EFFECT OF BETAINE SUPPLEMENTATION ON THE PRODUCTION PERFORMANCE OF BROILER DURING SUMMER SEASON

A THESIS

BY

MD. HAMIDUR RAHMAN
Registration No. 1805389
Semester: July-December, 2019

MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR – 5200

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Submitted to
The Department of Dairy and Poultry Science
Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfillment of the requirements for the degree of

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE
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Dedicated to

My

Beloved Parents

ACKNOWLEDGEMENT

The author express all praise due to the "Almighty Allah" who has created us to explore the hidden fact of the nature for the benefit of mankind and enabling the author to pursue his higher study and to submit his thesis for the degree of Master of Science in Poultry Science.

The author express his sincere appreciation, deep heartfelt gratitude and indebtedness to his reverend supervisor, Professor Dr. Tahera Yeasmin, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for his scholarly guidance, supervision, inspiration, instruction, constructive criticism, valuable advice and untiring assistance in all phases of the research work, and as well as successful completion of the thesis.

The author deeply indebted and sincerely grateful to his research co-supervisor Dr. Md. Kamruzzaman (Mithu), Assistant Professor, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for her constructive advices, encouragement, fruitful criticisms and scholastic supervision throughout the entire period of research work.

The author deeply indebted and sincerely grateful to Professor Dr. Tahera Yeasmin, Chairman, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for her constructive advices, encouragement, fruitful criticisms and scholastic supervision throughout the entire period of research work.

Finally, the author has much pleasure to express his heartfelt indebtedness and gratitude to his beloved parents, sisters specially his elder brother who opened the gate and paved the way for his higher study and relatives who had always sacrificed their causes of happiness for his constant inspiration throughout his academic life.

The author would also express his sincere thanks to all the lab staffs, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur for their sincere help and cooperation throughout the study period.

The Author

ABSTRACT

A study was conducted to investigate the effects of Betaine on live weight gain, feed consumption, FCR, meat yield traits of commercial broilers. A total of 96 day old broiler chicks (Cobb 500) fed diets T_0, T_1, T_2 and T_3 having three replications in each.T_0 (control diet), T_1 (0.03% betaine in water), T_2 (0.06% betaine in water) and T_3 (0.09% betaine in water).The birds were reared in litter management system. Body weight gains, feed conversion ratio (FCR), mortality and meat yield traits were recorded. The collected data were analyzed in CRD by using the SPSS software. Feed intake was almost similar (P>0.05) among the dietary groups. Body weight gain and FCR were significantly (P<0.05) different among the dietary groups. The highest body weight gain was (P<0.05) in T_2 (1632.83g), followed by T_1 (1566.64) and T_3 (1521.15g) and T_0 (1465.52g) respectively. The lowest FCR was found in T_2 (1.36) and the highest FCR in T_0 (1.45), the intermediate in T_1 (1.39) and T_3 (1.42) respectively. It was found that there was significant (P<0.05) difference among the dietary groups in case of carcass weight, live weight, thigh weight, breast weight but there was almost similar(P>0.05) among the dietary groups for heart weight, spleen weight, gizzard weight, head weight and intestine weight. Carcass weight in T_2 (980.43g) and live weight T_2 (1632.83g) were significantly (P<0.05) higher compared to control T_0 (766.89g) and T_0 (1465.52g) respectively. No mortality was found among the dietary groups during experimental period.

Keyword: Betaine, broiler chickens, heat stress, performance, survivability.

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LIST OF ABBREVIATION AND SYMBOLS

ANOVA	: Analysis of variance
CRD	: Completely Randomized design
DM	: Dry Matter
Dr.	: Doctor
et al.	: Associates
Fig.	: Figure
G	: Gram
HSTU	: Hajee Mohammad Danesh Science and Technology University
kcal	: Kilo-calorie
Ltd.	: Limited
ME	: Metaboilizable energy
ML	: Mille Litter
^{o}C	: Degree Celsius
Prof.	: Professor
SEM	: Standard Error of Means
Sl.	: Serial Number
Tk.	: Taka
%	: Percentage
&	: and
/	: Per/or
@	: At the rate of
+	: Plus/and
<	: Less than
>	: Greater than
±	: Plus-minus
µl	: Micro Liter
BBS	: Bangladesh Bureau of Statistics
pH	: Power of Hydrogen
ME	: Metabolizable energy
GIT	: Gastro Intestinal Tract
FCR	: Feed conservation Ratio

CHAPTER I

INTRODUCTION

In Bangladesh broiler rearing is very popular and available. It is one of the best earning sectors rather than other sectors. The farmers who have limited land with low capital can easily adopt broiler rearing with limited knowledge and also can contribute to the development of their own as well as can take part in national economy. But in broiler rearing farmers usually face some problems in hot weather.

Since broiler are homoeothermic and therefore can maintain their body temperature within a narrow range. Compared to other species of domestic animals, broilers are more sensitive to high ambient temperatures. Fast growing commercial broilers are highly sensitive to high temperature during growing-finishing phase. They have a thermo-neutral zone in environmental temperature in which body temperature maintenance is possible. Heat stress suppression remains a challenge for the broiler production in the summer season. The term “stress” is commonly used to describe the detrimental effects of a variety of situations on the health and performance of poultry, and may be defined as the set of responses to external demands that call upon the flocks to adapt to a new and an abnormal situation (Rosales, 1994). Various feed additives have been used in recent time to improve growth, feed efficiency, immune status and antioxidant capacity of broiler (Abudabos *et al.,* 2016; Alzawqari *et al.,* 2016; Shahid *et al.,* 2015; Chand *et al.,* 2014, Khan *et al.,* 2014). Efforts are under way to develop dietary measures that can inhibit heat stress to achieve optimum performance. Exposure to high ambient temperature causes serious physiological dysfunctions and triggers secretion of corticosteroids, which being catabolic in nature severely depress animal performance (Mujahid *et al.,* 2007; Beard, 1987). This adaptation process releases hormones and redistributes nutrient reserves at the cost of performance (Beck, 1991; Gross *et al.,* 1981; Freeman, 1987). The thermoneutral zone for poultry is 18°C–24°C in the tropics and 12°C–26°C in the temperate zones, but this often gets exceeded in the tropics, resulting in heat stress (Holik, 2009; Dei and Bumbie, 2011). By elevating circulatory corticosteroids and decreasing thyroid activity, heat stress impairs broiler performance, especially in adult birds, because the ability to dissipate heat decreases with age (Mahmoud *et al.,* 2014; Rosa *et al.,* 2007). Drastic decline in feed intake occurs in heat-stressed birds as a physiological response to minimize intrinsic heat production and to maintain the thermal homeostasis, thus bringing down feed efficiency, live weight gain, and survival rates (Geraert *et al.*, 1996; Koh *et al.*, 1999; Deaton *et al.*, 1982; Faria Filho *et al.,* 2007). Lower breast-meat yield and higher carcass-fat deposition are the other deleterious effects of heat stress that lower the economic value of broiler carcasses (Ain-Baziz *et al.*, 1996).

Many methods are also followed in poultry to reduce heat stress (Chand *et al.,* 2016). Such methods include the use of electric fans, cooling pad system and sprinkling of water through foggers (Khan *et al.,* 2014). As most of these methods cannot be practiced due to high expenses, other strategies such as nutritional therapies including the use of balancing nutrient contents, addition of vitamin C, sodium bicarbonate, potassium carbonate and aspirin in drinking water can be followed. One of these nutritional strategies for reducing stress in the broiler is the use of betaine as a feed additive in the poultry diet (Zimmermann *et al.*, 1996).

Betaine, the trimethyl derivative of the amino acid glycine, is a naturally occurring compound distributed widely in many plants and animal tissues (Ratriyanto *et al.,* 2009). Betaine is a hydrocarbon consisting of hydrogen and carbon. It contains amide group attached with three (CH3) methyl groups bound through the amino group of glycine amino acids. Betaine is made naturally as well as synthetically from a number of plants and animals (Boch *et al.*, 1994). Betaine is found naturally in higher quantities in beet roots (Wang *et al.*, 2004). Betaine has multiple functions that include donation of its labile methyl group, which is used in transmethylation reactions for synthesis of carnitine and creatine (Eklund *et al.*, 2005; Kidd *et al.*, 1997). Betaine in reaction with the homocysteine has methionine saving effect, where it donates methyl group instead of methionine (Paniz *et al.*, 2005). Betaine is an osmolyte and assists in cellular water homeostasis (Klasing *et al.,* 2002). Dietary supplementation of betaine presumably reduces the requirement for other methyl-group donors, such as methionine and choline (Siljander-Rasi *et al.,* 2003). Betaine supplementation in feed improves growth performance and feed intake under heat stressed condition (Hassan *et al.,* 2005). The positive effect of betaine is due to the fact that it reduces the body temperature in chickens (Klasing *et al.,* 2002). Being a methyl-group donor, betaine is thought to spare methionine from this function, thus allowing methionine to be utilized more for growth and muscle development (Paniz *et al.,* 2005).

This experiment was carried out according to the following objectives

- To find out the effect of betaine in prevention of heat stress during high environmental temperature.
- To know the effect of betaine on the better carcass characteristics of broiler.
- To assess the effect of betaine in the survivability of broiler.

CHAPTER II

REVIEW OF LITERETURE

Betaine is a natural osmolyte found in plants and animals grown in low rainfall areas. Its major functions in animals are as an osmolyte and methyl donor. Its osmolytic properties are useful to help maintain the gut mucus membrane during heat stress and digestive disorders. As a methyl donor, it is involved in the synthesis of methionine. Betaine is also reported to improve breast meat yield in broilers. Betaine has the potential to spare methionine and choline as methyl-group donors, and is thought to improve broiler performance under conditions of stress.

2.1 Betaine in metabolism and functions

Betaine in the purified form is absorbed by the mid-jejunum in chicks. Absorption of betaine is more rapid than choline or methionine. Choline and methionine are associated with plasma lipoprotein, whereas betaine remains in a free state in the plasma. Choline must be transported from the cytosol into the mitochondria where it is oxidised to betaine, which is transported to the cytosol. However, the efficiency of converting choline to betaine is reduced by polyether ionophore anticoccidials by interfering with mitochondrial membrane transport of the compound. The methyl group from betaine can be transferred to homocysteine to yield methionine via betaine-homocysteine methyl transferase. However, this reaction does not supply additional methionine to the cell, as the homocysteine is synthesised from methionine.

Betaine acts as a methyl donor and an osmolytes that assists in cellular water homeostasis. Dietary supplementation of betaine may decrease the requirement of methyl donors like methionine and choline. Betaine contributes methyl groups for the synthesis of carnitine via S-adenosyl methionine. Carnitine is required for transport of long-chain fatty acids across the mitochondrial membrane for oxidation. Betaine increases the concentration of S-adenosyl methionine and homocysteine in liver, which facilitates in-vivo methionine synthesis by utilising methyl groups from a single carbon pool. In chicks, betaine donates a methyl group (CH3) to homocysteine for the synthesis of methionine approximately three times more efficiently than choline. However, adequate concentration of cysteine is required to achieve the beneficial effects of betaine supplementation. Inadequate dietary concentration of cysteine may affect the activity of betaine by reducing the concentrations of homocysteine for methionine formation.

The osmo-protective property of betaine may be due to the dipolar zwitterions and its high solubility in water. Osmolytes are important during cellular dehydration. Betaine minimises water loss from cells against a prevailing osmotic gradient between cell and its surrounding environment. Betaine accumulation results in an increased water-binding capacity of the intestinal cells and it promotes changes in the structure of the gut epithelium that increase gut surface area. Betaine stimulates cell proliferation in the intestinal tissue, particularly the mucosal membrane. The enlarged gut wall epithelium may increase the surface for nutrient absorption. Reduced gut pH and intestinal villi height with betaine (hydrochloride) supplementation in the stomach may improve the digestibility of methionine and other nutrients (Eklund *et al.,* 2005; Attia *et al.,* 2005; Hassan *et al.,* 2005).

2.2 Betaine in nutrient utilisation

Betaine was reported to improve apparent digestibility of lysine, protein, fat and carotenoids and in-vitro methionine uptake in broilers challenged with coccidia. Improved cell integrity and surface area of gastrointestinal lumen with betaine supplementation appeared to enhance the digestibility of dietary nutrients. Dietary supplementation of betaine increased the activities of several hormones in serum such as luteinising hormone, follicle stimulating hormone, triiodo-thyronine, thyroxine, oestradiol and progesterone in laying hens. Increased activities of the hormones and mucosal surface area and cell integrity might have led the cell to an anabolic state and helped in cell multiplication and metabolic activity. Improved surface area and vitality of gut mucosa might have increased secretion of digestive enzymes and absorptive area in the intestine (Sakomura *et al.*, 2013; Eklund *et al.*, 2006).

2.3 Betaine as methyl donor

Betaine contains three methyl groups in its structure and donates these in several metabolic reactions. As a result, betaine can spare compounds such as methionine, choline and folic acid, thus betaine supplementation may reduce the need for supplementation of these nutrients. On a molecular weight basis, betaine contains about 3.75 and 0.90 times the methyl groups of methionine and choline, respectively. The first methyl group is donated to homocysteine in its enzyme-induced conversion to methionine in the liver. The other two-methyl groups are supplied to one-carbon pool, which will be handled by folic acid. Methyl tetra hydrofolate can also donate methyl group to homocysteine for methionine synthesis (Eklund *et al.*, 2005; Kidd *et al.*, 1997).

2.4 Betaine in osmolytic activity

Betaine functions as an osmolyte by most body tissues, including the liver. It helps to maintain osmotic equilibrium in small intestine epithelium by maintaining water balance in hyper-osmotic conditions like non-specific diarrhoea and coccidiosis. Under heat stress and dehydration, sodium ions (Na+) will be moved from the cellular fluid in to the cell. The higher the concentration of Na+, the greater is the inhibition of nutrient uptake by the cell. Higher concentrations of potassium (K+), magnesium and phosphorus within the cell also inhibit the activity of enzymes necessary for metabolism of nutrients.

To maintain normal enzyme activity, function and volume, cells raise the intracellular concentrations 'organic osmolytes' such as methyl amines, choline and betaine. In birds, myo-inositol, betaine and taurine are also major physiological organic osmolytes. Betaine prevents dehydration by increasing water-holding capacity of the cell. Utilisation of betaine as an osmolyte is more beneficial than inorganic electrolytes in terms of energy expenditure in osmo-regulation and compatibility with the cell organelles. The stability of mucosal cell structure increased and the movement of water from the mucosal cell decreased even at higher osmotic pressure in the gut lumen of birds fed a betaine-supplemented diet (Sun *et al.*, 2008).

Betaine supplementation also reduces the energy required for osmoregulation by reducing the activity of the 'Na/K' and 'calcium pump' by 64 and 73%, respectively. This primarily uses adenine triphosphate (ATP) as an energy source.

The concentration of electrolytes increases within the cell under dehydration. To regulate the desired concentration of water within the cell, K+ is pushed in to the cell against concentration gradient. To pump any ion against concentration gradient, one molecule of ATP is required. Higher concentrations of electrolytes in the cell are known to inactivate enzymes and proteins. The higher concentrations of the electrolytes bind with active sites of enzymes and thereby deactivate them. Movement of betaine across the cell membrane does not require energy and will not interfere with cell ecosystem or cell metabolism.

Betaine in feed or drinking water can control osmoregulatory conditions including diarrhoea, catharsis, diuresis and ascites.

2.5 Betaine in improved performance

The influence of betaine supplementation on performance of broilers primarily depends on the concentrations of other labile methyl groups in diet and magnitude of stress to the bird. Betaine supplementation was ineffective in influencing the bird's performance in diets with either adequate or severe deficiency of methionine. However, improvement in bodyweight, breast meat yield, feed conversion and decreased abdominal fat pad weights with betaine supplementation have been reported (El-Husseiny *et al.,* 2007). The results of our recent study indicate significant improvement in feed efficiency in broilers fed betaine (800mg/kg) either sub-optimal concentrations of methionine compared to those fed no betaine, irrespective of the concentration of the amino acid..

2.6 Betaine in reducing litter moisture

During intestinal stress conditions (including diarrhoea), the absorption of Na+ and chloride ions (Cl-) decreased through the intestinal villus membrane. This results in hyperosmolar solution in the intestinal lumen. Betaine supplementation increases intestinal mucosal cell integrity and allows the cells to function normally. This helps to optimise nutrient digestibility and reduce their excretion. Litter moisture content reduced at 6 days from 46 to 27% after feeding betaine in water (2.5g/litre). The dose of betaine to control diarrhoea in poults was 0.15-1.5g/kg bodyweight. Betaine is also reported to influence positively water balance in broilers exposed to coccidia and to reduce faecal moisture content.

2.7 Betaine in better carcass characteristics

Betaine has methionine-sparing activity and is involved in synthesis of carnitine. Thus, it has a role in protein and fat metabolism, respectively, and can alter carcass composition. Betaine reduces protein turnover, which results in higher nitrogen retention, which in turn has a positive effect on accretion of protein in muscle (carcass leanness). Betaine enhances lipid catabolism via its role in carnitine synthesis and leads to low carcass fat deposition.

The effect of betaine on abdominal fat deposition and carcass yields is inconsistent, depending on many other dietary factors (Esteve-Garcia and Mack, 2000).

Our recent data indicate significant improvement in breast meat yield with betaine supplementation (800mg/kg) to diets containing different concentrations of methionine compared without betaine supplementation, irrespective of methionine concentration. At higher concentrations of methionine (2.4% of crude protein), betaine had no effect on breast meat yield of commercial broilers.

2.8 Betaine in controlling coccidiosis

Effects of betaine as an osmolyte are clear when exposed to gut osmotic disorders. Coccidiosis decreases villus height and jejunal crypt:villus ratio, and increases the osmolarity in duodenum. The beneficial effects of betaine on birds infected with coccidiosis may be due to its osmolytic activity as well as increased phagocytic activity in the gut mucous membrane. Betaine (0.1%) decreased the osmolarity of duodenum and increased the number of leukocytes in the epithelium and lamina propria of chicks with coccidia infection. Betaine increased phagocytosis of coccidia by macrophages and nitric oxide release from heterophils and macrophages (Farooqi *et al.,* 2005).

The efficacy of certain anticoccidial compounds can be modulated by dietary betaine supplementation. Growth of Eimeria acervulina was reduced in chicks fed diets containing betaine (0.075%) plus salinomycin (66mg/kg) compared to those fed salinomycin only. Other anticoccidials like polyether ionophores interfere with conversion of choline to betaine.

2.9 Betaine in counteracting stress

Stress leads to release of more number of reactive oxygen species (ROS) in the system. These impair the cell structure, membrane integrity and ion pump in the gut lumen. Cell dehydration leads to disturbances in cell metabolism and its enzyme activities. Feed intake leads to movement of intracellular water in to the gut lumen, which causes shrinkage of the gut mucosal epithelium. Disturbance in cell structure impairs nutrient absorption, cell membrane transport and certain intracellular metabolic processes (metabolism of amino acid, ammonia, carbohydrates and fatty acids). In extreme circumstances, mucosal cell shrinkage may leads to disruption of intestinal mucosal integrity, which may permit the entry of pathogens and toxins in to the blood.

Betaine increases cytoplasmic osmotic pressure in stressed cells and tolerance to temperature and ionic disturbance (Attia *et al.*, 2009). Accumulation of conventional osmolytes like K+ in the cell may lead to disturbance in activity of cell enzymes. Accumulation of betaine protects the cells from osmotic stress and allows them to continue normal metabolic activities under stress conditions that would otherwise inactivate the cell. Betaine supplementation during heat stress reduces the heterophil:lymphocyte ratio, which is an indicator of stress in poultry (Freeman, 1988; Awad *et al.*, 2014).

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the research work

The experiment was conducted at the poultry farm of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, to investigate the effect of supplementation of Betaine on the performance and meat yield traits of broilers (Cobb 500) in summer season during the period from 13 September to 11 October, 2019.

3.2 Experimental birds

A total of 96 day-old broiler chicks (Cobb 500) were purchased from Kazi Farms Limited, Sador, Dinajpur, Bangladesh.

3.3 Layout of the experiment

Table 3.1: Layout showing the distribution of experimental broilers

Dietary Treatments		**Number of broiler in each replication**			**Total**
		R_1	**R_2**	**R_3**	
Control (without betaine)	**T_0**	8	8	8	24
Control+0.03% betaine	**T_1**	8	8	8	24
Control+0.06% betaine	**T_2**	8	8	8	24
Control+0.09% betaine	**T_3**	8	8	8	24
Total No. of broilers					**96**

The day-old chicks were reared at brooder house to adjust with the environmental condition up to 7 days. After 7 days, chicks were randomly allocated in four dietary treatment groups having three (3) replications in each and 8 birds per replications. The layout of the experiment is shown in Table 3.1.

3.4 Collection of feed ingredients

Feed ingredients used in the experiment were purchased from a feed shop of Dinajpur town.

3.5 Preparation of the experimental diet

Ready feed was used for the experimental study. At first required amount of ready feed ingredients were weighed by digital weighing balance. The experimental period were divided into two phases (broiler-starter and broiler-grower). The broiler chicks were fed broiler starter for 0 and 14 days and broiler grower for 15 to 28 days of age.

3.6 Calculated composition of experimental diets

Nutrients	Amount(kg/100kg feed)	
	Starter (1-14 days)	Grower (15-28days)
Crude protein (%)	22	21
Crude fiber (%)	3	3
Crude fat (%)	5	5-6
Lysine (%)	1.30	1.25
Methionine (%)	0.52	0.50
Calcium (%)	1	0.90
Phosphorus (%)	0.50	0.48
Moisture (%)	11	11
Metabolizable Energy, ME (kCal/kg)	3000	3100

3.7 Management of the experimental birds

Similar care and management in all treatment groups throughout the experimental period was practiced. At the initiation of the experiment, chicks were individually weighed recorded as initial body weight. The following management practices were followed during the whole experimental period and these management practices were identical for all dietary groups.

3.8 Managemental practices

3.8.1 Housing and equipment

An open sided house with two rooms will be used for rearing the experimental birds. The experimental house was properly cleaned and washed by forced water using a hose-pipe. Then, the room was disinfected by bleaching powder solution. After 15 days, the room was disinfected with Virkon solution (50 ppm). At the same time, all feeders, plastic buckets, waterer and other necessary equipments were also properly cleaned, washed and disinfected with bleaching powder solution, subsequently dried and left them empty for a week before the arrival of chicks.

3.8.2 Litter management

During the experiment period for the first 7 days litter was covered by clean newspaper and newspaper was removed when it becomes dirty. After that period the birds were reared on rice husk littered floor having a depth of 4 cm. Before use of litter calcium carbonate was spread on the floor. After first week, upper part of the litter with droppings were removed regularly and stirred three times a week up to the end of the experiment. The litter was disinfected with Virocid® solution in every other day. Litter materials, when found damp for any reason, were removed to prevent accumulation of ammonia and other harmful gases. At the end of each week, litter was stirred to break its compactness and maintain proper moisture. At the end of 2nd and 3rd weeks of age, dropping were cleaned from the surface of litter.

3.8.3 Brooding

The experiment was conducted in hot weather (September to October/2019). Additional heat was provided to brood the chicks when it was necessary. Brooding temperature was kept at 34^{o}C in the first 1 week of age and decreased gradually until they were adjusted to normal environmental temperature of the house and final temperature was 28^{o}C at the end of experiment. Additional heat was provided by fitting 100-walt electric bulb at the center of the pen about 12 inches above the floor from the 7-day old. The height of the bulbs was increased by raising the bulb gradually as per need of temperature. Paper was used on two sides of the house and in ventilators to protect cold and stormy wind. These sheets were removed partly or completely particularly at the later stage of finishing period when room temperature was found favorable. Daily room temperature (^{o}C) was recorded every six hours with a thermometer.

3.8.4 Lighting

All birds were exposed to continuous lighting of 23 hours and one hour dark period per day throughout the experimental period. The dark period was practiced to make the broilers familiar with the possible darkness due to electricity failure. Supplementary light at night was provided by electric bulb by hanging at a height of 2.8 miters to provide necessary lighting.

3.8.5 Temperature

The house temperature was maintained at 34°C for the first week. In the course of the trial period the temperature was gradually reduced from 34°C to 32°C during first week. Then the environmental temperature usually recorded as 38° from 11am to 5 pm, 32° from 5pm to 10pm and 28° from 10 pm to 11am.Thus the environmental temperature was recorded upto the last day of the experiment.

3.8.6 Floor, feeder and water space

An area of 8 sq. feet was allotted for 8 birds; therefore floor space for each bird was 1 sq. feet. One round feeder and one round waterer were provided for 8 birds; required feeding and drinking space was providing according to the number of birds in each replication.

3.8.7 Feed and water management

At the first week, feeds were supplied to the chicks on clean newspapers at three hours interval for the first 3 days. Linear feeder and round plastic drinker were used during brooding period. After that linear feeder were replaced by round plastic drinker. Feed and fresh water were supplied to the treatment T0. Feeds were supplied thrice daily (once at morning, at noon and again at night).Water was supplied also thrice daily (once at morning, at noon and again at night) at 0%, 0.03%, 0.06% and 0.09% betaine respectively in T_0, T_1, T_2 and T_3 treatment group. Feeders were cleaned at the end of each week and drinkers were washed daily.

Figure 3.1: Feeding and watering of broiler

3.8.8 Sanitation

Adequate sanitary measures were taken during the experimental period. The entrance point and veranda were kept clean and solution of bleaching powder and potassium permanganate ($KMnO_4$) was kept in foot bath alternatively.

3.8.9 Immunization

All birds were vaccinated against Baby chick Ranikhet Disease and Infectious Bronchitis at day one by the company. The birds were vaccinated against Ranikhet and Infectious Bursal (Gumboro) diseases by following schedule at the evening-

3.8.10 Applied vaccination program

Diseases	Day	Vaccine	Route	Time
Ranikhet	4	BCRDV	Eye	Evening
Gumboro	10	Gumborovac	Eye	Evening
Gumboro	16	Gumborovac	Eye	Evening
Ranikhet	21	NDLasota	Eye	Evening

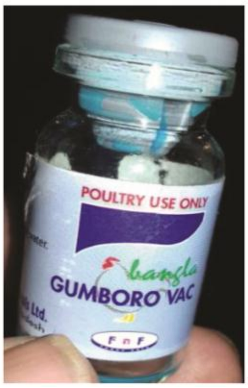

Figure 3.2: Vaccination

3.8.11 Clinical observation

The birds were critically observed twice a day for clinical sign if any (slow movement, infrequent sitting, lack of appetite, significant changes of feathering, paralysis etc.) and for monitoring other activities.

3.8.12 Medication

Immediately after unloading from the chick boxes the chicks were given Glucose and Vitamin-C to prevent the stress occurring during transport. Water soluble vitamin and normal saline were also provided for the first 3 days of brooding.

3.9 Data collection and record keeping

The following records were kept during 28 days of rearing period:

Live weight.

Feed consumption.

Feed conversion ratio.

Mortality

Temperature:

Five times daily during the experimental period.

Dressing yield:

At the end of the experiment one broiler was slaughtered from each replication to estimate dressing yield.

3.9.1 Live weight gain

Birds were weighed in a group at the beginning of the trial and then every week at the age of 7, 14, 21, 28 days. The weighing was done using pan balance.

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

Body weight gain = Final weight — Initial weight.

3.9.2 Feed intake

Feed offered daily and refusal at the end of each week was recorded. Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

$$\text{Feed Intake (g/bird)} = \frac{\text{Feed intake per replication}}{\text{No. of birds per replication}}$$

3.9.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

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

3.9.4 Temperature and relative humidity

During the experimental period the temperature and the relative humidity (RH) of the experimental house and pens at chick level were recorded two times a day (8AM and at 5 PM) with the help of an automatic thermo hygrometer. The environmental temperature usually recorded as 38° from 11am to 5 pm, 32°from 5pm to 10pm and 28° from 10 pm to 11am.Thus the environmental temperature was recorded upto the last day of the experiment.

3.9.5 Mortality

Mortality was recorded daily treatment wise when occurred.

3.9.6 Processing of broilers

After termination of the experiment, one bird weighing average of pen weight from each replication was selected randomly. Feed was withdrawn from the pens 24 hours prior to slaughter but water was available to facilitate proper bleeding. Birds were slaughtered according to halal method. Following slaughter, broilers were allowed to bleed for about 2 minutes. Then the birds were scaled in hot water (55-65^{o}C) for about 96 seconds in order to loosen the feather of the carcasses and weighed again. Breast meat, thigh meat, drumstic meat were separated from the carcass. Finally, processing was performed by removing head, shank, viscera, oil gland, kidney and giblets. As soon as these were removed the gall bladder was removed from the liver and pericardial sac and arteries were cut from the heart. Cutting it loose in front of the proventriculus and then cutting with both incoming and outgoing tracts removed the gizzard. Then, it was split open with knife, emptied and washed and the lining removed by hand.

3.9.7 Dressing yield

Dressing yield is based on the relationship between the dressed carcass weight and live bird weight after things like the skin and internal organs have been removed. Dressing yield can be calculated by taking weight of the carcass divided by weight of live bird.

$$\text{Dressing yield} = \frac{\text{Weight of the carcass}}{\text{Weight of live bird}}$$

3.10 Economy of broiler production

The cost of broiler production for each treatment group was calculated based on the market price of feed ingredients, cost of chicks, Betaine and management cost (labor, medicine, electricity and litter depreciation) to produce per kg of live broiler at the time of trial. The income from per kg of live broiler in different treatment groups was calculated by the selling price of per kg live broiler.

3.11 Statistical analysis

Data on different variables were subjected to analysis of variance (ANOVA) in a Complete Randomized Design (CRD) (Steel and Torrie, 1980). The significant differences between the treatment means were calculated from analysis of variance (ANOVA) table. All analyses were performed by using “IBM SPSS statistics 20” program.

CHAPTER IV

RESULTS

4.1 Performance of broiler of experimental birds

This experiment was conducted to study on feed consumption, feed conversion ratio, live weight gain and mortality of birds and carcass traits were used as criteria of response of broiler to different dietary levels of betaine are presented in different tables and discussed under the following subheadings.

4.1 Growth performance

4.1.1 Body weight

The effect of betaine on highest body weight gain is shown in table 4.1. The present study revealed that there was no significant (P>0.05) variation of initial body weight (g/broiler) among the dietary groups but final body weight (g/broiler) and body weight gain were significantly (P<0.05) differed among the dietary groups. The initial body weight (g/broiler) in T_0, T_1, T_2 and T_3 group was (39.50±0.04), (40.91±1.02), (40.45±1.05) and (39.33±0.60). At 7 days of age, the body weight was almost similar in different dietary groups. Significant different (p<0.05) were found at 14 days, 21 days and 28 days of age on body weight gain. The highest body weight was found in T_2 (1632.83g), followed by T_1 (1566.64g), T_3 (1521.15g) and T_0 (1465.52g) respectively.

However, there was a trend of increasing live weight with the increase of age (P<0.05). There was a tremendous (P<0.05) increase of live weight for increasing Betaine level at 14, 21, and 28 days of age in broilers. The result in this study revealed that supplementation of broiler with 0.03%, 0.06% and 0.09% Betaine gave rise to (P<0.05) improve in live weight than 0% Betaine of broilers. There was a tendency of increasing live weight broilers with increasing Betaine content up to 0.09% level.

Table 4.1: Body weight and mortality in different groups at different ages of birds

Age in days /parameter	Dietary groups (%)				Level of significance
	T_0	T_1	T_2	T_3	
Initial body weight	39.50±0.34	40.91±1.02	40.45±1.05	39.33±0.60	NS
7^{th}	168.34±0.60	171.71±0.63	169.78±0.85	174.42±0.62	NS
14^{th}	235.24±0.55^{a}	266.95±1.01^{c}	282.19±0.57^{d}	247.03±0.58^{b}	*
21^{th}	465.08±0.56^{a}	491.59±0.60^{c}	518.65±0.76^{d}	478.09±0.57^{b}	*
28^{th}	557.36±0.48^{a}	595.48±0.68^{c}	621.76±0.90^{d}	582.28±0.57^{b}	*
Total body weight (1^{st}-28^{th})	1465.52±2.53^{a}	1566.64±3.94^{c}	1632.83±4.13^{d}	1521.15±2.94^{b}	*
Mortality (%)	00.00	00.00	00.00	00.00	NS

T_0= Control

T_1= Control + 0.03% betaine

T_2= Control + 0.06% betaine

T_3= Control + 0.09% betaine

^{abc} means having different superscript in the same row differed significantly (P<0.05) *= 5% level of significance

NS= Non significant

4.1.2 Feed intake

The difference of feed intake was marked for broilers fed on (P<0.05) at 14 and 21 days of age. However, there were slightly variation in feed intake at 7 and 28 days of age on different dietary level. Feed intake at 14 and 21 days of age were differed on different diets. Feed intake appeared (P>0.05) significant at 14 and 21 days of age. Inclusion of betaine in water resulted in (p<0.05) increase in feed consumption at 14 and 21 days of age. It was found that at 0.03%, 0.06% and 0.009% treatment group consumed the highest amount whereas lowest in 0% treatment group.

Table 4.2: Feed intakes (g) in different groups at different ages of birds

Age in days /parameter	Dietary groups (%)				Level of significance
	T_0	T_1	T_2	T_3	
7th	175.07±0.54	174.42±0.46	183.36±0.54	181.39±0.73	NS
14th	326.98±1.03[a]	347.04±0.57[c]	355.56±0.61[d]	331.03±0.56[b]	*
21th	697.62±0.74[a]	707.89±0.51[c]	731.30±0.41[d]	702.80±0.37[b]	*
28th	930.93±21.46	946.93±3.46	951.09±3.06	943.09±2.87	NS
Total Feed Intake (1st-28th)	2130.6±23.77	2176.28±5.00	2221.31±4.62	2158.31±4.53	NS

T_0= Control

T_1= Control + 0.03% betaine

T_2= Control + 0.06% betaine

T_3= Control + 0.09% betaine

[abc] means having different superscript in the same row differed significantly (P<0.05) *= 5% level of significance

NS= Non significant

4.1.3 Feed conversion ratio (FCR)

Weekly feed conversion ratio (FCR) of broilers on different treatment group differed (P<0.05) during 14, 21and 28 days of age. At 7 days of age betaine levels obtained no difference which could be attributed to the alteration with the increase of betaine in feed conversion. Lowest FCR was obtained from 0.06% betaine level. It was (P<0.05) better in comparison with 0.00%, 0.003% and 0.009% betaine levels at 14, 21 and 28 days of age.

Table 4.3: Feed conversion ratio (feed intake/wt gain) of different birds of different groups

Age in days /parameter	**Dietary groups (%)**				**Level of significance**
	T_0	**T_1**	**T_2**	**T_3**	
7th	1.03±0.00	1.04±0.01	1.08±0.00	1.04±0.00	NS
14th	1.39 ± 0.00^{d}	1.29 ± 0.00^{b}	1.26 ± 0.00^{a}	1.34 ± 0.00^{c}	*
21th	1.50 ± 0.00^{d}	1.44 ± 0.00^{b}	1.41 ± 0.00^{a}	1.47 ± 0.00^{c}	*
28th	1.66 ± 0.04^{b}	1.59 ± 0.01^{ab}	1.53 ± 0.01^{a}	1.62 ± 0.01^{bc}	*
Total FCR (1st-28th)	1.45 ± 0.11^{d}	1.39 ± 0.79^{b}	1.36 ± 0.89^{a}	1.42 ± 0.65^{c}	*

T_0= Control

T_1= Control + 0.03% betaine

T_2= Control + 0.06% betaine

T_3= Control + 0.09% betaine

^{abc} means having different superscript in the same row differed significantly (P<0.05) *= 5% level of significance

NS= Non significant

4.2 Meat yield characteristics

It is found from the Table 4.5 that highest live weight (1632.83g) in group T_2 and lowest live weight (1465.52g) in group T_0 and group T_1 weight (1558.41g), other weight (1520.11g) group T_3 respectively which are significant. Carcass weights were significant and highest carcass weight (980.43g) found dietary groups T_2 and lowest carcass weight in dietary groups T_0 (766.89g)

Table 4.4: Effect of feeding Betaine to broilers on dressing parameters at different ages

Parameter	Dietary groups (%)				Level of significance
	T_0	T_1	T_2	T_3	
Live weight	1465.52±0.69^a	1558.41±0.60^c	1632.83±0.77^d	1520.11±5.81^b	*
Carcass weight	766.89±0.63^a	922.63±0.40^c	980.43±0.54^d	857.15±0.56^b	*
Breast weight	293.46±1.71^a	327.59±1.86^b	391.08±2.10^d	349.41±0.63^c	*
Thigh weight	244.32±0.61^a	263.48±0.65^c	283.84±0.84^d	255.09±0.56^b	*
Head Weight	34.29±0.54	35.88±0.52	37.04±0.59	33.51±0.52	NS
Shank weight	38.51±0.51	43.07±0.54	46.02±0.21	19.09±10.84	NS
Gizzard weight	32.04±0.63	42.57±3.02	43.13±0.54	34.33±0.40	NS
Liver weight	30.34±0.35	32.09±0.55	35.21±0.56	31.76±0.79	NS
Heart weight	4.92±0.12	5.44±0.06	5.53±0.08	5.03±0.17	NS
Spleen weight	2.56±0.13	2.63±0.26	2.79±0.06	2.52±0.10	NS
Intestine weight	96.67±0.45	98.07±0.56	105.13±0.56	91.93±0.97	NS

T_0= Control

T_1= Control + 0.03% betaine

T_2= Control + 0.06% betaine

T_3= Control + 0.09% betaine

^{abc} means having different superscript in the same row differed significantly (P<0.05) *= 5% level of significance

NS= Non significant

Significant differences were obtained for the percentage of breast meat and drumstick meat at different diets. Breast meat and drumstick meat of broilers almost increase in a linear fashion with the increase betaine levels. The tabulated result present that betaine levels had mark influence on meat characteristics.

CHAPTER V

DISCUSSION

5.1 Growth Performance

5.1.1 Live weight

The results of body weight gain of this experiment coincide with Attia *et al.* (2009) Haldar *et al.* (2011), Bowmaker and Gous (1991), Hassan *et al.* (2005), reported that broilers fed on 0.006% betaine in water had higher ($P<0.05$) live body weight and body weight gain. But contradict with Rostagno & Pack, (1996), Schutte *et al.* (1997).They reported that, broilers supplemented with more than 0.08% betaine in water level showed ($P<0.05$) lower live body weight. The improvement in weight gain of the broilers using betaine may probably be due to the fact that betaine is a source of amino acid and betaine acts as protein source. Supplementation of betaine influence carcass and parts weights due to its methyl-group donor property, which would increase methionine, cystine (McDevitt *et al.,* 2000), and glycine for protein synthesis and also contribute to reduce fat deposition in the carcass through several metabolic routes (Partridge, 2002).

5.1.2 Feed intake

This result is coincide with the findings of Bowmaker and Gous (1991) who found that feed consumption was highest ($P<0.05$) at 0.06% inclusion level of betaine in water at different stages of broiler. All the rest period of ages feed consumption of all groups there were significant effect for betaine level in water.

Results of the present research work showed that high ambient temperature significantly affected the growth performance and immune status of broilers while betaine supplementation improved these parameters. Under heat stress condition, there is reduction in feed intake which may be due to little energy requirement for heat preservation (Freeman, 1988). Awad *et al.* (2014) reported that feeding of betaine at the rate of 0.006% level in the water results in significantly higher feed intake as compared to control group. Similarly, Sakomura *et al.* (2013) also reported that betaine supplemented to broilers significantly increased feed intake as compared to the control group.

5.1.3 Feed conversion ratio (FCR)

Feed conversion ratio appeared to improve with increasing levels of betaine. Many investigators reported that, a inclusion level of 0.06% betaine enhanced live weight and FC in broilers. Our results showed that betaine supplementation significantly improved FCR at the rate of 0.03-0.08% in water. Findings of the present study are in line to the results of Attia *et al.* (2009) who stated that adding betaine at the rate of 0.08% in water could partially alleviate chronic heat stress in poultry as compared to the negative treatment. Similarly, El-Husseiny *et al.* (2007) revealed that addition of betaine at the levels of 0.75 g/L in water significantly improved FCR as compared to the control group.

But significant (P<0.05) differences was found among breast meat, drumstick meat, abdominal fat and skin. Our results showed that betaine supplementation at 0.06 % level significantly (P<0.05) improved dressing percentage. The increase in dressing percentage may be due to the osmotic effects of betaine, which increases water retention (Waldroup and Fritts, 2005). Our results are in line with El-Shinnawy (2015) who reported that supplementation of betaine significantly increased the dressing percentage in chicken. Esteve-Garcia and Mack (2000) observed significantly better dressing percentage at 32 days of age at the rate of 800 mg/L betaine.

CHAPTER VI

SUMMARY AND CONCLUSION

The experiment was conducted with 96 day old Cobb-500 broiler in Poultry Farm, Hajee Mohammad Danesh Science and Technology University, Dinajpur for a period of 28 days. The experiment was conducted to investigate the effect of betaine on the performance of broiler at appropriate level (%) of betaine in water to maximize the performance. The day old broilers were randomly distributed to 4 diets (0%, 0.03%, 0.06% and 0.09% betaine) with 3 replications each having 8 broilers. All broilers were supplied with feed and water. During 28 days of experiment growth, feed intake and dead broilers were recorded. Live weight, live weight gain, feed conversion, carcass characteristics, mortality (%), on different treatments were calculated for broilers.

Positive effect ($P<0.05$) of 0.06% betaine on growth was expressed from different diet along with different ages. Broilers reared on 0.03% and 0.006% betaine group also attained 1566.64g and 1632.83g live weight respectively which was higher than control. Feed intake was increased at 0.03% and 0.06% betaine levels and it was higher ($P<0.05$) than 0%. Better feed conversion was noticed during 14, 21 and 28 days of old at 0.006% betaine group at the end of the experiment. Meat yield characteristics showed significant differences for the percentage of breast meat and thigh weight. Carcass quality also improved by using different level of betaine.

Supplementation of betaine had a beneficial effect on growth and meat yield performance. Overall performance and quality was increased with increased amount of 0.06% betaine also leads to a better gain in monetary terms. It may be concluded that supplementation of betaine may be useful for economic and efficient production of broiler. Therefore, betaine supplementation at 0.06% may be suitable in broiler production.

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