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ORIGINAL PAPER

Effect of spraying quail eggs with different doses of riboflavin on reproductive and growth rates, blood metabolites, and economics

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ABSTRACT. The present study aimed to investigate the role of spraying fertilised quail eggs with riboflavin (SP) on hatchability, performance, carcass traits, liver and kidney function, and economic efficiency of growing quail. A total of 260 quail eggs were used for hatching and randomly divided into four treatment groups with five replicates (13 eggs/replicate) in a fully randomised design. The eggs were sprayed with riboflavin before incubation. The groups included a control group (no spraying), and three experimental groups sprayed with 3, 4 or 5 g/l riboflavin, respectively. The spray was applied for 2 min. After hatching, 128 Japanese quail chicks, aged 7 days, were randomly assigned to 4 treatment groups, 32 birds each. The study was conducted over 5 weeks. Compared to the control group, fertility and hatchability rates (%) significantly improved in groups sprayed with 3 g, 4 g, and 5 g of riboflavin (vitamin B_2). Quails hatched from eggs sprayed with vitamin B_2 showed significant increases in body weight, weight gain, feed intake, feed conversion ratio, and carcass traits at different growth stages compared to the control group. Additionally, all vitamin B_2-treated groups demonstrated significant improvements in blood indices. The economic efficiency was also significantly enhanced in treated groups, with the most favourable results observed in the group sprayed with 3 g/l vitamin B_2. In conclusion, spraying eggs with riboflavin at varying doses can be recommended to enhance growth performance, blood parameters, carcass traits, and economic efficiency in quails.

Introduction

In recent years, researchers have developed numerous feed additives to improve the metabolism and overall health of farm animals, while also enhancing their performance and reproduction capacity (Youssef et al., 2023; Abd Elzaher et al., 2023). Feed additives derived from natural sources, including organic acids, enzymes, probiotics, prebiotics, oils, and vitamins, have been proposed as safer alternatives to antibiotics (Alagawany et al., 2016; Youssef et al., 2023; Abd Elzaher et al., 2023). Li et al. (2023) reported that eggs absorbed only 25–30% of feed additives from basal diets, including vitamins and other components. Riboflavin, also known as vitamin B_2, is a water-soluble compound essential for animals and birds because it interacts with flavin enzymes involved in energy production and lipid metabolism (Cogburn et al., 2018). Riboflavin plays a critical role in aerobic cellular oxidation processes (Ciudad-Mulero et al., 2023). It is also crucial for the functioning of the nervous system, skin, and mucous membranes, thus its deficiency in animal diets can lead to stunted growth, nervous system disorders, and development of skin lesions (Surana et al., 2023). Moreover, riboflavin contributes to increasing final weight of birds, as well as improving food processing, feed intake, and egg production rate (Shastak and Pelletier, 2023). On the other hand, inadequate riboflavin intake from growing and breeding diets can cause reduced growth and hatchability of chicks (Matos et al., 2024). Riboflavin deficiency may also negatively affect keratin formation in feathers (Cogburn et al., 2018). In the poultry industry, product quality is just as important as maintaining animal health. According to Miao et al. (2023), the conversion rate of riboflavin from feed to eggs is high, ranging from 25 to 50%.

Tang et al. (2023), on the other hand, reported that riboflavin deficiency negatively affected energy metabolism and the antioxidant capacity of eggs. For instance, Arijeniwa et al. (1996) observed that providing breeder hens with insufficient riboflavin dose (6.5 mg/kg feed) resulted in higher embryonic mortality and reduced chick hatchability in tropical conditions; similar effects were also recorded in ducks (Tang et al., 2023). Riboflavin, originally known as the 'Yellow Enzyme' is required for normal cellular function, development, and growth. It plays a crucial role in energy production, as well as the metabolism of fats, drugs, steroids, and alcohol. Additionally, it acts as an antioxidant, neutralising harmful free radicals throughout the body. Given its nutritional value and biological functions, we hypothesised that riboflavin would significantly contribute to the growth and health of growing quails. Therefore, the objective of this study was to assess the impact of vitamin B_2 treatment, applied by spraying, on the performance, carcass traits, liver and kidney functions, and economic efficiency of growing quail methionine + cysteine.

Material and methods

The study was performed over 9 weeks in 2 distinct periods, divided into two stages: hatching period (March 21 to April 12, 2023) and the growth performance period (April 12 to May 17, 2023) at the Quails Research Unit, Animal & Poultry Production Department, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt. The study complied with ethical standards approved by the Institutional Animal Care and Use Committee (IACUC) at Zagazig University in Zagazig, Egypt (ZU-IACUC/2/F/313/2023).

Experimental design and management

A total of 260 Japanese quail eggs were randomly divided into four treatment groups, each consisting of 65 eggs. Each experimental group was further subdivided into five replicates, with 13 eggs per replicate. The experimental groups were as follows: (i) a control group, (ii) a group sprayed with 3 g of vitamin B_2/l, (iii) a group sprayed with 4 g of vitamin B_2/l, and (iv) a group sprayed with 5 g of vitamin B_2/l. The riboflavin spray was applied for 2 minutes prior to incubation. At seven days of age, 128 Japanese quail chicks were selected and randomly assigned to four treatment groups, with 32 birds and four replicates per group.

The basal diet used in this study was formulated according to the nutritional guidelines for quail chicks established by the National Research Council (NRC) in 1994. The composition of the basal diet is detailed in Table 1. Throughout the trial, all birds were offered free access to feed and fresh water.

Table 1. Composition and nutritional analysis of grower quail diet, %

Ingredients	
Yellow maize	51.80
Soybean meal	36.70
Maize gluten 60%	5.21
Cotton seed oil	2.90
Dicalcium phosphate	0.70
Limestone	1.65
NaCl	0.30
Premix[1]	0.30
L-lysine	0.13
DL-methionine	0.11
Choline chloride	0.20
Calculated content	
CP, %	24.00
ME, kcal/kg	2995
Ca, %	0.80
P, % available P	0.45
lysine, %	1.30
met + cys, %	0.92

CP – crude protein, ME – metabolizable energy, [1] premix added to 1 kg of diet includes: IU: vit. A 10 000, vit. D_3 2 000; mg: vit. E 15, vit. K_3 1, vit. B_1 1, vit. B_2 5, vit. B_6 1.5, niacin 30, pantothenic acid 10, folic acid 1, choline 300, zinc 50, copper 4, iodine 0.3, iron 30, selenium 0.1, manganese 60, cobalt 0.1; μg: vit. B_{12} 10, biotin 50

They also received routine vaccinations, medications, and standard veterinary care as needed. Environmental, hygienic, and management conditions in Sharkia Governorate, Egypt were consistently maintained for all bird groups.

Data collection and evaluated traits

The experimental data were collected, and the following criteria were calculated or identified:

Fertility and hatchability parameters

Japanese quail eggs (260) were randomly divided into 4 groups, with 65 eggs each. Each treatment group was replicated five times, with 13 eggs per replicate. The eggs were stored at a temperature of 15–18 °C and 70% relative humidity prior to incubation. For incubation and hatching, the eggs were maintained at a temperature of 36–37.5 °C and a relative humidity of 65–75% in a fully automated computerised hatchery located at the Quail Research Unit at the Animal and Poultry Production Department, Faculty of Technology and Development at Zagazig University. On day 7 of incubation, photogrammetry was used to assess fertility. After hatching, the number of hatched chicks was recorded, and the proportion of unhatched and pecked eggs was calculated. The ratio of fertile eggs to total number of eggs, fertility (%), and hatchability percentages were calculated following the methodology described by Alagawany et al. (2014).

Pecked chicks (%)

The proportion of pecked chicks was calculated after hatching using the following formula:

Pecked chicks (%) = [Number of pecked chicks / Total number of set eggs] × 100.

Growth performance

At 1, 3, and 5 weeks of age, the body weight (BW) of each bird was recorded. Body weight gain (BWG) was also measured over the course of the experiment. Feed intake was continuously monitored during the trial periods to estimate the feed conversion ratio (FCR) using the formula: FCR = g feed/g weight gain.

Carcass traits

After a six-week study period, three birds from each treatment were randomly selected and fasted for 12 h before slaughter. The quails were individually weighed prior to slaughter following halal practices. After bleeding, the feathers and bird bodies were separately weighed. Following evisceration, the head, giblets (liver and heart), and carcass percentage were weighed. According to Baéza et al. (2022), the dressing percentage was calculated based on the carcass, giblets, and head weights.

Blood collection and preparation

Blood samples were collected from the same three birds in each experimental group at day 42 of age (4 ml for each bird). Each sample was divided into two tubes: one with anticoagulant and the other without it. The blood samples with antico agulant were used to determine the haematological parameters, including red blood cells (RBC), haemoglobin (Hb), white blood cells (WBC), and platelets. The blood samples without anticoagulant were left to clot, and after clotting, the samples were immediately centrifuged at 3000 rpm for 15 min. The serum was then separated and stored at −20 °C for biochemical analysis of creatinine, albumen, total protein, globulin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Youssef et al., 2023).

Measurement of haematological parameters

Hb concentration, WBC and RBC counts, as well as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the methods of Dacie and Lewis (1991). The counts of WBC and RBC were determined using an A.O. bright line haemocytometer and a light microscope with 20× magnification, or an automatic cell counter (Hospitex Hema Screen 18, Sesto Fiorentino, Italy). Before counting RBC and WBC, blood samples were diluted 200 times with physiological saline (0.09% NaCl solution) for WBC and 20 times with a diluting fluid (1% acetic acid) containing a few drops of Fleishman's dye for RBC. Hb concentration was measured using the Shahly method, which involves comparison of acid hematin (produced from Hb) to normal hematin using 10% HCl and distilled water. The packed cell volume (PCV) was determined using Wintrobe haematocrit tubes. Blood samples were centrifuged at 1000 rpm for 20 m, and the PCV was calculated as a percentage based on the volume of packed cells in the graduated haematocrit tubes.

Determination of blood biochemical parameters

The albumin and total protein levels were determined following the methods of Peters et al. (1982) using Bio-diagnostic commercial kits (Giza, Egypt).

The globulin value was calculated by subtracting the albumen concentration from the total protein concentration. By utilizing kits from the Biodiagnostic Company (Giza, Egypt), the activity of ALT and AST were analysed spectrophotometrically. Creatinine levels were assessed using Giorgio's method (1974) and Bio-diagnostic commercial kits (Giza, Egypt).

Economic efficiency

The economic efficiency (EE) of the experiment was determined by dividing the net revenue by the total costs. Net income was calculated as the total cost of producing each chick, including all feed expenses, expressed in Egyptian pounds (EGP). Economic efficiency was then determined by dividing net revenue by the feed input. The cost of labour, housing, veterinary care, and the purchase price of the birds were not included in the calculation, as these costs were the same for all treatment groups.

Statistical analysis

All statistical analyses in the study were carried out using SAS (2004). The pen was considered the experimental unit for growth performance metrics, whereas individual birds served as the experimental units for all other parameters. A one-way ANOVA and Tukey's test were used to analyse the growth performance, carcass traits, serum components, and antioxidant indices. Significance was determined at $P < 0.05$.

Results

Fertility and hatchability parameters

Table 2 presents the impact of spraying Japanese quail eggs with varying concentrations of riboflavin on fertility and hatchability parameters. The results showed that Japanese quails hatched from eggs treated with 3, 4, and 5 g of vitamin B_2/l spray had significantly increased fertility (%) ($P \leq 0.01$) compared to the controls. The group treated with 4 g of vitamin B_2 demonstrated the highest percentage of fertility (95.39%), followed by Japanese quails hatched from eggs treated with 5 g of vitamin B_2 (93.85%), while the control group had the lowest fertility rate (84.62%). Additionally, the 5 g vit. B_2/l group had the highest percentages of unhatched eggs (13.6%), followed by the 4 g vitamin B_2/l group (12.2%). Conversely, the control group exhibited the lowest percentage of unhatched eggs (6.4%). Moreover, compared to the other groups, Japanese quails hatched from eggs treated with 4 g of vitamin B_2/l spray had the highest percentage of pecked chicks (9.2), but the difference was insignificant ($P > 0.05$). Treating Japanese quails with all vitamin B_2 concentrations significantly ($P \leq 0.05$) increased the unfertile egg percentage. The lowest percentage of unfertile eggs (4.62%) in group treated with 4 g of vitamin B_2, while the highest percentage of infertile eggs was recorded in the control group (15.39%). Additionally, hatchability rates of eggs treated with 3, 4, and 5 g of vitamin B_2/l spray significantly improved ($P < 0.01$), and the group administered 3 g of vitamin B_2 reached highest hatchability rate of all eggs (76.93%). The lowest hatchability values of all eggs were observed in the control group (72.31%), whereas the hatchability percentage of fertile eggs was found to be the highest in this group (85.58%). Furthermore, the findings demonstrated that the weight of the chicks at hatching (g) and the relative weight (%) in all experimental treatment groups were significantly ($P \leq 0.05$) lower compared to the control group. The group treated with 5 g of vitamin B_2/l spray had optimal values for chicks' weight in grams and weight percentage (12.85 g and 90.19%, respectively) among all treatment groups. In contrast, the lowest chick weight (8.27 g) and relative weight percentage (72.46%) were recorded in the control group.

Table 2. Effect of spraying Japanese quail eggs with different levels of riboflavin on fertility and hatchability parameters

Items	Spray-treated groups, g/l				Significance
	Control	3 g	4 g	5 g	
Fertility, %	84.62 ± 2.44^{b}	92.31 ± 2.44^{a}	95.39 ± 1.89^{a}	93.85 ± 1.54^{a}	**
Unhatched egg, %	6.40 ± 1.60^{c}	7.80 ± 2.38^{b}	12.20 ± 3.87^{ab}	13.60 ± 1.40^{a}	*
Pecked chicks	6.40 ± 1.60	7.80 ± 2.38	9.20 ± 2.79	4.80 ± 1.96	NS
Non-fertile eggs	15.39 ± 2.44^{a}	7.69 ± 2.44^{b}	4.62 ± 1.89^{b}	6.22 ± 1.56^{b}	**
Hatchability of total eggs, %	72.31 ± 1.89^{b}	76.93 ± 2.44^{a}	73.85 ± 1.89^{ab}	75.39 ± 2.88^{a}	**
Hatchability of fertile eggs, %	85.58 ± 2.02^{a}	83.44 ± 2.54^{a}	77.57 ± 2.68^{b}	80.39 ± 3.21^{ab}	*
Chick's weight, g	8.27 ± 0.27^{c}	11.61 ± 0.42^{b}	12.06 ± 0.35^{a}	12.58 ± 0.71^{a}	**
Chick's weight, %	72.46 ± 2.36^{c}	79.45 ± 1.40^{b}	87.45 ± 2.18^{a}	90.19 ± 1.73^{a}	**

Control – no spraying, 3 g – group sprayed with 3 g of vitamin B_2/l, 4 g – group sprayed with 4 g of vitamin B_2/l, 5 g – group sprayed with 5 g of vitamin B_2/l; NS – not significant, * – $P < 0.05$, ** – $P < 0.01$; data are presented as mean value ± SEM; ^{abc} – means with different superscripts in the same row are significantly different at $P < 0.05$

Growth performance

Body weight and body weight gain

Three spray treatments with increasing vitamin B_2 levels significantly influenced live body weight (LBW), BWG, feed intake (FI), and FCR in Japanese quails (Table 3). The data indicated a significant ($P \leq 0.01$) improvement in body weight at 1, 3, and 6 weeks of age for quail eggs treated with all vitamin B_2 concentrations compared to the control group.

Japanese quails hatched from eggs treated with a 5 g vitamin B_2/l spray group showed the lowest FI values (476.86 g), while the control group had the highest values of this parameter (482.22 g). In addition, there was a significant difference ($P \leq 0.01$) in the FCR between the treated and control groups. The group that hatched from eggs treated with a dosage of 4 g of vitamin B_2 per l of spray demonstrated the most favourable FCR of 2.91, exceeding the results of the other groups.

Table 3. Effect of spraying Japanese quail eggs with different levels of riboflavin on total body weight, body weight gain, feed intake and feed conversion ratio from 1 to 5 weeks of age

Items	Weeks	Spray-treated groups, g/l				Significance
		Control	3 g	4 g	5 g	
Body weight, g	1	24.25 ± 0.95^{c}	27.94 ± 0.52^{bc}	26.97 ± 0.49^{b}	27.87 ± 0.63^{a}	*
	3	135.34 ± 2.78^{c}	141.78 ± 1.11^{b}	148.37 ± 0.69^{a}	148.91 ± 0.81^{a}	**
	5	174.44 ± 3.55^{c}	178.97 ± 0.97^{b}	186.41 ± 1.20^{a}	186.53 ± 1.18^{a}	**
Body weight gain, g	1–3	111.09 ± 2.81^{d}	116.84 ± 1.19^{bc}	121.41 ± 1.05^{a}	121.03 ± 0.76^{ab}	**
	3–5	39.09 ± 3.46^{a}	37.19 ± 1.89^{c}	38.07 ± 0.97^{ab}	37.63 ± 0.69^{ab}	*
	1–5	150.19 ± 3.72^{d}	154.03 ± 1.14^{bc}	160.94 ± 1.12^{a}	158.66 ± 0.58^{a}	**
Feed intake, g	1–3	231.15 ± 1.75^{c}	234.88 ± 1.84^{ab}	232.53 ± 1.22^{a}	232.91 ± 1.82^{a}	**
	3–5	251.07 ± 2.07^{c}	237.23 ± 1.34^{a}	235.20 ± 1.51^{a}	243.95 ± 2.85^{a}	**
	1–5	482.22 ± 1.43^{b}	469.41 ± 1.21^{a}	467.73 ± 0.93^{a}	476.86 ± 1.87^{a}	**
Feed conversion ratio, g feed/g gain	1–3	2.09 ± 0.07^{a}	2.01 ± 0.03^{b}	1.92 ± 0.01^{b}	1.93 ± 0.01^{b}	**
	3–5	6.23 ± 0.51^{b}	6.35 ± 0.23^{a}	6.17 ± 0.04^{b}	6.25 ± 0.05^{a}	*
	1–5	3.21 ± 0.10^{a}	3.05 ± 0.03^{b}	2.91 ± 0.02^{c}	3.01 ± 0.01^{b}	**

Control – no spraying, 3 g – group sprayed with 3 g of vitamin B_2/l, 4 g – group sprayed with 4 g of vitamin B_2/l, 5 g – group sprayed with 5 g of vitamin B_2/l; * – $P < 0.05$, ** – $P < 0.01$; data are presented as mean value ± SEM; $^{a–d}$ – means with different superscript in the same row are significantly different at $P < 0.05$

Among the treated groups, quails hatched from eggs treated with 5 g vitamin B_2/l spray achieved the highest LBW (186.53 g), while the lowest LBW (174.44 g) was recorded for the control group.

The results in Table 3 demonstrate that quail eggs sprayed with 3, 4, or 5 g of vitamin B_2 per litre of spray gained significantly more BWG after hatching than the control group during the periods of 1 to 3 weeks, 3 to 6 weeks, and during the entire trial time (1 to 6 weeks of age). The group that was sprayed with 4 g of vitamin B_2/l had the highest BWG (160.94 g), while the control group had the lowest wight (150.19 g).

Feed intake and feed conversion ratio

Table 3 presents the FCR and FI (g) of Japanese quails hatched from eggs treated with varying amounts of vitamin B_2 per l of spray (3, 4, and 5 g) during the experimental periods. FI at 1–3 weeks, 3–6 weeks, and 1–6 weeks of age was significantly higher ($P \leq 0.01$) in quails hatched from eggs treated with 3, 4, and 5 g of vitamin B_2/l spray compared to the control group.

In contrast, the control group had the worst FCR, achieving a rate of 3.21.

Carcass traits

Table 4 presents the impact of different concentrations (3, 4, and 5 g) of vitamin B_2 per l of spray on the carcass traits. There was no significant impact of different levels of riboflavin on body weight before slaughter, body weight after slaughter, body weight after feather removal, head weight, non-giblet weight, giblet weight (heart, gizzard, and liver), leg weight, and empty body weight. The results indicate that, in comparison to the other groups, the Japanese quails in the control group showed the highest values of dressing weight % and carcass weight % (77.93% and 75.50%, respectively).

Blood parameters

Table 5 presents the impact of spraying eggs with 3, 4, and 5 g of vitamin B_2/l on blood parameters of hatched Japanese quails at the end of the trial. The findings suggest that blood parameters

Table 4. Effect of spraying Japanese quail eggs with different levels of riboflavin on carcass characteristics at 5 weeks of age

Items	Spray-treated groups				Significance
	Control	3 g	4 g	5 g	
Body weight before slaughter, g	166.33 ± 4.25	175.33 ± 9.35	183.33 ± 6.11	178.00 ± 7.50	NS
Body weight after slaughter, g	163.66 ± 4.48	171.66 ± 9.20	177.00 ± 5.29	174.66 ± 7.17	NS
Blood weight	2.66 ± 0.33[c]	3.66 ± 0.33[b]	6.33 ± 0.88[a]	3.33 ± 0.66[b]	*
Body weight after feathers removed	152.33 ± 4.97	161.33 ± 9.33	165.60 ± 1.70	159.30 ± 8.20	NS
Feather weight	11.33 ± 0.66[ab]	10.33 ± 0.88[ab]	11.40 ± 5.80[ab]	15.36 ± 2.00[a]	*
Head weight	7.76 ± 0.59	8.83 ± 0.49	8.80 ± 0.40	8.86 ± 0.29	NS
Non-giblet weight	5.60 ± 0.79	6.36 ± 0.03	7.56 ± 0.29	7.93 ± 0.84	NS
Giblet weight	4.03 ± 0.06	4.36 ± 0.37	4.66 ± 0.26	5.50 ± 0.51	NS
Liver weight	2.63 ± 0.08	3.13 ± 0.31	3.20 ± 0.15	3.86 ± 0.27	NS
Heart weight	1.40 ± 0.10	1.23 ± 0.12	1.46 ± 0.16	1.63 ± 0.24	NS
Testis weight	1.93 ± 0.40[a]	1.63 ± 0.53[b]	1.06 ± 0.06[b]	1.10 ± 0.05[b]	*
Leg weight	4.56 ± 0.77	3.70 ± 0.15	4.53 ± 0.43	4.03 ± 0.36	NS
Gizzard weight	3.33 ± 0.24	3.23 ± 0.13	3.66 ± 0.17	3.60 ± 0.40	NS
Empty body weight	125.46 ± 0.92	129.26 ± 6.87	137.46 ± 4.26	128.66 ± 5.66	NS
Carcass weight, %	75.50 ± 1.45[a]	73.73 ± 0.46[ab]	74.99 ± 0.20[a]	72.27 ± 0.20[b]	**
Dressing weight, %	77.93 ± 1.54[a]	76.21 ± 0.44[ab]	77.54 ± 0.32[ab]	75.35 ± 0.29[b]	**

Control – no spraying, 3 g – group sprayed with 3 g of vitamin B_2/l, 4 g – group sprayed with 4 g of vitamin B_2/l, 5 g – group sprayed with 5 g of vitamin B_2/l; Hot carcass (%) = [Empty body weight including head wt. / Pre-slaughter weight] × 100; Dressed (%) = [Hot carcass wt. + Giblets weight (liver + heart weights)] / Pre-slaughter weight × 100; NS – not significant, * – $P < 0.05$ and ** – $P < 0.01$; data are presented as mean value ± SEM; [abc] – means with different superscripts in the same row are significantly different at $P < 0.05$

of Japanese quails treated during egg development with vitamin B_2/l spray showed significant improvement ($P \leq 0.05$) in comparison to the control group. Quails from eggs sprayed with 5 g of vitamin B_2/l had the most optimal levels of RBC (×10^6), WBC (×10^3), PCV (%), total protein (g/dl), globulin (g/dl), platelets (U/l), MCV (Fi), MCHC (g/dl), ALT (U/l), and AST (U/l) when compared to the other groups.

Economic efficiency

Table 6 presents data on the percentage of economic efficiency (EE) for Japanese quails hatched from eggs sprayed with solutions containing 3, 4, and 5 g of vitamin B_2 per l of spray, measured over the experimental period from 1 to 6 weeks of age. The EE percentage was higher in all treated groups compared to the control group. The group that obtained the highest EE values hatched from eggs

Table 5. Effect of spraying Japanese quail eggs with different levels of riboflavin on blood characteristics at 5 weeks of age

Items	Spray-treated groups				Significance
	Control	3 g	4 g	5 g	
Total protein, g/dl	3.37 ± 0.13[d]	3.63 ± 0.03[c]	3.77 ± 0.17[b]	3.94 ± 0.04[a]	**
Albumin, g/dl	1.45 ± 0.05[b]	1.50 ± 0.03[a]	1.56 ± 0.08[a]	1.46 ± 0.01[b]	*
Globulin, g/dl	1.92 ± 0.08[c]	2.13 ± 0.05[ab]	2.21 ± 0.09[b]	2.49 ± 0.04[a]	**
A/G ratio	0.76 ± 0.01	0.70 ± 0.03	0.71 ± 0.01	0.59 ± 0.01	NS
Haemoglobin, g/dl	14.8 ± 0.70[c]	17.35 ± 0.15[a]	17.00 ± 0.11[b]	17.05 ± 0.25[b]	**
RBC, ×10^6	2.25 ± 0.05[c]	2.53 ± 0.03[b]	2.57 ± 0.04[b]	2.86 ± 0.06[a]	**
Platelets, U/l	6.35 ± 0.35[d]	7.25 ± 0.05[c]	8.50 ± 0.50[b]	10.10 ± 0.30[a]	**
WBC, ×10^3	130.00 ± 20.00[c]	175.00 ± 10.00[b]	184.00 ± 3.00[a]	192.00 ± 8.00[a]	*
PCV, %	34.15 ± 3.96[c]	41.70 ± 1.50[ab]	41.74 ± 0.50[ab]	44.20 ± 1.00[a]	*
MCH, Pg	57.30 ± 2.00[c]	60.10 ± 0.11[a]	57.30 ± 1.00[b]	57.30 ± 2.00[b]	*
MCV, Fi	90.45 ± 0.15[c]	107.15 ± 2.05[b]	112.65 ± 0.45[a]	115.40 ± 0.20[a]	**
MCHC, g/dl	35.00 ± 1.00[c]	36.70 ± 0.50[ab]	38.50 ± 0.50[a]	39.05 ± 0.16[a]	**
Creatinine, mg/dl	0.45 ± 0.05	0.45 ± 0.05	0.46 ± 0.02	0.45 ± 0.05	NS
ALT, U/l	6.68 ± 0.03[c]	7.25 ± 0.05[b]	7.38 ± 0.03[b]	8.25 ± 0.06[a]	**
AST, U/l	142.85 ± 2.45[c]	150.48 ± 0.13[b]	154.30 ± 1.00[a]	155.35 ± 0.75[a]	**

A/G – albumin to globulin, RBC – red blood cells, WBC – white blood cells, PCV – packed cell volume, MCH – mean corpuscular haemoglobin, MCV – mean corpuscular volume, MCHC – mean corpuscular haemoglobin concentration, ALT – alanine aminotransferase, AST – aspartate aminotransferase; Control – no spraying, 3 g – group sprayed with 3 g of vitamin B_2/l, 4 g – group sprayed with 4 g of vitamin B_2/l, 5 g – group sprayed with 5 g of vitamin B_2/l; NS – not significant, * – $P < 0.05$ and ** – $P < 0.01$; data are presented as mean value ± SEM; [a–d] – means with different superscripts in the same row are significantly different at $P < 0.05$

Table 6. Effect of spraying Japanese quail eggs with different levels of riboflavin on economic efficiency from 1 to 5 weeks of age, in Egyptian pound (EGP)

Items	Spray-treated groups			
	Control	3 g	4 g	5 g
Total number of chicks	47.00	50.00	48.00	49.00
Price/chick	5.00	5.00	5.00	5.00
Total revenue chick	235.00	250.00	240.00	245.00
Egg number	65.00	65.00	65.00	65.00
Price/egg	2.50	2.50	2.50	2.50
Total cost/egg	162.50	162.50	162.50	162.50
Fixed egg	0.60	3.43	4.57	5.71
Total cost	163.10	165.93	167.07	168.21
Net revenue	71.90	84.07	72.93	76.79
Economic efficiency	44.08	50.67	43.65	45.65

Control – no spraying, 3 g – group sprayed with 3 g of vitamin B_2/l, 4 g – group sprayed with 4 g of vitamin B_2/l, 5 g – group sprayed with 5 g of vitamin B_2/l; Total revenue hen = Number of chicks × Price/chick; Total cost/egg = Egg number × Price/egg; Total cost = Total cost/egg + Fixed bird; Net revenue = Total revenue chick − Total cost/egg, Economic efficiency = Net revenue/Total cost × 100

treated with 3 g of vitamin B_2/l spray. In contrast, the control group and the group hatched from eggs treated with 4 g of vitamin B_2 per l of spray had the lowest EE values.

Discussion

Supply of water-soluble vitamin B_2 is essential to maintain birds' health (Nemati et al., 2023). As the bird's body stores only small amounts of vitamin B_2, it must be supplied daily. According to Cogburn et al. (2018), B vitamins, including vitamin B_2, support energy-related cellular functions and participate in the production of red blood cells. Vitamin B_2 plays a major role in metabolism, by breaking down lipids and carbohydrates to generate the energy required for survival. It increases the overall vitality of the bird, as well as its hatchability and fertility parameters (Hocking et al., 2013). It also prevents gastrointestinal (GIT) diseases by maintaining and protecting the mucous membranes within it (Witten and Aulrich, 2018). Our results are consistent with those reported by Leber et al. (2022), who found that hens fed a diet containing 3.1 mg or 4 mg of vitamin B_2 per kg had significantly higher fertility ($P < 0.05$) compared to the control group. Similarly, Tang et al. (2019) reported that female ducks receiving riboflavin at concentrations of 10 mg/kg and 3 mg/kg in their diet showed a significant increase in fertility percentage ($P < 0.05$) during all trial periods. Consistently, Arijeniwa et al. (1996) observed a substantial ($P < 0.05$) increase in fertility percentage in laying hens administered 8.80 or 10.5 mg of vitamin B_2 per kg of diet compared to the control group.

Riboflavin acts as an antioxidant, protecting cells from irreversible damage caused by reactive oxygen species, whose production may rise in quail subjected to stress such as heat or crowding. Sufficient supply of riboflavin can help mitigate this effect by enhancing fertility and reducing the negative impact of stress (Akinyemi and Adewole, 2021).

According to Cogburn et al. (2018), the bird's stores vitamin B_2 in small quantities; therefore, it must be supplied daily. Vitamin B_2 is essential for maintaining the body's metabolism, which balances hormones and strengthens the immune system as the body breaks down fats and carbohydrates to produce energy. Vitamin B_2 increases the vitality of the bird population, hatchability %, fertility rate, and chick weight at hatching (Hocking et al., 2013). Additionally, Witten and Aulrich (2018) emphasise that preserving the health of mucous membranes in the digestive system plays a crucial role in preventing illnesses. Additionally, riboflavin improves the hardness and albumen structure of egg whites, and high-quality eggs may indirectly affect hatching success (Khillare and Chilkhalikar, 2022). Riboflavin is involved in various metabolic activities necessary for the proper development of embryos, including its role as a coenzyme in the electron transport chain, which produces energy for the growing embryo. As noted by Shastak and Pelletier (2023), a deficiency in riboflavin can impair energy production, stunt growth, and lead to abnormal development. Moreover, riboflavin is involved in albumen formation, which supplies vital nutrients and shields the growing embryo. These findings are consistent with those of Leiber et al. (2022), who observed a significant ($P < 0.05$) increase in hatchability, the percentage of fertile eggs that hatched, and chick weight in laying hens fed 3.1 mg or 4 mg of vitamin B_2/kg diet. In line with this, Tang et al. (2023) found that providing female ducks with 10 mg of riboflavin per kg of diet during different experimental periods led to an increase in both the number of fertile eggs and hatching success. Another study by Arijeniwa et al. (1996) demonstrated that laying hens administered 8.80 mg or 10.5 mg riboflavin/kg diet, respectively, had significantly higher percentages of total eggs set and chick weight (g) compared to the control group ($P < 0.05$).

In the present study, significant improvements ($P < 0.05$) in BWG were observed at 1, 3, and 6 weeks of age in quails hatched from eggs sprayed with vitamin B. These findings are in line with a work by Arijeniwa et al. (1996), who reported that chickens receiving varying doses of riboflavin exhibited the highest LBW and BWG compared to the control group. Moreover, the higher levels of riboflavin increased tissues' resistance against toxins and diseases (Witten and Aulrich, 2018; Leiber et al., 2022). In addition to facilitating the synthesis of red blood cells, vitamin B_2 and other B vitamins stimulate other cellular processes that generate energy for the body. Riboflavin also protects against digestive system disorders by maintaining and ensuring the integrity of the mucous membranes (Lambertz et al., 2021). The current results indicated that birds hatched from riboflavin-treated eggs had higher live body weight and weight gain compared to the control group. These findings may be associated with the active components of vitamin B_2, which is considered a valuable nutritional source due to its wide range of physiologically and nutritionally significant compounds (Tang et al., 2019). Throughout the trial period (from 1 to 6 weeks of age), Japanese quails hatched from eggs treated with 5 g of vitamin B_2/l spray showed the highest feed intake values, followed by those treated with 4 g vitamin B_2/l spray. These outcomes are consistent with those of Liber et al. (2022), who reported that FI in laying hens was significantly increased by different riboflavin levels compared to the control group. Adequate riboflavin doses can support efficient utilisation of feed energy, potentially improving FCR. Additionally, riboflavin is involved in protein synthesis, and research suggests that increased riboflavin supplementation may enhance protein utilisation in hens, leading to improved growth and feed efficiency (Jaroensuk et al., 2023).

The data indicated that adding riboflavin to quail diets did not adversely affect carcass characteristics. Lambertz et al. (2021) similarly reported no negative impact on carcass yield and offal. These results are also consistent with a prior study conducted by Leiber et al. (2022), who suggested that providing Japanese quails with a diet containing 2.5 mg and 4 mg of riboflavin per kg could potentially enhance the quality of their carcasses and reduce the fat deposition in their shoulders and internal organs. Selecting optimal dietary riboflavin levels for different poultry species have the potential to enhance growth efficiency and FCR of broiler chickens, indirectly benefiting carcass production. However, excessive supplementation does not provide additional advantages and may even be harmful (Tang et al., 2023).

In addition, the results demonstrated that the blood indices of quails hatched from eggs treated with a 5 g of vitamin B_2/l spray reached their most favourable values. This was consistent with findings of Goel et al. (2013), who observed that serum total protein and albumin levels were significantly higher ($P < 0.05$) in quails supplemented with B_2 compared to the control group. Similarly, El-Kholy et al. (2019) recorded significant increases ($P < 0.05$) in blood total protein, albumin, and globulin levels ($P < 0.05$) in quails treated with riboflavin. Goel et al. (2013) also found that the blood of vitamin-fed broiler chickens had higher haemoglobin levels, red blood cell counts and packed cell volume. To maintain healthy red blood cells, birds must consume an adequate amount of riboflavin. A deficiency in riboflavin can lead to anaemia, characterised by a decrease in haemoglobin levels, red blood cell count, and oxygen-carrying capacity. In addition to its role in energy production, riboflavin may also influence the development and generation of red blood cells and metabolic processes (Aljaadi et al., 2023). The current experiment showed that at 42 days of age, riboflavin treatment did not significantly affect ($P > 0.05$) blood creatinine concentrations compared to the control group, while significantly increasing ($P < 0.01$) liver enzyme activity (ALT and AST). The latter result was consistent with a study by Kitakoshi et al. (2007), who also found elevated AST and ALT levels following riboflavin administration.

The highest value of EE was recorded in the second group (3 g of vitamin B_2/l) when compared to the other groups. These findings align with those of Arijeniwa et al. (1996) who observed improved EE in Hubbard strain layers fed a riboflavin-supplemented diet. The latest authors reported that birds receiving vitamin B_2 per kg of diet achieved the highest net revenue and EE compared to the control groups.

Insufficient supply of riboflavin to quail can result in deformities in chicks and even mortality. Supplementation can mitigate such losses, supporting the healthy development and survival of chicks, thereby maximising flock productivity (Shastak and Pelletier, 2023).

Conclusions

Spraying Japanese quail eggs with varying doses of riboflavin improved the birds' growth performance, carcass traits, blood parameters, and economic efficiency. Furthermore, the results of the current study indicate that Japanese quail eggs treated with vitamin B_2 at a dose of 5 or 4 g/l via spraying improved blood biochemical parameters, hatchability, and fertility of hatched chicks.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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