Injury to garlic bulbs activates the enzymes allinase which Meta-bolizes allin to allicin (Koch and Lawson, 1996). Garlic appears to enhance the synthesis of nitric oxide, which accounts for its antihypertensive and coagulant effects (Masoud, 2006). Also, selenium in garlic accounts for its antioxidant and cancer preventive effects (Ross, 1999). In rabbits fed high cholesterol diet, garlic or allicin supplement significantly inhibited hypercholesterolemia, reduced tissue cholesterol, lowered low density lipoprotein concentration (LDL or bad cholesterol), raised high density lipoprotein concentration (HDL or good cholesterol) and reduced erythematous changes in aorta by 50% (Mirhadi *et al.,* 1992; Bordia *et al.,* 1975). Clinical studies in humans have revealed the hypocholeste-rolemic effect of garlic (Silagy and Neil, 1994; Warshafsky *et al.,* 1993). Egg yolk cholesterol was reduced

drastically by feeding 1 or 3% of garlic powder to laying hens for 3 weeks (Sharma *et al.*, 1979). Depressed hepatic cholesterol concentration in chicken was observed when 2% garlic was fed for 14 days (Sklan *et al.*, 1992). Masoud (2006) further reported that garlic powder when used as feed additive can activate the digestive process and this serves as an antibacterial alternative growth promoter. Inclusion of garlic to high fat diets enhanced triglyceride catabolism. Cullen *et al.,* (2005) reported that 1% garlic supplement in pigs increased growth, feed conversion and meat quality. Therefore, to dispel this growing concern for cholesterol in the average Nigerian, this study was designed with the objective of investigating the growth, serum cholesterol and hematological parameters of broilers fed varying dietary levels of garlic.

2.7.3. Garlic reduces metabolic disorders

The main chemical components in the volatile form of isolated garlic seedling are diallyl disul-phide (23.33%), 1, 3-dithiane (18.34%) and dibutyl phthalate (6.30%) (Jin *et al.*, 2007). Garlic and its preparations have been widely recognized as agents for prevention of various metabolic disorders such as atherosclerosis, hyperlipedemia, thrombosis, hypertension and diabetes. Several clinical reports have shown that garlic has cho-lesterol- lowering effect in animals due to the presence of sulphur-containing bioactive compounds in its homo-genates (Neil *et al.*, 1996; Chowdhury *et al.*, 2002). When raw garlic bulb is chopped or crushed, the enzyme allinase activates alliin, a non-protein amino acid present in the intact garlic, to produce allicin. Other important sulphur-containing compounds present in garlic homo-genates are allyl methyl thiosulphonate, l-propenyl allyl thiosulphonate and y-L-glutamyl-s-alkyl-L-cysteine (Banerjee and Maulik, 2002). Garlic products have become more popular in the last decade. Market research con-ducted in United States (1998) showed that garlic pro-ducts were the most popular of all dietary supplements (Wyngate, 1998). Dozens of brands on store shelves can be classified into four groups: garlic oil, garlic oil macerate, garlic powder and aged garlic extract (AGE). Epidemiological and medical studies suggest that individuals regularly consuming garlic have longer blood clotting times and show lower blood lipid levels which means a reduced risk of stroke and cardiovascular disease. Some other studies show that eating garlic regularly reduces risk of oesophageal, stomach, and colon cancer. Garlic has broad range of biological activity, including immune stimulation and anti-

tumor activity (Riggs *et al*., 1997). In the view of the above observations, the present investigation was done to study the alteration of garlic juice and powder supplementation on certain serum biochemical parameters of broilers.

2.7.4. Novel functions of garlic

Garlic contains at least 33 sulfur compounds (Alliin, Diallyl sulfides and Allicin), several enzymes, 17 amino acids and minerals such as selenium (Newall *et al.,* 1996) which are responsible for antibacterial, antifungal, antiviral (Ankari and Mirelman, 1999), antioxidant (Prasad *et al.,* 2009), anti parasitic, antithrombotic, anti cancerous and vasodilator characteristics (Canogullari *et al.,* 2010). The sulphur compounds of garlic are responsible for garlic's pungent odour and many of its medicinal effects like lowering cholesterol level (Chowdhury *et al.,* 2002). Positive effects of garlic on growth rate, feed conversion ratio (FCR), carcass characteristics and mortality rate have been studied earlier (Demir *et al.,* 2003; Lewis *et al.,* 2003; Tollba and Hassan, 2003). Thus, the present study was designed to observe the potential of incorporating at certain levels of garlic as a phytogenic growth promoter in commercial broilers.

The anti-oxidative influence of garlic in meat becomes more imperative in less developed nations, considering storage problems and increasing use of alternative feed resources without due consideration for meat quality (Onibi *et al*., 2007). Horton and Prasad (1991) reported that garlic as a natural feed additive, improved broiler growth, Feed Conversion Ratio (FCR) and decreased mortality rate. Similarly, Demir, (2005) in his experiment demonstrated that garlic may be used as alternatives to an antibiotic growth promoter in broiler production also Javandel *et al.,* (2008) reported that the use of natural feed additives like garlic has made it possible for one to avoid the harmful effects of synthetic antibiotics.

2.8. Importance of the study

- Use of onion and garlic as potential source of feed additives.
- Beneficial effects of onion and garlic weight gain and FCR.

Chapter III: Materials and Methods

3.1. Study period and location of the experimental shed

The experiments were carried out from January to July 2017, at the Department of Animal Science and Nutrition and feeding trial was conducted from March to May in the experimental farm and research laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

3.2. Preparation of poultry shed for the experiment

The selected broiler shed was carefully dry cleaning 3 times for 2 days then washed and cleaned up by using tap water with disinfectant. Phenyl solution was also spread on the floor and ceiling, then brushing was done by using steel brush along with clean water. Brooding boxes and broiler cages were also cleaned by using tap water with disinfectant for 2 times. After cleaning and disinfecting, the house was left for one week.

3.3. Experimental design

The experiment was carried out for a period of 42 days where we considered 0 to 14 days as starter and 15 to 42 days as grower. The statistical design used for the experiment was CRD (Completely Randomized Design). In this experiment, total 104 chicks were equally and randomly distributed in four treatment groups (T_0, T_1, T_2 and T_3) with two replications for each having 26 birds per treatment group and 13 birds per replication. Diet T_0 was the control diet formulated without the inclusion of onion and garlic. 1% onion, 1% garlic, and a mixture of 0.5% onion and 0.5% garlic were formulated for T_1, T_2 and T_3 dietary treatment, respectively. Diets for all treatment groups including control were iso-caloric and iso-nitrogenous both in starter (0-14 days) and grower periods (15-42 days) according to NRC (1994).

Table 3.1: Layout of the experiment

Dietary treatment groups	No. of broilers per replication		Total no. of broilers per treatment
T_0 (Control)	R_1	13	26
	R_2	13	
T_1 (1% Onion)	R_1	13	26
	R_2	13	
T_2 (1% Garlic)	R_1	13	26
	R_2	13	
T_3 (0.5% Onion & 0.5% Garlic)	R_1	13	26
	R_2	13	
Grand total			104

3.4. Collection of day-old chicks (DOC)

A total of 104 DOC Ross 308 strain of mixed sex was purchased from an agent of Nahar Agro Complex Limited, Jhautala Bazar, Khulshi, Chittagong, Bangladesh. All chicks were examined for any kind of abnormalities and uniform size during purchasing. Average body weight of purchased chicks was 42.00gm.

3.5. Collection of Feed ingredients

3.5.1. Collection of onion and garlic

Onion and garlic were collected from Jhautala Bazar, Khulshi, Chittagong Metropolitan. Then they were cut into small slice. The slices were sundried for one week separately and finally ground for further use in the experiments.

3.5.2. Collection of other Feed ingredients

Other feed ingredients were collected from Pahartoli Bazar, Khulshi, Chittagong Metropolitan after observing its quality through organoleptic test (color, odor, smell etc.).

3.6. Processing of onion and garlic powder

The sliced onion and garlic turned a couple of times during sundry session until they become crispy. Then they were dried in hot air oven to eradicate the moisture contents so that they can easily blend. They were ground until the finer powder form was found.

3.7. Feeding standard

Feeding standard followed in the experiment was that of Bangladesh standard of specification for poultry feed (2nd Revision, BDS 233: 2003). The birds were provided with dry mash feed throughout the experimental period. All the rations were iso-caloric and iso-nitrogenous. Feeds were supplied ad-libitum along with fresh clean drinking water for all the time.

3.8. Feed formulation and feeding the birds

The birds were supplied mash feed. Mash feed was prepared manually from raw feed ingredients, which was collected from retail and wholesale market. Four types of ration were used for two phases such as broiler starter for T_0 (Control), T_1 (1% Onion), T_2 (1% Garlic), T_3 (0.5% Onion and 0.5% Garlic) and broiler grower for T_0 (Control), T_1 (1% Onion), T_2 (1% Garlic) T_3 (0.5% Onion and 0.5% Garlic). Rations were formulated according to the requirement of birds (For broiler starter: ME=3000 kcl/kg, CP=22%, Ca=1% and P=0.5% and for broiler grower: ME=3100 kcl/kg, CP=21%, Ca=0.9% and P=0.4%). The composition of different feed ingredients and nutritive value of starter and finisher rations are given in Table 3.2 to 3.3.

Table 3.2: Feed ingredients used in experimental broiler diets (starter phase)

Ingredients (Kg/100kg)	Starter ration (0-14 days)			
	T_0	T_1	T_2	T_3
Maize	57	56	56	56
Rice polish	7.5	7.5	7.5	7.5
Soybean oil	2	2	2	2
Soybean meal	23	23	23	23
Protein concentrate(Provimi^R)	4.75	4.75	4.75	4.75
Fishmeal	4	4	4	4
Dicalcium phosphate	0.5	0.5	0.5	0.5
Onion	0	1	0	0
Garlic	0	0	1	0
Onion and garlic	0	0	0	1(0.5onion+0.5garlic)
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vit-mineral premix(Compfeed-B^R)	0.25	0.25	0.25	0.25
Toxin binder (Vtox-XL^R)	0.25	0.25	0.25	0.25
Enzyme(Cbt-XL^R)	0.25	0.25	0.25	0.25
Total	100	100	100	100

In table 3.2, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic ,T_3= Experimental diet with 0.5% onion and 0.5% garlic Vitamin Mineral Premix in Rations that mentioned in table 3.2: contains following ingredients per kg diet: Vitamin A = 5000 IU, Vitamin D_3 = 1000 IU, Vitamin K = 1.6 mg, Vitamin B_1 = 1 mg, Vitamin B_2 = 2mg, Vitamin B_3 = 16 mg, Vitamin B_6 = 1.6 mg, Vitamin B_9 = 320 µg, Vitamin B_{12} = 4.8 µg, H = 40 mg, Cu = 4 mg, Mn = 40 mg, Zn = 20 mg, Fe = 2.4 mg, I = 160 µg.

Table 3.3: Estimated nutritional composition (DM basis) of the experimental broiler starter diets

Traits	Calculated value (%)			
	T_0	T_1	T_2	T_3
ME (kcl/kg)	3041.25	3008.25	3008.25	3008.25
Crude Protein (CP)	21.65	21.56	21.56	21.56
Crude Fiber (CF)	3.98	3.95	3.95	3.95
Ether Extract (EE)	5.20	5.19	5.19	5.19
Calcium (Ca)	0.87	0.87	0.87	0.87
Phosphorus (P)	0.83	0.83	0.83	0.83

N.B: In table 3.3, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic, T_3= Experimental diet with 0.5% onion and 0.5% garlic

Table 3.4: Proximate composition of the experimental broiler diets (starter phase)

Traits	Proximate value (%)			
	T_0	T_1	T_2	T_3
Dry Matter (DM)	87.5	87.45	88	88.1
Crude Protein (CP)	21.6	21.5	21.54	21.45
Crude Fiber (CF)	3.41	3.27	3.72	3.68
Ether Extract (EE)	3.97	4.22	4.08	4.7
Ash	5.87	5.92	6.55	6.4
Nitrogen Free Extract (NFE)	52.65	52.54	52.11	51.87

N.B: In table 3.4, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic, T_3= Experimental diet with 0.5% onion and 0.5% garlic

N.B. The protocol of proximate analysis is attached in Annex part.

Table 3.5: Feed ingredients used in experimental broiler diets (grower phase)

Ingredients (Kg/100kg)	Grower ration (14-42days)			
	T_0	T_1	T_2	T_3
Maize	58	57.5	57.5	57.5
Rice polish	8	7.5	7.5	7.5
Soybean oil	2.5	2.5	2.5	2.5
Soybean meal	21.5	21.5	21.5	21.5
Protein concentrate(Provimi^R)	4.75	4.75	4.75	4.75
Fishmeal	3.5	3.5	3.5	3.5
Dicalcium phosphate	0.5	0.5	0.5	0.5
Onion	0	1	0	0
Garlic	0	0	1	0
Onion and garlic	0	0	0	1(0.5+0.5)
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vit-mineral premix (Compfeed-B^R)	0.25	0.25	0.25	0.25
Toxin binder (Vtox-XL^R)	0.25	0.25	0.25	0.25
Enzyme (Cbt-XL^R)	0.25	0.25	0.25	0.25
Total	100	100	100	100

In table 3.5, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic ,T_3= Experimental diet with 0.5% onion and 0.5% garlic Vitamin Mineral Premix in Rations that

mentioned in table 3.5: contains following ingredients per kg diet: Vitamin A = 5000 IU, Vitamin D_3 = 1000 IU, Vitamin K = 1.6 mg, Vitamin B_1 = 1 mg, Vitamin B_2 = 2mg, Vitamin B_3 = 16 mg, Vitamin B_6 = 1.6 mg, Vitamin B_9 = 320 µg, Vitamin B_{12} = 4.8 µg, H = 40 mg, Cu = 4 mg, Mn = 40 mg, Zn = 20 mg, Fe = 2.4 mg, I = 160 µg

Table 3.6: Estimated nutritional composition (DM basis) of the experimental broiler grower diets

Traits	Calculated value (%)			
	T_0	T_1	T_2	T_3
ME (kcl/kg)	3086.05	3055.05	3055.05	3055.05
Crude Protein (CP)	20.84	20.73	20.73	20.73
Crude Fiber (CF)	3.95	3.88	3.88	3.88
Ether Extract (EE)	6.39	6.31	6.31	6.31
Calcium (Ca)	0.89	0.89	0.89	0.89
Phosphorus (P)	0.72	0.71	0.71	0.71

N.B: In table 3.6, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic, T_3= Experimental diet with 0.5% onion and 0.5% garlic

Table 3.7: Proximate composition of the experimental broiler grower diets

Traits	Proximate value (%)			
	T_0	T_1	T_2	T_3
Dry Matter (DM)	88.66	88.3	89.1	88.5
Crude Protein (CP)	18.00	17.65	17.90	18.01
Crude Fiber (CF)	4.05	3.95	4.3	3.8
Ether Extract (EE)	7.42	7.96	8.05	7.65
Ash	6.8	6.7	7.6	7.2
Nitrogen Free Extract (NFE)	47.61	52.04	51.25	51.84

N.B: In table 3.7, T_0 = Control diet T_1 = Experimental diet with 1% onion, T_2 = Experimental diet with 1% garlic, T_3= Experimental diet with 0.5% onion and 0.5% garlic

N.B. The protocol of proximate analysis is attached in Annex part.

3.9. Managemental procedure

The following management procedures were followed during the whole experimental period and the uniformity in the management practices were maintained as much as possible.

3.9.1. Brooding of the chicks

After proper cleaning, washing and drying, the brooding boxes were kept for two weeks under strict hygienic conditions. Then they were ready to received broiler chicks. The experiment was performed in summer season. Dry and clean newspaper was also placed in the brooding box. Newspaper was changed three times in a day from the floor of the brooding box till 1^{st} week. During the brooding period chicks were brooded at a temperature of 90-95°F during 1^{st} week and 90-85°F during 2^{nd} week respectively with the help of electric bulbs. The key concern was the comfort of broiler birds. Electric bulbs and fans were used to maintain the temperature.

3.9.2. Brooder and cage spaces

Each box brooder having 2.38 ft. × 2.08 ft. was owed for 30 birds. After 14 days later broiler birds were transferred to cage having 3.5 ft. × 1.63 ft. for 13 birds. Therefore, floor space for each bird in the brooding box was 0.17 sq. ft. and cage was 0.44 sq. ft. respectively.

3.9.3. Feeder and drinker spaces

At the initial stage of brooding, feed and water were given to birds on paper and small drinker. Feeding and watering were performed by using one small round plastic feeder and one round drinker with a capacity of 1.5 liter in each brooding box. Three drinkers were given as far one drinker for ten birds. The feeders and drinker were fixed in such a way so that the birds could eat and drink conveniently. After 7^{th} day small round feeder was replaced by small liner feeder (2.21 ft. × 0.25 ft.) in each brooding box. During the period of cage rearing large liner feeder (3.5 ft. × 0.38 ft.) and large round drinker with a capacity of three litters was used for feeding and drinking.

3.9.4. Method of feeding, watering and lighting

Sufficient amount of formulated mash feed and fresh clean drinking water was supplied to the birds throughout the experimental period. Feed and drinking water were given three times a day. Starter ration was supplied for 0 to 14 days and grower ration for 15 to 42 days. During the early stage of growth feed and water were given to birds on paper and small drinkers. The birds were exposed to a continuous lighting of 24 hours of photo period for first two weeks. From three weeks they were given 23 hours light and one hour dark.

3.9.5. Litter management

Dry newspapers were used as litter materials at a considerable depth during the brooding period. After the ends of brooding period birds were replaced in the cage for rearing until the end of experiment. Litter materials were cleaned by brush and disinfected hygienically with detergent for one time in a day.

3.9.6. Bio-security/Sanitation

Strict bio-security measurement was taken by washing drinkers and drinkers in disinfectant, spraying savlon on floor. Regular litter disposal also performed to reduce methane gas in the experiment room.

3.10. Record keeping

Following parameters were recorded throughout the experimental period.

3.10.1. Body weight

Body weight of the chicks was recorded at first day and then regular basis at the weekly intervals by a digital weighing balance for whole experimental period.

Fig 3.1: Day old chicks in brooding

Fig 3.2: Mixing of feed ingredients

Fig3.3: Brooding of the chicks

Fig 3.4: Feeding of broiler

Fig 3.5: Weight measurement of broiler

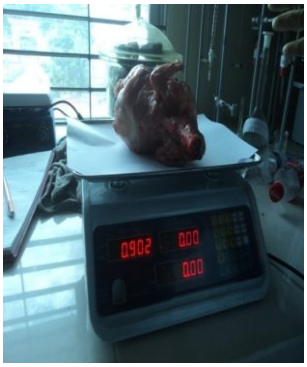


Fig3.6: Evaluation of carcass traits

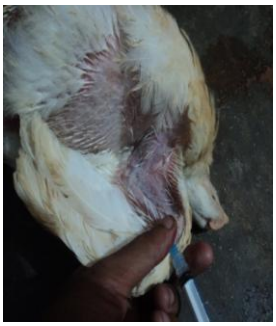


Fig 3.7: Collection of blood from broiler

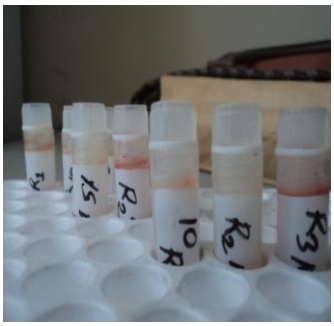


Fig 3.8: Serum samples for chemical analysis

3.10.2. Feed intake

Weekly feed intake was calculated by deducting the left over feeds from the total amount of supplied feed to the broilers.

3.10.3. Mortality

Mortality was recorded throughout the experimental period when death occurred in any replication.

3.11. Calculation of data

3.11.1. Body weight gain

The body weight gain was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final body weight - Initial body weight

3.11.2. Feed intake

Quantity of offered feed was weighed weekly. Refusal feed was recorded to determine the feed intake per week. Average feed intake was calculated weekly as gm/bird.

3.11.3. Feed conversion ratio (FCR)

The amount of feed intake per unit of weight gain is the feed conversion (FC) and the resulting ratio between them was measured as FCR. This was calculated by using following formula.

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

3.12. Evaluation of carcass traits

On day 42, five birds per experimental unit representative of average body weight were selected for the evaluation of carcass traits. Replicate groups were randomly selected for carcass and organ weight evaluation. The birds were weighed, slaughtered by severing the jugular vein and allowed to bleed thoroughly. Birds were defeathered and weighed to calculate. The dressed chicks were later eviscerated. The wings were removed by cutting anteriorly severing at the humero-scapular joint, the cuts were made through the rib head to the shoulder girdle, and the backs were removed intact by pulling anteriorly. Firstly thighs and then drum stick were dissected from each carcass and weighed separately. The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, shank,

chest, back, neck, wing, abdominal fat and head) were taken before dissecting out the organs. All the carcass traits were expressed as percentages of the live weight. The following traits were evaluated: carcass yield (CY), weight of primal parts (drumstick, thigh, breast, back, neck, wing) and weight of internal edible offal (gizzard, heart, liver, abdominal fat and neck fat).

$$\text{Carcass yield (CY)}\,\% = \frac{\text{Carcass weight} \times 100}{\text{Live weight}}$$

3.13. Chemical analysis of onion and garlic containing formulated feed

After processing of onion and garlic about 200 gm sample was collected for chemical analysis. After chemical analysis the rations were formulated as needed as experiment. After formulation of diets about 200 gm of sample (two samples) from each diet was taken for chemical analysis. These laboratory works were done before the arrival of DOC in poultry shed.

The experimental samples were also subjected for proximate analysis for moisture, dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF), total ash and insoluble ash in the Animal Nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh in accordance with standard methods described by the AOAC (2006).

3.14. Collection of blood and serum separation

On the day 42, two birds were selected from each replication randomly for collection of blood. About 2.5 ml of blood was collected from every bird by sterile syringe and put those syringe in refrigerator vertically. After 6 hours serum was collected in sterile plastic vial to estimate serum parameters.

Table 3.8: Proximate composition of Onion

Traits	Proximate value (%)
Dry Matter (DM)	18.8
Crude Protein (CP)	0.98
Crude Fiber (CF)	0.21
Ether Extract (EE)	0.85
Ash	0.17
Nitrogen Free Extract (NFE)	16.59

Table 3.9: Proximate composition of Garlic

Traits	Proximate value (%)
Dry Matter (DM)	21.42
Crude Protein (CP)	3.21
Crude Fiber (CF)	1.97
Ether Extract (EE)	0.51
Ash	2.31
Nitrogen Free Extract (NFE)	13.42

3.15. Blood parameter estimation

Blood was collected without anticoagulant from a total 6 birds from each group (2 birds from each replicate) at 42^{th} days of age of broilers. Serum was separated after centrifugation at 3,000 rpm for 15 minutes. Different blood parameters (cholesterol, glucose, triglyceride, LDL and HDL were measured in the post graduate laboratory under the department of Physiology, Biochemistry and Pharmacology, CVASU using standard kits (BioMereux, France) and automatic analyzer (Humalyzer 300, Merck®, Germany) according to the manufacturer's instruction (FVMAAU; Addis Ababa, Ethiopia).

3.16. Statistical analysis

All the data of live weight, weight gain, feed consumption and feed conversion etc., related to carcass parameters, blood parameters and chemical analysis of meat were entered into MS excel (Microsoft office excel-2007, USA). Data were compared among the groups by one way ANOVA in STATA version-12.1 (STATA Corporation, College Station, Texas) and subsequent Duncan's Multiple Range Tests (DMRT). Results were expressed as means and SEM. All P values of ≤ 0.05 and ≤ 0.01 were considered significant and highly significant, respectively.

Chapter IV: Results

The experiment was carried out to measure the effect of onion and garlic on the performance parameter and carcass characteristics of Ross308 broilers. The results obtained from the study have been described in this chapter.

4.1. Body weight gain per week

Table 4.1 represented that, significant difference (P<0.01) in weight gain of broilers among experimental dietary treatment groups were observed at 1st and 2nd weeks of age. From 3rd to 6th weeks of age, in live weight gain of broilers among dietary treatment groups were not significant (P>0.05).

Table 4.1: Weekly body weight gain of broilers of different dietary treatment (gm/broiler)

Age of bird	Dietary treatments				SEM	P value
	T_0	T_1	T_2	T_3		
1st week	71.7[a]	84.0[c]	85.0[c]	77.4[b]	0.42	0.00
2nd week	155.9[b]	151.2[a]	170.6[c]	185.3[d]	02.06	0.00
3rd week	281.4	294.6	301.7	285.8	05.06	0.45
4th week	440.0	501.0	468.0	514.0	12.76	0.12
5th week	275.0	298.0	321.5	334.0	09.20	0.36
6th week	278.0	274.5	352.5	334.0	14.86	0.10

T_0 = control feed; T_1 = feed contain 1% onion; T_2 = feed contain 1% garlic; T_3 = feed contain 0.5% onion & 0.5%garlic; SEM =Standard Error of Mean; Significant ($p \leq 0.05$); a,b,c and d= Means having different superscript in the same row differ significantly.

4.2. Feed consumption

Table 4.2 showed that the significant difference (P<0.05) in feed consumption of broiler in different groups were observed at 1st and 2nd week of age. At 3rd to 6th week of age, there was no significant difference (P>0.05) in feed consumption of broiler in different treatment groups.

Table 4.2: Weekly feed intake of broilers among different treatment groups (gm/broiler)

Age of bird	Dietary treatments				SEM	P value
	T_0	T_1	T_2	T_3		
1st week	76.7[c]	72.6[a]	77.5[c]	74.8[b]	0.72	0.00
2nd week	207.0[a]	203.9[b]	211.2[a]	217.8[d]	1.97	0.00
3rd week	405.5	411.5	413.0	387.3	5.80	0.46
4th week	750.5	813.0	778.0	822.0	12.99	0.16
5th week	607.0	605.0	626.5	576.5	17.97	0.87
6th week	826.0	756.0	818.0	818.5	19.44	0.66

T_0 = control feed; T_1 = feed contain 1% onion; T_2 = feed contain 1% garlic; T_3 = feed contain 0.5% onion & 0.5% garlic; SEM =Standard Error of Mean; Significant ($p \leq 0.05$), a,b,c and d= Means having different superscript in the same row differ significantly.

4.3 FCR

In 1st and 2nd weeks of age, weekly feed conversion ratio (FCR) of broilers among different dietary treatment groups were statistically significant ($P<0.05$). At 3rd to 6th week of age, there was no significant difference ($P>0.05$).

Table 4.3: Weekly feed conversion of broilers among different dietary treatment groups

Age of bird	Dietary treatments				SEM	P value
	T_0	T_1	T_2	T_3		
1st week	1.0[c]	0.8[a]	0.9[ab]	0.9[b]	0.88	0.00
2nd week	1.3[c]	1.3[abc]	1.2[b]	1.1[a]	1.20	0.00
3rd week	1.4[b]	1.3[ab]	1.3[a]	1.3[a]	1.35	0.06
4th week	1.7	1.6	1.6	1.5	1.60	0.09
5th week	2.2	2.0	1.9	1.8	1.83	0.35
6th week	2.9	2.7	2.3	2.4	2.37	0.06

T_0 = control feed; T_1 = feed contain 1% onion; T_2 = feed contain 1% garlic; T_3 = feed contain 0.5% onion & 0.5% garlic; SEM =Standard Error of Mean; Significant ($p \leq 0.05$), a,b,c and d= Means having different superscript in the same row differ significantly.

4.4 Effect of different diets on carcass quality of broilers

No significant differences ($P > 0.05$) were observed in weight of drumstick, thigh, breast, wing, neck, leg and head (table 4.4). Control group showed lower weight than other three groups. Significant differences ($P \leq 0.05$) were observed in weight of back in different dietary treatment groups. Internal edible parts (liver, heart, gizzard, abdominal fat and neck region fat) did not show significant result ($p > 0.5$) in different dietary treatments among the control T_0 and onion and garlic containing T_1, T_2 and T_3 groups.

Table.4.4 Weight percentage of primal parts and internal edible organs of broilers at 42 days of age (%)

Traits (%)	Mean				SEM	P value
	T_0	T_1	T_2	T_3		
Primal Parts						
Drumstick	8.2	8.6	8.3	8.7	0.13	0.74
Thigh	18.0	18.9	17.5	18.9	0.30	0.27
Breast	14.9	16.3	16.5	18.4	0.58	0.18
Back	11.2	12.1	11.4	13.1	0.30	0.06
Neck	4.3	3.4	3.5	3.9	0.21	0.51
Wing	5.7	6.1	5.6	5.3	0.22	0.80
leg	4.9	4.2	4.5	4.6	0.16	0.63
Head	2.3	2.0	2.4	3.1	0.08	0.57
Internal Edible Organ						
Liver	2.6	2.4	2.4	3.1	0.23	0.81
Heart	0.4	0.4	0.6	0.4	0.03	0.32
Gizzard	2.9	2.8	3.2	3.0	0.10	0.52

Abdominal fat	2.0	1.6	2.2	2.0	0.11	0.34
Neck region fat	0.9	0.7	1.0	0.7	0.14	0.91

T_0 = control feed; T_1 = feed contain 1% onion; T_2 = feed contain 1% garlic; T_3 = feed contain 0.5% onion & 0.5% garlic; SEM =Standard Error of Mean; Significant ($p \leq 0.05$), a,b,c and d= Means having different superscript in the same row differ significantly.

4.5. Effect of different diets on blood parameters of broilers

Table 4.5 represent that, there is no significant difference among the different serum constituents level of broilers at 42 days of age.

Table 4.5: Different serum constituents level of broilers at 42 days of age

Parameter	Serum constituents level (mg/dl)				SEM	P value
	T_0	T_1	T_2	T_3		
Cholesterol	96.2	85.8	90.4	93.8	3.80	0.86
Glucose	128.2	96.3	112.4	91.4	7.60	0.37
Triglyceride	71.0	87.5	99.7	65.8	10.20	0.73
LDL	179.1	163.1	171.1	152.3	6.60	0.63
HDL	96.0	77.6	99.3	81.7	5.10	0.44

T_0 = control feed; T_1 = feed contain 1% onion; T_2 = feed contain 1% garlic; T_3 = feed contain 0.5% onion & 0.5% garlic; SEM =Standard Error of Mean; Significant ($p \leq 0.05$), a,b,c and d= Means having different superscript in the same row differ significantly.

Chapter III: Discussion

5.1. Weight gain

Regarding the effect onion and garlic supplementation on productive traits during the experimental period, it was no evident that live weight gain was significantly increased by dietary onion and garlic supplementation as compared with control group. Results of the experimental study was not in accordance with previous findings of Goodarzi *et al.*, (2010) who investigated that broilers receiving 1% onion extract in drink water had higher weight gain (WG) compared to control group during grower and total period ($P<0.05$). Ressei *et al.,* (2010) who revealed that birds which received 1% garlic powder had greater weight gain in from 3rd to 6th weeks of age and he also found no significant differences 1 to 21 days of birds. But the study showed significant results in first two weeks of age of broiler and no significant result in 3rd to 6th weeks of age. The other researcher like Sies *et al.,* (1999) reported a positive effect of garlic meal on weight gain in broilers, even though some authors (Dey and Samantha, 1993; Javandel *et al.,* 2008; Choi *et al.,* 2010) found no significant effect of garlic dietary supplementation on daily weight gain in broiler chickens.

Rahmatnejad *et al.,* (2009), reported that garlic given at 1% did not affect weight gain in broiler chicken. The experimental study was in accordance at 3rd to 6th weeks of age with Rahmatnejad and Javandal *et al.,* (2008) who also reported that administration of garlic to broiler meat at 1% did not show any significant increase in their body weight gain instead at 2% .

5.2. Feed consumption

The treatment which was containing onion in diet tend to lower feed intake in contrary of garlic containing diet, though they had insignificant result between the groups. Goodrazi *et al.,* (2014) reported that daily feed intake increased in case of 1% onion /kg feed of diet. His report supports first two weeks of age of broiler because significant result was found at that period. The feed intake in this study tended to be higher in the chicks fed on solely mixture of garlic compared with control, onion, onion and garlic mixture group, but the differences were not statistically significant. These results were agreed with the finding of (Bamidele and Adejumo, 2012) who reported that, the mixture of garlic had no

significant effect on feed intake of broiler chick. Dieumou *et al.*, (2009); Amouzmehr *et al.*, (2013); Thakar *et al.*, (2004); Tuker (2002) Williams and Losa.(2001) and Zolikha (2014) found no significant effect of dietary garlic on the feed intake of broiler chicks. Like- wise EL-tazi (2014) indicated that the diet supplemented with garlic powder had significantly better feed intake compared to the control diet but these study indicates significant feed take was only found first two weeks of age of birds and no signification in 3rd to 6th weeks of age. Javandel *et al.*, (2008) who reported that feed consumption was significantly higher in birds fed diets with lower concentration of garlic 0.125 and 0.25% compared to higher level 0.5, 1 and 2%. No significant result in 3rd, 4th and 5th weeks between the treatment groups is also supported with the findings of Dieumou *et al.*, (2009); Amouzmehr *et al*., (2013) who showed no significant effect of garlic supplements on the feed intake.

5. 3. FCR

The weekly feed conversion at different ages in different dietary supplementation level improved the feed conversion of Ross 308 broiler strain. Though the significant result was found at the 1st and 2nd of age. Significant results were also found at 3rd and 6th weeks of age at $p \leq 0.05$ level in different dietary treatments. The result of this experiment was not supported by Goodrazi *et al.*, (2014) study's. He revealed that broiler receiving 1% onion in feed had higher significant effect to the broiler chicken. His study partially supports to the current experiment where significant result was found only in first two weeks of age. The insignificant result of different dietary treatments in 3rd, 4th, 5th and 6th weeks also supports the findings of Aji *et al*., (2011), Mansoub and Nezhady *et al.*, (2011) who have reported non-significant result of dietary garlic on FCR. Raeesi *et al.*, (2010) reported that, 1% garlic in supplementation lower the feed conversion ratio. He was also revealed that 3% garlic had better FCR than control group. In his experiment control group consumed more feed than other groups. Eglabiet *et al*., (2013) also report that feed conversion ratio was significantly lower in birds fed diet supplemented with 3% garlic.

5.4. Carcass quality and organ characteristics of broilers

Birds who received onion and garlic at 1% level did not show significant results in to primal and internal parts of the body. This study supports Kim *et al*., (2015) reported that the carcass traits and other edible parts dietary treatment containing onion and garlic had no significant effect. Aji *et al*., (2011) also replied that no significant effect was found in carcass yield obtained from broiler fed of onion and garlic. The results of this experiment is in line with Lydia *et al.*,(2001) who reported that there were no significant differences on carcass percentage and organ weight of birds fed varying levels of garlic. Treatment effect in this study was not significant on carcass dressing percentage. These results are in agreement with the finding of Sarica *et al.,* (2005); Dieumou *et al.,* (2009); Rahimi *et al.*,(2011); zolikha,(2014) and Amouzmehr,(2013) who reported that the dietary garlic did not have any significant effect on carcass dressing percentage of broiler chicks.

5.5. Blood parameters

The study revealed that the garlic containing dietary treatments (T_2 and T_3) had no significant effect on reduction of blood cholesterol level. But a lots of scholar indicated that garlic is a good source of reducing cholesterol in blood. The study also revealed that onion and garlic had no effect on blood glucose. No significant result found on blood triglycerides during this study. Non significant results in LDL and HDL to blood level also indicates that onion and garlic had no effect on them.

This study did not support Onyimonyi (2011) who reported that using of 0.75% garlic results least serum cholesterol 76.30 mg/dl. In a study supplemented of 2% garlic in diet reduced 24.2% total cholesterol in the blood of white meat (Stanaćev *et al.,* 2012). Manan *et al.,* (2012) reported that feeding garlic at two days interval may improves plasma lipid profile which is also supports this study. The study did not support Goodrazi (2013) who reported that use of onion in diet reduced the level glucose in blood. He mentioned hypoglycemia stimulates nervous system higher feed intake. Onion contains sulphar containing compunds likeS-Methylcysteine sulfoxide and Sallylcysteine suiloxide. These compounds are related to decreasing of blood lipid, liver protein and glucose.

The present study did not support Mirhadi *et al.*, (1992) who reported that, the cholesterol-reducing effect of garlic powder significantly inhibited hypercholesterolemia, reduced tissue cholesterol, lower low density lipoprotein concentration (LDL or bad cholesterol), raised high density lipoprotein concentration (HDL or good cholesterol This is also the findings of Vidica *et al*., (2011). The study did not support Horton *et al*., (1991) who reported that inclusion of 10 g/kg garlic in broiler diet could decrease cholesterol concentration, without any effect on HDL and TG.

Conclusion

Onion and garlic can be considered as promising source of feed additives. They have herbal medicinal effect on beneficial gut environment of poultry which helps absorption and digestion in optimum level. They helps in maintenances of biochemical profile of blood.

Recommendation and Future perspectives

As it is a pilot study, further studies may be conducted on parallel field to make a concrete remark. However, according to this research work, the following recommendations may be done:

- Onion and garlic percentage in feed can be increased (Instead of using 1% onion and garlic in diet).
- The ratio of onion and garlic mixture can be changed and recombined (Instead of onion : garlic = 0.5 : 0.5).

Limitations

During experiment following limitations were identified:

- Population size
- Infrastructure issue
- Biosecurity and
- Resources

References

Ahmad S (2005). Comparative efficiency of garlic, turmeric and kalongi as growth promoters in broiler. M.Sc.Thesis, Department of Poultry Science, University of Agriculture, Faisalabad, Pakistan.

Aji SB, Ignatuius K, Ado AY, Nuhu, JB and Abdulkarim A (2011). Effect of feeding onion (*Allium cepa*) and garlic (*Allium sativum*) on some performance characteristics of broiler chickens. Research Journal Poultry Science., 4: 22-27. http://dx.doi.org/10.3923/rjpscience.2011.22.27

Amagase H and Milner JA (1993). Impact of various sources of garlic and their constituents on 7, 12- dimethylbenzoa[a] anthracene binding to mammary cell DNA. Carcinogenesis, 14: 1627-1631.

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y (2001). Intake of garlic and its bioactive components. Journal of Nutrition., 131: 955–962.

Amouzmehr A, Dastar B, Nejad JG, Sung KI, Lohakare J, Forghani F (2012). Effect of garlic and thyme extracts on growth performance and carcass characteristics of broiler chicks. Journal of Animal Science and Technology., 54: 185–190.

Ankri S. and Mirelman D (1999). Antimicrobial properties of allicin from garlic. Microbes and infection., 1 (2): 125-129.

AOAC (1995) Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.

Balash J, Palacious LL, Musquer S, Ralomequer J, Menez MJ, Alemany M (1973). Comparative haematological values of several galliformes. Poultry Science., 52: 1531-1534

Banerjee KS, Maulik KS (2002). Effect of garlic on cardiovascular disorder: A review of Nutritional Journal., 1: 4.

Barnerje GC (2009). A Textbook of Animal Husbandry. Oxford and ICBLT Publishing Co. Put. Ltd. India. pp. 465-466.

Bedford M (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. World Poultry Science., 56: 347–365.

Begum IA (2008). Prospects and potentialities of vertically integrated contract farming in Bangladesh. Ph.D. Thesis, Department of Agricultural Development Economics, Hokkaido University, Japan.

Bordia A, Bansal HC, Arora SK, Signal SV (1975). Effect of the essential oils of garlic and onion on alimentary hyperlipidemia. Atherosclerosis, 21: 15-18.

Borek C (2001). Antioxidant health effects of aged garlic extract. Journal of Nutrition., 131: 1010S–1015S.

Bowker BC and Zhuang H (2013). Relationship between muscle exudate protein composition and broiler breast meat quality. Poultry Science ., 92: 1385–1392.

Buchanan NP, Hott JM, Cutlip SE, Rack AL, Asamer A and Mortiz JS (2008). The effect of anatural antibiotic alternative and and anatural growth promoter feed additive on broiler performance and carcass quality. Journal of Applied Poultry Research., 202-210.

Burgat V (1999). Residues of drugs of veterinary use in food. Review Part., 41: 985-990.

Burt S (2004). Essential oils: Their antibacterial properties and potential applications in food- A review. International Journal of Food Microbiology., 94: 223–253

Bywater RJ (2005). Identification and surveillance of antimicrobial resistance dissemination in animal production. Poultry Science., 48: 644-648.

Canogullari S, Baylan M, Erdogan Z, Duzguner V and Kucukgul, A (2010). The effect of dietary garlic powder on performance, egg yolk and serum cholesterol concentration in laying quails. Czech Journal of Animal Science., 55: 286-93.

Castanon JIR (2007). History of the use of Antibiotic as Growth Promoter in Euro feeds. Poultry Science., 11: 2466-2471.

Choi IH, Park WY, Kim YJ (2010). Effects of dietary garlic powder and α-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. Poultry Science., 89: 1724–1731.

Chowdhury SR, Chowdhury SD, Smith TK (2002). Effects of Dietary Garlic on Cholesterol Metabolism in Laying Hens. Poultry Science., 81:1856–1862.

Colligon PJ (1999). Vancomycin-resistant enterococci and use of a voparcin in animal feed :is there a link? Med. J. Aust.,171:144-146.Colonization and organ invasion in leghorn chicks. Avian Diseases., 38: 256-261.

Cullen SP, Monahan FJ, Callan JJ and O'doherty, JV (2005). The effect of dietary garlic and rosemary on grower-finisher pig performance and sensory characteristics of pork. Irish Journal of Agricultural Food Research., 44: 57-67.

Demir, E., Sarica, S., Ozcan, M.A. and Suicmez, M (2003). The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. Br. Journal of Poultry Science., 44: S44-S45.

Demir, E.; Kiline, K. and Yildirim, Y (2005). Use of antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat base broiler diets. South Africa animal Science., 35: 61-72.

Dey A and Samanta AR. (1993). Effect of feeding garlic(*Allium sativum*.) As a growth promoter in broilers.Indian Journal of Animal Health., 32: 17-19.

Dieumou FE, Teguia A, Kuiate JR, Tamokou JD, Fonge NB. and Donogmo MC (2009). Effect of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chicken. Livestock Research for Rural Development., 21(8): 21-33.

Dieumou FE, Teguia A, Kuiate JR, Tamakou JD, Doma UD, Abdullahi US, Chiroma AE (2011). Effect of supplemented diets with garlic organic extract and streptomycin sulphate on intestinal microflora and nutrients digestibility in broilers. Journal of Animal Food Science., 1:107–113.

DLS (2015). Annual report on livestock, Division of Livestock Statistics, Ministry of Fisheries and Livestock, Farmgate, Dhaka, Bangladesh.

Duncan DE (1955). New Multiple Range Test. Biometrics 11: 1– 42.

Ebesunun MO, Popoola OO, Agbedana EO, Olisekodiaka JM, Onuegbu JA and Onyeagala AA (2007). The effect of garlic on plasma lipids and lipoproteins in rats fed on high cholesterol enriched diet. Biokemistri., 19: 53-58.

Eltazi, MA (2014) Response of broiler chicks to diets containing different mixture levels of garlic and ginger powder as natural feed additives. International Journal of Pharmacy Research Allied Science., 3(4): 27-35.

Eyssen H. and De Somer P (1963). The mode of action of antibiotics in stimulating growth of chicks. Journal of Experimental Medicine., 117: 127-137.

Fadlalla LMT, Mohammed BH and Bakhiet AO (2010). Effect of feeding garlic on the performance and immunity of broilers. Asian Journal of Poultry Science ., 4(4): 182-89.

FMI, Food Marketing Institute (2006). low-level use of antibiotics in live stock and poultry. htt:// www.FMI.org/ media /bag /antibiotics.pdf accessed Aug., 2007.

Goodarzi M, Landy N, and Nanekarani SH (2013). Effect of onion (Allium cepa L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks. Health 5(8): 1210-1215.

Goodarzi M, Nanekaran SH. And Landy N (2014). Effect of dietary supplementation with onion (Allium cepa L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. Asian Pacefic Journal of Tropical Disease ., 4(Suppl 1): S297-S301

Griggs JP and Jacob JP (2005). Alternatives to Antibiotics for Organic Poultry Production. Journal of Applied Poultry Research., 14:750–756.

Griggs JP and Jacob JP (2005). Alternative: to antibiotics in organic poultry production. Journal of Applied Poultry Research.,14: 750-756.

Hanieh H, Narabara K, Piao M, Gerile C, Abe A. and Kondo Y (2010). Modulatory effects of two levels of dietary *Alliums* on immune responses. Animal Science Journal., 81: 673-680.

Harms RH, Ruiz N and Miles RD (1986). Influence of virginiamycin on broilers fed four levels of energy. Poultry Science., 65: 1984-1986.

Heinzl I, Borchardt T (2015). Secondary plant compound to reduce the use of antibiotics? Internatinal Poultry Production., 23: 15-17.

Horton GMJ, Fennel MJ, Prasad BM (1991). Effects of dietary garlic (*Allium sativum*) on performance, carcass composition and blood chemistry changes in broiler chickens. Canadian Journal of Animal Science., 71: 939–942.

Javandel FR, Navidshad R., Seifdavati J, Pourrahimi G.H., Baniyaghoub S (2008). The favorite dosage of garlic meal as a feed additive in broiler chickens rations. Pak. Journal of Biological Science., 11: 1746–1749.

Javed M, Durrani F, Hafeez A, Khan RU and Ahmed I (2009). Effect of aqueous extract of plant mixture oncarcass quality of broiler chicks. Journal of Agricutural And Biological Science., 4: 37-40.

Jennifer H (2002). Garlic Supplements Longwood Herbal Task force. Retrieved online from www.ukmi.nhs.uk.

Jin R, Cheng ZH, Tong F, Zhou YLS (2007). Chemical components and its allelopathy of volatile from isolated garlic seedling. Xibei Zhiwu Xuebao. 27(11): 2286-2291.

Jones FT and Ricke SC (2003). Observations on the history of the development of antimicrobials and their use in poultry feeds. Poultry Science., 82 (4): 613-7.

Kandil OM, Abdullah TH, Elkadi A (1987). Garlic and the immune system in humans: its effects on natural killer cells. Federation Proceeding., 46: 441.

Karangiya VK, Savsani HH, Patil SS, Garg DD, Murthy KS, Ribadiya NK, Vekariya SJ (2016). Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Veterinary World., 9: 245-250.

Khksar V, Golian A, Kermanshahi H, Movasseghi, Jamshidi A (2008). Effect of prebiotic fermacto on gut development and performance of broiler chickens fed diet low in digestible amino acids. Journal of Animal and Veterinary Advances., 7: 251-257.

Koch HP, Lawson LD (1996). Garlic: The science and Therapeutic Applications of *Allium sativum* and related species. Williams and Wilkins. Amazon. p. 329.

Kumar M, Berwal JS (1998). Sensitivity of food pathogens to garlic (*Allium sativum*). Journal of Applied Microbiology., 84: 213–215.

Kyo E, Uda N, Kasuga S, Itakura Y (2001). Immunomodulatory effects of aged garlic extract. Journal of Nutrition., 131: 1075S–1079S.

Lampe JW (1999). Health effects of vegetables and fruits: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition., 70: 475–90.

Lanzotti V (2006). The analysis of onion and garlic. Journal of Chromatography., 1112: 3–22.

Lau BHS (2001). Suppression of LDL oxidation by garlic. Journal of Nutrition., 131: 985S–988S.

Mansoub HN, Nezhad MAM (2011). The effects of using Thyme, Garlic and Nettle on performance, carcass quality and blood parameters, Annals of boiler Research., 2 (4): 315-320

Mansoub NH (2011). Comparative effects of using garlic as probiotic on performance and serum composition of broiler chickens. Annals of boiler Research., 2: 486-490.

Mansour SA, Bakr RFA, Mohamed RI, Hasaneen NM (2011). Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, MuscaDomestica L. The Open Toxicology Journal., 4: 1-13.

Masoud A (2006). Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. Journal of Poultry Science., 43(4): 378-383.

Melvin Joe, Jayochitra MJ, and Vijayapriaya M (2009). Antimicrobial activity of some common spices against certain human pathogens. Journal of Medicinal Plants Research., 3: 1134-1136

Miles RD, Janky DM. and Harms RH (1984). Virginiamycin and broiler performance. Poultry Science., 63: 1218-1221

Miles RD, Butcher CD, Henry PR and little RC (2006). Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters and quantitative morphology. Poultry Science., 85: 476-485.

Mirhadi SA, Singh S, Gupta PP (1992). Effect of garlic supplementation to cholesterol-rich diet on development of atherosclerosis in rabbits. Indian Journal of Experimental Biology., 29(2): 162-168.

Murray RK, Granner DK, Mayes PA, Rodwell VW (2003). Harpers Illustrated Biochemistry. 27th Edition. Appleton Lange. USA.

Narimani-Rad M, Nobakht A, Shahryar HA, Kamani J, Lotfi A (2011). Influence of dietary supplemented medicinal plants mixture (Ziziphora, Oregano and Peppermint) on performance and carcass characterization of broiler chickens. Journal of Medicinal Plants., 5(23): 5626-5629

Nasir Z and Grashorn MA (2006). Use of Black cumin (Nigella sativa) as alternative to antibiotics in poultry diets. Proc. 9th Tagung schweine-und geflügelernährung, Halle, Germany.

Newall CA, Anderson LA and Phillipson JD (1996). Herbal Medicines: A Guide for Health-Care Professionals. Vol. IX. Pharmaceutical Press, London, p. 296.

Niel HA, Silagy CA, Lancaster T, Hodgeman J, Vos K, Moore JW, Jones L, Catrill J, Fowler GH (1996). Garlic powder in the treatment of moderate hyperlipidaemia: a controlled trial and meta-analysis. Journal of Royal College of Physicians London., 30(4): 329-324.

NRC, (1994). National Research Council. Nutrient Requirements of Poultry. 9th edn., National Academy Press. Washington, DC., USA.

Ologhobo AD, Adebiyi FG, Adebiyi OA (2008). Effect of long term feeding of raw and sun-dried garlic (*Allium sativum*) on performance and lipid metabolism of broiler chicks. In: Proceedings of the Conference on International Research on Food Security, National Research and Management of Rural Development.,7-9.

Onibi EG, Adebisi EO, Fajemisin NA, Adetunji VA (2009).Response of broiler chickens in terms of performance and meat quality to garlic (Allium sativum) supplementation. African Journal of Agricultural Research., 4(5): 511-517.

Park SC, Grimers W, ferket JI and Fairchild AS (2000). The case for manna oligosaccharides in poultry diets. An alternate to growth promotant antibiotics.,56:535

Prasad R, Rose MK, Virmani M, Garg SL, Puri JP (2009). Effect of Garlic (Alium sativum) supplementation on haematological parameters in chicken (gallus domesticus). Indian Journal of Animal Research., 43(3): 157-162.

Puvaca N, Kostadinovic LJ, Ljubojevic D, Lukac D, Popovic S, Dokmanovc B and Stanacev VS (2014). Effects of dietary garlic addition on productive performance and blood lipid profile of broiler chickens. Biotechnology Animal Husbandry., 30(4): 669-676.

Qureshi AA, Din ZZ, Abuirmeileh N, Burger WC, Ahmad Y, Elson CE (1983). Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: impact on serum lipids. Journal of Nutrition., 113: 1746-1755.

Raeesi MS, Hoseini- Aliabad A, Roofchaee A, Zare Shahneh A and Pirali S (2010). Effect on Periodically Use of Garlic (*Allium sativum*) Powder on Performance and Carcass Characteristics in Broiler Chickens. World Academy of Science and Technology., 68: 1213-1219.

Rahimi S, Teymouri ZZ, Karimi TMA, Omidbaigi R and Rokni H (2011). Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. Journal of Agricultural Science and Tecnology., 13: 527-539.

Rahmatnejad EH, Roshanfekr O, Ashyayerzadeh M, Mamooeeand Ashyerizadeh A (2009). Evaluation the effect of several non-antibiotic additives on growth performance of broiler chicken . Journal of Animal and Veterinary Advance., 8: 1670-1673.

Ross IA (1999). Medicinal Plants of the World Chemical Constituents, traditional and modern medicinal uses. Humana Press. 3: p. 648.

Sahin O, Morishita TY. And Zhang Q (2002). Campyloacter colonization in poultry :sources of infection modes and transmission. Animal Health Research Revised., 3: 95-105.

Salim AB (2011). Effect of some plant extracts on fungal and aflatoxin production. International Journal of Academy Research., 3: 116-120.

Sallam KIM, Ishioroshi and Samejima K (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. Lebenson Wiss Technoloy., 37(8): 849–855.

Sarica S, Ciftci A, Demir E, Kilinc K, and Yildirim Y (2005). Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science., 35: 61–72.

Sarica S, Ciftci A, Demir E, Kiline K. and Yildirim Y (2005). Use of antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science., 35: 61-62.

SAS Institute (1994). SAS/STAT users guide. Version 6.1,3rd Ed. SAS Inst.,Cary,NC.

Sharma N (2007). Effect of supplementation of enzymes on growth performance in commercial broilers. A Thesis Submitted to Anand Agricultural University. p49.

Shoetan A, Augusti RT, Joseph PK (1984). Hypolipidemic effects of garlic oil in rats fed ethanol and a high lipid diet. Cellular and Molecular Life Science., 40(3): 261-263.

Sies H (1991). Oxidative stress:from basicresearch to clinical application. American Journal of Medicine., 91: 31-38.

Silagy CS, Neil NAW (1994). Garlic as lipid lowering agent-a meta analysis. Journal Royal College Physicians., 28(1): 39-45.

Sklan D, Berner YN, Rabinowitch HD (1992). The effect of dietary onion and garlic on hepatic lipid concentrations and activity of antioxidative enzymes in chicks. Journal of Nutritional Biochemistry., 3(7): 322-325

Soliman, N (2000). Histological and histochemical studies on the effect of garlic (*Allium sativum*) extract on the liver and lung of albino rat. M.Sc. Thesis, Histology Dept., Fac. Med., Ain Shams University, Cairo, Egypt.

SPSS (2009). Computer software SPSS Inc. Headquarters, Wacker Drive, Chicago, Illinois, U.S.A.

SPSS: Statistical Package for Social Sciences (1999). Suriya R, Zulkifli I and Alimon AR (2012). The effect of dietary inclusion of herbs as growth promoter in broiler chickens. Journal of Animal and Veterinary Advance., 11(3): 346-50.

Tatara RM, Sliwa E, Dudek K, Mosiewicz J, Studzinski T (2005). Effect of aged garlic extract and allicin administration to sows during pregnancy and lactation on body weight gain and gastrointestinal tract development of piglets. Part 1. Bull Veterinary Instruction Pulawy, 49: 349-355.

Thakar NM, Chairmam DM, McElro AR, Novak CL, Link RL (2004). Pharmacological screening of some medicinal plants as antimicrobial and feed additives. Msc Thesis. Department of Animal Science. Virginia Polytechnic Institute and State University, Blacksburg, Virgina USA. 73P.

Thorns CJ (2000). Bacterial food-born zoonoses. Revue Science and Technology., 19: 226-239.

Toghyani M, Toghyani M, Gheisari AA, Ghalamkari GH, Mohammadrezaei M (2010). Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Live Science., 129: 173-178.

Tollba AA (2003). Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions Thymus (Thymus vulgaris L) or fennel (Foeniculum vulgare L). Egypt Poultry Science., 23: 313-326.

Tollba, AAH and Hassan MH (2003). Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. Black cumin (niglla sativa) or Garlic (allium sativum). Poultry Science., 23: 327-340.

Tsao SM and Yin MC (2001). In vitro activity of garlic oil and four diallyl-sulfides against antibiotic resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Antimicrobial Chemotherapy., 47: 665-670.

Tuker L.(2002). Botanical broilers: Plants extract to maintain poultry performance. Feed International.,32: 26-29.

Vidica S, Dragan G, Niko M, Nikola P, Viadislav S, Nada P (2011). Effect of garlic (*Allium sativum*) in fattening chicks' nutrition. African Journal of Animal Science. 6(4): 943-948.

Vidyavati HG, Manjunatha H, Hemavathy J and Srinivasan K (2010). Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats. Journal of. Food Science and Technology. 47:55-60.

Warshafsky S, Kamer RS, Sivak SL (1993). Effect of garlic on total serum cholesterol. A metal-analysis. Annal International Medicine., 119(71): 599-605.

Williams P and Losa R (2001). The use of essential oils and their compounds in poultry nutrition. World Poultry-Elsevior., 17(4): 14-15.

Yang N and Jiang RS (2005). Recent advances in breeding for quality chickens. World's Poultry Science Journal., 61: 373-381.

Ziarlarimi A, Irani M, Gharahveysi S and Rahmani Z (2011). Investigation of antibacterial effect of garlic (*Allium sativum*), mint (*Menthe* spp.) and onion (*Allium cepa*) herbal extracts on *Escherichia coli* isolated from broiler chickens. African Journal of Biotechnology, 10 (50): 10320-10322.

Zolikha (2014). Response of broiler chicks fed on dietary garlic essential oil as natural growth promoter alternative to antibiotics.

Annex

❖ **Estimation of crude protein by Kjeldahl method:**

Procedure:

1. **Digestion:**
   a) 3.5 gm feed were weight.
   b) 5 gm digestion mixture was added.
   c) 20 ml concentrated H_2SO_4 was added.
   d) The digestion flask was placed on Kjeldahl digestion set.
   e) Heat was increased gradually and digested up to clear the residues.
   f) The flask was removed and cool.
2. **Distillation:**
   a) 20 ml distilled water was added.
   b) The content was transferred to distillation flask.
   c) 100 ml 40% sodium hydroxide was added and the condenser was set.
   d) 20 ml 2% boric acid was added and mixed in conical flask.
   e) The distillation flask was heated and continued up to collection of 100 ml of distillate.
3. **Titration:**
   a) The distillate was titrated against standard N/10 HCL solution.
   b) The titration volume was calculated and predicted.

$$\% \text{ Crude protein} = \frac{A \times B \times 0.014}{W} \times 6.25 \times 100$$

Here,

A= Volume of standard N/10 HCL solution

B= Normality of standard HCL solution

W= Weight of sample

❖ **Estimation of crude fiber:**

Procedure:

a) 3.5 gm ground sample was weighted.
b) 125 lm 1.25% H_2SO_4 solutions were added to the beaker.
c) 3 drops of n-octanol as antifoam agent was added.
d) Boiled for 30 minutes.
e) 3 times wash with distilled water.
f) 125 ml 1.25 % sodium hydroxide and 5 drops of antifoam were added.
g) Boiled for 30 minutes.
h) Filtrated and wash the residue.
i) Second wash was performed by 1% HCL.
j) The residue was dried at 105ºC.
k) Residue was cooled in desiccators and weighted.
l) The residue was burned.
m) They were ignited in muffle furnace at 550-600ºC.
n) The ash was weight and deducted.

$$\% \text{ Crude fiber} = \frac{W - W_1}{W_2} \times 100$$

Here,

W= Weight of crucible, crude fiber and ash

W_1= Weight of crucible and ash

W_2= Weight of sample

❖ **Estimation of Ether extract by SOXHLET apparatus**

Procedure:

a) Sample was dried to moisture free
b) The dry extraction flask weight carefully
c) 3.5g sample was weight and transferred to the thimble.
d) The thimble placed into extractor and closed the top by cotton
e) The extractor was fitted and poured ether up to siphoning.

f) Again ether poured half of the previous amount

g) Then boiled at 40-60ºc

h) Then dismantled the flask and dried on water bath

i) Heated at 100ºcup to constant weight

j) The flask was cooled in to desiccators and weight of the sample

$$\% \text{ Ether extract} = \frac{A - B}{W} \times 100$$

Here,

A= Weight of flask with ether extract

B= Weight of flask

W= Weight of sample

❖ **Estimation of ash:**

Procedure:

a) Crucible were cleaned and dried in hot air oven.

b) They were cooled in desiccator and weight.

c) 5 gm sample were placer into crucible and burned.

d) After cooling transferred to muffle furnace.

o) The sample was ignited at 550-600ºC.

e) Then they were cooled and weight.

$$\% \text{ Ash} = \frac{W - W_1}{W_2} \times 100$$

Here,

W= Weight of crucible and ash

W_1= Weight of crucible

W_2= Weight of sample

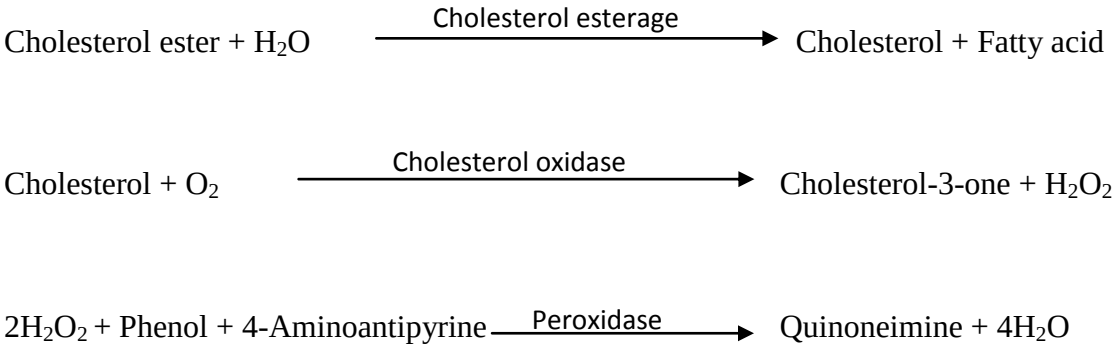
Method of estimating different biochemical parameters of serum (According to manufactures instruction):

❖ **Cholesterol assay**

Assay principle

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

Reaction



Materials and reagents

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was an enzmatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tube was taken. Then 10µl of cholesterol standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. 1000µl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

- **Triglyceride assay**

Assay Principle

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4–aminophenezone and 4–Chlorophenol under the catalytic influences of peroxidease.

Materials and reagent

1. Serum sample
2. TG conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves

Procedure

The sterile eppendorf tubes were taken. Then 1000 µl TG standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared as a tool.

Brief biography of the author

Mohammad Abdul Moyed Sharif completed his graduation degree on Doctor of Veterinary Medicine (DVM) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. As an intern student he received clinical training from Madras Veterinary College and Veterinary College & Research Institute, Namakkal, Tamilnadu, India. Abdul Moyed Sharif has a great enthusiasm in research and has done some nutritional and clinical research works. He has investigated the causes of naval ill in dairy farm during his internship at Chittagong. He has studied on clinical management of uterine torsion followed by complete post-partum uterine prolapse in murrah buffalo during his internship in VC&RI, Namakkal, Tamil Nadu, India. His research interest is to provide quality and less costly livestock and poultry feed by using unconventional feed ingredients.