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Effects of Adding Batcinel-K® Probiotic and Vaccine CEVAC SET-K® on Some Biochemical Markers of Broilers Parents' Stock

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ABSTRACT

The combined use of probiotics and vaccines offers a potential strategy to enhance poultry health and reduce dependence on antibiotics. However, the metabolic impact of such interactions in broiler breeder chickens remains insufficiently explored. This study aimed to evaluate the effects of Batcinel-K® (*Bacillus subtilis* BIM-B 454 D) and the inactivated *Salmonella* vaccine CEVAC SET-K® on lipid profile, carbohydrate metabolism, renal indices, and mineral status in broiler breeder chickens. Three hundred Ross 308 chicks were distributed into six equal groups: control (no treatment), probiotic only, vaccine only, and three combined probiotic + vaccine regimens: Regimen A (four probiotic cycles 1–35 days + vaccination at 42 and 98 days + booster probiotic 91–98 days), Regimen B (four probiotic cycles 1–35 days + recurrent 3-day probiotic dosing 42–91 days + vaccination 42 and 98 days), and Regimen C (two probiotic cycles 1–21 days + intermittent 2-day probiotic dosing 42–91 days + single vaccination 98 days). Serum collected at 56, 63, and 112 days was analyzed for total cholesterol, triglycerides, glucose, uric acid, creatinine, calcium, phosphorus, and magnesium. At 112 days, Regimen A produced a significant decline in total cholesterol and triglycerides ($P < 0.05$), accompanied by reduced uric acid and creatinine at 63–112 days. Glucose concentrations increased significantly in all probiotic groups, while magnesium was elevated in Regimen A at 112 days. Supplementation with Batcinel-K® particularly when applied under Regimen A in combination with CEVAC SET-K®, improved lipid metabolism and mineral status in broiler breeders. Structured probiotic–vaccine programs may therefore enhance metabolic resilience and overall flock performance.

Keywords: *Bacillus subtilis*, broiler breeder, CEVAC SET-K®, probiotic

INTRODUCTION

The global poultry industry faces increasing pressure to produce high-quality animal protein while minimizing reliance on antibiotic growth promoters, which are linked to antimicrobial resistance and residues in food products (**1**). Consequently, sustainable alternatives that enhance poultry health, performance, and food safety are critically needed. Among these, probiotics—live microorganisms that confer a health benefit to the host—

have gained prominence for their ability to modulate gut microbiota, improve nutrient absorption, and stimulate immune function (**2,3**).

In poultry, Bacillus subtilis-based probiotics have demonstrated efficacy in promoting growth, enhancing innate immunity, and increasing resistance to pathogens such as Salmonella (**4,5**). Concurrently, vaccination remains a cornerstone of disease prevention, with inactivated bacterins like CEVAC SET-K® (targeting *Salmonella* enteritidis and *S*. typhimurium) widely used to control salmonellosis in flocks (**6**). Notably, emerging evidence suggests that probiotics may act synergistically with vaccines, potentially augmenting humoral and cell-mediated immune responses (**7,8**).

While the individual effects of probiotics and vaccines are well-documented, their combined impact on the systemic metabolism of broiler breeder chickens remains insufficiently explored. Most studies focus on growth performance, gut health, or immune titers in broilers, with limited data on how structured probiotic–vaccine programs influence metabolic homeostasis in breeders—a critical aspect for long-term flock productivity and resilience.

In a preceding study, we evaluated the effects of Batcinel-K® and CEVAC SET-K® on hematological and basic biochemical parameters in Ross 308 broilers, finding significant improvements in hemoglobin, total protein, and globulin levels, particularly in groups receiving both probiotic and vaccine (**9**). These findings indicated enhanced natural resistance and protein metabolism. However, a detailed assessment of lipid profile, carbohydrate metabolism, renal function, and mineral status—key indicators of metabolic health—was not conducted.

We hypothesized that administering Batcinel-K®, alone or in combination with CEVAC SET-K®, would beneficially alter serum biochemical indices related to energy and mineral metabolism in broiler breeders. Therefore, the objective of this study was to quantify the effects of Batcinel-K® and CEVAC SET-K®—singly and in defined combined regimens—on serum cholesterol, triglycerides, glucose, uric acid, creatinine, calcium, phosphorus, and magnesium at 56, 63, and 112 days of age.

MATERIALS AND METHODS

Ethical Approval

All procedures conformed to the Guidelines for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee of the College of Veterinary Medicine, University of Al-Qadisiyah (Approval No. 1244; 1 August 2017).

Animals and Housing

A total of 300 one-day-old Ross 308 broiler chicks (straight-run, average initial body weight 40 ± 3 g) were obtained from the Vitebsk Broiler Chicken Farm (Belarus). Upon arrival, chicks were randomly allocated into six experimental groups of 50 birds each. All birds were housed in floor pens on dry wood shaving litter (5–8 cm depth) under standardized conditions. Ambient temperature was maintained at 32–34°C during the first week and gradually reduced to 20–22°C by the end of the trial. Relative humidity was kept between 50–70%. A lighting schedule of 23L:1D was provided initially, gradually adjusted to 18L:6D by week four and maintained thereafter. Birds had ad libitum access to water and a standard broiler diet formulated to meet NRC requirements (**10**). General health was monitored daily.

Probiotic and Vaccine

The probiotic preparation used in this study was Batcinel-K®, a liquid formulation containing Bacillus subtilis strain BIM-B 454 D. This product was developed by the Microbiology Institute of the Belarusian National Academy of Sciences in cooperation with the Institute of Experimental Veterinary Medicine. The vaccine used was CEVAC SET-K® (Ceva Santé Animale, France), a formalin-inactivated bacterin composed of *Salmonella* enteritidis and Salmonella typhimurium. It was administered subcutaneously at a dose of 0.5 mL per bird according to the manufacturer's instructions

Experimental Design

The study was designed to evaluate the effects of Batcinel-K® and CEVAC SET-K®, individually and in combination, on and physiological responses in broiler chicken breeder. Group 1 (G1) served as the negative control and received a standard balanced diet without any probiotic supplementation or vaccination. Group 2 (G2) received only the probiotic; birds were administered Batcinel-K® via drinking water at 0.1–0.2 mL per fowl per daily five consecutive day in nine cycles, each separated by 7–10 day intervals, continuing until the end of the trial. Group 3 (G3), designated as the vaccine-only group, received the standard ration and was vaccinated subcutaneously with CEVAC SET-K® at 47 days of age, followed by a booster dose at 98 days, both at 0.5 mL per bird.

Group 4 (G4) received both probiotic and vaccine following regimen A. From day 1 to day 35, birds were given Batcinel-K® at 0.1–0.2 mL per bird daily for five days, repeated in four cycles spaced 7–10 days apart. At 42 day of old, the probiotic was administered at 0.2 mL per bird per day for five days. Birds were then vaccinated at 42 day of old and revaccinated at 98 days with 0.5 mL per bird subcutaneously. In addition, probiotic supplementation resumed at day 91–98 at 0.3 mL per fowl daily for five days.

Group 5 (G5) received the same vaccine schedule as G4, but with a modified probiotic regime (Regime B). These birds received the probiotic from day 1 to day 35 in four cycles of five days each, spaced 7–10 days apart, at 0.1 to 0.2 mL per fowl per day. At 42 days, the dose was adjusted to 0.2 mL per bird per day for three days. From day 42 to 91, the birds received 0.3 mL per bird per day for three days, repeated every 7–10 days.

Group 6 (G6) followed regime C and was given the probiotic from day 1 to day 21 in two cycles of five days

each (7 days apart) at 0.1–0.2 mL per bird per day. At day 28, the probiotic was given at 0.2 mL per bird per day for two days. From day 42 to 91, birds received 0.3 mL per bird per day for two days per cycle, with each cycle separated by 7–10 days. A single vaccine dose of 0.5 mL per bird was administered subcutaneously at day 98.

Sampling and Biochemical Analyses

From each group, five birds were randomly selected at 56, 63, and 112 days. Approximately 5 mL of blood was collected from the axillary vein or by cardiac puncture using sterile syringes. Samples were allowed to clot at 18–20°C and centrifuged at 1,500 rpm for 10–15 min to obtain serum.

Serum total cholesterol and triglycerides were quantified using enzymatic assays (mmol/L). Uric acid and creatinine were measured colorimetrically (µmol/L and µmol/L, respectively); glucose was determined enzymatically (mmol/L). Calcium was determined by complexometric titration (EDTA), inorganic phosphorus by vanadate–molybdate colorimetry, and magnesium by enzymatic assay. Analyses were performed on a Eurolyser® analyzer (Cormay, Poland) with manufacturer-validated reagents.

Statistical Analysis

Statistical analyses were conducted using the pen as the experimental unit. For each sampling time (days 56, 63, and 112), data were summarized as pen means (n = 5 pens per treatment), and treatment groups were compared using one-way ANOVA for each biochemical parameter in SPSS software (version 19.0). When the ANOVA was significant, Dunnett's post-hoc test was applied to compare each treated group with negative control. Results are presented as mean ± SEM of pen means, and statistical significance was set at $P < 0.05$.

RESULTS

The results of blood tests on birds showed that the probiotic, during all growth periods, possessed a positive effect on some blood properties. The experimental groups that received the probiotic and vaccine CEVAC SET-K® against salmonellosis clearly demonstrated the obvious values.

The results of the first group presented in **Table 1** show numerical and not significant differences in cholesterol and triglycerides throughout the experiment. The experimental groups showed non-significant increase in these indicators up to 56 days of age, followed by non-significant decrease that started at 63 days. However, the concentration of total cholesterol in the 112 day significantly decreased in fourth group (2.09±0.15) in comparison with the first group (2.69±0.24). Furthermore, concentration of triglycerides at the same age was 0.58±0.13 which significantly ($P < 0.05$) less than that in the first group (0.92±0.09), also there were non-significant reduction in the levels of cholesterol and triglyceride in treated group compared with the first control group.

Table 1. Serum total cholesterol and triglycerides (mmol/L, Mean ± SEM) in broiler breeder chickens across treatment groups and time

Age (Days)	Group	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)
56	G1 (Control)	4.20 ± 0.24	1.16 ± 0.09
	G2 (Probiotic)	3.77 ± 0.14	1.04 ± 0.20
	G3 (Vaccine)	4.14 ± 0.10	1.07 ± 0.12
	G4 (Regimen A)	3.70 ± 0.26	0.95 ± 0.14
	G5 (Regimen B)	2.84 ± 0.95	1.08 ± 0.11
	G6 (Regimen C)	3.71 ± 0.12	1.00 ± 0.01
63	G1 (Control)	2.78 ± 0.24	1.12 ± 0.09
	G2 (Probiotic)	2.37 ± 0.14	0.93 ± 0.17
	G3 (Vaccine)	2.70 ± 0.12	1.01 ± 0.12
	G4 (Regimen A)	2.27 ± 0.26	0.85 ± 0.19
	G5 (Regimen B)	2.38 ± 0.12	0.97 ± 0.18
	G6 (Regimen C)	2.31 ± 0.11	0.91 ± 0.06
112	G1 (Control)	2.69 ± 0.24	0.92 ± 0.09
	G2 (Probiotic)	2.26 ± 0.14	0.73 ± 0.17
	G3 (Vaccine)	2.60 ± 0.12	0.81 ± 0.12
	G4 (Regimen A)	2.09 ± 0.15*	0.58 ± 0.13*
	G5 (Regimen B)	2.28 ± 0.12	0.77 ± 0.18
	G6 (Regimen C)	2.21 ± 0.12	0.71 ± 0.06

*Significant vs. Control (G1): $P < 0.05$

The levels of uric acid were significantly ($P < 0.001$) less than those in the first control group, there were numerical non-significant differences in all treated group in comparison with the first control group at 8 weeks. At 9 weeks, these levels were 378.07±7.84, 410.56±8.83, and 409.58±9.10 in the fourth, fifth, and sixth experimental groups, respectively. Additionally, the results of fourth, fifth and sixth groups were also significantly lower than those of the first control group at 16 weeks (**Table 2**). At nine weeks, the uric acid content reduced in the 2nd experimental group to 120.71%, and in the 3rd experimental group to 92.97%.

The levels of creatinine were significantly ($P < 0.001$) less than those in the first control group. These levels were 39.40±0.35, 41.10±1.26, and 43.08±1.49 in the fourth, fifth, and sixth experimental groups, respectively, at 16 weeks.

There were numerical non-significant differences in all treated group in comparison with the first control group at 8 weeks.

The concentrations of glucose were significantly ($P < 0.05$) increased in the second group as well as in the fourth ($P < 0.001$), fifth ($P < 0.05$), and sixth ($P < 0.01$) at 8 weeks. Also, these results were significant in the second group ($P < 0.05$) as well as the fourth ($P < 0.01$), fifth ($P < 0.05$), and sixth ($P < 0.05$) at 9 weeks. Moreover, these findings were significant in the second group ($P < 0.05$) as well as the fourth ($P < 0.001$), fifth ($P < 0.01$), and sixth ($P < 0.01$) at 16 weeks. However, results from Table 2 showed that the glucose content increased in the blood of all groups compared to group 1 (control), but some fluctuations in this indicator occurred at insignificant levels. During 9 weeks old, the content of this indicator increased in the 2nd group by 79.77% ($P < 0.01$), and in the 3rd experimental group by 69.23% ($P < 0.001$).

At the 8 weeks, concentration of calcium, phosphorus and magnesium as well as the calcium phosphorus ratio show non-significant changes among groups (**Table 3**). Also, these alterations were observed in 9 weeks old. At the 16 weeks, it was different numerically but non-significant changes in the mineral levels except the magnesium levels in the fourth group were significantly higher than those in the first control group ($P < 0.05$).

Table 2. Serum carbohydrate and renal metabolites in broiler breeder chickens across treatment groups and time (Mean ± SEM)

Age (Days)	Group	Glucose (µmol/L)	Uric Acid (µmol/L)	Creatinine (µmol/L)
56	G1 (Control)	9.84 ± 0.98	562.47 ± 13.39	39.29 ± 1.50
	G2 (Probiotic)	13.42 ± 1.32*	563.45 ± 22.51	44.32 ± 4.50
	G3 (Vaccine)	10.44 ± 1.15	552.57 ± 4.78	43.23 ± 2.55
	G4 (Regimen A)	15.19 ± 0.92***	544.74 ± 19.14	42.84 ± 2.10
	G5 (Regimen B)	13.51 ± 1.07*	552.44 ± 4.57	38.85 ± 0.20
	G6 (Regimen C)	13.55 ± 0.77**	553.50 ± 7.15	42.42 ± 2.91
63	G1 (Control)	11.52 ± 0.98	443.64 ± 13.17	46.28 ± 1.99
	G2 (Probiotic)	15.12 ± 1.32*	417.17 ± 31.41	44.85 ± 4.14
	G3 (Vaccine)	12.10 ± 1.15	432.61 ± 3.97	45.41 ± 0.93
	G4 (Regimen A)	16.15 ± 1.41**	378.07 ± 7.84***	42.59 ± 1.29
	G5 (Regimen B)	15.90 ± 1.38*	410.56 ± 8.83*	43.33 ± 1.38
	G6 (Regimen C)	15.82 ± 1.70*	409.58 ± 9.10*	44.27 ± 1.62
112	G1 (Control)	13.45 ± 0.92	379.08 ± 10.28	46.41 ± 0.38
	G2 (Probiotic)	17.18 ± 1.27*	365.72 ± 31.41	43.28 ± 3.66
	G3 (Vaccine)	14.46 ± 1.28	374.60 ± 4.44	44.22 ± 2.41
	G4 (Regimen A)	18.30 ± 1.72***	329.63 ± 20.26*	39.40 ± 0.35***
	G5 (Regimen B)	17.98 ± 1.23**	351.89 ± 7.17*	41.10 ± 1.26***
	G6 (Regimen C)	18.02 ± 1.21**	348.71 ± 9.29*	43.08 ± 1.49*

*Significant vs. Control (G1): $P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 3. Serum mineral levels in broiler breeder chickens across treatment groups and time (Mean ± SEM)

Age (Days)	Group	Calcium (mmol/L)	Phosphorus (mmol/L)	Ca:P Ratio	Magnesium (mmol/L)
56	G1 (Control)	2.48 ± 0.09	1.19 ± 0.12	2.11 ± 0.16	1.16 ± 0.10
	G2 (Probiotic)	2.41 ± 0.14	1.41 ± 0.12	1.74 ± 0.23	1.05 ± 0.12
	G3 (Vaccine)	2.34 ± 0.16	1.43 ± 0.30	1.73 ± 0.24	1.10 ± 0.18
	G4 (Regimen A)	2.37 ± 0.27	1.40 ± 0.10	1.70 ± 0.20	1.08 ± 0.24
	G5 (Regimen B)	2.44 ± 0.06	1.32 ± 0.16	1.90 ± 0.21	1.19 ± 0.36
	G6 (Regimen C)	2.44 ± 0.23	1.49 ± 0.14	1.67 ± 0.26	1.14 ± 0.16
63	G1 (Control)	3.78 ± 0.09	1.62 ± 0.27	2.48 ± 0.42	1.47 ± 0.09
	G2 (Probiotic)	3.71 ± 0.14	1.91 ± 0.12	1.97 ± 0.17	1.49 ± 0.05
	G3 (Vaccine)	3.64 ± 0.16	1.95 ± 0.32	1.94 ± 0.22	1.45 ± 0.19
	G4 (Regimen A)	3.34 ± 0.20	1.91 ± 0.11	1.75 ± 0.01	1.44 ± 0.19
	G5 (Regimen B)	3.74 ± 0.06	1.84 ± 0.15	1.87 ± 0.19	1.54 ± 0.32
	G6 (Regimen C)	3.07 ± 0.48	2.00 ± 0.15	1.58 ± 0.37	1.43 ± 0.15
112	G1 (Control)	5.78 ± 0.13	3.07 ± 0.26	1.91 ± 0.15	1.90 ± 0.08
	G2 (Probiotic)	5.72 ± 0.14	3.41 ± 0.13	1.68 ± 0.09	1.94 ± 0.01
	G3 (Vaccine)	5.63 ± 0.14	3.45 ± 0.33	1.66 ± 0.13	1.87 ± 0.15
	G4 (Regimen A)	5.34 ± 0.18	3.41 ± 0.09	1.56 ± 0.01	2.11 ± 0.06*
	G5 (Regimen B)	5.74 ± 0.11	3.35 ± 0.13	1.72 ± 0.10	1.99 ± 0.07
	G6 (Regimen C)	5.37 ± 0.29	3.60 ± 0.18	1.51 ± 0.16	1.96 ± 0.06

*Significant vs. Control (G1): $P < 0.05$

DISCUSSION

This study expands upon our previous findings (**9**) by demonstrating that the *Bacillus subtilis*-based probiotic Batcinel-K®, particularly when co-administered with the CEVAC SET-K® vaccine under a structured schedule (Regimen A), induces significant beneficial changes in key serum biochemical markers of metabolism in broiler breeder chickens.

At 112 days, the results showed that there was a significant improvement in lipid profile (reduced cholesterol and triglycerides) in the Regimen A group. This aligns with previous reports that probiotic supplementation can lower serum lipids in poultry (**11,12**).

The mechanism likely involves probiotic modulation of gut microbiota, leading to reduced cholesterol synthesis and absorption, increased bile salt deconjugation, and downregulation of hepatic lipogenic enzymes such as acetyl-CoA carboxylase (**13,14**). The superior effect observed in the combined regimen suggests a synergistic interaction where the vaccine may prime the immune system, and the probiotic reinforces gut barrier function and metabolic regulation.

The elevated serum glucose in current study in probiotic groups could indicate that there was an enhancement in the availability of energy, possibly due to improved nutrient digestion and absorption mediated by probiotic-derived enzymes and better gut health (**15**). This finding, coupled with the significant reduction in uric acid and creatinine, points toward improved protein metabolism and renal function. Lower uric acid suggests more efficient purine metabolism and renal filtration, while reduced creatinine may indicate better glomerular filtration rate or altered extrarenal elimination mediated by gut microbiota (**16,17**). These results contrast with some studies reporting increased uric acid with probiotics (**18**), highlighting that effects may be strain-specific and dependent on the overall health status of the host.

The results showed that there was an increase in serum magnesium in the optimal combined group (G4). Magnesium is a crucial cofactor in over 300 enzymatic reactions, including energy metabolism and protein synthesis (**19**). Enhanced magnesium status could contribute to improved metabolic efficiency and stress resilience. Probiotics are known to produce short-chain fatty acids that lower intestinal pH, potentially improving the solubility and absorption of minerals like magnesium (**22**).

The differential outcomes among the combined regimens (G4–G6) underscore the importance of application timing and schedule. Regimen A, with its prolonged initial probiotic colonization phase and strategic booster before vaccination, appeared most effective. This supports the concept that establishing a robust probiotic community before an immune challenge (vaccination) is critical for optimal synergistic effects (**21**).

Supplementation with Batcinel-K® probiotic, particularly according to Regimen A in combination with CEVAC SET-K® vaccination, significantly improved the serum biochemical profile of broiler breeder chickens by reducing cholesterol and triglycerides, enhancing glucose availability, supporting renal function, and improving magnesium status. These findings suggest that strategically designed probiotic-vaccine programs can enhance metabolic health and resilience, contributing to sustainable poultry production. Future research should focus on the underlying molecular mechanisms and the long-term impacts on reproductive performance and offspring quality.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL TRANSPARENCY

Akhil M Alsadwi serves as an Editorial Manager for The Iraqi Journal of Veterinary Medicine. Despite this role, the peer review process and the final publication decision were made independently and impartially, ensuring no influence from the author's editorial position.

ARTIFICIAL INTELLIGENT DECLARATION

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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