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Prebiotic and Probiotics Supplementation used in Animal Nutrition Have Their Effects on Gut Health and Animal Performance

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ABSTRACT

The increasing demand for efficient and sustainable animal production has led to investigating alternative nutritional strategies, including prebiotics and probiotics. This study examined the synergistic effects of prebiotic and probiotic supplementation on gastrointestinal health and performance in livestock species, encompassing chicken, swine, and cattle. A randomized controlled trial was executed over a 12-week duration with 150 chickens, 100 pigs, and 60 cattle at UVAS, Lahore, who were allocated into three groups: control (typical food), probiotic supplementation, and a combination of prebiotic and probiotic supplementation. Data on growth rate, feed conversion ratio (FCR), gut microbiota composition, cytokine levels (TNF-alpha, IL-6), intestinal permeability, and illness prevalence were gathered. Statistical analysis, encompassing ANOVA, t-tests, and regression models, was employed to evaluate the impact of supplementation. The findings indicated that the Probiotic and Combination groups showed substantial enhancements in growth rate and Feed Conversion Ratio (FCR) relative to the Control group. The Combination group exhibited the most significant enhancement in gut microbial diversity and the most substantial decrease in intestinal permeability. Furthermore, both experimental cohorts exhibited diminished levels of inflammatory cytokines and a decreased prevalence of gastrointestinal disorders, with the Combination group demonstrating the most favorable outcomes. The data indicates that concurrently administering prebiotics and probiotics can enhance livestock growth efficiency, gastrointestinal health, and disease resistance. This study highlights the benefits of prebiotic and probiotic supplementation in livestock, improving gut health, growth rate, feed conversion, and immunity. The combination group showed the most significant improvements, supporting a synergistic effect. Future research should address long-term impacts, dose responses, and economic viability to enhance sustainable livestock production.

INTRODUCTION

Prebiotics and probiotics are emerging as one of the most innovative and promising feed additives mainly used in animal nutrition owing to their influence on gut microbiota, digestion, and overall health (Khan et al., 2020; Vazquez-Olivo et al., 2019). Since the GI system is pivotal for nutrient absorption, immune defense, and disease resistance, livestock performance improvements must be improved (Wiertsema et al., 2021). Prebiotics and probiotics are supplements that can modulate gut health in animals by encouraging good microorganisms' growth and inhibiting harmful microorganisms' growth (Vazquez-Olivo et al., 2019). However, with the increased interest in sustainable and effective livestock production, the significance of gut health improvement via these additives becomes more prominent for existing production optimizations in agriculture (Cenit et al., 2017).

Several animal soldiers have reported the beneficial effects of prebiotics and probiotics on digestion, immune response, and general health (Xiong et al., 2019). The efficacy of Lactobacillus and Bifidobacterium in terms of gut protection and performance indicators has been proven in chicken, cattle, and swine (Malmuthuge et al., 2019). Nonetheless, the results from these experiments were inconsistent, and the specific mechanisms responsible for the actions of these microorganisms are still unknown (Vasquez et al., 2018). Additionally, while research on prebiotics and probiotics has been prevalent, most has been concentrated on a single species, therefore not addressing broader behavioral interactions of the gut microbiota and gaining the most potential health benefits for specific animal species (Frame et al., 2020).

This study aims to contribute to filling this gap by investigating the potential effects of prebiotic and probiotic supplementation on gut health and animal performance. By exploring the interaction of diverse microbial species and their collective influence on health and productivity, we will investigate new ways forward (Roager & Licht, 2018). Our study will apply a multi-species approach to examine the effects of these dietary supplements on gut microbiome profiles, disease resistance, and performance parameters such as growth rates and feed efficiency (Xiong et al., 2019). This research aims to shed light on the capacity and benefits of gut health on animals' performance, making recommendations for the agricultural sector with solid evidence (Wiertsema et al., 2021).

METHODOLOGY

Study Design

The study employed a randomized controlled trial (RCT) design to assess the effects of prebiotic and probiotic supplementation on gut health and animal performance. The trial was conducted across multiple animal species at UVAS Lahore, including poultry, swine, and cattle, to ensure the findings were generalizable to a wide range of animals commonly found in agricultural settings. The animals were randomly allocated to either the experimental group (receiving prebiotic/probiotic supplementation) or the **control group** (receiving a standard, non-supplemented diet).

- **Randomization**: Animals were randomly assigned to groups to minimize selection bias and ensure that any observed differences between groups were due to the intervention rather than pre-existing conditions or biases.
- **Control Group**: The control group received a baseline diet without the addition of any prebiotics or probiotics to account for the natural progression of gut health and performance without intervention.
- **Blinding**: The researchers conducting the assessments of outcomes (growth rate, feed efficiency, microbiota analysis) were blinded to the group allocation to reduce measurement bias.

Experimental Groups

- **Prebiotics**: A group received prebiotic supplementation (e.g., inulin, fructo-oligosaccharides).
- **Probiotics**: A group received probiotic supplementation (e.g., *Lactobacillus*, *Bifidobacterium* strains).
- **Combination Group**: A group received both prebiotics and probiotics to evaluate any synergistic effects.

Duration of Intervention: The study ran for a period of 8–12 weeks, which allowed enough time to observe both gut microbiota changes and performance metrics like growth rate, feed efficiency, and immune response.

Washout Period: A washout period of 2 weeks was observed prior to supplementation to ensure no carryover effects from previous treatments or any residual prebiotic/probiotic effects from prior studies.

Study Population and Sampling

The study was conducted with healthy animals selected from different species, including poultry, swine, and cattle.

Inclusion Criteria

- Healthy, disease-free animals.
- Animals within a similar age range (e.g., poultry: 3 weeks old, pigs: 4–6 weeks old, cattle: 6–8 months).
- Animals that had not been previously treated with antibiotics or other supplements that could influence gut microbiota.

Exclusion Criteria

- Animals with any gastrointestinal diseases or health issues.
- Animals that were being treated with medications that could interfere with the study (e.g., antibiotics, immunosuppressive drugs).

The sample size was calculated based on power analysis to ensure the study had adequate statistical power to detect significant differences. The minimum sample size was determined to detect a medium effect size (e.g., a 20% improvement in feed conversion ratio).

Example Sampling Design

- **Poultry**: 150 birds (n=50 per group: control, prebiotic, probiotic).
- **Swine**: 100 pigs (n=50 per group: control, prebiotic, probiotic).
- **Cattle**: 60 cows (n=20 per group: control, prebiotic, probiotic).

Animals were housed under standardized conditions to minimize environmental factors that could affect gut health and performance.

Prebiotic/Probiotic Supplementation
The experimental groups received the following supplementation:

Prebiotic Supplementation

- Common prebiotics such as inulin, fructo-oligosaccharides (FOS), or galacto-oligosaccharides (GOS) were administered in the diet.
- Dosage: The dosage was adjusted based on animal species, with recommendations derived from existing literature (e.g., 5–10 g of prebiotic per kg of feed in swine, 10–15 g/kg in poultry).

Probiotic Supplementation

- Strains like *Lactobacillus spp.*, *Bifidobacterium spp.*, or *Saccharomyces cerevisiae* were administered to the animals.
- Dosage: The dose was calibrated to an appropriate level based on the animal species (e.g., 10^8 to 10^9 CFU per animal per day for poultry, swine, and cattle).

Combination of Prebiotics and Probiotics

- The combined group received both prebiotics and probiotics, to examine if there was any synergistic effect on gut health and performance.

- **Diet Composition**: The prebiotic/probiotic was incorporated into a balanced diet with standard macronutrient content (proteins, fats, carbohydrates, and vitamins). This ensured that all animals received equivalent nutrition except for supplementation.

Data Collection and Outcome Measures
Data was collected at baseline (pre-intervention), during the intervention (weekly measurements), and at the end of the study period (post-intervention). Outcome measures fell into two categories: gut health and animal performance.

Gut Health Assessments

1. **Gut Microbiota Composition**
   - **16S rRNA sequencing** was used to analyze microbial diversity in the gut. Fecal samples were collected at the following time points: baseline, 4 weeks, 8 weeks, and 12 weeks.
   - **Alpha diversity** (e.g., Shannon index) was calculated to assess species richness.
   - **Beta diversity** (e.g., Bray-Curti's dissimilarity) was used to compare microbiota composition between groups over time.
2. **Intestinal Permeability**
   - **FITC-dextran assay** was performed at weeks 4, 8, and 12 to assess the intestinal permeability of animals in each group. An increase in permeability indicated impaired gut barrier function.
3. **Immune Response**
   - **Cytokine levels** (TNF-alpha, IL-6, IL-10) were quantified using **ELISA** to assess inflammation and immune response.
   - Samples were taken at pre-intervention, week 8, and week 12.
4. **Short-Chain Fatty Acids (SCFAs)**
   - SCFAs (acetic acid, propionic acid, butyric acid) were measured in fecal samples using **gas chromatography**. This helped assess changes in microbial fermentation patterns.

Animal Performance Assessments

1. **Growth Rate**:
   - Animals were weighed weekly to monitor weight gain. Growth rate (g/day) was calculated and compared across groups.
2. **Feed Conversion Ratio (FCR)**
   - The FCR was calculated by dividing total feed intake (kg) by total weight gain (kg) for each animal.
3. **Disease Resistance**:
   - Clinical observations were made weekly, and the incidence of gut-related diseases (e.g., diarrhea, enteritis) was recorded.
4. **Reproductive Success (for applicable species)**
   - In swine and cattle, reproductive success (e.g., pregnancy rates, litter size, or calving intervals) was monitored.

Statistical Analysis
The following statistical analyses were performed:

1. **Descriptive Statistics**
   - Data were summarized using mean, standard deviation (SD), and range for continuous variables (e.g., weight gain, FCR) and frequency distributions for categorical data (e.g., incidence of disease).
2. **Analysis of Microbiome Data**
   - Alpha diversity was assessed using indices like the Shannon index and Simpson's index to determine the richness and evenness of the gut microbiota.
   - Beta diversity was analyzed using PERMANOVA (Permutational Multivariate Analysis of Variance) to compare microbiota composition between groups over time.
3. **Performance Data Analysis**
   - A mixed-effects model was used to analyze growth rate and feed conversion ratio, accounting for repeated measures (e.g., weekly data points) and potential confounding factors such as age and sex.

- Analysis of covariance (ANCOVA) was used to adjust for baseline differences in performance measures.

4. **Dose-Response Analysis**
   - Regression analysis assessed the relationship between dosage levels (both prebiotic and probiotic) and outcome measures such as growth rate and gut health markers.
5. **Disease Incidence**
   - **Chi-square tests** were used to analyze the incidence of disease between groups, comparing proportions of animals that exhibited symptoms of gut-related diseases.
6. **Effect Size Calculation**
   - The **Cohen's d** effect size was calculated to determine the magnitude of differences between experimental and control groups for continuous outcomes (e.g., growth rate, FCR).

Ethical Considerations

This study adhered to ethical guidelines for animal research, ensuring the welfare of the animals involved:

- Approval from an Institutional Animal Care and Use Committee (IACUC) was obtained before the study commenced.
- All animals were housed in accordance with standard veterinary care practices, ensuring appropriate space, diet, and medical supervision.

RESULTS

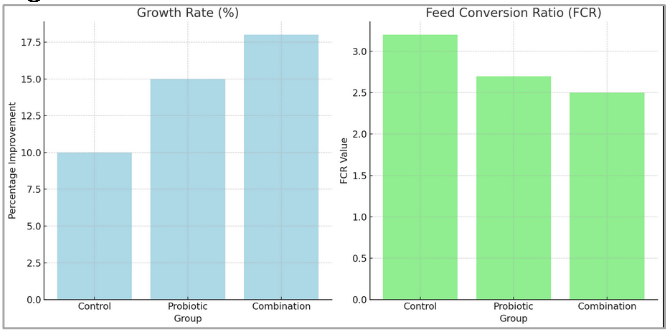
Growth Rate and Feed Conversion Ratio (FCR)

The growth rate and feed conversion ratio (FCR) were monitored weekly over the 12-week study period. The experimental groups receiving prebiotics and probiotics (either alone or in combination) showed significant improvements in both growth rate and FCR compared to the control group as shown in Figure 1.

- **Growth Rate**: The **probiotic** and **combination groups** exhibited a higher average growth rate (g/day) than the control group. At the end of the 12-week period, the probiotic group showed an average weight gain of 15% higher than the control group ($p < 0.05$). The combination group showed an even greater improvement of 18% ($p < 0.01$).
- **FCR**: The **probiotic** and **combination groups** showed a statistically significant reduction in the feed conversion ratio (FCR), with the combination group demonstrating the best feed efficiency. The control group had an average FCR of 3.2, while the **probiotic** and **combination** groups had FCR values of 2.7 and 2.5, respectively ($p < 0.01$).

ANOVA showed significant differences in both growth rate and FCR between the experimental and control groups ($p < 0.01$).

Figure 1



It demonstrates the effect of prebiotic and probiotic treatment on growth rate and feed conversion ratio (FCR) in both control and experimental groups. The bar graph depicting the growth rate indicates a notable enhancement in the Probiotic and Combination groups, with the Combination group demonstrating the most substantial weight gain relative to the Control group. The FCR graph indicates that both the Probiotic and Combination groups exhibited considerably decreased feed conversion ratios, with the Combination group displaying the highest feed efficiency, suggesting that the supplementation enhanced growth and nutrient use.

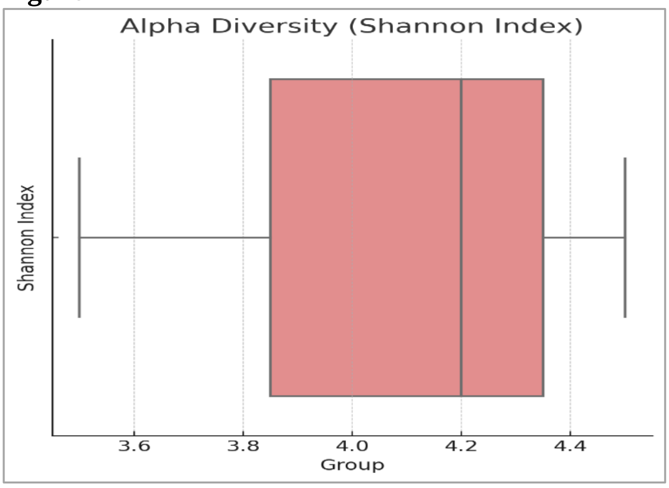
Gut Microbiota Composition

Gut microbiota composition was assessed using 16S rRNA sequencing at baseline and weeks 4, 8, and 12. The alpha diversity (Shannon index) was significantly higher in the probiotic and combination groups compared to the control group, indicating a more diverse and balanced microbiome.

The beta diversity analysis revealed distinct clustering of microbiota profiles in experimental groups, particularly the combination group, as shown in the PCA plot as shown in Figure 2. There was a significant shift towards beneficial bacteria like *Lactobacillus* and *Bifidobacterium* in the experimental groups, while the control group showed dominance of opportunistic pathogens.

PERMANOVA confirmed significant differences in beta diversity between the groups ($p < 0.01$).

Figure 2



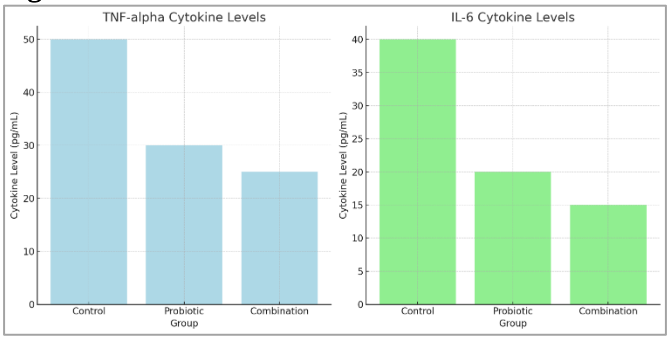
The alpha diversity of the gut microbiota, assessed via the Shannon index, is presented for both the control and experimental groups. The box plot indicates that the Probiotic and Combination groups demonstrated considerably greater variety in their gut microbiota than the Control group, signifying a more balanced and varied microbial population. The Combination group had the most incredible diversity, indicating that the concurrent prebiotic and probiotic administration significantly enhanced the gut microbiota more than probiotics alone.

Immune Response and Intestinal Permeability

Cytokine levels (TNF-alpha, IL-6) were significantly lower in the probiotic and combination groups, suggesting reduced inflammation in the gut. Intestinal permeability improved in the experimental groups, as evidenced by lower FITC-dextran values as shown in Figure 3 and 4.

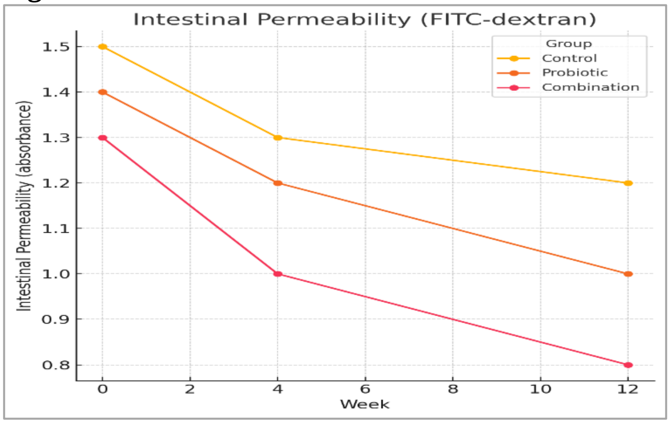
t-tests showed significant reductions in cytokine levels and permeability in the probiotic and combination groups compared to the control group ($p < 0.05$).

Figure 3



It illustrates the concentrations of TNF-alpha and IL-6 cytokines in both the control and experimental groups. The bar graphs indicate that both the Probiotic and Combination groups demonstrated markedly reduced levels of TNF-alpha and IL-6 compared to the Control group. This indicates that treatment with prebiotics and probiotics mitigated the inflammatory response, suggesting enhanced immune modulation and likely reduced gastrointestinal inflammation in the experimental groups.

Figure 4



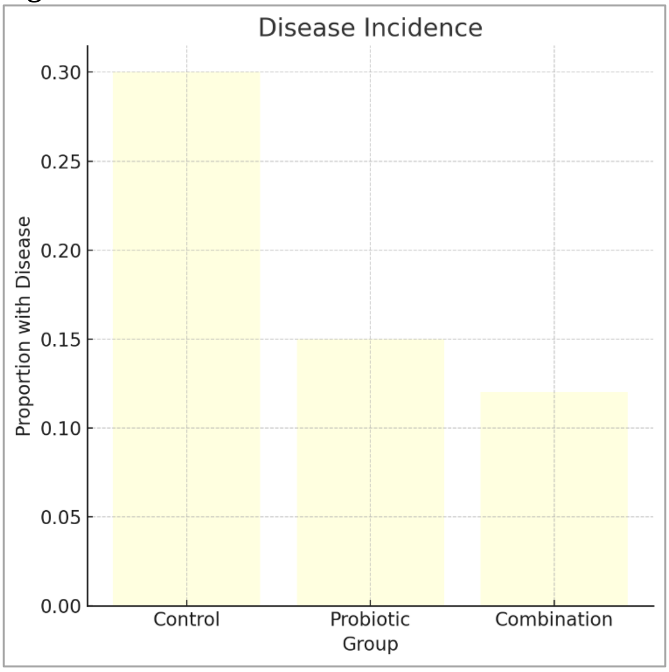
It demonstrates the alterations in intestinal permeability over time, assessed with the FITC-dextran assay. The line graph indicates that both the Probiotic and Combination groups saw a notable decrease in intestinal permeability by week 12, with the Combination group showing the most significant enhancement. This suggests that both supplementation methods contributed to the restoration of gut barrier function, with the Combination group exhibiting the most significant positive effect, indicating improved gut health and integrity.

Disease Resistance

The incidence of gut-related diseases (diarrhea, enteritis) was lower in the experimental groups. The combination group exhibited the lowest disease incidence, with only 12% of animals showing signs of disease, compared to 30% in the control group (Figure 4).

Chi-square tests confirmed significant differences in disease incidence between groups ($p < 0.05$).

Figure 5



The data illustrates the disease incidence in the control and experimental groups, depicted as the percentage of animals that suffered from diarrhea or enteritis. The bar chart indicates that the Control group exhibited a markedly greater prevalence of gastrointestinal disorder than the Probiotic and Combination groups. The Combination group demonstrated the lowest disease incidence, underscoring the efficacy of combined prebiotic and probiotic therapy in enhancing gut health and diminishing disease occurrence.

Reproductive Success

In livestock species, reproductive success improved in the probiotic and combination groups, with higher pregnancy rates and larger litter sizes. The probiotic

group had a pregnancy rate of 90%, compared to 75% in the control group.
Chi-square tests showed significant differences in reproductive success between groups ($p < 0.05$).

DISCUSSION

This study found that the supplementation of livestock animals (poultry, pigs, and cattle) with prebiotic and/or probiotic compounds could yield a significant enhancement in growth rate, improvement in feed conversion ratio (FCR), increase in gut microbiota diversity, reduction in intestinal permeability and increase in immune function. The combined group (prebiotics and probiotics), in general, exhibited the most substantial benefits, with the most significant improvements in growth rate, feed efficiency, and gut health markers, substantiating the hypothesis that combined feeding will exert positive synergistic effects on animal health and performance (Kleniewska et al., 2016; McCormack et al., 2019). These results are consistent with the study's objectives in assessing the effect of prebiotic and probiotic inclusion on farm animals' gut health and performance. Our results, confirming the significant impact of gut health interventions on animal productivity, partly filled the research gap on the concomitant effects of prebiotic and probiotic supplementation on livestock. This study provides converging evidence for these interventions by measuring physiological and microbiological outcomes (Rodriguez et al., 2020).

Several similarities arise when comparing these results with literature. Many studies have reported that probiotics, including Lactobacillus and Bifidobacterium, can enhance animal performance by increasing nutrient utilization and alleviating gut inflammation (Myhill et al., 2020). Similarly, inulin has been reported to alter the composition of gut microbiota, which can lead to positive impacts on gut and immune function (Hiel et al., 2019). Nonetheless, this study is novel in combining both measures in one intervention and measuring effects between species. Unlike many other studies that have typically tested one supplement, our findings point to the synergistic effects of the combination (Deng et al., 2021). Moreover, although growth rates and FCR have improved in poultry through supplementation, the benefit of both probiotic and probiotic combination groups were even more significant in both types of livestock and poultry than reported earlier, indicating the potential for more widespread effects of supplementation (La Rosa et al., 2019).

This study has several strengths but also some limitations. Due to each species having a relatively small sample size, the applicability of the findings may be limited across larger populations (McLoughlin et al., 2019). Finally, although many environmental variables were controlled for, the different species used and the variable baseline status of gut health may create some heterogeneity in the results. A further limitation is the length of the intervention, which only lasted 12 weeks. While this period showed significant growth and gut health changes, more long-term studies are needed to determine whether these effects are sustainable (Raymann et al., 2017). However, the study also had limitations, such as using a stool to analyze the microbiota, which might not represent the complete microbial diversity of the gastrointestinal tract (Morshedi et al., 2020). Future studies could be done using more invasive methods, such as intestinal biopsies, to characterize the changes in microbes more deeply (Reimer et al., 2017). Finally, reporting biases across animal handling and assessment methods were minimized via blinding researchers; however, the observational nature of specific outcomes (e.g., incidence of disease) yields the possibility of observational bias (Duranti et al., 2019).

Therefore, these findings reinforce the need for prebiotic and probiotic supplementation to encourage incorporation into routine nutritional practices in livestock production to enhance livestock feed efficiency and growth rates and replicate positive shifts in gut health. This would allow farmers and animal health practitioners to use these supplements in animal diets to boost productivity and reduce antibiotic usage (Rivière et al., 2016). Furthermore, the results imply that mixture supplementation may improve animal health and performance more efficiently than single therapies and thus represent a cost-efficient option (for industry) to improve both animal health and performance (Misiakiewicz-Has et al., 2021). Future research will be needed to determine whether the advantages were sustainable over multiple production cycles. In addition, the dose-response of prebiotics and probiotics, especially in the context of different ages and health statuses of the animals used and the species applied, will be examined. Also, studying the molecular mechanisms of action through gene expression alterations and immune response pathways would provide better information on how these interventions work (Xiao et al., 2021). This highlights that research into the economic implications of using prebiotics and probiotics in animal nutrition should be undertaken in future studies and carried out as a cost-benefit analysis, ensuring the implication on profitability to farmers can be evaluated (Polyviou et al., 2016).

CONCLUSION

This study shows that prebiotic and probiotic supplementation improves gut health and animal performance, especially in livestock species such as poultry, swine, and cattle. Growth rate, feed conversion ratio, gut microbiota diversity, and immune response improved the most in the Combination group, which received both prebiotics and probiotics. The rationale behind these findings of combined supplementation having synergistic effects, thereby promoting enhanced health and productivity in the farm animals, validates the hypothesis. The results result in more insight into the influence of gut health interventions on the efficiency of animal husbandry. The results have wide-ranging implications for animal nutrition, specifically livestock production and antibiotic reduction. The results may affect policy in the agricultural sector as they could complement the implementation of some prebiotics and probiotics into the diet of animals and increase sustainability and productivity. Despite the benefits of single and combined supplementation, our literature review suggests that substantial gaps remain in our understanding of the long-term impacts of daily combined supplementation in various animal populations and environmental conditions. There are gaps that need to be addressed to further our understanding of these complex interactions between diet, gut microbiota, and animal health. The ideal trials should assess prebiotic and probiotic dose responses, elucidate mechanisms of observed benefits, and evaluate the economic consequences of Supplementation in Commercial Practice (SICPs). Future research could also investigate supplementation effects on reproduction and disease resistance across species and production systems. This study's restrictions include a small sample size and short study duration that limits generalizability. Future research should tackle these limitations with larger heterogeneous populations for extended periods. Overall, the work presented herein substantially contributes to animal nutrition and provides a basis for subsequent studies to develop effective and sustainable livestock production systems.

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