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# *Enterococcus faecium* and *Clostridium butyricum* combined with selenium as alternatives to the antibiotic colistin: impacts on growth, cecal fermentation, and immune function in rabbits raised under hot environmental conditions

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**Introduction:** The current study was planned to evaluate the effect of using a combination of selenium (Se)-probiotic rabbit and colistin (COL) as a dietary supplement on growth, nutrient utilization, antioxidant and immune responses, blood metabolites, and cecal fermentation during summer conditions.

**Methods:** One hundred male New Zealand White rabbits aged 5 weeks with an initial body weight of (692.5 ± 5.19g) were randomly assigned to four groups (25 rabbits each) for 5–13 weeks of age experimental period. Four experimental groups in completely randomized design were used. (1) Control group: rabbits received a basal diet (BD) without any added supplements; (2) COL group: rabbits were given BD supplemented with 120 mg of COL/kg; (3) Se+EF group: rabbits consumed BD enriched with 0.3 mg of selenium plus $1 \times 10^8$ cfu of *Enterococcus faecium*/kg; and (4) Se+CB group: rabbits were fed BD fortified with 0.3 mg of selenium plus $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg.

**Results:** During overall period (5-13 weeks), body weight gain (BWG) and relative growth rate of rabbits consuming diets supplemented with COL, Se+EF, and Se+CB were greater to those of the control group. BWG (in groups Se+CB and Se+EF) and feed conversion ratio (FCR) (in groups COL and Se+EF) were improved compared with the control group. Malondialdehyde (MDA) values were declined in rabbits supplemented with Se plus probiotic (Se+CB or Se+EF) compared with the COL and control groups. Serum lipid profile in rabbits received selenium-probiotic combination (especially Se+EF) was improved. Furthermore, antioxidant levels were better in rabbits supplemented with Se plus probiotics compared to the control, and immune responses were significantly enhanced ($P < 0.05$).

**Conclusion:** Supplementing the diets of rabbits with a combination of selenium and probiotics (Se+CB or Se+EF) led to improvements in growth, antioxidant and immune responses, blood metrics, and cecal fermentation under heat stress conditions.

KEYWORDS

**selenium, probiotic, hot climate, antioxidant, immunity, cecal fermentation**

## 1 Introduction

The production of rabbits has become essential in meeting the growing demand for flesh and guaranteeing global food security (Elsayed et al., 2024). The rabbit may also serve as a model species, since it has been utilized in studies investigating the impact of feeding on productive and physiological indicators (Alagawany et al., 2023). The heightened sensitivity of rabbits to heat stress (HS) is due to their thick fur and the scarcity of sweat glands, which hinder their ability to adequately remove excess heat (El-Ratel et al., 2025a). The world is currently experiencing extremely high temperatures, which have recently reached unprecedented levels. The biggest threat to the rabbit industry and main cause of stress source is HS (Liang et al., 2022). Thus, HS impairs rabbit meat quality, immune response, antioxidant properties; gut microbiome and performance, as well as gut morphology represented by duodenal lumen and villi which were negatively affected by HS (Bassiony et al., 2021; Dalle Zotte et al., 2025). Heat stress can affect production by disrupting the endocrine system, decreasing nutrient absorption, increasing energy demands for production maintenance, and increasing oxygen-derived free radical levels, which cause oxidative damage (Collier et al., 2005; Oladimeji et al., 2022; El-Ratel et al., 2025b). The adverse impacts of HS on rabbits can be mitigated through the implementation of feeding techniques (Alagawany et al., 2023). Various natural feed additives have been employed to alleviate the adverse effects of HS in rabbits in a manner that is practical, safe, and cost-effective (Hashem et al., 2013; Al-Sagheer et al., 2023). Finding effective substitutes for antibiotics in rabbit feed was an urgent necessity. The use of environmentally friendly functional feed additives in diets to improve health and growth is becoming more popular (Miranda et al., 2024; Placha et al., 2022). Elazab et al. (2022) stated that using natural feed additives (rosemary and ginger essential oils) as environmentally friendly supplementation improved physiological status, meat nutritive value, feed utilization, and growth performance of rabbits.

Probiotics, or living beneficial bacteria, have drawn a lot of attention lately as a secure substitute for antibiotics. Studies have indicated that supplementing feed with probiotics can enhance feed utilization, improve gut health, and promote growth rates (Bhatt et al., 2017; Phuoc and Jamikorn, 2017; Jameel and Kalef, 2023, 2024).

The realization of these benefits can be achieved through: (1) the enhancement of antioxidative capacity; (2) the improvement of nutrient digestion and absorption; (3) the strengthening of immune response; (4) the maintenance of gut health and functionality; and (5) the establishment of a favorable balance of gut microbes (Liu et al., 2019; Xia et al., 2024). Dietary probiotics inhibit the growth of harmful opportunistic infections and promote the establishment of beneficial bacteria, which are crucial for feed degradation, antimicrobial peptide production, vitamin synthesis, and volatile fatty acid (VFA) production (Mancini and Paci, 2021). Probiotic dietary supplements have been shown to mitigate the detrimental effects of HS in growing rabbits (Bassiony et al., 2021; Fathi et al., 2017). Dietary probiotic supplements may exhibit anti-inflammatory, immunostimulatory, and antioxidant effects, enhancing nutrients digestibility and performance (Ebeid et al., 2023; Saeed et al., 2023).

*Enterococcus faecalis* (EF) is a Gram-positive, facultative anaerobic bacterium and is classified among the bacterial species known to produce lactic acid (Domann et al., 2007). *E. faecium* has been shown to significantly enhance gut health, improve feed efficiency, and promote body weight gain (BWG) by inhibiting harmful gut pathogens and supporting beneficial bacterial growth (Pajarillo et al., 2015). *Clostridium butyricum* (CB) is a Gram-positive anaerobic bacterium known for its capacity to synthesize butyric acid in the digestive system of healthy animals (Wang et al., 2019). It also exhibits greater endurance to extreme temperatures, low pH, and bile salt. As a result, CB is now acknowledged as a safe and effective feed supplement (Kong et al., 2011; Bassiony et al., 2021). The ability of CB to synthesize butyric acid and other short-chain fatty acids (SCFAs), coupled with its spore-forming capability, establishes it as an effective dietary supplement for animals. This promotes the release of digestive enzymes and improves the efficiency of nutrient transporters (Duan et al., 2018; Obianwuna et al., 2023). The increases in immunological response, blood components, and growth performance were obviously caused by dietary supplementation with EF and/or CB (Bassiony et al., 2021).

Selenium (Se) is a naturally occurring trace mineral that may directly influence the functioning of animals owing to its antiviral, anti-inflammatory, and antioxidant characteristics (Al-Sagheer et al., 2023). Selenium is essential for growth, immunological response, and antioxidant status as it resides in the active site of glutathione peroxidase (Eid et al., 2019). Research has suggested that dietary Se supplementation may positively influence the performance and carcass traits of rabbits subjected to HS conditions (Marai et al., 2007). Ayyat et al. (2018) reported that supplementing rabbit diets with organic Se (0.03 ppm) enhanced growth rates and mitigated the adverse effects of HS on rabbit growth during the summer months. Moreover, it was noted that giving growing rabbits supplemental dietary Se enhanced growth and carcass weight, raised the amount of Se in meat, stabilized anti-oxidative status, and strengthened immune function (Verma et al., 2012; Ebeid et al., 2013). On the other hand, the positive impacts of selenium-enriched probiotics resulted from the modulation of antioxidant activities and cytokines release. The antioxidant effect of selenium-enriched probiotics was attributed to significantly enhanced activities of intracellular antioxidant enzymes, like glutathione peroxidase, thioredoxin reductase, and glutathione peroxidase reductase (Kieliszek et al., 2020).

In order to maintain animal productivity at peak levels, optimal combinations of various strategies must be employed alongside effective management and husbandry practices. Therefore, selenium-probiotic combination can be used as a feed supplement to improved growth of rabbits under summer conditions. It is hypothesized that the positive impacts of the prior feed additives could mitigate the detrimental effects of HS on rabbit performance. Thus, the objective of this research was to evaluate how dietary supplementation with colistin (COL) and a combination of probiotics and selenium would affect the growth rate, feed utilization, antioxidant and immunological indices, blood biochemistry, and cecal fermentation of rabbits subjected to thermal stress during the summer months.

## 2 Materials and methods

### 2.1 Rabbit care and experimental design

The rabbits were housed in naturally ventilated rooms within galvanized wire cages measuring 40×35×60 cm. These cages were furnished with feeders and automated nipple drinkers, allowing for ad libitum access to water and feed. A one-week acclimatization period was provided before the commencement of the experiment, during which the rabbits were administered the basal diet (BD), serving as a control diet without additional supplements. The temperature-humidity index (THI) was calculated using data on relative humidity and air temperature gathered on the farm, in accordance with the equation outlined by Marai et al. (2001). The THI values were segmented into four categories: (1) <27.8 indicating an absence of HS; (2) 27.8 to < 28.9 representing a moderate level of HS; (3) 28.9 to 30.0 signifying a severe level of HS; and (4) >30.0 denoting an extremely severe level of HS. As shown in Table 1, rabbits were raised on BD that was formulated in accordance with Blas and Mateos (2010) recommendations. The AOAC (2006) methods were utilized to analyze the chemical composition of the BD. All groups' rabbits were housed in identical environmental, managerial, and hygienic conditions for duration of eight weeks.

One hundred male New Zealand White rabbits, aged five weeks, were arbitrarily assigned to four distinct groups. Each group consisted of 25 replicate cages, housing one animal per cage. The experimental groups were: (1) Control group: animals received the BD without any additional supplement; (2) COL group: animals were fed BD fortified with 120 mg of colistin per kg feed according to Romero et al. (2012). (3) Se+EF group: rabbits were fed BD fortified with 0.3 mg of selenium plus $1 \times 10^8$ cfu of *Enterococcus faecium* per of feed; and (4) Se+CB group: Rabbits were fed BD fortified with 0.3 mg of selenium plus $2.5 \times 10^6$ cfu of *Clostridium butyricum* per kilogram of feed according to Bassiony et al. (2021) and Jiao et al. (2022).

*Clostridium butyricum* (Clostri-mixVR) was sourced from Cheil Bio Co., Ltd., located in Youngdungpo-Gu, Seoul, South Korea. Similarly, *Enterococcus faecium*, originally from Probiotics International Ltd., UK, was procured from the same company. The doses of both probiotics used in this study were determined based on previous research findings (Bassiony et al., 2021; Al-Sagheer et al., 2023).

### 2.2 Growth parameters

Each rabbit was weighed separately at the start of the trial (5 weeks of age), and subsequently at 9 and 13 weeks of age, to determine BWG. The relative growth rate (RGR) was determined using the formula: $RGR = \frac{(Wt1 - Wt0)}{{}^{1}/_{2}(Wt0 + Wt1)} \times 100$. Where, W0 and W1 represent the initial and final body weights, respectively. The feed conversion ratio (FCR) was estimated by dividing total feed consumed (kg) by total weight gain (kg).

### 2.3 Carcass evaluation

On the 91$^{st}$ day of the trial, 32 rabbits (eight from each group) were weighted and subsequently slaughtered for carcass analysis. Hot carcass weight and the weights of various organs, including lungs, heart, kidneys, spleen, and liver were documented. The dressing percentage was calculated as the ratio of hot carcass weight to the live rabbit weight before to slaughter, represented as a percentage. Organ weights were expressed relative to live body weight (g/kg).

## 2.4 Blood sampling

On day 91 of the experiment, a total of 32 blood samples (eight rabbits per group) were obtained from the lateral ear vein. Two independent blood samples were obtained from each rabbit. The first sample, consisting of 2 ml, was collected into a tube containing an anticoagulant (EDTA). This sample was used to perform hematological analysis. The serum was separated from blood of the second sample after centrifugation for 20 min at 3000 rpm.

## 2.5 Hematological and biochemical parameters

Hematological parameters were assessed using whole blood samples to measure white blood cell count (WBC), hemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and hematocrit,. The analyses were conducted in accordance with the methodology outlined by Jain (1986) using an automated cell counter (model XE-2100, Sysmex, Kobe, Japan). Serum samples were analyzed to assess lipid profiles, immune parameters, antioxidant status, and serum selenium levels. Lipid profiles included low-density lipoprotein (LDL), total cholesterol, high-density lipoprotein (HDL), total triglycerides, and very low-density lipoprotein (VLDL). Immunological indices such as immunoglobulin M (IgM) and immunoglobulin G (IgG), as well as antioxidant markers including catalase, malondialdehyde (MDA), and glutathione (GSH), were evaluated using Diamond Diagnostic Co. kits (Giza, Egypt) in accordance with the manufacturer's instructions. Serum selenium was also quantified spectrophotometrically.

## 2.6 Cecal volatile fatty acid analysis

Following the slaughter of the rabbits, the cecal contents were promptly collected (n=8/group). The contents were extracted by applying gentle pressure and filtering through four layers of cheesecloth into a beaker. A 1 ml aliquot of the cecal content was mixed with 200 µL of 25% (w/v) meta-phosphoric acid in microcentrifuge tubes. The mixture was then centrifuged at a speed of 30,000×g (JA–17 rotor) for a duration of 20 minutes. The resulting supernatant was analyzed for VFA contents using gas chromatography (GC), following the methodology described by Palmquist and Conrad (1971).

## 2.7 Statistical analysis

Data (growth, nutrient utilization, antioxidant and immune responses, blood metabolites, and cecal fermentation) were subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test to evaluate mean differences, with significance established at $P < 0.05$. All statistical analyses were conducted using the statistical package SPSS (version 21, IBM Corporation, Armonk, NY, USA). Prior to ANOVA, the Shapiro-Wilk W test was used to confirm normality, and Levene's test was employed to assess the homogeneity of variances. The statistical model was:

$Y_{ij}$ = + $T_i$ + $e_{ij}$, where $Y_{ij}$ = observation, = general mean, $T_i$ = Treatment effect, and $e_{ij}$ = random error.

# 3 Results

Throughout the trial, a mean ambient temperature of 32.87 ± 0.91°C and a humidity level of 58.64 ± 1.02% were documented inside the farm. The average computed THI of 30.50 ± 0.39 indicates that the rabbits were under high heat stress.

## 3.1 Rabbit growth performance

Table 2 illustrates the impact of selenium-probiotic supplementation and colistin on growth performance and feed efficiency in rabbits during summer conditions. The findings indicated that after 13 weeks of age, rabbits receiving diets enriched with Se plus *Enterococcus faecium* or *Clostridium butyricum* exhibited a considerably greater body weight ($P = 0.001$) compared to the control group. No statistically significant difference in body weight was observed at week 9 of age between the control group and the supplemented groups. Rabbits received Se plus EF showed the highest BWG during the period from 9 to 13 weeks of age, followed by groups received COL and Se plus CB, while the lowest were obtained in the control group. Throughout the overall period (5-13 weeks), BWG of rabbits fed diets enriched with COL, SE+EF, and Se+CB was greater than that of rabbits in the control group. The relative growth rate of rabbits remained unaltered by treatments during the 5-9 and 9-13 weeks of age; however, it was significantly greater ($P = 0.003$) in rabbits receiving COL, SE+EF, and Se+CB over the overall period (5-13 weeks of age) compared to control rabbits. None of the treatments affected feed intake of rabbits at all the studied ages. The values of FCR were comparable across groups during the periods of 5-9 weeks and 9-13 weeks of age. Rabbits receiving a diet supplemented with COL and Se+ EF exhibited elevated FCR values throughout the entire duration (5-13 weeks of age) when compared with the control group. The FCR values between the control group and the SE+CB group showed no significant difference.

## 3.2 Slaughter traits

Table 3 presents the carcass traits of NZW rabbits influenced by dietary supplementation with Se-probiotic (CB or EF) and COL during

TABLE 1 Formulation and chemical analysis of the basal diet fed to rabbits.

| Item | Amount (%) |
|---|---|
| **Ingredient** | |
| Soybean meal | 14 |
| Wheat bran | 22 |
| Alfalfa hay | 34 |
| Barely grain | 17 |
| Yellow corn | 8 |
| Cane molasses | 3 |
| Di-calcium phosphate | 1 |
| NaCl | 0.5 |
| Premix[1] | 0.4 |
| DL-methionine | 0.1 |
| Total | 100 |
| **Calculated values[2], as fed** | |
| Digestible energy, MJ/kg | 10.30 |
| Lysine | 0.75 |
| Methionine + cysteine | 0.59 |
| **Analyzed composition, as fed** | |
| Dry matter | 91.50 |
| Crude protein (N × 6.25) | 16.38 |
| Ether extract | 2.73 |
| Organic matter | 83.60 |
| Neutral detergent fiber[3] | 31.56 |

[1]Premix provided the following vitamins and minerals per kilogram of diet: vitamin A, 8000 IU; vitamin $D_3$, 600 IU; vitamin E, 34 mg; vitamin $K_3$, 1.32 mg; vitamin $B_1$, 1.32; vitamin $B_2$, 4.0 mg; vitamin $B_6$, 1.32 mg; vitamin $B_{12}$, 0.01 mg; pantothenic acid, 13.32 mg; biotin, 0.13 mg; folic acid, 3.32 mg; choline chloride, 800 mg; manganese 32 mg; zinc 60 mg; iron 120 mg; copper 16 mg; iodine 2 mg; selenium 0.4 mg; and cobalt 0.4 mg.
[2]Calculated according to tables of ingredients (Maertens et al., 2002).
[3]Neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash.

summer conditions. The results displayed that neither the relative weights of internal organs (liver, lungs, spleen, heart, and kidney) nor dressing percentages were significantly affected by supplemental COL, Se and probiotics used in the present study (P > 0.05).

## 3.3 Hematological parameters

Table 4 presents the effects on blood hematology of growing rabbits under summer conditions of dietary supplementation with colistin and Se plus CB or EF. Non-significant increases were detected in hematological parameters including erythrogram (Hb, RBCs, MCV, MCH, MCHC, Hct and platelets) and leukogram (WBCs, lymphocyte, eosinophils, neutrophils and monocytes) pictures of blood in groups which received supplemental COL and Se probiotics (P > 0.05).

## 3.4 Biochemical parameters

Table 5 shows that dietary supplementation with Se-probiotic combination and colistin decreased the values (p= 0.030) of TC compared to the control group. Additionally, triglycerides and LDL values of the rabbit received diets containing Se plus EF significantly diminished compared to that of the control group (P=0.006 and P= 0.003, respectively). Moreover, values of VLDL were decreased with the treatment of COL and Se+CB (P=0.050). However, the findings exhibited that the influence of dietary treatments on HDL was not significant. Rabbit received the Se plus *E. faecium* and Se plus *C. butyricum* revealed the highest the blood Se content followed by the control and COL groups.

## 3.5 Antioxidant and immunological parameters

Figure 1 displayed impacts of dietary supplementation on serum concentrations of catalase, GSH, and MDA. The results illuminated that rabbits fed on diets supplemented with Se plus probiotic (Se+CB or Se+EF) displayed significantly higher levels of catalase than the COL and control groups. Additionally, GSH activity was increased in rabbits received COL and Se-probiotics (Se+CB or Se+EF) compared to the control. However, MDA values were declined in rabbits supplemented with Se plus probiotic (Se +CB or Se+EF) compared with the COL and control groups.

Serum concentrations of immunoglobulins (IgG and IgM) are summarized in Figure 2. The inclusion of the Se plus EF in rabbit feed had the highest value (p=0.034), followed by group treated with Se plus CB compared with control group. A notable increase in IgM levels was detected in the rabbits of the Se-probiotic group relative to the COL and control groups (p=0.133).

## 3.6 Cecal fermentation parameters

The cecal fermentation parameters of growing rabbits, influenced by the dietary selenium-probiotic combination and colistin during summer conditions, are presented in Table 6. The results demonstrated that cecal VFA concentration (acetic, propionic, butyrate, valerate and total VFA; mmol/100 ml) and cecal VFA proportions (%) were statistically nonsignificant (P>0.05) for all groups. Influences of dietary supplementation on cecal concentrations of ammonia-N were presented in Figure 3. The finding showed that cecal ammonia concentration was significantly declined with the inclusion Se plus probiotic (Se+CB or Se+EF) and COL in rabbit diets compared with the control rabbits.

# 4 Discussion

Under hot summer temperatures, HS is a significant environmental stressor that results in substantial economic losses for the rabbit industry (El-Ratel et al., 2025a, b). Therefore, the

TABLE 2 Growth performance and feed utilization of growing rabbits as affected by dietary supplementation with selenium-probiotic combination and colistin under summer conditions.

| Parameter | Experimental groups[1] | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | Control | COL | Se+CB | Se+EF | | |
| **Body weight (g)** | | | | | | |
| week 5 (Initial) | 705 | 688 | 692 | 685 | 5.19 | 0.633 |
| week 9 | 1297 | 1321 | 1327 | 1368 | 13.39 | 0.337 |
| week 13 (Final) | 1748 [c] | 1815 [bc] | 1828 [b] | 1907 [a] | 13.89 | 0.001 |
| **BWG (g/day)** | | | | | | |
| week 5–9 | 592 | 633 | 635 | 683 | 13.55 | 0.161 |
| week 9–13 | 452 [b] | 494 [ab] | 501 [ab] | 540 [a] | 10.15 | 0.035 |
| week 5–13 (Overall) | 1043 [c] | 1127 [b] | 1136 [b] | 1222 [a] | 14.91 | <0.001 |
| **Relative growth rate, %** | | | | | | |
| week 5–9 | 58.50 | 62.88 | 62.89 | 66.19 | 1.06 | 0.122 |
| week 9–13 | 29.96 | 31.61 | 31.85 | 33.11 | 0.70 | 0.529 |
| week 5–13 (Overall) | 84.80 [b] | 90.02 [a] | 90.21 [a] | 94.14 [a] | 0.86 | 0.003 |
| **Feed intake (g/day)** | | | | | | |
| week 5–9 | 70.89 | 66.97 | 70.18 | 68.92 | 0.86 | 0.410 |
| week 9–13 | 112.93 | 115.81 | 118.70 | 117.41 | 1.39 | 0.570 |
| week 5–13 (Overall) | 91.91 | 91.39 | 94.44 | 93.16 | 0.88 | 0.611 |
| **FCR (g feed/g gain)** | | | | | | |
| week 5–9 | 3.53 | 3.02 | 3.15 | 2.94 | 0.08 | 0.117 |
| week 9–13 | 7.09 | 6.68 | 6.93 | 6.19 | 0.15 | 0.193 |
| week 5–13 (Overall) | 4.99 [a] | 4.56 [b] | 4.68 [ab] | 4.31 [b] | 0.07 | 0.006 |

[1]The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet.

[a-c]Means in a column with different superscripts differ significantly. The level of significance was set at P ≤0.05. The experimental unit was the cage, n =20 cages per treatment.

BWG, body weight gain; FCR, feed conversion ratio; SEM, standard error of the mean.

TABLE 3 Carcass traits of growing rabbits as affected by dietary supplementation with selenium-probiotic combination and colistin under summer conditions.

| Parameter | Experimental groups[1] | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | Control | COL | Se+CB | Se+EF | | |
| Dressing (g/kg SW) | 576.7 | 575.4 | 574.2 | 583.4 | 2.27 | 0.458 |
| Liver (g/kg SW) | 29.27 | 26.63 | 27.27 | 26.65 | 0.57 | 0.429 |
| Lungs (g/kg SW) | 7.13 | 7.39 | 6.79 | 7.28 | 0.19 | 0.708 |
| Spleen (g/kg SW) | 0.65 | 0.47 | 0.65 | 0.46 | 0.04 | 0.135 |
| Heart (g/kg SW) | 2.57 | 2.32 | 2.56 | 2.72 | 0.08 | 0.390 |
| Kidney (g/kg SW) | 6.84 | 6.03 | 6.35 | 6.48 | 0.19 | 0.593 |

[1]The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet. The experimental unit was the individual rabbit.

TABLE 4 Hematological parameters of growing rabbits as affected by dietary supplementation with selenium-probiotic combination and colistin under summer conditions.

| Parameter | Experimental groups[1] | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | Control | COL | Se+CB | Se+EF | | |
| **Erythrogram** | | | | | | |
| Hb (g/dl) | 10.95 | 12.21 | 11.82 | 11.91 | 0.21 | 0.193 |
| RBCs ($10^6$/ml) | 4.56 | 4.90 | 4.91 | 4.93 | 0.10 | 0.532 |
| MCV (fL) | 77.34 | 80.32 | 76.86 | 76.20 | 0.78 | 0.241 |
| MCH (pg/dl) | 24.09 | 25.05 | 24.10 | 24.20 | 0.20 | 0.233 |
| MCHC (g/dL) | 31.26 | 31.23 | 31.36 | 31.76 | 0.19 | 0.751 |
| Hct (%) | 35.20 | 39.09 | 37.69 | 37.47 | 0.66 | 0.240 |
| Platelets ($10^3$/ml) | 292 | 372 | 350 | 397 | 20.10 | 0.318 |
| **Leukogram** | | | | | | |
| WBCs ($10^3$/ml) | 6.00 | 4.71 | 5.79 | 6.06 | 0.27 | 0.252 |
| Lymphocyte (%) | 64.55 | 65.69 | 66.25 | 64.68 | 1.26 | 0.961 |
| Eosinophils (%) | 2.00 | 2.44 | 2.00 | 2.33 | 0.16 | 0.682 |
| Neutrophils (%) | 29.71 | 28.87 | 29.08 | 30.43 | 1.34 | 0.978 |
| Monocytes (%) | 2.00 | 2.67 | 2.44 | 2.33 | 0.10 | 0.139 |

[1]The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of *Enterococcus faecium*/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet.

SEM stands for standard error of the mean; Hb, Hemoglobin; RBCs, Red blood cells; Hct, The hematocrit; MCV, Mean corpuscular volume; MCHC, Mean corpuscular hemoglobin concentration; WBCs, White blood cells. The experimental unit was the individual rabbit.

addition of some feed additives in the diet is necessary as growth promoters and immune booster for improving rabbit tolerance against these stressful conditions (Alagawany et al., 2022; Reda et al., 2022; El-Ratel et al., 2025b). Good health and improved performance reflect the beneficial effect of feed additive (El-Wardany et al., 2016; Oladimeji et al., 2022; Ebeid et al., 2023; Mohamed et al., 2025). The findings of the current study demonstrated that the addition of a selenium-probiotic combination to rabbit diets during summer conditions significantly enhanced BW and BWG in comparison to the control group. The observed growth-promoting effect may be ascribed to the synergistic interaction between Se and probiotics when administered together. Research indicates that the inclusion of dietary Se can significantly reduce the negative impacts of HS on the growth performance of animals. It was noted that growing rabbits' growth performance was enhanced by supplemental dietary Se (Ebeid et al., 2013). Some of the effects of Se were summarized as follows by Zheng et al. (2022): (I) Se can promote FCR by modulating the metabolism of carbohydrates,

TABLE 5 Lipid profile and selenium content in growing rabbits serum as affected by dietary supplementation with selenium-probiotic combination and colistin under summer conditions.

| Parameter | Experimental groups[1] | | | | SEM | P-Value |
|---|---|---|---|---|---|---|
| | Control | COL | Se+CB | Se+EF | | |
| TC (mg/dl) | 241.83[a] | 175.11[b] | 157.33[b] | 177.33[b] | 10.00 | 0.030 |
| TRIG (mg/dl) | 208.00[a] | 185.56[a] | 173.11[ab] | 135.22[b] | 7.72 | 0.006 |
| HDL (mg/dl) | 41.00 | 45.44 | 48.56 | 43.89 | 1.71 | 0.533 |
| LDL (mg/dl) | 119.37[a] | 111.53[a] | 87.04[ab] | 51.98[b] | 7.55 | 0.003 |
| VLDL (mg/dl) | 47.17[a] | 35.47[b] | 31.47[b] | 39.36[ab] | 1.97 | 0.050 |
| Se (mg/L) | 0.35[b] | 0.33[b] | 0.56[a] | 0.54[a] | 0.03 | <0.001 |

[1]The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet.

[a,b]Means in a column with different superscripts differ significantly. The level of significance was set at $P \leq 0.05$.

TC, total cholesterol; TRIG, total triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. The experimental unit was the individual rabbit.

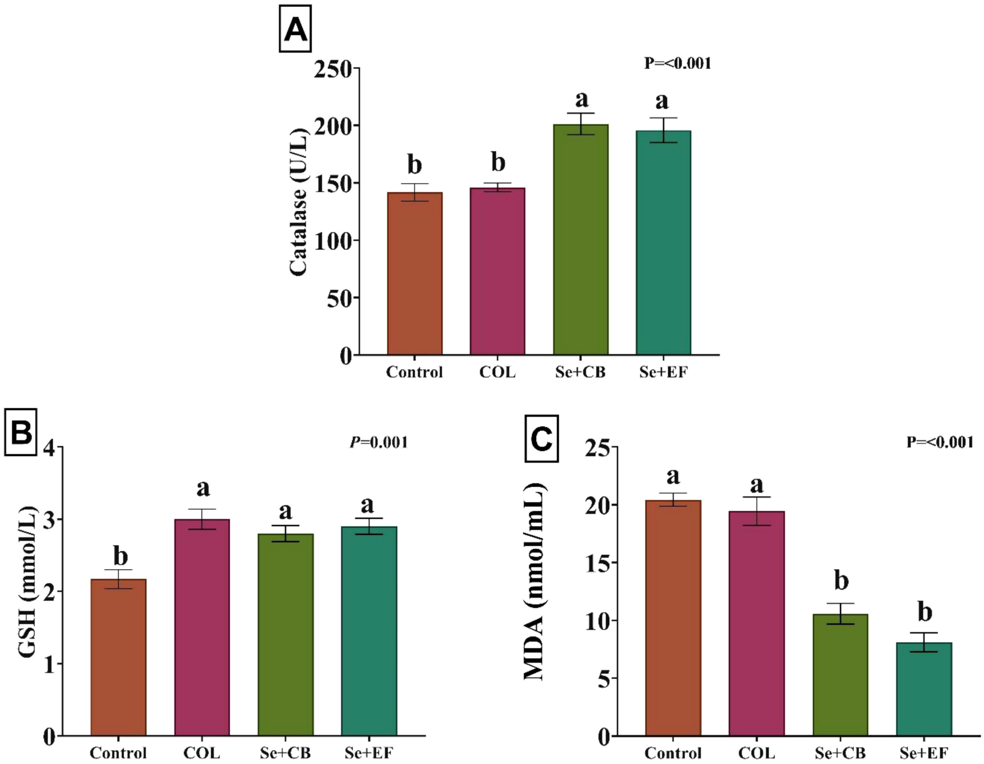


FIGURE 1
Effects of dietary supplementation on serum concentrations of catalase **(A)**, reduced glutathione [GSH; **(B)**], and malondialdehyde [MDA; **(C)**]. Different letters above the error bar indicate significant differences at P < 0.05. Values are expressed as means ± standard error. The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and 1 x $10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and 2.5 x $10^6$ cfu of *Clostridium butyricum*/kg diet.

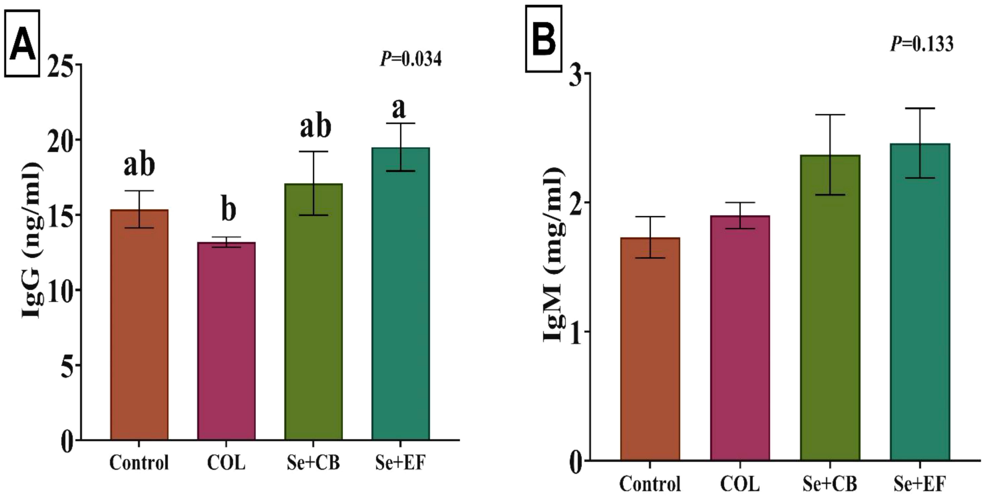


FIGURE 2
Effects of dietary supplementation on serum concentrations of immunoglobulin G [IgG; **(A)**] and immunoglobulin M [IgM; **(B)**]. Different letters above the error bar indicate significant differences at P < 0.05. Values are expressed as means ± standard error. The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and 1 × $10^8$ cfu of Enterococcus faecium/kg diet. (4) Se +CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and 2.5 × $10^6$ cfu of *Clostridium butyricum*/kg diet.

TABLE 6 Cecal fermentation parametersof growing rabbits as affected by dietary supplementation with selenium-probiotic combination and colistin under summer conditions.

| Parameter | Dietary treatments[1] | | | | SEM | p value |
|---|---|---|---|---|---|---|
| | Control | COL | Se+CB | Se+EF | | |
| **Cecal VFA concentration (mmol/100 ml)** | | | | | | |
| Acetic | 84.80 | 83.84 | 89.84 | 91.25 | 1.91 | 0.439 |
| Propionic | 7.66 | 7.66 | 8.02 | 9.00 | 0.28 | 0.254 |
| Butyrate | 14.57 | 18.99 | 19.80 | 17.60 | 0.74 | 0.096 |
| Valerate | 1.09 | 1.19 | 1.23 | 1.18 | 0.04 | 0.772 |
| Total VFA | 108.12 | 111.68 | 118.88 | 119.02 | 2.23 | 0.251 |
| **Cecal VFA proportions (%)** | | | | | | |
| Acetic (%) | 78.23 | 74.89 | 75.65 | 76.60 | 0.55 | 0.207 |
| Propionic (%) | 7.02 | 6.92 | 6.78 | 7.54 | 0.22 | 0.605 |
| Butyrate (%) | 13.75 | 17.12 | 16.53 | 14.86 | 0.60 | 0.210 |
| Valerate (%) | 1.01 | 1.07 | 1.04 | 0.99 | 0.04 | 0.876 |

[1]The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet. The experimental unit was the individual rabbit.

lipids, and proteins; (II) Se can augment the antioxidant defenses of animals, mitigating oxidative stress induced by heat and diminishing inflammatory responses, finally resulting in enhanced growth performance. Additionally, supplementation with probiotic *Enterococcus faecium* has been demonstrated to enhance broiler performance by improving FCR and increasing BWG, as reported by Samli et al. (2007). Hossain et al. (2015) found that broilers receiving diets supplemented with probiotic strains, including *C. butyricum*, exhibited a linear increase in BW and enhanced FCR compared to the control group. Ayyat et al. (2018) found that dietary Se and live yeast enhanced FCR in rabbits during summer conditions compared to the non-supplemented group. In the same context, values of FCR, net revenue and economic efficiency were improved with Nano-Se groups followed by organic Se (Se-probiotic) group compared to the control group (Moustafa et al., 2024).

Furthermore, Pogány Simonová et al. (2020) demonstrated that the inclusion of *E. faecium* in drinking water resulted in increased average daily body weight gain and final weight in rabbits compared to the control group. Bassiony et al. (2021) shown that the EF treatments enhanced FCR and BW in comparison to the COL treatment and control group. The supplementation of *C. butyricum* augmented BW of rabbits compared to control diet (Wang et al., 2023). Alagawany et al. (2023) indicated that providing the EF+CB complex to growing rabbits in hot summer conditions enhanced feed efficiency, as seen by FCR, and resulted in superior performance for BWG and final BW. Al-Sagheer et al. (2023) shown that the incorporation of selenium, inactivated yeast, and their combination in the diets of heat-stressed rabbits enhanced FCR over the period. The underlying mechanisms may involve several factors, including the production of intestinal enzymes, enhancement of the host immune system, increased resistance to colonization, reduction of toxin production, and lowering stress in rabbits (Abdel-Wareth et al., 2021; Elghandour et al., 2020). Overall, the productive performance improved due to this constructive action. The enhanced production of SCFAs in the caecum, especially butyric acid, which effectively improves feed utilization by stabilizing gut micro-ecological environments, may explain the observed performance improvements (Nakanishi et al., 2003). Furthermore, *C. butyricum* is purported to augment the activity

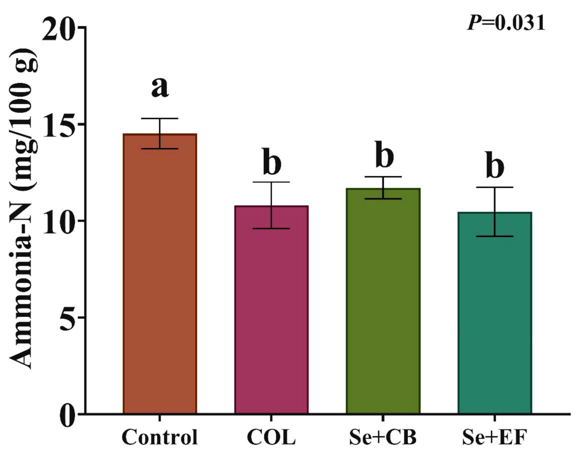


FIGURE 3
Effects of dietary supplementation on cecal concentrations of ammonia-N. Different letters above the error bar indicate significant differences at $P < 0.05$. Values are expressed as means ± standard error. The experimental groups consisted of (1) Control group: rabbits were fed a basal diet without any additions. (2) COL group: rabbits were fed the basal diet supplemented with 120 mg of colistin/kg. (3) Se+EF group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $1 \times 10^8$ cfu of Enterococcus faecium/kg diet. (4) Se+CB group: rabbits were fed the basal diet fortified with 0.3 mg of selenium and $2.5 \times 10^6$ cfu of *Clostridium butyricum*/kg diet.

of digestive enzymes, hence improving nutrient digestion and absorption (Fu et al., 2021). Finally, the enhancement in growth performance suggests a synergistic effect that facilitates a more efficient utilization of dietary Se and probiotics as feed additives.

Herein, the results indicate that the addition of a Se-probiotic combination did not affect carcass traits. Ayyat et al. (2018) found that rabbits kept in the summer days and supplemented with Se and live yeast did not affect carcass characteristics in comparison to the non-supplemented group. Bassiony et al. (2021) demonstrated that the relative organ weights and carcass yield percentages were not significantly influenced by all treatments. Al-Sagheer et al. (2023) reported that selenium supplementation did not influence carcass parameters. Consequently, the present findings confirm certain previous studies that indicated probiotics exerted no significant effect on rabbit organ and slaughter characteristics (Ayyat et al., 2018; Bhatt et al., 2017).

The current findings indicate that dietary supplements with colistin and Se, combined with either CB or EF, did not influence the blood variables in rabbits raised in summer environments. Al-Sagheer et al. (2023) shown that in heat-stressed growing rabbits, inactivated yeast and selenium did not significantly affect the levels of Hb, MCH, WBC, RBC, platelets, hematocrit%, and MCV. As well, Ayyat et al. (2018) found that under summertime conditions, Hb levels in growing rabbits were unaffected by dietary Se. This implies that the probiotics, at the supplemental doses administered, could maintain the regular hematopoietic function of rabbits. Conversely, Bassiony et al. (2021) reported that dietary addition with EF or EF+CB significantly increased the blood Hb content in growing rabbits raised in conditions of heat stress. The variances in results may stem from various factors, including the type of bioactive chemicals utilized, their origin, sample size, environmental stressors, and supplementation dosages.

In the current study, lipid profile in rabbits received selenium-probiotic combination (especially Se+EF) was improved. The improvement of lipid levels in rabbits fed diet containing Se-probiotic combination was due to that these supplements act as an effective antioxidant. The results presented here align with the findings of Amer et al. (2019), which shown that Se may reduce the blood concentrations of total cholesterol in growing rabbits. Guo et al. (2020) suggest that Se supplementation may exert an anti-cholesteremic effect by inhibiting adipose tissue degradation and reducing the diffusion of free fatty acids into the blood. Se-dependent antioxidant enzymes can aid in reducing hydrogen peroxide and lipid peroxide levels and may be beneficial in decreasing cholesterol levels (El-Deep et al., 2017). Wojcicki et al. (1991) found that decreasing the severity of atherosclerosis and lipid peroxidation was achieved by include Se in rabbits' diet. According to Yazhini et al. (2018), giving broilers probiotic supplements positively changed their lipoprotein metabolism, resulting in a more marked decrease in LDL and TC and an increase in HDL. Bassiony et al. (2021) showed that adding EF and CB in rabbit diets raised in the summer significantly reduced serum triglycerides and extremely LDL while raising HDL. This finding suggests that these supplements may have a hypocholesterolemic effect. Probiotics can influence blood cholesterol levels by incorporating cholesterol into their cells, converting it into coprostanol in the gut for excretion, inhibiting the rate-limiting enzyme of cholesterogenesis, or hydrolyzing bile salts (Nour et al., 2021). The synergistic effect of probiotic and minerals including selenium mitigates the adverse effects of heat stress on the rabbits. The hypolipidemic effects of probiotic with Se in reducing TG and TC, and increasing HDL level in serum were reported in rabbits (El-Ratel et al., 2023a). Supplemental probiotic with Zinc and/or Selenium decreased total cholesterol and LDL levels in blood serum (Hassan et al., 2021). Such effect may be attributed to the fact that all supplements could decrease absorbed and synthesized cholesterol in the poultry gut (El-Ratel et al., 2023b).

For rabbits to be healthy, microbial fermentation must be stable (Pogány Simonová et al., 2020). Rabbits employ bacterial fermentation in their digestive process and rely on the total VFA obtained from the cecum as a consistent energy source (Abu Hafsa and Hassan, 2021). The present study investigates the potential for enhancing cecal fermentation and intestinal health in rabbits. Results indicate that the concentrations of cecal VFA including acetic, propionic, butyrate, valerate, and total VFA—as well as their proportions, exhibited non-significant improvements when a selenium-probiotic combination was incorporated into rabbit feed during heat stress conditions. Al-Sagheer et al. (2023) stated that cecal VFA was not influenced by the dietary Se. Besides, *E. faecium* CCM7420's incorporation into rabbit feed resulted in an increase in fecal acetic acid levels in comparison to control rabbits. However, it did not affect the levels of other organic acids that were assessed, including butyric, succinic, and lactic acids (Simonová et al., 2008). Similarly to our findings, the molar proportion of VFA in the caecum was not influenced by the administration of additional probiotics (*E. faecium* CCM4231) to rabbits (Szabóová et al., 2011). Dietary *C. butyricum* supplementation augmented the levels of butyric acid, acetic acid, and total SCFAs in the intestinal digesta (Han et al., 2020). Furthermore, SCFAs benefit animals by providing intestinal mucosal epithelial cells with energy.

The capacity of natural feed additives to induce an overproduction of enzymes in the antioxidant defense system, which can neutralize reactive oxygen species (ROS) and reduce lipid peroxidation, may account for the improved antioxidant function (Mohammed et al., 2019). Wang et al. (2017b) elucidated that probiotics enhanced the activity of antioxidant enzymes such as SOD and GSH-Px, hence bolstering the system that protects against free radicals. Antioxidant enzymes help to degrade hydrogen peroxide and superoxide anions as well as inactivate ROS (Shen et al., 2014). Diets supplemented with *C. butyricum* augmented the concentration of GSH-Px, CAT, and SOD in gut of Rex rabbits and broiler chickens (Liao et al., 2015; Liu et al., 2019). Obianwuna et al. (2023) indicated that dietary probiotics supplementation significantly augmented GSH-Px, T-SOD, and CAT diminished MDA. Palkovicsné Pézsa (2023) summarized that probiotics have the potential to modify the redox status of the host in a number of ways, including: (1) decomposing ROS with their own antioxidant enzymes, (2) chelating metal ions, (3) controlling the host's enzymes producing ROS, (6) controlling the host's intestinal microbiota, (4) generating metabolites with antioxidant capacity, and (5) controlling cell signalling pathways. As for the role of selenium, it lowers lipid peroxidation and activates antioxidant enzymes, both of which are essential to the body's antioxidant defense system (Ebeid et al., 2013). The improvement of antioxidant state was positively correlated with the Se role in GSH-Px activation. According to Al-Sagheer et al. (2023), Se and inactivated yeast together have the ability to lower MDA growth levels and raise blood CAT and GPx levels. Supplementation with a combination of probiotic and SeNPs for five weeks pre-mating reduced MDA of heat-stressed rabbit does (El-Ratel et al., 2023a). Selenoproteins (TRxR and GSH-Px), which are crucial for the scavenging of ROS and the cellular redox system, have been linked to the primary antioxidant activity of Se (Brigelius-Flohé et al., 2001; Pilarczyk et al., 2012).

Immunomodulatory feed additives are primarily intended to reduce inflammation and prevent immune function impairment. The enhanced immune status in the current study is a sign of the immunomodulatory effects of the Se-probiotic combination under summer conditions and the potential for supporting the rabbit immune system component. It has been documented that probiotic microbes alter the host immunological response to infection by stimulating the creation of immunoglobulin (Fukushima et al., 1998). Wang et al. (2017a) observed that dietary supplementation with probiotics elevated serum levels of IgG and IgM. Some findings presented higher IgG and IgM levels and boosted cell-mediated immunity after probiotic administration to rabbits (Fathi et al., 2017; Wang et al., 2017a). Liu et al. (2019) showed that giving weaned rabbits the *Clostridium butyricum* probiotic resulted in improved immunoglobulin production and preserved intestinal barrier function. According to Wang et al. (2019), EF and CB regulate the structure of the intestinal flora to promote the production of VAFs, which in turn enhances immune function, augmenting the serum IgA, IgM and IgG levels. Han et al. (2020) discovered that *C. butyricum* can increase immunological function, allowing animals to fight off harmful microorganisms. They also showed that *C. butyricum* supplementation led to higher serum levels of IgM, IgG, and IgA than the control group. Improved immune function may partially contribute to the enhancement of antioxidant status and the suppression of ROS formation (Aviello and Knaus, 2018). Bassiony et al. (2021) shown that the inclusion of EF and CB enhanced immunological indicators in rabbits subjected to HS environments. The group of rabbits that received probiotics exhibited higher levels of serum lysozyme activity and complement component 3 compared to the control group (Bassiony et al., 2021). The synthesis of selenoprotein, which facilitates the proliferation and differentiation of immune cells, is generally associated with the biological effects of selenium on livestock (Pisoschi and Pop, 2015). Increased immunoglobulin levels can be attributed to the essential biological role of selenium in augmenting T helper cell activity and stimulating cytokine release. Diet Selenium-enriched probiotics enhanced antioxidant function, increased immune responses (Yanez-Lemus et al., 2022), and alleviated inflammation-induced intestinal injury (Liu et al., 2022). Furthermore, selenium-enriched probiotics significantly regulated intestinal flora, and significantly reduced the number of pathogenic bacteria (Kheradmand et al., 2014). Liu et al. (2023) stated that nano selenium-enriched probiotics play an important role in the treatment of immune modulation and diseases.

The sustainability and health of animals depend on the enhancement of environmental conditions and the management of excreted metabolic products (Emam et al., 2023). Reduced ammonia production by rabbits improves their growth and general health and lowers the mortality rate. According to Alagawany et al. (2023), rabbits that received (EF + CB) had the lowest concentrations of ammonia. Additionally, probiotic supplementation was linked to a lower cecum ammonia concentration, which may indicate that the liver is using more ammonia to produce protein (Alagawany et al., 2023). Ammonia impairs the structural integrity of cecal tissues and reduces the levels of butyrate, acetate, and propionate in rabbit cecums (Cui et al., 2021). Huang et al. (2021) reported that feeding rabbits with *C. butyricum* improves their intestinal morphology, gut microbiota, and growth performance.

## 5 Conclusion

The dietary supplementation of selenium has synergetic influence with probiotic (*Enterococcus faecium* and *Clostridium butyricum*) and enhanced growth performance antioxidant and immunological indices, blood parameters and cecal fermentation in growing rabbits reared under summer conditions. These results suggest that selenium-probiotic combination is a novel additive that can be included in the diet of rabbits to improve health parameters. Therefore, a better understanding of the relationship between probiotics and selenium in feed could enable the targeted improvement of nutritional strategies that mitigate heat stress, which is detrimental to the rabbit industry.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

The experimental protocols applied in this study were approved by Ethics Committee of the Local Experimental Animals Care of Zagazig University with the assigned approval number ZU-IACU/2/F/1/2024. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

MSE: Writing – original draft, Writing – review & editing. SSB: Writing – original draft, Writing – review & editing. AAA: Writing – original draft, Writing – review & editing. MA: Conceptualization, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. MEG: Writing – original draft, Writing – review & editing. EAE: Writing – original draft, Writing – review & editing. AL: Writing – original draft, Writing – review & editing. AAE: Writing – original draft, Writing – review & editing. MM: Writing – original draft, Writing – review & editing. MMA: Writing – original draft, Writing – review & editing. SSE: Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

## Publisher's note



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