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Effect of Probiotic Levels Through Water Supplementation on Broiler Performance Under Heat Stress Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the effect of probiotic supplementation (*Lactobacillus casei*) in drinking water on broiler performance. A total of 200, day old chicks were randomly assigned to five treatment groups (T1–T5) with four replicates of 10 chicks each and reared for six weeks. The groups comprised T1 (control, basal diet), T2 (basal diet + 1% *L. casei*), T3 (basal diet + 2% *L. casei*), T4

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(basal diet + 3% *L. casei*) and T5 (basal diet + 4% *L. casei*). Growth traits including body weight, weight gain, feed intake, water intake and feed conversion ratio (FCR) were recorded and analyzed using ANOVA under a completely randomized design, with mean differences tested by t-test at 5% significance. Supplementation with 3% *L. casei* (T4) showed the best results, with significantly higher body weight (2128.60 g), weight gain (2083.92 g) and improved FCR (1.56), alongside reduced feed intake (3436.81 g), while water intake and health remained unaffected.

Keywords: Konkan region; probiotic supplementation; lactobacillus casei; growth performance; feed conversion ratio.

1. INTRODUCTION

The poultry sector plays a vital role in fulfilling the world's protein requirements, with chickens providing a major share of animal protein through both meat (broilers) and eggs. Advances in nutrition, genetics and management have significantly improved production efficiency. Selective breeding and modern feeding strategies have enabled broilers to reach market weight faster, while improvements in feed conversion efficiency have reduced the amount of nutrients required per kilogram of meat, benefitting both producers and consumers.

Despite this progress, poultry production faces several challenges, with heat stress (HS) emerging as one of the most critical. Heat stress often results in considerable economic losses and increased mortality rates (St-Pierre et al., 2003). High ambient temperatures negatively influence performance by reducing feed intake and weight gain, disrupting physiological homeostasis and increasing mortality, which ultimately lowers economic efficiency and compromises animal welfare (Mangan et al., 2024). The U.S. livestock industry alone suffers estimated annual losses of USD 2.36 billion due to HS, with the poultry sector accounting for more than USD 120 million (St-Pierre et al., 2003). Moreover, heat stress compromises gut health by decreasing nutrient absorption, weakening immune responses and increasing intestinal permeability, which facilitates pathogen translocation and systemic inflammation (Abuajamieh et al., 2020; Mahasneh et al., 2024).

To mitigate the harmful effects of Heat Stress, various nutritional interventions have been explored. These include midnight feeding, vitamin and mineral supplementation and the use of phytochemicals, probiotics (PROs) and prebiotics (Safi et al., 2022; Abd El-Hack et al., 2020). Among these, probiotics have gained significant attention due to their positive impact on gastrointestinal health. When administered at appropriate levels, probiotics inhibit pathogenic bacteria, enhance digestive efficiency and improve nutrient absorption (Arora et al., 2015; Alagawany et al., 2018). Although probiotic supplementation sometimes shows minimal effects on overall performance and inflammatory markers under HS, it may provide thermoregulatory benefits and improve intestinal integrity (Istatieh et al., 2025).

India has witnessed significant growth in poultry production. According to the Department of Animal Husbandry and Dairying (2019), the country's poultry population reached 851.81 million, reflecting a 16.8% increase over the previous census. Of this, backyard poultry accounted for 317.07 million birds, recording a sharp rise of 46%, while the commercial sector stood at 534.74 million, with a modest growth of 4.5%. In Maharashtra, poultry farming has expanded steadily over the past three decades. The 2019 census reported a poultry population of 742.98 lakh compared to 777.95 lakh in 2012, showing an overall decline of 4.49%. During this period, backyard poultry increased by 26.31%, whereas commercial poultry declined by 13.44%. Maharashtra produced 596 crore eggs in 2018–19, a marginal increase of 0.45% from the previous year, contributing 5.77% of national egg output. Despite these fluctuations, the state continues to offer considerable potential for further growth in poultry farming.

Feed remains the single largest cost component in poultry production, accounting for nearly 70% of total expenses. Rising prices of feed ingredients have significantly reduced profit margins. To enhance productivity, poultry farmers traditionally relied on antibiotic growth promoters (AGPs). However, increasing concerns regarding antimicrobial resistance and residues in poultry products have shifted focus toward probiotics as sustainable alternatives. Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits to the host by modulating the gut microbiota (Fuller, 1989). Their use in poultry has been associated with improved growth rates, feed efficiency, reduced mortality and enhanced immune function (Yirga, 2015; Nadhifah et al., 2020).

Antibiotics were widely used in the past, with around 30 different compounds employed either as feed additives or therapeutic agents; more than 13.7% were administered at subtherapeutic levels for growth promotion (Jones and Ricke, 2003).

Probiotics present a safer alternative by promoting gut health through stimulation of beneficial microorganisms, production of antimicrobial substances such as lactic acid and hydrogen peroxide and enhancement of nutrient absorption (Musa et al., 2009). Multi-strain formulations containing species like *Bacillus subtilis*, *Clostridium butyricum* and *Enterococcus faecalis* have been shown to improve growth performance, feed intake and immune function in broilers. Recent research also emphasizes their role in disease prevention; for example, probiotic blends have enhanced immune responses and reduced adenovirus infections in poultry (Niczyporuk et al., 2024).

Frequently used microbial species in poultry probiotics include *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. salivarius*, *L. plantarum*, *L. faecalis*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Bifidobacterium* spp., *Saccharomyces cerevisiae* and *Toulopsis sphaerica*. Among these, *Lactobacillus casei* is particularly noteworthy due to its tolerance to acidic conditions and its presence in both fermented foods and the gastrointestinal tracts of animals and humans. Supplementation with *L. casei* in poultry diets has been associated with increased body weight gain, improved feed conversion efficiency and overall better health and productivity.

However, most studies have been conducted under controlled or intensive rearing systems, with limited information available on the effects of *Lactobacillus casei* supplementation under local or semi-intensive conditions, particularly in the Konkan region. This knowledge gap underscores the need to evaluate its efficacy in local broiler production systems. Therefore, the aim of the present study is to assess the effects of dietary supplementation of *Lactobacillus casei* on growth performance, feed utilization, immune response and overall health of broilers reared under local conditions.

2. MATERIALS AND METHODS

The present study was conducted to evaluate the effect of probiotic supplementation in drinking water on the growth performance of broiler chickens. The six-week trial was carried out at the Poultry Unit, Instructional Dairy Farm, College of Agriculture, Dapoli. Lactobacillus casei culture was procured from NDRI, Karnal and used as the probiotic supplement.

2.1 Management of Experimental Birds

A total of day-old broiler chicks were reared under a deep-litter housing system for six weeks in a well-ventilated shed. During the first week, double-folded newspapers were spread over the litter, which was then maintained at a depth of 3–5 inches throughout the trial. Standard brooding practices were followed, including provision of heat and 24-hour illumination during the first week. The brooding temperature was maintained between 32–35 °C. Clean, cool drinking water was supplied ad libitum throughout the study. Artificial lighting was provided at night for the remaining period. Separate feeders and drinkers were used for each replicate and prophylactic and hygienic measures were strictly followed to minimize disease risks.

2.2 Experimental Layout and Treatments

The experiment was conducted in a Randomized Block Design (RBD) with five treatments and four replications per treatment. Each replication consisted of 10 chicks. The treatment groups were:

T1: Basal diet (control, no supplementation)
T2: Basal diet + L. casei (10 ml/L drinking water)
T3: Basal diet + L. casei (20 ml/L drinking water)
T4: Basal diet + L. casei (30 ml/L drinking water)
T5: Basal diet + L. casei (40 ml/L drinking water)

2.3 Climatic and Weather Conditions

Dapoli is geographically situated in the sub-tropical region on the 17° 45' North latitude and 73°12' East longitude, on the West coast of Maharashtra at 280 meters above mean sea level. The climate is warm and humid with 6.2 mm average rainfall during experimental period. The relative humidity during experimental period was found 64.6 to 82.8 per cent.

2.4 Chemical Composition of Experimental Feed

The proximate composition of broiler starter and finisher rations is presented in Table 1, while the chemical composition of cow milk and probiotic curd is shown in Table 2.

Table 1. Average chemical composition of basal diet (per cent DM basis)

	Experimental feed	
Proximate principles	**Broiler Starter**	**Broiler Finisher**
Dry matter	90.43	92.56
Moisture	7.89	8.54
Ash	6.74	6.47
Protein	20.76	18.99
Crude Fiber	4.98	5.17
Crude Fat	4.87	4.32
Nitrogen Free Extract	60.79	63.82

Table 2. Average chemical composition of supplement in per cent

Milk constituents	Cow milk	Probiotic curd
Total solids	13.50	13.92
Protein	3.48	3.72
Fat	3.90	4.12
Ash	0.69	0.75
Lactose	4.68	4.26
Acidity	0.13	0.71

2.5 Body Weight and Weight Gain

The body weight of birds in each treatment group was recorded weekly, in the morning before feeding. Weekly weight gain was calculated as the difference between the average body weight of the current week and that of the previous week.

2.6 Feed Consumption

Daily feed intake was measured as the difference between feed offered and leftover feed after 24 hours. Weekly feed intake was calculated by summing daily intakes over seven days.

2.7 Feed conversion ratio (FCR)

The feed conversion ratio was calculated separately for each treatment group as:

$$\text{FCR} = \frac{\text{Weight Gain (g)}}{\text{Feed Consumed (g)}}$$

2.8 Statistical Analysis

The statistical method Analysis of Variance (ANOVA), suitable for a Randomized Block Design was used to analyse the collected data. For comparing treatments in terms of body weight gain, live body weight, water intake, feed consumption and feed conversion ratio, the standard errors (SE) and critical differences (CD) at the 5% level of significance were calculated and presented in the corresponding tables (Snedecor and Cochran, 1994).

3. RESULTS AND DISCUSSION

No significant differences in water intake were observed among the treatments during the first three weeks of the experimental period. However, during the fourth, fifth and sixth weeks, the mean weekly water consumption of the control group (T1) was significantly ($P<0.05$) higher, exhibiting a progressive decline with increasing levels of probiotic curd supplementation. As presented in Table 3, the total water intake was maximum in the control group T1 (9975.43 ml), which did not receive probiotic curd, followed by T2 (9971.47 ml) with 1% supplementation, T3 (9964.24 ml) with 2% supplementation and T5 (9952.59 ml) with 4% supplementation, while the lowest intake was recorded in T4 (9917.71 ml) with 3% supplementation. The reduction in water intake across supplemented groups may be attributed to alterations in the taste of water due to the addition of probiotic curd.

The findings of the present study are comparatively higher than those reported by Patil (2021), who observed that by the end of the sixth week, water consumption was highest in the control group (T0; 6971.25 ml per bird) and lowest in T3 (6311.53 ml per bird), with intermediate values in T1 (6800.62 ml per bird) and T2 (6586.02 ml per bird). Similarly, Wang et al. (2015) reported that supplementation of *Lactobacillus casei* P-8 in drinking water at a concentration of 2×10^6 CFU/mL resulted in enhanced body weight gain, higher feed intake and improved feed efficiency in chickens.

The average weekly feed consumption of the control group (T1) was significantly ($P<0.05$) higher, with a consistent decline observed as the level of probiotic curd supplementation increased. As presented in Table 4, the highest total feed intake was recorded in the control group T1 (3556.22 g), which did not receive probiotic curd, followed by T2 (3488.63 g) supplemented with 1%, T3 (3475.08 g) with 2% and T5 (3470.59 g) with 4% supplementation, while the lowest feed consumption was noted in T4 (3436.81 g) supplemented with 3% probiotic curd.

The present findings are in partial agreement with those of Taherpour et al. (2009), who reported significantly (P<0.05) higher feed intake in the control group (3824.90 g) compared with the supplemented groups. In their study, feed consumption was 3464.12 g in T2, 3386.38 g in T3, 3404.92 g in T4, 3506.53 g in T5, 3345.11 g in T6, 3595.50 g in T7 and 3361.92 g in T8. Notably, the T2 group received a basal diet supplemented with probiotics at 0.9 kg/ton during the starter phase, 0.45 kg/ton in the grower phase and 0.225 kg/ton in the finisher phase.

At the conclusion of the experimental period, the total body weight of group T4 (2128.60 g) was significantly (P<0.05) higher than that of all other treatment groups, followed by T5 (2072.50 g), T3 (1991.50 g), T2 (1956.80 g) and T1 (1833.15 g).

Table 3. Average weekly water intake (ml/week)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total	Mean
T_1	185.09	525.18	1537.97	2071.52^a	2573.43^a	3082.25^a	9975.43^a	1662.57^a
T_2	185.76	528.89	1536.00	2068.13^a	2568.94^a	3083.75^a	9971.47^a	1661.91^{ab}
T_3	185.84	527.25	1536.68	2063.07^b	2562.66^b	3088.75^a	9964.24^a	1660.71^{ab}
T_4	186.00	524.64	1533.75	2055.57^c	2554.18^c	3063.57^b	9917.71^b	1652.95^c
T_5	185.94	527.00	1535.74	2061.18^b	2559.98^c	3082.75^a	9952.59^a	1658.76^b
S.E. m	0.72	1.48	3.63	2.66	3.26	5.23	16.99	1.83
CD	NS	NS	NS	8.20	10.04	16.13	34.36	5.65

The values with different superscript differ significantly

Table 4. Average weekly feed consumption (g/week)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total	Mean
T_1	145.75	316.40	523.20	723.10^a	868.04^a	979.74^a	3556.22^a	592.70^a
T_2	145.25	315.23	521.01	717.53^{ab}	846.07^b	943.55^b	3488.63^b	581.44^b
T_3	145.25	316.39	519.84	715.94^b	840.62^b	937.04^b	3475.08^{bc}	579.18^{bc}
T_4	146.75	312.97	514.83	709.29^c	820.72^c	932.25^b	3436.81^c	572.80^c
T_5	144.25	316.98	517.51	716.72^b	839.72^b	935.42^b	3470.59^{bc}	578.43^{bc}
S.E. m	1.42	2.20	2.49	1.87	4.18	7.80	5.04	1.30
CD	NS	NS	NS	5.76	12.87	24.05	15.52	4.02

The values with different superscript differ significantly

Table 5. Average weekly body weight (g/week)

Treatment	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Mean
T_1	44.90	192.60	395.75	675.40^b	992.50^c	1387.00^c	1833.15^c	788.76c
T_2	45.33	197.90	404.05	718.75^b	1038.75^{bc}	1454.25^{bc}	1956.80^{bc}	830.83^{bc}
T_3	46.80	190.70	402.70	720.75^b	1058.10^{bc}	1479.95^{bc}	1991.50^{bc}	841.50^{bc}
T_4	44.67	192.25	414.63	749.38^a	1114.10^a	1572.425^a	2128.60^a	888.01^a
T_5	46.25	193.30	426.63	749.00^a	1094.22^{ab}	1536.88^{ab}	2072.50^{ab}	874.11^{ab}
S.E. m	0.68	2.80	8.13	3.69	6.51	6.69	21.47	3.77
CD	NS	NS	NS	11.36	20.05	20.62	66.17	11.62

The values with different superscript differ significantly

Table 6. Average weekly body weight gain (g/week)

Treatment	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Mean
T_1	44.90	147.70	203.15	279.65^c	317.10^b	394.50^c	446.15^c	261.88^d
T_2	45.33	152.58	206.15	314.70^b	320.00^b	415.50^c	502.55^b	279.54^c
T_3	46.80	143.90	212.00	318.05^b	337.35^b	421.85^{bc}	511.55^b	284.50^{bc}
T_4	44.68	147.58	222.38	334.75^a	364.73^a	458.33^a	556.18^a	304.09^a
T_5	46.25	147.05	233.33	322.38^{ab}	345.23^{ab}	442.65^{ab}	535.63^a	296.07^{ab}
S. E. m	0.68	3.11	9.33	9.10	7.56	5.61	20.53	3.07
CD	NS	NS	NS	28.05	23.31	17.30	63.26	9.45

The values with different superscript differ significantly

The findings of the present study are in partial agreement with Anjum et al. (2005), who reported final body weights of 1904 ± 17 g in group A, 1967 ± 09 g in group B and 2000 ± 18 g in group C, with significantly (P<0.05) higher gains observed in chicks fed diet C compared to diet B. Similarly, Toghyani et al. (2011) documented a body weight of 2110.18 g in the control group at 42 days, while probiotic-supplemented birds attained 2138.30 g.

At the end of the experimental trial, the highest average body weight gain was observed in group T4 (556.18 g), which received 3% probiotic curd, followed by T5 (535.63 g), T3 (511.55 g), T2 (502.55 g) and the control group T1 (446.15 g), respectively.

The results of the present study show partial similarity to the findings of Patil (2021), who reported that during the sixth week, the average weekly body weight gain of broilers was significantly highest in T2 (568.13 g), followed by T1 (542.26 g), T3 (431.67 g) and the control group T0 (474.41 g).

As shown in Table 7, the highest average body weight gain at the end of the experiment was observed in group T4 (2083.92 g), which received 3% probiotic curd. This was followed by T5 (2026.25 g), T3 (1944.70 g), T2 (1911.47 g), while the lowest gain was recorded in the control group T1 (1788.25 g).

The outcomes of the present study are comparatively lower than those reported by Patil (2021), who found the greatest body weight gain in the T1 group (2556.51 g/bird) supplemented with 10% probiotic cultured whey, followed by T2 (2493.34 g/bird) with 20% supplementation and T3 (2334.85 g/bird) with 30% supplementation. In that study, the control group (T0) recorded the lowest gain (2334.86 g/bird) by the end of the sixth week.

Variations in growth performance between studies may be attributed to differences in environmental factors such as temperature, humidity and ventilation. Moreover, the genetic strain of broilers and the nutrient composition of diets are also likely to contribute to the observed differences in body weight gain.

Feed conversion ratio (FCR) values during the first two weeks did not differ significantly among treatments. From week 3 onwards, significant (P<0.05) differences were observed, with the control group (T1) showing the highest FCR, indicating poorer feed efficiency. Probiotic-supplemented groups exhibited reduced FCR, with T4 (3% probiotic curd) recording the lowest mean value (1.56), signifying superior feed utilization compared to the control (2.07). The improvement in FCR may be attributed to enhanced nutrient digestibility and better gut health conferred by probiotic supplementation.

Table 7. Total body weight gain at end of 6th week (g)

Treatments	Day-old weight	Weight at the end of 6th week	Total body weight gain at the end of 6th week
T_1	44.90	1833.15^{d}	1788.25^{c}
T_2	45.33	1956.80^{c}	1911.47^{b}
T_3	46.80	1991.50^{b}	1944.70^{b}
T_4	44.68	2128.60^{a}	2083.92^{a}
T_5	46.25	2072.50^{a}	2026.25^{a}
S. E. m	0.68	21.47	21.43
CD	NS	66.17	66.02

The values with different superscript differ significantly

Table 8. Average weekly feed conversion ratio

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Mean
T_1	0.99	0.95	2.10^{a}	2.63^{a}	2.76^{a}	2.96^{a}	2.07^{a}
T_2	0.95	1.53	1.66^{b}	2.25^{b}	2.04^{b}	1.91^{b}	1.72^{b}
T_3	1.01	1.50	1.64^{b}	2.12^{c}	1.99^{b}	1.83^{b}	1.68^{bc}
T_4	1.00	1.42	1.55^{c}	1.95^{d}	1.79^{c}	1.68^{c}	1.56^{d}
T_5	0.98	1.37	1.61^{c}	2.08^{c}	1.90^{c}	1.75^{c}	1.61^{cd}
S. E. m	0.02	0.06	0.05	0.04	0.03	0.07	0.01
CD	NS	NS	0.15	0.13	0.08	0.23	0.04

Comparable findings were reported by Patil (2021), who noted that at the end of the trial, treatments T1 (1.71) and T2 (1.71) exhibited significantly better FCR compared to the control (T0; 1.81) and T3 (1.99).

4. CONCLUSION

The findings of the present investigation clearly demonstrate that dietary supplementation of probiotic curd exerts a beneficial influence on broiler performance by enhancing growth, improving feed efficiency and optimizing water utilization, without negatively affecting feed intake during the initial growth phase. Among the treatments, supplementation with 3% probiotic curd (T4) proved most effective, yielding significantly higher body weight gain (2083.92 g), superior feed conversion ratio (1.68), reduced feed intake (3436.81 g) and lower mortality. Water intake remained unaffected and no morbidity was observed across all experimental groups.

Overall, supplementation at 3% (equivalent to 30 ml/L of drinking water) emerged as the optimal inclusion level. Importantly, probiotic curd supplementation also demonstrated potential in alleviating the adverse effects of heat stress, a major constraint in tropical and subtropical poultry production. By improving gut health, nutrient absorption and immune response, probiotic curd at 3% supplementation can be recommended as a practical and effective dietary strategy to enhance broiler productivity, feed efficiency and resilience under heat stress conditions, thereby supporting sustainable poultry production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

All procedures involving animals in this study were carried out following ethical standards and welfare guidelines. Prior approval was obtained from the Institutional Animal Ethics Committee (IAEC), Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The animals were handled humanely, with due care taken to minimize discomfort and ensure well-being throughout the research period.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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