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Effect of Dietary Supplementation of *Bacillus Subtilis* and *Bacillus Clausii* based Probiotics alone or in combination on Performance in Jabalpur's Colour Birds

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ABSTRACT

The study was conducted to investigate the effect of dietary supplementation of *Bacillus subtilis* and *Bacillus clausii* based probiotics alone or in combination on performance in Jabalpur colour birds. A total of two hundred forty, 30 weeks age laying hens Jabalpur colour bird were selected from the poultry farm, adhartal Jabalpur. These layers were randomly distributed to five replicates of six layers each and allotted to 8 dietary treatments. The standard layer diet (T0) was formulated as per ICAR (2013) specification. T1: Control diet + antibiotic (avilamycin@100g/ ton of feed) T2: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @250g / ton of feed) T3: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @500g / ton of feed)T4: Control diet + *Bacillus clausii* (2× 10^9cfu/ g) @250g /ton of feed) T5: Control diet + *Bacillus clausii* (2× 10^9cfu/ g) @500g / ton of feed) T6: Control diet + *Bacillus subtilis* (125g) + *Bacillus clausii* (125g) (2× 10^9cfu/ g) @250g/ ton of feed) T7: Control diet +*Bacillus subtilis* (250g) + *Bacillus clausii* (250g) (2× 10^9cfu/ g) @500g/ ton of feed) respectively for 12 weeks of experimental period. Daily egg production recorded, there by hen day production was calculated. The result of 12 weeks (30^{th} to 42^{nd} weeks) of present study indicated that supplementation of *Bacillus subtilis* in combination with *Bacillus clausii* in layer diet significantly ($p<0.05$) improved overall production performance egg production and hen day production in Jabalpur colour birds.

Keywords: *Bacillus clausii, Bacillus subtilis,* Birds, Dietary supplementation, Probiotics

INTRODUCTION

Currently, the total Poultry population in our country is 851.81 million (as per 20^{th} Livestock Census and egg production is around 129.60 billion during 2021-22. The per capita availability during 2021-22 was around 95 eggs per annum. The Egg production has shown positive growth as 6.19% during 2021-22. For enhancing the productivity of laying hens and lower the incidence of disease commercially, a huge number of antibiotic growth promoters (AGPs) are given. Overuse of antibiotics at doses below therapeutic levels resulted in the development of superbugs, germs that are resistant to antibiotics and persists in both human and animal food chains (Xiang *et al,* 2019). Consequently, the European Union has restricted the use of AGPs in feed since 2006.Therefore; alternatives to antibiotics are urgently needed.

Probiotics of various commercial preparations are being considered worldwide for poultry as performance enhancers and suitable alternatives to antibiotics. The probiotics are considered as "direct-fed microbial" and they affect the host positively by balancing the intestinal microbial populations.

It is well recognized that probiotics organisms are beneficial for the host animals (Mazanko *et al,* 2018; Neijat *et al,* 2019) and the most commonly used probiotics organisms are *Lactobacillus spp., Bacillus spp., Enterococcus spp., Streptococcus thermophillus, Bifidobacterium spp., Escherichia coli* and fungal species. Spore-forming probiotics, (SFPs) especially Bacillus species, (like *Bacillus Subtilis, Bacillus clausii and Bacillus lechiformis)* have promising approach over other probiotics. Due to their encapsulation sporulation ability SFPs can reach the specific part of the gastrointestinal (GI) tract easily which is associated with their survival and colonization in the digestive tract (Khalid *et al,* 2022).

Of particular interest is a study By Adangle *et al* (2025) in which the authors demonstrated that dietary supplementation probiotic into four treatment groups, T0 (Diet without probiotic (Control), T1 (diet + 500 g probiotic (*Bacillus subtilis*)/ton of

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Table 1. Ingredient composition (% DM basis) of experiment diets.

Feed ingredient	Experimental diets (%)					
	T_0	T_1	T_2	T_3	T_4	T_5
Maize (kg)	450	450	450	450	450	450
Rice Polish (kg)	250	250	250	250	250	250
Soyaben meal (kg)	221	221	221	221	221	221
Shell grit (kg)	40	40	40	40	40	40
Calcite/LSP(kg)	25	25	25	25	25	25
Dicalcium phosphate (kg)	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix(kg)	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix (kg)	2.5	2.5	2.5	2.5	2.5	2.5
Salt (kg)	6.5	6.5	6.5	6.5	6.5	6.5
Total	1000	1000	1000	1000	1000	1000
Nutrient composition calculated (%)						
Crude protein	16.5	16.5	16.5	16.5	16.5	16.5
Ca	3.26	3.26	3.26	3.26	3.26	3.26
Total P	0.44	0.45	0.44	0.44	0.44	0.45
Energy (Kcal ME/kg diet)	2602	2599	2602	2602	2602	2599
Nutrient composition analysed (%)						
Crude protein	16.32	16.40	16.61	16.20	16.59	16.70
Ca	3.30	3.32	3.39	3.21	3.22	3.41
Total P	0.37	0.33	0.49	0.39	0.31	0.35

Table 2. Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups

	Treatment							
Age (weeks)	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
31st	4.06±0.20	4.08±0.05	4.10±0.14	4.12±0.12	4.12±0.10	4.16±0.09	4.22±0.08	4.26±0.12
32nd	4.06±0.16	4.16±0.06	4.12±0.12	4.20±0.22	4.04±0.11	4.12±0.13	4.40±0.18	4.50±0.32
33rd	4.10±0.19	4.10±0.13	4.04±0.07	4.26±0.19	4.10±0.08	4.32±0.18	4.24±0.11	4.48±0.14
34th	4.18±0.26	4.62±0.36	4.28±0.09	4.26±0.19	4.34±0.18	4.30±0.23	4.16± 0.09	4.48±0.23
35th	4.14^{b}±0.16	4.80^{ab}±0.20	5.10^{ab}±0.43	4.70^{ab}±0.34	4.56^{ab}±0.30	4.58^{ab}±0.29	5.10^{ab}±0.29	5.38^{a}±0.31
36th	4.20^{b}±0.21	4.62^{ab}±0.24	5.02^{ab}±0.31	4.90^{ab}±0.37	4.66^{ab}±0.15	4.80^{ab}±0.29	5.26^{a}±0.36	5.14^{ab}±0.37
37th	4.22±0.33	4.90±0.33	4.92±0.28	4.40±0.29	4.66±0.26	4.94±0.26	5.04±0.33	5.04±0.24
38th	4.12^{b}±0.24	4.84^{ab}±0.22	4.84^{ab}±0.27	4.94^{ab}±0.26	4.50^{ab}±0.38	4.58^{ab}±0.25	5.12^{a}±0.38	4.92^{ab}±0.29
39th	4.54±0.33	4.54±0.21	4.70±0.20	5.14±0.30	4.42±0.18	4.50±0.45	4.94±0.26	4.62±0.39
40th	4.44±0.31	4.32±0.19	4.70±0.25	5.10±0.29	4.52±0.27	4.44±0.28	4.88±0.32	4.70±0.25
41st	4.50±0.31	4.44±0.23	4.52±0.16	4.88±0.29	4.50±0.27	4.50±0.16	4.72±0.24	4.70±0.41
42nd	4.94±0.34	4.52±0.13	4.48±0.16	4.90±0.29	4.30±0.20	4.30±0.44	4.70±0.12	4.74±0.38
Avg.	4.29^{c}±0.09	4.50^{abc}±0.08	4.57^{abc}±0.09	4.65^{ab}±0.12	4.39^{bc}±0.06	4.46^{abc}±0.05	4.73^{a}±0.11	4.75^{a}±0.13

feed.), T2 (diet + 1000 g probiotic (*Bacillus subtilis*)/ton of feed) and T3 (Diet + 1500 g probiotic (*Bacillus subtilis*)/ton of feed) hens receiving *Bacillus subtilis* supplementation (especially T2) exhibited significantly higher feed intake and enhanced egg production, with HDEP. Supplementation of *Bacillus clausii* @ 0.03 ml/L as probiotic in drinking water significantly improved the liver function and humoral immunity of broiler chicks (Mushtaq *et al*, 2023).

Although a great deal of work has been done with probiotics, mostly with broiler chickens. However, studies examining the effect of *Bacillus clausii* on laying hens are limited and results of comparative effects of single strain- and multi-strain spore forming probiotics preparations on egg type chickens are scanty in the literature. Based on previous research, two types of probiotics bacteria were chosen to be administrated in a laying hen diet.

MATERIALS AND METHODS

A total of two hundred forty, 30 weeks age laying hens Jabalpur colour birds were selected from the poultry farm, adhartal Jabalpur. These layers were randomly distributed to five replicates of six layers each and allotted to 8 dietary treatments. The standard layer diet (T_0) was formulated as per ICAR (2013) specification. T_1: Control diet + antibiotic (avilamycin@100g/ ton of feed) T_2: Control diet + *Bacillus subtilis* (2× 10^9cfu/g) @250g / ton of feed) T_3:

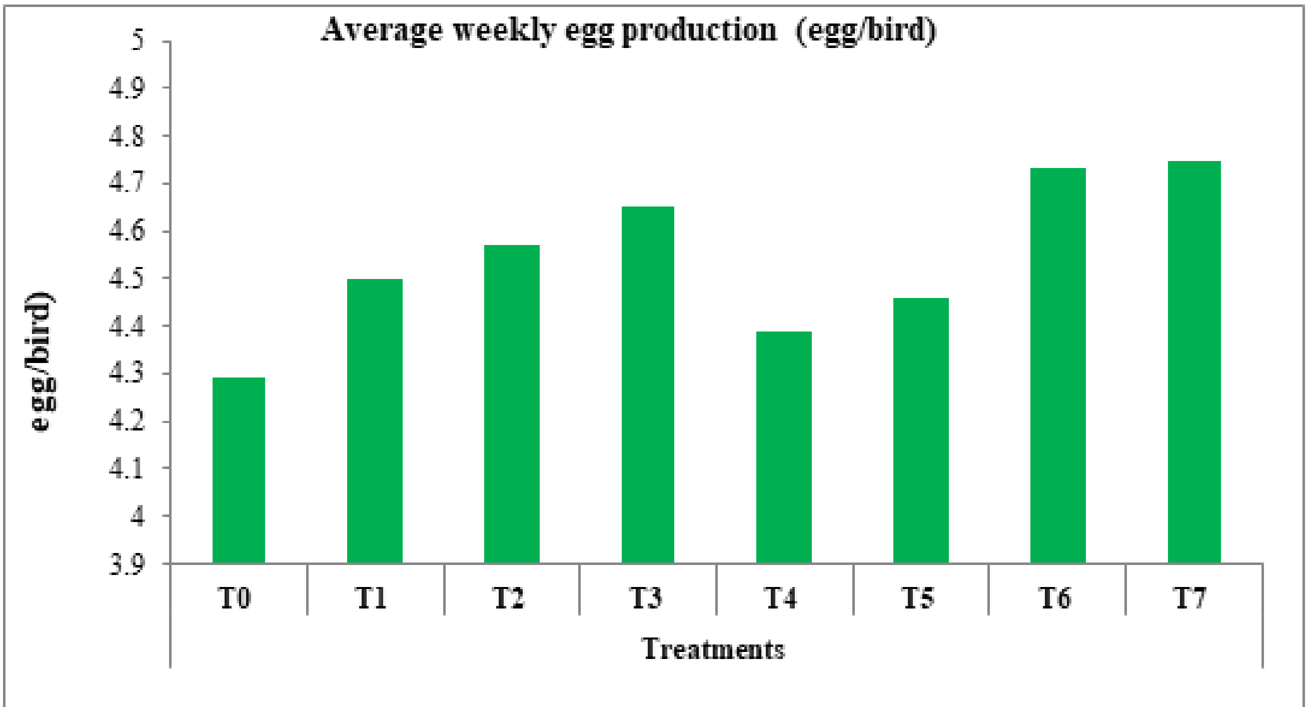

Fig 1.Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups

Control diet + *Bacillus subtilis* ($2\times 10+^{9}$cfu/g) @500g / ton of feed) T_4: Control diet + *Bacillus clausii* (2×10^{9}cfu/ g) @250g /ton of feed)T_5: Control diet + *Bacillus clausii* (2×10^{9}cfu/ g) @500g / ton of feed) T_6: Control diet + *Bacillus subtilis* (125g) + *Bacillus clausii* (125g) (2×10^{9}cfu/ g) @250g/ ton of feed) T_7: Control diet +*Bacillus subtilis* (250g) + *Bacillus clausii* (250g) (2×10^{9}cfu/ g) @500g/ ton of feed) respectively.

The feed ingredients were procured from the market and analyzed for proximate composition before formulation of diets. The experimental diets were formulated as per layer feed (ICAR, 2013), (16.5% CP and 2600 kcal ME/kg diet). The analyzed protein and ME values of feed ingredients were used for computation of rations. Composition of experimental layer diets used in the study is given in Table 1. The experimental was conducted for 12 weeks were Jabalpur colour birds managed under same environment. The production performance of birds closely monitors throughout trail periods. Replicate-wise weekly egg production was recorded and hen day production was calculated. Statistical analysis of the data was done by using analysis of variance using complete randomized design as per Snedecor and Cochran (1994). Difference among the treatments was tested for significance by Duncan's Multiple Range Test (1995).

RESULTS AND DISCUSSION

Production performance

The average weekly egg production (eggs/bird) and hen day production of Jabalpur colour birds under different treatments from the 31st to 42nd week was recorded summarized in Table 2 and Figure 1. At the beginning (31st week), egg production ranged from 4.06±0.20 (T_0) to 4.26±0.12 (T_7), with no significant difference ($p>0.05$) among groups. During the experimental period, weekly egg production gradually increased across treatments, reaching the highest values in the 35th 36th and 38th weeks, The weekly egg production significantly ($p>0.05$) increases in spore forming probiotics (*Bacillus subtilis* and *Bacillus clausii*) combination diets T_6 and T_7 as compared to control (T_0) fed on basal diet at 35th 36th and 38th weeks. At the end of the experiment (42nd week), the egg production was 4.94±0.34, 4.52±0.13, 4.48±0.16, 4.90±0.29, 4.30±0.20, 4.30±0.44, 4.70±0.12, and 4.74±0.38 for groups T_0, T_1, T_2, T_3, T_4, T_5, T_6, and T_7, respectively.

The overall average weekly egg production (egg/bird) showed wide variation among all dietary treatments. The overall average weekly egg production (egg/bird) was significantly ($p<0.05$) higher in birds allotted T_1 diet (antibiotic supplemented or positive control diet) (4.50±0.08), as compared to birds fed on

Table 3.Average weekly hen day production (%) of Jabalpur colour birds in different treatment groups

	Treatments							
Age in weeks	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
31st	67.67± 3.28	68.00± 0.82	68.33± 2.36	68.67± 1.93	68.67± 1.70	69.33± 1.45	70.33± 1.33	71.00± 1.94
32nd	67.67± 2.72	69.33± 1.00	68.67± 2.07	70.00± 3.61	67.33± 1.87	68.67± 2.20	73.33± 2.93	75.00± 5.38
33rd	68.33± 3.12	68.33± 2.17	67.33± 1.13	71.00± 3.10	68.33± 1.39	72.00± 2.95	70.67± 1.80	74.67± 2.32
34th	69.67± 4.33	77.00± 5.99	71.33± 1.43	71.00± 3.23	72.33± 3.05	71.67± 3.80	69.33± 1.55	74.67± 3.78
35th	69.00^{b}± 2.67	80.00^{ab}± 3.33	85.00^{ab}± 7.15	78.33^{ab}± 5.65	76.00^{ab}± 4.99	76.33^{ab}± 4.81	85.00^{ab}± 4.86	89.67^{a}± 5.12
36th	70.00^{b}± 3.50	77.00^{ab}± 3.96	83.67^{ab}± 5.15	81.67^{ab}± 6.12	77.67^{ab}± 2.45	80.00^{ab}± 4.89	87.67^{a}± 6.07	87.67^{a}± 6.23
37th	70.33± 5.46	81.67± 5.53	82.00± 4.61	73.33± 4.86	77.67± 4.37	82.33± 4.37	84.00± 5.44	84.00± 3.97
38th	68.67^{b}± 4.06	80.67^{ab}± 3.75	80.67^{ab}± 4.58	82.33^{ab}± 4.37	75.00^{ab}± 6.30	76.33^{ab}± 4.20	85.33^{a}± 6.35	82.00^{ab}± 4.75
39th	75.67± 5.54	75.67± 3.56	78.33± 3.33	85.67± 4.99	73.67± 2.95	75.00± 7.47	82.33± 4.30	77.00± 6.55
40th	74.00± 5.21	72.00± 3.22	78.33± 4.25	85.00± 4.86	75.33± 4.42	74.00± 4.61	81.33± 5.33	78.33± 4.25
41st	75.00± 5.24	74.00± 3.86	75.33± 2.66	81.33± 4.84	75.00± 4.56	75.00± 2.63	78.67± 4.03	78.33± 6.77
42nd	75.33± 5.74	75.33± 2.13	74.67± 2.66	81.67± 4.86	71.67± 3.33	71.67± 7.26	78.33± 2.04	79.00± 6.40
Avg.	71.53^{c}± 1.50	74.92^{abc}± 1.28	76.14^{abc}±1.44	77.50^{ab}± 1.93	73.22^{bc}± 1.05	74.36^{abc}±1.76	78.86^{a}± 1.79	79.11^{a}± 2.15

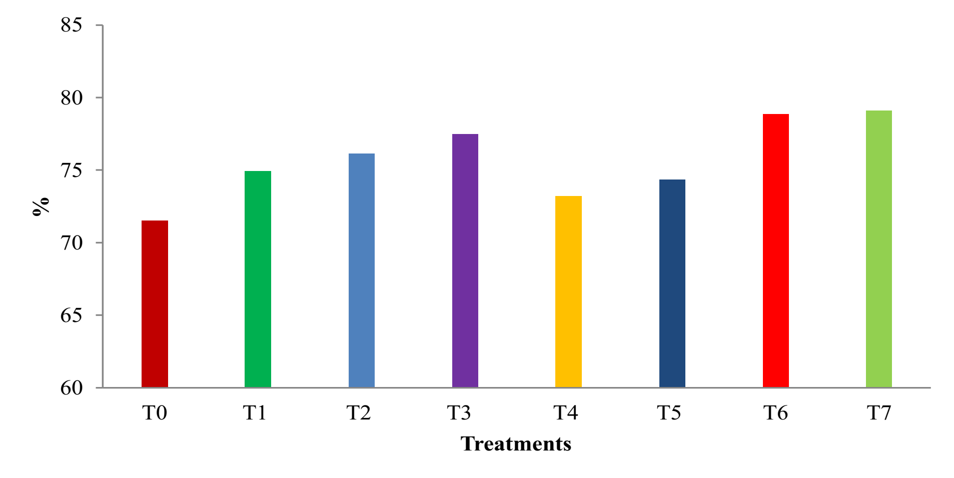

Fig 2. Average weekly hen day production (%) of Jabalpur colour birds in different treatment groups

control (T_0) or basal diet (4.29±0.09). Birds supplemented with different levels of *Bacillus subtilis and Bacillus clausii* strain alone or in combination preparations of spore forming probiotics caused significantly ($P<0.05$) improvement in the average weekly egg production (egg/bird). Among probiotic supplemented diets, maximum and significantly ($P<0.05$) higher average weekly egg production (egg/bird) were observed in birds allotted T_6 (4.73±0.11) and T_7 (4.75±0.13) diets. Probiotics combination diets (*Bacillus subtilis and Bacillus clausii* @125g and 250g/ton each). However, statistically it was comparable ($P>0.05$) to birds allotted T_2, T_3, T_5 and positive control T_1 diets. Minimum and significantly ($P<0.05$) lowest average weekly egg production (egg/bird) was found with those birds fed on T_0 and T_4 diets.

The average weekly hen-day production (%) of Jabalpur colour birds from the 31st to 42nd week of age under different dietary treatments (T_0–T_7) was recorded, summarized in Table 3. At the 31st week, the production percentage ranged from 67.67±3.28 in T_0 to 71.00±1.94 in T_7, with no significant differences among groups. A gradual increase was observed across treatments as the experiment progressed. By the 35th week, production was significantly higher ($p<0.05$) in supplemented groups, with values ranging from 76.00±4.99 in T_4 to 89.67±5.12 in T_7, compared to the control (T_0: 69.00±2.67). During the 36th to 38th weeks, production remained consistently higher in supplemented groups. The highest values were recorded in T_6 (87.67±6.07) and T_7 (87.67±6.23) at the 36th week and T_6 (85.33±6.35) at the 38th week, which were significantly higher ($p<0.05$) than the control (T_0: 68.67±4.06).

From the 39th to 42nd weeks, production levels stabilized across treatments, no marked differences ($p>0.05$) were observed among groups during this period, although the supplemented groups consistently maintained higher production compared to the control.

Birds supplemented with different levels of *Bacillus subtilis and Bacillus clausii* strain alone or in combination preparations of spore forming probiotics caused significantly (P<0.05) improvement in the HDP%. Among probiotic supplemented diets, maximum and significantly (P<0.05) higher HDP% were observed in birds allotted T_6 (78.86±1.79) and T_7 (79.11±2.15) diets (Probiotics combination diets (*Bacillus subtilis and Bacillus clausii* @125g and 250/ton each). However, statistically it was comparable (P>0.05) to birds allotted T_2, T_3, T_5 and positive control T_1 diets. Minimum and significantly (P<0.05) lowest HDP% were found with those birds fed on T_0 and T_4 diets.

The overall hen day egg production (%) showed wide variation among all dietary treatments. The overall HDP% was significantly (p<0.05) higher in birds allotted T_1 diet (antibiotic supplemented or positive control diet) (74.92±1.28), as compared to birds fed on control (T_0) or basal diet (71.53±1.50). Therefore, birds supplemented with different levels of *Bacillus subtilis* and *Bacillus clausii* strain alone or in combination preparations of spore forming probiotics improved HDP% of birds, significantly (P<0.05) when compared with non-supplemented groups and also statistically it was comparable (P>0.05) to birds allotted positive control (antibiotic supplemented) T_1 diets and thus suitable alternatives to antibiotics. Therefore, birds supplemented with different levels of *Bacillus subtilis* and *Bacillus clausii* strain alone or in combination preparations of spore forming probiotics can be considered as performance enhancers and suitable alternatives to antibiotics.

Average weekly egg production (egg/bird) of Jabalpur colour birds in different treatment groups was significantly higher in *Bacillus subtilis and Bacillus clausii* strain alone supplemented group as well as or in combination preparations of spore forming probiotics. In accordance with our findings, (Chen *et al*, 2020) reported the effect of dietary supplementation with *Bacillus subtilis* increased egg production and egg mass significantly compared to control group. (Liu *et al*, 2021) reported that laying hens fed diet that dietary *Bacillus subtilis* and essential oils (BSEO) supplementation on 900mg/kg (BSEO) significantly improve egg production. This improvement may be attributed to the action of several enzymes, including protease, amylase and cellulase, secreted in the gastrointestinal tract, whose activity can be enhanced by BS or EO supplementation (Li *et al,* 2018). Moreover, *Bacillus subtilis* has been reported to promote better gut morphology and stimulate the growth of beneficial intestinal microflora, which in turn enhances nutrient utilisation efficiency.

However, similarly (Ray *et al,* 2022) reported dietary supplementation of single-strain probiotics (SSP) containing *Bacillus Subtilis* (2×10^9cfu/g) and multi-strain probiotic (MSP) containing *Lactobacillus Acidophilus* $3 \times 10^7 - 10^8$ cfu/g, *Bacillus Subtilis* 3×10^7-10^8 cfu/g and *Saccharomyces Cerevisiae* $10^6 - 10^7$ cfu/g linearly increased egg production with dose increment in the MSP fed birds. The present results obtained are also in line with earlier reports (Darsi and Zhaghari, 2021) dietary *Bacillus subtilis* PB6 supplementation significantly improved egg production in broiler breeders at the late phase of production but, egg weight was not significantly influenced by probiotic supplementation. This effect could be attributed to the continuous administration of probiotics, which may have inhibited harmful microorganisms, thereby enhancing the overall health status.

In contast with our results (Sobczak and Kozłowski, 2015) suggested that there was no significant difference in egg production due to supplementation of *Bacillus subtilis* (1×10^8 CFU/kg) probiotics. Similarly, (Fathi *et al,* 2018) found that probiotics (*Bacillus subtilis*) supplementation in layer diets did not affect egg production traits compared with control group.

CONCLUSION

The overall production performance of the Jabalpur colour birds in terms of egg production and hen day egg production was improved by supplementation of with combination of *Bacillus subtilis* and *Bacillus clausii* probiotics (@125g each or @250g/ ton of feed, each) in comparison to those fed on control, positive control. Further investigations into optimal dosage levels of probiotics and synergistic combinations are essential for a more comprehensive understanding of their beneficial effects.

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