CHAPTER-I

INTRODUCTION

Thesis for MS in Poultry Science

Chapter-I

Introduction

The most popular, rapidly growing, emerging farming enterprise is now poultry production all over the world. It is popular that a very good and cheap sources of animal protein is now poultry meat compared to other meats include beef, mutton, chevon and pork. The shorter life span, no dietary restriction over poultry meat and egg amongst the different races of people, and its worldwide popularity for cheaper cost and availability, have made the poultry products more favorable and acceptable to the consumer world as a vital source of protein. The poultry production industry has grown rapidly in recent years, and it has become one of the most important sectors in the meat animal production enterprise, contributing job scope to people and providing the primary source of income for agricultural farmers (Mahfuz et al., 2020).

The broiler industry in Bangladesh is developing rapidly and its success depends on how rapidly a bird attains maximum marketable weight. The target of poultry production is to achieve high level of performance via efficient utilization of feed, and to keep high survivability as much as possible. The purpose of using feed additives is to enhance their growth rate, better feed efficiency, greater livability, and low mortality in poultry birds. These feed additives are termed as "growth promoters" and often called as non-nutritive feed additives (Singh and Panda, 2012). Growth promoters are chemical and biological substances, which are added to poultry feed with an aim to improve the growth rate of chickens, improve the utilization of feed, and thus it gives rise to better production and financial benefits (Kumar et al., 2021). Due to restrictions on the use of antibiotic growth promoters, indiscriminate application of antibiotics in poultry feed have already banned or usually limited in many countries, which has

prompted the search for potential replacement of using feed additives. In this regard, mushroom in poultry feed can act as a natural antibiotic, growth promoter, antimicrobial and antiviral agents for enhancing poultry productivity to an extent (Kumar et al., 2021).

Though tremendous improvement has been occurred in nutrition, genetics and environment for poultry industry, improving poultry performance is still a vital and challenging for the animal production, especially under certain adverse conditions. The application of different feed supplements in broiler diet has long been tried to attain the goal for optimum broiler production (Attia et al., 2005, Zhan et al, 2006, Kumar et al., 2021). The main goal of poultry farmers is to achieve optimum production with low investment. This trend is driving forces on the poultry researchers to find out alternative policies, for profitable poultry production. The restriction on animal by-products and indiscriminate uses of antibiotics in animal nutrition, has also driven a force over the poultry nutritionists and researchers for searching alternative feed supplements to enhance poultry performance (Adil et al., 2010).

The alternative might be using of mushroom in broiler diets to boost up the optimum productivity of the broiler chickens. It is believed that adding mushrooms to broiler diets is a great substitute for prophylactic antibiotics. It is reported that feeding certain mushrooms improves gut health in broilers and weight gain almost 5.2% when supplemented 5% of the *Hericium* caput-medusae mushrooms in the diet (Mahfuz et al., 2017). According to recent reports, Chinese herbal and mushroom extracts can be used in broiler chicken as an alternative to antibiotic growth promoters (Guo et al., 2004). There are about 140,000 species of mushrooms in nature, but only about 25 (*Agaricus bisporus, Pleurotus spp., Lentinus edodes, Auricularia spp.*) are commonly regarded as food, and only a few have reached the status of a commercial item.

Mushroom belongs to many properties such as a good protein source, unsaturated fatty acids, micro-nutrients (vitamins, minerals), and immunity (Tang et al., 2016). Mushrooms can be used as natural feed supplement that can improve the growth performance, health status and immunity of broiler chicken. Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Tang et al., 2016). Extracts obtained from diverse mushrooms are of special interest since they are known to offer health-promoting advantages on farm animals due to a variety of chemicals with antioxidant, antimicrobial, immune-enhancing, and stress-reduction capabilities (Dalloul et al., 2006). Natural antioxidants that could replace synthetic antioxidants and fulfill the requirements of consumers for food products free of chemical residues *Bisporus spp.* mushroom into chicken diets.

Mushrooms are recognized to offer a wide range of health-promoting characteristics due to the numerous antioxidant and antibacterial chemicals they contain. Antimicrobial activities, immune enhancement, and stress reduction in farm animals given natural medicinal products from fungi and herbs (Wang et al., 2001). Some previous studies were also done to assess the impact of mushroom in poultry feed (Guo et al., 2004, Mahfuz et al., 2018, Mahfuz et al., 2019). However, the findings of those studies are still contradictory, disagreement, inconsistent and not clear idea to apply on the certain dosages and mode of action of mushroom in the present poultry industry due to the gradual changes of genetic constitution and rearing strategies of poultry (Mahfuz et al., 2020).

Objectives of the study

Mushroom supplement in poultry diet might be economical or could have potential to improve the productivity by decreasing the production cost of broiler production. Provided the foregoing, the current study was conducted to achieve the following goals:

1. To ascertain the weight gain, feed consumption, feed conversion ratio (FCR) and mortality of broilers fed mushroom supplemented diet.
2. To investigate the carcass traits and meat quality of broiler chicken
3. To assess the bone morphology and blood metabolites of broiler.
4. To assess the cost benefit analysis of raising broiler chicken fed on test diet.

CHAPTER-II

Review of Literature

Thesis for MS in Poultry Science

Chapter-II

Review of literature

2.1 Introduction

Mushrooms (*Agaricus bisporus*) are basic plants that lack chlorophyll and hence are unable to manufacture their own nourishment. They depend upon other living on dead plants and organic matter. Mushrooms have a high amount of protein, vitamin, and mineral content. Mushrooms have a low carbohydrate and lipid content, making them a good diet for diabetics and anyone looking to lose weight. Mushrooms are also good source of energy about 454g of fresh mushrooms providing 120 kilocalories. Most of the edible varieties of mushroom belong to the family Agaricaceae of class basidiomycetes (Srivastava and Kumar, 2002). Mushroom can be used as a growth promoter in broiler production. The active ingredients found in mushrooms are antioxidants, phenolic compounds, tocopherols, carotenoids, and antibacterial compounds (Zhou et al., 2010; Hernandez et al., 2006). Additionally, mushrooms have been reported to have immune enhancing and stress reducing properties (Dalloul et al., 2006; Borchers et al., 2008).

Oyster mushrooms (*Pleurotus ostreatus*) are a type of farmed fungus that has antiviral and anticancer effects (Fard et al., 2014). Mushrooms can perform a variety of tasks (Guo et al., 2003). Due to the abundance of compounds with antioxidant, antibacterial, immune-boosting, and stress-reduction properties on farm animals, mushrooms have health-promoting properties (Dalloul et al., 2006). According to reports, Chinese herbal and mushroom extracts can be used in place of antibiotic growth promoters in broiler chicken (Guo et al., 2004a; Guo et al., 2004b).

Over the past 100 years, much research has persisted on coccidiosis because it represents a major disease problem demanding the attention of the poultry producers, feed manufacturers, and the poultry disease experts (Tsang et al., 1987), coccidiosis can be controlled by mushroom feeding.

Natural medicinal products originating from fungi or herbs have been used in animal feeding to improve performance through Amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Toghyani et al., 2012; Guo et al., 2003). Mushrooms have long been recognized as a valuable source of bioactive chemicals with therapeutic properties (Mazaheri et al., 2019). It's worth noting that varied quantities of mushroom-derived methanol extract can neutralize free radicals. This antioxidant property of mushrooms is because of the presence of phenolic compounds (Yanget al., 2002), which also possess antioxidant properties due to their renewal capacity as well as their chemical Structure that enables them to neutralize free radicals (Mazaheri et al., 2019).

As a result, scientists are refocusing their efforts on using our old medicinal system to uncover beneficial herbs and plants that may be utilized safely to boost poultry production. In light of the foregoing, the purpose of this study is to determine the influence of mushrooms (*Pleurotus ostreatus*) powder on commercial broiler growth performance as well as the effect of mushrooms (*Pleurotus ostreatus*) powder on commercial broiler carcass characteristics.

2.2 Definition

A mushroom, also referred to as a toadstool, is an above-ground, spore-bearing fruiting body of the fungus that grows on soil or its food source.

2.3 Oyster mushroom

A common edible fungus is the oyster mushroom. The fungi earned their name because they have an oyster-like shape and color. Oyster mushrooms are commonly ingested as a cuisine, although supplements are also available. Oyster mushrooms are used in various medical processes, including Traditional Chinese Medicine (TCM), to treat a variety of health conditions. Dietary fiber, beta-glucan, and other ingredients found in oyster mushrooms may improve health.

2.4 Types of oyster mushrooms

The traditional Oyster Mushroom is *Pleurotus ostreatus.* There are many different types of Oysters.

1. King oyster mushrooms: The largest oyster mushroom found in the Middle East, North Africa, and Asia

2. Pink oyster mushrooms: Bright pink color that fades when exposed to heat, with a pungent flavor.

3. Phoenix oyster: Similar to pearl oyster mushrooms in appearance and flavor, but smaller.

4. Golden oyster: Have a vivid gold hue and a flavor to match. Very fragile with a short shelf life.

5. Blue oyster: Have a bluish tint. Taste exactly like pearl oyster mushrooms.

6. Pearl oyster: The most common type and find in North America.

A

B

C

D

Figure 1: Edible Oyster Mushroom (*Pleurotus ostreatus*)

A. Mushroom on straw, B. Packaging of mushroom, C. Drying of mushroom, D. Dry mushroom with body. [source: BD Mushroom.com]

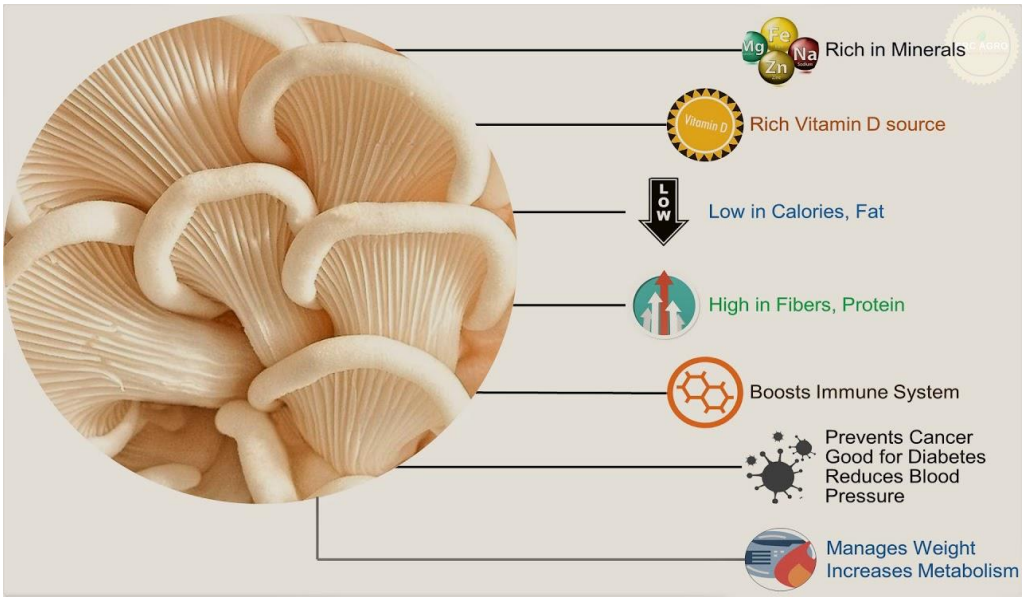

Figure 2: Different nutrients in Oyster mushroom (*Pleurotus ostreatus*)

2.5 The chemical composition of Mushroom and its structure

Researchers currently know the nutritional composition, active ingredients, and health benefits of medicinal mushrooms on both humans and animals. Active compounds for enhancing chicken growth performance have been found in a few common mushrooms. The approximate crude protein (CP) present in most medicinal mushrooms was between 178.9 and 279.5 g/kg (Akata, Ergonul, and Kalyoncu 2012; Pereira et al., 2012; Mahfuz et al., 2018). The major polysaccharide found in most mushrooms, -glucan, has been shown to have immune-modulating properties (Wu et al., 2014; Yang et al., 2015). The total mineral content in mushrooms varies between 50 and 120 g/kg of dry matter (DM) and about 72.5 to 104.0 g/kg of total ash (Beluhan and Ranogajec 2011; Pereira et al., 2012). Mushrooms have been reported to be potential source of several non-nutritional components. For example, it has been reported that phenolic compounds have antioxidant properties (Rahman, Abdullah, and Aminudin, 2015). On a dry matter basis, these phenolic components range from 9.0 to 26.0 g/g (Kim et al., 2008). Due to up-regulating cytokine gene expression, mushroom extract increased the concentration of interferon- (IFN), which has immunomodulatory and toxic effects on lymphoma cells (Lee et al., 2011; Li et al., 2011). In addition, it has been demonstrated that mushroom polysaccharides increase the weight of immunological organs in experimental mice and release a number of cytokines (Yin et al., 2010; Yan et al., 2014).

2.6 The role of medicinal mushrooms in monogastric animal research

A particular type of mushroom has recently been used in both in vitro and in vivo studies. The majority of the mushroom species that are connected to other, non-nutritional benefits belong to the phylum Basidiomycota (Bederska-Lojewska et al., 2017). Although the health benefits of mushrooms have long been emphasized in Asia

(Miles and Chang 2004), they are only viewed as a minor component of food in Europe (Giennenas et al., 2010; Bederska-Lojewska et al., 2017). Currently, researchers are emphasizing the potential of mycotherapy (Bederska-Lojewska et al., 2017). A list of the most popular medicinal fungi that have been used in animal studies over the years was presented in Table 1.

2.7 Effect of mushrooms on the production and performance of broiler chicken

Based on the results of previous research, there are lots of discussions over the performance and physiological responses of broilers fed various medicinal mushrooms. There are many variables due to species, doses, method of application (either non-fermented or fermented in combination with other beneficial organisms), the parts of mushrooms used (either fruiting bodies or stem base) and treatment period. Many experts believed, however, that mushrooms may play a significant function in boosting broiler chicken performance and health (Daneshm and, Sadeghi, and Karimi 2012; Guimaraes et al., 2014; Shang et al., 2016). The role of mushrooms on growth performance and physiological responses in broiler is summarized in the table 1. Feed intake was higher in broiler receiving mushrooms supplemented diet (*Agaricus brasiliensis*) compared with the control group during 1–21 d of age. In comparison to the other treatments, the group receiving diets containing 1.6 g/kg of mushrooms for 1-42 days gained more weight (Guimaraes et al,.2014). Spleen weight was higher in birds receiving the mushroom diet compared to the control group, but the bursa weight was lower in the mushroom-fed group (Guimaraes et al., 2014). Willis et al. (2007) used mushroom extract (Lentinus edodes) alone or in combination with probiotics to study the gender issue in broilers.

The extract of *Lentinus edodes* and *Tremella fuciformis* mushrooms did not influence weight gain, feed intake, FCR or the relative weights of organs in broilers compared to the birds' fed antibiotics (Guo et al., 2004).

Table 1: Different types of mushrooms with components and functions

Common/Local name	Scientific classification	Active components	Main functions	References
Golden needle mushrooms	K: Fungi	polysaccharide, ß-D-glucan, chitin, flammulinol	Enhance immune response	Yi et al., 2013
Winter mushrooms	P: Basidiomycota	flammulinolides sesquiterpenes	Antimicrobial, anti-tumour, lower fat	Yan et al., 2014, Rahman et al., 2015
Lily mushrooms	C: Agaricomycetes	Norsequiterpenes, lovastatin, phenolic	Plasma TC, LDL, triglyceride,	Kashina et al., 2016
Velvent shank/Enoki mushrooms	O: Agaricales F: Physalacriaceae G: Flammulina	compounds, protein, vit A, E, K, B-complex	Concentration, antioxidant properties, Low cholesterol, LDL	Jia et al., 2017
Monkey's head/red shank/ mushroom	Sp: *Flammulina velutipes* K: Fungi	polysaccharides, ß-D-glucans, α-glucans	Anti-tumour, anti-hyperlipidaemia, anti-infamantory	Zhang et al., 2012 , Zheng et al., 2015

2.8 The role of mushrooms on the body carcass yield and meat quality of broiler chicken

Poultry meat is a very popular food commodity around the world due to its low cost of production as compared to meat products as beef, lamb or pork, low fat content, high nutritional value and distinct flavor (Barbut, 2005; Chouliara et al. 2007; Patsias et al. 2008). Processed meat product consumption has also dramatically increased over the last decades (Bianchi et al. 2009). Food safety is an important aspect of food quality and efforts should be led to the safety of new functional products from poultry meat

(Burdock et al. 2016). Meat quality may be affected already by manipulation of animal feeding (Kennedy et al. 2005; Assi and King, 2007) or post mortem manipulation of carcass body. Meat quality is one of the economically important traits in chicken. Poultry meat is an important source of high-quality proteins, minerals and vitamins to balance the human diet. Specially developed varieties of chicken (broilers) are now available with the traits of quick growth and high feed conversion efficiency. Depending on the farm size, broiler farming can be the main source of family income or can provide subsidiary income and gainful employment to farmers throughout the year.

The water holding capacity (CWL) of meat and its products is an indicator of good quality, and it can protect meat from the harmful effects of chilling and freezing (Guimares et al., 2014). Furthermore, the formation of ice crystals within cells during preservation may protect against moisture loss. However, mushrooms can be utilized in poultry feeds without harming food quality (Guimares et al., 2014). Feeding dried mushrooms reduced malondialdehyde in the liver, breast and thigh tissues, and increased the synthesis of glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase production in broilers at 42 d of age (Giannenaset al., 2010a). Including oyster mush-rooms at 1.5% in feed has been reported to result in higher carcass weight and dressing percentage compared to a supplemented control group (Abro et al., 2016). Additionally, significant variations in meat quality, particularly in terms of texture and tenderness, were seen when oyster mushrooms were fed (Camay et al., 2016). In the experiment of male broilers fed oyster mushroom powder in comparison to a prebiotic supplement to examine carcass quality in male broilers, contrary to the former group of researchers fed oyster mushroom powder.

The addition of 20 g/kg of mushrooms to the diet had no effect on the weight of the carcass or the internal organs (Toghyani et al.,2012).

2.9 Effect of mushrooms on antioxidant, cholesterol and intestinal microbiota of broiler chicken

The antioxidant parameters, including superoxide dismutase (SOD) and catalase (CAT) values were higher while, MDA levels were low in the serum, liver and breast muscle in broilers fed different levels (6, 12 or 18 g/kg) of HCM (Shang et al.,2014). In a study, serum total cholesterol, triglyceride (TC) and low-density lipoprotein cholesterol levels were significantly lower Shang et al.,2014) with different levels (1, 3 or 5 g/kg) of HCM in broiler diets. For an example, serum TC was decreased from 3.72 to3.22 mmol/l for the diet containing 5 g/kg HCM on d 42, whereas it decreased from 51.6 to 37.3 mg/100 g in breast muscle sample for the same diet at the same age (Shang et al.,2014). Feeding broilers mush- rooms at 5% in feed resulted in higher *Bifidobacteria spp.* populations in the caecum compared to the control group (Hines et al., 2013). Combining *agaricus blazei* powder into the diet at a rate of 2 g/kg decreased total serum cholesterol levels while having no impact on TG levels in broiler chickens (Fanhani et al., 2016). Compared to birds fed control diets, birds fed the mushroom-supplemented diet had lower serum total cholesterol levels (Daneshm and et al., 2012). Meals containing mushrooms have no effect on plasma concentrations of TG, total cholesterol, low-density lipoproteins, or very low-density lipoproteins (Daneshm and et al.,2011). The author recommended using chickens fed mushrooms to study the mode of action on lipid metabolism (Daneshm and et al., 2012).

The lowest serum TG was found in chickens fed oyster mushrooms compared to the control diet (Toghyani et al., 2012). However, whether mushrooms have a hypo-cholesterolemic impact in poultry is currently being researched (Bederska-Lojewska et al., 2017) and other biochemical and blood parameters were not different among the

treatments. Broilers fed mushrooms had significantly higher intestinal villus height and a higher ratio of height to crypt depth than those fed controls (Shang et al., 2016). Additionally, broilers fed diets supplemented with mushrooms retained more protein from 66.5 to 69.8 g/kg than the control group (Shang et al., 2016). In this study, larger villus height and height to depth ratio were linked to higher nutrient retention. Giannenas et al., (2010b) investigated the effects of *Agaricus bisporus* supplementation on intestinal architecture and microbiology. The treatment did not significantly affect intestinal morphology in broilers, however, Lactobacilli spp. numbers were higher in broilers fed the 20 g/kg mushroom diets compared to the control group in the ileum, whereas, both Lactobacilli spp. and *Bifidobacter spp*. numbers the caecum were higher in the mushroom-fed group than the control. The authors concluded that mushroom supplementation may have beneficial effects on gut health in broiler (Giannenas et al., 2010b).

2.10. Effect of mushroom on the blood metabolites of broiler chicken

The dietary use of mushrooms reduces serum cholesterol indicated that under infectious conditions, the effect on mushrooms was more pronounced than under normal ones. A positive impact of mushrooms on lipid metabolism in male hamsters was reported in the literature by (Guo et al.,2004). According to research, hamsters' serum and liver tissue cholesterol levels can be lowered by using the extract and powder from needle mushrooms. In another study, rats fed exo-polymer from the *Hericium erinaceus* mushroom had lower levels of total cholesterol (TC), low-density lipoprotein cholesterol, and plasma triglycerides (Guo et al., 2004). The probiotic intestinal microflora that lowers acetyl-co-enzyme-A and carboxylase activity, resulting in a decrease in lipid synthesis (Toghyani et al., 2012). The effect on blood triglyceride concentration may also be explained by the mushroom's high lovastatin content,

particularly those from the Pleurotus family (Muszynska et al., 2017). According to research by Fanhani et al. (2016), broilers supplemented with 1.5 and 2 percent *Agaricus blazei* powder had significantly lower total serum cholesterol levels while having no impact on serum triglyceride levels.

2.11 Effect of mushroom on the survivability of broiler chicken

Survivability of broilers fed on different dietary treatments of mushroom was 100% and it was due to the presence of antioxidant (Selenium, Ergothioneine) (Ali et al., 2017).

In comparison to the control diet, broilers fed mushrooms (10 g/kg) had higher antibody titers against poultry red blood cells, according to Kavyani and Porreza (2012). However, there were no discernible variations in antibody titers against the virus that causes Newcastle disease (NDV). This finding indicated that mushroom supplementation could affect poultry immunological responses (Kavyani et al., 2012). Fanhani et al. found higher antibody titers against NDV in broilers fed diets with ablazei mushroom powder (2016). Similarly, the inclusion of oyster mushrooms at 2 g/kg in broiler diets could improve antibody response to NDV compared to both control and antibiotic fed birds (Daneshmand et al., 2012). In contrast, serum antibody responses against NDV, AI and sheep red blood cells were not affected in chickens supplemented with oyster mushrooms (Toghyani et al., 2012).

2.12 Effect of mushroom on the bone morphology of broiler chicken

Chemical composition of the bone tissue changes greatly depending on species, age, diet, nourishment and from bone to bone of the same animal. The chemical composition of chicken bone was 2.9% nitrogen corresponding to about 15.6% protein, 9.5% fat, 14.7% mineral and 57.5% moisture (Kettawan et al.,2002). In regard to the mineral composition, mushroom has the high content of calcium (39 mg/g), phosphorus (20 mg/g) and iron (1.8 mg/g) (Kakimov et al., 2021).

2.13 Effect of mushroom on the profitability of rearing broiler chicken

Feed cost or production cost represents the farm ‘s profitability. Higher profitability depends on production cost. So, production cost varies by multiple factors say-market price, feed, bird, processing method, mode of selling or marketing, house, labor, treatment and so on. Today’s broiler industries are adopting multiple polices for selling their finished product in the market with a view to earning higher profitability and cutting cost. Now broiler chicken in diversified forms such as live bird, dressed carcass, different meat cut, deboned or fillet meat etc., are available in the super shop to increase farm ‘s profitability by reducing production cost (Akter et al., 2020).

This sudden change in the market forms for poultry industry recently, from a whole live bird commodity to modern highly diversified processed products, has been an emerging issue to look ahead for quality poultry production along with low investment and cost. The result of previous investigator found increased profitability when broiler raised with mushroom fed diet (Ali et al., 2017).

2.14 Significance of the study

The study is undertaken to determine the usefulness of mushroom supplementation in poultry diets, by assessing growth performance, carcass yield, viability, blood metabolites, bone quality, and profitability of broiler fed mushroom -sourced diet. The results from the research study might act as guidelines for the poultry farmers, poultry researchers to formulate efficient ration formulation which could help poultry integrators to increase the broiler meat production with more efficiently, and thus could supply premium quality meat to the consumer across the globe.

The data regarding mushroom in poultry are not adequate. However, considerably more research study is needed to make available data. Besides, more improved, reliable, authentic and applied data are necessary to support those research findings by the poultry integrators, research scientists and so on across the globe. Further, still it has not been determined whether the mushroom has a reliable or available source or good product quality or not, which can be supplied to the market to create a new horizon of global mart for using this stuff in the poultry diet. After all, it is academic research, so, the research findings in the field of poultry science would be very useful for the higher studies of the broiler production including other biological sciences of the agricultural sciences.

The finding of the research study will help the poultry integrators to boost up their broiler meat production through reducing effect caused by environmental stress. Further it would help the farmers or poultry raisers to improve their socio-economic condition by income generation, employment creation, poverty alleviation, reduction of malnutrition, reduction of unemployment condition and female empowerment. After all, national economy and GDP of the country might be enhanced by this sort of research on poultry production.

2.15 Conclusion

In Bangladesh, the broiler industry is developing rapidly and its success depends on how rapidly a bird would achieve maximum marketable weight, and the farmers sell it to the market so quickly. For this reason, growth promoter is very conscious issue at this moment. As mushroom has the capability to be used as natural growth promoter in place of antibiotic in broiler chicken. Besides, mushroom is a good source of protein including other vital nutrients, which can be used as protein supplement in formulation of diet for the poultry and other livestock. Mushroom supplement in the poultry diet might be economical or could have potential to improve the productivity of broiler chicken under commercial farming condition.

CHAPTER-III

Materials and Methods

Chapter—III

Materials and Methods

3.1 Statement of the experiment

The experiment was carried out at the Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University (CVASU) to ascertain the efficacy of mushroom on the improvement of broiler chickens fed a diet supplemented with mushrooms. Feeding trial in broiler chicken was performed at the Poultry Research Shed of CVASU campus, from June to July, 2021. Laboratory analyses were performed in Poultry Nutrition Laboratory, Poultry Research and Training Centre (PRTC) and Biochemistry Laboratory of CVASU, Khushi, Chattogram.

3.2 Preparation of the experimental shed

Firstly, the experimental poultry shed was prepared by swiping and removing of dust dirt by broom. The battery cage was also washed and cleaned by whisk. Both shed and battery cages was then washed and cleaned properly with tap water containing detergent. The shed and cages were left for air drying for 3 days. After that, ceiling, wall and floor along with battery cages were treated with disinfectant with FAM 30^{R} (5ml/1L water) via sprayer and again left for drying for 1 week. The cage divided into 16 pens of equal size to accommodate broiler chicken. Before allowing the entrance of chick, the individual tube feeder, drinker and each pen were marked properly by sticker (bearing cage no. and treatment). An electric bulb (60 watt) was used to brood the chicks and set at the roof of each pen by hanging condition. The floor space provided for each bird was 0.5 sq. ft in the cage. The floor of each pan was covered with medium thick paper to reduce leg injury and to maintain warm temperature within each pen. All equipment was cleaned and disinfected accordingly outside the shed.

3.3 Collection of day-old broiler chicks and experimental design

A total of 96 (Ross 308) day-old broiler chicks of either sex was purchased from a local renowned hatchery (Kazi Farm Ltd.) on a pre-order basis to run the experimental trial from day 1 to 34d. The chicks were weighed just after having them and randomly distributed into four treatments *i.e.*, T_0, T_1, T_2, and T_3, and each treatment had four replicates with six broiler chicks per replicate cage in a completely randomized design (CRD). The layout of the experimental trial was demonstrated below in Table 2.

Table 2: Experimental design

Treatments	**Number of day-old chicks**				**No. of chicks per treatment**
	R_1	R_2	R_3	R_4	
T_0	6	6	6	6	24
T_1	6	6	6	6	24
T_2	6	6	6	6	24
T_3	6	6	6	6	24
Total	24	24	24	24	Grand Total=96

[T_0 refers to control diet, T_1, T_2 and T_3 refers to test diet / treatment which are supplemented with 0.5 %, 1.0 % and 1.5 %, mushroom, respectively, R_1, R_2, R_3 and R_4 refer to replicates 1, 2 and 3, 4, respectively]

3.4 Collection of the experimental feed and feedstuffs

Ready-made compound diet (crumble-starter) (Nourish^{TM}) was collected from the market by purchasing and fed the broiler chicks up to 2 weeks After that, finisher or test diets were prepared manually and feed the birds from 15-34 days. The macro-feed ingredients (maize, wheat, soybean meal, palm oil, and limestone) required for the feed formulation were purchased from the local market of Pahartali Bazar, Chattogram. Each macro-ingredient was purchased based on thorough selection by visual observation like organoleptic test (color, odor, moisture etc.). The micro-nutrients were procured from another local market (Hazari Lane, Terry Bazar, Chattogram). Particularly, test ingredient (mushroom) was collected from Savar Mushroom Institute, Dhaka, Bangladesh as order basis in ground form.

The composition of the ready-made diet (Nourish TM) was shown in Table 3 along with its approximate composition and reporting values.

Table 3: Chemical composition of starter diet

Nutrients	Proximate values (%)	Reporting values of Ready-made (Nourish feed)
ME (Kcal/kg)	-	3000
NFE	68.33	-
Moisture	14.17	12.0
DM	89.60	88.0
CP	21.18	20.0
CF	4.00	5.00
EE	1.79	-
Ash	4.70	6.00
Ca	1.00	0.95
Total P	0.45	0.45

3.5 Formulation of test diet

Four diets (T_0, T_1, T_2 and T_3) were formulated with the locally available feed ingredients as per the breeder manual or NRC (1994) in Table 4. All the formulated diets were iso-energetic and iso-nitrogenous. All feedstuffs were used to formulate control diet without mushroom, whereas T_1, T_2, and T_3 test diets were prepared with the supplementation of mushroom at the rate of 0.5 %, 1.0 % and 1.5 %, respectively. Starter diet (crumble) was procured from the local market which was provided to the chicks up to 14 days of age. Then, formulated diets (finisher mash) were allowed to feed the birds from d15-34 day. Throughout the duration of the trial, all of the birds had indefinite access to fresh, clean, and cool drinking water.

All formulated diets were supplemented with the multi-enzyme which contains microbial enzyme like amylase, xylanase, β-glucanase, cellulase, pectinase, protease and phytase activities, and supplemented to the basal diet according the level stated by

the manufacturing company. The composition and nutritional values of the formulated diets and test ingredient (mushroom) are shown below in the following Tables (4, 5)

Table: 4 Nutrient and ingredient composition of test diet of broiler chicken

Ingredients (%)	**Diets**			
	T_0	**T_1**	**T_2**	**T_3**
Maize	58.15	58.44	58.51	58.52
Soybean meal	32.00	32.00	31.87	31.51
Protein concentrate	2.10	2.10	2.10	2.13
Palm oil	3.80	3.70	3.74	3.83
DCP	0.90	0.56	0.61	0.51
Limestone	1.68	1.68	1.27	1.20
Table salt (NaCl)	0.36	0.25	0.25	0.25
Choline chloride	0.04	0.04	0.04	0.03
Vitamin min premix	0.25	0.25	0.25	0.25
L-lysine	0.18	0.18	0.18	0.18
DL-methionine	0.25	0.25	0.17	0.17
Mushroom	0.00	0.50	1,00	1.50
Toxin binder	0.05	0.05	0.01	0.01
Sand	0.24	0.00	0.00	0.00
Total	100	100	100	100
Nutrients (%) – calculated				
ME(Kcal/kg)	3075	3076	3076	3077
CP %	21.01	21.11	21.12	21.08
CF	3.48	3.46	3.50	3.47
EE	4.60	4.65	4.64	4.67
Ca	1.16	1.10	1.20	1.10
P	0.72	0.67	0.68	0.65

[[T_0 refers to control diet, T_1, T_2 and T_3 refers to treatment which are supplemented with 0.5 %, 1.0 % and 1.5 %, mushroom, respectively]]

Table 5: Nutrient compositions of the test ingredient (Oyster mushroom – *Pleurotus ostreatus)*

Nutrient Components	Analyzed value (%)
ME (Kcal/kg)	2930.00
NFE	49.37
DM	85.83
Moisture	14.17
CP	19.25
CF	20.78
EE	2.09
Ash	8.51
Ca	0.90
P	0.43

3.6 Feed grinding, mixing and preparing the diets

First of all, the macro ingredients collected from local market in ground form having a desirable particle size, weighed and mixed. Then micro-ingredients were also weighed by electric balance one by one and then put in a small bucket for each diet and mixed properly by turning layer by layer. After that, the weighed macro-ingredients were spread on the wide plastic paper kept on floor of house and mixed thoroughly by the help of shovel. After that, the micro-nutrients were mixed on feed mixture equally. Vegetable oil (Palm) was added at half of the required amount by sprinkling over the feed mixture and then mixed thoroughly with hand as well as shovel. Remaining half amount of vegetable oil was finally sprinkled over feed mixture and again mixed thoroughly by both hand and shovel. A thorough mixing was done manually with shovel after weighing all ingredients as per the requirement of individual diet. Finally, the mixed diets were stored in the bags with marking, and later used for feeding the bird as mash feed. Same procedures were followed for the preparation of all diets.

3.7 Management

Throughout the entire experimental period, the following management practices were used in an effort to maintain uniformity (similar feeding, lighting, environmental condition) in the management practices as much as possible.

3.7.1 Brooding

Day old chicks (DOC) were placed into the 16 equal sized pen of battery cage, a linear feeder and a drinker were provided for each pen. Electric bulb was used to brood the chicks. A 60watt electric bulb was hanged at a height of 45 cm in the upper middle of each pen roof in order to maintain brooding temperature. For the first two days, the birds were exposed to a temperature of 35 ℃. When the chicks arrived at 10 days old, the temperature was gradually lowered by 1 or 2 ℃ after every 1 or 2 days. Following that, the poultry shed's temperature was held at 25 ℃ for the rest of the experiment.

3.7.2 Floor space

Birds were reared in battery cage of 16 equal size pens. Each pen (4.4 sq. ft.) was marked out for 6 birds. Therefore, each bird had 0.7 square feet of floor space.

3.7.3 Feeder and drinker space

One drinker and feeder were kept in each pen. One feeder (4.5 linear inches per bird) and one round drinker with a capacity of 3.0 L were provided for each pen.

The tube feeder and drinker were arranged in a pattern to make it easy for the birds to intake food and water. Drinkers are cleaned and dried by detergent water 3-5 days interval. Birds were allowed to mash diet from linear feeder from d 15-34 days.

3.7.4 Feeding and watering

Feed and drinking water were supplied *ad-libitum to* the birds throughout the experimental period. Starter feed was supplied to birds up to 14d, once a day in the tube feeder in the early morning as an adjustment diet. Paper along with tube feeder and drinkers were used for feeding and watering the chicks during the early stages soon after coming from the hatchery. The finisher mash diets were given to the experimental birds from d15-34 days two times daily, where once in the morning at 6 AM and another in the afternoon at 6 PM. Fresh, clean and cool drinking water was supplied the birds three times a day *i.e.*, at 6 AM, 12 AM, and 6PM.

3.7.5 Lighting

The birds were exposed to a continuous lighting (23 h: 1h) in each 24 hrs. of photoperiod.

3.7.6 Immunization of birds

Birds were vaccinated against Ranikhet (New Castle Disease), and Gumboro disease (Figure 14), according to the schedule mentioned in Table 5. Ranikhet live vaccine (Cevac New L^R) and Gumboro live vaccine (Cevac Gumbo L^R) were procured from local veterinary medicine Dispensary. Vaccines were collected in ice contained air tight flask and individual vaccine was collected at the vaccination date. Nearly 25 ml distilled water was added to vaccine vial via syringe to make 500 dose diluted live vaccine. Vaccine was administered to individual birds via eye within 2 hours of collection. Individual vaccines were administered at the evening time of respective vaccination date (Table 6).

Table 6: Vaccination schedule

Age (Days)	Name and type of the Vaccine	Name of diseases	Route of administration
5	Cevac New L^R, Live	Newcastle disease	One drop in one eye
12	Cevac Gumbo L^R, Live	Gumboro	One drop in one eye
17	Cevac New L^R, Live	Gumboro	One drop in one eye
21	Cevac Gumbo L^R, Live	Newcastle disease	One drop in one eye

3.7.7 Medication

The chicks were given glucose and vitamin-C as soon as they were removed from the chick boxes to minimize any stress that might have occurred during transportation. Water soluble vitamin and normal saline were also provided for the first 3 days of brooding. During the course of experimental period, electrolytes and vitamin-C were added with the drinking water to combat stress due to high environmental temperature (33 ^oC to 37 ^oC).

3.7.8 Sanitation

Adequate and proper hygiene and sanitary measures were adopted and followed throughout the experimental period. Proper cleaning and disinfection of all equipment were done prior to the beginning of the trial. Potassium permanganate ($KMnO_4$) solution (1.5 %) was prepared and kept into a plastic bottle fitted with a sprayer at its opening mouth. It was kept at the entry point of poultry shed and used as disinfectant before entry into poultry shed. Hands and feet were also properly disinfected with 70% alcohol before entry into the shed.

3.7.9 Data and sample collection

Both pre-starter feed sample and test diets sample were collected prior to supplying birds for the assessment of the nutritive value of each diet. Body weight, feed intake and remaining feeds were recorded in record sheet in weekly basis to calculate body weight gain and feed conversion ratio (FCR). Blood samples of two birds from each replicate cage were collected on day 34 for measuring serum blood profile (total tissue, albumin, glucose, triglycerides, uric acid, creatinine). Besides, two healthy birds from each replicate were also selected randomly and then slaughtered halal way to collect bone sample, Meat yield traits like dressing percentage, breast weight, thigh weight, drumstick weight, shank weight, wing weight etc., were also recorded on d34. Abdominal fat content and individual weight of gastrointestinal organs (liver, pancreas, heart, small intestine, proventriculous, gizzard) was also recorded to ascertain the gastrointestinal organ development of the birds. Cost benefit analysis was calculated at the end of trial period.

3.7.10 Method for the broiler processing

At the end of trial period, two broilers were selected randomly, weighed and killed humanely from each replicate pen to assess carcass yield traits, abdominal fat accumulation and visceral organ weight. Feed and water were withdrawn from the pens 3 hours prior to killing in order to facilitate proper bleeding and skinning. After slaughter, birds were processed by removing the feather, skin, head, shank, viscera, oil gland, heart, kidneys, liver, lungs and small and large intestine of the carcasses. Heart and liver were removed from the gastro-intestinal tract by cutting and traction gently to let them loose. Gall-bladder was removed from liver. Gizzard and proventriculus was separated from gastro-intestinal tract by cutting it loose in front of the duodenum and behind the last end of esophagus.

Spleen was also collected by gentle cut and traction from liver parenchyma. Pancreas was collected from loop of duodenum by gentle cut and traction by scissors.

3.8 Recordkeeping

Throughout the entire experimental period, the following parameters were noted.

3.8.1 Mortality

Mortality record was kept when it happened. No mortality found.

3.8.2 Body weight

Live weight of broiler was taken replication wise for each treatment weekly. Average live weight of the broilers was also recorded at the beginning of the experiment and at the end of each weekend.

3.8.3 Feed intake

By calculating the left over from the total amount of feed given to birds

on each weekend, the quantity of feed consumed was determined.

3.9 Calculation of data

3.9.1 Weight gain

By subtracting the initial body weight from the final weight, the weight gain was calculated.

3.9.2 Feed conversion ratio (FCR)

The amount of feed needed for per unit of production is called feed conversion ratio. The efficiency of converting feed into meat called feed efficiency. It was calculated by using the following formula:

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

3.9.3 Mortality and livability

Mortality of birds was calculated on the basis of number of dead birds throughout the experimental period divided by the total number of birds housed at the start of experiment. Livability was calculated from mortality of birds per replicate cage. Using this formula, the mortality percentage was calculated.

$$\text{Mortality (\%)} = \frac{\text{Number of broiler died}}{\text{Toutal number of broiler housed}} \times 100$$

3.9.4 Dressing percentage

The dressing percentage of birds was calculated as follows:

$$\text{Dressing (\%)} = \frac{\text{Dressed Weight}}{\text{Body Weight}} \times 100$$

Slaughtering data ‘such as body weight, blood loss, feather loss, abdominal fat, shank weight, heart weight, gizzard weight, heart weight, small intestine, pancreas, bursa etc., were expressed in percentage.

3.10 Sample processing and analyses

3.10.1 Feed sample

Six feed samples were collected from ready-made and formulated test diets prior to feeding the birds. The samples were prepared by using a mortar and pestle to grind them, and they were then thoroughly mixed for laboratory analyses. About 500gm of each diet of finisher as well as starter diet were taken and sent to the Poultry Research and Training Center (PRTC) Lab for proximate analysis. Each analysis was done two times for each sample to minimize technical errors. The samples were tested for proximate analysis of dry matter (DM %), moisture %, crude protein (CP %), crude fiber (CF %), ether extract (EE %) and ash using standard laboratory procedures (AOAC, 2007). Dry matter estimation was done by oven dry method. Crude protein estimation was accomplished by Kjeldahl Method.

Ether Extract estimation was done by Soxhlet apparatus. Ash was measured by igniting the pre-asking sample on a Muffle furnace at a temperature of 600 °C for four to six hours. The percentages of Ca % and P % were determined using by atomic absorption and spectrophotometry (AOAC, 2007).

3.10.2 Measurement of Metabolizable Energy (ME)

The ME was determined indirectly on the basis of True Metabolizable Energy (TME) contents of the feed samples, assuming that TME was 8% higher than the ME, as it is reported that TME is 5 to 10 % higher than ME (Wiseman, 1987). The formula is for TME = 3951 + 54.4 EE - 88.7 CF - 40.8 Ash. Apart from this, another formula was used to calculate the ME of analytical feed, *i.e.* ME = 3.99*CP%+3.99*NFE+ 9.1*EE% or ME = (35×CP% + 85 ×EE% + 35 NFE%). NFE = 100 - (Moisture + Crude Protein + Crude Fat + Crude Fibre + Ash).

3.10.3 Serum biochemical parameters and analyses

One broiler was randomly selected from each replicate cage on day 34, weighed and killed humanely to collect blood sample for the assessment of blood metabolites such as total protein, glucose, albumin, uric acid, creatinine and triglycerides. The blood samples were then centrifuged at 3000 rpm at 4^{o}C for 15 minutes to obtain the serum, and these serum samples were collected in clean-plastic vials, and immediately frozen at -80^{o}C, until further chemical analyses were done in the lab. Serum total protein, albumin, triglyceride, glucose, uric acid and creatinine were determined using standard kits (Randox Laboratories Ltd., UK) and automatic analyzer (Humalyzer300, Merck®, Germany) as per the instructions given by the manufacturers company.

3.10.4 Processing bone sample and data collection

The right tibial bones were collected and boiled for 10 minutes in deionized water to facilitate the removing of the attached soft tissues and fat.

After that, bone characteristics such as bone length and head width were measured using digital callipers (In size, Japan) and the weight was recorded by digital balance. After taking the measurements of the bone traits, the samples were sent to the Department of Animal Science and Nutrition to analyse the bone mineral concentration. The samples were thoroughly dried before being ground and placed in a muffle furnace for four hours at 600°C to develop ash. The weight of bone ash samples was taken and then used to analyze the bone mineral concentration (Calcium and Phosphorus only) by atomic absorption and spectrophotometry respectively.

3.11 Evaluation of meat yield parameters

Two broilers were humanely dispatched on day 34, and various meat yield parameters including carcass weight, dressed weight, and abdominal fat content, as well as the weights of various cuts of meat (neck, thigh, wings, breast, back, shank, drumstick), and giblets (heart, liver, pancreas, proventriculus, and gizzard) were accurately recorded.

3.12 Production cost

All the costs involved for the production of broiler say costs for labor, feedstuffs, electricity, water, chicks, medication, vaccination, selling price of live broiler including others are taken into account for the cost benefit analyses.

3.13 Statistical analyses

All collected data were subjected to analysis by one way ANOVA procedure using Minitab software (Minitab, Minitab Version, 16, 2000). The significance of differences between means was tested using the Duncan ‘s multiple range tests (DMRT). Statistical significance was considered at $P \leq 0.05$.

Pictorial Presentation of Activities During Experiment

Thesis for MS in Poultry Science

Figure 3: Drying of feeders and drinkers

Figure 4: Disinfection of Pens

Figure 5: Mixing of feed ingredients

Fig. 6: Packaging of feed sample for testing

Figure 7: Packaging of test diet

Figure 8: Marking of brooding pen

Figure 9: Sexing of DOC

Figure 10: DOC in cage

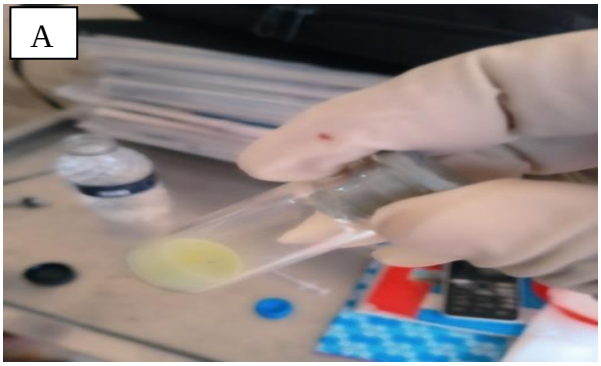

Figure 11: A. Vaccine, B. Vaccine preparation, C & D. Vaccination of birds

Figure 12: Feeding of birds

Figure 13: Cage rearing of birds

Figure 14: Weighing of feed

Figure 15: Weighing of birds

Figure 16: Evisceration

Figure 17: Weighing of body parts

Figure 18: Blood sample for analysis

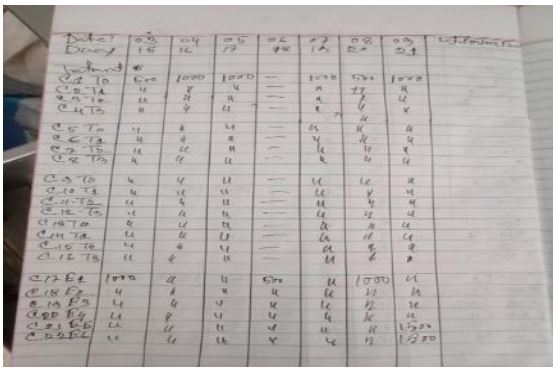

Figure 19: Record keeping

CHAPTER-IV

Results

Chapter-IV

Results

4.1 Growth responses of broiler chicken fed mushroom diet

The gross responses of broilers in terms of fed intake, body weight, FCR and viability are stated below in a tubular form. Apart from this, meat quality, carcass traits, organ weights, blood prolife, bone traits, gastro-intestinal development and profitability of broiler data also represented below in this section.

4.2 Feed intake

The feed intake (FI) result of broiler chicken up to 34 days of age was shown in Table 7. The data showed that the FI of broiler was not influenced ($P>0.05$) by the dietary treatment. Numerically greater FI was observed in the supplemented diets compared to control group.

Table 7: Feed intake (FI) (g/b) of broilers fed mushroom supplemented diets

Trait	Age (days)	Treatment				SEM	P –value
		T_0	T_1	T_2	T_3		
FI (g/b)	1-14	591.67	633.33	695.83	591.67	16.743	0.146
	1-21	1591.70	1633.30	1695.80	1591.70	16.750	0.146
	1-28	2658.33	2709.38	2736.46	2615.42	16.810	0.149
	1-34	3554.17	3626.04	3673.96	3573.75	16.351	0.173

[Data indicate mean values of 6 birds per replicate from day 1-34 days; T_0 refers to control diet with no supplemental mushroom, whereas T_1, T_2 and T_3 diets are supplemented with 0.5, 1.0, and 1.5% mushroom, respectively]

4.3 Live weight gain (LWG)

The LWG of broiler chicken is shown below in Table 8. It is evident from the LWG data that there was no significant ($P>0.05$) difference between treatment from 1- 14d, 1-21d, 1-28d and 1-34d, respectively, in this study. The LWG of broiler fed on

supplemented diets tend to be significant (P<.009) during d1-34 days of age. When compared to other dietary groups, T_3 group birds achieved the highest LWG (P =.009).

Table 8: Live weight gain (LWG) (g/b) of broiler chicken

Trait	Age (days)	Treatment				SEM	P-value
		T_0	T_1	T_2	T_3		
LWG (g/b)	1-14	532.96	551.54	543.67	536.50	4.010	0.405
	1-21	916.50	932.50	940.80	949.80	12.600	0.814
	1-28	1578.50	1520.10	1519.40	1607.60	21.400	0.404
	1-34	2127.20	2136.30	2186.30	2280.30	37.88	0.090

[Data indicate mean values of 6 birds per replicate from day 1-34 days; T_o refers to control diet with no supplemental mushroom, whereas T_1, T_2 and T_3 diets are supplemented with 0.5, 1.0, and 1.5% mushroom, respectively]

4.4 The feed conversion ratio (FCR)

The result show that FCR was not influenced significantly (P>0.05) among the treatment, as shown below in Table 9. Though no variation (P>0.05) was found in the FCR values between treatments, but numerically lower FCR value (1.57) was observed in the T_3 supplemented diets compared to other dietary group.

Table 9: The FCR of broiler fed mushroom supplemented diet

Trait	Age (days)	Treatment				SEM	P-value
		T_0	T_1	T_2	T_3		
FCR	1-14	1.11	1.15	1.28	1.11	0.031	0.199
	14-21	1.74	1.76	1.81	1.68	0.032	0.541
	21-28	1.69	1.79	1.80	1.63	0.027	0.110
	28-34	1.64	1.70	1.73	1.57	0.028	0.205

[Data indicate mean values of 6 birds per replicate from day 1-34 days; T_o refers to control diet with no supplemental mushroom, whereas T_1, T_2 and T_3 diets are supplemented with 0.5, 1.0, and 1.5% mushroom, respectively]

4.5 Survivability

The livability of broiler chickens fed mushroom supplemented diets on 34 days was not influenced (P>0.05) by dietary treatments (Figure 20). No mortality was observed entire the trial period.

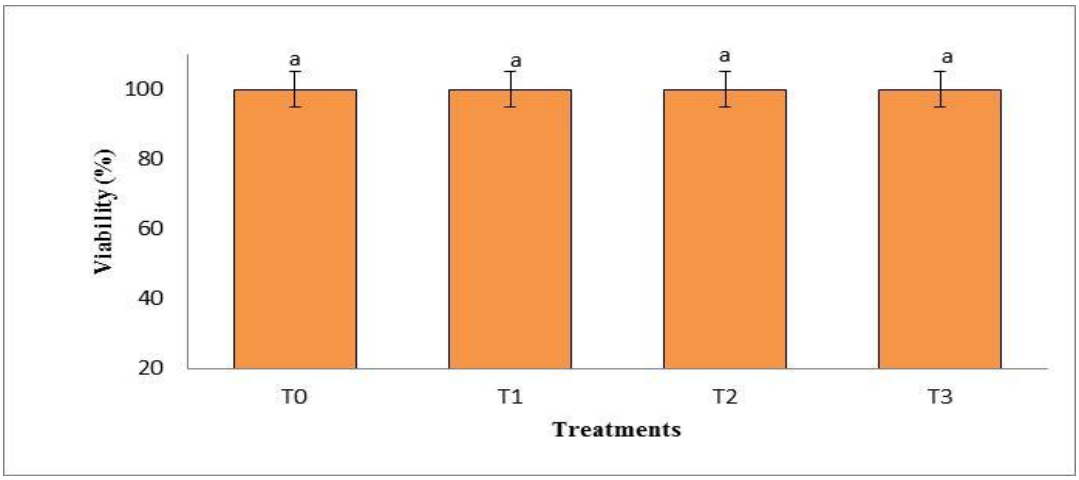

Figure 20: Survivability (%) of birds fed mushroom diets on day 34; Bar with similar letter has no significant differences (P>0.05) between treatments

4.6 Meat yield parameters of broiler chicken

Results of meat yield parameters shown in Table 10 demonstrated that the dietary treatment had no noticeable impact (P>0.05) on the weights of dressing percent, breast weight, drumstick weight, thigh weight, wing weight, fat, and neck weight percent, among other weights. Statistic (P>0.05), the T3 diet group's birds had the highest dressing (percent) and breast weight, while the T2 diet group's birds had the lowest dressing (percent) and breast weight but the highest drumstick and thigh weight.

Table 10: Meat yield traits (g/100g) of broilers fed mushroom on days 34

Traits (%)	Treatment				SEM	P-value
	T_0	T_1	T_2	T_3		
Dressing%	62.14	63.55	62.72	63.92	0.602	0.563
Breast weight	23.59	23.78	22.14	24.30	0.360	0.125
Drumstick weight	8.53	8.91	9.49	8.65	0.304	0.644
Thigh weight	9.69	9.77	9.92	9.69	0.364	0.991
Wing weight	5.32	4.94	5.32	5.78	0.233	0.676
Neck	2.52	2.82	2.17	1.96	0.097	0.111

[Data indicate mean values of 6 birds per replicate from day 1-34 days; T_o refers to control diet with no supplemental mushroom, whereas T_1, T_2 and T_3 diets are supplemented with 0.5, 1.0, and 1.5% mushroom, respectively, SEM-standard errors of mean]

4.7 Meat quality of broiler chicken fed mushroom diet

The abdominal fat content of broiler was measured herein this study to assess meat quality based on the fat deposition. The result showed that there was no difference (P>0.05) in the abdominal fat content (%) of broiler chicken between treatment (Figure 3). The data explains that the T0 diet had the lowest amount of fat (0.65%) followed by 0.77 %, 1.03 %, and 0.85% in T_1, T_2 and T_3, respectively (Figure 21).

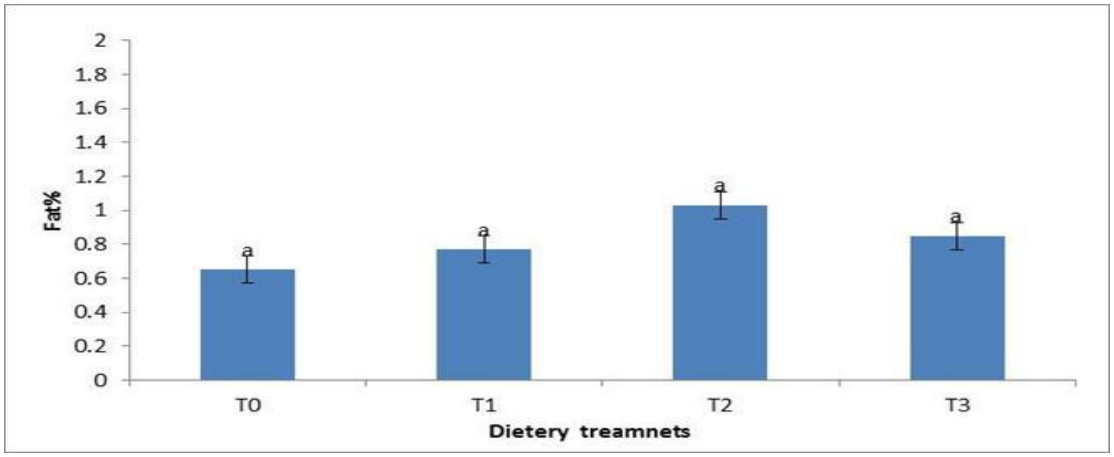

Figure 21: Fat (%) of broilers fed mushroom diets on day 34; Bar with similar letter has no significant differences (P>0.05) between treatments

4.8 Gastro-intestinal development of broiler

The relative weight of visceral organs of broiler chicken fed on the mushroom diet is shown in Table 11. The result demonstrates that the proventriculus, gizzard, liver, heart, and pancreas weights of the birds were parallel between treatments (P>0.05).

Table 11: Relative visceral organs weight (g/100g) of broiler chickens fed diet supplemented with mushroom on day 34

Traits (%)	Treatment				SEM	P-value
	T_0	T_1	T_2	T_3		
Gizzard weight	2.06	1.47	1.62	2.08	0.071	0.096
Liver weight	1.94	2.05	1.84	1.94	0.071	0.779
Heart weight	0.40	0.54	0.44	0.60	0.056	0.608
Pancreas weight	0.27	0.31	0.28	0.24	0.024	0.799

[Data indicate mean values of 6 birds per replicate from day 1-34 days; T_0 refers to control diet with no supplemental mushroom, whereas T_1, T_2 and T_3 diets are supplemented with 0.5, 1.0, and 1.5% mushroom, respectively, SEM-standard errors of mean]

4.9 Serum profile of broiler chicken fed mushroom diet

The broiler chickens' blood metabolite results, including total protein, glucose, albumin, uric acid, creatinine, and triglycerides are displayed in Table 12. The results of the serum metabolite analysis show that there was no significant impact (P>0.05) between treatments for all serum metabolites except for total protein, creatinine, and uric acid. In comparison to the control diet, the supplemental dietary groups' creatinine levels were significantly higher (P<0.01). Uric acid also shows significant (P<0.05) in the treatment. The total protein (P<0.053) was also tended to be significant between treatment.

Table 12: Blood serum profile of broiler chicken fed diet supplemented with mushroom

Traits	Treatment				SEM	P-value
	T_0	T_1	T_2	T_3		
Albumin (g/L)	14.00	13.60	14.80	14.70	0.218	0.298
Glucose (g/L)	249.60	247.45	268.35	256.50	6.664	0.703
Total protein (g/L)	27.4	23.95	24.60	27.75	0.382	0.054
Triglyceride (mg/dL)	55.00	61.30	64.55	65.70	3.581	0.731
Creatinine (mg/dL)	0.30^{c}	0.31^{c}	0.32^{b}	0.35^{a}	0.000	0.01
Uric acid (mg/dL)	2.70^{a}	1.00^{c}	1.95^{b}	2.75^{a}	0.143	0.041

[[Values (mean) bearing different superscript in a row differ significantly at *P<0.05; **P<0.01; SEM-standard errors of mean]]

4.10 Bone morphology of broiler

The relative length, weight and bone width of broiler chickens fed on the supplemented diet (mushroom) is shown in Table 13. The data showed that the weight of bone of birds was significant (P<0.05) among the treatments. Bone length tended to be significant (P<0.074).

Table 13: The bone morphology of broiler chicken fed mushroom diets

Traits	Treatment				SEM	P-value
	T_0	T_1	T_2	T_3		
Bone length (cm/b)	7.10	7.10	7.30	7.45	0.038	0.074
Bone weight (g/b)	16.25^b	15.00^c	14.50^c	17.48^a	0.140	0.003
Bone head width (cm/b)	6.15	6.30	6.45	6.45	0.082	0.569
Bone width (cm/b)	4.00	4.35	4.30	4.35	0.047	0.143
Ca (mg/dl)	62.85	61.80	60.75	65.35	0.597	0.179
P (mg/dl)	26.30	26.65	26.700	27.15	0.032	0.111

[Values (mean) bearing different superscript in a row differ significantly at *P<0.05; SEM-standard errors of mean]

4.11 Analyses of cost benefit

Cost benefit analyses data of broiler was provided in the Table 14. The cost of production was found to be lower in the birds fed supplemented diets than that of control or basal diets (T_0). The birds fed diets supplemented with mushrooms at 1.5% (T_3) had the lowest total cost of production (Tk/Kg live broiler). The birds fed no-mushroom diets at 0.0 % (T0) was counted a lower profit (P<0.05) than that of birds fed on mushroom diet.

Table 14: Cost of production and profit of broiler chicken

Traits	Treatments				SEM	P-value
	T_0	T_1	T_2	T_3		
Live weight (kg/b)	2.14	2.17	2.19	2.28	37.88	0.490
Viability (%)	100.00	100.00	100.00	100.00	0.554	0.418
Feed cost (Tk/ kg live weight)	60.35	58.43	58.81	56.36	-	-
Total production cost (Tk/kg live wt.)	117.39	112.35	111.57	108.26	-	-
Market price (Tk/kg live bird)	130.00	130.00	130.00	130.00	-	-
Profit (Tk/kg live bird)	12.61^{c}	17.65^{b}	18.43^{b}	21.74^{a}	0.433	0.04
Cost: benefit ratio	9.31^{a}	6.36^{b}	6.05^{b}	4.98^{c}	0.219	0.05

[*Detail total cost of production is given in the appendix Table]

CHAPTER-V

Discussion

Chapter-V

Discussion

5.1 Growth performances of broiler chicken fed mushroom supplemented diet

Generally, the growth rate, FCR, and carcass composition have been the most significant factors for measuring broiler performance (Rezaei et al, 2004). The gross response in terms of feed intake, body weight, FCR of broiler chickens has been considered as the primary criterion for determining the feed nutrient requirements, because the broiler chick is an ideal experimental subject with a limited nutrient store, high nutrient demand and rapid growth rate (Ammerman, 1995).The present study aimed to determine the effects of mushroom supplementation, and their relationships with growth performance, blood profiles, carcass yield, meat quality, gastro-intestinal development, bone morphology, meat quality of broiler chickens. The supplementation of mushroom in poultry diet has been known to influence the productivity. Addition of mushroom in feed has been the subject of studies on improvement of weight gain and feed efficiency (Mahfuz et al., 2019).

However, it is evinced from our current study that the live weight gain (LWG) of broiler was not influenced by the mushroom supplemented diet from d1-28, but poorly significant variation in LWG was noticed during d1-34 days of age which agreed with Shang et al., (2016) and Giannenas et al., (2010). Broiler fed on 1.5% mushroom supplemented diet (T_3) gained higher body weight than that of other dietary groups. It implies that increasing level of mushroom in broiler diet might enhance the growth performance of broiler which agreed with Kavyani et al., (2012) and Ashkan et al., (2014). The mushroom can act as growth promoter, anti-oxidant and these properties of

mushroom might stimulate the broiler to grow better than others (Lee et al., 2012). Besides, the numerically higher feed intake of broiler chicken of this diet group might be a result of increased body weight of broiler chicken fed mushroom diet, as is observed in this current study. Our result is agreed with the reports of previous investigators (Kavyani et al., 2012, Willis et al, 2013, Ashkan et al., 2014).

5.2 Feed Intake

Feed intake of broilers in different dietary treatments entire the trial period of the experimental periods was not affected by the dietary treatment. It indicates that broiler showed similar intention or preference or trend of consuming feed, irrespectively supplemented or non-supplemented feedstuffs. The similar result was also reported by the previous researchers (Giannenas et al., 2010, Guimaraes et al., 2014).

5.3 Feed conversion ratio (FCR)

The FCR in different dietary treatments during the whole experimental period was seen non-significant. At the end of the trial period, numerically lowest FCR (1.57) value was found in the T_3diet indicating that the best feed efficiency was due to optimum antioxidant activity of mushroom powder at the level of 1.5%. It seems that the broilers of T_3 dietary group are a bit efficient converter of feed to meat. The bird of this group grew better than that of otter dietary group and it might be as a result better feed efficiency or improved feed utilization. Mushroom is thought to hasten growth because it promotes better gut health, which leads to better nutrient utilization and feed conversion. Similar result was also found by other researches (Giannenas et al., 2010; Guo et al., 2004a; Ashkan et al., 2014), who reported that broilers that received diet 1.5% mushroom utilized their diets more efficiently. In accordance with a number of studies, the effect of dietary mushroom addition on performance varied according to the rearing stage. Willis et al., (2007) compared the addition of

mushroom extracts and the addition of probiotics using male broilers at 1–21 days of age and did not observe any significant effects on weight gain, food consumption, or efficiency among treatments. However, treatment with mushrooms in these studies at final phase 21-34 days has shown good results for dietary intake and feed to gain ratio, which were superior to mushroom treatments. These studies were similar to the positive control experiment performed in the present study. Additional studies have found that mushroom extracts did not restrain weight gain (Guo et al., 2004).

5.4 Effect of mushroom on the survivability of broiler chicken

No deaths were counted during the experiment. Survivability of broilers fed on different dietary treatments was 100 %. The immune-booster properties of mushroom might induce the survivability of broiler chicken (Tang et al., 2016). It is clear that the livability (%) of broilers was identical between treatment. The result of livability indicates that supplemental diets had no influence on the viability of the broiler chicks. The livability (%) of broilers was unaffected between treatments, as is observed from the current study. It can be assumed that mushroom-fed diet had no detrimental effect on the viability of broiler chicken for the growth and development of broiler. Further it can be assumed that mushroom in the broiler diets can be used undoubtedly, as it had no detrimental impact on the growth and survivability of the broiler chicken. It can be assumed that mushroom can act on the defensive mechanism of the body, as it is reported that mushroom could work positively on broiler chickens 'immune system as an antimicrobial and antiviral agent (Jedinak and Silva, 2008, Zhou et al., 2010). The humoral immunity of broiler chickens could be improved by dietary mushroom supplementation (Zhou et al., 2010). According to research, the poly and oligosaccharides found in mushrooms may promote cellular and humeral responses as well as innate and adaptive immunity (Deepalakshmi et al., 2014).

5.5 Effect of mushroom on carcass yield, meat cut traits and meat quality of broiler chicken

From the data it is obvious that there was no significant variation found in the dressing %, breast weight, thigh weight, neck, wing, drumstick weight % of broiler chicken in this study. The identical growth pattern of different organs of broiler carcass might be a result of uniform growth and development of the broiler chicken. With the report of previous researcher, our result is agreed who found similar results when broiler fed mushroom powder supplemented diet (Toghyani et al., 2012). Our result is in contrast with the result of Abro et al., 2016, who revealed that broiler feeding oyster mushroom (1.5%) had higher carcass weight and increased dressing percentage. The discrepancy of two experimental results might be due to number of factors say, age, rearing length, sex, feed composition, feed quality, strain, environment, temperature and so on.

This study also showed that fat content of broiler was also unaffected among treatments. The amount of fat content in the meat determines its quality. Extra fat accumulation in broiler carcass is generally considered as an unfavorable characteristic in the poultry industry (Remignon and LeBihan-Duval, 2003). Broiler chickens fed on mushroom supplemented diet deposited similar fat content in their carcasses. It implies that diet had no influence on the fat accumulation of broiler, though increasing trend of depositing fat (%) content was observed in the supplemented group (T_3). The probable reason for the increase in abdominal fat on the diets might be due to more rapid growth, leading to earlier transition from muscle to fat deposition (Hossain et al., 2013). Feed nutrients appear to be metabolized at different rates when supplied in diets to the birds. This influences deposition of fat. The rate of the later phases increases as the previous phase approaches completion, so that birds with rapid growth enter the fat deposition phase earlier than birds with slow growth (Hossain et al., 2015).

Lower the fat content gives rise to higher lean meat carcass yield. Generally, people prefer lean meat or fatless meat. So lean meat implies quality meat because it assures higher protein % than fat content in the carcass. Meat quality characteristics are correlated with meat pH tenderness, color (lightness, redness, and yellowness), and water holding capacities (Mahfuz et al., 2019).

5.6 Effect of mushroom on the relative weight of gastro-intestinal organs of broiler chicken

It is clear from the data that the relative weights of proventriculus, gizzard, liver, heart, and pancreas of birds were identical between treatments. It is generally known that visceral organs associated with digestive function develop most rapidly in the first 7 to 10 days of life (Nitsan et al.,1991; Iji et al.,2001 a, b). However, how the nature of the diets influences this development has not been adequately studied. The liver, heart, pancreas and gizzard are the main gastro-intestinal or secretory organs of the chicken. The uniform growth of visceral organ development might be due to similarity in body growth (Hossain et al., 2014). Feeding mushroom powder did not change the size of the liver, gizzard, or pancreas was in close agreement with the non-significant effect of mushroom powder on the weight of internal organs (liver, gizzard, heart, and pancreas) of broilers fed experimental rations (Giannenas et al., 2010).

5.7 Serum metabolites of broiler chicken fed mushroom diet

Except for creatinine and uric acid, the data of serum metabolites indicate that there was no significant influence between treatments. It is obvious from the data that inclusion of mushroom in the broiler diets caused a significant increase in serum creatinine and uric acid, and tend to increase higher protein level. The discrepancies of findings among the treatments might happen due to numerous factors such as species, age, duration, experimental condition, ration, dosage, mode of application and so on.

The total protein (TP) content was slightly increased in the treated group of broiler chicken in this study. The reason for the higher plasma protein accretion in blood vessel might be due to as a result of mushroom supplementation in broiler diets. Because it is reported that mushroom can increase the availability of amino acids (methionine and cysteine) level for synthesizing of protein formation in broiler chicken (Shang et al., 2014).

5.8 Bone characteristics and mineral concentration (Ca and P) of broiler chicken fed on mushroom diet

In the various bone characteristics of broilers between treatments, except for bone weight, there were no significant differences. However, birds on the T_3 diet group had the significantly highest bone weight (BW). Apart from this, bone length (BL) was also slightly increased in the broiler fed T_3 diet. The concentration of bone calcium (Ca %) and phosphorus (P %) was not influenced by treatment. It is obvious from the bone quality data that BW and BL were increased significantly in the broiler fed mushroom supplemented diet. The higher BL and BW might be a resultant of better growth performance of broiler chicken fed mushroom diet, as is observed in this study. Our result bit contradicts with the report of precious researchers (Konca et al, 2008), who observed decreased bone width when broiler fed mushroom supplemented diets.

The discrepancy of these two results likely might occurs due to many factors say, dosages, ration, bird age, environment, duration of trial, climate, sex, health condition, and so on. Besides, the different responses to mushroom supplementation across studies is probably due to varying mode of action of mushroom tested and the differences in animal health, and stress status between studies. (Schrama et al., 2003). Moreover, data are scarce regarding the effect of mushroom supplementation on bone quality of broiler, as limited studies were done so far this.

5.9 Profitability of broiler chicken fed mushroom diet

The increased body weight gain and lower production cost per treatment group in the mushroom diet group may be the cause of the higher profit margin. However, the birds' fed diets without mushroom diets were counted for lower profit (Tk/Kg live broiler). Our result is agreed with the findings of previous investigator (Ali et al., 2017), who found similar result in profitability when broiler raised with mushroom diet.

Today's broiler industries are flourishing rapidly with a goal of selling their finished products in the market in diversified forms such as live bird, dressed carcass, different meat cut, deboned or fillet meat etc. to increase farm 's profitability by reducing production cost (Akter et al., 2020). This sudden change in the market forms for poultry industry recently, from a whole live bird commodity to modern highly diversified processed products, has been an emerging issue to look ahead for quality poultry production along with low investment and cost.

The cost-benefit analysis shows that broilers fed a diet supplemented with mushrooms in this study gained more body weight at a lower cost. Higher body weight gain and lower production costs may have contributed to the higher profit margin. Additionally, net cost varied between the experimental groups. However, variations in feed intake, variations in feed cost (per kg), and variations in mortality across experimental groups were the main causes of variations in return or profit margin. The findings support earlier research that found broilers fed diets supplemented with mushrooms had significantly higher net profits.

However, current feed costs and market meat prices, as well as the criteria used to evaluate the cost, benefit, and performance of birds (live weight, carcass yield, or cut-up parts value, total production cost per kg live weight), could affect feed cost or production cost in relation to its financial returns.

In order to maximize profits, the relationship between feed ingredient costs and subsequent chick costs and processing yield must constantly be re-evaluated due to the fluctuating prices of meat, feed ingredients, and other costs associated with the broiler production.

CHAPTER-VI

Conclusion and Recommendations

Chapter VI

Conclusion and Recommendations

An overview of the study's findings showed that adding mushrooms to broiler diets slightly increased body weight without affecting feed intake, FCR, or livability. Besides, bone length, bone weight, creatinine, uric acid, total protein and profitability were also improved by mushroom diet fed to the birds. No significant improvement of abdominal fat accumulation was observed in the broiler fed diet regardless of supplemented or non-supplemented feed in this study. The current findings indicate that mushroom can play an effective role as an alternative feed additive for supplying lean meat via ameliorating growth performance with reduced cost of broiler chicken under farming condition.

From the result, considerable further research study is required regarding the limitations to the use of mushroom diets and how these diets could be used prudently for profitable livestock and poultry production. Despite the limitations of mushroom with the sources, appropriate strategies can be adopted to improve the quality of supplemented diets, eliminating their intrinsic problems for economic poultry production. To make supplemented diets better for profitable poultry operations, more research is needed.

Our present study suggests the following recommendations on further study regarding trials on mushroom diets:

1. Mushroom can be a substitute of feed additives as a non-synthetic source of feed supplement.
2. Further research should be conducted on point out the optimum level of mushroom in feed with in a larger population of broiler chickens.
3. Feed mixing should be done mechanically for proper assimilation.
4. Floor rearing of birds in an open sided house is strongly recommended.
5. More fund should allocate for the continual research studies.

CHAPTER-VII

References

Chapter VII

References

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Chapter-VIII

Appendix

Thesis for MS in Poultry Science

Table 1: Cost-Benefit analysis of broiler chicken fed mushroom diet

Parameters	Dietary treatments			
	T_0	T_1	T_2	T_3
Live weight (kg/b) on the last day of trial (34th day)	2.14	2.17	2.19	2.28
Livability (%) at the end of trial	100	100	100	100
No. of birds' survivability / treat.	24	24	24	24
Feed intake (kg/b) on 34th day	3.59	3.52	3.58	3.57
Feed cost (Tk/kg) on an average	36	36	36	36
Total Feed intake (kg)	86.08 kg (24 birds @ 3.59 kg)	84.53 kg (24 birds @ 3.52 kg)	85.86 kg (24 birds @ 3.58)	85.68 kg (24 bird @ 3.57 kg)
Total Feed cost (Tk)	86.08×36 = 3099 Tk	84.53 × 36 = 3043 Tk	85.86 ×36 = 3091 Tk	85.68 × 36 = 3084 Tk
Total live weight (kg) of birds per treatment	24 ×2.14 = 51.36 kg	24 × 2.17 = 52.08 kg	24 × 2.19 = 52.56 kg	24 ×2.28 = 54.72 kg
A). Feed cost (Tk/kg live weight)	3099/51.36 = 60.35	3043/52.08 = 58.43	3091/52.56 = 58.81	3084 /54.72 = 56.36
Day-old chick cost (Tk/bird)	25	25	25	25
B). Day-old chick cost (Tk/kg live bird)	25/2.14 = 11.42	25/2.17 = 11.52	25/2.19 = 11.42	25/ 2.28 = 10.96
Other costs include:				
i) Vaccination cost	300 Tk	300 Tk	300 Tk	300
ii) Medication cost	80	80	80	80
iii) Disinfectant cost (iosan & phenyl)	80	80	80	80
iv) Bulb & wire cost	100	100	100	100
v) Water & Electricity cost	80	80	80	80
v) Labour cost	1200	1200	1200	1200
vi) Transport cost	400	400	400	400
Total other cost (Tk) [i…..vi]	2240	2240	2240	2240
Other cost (Tk/kg live wt)	2240/51.36 = 43.61	2240/52.08 = 43.01	2240/52.56 = 42.62	2240/54.72 = 40.94
C). Other cost (Tk/kg live weight)	43.61	43.01	42.62	40.94
D). Total production cost (Tk / kg live wt.) [A+B+C]	117.39	112.35	111.57	108.26
E). Selling live bird market price (Tk /kg live bird)	130.00	130.00	130.00	130.00
Profit (Tk/kg live bird) [E-D]	12.61	17.65	18.43	21.74

Table 2: Nutrient compositions of the formulated diets (analytical values)

Nutrient components (%)	T_0	T_1	T_2	T_3
DM	82.40	82.52	82.09	82.83
Moisture	17.60	17.48	17.91	17.17
CP	20.20	20.45	20.30	20.40
CF	5.87	6.51	6.55	6.04
EE	1.00	1.47	1.93	2.19
Ash	9.00	5.77	5.78	5.36
Ca	1.30	1.00	1.20	1.20
P	0.43	0.46	0.45	0.45

T_0 = Control diet (0.0 % Mushroom)

T_1 = Test diet (0.5 % Mushroom)

T_2 = Test diet (1.0 % Mushroom)

T_3 = Test diet (1.5 % Mushroom)

Brief Bio-data of the Author

Rupom Devnath, the author of this manuscript, was born on 24th September, 1993 in Feni district of Bangladesh. He is the 4th son of Manik Lal Nath and Bela Rani Nath. He passed Secondary School Certificate Examination (SSC) in 2009 from Feni Government Pilot High School, Feni and Higher Secondary School Certificate Examination (HSC) in 2011 from Rifles Public College, Dhaka. He obtained his Doctor of Veterinary Medicine (DVM) degree in 2018 from Chattogram Veterinary and Animal Sciences University (CVASU). He did his clinical training in Veterinary Clinical Medicine from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), India and Khon Kaen University (KKU), Thailand in the year of 2018. Now he is a candidate for the Master Degree in Poultry Science under the Department of Dairy and Poultry Science, Faculty of Veterinary Medicine, CVASU. The author got NST Fellowship for his MS research. He has a great interest to work in the field of Poultry Science.