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Investigating the effect of quail egg supplementation enriched with marine macroalgae *Eucheuma spinosum* on hematological indices, lipid profile parameters, and blood glucose level of Sprague Dawley rats during lactation and offspring growth

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ABSTRACT

Background: The lactation period is a crucial period where the nutritional status and the mother's environment influence milk production, impacting organ differentiation, function, and structure in the baby's body.

Aim: The study aimed to determine the impact of providing lactating rats with quail egg supplements enriched with marine macroalgae *Eucheuma spinosum* on their physiological condition (blood cells, lipids, blood glucose, antioxidant activity, and prolactin hormone levels) and the growth of their offspring.

Methods: The study involved 25 lactating Sprague Dawley white rats aged 3 months old and weighing approximately 200 g divided into five treatment groups thus; T0 as the control, T1 with quail eggs enriched with commercial feed, T2 with quail eggs enriched with 3% of marine macroalgae, T3 with quail eggs enriched with 4% of marine macroalgae, and T4 with quail eggs enriched with 5% of marine macroalgae, which received one quail egg for 21 days. At the end of the study period, the following parameters were assayed: vitamin A, iodine, weight and body length of rat pups, red blood cell count, hemoglobin levels, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, lymphocytes, neutrophils, red blood cell distribution width, platelet distribution width, and mean platelet volume. Also, cholesterol levels, high-density lipoprotein, low-density lipoprotein, blood glucose levels, prolactin hormone, antioxidant activity with SOD and MDA.

Results: The study result found that adding marine macroalgae to the quail feed significantly increased the vitamin A and iodine content in the quail egg yolks. Furthermore, the weight and body length of the rat pups in the supplemented groups significantly increased ($p < 0.05$) compared to the control group. However, it did not have a significant impact ($p > 0.05$) on the rats' blood parameters, cholesterol, blood glucose, antioxidant activity, and prolactin hormone levels.

Conclusion: In conclusion, providing lactating rats with quail eggs enriched with marine macroalgae *E. spinosum* support the growth and development of the rat offspring without negatively impacting the rats' overall health parameters.

Keywords: Marine macroalgae, Quail eggs, Lactation, Haematological, Antioxidant activity.

Introduction

Lactation is a crucial period where the nutritional status and the mother's environment influence milk production, impacting organ differentiation, function, and structure in the baby's body (Marshall *et al.*, 2022; Carretero-Krug *et al.*, 2024). The lactation period begins with milk secretion and includes all changes in the mammary glands from the beginning of pregnancy to the breastfeeding period (Neville *et al.*, 2023).

During lactation, nutritional needs increase compared to normal conditions. These nutrients are utilized to increase blood volume, as well as the growth of mammary glands in preparation for breastfeeding and to increase body metabolism. Inadequate nutrition during lactation can lead to decreased milk production, complications for the mother and baby, an increased risk of cardiovascular disease (Crozier *et al.*, 2009; Dos *et al.*, 2014; Biberoglu *et al.*, 2016), and causes

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oxidative stress due to excessive metabolic load during lactation (Toy *et al.*, 2009; Berchieri-Ronchi *et al.*, 2011). These nutritional needs must be maintained from pregnancy to lactation. Complete nutrition is essential for recovery after childbirth and preparation for milk production. The nutritional requirements for milk formation are particularly high because breast milk is the sole source of nutrition for children's growth and development (Prawitasari *et al.*, 2019). Under normal conditions, breast milk provides all the nutritional needs for growth, development, and the child's immunological response (Lokossou *et al.*, 2022). The calories expended during lactation by the mother must be balanced with nutritional intake for the energy obtained from food to be used for milk production (Kim and Yi, 2020).

Balanced energy and protein supplementation can increase fetal growth and reduce the risk of fetal mortality at birth, low birth weight, and delivery at early gestational age, particularly in pregnant and lactating mothers who are malnourished (Imdad and Bhutta, 2011; Salmuth *et al.*, 2021). Furthermore, various important micronutrients are necessary for achieving nutritional needs during breastfeeding, including folic acid, calcium, iron, zinc, iodine, and vitamins (Delange, 2007; Mousa *et al.*, 2019). Most nutritional needs are provided through the food consumed by the parent. Quail eggs known for their high nutritional content, are a readily available functional food source that can be further enriched through the feed provided, offering essential nutrients for the growth and development of the fetal (Miranda *et al.*, 2015; Jeke *et al.*, 2018). The nutritional content of quail eggs includes fat, protein, carbohydrates, fiber, essential fatty acids, vitamins, minerals, lipids, and bioactive compounds as antioxidants (Basri *et al.*, 2018; Liu *et al.*, 2024). The nutritional value of quail eggs can be enhanced by using appropriate feed additives, such as macroalgae. Macroalgae can be used as a valuable source of feed additives due to their abundant availability and easy access of the resource. Furthermore, the nutritional composition of macroalgae comprises carbohydrates, protein, lipids, minerals, dietary fiber, amino acids, and bioactive compounds, all of which are potentially suitable for inclusion in quail feed (Øverland *et al.*, 2019; Kulshreshtha *et al.*, 2020). Macroalgae can be used as feed additives to improve health, productivity, and the overall quality of meat and eggs (Hajati and Zaghari, 2019). Previous studies on the addition of macroalgae, specifically *Macrocystis pyrifera* flour, has been found to increase the nutritional content of chicken eggs (Rendón *et al.*, 2003).

Limited research has been conducted on the impact of quail eggs enriched with marine macroalgae as a nutritional supplement during lactation. Previous studies have found that consuming chicken eggs and milk during lactation can significantly increase breastmilk production (Achalapong, 2016). Similarly,

studies on administering egg protein have indicated a significant increase in the weight and body length of rat pups (Naeem *et al.*, 2023). Furthermore, a study involving the supplementation of one organic quail egg per day to hematology showed no significant differences in results; however, it was observed that organic quail egg supplementation could maintain the hematological status of erythrocytes, hemoglobin (Hb), and blood pH in lactating white rats (Basri *et al.*, 2018). Based on these findings, the objective of this study is to investigate the effects of quail eggs enriched with *Eucheuma spinosum* on the physiological condition of lactating rats including hematological indices, lipid profile parameters, blood glucose, antioxidant activity, and the hormone prolactin, the weight and body length of rat pups.

Materials and Methods

Study area

The research was conducted at the Integrated Research and Testing Laboratory, Tropical Livestock Research Center Laboratory, Faculty of Animal Husbandry, Gadjah Mada University.

Procedures

Female Sprague Dawley rats were used in the study. The rats were housed at 26°C with 70% humidity under artificial lighting (12 hours light and 12 hours dark) (Gopalakrishnan *et al.*, 2018). They were fed for 15 hours (17:00–07:00), a 9-hour fasting period (08:00–16:00), and ad libitum access to feed and drinking water. The rats were given standard feed (Rat Bio) produced by PT. Citra Ina Feed Mill contains 60% carbohydrates, 20% protein, 4% fat, 4% crude fiber, 12% calcium, and 0.7% phosphorus. The study treatment involved quail eggs enriched with marine macroalgae of the *E. spinosum* (Fig. 1). Each rat was given one quail egg per day for 21 days, with the eggs boiled for 2 minutes and then cut into small pieces before being given to the rats every day at 16:00 (Basri *et al.*, 2018).

Research design

This is an experimental study with a completely randomized design. The study used 25 lactating white Sprague-Dawley rats aged 3 months old and weighing around 200 g. The rats were divided into 5 treatments:

- T0: control treatment
- T1: treatment with quail eggs enriched with commercial feed
- T2: treatment with quail eggs enriched with commercial feed + 3% marine macroalgae
- T3: treatment with quail eggs enriched with commercial feed + 4% marine macroalgae
- T4: treatment with quail eggs enriched with commercial feed + 5% marine macroalgae.

Each treatment group consisted of 5 replications, with each replication including 1 lactating white rat. At the end of the lactation period, blood sampling was performed. Blood samples were collected from the



Fig. 1. Marine macroalgae *E. spinosum*.

orbital sinus of the eye after prior anesthesia using ketamine and xylazine. A 1 ml blood sample was collected using microcapillary hematocrit and placed in an ethylen diamine tetra acetic acid vacutainer tube. The blood samples were then centrifuged at 3,000 rpm speed for 5–15 minutes.

The mating process of the rats

The mating process of white rats commences by placing male and female rats in separate cages with transparent bases for 1 day. The initial step involves placing three female white rats, which have undergone the estrus phase, along with one male white rat in a cage measuring 37 × 30 × 20 cm. The mating process typically takes place during nighttime and extends over a period of 5 days.

Sample analysis method

The UV-Vis Spectrophotometer method was used to analyze the Vitamin A content (Nielsen, 2010; Nisa et al., 2017). Additionally, the iodine concentration in egg yolk was determined using the spectrophotometer method at 440 nm (Sumaiya et al., 2016). A complete blood analysis, including RBC, HGB, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, white blood cell, lymphocytes, neutrophils, red blood cell distribution width, platelet distribution width, and mean platelet volume, was conducted using the hematology analyzer method (Binev, 2023). Cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were analyzed using the CHOD-PAP method. Blood glucose was

measured using the GOD-PAP method, using UV-Vis spectrophotometry (Apriyanto et al., 2021).

SOD, MDA, and prolactin hormone levels were measured using an ELISA kit from the Bioassay Technology Laboratory (Shanghai, China). The CAT numbers of each kit were: SOD CAT No. E0168Ra, MDA CAT No. E0156Ra, Prolactin CAT No. E0190Ra. The development of rat pups was observed, including body weight gain measured using a digital scale with an accuracy of 0.01 g and body length observed using a caliper (Mahmood et al., 2012; Iwansyah et al., 2017).

Data analysis

The data obtained were analyzed using variance, and if a significant difference was found, the Duncan test was conducted at a 95% significance level. The analysis was performed using SPSS 22 for Windows software.

Ethical approval

The study was carried out with approval from the Ethical Clearance Commission of the Integrated Research and Testing Laboratory (LPPT) at Gadjah Mada University, with the number 00026/04/LPPT/VIII/2023.

Results

The vitamin A and iodine content in quail eggs significantly increased ($p < 0.05$) in the T1, T2, and T3 groups compared to the control group (Table 1). The highest increase in vitamin A content was observed in the T2 group. This increase in vitamin A levels was attributed to the addition of *E. spinosum* to the quail feed. The vitamin A content in *E. spinosum* was 31.56 µg/100 g, and it increased the highest vitamin

A content to $87.12 \pm 1.71 \mu\text{g}/100 \text{ g}$ in quail-fed 4% marine macroalgae. The marine macroalgae species *E. spinosum* contains 57.77 ppm of iodine. Quail eggs supplemented with 4% marine macroalgae feed have the highest iodine content at $12.01 \pm 0.1 \text{ ppm}$ (Table 1).

The body weight and body length of the rat pups were measured every week to determine the effects of the treatments (Table 2). Results of the body weight and body length of the rat pups on days 7, 14, and 21 showed a significant increase ($p < 0.05$) compared to the control group (Table 2). The administration of quail eggs to groups T1, T2, T3, and T4 significantly increased the body weight and body length of the rat pups compared to treatment T0. The highest increase in body weight and body length of the rat pups was found in group T4, while the lowest values were found in the control group.

Providing lactating rats with quail egg supplements enriched with marine macroalgae did not result in any significant changes in the levels of various hematological parameters compared to the control group (Table 3). The lipid components examined in this study were cholesterol, HDL, LDL, blood glucose, SOD, MDA and prolactin hormone. According to the results, cholesterol, HDL, LDL, blood glucose, SOD, MDA, and prolactin hormone levels did not show significant changes compared to the control group (Table 4).

Discussion

Vitamin A and iodine content

The concentration of vitamin A in egg yolk is closely linked to the concentration of vitamin A in the feed consumed by poultry (Lima and Souza, 2018; Meléndez-Martínez, 2019). Previous studies have shown that providing macroalgal extract can increase the vitamin A content in broilers egg (Ivarsson *et al.*, 2023). It is important to note that vitamins are required in small amounts but play a critical role in primary biological functions such as growth, embryonic development, epithelial cell differentiation, and immune function. Vitamin A deficiency can lead to various disorders, including lack of appetite, decreased development and immunity, and increased disease susceptibility (Khan *et al.*, 2023). The process of transferring vitamin A from the feed to quail eggs involves the absorption of vitamin A from the feed consumed by the quail, which is then stored in the egg yolk. Since vitamin A is fat-soluble, it is absorbed along with the fat in the feed and carried to the liver. During egg formation, vitamin A is transferred through the bloodstream to the ovaries for the synthesis of egg yolk precursors (Khan *et al.*, 2023).

The high level of iodine in the quail eggs is due to the high transfer of iodine from the feed to the eggs. Previous research has shown that feed containing 13 ppm iodine can increase the iodine content in chicken egg yolks (Sumaiya *et al.*, 2016). Similarly, other

Table 1. Vitamin A ($\mu\text{g}/100 \text{ g}$) and iodine (ppm) content in quail eggs enriched with macroalgae *E. spinosum*.

Parameters	T0	T1	T2	T3
Vitamin A	52.57 ± 1.88^a	86.48 ± 1.35^c	87.12 ± 1.71^c	85.03 ± 1.21^{bc}
Iodine	6.66 ± 0.36^a	11.29 ± 0.67^c	12.01 ± 0.61^c	9.46 ± 0.57^b

Note: Different superscripts on the same line indicate significant differences ($p < 0.05$). T0: given commercial feed; T1: fed commercial feed + 3% marine macroalgae; T2: fed commercial feed + 4% marine macroalgae; T3: fed commercial feed + 5% marine macroalgae.

Table 2. Body weight (g) and length (mm) of rat pups supplemented with quail eggs enriched with *E. spinosum*.

Parameters	T0	T1	T2	T3	T4
Weight					
0–7 day	11.64 ± 0.92^a	15.60 ± 1.40^b	16.21 ± 1.44^b	16.55 ± 0.56^b	16.69 ± 0.51^b
8–14 day	17.13 ± 1.11^a	23.04 ± 2.78^b	24.22 ± 2.78^{bc}	25.46 ± 0.84^{bc}	26.03 ± 1.18^c
15–21 day	22.56 ± 2.00^a	30.38 ± 2.75^b	32.90 ± 2.93^b	33.44 ± 2.37^b	33.35 ± 2.62^b
Length					
0–7 day	61.98 ± 4.51^a	69.32 ± 2.85^b	70.95 ± 0.53^b	71.63 ± 0.96^b	70.67 ± 0.76^b
8–14 day	75.01 ± 2.49^a	79.57 ± 5.37^{ab}	83.10 ± 3.84^b	83.36 ± 0.90^b	83.29 ± 1.49^b
15–21 day	81.83 ± 1.54^a	92.74 ± 3.35^b	94.23 ± 2.75^b	95.01 ± 2.87^b	95.28 ± 3.78^b

Note: Different superscripts on the same line indicate significant differences ($p < 0.05$). T0: control treatment; T1: treatment with quail eggs enriched with commercial feed; T2: treatment with quail eggs enriched with commercial feed + 3% marine macroalgae *E. spinosum*; T3: treatment with quail eggs enriched with commercial feed + 4% marine macroalgae *E. spinosum*; T4: treatment with quail eggs enriched with commercial feed + 5% marine macroalgae *E. spinosum*.

Table 3. Hematology profile of lactating rats given quail eggs supplementation enriched with macroalgae *E. spinosum*.

Parameters	T0	T1	T2	T3	T4
RBC ($\times 10^6/\mu\text{l}$)	7.22 \pm 0.30 ^a	7.09 \pm 0.28 ^a	7.19 \pm 0.30 ^a	7.28 \pm 0.21 ^a	7.37 \pm 0.23 ^a
Hb (g/dl)	14.22 \pm 0.20 ^a	14.06 \pm 0.27 ^a	14.18 \pm 0.42 ^a	14.64 \pm 0.35 ^a	14.46 \pm 0.44 ^a
HCT (%)	41.06 \pm 0.96 ^a	44.68 \pm 7.63 ^a	41.76 \pm 1.12 ^a	42.80 \pm 1.53 ^a	43.04 \pm 1.44 ^a
MCV (fl)	57.60 \pm 1.25 ^a	58.97 \pm 1.69 ^a	57.52 \pm 1.24 ^a	58.57 \pm 0.73 ^a	58.27 \pm 0.25 ^a
MCH (pg)	19.52 \pm 0.53 ^a	19.92 \pm 0.72 ^a	19.87 \pm 0.78 ^a	19.95 \pm 0.24 ^a	19.67 \pm 0.17 ^a
MCHC (g/dl)	34.66 \pm 0.87 ^a	33.90 \pm 0.38 ^a	33.96 \pm 0.67 ^a	34.22 \pm 0.68 ^a	33.60 \pm 0.25 ^a
WBC $\times 10^3/\mu\text{l}$	4.22 \pm 0.69 ^a	5.08 \pm 1.21 ^a	5.10 \pm 1.07 ^a	5.04 \pm 0.65 ^a	5.12 \pm 0.86 ^a
LYM ($\times 10^3/\mu\text{l}$)	2.60 \pm 0.48 ^a	3.92 \pm 1.10 ^a	3.44 \pm 0.62 ^a	3.60 \pm 0.60 ^a	3.42 \pm 0.25 ^a
NEUT ($\times 10^3/\mu\text{l}$)	1.62 \pm 0.31 ^a	1.16 \pm 0.50 ^a	1.66 \pm 0.74 ^a	1.44 \pm 0.29 ^a	1.70 \pm 0.68 ^a
RDW (fl)	28.22 \pm 0.82 ^a	29.32 \pm 5.67 ^a	29.86 \pm 1.60 ^a	29.62 \pm 1.28 ^a	30.18 \pm 1.79 ^a
PDW (fl)	7.08 \pm 0.27 ^a	7.54 \pm 0.62 ^a	7.18 \pm 0.47 ^a	7.74 \pm 0.35 ^a	7.32 \pm 0.61 ^a
MPV(fl)	6.20 \pm 0.14 ^a	6.50 \pm 0.31 ^a	6.24 \pm 0.13 ^a	6.48 \pm 0.19 ^a	6.32 \pm 0.34 ^a

Note: The same superscript on the same line shows that it is not significantly different ($p > 0.05$). T0: control treatment; T1: treatment with quail eggs enriched with commercial feed; T2: treatment with quail eggs enriched with commercial feed + 3% marine macroalgae *E. spinosum*; T3: treatment with quail eggs enriched with commercial feed + 4% marine macroalgae *E. spinosum*; T4: treatment with quail eggs enriched with commercial feed + 5% marine macroalgae *E. spinosum*.

Table 4. Serum biochemistry, oxidative stress status and prolactin hormone in lactating rats supplemented with quail egg enriched with macroalgae *E. spinosum*.

Parameters	T0	T1	T2	T3	T4
Cholesterol (mg/dl)	73.25 \pm 14.00 ^a	77.50 \pm 7.97 ^a	80.97 \pm 2.96 ^a	70.27 \pm 10.16 ^a	87.45 \pm 17.10 ^a
HDL (mg/dl)	31.57 \pm 2.76 ^a	34.83 \pm 2.35 ^a	35.67 \pm 0.93 ^a	33.23 \pm 1.35 ^a	39.87 \pm 4.10 ^a
LDL (mg/dl)	45.47 \pm 4.71 ^a	51.63 \pm 9.62 ^a	59.23 \pm 1.40 ^a	52.67 \pm 3.08 ^a	55.90 \pm 8.46 ^a
Glucose (mg/dl)	52.93 \pm 3.07 ^a	66.80 \pm 7.97 ^a	89.57 \pm 10.60 ^a	45.17 \pm 15.47 ^a	90.40 \pm 2.14 ^a
MDA (nmol/ml)	1.37 \pm 0.10 ^a	1.26 \pm 0.06 ^a	1.25 \pm 0.64 ^a	1.25 \pm 0.47 ^a	1.27 \pm 0.05 ^a
SOD (ng/ml)	2.25 \pm 0.15 ^a	1.93 \pm 0.38 ^a	1.87 \pm 0.23 ^a	2.06 \pm 0.18 ^a	2.01 \pm 0.20 ^a
Prolactin Hormone	86.69 \pm 5.49 ^a	92.87 \pm 10.60 ^a	84.75 \pm 6.30 ^a	91.95 \pm 7.56 ^a	83.96 \pm 5.92 ^a

Note: The same superscript on the same line shows that it is not significantly different ($p > 0.05$). T0: control treatment; T1: treatment with quail eggs enriched with commercial feed; T2: treatment with quail eggs enriched with commercial feed + 3% marine macroalgae *E. spinosum*; T3: treatment with quail eggs enriched with commercial feed + 4% marine macroalgae *E. spinosum*; T4: treatment with quail eggs enriched with commercial feed + 5% marine macroalgae *E. spinosum*.

studies have indicated a linear correlation between the iodine content in feed mixtures of up to 5 mg/kg and the iodine content in egg yolks (Słupczynska *et al.*, 2014). The iodine in feed is absorbed through the digestive tract and transported via the bloodstream to various tissues and organs, including the ovaries (Andersson and Braegger, 2022). The developing egg follicle (ovum) accumulates iodine in the ovary. As the mature egg follicle moves to the oviduct, where the shell and egg white are formed, iodine accumulates in the egg components. Iodine is a micronutrient necessary to produce thyroid hormones, which are essential for regulating metabolism, growth, and development (Andersson and Braegger, 2022). Sufficient iodine concentration in breast milk is crucial for providing optimal neonatal thyroid hormones and preventing

impaired neurological development in breastfed babies (Azizi and Smyth, 2009).

Body weight and length of rat pups

There was a linear increase in the body weight and body length of the rat pups starting on days 7, 14, and 21 in the groups given quail egg supplementation. The highest increase in body weight and body length of the rat pups was found in group T4, while the lowest values were found in the control group. Based on these results, it was found that supplementation of quail eggs enriched with *E. spinosum* was able to increase the body weight and body length of the rat pups (Fig. 2). Quail eggs enriched with *E. spinosum* contain high levels of nutrients, particularly protein. Body length and weight are crucial parameters that need to be measured to understand the impact of high protein intake on the



Fig. 2. Rat pups aged 21 days after being given treatment, including: T0: control treatment; T1: treatment with quail eggs enriched with commercial feed; T2: treatment with quail eggs enriched with commercial feed + 3% marine macroalgae *E. spinosum*; T3: treatment with quail eggs enriched with commercial feed + 4% marine macroalgae *E. spinosum*; T4: treatment with quail eggs enriched with commercial feed + 5% marine macroalgae *E. spinosum*.

growth of the offspring (Pastuszewska *et al.*, 2008). Previous research has also reported that providing egg protein to mothers significantly increased the body weight and length of rat pups compared to the control group (Naeem *et al.*, 2023). Consistent with the previous statement, a high correlation was found between body length and weight with high protein consumption (Pastuszewska *et al.*, 2008). Another study reported that providing one egg per day for 6 months to children in Ecuador resulted in a 47% reduction in stunting prevalence and a 74% reduction in underweight, thus helping the offspring achieve normal growth (Iannotti *et al.*, 2017).

Hematological indices

Hematological examination is valuable for diagnosing and monitoring metabolic diseases (Dunlea *et al.*, 2023). Hematological status is a useful indicator for assessing health conditions during lactation (Wrzecińska *et al.*, 2023). Concerning the levels of various hematological parameters, the study results showed that there are no significant changes in the treatment groups compared to the control. Basri *et al.*, (2018) found that there

is no impact on red blood cell and Hb levels in lactating white rats when given organic quail eggs and suggested that supplementing lactating rats with quail eggs enriched with marine macroalgae can help maintain their hematological condition and prevent physiological disorders during the lactation period. Normal hematological levels indicate the absence of metabolic disorders, diseases, organ damage, drug influence, or stress in rats (Lager and Jordan, 2012; Kuhn *et al.*, 2017; Gwozdziński *et al.*, 2021).

Serum biochemistry, oxidative stress status, and prolactin hormone

This study showed a similar result to the previous research that providing organic quail egg supplements during the lactation period had no impact on the lipid profile parameters such as total cholesterol, LDL, HDL, and triglycerides (Purba *et al.*, 2018). The lack of effect on increasing plasma lipid levels during lactation is attributed to increased distribution of lipids to the placenta and mammary glands to meet the lipid needs of rat offspring (Zhang *et al.*, 2017). Additionally, the similar feed consumed may have

caused the lack of effect of quail egg supplementation on cholesterol, LDL, and HDL levels in lactating rats. The cholesterol content influences cholesterol levels synthesized in the liver and small intestine in the diet (Caponio *et al.*, 2020). The body has a specific mechanism for managing excess cholesterol from food intake, involving reducing endogenous absorption and synthesis, and increasing cholesterol excretion through bile (Fernandez and Murillo, 2022). Excess cholesterol in cells inhibits HMG-CoA activity (Altmann *et al.*, 2004), thus causing the body to maintain a balance in plasma cholesterol levels (Fernandez and Murillo, 2022). Cholesterol is crucial for the development of brain tissue, nerve myelination, and serves as a fundamental ingredient for many enzymes (Lawrence and Lawrence, 2011).

The research on the effect of quail eggs on blood glucose levels revealed no significant difference compared to the control group. However, in group T3, glucose levels decreased, possibly due to metabolic changes (Hussein, 2013). Metabolic changes indicate an increase in the rat's nutritional requirements for the growth of her offspring (Zhu *et al.*, 2015). Giving quail eggs may not significantly impact blood glucose levels. A homeostasis process occurred in groups T1, T2, and T4, maintaining normal blood glucose levels. Homeostasis is achieved when glucose enters the liver. The small intestine and liver absorb glucose, and excess glucose is converted into glycogen and stored in the liver and muscles, with a small amount stored in the brain (Kanungo *et al.*, 2018; Zhang *et al.*, 2019).

Findings from this investigation of MDA's lipid peroxidation indicate no significant increase compared to the control, and there was no decrease in SOD levels across all treatments. Throughout the study, SOD and MDA levels remained constant, indicating the absence of significant oxidative stress. MDA and SOD levels fluctuate in response to physiological imbalances during the lactation period. Previous research also supports the observation that MDA levels in lactating rats do not significantly increase compared to controls (Zheng *et al.*, 2015). This absence of oxidative stress in lactating rats may be attributed to the direct energy metabolism to the offspring via milk (Garratt *et al.*, 2011). The physiological regulation of antioxidant systems plays a crucial role in maintaining the oxidant-antioxidant balance, thus preventing oxidative damage to macromolecules (Garratt *et al.*, 2011). The data suggests that antioxidants are physiologically regulated in response to reactive oxygen species (ROS) in various tissues of lactating rats, contributing to oxidant-antioxidant balance during peak lactation (Zheng *et al.*, 2015). The increase in metabolic rate during lactation leads to a rise in the production of ROS, potentially disrupting macromolecules, which can be mitigated by antioxidants (Selman *et al.*, 2000; Salmon *et al.*, 2001; Dowling and Simmons, 2009; Garratt *et al.*, 2011; Xu *et al.*, 2014).

According to the research findings, the treatment group given quail eggs did not show a significant increase in prolactin hormone levels compared to the control. This contrasts with previous research, suggesting that high-protein foods can significantly stimulate prolactin secretion (Velázquez-Villegas *et al.*, 2015). In rats, the lactation period lasts for 21 days after birth (Grases-Pintó *et al.*, 2021). The levels of secreted prolactin are influenced by various factors, including the frequency and duration of offspring breastfeeding (Cregan *et al.*, 2002; Wati *et al.*, 2023), maternal psychology (Nagel *et al.*, 2022), and the length of the lactation period. Prolactin hormone levels naturally decrease over time (Powe *et al.*, 2010). Given that blood sampling in this study was conducted at the end of the third week, which also marks the end of the lactation period, no increase in prolactin hormone levels would likely be observed in the blood samples.

Conclusion

The research results indicate that the addition of 4% of marine macroalgae *E. spinosum* to quail feed can effectively increase vitamin A and iodine levels in quail egg yolks. Furthermore, the T4 treatment, which involves enriching quail eggs with 5% marine macroalgae, is shown to increase the weight and body length of rat pups. However, this treatment did not have a significant impact on hematological levels, lipids, antioxidant activity, and the hormone prolactin compared to the control treatment. Overall, the study suggests that incorporation of quail eggs enriched with marine macroalgae *E. spinosum* support the growth and development of the rats offspring without negatively impact the health of the rats. Further research is needed to explore the potential beneficial effects of quail eggs enriched with marine macroalgae on other health parameters in lactating rats.

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Conflict of interest

The authors have declared no conflicts of interest.

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Authors' contributions

HB and SM concept and design, HB analisis/interpretasi data and drafting manuscript, SM HG ZZ critical revision of manuscript, HB HG statistical analysis, HB admin, technical or material support, SH HG ZZ Supervision and final approval.

Data availability

All data from this study are provided in the manuscript.

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