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Studies on the effect of dietary supplementation of probiotics on immune status, faecal microflora and hematological parameters of crossbred pigs

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Abstract
Pig farming is considered an essential component of the livestock sector, and pork is a significant trade commodity globally. According to the Food and Agriculture Organization (FAO), global pork production reached 97 million metric tons in 2020. In India, an estimated half a million people are involved in Pig farming. It is more concentrated in certain states of the country, including Assam, Meghalaya, Nagaland, Manipur, and Mizoram. According to the 20th Livestock Census, the total pig population in the country is 9.06 million, which accounts for 1.7% of the total livestock population. Several studies have been conducted to enhance the growth performance of grower pigs through the use of various feed additives. The different feed additives added to pig feed include enzymes, antioxidants, antibiotics, prebiotics, and probiotics. Probiotic is added to increase the population of beneficial microorganisms, such as *Lactobacilli* and *Bifidobacteria*. These healthy bacterial colonies then inhibit the growth of harmful microorganisms by producing substances (e.g., bacteriocins and/or organic acids). Probiotic feed additives stimulate antibody production, thereby naturally boosting the immune system. So, probiotics are given in all different stages of growth of pigs (Pre-weaning, post-weaning, and in Reproduction). It boosted the immune response, and Hematological indices and gut microbiota balance improved, with higher Lactobacillus counts in the supplemented groups.

Keywords: Crossbred pigs; Probiotics; Immune status; Microbial count; Hematological parameters

1. Introduction
Pig farming plays a vital role in the livestock industry, with pork ranking among the most widely traded meat products worldwide. Data from the Food and Agriculture Organization (FAO) indicates that global pork production stood at around 97 million metric tons in 2020. China emerged as the dominant producer, contributing more than half of the total output. To meet the rising demand for pork, major exporting nations rely on large-scale, intensive production systems designed to optimize efficiency (Bhaduaria *et al.,* 2023) [1]. In India, pig farming is mainly unorganized, with minimal use of additional nutrient supplements. However, over the past few decades, this sector has gained greater acceptance due to several factors, including the rising demand for pork products, shifts in dietary habits, rapid urbanization, increasing disposable income among the middle class, and the entry of international food chains that use pork for items such as bacon and sausages (Shastry and Thomas, 2021) [2]. Nevertheless, pigs compete directly with humans for conventional feed resources, which has led to the exploration of alternative feed options aimed at lowering costs and improving feed conversion efficiency (Shastry and Thomas, 2021) [2]. Various feed additives such as enzymes, antioxidants, antibiotics, prebiotics, and probiotics are commonly incorporated into pig diets to enhance growth and health (Al-Jaf& Del, 2019) [3]. Likewise, probiotics help maintain a healthy gut microbiota by enhancing populations of beneficial microorganisms like Lactobacil0lus and Bifidobacterium, which in turn suppress harmful microbes through the production of bacteriocins and organic acids (Al-Jaf& Del, 2019) [3]. Probiotics are described as live microbial feed additives that exert beneficial effects on the host

by enhancing growth performance, strengthening immunity, and maintaining intestinal microbial equilibrium (Fuller, 1989)[4]. Similarly, yeast-based products such as yeast culture, yeast extract, and yeast hydrolysates (YH) act as effective probiotics because they are rich in nucleotides, B-complex vitamins, amino acids, and cell wall polysaccharides like β-glucans and mannans. These bioactive components contribute to enhanced growth, immune regulation, gut development, and tissue repair in animals (Sauer, 2010) [5].

2 Materials and methods

2.1 Place of the experiment: The present work is carried out at the Instructional Piggery Unit, Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Rewa (M.P.)

2.2 Management of Pigs

The experimental pens were prepared according to standard practice before separating the weaned piglets. All healthy experimental piglets were taken and kept in similar management conditions, provided with normal routine healthcare, and vaccinated. The experiment was conducted in 40 weaned crossbred piglets (Large white Yorkshire x Desi pigs) weaned at 60 days of age, born in the same season (±2 months) from different sows, and were randomly divided into 4 experimental groups, each containing 10 piglets. The experimental groups consist of one control group (only a basal diet is given) and three treatment groups (basal diet + probiotics) with varying degrees of probiotics added to the basal diet. Table 1 illustrates the experimental design.

Table 1: Design of experimentation details

Experimental Animals			
Group 1	**Group 2**	**Group 3**	**Group 4**
Control group (Basal diet) NRC (1980) feeding standard with CP 22% and 18%	Basal diet + Probiotic @ 200g/ tonne of feed [*Bacillus subtilis* and *B. lichenformis*@ 3x10^6 CFU per gram, *Saccharomyces cerevisiae*@ 6x10^{6}CFU / gram] (from 60 days of age upto120 days)	Basal diet + Probiotic @ 400g/ tonne of feed [*Bacillus subtilis* and *B. lichenformis* @ 6x10^6 CFU per gram, *Saccharomyces cerevisiae*@ 12x10^{6}CFU / gram] (from 60 days of age upto120 days)	Basal diet + Probiotic @ 600g/ tonne of feed [*Bacillus subtilis* and *B. lichenformis*@ 9x10^6 CFU per gram, *Saccharomyces cerevisiae* @ 18x10^{6}CFU / gram] (from 60 days of age upto120 days)
10 piglets	10 piglets	10 piglets	10 piglets

2.3 Parameter / Observations recorded

Biochemical analysis of serum
1. Immunity Status
2. Fecal microbiota count
3. Blood parameters
a. RBCs (million/ mm³)
b. WBCs (Thousand/ mm³ or Thousand/µl³)
c. DLC (%) d. Hb (g/dl)
e. PCV (%)
f. MCV (µ³) = Vol of RBC in 100 ml of blood (PCV) /No of RBC in 100 ml of blood (Count)
g. MCH (pg/cell) = Hb % in 100 ml of blood /No of cells in 100 ml of blood
h. MCHC (g/dl) = Average of Hb × 100 /Vol. of RBC containing the Hb

2.4 Immunity Status

The humoral immune response was evaluated using the microhemagglutination assay. For this, sheep blood was collected aseptically from the jugular vein into an EDTA vial and carefully transported to the laboratory. The samples were centrifuged at 3000 rpm for 5 minutes to separate the red blood cells (RBCs) from other components. The supernatant was discarded, and the packed RBCs were washed three times with phosphate-buffered saline (PBS). A 2% suspension of RBCs was then prepared and further diluted with PBS at a ratio of 1:9 to obtain a final 20% RBC suspension. Pigs maintained on probiotics-supplemented diets for 120 days were injected intramuscularly in the ham region with 1 ml of the 20% sheep red blood cell (SRBC) suspension, following the method of Wagmann and Smithies (1966)[6]. Blood samples were collected by venipuncture on days 0, 14, and 21 postinjection. The serum was separated from the blood samples by centrifugation and used for the hemagglutination assay. For the assay, V-bottom microtiter plates were used. Each well was filled with 50 µL PBS, followed by the addition of 50 µL of the serum sample. Serial 10-fold dilutions of sera from each sampling day (0, 14, and 21) were prepared. Subsequently, 50 µl of SRBC suspension was added to each well and mixed thoroughly. The plates were incubated at 37 °C for 3–5 hours. The antibody titer was determined as the log2 value of the reciprocal of the highest serum dilution exhibiting visible agglutination of SRBCs (Kumar *et al*., 2012) [7].

2.5 To Study Fecal Microbiota Count

Fresh fecal samples were collected from two pigs per pen by gently massaging the rectum after 120 days of probiotic feeding. The samples were immediately placed on ice packs and transported to the laboratory for microbiota analysis. Fecal material from each group was plated on media, specifically Lactobacillus MRS Agar for Lactobacillus spp. and MacConkey/TBX Agar for *E. coli*. For sample preparation, 1 g of fecal matter from each group was homogenized with 9 ml of sterile distilled water in a conical flask to obtain the initial suspension. Tenfold serial dilutions were prepared by transferring 1 mL of the suspension into 9 mL of sterile distilled water in a series of test tubes. The solid portion of feces was thoroughly crushed and mixed in 90 ml of normal saline before dilution. For each dilution step, fresh micropipette tips were used to prevent cross-contamination. From dilutions of 10^7, 10^8, 10^9, and 10^{10}, 1 ml aliquots were spread onto sterile Petri plates. Plates for E. coli isolation were incubated anaerobically at 39 °C for 24 hours, while those for Lactobacillus spp. were incubated aerobically at 37 °C for 48 hours.

2.6 Collection and processing of blood

Blood (2 ml/pig) was drawn in a sterile syringe/ Normal 2ml syringe from ear vein puncture, posing minimum disturbance to the pig on 60th, 120th day, and at the 180th day. Immediately after collecting, the tubes were transported to the laboratory on ice for further processing. A part of the blood sample was used for hematological studies (RBCs, WBCs, DLC, Hb, PCV).

2.7 Statistical Analysis
The recorded data were analyzed using two-way analysis of variance (Snedecor and Cochran, 1994) with SPSS software version 25.

3 Result and Discussion
3.1 Hemaglutinationtitre
The effect of probiotic supplementation at different doses in treatment groups (groups 1 to 4) across 0th, 14th, and 21st days on the microhemagglutination titre of crossbred pigs was evaluated. The study showed that microhemagglutination (HA) titres differed significantly ($p < 0.05$) among groups and across days, with all groups displaying comparable patterns of immune response. On day 0, Group 1 recorded the highest baseline titre, though the range remained similar across groups. By day 14, all groups showed a significant increase in HA titers, with Group 3 exhibiting the strongest immune response, followed by Groups 4, 2, and 1. A slight decline occurred on day 21, with Group 2 showing the highest titre, while Group 1 demonstrated the weakest sustained response.
These results suggest that probiotic supplementation enhances intestinal immunity by promoting immune cell activity and improving antigen clearance. The findings align with previous studies (Sarkar *et al.,* 2023; Konieczka *et al.,* 2023; Mun *et al.,* 2021; Kumar *et al.,* 2014; Joyoswol *et al*., 2021) [8, 9, 10, 11, 12], which consistently report improved humoral and cellular immune responses in animals supplemented with probiotic or Bacillus-based diets.

3.2 Fecal microbial count
The effect of probiotics supplementation at different doses in treatment groups (Groups 1 to 4) on the fecal microbial count of crossbred pigs at the end of the trial was evaluated. The analysis showed that faecal E. coli counts did not differ significantly between the treatment groups ($p > 0.05$), indicating that the interventions had little effect on this bacterium, with values remaining relatively stable across the groups. In contrast, Lactobacillus counts differed significantly ($p < 0.05$), with Group 3 showing the highest levels, suggesting that its diet, likely enriched with probiotics, was most effective in promoting beneficial gut flora.
These findings align with previous studies reporting increased Lactobacillus counts and reduced or unchanged E. coli or coliform levels following probiotic supplementation in pigs (Wang & Kim, 2021; Kumar *et al.,* 2014; Nguyen *et al*., 2019; Rybarczyk *et al.,* 2021; Sampath *et al.,* 2021; Mishra *et al.,* 2014) [13-17]. Overall, the results reinforce the positive role of probiotics in enhancing beneficial microbes without significantly affecting E. coli populations.

3.3 Hematological parameters
3.3.1 Hemoglobin
The effect of probiotic supplementation at different doses in treatment groups (Fig.1) (Groups 1 to 4) across 60,120th and 180th day on hemoglobin of crossbred pigs was evaluated. Hemoglobin levels remained statistically non-significant ($p>0.05$) across all groups throughout the 180-day trial, with mean values showing minor, uniform fluctuations from 14.10–14.80 g/dl on day 60, 14.28–14.70 g/dl on day 120, and a slight decline to 14.00–14.10 g/dl by day 180, indicating no probiotic-related effect. These stable values suggest that probiotic supplementation did not significantly influence hemoglobin concentrations in crossbred pigs, although the steady levels may reflect a supportive role in maintaining general health and metabolic balance.

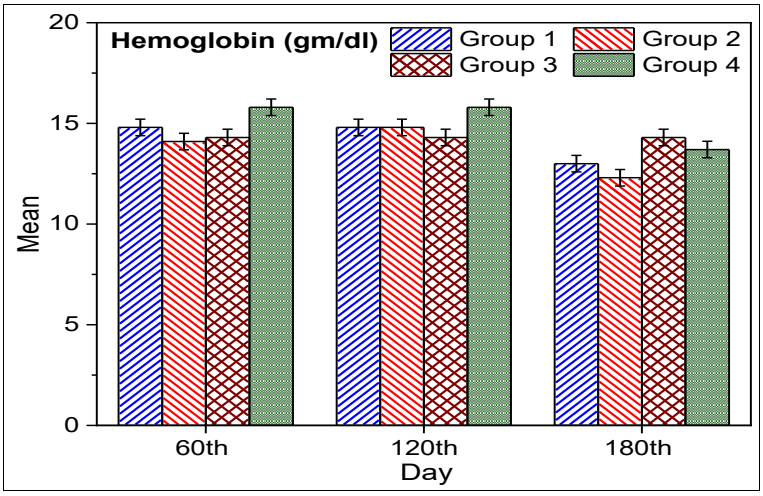

Fig 1: Hemoglobin of Crossbred pigs Mean(±SE) in treatment groups and across 60th, 120th and 180th day

The results align with Bhaskar *et al.* (2023) [18], who also observed no significant hematological differences with probiotic use, and partially agree with studies suggesting probiotics may aid nutrient absorption and gut health, though they contrast with findings by Giang *et al.* (2011) [19] and Ajeyegoro*et al*. (2017) [20] that reported increased hemoglobin in pigs receiving Lactobacillus-based diets.

3.3.2 Packed cell volume
The effect of probiotic supplementation at different doses in treatment groups (Fig.2) (Group 1 to 4) across 60,120th and 180th day on PCV of crossbred pigs was evaluated. The PCV values showed minor fluctuations across groups during the study. On the 60th day, Groups 1, 2, 3, and 4 recorded PCV levels of 44 ± 1.21, 41.2 ± 1.19, 41.6 ± 1.25, and 46.6 ± 1.00, respectively. By the 120th day, Group 4 continued to show the highest PCV (46.6±1.23), followed by Group 2 (44.18±1.23) and Group 1 (43±1.23), while Group 3 remained lower at 41.6±1.22. At the 180th day, PCV values stabilized across all groups, ranging narrowly between 41.8 ± 1.26 and 42.3 ± 1.21.

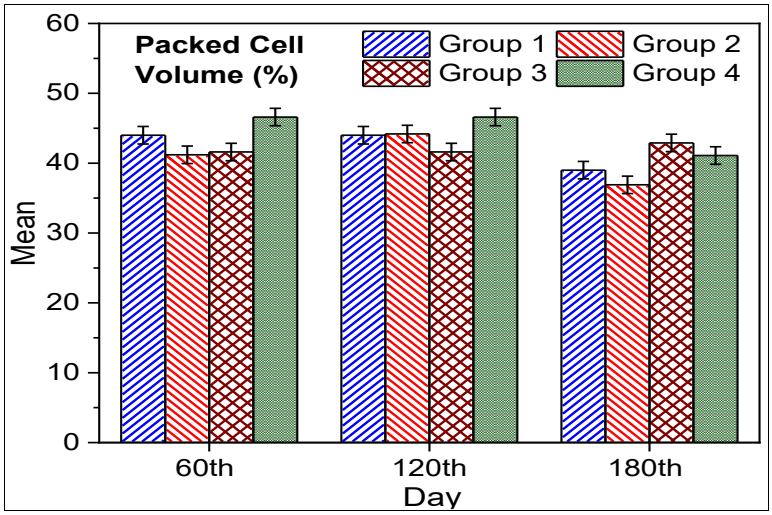

Fig 2: PCV of Crossbred pigs Mean (±SE) in treatment groups and across 60th, 120th and 180th day

These findings align with earlier reports (Bhaskar *et al.,* 2023) [18] showing no significant differences in major hematological indices with probiotic supplementation, although previous studies (Kritas *et al.,* 2015; Lie *et al*., 2021; Giang *et al*., 2011; Dowarah*et al*., 2017) [21, 22, 19, 23] have noted that probiotics can enhance blood profiles through improved nutrient utilization, gut health, and immune function.

3.4 Total Erythrocyte Count

The total erythrocyte count (TEC) showed statistically significant differences among groups (Fig. 3) on the 60th day, with values ranging from 6.18 ± 0.18 to 6.24 ± 0.19 in Groups 1-3 and slightly lower in Group 4 (5.86 ± 0.17). By the 120th day, Groups 3 and 4 exhibited significantly lower TEC (5.86 ± 0.15 and 5.90 ± 0.15) compared to Groups 1 and 2 (6.16 ± 0.15 and 6.20 ± 0.12). However, by the 180th day, TEC increased across all groups, with no significant differences observed, indicating comparable levels among treatments.

These findings are consistent with Bhaskar *et al*. (2023b) [24], who reported no significant changes in major hematological parameters with probiotic supplementation, as well as previous studies in pigs and other livestock showing that probiotics primarily support gut health and overall physiological stability without markedly altering blood indices.

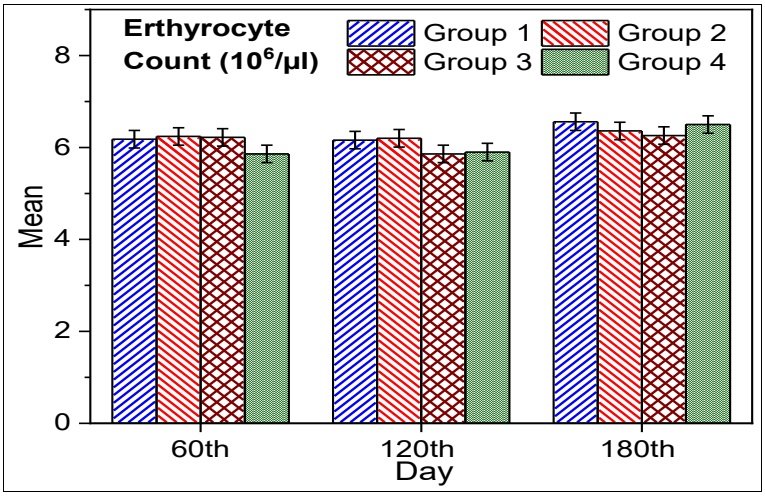

Fig 3: Total Erthyrocyte count of Crossbred pigs Mean(±SE) in treatment groups and across 60th, 120th and 180th day

3.5 Total Leucocyte count

The study showed that total WBC counts remained stable across all groups throughout the 60th, 120th, and 180th days (Fig. 4), with no statistically significant differences ($p > 0.05$) either between groups or within groups over time. This suggests that probiotic supplementation did not adversely affect leukocyte levels and helped maintain a stable immune profile throughout the trial.

These findings align with Bhaskar *et al*. (2023) [24], who also reported no significant changes in major hematological parameters with probiotic supplementation. Similar reports by Sarkar *et al.* (2023) [8] and Kritas *et al.* (2015) [21] suggest that probiotics such as Lactobacillus spp. and Bacillus spp. Support normal hematological values and contribute to immune homeostasis by improving gut microbiota and reducing systemic inflammation, thereby indirectly stabilizing leukocyte dynamics.

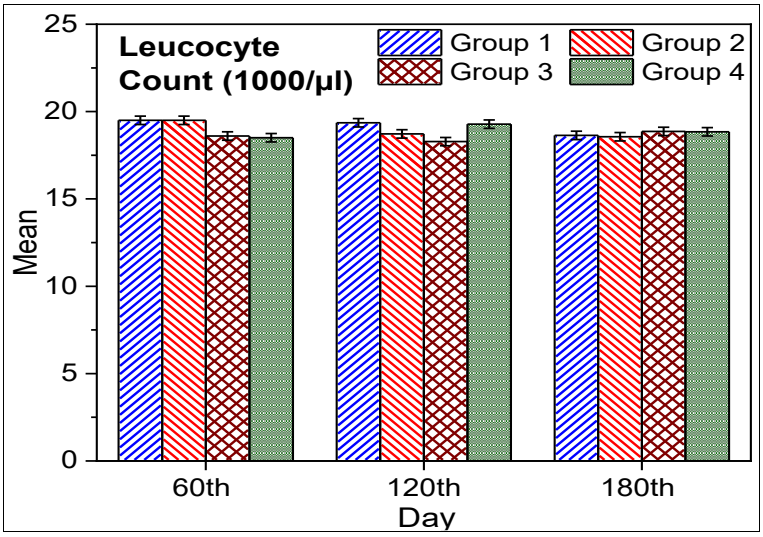

Fig 4: Total Leucocyte count of Crossbred pigs Mean(±SE) in treatment groups and across 60th, 120th and 180th day

3.6 Differential leucocyte count

The differential leucocyte count values across all four pig groups remained statistically non-significant ($p > 0.05$), indicating that the treatments did not induce significant shifts in immune cell proportions. Lymphocyte percentages increased slightly from day 0 to day 180 in all groups, with higher values noted in Groups 3 and 4 by day 120 and 180, suggesting mild enhancement of adaptive immunity. Neutrophil levels remained stable across all periods, reflecting consistent innate immune function without inflammatory overstimulation. Eosinophils showed slight increases by day 180, most notably in Groups 2 and 3, indicating improved immune surveillance, while basophils and monocytes exhibited only minor, non-significant fluctuations throughout the study.

Overall, the treatments maintained stable immune profiles with modest improvements in lymphocyte and eosinophil activity, aligning with earlier reports that probiotics modulate immunity without inducing excessive neutrophil responses (Wang & Kim, 2021; Kiros *et al.,* 2016; Ajeyegoro *et al*., 2017) [13, 25, 20]. However, these findings differ from Bhaskar *et al.* (2023) [18], who reported no significant changes in major haematological parameters with probiotic supplementation.

4 Conclusion

The study demonstrated that probiotic supplementation in the diet of crossbred pigs positively influenced growth performance, feed efficiency, immune response, antioxidant status, hematological stability, gut microbial balance, and nutrient digestibility. Probiotics also enhanced immune responses, maintained healthy hematological parameters, and promoted beneficial Lactobacillus populations while supporting nutrient absorption and utilization. These findings confirm that probiotics are a viable and sustainable alternative to antibiotic growth promoters, supporting both productivity and animal health in pig production.

Conflict of Interest

The author has no competing interests to declare.

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