Poultry Science

The anticoccidial effects of probiotics and prebiotics on the live coccidia vaccine and the subsequent influence on poultry performance post-challenge with mixed *Eimeria* species

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ABSTRACT Live vaccines containing *Eimeria* oocysts are commercially available to protect against avian coccidiosis. Additionally, probiotics (**PRO**) and prebiotics (**PRE**) improve the poultry productivity and health and can be used as anticoccidial substitutes. However, the impact of PRO and PRE on reproductive potential, lesion score, intestinal health, and immunization outcomes of the live coccidia vaccines has not received adequate attention. Five groups of unsexed 1-day-old broiler chicks were used as follows: negative control (**NC**); challenged control (**CC**); vaccinated and challenged (**VC**); vaccinated, PRO-treated, and challenged (**V-PRO**); and vaccinated, PRE-treated, and challenged (**V-PRE**). At 21 d post-vaccination (**pv**), the vaccine increased the count of cecal anaerobes ($P \leq 0.05$) and coliforms ($P > 0.05$) as well as harmed body weight gain (**WG**) ($P \leq 0.05$), cecal lactic acid bacteria ($P \leq 0.05$), and plasma carotenoid level ($P > 0.05$). None of the additives decreased oocyst shedding after vaccination, although they lowered the middle intestine and cecal lesion scores ($P > 0.05$). Compared to VC (2.68 ± 0.12) and V-PRE (2.66 ± 0.05), the V-PRO group showed an improved carotenoid level pv (2.96 ± 0.05) ($P \leq 0.05$). V-PRE exhibited higher WG (822.95 ± 18.25) ($P > 0.05$) and FI (1153.01 ± 10.02) ($P \leq 0.05$) than VC (781.86 ± 25.16 and 1109.85 ± 33.68) and V-PRO pv (787.61 ± 19.92 and 1077.43 ± 15.99). Following the homologous coccidia challenge, coccidia-vaccinated broilers adminstered the PRO or PRE continued to exhibit protection levels comparable to those received the vaccine alone. During 2 weeks post-challenge, VC, V-PRO and V-PRE improved bird performance and reduced oocyst shedding and lesion scores compared to CC. Ultimately, PRO and PRE treatments did not significantly reverse the reduction in growth performance in broiler chickens vaccinated against coccidia during the 1st three weeks of age.

Key words: anticoccidial, broilers, coccidiosis, chickens, feed additive, growth performance, probiotics, vaccine

2024 Poultry Science 103:104283
https://doi.org/10.1016/j.psj.2024.104283

INTRODUCTION

Avian coccidiosis is one of the most prevalent infections affecting chickens worldwide. Therefore, the main obstacle that impedes the development and prosperity of developing agricultural nations remains (El-Shall et al., 2022). The intracellular protozoa of *Eimeria* species infect different sections of the intestinal tract with diverse degrees of severity. Coccidiosis adversely causes high death rates, negatively affects growth and feed utilization, and increases the susceptibility of birds to subsequent diseases (Ritzi et al., 2016; Rodgers et al., 2015; Liao et al., 2024). *Eimeria* infection also exacerbates the intestinal proliferation of pathogens such as *Clostridium, perfringens, Salmonella enterica serovars Enteritidis,* and *Salmonella enterica serovars Typhimurium*

Received June 25, 2024.

Accepted August 28, 2024.

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(Arakawa et al. 1981; Timbermont et al., 2011; Williams, 2002).

The 2 main approaches used in commercial chicken production to manage *Eimeria* are chemoprophylaxis, which is mostly applied to broiler chicks, and immunization with live, virulent, or attenuated oocysts (egg-laying chickens) (Soutter et al., 2020).

Anticoccidial drug administration has been a successful control technique for a long time. Still, as drug resistance increases, there is a need to offer drug-free products and decrease their use in animal production (Peek and Landman, 2011). In contrast, coccidia vaccines offer a safe alternative method for chemotherapy (Gao et al. 2024), which contain live sporulated oocysts of various combinations and concentrations of *Eimeria* species (Dalloul and Lillehoj, 2005; Williams, 2002). Vaccine administration at an earlier age reduces the infection level, thereby inducing an immune response and decreasing *Eimeria's* resistance to anticoccidial drugs. However, coccidia vaccines, particularly wild strains, can trigger severe hemorrhagic reactions or malabsorptive coccidiosis, which results in growth reduction and might make chicks more vulnerable to secondary infectious diseases, including necrotic enteritis (Dalloul and Lillehoj, 2005; Li et al., 2005).

Therefore, in addition to properly administering the coccidia vaccine, ensuring that chicks have a healthy intestinal tract inhabited by a normal beneficial microbiota could prevent any potential side effects when given to young birds. It also acts as a vital barrier preventing the entry of potentially harmful pathogens (Stringfellow et al., 2011; Williams, 2002). In this context, nutritional intervention with additives to maintain a healthy intestinal tract is a crucial complementary technique that showed partial control of coccidiosis. They can provide dietary requirements for vaccinated birds, maintain intestinal integrity, provide a stabilizing influence on the gut microbiota, improve nutrient digestion and absorption, and have an immune-stimulating effect (Arczewska-WŁOsek and ŚWiĄTkiewicz, 2014). This includes utilizing probiotics (Ritzi et al., 2016; Stringfellow et al., 2011), botanicals (Abbas et al. 2012), and exogenous enzymes such as protease (Peek et al., 2009). Previous reports have documented the effectiveness of several probiotics in reducing oocyst shedding and increasing T and B cell-specific cytokines (Dalloul et al. 2005), improving bird performance against *Eimeria* infection (Lee et al., 2007), and reducing microscopic lesions in infected birds (Chen et al., 2016).

The probiotic strains exerted an anticoccidial action against *Eimeria* species *in vitro* (Henikl et al., 2010) and *in vivo* (Giannenas et al., 2012). Moreover, it was shown by (Elmusharaf et al., 2006,2007) that giving prebiotics (mannan-oligosaccharide) to *Eimeria*-challenged broiler chickens decreased the excretion of oocysts.

In light of the previously mentioned considerations, it is imperative to assess the anticoccidial potential of beneficial microorganisms and their derivatives against coccidia-vaccinal strains to prevent performance decline induced by early vaccination, promote immune development, and improve the vaccine's ability to protect against coccidia challenge. Before the challenge, the study assessed the effects of probiotic and prebiotic supplements on the expression of the live coccidia vaccine in broiler chickens. The coccidia challenge was then performed to demonstrate how these supplements affected the development of the immune response.

MATERIALS AND METHODS

Experimental Birds and Design

The current study was conducted at the Faculty of Veterinary Medicine, Alexandria University, Egypt. The Faculty of Veterinary Medicine's Animal Care and Ethics Committee approved all the management procedures followed during this experiment (AU 013-2022/11/3-4-162).

This study used one hundred and fifty 1-day-old broiler chicks (ROSS 308). Upon the arrival of chicks, they were tagged with wing tags, individually weighed at the beginning of the trial (average BW 42.9 g), and randomly divided into 5 groups with 3 replicates per group (10 birds/replicate) as follows: NC: unvaccinated, untreated, and unchallenged group (Negative Control); CC: unvaccinated, untreated, and challenged group (Challenged Control); VC: vaccinated, untreated, and challenged group; V-PRO: vaccinated, probiotic-treated, and challenged group; and V-PRE: vaccinated, prebiotic-treated, and challenged group. The experimental design is shown in (Figure 1). The birds had *ad libitum* access to feed and water. They were kept in floor pens with wood shavings about 5 cm deep throughout a 35-d grown-out period. Ventilation and heating were automatically regulated. The birds received a commercial starter (1−14 d) (ME: 2,930 kcal/kg diet and CP: 23%), grower (15−27 d) (ME: 3041 kcal/kg diet and CP: 21%), and finisher (28−35 d) (ME: 3100 kcal/kg diet and CP:19%) feed that was free from antimicrobials and anticoccidial drugs.

Coccidian Vaccine, Probiotic, and Prebiotic

Vaccination groups received eye drops of the Fortegra vaccine on the first day of life. Fortegra vaccine contains 5 strains of 4 *Eimeria* species (*E. tenella, E. acervuline, E. maxima, E. maxima MFP, and E. mivati*), Intervet Inc., Omaha, NE 68103, U.S. Vet. Lic. No. 165A., Ser. No: 94320092.

Probiotic (PRO): BACTO SAC sol is composed of 1×10^9 CFU of each of *Lactobacillus (L) acidophilus, L. plantarum, Pediococcus pentosaceus, Saccharomyces cerevisiae, Bacillus (B) subtilis, B. licheniformis*/1 liter, K.M.P. BIOTECH. CO., LTD, Thailand, Lot No. 20109. It was administered via drinking water (1ml/5L).

Prebiotic (PRE): Beta Fructan was applied via drinking water (0.5ml/L). It comprises 100 gm of 2.6 *Beta Leva Fructan*/1L solution, GENCORE INT. INC., ANN. ARBOR. MI., Lot NO. 42017.

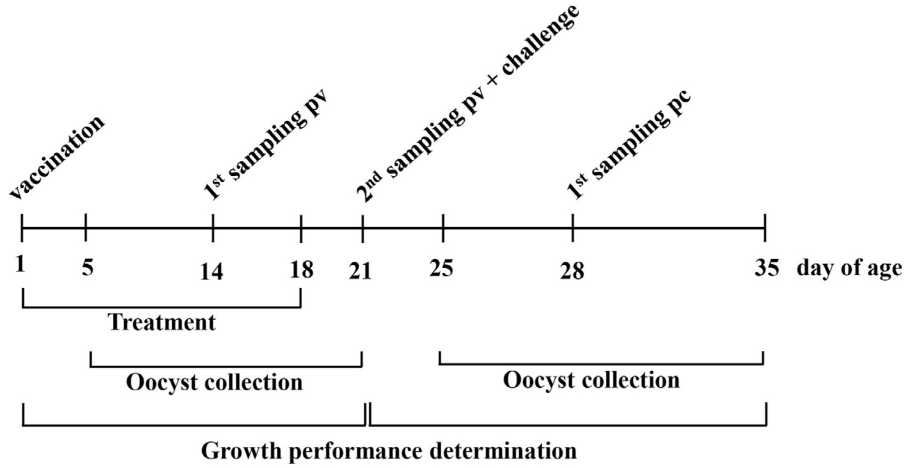

Figure 1. Schematic outline of the experimental design. pv: post-vaccination. pc: post-challenge. Sampling: for lesion scoring, organ indexing, plasma biochemical measuring, and bacterial counting.

PRO and PRE products were administered to the corresponding groups between the first and eighteenth day of age.

On day 21, a 25-fold dosage of the Fortegra vaccine was administered via crop gavage to challenge the birds in the second to fifth groups.

Growth Performance

Weekly body weight and feed intake (**FI**) were recorded. Body weight gain (**WG**) and feed conversion ratio (**FCR**) were computed during periods of postvaccination (**pv**), postchallenge (**pc**), and throughout the experiment.

Intestinal Lesion Score

Three birds per group were randomly chosen and humanely euthanized on d 14 and 21 pv and d 7 pc. Lesions scores were assessed in the duodenum (upper), mid-intestine (middle), and cecum for *E. acervulina, E. maxima,* and *E. tenella*, respectively, according to (Johnson and Reid, 1970).

Oocyst Count

From days 5 to 21 pv and 4 to 14 pc, fresh fecal samples were collected, pooled for each group, fully homogenized, and stored in the refrigerator until use. Oocyst counting was performed using the McMaster slide and expressed as oocyst per gram of feces.

Plasma Carotenoid level

Three blood samples per group were collected on d 14 and 21 pv and d 7 pc. A spectrophotometer was used to measure plasma carotenoid concentration using commercial kits obtained from Bio-diagnostic Co., Giza, Egypt.

Ileal and Cecal Bacterial Count

On days 21 pv and 7 pc, the fecal content from each segment (1 gram) was independently mixed with 9 mL of peptone water (HIMEDIA, India) and incubated for 1 h at room temperature. The samples were then subjected to 10-fold serial dilution using peptone water up to 10^{-6}. Next, each suitably diluted sample (0.1 mL) was plated onto MRS agar (de Man Ragosa and Sharpe agar, Lab M Ltd, UK) and incubated under anaerobic conditions at 30°C for 48 h for enumeration of lactic acid bacteria (Shazali et al., 2014). The coliforms were isolated on MacConkey agar (HIMEDIA, India) at 37 °C for 24 h. Anaerobe's count was detected on the reinforced clostridial agar (HIMEDIA, India) after anaerobic culture at 37 °C for 48 h. The colony forming unit (**cfu**) per gram was determined using duplicate agar plates, and the result was expressed as Log_{10} cfu/g.

Economic Assessments

The economic effectiveness of the current study was evaluated using input-output analysis, primarily focusing on the overall total feed cost and the prevailing market price for live bird weight. The cost of the supplements was (0, 0, 0.80, 0.90, and 1.12 EGP/bird) for NC, CC, VC, V-PRO, and V-PRE groups, respectively. Multiplying the total amount of feed used by each bird by the feed's price yielded the overall feed cost. The total variable cost is the combined expense of supplement and feed costs. Other costs that were the same for all groups and not included in our calculations were the cost of a day-old chick, utilities, and veterinary care. Feed cost per kilogram gain = feed cost/kilogram body weight gain. Average body weight multiplied by meat price yields the overall return. Net return = total return minus variable cost.

Statistical Analysis

Data were analyzed using SPSS version 20. A 1-way ANOVA test was followed by Duncan's multiple

Table 1. Growth performance and mortalities in response to probiotic and prebiotic supplementation in coccidia-vaccinated broiler chickens.

	NC	CC	VC	V-PRO	V-PRE
Final BW (g)	2,373.65 ± 67.38[a]	2,091.98 ± 59.75[b]	2,179.03 ± 51.53[b]	2,215.25 ± 53.97[ab]	2,171.09 ± 61.38[b]
Body weight gain (g)					
Age (d)					
1st−21st*	873.43 ± 20.90[a]	857.24 ± 23.40[a]	781.86 ± 25.16[b]	787.61 ± 19.92[b]	822.95 ± 18.25[ab]
21st−35th†	1,431.25 ± 47.93[a]	1,172.73 ± 50.15[b]	1,310.85 ± 39.97[a]	1,369.43 ± 37.11[a]	1,294.54 ± 52.30[ab]
1st−35th	2329.11 ± 67.32[a]	2049.23 ± 59.59[b]	2135.95 ± 51.45[b]	2172.33 ± 54.04[ab]	2,127.98 ± 61.31[b]
Feed intake (g)					
1st−21st	1,125.19 ± 7.49[ab]	1,126.74 ± 5.74[ab]	1,109.85 ± 33.68[b]	1,077.43 ± 15.99[c]	1153.01 ± 10.02[a]
21st−35th	2,679.63 ± 41.93[a]	2,577.92 ± 1.01[a]	2,742.69 ± 143.99[a]	2,607.61 ± 331.92[a]	2,655.03 ± 193.10[a]
1st−35th	3,804.82 ± 49.42[a]	3,504.65 ± 6.75[a]	3,852.53 ± 177.66[a]	3,685.04 ± 347.91[a]	3,808.04 ± 183.08[a]
FCR (g/g)					
1st−21st	1.30 ± 0.03[a]	1.34 ± 0.04[a]	1.42 ± 0.05[a]	1.42 ± 0.03[a]	1.42 ± 0.03[a]
21st−35th	1.89 ± 0.06[b]	2.24 ± 0.10[a]	2.14 ± 0.06[a]	1.89 ± 0.07[b]	2.10 ± 0.09[ab]
1st−35th	1.64 ± 0.04[b]	1.72 ± 0.05[ab]	1.82 ± 0.04[a]	1.70 ± 0.04[ab]	1.81 ± 0.05[a]
Mortalities	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \leq 0.05$). NC: unvaccinated, untreated, and unchallenged (Negative Control); CC: unvaccinated, untreated, and challenged (challenged Control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged.

*Three weeks post-vaccination.

†Two weeks post-challenge.

comparisons to test the significant differences at $P \leq 0.05$. The findings are displayed as the average ± standard error of the mean (SEM). GraphPad Prism 6 (Graph Prism Software, La Jolla, CA) was used to design the figures.

RESULTS

Growth Performance

Table 1 shows the effect of probiotic and prebiotic supplementation on the growth performance of coccidia-vaccinated broiler chickens. The final BW ($P \leq 0.05$), total WG ($P \leq 0.05$), and total FI ($P > 0.05$) of the CC group were lower than the NC group. In the post-vaccination period (3 wk pv), the vaccinated (VC) and the V-PRO groups showed lower WG than NC ($P \leq 0.05$) and V-PRE ($P > 0.05$). All vaccinated groups, whether supplemented or not, had WG equivalent to the NC group at 2 wk pc ($P > 0.05$). However, throughout the whole experiment, all challenged groups (CC [$P \leq 0.05$], VC [$P \leq 0.05$], V-PRO [$P > 0.05$], and V-PRE [$P \leq 0.05$]) showed lower total WG than the NC group.

There was no difference in FI between all groups throughout the post-challenge period and the whole duration. However, the VC and V-PRO groups had significantly reduced FI during the post-vaccination period compared to other groups ($P \leq 0.05$).

FCR did not differ ($P > 0.05$) between groups for 3 wk pv. The CC group had the worst FCR for 2 wk pc ($P \leq 0.05$), followed by the VC group. V-PRO group showed the best FCR on the 1st−35th d when compared to the NC group ($P > 0.05$), while that of VC and V-PRE groups were worse than the NC group ($P \leq 0.05$).

Plasma Carotenoids

The treatments had distinct effects on the concentration of plasma carotenoids ($P \leq 0.05$) (Table 2). The V-PRE group showed a significant drop in carotenoid concentration on d 14 pv compared to other groups ($P \leq 0.05$). VC and V-PRE groups had lower values on d 21 pv than CC and V-PRO groups ($P \leq 0.05$). In contrary, during the post-challenge period, the plasma carotenoid level was increased in VC and V-PRE groups compared to the CC group, particularly on d 7 pc ($P \leq 0.05$). The V-PRO group also showed a numerical rise in plasma carotenoids pc ($P > 0.05$).

Postmortem Examination of the Intestinal Tract and Lesion Scoring

The digestive tract had several anomalies, including thickening, bleeding, and congestion of the serosa and mucosa. Following vaccination and challenge, the lesion scores of the upper intestine (Figure 2A), the middle intestine (Figure 2B), and the cecum (Figure 2C) are displayed.

During the post-vaccination period, the V-PRE group had a decreased average lesion score of the middle intestine ($P \leq 0.05$) and cecum ($P > 0.05$) on d 14 pv (the second week of age) as well as of the upper ($P \leq 0.05$) and middle intestine ($P > 0.05$), and cecum ($P > 0.05$) on d 21 pv (the third week of age) compared to the VC group. Except for the upper intestinal section on day 21 pv, where the V-PRE group displayed a lower lesion score ($P \leq 0.05$) than the V-PRO group, there were no differences ($P > 0.05$) in the lesion scores at different intestinal parts between the 2 groups. On the other hand, on d 7 pc (the fourth week of age), the V-PRO group showed the lowest lesion score in the upper intestine ($P \leq 0.05$) and cecum ($P > 0.05$).

Oocyst Shedding

The V-PRE group had the maximum level of oocyst shedding during the post-vaccination period ($P \leq 0.05$).

Table 2. Plasma carotenoid concentrations in response to probiotic or prebiotic supplementation in coccidia vaccinated broiler chickens.

Week of age	NC	CC	VC	V-PRO	V-PRE
2nd (14-d pv)	2.98 ± 0.04^{a}	3.04 ± 0.04^{a}	3.00 ± 0.06^{a}	2.96 ± 0.01^{a}	2.75 ± 0.06^{b}
3rd (21-d pv)	2.92 ± 0.05^{ab}	2.95 ± 0.09^{a}	2.68 ± 0.12^{bc}	2.96 ± 0.05^{a}	2.66 ± 0.05^{c}
4th (7-d pc)	2.92 ± 0.05^{ab}	2.73 ± 0.09^{b}	3.00 ± 0.04^{a}	2.87 ± 0.01^{ab}	2.95 ± 0.08^{a}

Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \le 0.05$). NC: unvaccinated, untreated, and unchallenged (negative control); CC: unvaccinated, untreated, and challenged (challenged control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged; d pv: day post vaccination. d pc: day post challenge.

The additive-supplemented birds (V-PRO and V-PRE) demonstrated a reduction in oocyst excretion after the challenge. Moreover, the V-PRO group had the lowest excretion pc when compared to the CC group ($P \le 0.05$), but there was no difference between them and the VC and V-PRE groups ($P > 0.05$) (Table 3).

Intestinal Bacterial Count

AT d 21 pv (the third week of age), the coccidia vaccine numerically increased the ileal coliform and anaerobic bacterial count. In contrast, the ileal lactic acid bacterial count decreased compared to the NC group ($P > 0.05$) (Table 4). The 2 additives (V-PRO and V-PRE) showed non-significant changes in ileal bacterial count compared to NC and VC ($P > 0.05$). Moreover, the VC group showed lower levels of cecal lactic acid bacteria ($P \le 0.05$) and higher levels of cecal coliforms ($P \le 0.05$) and cecal anaerobes ($P > 0.05$) than the NC group. The 2 additives increased cecal lactic acid bacteria ($P > 0.05$) and decreased anaerobic count ($P \le 0.05$) and coliforms ($P \le 0.05$) in comparison to the vaccine alone (**VC**).

On d 7 pc, the ileal bacterial counts (coliforms, anaerobes, and lactic acid bacteria) of the CC group and the NC group were similar ($P > 0.05$) (Table 4). The VC group's ileal coliform count was higher ($P \le 0.05$) than the CC group; however, there was no difference ($P > 0.05$) in the ileal anaerobic or lactic acid bacterial count. The differences in cecal microbiology between the groups on d 7 pc were not statistically significant ($P > 0.05$).

Economic Evaluation

The effects of probiotic and prebiotic supplementation on cost and return metrics in coccidia-vaccinated broiler chickens are shown in Table 5. There were no discernible variations between the groups' feed and variable costs ($P > 0.05$). However, there were observable differences in the feed cost per kilogram gain among the groups; the

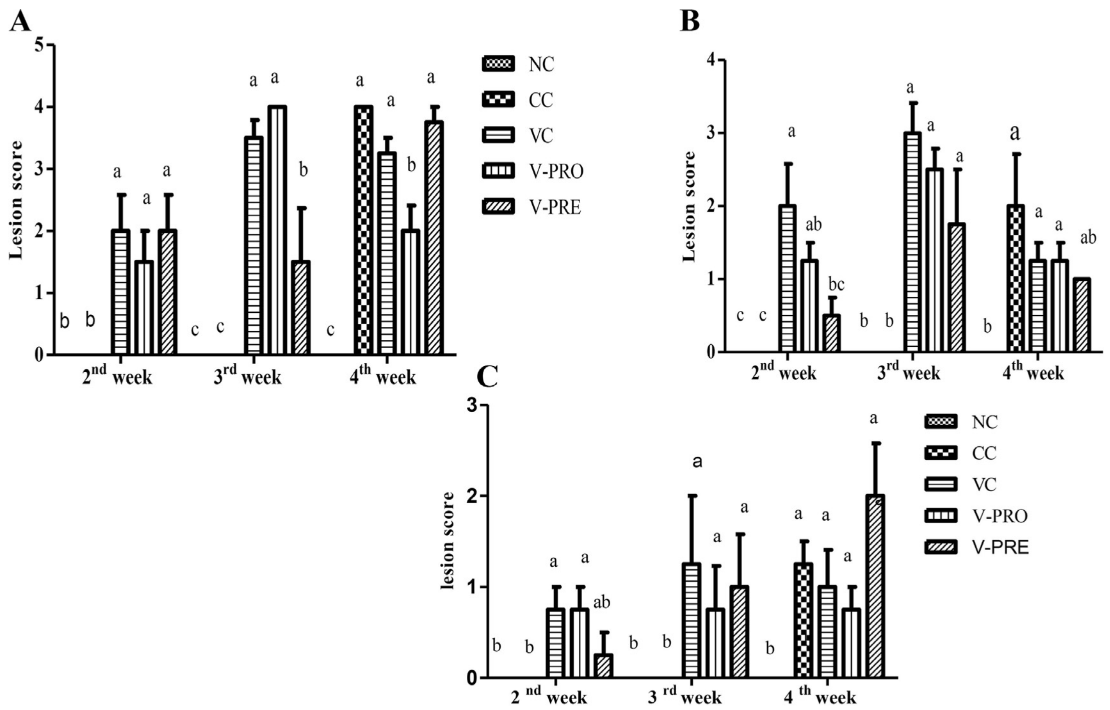

Figure 2. Lesion scores of the upper intestine (A), mid intestine (B), and cecum (C) in response to probiotic and prebiotic supplementation in coccidia-vaccinated broiler chickens. Statistical difference is considered at ($P \le 0.05$). NC: unvaccinated, untreated, and unchallenged (Negative Control); CC: unvaccinated, untreated, and challenged (Challenged Control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged.

Table 3. Cumulative oocyst shedding per gram ($\log_{10}$) in response to probiotic or prebiotic supplementation in coccidia-vaccinated broiler chickens.

Period	NC	CC	VC	V-PRO	V-PRE
Post vaccination					
1st wk	0^b	0^b	3.98 ± 0.26^a	3.83 ± 0.19^a	3.90 ± 0.54^a
2nd wk	0^c	0^c	4.42 ± 0.42^b	4.75 ± 0.31^{ab}	5.43 ± 0.36^a
3rd wk	0^b	0^b	5.33 ± 0.01^a	5.42 ± 0.11^a	5.28 ± 0.08^a
Total	0^c	0^c	5.44 ± 0.05^b	5.57 ± 0.02^{ab}	5.77 ± 0.15^a
Postchallenge					
1st wk	0^b	3.91 ± 1.96^a	4.57 ± 0.11^a	4.48 ± 0.18^a	4.53 ± 0.14^a
2nd wk	0^c	4.16 ± 0.06^a	4.08 ± 0.13^a	3.67 ± 0.10^b	3.88 ± 0.11^{ab}
Total	0^c	5.80 ± 0.08^a	4.69 ± 0.11^b	4.55 ± 0.17^b	4.61 ± 0.13^b

Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \leq 0.05$). NC: unvaccinated, untreated, and unchallenged (negative control); CC: unvaccinated, untreated, and challenged (challenged control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged; wk: week.

NC group had the lowest value, and the VC group had the highest. Moreover, the NC group showed the highest ($P \leq 0.05$) total return and net return compared to other groups.

DISCUSSION

The growth performance of the VC group on d 21 pv was adversely impacted. There have been reports of low performance in broiler chicks given coccidia vaccination. According to Da Silva et al. (2009), there was a reduction in BW in the early stages following coccidia vaccination. The coccidia vaccination led to reduced body weight gain, decreased body weight and higher FCR, or an enhanced FCR without impacting weight, according to studies by Cowieson et al. (2020), Lee et al. (2011), Wang et al. (2019).

Cowieson et al., (2020) hypothesized that the nutritional shift from muscle growth to increased immunity to the vaccine is related to the growth reduction associated with coccidia vaccination, especially in the grower phase. From a different perspective, gut damage and malabsorption resulting from coccidia vaccination may lower growth performance. In the current study, this might manifest as a decrease in plasma carotenoid levels, an imbalance of intestinal flora, and an increase in gross lesion scores.

A potential biomarker for coccidia infection is the concentration of plasma carotenoids (Cowieson et al., 2020). Plasma carotenoid' decline might be related to the intestine's diminished capacity to absorb nutrients and the disruption of these substances by reactive oxygen species generated due to the *Eimeria* infection (Allen, 1997). In this investigation, coccidia vaccination had the worst effect on plasma carotenoid levels at d 21 pv, which coincided with a considerable increase in intestinal lesion scores. This is related to the recycling of vaccinal Eimeria oocysts, as evidenced by reinfection and increased oocyst shedding. Continual exposure to oocysts in the litter is essential for the immune response against coccidiosis (Chapman et al., 2002). However, the *Eimeria*

Table 4. Ileal and cecal bacterial counts (Log_{10} cfu/g) in response to probiotic or prebiotic supplementation in coccidia-vaccinated broiler chickens.

Week of age	NC	CC	VC	V-PRO	V-PRE
Ileum					
Coliforms					
3rd*(21-d pv)	7.81 ± 0.12^{ab}	7.16 ± 0.06^b	8.52 ± 0.14^a	7.69 ± 0.07^{ab}	8.49 ± 0.58^a
4th (7-d pc)	7.55 ± 0.12^b	7.41 ± 0.42^b	8.59 ± 0.14^a	8.64 ± 0.17^a	7.99 ± 0.52^{ab}
Lactic acid bacteria					
3rd	8.45 ± 0.4^a	8.30 ± 0.42^a	7.84 ± 0.1^a	7.85 ± 0.23^a	8.61 ± 0.32^a
4th	8.32 ± 0.34^a	8.00 ± 0.22^a	7.93 ± 0.36^a	7.47 ± 0.27^a	8.14 ± 0.26^a
Anaerobes					
3rd	8.19 ± 0.19^a	8.30 ± 0.42^a	8.34 ± 0.2^a	8.14 ± 0.17^a	8.44 ± 0.63^a
4th	8.26 ± 0.05^a	8.30 ± 0.11^a	8.27 ± 0.15^a	8.00 ± 0.24^a	8.56 ± 0.19^a
Cecum					
Coliforms					
3rd	9.43 ± 0.31^a	8.31 ± 0.41^{bc}	8.63 ± 0.29^{ab}	7.55 ± 0.18^c	7.57 ± 0.10^c
4th	8.86 ± 0.15^a	8.62 ± 0.2^a	8.50 ± 0.14^a	8.71 ± 0.16^a	8.78 ± 0.17^a
Lactic acid bacteria					
3rd	9.81 ± 0.23^a	8.56 ± 0.47^{ab}	7.91 ± 0.49^b	8.69 ± 0.45^{ab}	7.90 ± 0.33^b
4th	8.69 ± 0.22^a	8.30 ± 0.21^a	8.34 ± 0.42^a	7.86 ± 0.22^a	8.37 ± 0.36^a
Anaerobes					
3rd	7.91 ± 0.49^b	8.56 ± 0.47^{ab}	9.81 ± 0.23^a	8.68 ± 0.45^{ab}	7.92 ± 0.33^b
4th	8.69 ± 0.22^a	8.29 ± 0.21^a	8.34 ± 0.42^a	7.85 ± 0.22^a	8.37 ± 0.36^a

Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \leq 0.05$). NC: unvaccinated, untreated, and unchallenged (negative control); CC: unvaccinated, untreated, and challenged (challenged control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged.

*At which coccidian challenge was performed. nd: not done. dpv: day post vaccination. dpc: day post challenge.

Table 5. Economic parameters in response to probiotic or prebiotic supplementation in coccidia-vaccinated broiler chickens.

Groups	Feed cost (EGP/bird)	Variable cost (EGP/bird)	Feed cost/kg gain cost (EGP/kg	Total return (EGP/bird)	Net return (EGP/bird)
NC	87.51 ± 0.66^{a}	87.51 ± 0.66^{a}	37.34 ± 0.63^{c}	162.54 ± 3.91^{a}	75.03 ± 3.25^{a}
CC	80.61 ± 0.09^{a}	80.61 ± 0.09^{a}	39.48 ± 0.78^{abc}	146.52 ± 5.34^{b}	65.91 ± 5.43^{ab}
VC	88.61 ± 2.36^{a}	89.41 ± 2.36^{a}	41.51 ± 0.82^{a}	148.04 ± 0.99^{b}	58.63 ± 1.37^{b}
V-PRO	84.76 ± 4.62^{a}	85.65 ± 4.62^{a}	38.96 ± 0.96^{bc}	150.64 ± 4.42^{b}	64.98 ± 0.20^{b}
V-PRE	87.58 ± 2.43^{a}	88.71 ± 2.43^{a}	41.23 ± 0.46^{ab}	147.32 ± 2.39^{b}	58.61 ± 0.04^{b}

Values are means ± standard error. Means within the same column of different superscript letters are significantly different at ($P \leq 0.05$). NC: unvaccinated, untreated, and unchallenged (Negative Control); CC: unvaccinated, untreated, and challenged (Challenged Control); VC: vaccinated, untreated, and challenged; V-PRO: vaccinated, probiotic-treated, and challenged; V-PRE: vaccinated, prebiotic-treated, and challenged.

infection and vaccination induce enterocyte damage that decreases food digestion and absorption and increases the amount of mucin and plasma proteins. As a result, bacterial infections proliferate more frequently (Kiarie et al., 2019; Williams, 2005). Moreover, the elevated pH of the cecal content resulting from an *Eimeria* infection promotes the growth of C. perfringens (Williams, 2005). The present study showed that the vaccinated chickens' ileal bacteria were not changed noticeably on d 21 pv, but their cecum exhibited high concentrations of coliform and anaerobic bacteria. This may be explained by the pH changes brought on by live coccidia vaccine, which resulted in a rise in the cecal pH and a drop in the small intestinal pH (Ruff et al., 1974). Comparably, Fox et al. (1987) showed that although the duodenum in healthy birds has a pH of 6.0 or above, infected birds with coccidiosis have a pH of less than 5.

Economics showed that the VC group had the highest feed cost per kilogram growth. Moreover, the net return and total return decreased in all treated groups compared to the NC group. According to (Mathis et al., 2014), to optimize bird performance, the coccidia cycle must be restricted post-vaccination during the latter starter and grower stages of production with anticoccidial medicine. Therefore, to enhance the immune response, lessen the negative consequences of coccidiosis, and promote bird performance, live coccidiosis vaccination should be given in conjunction with anticoccidial dosages that let a sufficient number of coccidia leak out.

Probiotics and prebiotics are substitutes for anticoccidial medications to control coccidia infections. Studies have demonstrated that probiotics can decrease oocyst excretion (Mohsin et al., 2021; Tewari and Maharana, 2011), lessen the severity of coccidia lesions (Chen et al., 2016; Elmusharaf et al., 2007), improve the cell-mediated (Chen et al., 2016; Dalloul et al., 2005) and humeral immunity (Lee et al., 2007), and improve the bird performance (Wang et al., 2021). Similarly, prebiotics improved local mucosal IgA secretions, humoral and cell-mediated immune responses, and oocyst excretion in feces (Gómez-Verduzco et al., 2009). Remarkably, compared to un-supplemented vaccinated controls, V-PRE ($P \leq 0.05$) and V-PRO ($P > 0.05$) showed an increased number of oocysts produced across 21 d pv; these results were consistent with the carotenoid levels and lesion scores pv. According to (McCann et al., 2006), the severity of infection of broilers with a mix of *E. acervulina, E. maxima,* and *E. tenella* was not reduced when given prebiotics (0.5 g/kg of yeast cell wall). Furthermore, compared to salinomycin and vaccination, (Behnamifar et al., 2019) reported that probiotics and prebiotics are ineffective in reducing coccidiosis and its related repercussions.

Our results revealed that, as evidenced by the lesion scores pv, vaccinated birds that received additives were parasitized by the vaccinal oocysts, and these organisms subsequently completed their life cycles. As a result, weight gain on d 21 pv responded proportionally in the additive-supplemented groups. However, the FCR wasn't improved compared to the vaccine alone. Previous studies have found that prebiotics had no discernible effect on the chicken's growth performance after a challenge with coccidia (Wang et al., 2019), either with (Cox et al., 2010; Elmusharaf et al., 2007) or without reducing the severity of lesions (Elmusharaf et al., 2006). Similarly, the probiotic administration had no discernible effect on the *Eimeria*-infected chickens' growth (Mohsin et al., 2021; Wang et al., 2019). Variations in the probiotic or prebiotic's cellular and functional components, dosages, administration methods and duration, and inoculation doses of *Eimeria* spp. may account for several contradictory findings between the studies. In addition to the bird's age, feed, and the upkeep and sanitary circumstances of its farm (Crittenden et al., 2005; Mountzouris et al., 2007).

Probiotics and prebiotics altered the pattern of gut bacteriology at d 21 pv when compared to the vaccine alone. It was noticeable in the cecum, where the lactic acid bacteria count rose and coliforms and anaerobe count decreased, with few variations between the 2 additives. Numerous studies have demonstrated the competitive exclusion impact of probiotics (Giannenas et al., 2014; Memon et al., 2022; Wang et al., 2021) and prebiotics (Lan et al., 2004) in altering the intestinal microbiota of coccidia-infected chickens. The development of the immune system, the decrease in enteric pathogen adhesion, the stimulation of the growth of the gut epithelium, and the absorption of dietary nutrients and energy are all influenced by the gut microbiota (Kelly et al., 2005). Therefore, rapid intestinal mucosal repair after infection is made possible by microbe recovery and maintaining intestinal homeostasis (Madlala et al., 2021).

The homologous Eimeria species challenge was used to assess the impact of the additives on the immunological status of the vaccinated broilers. The coccidia challenge had a detrimental effect on the body weight gain, feed intake, and FCR of the CC group. This is due to coccidiosis effectively reduces the intestinal lining's functional and structural damage as well as the digestion and absorption of nutrients linked to anorexia (Kiarie et al., 2019). Enhancements in the levels of plasma carotenoids in all vaccinated groups on d 7 pc indicated that the vaccine acted as a preventive measure by enhancing the absorption and digestion of nutrients. However, the lesion score of VC birds decreased numerically on d 7 pc. Following a challenge, Williams (2003) demonstrated that both vaccinated and non-vaccinated birds developed macroscopic coccidia lesions without suffering any detrimental consequences in their performance. Because of this, the chickens who received the vaccine showed clinical immunity as indicated by weight gain between d 21 and 35, but the CC group did not. Moreover, the FCR and FI of the V-PRO and V-PRE groups were lower than those of the VC group during the interval 21 to 35 d of age and were comparable to the NC group, particularly for the V-PRO group. Similar findings were previously reported by (Ritzi et al., 2016), who revealed that the administration of Poultry Star (*Bifidobacterium animalis, Lactobacillus salivarius, and Enterococcus faecium*) in addition to the live coccidia vaccine to broiler chickens reduced FCR and FI significantly compared to the vaccine alone.

Despite the findings of non-significant differences in gross lesions post-challenge between the birds that received the vaccine alone and the birds that co-administered PRO or PRE, there were considerably fewer oocysts excreted than in the CC group, suggesting a host immunological response (Williams, 2003). Based on these findings, PRO and PRE did not affect the immunization process or the subsequent immunological response.

CONCLUSIONS

Collectively, this study indicated that giving additional probiotics or prebiotics to coccidia-vaccinated chickens during the first eighteen days of their life reduced the amount of intestinal damage and altered cecal microbiota during their starter phase but did not enhance growth performance and profitability. Furthermore, the effectiveness of the coccidia vaccines against the coccidiosis challenge was not affected by additive supplementation in an antagonistic or additive manner. It is recommended that additional research be conducted on using the additives as a feed supplement in place of drinking water, as well as at greater doses and longer administration times.

ACKNOWLEDGMENTS

This study was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R30), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. This research was funded by the Researchers Supporting Project number (RSPD2024R811), King Saud University, Riyadh, Saudi Arabia.

DISCLOSURES

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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