CHAPTER-I

INTRODUCTION

Poultry serves as one of the means for satisfying the increased demand for animal protein. Presently, chicken meat is on demand as a cheap source of protein with low cholesterol value. Therefore, number of broiler farming is increasing day by day. As 70% of total cost of production, is contributed by feed only, improvement of feed conversion ratio will significantly enhance the net profit. A number of feed additives like antibiotics, steroids, vitamin, minerals and other growth promoters are used to improve the performance of broiler growth. Antibiotics have long been used as growth promoters, and now indiscriminate use of antibiotics in animals is being restricted by the scientists.

It is reported that, the use of antimicrobial growth promoters (AGP) in animal nutrition has been found beneficial for the improvement of growth performance and prevention of diseases (Barton, 2000; Snel *et al.,* 2002). The excessive dependency on medications threatens the mankind in antibiotic resistance. It is also discouraged to use growth promoters, because of their residual effect in boiler meat. In this regard, the authorities of several regions of the world are restricting the use of antibiotics in livestock, and establishing routine surveillance and monitoring programs, and suggest that antimicrobials belonging to the same class as those used for humans be banned, except if risk analyses are performed.

Probiotics are one sort of feed additive that contain live microorganisms and promote beneficial effects on the host of favoring the balance of the intestinal microbes (Fuller 1989). The probiotics include live bacteria, yeast, their metabolites and pH adjusters, which contribute to maintain balance in intestinal microflora (Islam *et al.*, 2004). Probiotic microorganisms are responsible for the production of vitamin B complex and digestive enzymes for stimulation of intestinal immunity, increasing protection against toxins produced by pathogenic organisms. Probiotic acts as a mono or mixed culture of living microorganisms, which beneficially affect the host by improving the properties of the indigenous microflora. Probiotic organisms help

to improve the environment of the intestinal tract. It may also be defined as living microorganisms, which is given to animals assist in the establishment of an intestinal population, which is beneficial to the animal and antagonistic to harmful microbes (Mathivanan *et al.,* 2007).

Probiotic feeding assists in preventing colonization of pathogens in the intestinal tract and in producing certain enzyme like substances (Lee *et al.*, 2007). Probiotics are claimed to exert beneficial effects on live weight gain, feed consumption, feed conversation ratio and livability (Watkins *et al.,* 1982). The nutritive value of available feed stuffs such as wheat, maize, rice polish, til oil cake, soybean meal etc., in Bangladesh contain more indigestible part. (Jin *et al.,* 2000). Most of the feed ingredients contain some anti-nutritional factors and non- digested part, which inhibit feed utilization. The ant-nutritive effect is manifested by depressed nutrient utilization accompanied by poor growth. This adverse effect can be overcome by supplementation of exogenous carbohydrase (xylanase) enzymes improve digestibility of starch, protein, fat and apparent metabolisable energy in broiler feed (Choct *et al.,* 1995). Moreover, the growing concern arising among the people about food safety, environmental contamination, and general health issues due to the presence of residual antibiotics in poultry meat has driven a way to find out a solution to the use of antibiotic growth promoter. Considering these facts in mind the feeding of other non-antibiotic growth promoters such as prebiotics, probiotics, and synbiotics finds a potential substitute for antibiotics..

For example, probiotic efficacy may depend on factors such as microbial species composition (e.g., single or multi-strain) and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors. Considering the above facts, the current study was under taken to fulfill the following objectives:

1. To investigate the effects of probiotics on gut health and production performance of broiler

2. To determine the gut microbial population and assessing gut health condition of the broilers fed probiotics.

CHAPTER-II

MATERIALS AND METHODS

2.1 Statement of the experiment

The experiment was conducted at poultry unit in Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, to investigate the productivity of broiler chickens fed probiotics. Biological trial was conducted at the Poultry Research shed of CVASU campus during the period of 31th May to 28th June, 2017. The laboratory analyses were rendered at the Poultry Research & Training Center (PRTC) in CVASU, Khulshi, Chittagong.

2.2 Preparation of the experimental house

The research shed was prepared by cleaning and washing properly using tap water. Ceiling, walls and floor were disinfected using Fam30 solution (1ml/2ltr water). The house was left for one week after cleaning and disinfection to dry up properly. After drying the room, all doors and windows were closed, and later fumigated (adding 35ml of formalin to 10g potassium permanganate per cubic meter) and sealed for 24 hours. Lime was spread on the floor and around the shed for strictly maintaining bio-security.

2.3 Layout of the experiment

A total of 96 (Ross 308) day-old broiler chicks of either sex were collected from the local hatchery (Nahar Agro Farm Ltd., Chittagong) on a pre-order basis to the run the experimental trial from d1 to 28 days. The chicks were weighed (46.33±0.01g/b) on receipt and then randomly assigned into four dietary treatments groups (D_1 D_2, D_3, and D_4); each treatment was replicated 3 times with 8 birds per replicate in a completely randomized design. The layout of the experiment was shown below in Table 1.

Table 1: Layout of experiment

Treatment	Number of birds per replication			No. of birds
	R_1	R_2	R_3	
D_1(Control)	8	8	8	24
D_2(Poultry Starsol 1gm/L)	8	8	8	24
D_3 (Avilac plus 1ml/L)	8	8	8	24
D_4 (Avibac 1gm/L)	8	8	8	24
Total	32	32	32	Grand total= 96

[D_1 refers to Control diet without probiotics, whereas D_2 refers to probiotics Poultry Starsol 1gm/L; D_3 = probiotics Avilac+ 1ml/L and D_4 denotes =Avibac 1gm/L. respectively]

2.4 Collection of the experimental diet and test ingredient

The ready-made (crumble-pellet) broiler diets (starter and grower) marketed by CP were procured from the local market of Chittagong, and fed the birds' entire the trial period (d1-28). The samples were collected and sent to the laboratory, and later analyzed the collected feed samples as per the methods described by AOAC (2007). The proximate value and reporting value (found on the bag) are shown below in Table 2.

Table 2: Nutrient Composition of ready-made diet (CP)

Nutrient (%)	Reported value	Analytical value
Dry Matter		87.74
Crude Protein	21.5	23.05
Crude Fiber	5.0	5.73
Ether Extract	3.5	7.4
Ash	6.0	5.65
Calcium	1.0	1.11
Phosphorus	0.50	0.66
Metabolisible Energy (Kcal/kg)	2950	3253.25

The broiler chicks were fed with starter diets up to 14 days, after that, grower diets were used for the remaining period (d15-28d). The name of test ingredient used for this experiment is probiotics, *i.e.* Poutry Starsol, Avilac Plus, and Avibac etc., were collected from the nearby market of Chittagong. The manufacturing or marketing companies of these probiotics are Renata Ltd. (Poultry Starsol), Orion Pharma Ltd. (Avilac Plus) and Opsonin Pharma Ltd. (Avibac), respectively.

The probiotics were treated with water at the particular doses (shown above in Table 1), and this treated water was used for drinking the broilers.

2.5 Management

The following management procedures were followed during the experimental period and the uniformity in the management practices was maintained.

2.5.1 Housing and brooding

The collected 96 chicks were randomly distributed in the 16 pans of equal size which were cleaned and disinfected previously. Chicks were brooded with electric bulbs (60 wt) that were placed in the middle of the pen above the chicks by hanging condition. For the first two days, the birds were provided with a temperature of 33^{o}C. The temperature then was gradually reduced by 1 or 2^{o}C every 1 or 2 days until the chicks were 19 days old at which point the temperature was maintained at 24^{o} C for the rest of the trial.

2.5.2 Floor space

Bird was raised in battery cages by dividing 16 pens of equally sizes. Each pen was labeled properly with different treatment groups before entering chicks into the cage. Dry and clean newspaper was placed on the floor of each pens as bedding materials prior to allowing chicks in the room, and the paper was replaced with new one as or when it get too dirt during the whole brooding period. Each pen having size (3.5 ft. × 1.63 ft.) was allotted for 8 birds. Therefore, floor space for each bird was 0.71 sq. ft.

2.5.3 Feeder and drinker space

Each pen was furnished with a feeder and one drinker. One feeder (60 cm × 8 cm × 5 cm) and one round drinker with a capacity of 2.5 liters were provided for each pen. The feeder and drinker were placed in such a way so that the birds were able to eat and drink conveniently.

2.5.4 Feeding and watering

Feed and drinking water were supplied *ad-libitum* to the birds throughout the experimental period. Feed was supplied to the experimental birds daily once in the morning at 6A.M. and again in the afternoon at 6 PM, and fresh drinking water was supplied the birds three times a day *i.e.* at 6 AM., 02 PM, and 6 PM. Paper and pot drinkers with small plate were used to feeding and watering the chicks during the early stages soon after coming from the hatchery. Bird was fed starter broiler diet for the first 14 days, and then grower diet in pellet form was used to feed the broilers entire trial period from the rest of the trial period (d 15 to 28 days). During brooding period, feeding was done by using one round feeder and waterer having a capacity of 1.5 liter. The feeders and drinker were fixed in such a way so that the birds could eat and drink conveniently. After brooding period, large liner feeder (3.5 ft. × 0.38 ft.) and large round waterer with a capacity of three liters were used for serving the purposes

2.5.5 Lighting:

The birds were exposed to a continuous lighting of 23 hours and a dark period of 1 hour in each 24 hours of photoperiod.

2.5.6 Immunization and medication:

All the birds were vaccinated against Newcastle Disease (ND), Infectious Bursal Disease (IBD) as per schedule given in Table 2. After each vaccination, multivitamin (Rena-WS, Renata; 1g/ 5 liter of drinking water) was supplied along with vitamin-C to overcome the stress due to vaccination and cold shock.

During the first day of the experiment the day old chicks are supplied with glucose (Glucose-D) @ 1gm/2 litre drinking water. All the vaccines were collected from the DLS, Chittagong.

Table 3: Vaccination schedule

Age	Name and type of vaccine	Name of disease	Route of administration
4th day	BCRDV (Live)	New Castle Disease	One drop in each eye
10th day	IBD (Live)	Infectious Bursal Disease	One drop in each eye
18th day	IB + RDV (Live), Booster dose	New Castle Disease	Two drop, orally

2.5.7 Data and sample collection

Body weight and feed intake were recorded weekly for the calculation of body weight gain and feed conversion ratio (FCR). On day 28, one bird was selected randomly, weighed and killed humanly to collect caecal sample for microbial count (total viable count, total lactobacilli count). Feed samples were also collected prior supplying the birds to assess the nutritive value of the feeds.

2.6 Record keeping:

The following parameters were recorded throughout the experimental period.

2.6.1 Body weight:

The live weight of day old chicks was recorded at the first day of the experiment and at the beginning of each week the birds of each replication of each treatment was weighted and recorded properly.

2.6.2 Feed intake:

Weekly feed intake was recorded by deducting the left over feeds from the total amount supplied to birds each week.

2.6.3 Temperature and relative humidity of house:

During the experiment the temperature and relative humidity were recorded four times a day i.e. 6 A.M., 2 P.M., 6 P.M. and 12 A.M. The temperature and relative humidity was recorded with the help of hygrometer.

2.7 Calculation of data:

2.7.1 Weight gain:

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

2.7.2 Feed conversion ratio (FCR):

The amount of feed consumed per unit of weight gain is called feed conversion ratio. This was calculated by using the following formula:

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

2.8 Statistical analysis

All recorded and calculated data were statistically analyzed for analysis of variance in a Completely Randomized Design (CRD) using the Minitab statistical computer package program (Minitab, 2000). The significance of differences between means was tested using the Duncan multiple-range test. Statistical significance was considered at $P \leq 0.05$.

Pictorial presentation of some activities during experiment

Fig 1: Inside View of Cage

Fig 2: Outside View of Cage

Fig 3: Baby Chicks in Carrier Box

Fig 4: Feeding the birds

Fig 5: Weighing of Baby Chicks

Fig 6: Weighting of feed

CHAPTER-III

RESULTS

The experiment was carried out to find out the effect of probiotics treated with water on the gross responses (body weight gain, feed intake, FCR) of Ross-308 broiler chickens. The results obtained from the study are stated below in this chapter.

3 The gross responses and livability of broiler chickens fed probiotics

3.1 Body weight gain

The results of body weight (BWG) of broiler chickens are shown in Table 4. The body weight gain (BWG) of broilers fed probiotics differed significantly between treatments entire the trial period, except first week (Table 4). Significantly highest BWG (1728.0 g/b) was found in the bird fed D_3 group, whereas D_1 being the lowest BWG (1584.70 g/b) from d1-28. The BWG up to 21d, was found significantly ($P<0.05$) increased in D_2 D_3 and D_4 groups of birds, and it was similar between the group.

Table 4: Body weight gain (g/bird) of broiler fed probiotic from d1- 28 days

Parameter	Age (days)	Treatments				Pooled SEM	P-values
		D_1	D_2	D_3	D_4		
BWG(g/b)	1-7	163.33	167.33	166.33	165.00	0.750	0.330
	1-14	377.00^{b}	386.67^{a}	390.00^{a}	392.67^{a}	1.674	0.047
	1-21	943.00^{b}	957.00^{a}	963.67^{a}	969.00^{a}	2.480	0.028
	1-28	1584.70^{c}	1671.00^{b}	1728.00^{a}	1724.70^{a}	7.159	0.01

[Data represent mean values of 8 birds per replicate groups during 1- 28 days; ^{a,b,c} Means bearing uncommon superscripts within a row are significantly different at the levels shown above; SEM= Pooled standard error of means].

3.2 Feed intake

The feed consumption of birds during different stages of growth in different treatments is given in Table 5. Results show that feed intake had no significant

difference (P>0.05) between the treatment groups from d1-28 days. Numerically, birds on D_2 group consumed more feed than that of other diet group on day 28d, though no difference (P>0.05) was observed between treatments. The FI of D_3 group is 22247.70g followed by 2245.70, 2243.70g, and 2251.70 g in D_4, D_2 and D_1, respectively, on the last day of trial period (d28).

Table 5: Feed Intake (FI) of broiler fed probiotic from d1- 28 days

Parameter	Age (days)	Treatments				Pooled SEM	P-values
		D_1	D_2	D_3	D_4		
FI (g/b)	1-7	168.67	170.67	168.67	170.33	1.348	0.923
	1-14	633.00	629.33	628.67	629.67	2.520	0.929
	1-21	1396.0	1376.7	1375.7	1377.7	4.965	0.458
	1-28	2251.7	2243.7	2247.7	2245.7	6.350	0.973

3.3. Feed Conversion Ratio (FCR)

The FCR of broiler chickens of different dietary treatment groups recorded during different stages of growth are presented in Table 6. Data revealed that the FCR of broilers differed significantly (P<0.01) during last week (28d) only, except for others. Improved FCR values were observed in the broilers of D_2, D_3, and D_4 groups compared to those of other group (D_1).

Table 6: Feed Conversion Ratio (FCR) of broiler fed probiotic from d1- 28 days

Parameter	Age (days)	Treatments				Pooled SEM	P-values
		D_1	D_2	D_3	D_4		
FCR or feed gain ratio	1-7	1.03	1.02	1.01	1.032	0.008	0.786
	1-14	1.68	1.63	1.61	1.60	0.014	0.272
	1-21	1.48	1.44	1.43	1.42	0.008	0.111
	1-28	1.42^a	1.34^b	1.30^b	1.30^b	0.006	0.01

CHAPTER-IV

DISCUSSIONS

4 Gross responses of broilers fed Probiotic:

4.1 Body weight gain of broilers

It is obvious from the data that the body weight gain of broilers fed probiotics treated group was found to be significantly improved as compared to control group throughout the experiment except for 1st week, in this study. The probiotics are one kind of feed additives. The main function of feed additives is to enhance feed utilization efficiently. Once after applied in the broilers, the feed additives start to act upon their digestibility of the ingested feed materials, which are then help the birds to utilize feed more efficiently. It can be assumed that, the efficient utilization of feed by the broilers might give rise to better growth responses of the broiler chickens fed probiotics treated diets. In broiler nutrition, probiotics have a multiple beneficial effect on broiler performance, which includes modulation of intestinal microflora and pathogen inhibition, intestinal histological changes, immunomodulation, certain haemato-biochemical parameters, improving sensory characteristics of dressed broiler meat, and promoting microbiological meat quality of broilers.

However, our findings agree with the report of Dhande *et al.,* (1993), who also observed that probiotic fed broilers had higher body weight and better feed conversion efficiency. The improvement in weekly body weights due to supplementation of probiotic indicated that the inclusion of probiotic beneficially affects the host by improving its intestinal microbial balance as reported by Fuller (1989). Similar results that supplementation of probiotic in feed promoted the growth and feed efficiency for better production of meat and egg were also reported by Verma (1992).

The present findings are also similar with the reports of previous investigators (Jayakumar *et al.,* 1996; Kalbande *et al.,* 1992, Mishra *et al.,* 1994), who also reported the increase in weight and better quality of broiler meat with the use of

probiotics in poultry rations. The report on effect of probiotics on body weight of broilers is contradictory. While several workers have claimed a significant improvement in body weight of broilers following probiotic supplementation in broiler diet (Kumararaj *et al*., 1997; Gohain and Sapcota, 1998), there are others who have concluded based on their studies that addition of probiotics did not significantly affect the body weight of broilers (Samanta and Biswas, 1995). The beneficial effect of probiotic supplementation to broiler diet in terms of increased body weight and body weight gain is well documented in study of Singh *et al.* (1999).

4.2 Feed intake

There is no difference between feed intakes of broiler chickens fed probiotics supplements, in present study. It denotes that broilers consumed feed uniformly entire the trial period, as no significant differences are found in feed consumption of bird between treatments. The uniform feed intake of broilers on probiotics might be due to providing same diet and the mode of application of probiotics to the birds. The probiotics was applied the birds via water, not with feed, as it is liquid in form. This mode of application of growth probiotics might be a reason for similar feed intake of the broilers. Besides, all birds had a free access to same diet (crumble-pellet) entire the trial period. It was observed that there were no adverse effects of the probiotics on feed consumption, palatability and thereby performance of broiler birds. Our results might contradicts with the reports of previous researchers (Kim *et al.,* 2001; Gupta *et al.,* 2003; Sharma *et al.,* 2003), who observed that broilers diet supplemented with probiotic showed improved feed intake than the control.

The difference between two studies might be due to variation in supplying probiotics to birds and sources of probiotics from which these were prepared. In our study, we supplied probiotics though water, whereas they applied probiotics with diet.

4.3 Feed conversion ratio (FCR)

It is obvious that the FCR of broiler was improved significantly by probiotics supplements during 1-28 days .The data of FCR value indicates that birds of D_2, D_3 and D_4 groups assumed to be more efficient in converting feed to meat than the broilers of D_1 or control group during 1-28 days. It shows that the birds of these groups assume to be more efficient than that of others, as the broilers of this groups

had received superior FCR values (1.30; 1.34) than that of control groups. The results of present study are in agreement with the previous reported by Dhande *et al*. (1993) and Verma (1992), who found significant differences in FCR of birds fed probiotics. The inclusion of probiotics showed increased feed efficiency, better quality of broiler meat and extensive decrease in infectious diseases were also reported by Jayakumar *et al.* (1996). The present findings are in agreement with the findings of Wiseman (1990) and Mudalgi *et al.* (1993).

CHAPTER-V

CONCLUSION

The present study shows that the supplementations of probiotics in the feed of broiler enhanced the body weight with improved feed efficiency, net profit without affecting feed intake. So, from a practical point of view, this study highlights the need for probiotic inclusion in the broiler diet to achieve the desired beneficial outcome.

However, an overview of the results obtained in this study revealed that, broiler chickens fed probiotics supplements responded positively as a result of increased body weight gain, better FCR without affecting feed intake. From the results, it could be assumed that, application of supplemental products on the broilers chickens, might enhance broiler production. Supplementation of probiotics on broiler chicken might be economical, and it could enhance commercial production. Further, it might enhance nutrient availability of bird and thus increase the productivity of broiler chickens.

Broilers rearing on probiotics might change the consumer view and could draw the attention of the consumer world to ingest broiler meat produced from antibiotics free components, because it has no health hazard or residual effect, as is seen in the antibiotics.

Broilers were reared in cage system in this study, which might be changed to floor system for conducting commercial production. Some abnormalities such as breast blister and leg problem might arise from rearing broilers in cages, and it might downgrade the quality of bird.

CHAPTER-VI

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ACKNOWLEDGMENTS

The author is ever grateful and indebted to the Almighty God without whose grace it would have never been possible to pursue this study in this field of science and to complete this production report writing for the Degree of Doctor of Veterinary Medicine (DVM).

The author would like to thanks his reverend and beloved teacher and supervisor **Professor Shahnaz Sultana,** Department of Agricultural Economics & Social Science, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University for his valuable advice, suggestions and kind co-operation during the study period.

The author would like to thanks to the Director of External affairs **Professor Dr. A.K.M. Saifuddin,** Department of Physiology, Biochemistry & Pharmacology**,** Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University for his suggestion.

The author expresses his sincere gratitude and gratefulness to **Dr. Sukanto Das** for giving an opportunity to participate his thesis activities and for his valuable advice, inspiration, cordial co-operation, valuable suggestion during the study period.

The Author

September, 2018

Biodata

Ashik Hazra, Son of **Binoy Krishna Hazra** and **Basanti Hazra**. He is an interned veterinary doctor under the faculty of Veterinary Medicine (FVM) in Chittagong Veterinary and Animal Sciences University (CVASU). He passed his Secondary School Certificate (SSC) Examination in 2010 followed by Higher Secondary Certificate (HSC) Examination in 2012 from Jessore board. In future he would like to do Research work about poultry sciences and poultry nutrition.