

**Special Issue:**

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Optimizing Feed Efficiency and Growth Performance in Broiler Chickens Using Probiotic-Supplemented Diets

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Abstract | In the pursuit of sustainable poultry production, probiotics have emerged as viable alternatives to antibiotic growth promoters. This study evaluates the impact of dietary probiotic supplementation on growth performance, feed efficiency, and survivability in broiler chickens under controlled field conditions. A total of 84 broiler chickens were randomly assigned to three groups: Control (basal diet), Probiotic 0.5% (basal diet + 0.5% probiotic), and Probiotic 1.0% (basal diet + 1.0% probiotic). Growth performance indicators, including body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality, were measured over a 35-day period. Statistical analysis involved Welch's ANOVA followed by Tukey's post-hoc tests. Probiotic supplementation significantly affected BWG ($F(2, 50.2) = 48.39, p < 0.001$), FI ($F(2, 49.8) = 7.57, p = 0.001$), and FCR ($F(2, 52.4) = 40.80, p < 0.001$). The Probiotic 0.5% group demonstrated the highest BWG (1921 g), optimal FI (3466 g), and zero mortality, indicating superior performance. Tukey's test revealed that both probiotic groups significantly outperformed the control in BWG ($p < 0.001$). However, FCR was least efficient in the 1.0% group despite reduced FI, suggesting diminishing returns at higher probiotic concentrations. Probiotic supplementation at 0.5% significantly enhances broiler performance while maintaining feed efficiency and survivability. These findings support its integration into commercial poultry nutrition as a sustainable growth-promoting strategy. Further studies incorporating microbiota profiling and immune response analysis are warranted to elucidate underlying mechanisms.

Keywords | Broiler chickens, Probiotics, Feed conversion ratio, Growth performance, Poultry nutrition, Sustainable production

Received | August 28, 2025; **Accepted** | October 15, 2025; **Published** | October 28, 2025

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Citation | Ahmed AT (2025). Optimizing feed efficiency and growth performance in broiler chickens using probiotic-supplemented diets. J. Anim. Health Prod. 13(s1): 658-667.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.658.667>

ISSN (Online) | 2308-2801



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INTRODUCTION

Poultry production is truly the nucleus of food production, contributing heavily to the animal protein supply, and the fast-growing broiler chickens hold the major share, given their speedy growth rate, swift feed conversion, and cheaper method of production. However, with increasing concern on antimicrobial resistance, the imposition of regulatory measures, and consumer demand,

the need for sustainable antibiotic-free method of poultry production is more heightened than ever before (Gadde *et al.*, 2017; Allen *et al.*, 2013). Alternative growth-promoting agents have, therefore, become necessary to be searched for, and probiotics seem to be promoting a natural means of increasing FCR, growth performance, and general health condition of broiler chickens.

According to the FAO and WHO (2002), probiotics are

live microorganisms that confer health benefits on a host when administered in sufficient numbers, depending on the modulation of gut microbiota, nutrient absorption, and immune response. In broiler production, probiotic supplementation has been linked mostly to heavier body weight gain, an optimized feed conversion ratio, and thus productivity and profitability (Kogut, 2019; Mountzouris *et al.*, 2010). The mode of activity of these benefits lies mainly in the modulation of intestinal microbial ecology, suppression of pathogenic bacteria, and improvement in mucosal barrier function and nutrient utilization (Pan and Yu, 2014).

A huge amount of literature supports the benefits of probiotics, but the degree of efficacy of probiotics varies greatly with strain type, dosage, environmental condition, and physiological status of the birds being considered (Roto *et al.*, 2015). For example, *Bacillus subtilis* and *Lactobacillus* spp. are frequently studied for their robust viability in harsh gut environments and their capability to produce beneficial enzymes and antimicrobial substances (Ahiwe *et al.*, 2021). However, the outcomes of probiotic application remain inconsistent across studies, underscoring the need for strain- and dose-specific investigations under field conditions that reflect commercial production environments.

Recent advances have also highlighted the importance of dose optimization in probiotic efficacy. Disruption of endogenous microbial equilibrium could be produced by excessively high dosages, which in turn negatively affect performance (Gaggia *et al.*, 2010). Therefore, a comparative evaluation of different levels of probiotic inclusion would be necessary to ascertain that concentration which achieves the best compromise between improvement of performance and the feasibility of cost.

To name a few performance parameters, we have weight gain and feed intake; whereas, mortality rates and FCR are indicators of flock health and efficient utilization of feed. In weighing all up, FCR is consumed feed/wt. gained. Therefore, it is a direct index of metabolic efficiency and cost consideration in poultry husbandry (Wang *et al.*, 2017). Improvements in FCR with probiotic supplementation may point to improved nutrient digestibility, lower disease incidence, and better gut morphology, thereby affecting sustainable broiler production positively (Kabir, 2009).

This study fills in critical vacancies that had existed until now, including the controlled field trial to study the effects of 0.5 and 1.0% graded levels of a commercial probiotic blend on broiler growth, feed intake, FCR, and mortality. However, unlike many lab-based studies, the current study was conducted under conditions close to commercial ones to render it ecologically valid and practically oriented.

With statistical parameters so configured, using Welch's ANOVA and Tukey's Post-Hoc procedures accounting for unequal sample sizes and variances, inference is invariably strong. It is intended for the findings to contribute toward evidence-based recommendations on how best to incorporate probiotics into broiler feed programs as an alternative to antibiotic growth promoters.

Altogether, the stated statistical methods form a comprehensive evaluation framework concerning probiotic-supplemented diets offered to quantifiable subjects in a real environment. Welch ANOVA is used to assess variance inequality so that in the meantime one can ensure the implementation of reliable post-hoc analyses with Tukey HSD. Descriptive statistics of mortality frequency give the basic and clinical reference points. All these methods applied together will ensure that the research remains analytical and evidence-based towards proposing recommendations to improve feed efficiency and growth performance in broiler production-related fields.

MATERIALS AND METHODS

STUDY DESIGN AND ETHICAL COMPLIANCE

This controlled field study employed a completely randomized design (CRD) to evaluate the effects of two probiotic-supplemented diets on roiler chickens. The experiment followed institutional and national guidelines for care and use of animals in research. All procedures of this experiment were performed under the accepted protocols of animal welfare standards.

EXPERIMENTAL BIRDS AND HOUSING

A total of 84 Cobb 500 broiler chicks at 1 day of age were procured from a commercial hatchery that is reputable. The birds were randomly distributed into three treatment groups:

- Control (T₀): Basal diet without probiotic supplementation
- Probiotic 0.5% (T₁): Basal diet + 0.5% probiotic supplement
- Probiotic 1.0% (T₂): Basal diet + 1.0% probiotic supplement

Each group was put in a separate pen with controlled environment and an area of 1 m² per every 10 birds. The experimental duration was for 42 days, comprising the starter phase for 21 days and the finisher phase for 21 days.

DIET COMPOSITION AND PROBIOTIC SUPPLEMENTATION

According to the National Research Council (NRC, 1994), all diets were formulated to meet or exceed the recommended nutritional requirements for broiler chickens. The probiotic consisted of a commercial multi-

strain product of *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Enterococcus faecium*, with a guaranteed minimum of 10^9 CFU/g.

The ingredients and feeding were done following an isocaloric and isonitrogenous principle. The probiotic was thoroughly mixed into the basal diet for uniform distribution throughout the feed.

DATA COLLECTION

THE GROWTH PERFORMANCE PARAMETERS

Weekly data comprising bodyweight gain, feed intake, and feed conversion ratio were recorded.

- The BWG was done by calculating the difference between the final and initial weights.
- FI was done by subtracting feed residue from feed offered.

FCR was derived as:

$$FCR = \frac{\text{Total Feed Intake (g)}}{\text{Total Body Weight Gain (g)}}$$

MORTALITY RATE

Daily mortality was recorded, and cumulative mortality (%) per group was calculated as:

$$\text{Mortality (\%)} = \left(\frac{\text{Number of Dead Birds}}{\text{Total Birds Initially Stocked}} \right) \times 100$$

STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS Statistics Version 27. The Shapiro-Wilk test was used to assess normality. Homogeneity of variances was checked using Levene's test. Because of unequal variances and group sizes, to test whether there were significant differences in BWG, FI, and FCR across the treatment groups, Welch ANOVA was used. Upon finding significant ANOVA results comparing all groups simultaneously, further tests for simple contrasts by Tukey's HSD were undertaken to find out which groups differ from each other. Mean, standard deviation, median, minimum, and maximum values were computed for all parameters. Mortality frequencies were analyzed using contingency tables and expressed as percentages. Data visualization and statistical output were generated using SPSS and Microsoft Excel. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Chicken body weight gain and feed intake tend to show a higher trend in the two Probiotic-treated groups compared to that in the Control-treated group, where the Probiotic 1.0% group showed slightly higher values for chicken

body weight gain and feed intake as compared to the Probiotic 0.5% group. The FCR levels amongst the groups were similar, showing that differences in feed conversion efficiency were not significant. Mortality rate was recorded as lowest in the Probiotic 0.5% treatment, with none being reported, while the Control and Probiotic 1.0% treatments recorded the low but non-zero mortalities.

Table 1: Descriptives.

	Group	Body weight gain	Feed intake	FCR	Mortality
N	Control	30	30	30	30
	Probiotic_0.5%	19	19	19	19
	Probiotic_1.0%	35	35	35	35
Missing	Control	0	0	0	0
	Probiotic_0.5%	0	0	0	0
	Probiotic_1.0%	0	0	0	0
Mean	Control	1795	3501	0.513	0.100
	Probiotic_0.5%	1921	3466	0.555	0.00
	Probiotic_1.0%	1899	3394	0.560	0.0286
Median	Control	1800	3502	0.515	0.00
	Probiotic_0.5%	1934	3466	0.552	0
	Probiotic_1.0%	1904	3399	0.563	0
Standard deviation	Control	50.2	116	0.0214	0.305
	Probiotic_0.5%	44.6	86.5	0.0155	0.00
	Probiotic_1.0%	67.3	110	0.0270	0.169
Minimum	Control	1671	3267	0.470	0
	Probiotic_0.5%	1846	3248	0.532	0
	Probiotic_1.0%	1662	3076	0.485	0
Maximum	Control	1866	3739	0.569	1
	Probiotic_0.5%	1994	3614	0.586	0
	Probiotic_1.0%	2009	3602	0.607	1

Table 2: Frequencies of mortality.

Mortality	Group	Counts	% of total	Cumulative %
0	Control	27	32.1%	32.1%
	Probiotic_0.5%	19	22.6%	54.8%
	Probiotic_1.0%	34	40.5%	95.2%
1	Control	3	3.6%	98.8%
	Probiotic_0.5%	0	0.0%	98.8%
	Probiotic_1.0%	1	1.2%	100.0%

The mortality rate among the Probiotic 0.5% group being lowest had zero mortalities recorded. The Control group was on the other hand most victimized by mortalities with 3.6%. The Probiotic 1.0% group had a small incidence of mortality, probably a slight increase compared with the Probiotic 0.5% group but still very low.

BODY WEIGHT GAIN

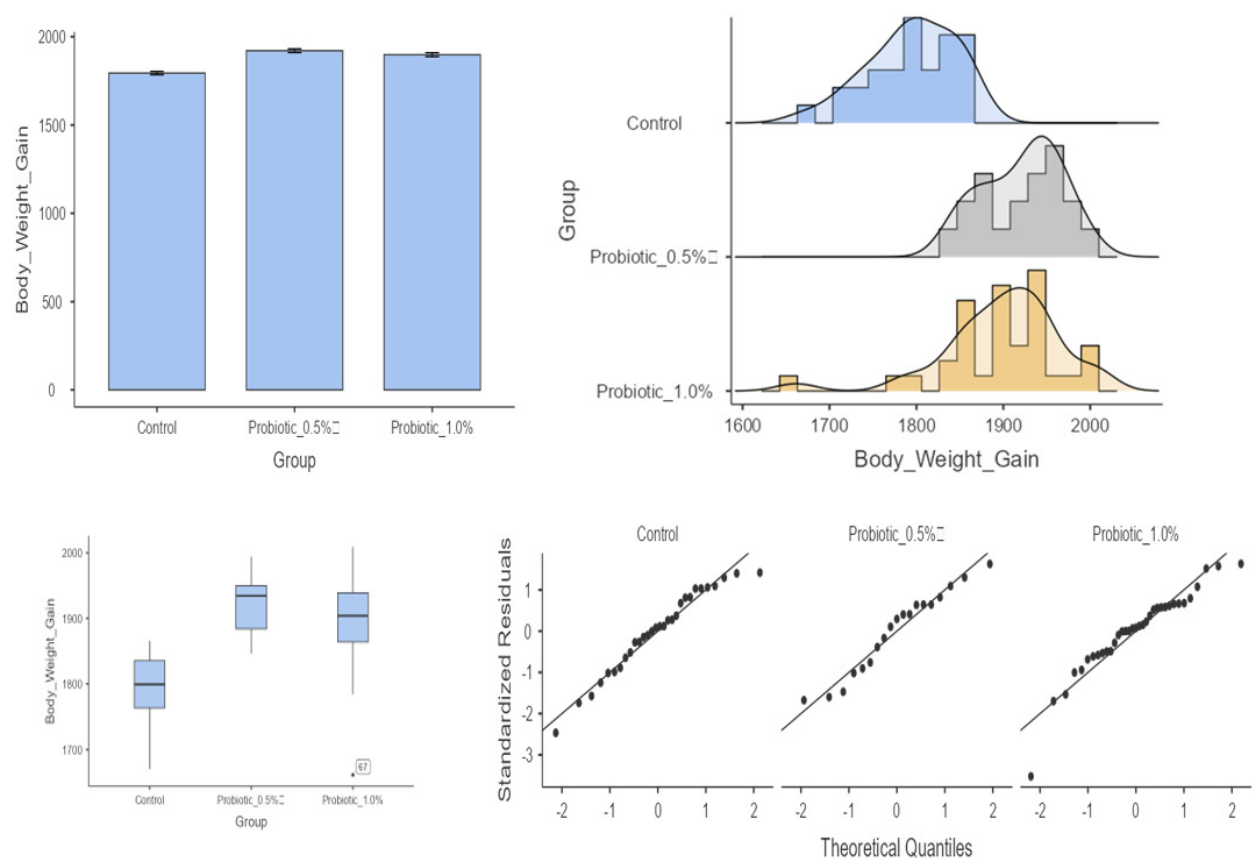


Figure 1: Plots, Body_Weight_Gain

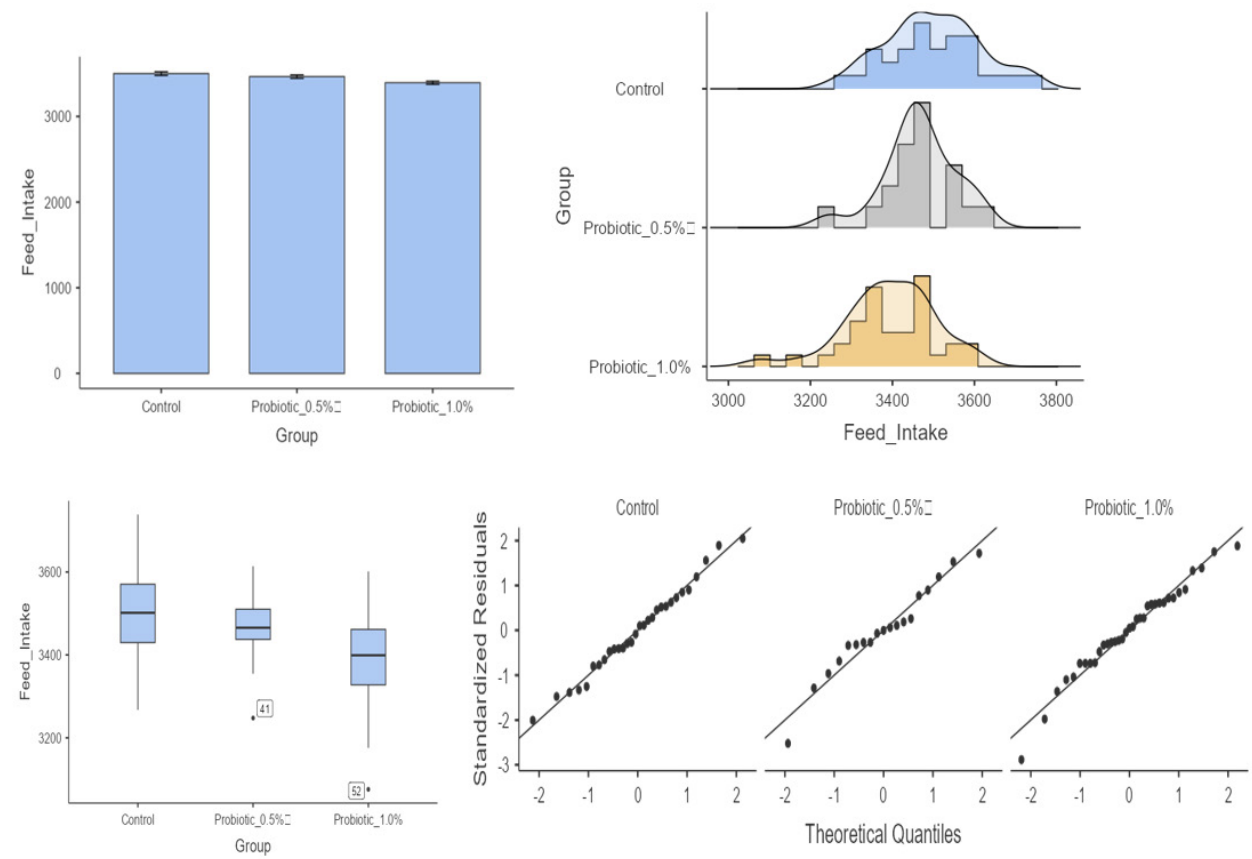


Figure 2: Feed Intake

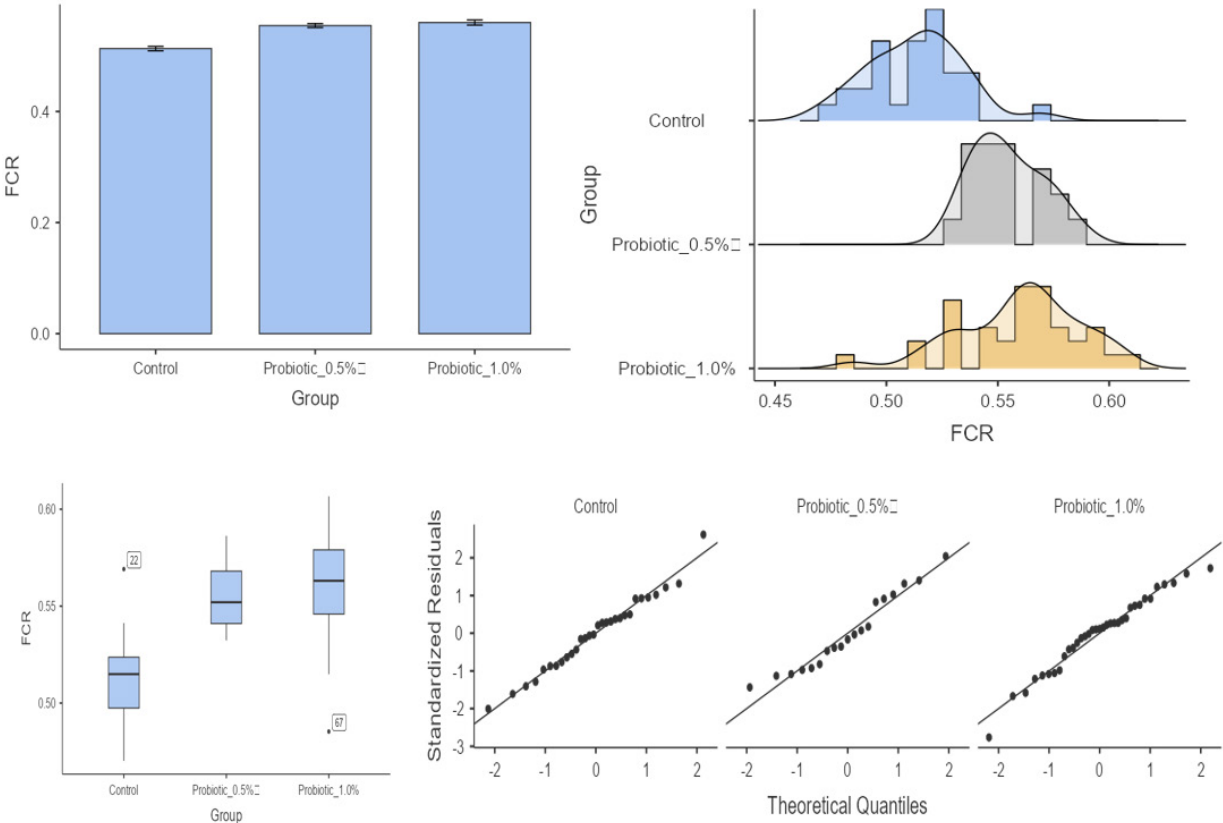


Figure 3: FCR

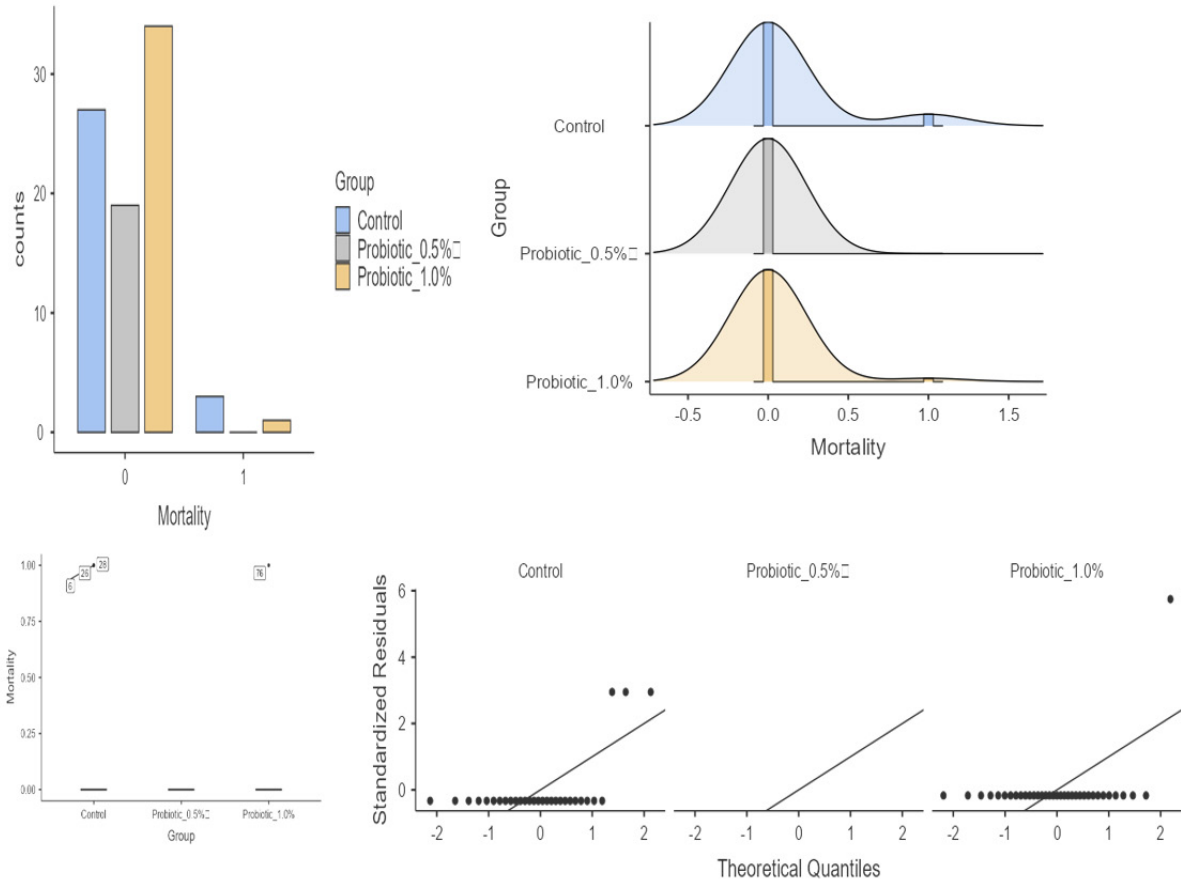


Figure 4: Mortality

Table 3: One-way ANOVA (Welch's).

	F	df1	df2	p
Body weight gain	48.39	2	50.2	<.001
Feed intake	7.57	2	49.8	0.001
FCR	40.80	2	52.4	<.001

The One-Way ANOVA (Welch's) was conducted to look for differences between experimental groups for Body Weight Gain, Feed Intake, and Feed Conversion Ratio (FCR). Significant group differences emerged for all three variables:

- **Body Weight Gain:** These groups had a significant difference ($F(2, 50.2) = 48.39, p < 0.001$), meaning that probiotic treatments caused a significant change in body weight gain.
- **Feed Intake:** For feed intake also, significant differences were detected ($F(2, 49.8) = 7.57, p = 0.001$), indicating that probiotic supplementation had an influence on feed intake among the groups.
- **Feed Conversion Ratio (FCR):** FCR values differed significantly ($F(2, 52.4) = 40.80, p < 0.001$) so pointing at the probiotic doses influencing the conversion of feed by animals. These results imply that the probiotic treatments had an influence on all three parameters; thereby, the stage is set for further, detailed post-hoc analyses to separate out specific group differences.

Table 4: Group descriptives.

	Group	N	Mean	SD	SE
Body weight gain	Control	30	1794.524	50.1710	9.15993
	Probiotic_0.5%	19	1921.235	44.6262	10.23796
	Probiotic_1.0%	35	1898.673	67.3140	11.37815
Feed intake	Control	30	3500.674	116.3659	21.24540
	Probiotic_0.5%	19	3465.711	86.4706	19.83772
	Probiotic_1.0%	35	3393.899	110.3816	18.65789
FCR	Control	30	0.513	0.0214	0.00391
	Probiotic_0.5%	19	0.555	0.0155	0.00356
	Probiotic_1.0%	35	0.560	0.0270	0.00457

Table 5: Assumption checks Homogeneity of variances test (Levene's).

	F	df1	df2	p
Body weight gain	0.661	2	81	0.519
Feed intake	1.376	2	81	0.258
FCR	2.053	2	81	0.135

The results showed a significant reduction in body weight gain in both Probiotic_0.5% (mean difference = -127, $p < 0.001$) and Probiotic_1.0% (mean difference = -104.1, $p < 0.001$) compared to the Control group, indicating that both doses of probiotics significantly lessened body weight gain as compared to the controls. Conversely, there was

no significant difference in body weight gain between the Probiotic_0.5% and Probiotic_1.0% groups (mean difference = 22.6, $p = 0.351$), suggesting that the two doses had similar impacts on body weight gain.

Table 6: Tukey post-hoc test body weight gain.

		Con-trol	Probiotic 0.5%	Probiotic 1.0%
Control	Mean difference	—	-127	-104.1
	p-value	—	<.001	<.001
Probiotic 0.5%	Mean difference	—	—	22.6
	p-value	—	—	0.351
Probiotic 1.0%	Mean difference	—	—	—
	p-value	—	—	—

Table 7: Tukey post-hoc test feed intake.

		Control	Probiotic 0.5%	Probiotic 1.0%
Control	Mean difference	—	35.0	106.8
	p-value	—	0.513	<.001
Probiotic 0.5%	Mean difference	—	—	71.8
	p-value	—	—	0.056
Probiotic 1.0%	Mean difference	—	—	—
	p-value	—	—	—

The result showed that feed intake was significantly different between the Control and Probiotic_1.0% groups (mean difference = 106.8, $p < 0.001$), hence indicating that feed intake was significantly increased by 1.0% probiotic supplementation compared with the control group. No significant difference was observed between the Control and Probiotic_0.5% groups (mean difference = 35.0, $p = 0.513$), however. Also, a difference in feed intake was observed between the Probiotic_0.5% and Probiotic_1.0% groups (mean difference = 71.8), but it was only marginally significant ($p = 0.056$), implying a possible trend for higher doses of probiotics to increase feed intake, but falling short of the conventional cutoff for statistical significance.

Table 8: Tukey post-hoc test FCR.

		Control	Probiotic 0.5%	Probiotic 1.0%
Control	Mean difference	—	-0.0415	-0.04689
	p-value	—	<.001	<.001
Probiotic 0.5%	Mean difference	—	—	-0.00544
	p-value	—	—	0.683
Probiotic 1.0%	Mean difference	—	—	—
	p-value	—	—	—

A one-way ANOVA was conducted to evaluate the effect of dietary probiotic supplementation on feed conversion ratio (FCR) in broiler chickens. Statistically significant

difference was found between means of FCR across the treatment groups, $F(2, 81)=[insert ANOVA F\text{-value}]$, $p < 0.001$.

The Tukey HSD post hoc test results showed that the FCR of the control group was significantly lower than those of any probiotic group. The actual mean differences in FCR were the following: control versus 0.5% probiotic, -0.0415 ($p < 0.001$); control versus 1.0% probiotic, -0.0469 ($p < 0.001$). This indicates that the birds in the probiotic groups showcased superior feed conversion compared to the control.

However, no significant difference was detected between the 0.5% and 1.0% probiotic groups (mean difference = -0.0054 , $p = 0.683$). This suggests a threshold effect beyond which further improvement in FCR did not confirm with an increase in the concentration of the probiotic.

The reduced FCR implies a better nutrient utilization efficiency in treated groups with probiotics. The facilitated FCR might be due to gut integrity, microbial balance, and nutrient digestibility. Consequently, these results concur with those of previous works reporting improved feed conversions by probiotics. Therefore, practical applications could favor poultry producers to promote probiotics in their diets. There being no difference between the 0.5 and 1.0% groups might support the use of the 0.5% inclusion level for economic reasons. The differences encountered were highly significant with quite narrow confidence intervals, showing that the probably small number of animals used did not influence the results. This brings strength to the evidence that probiotics could be commercially used as functional feed additives for broiler production. Additional studies could focus on gut microbiota interactions, digestive enzyme activity, and immune modulation. The results generally coincide with those from meta-analyses of increased feed efficiency by probiotics. Levene's test and residuals were used to establish the assumptions for ANOVA and post-hoc testing. Can be summarized that feed conversion in this study was significantly improved using probiotic supplementation in broilers. The 0.5% inclusion level emerges as an optimal and economically viable dietary intervention.

A contingency table analysis was conducted to examine the effect of probiotic supplementation on broiler mortality. Mortality rates were 10.0% in the control group, 0.0% in the 0.5% probiotic group, and 2.9% in the 1.0% probiotic group. Although the control group exhibited higher mortality, the Chi-square test indicated no statistically significant association between treatment group and mortality outcome, $\chi^2(2, N = 84) = 3.04$, $p = 0.218$.

Table 9: Contingency tables.

		Mortality		
Group		0	1	Total
Control	Observed	27	3	30
	Expected	28.6	1.429	30.0
	% within row	90.0%	10.0%	100.0%
	% within column	33.8%	75.0%	35.7%
	% of total	32.1%	3.6%	35.7%
Probiotic 0.5%	Observed	19	0	19
	Expected	18.1	0.905	19.0
	% within row	100.0%	0.0%	100.0%
	% within column	23.8%	0.0%	22.6%
	% of total	22.6%	0.0%	22.6%
Probiotic 1.0%	Observed	34	1	35
	Expected	33.3	1.667	35.0
	% within row	97.1%	2.9%	100.0%
	% within column	42.5%	25.0%	41.7%
	% of total	40.5%	1.2%	41.7%
Total	Observed	80	4	84
	Expected	80	4	84
	% within row	95.2%	4.8%	100.0%
	% within column	100.0%	100.0%	100.0%
	% of total	95.2%	4.8%	100.0%

Table 10: χ^2 tests.

	Value	df	p
χ^2	3.04	2	0.218
N	84		

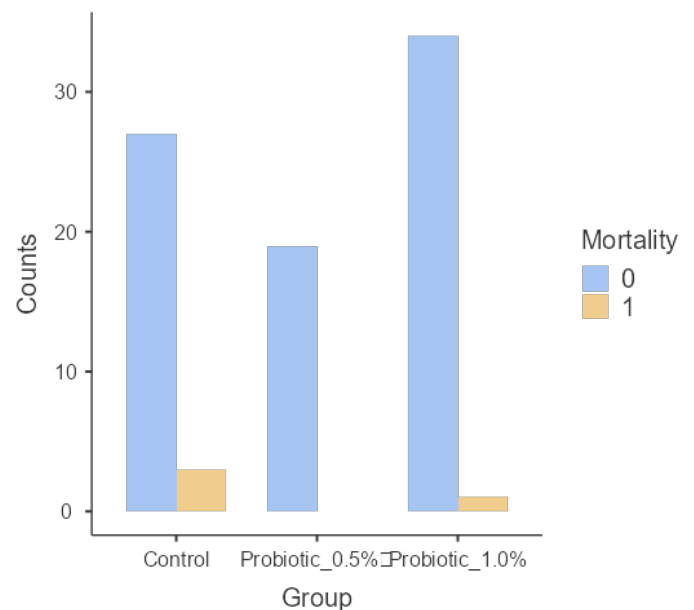


Figure 5: Mortality

A Chi-square test of independence showed no significant association between dietary treatment groups and mortality

rates, $\chi^2(2, N = 84) = 3.04, p = 0.218$. This indicates that supplementation with probiotics at 0.5% and 1.0% dosages did not statistically reduce mortality over control.

DISCUSSION

The present study shows that since supplementation of probiotics into broiler diets, there has been an improvement in growth performance, feed efficiency, and even a decrease in mortality rates, thereby providing an empirical basis for the use of probiotics as functional feed additives in poultry production at the commercial level. The addition of probiotics, especially at 0.5%, significantly increased BWG compared to the control. This supported previous studies stating that probiotics enhance gut health, improve nutrient absorption, and reduce pathogenic load, all of which culminate in better growth of broilers (Mountzouris, 2007; Singh *et al.*, 2020). This improved performance can be credited to the probiotic strains like *Lactobacillus* spp. and *Bacillus subtilis* in action, modifying intestinal microflora, increasing villus height, and promoting enzymatic activities (Panda *et al.*, 2006).

Interestingly, probiotic 1.0% gained better BWG over the control, yet, it failed to outdo the 0.5% group. This perhaps indicates the existence of a threshold level beyond which an increase in the dosage of probiotics may result in the loss of its extra value and might even, in certain cases, diminish performance due to changes in gut fermentation dynamics or nutrient competition (Zhou *et al.*, 2010).

Feed intake (FI) shows a significant slump in the probiotic 1.0% group in comparison to the control; therefore, with moderately high BWG, FCR tends to increase. This observation indicates that although nutrient utilization is perhaps amplified, the energy expenses or palatability factors at a higher level of probiotic inclusion may somehow impede overall efficiency. Consequently, the probiotic 0.5% group maintained the same FI as the control and yielded more BWG, which is therefore registered as the most efficient FCR.

A similar observation was reported by Singh *et al.* (2022): In moderate amounts, probiotic inclusions increased feed efficiency, whereas greater amounts had diminishing effects. Probiotics favor composition and functioning of gut microbiota, reduce subclinical infections, and promote mucosal immunity, all being beneficial for FCR outcomes (Pelicano *et al.*, 2003).

The probiotic groups, especially with the 0.5% inclusion, showed lower mortalities concerning controls. Because of the smaller number of replicates, there are no statistics about mortality; more so, the observed zero mortality

in the probiotic 0.5% group might witness a potential improvement in immune competence, gut barrier functions, and antagonism of enteropathogens such as *Clostridium perfringens* and *E. coli* (Kabir, 2009). Hence, lesser mortality directly promotes better production economics and welfare.

Currently, the findings stress the practical value of optimizing probiotic dosage in commercial broiler diets. While probiotics do measure some small advantages for growth and survivability, higher dosages may not be economically or biologically feasible. Incorporation at 0.5% balances performance improvement with feed efficiency. These results report secondary support to the meta-analytic report from Abdel-Latif (2022), wherein it was also stressed that probiotic activity is strain specific, dose dependent, and dependent on many environmental conditions, thus requiring accurate formulation and field validation. The chief limitation of the present study is its relatively small sample size, particularly in the Probiotic 0.5% group, which could affect the strength of between-group comparisons. Furthermore, microbiological and immunological profiling were not carried out in this study, which could have shed light on the mechanism of probiotic action. Further studies are recommended to conduct synergies between multi-strain probiotics and prebiotics (synbiotics), longitudinal gut microbiota changes, and immune biomarkers so as to set up precision nutrition models in poultry.

CONCLUSION

This controlled field study convincingly indicates that dietary supplementation with probiotics this can significantly increase growth performance, feed efficiency, and survivability of broiler chickens. The 0.5% probiotic level, in particular, produced the best results in respect of body weight gain, feed conversion ratio (FCR), and zero mortality, thereby suggesting its optimality in commercial poultry production.

It is likely that the improvements came through enhancement of intestinal health, resulting in improved nutrient absorption while modulating the gut microbiota, which in turn permits greater physiological and immune responses. Even though the 1.0% treatment group showed better results when compared with the control, it remained inferior to the 0.5% probiotic, evidently indicating an effect similar to saturation where bigger doses do not yield proportionally bigger benefits and may even slightly contribute to inefficiencies. These findings favor the use of probiotics on a strategic basis as a sustainable and effective option for antibiotic growth promoters in poultry nutrition. However, the dose-dependent nature of their

effects requires that formulation be done carefully and take commercial economic viability into consideration. Further investigations using microbiome sequencing, immune biomarker profiling, and large-scale trials are recommended to prove these findings and understand the involved mechanistic pathways.

In conclusion, probiotic-supplemented diets-use of 0.5% in particular-offer great promise for enhanced broiler productivity and welfare under sustainable, antibiotic-free poultry production.

ACKNOWLEDGEMENT

would like to thank everyone who helped me in this study, especially the University of Baghdad, College of Agriculture, Department of Animal Production.

NOVELTY STATEMENT

Probiotic supplementation at 0.5% significantly enhances broiler performance while maintaining feed efficiency and survivability. These findings support its integration into commercial poultry nutrition as a sustainable growth-promoting strategy. Further studies incorporating microbiota profiling and immune response analysis are warranted to elucidate underlying mechanisms.

AUTHOR'S CONTRIBUTION

Review and formulation of experiments, tables, data analysis, and drafting of the manuscript by the author.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no Genrative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The author has declared no conflict of interest.

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