Effect of Aqua Blok and Poultry Tonic supplementation on production potentials during transportation in broiler chicks arriving on farm.

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Roll No.: 0118/01

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Pharmacology

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June, 2020

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

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DEDICATION

TO RESPECTED TEACHERS AND BELOVED FAMILY MEMBERS

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The Author

June, 2020

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List of Abbreviations

Content	Elaboration
ADWG	Average Daily Weight Gain
ANOVA	Analysis of Variance
BCRDV	Baby Chick Ranikhet Disease Virus
BW	Body Weight
CI	Confidence Interval
℃	Degree Celsius
DOC	Day Old Chick
EU	European Comission
EPEF	European Production Efficiency Factor
EU	European Union
FCR	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
FU	Flock Uniformity
FWM	First Week Mortality
H : L	Heterophil : Lymphocyte
IBD	Infectious Bursal Disease
K	Potassium
mcg	Microgram
mg	Miligram
NADP	Nicotinamide Adenine Dinucleotide Phosphate
N/A	Not Applicable
Na	Sodium
ND	Newcastle Disease
SD	Standard Deviation
T_1	Treatment Group 1
T_2	Treatment Group 2
T_3	Treatment Group 3
T_4	Treatment Group 4
WBWG	Weekly Body Weight Gain
TDS	Total Digestible Solids

WEPEF	Weekly European Production Efficiency Factor
WFCR	Weekly Feed Conversion Ratio
WFI	Weekly Feed Intake
WM	Weekly Mortality
WWI	Weekly Water Intake

ABSTRACT

In this study, we have determined the effects of Aqua Blok and Poultry Tonic supplementation in an intensive broiler production business by assessing average live weight gain (ALWG), feed conversion ratio (FCR), mortality rate, feed and water intake, stocking density, European production efficiency factor (EPEF). To achieve these goals a total of 3000 day old broiler chicks Cobb-500® were allocated into four different treatment groups with equal number of chicks (n=750) where T_1 (Standard farm managements), T_2 (Aqua Blok + Poultry tonic + Feed), T_3 (Aqua Blok + Feed), T_4 (Chick tonic + Feed) and reared for a period of 30 days in farm by following standard intensive broiler production system. Performance differences among different treatment groups were recorded and descriptive statistics including mean, standard deviation, percentage and one way ANOVA were performed. The highest average live weight gain (1492.4g) in T_4, the lowest FCR (1.61) in T_2, the highest mortality rate (5.20%) in T_3 were found. As an indicator of stress heterophil-lymphocyte ratio and some biochemical parameters were calculated. The highest heterophil-lymphocyte ratio (0.40) was found in the treatment group 4 (T_4) by the method of differential leucocyte count. The concentration of uric acid (mg/dl) is higher in group T_4 (8.55±3.35) and lower in group T_3 (6.6±1.4).The albumin level (g/dl) was the lowest (24.15±1.91) in T_2 among the groups. The production cost per kg broiler was also calculated where the lowest value found in the T_2 group as compared to other groups. Finally, we can suggest Aqua Blok supplementation during transportation to reduce stress of broiler chick and combination of Aqua Blok and Poultry Tonic might be used to lessen feed cost as it decreased the value of FCR.

Key words: Aqua Blok, Poultry Tonic, Broiler, Cobb -500®, FCR, Heterophil-lymphocyte ratio.

CHAPTER I

INTRODUCTION

CHAPTER 1: INTRODUCTION

Bangladesh is an agriculture based tropical country where more than 80% people are living in rural areas and highly dependent on agriculture. Poultry farming is one of the major activities in rural areas as it provides immense employment opportunities to the local communities especially for youth & women that helps in poverty reduction, ensures food security and improves the nutritional status of the people. Poultry industry can produce very high-quality proteins for human nutrition as well as a source of income for the community in many countries, therefore poultry production plays very important role in economic development of any country (Tarhyel et al., 2012). Poultry is a promising sector in Bangladesh which is increasing day by day. But the future development of the chicken meat business in hot places of the world is hampered by a number of factors. The first and most important factor is financial availability.

A number of factors are associated with poultry production systems which may cause stress on birds as well as may reduce production. According to Elrom (2000), the stressors classified into mental, physical or mixed. Extreme temperature (heat or cold), overcrowding or high stocking density, starvation (feed and/or water deprivation or withdrawal), and harmful handling such as restraint, noise, and transportation all can cause physical stress in animals, where as social mixing, food and water deprivation, fear, and pain can cause mental stress. Physical stressors could be categorized into three phases (Nilipour, 2002) such as handling of the bird during catching most are injured, physical injury during loading into coops and the environment's impact on the bird during travel. Physiological stress can harm the overall performance and body growth of meat-type chicken (Mckee and Sams, 1997; Mashaly et al., 2004), and this is still a difficult topic for poultry producers and academics to understand.

Heat stress results in decreased feed consumption and increased water consumption. As temperature rises, the bird has to maintain the balance between heat production and heat loss and so will reduce its feed consumption to reduce heat from metabolism. Earlier study revealed that feed consumption is reduced by 5% for every 1 ºC rise in temperature between 32-38ºC. Hot climate has a severe impact on poultry performance. Heat stress depresses growth rate and production as a result of a downturn in voluntary feed intake in birds (Sahin et al., 2001). In a recent study (Sohail et al., 2012), broilers subjected to chronic heat stress had significantly reduced feed intake (16.4%), lower body weight (32.6%) and higher feed conversion ratio (+25.6%) at 42 days of age. In addition to decreased feed intake, it has been shown that heat stress leads to reduced dietary digestibility and decreased plasma protein and calcium levels (Zhou et al., 1998).

Stressful situations in which birds are exposed can have immunological or metabolic implications. After experimental stress induction, chickens' immune-protective organs (such as the thymus, bursa, and spleen) regressed, mitochondrial metabolic oxidative capacity shrank, avian uncoupling proteins were up regulated, antioxidant reserves were depleted, and the pattern of antioxidant enzyme activities changed (Akbarian et al., 2016).Weight loss, higher susceptibility to illnesses, increased feed conversion ratio, infertility or sub fertility, lower livability, and immunological suppression are the most prevalent stress symptoms in broilers. Stress can lead to decreased productivity and even mortality if it is experienced frequently and for an extended period of time (Gebregeziabhear et al., 2015).

Unlike, traditional poultry productions, different types of supplements are provided in commercial poultry diets to make-up the losses during transportation because of many stress factors and to obtain rapid weight gain and reduction of production cost (Onyimonyi et al., 2009).Usually, supplements are not only used as protein source but also used as source of some essential vitamins and minerals (Ravindran et al., 1986).

Nucleotides are considered to be semi-essential nutrients in man and animals but they may become essential nutrients in certain situations where intense nucleic acids and protein synthesis is needed, such as the rapid growth and repair of gastrointestinal mucosa (Holen and Jonsson, 2004) the formation of the various cell lines of the immune system (Jyonouchi et al., 1996; Burrells et al., 2001) as well as the normal functioning of the brain and liver (Perez et al., 2004).

Present study has been conducted with two drugs such as Aqua Blok and Poultry tonic. Aqua-Blok is a concentrated liquid gel block containing vitamins, trace elements, essential fatty acids, essential amino acids, electrolytes, nucleotides on a base of prebiotics and rapidly absorbed carbohydrates in a water base. When used alone or in combination as part of a general feeding strategy in broiler and hatching operations, the product appears to have a positive influence on broiler growth and performance (unpublished data). On the other hand, Poultry Tonic is a completely water soluble, liquid vitamin, mineral, electrolyte and nucleotide supplement for poultry intended for use in the drinking water and is unique in that it is totally soluble in water, does not separate out on standing and does not contain any sugar, thereby reducing the risk of microorganism growth and biofilm formation in header tanks, water lines and drinking bells and nipples.

The objective of the present study was to evaluate the effect of Aqua-Blok and Poultry Tonic supplementation at the time of transportation and to measure the performance and carcass yield of broilers.

Specific objectives:

To accurately determine the effects (if any) of routine application of Aqua-Blok and Poultry Tonic to intensively reared broiler chicks and to measure following production parameters:

- Average Weekly Weight Gain (AWWG)
- Feed Conversion Ratio (FCR)
- Mortality rates
- European Production Efficiency Factor (EPEF)
- Water intake
- Uniformity
- Heterophil to Lymphocyte ratio
- Biochemical parameters

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2: REVIEW OF LITERATURE

2.1 World broiler market

In both industrialized and emerging countries, chicken meat production has increased at a higher rate than any other meat since the 1960s. Because of the inherent efficiency in feed conversion and the reduced production costs associated with intensive poultry farming, this growth pattern is projected to continue. Such efficiency in production is especially important to emerging nations, which frequently have limited agricultural resources but large, often impoverished populations (Chang, 2007). Three variables primarily influence the structure of the global broiler market: endowment of resources (as evidenced by, for example, agro-climatic conditions, as well as the availability and accessibility the price of main inputs including land, capital, and labor consumer preferences, feed, and technology), as well as government policies. In terms of volume, the United States, China, Brazil, the European Union (EU 15), Mexico, India, Thailand, Japan, and Canada were the world's top broiler producers. The United States, Brazil, the EU 15, Thailand, China, Canada, Argentina, Hong Kong, and Hungary were the top exporters in the world. Russia, Japan, China, Saudi Arabia, the EU 15, Mexico, Hong Kong, the United Arab Emirates, South Korea, Canada, and Romania were the top importers. The United States, China, Brazil, the EU 15, Mexico, Japan, India, Russia, Saudi Arabia, and Canada were the top consumers. There are exceptions to the general rule that output is positively associated with consumption and exports.

2.2 Bangladesh and Poultry farming

Poultry subsector is an integral part fostering agricultural growth in Bangladesh. Poultry also plays an important role in the subsistence economy and contributes 1.6% in GDP (Alam et al., 2014) in Bangladesh. The per capita meat and egg consumption in Bangladesh is one of the lowest in the world. The average per capita meat and egg recruitment is 43.25 kg and 104 numbers, respectively and the available values are only 9.12 kg and 36 numbers per year (FAO/APHCA 2008). To reduce the gap between the demand and supply of animal protein, poultry can play an important role. Poultry sub-sector is an integral part of the farming system in Bangladesh and has contributed in the direct and indirect economic viability of about 6 million people in Bangladesh. At present this sector is contributing about 14% of gross livestock output which alongside dominating about 37% of the entire meat production in this country (Hamid et al., 2017). Therefore, this has gained an immense growth pace considering the need to supply protein requirement of densely populated developing country like Bangladesh.

According to the recent statistics in 2018-2019, there are about 262.6 million poultry in Bangladesh which is principally dominated by 221.3 million chicken populations (DLS, 2019). But, one of the main limiting factors for poultry production is the environmental stress and insufficiency of balanced feed including vitamins and micronutrients leading unacceptable lack in the output from poultry sector in Bangladesh. So, this is therefore a pre-requisite to establish an appropriate feed selection strategy using suitable growth promoters to limit the stress factors for ensuring sustainable poultry industries in Bangladesh.

2.3 Major issues regarding broiler production

Technological advances in broiler production and marketing have contributed greatly to the success of the poultry industry, as discussed earlier. However, there has been a steady rise in government regulations on, and consumer dissatisfaction with, industrialized poultry production systems (McMullin 2003). Among the particular concerns are:

- The use of antimicrobial growth promoters, animal protein, and genetically modified materials in feeds;
- The impact on the environment;
- Animal welfare; and
- Disease control.

Meeting these extra consumer and government criteria will, of course, have considerable ramifications for future broiler production and marketing, which will, in turn, have implications for cost of production and market competitiveness. Ellendorff (2003) described to these challenges as an "area of conflict in broiler production" are depicted below:

- Price, product quality
- Animal welfare and health
- Environment
- Cost of production
- Market competition

2.4 Transport and poultry

Poultry are imposed by many stressors when they are transported for a long duration of time. For example, thermal changes, acceleration, motion, vibration, fasting, withdrawal of water, social disruption, noise and internal vehicle thermal microenvironment (Abeyesinghe et al., 2001) disturbed the normal micro flora and increased susceptibility to pathogens such as *Salmonella* that binds and colonizes the intestinal epithelium in poultry (Burkholder et al., 2008) and also induced changes in blood composition as well as heart rate, electrolyte concentration, hormone levels, metabolites enzymes, live weight and meat quality.

2.5 Production potentials of broiler

In many researches, average daily feed intake (ADFI), average daily gain or average daily growth (ADG) and feed conversion ratio (FCR) are frequently used growth performance indices (Leeson et al., 2005; Hu and Guo, 2007; Panda et al., 2009; Irani et al., 2011; Zhang et al., 2011b; Chamba et al., 2014; Kaczmarek et al., 2016). Growth performance parameters influence profitability in broiler production; hence they are termed as the economic traits of broilers.

2.5.1 Average daily growth, daily feed intake and FCR

Body weight, feed efficiency, livability and dressing yield are commonly used economic traits in broiler farming (Prabakaran, 2003). Improvement in average daily feed intake and average daily growth consequently results in good FCR. FCR is obtained by calculating by the ratio of total input to total output, considered as the most important performance index in broiler farming because it largely determines the profit margin (Prabakaran, 2003).

2.5.2 Water consumption

Water is a vital nutrient of all living organism. It is involved in many metabolic processes including body temperature control, digestion and absorption of food, transport of nutrients, and the elimination of water products, via urine, from the body of poultry (Jafari et al., 2006). A number of differing and even cumulative factors have influential effect on the volume of water consumed by birds (Manning et al., 2007).To monitor the birds' water consumption by house in liters/1000 birds per day is a well-accepted practice (Defra 2002 and ACP, 2006). An expected level of water consumption may be increased or decreased which is an indication of a health problem (Butcher et al., 1999). If the concentration of ammonia in the air exceeds certain levels, inadequate control of moisture in the house environment can lead to various health and welfare issues including contact dermatitis, ascites and respiratory disease (Manning et al., 2007).

External air humidity, type and management of drinker system, water consumption, stocking density, bird age and weight, ventilation rates, temperature profile and disease status of the birds are the factors which have an impact on internal air humidity (Scahaw, 2000). 1000 mature birds can excrete half a ton of water per day (Pattison et al., 2007) thus management procedures which effectively manage air and moisture of litter are crucial to bird health and welfare. The dry matter content of chicken faeces is around 20% and the moisture content is driven by water consumption (Broadbent and Pattison, 2003). There is a relationship between feed and water consumption has been demonstrated by research (Georgia, 2001, and Lott et al., 2003). Further it has been argued that water consumption increases by 6% for every 1ºC rise in temperature from 20ºC where it approximates to 1.8-2.0 times feed quantity and that feed intake is reduced by 1.23% for every 1ºC rise in temperature and by 5% for every 1ºC rise between 32-38ºC (Singleton, 2004).

The air velocity in the poultry houses has also been shown to have an impact on broiler performance and also there on feed and water consumption (May et al., 2000).

2.5.3 Stocking density

The optimum stocking density for broilers is debated among broiler companies, farmers, feed retailers, whole sellers and animal welfare activists (FMI-NCCR, 2003). Maintaining a high stocking density is a common practice as it allows an increase economic return per unit or space. High stocking density increase of ammonia production, foot pad lesions and decrease feed consumption (Dozier et al., 2005; Bandyopadhyay et al., 2006). There was no significant difference among three stocking densities at day 1st and 7th observations. However, significant differences in the least square means of body at day 14, day 28 and day 42 have been recorded (Singh et al., 2014).

According to Simitzis et al., (2012) the higher stocking density negatively affects final body weight and feed intake but not cumulative feed conversion rate. Muscle color traits, pH, cooking loss and shear values are not affected. Birds reared at the lower density shows higher intramuscular fat, liver weight, liver NADP-isocitrate and NADP-malate dehydrogenase activity. Higher stocking density is associated with decreased locomotors activity and increased physiological (H: L ratio and bursa weight) and oxidative (glutathione concentrations and reduced: oxidized glutathione ratios) stress indicators. There are differing opinions if stocking density affects feed consumption, mortality rate and carcass quality (Feddes et al., 2002), but high stocking density reduces access to feed and water (Sikder et al., 2012).

2.5.4 Mortality rate

Factors such as rearing season (Imaeda, 2000), shipping distance and delivery route (Chou et al., 2004), stocking density, flock size, feeding management, drinking system, ventilation, and floor insulation at the broiler farm (Heier et al., 2002) are related to first week mortality (FWM). According to Heier et al. (2002), the mortality of large flocks and flocks with a high stocking density was significantly lower than in small flocks and flocks with small density. In addition, Chou et al. (2004) found the lowest cumulative FWM in broiler chicks raised in rooms with open-curtain ventilation (1.30%) than those raised in rooms with negative-pressure ventilation (1.42%) and water-cooled ventilation (1.37%).

2.6 Nutritional Supplement of poultry

Nutraceuticals are the nutrients or constituents of animal diet that have nutritional and pharmaceutical importance by preventing various diseases, possessing immunomodulatory potential, providing health benefits and consequently increasing productivity (Dhama et al., 2015; Aronson 2017; Helal et al., 2019; Janabi et al., 2020). They include nutrients and non-nutrients, like amino acids, minerals, vitamins, fatty acids, enzymes, prebiotics, probiotics, synbiotics, pigments, medicinal herbs, herbal extracts, antioxidants, organic acids, flavouring agents, etc. (Alagawany et al., 2018a; Elgeddawy et al., 2020).

2.6.1 Vitamins as nutraceuticals

Vitamins are essential nutraceuticals, required for the optimum general health and physiological functions such as development, growth, maintenance and reproduction.

Vitamins exert catalytic functions that facilitate nutrient synthesis, thus controlling metabolism and affecting the performance and health of poultry. Vitamins in poultry feeds have two origins; they are natural components of the ingredients used to prepare the diet and they can be added as a supplement in a concentrated form (Whitehead 2002). There are many vitamins (fat-soluble vitamins: A, D, E and K; and water-soluble vitamins: B1, B2, B6, B12, folic acid, pantothenic acid, biotin, niacin and vitamin C) needed for optimal poultry health. The use of these nutrients in sufficient quantities can improve animal health. Most vitamins cannot be synthesized by birds and must be provided by feed, however, the feed alone is not sufficient to cover vitamin requirements. Diets supplemented with vitamins play an important role in disease treatment and prevention; because vitamins allow an animal to use proteins and energy for health improvement, FCR, growth, and reproduction (Whitehead 2002; McDowell and Ward 2008).

2.6.2 Amino acids as nutriceuticals

Ten amino acids classified as essential (lysine, methionine, tryptophan, threonine, arginine, isoleucine, leucine, histidine, phenylalanine and valine) must be provided in the diet for maximum performance. Out of these 10 essential amino acids, lysine and methionine are the first two limiting amino acids for broilers (Corzo et al., 2007; Rehman et al., 2019), while threonine is the third limiting amino acid (Kidd and Kerr 1996). Glycine is considered to be essential for young birds. Glycine and serine are the non-essential limiting amino acids in the diet of poultry (Siegert and Rodehutscord, 2019). Cysteine and tyrosine are recognized as semi-essential amino acids because they can be synthesized from methionine and phenylalanine, respectively (Ravindran 2012). The development of immune function in poultry will be promoted if they receive sufficient amino acids in their diets.

2.6.3 Minerals as nutraceuticals

In poultry, minerals are required as a part of an activator of hormones and enzymes, for the skeleton and eggshell formation and replacement, and for the maintenance of acid-base balance (sodium (Na), potassium (K) and chloride (Cl)) and osmotic homeostasis (Ravindran 2012). Organic minerals in small amounts can be added to bird diets as minerals are better assimilated by poultry than mineral salts (Nollet et al., 2007; Ravindran 2012).

Trace minerals

Mn, Zn and Cu are structural constituents and catalytic of the antioxidant enzyme - superoxide dismutase (SOD) and also act on immunity mediators such as thymus peptides, cytokines and enzymes (Silva et al., 2015). Zn and Mn are co-factors involved in the carbonates and mucopolysaccharides synthesis which is necessary for bone formation (Swiatkiewicz et al., 2014).

2.7 Risk factor associated with poultry production

Each individual farm or flock has its own risk profile for the introduction of pathogens, subsequent development of disease, and spread of disease to other farms. This is influenced by a number of factors, including: the density of farms (Marangon et al., 2004), especially for agents in which rate of transmission is density dependent (e.g. airborne spread) (Truscott et al., 2007); and the linkages between different farms through production and market chains, which may lead to disease transmission that is density independent (e.g. spread via fomites).

2.8 Stress indicators

Stress can suppress the growth rates of animals. Measurement of corticosterone levels as an indicator of stress (Lentfer et al., 2015; Matur et al., 2016). During stress condition in broiler, glucocorticoid hormones secretion is increased, which in turn affect in decrease in the level of lymphocyte in the blood. Number of leucocytes increase during mildly or moderately stressful conditions and consequently the heterophil and lymphocyte ratio can be used to detect the physiological stress for most stressors (Maxwel and Robertson, 1998). Differential leucocyte counts include proportion of heterophil (neutrophil), lymphocyte, monocyte, eosinophil, and basophil. Heterophil acts against bacterial infection in any infected area and destroys the pathogens through oxygen channels both independently and dependently. This value of broiler is highly influenced by animal genetics, stress level, environmental conditions (maintenance and overall transportation process), and adequacy in feed nutrients. In contrast, lymphocyte produces circulating antibodies in the blood and cellular immune response.

The stress level of broilers can be measured by various means, and the heterophil to lymphocyte ratio (H/L ratio) is the most reliable methods (Mashaly et al., 2004). Several studies concluded that H/L ratio is a more reliable indicator of the perceived magnitude of stress than plasma corticosterone values in avian species. Heterophils are important phagocytic cells in avian species. Matur et al. (2016) reported that reflection of social stress was evaluated by heterophil and lymphocyte percentage and H/L ratio. Gross and Siegel (1983) reported that the H/L ratios of 0.2, 0.5 and 0.8 corresponds to low, medium and high degrees of stress in chickens, respectively. Physiological and physical stressors tend to increase the H/L ratio (Maxwell et al., 1998). During stress conditions, the heterophil percentage is higher, the H/L ratio is higher and lymphocyte percentage is lower.

2.9 Production cost

In the prevailing market conditions, the reduction in final stocking density will result in a decline in net revenue from broiler production, according to the findings of the study of Utnik-banas et al., (2014). Small farms have greater fixed costs per production area unit than large ones. Fixed expenses were highest in farm with 950 m^2, accounting for 10.7% of expenditures involved in producing 1 kilogram of cattle, and a drop in stocking density to 33 kg m–2 would raise this proportion to 14.3%. The profitability of broiler production is determined by the meat price and the cost of production inputs, which are determined by production performance and market circumstances (Gallot and Champagne, 2010).

CHAPTER 3

METHODOLOGY

CHAPTER 3: METHODOLOGY

3.1 Study period and study area

The study was conducted from March to April 2020.In this study, chicks were reared for a period of 30 days where daily food and water intake, sick and dead mortality rate were measured and body weight of chicks was taken weekly basis i.e., at day 0, 7,14, and 28.

For fulfilling the objectives of our study, we selected a farmer, who is experienced in broiler farming for 20 years at Chotodarogar Hat, Shitakunda, Chattogram. To maintain a similar environment, we made a deal with the farmer to use his three broiler rearing sheds.

3.2 Study Design

This study was in the form of a randomized, partially blinded, controlled clinical study consisting of four groups. The chicks were categorized into four different groups: Group 1(standard farm management), Group 2 (Aqua Blok®& Poultry tonic®), Group 3 (Aqua Blok®) and Group 4 (Chick tonic®) of 750 chicks in each group.

Group 1 was received all treatments, vaccinations, management comprising normal practice by one farm.

Group 2 was supplemented with Aqua Blok® at the hatchery at the rate of 1 gm per chick, i.e., 50 g per 50 chicks in transport crates/box until releasing within the chick guard in the next morning. Later on, Poultry tonic® was offered for 5 days from day 1 to day 5 treatment period. Poultry tonic was mixed in the bulk water container before offering in the Bell drinker.

Group 3 was supplemented with Aqua Blok® at the hatchery at the rate of 1 gm per chick, i.e., 50g per 50 chicks in transportable crate.

Group 4 was supplemented with commercial Chick Tonic® for 5 days from day 1 to day 5.

Table 3. 1: Treatment Groups

Group	No. of Birds	Treatment	Dosages	Treatment Time
1	750	Standard farm management + Feed	1gm/2 liters of water.	Standard Farm management. Brood Care was administered to chicks from day 1 to day 5.
2	750	Aqua Blok+ Feed	1 gm/bird	The distance of farm from hatchery is about 1 km but the chicks were travelled for 5 hours before reaching in the farm, the chicks were remaining in the crate overnight, and the brooding of chicks was started in the next morning (8.0 am). Aqua Blok was remained in the crate until starting of brooding.
		Poultry Tonic + Feed	1:500	Poultry tonic was treated for 5 days since starting of brooding (day 1 to day 5).
3	750	Aqua Blok+ Feed	1gm/bird	Same as group 2.
4	750	Chick Tonic	1:500	Chick tonic was treated for 5 days since starting of brooding (day 1 to day 5).

Note: We have tried to accommodate 3000 birds with the same environment in different sheds.

The feed changing schedule and vaccination schedule are summarized in table 3.4 and table 3.5 respectively. Average weekly weight gain, FCR, mortalities and EPEF were calculated as per house at the end of treatment period (30 days).

Table 3. 2: Active ingredients of Aqua Blok and Poultry tonic

Active ingredients	Aqua Blok	Poultry tonic
Vit. A	5000iu	5lac
Vit. D_3	750iu	75000iu
Vit.E	100mg	10000mg
Vit. B_1	12.5mg	1250 mg
Vit. B_2	5mg	500mg
Vit. B_6	6.25 mg	625mg
Vit. B_{12}	53mcg	5300mcg
Vit. K_3	3.5mg	350mg
Biotin	12.5 mcg	1250mcg
Vit. C	10mg	1000mg
Niacin	14mg	1400mg
Folic acid	6.25mg	625mg
Pantothenic acid	6.25mg	625mg
Lysine HCl	10mg	1000mg
Methionine	15mg	1500mg
Amino acid profile	10mg	1000mg
Iodine	5mg	500mg
Selenium	1.25mg	125mg
Cobalt	5mg	500mg
Copper	20mg	2000mg
Manganese	10mg	1000mg
Zinc	30mg	3000mg
Sodium salt	98mg	9.815g
Potassium salt	884mg	82.430 g
Magnesium salt	4.6mg	455mg
Citric acid	125 mg	3.28 g
Essential fatty acid	5mg	500mg
Nucleotides	50 mg	5000mg

3.3 Management of experimental birds

3.3. a. Housing& Brooding of chicks

Before arrival of chicks to the house, we had prepared sheds according to standard management systems. A total of 3000-day old Cobb 500® chicks were purchased from a commercial hatchery (CP hatcheries Bangladesh Limited), located in Sitakunda, Chattogram. A total of 60 crates were taken from the hatchery where each crate contained 50 chicks at 5 P.M. A total of 50 chicks were contained in each crate. Immediately before starting transportation of chicks by open motor van from the hatchery to the farm, almost 50 g Aqua Blok® @ 1gm/piece/chick was placed in four corners of the crate (each crate contains four small compartment) at the premises of the hatchery and we had brought the chicks in the farm after traveling nearly 5 hours and kept the crates within the respective shed, as per calculation previously, for overnight. In next morning we had released the chicks in the brooder from crates. The chicks were brooded in a deep litter house which was properly cleaned by using disinfectant before the arrival of the chicks. Wood shavings were used as litter material and drinkers were provided as per standard protocol. Feed was manually supplied by sprinkling for first 12 hours and then feeder is introduced to chicks. We measured the body weight of a total of 50 chicks /group at day 2 of chicks reared since unavailability of electronic balance at day 1. The study period was 30 days long. A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding which was changed to 200-watt energy bulb from day10.

3.3. b. Feeds and Feeding

All the broilers were fed with broiler pre-starter for 10 days (from 0 to 10days of age) and broiler starter for10 days (from 11 to 20 days) and broiler grower for 10 days (from 21 to 30 days). Feed and water were provided adlibitum (using plastic drinkers and galvanized feeder). On Day 1 of rearing, feeds were offered on paper in sprinkle manner for 8 hours (From 8.0 A.M. to 4.0 P.M.). Auto feeder and drinker were not used. Both feed and water were offered for two times (8.0 A.M. and 4.0 P.M.).

The remaining feed and water in the feeder and drinker were deducted from the offered amount. We recorded the data and events in record keeping sheet attached at Annex I.

Table 3. 3: Feed Composition

Feed	Crude protein (%)	Fat (%)	Crude fiber (%)	Moisture (%)
Broiler Pre-Starter	21.50	3.50	5.00	12.00
Broiler Starter	20.00	3.00	5.00	12.00
Broiler Grower	19.00	3.00	5.00	12.00

Table 3. 4: Feed changing schedule

Day	Change from	Change to
10	Broiler Pre-Starter	Broiler Starter
20	Broiler Starter	Broiler Grower

3.3. c. Vaccination

Vaccine was given for commonly found diseases in broiler like Newcastle Disease, Infectious bursal disease etc. The chicks were vaccinated following the mentioned below:

Table 3. 5: Vaccination schedule

Day	Name of vaccine
4	ND/BCRDV
10	IBD
17	ND/BCRDV

3.4 Facilities, equipment and product required

Facilities: All trial houses in the same compound and in the similar environment. The products were in cool, secure place (or within a convenient distance from) each study site in which product may be stored.

Equipment:

- Ambient temperature thermometers×2
- Clipboards
- Electronic balance
- Brooder/Chick guard
- Circular feeder
- Bell drinker
- Rake

Product:

- Aqua-Blok

- Poultry tonic
- Chick tonic
- Brood care

All products were delivered to farms by investigator and products were received by the responsible farm staff on the Product Receipt Form in Annex II. Products were kept in a secure, locked facility at all times.

3.5 Data collection, Growth performance calculation

Study personnel were not blinded as they were directly responsible for ensuring that the correct products were administered to each house in the various groups. Staff responsible for recording and calculating parameters such as daily feed intake, water intake, weekly weight gain, and mortalities was blinded as far as possible. Weekly feed conversion ratio (WFCR), Weekly feed intake (WFI), Weekly body weight gain (WBWG), Weekly water intake (WWI), Weekly European production efficiency factor (WEPEF), and Weekly mortalities (WM) were assessed and recorded for each group according to the modified formula by Ayssiwede et al., (2011), such as-

WFI (g/bird/week)={(Quantity of feed offered-Quantity of feed left)/week ÷ Number of birds}

WBWG (g/day) =Weight Gain of the period (g) ÷ Length of the period (days)

WFCR=Feed intake during a period (g) ÷Weight gain of the period (g)

Flock uniformity %=100-(Standard deviation (g)/average body weight (g) ×100).

European Production Efficiency Factor (EPEF):

To compare broiler performance from different flocks we have used the European Production Efficiency Factor (EPEF). This factor standardized technical results, taking into account feed conversion, mortality and daily gain. The formula is: (Average grams gained/day X % survival rate)/Feed Conversion X 10.

3.6 Blood collection and differential leucocyte count

Ten, day 2 aged chicks from every group were collected and had been sacrificed to collect blood. Then blood of chicks(5 ml) was taken into vial with anticoagulant and brought to research laboratory under department of Physiology, Biochemistry and Pharmacology, CVASU for counting different leucocyte and the methods are attached in annex.

3.7 Biochemical analysis

Blood was collected by sacrificing of five, day 2 aged baby chick in vial without anticoagulant then shifted to research laboratory. Proper aseptic measures were followed during the time of serum analysis and kept in refrigerator until analysis had done. Before analysis of serum, specific reagents were added to serum then kept for 5-10 minutes. All the samples were then vortexed to make the sera homogenous in nature. The sera were assayed for different biochemical parameters such as albumin, uric acid, sodium and potassium level were measured using a biochemical analyzer (Humalyzer 3000, Germany) using commercial kits.

3. 8 Data analysis

All recorded and collected data were entered into the MS Excel-2010, sorted out and exported into Statistical Package for Social Sciences 2020(SPSS-20). Descriptive statistics were performed including mean, standard deviation, percentage. A one way analysis of variance was done.

CHAPTER 4

RESULTS

CHAPTER 4: RESULTS

The findings of different production parameters of broiler chicken (Cobb 500®) reared at intensive production systems are shown weekly basis and finally at marketing age on day 30.

Table 4. 1: Status of production parameters in different treatment groups at the end of 1^{st} week of the study period (n=750)

Parameter	T_1	T_2	T_3	T_4
Day 2 old chicks' body weight (Kg)/flock	46.6	47.6	47.3	47.3
Total body weight at end of 1^{st}week (Kg)/flock	136.6	135.7	136.6	140.7
Total body weight gain at end of 1^{st} week (Kg)/ flock	89.9	88.1	89.3	93.4
Av. Daily weight gain (Kg) up to 7 days/flock	14.9	14.7	14.9	15.6
Av. Daily weight gain (g) up to 7 days/bird	19.9	19.6	19.8	20.8
Cumulative Feed Intake (Kg) up to 7 days/ flock	121	122	122	124
Cumulative Feed Intake (Kg) per bird up to 7 days /bird	0.1613	0.1626	0.1626	0.1653
Average daily feed intake(g)/bird/day	23.0	23.2	23.2	23.6
Feed conversion ratio (FCR)	1.35	1.38	1.37	1.33
Cumulative Mortality Rate (n, %)	7(0.93)	9(1.2)	14(1.87)	9(1.2)
Survival Rate (n, %)	743(99.07)	741(98.8)	736(98.134)	741(98.8)
European production efficiency factor (EPEF)	147.35	140.32	142.4	154.5

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

The production parameters obtained at the end of first week of broiler rearing is shown in table 4.1. Total body weight at day 2 old chicks' body weight/flock was around 46.6 Kg in T_1 (lowest) and the highest total body weight at the same day was found 47.6 kg in T_2. The other flocks (T_3 and T_4) total body weight per flock was found 47.3 kg. Average daily weight gain/bird up to day 7 was found 19.9 g, 19.8 g, 19.8g and 20.8 g in T_1, T_2, T_3 and T_4 respectively. The feed conversion ratio was found the lowest in T_4 whereas the highest FCR was found in the T_2. At the end of the week, the European production efficiency factor was found the highest in T_4 and the lowest in the T_2.

Table 4. 2: Status of production parameters at different treatment groups at the end of 2nd week of the study period (n=750)

Parameter	T_1	T_2	T_3	T_4
Day 2 old chicks' body weight (Kg)/flock	46.6	47.6	47.3	47.3
Total body weight at end of 2nd weeks (Kg)/flock	390	390	390.975	411.975
Total body weight gain at end of 2nd weeks (Kg)/ flock	343. 4	342. 4	343.6	364.7
Av. Daily weight gain (Kg) up to 14 days/flock	26.4	26.3	26.4	28.1
Av. Daily weight gain (g) up to 14 days/bird	35.2	35.1	35.2	37.4
Cumulative Feed Intake (Kg) up to 14 days/flock	435	437	445	446
Cumulative Feed Intake (Kg) per bird up to 14 days/bird	0.58	0.5826	0.5933	0.5946
Average daily feed intake(g)/bird/day	41	41.6	42.3	42.4
Feed conversion ratio (FCR)	1.27	1.28	1.29	1.22
Cumulative Mortality Rate n (%)	12(1.6)	15(2)	18(2.4)	12(1.6)
Survival Rate n (%)	738(98.4)	735(98)	732(97.6)	738(98.4)
European production efficiency factor (EPEF)	273.69	269.7	266.32	301.65

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

The production parameters obtained at the end of second week of broiler rearing is shown in table 4.2. Average daily weight gain/bird up to day 14 was found 35.2 g, 35.1 g, 35.2 g and 37.4 g consecutively in T_1, T_2, T_3 and T_4. The feed conversion ratio was found the lowest in T_4 whereas the highest FCR was recorded in the T_3. At the end of the week, the European production efficiency factor was found the highest in T_4 and the lowest in the T_3.

Table 4. 3: Status of production parameters at different treatment groups at the end of 3rd week of the study period (n=750)

Parameter	T_1	T_2	T_3	T_4
Day 2 old chicks' body weight (Kg)/flock	46.61	47.6	47.3	47.3
Total body weight at end of 3rd weeks (Kg)/flock	664.5	662.9	677.85	707.55
Total body weight gain at end of 3rd weeks (Kg)/ flock	617.9	615.3	630.5	660.3
Av. Daily weight gain (Kg) up to 21 days/flock	30.9	30.8	31.5	33.0
Av. Daily weight gain (g) up to 21 days/bird	41.2	41.1	42.0	44.02
Cumulative Feed Intake (Kg) up to 21 days/flock	970	942	955	976
Cumulative Feed Intake (Kg) per bird up to 21 days/bird	1.2933	1.256	1.273	1.3013
Average daily feed intake(g)/bird/day	61.5	59.8	60.6	61.9
Feed conversion ratio (FCR)	1.57	1.53	1.51	1.48
Cumulative Mortality Rate n (%)	22(2.93)	24(3.2)	24(3.06)	18(2.4)
Survival Rate n (%)	728(97.07)	726(96.8)	726(96.94)	732(97.6)
European production efficiency factor (EPEF)	254.8	260.03	269.25	290.29

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

The production parameters recorded at the end of 3rd week of broiler rearing is shown in table 4.3. Average daily weight gain/bird up to day 21 days was found 41.2 g, 41.1 g, 42 g and 44.02 g consecutively in T_1, T_2, T_3 and T_4. The feed conversion ratio was found the lowest in T_4 whereas the highest FCR was found in the T_1. At the end of the week, the European production efficiency factor was found the highest in T_4 and the lowest in the T_2.

Table 4. 4: Status of production parameters at different treatment groups at the end of 4^{th} week of the study period (n=750)

Parameter	T_1	T_2	T_3	T_4
Day 2 old chicks' body weight (Kg)/flock	46.6	47.6	47.3	47.3
Total body weight at end of 4^{th}weeks (Kg)/flock	1039.6	1006.6	1001.8	1057.04
Total body weight gain at end of 4^{th}weeks (Kg)/ flock	992.95	959.04	954. 47	1009.79
Av. Live weight at marketing age (g) per bird	1386.13	1342.13	1335.73	1049.39
Av. Daily weight gain (Kg) up to 28 days per flock	36.78	35.52	35.35	37. 40
Av. Daily weight gain (g) up to 28 days per bird	49.0	47. 4	47.1	49.9
Cumulative Feed Intake (Kg) up to 28 days/flock	1550	1512	1525	1521
Cumulative Feed Intake (Kg) per bird up to 28 days/bird	2.0666	2.016	2.0333	2.028
Average daily feed intake(g)/bird/day	73.8	72	72.6	72.4
Feed Conversion Ratio	1.56	1.58	1.60	1.51
Cumulative Mortality Rate n (%)	33(4.4)	32(4.27)	38(5.07)	29(3.87)
Survival Rate n (%)	717(95.6)	718(95.73)	712(94.93)	721(96.13)
European Production Efficiency Factor (EPEF)	300.28	287.19	279.45	317.67

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

The production parameters recorded at the end of 4^{th} week broiler rearing is shown in table 4.4. Average daily weight gain/bird up to day 28 days was found 49 g, 47.4 g, 47.1 g and 49.9 g respectively in T_1, T_2, T_3 and T_4. The feed conversion ratio was found the lowest in T_4 whereas the highest FCR was found in the T_2. At the end of the week, the European production efficiency factor was found the highest in T_4 and the lowest in the T_2 group respectively.

Table 4. 5: Water intake of different treatment group (n=750)

Treatment group	CWI/flock/day up to 1st week (L)	DWI /bird up to 1st week(L)	CWI/Flock/day up to 2nd week (L)	DWI /bird/day up to 2nd week(L)	CWI/Flock/day up to 3rd week (L)	DWI/bird /day up to 3rd week (L)	CWI/Flock/day up to 4th week (L)	DWI/bird /day up to 4th week (L)
T_1	46.85	0.062	80.15	0.1068	118. 42	0.1579	146.86	0.1958
T_2	47.43	0.063	80.57	0.1074	117.76	0.15701	144.93	0.1932
T_3	46.86	0.062	80.07	0.1067	117. 43	0.15657	145.39	0.1938
T_4	46.86	0.062	79.86	0.1065	119.902	0.1598	145.64	0.1942

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed); CWI, Cumulative water intake; DWI, Daily water intake

Weekly water intake in different treatment groups is portrayed in table 4.5. The cumulative water intake at the end of 1st week of treatment period was observed in treatment group 1 (47.43 L/flock) whereas the water consumption was almost equal (about 46.86 liter) in the remaining treatment groups. At the end of 4th week of treatment period the cumulative water intake/flock/day was found the highest in the T_1 (146.86 L/flock/day) and the lowest water intake was observed in T_2. The cumulative water intake per bird/day was 0.1932 L in the group of birds treated with Aqua Blok and Poultry tonic.

Table 4. 6: Environmental temperature and air relative humidity during the experimental period

Week of age	Environmental temperature (°C)			Air relative humidity (%)		
	Minimal	**Maximal**	**Mean ± SD**	**Minimal**	**Maximal**	**Mean ± SD**
1	29	34	30.86	-	-	-
2	24.7	36	29.87	50	87	64.43
3	31	36	33.86	33	74	48.57
4	32	35	33.71	41	82	53.29
Over all	**24.7**	**36**	**32.34 ± 2.94**	**33**	**87**	**56.71 ± 15.28**

The recorded environmental temperature and relative humidity in the farm houses are shown in table 4.6. The overall environmental temperature was 32.34° C with the range between 24.7 to 36° C and the overall relative humidity was recorded 56.71% that ranged between 33 to 87%.

Table 4. 7: Mortality rate of broiler chicks in different treatment groups during study period (n, %)

Treatment Group	1st week	2nd week	3rd week	4th week	Cumulative mortality up today 30
T_1	7(0.93)	5(0.67)	10(1.33)	11(1.47)	33(4.4)
T_2	9(1.2)	6(0.8)	9(1.2)	8(1.07)	32(4.23)
T_3	14(1.87)	4(0.53)	6(0.8)	14(1.87)	39(5.2)
T_4	9(1.2)	3(0.4)	6(0.8)	11(1.47)	30(4.0)
Over all	39(1.3)	18(0.6)	31(1.03)	44(1.47)	134(4.47)

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

Mortality rate of broiler chicks during 30 days of rearing period is shown in table 4.7. The overall mortality rate considering all study population had been fluctuating in different weeks during 30 days of production cycle. The highest mortality rate was found (1.47%) in the 4^{th} week of rearing whereas the lowest mortality rate was observed in the 2^{nd} week of rearing (0.6%). At the end of the study period the highest mortality rate was recorded in T_3 (5.2%) while the mortality rate was found the lowest in T_4 (4.0%).

Table 4. 8: Body weight and Flock uniformity during the treatment period of Aqua Blok in Broiler chicken

Treatment Group	Day -2 (n=50)		1st week (n=150)		3rd week (n=150)		4th week (n=150)	
	BW(g) Mean ± SD	FU (%)	BW(g) Mean ± SD	FU (%)	BW(g) Mean ± SD	FU (%)	BW(g) Mean ± SD	FU (%)
T_1	62.14±4.49	92.77	182.13±14.34	92.13	885.33±113.74	87.15	1458±99.18	93.2
T_2	63.46±4.25	93.30	180.99±15.66	88.41	883.8±113.33	81.68	1419.8±140.79	90.08
T_3	63.12±4.66	92.62	182.13±15.44	91.52	903.8±120.45	86.67	1419±102.99	92.74
T_4	63±5.47	91.32	187.55±15.04	91.98	943.4±94.82	89.95	1472.2±139.32	90.54

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed), BW, Body weight; FU, Flock uniformity.

The body weight and flock uniformity during 28 days of treatment period of Aqua Blok and Poultry tonic is depicted in table 4.8. The average initial body weight at Day 2 was found the lowest in T_1 **(**62.14g) and found the highest in T_2 (63.46g). The lowest flock uniformity at Day 2 of rearing was 91.32 % in the T_4 on the other hand it was the highest in the T_2 (93.3%). At the end of 4th week of treatment of Aqua Blok and Poultry tonic, the highest average live weight gain (1472g/bird) was found the highest in the T_4 while the lowest average live weight (1419 g/bird) was recorded in the T_3. At the end of 4th week of treatment period the highest flock uniformity was observed 93.2 % in T_1 but lowest flock uniformity (90.08%) was recorded in the T_2.

Table 4. 9: Final Production Figure

Treatment group	DOA's(n/flock)	ALM(g)	Mortalities (%)	ADG (g)	FCR	Mortalities Total	Total mass (Kg)	PEF	Square feet/bird(birds/meter^2)
T_1	2	1455.5	4. 4	48.1	1.67	33	1091.6	275.4	1.09(9.61)
T_2	1	1386.4	4.23	47.8	1.61	32	1039.8	287.3	0.54(19.81)
T_3	1	1349.6	5.20	44. 4	1.67	39	1012.2	252.1	0.54(19.81)
T_4	0	1492.4	4.00	49.3	1.64	30	1119.3	288.58	1.12(9.91)

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

ALM: Average Live Mass
ADG: Average Daily Gain
FCR: Feed Conversion Ratio
PEF: Production Efficiency Factor
DOA's: Dead on Arrival

Final production parameters at the end of 30 days treatment are shown in table 4.9. In T_1, initial mortality of chicks on arrival at the farm was 2, while inT_4 dead on arrival of chicks was observed zero (0). The highest mortality rate was observed in T_3 (5.2%), and the lowest mortality was found in T_4 (4.00 %). Although the highest average live mass was observed in T_4, the lowest feed conversion ratio was found in T_2 (1.61), which means that the chick took 1.61 kg of feed to produce 1 kg of meat. And in the T_4, the chick took 1.64 kg feed to produce 1 kg of meat.

Table 4. 10: Production cost in different treatment groups at the marketing age (on day 30) considering the feed cost (n=750)

Parameter	T_1	T_2	T_3	T_4
Total body weight gain at marketing age (Kg)/flock	1091.6	1039.8	1012.2	1119.3
Total Feed Intake (Kg)/flock	1740	1595	1610	1755
Total feed cost @ 35TK per Kg/flock	60,900	55,825	56,350	61,425
Total cost/Kg broiler product (TK.) at the marketing age	55.79	53.69	55.67	54.89
Difference of per kg broiler production cost (TK.) as compare to T_2 group	2.1	0	1.98	1.2
Amount of increased production cost per flock as compare to T_2 group (@BDT)	2292.36	0	2004.16	1343.16

Note: The remaining operational costs are same for all flocks. T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed**)**

The table 4.10 depicted the production cost @ BDT of broiler product considering feed cost without other operational costs. The cost of per kg broiler at the marketing age is Tk. 55.79, Tk. 53.69, Tk. 55.67, and Tk. 54.89 respectively in Treatment group 1, 2, 3 and 4. The lowest production cost per kg broiler was found in treatment group 2 as compare to other treatment groups. Comparatively the highest production cost per flock was found in control group (T_1), followed by T_3 (Aqua Blok + Feed), T_4 (Chick tonic + Feed) thanT_2 (Aqua Blok + Feed + Poultry tonic) group based on feed cost since remaining costs were the same for all treatment groups.

Table 4. 11: Differential Leukocyte Counts (DLC) in different treatment groups (N=17)

Parameter	Treatment Group	N	Mean (%)	95%CI (%)		P-value
				Lower Bound	Upper bound	
Lymphocyte	T_1	4	70.25	66.97	73.53	.286
	T_2	4	68.75	61.13	76.37	
	T_3	5	71.00	65.81	76.19	
	T_4	4	62.50	43.56	81.44	
Heterophil	T_1	4	16.25	10.68	21.82	.138
	T_2	4	13.75	6.13	21.37	
	T_3	5	13.00	9.59	16.40	
	T_4	4	23.00	5.14	40.86	
Eosinophil	T_1	4	6.25	2.27	10.23	.113
	T_2	4	6.75	1.99	11.50	
	T_3	5	11.00	5.81	16.19	
	T_4	4	6.25	2.27	10.23	
Monocyte	T_1	4	5.50	3.91	7.09	.443
	T_2	4	6.00	1.69	10.31	
	T_3	5	5.00	5.00	5.00	
	T_4	4	7.00	3.10	10.89	
Basophil	T_1	1	3	-	-	
	T_2	2	2	2	2	
	T_3	0	-	-	-	
	T_4	0	-	-	-	
Heterophil and Lymphocyte Ratios	T_1	4	0.23	0.15	0.32	.125
	T_2	4	0.20	0.07	0.33	
	T_3	5	0.18	0.13	0.25	
	T_4	4	0.40	-0.01	0.82	

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

Differential leukocyte counts in different treatment groups of broiler chicken were shown in table 4.11. The lymphocyte counts found the lowest in T_4 treatment group as compared to other treatment groups, suggesting that the chicks of this group were under stress as compared to other treatment groups. The ANOVA test result for Heterophil - lymphocyte ratio were not differ significantly (P>0.05) between the 4 treatment groups. However, the H-L ratio found higher in T_4 than the other counterpart groups. This finding suggesting that the T_4 group was more in stressed conditions.

Table 4.12: Bonferroni Pairwise Comparison of Heterophil and Lymphocyte Ratio

Mean	Vs	Mean	P-value	Mean Difference	Lower bound	Upper Bound
0.23^{T1}	Vs	0.20^{T2}	1.00	2.5	-2.69	.33
0.23^{T1}	Vs	0.18^{T3}	1.00	3.25	-.24	.33
0.23^{T1}	Vs	0.40^{T4}	.573	-6.75	-.47	.13
0.20^{T2}	Vs	0.18^{T3}	1.00	.75	-.27	.097
0.20^{T2}	Vs	0.40^{T4}	.337	-9.25	-49	.097
0.18^{T3}	Vs	0.40^{T4}	.185	-10.0	-.50	.06

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)

Bonferroni pairwise comparison test results showed higher difference between T_3 and T_4 groups, though there were no statistically significant difference (p=0.185) (Table 4.12). We noticed that there was no difference in H_L ratio between T_1 and T_2, T_1 and T_3, T_2 and T_3 but difference was present in between T_1 and T_4, T_2 and T_4, T_3 and T_4.

Table 4.13: Biochemical analysis of serum of broiler chick at 2nd day

Parameter	T_1	T_2	T_3	T_4	P value
Uric acid(mg/dl)	8.1 ± 1.4	8.3 ± 0.28	6.6 ± 1.98	8.6 ± 4.14	NS
Albumin (g/dl)	32.1 ± 1	24.15 ±1.91	30.25 ± 6.72	41.05 ± 22.27	NS
Na(mmol/l)	150.25 ± 14.07	135.45 ± 7.56	147.75 ± 17.32	175.05 ± 7	NS
K (mmol/l)	25.15 ± 5.59	24.9 ± 2.26	27 ± 1.13	33.3 ± 3.54	NS

Note: T_1 (Control, Standard farm managements + Feed); T_2 (Aqua Blok + Feed + Poultry tonic); T_3 (Aqua Blok + Feed); T_4 (Chick tonic + Feed)
NS= Not significant

Result showed in mean ±standard deviation. Uric acid concentration lowest in T_3 (6.6 ± 1.98) and highest in T_4 (8.6 ± 4.14) were observed from above table but statistically were not significant (p=0.8803, >0.05). The albumin content in serum sample was lowest (24.15 ± 1.91) in T_2, highest value (41.05 ± 22.27) was in T_4 which was not also statistically significant (p=0.7230, >0.05). The Na content of serum was lowest in T_2 group (135.45 ± 7.56) and highest value was in T_4 (175.05 ± 7) though the value were not significantly differ to each other (p=0.1218, >0.05). The K level was lowest in T_2 (24.9 ± 2.26), highest in T_4 (33.3 ± 3.54) which was not significant statistically (p=0.2028, >0.05).

CHAPTER 5

DISCUSSION

CHAPTER 5: DISCUSSION

The goal of the present study is to find out the performance of Aqua Blok and Poultry tonic in commercial broiler chicken Cobb 500® in intensive production system during 30 days of treatment period. The hypothesis for this study was that dietary supplementation of Aqua Blok and Poultry tonic could improve growth performance along with gastrointestinal development and immune function of broilers. Earlier study showed that, yeast nucleotides added to bird feed led to higher body weight, higher daily body weight gains, and better feed conversion ratios (Esteve-Garcia et al., 2007; Jung and Batal, 2012; Wang et al., 2009). Improvements in weight gain were especially noticed when nucleotides were fed the first 3 weeks of life, indicating optimal early development of the birds will support performance later on (Esteve-Garcia et al., 2007; Rutz et al., 2008).

As Aqua Blok and Poultry Tonic contained vitamins, trace elements, essential fatty acids, amino acids, electrolytes and nucleotides could have the influential effect on performance of broiler. One possible explanation for the above beneficial effects is that nucleotide supplementation may conserve amino acids such as glutamine, aspartate, and glycine for de novo synthesis, resulting in the use of these amino acids for growth. Hu et al., 2016stated that increased glutamine availability is particularly important for high-glutamine-demand processes and organs such as the pectoralis major muscle (0.06 g glutamine/g protein of muscle). It is well known that the dramatic increase in growth rate of broilers is mainly occurred in the first three weeks after hatching.

5.1 Production potential

Recently, Leung et al. (2019a) observed improved feed conversion during pre-challenge and increased body weight gain (BWG) and villus height (VH) in *Eimeria*-challenged broilers fed diet supplemented with nucleotides. In a subsequent study, Leung et al. (2019b) concluded that, regardless of *Eimeria* challenge, supplementation with nucleotides had no effect on growth performance and intestinal function. Present study showed that there were no significant differences among treatment group in feed intake, body weight gain, production efficiency and feed conversion ratio. Maribo (2003) observed that supplementing broiler chickens' diet with a yeast product containing high nucleotides have no positive effect on growth performance.

Pelícia et al., (2010) found that the addition of nucleotides to broiler feed by 0.05, 0.06 and 0.07% did not have any effect on the broiler performance or carcass yield.

5.1.1 Feed conversion ratio

Though body weight gain, feed intake, production efficiency was found higher in T_4 than other treated group. In final production figure (table 4.8), the overall feed conversion ratio is lowest in T_2 (1.61) which indicated the lower production cost among the treatment groups.

However Pelícia et al., (2010), Jung and Batal (2012) reported that nucleotide supplementation in the diets had no significant effects on WG, FI, and FCR in broiler chickens. This finding showed that the nucleotide supply in the basal diets is sufficient to meet the broiler's requirements. Therefore, he stated that nucleotide supplementation may be beneficial under challenges and infectious conditions. This statement is similar to the findings of present study because there was no significant difference among the group. Present study showed that the supplementation of aqua Blok and Poultry Tonic improved feed conversation ratio throughout the experimental period. Similar finding observed in Esteve-Garcia et al., (2007) who reported that addition of nucleotide at 500 mg/kg feed significantly improved body weight and feed to gain ratio at 21 days though body weight were increased. Daneshmand et al., (2017) also reported that the combination of commercially available nucleosides (adenosine, guanosine, uridine and cytidine) at level of 1g/kg significantly increased growth rate and improved FCR. The result of this study differed with Owens and McCracken (2007) who demonstrated that supplementation of yeast extract to a broiler diet had a beneficial effect on feed intake and BW gain from 7 to 14 d of age.

5.1.2 Mortality rate

Mortality plays an important role in determining the overall profitability of a flock. An increase in mortality from 2.5 to 10% reduced net profit per broiler (Kitsopanidas and Manos, 1991) where present study showed that overall mortality rate in all flocks' ranges from 4.0 to 5.2%. Though there was no significant differences in mortality with different stocking density, the highest mortality in T_3 (5.2%) could also be attributed to stress and increased microbial load in the litter. During heat stress period most of the production energy is diverted to maintain thermoregulatory mechanism that results in to decreased weight gain, poor immunity, oxidative stress predisposing birds to various infectious diseases and high mortality rates (Cahaner and Leenstra, 1992).

5.1.3 Feed and water intake

According to Lucke et al., (2018) water intake and dry matter intake in broiler are correlated with stress. When they had experimented with deoxynivalenol (DON), a mycotoxin on feed, they observed the water intake of flock excessively increased and feed intake decreased. In this study, there was no obvious difference among the treatment groups though the feed intake was higher in group 4 and group 1 successively. It might be occurred for less stocking density (9.91 and 9.61 birds/ meter^2 respectively) in these two groups where group 2 and 3 were kept in higher stocking density (19.81bird/meter^2). Here stocking density imposed stress to later groups. To maximize earnings, commercial broiler producers frequently grow broilers in high stocking density farming condition. However, this method may have the opposite impact than they predicted, resulting in increased mortality and reduced body growth. (Esteve-Garcia et al., 2007; Mtileni et al., 2007; Hall, 2001).

5.1.4 Stocking density and body weight

Ghosh et al (2012) reported that highly significant ($p<0.01$) body weight gain observed at 1.5sq.ft/bird in both 4th and 6th week of rearing period. Birds of that group gave highest body weight in 4th and 6th week. Present study depicted that with a stocking density of 1.12 sq.ft/ bird had achieved 1119.3 kg/ flock at 4th week of age where as bird with 1.09sq ft/bird gained 1091.6 kg/ flock. The body weight gain of T_3 group (0.54 sq. ft per bird) is least in 4th (1012.2) this could be due to the effect of stress, induced by high bird density. More bird density leads to more litter moisture, air ammonia and microbial counts in the house (Jayalakshmi et al., 2009) and consequently body weight gain could be restricted. This result is consistent with the findings of Proudfoot et al., (1979).

5.2 Heterophil to Lymphocyte ratio

Nerve fibers release nor adrenaline into the bloodstream in response to stress, which causes signals in the bone marrow to enhance production of hematopoietic stem cells, particularly white blood cells. (Heidt et al., 2014). Depending on the stressor, the heterophil and lymphocyte numbers, and hence the heterophil to lymphocyte ratio, might change (Simitzis et al., 2012, Ajakaiye et al., 2010, Honda et al., 2015, Scanes 2016 and Osti et al., 2017). Increased H/L ratios in bird species under heat stress have been reported in numerous studies such as (Huff et al., 2005). In present study, determination of the heterophil - lymphocyte ratio was calculated by DLC method as a stress marker. Here, T_2 and T_3 groups were treated with Aqua Blok gel during transportation to compensate the transportation stress. That's why, Aqua Blok treated groups (T_2 and T_3) were found with lowest heterophil-lymphocyte ratio. On the other hand, higher ratio was found in (T_4) group. It directed that, T_4 group was in more stressful condition though it was not statistically significant ($P > 0.05$). Another study showed that heterophil - lymphocyte ratio in broiler was at 0.5 to 0.6 which belongs to normal and indicated that the broiler were not in stress during transportation process (Wicaksono et al., 2020). In their study they showed that result for tropical region but the present study was conducted just after winter season when the climate was not as hot as tropical region. This might be only reason for the dissimilar findings (0.18 to 0.40 in present study). According to Gross and Siegel (1983), present treatment group T_4, T_1 were in low to medium level of stress and T_2 and T_3 were in normal condition at day 2 of their rearing period (as the blood were collected at second day of rearing)

5.3 Eosinophil level

The significantly decreased eosinophil levels in broilers subjected to stocking density stress were consistent with data that demonstrate a decrease in circulating eosinophil counts was a marker of animal stress (Sarjan et al., 2017; Belden et al., 2005). The quantity of eosinophils in the blood can be used to distinguish between leukocyte responses caused by infection and those caused by stress. (Tornquist and Rigas., 2010). The eosinophil count in T_3 was higher among treatment groups meant that the group was less stressed in day 2 of age after transport.

5.4 Uric acid level

The high stocking density group had significantly greater serum uric acid concentrations in post-stress than the other groups (Nwaigwe et al., 2020). Uric acid in the blood is a marker of protein catabolism, and a rise in it indicates greater protein or amino-acid catabolism (Carsia, 2015). In this study, higher concentration of uric acid was found in group 4 and lower concentration was in group 2 though the value was not statistically significant (p=.8803). From the above statement it was cleared that later group was in less stress condition than group 2 at 2days of old.

5.5 Production cost

Farmers strive to keep production costs low, which, when combined with low food prices, makes their output more competitive. In today's market, customers place a high value on the quality and pricing of farm products. (Vanhonacker and Verbeke, 2009; Napolitano et al., 2013). The lowest production cost per kg broiler was found lowest in the T_2 group as compared to other groups since other management costs are same for all flocks except treatment cost. In present study, the cost of 1 kg commercial feed is Tk. 35, and the total cost of 1 kg broiler production in T_2 is Tk. 53.69 while it was highest in T_1 group. , the net profit will be high in T_2 since reduced amount of feed consumptions per unit of meat production.

CHAPTER 6

CONCLUSION

CHAPTER 6: CONCLUSION

Transportation of DOC from hatchery to farm premises causes stress which hampers the growth performance of broiler which can be reduced by using Aqua Blok during the shipment period. The lowest Heterophil to Lymphocyte ratio of blood sample from T_3 and T_2 group respectively provided that the group were faced less stress condition than other treatment groups. In final production figure, we observed lower FCR in T_2 (Aqua Blok & Poultry tonic) which directed us for minimizing the overall cost of production we can suggest to use this product. Though the product does not show satisfactory result in body weight gain, mortality, production efficiency, FCR in weekly basis. This might be due to higher stocking density and housing condition of that farm. Now we can conclude that combined supplementation of Aqua Blok and Poultry tonic would give better efficiency under proper management. New studies are highly encouraged to evaluate the effect of Aqua Blok and Poultry tonic added to broiler diets under the challenges typically found in commercial farms.

CHAPTER 7
RECOMMENDATIONS

CHAPTER 7: RECOMMENDATIONS

1. Laboratory analysis of drinking water to determine the pH and physical parameters (such as color and specific gravity) and TDS (Total Digestible Solids) could be done
2. Biochemical analysis of blood at weekly basis up to marketing age also could be performed
3. Histopathology of intestine to observe the villi growth in different age and in different treatment groups to check the efficacy of drugs in digestion as well as in absorption process.

CHAPTER 8

STRENGTH AND LIMITATION

CHAPTER 8: STRENGTH AND LIMITATIONS

Strength:

- Trained co-worker
- Large number of chicken populations
- Unbiased data collection

Limitations:

- Due to Covid-19 , sample collection for histopathology and biochemical analysis were not done in weekly.
- T_2 and T_3 group were not reared separately because of deficiency of sufficient house.

CHAPTER 9

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ANNEX

Annex I

Record sheet

Group	Feed intake (Kg)																											
	D-1		D-2		D-3		D-4		D-5		D-6		D-7		D-8		D-9		D-10		D-11		D-12		D-13		D-14	
G-1	I	F																										
G-2																												
G-3																												
G-4																												

Group	Water intake (Liter)																											
	D-1		D-2		D-3		D-4		D-5		D-6		D-7		D-8		D-9		D-10		D-11		D-12		D-13		D-14	
G-1	I	F																										
G-2																												
G-3																												
G-4																												

Group	Mortality																												
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D23	D24	D25	D26	D27	D28	D29	D30
G-1																													
G-2																													
G-3																													
G-4																													

Group	Used bird				
	D-1	D-7	D-14	D-21	D-28
G-1					
G-2					
G-3					
G-4					

	Body weight(gm)																			
	Day-1				Day-7				Day-14				Day-21				Day-28			
SL. No.	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4
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Annex II

Product description

<table>
<tr><td colspan="2">Protocol number: 01</td><td colspan="3">Protocol title: Effect of Aqua Blok and Poultry Tonic supplementation on production potentials during transportation in broiler chicks arriving on farm.</td></tr>
<tr><td colspan="2">Principal investigator: Dr. AKM Saifuddin</td><td colspan="3">Technologist:</td></tr>
<tr><td colspan="5">Investigational product received from sponsor:</td></tr>
<tr><td colspan="5">Formulation and description:</td></tr>
<tr><td colspan="5">Name of product: Aqua Blok & Poultry Tonic</td></tr>
<tr><td colspan="5">Manufacturer:Ashkan</td></tr>
<tr><td>Formulation</td><td>Active ingredient(s) and concentration</td><td>Vitamins, amino acids, electrolytes, nucleotides, prebiotic.</td><td>Batch number</td><td></td></tr>
<tr><td>Description</td><td colspan="4"></td></tr>
<tr><td>Expiry date:</td><td colspan="4">06/2021</td></tr>
<tr><td>Quantity /amount received</td><td>3000 gm</td><td>Date of receipt
08/03/2020</td><td colspan="2">Received by:
Dr AKM Saifuddin</td></tr>
<tr><td>Date</td><td>Amount or quantity dispensed</td><td colspan="2">Comments</td><td>Signature(PI)</td></tr>
<tr><td>15/3/2020</td><td>1500 gm</td><td>Aqua Blok</td><td></td><td></td></tr>
<tr><td colspan="5">Disposal or investigational product:</td></tr>
<tr><td colspan="4">Total amount/quantity of product received</td><td>3000 gm</td></tr>
<tr><td colspan="4">Total amount/quantity of product used or dispensed</td><td>1500 gm</td></tr>
<tr><td colspan="4">Total amount/quantity of product remaining</td><td>1500gm</td></tr>
<tr><td colspan="5">Disposal of remaining investigational product:</td></tr>
<tr><td colspan="4">Signature: Principal investigator:</td><td>Date</td></tr>
</table>

Annex III

<u>Experimental procedure of DLC</u>

Preparation of blood smear

1. Several clean grease free slides with smooth unbroken end were selected, then a drop of blood was placed at right end of the slide.
2. The slide was taken on a piece of paper on the table holding firmly by the force or thumb finger on left hand.
3. The even edge of the second slide was placed near the drop of blood towards the middle of slide.
4. The second slide was drawn towards the drop of the blood at an angle of 45 and the blood was spread along the edge.
5. Keeping the second slide in the same angle a quick even push was given with a uniform force toward the other end of slide.

Staining of blood film

1. When the blood film dried, it was placed in the staining rack.
2. 8-10 drops of Wright's stain was poured over the slide to cover the blood smear and allowed to stain for 2 minutes.
3. Distilled water above double quantity of stain was added and mixed by blowing pipe and allowed to stand for 5 minutes.
4. Then the film was washed with distilled water without much distribution the slide till the slide film become pinkish.
5. The slide was placed against the support and allowed to dry in air.

Counting of WBC

The slide was placed on a fixed place and a drop of immersion oil on the slide. Then the cells were identified by using high power objective 100x following parallel stripe method. The counting was continued till 100 or 200 cells were counted. Different types of cells were counted by tally method. 200 cells counting is the best method.

Biography

This is Mosammat Moonkiratul Zannat, daughter of late Delwar Hossain and Hosne Ara Begum from Chakaria upazila under Cox's bazar district of Bangladesh. She passed the Secondary School Certificate examination in 2008 followed by Higher Secondary Certificate examination in 2010. She obtained her Doctor of Veterinary Medicine Degree in 2016 (held in 2017) from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Presently, she is a candidate for the degree of MS in Pharmacology under Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, CVASU. With her best knowledge and expertise, she hopes to deliver competent veterinary medical treatment and sustains the norms of professionalism in the future.