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ORIGINAL RESEARCH

Effect of *Lactobacillus* Species Probiotics on Growth Performance of Dual-Purpose Chicken

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Introduction: In-feed probiotics are becoming attractive alternatives to antibiotics in the poultry industry due to the ever-growing strict prohibitions on antibiotic growth promoters (AGP) in animal production.

Methods: The study was conducted to investigate the effects of *Lactobacillus paracaseis sparacasei* and *Lactobacillus rhamnosus* on the growth performance of 120 day-olds randomly selected Sasso dual-purpose chicken. They were divided into four groups with two replicates per group and 15 chicks per replicate. The treatments were T_1 (control), T_2 (supplement diet with 4g probiotic), T_3 (supplement diet with 2g probiotic), T_4 (supplement diet with 1g probiotic). The experimental feeding trials were conducted after two weeks adaptation period.

Results: The present findings revealed that the chickens supplemented with *Lactobacillus* species probiotics during the first week of age have shown higher body weight than control ($p < 0.05$). The feed intake of week one of T_2 and T_3 were significantly higher ($p< 0.05$) than the T_1 (control). However, there was no significant difference ($p> 0.05$) in feed intake in the 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} weeks of all treatment groups. The present result showed that there was a significant body weight gain ($p< 0.05$) in all probiotic fed groups than the control group. The highest body weight gain was observed in chickens found in the T4 treatment group. Whereas the body weight gains significantly higher and improved the feed conversion ($p<0.05$) in the T_2 and T_4 than the T_1 (control). However, the feed conversion ratio was significantly influenced by probiotic inclusion in T_3 as compared to the control group.

Conclusion: Overall, the results suggest that *Lactobacillus paracaseis sparacasei* and *Lactobacillus rhamnosus* have a positive effect on the growth performance of broilers.

Keywords: Body weight gain, Broiler, Chicken, *Lactobacillus* species, Probiotics

Introduction

Ethiopia possesses an estimated number of 60.51 million poultry. Poultry production has great importance as a primary supplier of eggs and meat, sources of cash income. The sub-sector remains one of the most common and economically rewarding means of providing animal proteins that contribute significantly to the protection of food and nutrition.[1]

The demand for chicken meat is evolving. Consumers are now becoming more conscious and more aware of items viewed as "naturally or organic" products.[2,3] Commercial poultry production is a leading source of animal protein, and the poultry industry has grown in scale faster than other livestock producers,[4,5] however, there is a major challenge on sustainable availability of high-quality feed at a minimum cost. A feed is a significant component of the overall production cost of

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poultry. Enteric conditions in the poultry industry are also one of the main issues. These diseases are responsible for lower rates of growth and consequent economic gains due to a lower weight gain, higher death rates, lower feed conversion rates (FCR), and higher prescription prices.[6,7] Antibiotic supplementation as sub-therapeutics improves the efficiency of chicken feed and preserves gut health, growth, and development.[8]

Birds provide a comparatively robust and durable intestinal microbial composition across time. It is necessary to avoid pathogenic infections, to allow complex plant fibers to be digested into short chains of fatty acids, to synthesize essential vitamins and amino acids, to control fat metabolism, and to form the production of the immune system.[9–11] The Intestinal microbial population is a healthy dietary alterations and plays important roles in the physiology and health of the host.[12–14] However, various environmental factors such as feed, stress, viruses, and medicines, especially antibiotics, may trigger intestinal dysbacteriosis and dysregulation of the immunity in birds. Hence, bird health and bird development may be impacted and intestinal necrotic diseases may occur.[15–18]

In the poultry industry, antibiotics have been first used as a feed additive in the 1940s and were commonly used to stimulate development over the last few decades. In-feed antibiotic growth promoters (AGP) have proved to be successful in improving food-animal health and production by avoiding gastrointestinal infection, reducing morbidity and mortality, and maximizing the quality of feed usage.[19–21] Also, the widespread use of antibiotics in poultry products may produce antibiotic-resistant bacteria.[22] The use of antibiotics as a promoter of poultry growth in animal diets has been restricted in many countries.[23]

With the strict prohibition of antibiotics, the search for healthy substitutes for antibiotics in the processing of food animals is becoming exceedingly important. During the past several decades, the efficiency and overall health of food animals are improved by many types of dietary substitutes, such as directly feed microbials such as probiotics, prebiotics, and natural minerals; these dietary alternatives were seen as "Generally Recognized as Safe (GRAS)" alternatives to antibiotics.[24–26]

Probiotics are defined as a culture of live microorganisms that are beneficial to the host by improving the properties of the indigenous microbiota when applied to animals.[22]

Non-pathogenic, non-toxic, and able to exert a beneficial effect on the host animal should be the characterstics of a healthy probiotic. They should be present in the gut environment as viable cells and capable of survival and metabolization. Also under storage and field conditions, it should be stable and able to remain viable for longer periods. By promoting the development of beneficial microorganisms, the value of probiotic consumption reduces the risk of gastrointestinal diseases. Probiotic supplementation enhances the bioavailability of nutrients, and avoidance or elimination of allergies in susceptible individuals.[27]

Due to pathogen inhibition, the observed results after probiotic supplementation are linked to a more favorable microbial community in the gut. Pathogen inhibition mechanisms may involve immune system activation, competition for available nutrients, and direct antimicrobial effects through secretion of inhibitory substances or competition for intestinal epithelium adhesion receptors.[28,29]

Lactobacilli species are microaerophilic gram-positive bacteria typically contained in milk, fruits, and soil. They retain the natural equilibrium in chicken intestines while preserving the natural stability of the microflora.[30,31] They decrease the fat content in the body and serum and increase the efficiency of poultry. It may also be used as an alternative for antibiotics in the form of supplements. There are many reasons to concentrate on the use of Lactobacilli species: (1) Lactobacilli showed "competitive exclusion", (2) Lactobacilli bind quickly to the gut epithelial cells and (3) Lactobacilli affect the immune system and understand that they are healthy symbionts for the host.[32]

Several previous reports had shown that commercial probiotics that contain at least 1×10^8 CFU g^{-1} *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium thermophilum*, and *Enterococcus faecium* improves the growth performance, feed efficiency, the immune response of broiler chickens and act as a replacement for antibiotic growth promoters.[33–38] The ability of probiotics can be increased by choosing potent strains, gene manipulation, combining two or more strains, combining probiotics, and synergistic components that affect the host.[4]

As attractive replacements to AGP, numerous studies have reported that probiotics are beneficial for the growth performance and animal health through enhancing the intestinal development and nutrients absorption, regulating the mucosal immune system, inhibiting intestinal pathogen colonization and infection, and reshaping intestinal microbiota.[24,39] Lack of evidence as to their mechanism of action and the impact on host animals is the issue with probiotics. In some cases, probiotics are effective

particularly in newborn animals or those that have been treated with antibiotics, where they have the same effect as products of competitive exclusion. They can also be effective in improving weight gain and feeding conversion rates.[40] The search for new, effective, and non-toxic feed additives on poultry growth against unexpected hazards is still continuing. From this point of view, the research was conducted to study the effects of probiotic supplementation in broiler chickens on growth efficiency.

Materials and Methods

Experimental Animals

Day-old broilers Sasso breed were purchased and selected for the current study from EthioChicken. The deep litter experimental house was prepared, cleaned, and disinfected before the introduction of experimental chickens. Chicks were vaccinated against Infectious bursal disease, Marek's disease, and Newcastle disease. Feed was measured and offered twice a day and fresh and clean water were also provided daily. Daily feed refusals in each replicate were collected, weighed, and recorded before the daily feed offer.

Feed Supplements

A commercial diet was used in the study. The nutritional composition of diets fed during the starter (1–21 days of age), grower (22–42 days of age), and finisher (42–56 days of age) periods (Table 1).

Experimental Design and Dietary Treatment

A total of 120 chickens were used during this experimental study. Fifteen chickens were randomly assigned to each of two replicates of the four treatment groups to adapt the experimental diet and procedure before the actual research started. The experiment was conducted in a completely randomized design with four dietary treatments each with two replications. A total of 120 chickens were randomly distributed to 8 pens having four groups with two replicates per each group and 15 chicks per replicate and two of the pens were received the same diet which was allocated to the pen.

Table 1 Common Ingredients of Commercial Diet

Ingredients	Broiler Starter (1–21 Days)	Broiler Grower (22–42 Days)	Broiler Finisher (43–56 Days)
Protein	20.9%	19.7%	18%
Fat	3%	2.50%	8%
Fiber	4.5%	5%	5.5%
Calcium	1.15%	1.10%	0.65%
Energy (kcal/kg)	3035	2958	3250
Moisture	10%	10%	10%

Probiotics Preparation and Sample Collection

Lactobacillus paracasei is a rod-shaped (bacillus shape) bacterium with a width of 2.0 to 4.0 μm and a length of 0.8 to 1.0 μm. It is commonly found in many human and animal habitats such as human intestinal tracts and mouths as well as sewages, silages, and dairy products. It helps to strengthen the immune function, oxidative stress, body fat reduction.[41,42] *Lactobacillus rhamnosus* is a kind of friendly bacteria found in the intestines. Its health advantages include relief of Irritable bowel syndrome (IBS) symptoms, the treatment of diarrhea, enhancement of gut health, and cavity defense. *L. rhamnosus* is present in some milk products as a probiotic supplement.[43] Some strains of *L. rhamnosus* bacteria are particularly useful in treating infections of the female urogenital tract and endocarditis.[44,45]

The strains *Lactobacillus paracaseis sparacasei and Lactobacillus rhamnosus* were isolated from the gut region of the chick and confirmed at species level using the Biolog^{TM} Identification system and passed the necessary *in vitro* probiotic screening test were obtained from Microbiology laboratory, School of Veterinary Medicine. A co-culture of *Lactobacillus* strains (equal volume of 0.5 McFarland turbidity standards) was propagated on sterile de Man, Rogosa, and Sharpe (MRS) broth and harvested to prepared experimental feed. The probiotic-containing feed was prepared as treatment groups were T_1 (control), T_2 (supplement diet with 4g probiotic/kg feed), T_3 (supplement diet with 2g probiotic/kg), T_4 (supplement diet with 1g probiotic/kg). The chickens were provided with the experimental feed every three days for five consecutive weeks.

On day 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} weeks of age, three birds from each replicate were chosen randomly and weighed after deprivation of feed overnight.

Data Management and Statistical Analysis

The data collected during the study period were subjected to statistical analysis using STATA version 13. Descriptive

Table 2 Effects Probiotic Supplementation on Broiler Body Weight

Week	Treatment Groups				
	T_1	T_2	T_3	T_4	P-value
Initial	129.8±5.87	133.07±0.71	125.31±4.70	134.50±7.35	0.417
1st	459.85±14*	482.09±11.12*	455.47±14.67	470.41±6.46	0.000
2nd	691.67±12*	740.02±9.45*	692.665±10.37	738.33±7.07*	0.011
3rd	925.89±2.5*	959.28±49.2*	907.09±14.87	933.94±11.59	0.000
4th	1173.35±3.7	1210.65±5.45	1112.05±13.93	1216.55±59.89	0.1184
5th	1435.87±27*	1557.10±104.0*	1397.05±20.22	1556.17±133.4*	0.0000

Note: Means bearing *Superscript were significant values of the Post-hoc Honest Significant Difference (HSD) test ($p< 0.05$).

statistics were employed to summarize the data and expressed in terms of mean and standard deviation. One-way ANOVA and the post hoc Tukey's test were performed to identify significant differences between the four feeding treatments. Levels of significant differences were detected at 95% confidence interval and $P < 0.05$.

Results

In this study to evaluate the effect of probiotics on the growth performance of broiler chickens on body weight, body weight gain, feed intake, and feed conversion ratio were recorded. Bodyweight at the beginning of the experiment was not a significant difference among groups ($P> 0.05$). Bodyweight of the broilers' week wise showed that at the first week of treatment the bodyweight of broilers with Probiotic supplemented diet results numerically higher body weight than without probiotic ($P< 0.05$) despite the Bodyweight of T_2 and T_4 treatment groups were found significantly different from T_1 and T_3 treatment groups ($p< 0.05$). In the 2nd week, there was a significant difference between T_1 (control) and other treatment groups T_2, T_3, and T_4. Even though T_2 and T_4 showed slightly higher body weight than T_1 and T_3 treatment groups. Whereas in the 4th week T_2 and T_4 showed slightly higher body weight than T_1 and T_3 treatment groups and in the 5th week T_3 slightly lower than treatment groups (Table- 2).

The feed intake of week one of T_2 and T_3 were significantly higher ($p< 0.05$) than the T_1 (control). However, there was no significant difference ($p> 0.05$) in feed intake in the 2nd, 3rd, 4th, and 5th week of the treatment groups (Table 3).

The present result showed that there was a significant difference ($p< 0.05$) between the control and probiotic fed group in the first and second week on body weight gain and in the 3rd week T_1 (control) higher value than probiotic supplementation treatment groups. While in the fourth week, T_4 was higher body weight gain than the control and other treatment groups. In the fifth week, there was a significant difference ($P< 0.05$) among different treatments, and the highest body weight gain was observed T_4 with probiotic supplementation treatment groups. Whereas the final body weight gains significantly higher ($p< 0.05$) in the T_2 and T_4 than the T_1 (control) (Table 4).

The current study shows that the data on total feed intake, total body weight gain (BWG), and FCR in broilers were influenced by probiotic feed treatments. The results indicated that there was a significant ($P< 0.05$) difference in feed intake in the treatment groups than in the control. The T_2 and T_4 treatment groups were significantly improved final body weight during the experimental period. The feed

Table 3 Effects Probiotic Supplementation on Broiler Feed Intake

Week	Treatment Groups				
	T_1	T_2	T_3	T_4	P-value
1st	688.63±62.84	802.89±40.55	828.93±58.9	799.98±28.78	0.000
2nd	955.95±59.47	943.69±51.79	961.07±42.23	979.1±93.82	0.782
3rd	1072.96±93.7	1132.09±83.2	1190.57±86.53	1185.1±53.98	0.0502
4th	1414.06±144.06	1426.2±123.02	1411.3±85.94	1448.65±130.1	0.939
5th	1711.7±60	1725.6±53.69	1727.1±57.08	1730.04±52.3	0.929

Table 4 Effects Probiotic Supplementation on Broiler Body Weight Gain

Week	Treatment Groups				
	T_1	T_2	T_3	T_4	P-value
1st	330.05±11.98*	349.02±11.82*	330.17±19.37	335.91±13.82	0.002
2nd	231.81±15.68*	257.93±8.34*	237.20±4.29	267.92±10.61*	0.000
3rd	234.22±9.43*	219.26±58.87	214.42±4.50	195.6±4.52*	0.026
4th	247.465±11.37*	251.37±24	204.96±10.94*	282.61±18.30	0.000
5th	262.52±12.04*	276.44±29.26	284.96±6.29	339.62±10.84*	0.000
Final BWG	1306.07±27.56*	1424.03±105.41*	1271.70±24.92	1421.67±141.09*	0.000

Note: Means bearing *superscript were significant values, values of Post-hoc HSD test (p<0.05).

conversion ratio was significantly ($P< 0.05$) improved in T_2 and T_4 than T_1 (control). However, the feed conversion ratio was significantly influenced by probiotic inclusion in T_3 compared to the control group (Table 5).

Discussion

Probiotics have been considered as an attractive alternative to in-feed antibiotics for their unique functions, including preventing intestinal infectious diseases, enhancing overall health and performance of poultry, and improving the quality of poultry products.[24,26,46] The present study also revealed that the supplementation of two selected probiotics, *Lactobacillus paracaseis sparacasei*, and *Lactobacillus rhamnosus*, are also beneficial in improving the growth performance (BW, weight increments, and FCR) of dual chickens. Similarly, previous studies by Singh et al[47] Banday and Risam,[48] Forte et al[3] Park et al[49] Ramlucken et al[50–53] had also reported that dietary probiotic supplementation may increase broiler growth efficiency by increasing feed conversion and improving gut health. The reason for the growing interest in dietary probiotics is to limit or eradicate the use of feedback antibiotics in the development of food animals.

Initially, a positive effect was expected in light of previous reports showing that dietary probiotics increased growth performance and nutrient utilization in broiler chickens.[54–57] Mookiah et al[58] reported a significant increase in body weight gain and feed efficiency when birds were fed diets supplemented with isomalto-oligosaccharides and 11 strains of Lactobacillus species. Probiotics can protect the integrity of the intestinal structure, deter infections from proliferating, generate digestive enzymes, and improve nutrient consumption, all of which can stimulate animal growth and development.[59,60]

Moreover, the results of the current investigation is in accordance with those obtained in studies by Forte et al[3] that used *Lactobacillus* species on local or rural breed chickens, Salarmoini and Fooladi,[61] that used *Lactobacillus* species alone or in combination with other *Lactobacillus* strains.[62–67] The findings obtained in this analysis in particular are in line with those obtained by Khan et al[68] using *Lactobacillus* strains in Kabir chickens. The findings resulting from dietary supplementation confirm the positive effects found in the production of chickens included in this study.

The present outcomes revealed that there was a statistically significant ($p< 0.05$) difference between probiotics groups and the control group in Bodyweight gain. Dhande et al[69] have reported similar body weight gain was observed in the chicks fed on probiotic supplemented diet than chicks fed on the diet without probiotics. The findings of this research also coincide with the previous findings of Kabir et al[70]. Bai et al[71] and Anjum et al[72] who reported that the growth performance of broilers was increased after feeding probiotics. On the other hand reports by Poorghasemi et al[73] demonstrated that there was both a significant increase and decrease in body weight gain of the birds after lactofeed probiotic as compared to the control group. Another study by

Table 5 Effects Probiotic Supplementation on Broiler Feed Conversion Ratio

Parameter	T_1	T_2	T_3	T_4	P-value
Total FI	2726.90±22.82*	2760.53±22.90*	2800.311±21.96*	2813.392±22.7*	0.000
Total BWG	1306.07±27.56*	1424.03±105.41*	1271.70±24.92	1421.67±141.09	0.000
FCR	2.088±0.054*	1.94±0.08*	2.21±0.046*	1.98±0.13*	0.000

Note: Means bearing *superscript were significant values, values of Post-hoc HSD test (p<0.05).

Poorghasemi et al[74] also revealed that there was significant variation (P< 0.05) in terms of body weight gains in all treatment groups (except the probiotic group) during the starter period as compared to the control group. While during the grower period, no significant variation was observed between the treatment groups and the controls one.

However, the current finding was in contrast to the previous finding of Yu et al (2007) that reported, during the growing or finishing periods, the probiotic inclusion did not significantly affect the body weight gain, feed intake, and feed conversion. It is also similar to the finding of Nafees and Pagthinathan[75] and Biernasiak and Slizewska[76] who reported that probiotics did not affect the growth improvement of the broilers. Similarly, Samanta,[77] Awad et al[56] Roshanfekr and Mamooee,[78] Ramarao et al[50] and Yalcinkayal et al[79] reported that probiotic supplementation in broilers ration had no significant effects on body weight gain.

The reason for the discrepancy between the results observed in the aforementioned studies could be related to the duration and time of feeding, diversity of probiotic formulations (mono-species/mono-strain, or mono-species /multistrain, or multispecies, or even multi genera), administration methods (specific dosages in feed and/or in water), general characteristics of probiotic, such as the production of lactic acid, the competitive elimination of pathogenic bacteria, and the improvement of the condition of the intestine, chicken genotypes and rearing systems.[80,81] Besides, pure broiler chicken fed with probiotics starting from day-old may show a positive response to their growth performance. The differences in the environmental conditions, as well as management (nutritional constituents, humidity, light, ventilation capacity, feeding process, drinking water quality, and other physiological parameters) existing during the experiments, may also contribute to the variation.

In the present study, it was observed that the broilers fed with probiotic supplemented diet throughout the experimental period had consumed significantly more feed as compared to the control group. The findings of Poorghasemi et al[74] Kalavathy et al[62] and Ramarao et al[50] have shown that broilers diet supplemented with probiotics showed improved feed intake compared to the control group. This can be substantiated from the fact that the experimental broilers had consumed significantly more feed than control ones due to increased digestive efficiency. These findings in line with the different research conducted by Onderci et al[82] Gunal et al[83] and Onderci et al[84]. Moreover, Probiotics improve the digestive process via increase of the useful microbial population, enzymatic activity of bacteria, and the improvement of intestine microbial balance with consequent effects on food digestion, absorption, and intake.[85]

The present findings also indicated a significantly better feed conversion ratio (FCR) on probiotic supplementation in the diet of commercial dual-purpose chickens and unlike the result reported by Mohan et al[86] there were no significant differences in the final weight and FCR among the treatment groups with different concentrations of probiotic. This result was also not supported by Ignatova et al[87] and Sen et al[88] whereas Rahimi et al[89] are reported that there was no significant benefit with probiotic addition to the broilers feed. The differences could be due to different factors that could alter the efficacy of a probiotic, such as strains of bacteria utilized, composition and viability of the probiotic bacteria, and the preparation methods.

In general, during comparison of studies regarding probiotics, it is essential to consider that mechanisms of action and beneficial effects are suggested to be specific for genus, species, and strain of the examined microorganisms.[90] Furthermore, the variation of a probiotic's efficacy could be due to external experimental conditions, other than to the differences in the preparation itself.[91]

Conclusion

In conclusion, the finding of the current study has shown that the two screened probiotics, *Lactobacillus paracaseis sparacasei* and *Lactobacillus rhamnosus*, were beneficial for the growth performance by improving body weight gain, feed conversion ratio, feed intake, and positively affects the growth of the chicken. Furthermore, the adaptability of *Lactobacillus paracaseis Sparacasei* and *Lactobacillus rhamnosus* could provide a solution to address the needs of the current generation of ecologically aware consumers. More studies should be done with different rurally reared chickens and a different management system. Further studies characterized by a systematic approach and the use of advanced technologies will be needed to fully comprehend the mechanisms of action of the probiotic strains and to better assess their use in poultry nutrition.

Ethics Approval and Consent to Participate

All experimental procedures (animal care, sampling) were conducted following the standards established by the College Research Ethics Committee at the Wolaita Sodo University, Ethiopia. Animal experimentation in the study was approved by the University Research Board.

Disclosure

All authors declared no conflicts of interest in this work.

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