International Journal of Veterinary Science and Agriculture Research
Volume 8 Issue 5, September-October 2026
ISSN: 2582-4112, Available at www.ijvsar.com

National Assessment of Enteric Lesions and the Efficacy of Multi-Strain Probiotics–Phytocompound Combination in the Bangladesh Broiler Industry.

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Keywords: *Broiler, Coccidiosis, Dysbacteriosis, Gut Health, Lesion Scoring*

Abstract: *The poultry industry in Bangladesh is a vital source of animal protein and economic stability; however, it faces persistent challenges from enteric diseases, such as coccidiosis and dysbacteriosis, particularly following the ban on antibiotic growth promoters (AGPs). These conditions compromise gut health, feed efficiency, and overall flock performance, with seasonal stressors such as high humidity and temperature fluctuations further exacerbating disease prevalence. This study aimed to evaluate the national status of coccidiosis and dysbacteriosis in broiler farms and assess the efficacy of a probiotic-based solution, combination of Bacillus subtilis PB6, Bacillus coagulans, and phytocompound, under field conditions. A nationwide field investigation was conducted in 2024 across 29 districts, covering Bangladesh's major broiler-producing regions. A total of 655 farms were visited, and 393 farms were randomly selected for systematic lesion scoring. Intestinal health parameters were assessed in birds aged 15–35 days using standardized scoring systems for coccidiosis and dysbacteriosis. Seasonal temperature and humidity data were recorded and correlated with lesion trends. Farms using combination of Bacillus subtilis PB6, Bacillus coagulans, and phytocompound, were compared with non-user farms to assess year-round and seasonal effects. Results revealed that coccidial lesion scores remained statistically similar between supplemented and non-supplemented flocks ($p>0.05$), although supplemented flocks exhibited numerically lower*

coccidial challenges. In contrast, dysbacteriosis scores were significantly reduced in combination of Bacillus subtilis PB6, Bacillus coagulans, and phytocompound, users (0.70 ± 0.02) compared with non-users (0.92 ± 0.03; p<0.001). Dysbacteriosis prevalence peaked during the rainy and early winter periods, coinciding with elevated humidity and moderate ambient temperatures. Seasonal analysis demonstrated pronounced benefits of supplementation during the rainy and winter seasons, where dysbacteriosis scores were markedly lower in supplemented flocks (p<0.05). Seasonal variation had a stronger impact on dysbacteriosis than on total mean lesion scores (TMLS). In summer, combination of Bacillus subtilis PB6, Bacillus coagulans, and phytocompound users showed numerically lower TMLS (0.69 vs. 0.72; p > 0.05) and dysbacteriosis scores (0.69 vs. 0.82; p > 0.05) compared with non-users. During the rainy season, TMLS remained comparable between groups (0.86 vs. 0.84; p > 0.05), whereas dysbacteriosis was significantly reduced in users (0.75) relative to non-users (0.95; p < 0.05). A similar pattern was observed in winter, with lower TMLS in users (0.71 vs. 0.80; p > 0.05) and a marked reduction in dysbacteriosis (0.64 vs. 0.98; p < 0.05). Additionally, supplemented flocks exhibited improved fecal consistency and enhanced intestinal integrity, reflecting superior gut health and nutrient absorption. The study demonstrates that dysbacteriosis is a major, seasonally driven gut health challenge in Bangladesh broiler production. Combination of Bacillus subtilis PB6, Bacillus coagulans, and phytocompound, effectively improved microbial balance and intestinal integrity, particularly during high-risk seasons, offering a sustainable alternative to antibiotic growth promoters while supporting performance, resilience, and profitability in tropical poultry systems.

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I. Introduction

The poultry industry in Bangladesh plays a crucial role in providing animal protein through meat and eggs, making a significant contribution to the national economy. Small-scale commercial poultry farms account for 50-60% of the poultry meat consumed in the country [1,2]. Despite the industry's growth, it faces significant challenges from diseases such as coccidiosis, necrotic enteritis (NE), dysbacteriosis, colibacillosis, and chronic respiratory disease, which affect both production and profitability [3,4]. These diseases particularly impact broiler health, where intestinal health is directly linked to digestion and nutrient absorption, with any deviations leading to digestive disorders, such as bacterial enteritis and NE [5].

Dysbacteriosis, also known as small intestine bacterial overgrowth (SIBO), is characterized by abnormal bacterial populations in the small intestine, which can result from both infectious and non-infectious factors. One of the most prevalent diseases caused by this imbalance is NE, which is induced by *Clostridium perfringens* [6]. This Gram-positive, anaerobic bacterium is responsible for significant economic losses worldwide, estimated to be between 2 and 6 billion USD annually [7]. NE reduces broiler performance by lowering body weight (12% decrease) and increasing feed conversion ratio (11%) compared to healthy flocks [7]. NE can manifest clinically with high mortality and subclinically with weight loss, poor feed conversion, and gastrointestinal symptoms, such as greasy droppings and decreased feed intake [8].

Environmental factors like seasonal changes and heat stress also play a role in gut health, as high temperatures increase the risk of digestive disorders [9]. Heat stress, which reduces feed intake and growth performance, leads to changes in gut microbiota, further compromising intestinal health [10]. The prevalence of NE increases during the rainy and winter seasons when relative humidity is higher, exacerbating the effects on [11].

The use of unconventional feed ingredients and the excessive administration of antimicrobial growth promoters (AGPs) in poultry feed have contributed to gut health issues. This practice has led to antibiotic resistance and the

reduction of beneficial microflora, culminating in a ban on AGPs in many countries [12]. Consequently, dysbacteriosis and NE have become global concerns. Despite regulations, antimicrobial resistance (AMR) remains a significant issue in the poultry sector, which necessitates sustainable alternatives to AGPs [12,13]. Probiotics, specifically direct-fed microbes, offer a promising solution [13]. They enhance gut health by promoting beneficial bacteria and suppressing pathogens such as *Clostridium perfringens* [14]. *Bacillus subtilis* PB6 has shown efficacy in improving intestinal morphology, growth performance, and controlling NE in broilers [13]. As a result, probiotics present a viable strategy for maintaining gut health while reducing reliance on antibiotics.

This study aims to assess the overall condition of coccidiosis and dysbacteriosis in Bangladesh's broiler farms. Additionally, it compares the efficacy combination of *Bacillus subtilis* PB6, *Bacillus coagulans,* and Phytocompound in farms using the product versus those that do not, specifically in relation to the occurrence of these conditions. The study also examines the seasonal impacts on the effectiveness of combination *Bacillus subtilis* PB6, *Bacillus coagulans,* and Phytocompound in managing dysbacteriosis and coccidiosis in these farms.

II. Methodology

A field study was conducted on broiler farms in major broiler production areas of Bangladesh. The feed producers employed various techniques for gut health management through their feed programs. A total of 19 feed companies across 29 districts in Bangladesh (Fig. 1) were visited one or more times in 2024. In total, 655 broiler farms were visited, with 393 of these farms randomly selected for lesion scoring throughout the year. For each selected farm, three healthy birds were humanely sacrificed by cervical dislocation, performed by an experienced veterinarian, and immediately necropsied. The intestinal parameters were scored for lesions related to Coccidiosis and Dysbacteriosis. This study focused on broiler chickens aged between 15 and 35 days, with an average age of 23.73 days, as this age range was associated with the highest prevalence of both Coccidiosis and Dysbacteriosis. The lesion scoring procedure for coccidiosis followed the established method [15], while dysbacteriosis was assessed using the scoring system developed by the Kemin technical team, based on a previously described macroscopic scoring method [16].

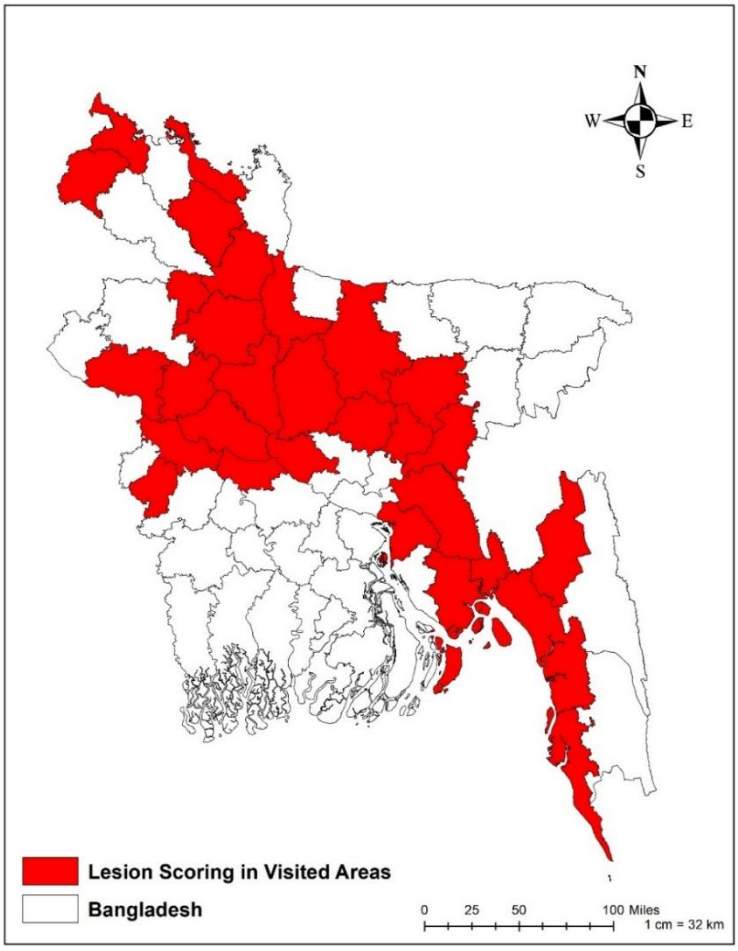


Figure 1: Location of the field visit and lesion scoring districts of Bangladesh marked in red. The map is created in ArcGIS 10.8.2. ArcGIS Enterprise, ESRI, Redlands, California, USA.

III. Result:

3.1 Records of Temperature and Humidity in the year 2024:

During field visits and lesion scoring across different areas and districts in Bangladesh, temperature and humidity were recorded (Table 1 and Fig. 2). The results show a clear pattern of seasonal temperature variations around poultry farms in 2024. From March onward, the environmental temperature increases and continues up to October, covering the summer and monsoon seasons with high humidity. In contrast, temperatures are lower from January to February and from November to December. In tropical countries, broiler chickens are optimally reared within a thermal comfort range of approximately 18–24°C, depending on age, with exposure to higher ambient temperatures commonly resulting in heat stress, reduced feed intake, and impaired growth performance [17,18].

Table 1: Records of temperature and humidity in the year 2024

Month	Avg Air Temp (°C)	Avg RH (%)	Avg Feels-Like Temp (°C)
January	15.6	65	18.5
February	22.6	68.8	28.0
March	26.8	66	33.8
April	33.6	65.5	38.4
May	34.5	70.5	48.5
June	29.5	83	37.5
July	32.5	78	42
August	32	71.5	40.5
September	31	79.5	40.8
October	31.5	66.5	38
November	25.4	52.67	28
December	22.8	62.6	22.7

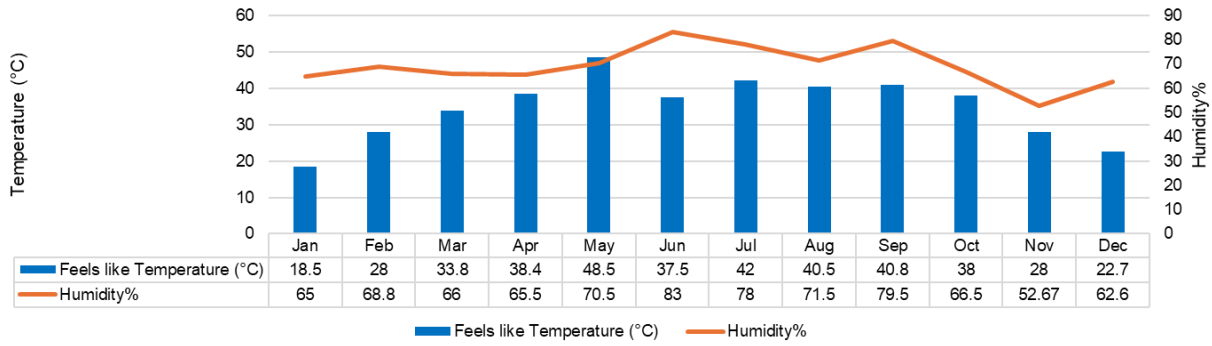


Figure 2: Records of temperature and humidity in 2024

3.2 IH Lesion Scoring Trends Y2024 – Bangladesh

Fig. 3 illustrates monthly trends in TMLS and dysbacteriosis scores throughout 2024. TMLS values remained relatively stable, ranging from 0.61 in July to 0.99 in October, with minor peaks in February (0.86) and early autumn. Dysbacteriosis exhibited greater variability, increasing steadily from 0.64 in January to a maximum of 1.03 in October before slightly declining to 0.97 in December. Across all months, dysbacteriosis scores were consistently higher than TMLS, indicating greater susceptibility to microbial imbalance compared to lesion severity. The sharp rise during late rainy and early winter periods suggests seasonal influence on gut health, highlighting critical intervention windows.

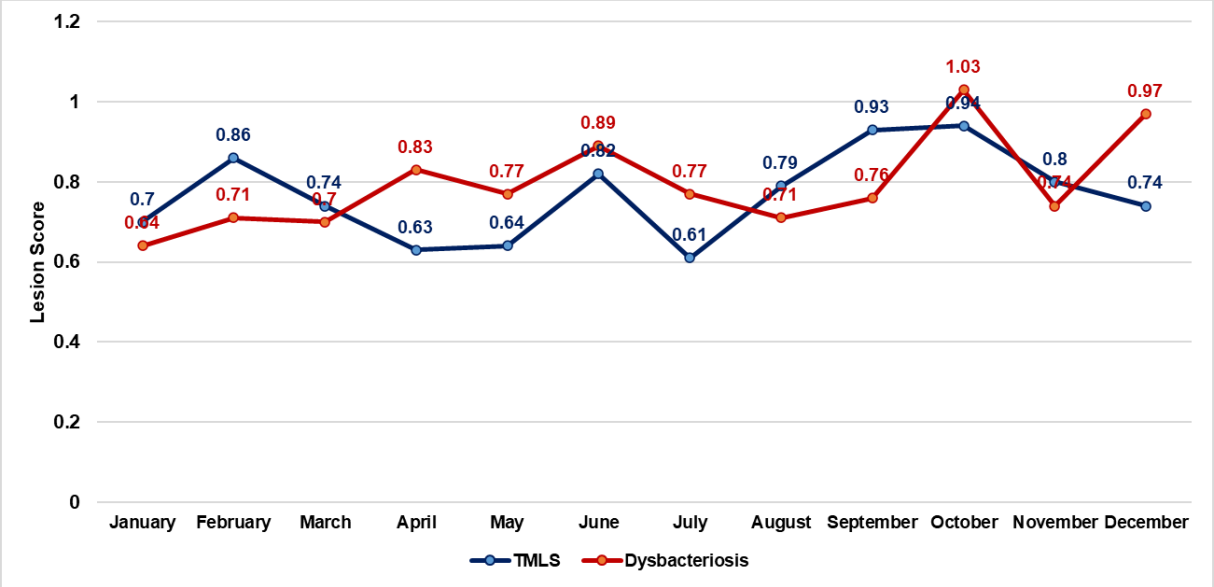


Figure 3: IH Lesion Scoring Trends Y2024

3.3 Effect of Seasonal Variation on TMLS and Dysbacteriosis in Users vs. Non-Users of a Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and Phytocompounds

Seasonal variation influenced dysbacteriosis more than TMLS. In summer, TMLS was slightly lower in combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound users (0.69) compared to non-users (0.72), but differences were not significant ($p > 0.05$). Dysbacteriosis also showed no significant variation, though Users had lower scores (0.69 vs. 0.82; $p > 0.05$). During the rainy season, TMLS remained similar between groups (0.86 vs. 0.84; $p > 0.05$), whereas dysbacteriosis was significantly reduced in Users (0.75) compared to non-users (0.95; $p < 0.05$). Winter showed a comparable trend: TMLS was lower in Users (0.71) than non-users (0.80; $p > 0.05$), while dysbacteriosis was markedly lower in Users (0.64) versus non-users (0.98; $p < 0.05$). Overall, combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound supplementation consistently improved gut microbial balance during rainy and winter seasons, while lesion scores remained unaffected across all seasons (Table 2 & Fig. 4).

Table 2: Effect of seasonal variation on TMLS and dysbacteriosis scores in users and non-users of a combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompounds in 2024

Season	Parameters	Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound User (Mean ± SE)	Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound non-user (Mean ± SE)	P-value
Summar Season	TMLS	0.69 ± 0.064^{a}	0.72 ± 0.060^{a}	0.78
	Dysbacteriosis	0.69 ± 0.044^{a}	0.82 ± 0.056^{a}	0.09
Rainy Season	TMLS	0.86 ± 0.068^{a}	0.84 ± 0.064^{a}	0.82
	Dysbacteriosis	0.75 ± 0.031^{b}	0.95 ± 0.039^{a}	0.00021
Winter Season	TMLS	0.71 ± 0.072^{a}	0.80 ± 0.076^{a}	0.378
	Dysbacteriosis	0.64 ± 0.042^{b}	0.98 ± 0.053^{a}	0.00002

[a, b]: Different superscripts in the same row indicate significant differences ($p<0.05$).

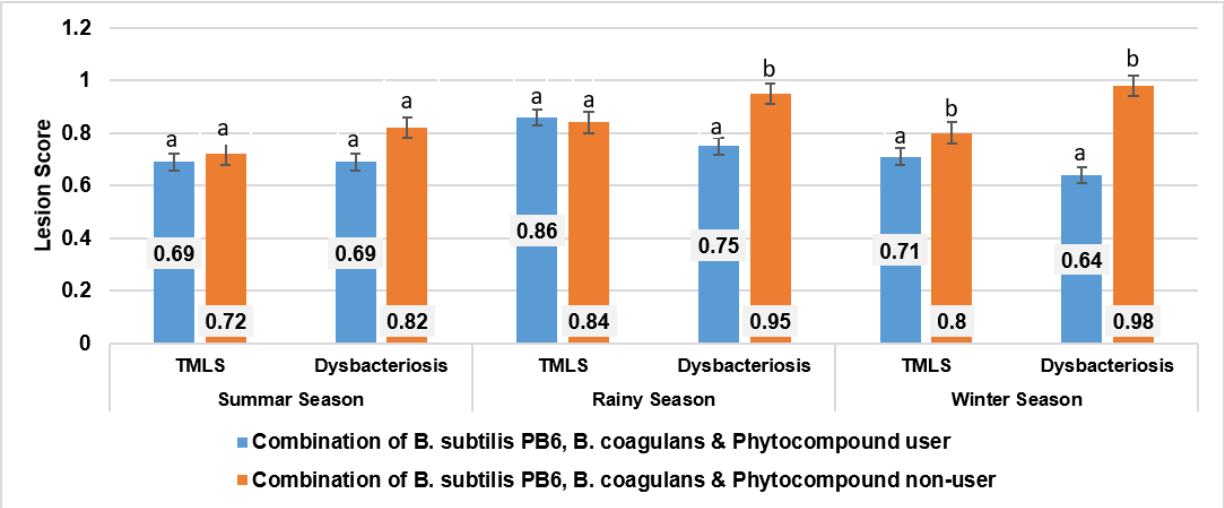


Figure 4: Effect of seasonal variation on TMLS and dysbacteriosis scores in users and non-users of a combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompounds in 2024

3.4 Effect of a Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and Phytocompounds on Gut Health, Coccidiosis, and Dysbacteriosis in Users and Non-Users

Lesion scoring activities conducted in Bangladesh during 2024 revealed that Eimeria species scores were comparable between combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound users and non-users (Table 3 & Fig. 5). *E. acervulina* averaged 0.07 in users versus 0.08 in non-users ($p > 0.05$), *E. maxima* were 0.45 versus 0.47 ($p > 0.05$), and *E. tenella* was 0.23 versus 0.27 ($p > 0.05$), indicating no significant differences. Similarly, TMLS remained statistically non-significant ($p > 0.05$), though slightly lower in users (0.77) compared to non-users (0.80). In contrast, dysbacteriosis scores showed a marked reduction in combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound users (0.70) compared to non-users (0.92), with a highly significant difference ($p<0.001$). These findings suggest that while in combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound supplementation did not significantly affect coccidial lesion scores, it substantially improved gut microbial balance, particularly reducing dysbacteriosis under field conditions.

Table 3: Effect of a combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompounds on gut health, coccidiosis, and dysbacteriosis in users and non-users

Parameters	Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound user (Mean ± SE)	Combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound non-user (Mean ± SE)	P-Value
Eimeria acervulina	0.07 ± 0.02^{a}	0.08 ± 0.01^{a}	0.55
Eimeria maxima	0.45 ± 0.03^{a}	0.47 ± 0.03^{a}	0.76
Eimeria tenella	0.23 ± 0.02^{a}	0.27 ± 0.03^{a}	0.31
TMLS	0.77 ± 0.04^{a}	0.80 ± 0.04^{a}	0.61
Dysbacteriosis	0.70 ± 0.02^{b}	0.92 ± 0.03^{a}	0.000

[a, b]: Different superscripts in the same row indicate significant differences ($p<0.05$).

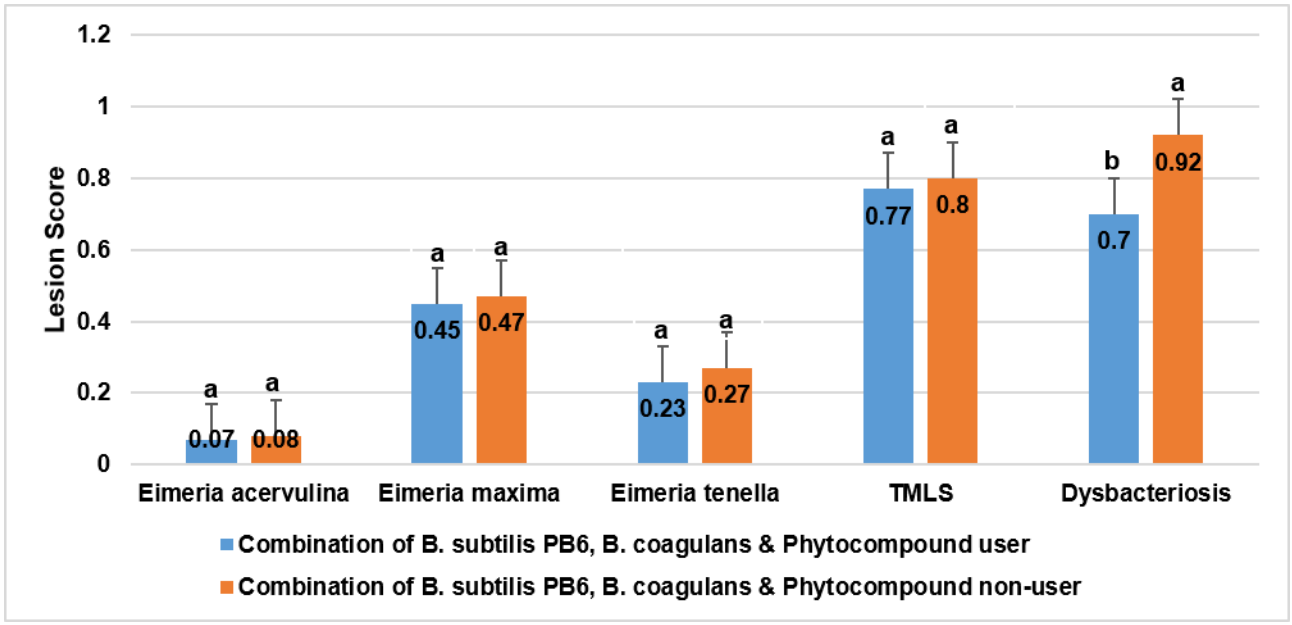


Figure 5: Effect of a combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and Phytocompounds on gut health, coccidiosis, and dysbacteriosis in users and non-users

3.5 Evaluation of Fecal Characteristics

The consistency of poultry droppings was systematically observed throughout the lesion scoring time, and the farm was visited in different districts via visual examination, as depicted in Fig. 6. The combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound non-user group exhibited significant abnormalities, including loose droppings, foamy content, and undigested feed particles accompanied by orange-colored mucoid matter, indicative of poor digestive health. In contrast, combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound demonstrated substantial improvements in excreta consistency, characterized by normal, well-formed droppings, reflecting enhanced gut health and nutrient absorption. These findings align with the poultry producer's observations and highlight the efficacy of the treatment in mitigating gastrointestinal disturbances.

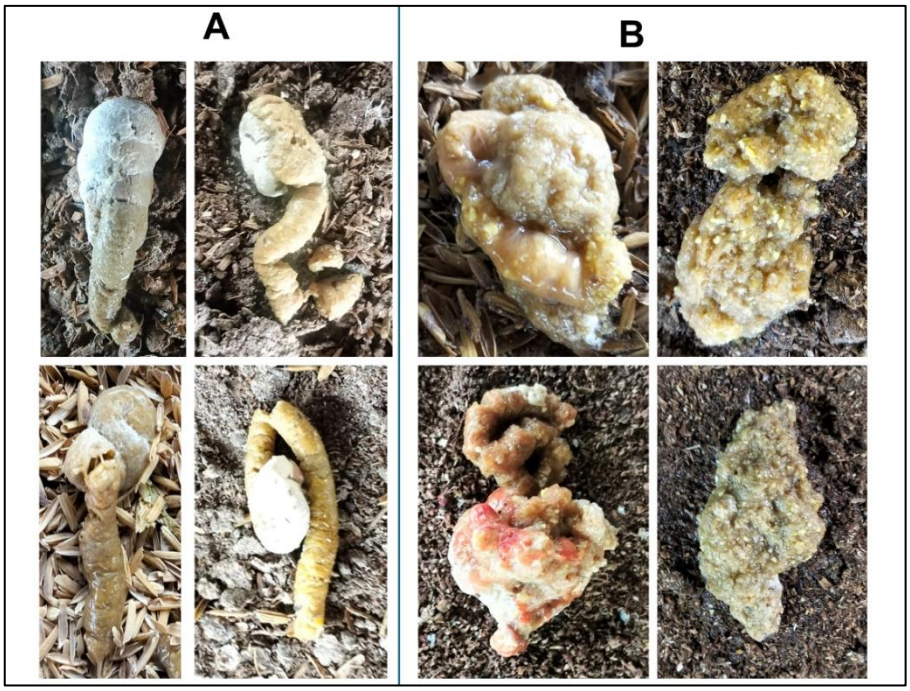


Figure 6: In A: combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound user (normal, well-formed droppings) and in B: combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound non-user (loose droppings, foamy content, and undigested feed particles accompanied by orange-colored mucoid matter [19]

3.6 Intestinal lesion & gut health Status:

The intestinal lesion and dysbacteriosis scores were assessed for both the combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound user and non-user groups, and the observation is presented in Figure 7, showing that the intestinal wall is more inflamed, contains frothy, foamy, ballooning, and orange mucoid content compared to the combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound user group. These findings suggest that treatment with combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound not only ameliorated the intestinal lesion score but also helped preserve gut integrity, leading to a substantial improvement in the overall intestinal health score.



Figure 7: In A: combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound Dry user (Intestinal wall good thickness, no inflammation, and no feed particles in the feed passage, no orange-colored mucus). In B: combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound non-user group (Intestinal wall is more inflamed, ballooning, and contains frothy, foamy, orange mucoid content) [19].

IV. Discussion:

Bangladesh's poultry sector, essential for food security and rural incomes, faces major constraints affecting performance and profitability. Rising feed ingredient costs, declining raw material quality, mycotoxin contamination, compromised gut health, and heat stress under hot–humid conditions are key challenges [20]. Among enteric diseases, coccidiosis and necrotic enteritis (dysbacteriosis) are most prevalent; this study evaluated their national status and seasonal influences.

Antibiotic growth promoters (AGPs) are now formally prohibited in Bangladesh. In the past, the inclusion of antibiotics at subtherapeutic levels in poultry feed was widely practiced, enhancing growth efficiency and controlling enteric pathogens, particularly *Clostridium perfringens*, thereby lowering disease incidence [21,22,24]. The beneficial outcomes were associated with improved nutrient availability, reduced intestinal inflammation, suppression of bacterial toxin production, and fewer subclinical intestinal infections [13,21]. Nevertheless, growing global concern over antimicrobial and cross-resistance, and the subsequent threat to human therapeutics, has led to strict regulatory measures [24]. Following AGP removal in Bangladesh, gut health challenges have intensified, with persistent subclinical coccidiosis and dysbacteriosis attributed to inferior feed quality, inadequate anticoccidial programs, immune suppression, and emerging pathogens [25,26]. As a result, non-antibiotic strategies are increasingly emphasized, and probiotics—particularly *Bacillus subtilis* PB6 have demonstrated notable effectiveness under local field conditions [14,27].

Bangladesh's tropical environment, marked by persistently high temperature and humidity, further intensifies enteric health challenges by inducing heat stress, depressing feed intake, compromising intestinal barrier function, and predisposing birds to leaky gut syndrome. Seasonal transitions, particularly during monsoon and winter, create favorable conditions for microbial imbalance. This pattern was reflected in 2024 lesion scoring data from

Bangladesh, where coccidiosis and dysbacteriosis scores consistently exceeded TMLS thresholds, with pronounced peaks during rainy and winter months. The present findings align with earlier reports showing a higher winter prevalence of necrotic enteritis or dysbacteriosis (11.67%) and the lowest incidence in summer (3.2%) [11]. In this study, supplementation with combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound did not significantly affect coccidial TMLS; however, scores were numerically lower in supplemented flocks throughout the year, suggesting an immunomodulatory role of *Bacillus subtilis* PB6. By supporting gut integrity and reducing coccidial challenge, probiotic use may indirectly limit *C. perfringens* proliferation and subsequent necrotic enteritis, as coccidial-induced mucosal damage is a known predisposing factor for NE [28].

The present study demonstrated that supplementation with combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound, significantly reduced dysbacteriosis scores and improved overall microbial balance in broilers. Dysbacteriosis scores decreased from 0.92 in non-supplemented flocks to 0.70 in supplemented flocks ($p < 0.05$), with the most pronounced improvements observed during the rainy season (0.75 vs. 0.95; $p < 0.05$) and winter (0.64 vs. 0.98; $p < 0.05$). Although numerical reductions were also evident during summer (0.62 vs. 0.82), these differences were not statistically significant ($p > 0.05$). These findings underscore the role of *B. subtilis* PB6 in alleviating seasonal gut health stress, consistent with previous observations [14]. The beneficial effects of PB6 are primarily attributed to its ability to produce antimicrobial substances typical of Gram-positive bacteriocins, which exhibit broad-spectrum inhibitory activity against *Clostridium* species. In particular, PB6 produces antimicrobial peptides such as surfactins that suppress *Clostridium perfringens*, promote microbial homeostasis, and support intestinal integrity. Enhanced gut morphology, characterized by increased villus height and an improved villus height-to-crypt depth ratio, has been associated with superior nutrient absorption and feed efficiency [29,30,31]. Several studies have confirmed that *B. subtilis* PB6 supplementation improves intestinal morphology and effectively controls necrotic enteritis in broilers [14], while its inhibitory effects against enteric pathogen invasion are well documented. Improved body weight gain and reduced feed conversion ratio (FCR) were reported in broilers, particularly under *C. perfringens* challenge [32]. In addition to direct antimicrobial action, PB6 supplementation has been associated with increased production of short-chain fatty acids, notably acetic and butyric acids, which support intestinal energy metabolism, epithelial development, and villus maturation, thereby contributing to reduced necrotic enteritis severity [33]. The spore-forming nature of *B. subtilis* PB6 enhances its stability during feed processing and resilience within the gastrointestinal tract, making it a practical, poultry producers facing escalating feed costs and performance losses [34]. Supporting evidence from related Bacillus species further strengthens these findings, as dietary supplementation with *Bacillus coagulans* has been shown to increase sIgA-positive cells, improve villus height-to-crypt depth ratios, and upregulate immune-related genes such as IFN-α (Interferon-alpha), TLR3 (Toll-like receptor 3), and MDA5 (Melanoma Differentiation-Associated protein 5), collectively enhancing mucosal immunity and barrier function [35,36]. Moreover, *B. coagulans* supplementation reduced pro-inflammatory cytokines while increasing anti-inflammatory mediators, antimicrobial peptides, and T-cell responses. Both *B. subtilis* PB6 and *B. coagulans* have also been reported to maintain the expression of key tight junction proteins, including claudins, Occluding, and

ZO-1 (Zonula Occludens-1), thereby counteracting leaky gut syndrome under pathogenic stress [30,37]. Complementary evidence indicates that dietary phytocompounds exert antioxidant and anti-inflammatory effects that further enhance intestinal integrity and immune balance in poultry [36]. Collectively, these mechanisms explain the observed reduction in dysbacteriosis and highlight the value of PB6-based probiotic strategies for sustainable poultry production systems.

V. Conclusion

Bangladesh's poultry industry faces persistent gut health challenges, particularly coccidiosis and dysbacteriosis, exacerbated by seasonal stress and the ban on antibiotic growth promoters. Field data from 2024 confirm that combination of *Bacillus subtilis* PB6, *Bacillus coagulans*, and phytocompound, significantly reduced dysbacteriosis scores and improved microbial balance, especially during rainy and winter seasons. While coccidial lesion scores remained unaffected, PB6 supplementation enhanced intestinal integrity, villus morphology, and nutrient absorption, offering a sustainable alternative to antibiotics. These findings underscore the role of PB6-based probiotics in mitigating enteric disorders and supporting performance under tropical conditions, ensuring profitability and food security.

Acknowledgements

The present study was supported by Kemin Industries South Asia Pvt. Ltd.

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