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Research Article

Effects of Dietary Supplementation of Probiotic and Synbiotic on Post-hatch Splenic Development in Broiler Chicken

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ABSTRACT

Introduction: The use of antibiotics in poultry feed is becoming more prohibited globally, it is important to consider the potential of natural feed additives as antibiotic substitutes to support the development and operation of chickens' immune systems. The study aimed to assess the effect of probiotic and synbiotic supplementation in broiler feed on the post-hatch development of broiler spleen.

Materials and methods: A total of 270-day-old broiler chickens of both sexes were randomly assigned to three dietary treatments each consisting of three replicates and each replicates had 30 chickens. The dietary treatments included the control group receiving a basal diet, the second group receiving a basal diet supplemented with probiotic (Pro.B® with 0.5 gm/Kg feed), and the third group receiving a basal diet supplemented with synbiotic (SynBac® with 1 gm/4-liter water). Probiotic and synbiotic supplementation in feed was started from day seven. Samples (n=15) were collected on the first day and every week until five weeks of age, and the biometry and histomorphometry parameters of the spleen in different groups were studied.

Results: The biometrical values of spleen were higher at every age group in both probiotic- and synbiotic-treated broiler chickens compared to the control broilers, but significantly higher biometrical values were observed only in synbiotic-treated broiler chickens at five weeks of age compared to other groups. Regarding histomorphometry parameters, capsule thickness was insignificantly lower until day 28 and significantly lower at day 35 in both probiotic and synbiotic-treated broiler chickens compared to the control group. Moreover, the length and width of lymphatic nodules were higher in both probiotic and synbiotic-treated broiler chickens compared to the control broiler chickens. Remarkably, no significant differences were observed in the biometrical and histomorphometric parameters of the spleen between the probiotic and synbiotic-treated broiler chickens.

Conclusion: Dietary inclusion of probiotics and synbiotics in broiler feed could improve the post-hatch growth of the spleen. However, the supplementation of both synbiotics, along with probiotics in the broiler diet had a profound influence on the post-hatch growth of the spleen which has been indicated by a significant increase in biometrical values.

1. Introduction

The total performance of broiler immune systems is strongly influenced by the growth and development of their immune organs. Maintaining immune function is interrelated to the development of peripheral lymphoid organs, such as the spleen in chickens[1]. The spleen, the largest immunological organ, aids in the immune response by removing damaged or infected cells from the bloodstream and providing immunity to infection for the

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host[2]. Spleen size is used as an indicator of immune system response in different conditions[3].

Considering the food safety issue, poultry farmers show a great interest in natural antibiotic alternatives including probiotics, prebiotics, synbiotics, phytobiotics, etc. Probiotics have a number of positive health impacts on human and animal health and modulate intestinal microflora inhibit pathogens[4], and improve the quality of meat and bone[5]. Probiotic-fed birds had a greater tibial length, weight, and strength as well as higher plasma levels of calcium and phosphorus compared with the controls. In addition, probiotic-fed birds' leg muscles had higher color lightness at both 30 min and 5 h postmortem and greater water-holding capacity with a trend for less cooking loss and lower pH values at 5 hours postmortem[5]. Probiotics in poultry work by producing bacteriocins, stimulating the immune system linked to the gut, lowering pH through acid fermentation, competing for nutrients and mucosal attachment, promoting the production of short-chain fatty acids, and maintaining normal intestinal microflora[6]. Probiotics provide all the necessary activity needed to enhance the production and performance of poultry. Moreover, probiotics meet all the food safety and eco-friendly parameters. Probiotics enhance the gut flora, improve immunity, increase the activity of digestive enzymes by decreasing the production of ammonia, bind with enterotoxins and neutralizing them and as a result, improve feed intake which directly affects the performance and production of the poultry[7]. A blend of prebiotics and probiotics called synbiotics can potentially enhance the effectiveness of probiotic preparations by enhancing the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. Dietary synbiotic supplementation improves leg health quality[8], and intestinal integrity[9] in broiler chickens.

Aside from genetics, the environment and feed can modulate the development of the immune system. The immune system's maturity is greatly influenced by interactions with a healthy microbiome[10]. Potential and beneficial intestinal microflora plays an important function in the control of innate and adaptive immunity and the development of immunological organs[11]. This is especially important at hatching when gut-associated lymphoid tissue is immature and requires early stimulation. Otherwise, it will weaken the health status and performance of the animal[12]. Supplementing broilers with probiotics and synbiotics may be a major factor in supplying the beneficial microbes in the broiler gut, which will ultimately enhance the development of their immune systems. Therefore, the present study was undertaken to assess the dietary effect of probiotics and symbiotic on the post-hatch development of the spleen of broiler chickens using a biometric and histomorphometric approach.

2. Materials and Methods

2.1. Ethical approval

The rules established by the University of Rajshahi, Bangladesh's Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee were followed for conducting animal research (Memo No. 455(13)/ 320/IAMEBBC/IBSc).

2.2. Animals

A total of 270 day-old broiler chicks (Efficiency Plus [EP]) were procured from Aftab Bahumukhi Farms Limited, Bangladesh. The day-old chicks were physically and physiologically normal. With unrestricted access to food and water, the experimental broilers were kept in sanitary settings. From days 1 to 14, the broiler starter; from days 15 to 28, the broiler grower, and from days 29 to 35, the broiler finisher (Nourish Feeds Limited, Bangladesh) was fed to the experimental chickens. The recommended immunization schedule was followed. Throughout the whole trial period, every effort was made to maintain the uniformity of the management practice including a lighting period of 23 hours and a 1-hour dark phase each day; a temperature of 21-25°C and 55-65% broiler house's relative humidity. A total of 49 square feet (7 feet × 7 feet) was allotted for each pen for 32 broiler chickens. In light of this, each chicken was given a 1.5-square-foot habitat.

2.3. Experimental groups

Three experimental units, comprising control, probiotic-treated, and synbiotic-treated groups, were randomly assigned to the day-old broiler chicks. There were six age sub-groups (n=15) for each group (n=90): post-hatch day 1, 7, 14, 21, 28, and 35.

2.4. Treatments

The dietary treatments were including, (1) control with basal feed, (2) basal feed enhanced with probiotic (Pro.B® @ 0.5 gm/Kg feed), and (3) the basal feed enhanced by synbiotic (SynBac® @ 1 gm/4 liter water). Probiotic and synbiotic supplementation with feed was started from day 7 and continued until day 35. The composition of probiotics and synbiotic used in the broiler feed are presented in Table 1. The probiotics and synbiotic were manufactured by K.M.P. BIOTECH CO., LTD. Thailand, and PVF Agro Limited in Dhaka, Bangladesh, handled the marketing.

2.5. Tissue collection and preservation

On day 1, 7, 14, 21, 28, and 35, chickens from various

Table 1. The probiotic and synbiotic supplements were used in the broiler chicken diet from day 7 of age until day 35

Composition of probiotic (Pro.B®) per Kg		
1.	*Bacillus subtilis*	1.0×10^{10}cfu
2.	*Bacillus licheniformis*	1.0×10^{10}cfu
3.	*Bacillus pumilus*	1.0×10^{10}cfu
Composition of synbiotic (SynBac®) per Kg		
1.	*Bacillus subtilis*	5×10^{9}cfu
2.	*Pediococcusacidilacticil*	5×10^{9}cfu
3.	*Streptococcus faecium*	5×10^{9}cfu
4.	*Saccharomyces cerevisiae*	1×10^{9}cfu
5.	Xylo-oligosaccharide (XOS)	35 grams

experimental groups were killed by the cervical subluxation method. Spleen samples were obtained by dissection and cleaned in physiological saline solution following the sacrificing of the broilers. Immediately following biometric examination, the spleens that were collected were put in a fixative (10% formalin solution) to facilitate subsequent histomorphometric analysis.

2.6. Biometric study

The spleens of all experimental groups of broiler chickens were measured for biometric (weight, length, breadth, and thickness) values. Using sliding calipers on a millimeter (mm) scale, the spleen's size was ascertained, and its weight was measured in grams (gm) on an electronic digital weighing scale (Model: PS.P3.310, Taiwan).

2.7. Histomorphometric study

Formalin-fixed spleens of all experimental groups were processed in the lab using the standard paraffin section histology technique. Sections were cut to 5 µm thickness and, in accordance with Gofur *et al.*[13], stained using the standard Mayer's Hematoxylin and Eosin (H & E) stain, albeit with a few alterations. Stained spleen sections from the experimental groups were examined thoroughly at magnifications of 10 and 40 using compound microscopes. A photographic microscope system (digital camera model: C-B5, OPTIKA, Italy, equipped with a microscope, Model B-293PLi, OPTIKA, Italy) was used to capture the photographs of the stained tissue sections. The capsular thickness and the length and width of the lymphatic nodule were measured as histomorphometric features using a pre-calibrated ocular micrometer (Erma, Japan) in µm.

2.8. Statistical analysis

For every observation, the mean ± SD was used. Turkey HSD posthoc analysis was used in conjunction with one-way analysis of variance (ANOVA) using SPSS version 26 to assess differences in the biometrical and histomorphometric values of the spleen among the three experimental groups (control, probiotic- and synbiotic-treated) of broiler chickens of the same age according to Gofur *et al.*[14]. P values of 0.05 or less were used to represent significant differences.

3. Results

3.1. Effects of probiotic and synbiotic on biometry of spleen

The feed additives (synbiotic and probiotic) were added in broiler feed from day 7. So the comparative effect of dietary probiotics and synbiotics with the control was measured from day 14. Spleen's biometric measurements included its length, breadth, thickness, and weight. It was discovered that post-hatch age had an impact on the spleen growth of broiler chickens based on the biometrical parameters of the broiler spleen in all experimental groups. With the progression of post-hatch growing age, the spleen's biometric parameters grew throughout the whole experimental period in all experimental groups (Figure 1).

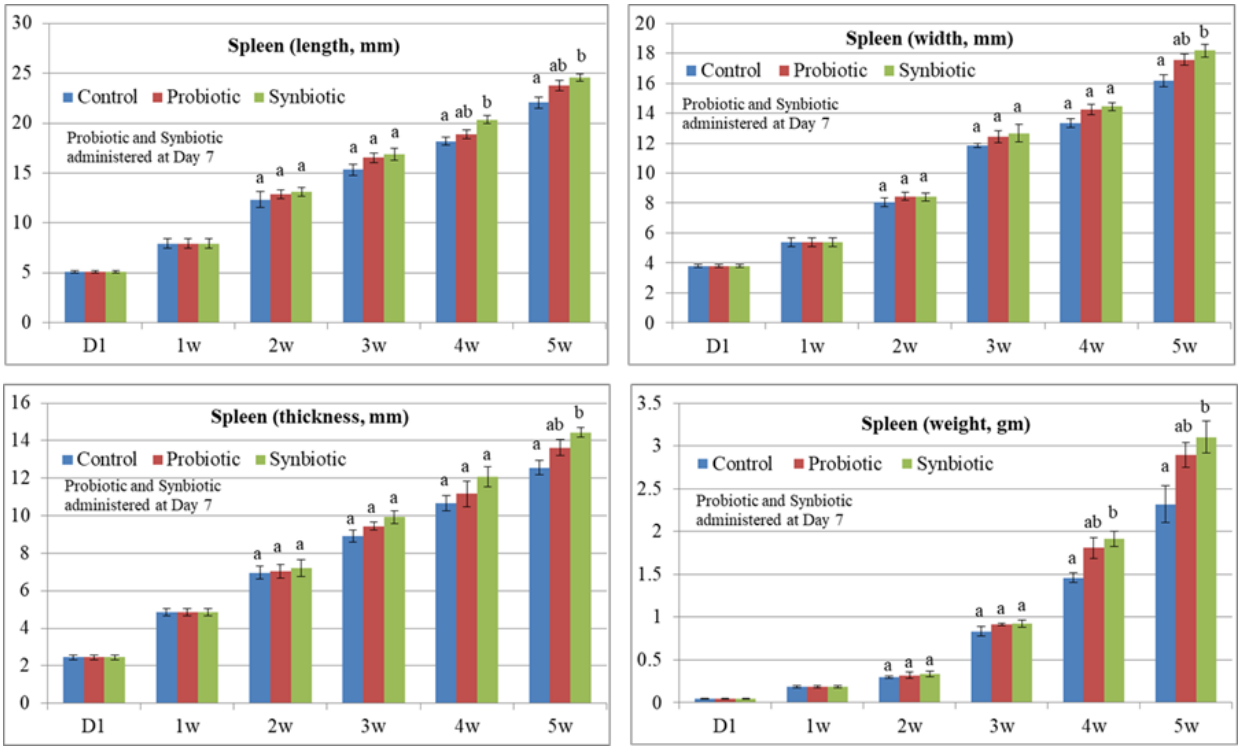

Figure 1. Comparative biometric measurements of the spleen in control, probiotic- and synbiotic-treated broiler chickens. Significant differences among the experimental groups of broiler chickens at a particular post-hatch age group are indicated by values within a group of columns with different superscripts ($p < 0.05$).

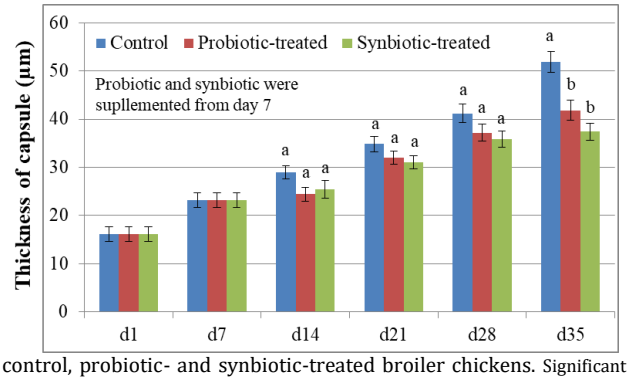

Figure 2. Thickness of splenic capsule in control, probiotic- and synbiotic-treated broiler chickens. Significant differences among the experimental groups of broiler chickens at a specific post-hatch age group denote values within a group of columns with different superscripts ($p < 0.05$).

The splenic biometrical values were insignificantly ($p > 0.05$) higher at every age group (from day 14 to day 35) in probiotic-treated broiler chickens compared to the control broilers, whereas in synbiotic-treated broiler chickens, all biometrical splenic values were higher at every age group compared to the control broilers but the length and weight were significantly ($p < 0.05$) higher from day 28 and width and thickness were significantly ($p < 0.05$) higher from day 35 (Figure 1) indicating when prebiotic was supplemented along with probiotic in the broiler feed, *i.e.*, synbiotic supplementation had a profound influence on the post-hatch growth of spleen. However, there was no discernible change between the biometrical values of the spleen of probiotic- and synbiotic-treated broiler chickens (Figure 1) though the synbiotic-treated broiler chickens had higher splenic biometric values than the probiotic-treated broiler chickens.

3.2. Effects of probiotic and synbiotic on histomorphometry of spleen

Broiler chickens in the control, probiotic- and synbiotic-treatment groups, all had similar histological architectures in their spleens. Post-hatch day 1 broiler chickens were characterized by a thin capsule surrounding the spleen made of collagen and smooth muscle fibers without any trabeculae. Splenic parenchyma consisted of red and white pulp. There was not a noticeable border between the pulps, unlike in mammals. After seven days of hatching, the spleens of broiler chickens were analyzed, and the results showed that they were the same as on the first day. In all experimental groups, the spleen parenchyma featured a noticeable lymphatic nodule during this stage of development. The histological properties of the spleen in all groups at days 14, 21, 28, and 35 of post-hatch development were the same as those at day 7, with histological structures more developed in all experimental groups than in earlier groups.

The histological traits of the broiler spleen during its post-hatch development were improved when probiotic and synbiotic supplements were added to the base feed. Across all experimental groups' broiler chickens, there was a rising trend in the thickness of the spleen capsule until post-hatch day 35. However, when compared to control broilers, the thickness of the capsule was insignificantly ($p > 0.05$) lower until day 28 and significantly ($p < 0.05$) lower at day 35 in both probiotic- and synbiotic-treated broiler chickens (Figure 2). Furthermore, no discernible variation in the spleen capsular thickness was found between broiler chickens treated with probiotics and synbiotics (Figure 2) though the synbiotic-treated broiler chickens had comparatively lower capsular thickness than the probiotic-treated broiler chickens.

During the trial, until post-hatch day 35, broiler chickens of all experimental groups showed a rising trend in the length and width of lymphatic nodules. Though the findings between the experimental groups were not statistically significant, the length and width of lymphatic nodules were greater in broilers treated with probiotics and synbiotics than in the control group ($p > 0.05$, Figure 3 and 4)

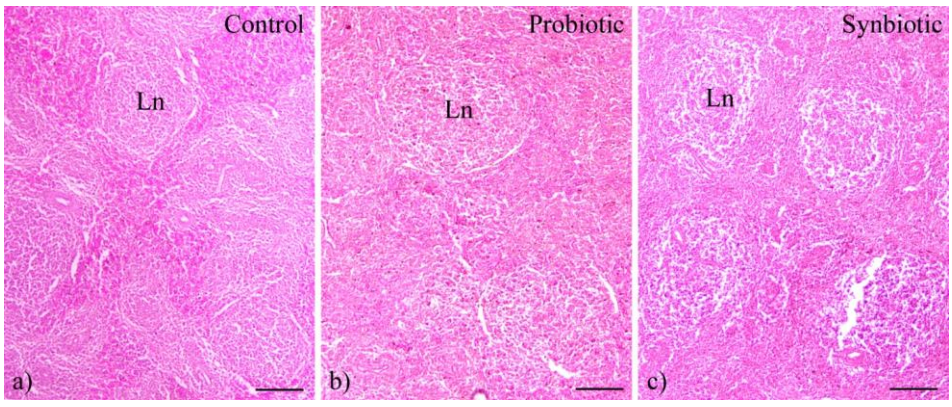

Figure 3. Lymphatic nodules of the spleen in a) control, b) probiotic- and c) synbiotic-treated 35-day-old broiler chickens. Ln, Lymphatic nodule. Scale bar = 100 µm.

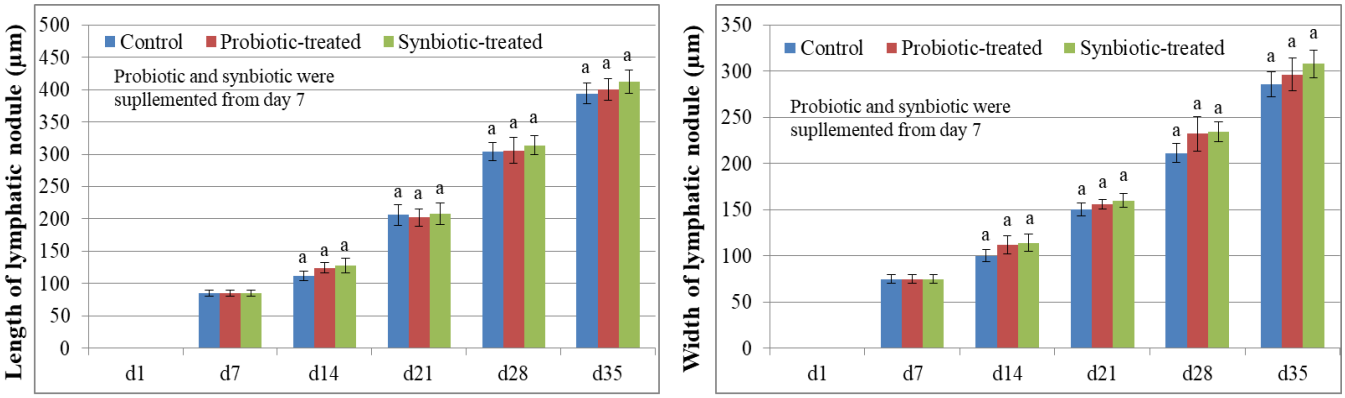

Figure 4. Comparative values of lymphatic nodules of the spleen in control, probiotic- and synbiotic-treated broiler chickens. Similar superscripts within a group of columns indicate the differences are non-significant ($p > 0.05$) among the experimental groups at a particular post-hatch age group of broiler chickens.

4. Discussion

As a useful indicator of the immune system's reactions under different circumstances, the biometry, especially the size, of the spleen can be employed in broiler chickens[3]. In the present research, broiler chickens' spleen biometrical parameters of all experimental groups were significantly increasing by day 35, indicating normal developmental progress. This is in line with findings in Sonali chickens, where Ayman et al.[15] reported that the spleen's weight, length, width, and thickness were all noticeably expanding until day 56. In Deshi chickens[16], it was also observed that the biometric values of the spleen increased as post-hatch age increased. It is reported that the weight and size of the spleen change with age in different breeds and even within the same breed in different situations[3]. Additionally, feed additives increase the biometric values of the internal organs of chickens[17–19]. The current study observed a significant increase in the biometric values of the spleen in the synbiotic-treated group when compared to the control group. However, Dizaji et al.[17] discovered that the prebiotic-supplemented group had a greater spleen weight when compared to other supplemented groups (probiotic, synbiotic). Probiotic and synbiotic inclusion to the broiler feed, however, did not significantly affect the weight of the spleen as compared to the control group, according to Awad et al.[20]. Probiotic addition to the broiler chicken diet has not been shown to significantly alter splenic values, according to previous reports[21–23]. The current study also found similar results with dietary probiotic supplementation. There were no significant differences in the biometric values of spleens between probiotic- and synbiotic-treated broiler chickens in the current study, in contrast to Dizaji et al.[17] who found that the weight of the spleen was significantly different between the probiotic and synbiotic group.

Numerous advantageous impacts on growth performance, intestinal morphology, and microbiota have been documented for supplementation of prebiotics, probiotics, and synbiotics[17,24,25]. Additionally, feeding broiler chickens prebiotic supplements improves their commensal gut bacteria, which act as growth boosters for the microflora and modifies the immunological response[26,27]. Because they have been shown to improve broiler performance, increase resistance to intestinal bacterial infection, and boost immunological status in chickens, synbiotics have become increasingly important as growth promoters[28]. Serum levels of IgG and IgA were elevated by dietary synbiotic treatment[29]. According to Janardhana et al.[26], the broiler chickens treated with synbiotics showed an increase in IgM and IgG plasma titers. The most efficient way to boost the chicken immune system was through nutritional supplementation with synbiotics, according to Szczypka *et al.*[30]. Nevertheless, no research has been done on how probiotics and synbiotics affect the histomorphometry of immunological organs like the spleen.

The basic histological structure of the spleens of broiler chickens in all experimental groups was found to be similar to other breeds of chickens, such as Sonali chickens[14], Deshi chickens[16], and native ducklings of Bangladesh[31]. Supplementing broiler feed with probiotics and synbiotics enhances the histomorphometric characteristics of the broiler spleen throughout post-hatch development. When probiotic- and synbiotic-treated broiler chickens were compared to control broiler chickens, both groups showed somewhat greater histomorphometric values for the length and width of lymphatic nodules and lower values for capsular thickness; however, only the synbiotic-treated broiler chickens showed statistically significant differences. Furthermore, no appreciable variations were found in the spleen histomorphometric values of broiler chicks treated with probiotics versus synbiotics, though the synbiotic-treated broilers showed better results than the probiotic-treated broilers. The results of the present research showed that adding prebiotics to broiler feed in addition to probiotics, i.e., synbiotic supplementation, significantly impacted the spleen's growth after hatch. The results of this investigation were consistent with the Dizaji et al. research[17]. According to Dizaji et al.[17], adding prebiotics, probiotics, synbiotics, and acidifiers to the broiler feed enhanced growth performance when compared to the control group, and comparing growth performance among the experimental groups, synbiotics had the biggest impact. According to Ghahri et al.[32], chickens treated with synbiotics had noticeably higher antibody titers against NDV in comparison to the control group.

5. Conclusion

The results of the study showed that adding probiotics to the diet of broiler chickens had a non-significant effect, but adding synbiotics boosted the post-hatch development of the spleen of the broiler chickens in the biometrical and histomorphometric aspects. The findings imply that adding multispecies synbiotic supplements to the meal could be an alternate management approach for successfully enhancing the immunity and overall health of broiler chickens. There is more study needed to determine the effects of probiotics and synbiotics at different ages and conditions.

Declarations

Competing interest

There is no conflict of interest.

Authors' contribution

Md. Royhan Gofur was responsible for conceiving and designing the experiments. Sample collection was carried out by Md. Royhan Gofur, Shabnaz Aktar, and Mst. Aesha. The experiments were performed by Shabnaz Aktar, Mst. Aesha, and Most. Rukhsana Akter. Statistical analysis was conducted by Md. Royhan Gofur. Fund acquisition was managed by Md. Royhan Gofur and Afia Khatun. Supervision was provided by Md. Royhan Gofur and Khandoker Mohammad Mozaffor Hossain. The main draft of the writing was completed by Md. Royhan Gofur. Review and editing were performed by Khandoker Mohammad Mozaffor Hossain, Afia Khatun, Sordar Mohammed Kamruzzaman, and Md. Emtiaj Alam. All authors read and apprvoded the final version of the manuscript.

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Availability of data and materials

The data are available with a reasonable request.

Ethical considerations

The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification.

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