CVJ CLUJ VETERINARY JOURNAL

Research article

Impact of early phytobiotic and prebiotic supplementation on growth performance trajectories in broiler chickens: a time × treatment interaction study

Cîmpean Adrian [1*], Pojar Tudor Nicolae [1], Giulia Mariș [1], Ilie Antal [1], Borzan Mihai Marian [1]

[1] Department of Animal breeding and Animal Productions, University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine Cluj-Napoca, Romania, adrian.cimpean@usamvcluj.ro
* Correspondence: adrian.cimpean@usamvcluj.ro

Abstract: This study evaluated the effects of early dietary supplementation with phytobiotic (nettle flour) and phytobiotic–prebiotic (alfalfa flour) additives on the growth performance of Ross 308 broiler chickens. A total of 90 one-day-old chicks were allocated to three dietary treatment groups (control, nettle, and alfalfa), each consisting of 30 birds in three replicates of 10 birds. Feed additives were administered during the first 7 days post-hatch, followed by a common basal diet across all groups until day 35. Body weight was recorded on days 7, 21, and 35. Statistical analyses included one-way ANOVA, two-way ANOVA with interaction, Ordinary Least Squares (OLS) regression, and Welch's ANOVA to account for heteroscedasticity and non-normal distributions. Significant treatment effects were observed at all time points, with a marked divergence emerging by day 35 ($F(2, 87) = 138.78$, $p < 0.0001$). Post-hoc analyses confirmed that nettle flour led to the greatest increase in body weight compared to both the control and alfalfa groups ($p < 0.001$). The alfalfa group exhibited a moderate but statistically significant effect ($p < 0.05$). Two-way ANOVA revealed a strong treatment × time interaction ($F = 90.93$, $p < 0.0001$), indicating that the efficacy of the additives varied across developmental stages. OLS regression confirmed the robustness of the model ($R^2 = 0.957$), with the "Nettle × Day 35" interaction contributing an estimated +715 g gain in body weight over the control. These findings highlight the potential of short-term early phytobiotic and prebiotic supplementation to produce long-term enhancements in broiler growth performance. Nettle flour, in particular, demonstrated superior efficacy, suggesting its potential as a functional feed additive in intensive poultry systems.

Keywords: broiler chickens; phytobiotics; prebiotics; early-life nutrition; growth performance

Received: 11.09.2025
Accepted: 01.10.2025
Published: 05.03.2026

DOI:10.52331/v31i13975

1. Introduction

The increasing global demand for poultry meat has accelerated the development of intensive broiler production systems. Within these systems, maximizing growth performance while maintaining animal health and minimizing the use of synthetic growth promoters is a core challenge. Traditional reliance on antibiotic growth promoters has diminished due to regulatory restrictions and public health concerns, prompting the search for effective and sustainable nutritional alternatives [3].

Among the most promising candidates are phytobiotics and prebiotics, which are increasingly used in poultry nutrition to improve growth rates, feed efficiency, and gut health [5,6]. Phytobiotics—bioactive compounds derived from plants—are known to exhibit antioxidant, anti-inflammatory, and antimicrobial properties [1,5]. These effects can support the immune system, enhance nutrient absorption, and promote gastrointestinal stability in broilers [4,5]. Prebiotics, defined as non-digestible dietary components that selectively stimulate the growth of beneficial intestinal bacteria, can further modulate the gut microbiota and contribute to improved feed conversion and growth performance [2,7].

Despite extensive research on these compounds, most studies focus on their continuous inclusion in the diet throughout the entire production cycle. However, the early post-hatch period represents a critical window in broiler development, during which the digestive and immune systems undergo rapid maturation [8]. Short-term nutritional interventions during this phase may have long-lasting effects on growth performance due to developmental programming mechanisms [8]. The concept of early-life feed additive application—particularly during the first week—has therefore gained increasing interest for its potential to trigger positive physiological trajectories.

Additionally, many nutritional studies in poultry apply statistical methods that examine treatment effects at isolated time points, without considering potential interaction effects between treatment and time. Such an approach may overlook the dynamic and cumulative nature of dietary interventions. Incorporating a time × treatment interaction analysis can provide a more comprehensive understanding of how the timing and duration of supplementation influence growth outcomes [9].

The present study aimed to evaluate the effects of early dietary supplementation (during the first 7 days of life) with phytobiotic (nettle flour) and phytobiotic–prebiotic (alfalfa flour) feed additives on the growth performance trajectory of Ross 308 broiler chickens. Nettle (Urtica dioica) has been previously investigated as a natural phytogenic additive capable of improving productive performance and meat quality in broilers [10]. Body weight was monitored on days 7, 21, and 35 to assess both immediate and long-term impacts of the interventions. In addition to conventional statistical analyses, advanced modeling techniques—including two-way ANOVA with interaction terms and regression modeling—were used to detect time-dependent effects and quantify their magnitude.

It was hypothesized that short-term inclusion of plant-based feed additives in the early post-hatch period would result in significant and sustained improvements in growth performance, with measurable interaction effects depending on treatment type and developmental stage.

2. Materials and Methods

The experiment was conducted over a 35-day period on Ross 308 broiler chickens, maintained under controlled environmental conditions. Ninety one-day-old chicks were randomly assigned to three dietary treatment groups, each consisting of 30 birds divided into three replicates of 10 birds per pen. The experimental layout followed a completely randomized design to ensure unbiased allocation.

Chicks were housed in deep-litter floor pens within a temperature- and humidity-controlled facility. They had unrestricted access to feed and water throughout the trial. Environmental parameters—including temperature, humidity, and ventilation—were continuously monitored and adjusted according to the Ross 308 commercial management guidelines. Lighting was set to 23 hours light and 1 hour darkness during the first week and was later adjusted to 18 hours light and 6 hours darkness from day 8 until the end of the study.

All diets were formulated to meet or exceed the nutritional recommendations of the NRC (1994) for broiler chickens. Three dietary treatments were tested. The control group received a standard basal diet with no additives. A second group received the same basal diet supplemented with 0.5% nettle flour (*Urtica dioica*), and the third group received 0.5% alfalfa flour (*Medicago sativa*) added to the basal diet. These additives were included only during the first 7 days post-hatch, after which all groups were fed the same standard basal diet. Feed and water were provided ad libitum throughout the entire period.

Body weight was recorded individually on days 7, 21, and 35 using a digital scale with 1-gram precision. These time points were selected to capture the early growth response, intermediate phase development, and final body weight at market age. Birds were monitored daily for feed intake, general behavior, and signs of illness. No clinical issues or mortalities were observed during the study.

Statistical analysis was performed using Python 3.11 and relevant scientific libraries including statsmodels, scipy, and pingouin. Data were expressed as means ± standard deviation, and statistical significance was set at $p < 0.05$. One-way ANOVA was used to evaluate treatment effects at each time point, followed by Tukey's Honest Significant Difference (HSD) test for pairwise comparisons. To assess the combined influence of treatment and time, a two-way ANOVA with interaction was conducted on the full dataset. In addition, Ordinary Least Squares (OLS) regression modeling was applied to quantify the contributions of each factor and their interaction to final body weight. When the assumptions of normality and homogeneity of variances were not met, as determined by the Shapiro–Wilk and Levene's tests, Welch's ANOVA was used as a robust alternative. Graphical tools, including boxplots and interaction plots, were employed to visually support the statistical interpretations and highlight growth trends across treatments.

3. Results

Statistical Results and Scientific Interpretation — Effects of Phytobiotic Supplementation on Body Weight in Ross 308 Broiler Chickens. To assess the impact of dietary supplementation with phytobiotic (nettle flour) and phytobiotic–prebiotic (alfalfa flour) additives on growth performance, we evaluated body weight progression at three critical time points: day 7, day 21, and day 35. The data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc tests, and further validated through two-way ANOVA with interaction and linear regression modeling.

Day 7 – Early Onset of Growth Differentiation. At 7 days of age, a statistically significant effect of treatment was observed ($F(2, 87) = 4.28$, $p = 0.0168$). Post-hoc comparisons revealed a significant increase in body weight in the alfalfa group compared to the control ($p < 0.05$). The nettle group showed no significant difference from the control, although the contrast with the alfalfa group approached marginal significance ($p \approx 0.05$). These findings suggest that the alfalfa-based phytobiotic–prebiotic had an early stimulatory effect on body weight, while nettle exhibited a moderate, less pronounced response.

Day 21 – Intermediate Phase with Borderline Significance. By day 21, the differences between groups remained statistically borderline ($F(2, 87) = 3.11$, $p = 0.0498$). Tukey's test identified a marginally significant difference between the control and nettle groups, whereas comparisons involving the alfalfa group did not reach significance. These results indicate a tendency toward improved body weight in the nettle group, yet without sufficient statistical robustness to confirm a definitive effect at this stage.

Day 35 – Clear and Highly Significant Divergence. At the end of the feeding trial (day 35), treatment effects became highly significant ($F(2, 87) = 138.78$, $p < 0.0001$). All pairwise comparisons were statistically significant ($p < 0.001$), confirming a strong divergence among treatments. Chickens in the nettle group achieved the highest final body weights, significantly surpassing both the control and alfalfa groups. The alfalfa group demonstrated an intermediate effect, indicating a positive but less potent influence compared to nettle supplementation (Table 1).

Advanced Statistical Analysis — Two-Way ANOVA and Linear Regression. A two-way ANOVA with interaction was conducted to evaluate the combined effects of dietary treatment and age on broiler body weight (Fig. 1). The results confirmed highly significant main effects for both treatment ($F = 118.45$, $p = 2.48 \times 10^{-37}$) and time ($F = 2591.75$, $p = 6.74 \times 10^{-173}$), as well as a highly significant interaction between the two factors ($F = 90.93$, $p = 2.62 \times 10^{-48}$). This interaction indicates that the efficacy of dietary additives is time-dependent and varies across growth stages.

To quantify these effects, an ordinary least squares (OLS) regression model was applied, yielding an R^2 value of 0.957, suggesting that the model explained 95.7% of the total variance in body weight. Notably, the interaction term "Nettle × Day 35" emerged as a major contributor, associated with an estimated +715 g increase in body weight compared to the baseline (control at 21 days). This underscores the potent and time-amplified effect of nettle supplementation in the final phase of growth.

Dietary inclusion of plant-based feed additives—specifically, nettle flour (phytobiotic) and alfalfa flour (phytobiotic–prebiotic)—exerted a statistically significant influence on body weight in Ross 308 broiler chickens. A cumulative, time-dependent effect was evident, with the most pronounced differences occurring at day 35. Among the tested treatments, nettle flour led to the greatest weight gain, contributing an additional 715 g over the baseline. These findings demonstrate the potential of phytobiotic feed strategies as functional growth promoters in broiler production, particularly in enhancing performance during the later stages of development.

The boxplot illustrates the distribution of body weight across three dietary treatments: control (standard feed), phytobiotic (nettle flour), and phytobiotic–prebiotic (alfalfa flour) over a 35-day rearing period. At 7 days, group differences were minor; however, by day 21, a moderate increase in median weight was observed in the Nettle group. A substantial divergence appears at day 35, where Nettle flour supplementation led to a significantly higher median weight and narrower interquartile range, suggesting both enhanced growth and reduced variability. The Alfalfa group showed intermediate performance, while the control group consistently recorded the lowest weight values. These results support a time-dependent effect of phytobiotic interventions on growth performance in broilers.

The interaction plot displays the mean body weight (± SD) of Ross 308 broiler chickens subjected to three dietary treatments: control, phytobiotic (nettle flour), and phytobiotic–prebiotic (alfalfa flour), assessed at 7, 21, and 35 days of age.

Table 1. Body weight (g) of Ross 308 broiler chickens at 7, 21, and 35 days after population under dietary supplementation with phytobiotic and phytobiotic–prebiotic feed additives.

No.	Control (7 d) (*g*)	Nettle Flour (7 d) (*g*)	Alfalfa Flour (7 d) (*g*)	Control (21 d) (*g*)	Nettle Flour (21 d) (*g*)	Alfalfa Flour (21 d) (*g*)	Control (35 d) (*g*)	Nettle Flour (35 d) (*g*)	Alfalfa Flour (35 d) (*g*)
1.	288	300	368	1124	952	1094	1325	2210	1545
2.	267	263	285	1074	1102	996	1680	2682	1645
3.	315	302	276	442	1204	1070	1685	2364	1730
4.	304	284	333	1296	1196	1028	1332	2722	1400
5.	325	310	318	1080	1407	1110	1588	2534	1688
6.	290	294	287	1182	1072	1042	1560	2284	1608
7.	275	337	324	1056	1116	966	1455	2686	1760
8.	281	336	302	1302	1098	1200	1750	2324	1528
9.	226	315	273	1136	1334	1284	1636	2022	1450
10.	286	303	346	954	1132	992	1910	2146	1525
11.	222	325	310	1242	1148	890	1335	2372	1530
12.	321	334	283	1000	1264	1186	495	2010	1721
13.	306	280	352	838	1064	1112	1570	2102	1655
14.	286	317	326	1176	1188	1134	1470	2690	1705
15.	301	300	300	1190	1092	1008	1625	2520	1505
16.	198	242	332	822	1244	1166	1723	2426	1450
17.	240	292	340	1214	1296	1135	1790	2316	1620
18.	318	279	312	992	1194	1154	1575	2216	1610
19.	296	317	283	1140	1164	1096	1490	2244	1725
20.	320	284	358	1135	1155	1124	1370	2516	1700
21.	245	298	318	1076	1088	1134	1480	2084	1710
22.	281	328	334	1126	1204	1012	1610	2350	1210
23.	308	330	329	1166	1166	1172	1640	2226	1440
24.	337	275	264	1182	984	1204	1635	2454	1340
25.	296	280	235	1056	1030	1048	1605	2506	1325
26.	310	289	280	1148	1386	1169	1850	2360	1420
27.	300	292	310	1110	964	876	1825	2204	1710
28.	283	275	302	876	1050	1214	1635	2206	1740
29.	276	299	260	1102	1216	1135	1782	2140	1660
30.	290	288	312	1044	1185	1192	1555	2741	1800
Media	286.37	298.93	308.40	1076.03	1156.50	1098.10	1566.03	2355.23	1581.83

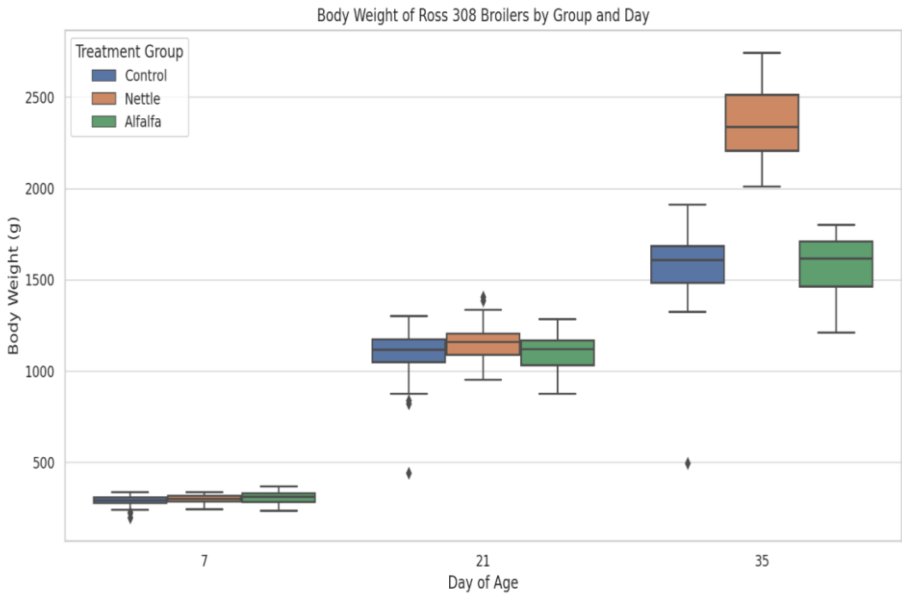

Figure 1. Distribution of body weight (g) of Ross 308 broiler chickens at 7, 21, and 35 days post-population under different dietary treatments.

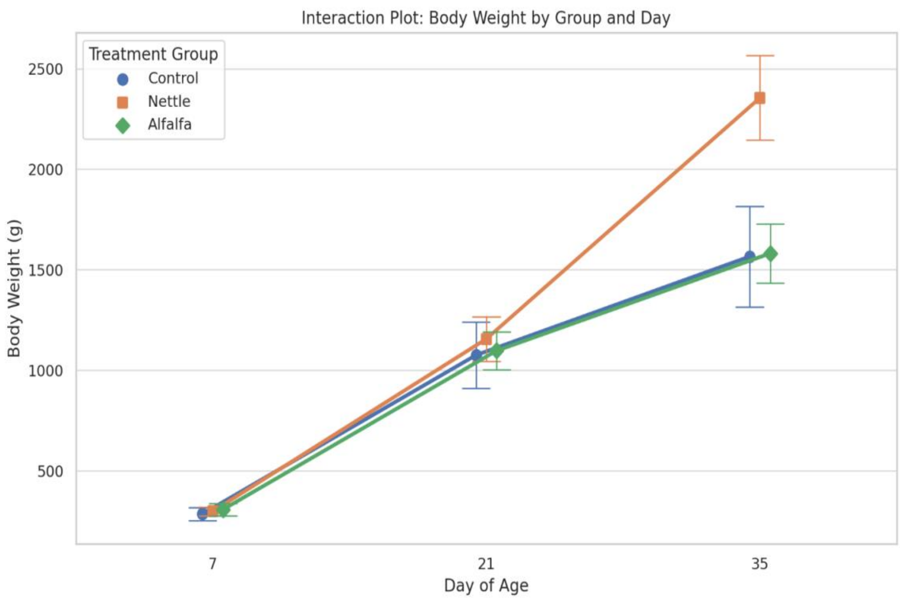

Figure 2. Interaction plot showing the effect of dietary treatment and age on the body weight of Ross 308 broiler chickens.

A marked interaction effect is evident, particularly at day 35, where the Nettle group diverges sharply, exhibiting the highest growth trajectory. The lines representing the three groups are non-parallel, indicating a significant treatment × time interaction, as supported by ANOVA ($p < 0.0001$).

General Statistical Analysis – Overall Comparison of Treatment Groups. To evaluate the overall effect of dietary supplementation on broiler growth, we conducted a one-way ANOVA on the full dataset ($n = 270$), combining data from all time points. This approach allowed us to assess the average impact of each dietary treatment—control, alfalfa (phytobiotic–prebiotic), and nettle (phytobiotic)—regardless of the age of the birds.

The analysis revealed a statistically significant effect of treatment group on body weight ($F(2, 267) = 5.45$, $p = 0.0048$), indicating that, when considered across all time points, the type of feed additive influenced growth performance. Post-hoc comparisons using Tukey's HSD test showed that both supplemented groups had higher mean body weights compared to the control group. Specifically, the alfalfa group exhibited a moderate but statistically significant increase ($p = 0.045$), while the nettle group showed a more pronounced effect ($p = 0.001$). Although the nettle group also outperformed the alfalfa group in terms of mean body weight, this difference did not reach statistical significance ($p = 0.097$) (Fig. 2).

These findings suggest that both phytobiotic and phytobiotic–prebiotic supplementation support enhanced growth in broiler chickens, with nettle flour appearing to have a stronger effect overall.

To verify the validity of the ANOVA results, we tested the underlying assumptions of normality and homogeneity of variances. The Shapiro–Wilk test revealed significant deviations from normality in all three groups ($p < 0.05$), indicating that body weight data were not normally distributed. Additionally, Levene's test for homogeneity of variances also failed ($p < 0.001$), showing that the variability in body weight differed significantly between groups.

Given these violations of ANOVA assumptions, we applied Welch's ANOVA—a robust alternative that does not assume equal variances. The results remained statistically significant ($F(2, 172.68) = 4.09$, $p = 0.0183$), confirming the presence of treatment effects on body weight. Mean values across all time points further supported this conclusion: chickens in the nettle group had the highest average body weight (1270.2 g), followed by alfalfa (996.1 g), and control (976.1 g).

In summary, the statistical evidence consistently indicates that dietary supplementation, particularly with nettle flour, contributes to improved growth performance in Ross 308 broilers. These effects persist even when model assumptions are violated, reinforcing the reliability of the observed treatment differences.

While early-stage differences (day 7) are minimal, the later-stage divergence highlights the time-dependent synergistic effect of the phytobiotic intervention, particularly in the Nettle group.

4. Discussion

The increasing global demand for poultry meat has accelerated the development of intensive broiler production systems. Within these systems, maximizing growth performance while maintaining animal health and minimizing the use of synthetic growth promoters is a core challenge. Traditional reliance on antibiotic growth promoters has diminished due to regulatory restrictions and public health concerns, prompting the search for effective and sustainable nutritional alternatives [3].

Among the most promising candidates are phytobiotics and prebiotics, which are increasingly used in poultry nutrition to improve growth rates, feed efficiency, and gut health [5,6]. Phytobiotics—bioactive compounds derived from plants—are known to exhibit antioxidant, anti-inflammatory, and antimicrobial properties [1,5]. These effects can support the immune system, enhance nutrient absorption, and promote gastrointestinal stability in broilers [4,5]. Prebiotics, defined as non-digestible dietary components that selectively stimulate the growth of beneficial intestinal bacteria, can further modulate the gut microbiota and contribute to improved feed conversion and growth performance [2,7].

Despite extensive research on these compounds, most studies focus on their continuous inclusion in the diet throughout the entire production cycle. However, the early post-hatch period represents a critical window in broiler development, during which the digestive and immune systems undergo rapid maturation [8]. Short-term nutritional interventions during this phase may have long-lasting effects on growth performance due to developmental programming mechanisms [8]. The concept of early-life feed additive application—particularly during the first week—has therefore gained increasing interest for its potential to trigger positive physiological trajectories.

Additionally, many nutritional studies in poultry apply statistical methods that examine treatment effects at isolated time points, without considering potential interaction effects between treatment and time. Such an approach may overlook the dynamic and cumulative nature of dietary interventions. Incorporating a time × treatment interaction analysis can provide a more comprehensive understanding of how the timing and duration of supplementation influence growth outcomes [9].

The present study aimed to evaluate the effects of early dietary supplementation (during the first 7 days of life) with phytobiotic (nettle flour) and phytobiotic–prebiotic (alfalfa flour) feed additives on the growth performance trajectory of Ross 308 broiler chickens. Nettle (Urtica dioica) has previously been reported as a plant-based additive capable of improving productive performance and meat quality in broilers [10]. Body weight was monitored on days 7, 21, and 35 to assess both immediate and long-term impacts of the interventions. In addition to conventional statistical analyses, advanced modeling techniques—including two-way ANOVA with interaction terms and regression modeling—were used to detect time-dependent effects and quantify their magnitude.

It was hypothesized that short-term inclusion of plant-based feed additives in the early post-hatch period would result in significant and sustained improvements in growth performance, with measurable interaction effects depending on treatment type and developmental stage.

5. Conclusions

This study demonstrates that early-life supplementation with plant-derived feed additives—specifically nettle flour (phytobiotic) and alfalfa flour (phytobiotic–prebiotic)—can exert significant, time-dependent effects on growth performance in Ross 308 broiler chickens.

Although the additives were included only during the first 7 days post-hatch, their influence persisted and intensified over time, culminating in significant body weight differences at day 35. Among the tested treatments, nettle flour produced the highest final body weight (2355.2 g), exceeding both the control (1566.0 g) and alfalfa (1581.8 g) groups ($p < 0.001$). The early administration of nettle was associated with a +715 g weight gain, as quantified by OLS regression modeling, underscoring its long-term anabolic effect.

While alfalfa flour induced a moderate improvement in early growth (day 7), its effect diminished by day 21 and did not translate into a significant long-term gain. This suggests a transient prebiotic effect, less effective than the more persistent action observed in the nettle group.

The statistical robustness of these findings was confirmed through two-way ANOVA, Welch's ANOVA, and regression modeling, revealing a strong treatment × time interaction and validating the efficacy of early phytobiotic interventions.

In conclusion, short-term phytobiotic supplementation during the early post-hatch period may represent a viable strategy to enhance broiler productivity without continuous additive use. Nettle flour, in particular, emerges as a promising candidate for improving final body weight in intensive poultry systems, supporting its potential integration into functional feed programs aimed at optimizing performance while reducing reliance on synthetic growth promoters.

Author Contributions: Conceptualization, C. A. and P. T. N.; Methodology, C. A. and P. T. N.; Validation, C. A., P. T. N. and B. M. M.; Data Analysis, C.A.; Investigation, C. A. and P. T. N.; Resources, P.T.N.; Writing – Original Draft Preparation, C.A.; Writing – Review & Editing, P.T.N. and B.M.M.; Visualization, C.A.; Supervision, P.T.N.; Project Administration, P.T.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: This research was supported by the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Khatun, A.; Das, S.C.; Ray, B.C.; Ahmed, T.; Hashem, M.A.; Khairunnesa, M.; Roy, B.C. Effects of feeding phytobiotics on growth performance, breast meat quality, blood biochemical indices, and liver enzymes of broiler chickens. *Europ Poult Sci* **2023**, *87*: Article 374.
2. Naeem, M., Bourassa, D. Probiotics in poultry: Unlocking productivity through microbiome modulation and gut health. *Microorganisms* **2025**, *13(2)*, 257.
3. Wickramasuriya, S. S., Ault, J., Ritchie, S., Gay, C. G., Lillehoj, H. S. Alternatives to antibiotic growth promoters for poultry: A bibliometric analysis of the research journals. *Poult Sci* **2024** *103(9)*, 103987.
4. Peng, H., Zhang, L., Zhao, L., Xing, T., Gao, F. Benzoic acid and oregano essential oil interact to increase the immune function and intestinal development of Langshan chickens. *Br Poult Sci* **2026,** 1-12.
5. Obianwuna, U. E., Chang, X., Oleforuh-Okoleh, V. U., Onu, P. N., Zhang, H., Qiu, K., Wu, S. Phytobiotics in poultry: Revolutionizing broiler chicken nutrition with plant-derived gut health enhancers. *J Anim Sci Biotechnol*, **2024**, *15*, 169.
6. Abd El-Ghany, W. Phytobiotics in poultry industry as growth promoters, antimicrobials and immunomodulators: A review. *J World's Poult Res* **2020**, *10(4)*, 571–579.
7. Patterson, J. A., Burkholder, K. M. Application of prebiotics and probiotics in poultry production. *Poult Sci* **2003**, *82(4)*, 627–631.
8. Gaweł, A., Madej, J. P., Kozak, B., Bobrek, K. Early post-hatch nutrition influences performance and muscle growth in broiler chickens. *Animals* **2022**, *12(23)*, 3281.

9. Marx, F. O., Alvarez, M. V. N., Bassi, L. S., Félix, A. P., Krabbe, E. L., Oliveira, S. G., Maiorka, A. Use of statistical models to determine the optimal concentration of metabolizable energy for growth performance of broiler chickens. *Livest Sci* **2023**, *274*, 105268.
10. Mierlița, D., Davidescu, M. A., Doliș, M. G., Simeanu, D., Pop, I. M. The effect of nettle flour (Urtica dioica) in diets for broiler chickens on productive performance, lipid quality and oxidative stability of meat. *Ann Univ Oradea*, **2023**, Fascicle: Ecotoxicology, Animal Science and Food Science and Technology.