Effects of Supplementing Different Levels of Meat and Bone Meal on Productive Performance, Carcass Characteristics and Hematobiochemical Parameters in Broiler

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Examination Roll No. 0116/09; Registration No. 292
Semester: July-December 2017

A thesis submitted in partial of the requirements for the fulfillment of the degree of Master of Science in Animal and Poultry Nutrition

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

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December 2017

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Abbreviations

ANOVA	-	Analysis of variance
BBS	-	Bangladesh Bureau of Statistics
BCRDV	-	Baby Chick Ranikhet Disease Vaccine
BMD	-	Bangladesh Meteorological Department
CF	-	Crude fibre
CP	-	Crude protein
CVASU	-	Chittagong Veterinary and Animal Sciences University
DM	-	Dry matter
EE	-	Ether extract
FAO	-	Food and agriculture organization
FCR	-	Feed conversion ratio
g	-	Gram
IBD	-	Infectious Bursal Disease
IBD	-	Infectious Bronchitis Disease
Kg	-	Kilogram
LW	-	Live weight
ME	-	Metabolizable energy
NFE	-	Nitrogen free extract
NS	-	Non-significant
SEM	-	Standard error of mean
SGOT	-	Serum Glutamic Oxaloacetic Transaminase
SGPT	-	Serum Glutamic Pyruvic Transaminase

Abstract

Meat and bone meal (MBM) is a potential source of dietary protein for broiler. One hundred Cobb 500™ unsexed day old commercial broiler chicks were used in a 28-day trial to investigate the effects of different levels MBM on productive performance, carcass characteristics and blood parameters in commercial broiler. Birds were randomly distributed into five dietary treatment groups designated as T_0, T_1, T_2, T_3 and T_4 and supplemented with 0, 2, 4, 6 and 8% MBM for T_0, T_1, T_2, T_3 and T_4 treatment groups, respectively. All birds had free access to feed and water. Results indicated that, weekly average weight gain increased significantly ($p<0.05$) from 15.7 to 24.3 g/d at 1st week, 23.6 to 30.0 g/d at 2nd week, 32.9 to 43.6 g/d at 3rd week and 30.7 to 52.2 g/d at 4th week as the level of MBM supplementation increased from 0 to 8%. The highest average weight gain (52.2 g/d) was recorded in T_4 group at 4th week. Similar to weight gain, average weekly feed intake differed significantly ($p<0.05$) from 1st to 4th weeks of age. Feed intake increased ($p<0.05$) from 56.4 to 82.2 g/d at 4th week as the level of MBM supplementation increased from 0 to 8%. The highest weekly average feed intake (82.2 g/d) was recorded in T_4 group at 4th week. Unlike feed intake, FCR remained unchanged ($p>0.05$) at 2nd and 3rd weeks but differed ($p<0.05$) at 1st and 4th weeks. The best FCR (1.56) was recorded in T_4 group at 4th week. Drumstick weight ($P<0.05$), neck weight ($P<0.05$), breast weight ($p<0.001$) and ether extract content ($P<0.05$) increased linearly with increasing levels of MBM supplementation. Supplementation of MBM had no influence ($p>0.05$) on blood parameters. Total protein differed ($P<0.01$) at 4th week. It was concluded that, increasing levels of supplemental MBM substantially improved performance parameter and carcass characteristics without interfering hematobiochemical parameters in broilers.

Keywords: Blood parameter, carcass characteristics, feed conversion ratio, meat and bone meal, weight gain, commercial broiler

Chapter I: Introduction

Commercial broiler farming is a rapidly growing and highly demanding agricultural sector in Bangladesh. Broiler provides tender meat for human consumption within a short period of time. The popularity of broiler meat is increasing day by day and the number of dressed broilers has been increasing generating large volumes of animal residues which may be used as an alternative feedstuff in broiler diets **(Caires *et al*., 2010)**. The most common type of animal byproducts used in poultry diets are meat and bone meal (MBM), fish meal, blood meal, feather meal and poultry offal meal (**Caires *et al*., 2010)**.

In commercial poultry farming systems, feed costs alone account for 65-70% of the total cost of production and protein costs account 50% of the cost of feed **(Banerjee, 1992).** Meat and bone meal is of one of the cheapest and nutritionally balanced animal protein source produced by recycling animal by-products from slaughter house wastes including carcass trimmings, residues, condemned livers, offals, bones, fats, fetuses and condemned carcasses of dead animals after removing blood, hair, hoofs, horns, manure, stomach contents and hide trimmings **(Miles and Jacob, 2011)**.

Meat and bone meal has been used as an ingredient in poultry diets since 1950 **(Patrick, 1953)**. In recent years, more attention has been paid to MBM supplementation in diets for poultry feed industry. Meat and bone meal is not a single feed ingredient, but an ingredient resulting from a variety of different products **(Miles and Jacob, 2011).** There is no fixed proportion among these components used by manufacturers; hence the resulting products are variable in chemical composition **(Liu, 2000).** However, meat and bone meal is rich in macro and microelements as well as in organic substances. In addition of being a protein source, it is also an excellent dietary source for vitamin B, calcium, phosphorus and available energy **(Waldroup and Adams, 1994; Sell, 1996; Sell and Jeffrey, 1996)**.

Meat and bone meal contains 6.0% moisture, 2111 kcal/kg metabolisable energy, 55.5% CP, 14.5% EE, 28 to 36% ash, 7 to 10% calcium and 4.5 to 6% phosphorus **(Dolz and De Blas 1992).** Digestibility of meat and bone meal protein ranges from

81.0 to 87.0% **(Parsons *et al*., 1997).** Lysine and cystine available in meat and bone meal has also better bioavailability **(Summers *et al*., 1964; Liu, 2000)**. Meat and bone meal can be used in poultry up to 5 to 10% in basal diet of the formulated ration **(Waldroup, 2002; Dolz and De Blas, 1992).** However, there are some limitations to use meat and bone meal in poultry diets due to variability in protein quality **(Wang *et al*., 1997; Parsons *et al*., 1997)**, lower feed conversion efficiency **(Drewyor, 2000; Caires *et al*., 2010; Liu, 2016)** and poor nitrogen utilization as compared to soybean meal **(Kim *et al*., 1993; Knaus *et al*., 1998).**

1.1 Justification of the study

Meat and bone meal is an important feedstuff in poultry nutrition because of high protein content and comparatively less cost than other animal protein sources. Thus, incorporation of the increased levels of meat and bone meal instead of other animal protein sources in poultry diets might reduce feed cost. Limited studies are available regarding optimum levels of meat and bone meal for high performing broiler in the contemporary environment of Bangladesh. Additionally, carcass quality and hematobiochemical parameter of broiler birds fed diet supplemented with varying levels of meat and bone meal as the sole source of animal protein are scarce.

1.2 Research questions

1.2.1 What is the effect of MBM on productive performance of broiler?
1.2.2 Which level of MBM is optimum to improve performance of broiler?
1.2.3 Does MBM have any effect on carcass characteristics of broiler?
1.2.4 What is the impact of MBM on blood parameters of broiler?

1.3 Scope of the study

Development of sustainable feeding strategy to use various levels of MBM for the improvement of production performance and carcass characteristics in commercial broiler. Studying the feasibility and effectiveness of using higher than traditional levels of MBM in diets for broiler chickens and to elucidate possible problems that might be associated with such practice in Bangladesh.

1.4 Specific objectives

1.4.1 To estimate the effects of different levels of MBM on feed intake, weight gain and FCR in commercial broiler.

1.4.2 To measure the effects of various levels of MBM on carcass characteristics in commercial broiler.

1.4.3 To quantify the hematological and biochemical effects of various levels of MBM in commercial broiler.

Chapter II: Review of Literature

2.1 Background

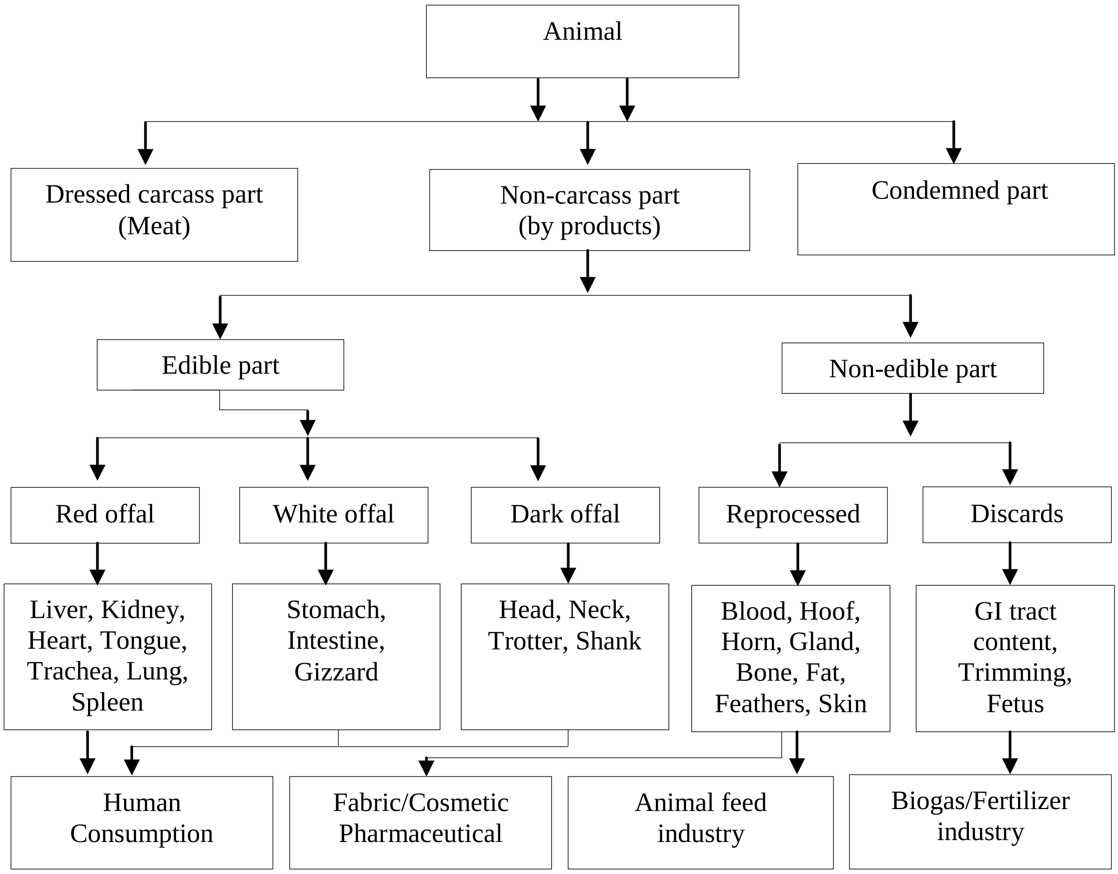
The chapter reviews background, classification, chemical composition, microbiology, rendering process and inclusion levels of meat and bone meals. The animals which are now the principal sources of meat were domesticated during early civilization **(Fernando, 1992).** The primary propose of farm animal production was to produce food for human consumption **(Liu, 2000).** This process of converting livestock to meat in abattoirs usually generates a lot of by-products which can be utilized further by humans as food or reprocessed as secondary by-products for both agricultural and industrial uses **(Liu, 2002).** Should these materials be of no intrinsic value, disposal would not only cause an increased burden to the environment but also represent an additional cost to the food industry **(Brooks, 1991).** The yield of these by-products has been reported to account 10% to 15% of the value of the live animal in developed countries, although, animal by-products account for about two-third of the animal after slaughter **(Irshad and Sharma, 2015)**. Therefore, adequate rendering of these residues into animal protein meal will contribute substantially to the human food industry and the environment as well as providing an alternative protein source to meet the demand of a fast growing animal industry **(Liu, 2000).**

Meat meaning "food, nourishment" is especially solid food as opposed to drink. As a noun, meaning of meat is the flesh of animals used as food **(Adedokun *et al*., 2005).** Generally, this means the skeletal muscle and associated fat and other tissues, but it may also describe other edible tissues such as offal. There is an increasing demand for quality in animal products especially broiler meat, as well as concerns about the effects of these products on human health. Therefore, animal production systems will have to focus not only on obtaining high production, but also on their impact on the environment as well as on human and animal health**.**

MBM has been used as an ingredient in broiler diets for almost 100 years **(Prange *et al*., 1927; 1928a; Kratzer and Davis, 1959; Skurray, 1974)**. It is an important alternative source of protein. Its production increases with annual increase in meat production in many parts of the world. Meat and bone meal is a type of animal protein feed obtained by rendering animal offal, bones, heads, hooves and soft tissues such as meat residues, organs, connective tissues as well as whole condemned carcasses. In general, animal hair and blood are not present in MBM. Meat and bone meal consists of residues from pork, beef and sheep, either as individual or a mixture of all different animal species depending on the raw materials available. Depending on the level of protein in the final product meat and bone meal can be divided into meat meal (protein content >55%) and meat and bone meal (protein content around 40-55%). The proportion of bone to soft tissues used in the manufacturing process in the finished products are known as low ash (<20%) or high ash (>20%) meal **(Liu, 2000).**

2.2 Classification of animal by-products

Animal by-products include all parts of a live animal that are not part of the dressed carcass such as liver, heart, rumen contents, kidney, blood, fats, spleen and meat trimmings. In this sense, the production of these animal by-products can be grouped into non-carcass meat and non-meat products.



Flow diagram 1. Manufacturing process of meat and bone meal

Edible parts are products that are approved by registered public health inspector and considered safe for human consumption after inspection in the abattoir. In contrast, inedible parts cannot be consumed by humans and are condemned as discards or re-processed and used as secondary by-products. Edible parts contain essential nutrients such as vitamins (B1, B2, B6, and folic acid), proteins, minerals and fat, with important poly-unsaturated fatty and amino acids which comparable to those in muscular tissue**.** On the other hand, inedible parts such as bones, hides and skin, feathers, hooves, horns, hair, bristles and rumen digesta can be transformed into useful and valuable products for human and livestock consumption (**Irshad and Sharma, 2015).** It is widely accepted that bone can be re-processed into livestock feed while hide/skin and feathers can be processed and utilized in the upholstery, leather and textile industry **(Elfaki *et al*., 2014).**

2.3 Utilization of inedible by-Products

Production of animal feed through recycling of animal waste to ease cost of feed has been in operation for over forty years **(Bhattacharya and Taylor, 1975).** Inedible parts as stated earlier represent discards that are unsuitable for human consumption and rejected as wastes or reprocessed into secondary products i.e., gelatine and keratin extraction, belts, footwear, and pharmaceuticals. Most inedible animal by-products consist of hides, hair, horns, teeth, blood, fats, bone, ligaments and cartilage, feet, manure, trimmings, rumen contents and glands. They can further be separated into parts such as elementary and secondary by-products. Elementary by-products are by-products which are described as being collected after slaughter and include blood, bones, pancreas, intestine, hides and skins, hoofs and horns. Besides, many cultures also consume blood as food or in combination with meat and other ingredients such as in blood sausage, black puddings and pancakes. In feed industry, blood is used in the production of blood meal for feeding livestock and pet **(Toldra *et al*., 2012; Bah *et al*., 2016).**

Meat and bone meal in animal diet remains a protein source because of its available essential amino acids, minerals and vitamins. On the other hand, the use of meat and bone meal became restricted in countries that experienced mad cow disease epidemics due to health concerns. Bone meal is a mixture of finely and coarsely ground animal bones and slaughter-house waste products. It is used as an organic fertilizer for plants and as a nutritional supplement for animals. Bone meal is primarily used as a source of phosphorus and protein. Finely ground bone meal may provide a quicker release of nutrients than the coarser ground version of bone meal**.** Bone meal, along with a variety of other meals, especially meat meal, is used as a dietary/mineral supplement for livestock. It is used to feed monogastric animals with bone meal from ruminants, and vice versa, to prevent the spread of bovine spongiform encephalopathy or "Mad cow disease". Proper heat control can reduce salmonella contaminants. Bone meal once was often used as a human dietary for calcium supplement.

2.4 Types of meat and bone meal

There are a number of different types of meat and bone meal. Differences in the types of raw materials incorporated with beef and pork by-products, together with differences in processing, method and conditions, result in variations in the nutrient profile of meat and bone meal. This variability in the nutrient profile of meat and bone meal can lead to unwanted variability in poultry performance **(Miles and Jacob, 2011).** High quality meat and bone meal is usually guaranteed to contain a minimum of 50% protein content. Lower quality meat and bone meals are available that contain a minimum of 45% protein content. Meat and bone meal is an excellent source of protein. In poultry diets, meat and bone meal is typically limited to less than 5% of the diet content because of the high calcium, phosphorus, and lysine content of the meal.

Table 1. Chemical composition of meat and bone meal[1]

Ingredient	Nutrients (%)							
	DM	**ME**	**CP**	**EE**	**CF**	**Ca**	**Met**	**Lys**
MBM 45%	92.0	1080.0	45.0	8.5	2.5	11.0	0.5	2.2
MBM 50%	93.0	1150.0	50.0	8.5	2.8	9.2	0.7	2.6
MBM 55%	93.0	1220.0	55.0	7.2	2.5	7.6	0.8	3.0

Source: Jacob, 2015; DM=Dry matter; ME=Metabolizable energy (kcal/lb); CP=Crude protein; EE = Crude fat, CF=Crude fiber, Ca=Calcium, Met=Methionine, Lys= Lysine

2.5 Chemical composition of meat and bone meal

Large amounts of MBM are produced in the World annually. **Johnston and Coon (1979)** showed that high quality meat and bone meal can be produced from rendered materials. However, because of the nature of the raw materials and processing methods, the quality of animal protein meals can vary markedly **(Wilder, 1973; Skurray, 1974; Johnston and Coon, 1979)**. Differences in the types of raw materials incorporated, together with differences in processing, method and conditions, result in variations in the nutrient profile of meat and bone meal. The variability in protein quality of meat and bone meal is one of the most important concerns, and often limitation, in its use in poultry and livestock rations. **Johnston and Coon (1979)** reported that reducing the pepsin concentration from 0.2 to 0.002% increased the accuracy of the pepsin N digestibility assay as a predictor of in vivo meat and bone meal protein quality.

Meat and bone meal contains 3.0-11.2% moisture, 49.0-52.8% crude protein, 8.5-14.8% crude fat, 6.0-12.0% calcium, 3.5-5.0% phosphorus, 2.2-3.0% lysine and 1770-2420 KCal/kg metabolizable energy for poultry **(Miles and Jacob, 2011).** Other amino acids are arginine (3.17-5.15%), histidine (0.48-1.85%), isoleucine (0.84-2.56%), leucine (1.82-5.21%), lysine (1.73-4.28%), methionine (0.44-1.54%), phenylalanine (1.07-3.22%), threonine (1.23-2.70%) and valine (1.31-3.62%) **(Hendriks *et al*., 2002).** Chemical properties of meat and bone meal vary a lot from different raw materials. On average, the pH tends to be acidic, about 6.5. Organic matter in content is about 50% (**Chen, 2008).**

2.6 Microbiology and palatability

The variability in the nutrient profile of meat and bone meal can lead to unexpected changes in poultry performance. Concerns about microbiological quality and palatability often limit the use of higher dietary quantities. As with most feed ingredients used by the animal industry, meat and bone meal is a relatively dry material which has been rendered and heat-sterilized. It is important to protect all feed ingredients from contamination. There is a misconception that only products of animal origin may be contaminated with micro-organisms such as Salmonella. This is not true. It is also important to routinely rotate inventory. Long-term storage of meat and bone meal and other animal by-product meals is not possible, primarily due to the possibility of oxidative spoilage. Meat and bone meal has a high fat content. With prolonged storage, this fat can become rancid. Palatability problems with meat and bone meal are usually associated with a high fat-rancid meal that has not been properly treated with an antioxidant. Today, most of the high quality meat and bone meal products are adequately treated with an anti-oxidant **(Miles and Jacob, 2011).**

2.7 Rendering process

Millions of tons of animal by-products are produced in the U.S. each year in the agricultural and animal industries. The rendering process allows for the recycling of animal slaughtering wastes that would otherwise be disposed of in landfills. The animal processing industry provides meat, eggs and milk to stores and thus to people around the world. But many of the by-products of this industry are not suitable for human consumption. The large amounts of waste from the animal agriculture industry could fill landfills to capacity and wreak havoc on the environment's delicate balance. Fortunately, though, these by-products can be recycled into meal and used in the production of animal feed to supply additional nutrients to livestock, poultry and even pets. Common rendering processes are dry (105-130 ^{0}C for batch type, 105-140 ^{o} C for continuous type) and wet (90-140 ^{o} C for batch type, 60-95 ^{0}C for continuous type).

Unmarketable animal tissues are typically processed by rendering plants, which transform them into meat and bone meal or similar products. Meat and Bone Meal's traditional use as animal feed has become increasingly threatened, but meat and bone meal has potential for non-feed applications (**Gracia *et al*., 2004).**

2.8 Uses of meat and bone meal

Bone meal is a mixture of finely and coarsely ground slaughterhouse waste products. The most common sources of these waste by-products are beef, pork, sheep and poultry. This mixture can be used as an organic fertilizer for plants or as a nutritional supplement for livestock and other animals. The use of bone meal in livestock feed can even prevent "Mad cow disease." Similarly, meat meal is an animal feed produced by recycling animal by-products. These by-products are cooked, or "rendered," to produce a nutritional and economical feed ingredient. When bones are added to meat meal, it becomes a product known as MBM. MBM is an excellent source of protein, calcium phosphorous, vitamin B-12 and numerous other minerals that are necessary to an animal's health.

In order for animal by-products to be used in other materials and processes, the by-products must first be treated. Bone meal, meat meal and blood meal are produced in a process known as rendering. In this process, the raw material is heated to remove moisture and release fat. The dry rendering process often begins with crushing and grinding the material, followed by heat treatment to reduce moisture content and eliminate any microorganisms. The melted fat is then separated from the solid protein through draining and pressing, and the solid material is ground into powder, such as meat meal, meat and bone meal, feather meal and blood meal.

The rendering process transforms waste by-products from the animal industry into stable, valuable and safe-to-use materials. The majority of the waste material that is processed in rendering comes from slaughterhouses and can include fatty tissue, bones and other processing offal. Offal is the parts of an animal that are not fit for human consumption, such as organs, blood and feathers. Almost 30 percent of an animal's live weight ends up as offal, which would be expensive to dispose of and wasted if not for the rendering process.

In the rendering process, the inedible parts of slaughtered livestock are transported to a rendering plant, where the material is ground, cooked and pressed into meat and bone meal. The different grades of meal are blended back into poultry and slime feeds, like fats from the process. There are 3,600 calories per pound of fat, making the material valuable for adding energy to the feed. A rotary dryer is often used in the process to remove moisture from the raw material. In addition to producing meat and bone meal, a rotary dryer can also be used to coagulate and dry blood for blood meal and to produce feather meal, which can be used as a feed ingredient.

Anywhere from one-third to one-half of each animal produced for meat, milk, eggs and fiber is not consumed by humans. About 49 percent of the live weight of cattle, 44 percent of the live weight of pigs, 37 percent of the live weight of broilers and 57 percent of the live weight of most fish species are materials that are not consumed by humans. The current volume of raw material generated each year is nearly 54 billion pounds. When these raw waste materials are subjected to the rendering process, the result is many valuable and useful products, from bone meal to poultry meal, which are then used as feed ingredients for cattle, poultry and pets.

The composition requirements for meat and bone meal and all other meals and animal feed ingredients used in the U.S. are established and regulated by the Association of American Feed Control Officials **(AAFCO)**. Meat and Bone Meal, for example, must contain a minimum of four percent phosphorous with a calcium level not to exceed 2.2 times the actual phosphorous level.

Millions of tons of animal by-products are produced in the U.S. each year in the agricultural and animal industries. If these waste products are not recycled or reused, they must be disposed of in landfills, causing huge economic losses for the animal processing industries, as well as issues in the environment. Meat and bone meal and blood meal are valuable products that can be sold for use in the pet food industry, feed industry and other industries. The fats from the rendering process can also be used in the pharmaceutical, chemical and oil industries, as well as many others.

The rendering process allows for the recycling of animal slaughtering wastes that would otherwise be disposed of in landfills. These recycled products can then be used in animal feed or as organic fertilizers. Recycling these waste materials is not only beneficial for the environment; it also prevents substantial loss of money for those in the animal agriculture industries. Making meal from raw waste materials is an economically beneficial process that produces valuable products for the agricultural industries.

2.9 Inclusion levels of meat and bone meal

The use of meat and bone meal in poultry diets is often restricted to less than 5%. The poultry is the predominant consumers of meat and bone meal because of its high calcium, available phosphorus, and lysine contents. Upper acceptable limit of meat and bone meal in the diet is about 10 percent **(Miles and Jacob, 2011).** Feeding higher levels in the diet will cause imbalances of calcium and phosphorus. In poultry diets, most nutritionists consider levels between 2-7 percent acceptable **(Miles and Jacob, 2011).**

2.10 Conclusion

Chemical composition of meat and bone meal is widely variable. Factors responsible for these variations are discussed. Despite variability, this is a potential source of protein, calcium, phosphorus and other trace minerals. Taking into consideration of the nature of variation, inclusion levels and its subsequent consequences on productive performance, carcass characteristics and hematobiochemical parameters in broilers could be explored as a noble study.

Chapter III: Materials and Methods

3.1 Study area

The experiment was conducted during May to June 2017 in the experimental farm and research laboratories of the Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh. May-June is considered as summer season in Bangladesh. In May, average temperature was 31.5^{o}C, average humidity was 82.0% and average precipitation was 184.8 mm. In June average temperature was 32.8^{o} C, humidity was 88.0% and average precipitation was 67.5 mm **(BMD, 2015; Weatherbase, 2013; BBC weather, 2013).**

3.2 Design of the experiment

The experimental birds were assigned to a Completely Randomized Design. A total of 100 birds were randomly distributed into five dietary treatment groups designated as $T_{0,}$ $T_{1,}$ $T_{2,}$ T_3 and T_4 and supplemented with 0%, 2%, 4%, 6% and 8% MBM for $T_{0,}$ $T_{1,}$ $T_{2,}$ T_3 and T_4 groups, respectively. Each treatment was further divided into two replicates having 10 birds per pen.

3.3 Animals and housing

One hundred Cobb 500 day old unsexed broiler chicks were purchased from Nahar Agro Complex Limited, Chittagong, Bangladesh. All chicks were examined for abnormalities and uniform size. Average body weight of the chicks was 48.74±0.26 g. The experimental shed was brick cemented with corrugated metal wiring. Floor space for each bird was 0.17 square feet in brooding box and 0.75 square feet in the cage. The cage was further divided into 20 pens. The pens were selected in an unbiased way for uniform distribution of chicks. The chicks were brooded in the wooden box. After 14 days, birds were transferred to the respective pens. Each pen was allocated for 10 birds. Dry and clean newspaper was placed in the brooding box and changed for every 6 hours. Room temperature and humidity was maintained using 200 watt incandescent lamps and ceiling fans. The birds were exposed to continuous lighting. During brooding period, chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Temperatures were measured by using thermometer.

3.4 Cleaning and sanitation

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

3.5 Experimental diets

Feed ingredients were purchased from Pahartali market, Chittagong, Bangladesh. During purchase, cleanliness and date of expiry were checked. Meat and bone meal was supplemented at 0%, 2%, 4%, 6% and 8% to prepare the experimental mash diets. Dry mash was provided to the birds throughout the whole experimental period. Five different types of rations were formulated. Each ration had two different types i.e., starter (0 to 14 days) and finisher (15 to 28 days). All rations were iso-caloric and iso-nitrogenous. The composition of different feed ingredients and nutritive value of starter and grower rations are given in Table 2 and 3.

Table 2. Ingredient and nutrient composition of the broiler starter ration (0-14 days)

Ingredients (%)	Dietary treatments				
	T_0	T_1	T_2	T_3	T_4
Maize	59.50	59.50	59.35	59.80	60.70
Rice polish	1.50	2.80	3.45	4.00	4.20
Soybean meal	33.20	30.50	28.00	25.50	22.70
Vegetable oil	2.25	2.00	1.87	1.75	1.42
MBM[1]	0.00	2.00	4.00	6.00	8.00
Molasses	0.30	0.40	0.90	0.60	1.20
Limestone	1.50	1.20	1.00	0.80	0.40
Vit-min. premix[2]	0.20	0.30	0.30	0.35	0.35
Common salt	0.25	0.25	0.25	0.25	0.25
DCP[3]	1.00	0.70	0.53	0.56	0.40
DL-Methionine[4]	0.25	0.27	0.28	0.28	0.28
L-Lysine[5]	0.02	0.03	0.02	0.05	0.05
Toxin binder[6]	0.03	0.05	0.05	0.06	0.05
Total	100	100	100	100	100
Estimated chemical composition (%)					
Met. energy[7]	2965.17	2965.11	2965.82	2965.15	2965.17
Crude protein	20.60	20.61	20.64	20.69	20.63
Crude fibre	4.95	4.95	4.98	5.03	4.82
Calcium	0.97	1.03	1.14	1.30	1.35
Phosphorus	0.71	0.78	0.85	0.97	1.04
Lysine	1.13	1.14	1.14	1.17	1.17
Methionine	0.54	0.56	0.57	0.57	0.57
Cysteine & Methionine	0.78	0.75	0.73	0.70	0.67
Tryptophan	0.26	0.24	0.24	0.30	0.22

T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8%; [1]MBM (52.0% CP, imported from Australia by Rahman and Brothers, Asadgonj, Chittagong, Bangladesh); [2]Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D_3 1000 IU, K 1.6 mg, B_1 1 mg, B_2 2 mg, B_3 16 mg, B_6 1.6 mg, B_9 320 µg, B_{12} 4.8 µg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 µg); DCP[3] (18% P, 23% Ca); [4]DL-Methionine (Purity 99.0%); [5]L-Lysine (Purity 99.0%); [6]Toxin Binder (Purity 98%, all imported from Poland); [7]Metabolizable energy (kcal/kg).

Table 3. Ingredient and nutrient composition of the broiler finisher ration (14-28 days)

Ingredients (%)	Dietary treatments				
	T_0	T_1	T_2	T_3	T_4
Maize	60.10	60.55	61.35	60.9	61.08
Rice polish	1.85	2.80	2.85	4.90	5.00
Soybean meal	31.00	28.40	25.80	23.00	20.50
Vegetable oil	3.50	3.20	2.98	2.70	2.60
MBM[1]	0.00	2.00	4.00	6.00	8.00
Molasses	0.30	0.30	0.60	0.60	1.20
Limestone	1.40	1.00	0.95	0.80	0.50
Vit-min. premix[2]	0.20	0.20	0.25	0.30	0.30
Common salt	0.25	0.25	0.25	0.25	0.25
DCP[3]	1.10	1.00	0.60	0.20	0.20
DL-Methionine[4]	0.25	0.25	0.29	0.29	0.29
L-Lysine[5]	0.02	0.02	0.03	0.02	0.02
Toxin binder[6]	0.03	0.03	0.05	0.04	0.06
Total	100	100	100	100	100
Estimated chemical composition (%)					
Met. Energy[7]	3057.89	3057.82	3057.25	3057.00	3057.57
Crude protein	19.71	19.76	19.76	19.78	19.78
Crude fibre	6.19	6.11	6.00	6.05	6.05
Calcium	0.95	1.00	1.12	1.20	1.32
Phosphorus	0.72	0.80	0.84	0.89	0.99
Lysine	1.07	1.08	1.09	1.08	1.08
Methionine	0.53	0.53	0.57	0.57	0.57
Cysteine & Methionine	0.74	0.72	0.70	0.67	0.64
Tryptophan	0.24	0.24	0.23	0.22	0.21

T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8%; [1]MBM (52.0% CP, imported from Australia by Rahman and Brothers, Asadgonj, Chittagong, Bangladesh); [2]Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D_3 1000 IU, K 1.6 mg, B_1 1 mg, B_2 2 mg, B_3 16 mg, B_6 1.6 mg, B_9 320 µg, B_{12} 4.8 µg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 µg); DCP[3] (18% P, 23% Ca); [4]DL-Methionine (Purity 99.0%); [5]L-Lysine (Purity 99.0%); [6]Toxin Binder (Purity 98%, all imported from Poland); [7]Metabolizable energy (kcal/kg).

3.6 Feeding of birds

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

3.7 Medications

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

3.8 Carcass measurement

On 4th week of the study period, four birds were randomly selected from each replicate and killed by severing the jugular vein and carotid artery. Once a bird was adequately bleed out, it was scalded and feather was removed. After defeathering, the birds were eviscerated and the head and feet were removed as per technique described by **Jones (1984)**. During evisceration process, abdominal fat, lung, liver, kidney, spleen, gizzard and proventriculus were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.

3.9 Analysis of feed and meat

From each treatment, 100 g of prepared mash feed was taken and preserved in an air tight bag to carry them in the laboratory for analysis during the experimental period. After slaughter, 120 g of meat was collected in the air tight bag from each carcass for estimation of the chemical composition of meat. Feed and meat samples were dried at 80°C and ground to powder. After drying, chemical analyses of the feed and meat samples were carried out in triplicate for dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash (TA) in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong as per **AOAC (2006).**

3.10 Hematological analysis

Blood samples were collected from the brachial vein of four birds from each group (Two birds from each replicate) using a 3 ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant, ethylene diamine tetra acetic acid. The total red blood cell counts were performed in a 1:200 dilution of blood in Hayem's solution. The differential leukocyte counts were determined by preparation of blood smears stained with Wright's stain. The hemoglobin concentration was estimated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobinometer. Packed cell volume was measured after centrifugation of a small amount of blood using micro-hematocrit capillary tubes.

3.11 Serum analysis

Blood was collected without anticoagulant from a total of four birds from each group at 21^{st} and 28^{th} days of age. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the ependroff tube by micropipette. Sera samples were marked and stored in -20°C until being analyzed for glucose, total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) by Humalyzer 3000 (Semi-automatic, microprocessor-controlled photometer with large graphic LCD screen, Wisbaden, Germany). Randox® veterinary reagent kits were used for determination of the blood parameter of interest. Serum sample was mixed with the respective reagents in an ependroff tube. The serum with reagent was aspired by spectrophotometric method which measured the target parameter and immediately the printed result was recorded.

3.12 Data collection

Weight gain, feed intake and FCR were recorded at weekly intervals. Carcass characteristics, hematological and biochemical parameters were recorded at 3^{rd} and 4^{th} weeks. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total amounts of feed supplied to the birds. FCR was calculated dividing feed intake by the weight gain.

3.13 Statistical analysis

Data were compiled in MS Excel. Raw data related to weight gain, feed intake, FCR, carcass characteristics, hematological and biochemical parameters were tested for normality by using normal probability plot and analyzed for ANOVA by using **Stata (2017).** Means showing significant differences were compared by Duncan's New Multiple Range Test **(Duncan, 1955).** Statistical significance was accepted at $p<0.05$ for F-tests.

Chapter IV: Results

The experiment was carried out to investigate the effects of various levels of meat and bone meal on the performance parameters, carcass characteristics and hematobiochemical parameters of Cobb-500 broilers. The results obtained from the present study have been presented in this chapter.

4.1 Live weight

Results indicated that, weekly average live weight differed significantly ($p<0.05$) among different dietary treatment groups (Table 4). Highest (1062.0 g/bird) and lowest (758.3 g/bird) average live weights were recorded in T_4 and T_0 groups, respectively at 4th week.

Table 4. Live weight (g/bird), weight gain (g/bird/d), feed intake (g/bird/d) and FCR of the experimental broiler birds fed diets supplemented with different levels of MBM from 1st to 4th weeks of age

Variable	Age	Dietary treatments					SEM	Sig.
		T_0	T_1	T_2	T_3	T_4		
Live weight	1st wk	154.7	187.9	215.4	218.7	212.3	8.06	**
	2nd wk	322.4	351.4	415.4	429.8	394.2	13.39	**
	3rd wk	547.5	643.1	693.6	717.2	701.9	20.61	**
	4th wk	758.3	930.5	948.2	969.0	1062	32.90	**
Weight gain	1st wk	15.7	20.0	24.3	24.3	23.6	1.12	**
	2nd wk	24.3	23.6	28.6	30.0	26.4	0.82	***
	3rd wk	32.9	41.4	40.0	41.4	43.6	1.25	***
	4th wk	30.7	41.4	37.9	35.7	52.2	2.40	**
Feed intake	1st wk	18.6	24.3	27.1	27.9	27.9	1.19	**
	2nd wk	33.6	35.8	40.0	41.4	37.9	1.15	**
	3rd wk	55.0	63.6	65.0	65.7	67.1	1.14	**
	4th wk	56.4	74.3	67.8	65.0	82.2	2.89	**

Variable	Age	Dietary treatments					SEM	Sig.
		T_0	T_1	T_2	T_3	T_4		
FCR	1st wk	1.18	1.01	1.12	1.15	1.18	0.02	***
	2nd wk	1.38	1.35	1.40	1.38	1.44	0.01	NS
	3rd wk	1.68	1.54	1.63	1.59	1.55	0.02	NS
	4th wk	1.83	1.79	1.80	1.82	1.56	0.03	**
	0-4 wk	1.51	1.44	1.48	1.48	1.43	0.02	***

T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8% MBM; SEM=Standard error of mean; NS=Non-significant (P>0.05); **=Significant (p<0.01); ***=Significant (p<0.001)

4.2 Weight gain

Average weekly weight gain differed significantly ($p<0.05$) among different dietary treatment groups (Table 4). Maximum (52.2 g/d) and minimum (30.7 g/d) average weight gains were recorded in T_4 and T_0 groups, respectively at 4th week.

4.3 Feed intake

Similar to weight gain, average weekly feed intake differed significantly ($p<0.05$) among various dietary treatment groups (Table 4). Highest (82.2 g/bird/d) and lowest (56.4 g/bird/d) average feed intakes were recorded in T_4 and T_0 groups, respectively at 4th week.

4.4 Feed Conversion Ratio

FCR did not differ ($p>0.05$) within experimental birds at 2nd and 3rd weeks irrespective of the levels of MBM supplementations (Table 4). However, the difference was significant ($p<0.05$) at 1st and 4th weeks. The best (1.56) and worst (1.81) FCR was recorded in the T_4 and T_0 groups, respectively at 4th week.

4.5 Blood parameters

Blood parameters remained within normal ranges irrespective of the levels of meat and bone meal supplementation throughout the whole experimental periods (Table 5).

Table 5. Blood parameters of the experimental broiler birds fed diets supplemented with different levels of meat and bone meal at 3rd and 4th weeks of age

Parameters (%)	Week	Dietary treatments					SEM	Sig.
		T_0	T_1	T_2	T_3	T_4		
Lymphocyte	3rd	91.9	92.0	91.9	93.7	93.7	0.51	NS
	4th	89.5	91.5	90.1	91.0	89.5	0.52	NS
Heterophil	3rd	1.3	1.4	1.5	0.97	1.5	0.09	NS
	4th	1.6	1.8	1.8	1.7	1.4	0.19	NS
H/L ratio	3rd	0.015	0.015	0.015	0.010	0.015	0.002	NS
	4th	0.015	0.025	0.020	0.020	0.010	0.005	NS
Eosinophil	3rd	1.3	2.1	1.5	1.5	1.5	0.19	NS
	4th	1.6	2.6	2.1	1.7	1.9	0.22	NS
Monocyte	3rd	3.4	2.1	2.5	2.0	2.3	0.35	NS
	4th	3.7	2.9	3.9	3.4	3.8	0.33	NS
Basophil	3rd	1.9	2.7	2.5	1.9	1.0	0.21	NS
	4th	1.6	3.2	2.1	2.2	3.4	0.29	NS
Hemoglobin	3rd	4.3	4.3	5.2	4.9	4.9	0.16	NS
	4th	5.5	4.5	5.2	6.1	5.1	0.23	NS
PCV	3rd	31.5	33.0	32.5	33.5	31.5	1.23	NS
	4th	25.0	32.5	32.0	35.5	30.5	1.52	NS
ESR	3rd	1.3	1.8	1.5	1.5	1.8	0.17	NS
	4th	3.0	2.0	2.3	2.0	2.5	0.22	NS
TEC	3rd	3.4	3.8	4.3	2.3	4.8	0.33	NS
	4th	2.6	2.8	4.2	4.5	4.6	0.45	NS

T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8% MBM; SEM=Standard error of mean; NS=Non-Significant (P>0.05)

4.6 Serum parameters

Serum parameters exhibited normal ranges among different treatment groups (p>0.05) except for total protein which differed significantly (p<0.01) at 4th week. Maximum (3.9) and minimum (2.7) average values were recorded in T_3 and T_0 groups, respectively at 4th week.

Table 6. Serum parameters of the experimental broiler birds fed diets supplemented with different levels of meat and bone meal at 3rd and 4th weeks of age

Parameter	Dietary treatments						SEM	Sig.
	Age	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**		
Glucose (g/dl)	3rd	0.2	0.2	0.3	0.2	0.2	25.68	NS
	4th	0.2	0.2	0.1	0.2	0.2	17.15	NS
Total protein (g/dl)	3rd	2.6	2.8	2.9	2.6	2.7	0.25	NS
	4th	2.7	3.0	2.4	3.9	3.1	0.18	**
Albumin (g/dl)	3rd	3.5	2.8	3.9	2.8	3.3	0.19	NS
	4th	3.4	3.2	4.0	3.6	3.2	0.14	NS
Cholesterol (g/dl)	3rd	0.1	0.1	0.1	0.1	0.1	6.93	NS
	4th	0.9	1.2	1.1	1.3	1.1	6.12	NS
SGPT (U/L)	3rd	11.8	52.3	25.4	50.8	11.9	10.17	NS
	4th	36.7	44.5	39.6	13.4	11.9	6.57	NS
SGOT (U/L)	3rd	130.4	198.9	122.8	121.9	64.8	16.28	NS
	4th	109.6	106.7	118.9	99.8	96.5	9.93	NS

T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8% MBM; SEM=Standard error of mean; NS=Non-significant (P>0.05); **=Significant (P<0.01)

4.7 Carcass characteristics

The carcass parameters differed (p<0.05) in terms of drumstick weight, neck weight and breast weight at 4th week (Table 7). Breast weight increased markedly (p<0.001) at 4th week due to increasing levels of meat and bone meal supplementation. However, other carcass parameters remained unchanged (p>0.05) throughout the entire experimental periods.

Table 7. Carcass characteristics (%) of the experimental broiler birds fed diets supplemented with different levels of meat and bone meal at 4th week of age

Parameters (%)	Dietary treatments					SEM	Sig.
	T_0	T_1	T_2	T_3	T_4		
Dressed weight	63.9	65.6	63.0	66.2	64.9	0.59	NS
Drumstick weight	7.5	7.6	8.9	8.9	8.1	0.22	*
Thigh weight	10.1	10.0	10.1	10.2	10.0	0.06	NS
Breast weight	17.4	17.7	21.1	20.9	20.9	0.57	***
Neck weight	4.5	4.7	4.5	4.6	4.6	0.03	*
Back weight	10.5	11.3	12.3	13.5	13.2	0.47	NS
Wing weight	4.6	4.8	4.8	4.8	4.6	0.05	NS
Feet weight	5.3	5.1	5.6	5.5	5.1	0.08	NS
Liver weight	4.0	3.7	3.7	4.2	3.7	0.07	NS
Heart weight	0.7	0.7	0.8	0.8	0.7	0.03	NS
Ab. fat weight	0.3	0.4	0.4	0.4	0.4	0.02	NS
Neck fat weight	0.3	0.2	0.3	0.4	0.3	0.02	NS
Gizzard weight	2.7	2.7	2.8	2.8	2.7	0.02	NS
Proven. weight	0.7	0.8	0.8	0.8	0.7	0.02	NS

Ab. =Abdominal; Proven. =Proventriculus; T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6% MBM; T_4=Diet containing 8% MBM; SEM=Standard Error of Mean; NS=Non-Significant (P>0.05); *=Significant (P<0.05); ***=Significant (p<0.001)

4.8 Chemical composition of meat

The chemical composition of meat significantly differed (p<0.05) in terms of EE content in MBM supplemented groups (Table 8). There were no marked (p>0.05) changes in the chemical composition of meat in terms of DM, CP and TA contents.

Table 8. Chemical composition of meat of the experimental broiler birds fed diets supplemented with different levels of meat and bone meal

Parameters (%)	**Dietary treatments**					**SEM**	**Sig.**
	T_0	**T_1**	**T_2**	**T_3**	**T_4**		
DM	26.7	25.1	26.9	25.3	24.2	0.52	NS
CP	67.9	66.7	67.6	69.2	69.6	0.53	NS
EE	18.6	11.9	23.2	24.4	18.4	1.57	*
TA	4.5	4.6	4.3	4.5	4.3	0.06	NS

DM=Dry Matter; CP=Crude Protein; EE=Ether Extract; T_0=Diet without MBM; T_1=Diet containing 2% MBM; T_2=Diet containing 4% MBM; T_3=Diet containing 6%; MBM; T_4=Diet containing 8% MBM; SEM=Standard error of mean; NS=Non-significant (P>0.05); *=Significant (P<0.05)

Chapter V: Discussion

The study investigated the effects of meat and bone meal supplementation below and above recommended levels to investigate its effects on productive performance, carcass characteristics and hematobiochemical parameters in commercial broiler for a typical period of 28 days.

5.1 Weight gain

Supplementation of meat and bone meal from 1st to 4th weeks of age in commercial broiler birds indicated that, weight gain substantially improved in treatment groups compared to control. The result is closely consistent with previous studies where, increasing levels of dietary protein had significant positive effects on body weight gain in broilers**.** In present study, highest weight gain was recorded in 8% MBM supplemented group which is aligned with other studies **(Liu, 2000; Drewyor and Waldroup, 2000; Wang and Parsons, 1998; Karakas *et al*., 2001).** Increased weight gain in intervention groups achieved in earlier studies could have been due to potential effect of MBM to improve the digestibility of other nutrients of the ration **(Liu, 2000).**

In compliance with present study, formulation of diet with 10% MBM had better performance in terms of weight gain **(Wang and Parsons, 1998)**. In another study, 5.0% MBM supplemented diet showed consistent results compared to diets formulated with blood meal in same the ratio (**Caires, 2010)**. However, diets formulated with 4.0% MBM, 3.0% poultry offal meal and vegetable proteins did not exhibit better performance in 21-day-old broiler birds **(Bellaver *et al*., 2005)**. Additionally, diets containing MBM, poultry offal meal or a combination of MBM and bone meal, feather meal, and poultry offal meals presented better weight gain in broiler as compared with simply soybean meal based diet. Increased weight gain in MBM supplemented group had much higher protein digestibility and less anti-nutritional substances as compared to soybean meal (**Beski *et al*., 2015).**

The results obtained in the present study, however, were inconsistent in few cases where addition of 6% MBM in broiler diets exhibited minimum or no positive changes on growth performance in broiler birds **(Faria Filho *et al*., 2002)**. Similar results were obtained from other studies **(Sartorelli, 1998; Junqueira *et al*., 2000)** where addition of MBM did not exhibit marked changes in weight gain of broilers in treatment groups compared to control. In another study addition of 6% MBM in broiler diets exhibited minimum or no positive changes on growth performance in broiler birds **(Faria Filho *et al*., 2002)**. Similar results were obtained from previous studies **(Sartorelli, 1998; Junqueira *et al*., 2000)** where addition of MBM did not exhibit marked changes in weight gain of broilers in intervention groups compared to control. The reasoning for this failure of MBM to accelerate target gain was not clearly elucidated. However, the most probable reason could be the differences in the production processes and origins of the indigestible residues available in the MBM (**Cruz *et al*., 2009)**.

5.2 Feed intake

In present study, gradually increasing levels of MBM had remarkable positive effects on feed intake in commercial broiler. It was evident that, inclusion of 8.0% MBM increased ($p<0.05$) feed intake in treatment groups compared to control at 4th week. Birds consumed relatively more feed during finisher phase despite reduced total feed intake (**Karakas *et al*., 2001; Liu, 2000; Faria Filho *et al*., 2002).** The physiological state of the birds and other sources of variation in MBM may interfere feed intake **(Pesti and Edwards, 1983).** In contrast to the present study, feed intake in MBM supplemented group was inferior in a corn-soybean based diet **(Liu, 2000).** Similarly, 6% MBM supplemented diet reduced feed intake (**Faria Filho *et al*., 2002)**. Decreased feed intake could have been due to high ambient temperature **(Ojano-Dirain and Waldroup, 2002)**. The reduced feed intake in broiler due to environmental stress has been elucidated. Interestingly, feed intake was not influenced ($p>0.05$) by the sources of dietary protein while the diets were iso-caloric with minimum energy levels **(Mbajiorgu *et al*., 2011).**

5.3 Feed conversion ratio

It was speculated that, FCR at different ages of broilers fed diets supplemented with MBM markedly improved during 1st to 4th weeks of age. These results are in compliance with previous studies **(Nworgu *et al*., 2001)** where supplementation of broiler diets with MBM improved FCR in treatment groups compared to control. Similarly, pronounced impact on FCR was reported in diets supplemented with 120 g/kg MBM compared to 60 g/kg in commercial broilers (**Liu *et al*., 2017).** It was reported that, the effects of feeding diets at 21 days containing various levels of phosphorus from low-ash or high-ash MBM did not impair feed utilization in broilers (**Drewyor and Waldroup, 1998)**.

5.4 Haematological changes

Despite substantial increment of meat and bone meal from 0 to 8%, no statistical variations in blood parameters among different treatment groups were evidenced. However, feeding supplemental meat and bone meal resulted in increase of eosinophil in the supplemented groups compared to control. In general, increased levels of eosinophil percentage indicate internal parasitic problem present in bird. In present study, it was not detected due to short period of study.

5.5 Biochemical changes

In this study, total protein and glucose level were in normal range at 3rd and 4th weeks of age. Cholesterol level was lower in 3rd week than in 4th week. In younger age, cholesterol level remained low due to higher demand of energy caused for body development **(Almeida *et al*., 2006**). Albumin does not vary with age which is similar to present study. In fact, life is the continuation of a series of complex biochemical reactions supported by enzymes. Therefore, changes in enzyme activities are considered as an indication of health**.** In present study, despite various levels of supplemental MBM, all biochemical parameters remained unchanged ($p>0.05$) except total protein ($p<0.05$). Normally, total protein value remained high in 28 days than 35 days. In contrast to present study, feeding MBM resulted an increase in cholesterol levels in turkey **(Slepickova *et al*., 2008).**

Liver is the main organ for controlling metabolism in entire body. Of all the enzymes, SGPT and SGOT are the most specific types of enzymes of the liver which increase in the plasma due to destruction of cell membrane and cell necrosis in acute liver disease and also due to accumulation of toxic substances in liver **(Meyer and Harvey, 1998).** In present study, SGOT and SGPT remained normal in MBM supplemented groups. Liver transaminases, SGOT and SGPT are essential in protein biosynthesis and normal range in their concentration reflects better liver function and normal health.

5.6 Carcass characteristics

Increasing levels of MBM supplementation substantially improved carcass quality in terms dressed weight, breast weight, drumstick weight and neck weight of birds. These results are consistent with previous study (**Caires, 2010)** where MBM supplementation substantially increased thigh and drumstick weight in treatment group compared to control. On the other hand, increasing dietary energy can cause the deposition of excess abdominal or carcass fat in broilers **(Min *et al.*, 2007)** which was not evident in this study. However, contrasting results were reported in other studies where all carcass parameters were not influenced ($p>0.05$) by the use of MBM **(Junqueira *et al*., 1992; Faria Filho *et al*., 2002; Caires., 2010).**

5.7 Chemical composition of meat

In this study, supplementation of MBM had no effects on the chemical composition of broiler meat in terms of DM, CP and TA except for EE ($P<0.05$). These results are consistent with another study **(Adela *et al* ., 2013)** where increasing dietary protein contents in isocaloric diets increased protein content and decreased fat percent in broiler carcass. It indicated that, increased carcass protein and decreased fat resulted due to elevated dietary protein and decreased dietary energy.

5.8 Limitations of the study

5.8.1 The sample size was only 100 birds due to resource limitations.

5.8.2 Seasonal variations were not observed due to limited study period.

Chapter VI: Conclusion

The study investigated the effects of meat and bone meal supplementation on performance parameters, carcass characteristics and blood parameters in commercial broiler under intensive rearing system. It was evident that, there was a positive relationship between gradually programme in meat and bone meal supplementation and performance of commercial broiler without notable changes in blood parameters. Highest weight gain, optimum feed intake and best FCR were observed in birds fed diet containing 8% meat and bone meal supplement. There were no unusual changes in the blood and serum parameters in comparison to the reference level. Similar to performance parameter, carcass characteristics were improved in terms of breast muscles yield in meat and bone meal supplemented group. The study, therefore, suggests that, meat and bone meal is a potential feed supplement with basal diet at an inclusion level of 8%. However, a long term investigation with larger sample size and multi-dimensional temporal pattern is suggested for increasing sensitivity and validity of the study under field condition.

Chapter VII: Recommendations and Future Direction

Meat and bone meal is comparatively cheaper than other animal protein sources. It is readily available product having significant positive effect in terms of weight gain, FCR and carcass characteristics without notable pathological changes in blood parameters of broiler birds. Therefore, meat and bone meal could be an important and economical solution for high performing broiler production in tropical environment of Bangladesh.

Inclusion of 8.0% meat and bone meal is recommended in regular broiler diet for better growth, optimum FCR and desirable carcass characteristics. Further investigations are required to determine the bioavailability of the organic phosphorus and calcium in MBM. However, the long term effect of meat and bone meal supplementation on productive performance of broilers should be investigated in future for validation of the study for human health.

Due to financial constraints and technical limitations, some vital blood parameters specially High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), White blood cell count (WBC), calcium, phosphorus and other trace minerals both in meat and feed were not analyzed. These parameters could have vital impact on human health. The study explores new horizon for investigating those parameters with larger sample size and variable temporal pattern as future study.

References

AAFCO (The Association of American Feed Control Officials). 2000. Official Publication of the Association of American Feed Control Officials. Atlanta, Washington, DC.

Adedokun SA, Adeola O. 2005. Metabolizable energy value of meat and bone meal for pigs. Animal Science. 83(11): 2519-2526.

Adedokun SA, Adeola O. 2005. Metabolizable energy value of meat and bone meal for ducks. Poultry Science. 84: 1539-1546.

Adela M, Vacaru-Opris I, Dumitrescu G, Marcu A, Ciochina LP, Nicula M, Dronca D, Kelciov B. 2013. Effects of diets with different energy and protein levels on breast muscle characteristics of broiler chickens. Animal Science and Biotechnology. 46: 333-340.

Almeida JG, Vieira SL, Gallo BB, Conde ORA, Olmos AR. 2006. Period of incubation and posthatching holding time influence on broiler performance. Brazilian Journal of Poultry Science. 8: 153-158.

Anderson JO, Warnick RE. 1971. Relative value of three animal protein supplements in chick rations. Poultry Science. 50: 1545.

AOAC (Association of Official Analytical Chemists International). 2005. Official Methods of Analysis, 18th edition. AOAC International, Washington, DC.

Banerjee GC. 1992. Poultry (3rd edition). Oxford and IBH publishing Company Private Limited. New Delhi.

Batterham ES, Darnell RE, Herbert LS, Major EJ. 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal determined by slope-ratio assay with growing pigs, rats and chicks and by chemical techniques. British Journal of Nutrition. 55(2): 441-453.

Bellaver C, Costa CAF, Avila VS, Fraha M, Lima GJMM, Hackenhar L, Baldi P. 2005. Substitution of animal by-product meal by vegetable ingredients in diets for broilers. Scielo. 35: 671-677.

Bellaver C, Costa CAF, Avila VS, Fraha M, Lima GJMM, Hackenhar L, Baldi P. 2005. Replacement of flour animal origin by ingredients of plant origin in diets for broilers. Scielo. 35(3): 671- 677.

Beski SSM, Swick RA, Iji PA. 2015. Specialized protein products in broiler chicken nutrition: A review. Animal Nutrition. 1: 47-53.

Bhattacharya AN, Taylor JC. 1975. Recycling Animal Waste as a Feedstuff. Animal Science. 41: 1438-1457.

BMD (Bangladesh Meteorological Department). 2015. Weather Forecast. Ministry of Defense of the Government of Bangladesh.

Brooks P. 1991. Meat and bone meal: The underutilized raw material. Feedstuffs. pp. 13-15.

Burnham FA. 1996. The rendering industry: A historical perspective. In The Original Recyclers, National Renderers Association, Alexandria.

Caires CM, Fernandes EA, Fagundes NS, Carvalho AP, Maciel MP, Oliveira BR. 2010. The use of animal byproducts in broiler feeds. Use of animal co-products in broilers diets. Revista brasileira de ciencia avicola. Brazil. Scientific Electronic Library Online. 12(1).

Chen L, Helenius J, Kangus A. 2009. Nordic Baltic Fusarium Seminar 422: Meat bone meal as nitrogen and phosphorus fertilizer. Nordic Association of Agricultural Scientists. 5(2): 26.

Cruz VC, Ducatti C, Araujo PC, Sartori JR, Piccinin A. 2009. Effect of poultry viscera meal inclusion in broiler diets in different rearing periods on performance, carcass and parts yields. Brazilian Journal of Poultry Science. 11: 161-165.

Dale N. 1997. Metabolizable Energy of Meat and Bone Meal. Applied Poultry Research. 6(2): 169-173. Available from: http://doi.org/10.1093/japr/6.2.169

Dolz S, De Blas C. 1992. Metabolizable Energy of Meat and Bone Meal from Spanish Reendering Plants at Influceced by Level of Substitution and Method of Determination. Poultry Science. 71: 316-322.

Drewyor ME, Waldroup PW. 1998. Utilization of high levels of meat and bone meal in broiler diets. Poultry Science. 77(1): 30.

Duncan's DB. 1955. Multiple Range and Multiple F Test. Biometrics. 11: 1-42.

Elfaki MOA, Abdelatti KA, Malik HEE. 2014. Effect of Dietary Dried Rumen Content on Broiler Performance, Plasma Constituents and Carcass Characteristics. Global Journal of Animal Scientific Research. 3(1): 264-270.

Faria Filho DE, Faria DE, Junqueira OM, Rizzo MF, Araujo LF, Araujo CSS. 2002. Evaluation of Meat and Bone Meal in Broiler Chickens Feeding. Revista Brasileira de Ciencia Avicola. 4(1): 01-09.

Fernando T. 1992. Blood meal, meat and bone meal and tallow in inedible meat by-products. Essex, England: Elsevier Science. pp. 81-112.

Gracia RA, Rosentrater KA, Flores RA. 2006. Characteristics of North American Meat and Bone Meal Relevant to the Development of Non-Feed Applications. Applied Engineering in Agriculture. 22(5): 729-736.

Hendriks WH, Butts CA, Thomas DV, James KAC, Morel PCA, Verstegen MWA. 2002. Asian-Australian Journal of Animal Sciences. 15(10): 1507-1516. Available from: https://doi.org/10.5713/ajas.2002.1507

Irshad A, Sharma BD. 2015. Abattoir By-Product Utilization for Sustainable Meat Industry: A Review. Journal of Animal Production Advances. 5(6): 681-696.

Jacob J. 2015. Feeding Meat and Bone Meal to Poultry, Small and Backyard Flocks. University of Kentuchy.

Johnston J, Coon CN. 1979. A comparison of six protein quality assays using commercially available protein meals. Poultry Science. 58: 919-927.

Jones R. 1984. A standard method of dissection of poultry for carcass analysis. Technical Bulletin. 222. West of Scotland Agricultural College, Scotland.

Junqueira OM, Knoop R, Sakomura NK, Faria DE. 1992. Meat and bone meal and dicalcium phosphate as a source of phosphorus for broilers. Zootechnical Science. 7: 12-14.

Karakas P, Versteegh HAJ, Honing YV, Kogut J, Jongbloed AW. 2001. Nutritive Value of the Meat and Bone Meals from Cattle or Pigs in Broiler Diets. Poultry Science. 80(8): 1180-1189.

Kim EJ, Utterback PL, Parsons CM. 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among three different bioassays. Poultry Science. 91(6): 1350-1355.

Knaus WF, Beermann DH, Robinson TF, Fox DG, Finnerty KD. 1998. Effects of a dietary mixture of meat and bone meal, feather meal, blood meal, and fish meal on nitrogen utilization in finishing Holstein steers. Journal of Animal Science. 76(5): 1481-1487.

Kratzer FH, Davis PN. 1959. The feeding value of meat and bone meal protein. Poultry Science. 38(6): 1389-1393.

Liu DC. 2002. Better Utilization of By-Products from the Meat Industry. Food and Fertilizer Technology Center, Taipei, Taiwan.

Liu M. 2000. Nutritional Evaluation of High Ash Meat and Bone Meal for Poultry. MS Thesis, University of Manitoba, Canada.

Liu SY, Cowieson Aj, Selle PH. 2016. The influence of meat-and-bone meal and exogenous phytase on growth performance, bone mineralisation and digestibility coefficients of protein (N), amino acids and starch in broiler chickens. Animal Nutrition. 2(2): 86-92.

Mbajiorgu CA, Ng JW, Ambi, Norris D. 2011. Effect of varying dietary energy to protein ratio level on growth and productivity of indigenous Venda chickens. Asian Journal of Animal and Veterinary Advances. 6: 344-352.

Meyer DJ, Harvey JW. 1998. Veterinary Laboratory Medicine. Interpretation and Diagnosis. 2nd ed., W. B. Saunders Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. pp. 157-187.

Miles RD, Jacob JP. 1998. Using Meat and Bone Meal in Poultry Diets. Institute of Food and Agricultural Sciences, University of Florida. Reviewed March 2011. Available from: http://edis.ifas.ufl.edu.

Min YN, Hou SS, Gao YP, Huang W, Liu FZ. 2007. Effect of Dietary Crude Protein and Energy on Gosling Growth Performance and Carcass Trait. Poultry Science. 86: 661-664.

NRC. 1994. Nutrient Requirements of Poultry. 9th review and edition. National Academy Press, Washington, DC.

Nworgu FC, Egbunike GN, Ogundota FI, Salako RA and Fakeye OE. 2001. Effects of different dietary protein levels on the performance and carcass characteristics of broilers. ASSET. 1(2): 75-87

Ojano-Dirain CP, Waldroup PW. 2002. Protein and amino acid needs of broilers in warm weather: A review. International Journal of Poultry Science. 1(4): 40-46.

Parsons CM, Castanon F, Han Y. 1997. Protein and amino acid quality of meat and bone meal. 76(2): 361-368.

Partrick H. 1953. Supplements for a meat-scraps type chick ration. Poultry Science. 32: 570-572.

Pesti GM, Edwards HM, Jr. 1983. Metabolizable energy nomenclature for poultry feedstuffs. Poultry Science. 62: 1275-1280.

Prange RW, Carrick CW, Hauge SM. 1927. The Study of the Protein in a Commercial Meat Products. Poultry Science. 6: 302.

Prange RW, Carrick CW, Hauge SM. 1928. Growth Values of Proteins from Commercial Animal Products 1. Commercial Meat and Bone Scraps. Poultry Science. 7(3): 95-105. Available from: http://doi.org/10.3382/ps.0070095

Prange RW, Carrick CW, Hauge SM. 1928. Growth Values of Proteins from Commercial Animal Products III. A Commercial Meat Meal. Poultry Science. 7(5): 233-238. Available from: http://doi.org/10.3382/ps.0070233

Sartorelli SA, Bertechini AG, Fassani EJ, Kato RK, Fialho ET. 2003. Nutritional and microbiological evaluation of meat and bone meal produced in the state of Minas Gerais. Revista Brasileira de Ciencia Avicola. 5(1): 51-60.

Sell JL, Jeffrey MJ. 1996. Availability for poults of phosphorus from meat and bone meals of different particle sizes. Poultry Science. 75(2): 232-239.

Sell JL. 1996. Influence of dietary concentration and source of MBM on performance of turkeys. Poultry Science. 75(9): 1076-1079.

Skurray GR. 1974. The nutritional evaluation of meat meals for poultry. World's Poultry Science Journal. 30: 129-136.

Slepickova M, Vorlova L. 2008. Effect of Meat and Bone Meal Substitutes in Feed Mixes on Quality Indicatorsof Turkey Breast Meat. Acta Veterinaria Brno. 77: 297-304.

Summers JD, Slinger SJ, Ashton GC. 1964. Evaluation of meat meal as a protein supplement for the chick. Canadian Journal of Animal Science. 44: 228-234.

Stata 2017. Stata/SE 14.1, StataCorpLP, 4905 Lakeway Drive, College Station, TX77845, USA.

Swan JE. 1992. Animal by-product processing. Encyclopedia of Food Science and Technology, Wiley, New York. pp. 42-49.

Toldra F, Aristoy MC, Mora L, Reig M. 2012. Innovations in Value Addition of Edible Meat By-Products. Meat Science. 92: 290-296.

Waldroup P. 2002. The future of poultry nutrition. Poultry International. 41(7): 12-19.

Waldroup PW, Adams MH. 1994. Evaluation of the phosphorus provided by animal proteins in the diet of broiler chickens. Applied Poultry Research. 3: 209-218.

Wang X, Parsons CM. 1998. Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acids basis. Poultry Science. 77: 1010-1015.

Wang X, Parsons CM. 1998. Effect of raw material source, processing systems and processing temperatures on amino acid digestibility of meat and bone meal. Poultry Science. 77(6): 834-841.

Wang X, Castanon F, Parsons CM. 1997. Order of amino acid limitation in meat and bone meal. Poultry Science. 76(1): 54-58.

Wang X, Parsons CM. 1998. Bioavailability of the digestible lysine and total sulfur amino acids in meat and bone meals varying in protein quality. Poultry Science. 77(7): 1003-1009.

Wilder OHM. 1973. Effect of processing on meat meal and other meat products. pp. 282-285 in: Effect of Processing on the Nutritional Value of Feeds. National Academy Press, Washington, DC.

Winer BJ, Brown R, Michels KM. 1991. Statistical Principles in Experimental Design. Mcgraw Hill, NewYork.