CHAPTER-1: INTRODUCTION

The plant-based poultry diet contains an important anti-nutrient factor called phytate which is the major storage site of phosphorus (P). Phytate not only limits the availability of P but also other minerals and nutrients like protein, carbohydrate, etc. Poultry is unable to hydrolyze phytate due to a lack of effective endogenous phytase activity (Broch *et al.*, 2018). Moreover, the endogenous intestinal phytase poorly hydrolyzes the phytate due to the different pH level and cation concentration of the gastrointestinal tract (GIT) of poultry (Cowieson *et al.*, 2019). Therefore, exogenous phytase is routinely added to the poultry diet to improve the availability of phytate-bound minerals and nutrients. A significant amount of literature has already reported the beneficial effect of conventional dose of phytase (500 FTU/kg) on growth performance, nutrient utilization, and bone quality of broiler chickens (Simons *et al.*, 1990; Ravindran *et al.*, 1999; Selle *et al.*, 2000; Dilger *et al.*, 2004; Selle *et al.*, 2006; Selle & Ravindran, 2007; PRavindran *et al.*, 2008; Selle *et al.*, 2011; Adeola & Cowieson, 2011; Akter *et al.*, 2016; Attia *et al.*, 2020).

The corn-soybean-based poultry diet contains around 28 % phytate which stores 60-80 % of the total P (Cheryan, 1980). It has been reported that 500 FTU/kg of phytase could only hydrolyze 62 % of the total phytate and released only 0.15% phytate-P (Walk *et al.*, 2013). Due to several extrinsic and intrinsic factors the conventional dose (500 FTU/kg) of phytase cannot completely dephosphorylate the phytate (Karimi *et al.*, 2011). It has been assumed that benefit from phytase supplementation can be maximized by increasing the phytase level more than 500 FTU/kg of poultry diet. Selle and Ravindran (2007) claimed that the super dosing effect of phytase supplementation is more pronounced with increasing dose of this phytase leading to the idea of phytase super dosing in poultry diet.

The first work of phytase super-dosing was reported by Nelson *et al.*, (1971) where the effect of phytase super dose (950 to 7600 FTU/kg) was evaluated. The authors observed that the phytate-P disappearance increased by 55.5% when the phytase dose increased from 950 to 7600 FTU/kg. The weight gain and ash content of bone at 21 d were highest at 7,600 FTU/kg (Nelson *et al.*, 1971). Another study stated that supplementation of

phytase between 1000FTU/kg and 5000FTU/kg of diet significantly improved length, width, and mineral content of tibia bone compared to a diet with 500FTU/kg of phytase (Manobhavan *et al.*, 2016). According to Shirley and Edwards (2003) supplementation of 12000FTU phytase/kg of diet effectively hydrolyzed 95% of phytate-P. This enhanced efficacy of phytase super dosing could be due to the complete hydrolysis of phytate and release of minerals (P, Ca, Zn, Fe, etc.) and other nutrients, like protein and energy (Zyla *et al.*, 2004; Cowieson *et al.*, 2013).

Most of the study stated that the benefits of phytase super dosing become more pronounce when supplemented to non-phytate phosphorus (NPP) deficient diet (Pirgozliev *et al.*, 2008a; Walk *et al.*, 2012c; Manobhavan *et al.*, 2016; Pieniazek *et al.*, 2017; Broch *et al.*, 2018; Leyva-Jimenez *et al.*, 2019b). Although these aforementioned studies reported the positive effect, the impact of phytase super dosing is still inconsistent as the phytase dose and non-phytate phosphorus (NPP) level of the diet varied over the literature. Moreover, the amount of literature on phytase super dosing is very limited in Bangladesh context. Therefore, the present study was undertaken to evaluate the effect of phytase super dosing on the performance, bone quality, and serum profile of broiler chickens.

Objectives:

1. To evaluate the effect of phytase super dosing on the growth performance of the broiler chickens.
2. To assess the effect of phytase super dosing on blood mineral contents and bone quality of broiler chickens
3. To evaluate the profitability of phytase super dosing on broiler diets.

CHAPTER-2: LITERATURE REVIEW

Before conducting a research by following experimental procedures, it is important to have a look on the previously conducted research activities on the related topics. A review of the literature relevant to the present research work has been given below.

2.1 Phytate

Phytate (Myo-inositol-1,2,3,4,5,6-hexakisdihydrogenphosphate) is a salt that contain derivative of the myo-inositol family of cyclitols which derived from glucose and 6 phosphate molecules (Loewus and Murthy, 2000). Myo-inositol 1-phosphate is synthesized by inositol 1-phosphate synthetase enzyme. Then the myo-inositol 1-phosphate is dephosphorylated into free myo-inositol with the help of inositol 1-phosphate phosphatase which generally creates phytate (Bohnert *et al.,* 1995).

Phosphorus is stored in plant or seed as phytate which shows significant anti-nutritional impact in monogastric animals due to its ability to chelates different ions such as Ca, Mg, K, Zn or P that forms mineral-phytate complex. Phytate dephosphorylation mainly occurs in the fore stomach in broiler (Selle *et al.,* 2011). In monogastric animals or birds, endogenous phytase secretion has a limited ability to break the phytate compound. The efficacy of the endogenous phytase reduces due to insoluble complex formation by phytate and dietary nutrients interaction (Selle *et al.,* 2009; Gupta *et al.,* 2015). So, exogenous phytase is supplemented to broiler diet at a certain level to maximize the dephosphorylation of phytate complex.

2.2 Phytase

Phytase or myo-inositol hexakisphosphate phosphohydrolase is a protein enzyme which catalyzes the stepwise removal of P from phytate (Dersjant-Li *et al.,* 2015). This stepwise dephosphorylation process of phytate increases the concentration of lower myo-inositol phosphate 1 to 5 (InsP5, 4, 3, 2, 1) esters (Selle and Ravindran, 2007) which is readily soluble in the GIT of poultry than myo-inositol phosphate 6 (InsP6) thus reduces the anti-nutritional effects of phytate (Dersjant-Li *et al.,* 2015).

2.3 Response of broiler chicken to super dosing of phytase

Phytase is supplemented at 500FTU/kg diet commercial and considered as an economical dose which improved the performance of broiler chickens (Selle and Ravindran, 2007; Pirgozliev *et al.*, 2012; Lalpanmawia *et al.*, 2014; Cowieson *et al.*, 2009). The idea of super-dosing (>1000FTU) arises from the "extra-phosphoric effects" of elevated phytase dose (Cowieson *et al.*, 2011). The use of higher phytase enzyme dose has been gaining importance not only because it would release more P for body utilization leaving less residual phytate but also it would generate myoinositol (Shirley and Edwards, 2003). The supplementation with super dose of phytase showed beneficial effects in many studies (Shirley and Edwards, 2003; Augspurger and Baker, 2004; Cowieson *et al.*, 2006; Pirgozliev *et al.*, 2007; Manobhavan et al., 2016; Kies *et al.*, 2006).

However, in previously published articles the following effects were observed at different level of phytase supplementation on broiler chicken:

2.3.1 Growth performance

Phytase showed better effect on phytase super dose supplementation in many published articles. Raut *et al.*, (2018) reported that the feed conversion ratio of broiler chicken fed diet supplemented with 1000, 1500 and 2000 FTU phytase/kg of feed were (1.56, 1.57, and 1.58, respectively) better than those on diet with 500FTU/kg of phytase (1.660). A study by Karadas *et al.*, (2010) showed that phytase supplemented at 12500 FTU/kg significantly increased the BWG and FCR compared to 500FTU/kg phytase.

Raut *et al.*, (2018) stated that BWG at 4th week of age was better at super dose (>1500FTU/kg diet) of phytase than non-phytase group. At d 21, supplementation of 7,600 FTU phytase/kg of diet improved the weight gain and bone ash content of broiler chicken (Nelson *et al.*, 1971). Raut *et al.*, (2018) observed that feed intake (FI) decreased at super dose of phytase supplementation than control group. Supplementation of 2000 FTU phytase/kg of diet decreased the FI by 78 gm in broilers than those offered 500 FTU phytase/kg of diet (Raut *et al.*, 2018).

Pirgozliev *et al.*, 2010 observed 9.4% better FCR in low P diet by phytase supplementation at 500 FTU/kg and even 10.1% better FCR compared to normal P diet when phytase was supplemented at 12,500 FTU/kg to low P diet. Shirley and Edwards (2003) also reported that BW gain of broiler chicken was higher in normal P diet (501 g/chick) than in low P diet with super dose of phytase (515 g/chick). Cowieson *et al.*, (2006) stated that the chicken fed with either 2400 or 24,000 FTU had 14% better feed efficiency.

2.3.2 Bone development and mineralization (Bone calcium and phosphorus)

Bone mineralization is sensitive to the bioavailability of minerals within a diet and is directly correlated to the phosphorous and calcium deposition (Viveros *et al.*, 2002; Hall *et al.*, 2003). A study by Manobhavan *et al.*, (2015) showed that phytase supplementation between 1000 FTU/kg and 5000 FTU/kg significantly increased the bone length *($P<0.001$),* bone width and mineral content of bone (Ca, P, Mg and Zn) compared to broilers supplemented with the standard 500FTU/kg phytase. Cowieson *et al.*, (2011) stated that 500FTU/kg phytase liberated Ca and P to a ratio of, or greater than, 2:1. Increasing the phytase dose from 500FTU/kg to 1000FTU/kg resulted in a further 30% increase in phytate degradation. This translated into further increases in bone mineralization (Lee *et al.*, 2003).

2.3.3 Carcass quality and visceral organ development

Sharma *et al.*, (2016) observed no effect of supplementation of different level of phytase (500FTU, 1000FTU and 1500FTU/kg of diet) on the relative weight of breast meat, liver, spleen, abdominal fat, small intestine, bursa of broiler chicken at 35 days of age. The increment of breast weight was 4.94% at 1000 FTU phytase /kg of diet. They also stated that phytase supplementation decreased the relative weight of small intestine by 8% at d 24 at 1000 and 1500 FTU/kg but there was no effect at 35 days of age. Broch *et al.*, 2018 also indicated the non-relative effect between phytase and carcass quality. Effect of phytase super dosing on visceral organ development is very limited, therefore, further research is needed for filling this gap.

2.3.4 Effect on blood mineral contents

Shim *et al.*, (2012) claimed that phosphorus (P) also has important role in ATP production, serum mineralization, phosphoglycerates and acid-base balance to the birds and animal. An observations of improved serum P with phytase supplementation had been reported by previous studies (Augspurger *et al*., 2004; Bhanja *et al.*, 2005; Kozlowski and Jeroch, 2011, Jalani *et al.*, 2012; Rutherford *et al.*, 2012;; Beiki *et al.*, 2013; Arabi *et al.*, 2013). A study conducted by Raut *et al.*, (2018) indicated that serum Ca percentage improved significantly by phytase supplementation. The study also observed that supplementation of 2000FTU phytase/kg of diet increased serum Ca percentage than 500FTU/kg and 1000FTU phytase/kg of diet. Augspurger *et al.*, (2004) observed significant effect on Total protein (TP %) in serum by inclusion of phytase in broiler diet.

2.3.5 Profitability due to phytase supplementation

The net profit tended to increase due to decreased production cost when level of phytase supplementation increased (Raut *et al.*, 2018). If the feed cost and DOC cost were the major inputs considered, super dosing of phytase could make a reasonable profit margin. Super dosing of phytase improved digestibility and better utilization of nutrients, thus improved the net profit per kg of broiler significantly at supplementation of 1500 FTU phytase/kg (Khose *et al.*, 2003; Dhore *et al.*, 2012; Jadhav *et al.*, 2011).

2.4 Justification of the present study

From the above discussion, it can be said increasing the phytase level more than 500 FTU/kg in diet can positively influence the growth response of birds. However, the level of phytase super dosing varies from 1000 to 7600 FTU/kg over the published works. Besides, the effect of increasing level of phytase on visceral organ development, carcass quality and serum profile are very limited. Therefore, further research is needed to identify the precise level of phytase dosing and its impact on overall performance, carcass quality, serum profile and economic profitability.

CHAPTER 3: MATERIALS AND METHODS

The experiment was carried out at the Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University (CVASU) to ascertain the effects of increasing level of phytase on performance, plasma mineral contents, and bone mineralization in the broiler. Feeding trial in broiler chicken was performed at the Poultry research shed of CVASU campus, during September-October 2019. Laboratory analyses were performed in Poultry nutrition laboratory and Biochemistry laboratory of CVASU, Khulshi, Chattogram.

3.1 Experimental design and collection of day-old broiler chicks

A total of 96 Cobb 500 day-old broiler chicks of either sex was purchased from a renowned hatchery (M M Aga Farm Ltd) on a pre-order basis to run the experimental trial from day 1 to 28days. The chicks were weighed on receiving day and then randomly assigned into four dietary treatment groups (D0, D1, D2, and D3), where each treatment was replicated 4 times with 6 birds per replicate in a completely randomized design (CRD). The layout of the experimental trial was demonstrated below in Table 1.

Table 1: Layout of the experiment

Treatment	**No. of birds per replicate**				**No. of birds per**
	R_1	**R_2**	**R_3**	**R_4**	**treatment**
D_0	6	6	6	6	24
D_1	6	6	6	6	24
D_2	6	6	6	6	24
D_3	6	6	6	6	24
Total	24	24	24	24	Grand Total=96

3.2 Formulation of experimental diets

Starter diet (crumble) was procured from the local market which was provided to the chicks up to 12 days of age as an adjustment period. The proximate composition of the ready-made starter diet (NaharTM) according to manufactured company were shown in Table 2. Four different test diets (D0, D1, D2, and D3) were formulated with the locally available feed ingredients to fulfill or exceed the requirements of NRC (1998), as shown below in Table 3, where diets were iso-caloric and iso-nitrogenous. The experimental enzyme-phytase (Renaphytase®) was collected from a medicine company (Renata Pharmaceuticals Ltd.). Samples were taken from the handmade diets before supplying the chicks in trial pen and sent to the lab for proximate analyses. All feedstuffs were used to formulate a control diet without phytase (D0), whereas D1, D2, and D3 test diets were prepared with the supplementation of phytase at the rate of 500 FTU, 1500 FTU, and 2500 FTU, respectively. After that, formulated diets were offered to the birds from day13-28. All the birds had free access to diets and fresh, clean, and cool drinking water during the entire trial period. The composition and nutritive values of formulated finisher test diets are shown in Tables 3 and 4 respectively.

Table 2: Nutrient composition of ready-made starter diet (Nahar starter feed™)

Nutrient components (%)	Proximate values
ME (kcal/kg)	3035
Moisture	11
DM	89
CP	22
CF	3
EE	5.70
Ash	6.20
Ca	0.9
P	0.45
Lysine	1.32
Methionine	0.5

Table 3: Composition of finisher diet for broiler chickens (13 -28 days)

Feed Ingredients (g/kg)	Diets			
	Do	**D_1**	**D_2**	**D_3**
Maize	62	62	62	62
Palm oil	3.75	3.725	3.725	3.725
Protein concentrate (Propack®)	3.80	3.80	3.7875	3.75
Soybean meal	27.84	27.84	27.84	27.84
Limestone	1.13	1.13	1.13	1.13
DCP	0.5	0.5	0.5	0.05
NaCl	0.25	0.25	0.25	0.25
L-lysine	0.16	0.16	0.16	0.16
DL-methionine	0.25	0.25	0.25	0.25
Vitamin min premix	0.25	0.25	0.25	0.25
Toxin Binder	0.03	0.03	0.03	0.03
Choline chloride	0.04	0.04	0.04	0.04
Phytase	0	0.025	0.0375	0.075
Total	100	100	100	100

[Control diet (Do) with no Phytase, whereas D1, D2 and D3 diets are supplemented with 500 FTU, 1500 FTU and 2500 FTU Phytase per kg of ration respectively]

Table 4: Calculated and analyzed value of the nutrient components (%) of finisher diet:

Nutrients	Finisher diets			
	Do	**D_1**	**D_2**	**D_3**
	Calculated value			
ME (kcal/kg)	3121.021	3118.78	3118.42	3117.33
CP	20.10	20.13	20.07	20.09
Ca	0.91	0.91	0.91	0.91
P	0.52	0.52	0.52	0.52
Lysine	1.22	1.22	1.22	1.22
Methionine	0.34	0.34	0.34	0.33
CF	3.27	3.27	3.27	3.27
EE	3.51	3.48	3.48	3.48
	Analyzed value			
DM	85.25	85.85	91.20	86.90
Moisture	14.75	14.15	8.80	13.10
CP	20.15	20.19	20.20	20.18
CF	3.20	3.50	3.50	3.20
EE	3.78	3.77	3.76	3.75
Ash	5.40	6.60	6.50	5.20

3.3 Management of birds

A total of 96 day-old-chicks (DOC) was randomly distributed into the 16 equal-sized, clean, and disinfected pens which were furnished with a feeder and a drinker. Initial average weight was taken at 1st day of brooding. Each pen (4.4 sq. ft.) was allotted for 6 birds. Therefore, floor space for each bird was 0.73 sq. ft. A 60-watt electric bulb was hanged at a height of 45 cm in the upper middle of each pen roof to maintain brooding temperature where each DOC was provided 0.3 watt light. The birds were exposed to a temperature of 35° C for the first two days. Then the temperature was gradually reduced by 1 or 2° C after every 1 or 2 days until the chicks arrived at 10 days of old. Afterward, the poultry shed temperature was maintained at 25° C for the rest of the trial.

Feed and drinking water were supplied ad-libitum to the birds throughout the experimental period. Starter feed was supplied to birds from day 1 to day 12 once per day in the tube feeder in the early morning as an adjustment diet. Paper along with tube

feeders and drinkers were used for feeding and watering the chicks during the early stages soon after coming from the hatchery. The finisher mash diets were given to the experimental birds from D 13-28 days. Birds were vaccinated against Ranikhet or New-castle disease and Gumboro disease according to the schedule mentioned in Table 5. Adequate and proper hygiene and sanitary measures were adopted and followed throughout the experimental period. Proper cleaning and disinfection of all equipment were done before the beginning of the trial.

Table 5: Vaccination schedule

Age (Days)	Name and type of the vaccine	Name of disease	Route of administration
5	Cevac New L^{R}, Live	Newcastle disease	One drop in one eye
12	GumboMed Plus™, Live	Gumboro	One drop in one eye
19	Cevac New L^{R}, Live	Gumboro	One drop in one eye

3.4 Sample collection

On d 28, two birds were selected randomly from each replicate for sample collection. The birds were slaughtered humanely by cutting the jugular vein. Blood samples were collected in a falcon tube separately. After centrifugation at 5000 revolutions per minute, the serum samples were taken into the 2ml eppendorf tube and stored at -20 ^{o}C until further analysis. The tibia bones were also collected from the same birds and stored at -20 ^{o}C for further processing and analysis. Different meat yield parameters such as carcass weight, dressed weight, weights of different meat cuts (neck, thigh, wings, breast, drumstick), and giblets weights (heart, lungs, liver, shank, proventriculus and gizzard and abdominal fat) were recorded. Besides, weights of other samples such as small intestine, pancreas, proventriculus. Meat yields and cuts were also recorded from the same birds to evaluate carcass yields. Bodyweight, feed intake, and remaining feeds

were recorded weekly basis and the FCR was calculated accordingly. As there was no occurrence of death in the bird population during the trial period, so mortality was not recorded.

3.5 Sample processing and chemical analysis

Feed samples were collected from formulated test diets before feeding the birds. The samples were processed by grinding with the help of mortar and pestle and then mixed thoroughly for lab analyses. About 500 gm of each diet of finisher were taken for proximate analysis. The samples were tested for proximate analysis having dry matter (DM %), moisture %, crude protein (CP %), Crude Fiber (CF %), and ash using standard laboratory procedures (AOAC, 2007). Dry matter estimation was done by the oven-dry method. Crude protein estimation was accomplished by the Kjeldahl Method. Ash was measured by igniting the pre-ashing sample on a muffle furnace at a temperature of 600°C for four to six hours. The serum total Protein (TP), Calcium (Ca), Phosphorus (P), alkaline phosphatase (AP), GPT (glutamic pyruvic transaminase), GOT (glutamic oxaloacetic transaminase) level were analyzed by using their respective standard assay kit (Randox Laboratories Ltd, UK) and semi-automated Humalyzer (Humalyzer 4000 Merck®, Germany). For bone sample analysis, the left tibia from each sampled bird was removed between the tibial-tarsal joint and the tibial-femoral joint. Firstly, the bones were defleshed of muscle and tissue by hand using a scalpel and then weighed. Length and width were also measured for each tibia. The tibia bones then were dried in a force draft oven (95°C) to reach a constant weight. The dried tibia bones were ashed at 650◦C for 23 h. The bone ash for each tibia was then digested with aqua regia and analyzed for Ca and P content using standard laboratory procedures (AOAC, 1990).

3.7 Cost benefit analysis

Cost of production was calculated considering the expense on chick, feed, medicine, labor, etc. Chick cost was calculated from the purchasing cost. Feed cost was considered from the sale price of the feed marketed through dealers. Cost-benefit analysis is shown in Table 12.

3.8 Statistical analysis

All collected data were subjected to analysis by one-way ANOVA procedure using SPSS software V.25. The significance of differences between means was tested using the least significance difference (LSD). Statistical significance was considered at $P \leq 0.05$.

Fig 1: Renaphytase®

Fig 2: Weighing micro nutrients

Fig 3: Weighing of feed ingredients

Fig 4: Initial mixing of feed ingredients

Fig 5: Mixing of micro nutrients

Fig 6: Hand mixing of ration ingredients

Fig 7: Placing the DOC in the prepared brooding pan

Fig 8: Floor space for 6 birds

Fig 9: Immunization

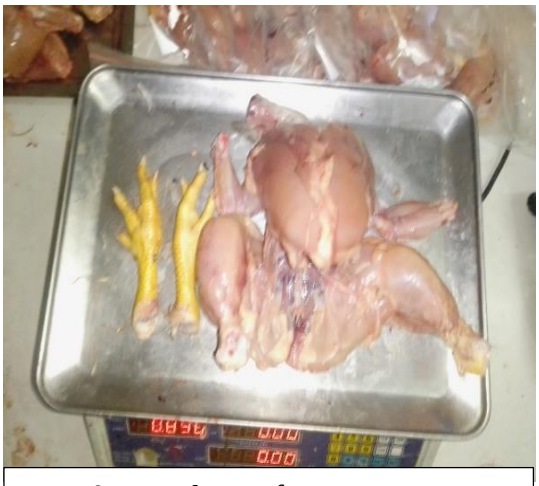

Fig 10: Weighing of carcass

Fig 11: Sample preparation for Bio-chemical test

Fig 12: Biochemical analysis

CHAPTER 4: RESULTS

4.1 Gross responses

The effect of the increased level of phytase supplementation on gross responses of broiler chickens is summarized in Table 6. There was no effect *(P > 0.05)* of phytase on BWG and FI of broiler chickens from d 13 to 28. However, the highest weight gain was recorded for the diet group D2 followed by D1, D0, and D3. FI was tended *(P = 0.056*) to increase by the phytase supplementation to diets. Birds that receive the D3 diet consumed more feed compared to those on other diets. The FCR of broilers was significantly influenced by dietary treatment from d 13 to 28. Birds on the D1 and D2 diets showed better *(P < 0.05)* FCR than those on D0 and D3 diets.

Table 6: Effect of different level of phytase on growth performance (d 13-28)

Diets	Phytase (FTU/kg)	BWG	FI	FCR
D0	0	916.04	1640.83	1.83[a]
D1	500	937.04	1730.92	1.73[b]
D2	1500	966.21	1620.63	1.67[b]
D3	2500	892.83	1899.46	1.85[a]
SEM		22.11	67.57	0.02
P value		0.275	0.056	0.001

Data represent the means of 4 replicate cages (*n*=4); SEM= Standard Error Mean

[a-b] Means with different letters within the same column differ significantly *(P < 0.05).*

4.2 Tibia bone development

The effect of phytase supplementation on tibia bone development of broiler chickens is summarized in Table 9. There was no effect *(P > 0.05)* of phytase inclusion on the weight of the tibia bone of broiler chickens from d 13 to 28. However, the length and width of tibia bone was significantly influenced by phytase supplementation. Birds offered D3 diets showed minimum length and width of the tibia bone compared to birds that received other diets. The highest *(P < 0.05)* Ca deposition was observed in birds fed the D2 diet than those on other diets. Supplementation of phytase did not affect *(P > 0.05)* the P concentration of tibia bone of broiler chickens.

Table 7: Effect of dietary phytase level on tibia bone quality of birds at d 28

	D0	D1	D2	D3		
	Phytase (FTU/kg)					
	0	500	1500	2500	SEM	P value
Weight (gm)	16.55	17.95	17.76	17.42	0.24	0.185
Length (mm)	77.12[a]	77.81[a]	77.99[a]	74.59[b]	0.40	0.001
Width (mm)	7.96[a]	8.11[a]	8.10[a]	6.92[b]	0.13	0.001
Ca %	11.67[b]	12.20[b]	14.26[a]	11.89[b]	0.32	0.002
P %	7.32	7.29	7.55	6.83	0.12	0.206

Data represent the means of 4 replicate cages (*n*=4); SEM= Standard Error Mean

[a-b] Means with different letters within the same row differ significantly *(P < 0.05).*

4.3 Serum biochemistry

The effect of phytase supplementation on serum contents of broiler chickens from d 13 to 28 is summarized in Table 10. The serum TP and P levels were increased *(P < 0.001)* in birds consumed D1 and D2 birds than those on D0 and D3 diets. Dietary treatment had no significant effect on serum Ca, GPT, and GOT levels in broiler chickens.

Table 8: Effect of different level of phytase on blood parameters of broiler chicken (d 13 to 28)

	D0	D1	D2	D3	SEM	P value
	Phytase (FTU/kg)					
Traits	**0**	**500**	**1500**	**2500**		
TP (g/dl)	3.22[b]	4.08[a]	4.32[a]	3.29[b]	0.14	0.001
P (mg/dl)	4.98[b]	5.55[a]	5.67[a]	4.08[b]	0.50	0.001
Ca (mg/dl))	9.07	11.28	11.77	10.42	0.11	0.528
ALT (U/L)	56.01	51.75	55.38	56.38	1.38	0.669
AST (U/L)	204.02	201.47	219.28	202.28	2.82	0.068
AP ((U/L)	1200.88	1201.56	1229.31	1200.94	39.38	0.994

Data represent the means of 4 replicate cages (*n*=4); SEM= Standard Error Mean

[a-b] Means with different letters within the same row differ significantly *(P < 0.05)*.

TP = Total protein; P = Phosphorus; Ca= Calcium; ALT = Alanine aminotransferase

AST= Aspartate aminotransferase; AP = Alkaline phosphatase

4.4 Carcass yield parameters

The effect of phytase supplementation on carcass yield and cuts of broiler chickens is summarized in Table 7. There was no significant effect of phytase supplementation on dressing % and meat yields except for drumstick. Birds consumed the D0 and D3 diets had a smaller *(P < 0.022)* drumstick than those on other diets.

Table 9: Effect of different level of phytase on carcass characteristics of birds (d 13 to 28)

Carcass traits (%)	**D0**	**D1**	**D2**	**D3**	**SEM**	**P value**
	Phytase (FTU/kg)					
	0	**500**	**1500**	**2500**		
Dressing	67.24	65.61	66.63	65.67	0.55	0.136
Breast	22.66	22.47	20.88	22.73	0.49	0.534
Drumstick	8.00[b]	8.85[a]	8.80[a]	8.02[b]	0.12	0.022
Thig	10.28	10.24	10.44	10.36	0.15	0.932
Neck	2.51	2.68	10.50	10.36	0.09	0.833
Shank	4.29	4.15	4.11	4.20	0.07	0.895
Wing	5.28	5.30	4.79	5.23	0.11	0.376

Data represent the means of 4 replicate cages (*n*=4); SEM= Standard Error Mean

[a-b] Means with different letters within the same row differ significantly (*P*<0.05).

4.5 Visceral Organs development

Table 8 summarizes the effect of phytase supplementation on visceral organ development of broiler chickens. Supplementation of phytase influenced *(P < 0.05)* the weight of the liver, heart, and spleen. Birds that consumed the D1 diet showed bigger liver (2.72gm) followed by D2, D3 and D0. The size of the heart increased *(P < 0.036)* in birds fed D0 and D3 diets than those received D1 and D2 diets. The weight of the

spleen was highest in birds consumed D3 diet than birds on other diets. There was no significant effect of phytase supplementation on the weight of the small intestine, proventriculus, gizzard, pancreas, bursa, and abdominal fat.

Table 10: Effect of different level of phytase on visceral organ development of birds (d 13 to 28)

Visceral organ weight (g/100 g BW)	D0	D1	D2	D3	SEM	P value
	Phytase (FTU/kg)					
	0	500	1500	2500		
SI	2.46	2.43	2.65	2.67	0.99	0.790
Proventriculus	0.58	0.64	0.58	0.71	0.05	0.844
Gizzard	2.95	3.33	3.37	3.30	0.12	0.637
Liver	2.10^{b}	2.72^{a}	2.52^{ab}	2.43^{ab}	0.08	0.019
Heart	0.58^{a}	0.45^{b}	0.48^{b}	0.63^{a}	0.02	0.001
Spleen	0.07^{b}	0.08^{b}	0.08^{b}	0.13^{a}	0.01	0.009
Pancreas	0.30	0.30	0.25	0.23	0.01	0.153
Bursa	0.05	0.05	0.06	0.05	0.003	0.812
Abdominal fat	2.09	2.54	2.24	2.66	0.13	0.405

Data represent the means of 4 replicate cages (n=4); SEM= Standard Error Mean

$^{a-b}$ Means with different letters within the same row differ significantly ($P < 0.05$)

SI= Small intestine

4.6 Cost Benefit analysis

Table 12 shows the cost-benefit analysis of broiler chickens fed diets supplemented with different level of phytase. The cost-benefit parameters were significantly better in birds fed D1 and D2 diets than those received D0 and D3 diets. The feed costs and production cost /kg live bird were reduced *($P < 0.001$)* in birds fed D1 and D2 diets than birds consumed D0 and D3 diets. The highest *($P < 0.001$)* profit /kg live bird was observed in birds fed D2 diet (20.38Tk) followed by diet D1, D0 and D3. The D1 and D2 diets showed better *($P < 0.001$)* cost: benefit ratio than other diets.

Table 11: Economics of broiler production supplemented with varying levels of phytase

Diets	Phytase (FTU/kg)	Feed cost/kg live bird	Production cost/kg live bird	Profit (Tk)/kg live bird	Cost: Benefit ratio
D0	**0**	67.17[a]	117.17[a]	12.83[b]	9.18[a]
D1	**500**	59.87[b]	109.87[b]	20.13[a]	5.80[b]
D2	**1500**	59.62[b]	109.62[b]	20.38[a]	5.38[b]
D3	**2500**	69.33[a]	119.33[a]	10.67[b]	10.89[a]
SEM		1.32	1.32	1.32	0.65
P value		0.001	0.001	0.001	0.001

Data represent the means of 4 replicate cages (*n*=4); SEM= Standard Error Mean

[a-b] Means with different letters within the same row differ significantly *(P < 0.05)*

CHAPTER 5: DISCUSSION

5.1 Gross response

In this study, dietary supplementation of phytase did not affect BWG of broiler chickens from d 13 to 28. This is consistent with the previous findings (Dos Santos *et al.*, 2013; Walk *et al.*, 2013). However, the overall BWG of broiler chickens of different diet groups at d 28 was below the standard weight of cobb broilers (Cobb 500, 2013). This could be due to the use of mash diets instead of pellet diets for birds (Scholey *et al.*, 2018).

Phytase supplementation tended *($P = 0.056$)* to affect the FI of broiler chickens at the end of 28 days. Birds received a diet with the highest level of phytase (2500 FTU /kg) consumed more feed which is in accordance with a similar study by Pirgozliev *et al.*, (2008b). These authors reported that FI increased by 22% when phytase level was increased to 2500 FTU/kg. Besides, the lowest FI was observed in birds fed a D1 (500 FTU/kg) diet which agrees with the previous study (Augspurger *et al.*, 2007; Santos *et al.*, 2008; Saima et al., 2009). The possible explanation of improved feed consumption can be due to the increased availability of dietary P by phytase supplementation (Scholey *et al.*, 2018).

In the current study, birds that consumed D1 and D2 diet showed better FCR than those on the D0 and D3 diets. Moreover, D1 and D2 diet also non-significantly reduced the feed consumption but improved the weight gain. This improvement in growth response therefore could be the result of the beneficial effect of phytase that releases phytate-bound nutrients and make them available for the utilization of the birds (Pieniazek *et al.*, 2017; Raut *et al.*, 2018; Leyva-Jimenez *et al.*, 2019).

5.2 Tibia bone development

In this study, the maximum width and length of the tibia bone was observed in birds offered a D1 and D2 diet with 500 and 1500 FTU phytase /kg, respectively. These groups of birds also showed an increased concentration of Ca in tibia bone than birds on other diets. These findings are consistent with previous studies (Pieniazek *et al.*,

2017; Leyva-Jimenez *et al.*, 2019). Interestingly, supplementation of 2500 FTU phytase/kg of diet reduced the Ca deposition, length, and width of tibia bone of broiler chickens. However, it has been claimed that a higher dose of phytase cause complete hydrolysis of phytate, thus releases more Ca than P whereas a commercial dose of phytase liberates more P than Ca (Scholey *et al.*, 2018). Therefore, there is a possibility of occurring imbalance in the Ca:P ratio when an increased level of phytase is added to the diet that already contained enough available P. The imbalance of the Ca and P ratio consequently leads to the formation of the Ca-phytate complex and reduce the phytase activity (Walk *et al.*, 2013).

5.3 Serum biochemistry

The concentration of serum P was increased in birds consumed D1 and D2 diet supplemented with 500 and 1500 FTU phytase/kg of diet which is in line with the growth performance of these groups of birds. Supplementation of phytase improved the serum TP level in birds received D1 and D2 diets than those on D0 and D3 diets. The improvement in serum P level could be the result of phytase-induced hydrolysis of phytate-mineral complex (Simons *et al.*, 1990). Previous studies also reported increased concentration of serum P level by phytase supplementation to the diet (Augspurger *et al.*, 2004; Bhanja *et al.*, 2005; Arabi *et al.*, 2010; Kozlowski and Jeroch, 2011; Farzinpour *et al.*, 2011; Jalani *et al.*, 2012; Kuhn *et al.*, 2012; Rutherford *et al.* 2012; Wang *et al.*, 2013, Beiki *et al.*, 2013; Arabi *et al.*, 2013). The serum GOT, GPT, and AP level to act as an indicator for liver health. The non-significant effect of treatment on serum enzymes suggested that the liver functions were not affected by phytase supplementation in this study. A similar finding was also reported by (Ciurescu *et al.*, 2020).

5.4 Carcass yield

In this study, the maximum weight of the drumstick was observed in birds offered D1 and D2 diets than those on D0 and D3 diets. Non-significant effects of phytase were recorded for breast, thigh, wing, and neck development which is consistent with the previous study (Sharma *et al.*, 2016).

5.5 Visceral organ development

In this study, the weight of the spleen was recorded highest for the D3 diet than the D0 diet, and this agrees with the findings of (Sharma *et al.*, 2016). Birds fed phytase supplemented diets (D1, D2, D3) showed larger liver than those on diet without phytase. The increased weight of the heart in the birds fed diet without phytase in this trial can be due to the lack of available P. It has been reported that lack of available P supply causes hyperphosphatemia resulting in heart hypertrophy (Sousa *et al.*, 2015). The lack of phytase effect on the relative weight of bursa and abdominal fat is also consistent with the previous study (Sharma *et al.*, 2016; Broch *et al.*, 2018).

5.6 Cost-benefit analysis

It was observed that the total profit/ kg live bird was significantly better in broiler chickens fed D1 and D2 diets supplemented with 500 and 1500 FTU/kg, respectively. These findings agree with Raut *et al.* (2018). The reduced total feed and production cost of the birds on D1 and D2 diets support this finding. Because phytase supplementation releases minerals like P Ca, Zn, etc. and other nutrients (protein) and alleviated the anti-nutrient effects of phytate which improve the FCR and thus reduce the feed cost. However, supplementation of 2500 FTU phytase/kg of diet showed the lowest total profit/kg live bird and highest cost: benefit ratio that is comparable to those on phytase free diet. This can be explained by the worst FCR of this diet group of birds.

CHAPTER-6: CONCLUSION

Supplementation of 500 and 1500 FTU/kg of phytase improved the FCR of birds. The width, and length of the tibia bone were significantly highest in birds fed diet supplemented with 500 and 1500 FTU phytase/kg. Supplementation of 1500 FTU/kg to diet also improved the concentration of Ca in tibia bone. The serum P and TP level was improved in birds fed diets supplemented with 500 and 1500 FTU phytase/kg of diet. Diet supplemented with 500 and 1500 FTU/kg also reduced the total feed and production cost/kg live bird, thus improved the total profit/kg of bird. However, there was no improvement in growth response and other variables when the highest level (2500 FTU/kg) of phytase was supplemented to diets. In conclusion, the study results indicate that the use of 500 and 1500 FTU phytase per kg of diet could potentially improve the overall performance and consequently increase the total profit/kg live bird. Further research is needed to examine the effect of super-dosing phytase on a marginally P deficient diet.

LIMITATION AND RECOMMENDATION

Several problems were confronted during the period of the research study. The following recommendations would be suggested to carry out the further research study successfully.

1. The effect of phytase on the digestibility of nutrients warrants further research.
2. Due to a lack of required lab facilities and infrastructure, it was not possible to measure the phytase level in the diet after mixing in the current study.
3. Pellet feed is more homogenous in nutrients contents and utilized by birds more efficiently than mash feed. There is a lack of facility for mechanical feed mixing and pelleting. Therefore, machine mixing, and pelleting facilities could be installed for efficient feed formulation and the facilitation of further research works in the future.

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Appendix A: Gross responses of birds from d 13-28

Cage no.	Treatment	BWG (gm) @ d 13	BWG (gm) @ d 26	BWG(gm) @ (d 13-26)	Feed intake (gm) (d13-26)	FCR (d 13-26)
1	DoR1	2740	9000	6260	9415	1.50399361
2	DoR2	2600	8400	5800	10270	1.770689655
3	DoR3	2620	8475	5855	10720	1.830913749
4	DoR4	2695	8530	5835	10604	1.81730934
5	D1R1	2670	8400	5730	10754	1.876788831
6	D1R2	2580	7300	4720	9016	1.910169492
7	D1R3	2570	8030	5460	9264	1.696703297
8	D1R4	2540	7900	5360	9864	1.840298507
9	D2R1	2670	8000	5330	9214	1.728705441
10	D2R2	2590	8000	5410	10079	1.863031423
11	D2R3	2575	8260	5685	9348	1.644327177
12	D2R4	2400	5550	5680	9200	1.61971831
13	D3R1	2464	8300	5836	10500	1.799177519
14	D3R2	2530	8050	5520	12645	2.29076087
15	D3R3	2600	8300	5700	10245	1.797368421
16	D3R4	2530	8000	5470	9744	1.781352834

Appendix B: Carcass weight (gm) on day28

Cage no.	Treatment	Live weight (gm)	Carcass(gm)	Dressing %	breast(gm)	Drumstics	Thigh	Shank	Neck	Wings
1	DoR1	1515	1026	67.722772	321	142	155	66	35	80
2	DoR2	1435	927	64.599303	320	115	155	55	37	75
3	DoR3	1525	1052	68.983607	375	135	150	63	35	80
4	DoR4	1530	1035	67.647059	345	146	157	74	44	82
5	D1R1	1560	1024	65.641026	366	122	150	65	36	70
6	D1R2	1336	900	67.365269	330	105	150	52	35	67
7	D1R3	1480	932	62.972973	285	122	145	62	42	85
8	D1R4	1513	1006	66.490416	340	122	156	66	45	90
9	D2R1	1490	965	64.765101	324	126	166	53	45	72
10	D2R2	1415	890	62.897527	296	126	135	62	30	72
11	D2R3	1300	847	65.153846	285	116	132	55	34	65
12	D2R4	1300	802	61.692308	246	116	142	56	33	55
13	D3R1	1504	966	64.228723	316	142	154	66	35	84
14	D3R2	1455	965	66.323024	344	133	143	65	43	66
15	D3R3	1500	950	63.333333	303	122	156	56	40	82
16	D3R4	1500	1032	68.8	391	135	173	65	41	80

Appendix C: Tibia bone on day 28

Cage no.	Treatment	Weight of tibia/bird (gm)	Average length (mm)	average width (mm)
1	DoR1	16.07	78	8.1
2	DoR2	16.21	78.2	8.12
3	DoR3	16.44	76.15	8.12
4	DoR4	16.5	76.13	8.14
5	D1R1	16.9	77.2	7.95
6	D1R2	17.71	78.13	8.1
7	D1R3	19	75.1	8.7
8	D1R4	18.2	77.79	7.8
9	D2R1	17.2	77.1	8.1
10	D2R2	14.91	76.42	8.33
11	D2R3	18.58	78.51	7.35
12	D2R4	17.5	77.55	8

13	D3R1	19.01	76.15	7
14	D3R2	21.98	78.12	6.84
15	D3R3	18.1	74.1	6.9
16	D3R4	15.8	72	6.94

Appendix D: Visceral organ (gm) traits on day 28

Cage no.	Treatment	Small intestine	Proventri-ulus	Gizzard	Liver	Heart	Spleen	Pancreas	Bursa
1	DoR1	2.35	.59	3.28	2.34	0.62	0.09	0.27	0.05
2	DoR2	2.83	.66	3.09	2.28	0.67	0.09	0.39	0.04
3	DoR3	2.00	.65	3.15	2.09	0.62	0.06	0.27	0.04
4	DoR4	2.65	.43	2.29	1.69	0.63	0.11	0.26	0.06
5	D1R1	2.88	.72	3.53	2.57	0.50	0.08	0.32	0.05
6	D1R2	2.05	.60	3.18	2.74	0.45	0.09	0.24	0.04
7	D1R3	2.79	.71	3.53	3.15	0.44	0.05	0.37	0.06
8	D1R4	1.99	.54	3.08	2.44	0.53	0.07	0.29	0.06
9	D2R1	2.06	.70	2.95	2.55	0.44	0.07	0.24	0.03
10	D2R2	2.65	.59	3.45	2.69	0.44	0.05	0.24	0.06
11	D2R3	2.42	.38	2.70	2.40	0.49	0.09	0.27	0.07
12	D2R4	3.48	.67	4.38	2.47	0.43	0.10	0.23	0.08
13	D3R1	2.57	.52	3.53	2.40	0.53	0.11	0.19	0.05
14	D3R2	2.77	1.34	3.09	2.50	0.63	0.11	0.19	0.04
15	D3R3	2.63	.47	3.81	2.61	0.53	0.14	0.23	0.06
16	D3R4	2.69	.51	2.76	2.23	0.63	0.14	0.31	0.06

Appendix E: Cost benefit analysis

Cage no.	1	2	3	4	5	6	7	8
Treatment	D0R1	D0R2	D0R3	D0R4	D1R1	D1R2	D1R3	D1R4
Feed intake(kg)	9.415	10.27	10.72	10.604	10.754	10.016	9.264	9.914
Feed price per kg(tk)	37.1	37.1	37.1	37.1	37	37	37	37
Total Feed cost (tk)	349.30	381.02	397.71	393.41	397.90	370.59	342.77	366.82
LWG (kg)	6.26	5.80	5.86	5.84	5.73	5.00	5.46	5.36
Feed cost (tk)/kg live bird	55.80	65.69	67.93	67.42	69.44	74.12	62.78	68.44
DOC price (tk)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
others cost/kg live bird (tk)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Total production cost/kg live bird (tk)	105.80	115.69	117.93	117.42	119.44	124.12	112.78	118.44
Market price/kg live bird (tk)	130.00	130.00	130.00	130.00	130.00	130.00	130.00	130.00
Profit /kg live bird (tk)	24.20	14.31	12.07	12.58	10.56	5.88	17.22	11.56
Cost : benefit	4.37	8.09	9.77	9.34	11.31	21.10	6.55	10.24

9	D2R1	9.214	36	331.70	5.33	62.23	25.00	25.00	112.23	130.00	17.77	6.32
10	D2R2	10.079	36	362.84	5.41	67.07	25.00	25.00	117.07	130.00	12.93	9.05
11	D2R3	9.348	36	336.53	5.69	59.20	25.00	25.00	109.20	130.00	20.80	5.25
12	D2R4	9.65	36	347.40	5.10	68.12	25.00	25.00	118.12	130.00	11.88	9.94
13	D3R1	10.49	35	367.15	5.84	62.91	25.00	25.00	112.91	130.00	17.09	6.61
14	D3R2	12.645	35	378.12	5.52	68.50	25.00	25.00	118.50	130.00	11.50	10.30
15	D3R3	10.245	35	358.58	5.70	62.91	25.00	25.00	112.91	130.00	17.09	6.61
16	D3R4	9.744	35	341.04	5.47	62.35	25.00	25.00	112.35	130.00	17.65	6.36

Brief biography of the author

Sajjad Hossain graduated in 2017 from the Faculty of Veterinary Medicine, Chattogram Veterinary & Animal Sciences University (CVASU) Khulshi-4225, Chattogram, Bangladesh. He is doing his Master of Science in Poultry Science, Faculty of Veterinary Medicine, CVASU. He is looking forward to carrying out research in his area of interest and enormous enthusiasm to develop his skills and expertise in the area of Poultry. In order to complete his thesis, he conducted a research work at Poultry Science laboratory and Bio-Chemistry laboratory, Chattogram Veterinary and Animal Sciences University.