

## Dose-response effects of aqueous garlic extract supplementation via drinking water on growth, hematology, immunity, and coccidiosis in Japanese Quails (*Coturnix Japonica*)

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### ABSTRACT

This research evaluated garlic extract addition to drinking water on growth performance, hematological characteristics, survivability, and coccidial infection in Japanese quail (*Coturnix japonica*). There were 500 quails randomly allotted to four groups receiving 0, 1, 2, or 4 mL/L garlic extract for 35 days. Survival improved significantly in all garlic groups, especially 4 mL/L group, which kept 100% survivability within early duration and 99.2% upon termination of the experiment. Weight gain rose considerably with garlic addition, where 4 mL/L group achieved  $196.08 \pm 1.99$  g at 35 days compared to  $183.22 \pm 1.02$  g control group. Conversely, daily intake tended to be low in garlic groups, especially in the finishing phase (27–35 days), in support of improved efficiency in feed intake. Hematological findings indicated red blood cell and hemoglobin declined in a dose-related manner, yet white blood count significantly elevated, showing garlic's immunostimulation role. Additionally, shedding of oocyst considerably diminished in garlic-innoculated birds. At 35 days, 4 mL/L group showed  $3.6 \pm 0.2$   $\log_{10}$  oocysts/g feces.oocyst count, whereas  $4.9 \pm 0.3$   $\log_{10}$  were counted in 4 mL/L control, affirming a dose-related anti-coccidial action. These data indicate garlic extract from 2 to 4 mL/L can improve growth, immunity, and disease resistance of Japanese quail without any negative impact effects.

**Keywords:** *Coccidial infection, Coturnix japonica, Garlic extract, Growth performance, Quails*

### INTRODUCTION

Modern poultry production increasingly emphasizes the importance of aligning farming practices with the physiological and biological needs of birds to ensure optimal health and productivity. A robust and well-performing poultry population is essential for maintaining both the profitability and sustainability of the industry. To achieve this, contemporary approaches are shifting toward the use of science-based interventions that promote growth, strengthen the immune system, and enhance overall disease resistance. In this context,

considerable attention has been directed toward feed additives of natural-origin, which are being explored as promising tools for improving animal nutrition and performance (Razanova, 2018; Chudak *et al.*, 2019; Cherniy *et al.*, 2021).

Among various poultry species, Japanese quail (*Coturnix japonica*) has gained increasing attention due to its favorable biological characteristics. These include rapid growth, early sexual maturity, efficient feed conversion, and adaptability to intensive production environments. The global expansion of quail farming is largely attributed to these traits, combined with rising consumer demand for quail

meat and eggs (Lukanov, 2019). In Vietnam, quail farming has developed quickly, ranging from smallholder farms to large-scale operations, thanks to its relatively low risk and simplicity in rearing techniques (Khang *et al.*, 2021). As noted by Rehman *et al.* (2022), this species is referred to by different local names, such as “marai” in Iraq and “ferry” in Syria. Japanese quails are easily recognizable due to their compact body size, generally not exceeding 20 cubic centimeters.

Growing public concerns over antimicrobial resistance and the demand for residue-free animal products have accelerated the search for effective natural alternatives to antibiotic growth promoters. Quails are particularly susceptible to environmental stressors, with heat stress being a significant limiting factor in their reproductive performance, especially in tropical and subtropical regions (El-Tarabany, 2016). Garlic has been historically used for the treatment of respiratory ailments such as colds, coughs, and asthma, and more recent studies have highlighted its potential in enhancing immune system responses (Lawrence and Lawrence, 2011). Additionally, garlic possesses a wide array of biological activities including antibacterial (Shobana *et al.*, 2009), antifungal (Pai and Platt, 1995), antiviral (Weber *et al.*, 1992), anticancer (Kaschula *et al.*, 2010), antioxidant (Galano and Francisco-Marquez, 2009), hypocholesterolemic, antiplatelet, and anti-inflammatory effects (Piscitelli *et al.*, 2002). These properties are attributed to a variety of bioactive compounds such as phenols, flavonoids, polysaccharides, and proteins (Bianchini and Vainio, 2001; Nishimura *et al.*, 2004). Garlic (*Allium sativum L.*), a member of the Alliaceae family, has been used in traditional medicine for more than 5,000 years and is now being widely recognized as a potent phytochemical feed additive due to its functional compounds, especially allicin, which offers proven antimicrobial, antioxidant, and immunomodulatory effects (Amagase *et al.*, 2001). Although the extraction of bioactive constituents from garlic has been widely studied both internationally and in Vietnam, many conventional methods rely on toxic solvents like chloroform and methanol, which limit the applicability of garlic extract in food, nutraceutical, and pharmaceutical products (Jang

*et al.*, 2017). Despite these challenges, numerous studies continue to highlight garlic’s multifunctional roles in enhancing gastrointestinal and liver health, as well as its antimicrobial and antioxidant potential (Chung, 2006; Ziarlarimi *et al.*, 2011).

Coccidiosis remains one of the most economically damaging parasitic diseases in poultry, caused by protozoa of the genus *Eimeria* (Kemp *et al.*, 2013). With over 1,800 identified species, *Eimeria* parasites affect a wide range of avian and mammalian hosts by infecting the gastrointestinal tract (Haug *et al.*, 2008). These parasites have complex life cycles that occur entirely within a single host and involve both intracellular and extracellular developmental stages. The replication of *Eimeria* species within the intestinal lining leads to the production of oocysts, which are shed into the environment through feces. When birds ingest sporulated oocysts, the cycle begins anew in the gut (Chapman, 2014). In quails, the most prevalent species include *Eimeria tsunodai*, *E. uzura*, and *E. bateri* (Lu *et al.*, 2021). Japanese quails are particularly vulnerable, and coccidiosis significantly affects their health and productivity, especially during early growth stages (El Bakrey *et al.*, 2024). The disease results in decreased weight gain, poor feed conversion, intestinal lesions, and increased mortality, causing substantial economic losses (Umar *et al.*, 2014). Yet despite its significance, coccidiosis in quails has received limited research attention, particularly regarding its specific clinical manifestation and underlying genetic factors (Arafat and Abbas, 2018). Conventional management strategies rely heavily on synthetic anticoccidials, but long-term use has led to the emergence of drug-resistant strains and the risk of chemical residues in poultry products. This has prompted growing interest in natural alternatives that can offer effective disease control while preserving food safety.

The present study was designed to evaluate the dose-dependent effects of aqueous garlic extract supplementation through drinking water on growth performance, survival rate, hematological parameters, and coccidial infection levels in Japanese quails. Unlike previous studies focusing on dietary inclusion of garlic powder, this work highlights the novelty of

using aqueous extract via water administration to enhance growth, immunity, and disease resistance under tropical rearing conditions.

## MATERIALS AND METHODS

### Location and Time

This experiment was conducted at a poultry breeding farm located in Phong Dien commune, Can Tho City, Vietnam, from August to October 2024.

### Animals and Experimental design

The experiment was conducted on 500 Japanese quails (*Coturnix japonica*) during the period from 1 to 35 days of age. The birds had an initial average body weight of  $10.96 \pm 0.15$  grams per bird and were healthy and uniform in terms of breed, age, and body weight. Prior to the formal experimental period, garlic extract was administered in the drinking water for five consecutive days to monitor any initial physiological or behavioral reactions. During this preliminary phase, the birds were closely observed, and no abnormal signs or adverse responses were recorded. Upon confirmation of safety, the main experiment was initiated.

The quails were assigned to a completely randomized design with one experimental factor, which was the level of garlic extract supplementation in drinking water. Four treatment groups were established: 0 mL/L (control), 1 mL/L, 2 mL/L, and 4 mL/L. Each treatment consisted of 25 replicate pens, and each pen housed five birds, totaling 125 birds per group.

The experiment was carried out in a semi-open poultry house with natural ventilation. Pens were arranged in a single row to minimize environmental variation across treatments. Each pen measured 60 cm in length, 40 cm in width, and 30 cm in height, and was equipped with one round plastic feeder and one nipple drinker. Rice husk was used as bedding material and was replaced weekly. The house was cleaned and disinfected before bird placement, and strict biosecurity measures were maintained throughout the trial.

The ambient temperature was maintained at 33–35°C during the first week and gradually decreased by 2°C per week until reaching 26°C. Lighting was provided for 23 hours per day in

the first week and adjusted to 16 hours per day from the second week onward.

### Feeds and Feeding

All quails were fed a commercial mash diet formulated for growing quails (FMC 332, Fame Feed Company, Vietnam). According to the manufacturer's specifications, the diet contained approximately 22% crude protein, 3150 kcal/kg metabolizable energy, 5% crude fat, 5% crude fiber, 0.8–1.1% calcium, 0.45% available phosphorus, and 1.0% lysine. Feed and clean drinking water were provided ad libitum throughout the experimental period.

Fresh garlic bulbs were sourced from a local supplier and selected based on uniform size, absence of mold, sprouting, or mechanical damage. The cloves were peeled, washed thoroughly with clean water, and air-dried before being ground using a household blender. The crushed garlic was then mixed with distilled water at a 1:1 weight-to-volume ratio, corresponding to 1 kg of garlic per 1 liter of water. The mixture was sealed and allowed to macerate at room temperature for 24 hours to facilitate the extraction of bioactive compounds. After the incubation period, the mixture was filtered through a fine cloth or membrane to remove the solid residues, and the clear extract was collected. The filtered garlic extract was stored in dark-colored glass bottles at 4 to 8°C in a refrigerator and used within three days to preserve its biological efficacy.

Each morning, the appropriate volume of garlic extract was diluted in the daily drinking water according to the designated treatment levels (0 mL/L, 1 mL/L, 2 mL/L, and 4 mL/L). Drinking water was freshly prepared each day and distributed in the morning. Any remaining water was recorded to estimate actual water intake per pen.

### Ethical Approval

The experimental procedures in this study did not involve any invasive interventions and therefore did not require formal approval from an institutional ethics committee. All activities related to animal care, handling, and sample collection were conducted in accordance with the Law on Animal Husbandry (No. 32/2018/QH14), promulgated by the National Assembly of the Socialist Republic of Vietnam. Animal welfare

and health status were monitored daily, and all necessary measures were taken to minimize stress and ensure humane treatment throughout the experimental period.

### Measurements

Throughout the experimental period, the following parameters were monitored to evaluate the effects of garlic extract supplementation (0, 1, 2, and 4 mL/L) on the growth performance, physiological health, and coccidial resistance in Japanese quail.

Survival rate (%):

Survival was monitored in four periods (days 6–13, 13–20, 20–27, and 27–35). The survival rate was calculated using the formula:

Survival rate (%) =

$$\frac{N_s}{N_o} \times 100$$

$N_s$ : Number of surviving birds at the end of the period

$N_o$  = Initial number of birds at the beginning of the period

Body weight (g):

Individual body weight was measured on days 6, 13, 20, 27, and 35 using a high-precision digital balance (GS3203A, 320 g / 0.001 g, GS-Shinko, Japan). Results were expressed as mean  $\pm$  standard deviation for each treatment group.

Daily feed intake (g/bird/day):

Feed consumption was recorded weekly per cage. The total feed offered minus the feed residue was used to calculate feed intake. Daily feed intake per bird was determined using the following equation:

Feed intake (g/bird/day) =

$$\frac{\text{Total feed consumed in period (g)}}{\text{Number of birds} \times \text{Number of days}}$$

Hematological parameters:

On day 35, blood samples were collected from 10 randomly selected birds per treatment group via brachial vein puncture. Hematological analyses were conducted using a fully automated hematology analyzer (Celltac  $\alpha$  MEK-6550, Nihon Kohden, Japan) suitable for avian samples. The measured parameters included red blood cell count (RBC,  $\times 10^6/\text{mm}^3$ ), white blood cell count (WBC,  $\times 10^3/\text{mm}^3$ ), and hemoglobin concentra-

tion (Hb, g%).

Oocyst count ( $\log_{10}$  oocyst/g):

Fecal samples were collected on days 20, 27, and 35. The number of oocysts per gram of feces was determined using the McMaster technique, and results were expressed in base-10 logarithmic scale ( $\log_{10}$  oocyst/g) for statistical analysis.

### Statistical Analysis

Data were recorded in Microsoft Excel 2016 and analyzed using Minitab version 16.0. Normality of data distribution was checked using the Shapiro–Wilk test before analysis. For all normally distributed data, one-way analysis of variance (ANOVA) was performed to determine the effects of different garlic extract levels. When a significant difference was detected ( $p < 0.05$ ), means were separated using Tukey's HSD post-hoc test. Descriptive statistics (mean  $\pm$  standard deviation) were also presented for all parameters to summarize data variability.

## RESULTS AND DISCUSSION

### Survival rate (%) of Japanese quail across periods with varying garlic extract concentrations

Survival rate (%) in Japanese quail showed significant variation among treatment groups over time, as illustrated in Figure 1. During day 6–13, the control group (0 mL/L garlic extract) recorded a survival rate of 98.4%, whereas the 4 mL/L group achieved 100%. This positive trend persisted throughout the experimental period. From day 13–20, survival in the control group decreased to 97.6%, while all treated groups maintained 99.2%. The largest gap appeared by day 27–35, when the control group showed the lowest survival at 95.7%, in contrast to the stable 99.2% observed in the 2 and 4 mL/L groups. These results suggest that garlic extract improved survival in a dose-responsive manner.

The improvement in survival is likely attributable to the antimicrobial and immunostimulatory effects of garlic. In the control group, the progressive decline in survival may reflect increased vulnerability to stress and infection in the absence of phytogetic support. Modern poultry systems expose birds to multiple challenges including oxidative stress, commonly due to high

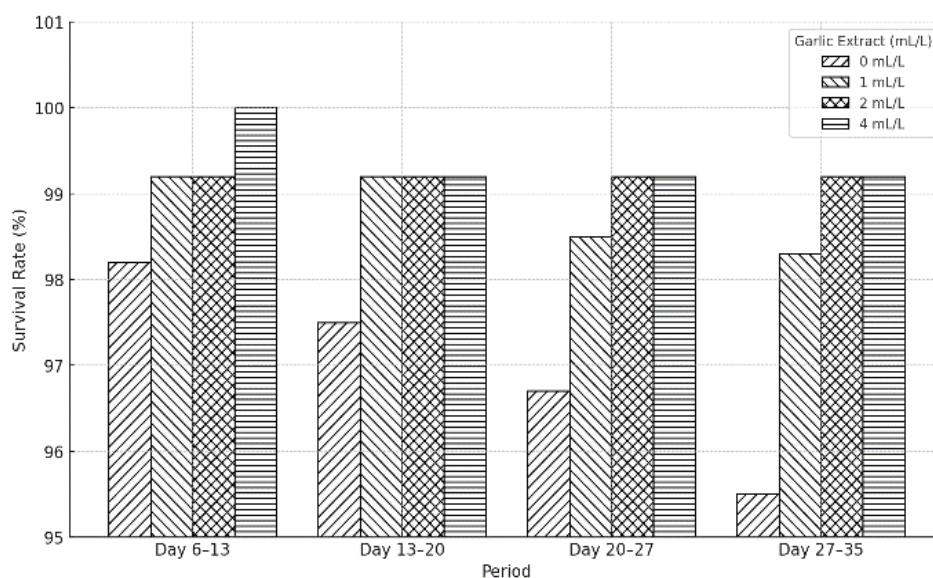


Figure 1. Survival rate (%) of Japanese quail across different time periods under various garlic extract concentrations (0, 1, 2, and 4 mL/L). Values represent total group survival rates; standard deviation is not applicable.

stocking density and accelerated growth (Oke *et al.*, 2024). Dexamethasone-induced oxidative stress disrupts gut and metabolic functions and impairs immunity (Miao *et al.*, 2021), which can ultimately lower performance and survival (Berenjian *et al.*, 2021). In contrast, the garlic-treated groups showed stable survival across all periods, likely due to improved gut integrity, reduced infection pressure, and better control of oxidative pathways. Together, these findings highlight the functional role of garlic extract as a natural additive that enhances survivability during critical growth phases in quail production. Yalçin *et al.* (2007) reported, incorporating garlic powder into the diets of laying quails did not produce notable changes in key performance parameters such as body weight, egg production rate, feed intake, feed conversion ratio, eggshell thickness, similarly.

### Growth performance of Japanese quail under different levels of garlic extract supplementation

Table 1 presents the average body weight of Japanese quail throughout the experimental period. On day 6, no significant differences were observed among treatment groups, indicating homogeneous initial conditions. However, by day 13, body weight had begun to diverge among treatments, with the 2 and 4 mL/L groups reaching  $52.95 \pm 0.25$  g and  $52.98 \pm 0.14$  g, respectively,

compared to  $48.27 \pm 0.24$  g in the control group. Statistically significant differences ( $p < 0.05$ ) were observed from day 20 onward. On day 20, birds in the 4 mL/L group recorded the highest body weight ( $102.24 \pm 0.98$  g), followed by the 2 mL/L ( $101.06 \pm 1.07$  g), 1 mL/L ( $94.17 \pm 1.03$  g), and the control ( $92.61 \pm 0.15$  g). This same trend persisted through day 35, with final weights of  $196.08 \pm 1.99$  g in the 4 mL/L group and  $183.22 \pm 1.02$  g in the control group.

As shown in Table 2, feed intake per bird increased over time among all groups. Yet from day 13 onward, birds in the garlic-treated groups consumed less than those in the control group. During the final period (day 27–35), the control group had the highest daily intake ( $25.12$  g/day), whereas the 4 mL/L group consumed the least ( $22.02$  g/day), suggesting that garlic supplementation enhanced feed conversion efficiency.

Growth performance is a fundamental trait in poultry production, influenced by both genetic and environmental factors (Sezer and Tarhan, 2005). Modeling of growth curves provides practical tools for predicting optimal slaughter age, evaluating health, and managing genetic progress (Haqani *et al.*, 2021; Kaplan and Gürçan, 2018). The present results, supported by Table 1 and Table 2, clearly indicate that garlic extract exerted a positive effect on growth, particularly in the 2 and 4 mL/L groups. According to Reda *et al.* (2024), mature male quails typically weigh 190–

Table 1. Average Body Weight (G) of Japanese Quail at Different Ages Under Garlic Extract Treatments (Mean  $\pm$  SD)

Period	0 mL/L	1 mL/L	2 mL/L	4 mL/L
Day 6	22.11 $\pm$ 0.13	23.81 $\pm$ 0.12	23.84 $\pm$ 0.13	23.83 $\pm$ 0.13
Day 13	48.27 $\pm$ 0.24	50.07 $\pm$ 0.05	52.95 $\pm$ 0.25	52.98 $\pm$ 0.14
Day 20	92.61 $\pm$ 0.15 <sup>c</sup>	94.17 $\pm$ 1.03 <sup>bc</sup>	101.06 $\pm$ 1.07 <sup>b</sup>	102.24 $\pm$ 0.98 <sup>a</sup>
Day 27	140.06 $\pm$ 1.32 <sup>c</sup>	142.65 $\pm$ 1.09 <sup>bc</sup>	150.13 $\pm$ 0.64 <sup>b</sup>	153.17 $\pm$ 0.91 <sup>a</sup>
Day 35	183.22 $\pm$ 1.02 <sup>c</sup>	185.16 $\pm$ 1.42 <sup>bc</sup>	193.42 $\pm$ 1.71 <sup>b</sup>	196.08 $\pm$ 1.99 <sup>a</sup>

<sup>abc</sup> Mean values within rows with different superscripts are different at  $p < 0.05$

Table 2. Daily Feed Intake of Experimental Japanese Quail by Age (g/bird/day)

Period (days)	0 mL/L	1 mL/L	2 mL/L	4 mL/L
6–13	7.75	7.74	7.74	7.72
13–20	15.89	15.86	15.86	15.82
20–27	20.49	20.47	20.41	20.35
27–35	25.12	23.71	22.19	22.02

250 g and females 225–300 g. Birds in the 4 mL/L group approached this upper range by day 35, despite consuming less feed. Previous studies have reported that female quails exhibit greater feed utilization efficiency, contributing to their enhanced growth performance. Research by Narinc *et al.* (2014) and Elkomy *et al.* (2019) confirms that females consistently attain higher body weight and growth rates than males. This is consistent with the principle of sexual dimorphism observed in Japanese quail, which has been extensively documented in the works of Hyánková *et al.* (2001), Aggrey (2003), and Kizilkaya *et al.* (2006). In this study, birds in the 4 mL/L group exhibited reduced feed intake yet superior weight gain, reflecting improved nutrient conversion. This effect may be attributed to the bioactive compounds in garlic, particularly sulfur-containing constituents like allicin, which stimulate digestive enzymes, suppress pathogenic gut flora, and reduce oxidative stress (Shang *et al.*, 2019). These findings support the conclusion that garlic extract acts as a functional additive with growth-promoting properties in quail nutrition.

### Effects of Garlic Extract on Physiological and Blood Biochemical Parameters in Japanese Quails

The hematological parameters of Japanese quail in response to garlic extract supplementation are presented in Table 3. Red blood cell (RBC) count showed a significant decrease as garlic concentration increased ( $p < 0.05$ ). The

control group recorded the highest RBC count at  $2.62 \pm 0.09 \times 10^6/\text{mm}^3$ , whereas the 4 mL/L group had the lowest at  $1.89 \pm 0.21 \times 10^6/\text{mm}^3$ . Hemoglobin concentration followed a similar pattern. The control group maintained the highest level at  $7.06 \pm 0.28 \text{ g}\%$ , while birds in the 4 mL/L group recorded  $6.21 \pm 0.18 \text{ g}\%$ . Although these declines were statistically significant, hemoglobin levels remained within physiological limits (Agina *et al.*, 2017). The downward trend in RBC and hemoglobin may be linked to the mild hemolytic effects of organosulfur compounds found in garlic, particularly diallyl disulfide, which can alter red blood cell membrane stability at higher concentrations (Amagase *et al.*, 2001). Despite this effect on erythrocyte indices, it is important to note that the values did not indicate pathological anemia and are unlikely to impair oxygen-carrying capacity in the short term. In contrast, white blood cell (WBC) count increased with higher levels of garlic extract. The control group had the lowest WBC count at  $16.41 \pm 0.74 \times 10^3/\text{mm}^3$ , while the 4 mL/L group exhibited the highest at  $20.09 \pm 0.11 \times 10^3/\text{mm}^3$ , with significant differences among all groups ( $p < 0.05$ ).

This elevation in leukocyte numbers suggests improved immunological readiness in the treated birds. The increase may be attributed to bioactive compounds such as allicin and S-allyl cysteine, which have been shown to enhance the proliferation and function of innate immune cells. Garlic supplementation appears to promote

Table 3. Effect of Garlic Extract Supplementation on Hematological Parameters of Japanese Quail (Mean  $\pm$  SD)

Parameter	0 mL/L	1 mL/L	2 mL/L	4 mL/L
Red blood cells (RBC) ( $\times 10^6/\text{mm}^3$ )	2.62 $\pm$ 0.09 <sup>a</sup>	2.15 $\pm$ 0.36 <sup>b</sup>	1.91 $\pm$ 0.47 <sup>bc</sup>	1.89 $\pm$ 0.21 <sup>c</sup>
White blood cells (WBC) ( $\times 10^3/\text{mm}^3$ )	16.41 $\pm$ 0.74 <sup>c</sup>	19.03 $\pm$ 0.72 <sup>b</sup>	19.76 $\pm$ 0.14 <sup>ab</sup>	20.09 $\pm$ 0.11 <sup>a</sup>
Hemoglobin (g%)	7.06 $\pm$ 0.28 <sup>a</sup>	7.01 $\pm$ 0.16 <sup>ab</sup>	6.74 $\pm$ 0.05 <sup>b</sup>	6.21 $\pm$ 0.18 <sup>c</sup>

<sup>abc</sup> Mean values within rows with different superscripts are different at  $p < 0.05$

Table 4. Serum Total Protein and Its Fractions (g/L) (Mean  $\pm$  SD)

Parameter	0 mL/L	1 mL/L	2 mL/L	4 mL/L
Total Protein	31.15 $\pm$ 1.18 <sup>a</sup>	25.24 $\pm$ 1.12 <sup>b</sup>	24.53 $\pm$ 1.08 <sup>bc</sup>	22.19 $\pm$ 1.15 <sup>c</sup>
Albumin	16.02 $\pm$ 1.06 <sup>a</sup>	15.08 $\pm$ 1.14 <sup>ab</sup>	13.11 $\pm$ 0.96 <sup>b</sup>	12.76 $\pm$ 0.93 <sup>c</sup>
Globulin	14.89 $\pm$ 0.69 <sup>a</sup>	14.01 $\pm$ 0.72 <sup>ab</sup>	12.14 $\pm$ 0.69 <sup>b</sup>	11.91 $\pm$ 0.71 <sup>c</sup>

<sup>abc</sup> Mean values within rows with different superscripts are different at  $p < 0.05$

immune activation while mildly suppressing erythropoiesis, indicating a physiological shift toward enhanced immune defense. These hematological changes highlight the importance of establishing reliable reference values for quail, as emphasized by Agina *et al.* (2017), especially when evaluating the effects of phyto-genic additives in production and reproduction systems. Serum total protein, albumin, and globulin concentrations decreased significantly ( $p < 0.05$ ) with increasing levels of garlic extract in drinking water (Table 4). The 4 mL/L group exhibited the lowest values across all protein parameters, suggesting a dose-dependent suppressive effect on protein synthesis or mobilization. This reduction might be associated with the metabolic responses to organosulfur compounds present in garlic, which can influence hepatic protein metabolism. Allicin and its derivatives are known to modulate liver enzyme activity, potentially redirecting amino acid utilization toward antioxidant and immune pathways rather than plasma protein synthesis. Moreover, the mild decline in serum proteins may reflect enhanced utilization of globulins during immune stimulation or increased catabolic turnover associated with detoxification of bioactive sulfur metabolites. Similar findings were reported by Canogullari *et al.* (2010) and Cherniy *et al.* (2021), who observed reduced serum protein fractions in quails and chickens supplemented with high levels of garlic-based phyto-genic additives.

#### Oocyst count ( $\log_{10}$ ) in quail feces at days 20, 27, and 35 under garlic extract supplementation.

The oocyst count ( $\log_{10}$ ) per gram of feces in

Japanese quail differed significantly among the experimental groups and over time, as shown in Figure 2. On day 20, the control group receiving 0 mL/L garlic extract exhibited the highest oocyst excretion, with an average of  $4.5 \pm 0.3 \log_{10}$  oocysts/g. In comparison, birds receiving 1, 2, and 4 mL/L recorded significantly lower counts of  $4.2 \pm 0.2$ ,  $3.8 \pm 0.2$ , and  $3.5 \pm 0.2 \log_{10}$ , respectively ( $p < 0.01$ ). This pattern throughout the experimental timeline. By day 27, the oocyst count rose slightly in all groups but remained lower in the garlic-supplemented treatments, with values of  $4.8 \pm 0.3$  in the control, and  $4.4 \pm 0.2$ ,  $4.0 \pm 0.2$ , and  $3.6 \pm 0.2 \log_{10}$  in the 1, 2, and 4 mL/L groups, respectively. A similar trend was evident on day 35. At this point, the control group reached its peak at  $4.9 \pm 0.3 \log_{10}$  oocysts/g, whereas the 4 mL/L group maintained the lowest count at  $3.6 \pm 0.2 \log_{10}$  ( $p < 0.01$ ). These findings indicate a clear dose-dependent reduction in oocyst output associated with garlic supplementation, suggesting an inhibitory effect of garlic on coccidial development.

Coccidiosis in quails is often subclinical yet still compromises growth, feed efficiency, and survival (Simiyoon *et al.*, 2018). As Seok *et al.* (2003) reported, quails are highly susceptible to infectious diseases, and coccidiosis remains one of the most challenging. Current control measures rely primarily on anticoccidial agents that block both asexual and sexual stages of *Eimeria spp.* (Odden *et al.*, 2018). However, the results in this study support the potential use of garlic extract as a natural alternative. Birds in the garlic-treated groups consistently demonstrated reduced oocyst shedding, with suppression most notable in the 2 and 4 mL/L treatments. This re-

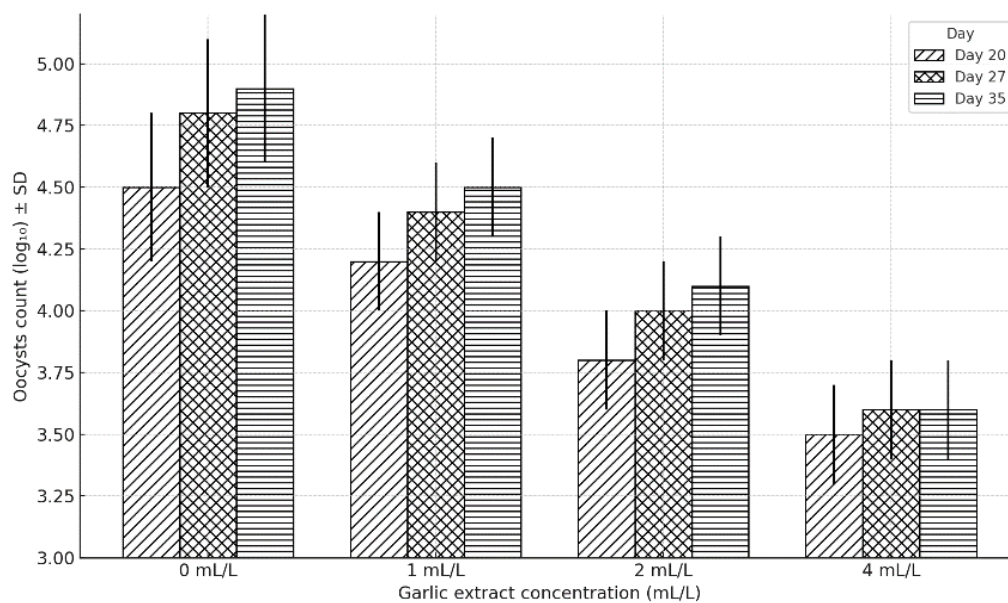


Figure 2. Number of oocysts per gram of feces ( $\log_{10}$ ) in Japanese quail at days 20, 27, and 35, across different garlic extract levels (0, 1, 2, 4 mL/L).

duction may result from both a direct effect on parasite development and immune enhancement in the host. The relative stabilization of oocyst counts between days 27 and 35 may reflect acquired immunity. Given that a one-log reduction represents a tenfold decrease in oocyst burden, the biological significance of this reduction is considerable. *Coccidial* infections are known to damage intestinal mucosa, limiting nutrient absorption and weight gain (Baba *et al.*, 1982; Bujmehrani, 2011). Al-Zarkoushi and Al-Zubaidi (2021) detailed the extent of tissue damage, noting parasite presence throughout mucosal and submucosal layers. Clinical signs in quails include reduced appetite, weakness, pallor, imbalance, diarrhea with possible blood, and reduced egg laying in hens (Teixeira *et al.*, 2004; Simiyoon *et al.*, 2018). Garlic (*Allium sativum* L.) contains numerous biologically active compounds including sulfur-based molecules, enzymes, amino acids, and trace minerals like selenium. Allicin, ajoene, dialkyl polysulfides, S-allylcysteine, diallyl sulfide, and others contribute to its pharmacological profile (Canogullari *et al.*, 2010). Additionally, Ziariarimi *et al.* (2011) demonstrated that garlic extract has potent antibacterial activity, effectively inhibiting *Escherichia coli* in poultry. Taken together, these findings highlight the potential role of garlic extract

as a natural, effective alternative to synthetic anticoccidial drugs in quail farming systems.

## CONCLUSION

Garlic extract supplementation enhanced survival, growth performance, immune response, in Japanese quail. Higher doses showed greater benefits without adverse effects, supporting its use as a natural additive in quail production. Based on the observed benefits, garlic extract at 2–4 mL/L is recommended as a safe and effective phyto-genic supplement in Japanese quail production, particularly in antibiotic-free systems.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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