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Dietary supplementation of microbiota inoculum and single clove garlic extract on growth performance, egg quality, reproductive organ, and hematological trait in laying quail

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ABSTRACT

Background: Several alternative feed additives to replace AGP to maintain good quail performance include the use of probiotics and herbal extracts.

Aim: In this study, the researchers want to find out the best dosage of microbiota inoculum as probiotics (*Lactobacillus acidophilus, Bifidobacterium,* and *Lactobacillus plantarum*), single garlic extract (*Allium Sativum L*), and combination groups to improve laying quail egg production, hematology profile (erythrocytes, hemoglobin, hematocrit, leukocytes, and platelets), and reproductive organs in terms of length and weight and have better egg quality on the internal (Haugh units, yolk score, albumin index, yolk index) and external (shell thickness, egg weight) while laying phase of laying quail.

Methods: A total of 100 4-week-old laying quails of uniform body weight were randomly distributed into five treatments with four replicates each and five quails in each replicate. There were 5 treatment groups: T0 quails were given basal feed; T1 quails were given basal feed and drinking water added with probiotics at a dose of 4 ml/l; T2 quails were given feed added with a single garlic extract at a dose of 2 ml/g and ordinary drinking water; T3 quails were given feed with a single garlic extract at a dose of 2 ml/g and drinking water supplemented with probiotics at a dose of 4 ml/l; and T4 quails were given a single garlic extract at a dose of 1 ml/g and drinking water supplemented with probiotics at a dose of 2 ml/l. The study investigated the effects of microbiota inoculum, including probiotics, garlic extract, and a control group, on reproductive organ morphologies in chickens. Results showed significant improvements in weight vagina, uterus, oviduct, ovarium, weight cloaca, growth performance, body weight, egg production, internal egg quality, yolk color score, yolk index, albumin index, external egg quality, Haugh unit, egg height, shape index, egg cell weight, and egg cell thickness.

Results: The number of erythrocytes was significantly higher in the chickens treated with the microbiota inoculum compared to the control group. Hemoglobin levels were not significant in all treatments, but hematocrit levels were significant in the chickens treated with the microbiota inoculum. Leukocytes were also significantly higher in the chickens treated with the microbiota inoculum compared to the control group.

Conclusion: The use of a combination of microbiota inoculum in drinking water and garlic extract in feed has been proven to be effective in reducing feed consumption, maintaining hematology, increasing reproductive organs, and boosting the number of laying quail productions, thereby reducing feed conversion ratio.

Keywords: Probiotics, Good Health, Growth performance, Quality eggs, Reproductive organs, Quail.

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Introduction

Antibiotics have been used in animal production as growth promoters and therapeutic and prophylactic/metaphylactic agents for over 50 years, conferring a health benefit on the host. Health benefits have mainly been demonstrated for specific probiotic strains of the following genera: *Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus, Streptococcus, Pediococcus,* and *Leuconostococcus* (Al Sattar *et al.*, 2023). The inappropriate and hazardous application of antibiotics as antimicrobials on farms enables microorganisms to develop resistance to specific drugs, such as antibiotics, rendering them ineffective against diseases. This results in the emergence of antimicrobial-resistant pathogens and the contamination of animal-derived foods with undesirable drug residue levels (Chantziaras *et al.*, 2014). A study in seven European countries revealed a significant correlation between the prevalence of antimicrobial-resistant *Escherichia coli* bacteria in pigs, poultry, and cattle and the consumption of eight antimicrobial classes (Van Boeckel *et al.*, 2015).

Probiotics, in sufficient quantities, are microorganisms that can exert a beneficial influence on the host organism's health (Fijan, 2014). Probiotics provide numerous benefits to livestock, including growth promotion, inflammation mitigation, gastroenteritis prevention, and egg quality enhancement (Al-Shawi *et al.*, 2020; Agustono *et al.*, 2023). Probiotics, including *Bifidobacterium* sp., *Lactobacillus acidophilus*, and *Lactobacillus plantarum*, are added to animal feed to inhibit harmful microorganism growth and enhance nutrient absorption (Al-Shawi *et al.*, 2020; Anee *et al.*, 2021; Agustono *et al.*, 2023). Research shows that probiotic supplementation boosts egg production, feed conversion efficiency, poultry performance, and eggshell quality, promoting reproductive organ growth and maturation and ultimately improving egg quality.

A single garlic extract (*Allium sativum L.*) is among the botanical species that are employed as feed additives in potable water and animal feed (Shang *et al.*, 2019). Batiha *et al.* (2020) revealed that a single garlic extract, rich in active compounds such as allicin, has been found to reduce pathogen bacteria, allergies, tumors, and inflammation, while also containing antioxidant and antibacterial properties. Flavonoids function by denaturing bacterial proteins (Tesfaye, 2021). Minerals and vitamins are significant components of single garlic extract and contribute significantly to its health benefits (Obochi *et al.*, 2009). A single garlic extract can stimulate ovaries-produced hormones and gonadotropins while inhibiting the proliferation of cancer cells (Chen *et al.*, 2021). Research indicates that by increasing growth performance and improving the health of birds, the use of garlic extract as a growth promoter lowers production costs. The performance of quails is closely linked to their physiological state, which can be assessed through their blood profile, which is a crucial indicator of livestock health (Kairalla *et al.*, 2022). This research aims to provide initial information regarding the effective dosage of single use of probiotics and single garlic extract as well as their combination, which is expected to provide beneficial effects and improve the performance, productivity, and quality of pre-layer laying quail eggs to prepare laying quail during the layer period.

Materials and Methods

Ethical approval

The study was approved by the Ethical Committee of Universitas Airlangga, Indonesia, with number 2.KEH.128.08.2023.

Study period and location

The study spanned a duration of 36 days, specifically from August to September 2023. The treatment for research animals was allowed in research animal cages at Airlangga University; an assessment of productivity performance (including pubertal characteristics, growth of reproductive organs, and egg quality at puberty) was evaluated at the Feed and Nutrition Laboratory of Airlangga University.

Materials

The research materials comprised 5-week-old Japanese Quails (*Coturnix coturnix japonica*), probiotics (1.2×10^9 CFU/ml) of *Lactobacillus acidophilus, Lactobacillus plantarum,* and *Bifidobacterium sp*., 30% single garlic extract, 96% ethanol, distilled water, CMC Na, and commercial egg-laying quail feed (BP104) from PT Charoen Pokphand Indonesia, and 10% benzalkonium chloride disinfectant were used to disinfect the cages.

Experimental design

The present research employed a completely randomized design comprising 5 treatments, with 4 replications per treatment and 5 birds per replication, for an overall number of 100 animals. There were 5 treatment groups: T0 quails were given basal feed; T1 quails were given basal feed and drinking water added with probiotics at a dose of 4 ml/l; T2 quails were given feed added with a single garlic extract at a dose of 2 ml/g and ordinary drinking water; T3 quails were given feed with a single garlic extract at a dose of 2 ml/g and drinking water supplemented with probiotics at a dose of 4 ml/l; and T4 quails were given a single garlic extract at a dose of 1 ml/g and drinking water supplemented with probiotics at a dose of 2 ml/l.

Data collection technique

An investigation was conducted into the growth performance, development of reproductive organs, attributes of puberty, and quality of eggs. Daily assessments were conducted on health conditions, mortality, feed consumption, laying performance, and egg quality. Following the conclusion of the experiment at 10 weeks of age, three viable laying quails were chosen from each group replicate, for a total of 60 laying quails. These quail were in good health and were

sacrificed; the weight of the carcass was determined by collecting the organs.

Egg quality

Egg quality is evaluated from 2 parameters: internal and external. Internally, egg quality can be calculated using the following parameters: egg yolk height, egg yolk color (Roche yolk color fan), albumin thickness, egg white index (determined by caliper readings of thick albumin), egg yolk index (calculated as the ratio of the height to the diameter of the yolk egg), and Haugh units (HU) = 100log (H+7,57–1,7.$W^{0.37}$) (Raymond Haugh, 1937). Externally, egg quality can be determined by examining the eggshell, egg mass, egg height, and egg width.

Hematology profile

Blood samples were taken after treatment at the end of week 9. Twenty blood samples were taken from each treatment group. Testing blood samples to determine the number of erythrocytes, leukocytes, hemoglobin levels, hematocrit, and platelets was carried out using a hematology analyzer (Haribo).

Reproductive organs

Collecting of organs and reproductive tract after necropsy in laying quail: organs and reproductive tract can be removed after previous removal of the

Table 1. Feed ingredients and nutrient content of basal diets.

Ingredients (g/kg)	Diets	
	Starter phase (1-21 days)	Grower phase (21-35 days)
Corn	530	589
Soybean meal	336	322
Corn oil	60	50
Dicalcium phosphate	16	16
Calcium carbonate	17	13
Methionine	2.0	1.0
Vitamin premix	25	25
a. Vitamin A (IU)	15,000	15,000
b. Vitamin D3 (IU)	3,750	3,750
c. Vitamin E (mg)	37.5	37.5
d. Vitamin K3 (mg)	2.55	2.55
e. Thiamin (mg)	3	3
f. Riboflavin (mg)	7.5	7.5
g. Vitamin B6 (mg)	4.5	4.5
h. Vitamin B12 (µg)	24	24
i. Niacin (mg)	51	51
j. Folic acid (mg)	1.5	1.5
k. Biotin (mg)	0.2	0.2
l. Pantothenic acid (mg)	13.5	13.5
m. Choline chloride (mg)	250	250
n. Antioxidant (mg)	100	100
Mineral mix	25	25
a. Zinc (mg)	37.5	37.5
b. Manganese (mg)	37.5	37.5
c. Iron (mg)	37.5	37.5
d. Copper (mg)	3.75	3.75
e. Iodine (mg)	0.83	0.83
f. Sulfur	62.5	62.5
g. Selenium (mg)	0.23	0.23
Salt	4.0	4.0

digestive tract. Once it has been taken, the length, width, and weight of the organ can be measured by sorting each organ. Measurements of the reproductive organs and tract can be done by dividing the parts of the organ (ovaries, oviduct, uterus, vagina, and cloaca). Respective organs were measured using a Vernier caliper.

Data analysis

The information was gathered and analyzed using SPSS Version 22.0. The acquired data were subjected to analysis of variance with a 5% confidence level; if a significant difference was identified, the distance test by Duncan test was conducted.

Results

Growth performance

The following measures of the variable indicator can describe the growth performance condition of laying quail: body weight, initial weight, feed consumption, weight gain, feed conversion ratio of body weight, and feed conversion ratio of egg production. The measurements of growth indicators, such as more efficient feed consumption, showed that the treatment groups (T1, T2, T3, and T4) were significantly different from the control group (T0) (Table 2). The results showed that feed consumption in the combination treatment group of probiotics and single garlic extracts (T3, T4) had a high efficacy value compared to the single treatment group with probiotics and single garlic extracts (T1, T2), while the control group (T0) had the lowest value. The same conditions also occur when administering probiotics, extracts, and their combinations (T1, T2, T3, and T4), which can have a significant effect on final weight, feed conversion ratio (FCR) body weight, and FCR egg production in laying quail compared to the control group (T0).

Hematology profile

Erythrocyte

Research on quail blood samples revealed that the T0 treatment group significantly differed from T1 and T4, but not from T2 and T3. The T1 treatment group showed no significant difference from T4, but from T0. The T2 treatment group showed no significant difference but from T0, T1, and T3. The average number of erythrocytes based on the data obtained is still within normal standards.

Hemoglobin

The results of research on quail blood samples to see hemoglobin levels showed results that were not significantly different between treatment groups T0, T1, T2, T3, and T4. The average hemoglobin level based on the data obtained is still within normal standards.

Hematocrit

The study on quail blood samples revealed that the T0 treatment group was significantly different from T1, T2, and T3, while the T1 treatment group was not significantly different from T2, T3, and T4, but significantly different from T0. The average hematocrit level based on the data obtained is still within normal standards.

Leukocyte

Research on quail blood samples revealed significant differences in leukocyte levels between different treatment groups, with the T0 group showing the most significant differences. Treatment groups T1, T2, T3, and T4 showed no significant differences, but T0 showed the most significant differences. The average leukocyte level based on the data obtained is still within normal standards.

Platelet

Research on quail blood samples revealed significant differences in platelet levels between different treatment groups. The T0 treatment group showed significant differences from T1, T2, T3, and T4. The T1 treatment group showed no significant difference but was significantly different from T4 and T0. The average platelet level based on the data obtained is still within normal standards.

Reproductive organs

In this study, probiotics, extracts, and their combinations (T1, T2, T3, and T4) could have more efficacy on reproductive organ morphology, such as length and weight morphology than the control group (T0). The statistical analysis's results on reproductive organ weight, including vagina, uterus, oviduct, ovarium, and cloaca. Especially treatment group's combinations

Table 2. Growth performance parameters of laying quail.

Variables	T0	T1	T2	T3	T4
Initial weight (g)	220 ± 7.58^{a}	220.60 ± 1.51^{a}	220.10 ± 6.48^{a}	220.32 ± 6.45^{a}	220.96 ± 4.30^{a}
Final weight (g)	238.36 ± 4.06^{a}	244.11 ± 2.18^{b}	246.68 ± 4.36^{b}	246.58 ± 3.87^{b}	246.80 ± 5.58^{b}
Feed consumption (g)	$37.10^{a} \pm 0.16$	$34.12^{b} \pm 0.50$	$32.77^{c} \pm 0.50$	$29.85^{e} \pm 1.00$	$31.40^{d} \pm 0.81$
Weight gain (g)	$8.57^{a} \pm 0.42$	$9.02^{b} \pm 0.53$	$9.30^{b} \pm 0.82$	$9.52^{b} \pm 0.99$	$9.12^{b} \pm 0.89$
FCR body weight	$4.32^{a} \pm 0.22$	$3.75^{b} \pm 0.26$	$3.55^{bc} \pm 0.31$	$3.15^{c} \pm 0.31$	$3.42^{bc} \pm 0.33$
FCR egg production	$4.55^{a} \pm 0.57$	$3.97^{b} \pm 0.95$	$3.90^{b} \pm 0.81$	$3.27^{d} \pm 0.09$	$3.65^{c} \pm 0.43$

[a,b,c,d,e] The different superscripts on the bar chart show significant differences ($p < 0.05$). (T0) 100% basal feed, (T1) basal feed + probiotics at a dose of 4 ml/l, (T2) basal feed + single garlic extract at a dose of 2 ml/kg, (T3) basal feed + single garlic extract at a dose of 2 ml/kg + probiotics at a dose of 4 ml/l, (T4) basal feed + single garlic extract at a dose of 1 ml/kg + probiotics at a dose of 2 ml/l.

of probiotics and single garlic extract (T3, T4) have a greater effect on the vagina and oviduct organs than other treatment groups (T1, T2).

In the same condition on reproduction organ length morphological measurement (Table 3), the treatment group's combinations of probiotics and single garlic extract on the uterus, oviduct, ovarium, and cloacal organs showed differences with the basal feed group (T0), whereas on vaginal organ length morphological variable was not significantly difference between the other groups.

Internal quality eggs

In this research, the effect of treatment supplementation, such as probiotics, single garlic extracts, and combinations of the others, can generally increase the internal egg's value than a control group. The statistical analysis result showed that the yolk color score, yolk index, albumin index, and Haugh unit for combination probiotic and single garlic extract groups (T3, T4) showed significant differences with other groups (T0, T1, T2). The supplementation probiotic group (T1) and single garlic extract group (T2), on measurement variable of yolk color score and Haugh unit, showed significant differences with a control group (T0). For the yolk index, the probiotic and single garlic extract groups (T1 and T2) did not have significant differences with the control group (T0).

External quality eggs

Variables measuring the external quality of eggs are egg weight, shape index, egg cell weight, and egg cell thickness. In general, probiotic supplementation, single garlic extract, and other combinations in the treatment group can provide benefits for increasing egg weight, egg cell weight, and egg cell thickness (Table 3). Meanwhile, the measurement of the egg shape index variable showed no significant differences between groups (T0, T1, T2, T3, and T4).

Discussion

In this study, the addition of feed additives with probiotics, single garlic extract, and their combination compared with the control group, which was only given basal feed with no treatment, was able to provide a positive increase in the performance of laying quail.

Table 3. Reproduction organ weight, reproduction organ length, internal egg quality, and external egg quality of laying quail.

Variables	T0	T1	T2	T3	T4
Reproduction organ weight (g)					
Vagina	$1.03^{a} \pm 0.42$	$1.51^{ab} \pm 0.36$	$1.49^{ab} \pm 0.35$	$1.75^{c} \pm 0.24$	$1.67^{c} \pm 0.17$
Uterus	$1.57^{a} \pm 0.28$	$2.2^{ab} \pm 0.38$	$2.05^{ab} \pm 0.56$	$3.00^{b} \pm 0.93$	$2.4^{ab} \pm 0.65$
Oviduct	$3.44^{a} \pm 0.38$	$4.20^{ab} \pm 0.41$	$4.24^{ab} \pm 0.32$	$5.38^{c} \pm 0.88$	$4.55^{b} \pm 0.37$
Ovarium	$4.82^{a} \pm 1.53$	$7.14^{b} \pm 0.58$	$7.29^{b} \pm 0.53$	$8.63^{b} \pm 1.39$	$7.48^{b} \pm 0.40$
Cloaca	$1.22^{a} \pm 0.48$	$1.72^{ab} \pm 0.19$	$1.47^{ab} \pm 0.36$	$1.99^{b} \pm 0.50$	$1.50^{ab} \pm 0.65$
Reproduction organ length (cm)					
Vagina	$3.22^{a} \pm 33$	$4.00^{a} \pm 0.70$	$4.05^{a} \pm 0.84$	$5.15^{a} \pm 1.38$	$4.09^{a} \pm 1.22$
Uterus	$4.37^{a} \pm 0.85$	$4.82^{ab} \pm 1.3$	$5.15^{ab} \pm 0.50$	$6.65^{c} \pm 0.85$	$5.92^{bc} \pm 0.29$
Oviduct	$23.3^{a} \pm 2.9$	$27.37^{ab} \pm 2.1$	$27.90^{ab} \pm 2.8$	$31.82^{b} \pm 5.1$	$28.650^{b} \pm 1.16$
Ovarium	$10.69^{a} \pm 0.951$	$12.37^{ab} \pm 2.09$	$13.95^{bc} \pm 1.6$	$15.17^{c} \pm 0.6$	$13.8^{bc} \pm 0.8$
Cloaca	$1.500^{a} \pm 0.40$	$1.950^{ab} \pm 0.10$	$1.700^{ab} \pm 0.29$	$2.750^{c} \pm 0.50$	$2.225^{bc} \pm 0.51$
Internal egg quality					
Yolk color score	$5.20^{a} \pm 0.76$	$6.10^{b} \pm 0.64$	$5.95^{b} \pm 0.68$	$6.35^{c} \pm 0.58$	$5.70^{b} \pm 0.80$
Yolk index	$0.400^{a} \pm 0.061$	$0.426^{ab} \pm 0.062$	$0.425^{ab} \pm 0.057$	$0.459^{b} \pm 0.052$	$0.450^{b} \pm 0.047$
Albumin index	$0.072^{a} \pm 0.027$	$0.085^{a} \pm 0.022$	$0.108^{b} \pm 0.032$	$0.116^{b} \pm 0.017$	$0.112^{b} \pm 0.021$
Haugh unit	$76.57^{a} \pm 4.68$	$79.38^{b} \pm 3.64$	$80.82^{b} \pm 5.34$	$80.99^{b} \pm 2.74$	$81.83^{b} \pm 2.30$
External egg quality					
Egg weight (g)	$11.30^{a} \pm 0.70$	$11.85^{b} \pm 0.73$	$11.64^{ab} \pm 0.65$	$12.65^{c} \pm 0.65$	$11.97^{b} \pm 0.47$
Shape index	$78.24^{ab} \pm 0.55$	$76.53^{a} \pm 0.61$	$78.71^{b} \pm 0.53$	$77.70^{ab} \pm 0.62$	$78.63^{b} \pm 0.93$
Egg cell weight (g)	$0.966^{a} \pm 0.096$	$1.061^{b} \pm 0.077$	$1.007^{ab} \pm 0.130$	$1.066^{b} \pm 0.065$	$1.012^{ab} \pm 0.092$
Egg cell thick (cm)	$0.745^{a} \pm 0.047$	$0.803^{b} \pm 0.036$	$0.786^{b} \pm 0.027$	$0.806^{b} \pm 0.027$	$0.790^{b} \pm 0.029$

[a,b,c] The different superscripts on the bar chart show significant differences ($p < 0.05$). (T0) 100% basal feed, (T1) basal feed + probiotics at a dose of 4 ml/l, (T2) basal feed + single garlic extract at a dose of 2 ml/kg, (T3) basal feed + single garlic extract at a dose of 2 ml/kg + probiotics at a dose of 4 ml/l, (T4) basal feed + single garlic extract at a dose of 1 ml/kg + probiotics at a dose of 2 ml/l.

This is in line with the opinion of (Lokapirnasari *et al.*, 2017); probiotics in feed increase feed consumption by aiding digestion and enzymatic activity, enhancing feed efficiency and digestibility. This leads to complete food absorption, allowing birds to use well-absorbed food for tissue growth and increased body weight (Yeo and Kim, 1997). Probiotics play a role in increasing acidity in the poultry digestive tract (Kabir, 2009). Pathogenic bacteria are not able to grow well in the digestive tract, which has an acidic atmosphere; thus, probiotic bacteria can compete and dominate well in the digestive tract (Khemariya *et al.*, 2017; Lokapirnasari *et al.*, 2019).

Apart from that, the role of single garlic extract, which contains allicin as a bioactive substance that acts as an antibacterial, shows that single garlic extract is effective in inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium, and Pseudomonas aeruginosa* bacteria so that the body's metabolism and food absorption will improve better (Prihandani, 2015). The use of a single garlic extract in the diet can increase bird weight gain. This is due to the presence of phytochemical compounds in the material. In a single garlic extract, the allicin compound will trigger the change of precursor components into sulfur components, and this is then reported to be efficacious in stimulating growth (Tudu *et al.*, 2022). The sulfur component in a single garlic extract will increase the availability of sulfur-containing amino acids, such as methionine, in the bird's body. According to Zhang *et al.* (2023), increasing the concentration of the amino acid methionine in the body will increase poultry growth. In other performance indicators, namely FCR, the addition of probiotics and a single garlic extract showed the best FCR value for body weight, 3.15 ± 0.31. Khalil (2015) stated that the FCR value for quail body weight is 3.3–4.9, which is higher compared to broilers, which is 1.3–2.2. The addition of probiotics and a single garlic extract significantly affected the FCR value of egg production in laying quail. The T3 treatment group produced the best FCR value, which was a 3.27 value for a probiotic and single garlic extract combination.

Probiotics work to reduce the feed conversion ratio by expanding the area of absorption by the intestine so that the feed consumed can be absorbed properly (Anee *et al.*, 2021). According to Puspitaningrum (2021), probiotics can be combined with a single garlic extract because lactic acid bacteria (LAB) can utilize fructose from single garlic extract. The addition of synbiotics from a single garlic extract and LAB in poultry rations can increase the LAB population and lactic acid production. Increasing the LAB population in the intestine can reduce the pH in the digestive tract, suppress pathogenic bacteria, and activate digestive enzymes (Bedford and Apajalahti, 2022) so that the bird's body metabolism is better and the absorption of food substances is better, which causes increased body weight gain, a decreased feed conversion ratio of body weight, and a decreased feed conversion ratio of egg production in laying quail.

The study focuses on the health status of quail and their nutritional needs. The average number of erythrocytes in quail ranges from 2.55 to 2.82 × 10^6/mm^3, which is within the normal range. Probiotics as a feed additive can improve digestive conditions, allowing for better blood cell formation. Probiotics contain proteolytic bacteria that synthesize protease enzymes, producing keratinase, which breaks down keratin into amino acids (Ali *et al.*, 2013). Erythrocyte production is also influenced by high and low oxygen levels, which induce growth and differentiation (Adeyemo *et al.*, 2010).

Garlic extract does not cause toxic effects on erythrocyte numbers, indicating healthy quail. Probiotics and garlic extract in feed and water do not interfere with erythrocyte numbers. Hemoglobin levels are within normal range (Jomova *et al.*, 2023). Increased hemoglobin levels result in a better ability to carry oxygen to tissues and more efficient excretion of carbon dioxide, affecting the condition and function of cells and tissues (Tombarkiewicz *et al.*, 2020). The addition of LAB to the feed can also increase the average amount of hemoglobin in the quail. Protease enzymes are needed to break down proteins into amino acids needed in the hemopoiesis process, leading to

Table 4. Hematological parameters of laying quail.

Treatment	Mean ± SD				
	Erythrocyte (10^6/mm^2)	Hemoglobin (g dl^{-1})	Hematocrit (%)	Thrombose (10^3/mm^3)	Leukocytes (10^3 /mm^2)
T0	2.55^{a} ± 0.05	11.72^{a} ± 0.41	31.92^{b} ± 0.15	258.75^{c} ± 11.2	27.15^{b} ± 0.15
T1	2.67^{b} ± 0.05	11.52^{a} ± 0.25	30.75^{a} ± 0.82	238.50^{b} ± 2.38	21.57^{a} ± 0.82
T2	$2.62^{a,b}$ ± 0.05	11.32^{a} ± 0.45	30.32^{a} ± 0.27	225.25^{ab} ± 3.77	21.07^{a} ± 0.27
T3	$2.60^{a,b}$ ± 0.08	11.57^{a} ± 0.45	30.77^{a} ± 0.60	236.75^{ab} ± 6.23	21.12^{a} ± 0.60
T4	2.65^{b} ± 0.05	11.80^{a} ± 0.35	31.05^{ab} ± 0.81	222.50^{a} ± 16.46	22.12^{a} ± 0.81

^{a,b,c} The different superscripts on the bar chart show significant differences ($p < 0.05$). (T0) 100% basal feed, (T1) basal feed + probiotics at a dose of 4 ml/l, (T2) basal feed + single garlic extract at a dose of 2 ml/kg, (T3) basal feed + single garlic extract at a dose of 2 ml/kg + probiotics at a dose of 4 ml/l, (T4) basal feed + single garlic extract at a dose of 1 ml/kg + probiotics at a dose of 2 ml/l.

an increase in hemoglobin with increasing probiotics (Livingston *et al.*, 2020).

The average number of leukocytes in quail ranges from 21.07 to 27.15 × 10^3/mm^3. Probiotics, used as feed additives, maintain digestive tract microbiota balance by eliminating pathogenic microorganisms by competing for nutrients and creating an uncomfortable ecosystem (Jha *et al.*, 2020). Platelets, also known as platelets or blood platelets, are fragments of megakaryocyte cytoplasm formed in the bone marrow (Hartwig and Italiano, 2003). The average number of platelets in all treatments was in the normal range. Flavonoids in garlic have antibacterial, antiviral, and inflammatory properties, which can increase platelet levels (Batiha *et al.*, 2020). Garlic extract and probiotics have shown potential benefits in maintaining the health of quail and their nutritional needs.

Probiotics have a role in increasing the acidity of the digestive tract (Plaza-Diaz *et al.*, 2019). Pathogenic bacteria are not able to grow well in the digestive tract, which has an acidic atmosphere; thus, probiotic bacteria can compete and dominate well in the digestive tract (Khemariya *et al.*, 2017). According to Agustono *et al.* (2023), nutrient assimilation in the intestine is crucial for cell regeneration and function maintenance, as it aids in the digestion of proteins and lipids, contributing to tissue formation and cell proliferation. The intestine's ability to regenerate cells increases with higher protein and fat digestion, leading to longer villi sizes and larger intestinal lumen (Ravindran and Reza Abdollahi, 2021). A single garlic extract on organ size and the reproductive tract functions as an antioxidant, catalase, and glutathione peroxidase (Asadpour *et al.*, 2013).

To optimize oviduct gland cell performance without causing oviduct gland cell proliferation, antioxidants for the size of the reproductive organs and tract can help neutralize free radical products (Grzesiak *et al.*, 2022). gonadotropin-releasing hormone is released when the body absorbs nutrients properly. This hormone then triggers the release of FSH and LH from the pituitary gland and estrogen from the ovaries (Zhao; *et al.*, 2023). FSH stimulates ovarian follicle development, maturation, and vascularization, while LH induces ovulation. Follicle development and estrogen secretion are influenced by the increased granulosa cells (Hlokoe *et al.*, 2022; Zhao; *et al.*, 2023). A higher level of estrogen will help the oviduct grow so that albumin, protein, and egg fat can be made. It will also help the body absorb calcium, vitamins, and minerals that are needed for egg formation, which is what determines egg formation in the end (An *et al.*, 2016).

The study found that the addition of probiotics and garlic extract to drinking water and feed can increase the size of reproductive organs and tracts in Japanese quail. According to Hadiani *et al.* (2022), the average quail egg production ranges from 60.35% to 61.07%. The T3 treatment group had higher quail-day production (QDP) numbers than the T1, T2, T3, and T4 groups, with a score of 78.57%. This was because they were given 4 ml/l of probiotic drinking water and 30% (2 ml/kg) of single garlic extract in their feed. Chen *et al.* (2022) Lactobacillus acidophilus bacteria can prevent white chalk defecation in birds, improve feed efficiency and QDP, suppress pathogenic bacteria, and increase the immune response in livestock, while also suppressing *Salmonella pullorum*.

Muhammad *et al.* (Mahi *et al.*, n.d.) classified laying quail eggs into three distinct weight categories in a 2012 study: light (8.5–9.5 g per item), medium (9.6–10.5 g per item), and heavy (10.6.5–11.5 g per item). The findings about the mean egg weight across all experimental conditions were incorporated into the weight category. Variations in the weight of eggs may be caused by genetics, the parental body weight, the ratio ingested, the species of bird that lays the eggs, or other factors (You *et al.*, 2009). The weight of an egg can be influenced by the substances it contains. Protein accounts for approximately 50% of the egg's weight, and the ingestion of diverse substances, such as probiotics and single garlic extract, can contribute to this variation. The intestinal absorption of a substantial quantity of nutrients from feed enhances the distribution of material necessary for egg formation to the ova (Khan *et al.*, 2023). (Peric *et al.*, 2012) state that quail eggs are considered round if their shape index value is greater than 77, whereas ovoid (or "normal") eggs have a shape index value between 69 and 77. The investigation found that the mean shape index for all treatments was normal, with calcium content in feed and egg formation being significant determinants. (Yousefi, 2019). To increase calcium availability, probiotics present in drinking water stimulate the quantitative or qualitative composition of intestinal microflora (Raveschot *et al.*, 2020).

The outcomes of the thickness and weight assessments of the shell failed to meet typical criteria due to the inclusion of the inner membrane in this investigation, which increased the mean value. An analogous investigation conducted by(Baylan *et al.*, 2018) examined the effects of incorporating a single garlic extract into laying quail feed on the internal and external quality of eggs. The findings of this study indicated that the weight of the eggshells was significantly affected ($p < 0.05$). The reason for this in laying quail is the inclusion of a single garlic extract in the diet, which is rich in calcium and phosphorus. (Zhao, *et al.*, 2023) state that the quantity of ovomucin secreted by the magnum has a substantial influence on the quality of egg whites. The egg white index is influenced by protein intake, as increased protein content enhances ovomucin secretion, thereby increasing the egg white height (Omana and Wang, 2020; Zhao; *et al.*, 2023).

The results acquired for the mean IKT across all regimens were classified as normal. The egg yolk index results indicate that T0 differs substantially from T3

and T4. Protein significantly impacts the development of the vitelline and chalaza membranes, which are responsible for fortifying and sustaining the egg yolk throughout the process of egg formation (Pribadi and Kurtini, 2015). A high egg yolk index is influenced by optimal nutrient absorption; therefore, supplementing feed and drinking water with a mixture of probiotics and a solitary single garlic extract facilitates the absorption of incoming nutrients.

As per the findings of (Dansou *et al.*, 2023; Zhao; *et al.*, 2023), eggs falling within the AA category have an HU value exceeding 79. Eggs falling within the class A category have HU values below 79 and above 55, while eggs falling within the class B category have HU values below 31. The research findings on the T0 mean are classified under category A, whereas the T1, T2, T3, and T4 means are classified under category AA. Superior egg quality is indicated by the high average Haugh unit value of each treatment (Goodwin *et al.*, 1961). In this investigation, the egg yolk scores T1, T2, T3, and T4 were found to be statistically distinct from T0. (Dansou *et al.*, 2023) posit that egg yolk scores are susceptible to the effects of feed containing carotene pigments resembling vitamin A, such as zeaxanthin, xanthophyll, and lutein, which are present in yellow maize, one of the feed compositions utilized in this study. An additional benefit of combining probiotics with a single garlic extract is that it facilitates the assimilation of nutrients, including xanthophylls, which are subsequently transported to the reproductive organs of poultry in preparation for egg formation.

Conclusion

Probiotics in drinking water and garlic extract in feed are effective for quail nutrition, reducing body weight and egg production, maintaining blood profile, increasing reproductive organs, and improving egg quality. Further research is needed on digestive enzymes and the nutritional value of feed.

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Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

BA, WPL: Overseeing research. BA, MNY: Conducting research. SHW, BA, WPL: Statistical analysis of data. BA, SW: Drafting the manuscript. SW, TDM: Revise the manuscript. All authors read and approved the final manuscript.

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