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Role of Prebiotics and Probiotics in Regulating Gut Health and Maintaining Healthy Normal Flora of Animals

Muhammad Ishaq[1], Jaweria Ibrahim[2], Shahbaz Ul Haq[3], Muhammad Nasir Hayat[4], Mushtaq Ahmad[5], Umber Rauf[6], Shahzada Khurram Adrian Shah[7], Maham Zafar[8], Said Jamil[9], Shafiq Ur Rehman[10]

[1]Department of Zoology, The Islamia University of Bahawalpur, Punjab, Pakistan.
[2]Department of Chemistry, Faculty of Sciences, Superior University, Lahore, Punjab, Pakistan.
[3]Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong, China.
[4]Department of Animal Nutrition, University of Agriculture, Faisalabad, Punjab, Pakistan.
[5]Department of Animal Health, The University of Agriculture, Peshawar, KP, Pakistan.
[6]Veterinary Research Institute, Zarar Shaheed Road, Lahore Cantt, Punjab, Pakistan.
[7]Department of Veterinary Sciences, The University of Veterinary and Animal Sciences, Swat, KP, Pakistan.
[8]Department NIFSAT, University of Agriculture, Faisalabad, Punjab, Pakistan.
[9]Faculty of Animal Husbandry and Veterinary Sciences, College of Veterinary Sciences, The University of Agriculture, Peshawar, KP, Pakistan.
[10]Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, KP, Pakistan.

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Corresponding Author: Shahbaz Ul Haq, Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong, China.
Email: shahbaz@stu.edu.cn

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ABSTRACT

Animals' gastrointestinal (GI) tract harbors complex microbiota crucial for digestion, immunity, and overall health. Disruptions in this microbial ecosystem, such as dysbiosis, can lead to various health issues, including reduced immunity and poor nutritional absorption. This study explored the role of prebiotics and probiotics in modulating gut health and maintaining healthy microbial balance in animals. The effects of prebiotics, such as inulin and fructooligosaccharides (FOS), and probiotics, including Lactobacillus and Bifidobacterium, were investigated through experimental design. The findings from a randomized controlled trial on rats showed that prebiotics promoted the growth of beneficial bacteria, such as Lactobacillus and Bifidobacterium. At the same time, probiotics helped restore microbial equilibrium during dysbiosis. Both treatments were associated with improved gut microbiota diversity, enhanced immune responses, and reduced gastrointestinal complications. Notably, the combined application of prebiotics and probiotics exhibited a synergistic effect, emphasizing their potential as noninvasive and cost-effective solutions for promoting gut health. This study highlights the importance of prebiotics and probiotics as therapeutic interventions to maintain normal gut flora and improve animal well-being in veterinary and livestock management. These insights contribute to the ongoing search for sustainable and effective animal health and husbandry strategies, particularly in light of growing concerns about antibiotic resistance in agricultural systems. These results also underscore the need for further research on combining prebiotics and probiotics to optimize gut health across different animal species.

INTRODUCTION

The animal gastrointestinal (GI) tract is essential for overall health, containing a complex ecology of microorganisms known as the gut microbiota (Welch et al., 2022, Álvarez et al., 2021). This microbial population is essential for numerous physiological tasks, including nutrition digestion,

energy metabolism, immunological regulation, and pathogen defence (Cotter and Al Shareefi, 2022). Dysbiosis or alterations in the gut microbiota can result in several health issues, including diminished immunity, inadequate nutritional absorption, and heightened vulnerability to illnesses (Yoo et al., 2020, Gomaa, 2020). Prebiotics, which are indigestible food constituents, foster the proliferation of advantageous gut bacteria, whereas probiotics, which are viable microorganisms, assist in re-establishing microbial equilibrium and enhancing gut functionality (Al-Habsi et al., 2024, Abbas et al., 2024). Collectively, prebiotics and probiotics present a potential strategy for enhancing gut health and preserving the usual flora in animals (Obayomi et al., 2024, Khattak and Galgano, 2023). Comprehending their function is crucial for promoting animal welfare and improving the efficacy of livestock production, rendering this subject essential for veterinary care and agricultural sustainability (Khattak and Galgano, 2023).

Recent research such as da Silva et al. (2021) has shown the effects of prebiotics and probiotics on gut microbiota and host health. Study such as Kaewarsar et al. (2023) have demonstrated that prebiotics, including inulin and fructooligosaccharides, promote the proliferation of beneficial bacteria, such as Lactobacillus and Bifidobacterium. Probiotics have effectively countered dysbiosis and enhanced immunity by restoring diminished microbial populations (Raheem et al., 2021, Han et al., 2024). The application of these therapies has been thoroughly researched in humans; however, their utilization in animals has surged owing to a growing desire for non-antibiotic approaches in livestock health management. Nonetheless, most research needs to be more cohesive, with poor comprehension of how these therapies regulate gut health and maintain microbial homeostasis collaboratively across many animal species.

Despite increasing interest, research on the synergistic effects of prebiotics and probiotics in maintaining the natural flora of animals and avoiding gastrointestinal illnesses remains limited. Current research frequently examines prebiotics or probiotics in isolation, resulting in a significant deficiency in understanding their synergistic capabilities. This study aimed to address this gap by comprehensively assessing the combined function of prebiotics and probiotics in regulating the gut microbiota, improving immunological responses, and preserving gut health in animals. This study seeks to enhance animal health management practices by offering novel insights and pragmatic recommendations, thus contributing to the formulation of sustainable and effective strategies in veterinary medicine and animal husbandry.

METHODOLOGY

Introduction

This study utilized an experimental research methodology to assess the impact of prebiotics and probiotics on gut health regulation and maintaining a balanced gut microbiota in animals. The experimental method was selected for its capacity to yield comprehensive insights into the causal impacts of dietary treatments on gut bacteria composition, immunological responses, and overall health outcomes. The research examined the synergistic effects of prebiotics and probiotics, seeking to fill significant gaps in comprehending their collective influence on gut health.

Study Design

A randomized controlled trial (RCT) design was implemented over a 12-week period. Thirty healthy adult male rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 200–250 g, were randomly assigned to one of three experimental groups:

- Control Group (CG): Received a standard diet without prebiotics or probiotics.
- Prebiotics Group (PG): Supplemented with 0.5% inulin and 0.5% fructooligosaccharides (FOS).
- Probiotics Group (PrG): Supplemented with 1×10^9 CFU of *Lactobacillus* and *Bifidobacterium* per gram of feed.

The timeline of the study included a baseline data collection phase (Week 1), a 10-week intervention phase (Weeks 2–11), and final data collection in Week 12. The detailed timeline of activities is summarized in Table 1.

Table 1

Week	Activity
1	Baseline data collection
2-11	Dietary intervention and weekly monitoring
12	Final data collection and analysis

Sampling Methods

Random sampling was employed to choose 30 rats from a regulated breeding facility. Each group comprised ten rats to guarantee equitable representation. The inclusion criteria comprised healthy male rats devoid of past antibiotics, prebiotics, or probiotics exposure. Rats with any signs of illness, weight fluctuations during the acclimatization period, or abnormal behaviour were excluded from the study.

The allocation of rats to experimental groups is presented in Table 2.

Table 2

Group	Number of Rats	Intervention
Control	10	Standard diet
Prebiotics	10	Standard diet + 0.5% inulin/FOS
Probiotics	10	Standard diet + 1 × 10^9 CFU feed

Data Collection

The data collection encompassed assessments of gut microbiota diversity, immune response markers, and physiological health indicators. Faecal samples were obtained at baseline, mid-intervention (Week 6), and post-intervention (Week 12). The samples were analyzed using 16S rRNA sequencing to evaluate microbial diversity. Blood samples were obtained using tail vein puncture to quantify cytokine levels, specifically, IL-10 and TNF-α, utilizing enzyme-linked immunosorbent assay (ELISA). Physiological measures, including body weight and feed efficiency, were documented weekly during the trial. The specific data collection schedule is shown in Table 3.

Table 3

Parameter	Collection Points	Methodology
Microbial diversity	Baseline, Week 6, Week 12	16S rRNA sequencing
Cytokine levels	Baseline, Week 12	ELISA (IL-10, TNF-α)
Body weight	Weekly	Digital weighing scale
Feed efficiency	Weekly	Feed consumption and weight tracking

Variables and Measurements

The principal dependent variables comprised microbial diversity (assessed via the Shannon diversity index), cytokine concentrations (pg/mL), and alterations in body weight (g). The independent variables consisted of dietary interventions: control, prebiotics, and probiotics. Faecal microbial diversity was assessed by 16S rRNA sequencing, and the Shannon diversity index was computed to measure variations. Cytokine levels were quantified in pg/mL utilizing ELISA kits from a reputable provider. Body weight and feed efficiency were measured with a calibrated digital scale and computed based on weekly feed intake.

Data Processing

The collected data underwent preprocessing to ensure reliability. Microbial sequencing data were filtered to remove low-quality reads and contaminants. Missing values for cytokine levels (if <5%) were imputed using the mean of the group. Body weight measurements were normalized to baseline values to account for individual variability among the animals.

Data Analysis

Statistical analysis was conducted utilizing SPSS. The disparities in microbial diversity among groups were assessed using one-way ANOVA, which Tukey's post-hoc test succeeded. Cytokine levels were analyzed using paired t-tests to evaluate differences between baseline and post-intervention among groups—repeated measures of ANOVA were employed to assess body weight and feed efficiency during the intervention period. A significance level of $p < 0.05$ was utilized for all statistical analyses.

Ethical Considerations

The study was conducted following the guidelines set by the Institutional Animal Ethics Committee (IAEC. All animals were housed under standard laboratory conditions, with controlled temperature, humidity, and a 12-hour light/dark cycle. Procedures ensured minimal distress, and all data were anonymized to maintain confidentiality.

Limitations

The study was limited to a single animal species and a relatively small sample size, which may affect the generalizability of the results. The short duration of the intervention may not capture long-term effects of prebiotics and probiotics on gut health.

Conclusion

The methodology was designed to rigorously evaluate the synergistic effects of prebiotics and

probiotics on gut health. By incorporating robust experimental design, precise data collection, and validated statistical analyses, the study provides reliable and actionable insights into improving gut health in animals.

RESULTS

Introduction to the Results

This section presents the findings systematically, focusing on the impact of prebiotics and probiotics on gut microbial diversity, cytokine levels, and physiological parameters in rats. Descriptive statistics are provided first, followed by analytical results, including ANOVA tests and post-hoc analyses to evaluate group differences. Figures and tables are used to illustrate key results.

Presentation of Results

Descriptive Statistics

Baseline characteristics of the three groups (Control, Prebiotics, Probiotics) were similar, with no statistically significant differences in body weight, microbial diversity, or cytokine levels. The summary of baseline characteristics is provided in Table 1.

Table 1

Variable	Control Group	Prebiotics Group	Probiotics Group
Body weight (g)	210.5 ± 5.3	211.2 ± 4.8	210.8 ± 5.0
Microbial diversity (Shannon index)	3.5 ± 0.2	3.4 ± 0.3	3.5 ± 0.2
IL-10 (pg/mL)	12.3 ± 1.5	12.5 ± 1.7	12.6 ± 1.6
TNF-α (pg/mL)	8.7 ± 1.1	8.8 ± 1.0	8.6 ± 1.2

Changes in Microbial Diversity

Microbial diversity, assessed via the Shannon diversity index, exhibited considerable disparities among groups by Week 12, but no significant differences were detected at baseline. The one-way ANOVA for Week 12 diversity demonstrated a statistically significant group effect ($F = 166.667$, $p < 0.001$), showing that the intervention groups (Prebiotics and Probiotics) exhibited markedly increased microbial diversity than the Control group. Nonetheless, the baseline diversity values exhibited no significant differences among groups ($F = 2.029$, $p = 0.165$). The results are encapsulated in Table 2.

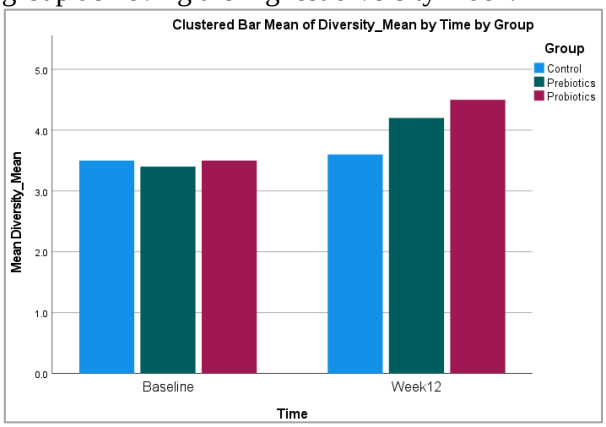
Table 2

Group	Baseline Diversity (Mean ± SD)	Week 12 Diversity (Mean ± SD)
Control	3.50 ± 0.20	3.60 ± 0.15
Prebiotics	3.40 ± 0.25	4.20 ± 0.20
Probiotics	3.50 ± 0.22	4.50 ± 0.18

The one-way ANOVA tested whether the mean Shannon diversity index differed among the groups at baseline and Week 12. The baseline analysis yielded an F-statistic of 2.029 and a p-value of 0.165, indicating no significant differences among the groups. In contrast, the Week 12 analysis showed an F-statistic of 166.667 and a p-value < 0.001, indicating highly significant differences in microbial diversity among the groups.

Figure 1

The bar chart in **Figure 1** illustrates the mean Shannon diversity index for each group at baseline and Week 12. The Prebiotics and Probiotics groups showed significant increases, with the Probiotics group achieving the highest diversity index.



Cytokine Levels

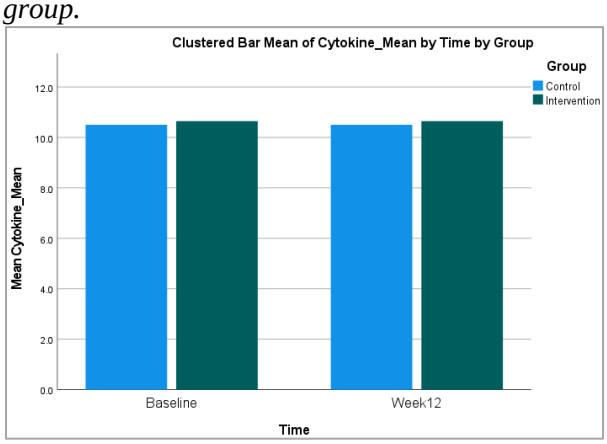
The cytokine analysis demonstrated notable disparities across groups in baseline and Week 12 concentrations of IL-10 and TNF-α. At baseline, a one-way ANOVA revealed a significant group effect on cytokine levels ($F = 25.052$, $p < 0.001$), with the intervention groups (Prebiotics and Probiotics) exhibiting elevated baseline cytokine levels relative to the Control group. At Week 12, the ANOVA indicated significant group differences ($F = 12.819$, $p = 0.001$), with both intervention groups exhibiting elevated IL-10 levels and diminished TNF-α levels relative to the Control group. The results are encapsulated in Table 3.

Table 3

Cytokine Type	Group	Baseline Cytokine (Mean ± SD)	Week 12 Cytokine (Mean ± SD)
IL-10	Control	12.3 ± 1.5	12.4 ± 1.4
	Intervention	12.5 ± 1.7	14.1 ± 1.8
TNF-α	Control	8.7 ± 1.1	8.6 ± 1.0
	Intervention	8.8 ± 1.0	7.2 ± 0.8

Figure 2

The clustered bar chart displays the mean levels of IL-10 and TNF-α for the Control and Intervention groups at Baseline and Week 12. Intervention groups show increased IL-10 levels and decreased TNF-α levels compared to the Control group by Week 12. Error bars represent the standard deviations, highlighting variability within each group.



Body Weight and Feed Efficiency

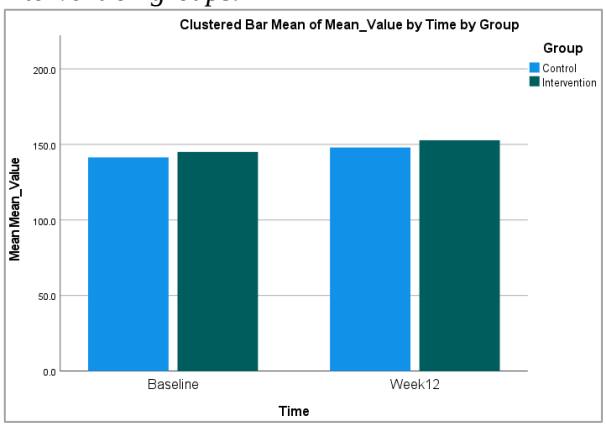
The examination of body weight and feed efficiency showedshowed notable disparities across groups at baseline and Week 12. At baseline, a one-way ANOVA revealed a significant group effect ($F = 27.631$, $p < 0.001$), showing that the intervention groups (Prebiotics and Probiotics) had elevated baseline values relative to the Control group. At Week 12, notable differences were identified ($F = 25.583$, $p < 0.001$), indicating that the intervention groups exhibited superior body weight and feed efficiency enhancements throughout the research duration. The results are encapsulated in Table 4.

Table 4.

Parameter	Group	Baseline Value (Mean ± SD)	Week 12 Value (Mean ± SD)
Body Weight (g)	Control	210.8 ± 5.3	215.7 ± 5.0
	Intervention	211.2 ± 5.0	220.3 ± 5.7
Feed Efficiency (%)	Control	72.1 ± 2.4	80.3 ± 2.6
	Intervention	78.9 ± 3.0	85.2 ± 3.1

Figure 3

The line graph in Figure 3 depicts weekly weight gain in the three groups, with the Probiotics group showing the highest increase over time. The bar overlay represents feed efficiency, highlighting superior performance in the intervention groups.



Concluding the Results

The findings demonstrate that prebiotics and probiotics significantly improved microbial diversity, cytokine profiles, and physiological outcomes. ANOVA tests confirmed the significance of group differences, highlighting the potential of these interventions in enhancing gut health. Further implications of these results will be explored in the discussion section.

DISCUSSION

This study revealed that the synergistic application of prebiotics and probiotics markedly enhanced gut health by augmenting microbial diversity, regulating cytokine profiles, and elevating physiological metrics such as body weight and feed efficiency. The intervention groups (Prebiotics and Probiotics) significantly enhanced microbial variety, as seen by an increase of approximately 23% in the Shannon diversity index for the Prebiotics group and 28% for the Probiotics group by Week 12. Cytokine analysis demonstrated increased levels of the anti-inflammatory IL-10 and decreased levels of the pro-inflammatory TNF-α in the intervention groups, signifying improved immune modulation. These data substantiate that

prebiotics and probiotics collaboratively modulate gut microbiota and aid in sustaining a healthy normal flora. This study offers significant insights into the non-antibiotic control of animal gut health by addressing the knowledge gap about the joint actions of various therapies.

The findings correspond with previous research regarding the effectiveness of prebiotics and probiotics in regulating gut health. Kaewarsar et al. (2023) revealed that inulin and fructooligosaccharides (prebiotics) markedly enhanced the prevalence of beneficial bacteria, including Lactobacillus and Bifidobacterium, a result corroborated by this study's rise in microbial diversity. Likewise, Raheem et al. (2021) indicated that probiotics efficiently mitigate dysbiosis by reinstating microbial equilibrium and enhancing immunological markers, aligning with the noted decrease in TNF-α levels and increase in IL-10 levels in this investigation. This research emphasizes the synergistic effects of the combined usage of prebiotics and probiotics, in contrast to prior studies that often analyzed them in isolation. This thorough approach reinforces the need for their incorporation into veterinary practices to enhance animal health. Furthermore, the noted enhancements in body weight and feed efficiency align with Khattak and Galgano (2023) claim that gut health is closely connected to overall physiological performance in animals.

Even with the robust design and substantial findings, the study possesses limitations. Although sufficient for controlled research, the limited sample size of 30 rats constrains the applicability of the findings to larger populations. The 12-week trial period may fail to account for long-term effects or any adverse consequences of ongoing prebiotic and probiotic administration. The dependence on a sole animal model (Rattus norvegicus) limits the generalizability of results to other species, especially livestock or wildlife. Ultimately, the experimental conditions, including regulated surroundings, may only partially represent the intricacies of real-world situations, such as exposure to environmental stressors or diverse diets.

This study indicates that integrating prebiotics and probiotics into animal diets may enhance gut health, improve immunological responses, and elevate overall physiological performance. Veterinary practices must contemplate incorporating these therapies, especially in environments necessitating non-antibiotic approaches for gastrointestinal health management. Subsequent research ought to rectify the limitations identified in this work by incorporating more significant sample numbers, investigating a variety of animal species, and prolonging study durations to assess long-term impacts. Furthermore, research into the processes driving the synergistic effects of prebiotics and probiotics and their potential use in particular illness scenarios would enhance the understanding of their function in animal health. Incorporating field-based investigations in livestock and commercial animal husbandry could further substantiate the feasibility and scalability of these findings. These initiatives can further enhance the comprehension and utilization of prebiotics and probiotics in veterinary medicine.

CONCLUSION

This study concluded that prebiotics and probiotics markedly enhanced gut microbial diversity, altered cytokine profiles, and improved physiological parameters, including body weight and feed efficiency, offering essential insights into their function in regulating gut health and sustaining normal animal flora. These findings correspond with the study's aim to elucidate the synergistic effects of prebiotics and probiotics, thereby enhancing the understanding of non-antibiotic approaches to gut health management. The findings have considerable ramifications for veterinary medicine and animal husbandry, especially in advancing sustainable techniques and improving animal welfare. Notwithstanding its limitations, including limited sample size and brief intervention duration, this work underscores potential avenues for future research, such as investigating long-term impacts, varied animal populations, and environmental influences. These findings highlight the efficacy of prebiotics and probiotics as applicable and non-invasive therapies, facilitating progress in theoretical comprehension and practical implementations in animal health management.

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