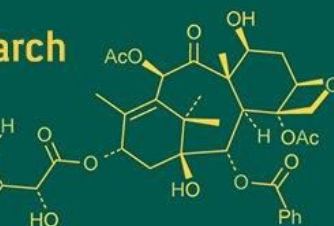
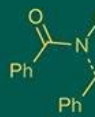


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
NAAS Rating (2026): 5.29  
IJABR 2026; SP-10(3): 410-417  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 15-12-2025  
Accepted: 17-01-2026

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## Dietary Phytogetic Botanical Blend (PBB) modulates intestinal barrier integrity and histopathological goblet cell–mucin dynamics in broiler

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3Sf.7894>

### Abstract

In a 42-day research trial, a total of 1,760 day-old “Cobb-430Y” broiler chicks were randomly assigned to four experimental groups: NC, PBB1, PBB2, and CS. Each group consisted of 440 birds, subdivided into four replicates of 110 birds each. The present study aimed to comparatively assess the effects of a phytogetic botanical blend (PBB) and a conventional synbiotic (CS) on the European Production Efficiency Factor (EPEF), tight junction protein (TJ) specifically claudin-2 (CLDN-2) and zonula occludens-1 (ZO-1) and histopathological findings of PAS-stained ileum in broiler chickens. The PBB was formulated as a blend of *Ocimum sanctum*, *Allium cepa*, *Trachyspermum ammi*, *Mangifera indica*, *Curcuma longa*, and *Piper longum*. The conventional synbiotic comprised probiotic strains (*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, and *Lactobacillus acidophilus*) along with prebiotic components, namely  $\beta$ -glucans and mannan oligosaccharides. The NC group received only the basal diet. Groups PBB1 and PBB2 were supplemented with PBB at 500 g/MT and 1000 g/MT of finished feed, respectively, while the CS group received the synbiotic at 250 g/MT. Compared with the NC group, birds in PBB1, PBB2, and CS exhibited significant increases ( $P < 0.05$ ) in EPEF of approximately 11.78%, 16.24%, and 15.38%, respectively. Furthermore, PBB2 and CS supplementation significantly ( $p < 0.05$ ) enhanced TJ protein relative to NC. Goblet cell number showed no significant difference ( $p > 0.05$ ) among the groups. However, goblet cell area increased across all supplemented groups, with elevations of 28.60%, 93.50%, and 110.92% in PBB1, PBB2, and CS, respectively. Mucin-associated staining intensity demonstrated slight increases of 2.97%, 1.30%, and 0.63%, while integrated optical density (IOD) increased by 29.18%, 85.76%, and 90.99% in PBB1, PBB2, and CS, respectively. Overall, PBB inclusion at 1000 g/MT effectively improved EPEF, TJ proteins, goblet cell morphometry, and mucin-associated optical parameters, reinforcing mucosal defence. Similar benefits were observed with CS at 250 g/MT.

**Keywords:** Phytogetic botanical blend, broiler, conventional synbiotic, mucin, goblet cells, tight junction proteins

### Introduction

Since the 1970s, poultry meat and egg production have expanded rapidly, with the meat sector being more dynamic than eggs, and accompanied by rising global trade <sup>[1]</sup>. In poultry, the gastrointestinal tract forms the most extensive exposed surface, acting as a selective barrier between the bird and its luminal environment. This barrier, made up of epithelial cells joined by intercellular junctions, limits pathogen and toxin entry while regulating nutrient and ion passage <sup>[2]</sup>. Tight junctions (TJs) are central to this function, not only controlling paracellular permeability but also participating in cell signalling, proliferation, and gene expression. Goblet cells further strengthen this defence by secreting mucins that form a protective mucus layer, preventing pathogen adhesion and maintaining gut homeostasis <sup>[3]</sup>. However, stressors such as heat stress, dietary challenges, and infections can compromise epithelial integrity, leading to increased intestinal permeability or “leaky gut.” Pathogens like *E. coli*, *Clostridium perfringens*, and *Salmonella* can disrupt TJs by altering proteins such as occludin, claudins, and ZO-1, thereby weakening the barrier <sup>[1]</sup>. Among these proteins, claudin-2 forms ion and water-permeable pores that reduce epithelial tightness, while ZO-1 acts as a scaffolding protein linking TJs to the actin cytoskeleton, maintaining epithelial stability and barrier function <sup>[4]</sup>.

In recent years, several phytogetic feed additives (PFAs) and functional ingredients have been explored for their ability to enhance these defence mechanisms. Among them, *Curcuma longa* (curcumin) has been widely studied for its role in protecting the epithelial barrier and supporting mucus secretion [5], [6]. *Thymus vulgaris* (rich in thymol and carvacrol) has been linked in broilers to increased MUC2 expression, higher goblet cell counts [7], and improved tight junction activity [8]. Onion (*Allium cepa*), rich in phenolic compounds with antioxidant and antimicrobial properties, has been shown to enhance gut health in poultry by increasing goblet cell density and mucin secretion. These effects are further linked to the regulation of TJ proteins such as claudin-2 (CLDN-2) and zonula occludens-1 (ZO-1), which are critical for maintaining epithelial integrity [9]. Similarly, *Ocimum sanctum* (tulsi), containing eugenol, has been reported to reduce intestinal permeability under stress and to promote beneficial gut bacteria [10]. *Trachyspermum ammi* (ajwain) has also demonstrated improvements in villus architecture and a healthier balance of *Lactobacillus* to *E. coli* populations, both of which contribute to a stronger mucus barrier [11].

In addition to these, other additives have shown promising but less direct evidence. *Piper longum* (through its active compound piperine and evidence from related species) has been associated with improved villus morphology and upregulation of tight junction proteins including Occludin, ZO-1, and claudins [12]. *Mangifera indica* (mango) extracts rich in mangiferin have been reported to restore ZO-1 and claudin expression and reduce epithelial leakage in experimental models, while also improving growth and caecal microbiota in poultry [13].

Taken together, these findings indicate that both phytogetic and functional additives may strengthen the gut barrier by acting on tight junction proteins, mucin secretion, and goblet cell function. This study investigates a phytogetic blend containing *Ocimum sanctum*, *Allium cepa*, *Trachyspermum ammi*, *Mangifera indica*, *Curcuma longa* and *Piper longum*, focusing on its effects on tight junction proteins (CLDN-2, ZO-1), mucins, and goblet cells morphometry in broilers. These plant-derived additives, valued for their antimicrobial, antioxidant, and gut-protective properties, are compared with synbiotics (prebiotics + probiotics). Unlike synbiotics, which may face challenges such as heat instability and strain specificity, phytogetics are naturally stable and multifunctional. Our objective is to assess whether this blend can serve as a reliable alternative to synbiotics for supporting gut health and performance in broiler production.

## Materials and Methods

### Housing and Vaccination

The experiment was conducted at the Dr. B.V. Rao Institute of Poultry Management and Technology (IPMT), Pune, Maharashtra, India. A total of 1,760 one-day-old mixed-sex "Cobb 430Y" broiler chicks were procured from Venkateshwara Hatcheries Pvt. Ltd. Birds were reared in open-sided, non-environmentally controlled sheds and allocated to separate pens, providing 2 ft<sup>2</sup> floor space per bird. Rice husk litter (2–3 inches depth) was uniformly spread over a cemented floor.

Upon arrival, chicks were individually weighed and randomly assigned to pens following a completely randomized design. Venky's Aminovit Gold was supplied via drinking water during the first five days. Birds were

immunized against Newcastle disease using the Super LaSota vaccine on day 7, with a booster administered on day 21. Vaccination against infectious bursal disease (intermediate strain) was performed on day 14, followed by a booster on day 28. Primary vaccinations were given intranasally, while booster doses were delivered through drinking water.

### Phytogetic botanical blend (PBB)

A phytogetic formulation, designated as PBB, was evaluated in the present study. The blend comprised *Ocimum sanctum*, *Allium cepa*, *Trachyspermum ammi*, *Mangifera indica*, *Curcuma longa* and *Piper longum*. The individual plant materials were first passed through a vibratory sieve to eliminate extraneous impurities, followed by thorough blending to achieve a uniform mixture prior to incorporation into the feed.

### Experimental design

A total of 1,760 broiler chicks were randomly allocated to four treatment groups: NC, PBB1, PBB2, and CS. Each treatment comprised 440 birds, further subdivided into four replicates of 110 birds each. Replicates were distributed uniformly within the available housing facilities in accordance with the experimental layout and standard management practices of the farm. The study was conducted over a period of 42 days.

### The experimental treatments were structured as follows (Table 1)

Group NC: Negative control group receiving basal diet  
Group PBB1: Basal diet + 500 g PBB/MT of feed  
Group PBB2: Basal diet + 1000g PBB/MT of feed  
Group CS: Basal diet + 250 g Conventional synbiotic/MT of feed

The conventional synbiotic used in this study comprised a synergistic combination of probiotic strains, including, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, and *Lactobacillus acidophilus*, with a minimum total viable count of  $4 \times 10^9$  CFU/g. The formulation was supplemented with prebiotic components, namely  $\beta$ -glucans and mannan oligosaccharides.

### Feed and water

Mash-form feed was manufactured at the IPMT feed facility specifically for the study. Birds had unrestricted access to clean drinking water throughout the experimental period. The feeding program was implemented in three phases: pre-starter (weeks 1–2), starter (weeks 3–4), and finisher (weeks 5–6). Proximate composition of the diets was analysed at Envirocare Labs Pvt. Ltd., and the analytical results are summarized in Table 2.

### Methodology for chicken claudin-2 (CLDN-2) and zonula occludens-1 (ZO-1)

The concentration of tight junction proteins Claudin-2 (CLDN-2) and Zonula Occludens-1 (ZO-1) in intestinal tissue samples were measured using chicken-specific sandwich ELISA kits from Bioassay Technology Laboratory, China (CLDN-2: Cat No. E0363Ch; ZO-1: Cat No. E0141Ch). Before analysis, tissue samples from the intestine were subjected to protein extraction. Standards and samples were prepared and added to the microplate wells according to

manufacturer's protocol. For sample wells, 40 µL of the extracted protein sample was mixed with 10 µL of specific detection antibody, followed by addition of 50 µL of streptavidin-HRP reagent. For the standard wells, 50 µL of standard solution was added to the well. Plates were incubated at 37°C for 60 minutes to allow antigen-antibody interaction. Following incubation, wells were washed five times to remove unbound components. Subsequently, 50 µL of substrate solution A and 50 µL of substrate solution B were added to each well and the plates were incubated in the dark at 37 °C for 10 minutes. The enzymatic reaction was terminated by adding 50 µL of stop solution, resulting in a color change from blue to yellow. Absorbance was immediately measured at 450 nm using a microplate reader (Epoch 2, Microplate reader, BioTek (Agilent) USA). The concentrations of CLDN-2 and ZO-1 were calculated from standard curve generated by plotting optical density (OD) values against known concentrations using GraphPad Prism Software (Version 6.01). Sample concentrations were interpolated using a quadratic regression model.

### Histo-pathological evaluations of pas-stained (PAS) ileum in chickens

On day 42 of the experiment, six birds from each replicate were selected randomly and slaughtered according to the halal procedure. From the slaughtered birds (n=96), ileum was collected aseptically and fixed in 10% neutral buffered formalin. These tissues were trimmed and routinely processed. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax <sup>[14]</sup>. Paraffin wax embedded tissue blocks were sections at 5 µm thicknesses with the Rotary Microtome (Leica, Germany). Slides of ileum are stained with Periodic Acid Schiff (PAS) stain <sup>[15]</sup> and following parameters were evaluated:

1. Count of goblet cells (Nos)
2. Measurement of size individual goblet cells (area in µm<sup>2</sup>)
3. Staining intensity of mucin in individual goblet cells (The average concentration of PAS stain in the cells)
4. Integrated optical density- IOD of mucin in individual goblet cells (The total amount of PAS stain within the cell, a measure of content/concentration.)

### Histo-morphological and PAS-Mucin Quantification Analysis

For histological evaluation, 15 non-overlapping images per slide were captured using a Magnus microscope equipped with a Magcam 5.0 digital microscopic camera. The captured images were analyzed using ImageJ software (Fiji distribution, NIH, USA). Colour separation of histological stains was performed using the Colour Deconvolution plugin following the method described by <sup>[16]</sup>.

### Image Processing and Quantification of PAS-Stained Mucin Glycoprotein

The quantification of mucin-positive goblet cells from Periodic Acid-Schiff (PAS)-stained sections was performed following standardized image analysis procedures:

#### 1. Image Acquisition and Preparation

PAS-stained images were opened in Fiji (ImageJ). When necessary, duplicate images were created to preserve the original files for reference.

#### 2. Scale Calibration

The image scale was calibrated using the scale bar embedded in the micrograph. A straight-line selection was drawn along the scale bar, and the known distance was entered under the “Set Scale” option to obtain measurements in micrometers (µm).

#### 3. Color Deconvolution

Color separation was performed using the Color Deconvolution plugin. Regions of interest (ROIs) were manually selected to define the staining vectors for hematoxylin (blue), PAS/Schiff reagent (magenta), and residual background staining. The PAS-specific channel was isolated for subsequent analysis.

#### 4. Image Thresholding

The selected PAS channel image was converted to 8-bit format. Thresholding was applied using the Shanbhag method with red threshold settings to selectively highlight mucin-positive goblet cells. Threshold intensity was adjusted uniformly across samples to ensure consistent detection.

#### 5. Quantitative Analysis

Measurements were obtained using the “Set Measurements” function, selecting area, integrated density, and mean gray value. Goblet cells were quantified using the “Analyze Particles” function, with particle size parameters adjusted according to the defined threshold (e.g., 3–infinity or 5–infinity µm<sup>2</sup>) to accurately capture mucin-positive cells.

#### Statistical analysis

Data were expressed as mean ± standard error (SE). Treatment differences were analysed using one-way analysis of variance (ANOVA) <sup>[17]</sup>, followed by Tukey's multiple comparison test <sup>[18]</sup> to identify significant differences among groups. Statistical significance was established at  $p < 0.05$ . All statistical computations were performed using GraphPad Prism.

### Results

#### European production efficiency factor

Table 3 shows the effect of PBB and CS on European production efficiency factor (EPEF) in broilers. The EPEF in groups PBB1, PBB2, and CS was significantly ( $p < 0.05$ ) higher than that of the control group NC. The EPEF in groups PBB1, PBB2, and CS increased by 11.78%, 16.24%, and 15.38%, respectively, which were significantly ( $p < 0.05$ ) higher compared with the control group NC. The group PBB2 at a dose of 1000g/MT of feed showed the numerically highest EPEF among the four groups.

#### Tight junction proteins

##### Claudin 2 and Zonula occludens – 1

Table 4 presents the effect of PBB and CS on tight junction proteins (CLDN-2 and ZO-1) in broiler birds. The supplementation with 250 g of CS per MT of feed resulted in a significant ( $p < 0.05$ ) upregulation of claudin-2 and zonula occludens-1 level in birds, relative to the control group (NC) and those receiving 500g (PBB1) or 1000g (PBB2) of the phytogetic botanical blend per MT of feed. Claudin-2 levels in groups PBB1 and PBB2 were significantly ( $p < 0.05$ ) higher by 28.20% and 109.15%, respectively, than those observed in the control group NC.

In the case of zonula occludens-1, birds in groups PBB2 and CS exhibited significant ( $p < 0.05$ ) increases of 17.80% and 35.60%, respectively, relative to the control group NC.

#### **Goblet cell number and area, with mucin-associated staining intensity and integrated optical density (IOD) of individual goblet cells**

Table 5 shows the effect of PBB and CS on histopathological evaluations of PAS-stained (PAS) ileum in broilers. No significant differences ( $p > 0.05$ ) were observed in goblet cell numbers among the experimental groups. Regarding goblet cell area, the PBB2 and CS groups exhibited significantly greater values ( $p < 0.05$ ) compared with the NC and PBB1 groups. However, no statistically significant difference ( $p > 0.05$ ) was observed between the PBB2 and CS groups. The goblet cell area was elevated in all treatment groups relative to the NC group, with increases of 28.60%, 93.50%, and 110.92% observed in PBB1, PBB2, and CS, respectively. In comparison with NC group, a statistically significant increase ( $p < 0.05$ ) in staining intensity was observed in PBB1 group. Relative to NC group, staining intensity demonstrated marginal enhancements of 2.97% in PBB1, 1.30% in PBB2, and 0.63% in CS group. Furthermore, when contrasted with the NC group, percentage increases in IOD of 29.18%, 85.76%, and 90.99% were recorded in PBB1, PBB2, and CS groups, respectively.

The NC group, which received no supplementation, displayed fewer PAS-positive goblet cells, as illustrated in Fig 1. The PBB1 group demonstrated a numerical increase in IOD compared with the NC group (Fig 2). In contrast, the PBB2 and CS groups showed pronounced PAS-positive goblet cell staining, reflecting enhanced mucin deposition, as depicted in Fig 3 and Fig 4, respectively.

## **Discussion**

### **European production efficiency factor**

The improvement in EPEF may be attributed to enhanced nutrient utilization and improved intestinal functionality, as EPEF reflects growth rate, feed conversion efficiency, and liveability [19]. Phytogetic and synbiotic supplementation may support digestive efficiency and reduce subclinical intestinal stress, thereby optimizing energy allocation toward growth performance.

The findings of this trial are consistent with those, who showed that essential oils containing spearmint, lemongrass, thyme, sage, black cumin, and clove were economically advantageous for broiler production [20]. In line with our results, Belali *et al.*, (2022) [21] observed that dietary inclusion of thyme extract at 50 and 100 mg/kg of feed, as well as thyme powder at 150 and 250 mg/kg of feed, significantly ( $p < 0.05$ ) improved the European production index compared with the control group in broilers. In contrast, El-Ashram and Abdelhafez (2020) [22] showed that due to incorporation of the feed additive at 150 and 300 mg/kg, containing thyme and anise essential oils, did not result in a significant ( $p > 0.05$ ) improvement in the EPEF when compared with the non-supplemented group in broiler chicken. The findings of the present study were in disagreement with those of Metwally (2022) [23], who reported that dietary provision of curcumin at 100 and 200 g/kg of feed had no effect ( $p < 0.05$ ) on the EPEF in quails over a 42-day trial period. Similarly, Dev *et al.*, (2020) [24] reported that synbiotics supplementation (MOS +

*Lactobacillus acidophilus*) significantly improved growth performance and EPEF values ( $P < 0.01$ ) compared to control and antibiotic-fed birds, indicating enhanced overall productivity in broilers.

### **Tight junction proteins**

#### **Claudin 2 and Zonula occludens – 1**

Tight junctions (TJs) are crucial cellular structures found in epithelial and endothelial tissues and playing essential roles in the physiological functions of broilers. These specialized junctions act as selective barriers that regulates the passage of ions, small molecules, and cells between adjacent cells [25]. By sealing intercellular spaces, TJs maintain tissue integrity and prevent fluid and solute leakage, thereby ensuring homeostasis and contributing to the structural stability of endothelial tissues [26].

The intestinal epithelial barrier plays a vital role in maintaining homeostasis by enabling nutrient and water absorption while protecting against pathogens and other harmful agents [27]. Disruption of tight junction proteins may impair nutrient absorption, increase intestinal permeability, promote bacterial translocation, and trigger inflammation and tissue damage [28]. Moreover, TJs play roles in cell signaling and immune responses, highlighting their significance in the health and development of broilers [25].

The present research trial evaluated the influence of phytogetic botanical blend on tight junction proteins, specifically ZO-1 and CLDN-2. It was found that, PBB and CS supplementation significantly ( $p < 0.05$ ) improved the levels of ZO-1 and CLDN-2 in comparison with the NC group. The upregulation of ZO-1 and claudin-2 suggests improved epithelial barrier integrity and regulated paracellular permeability. Phytogetic and synbiotic supplementation may enhance tight junction expression through antioxidant and microbiota-mediated mechanisms, thereby reducing inflammation-associated disruption of the intestinal barrier [29].

Although Claudin-2 is often characterized as an inflammation-associated tight junction molecule due to its upregulation in various inflammatory conditions [30], its dose-dependent increase in the present study, in parallel with ZO-1, indicates coordinated modulation of tight junction components. This pattern suggests that phytogetic and synbiotic supplementation may induce adaptive remodelling of tight junctions rather than epithelial barrier disruption.

Such coordinated regulation may reflect physiological optimization of epithelial function rather than pathological permeability changes [31]. Consistently, pomegranate peel supplementation in broilers has been reported to upregulate claudin-2 expression concurrently with improvements in intestinal integrity and growth performance, supporting a nutritionally mediated regulatory effect on tight junction dynamics [32]. Mechanistically, experimental models demonstrate that claudin-2 upregulation can be associated with enhanced epithelial turnover, regulated paracellular transport, and mucosal repair processes, reflecting physiological epithelial adaptation rather than pathological permeability alterations [31].

Increase in TJs proteins that observed in the present study may be attributed to carotenoids, which possess antioxidant and anti-inflammatory properties that enhance barrier function [33]. In this context, dietary thyme essential oil helps maintain intestinal integrity and strengthens the mucosal

barrier (34). Consistent with Chen *et al.* (2024) [35], dietary supplementation of curcumin at 200 mg/kg feed markedly upregulated ( $p < 0.05$ ) the mRNA expression of ZO-1 and occludin on day 7 post-infection, relative to the group challenged with  $5 \times 10^4$  *Eimeria tenella* oocysts in broiler chicken on day 14 of the experiment. Likewise, oral administration of carvacrol essential oil at a dose of 200  $\mu$ L per bird for 15 consecutive days in 28-day-old broilers significantly upregulated ( $p < 0.05$ ) the expression of claudin-1, ZO-1, and ZO-2 genes in the small intestinal mucosa [36]. The present findings differed from Guo *et al.*, (2023) [37], who reported no significant ( $p > 0.05$ ) effect of curcumin and pueraria extract (200 mg/kg) on jejunal mRNA expression of claudin-1, occludin, or ZO-1. Similarly, the probiotic *Lactobacillus plantarum* GX17 has been shown to enhance intestinal barrier function by upregulating key tight junction-related genes, including CLDN, MUC2, and TLR2. This finding is consistent with the effects observed with commercial probiotic treatments, indicating a potential role for GX17 in strengthening epithelial barrier integrity [38].

The expression of tight junction proteins (ZO-1 and CLDN-2) was upregulated by the phytogenic botanical blend (PBB) and conventional synbiotic (CS). This increase may be due to improved intestinal barrier function.

#### Goblet cell number and area, with mucin-associated staining intensity and integrated optical density (IOD) of individual goblet cells

Mucin represents a primary immune defence in the gut, and increased secretion may aid in preventing pathogen and toxin invasion [39]. By releasing mucin, goblet cells contribute to intestinal lubrication and safeguard the epithelial wall. The number of goblet cells indicates the capability of the GI tract for mucus secretion; the function of goblet cells is to produce and secrete mucins in the GI tract [3]. IOD, which reflects the relative mucin content within stained goblet cells, together with the observed increase in goblet cell size, indicates enhanced mucin accumulation and secretory activity in the supplemented groups [40].

In the present study, although goblet cell number was not significantly altered ( $P > 0.05$ ), goblet cell size and IOD were significantly increased in the supplemented groups ( $P < 0.05$ ), indicating enhanced mucin synthesis and accumulation within individual goblet cells. The elevated IOD values, under standardized staining conditions, reflect greater intracellular mucin content per cell, suggesting increased secretory capacity and improved mucosal barrier function [41].

The increase in goblet cell size and mucin content, without a statistical change ( $p > 0.05$ ) in cell number, suggests functional activation rather than a hyperplastic response. This qualitative enhancement of mucin production may strengthen the chemical barrier of the intestine and reduce pathogen adherence and epithelial stress [3].

In the current study, a mixture of Mannan-oligosaccharide with *Lactobacillus* and *Bacillus spp* was used as a synbiotic. Our results differ from those reported by Kritdayopas *et al.*, (2019) [42], who found that, MOS and synbiotic supplementation significantly increased ileal goblet cell counts ( $P < 0.05$ ), which may be attributed to the proliferation of beneficial bacteria and enhanced mucus secretion, thereby strengthening the intestinal barrier under

high stocking density conditions. Consistent with our findings, Hashemipour *et al.*, (2013) [43], reported that supplementation with thymol and carvacrol (100 and 200 mg/kg feed), exhibited no significant ( $p > 0.05$ ) alteration in jejunal goblet cell counts compared to the control up to 42 days of age.

In dissimilarity with our findings, Harman *et al.*, 2024 [44] observed that dietary supplementation with 1% onion powder significantly ( $p < 0.05$ ) increased goblet cell numbers relative to the control group. While the provision of microencapsulated essential oil containing 50% thyme oil and 12.5% each of peppermint and black pepper seed significantly ( $p < 0.05$ ) reduced goblet cell counts when administered at 0.5, 1, and 2 kg/ton of feed in broilers challenged with *Salmonella enteritidis* [45]. In divergence with our findings, Omar *et al.*, 2020 [46] reported that inclusion of phenolic-rich onion extract (2 and 3 g/kg feed) notably enhanced ( $p < 0.05$ ) duodenal and jejunal goblet cells in broilers relative to the control group.

A significant ( $p < 0.05$ ) increase in goblet cell size and mucin-associated IOD was observed following the provision of PBB and CS, suggesting that PBB shows comparable efficacy in improving mucosal barrier function. The concurrent improvement in tight junction expression and mucin dynamics further supports a coordinated reinforcement of both the physical and chemical components of the intestinal barrier.

**Table 1:** Dietary treatment groups of experimental birds

| NC                                                                   | PBB1                                                                         | PBB2                                                                          | CS                                                                       |
|----------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Control broiler diet without inclusion of Phytogenic botanical blend | Inclusion of Phytogenic botanical blend at dose of 500 g/mt of finished feed | Inclusion of Phytogenic botanical blend at dose of 1000 g/mt of finished feed | Inclusion of Conventional Synbiotic at dose of 250 g/mt of finished feed |

**Table 2:** Proximate compositions of pre-starter, starter and finisher diets

| Chemical Parameters      | Pre-starter | Starter | Finisher |
|--------------------------|-------------|---------|----------|
| ME (Kcal/kg)             | 3016        | 3096    | 3209     |
| Loss on drying           | 11.4        | 12.1    | 10.9     |
| Crude protein%           | 23.4        | 21.4    | 18.7     |
| Total ash%               | 7           | 6.2     | 6.5      |
| Ether extract%           | 3           | 4.9     | 7.6      |
| Soluble dietary fibre%   | 0.9         | 1.7     | 1.8      |
| Insoluble dietary fibre% | 18.2        | 15.9    | 13.3     |

**Table 3:** Effect of PBB and CS on European production efficiency factor of different experimental groups

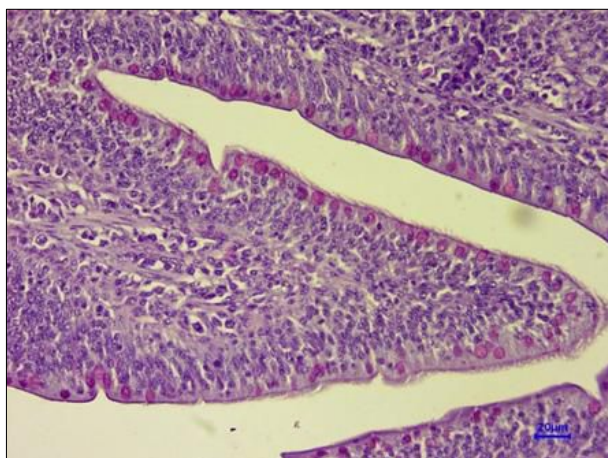
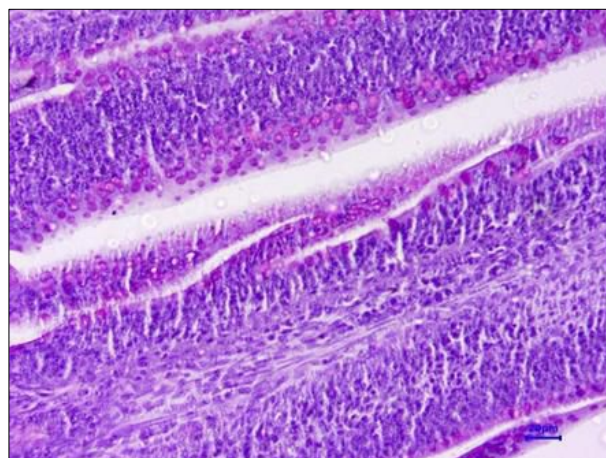
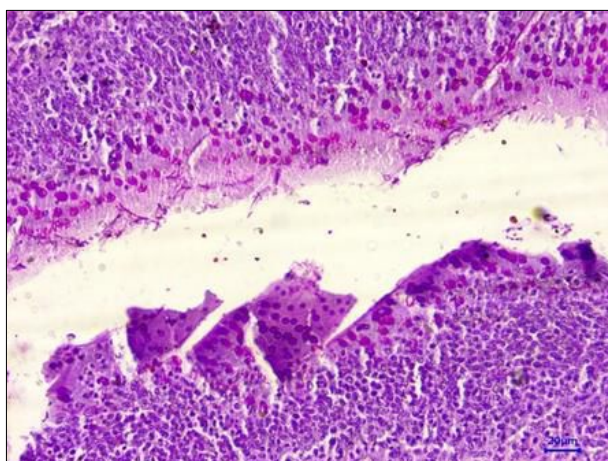
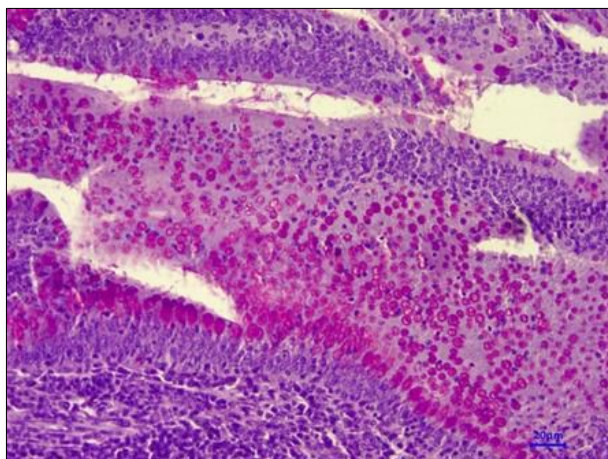
| European Production efficiency factor | Experimental groups        |                            |                            |                            |
|---------------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                       | NC                         | PBB1                       | PBB2                       | CS                         |
|                                       | 338.09 <sup>b</sup> ± 4.78 | 377.91 <sup>a</sup> ± 3.39 | 392.98 <sup>a</sup> ± 3.74 | 390.09 <sup>a</sup> ± 5.76 |

**Table 4:** Effect of PBB and CS on Claudin-2 and Zonula occludens-1 in birds from different experimental groups

| Week               | Experimental groups        |                             |                              |                              |
|--------------------|----------------------------|-----------------------------|------------------------------|------------------------------|
|                    | NC                         | PBB1                        | PBB2                         | CS                           |
| Claudin-2          | 668.31 <sup>d</sup> ± 8.19 | 856.68 <sup>c</sup> ± 22.03 | 1397.78 <sup>b</sup> ± 21.89 | 1713.37 <sup>a</sup> ± 44.06 |
| Zonula Occludens-1 | 5.45 <sup>c</sup> ± 0.11   | 5.92 <sup>bc</sup> ± 0.16   | 6.42 <sup>b</sup> ± 0.12     | 7.39 <sup>a</sup> ± 0.09     |

**Table 5:** Effect of PBB and CS on histopathological evaluation of PAS-stained ileum in broilers

| Parameters                                   | Experimental Groups          |                               |                               |                              |
|----------------------------------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|
|                                              | NC                           | PBB1                          | PBB2                          | CS                           |
| Goblet cells (Number)                        | 90.93±5.52                   | 90.63±6.14                    | 95.53±4.63                    | 101.23±5.55                  |
| Area/Size of goblet cells (µm <sup>2</sup> ) | 14.93 <sup>b</sup> ±2.14     | 19.20 <sup>b</sup> ±2.05      | 28.89 <sup>a</sup> ±2.86      | 31.49 <sup>a</sup> ±1.60     |
| Staining Intensity                           | 223.96 <sup>b</sup> ±0.77    | 230.62 <sup>a</sup> ±1.96     | 226.86 <sup>ab</sup> ±2.08    | 225.37 <sup>ab</sup> ±1.20   |
| Integrated optical density (IOD)             | 3398.20 <sup>c</sup> ±476.06 | 4389.68 <sup>bc</sup> ±459.79 | 6312.53 <sup>ab</sup> ±575.34 | 6490.55 <sup>a</sup> ±533.22 |

**Fig 1:** Photomicrograph of the ileum from control group showing intact villi and normal mucin-filled goblet cell distribution. PAS, 40X.**Fig 4:** Photomicrograph of the ileum from the CS group bird showing abundant mucin-filled goblet cells with intact villus architecture, similar to PBB2 group. PAS, 40X.**Fig 2:** Photomicrograph of the ileum from PBB1 bird showing prominent mucin-filled goblet cells compared to control group. PAS, 40X.**Fig 3:** Photomicrograph of the ileum from PBB2 bird showing abundant mucin-filled goblet cells with intact villus architecture. PAS, 40X.

### Conclusion

The findings of the present study demonstrate that dietary supplementation with phytogenic botanical blend (PBB) at 1000 g/MT of feed and conventional synbiotic (CS) at 250 g/MT of feed significantly enhanced the EPEF relative to the control group. Similarly, both PBB2 and CS groups exhibited comparatively higher upregulation of key TJs, such as CLDN-2 and ZO-1, suggesting enhanced epithelial barrier modulation. Moreover, elevated mucin-associated staining intensity and IOD observed in both PBB2 and CS groups, together with favourable changes in goblet cell morphometry, indicate strengthened mucosal defence and improved intestinal barrier integrity. Collectively, these findings suggest that the PBB (1000 g/MT of feed) demonstrated comparable efficacy to a CS (250 g/MT of feed).

### Acknowledgments

The authors sincerely acknowledge the support provided by the Dr. B. V. Rao Institute of Poultry Management and Technology (IPMT), Uruli Kanchan, Maharashtra, India, and Verity Vet Laboratories – Molecular Veterinary Diagnostic Laboratory, Baner, Pune, Maharashtra, India.

### References

1. Yegani M, Korver DR. Factors affecting intestinal health in poultry. *Poultry Science*. 2008;87(10):2052-2063. doi:10.3382/ps.2008-00091.
2. Ducatelle R, Goossens E, Eeckhaut V, Van Immerseel F. Poultry gut health and beyond. *Animal Nutrition*. 2023;13:240-248. doi:10.1016/j.aninu.2023.03.005.
3. Duangnumsaeng Y, Zentek J, Goodarzi Boroojeni F. Development and functional properties of intestinal mucus layer in poultry. *Frontiers in Immunology*. 2021;12:745849.
4. Awad WA, Hess C, Hess M. Enteric pathogens and their toxin-induced disruption of the intestinal barrier

- through alteration of tight junctions in chickens. *Toxins*. 2017;9(2):60.
5. Farahat M, Ibrahim D, Kishawy AT, Abdallah HM, Hernandez-Santana A, Attia G. Effect of cereal type and plant extract addition on the growth performance, intestinal morphology, caecal microflora, and gut barrier gene expression of broiler chickens. *Animal*. 2021;15(3):100056. doi:10.1016/j.animal.2020.100056.
  6. Kamali SA, AA M, SA H. The effects of herbal plants on mucin 2 gene expression and performance in ascitic broilers.
  7. Liu R, Ding X, Dang M, Wang J, Zhu W. Effects of hesperidin, thymol, rosmarinic acid and their combined effect on growth performance, intestinal barrier function and cecal microbiota in broilers. *Poultry Science*. 2024;103(12):104247. doi:10.1016/j.psj.2024.104247.
  8. Jamroz D, Wiertelcki T, Houszka M, Kamel C. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *Journal of Animal Physiology and Animal Nutrition*. 2006;90(5-6):255-268. doi:10.1111/j.1439-0396.2005.00603.x.
  9. Omar AE, Al-Khalaifah HS, Mohamed WA, Gharib HS, Osman A, Al-Gabri NA, *et al.* Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behavior, intestinal histology, amino acid digestibility, antioxidant activity, and immune status of broiler chickens. *Frontiers in Veterinary Science*. 2020;7:582612. doi:10.3389/fvets.2020.582612.
  10. Islam R, Sultana N, Bhakta S, Haque Z, Hasan A, Siddique MP, *et al.* Modulation of growth performance, gut morphometry, and cecal microbiota in broilers by clove (*Syzygium aromaticum*) and tulsi (*Ocimum sanctum*) supplementation. *Poultry Science*. 2023;102(1):102266. doi:10.1016/j.psj.2022.102266.
  11. Kolbadinejad A, Rezaei pour V. Efficacy of ajwain (*Trachyspermum ammi* L.) seed at graded levels of dietary threonine on growth performance, serum metabolites, intestinal morphology and microbial population in broiler chickens. *Journal of Animal Physiology and Animal Nutrition*. 2020;104(5):1333-1342. doi:10.1111/jpn.13357.
  12. Zhou L, Lin Y, Chang Y, Abouelezz KF, Zhou H, Wang J, *et al.* The influence of Piper sarmentosum extract on growth performance, intestinal barrier function, and metabolism of growing chickens. *Animals*. 2023;13(13):2108. doi:10.3390/ani13132108.
  13. Saleem K, Hayat Z, Latif HR, Tariq Z, Riaz T, Ullah S, *et al.* Growth performance and gut microbiota of broilers administered different levels of mango seed kernel extract. *Brazilian Journal of Poultry Science*. 2024;26(2):eRBCA-2023. doi:10.1590/1806-9061-2023-1843.
  14. Layton C, Bancroft JD, Suvarna SK. Fixation of tissues. In: Suvarna SK, Layton C, Bancroft JD, editors. *Bancroft's theory and practice of histological techniques*. 8th ed. 2018. p. 40-63.
  15. McManus JF. Histological and histochemical uses of periodic acid. *Stain Technology*. 1948;23(3):99-108.
  16. Ruifrok AC. Quantification of histochemical staining by color deconvolution. *Analytical and Quantitative Cytology and Histology*. 2001.
  17. Quirk TJ. One-way analysis of variance (ANOVA). In: *Excel 2019 for social science statistics: A guide to solving practical problems*. Cham: Springer International Publishing; 2021. p. 167-184. doi:10.1007/978-3-030-64333-1\_8.
  18. Abdi H, Williams LJ. Tukey's honestly significant difference (HSD) test. *Encyclopedia of Research Design*. 2010;3(1):1-5.
  19. Dardouri M, Mtibaa MM, Azaiez S, Khachroub AM, Mansour W. Herbs impact on poultry health and antimicrobial resistance: A scoping review with one health perspective. *BMC Veterinary Research*. 2025;21(1):499.
  20. Vlaicu PA, Untea AE, Turcu RP, Saracila M, Panaite TD, Cornescu GM. Nutritional composition and bioactive compounds of basil, thyme and sage plant additives and their functionality on broiler thigh meat quality. *Foods*. 2022;11(8):1105.
  21. Belali M, Seidavi A, Bouyeh M. Effects of combined use of thyme powder and aqueous extract on growth performance, carcass and organ characteristics, blood parameters, enzymes, immune system and jejunum morphology in broilers. *Journal of the Hellenic Veterinary Medical Society*. 2022;73(3):4465-4476.
  22. El-Ashram S, Abdelhafez GA. Effects of phytogenic supplementation on productive performance of broiler chickens. *Journal of Applied Poultry Research*. 2020;29(4):852-862.
  23. Metwally MM. Influence of curcumin and ground anise seeds as medical herbs on the performance, European production efficiency factor, carcass characteristics and some blood constituents of Japanese quails. *Egyptian Journal of Nutrition and Feeds*. 2022;25(3):387-406.
  24. Dev K, Mir NA, Biswas A, Kannoujia J, Begum J, Kant R, Mandal A. Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. *Animal Nutrition*. 2020;6(3):325-332.
  25. Hossain ME, Munni MB, Islam S. Architecture, permeability, disruption dynamics and remodeling of tight junction proteins: Implications for performance, health, and welfare of the broiler chicken. *Poultry Science*. 2025;106117. doi:10.1016/j.psj.2025.106117.
  26. Oni AI, Oke OE. Gut health modulation through phytogenics in poultry: mechanisms, benefits, and applications. *Frontiers in Veterinary Science*. 2025;12:1616734.
  27. Stevens Y, Pinheiro I, Salden B, Duysburgh C, Bolca S, Degroote J, *et al.* Effect of a carotenoid-producing *Bacillus* strain on intestinal barrier integrity and systemic delivery of carotenoids: A randomised trial in animals and humans. *Journal of Functional Foods*. 2021;80:104445.
  28. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*. 2014;14(3):141-153.
  29. Shehata AA, Yalçın S, Latorre JD, Basiouni S, Attia YA, Abd El-Wahab A, *et al.* Probiotics, prebiotics, and phytogenic substances for optimizing gut health in poultry. *Microorganisms*. 2022;10(2):395. doi:10.3390/microorganisms10020395.
  30. Horowitz A, Chanez-Paredes SD, Haest X, Turner JR. Paracellular permeability and tight junction regulation

- in gut health and disease. *Nature Reviews Gastroenterology & Hepatology*. 2023;20(7):417-432.
31. Ahmad R, Chaturvedi R, Olivares-Villagómez D, Habib T, Asim M, Shivesh P, *et al*. Targeted colonic claudin-2 expression renders resistance to epithelial injury, induces immune suppression, and protects from colitis. *Mucosal Immunology*. 2014;7(6):1340-1353.
  32. Zhang R, Yang J, Wang Q, Hu D, Zhao Q, Zhu S, *et al*. Comparative efficacy of plant extracts and probiotics on growth and gut health in chickens with necrotic enteritis. *Animals*. 2024;14(22):3312. doi:10.3390/ani14223312.
  33. Cheng J, Balbuena E, Miller B, Eroglu A. The role of  $\beta$ -carotene in colonic inflammation and intestinal barrier integrity. *Frontiers in Nutrition*. 2021;8:723480.
  34. Wlodarska M, Willing BP, Bravo DM, Finlay BB. Phytonutrient diet supplementation promotes beneficial *Clostridia* species and intestinal mucus secretion resulting in protection against enteric infection. *Scientific Reports*. 2015;5(1):9253.
  35. Chen Y, Liu L, Yu L, Li S, Zhu N, You J. Curcumin supplementation improves growth performance and anticoccidial index by improving the antioxidant capacity, inhibiting inflammatory responses, and maintaining intestinal barrier function in *Eimeria tenella*-infected broilers. *Animals*. 2024;14(8):1223.
  36. Liu SD, Song MH, Yun W, Lee JH, Kim HB, Cho JH. Effect of carvacrol essential oils on growth performance and intestinal barrier function in broilers with lipopolysaccharide challenge. *Animal Production Science*. 2020;60(4):545-552. doi:10.1071/AN18326.
  37. Guo S, Hu J, Ai S, Li L, Ding B, Zhao D, *et al*. Effects of pueraria extract and curcumin on growth performance, antioxidant status and intestinal integrity of broiler chickens. *Animals*. 2023;13(8):1276.
  38. Yin Y, Liao Y, Li J, Pei Z, Wang L, Shi Y, *et al*. *Lactobacillus plantarum* GX17 benefits growth performance and improves functions of intestinal barrier and intestinal flora among yellow-feathered broilers. *Frontiers in Immunology*. 2023;14:1195382. doi:10.3389/fimmu.2023.1195382.
  39. Murai A, Kitahara K, Terada H, Ueno A, Ohmori Y, Kobayashi M, *et al*. Ingestion of paddy rice increases intestinal mucin secretion and goblet cell number and prevents dextran sodium sulfate-induced intestinal barrier defect in chickens. *Poultry Science*. 2018;97(10):3577-3586.
  40. Biasato I, Ferrocino I, Biasibetti E, Grego E, Dabbou S, Sereno A, *et al*. Modulation of intestinal microbiota, morphology and mucin composition by dietary insect meal inclusion in free-range chickens. *BMC Veterinary Research*. 2018;14(1):383. doi:10.1186/s12917-018-1690-y.
  41. Forder RE, Howarth GS, Tivey DR, Hughes RJ. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. *Poultry Science*. 2007;86(11):2396-2403.
  42. Kridtayopas C, Rakangtong C, Bunchasak C, Loongyai W. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. *Poultry Science*. 2019;98(10):4595-4605. doi:10.3382/ps/pez152.
  43. Hashemipour H, Kermanshahi H, Golian A, Raji A, Van Krimpen MM. Effect of thymol + carvacrol by Next Enhance 150® on intestinal development of broiler chickens fed CMC containing diet. *Iranian Journal of Applied Animal Science*. 2013;3(3):567-576.
  44. Harman H, Eşki HT, Ündağ İ, Akbulut K, Başer E, Kırıkçı K, *et al*. The effect of onion and garlic powders on growth performance, viability, intestine histomorphology, and some blood parameters in Chukar partridge chicks. *Journal of the Hellenic Veterinary Medical Society*. 2024;75(3):7825-7832. doi:10.12681/jhvms.35607.
  45. Moharreri M, Vakili R, Oskoueian E. Evaluation of microencapsulated essential oils in broilers challenged with *Salmonella enteritidis*: A focus on the body's antioxidant status, gut microbiology, and morphology. *Archives of Razi Institute*. 2022;77(2):629-637. doi:10.22092/ARI.2021.354334.1634.
  46. Omar AE, Al-Khalaifah HS, Mohamed WA, Gharib HS, Osman A, Al-Gabri NA, *et al*. Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behavior, intestinal histology, amino acid digestibility, antioxidant activity, and immune status of broiler chickens. *Frontiers in Veterinary Science*. 2020;7:582612.