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Seasonal Variations and Dietary Protein-Energy Levels: Effects on Laying Quails under Subtropical Conditions

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ABSTRACT: Optimizing the diet of laying Japanese quail (*Coturnix japonica*) is essential to enhance their productivity. The experiment evaluated the efficiency, digestive parameters, and nutrient composition of laying Japanese quail during warm and cold months under subtropical conditions, in relation to the amounts of crude protein (CP) and metabolizable energy (ME). A randomized experimental design was used, involving 360 laying Japanese quail assigned to nine treatment groups, each with five replications of eight hens each across two independent experiments conducted during cold and warm seasons. Variations in protein and energy concentrations significantly impacted multiple production performance metrics, such as egg mass (EM), cost-effectiveness and feed conversion ratio (FCR). Quails showed the highest FCR and EM measurements when fed a diet with 2900 kcal/kg and 22% CP during the cold season, and 3000 kcal/kg with 20% CP during the warm season. Protein and energy levels significantly impacted egg quality and digestion ratios of dry matter (DM) and ether extract (EE). For optimal growth and overall health, Japanese quail should be fed a summer diet containing approximately 20% crude protein and 3000 kcal ME/kg. Adjusting the protein and energy content in the feed can enhance the performance and nutrient efficiency of laying Japanese quail, particularly in seasonal conditions, thereby informing feeding strategies for better production outcomes.

Keyword: digestibility coefficients; Japanese quail; metabolizable energy; seasonal variation; nutrient digestibility.

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INTRODUCTION

The Japanese quail (*Coturnix coturnix japonica*), is a small, fast-growing, and low-maintenance laboratory animal, is increasingly raised for meat and eggs due to genetic improvements (Lansford and Cheng, 2024). Quail farming is thriving globally, particularly in hot climates like Egypt, where these birds thrive better than chickens (El-Kelawy and Refaie, 2024). High ambient temperature is one of the major environmental stressors in avian production in many regions of the world (Vakili and Ebrahimnezhad, 2022). Subtropical conditions, with high temperatures and humidity, affect quail production, leading to reduced growth, reproductive performance, and egg quality. As environmental temperature increases above the thermo-neutral temperature, heat stress occurs. (Vakili and Ebrahimnezhad, 2022). Heat stress decreases feed intake, growth rates, and feed conversion efficiency (Alagawany et al., 2017). Reproductive efficiency, including egg production and fertility, is also compromised (Santana et al., 2021). Eggshell quality weakens, leading to breakage due to reduced calcium deposition (Mashaly et al., 2004). Quails exposed to heat also experience higher mortality and increased disease susceptibility (Videla et al., 2020). These challenges highlight the need for adaptive strategies to maintain productivity in subtropical conditions (Renaudeau et al., 2012). In recent years, global warming has been the subject of interesting study regarding its impact on animals (Varis, 2024).

Climate change threatens quail production, requiring solutions to mitigate health, growth, and reproduction issues (El-Sabry et al., 2021; Gomaa et al., 2024). Different nutritional strategies are used to reduce the negative effects of high temperatures (Ribeiro et al., 2021). Diet modification can reduce the adverse effects of thermal stress on quails (Mangan and Siwek, 2024). Energy and protein are key nutritional factors affecting quail production (Korver, 2023; Barszcz et al., 2024). Studies show that diets with 22.42% CP and 2850 Kcal ME/kg provide the best outcomes (Pinto et al., 2002). Research aims to identify the optimal energy and protein needs of laying Japanese quail in different seasons.

MATERIALS AND METHODS

The experiment of this research was conducted at the Poultry Research Farm, Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The care and management of the animals were performed following the guidelines of Zagazig

University for the use and management of laboratory animals, as well as those of the Egyptian Research Ethics Committee. The analyses were performed in the laboratory of the animal husbandry department to determine the nutritional value of the experimental diets and the external and internal quality of the eggs.

Experimental Design, Diets and Birds

In an experiment with a randomized method involving two separate trials conducted during the cold and warm seasons, each with five replicates of eight hens, 360 eight-week-old laying Japanese quails were allocated at random to nine different treatment groups. The laying Japanese quails received the following treatment diets for both the cold and warm seasons: T1 was fed a diet consisting of 18% CP and 2800 Kcal ME/kg, T2 received 20% CP and 2800 Kcal ME/kg, T3 was provided with 22% CP and 2800 Kcal ME/kg, T4 consisted of 18% CP and 2900 Kcal ME/kg, T5 had 20% CP and 2900 Kcal ME/kg, and T6 was given 22% CP and 2900 Kcal ME/kg, T7 was fed a diet with 18% CP and 3000 Kcal ME/kg, T8 received 20% CP and 3000 Kcal ME/kg, and T9 was provided with 22% CP and 3000 Kcal ME/kg. For a period of three months, starting at two months of age, the adult Japanese quail birds were assessed during both cold and warm months. During the study, the quails from both groups (Exp. 1 and Exp. 2) were kept in suitable trial enclosures. Eight quails were accommodated in a cage measuring 90 x 40 x 40 cm, equipped with trough feeders and nipple drinkers. Birds were raised in open houses in cages during the winter and summer. Average temperatures and relative humidity in winter (January - February and March) were 18.6, 20.7, and 21.8 °C, and 44.8, 48.3 and 42.2%. The temperatures and relative humidity during the summer months (June - July - August and September) were 33.6, 35.9, 34.2, 31.8 degrees Celsius, 60.7, 66.3, and 64.6. 59.6%, respectively. During the experimental phase, the quails received 16 hours of light daily during the laying phase. They were given unlimited entry to fresh water and fed for the entire duration of the trials. Table 1 presents the composition of feed components along with the projected nutrient analysis for diets designed for laying quail. The protein amounts under evaluation had similar energy content. The composition of each diet was adjusted and modified to develop experimental diets, reflecting variations in calorie and protein content. For high-energy diets, the oil content was increased by substituting wheat bran, soybean meal, and corn, while in high-protein diets,

Table 1. Composition and calculated analysis of layer quail diets

Ingredients %	T1	T2	T3	T4	T5	T6	T7	T8	T9
Yellow corn	55.15	53.20	52.94	58.45	59.25	57.45	64.25	64.25	62.85
Soybean meal 44%	28.00	27.75	24.00	28.00	22.10	22.10	24.10	20.10	19.00
Corn gluten 60%	8.00	8.60	11.30	4.40	8.70	9.00	3.00	6.00	7.00
Cotton seed oil	0.50	2.10	3.48	0.70	1.50	3.00	0.00	1.00	2.50
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine HCL	0.15	0.15	0.18	0.15	0.25	0.25	0.25	0.35	0.35
DL-Methionine	0.10	0.10	0.00	0.20	0.10	0.10	0.30	0.20	0.20
Total	100	100	100	100	100	100	100	100	100
Chemical and Calculated Analysis**									
DM	90.3	91.1	90.7	90.1	91.6	90.8	89.8	90.4	90.8
CP% (as Fed) **	18.75	18.94	18.98	16.58	16.69	16.62	14.64	14.82	14.77
ME Kcal /Kg **	2740.1	2891.8	2996.6	2763.9	2903.5	3055.2	2876.8	2893.7	2991.4
TDN%	65.20	68.80	71.30	65.80	69.10	72.70	68.40	68.80	71.20
CP%***	21.95	22.03	22.15	20.10	20.13	20.07	18.10	18.08	17.97
ME Kcal/Kg***	2800	2904	3014	2802	2920	3005	2804	2911	3006
C/P ratio	127.2	131.8	136.3	140.0	145.00	150.0	155.5	161.1	166.6
Ca%	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
P% Avail. Phos.	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysine %	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
Methio. +Cyst. %	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Price/ton diet, \$ ****	414.63	432.32	452.76	392.69	414.32	429.66	369.12	388.27	405.71

* Layer Vit. & Min. premix: Each 2.5kg of vitamin and mineral premix (commercial source Pfizer co.) contains Vit A. 12 Miu., E. 15 IU., Vit. D3 4 Miu., Vit. B1 1 g, Vit. B2 8g, pantothenic acid 10.87g, Nicotinic acid 30g, Vit. B6 2g, Vit. B12 10 mg. Folic acid 1g, Biotin 150 mg, Copper 5g, Iron 15 g, Manganese 70g, Iodine 0.5 g, Selenium 0.15g, Zinc 60g, Antioxidant 10g. ** As Fed values refer to the analyzed composition of the diets according to NRC (1994). *** Calculated values estimated according to NRC (1994). **** Calculated according to the price of feed ingredients when the experiment was started.

the substitution was the opposite. The entire trial feeding regimen was adjusted for the amino acid lysine and methionine, as well as essential minerals and vitamins, to align with the guidelines of the NRC (1994), as shown in Table 1.

Measurements investigated

Live body weight (LBW)

The birds were weighed separately to the nearest gram in dawn before they received any feed or water, starting from 2 months to 5 months of age at the conclusion of the research. The total live body weight for each treatment group was recorded and calculated by the number of birds in that group to compute the mean LBW.

Egg production traits

Egg number (EN)

Starting from reproductive maturity and continuing until the end of the experiment, the number of eggs produced by each female in every pen was recorded.

Egg weight (EW)

The weight of eggs in each pen was measured to the nearest gram for each female (aged 8–20 weeks), from reproductive development until the conclusion of the trial.

Eggs mass (EM)

The egg mass (g) was determined by the product the mean weight of egg laid each day in each pen by the total number of eggs produced.

Feed conversion ratio (FCR)

The FCR during the trial laying phase (2–5 months of age) was calculated using this formula:

$$\text{Feed conversion for egg produced} = \frac{\text{g feed}}{\text{g egg produced}}$$

Egg quality

Egg quality assessments were conducted two times throughout the second and fourth weeks of every month. To analyze the egg constituents, four eggs from each treatment replicate were used. The egg shape index (ESI) was calculated utilizing the equation: $\text{Width (mm)} / \text{Length (mm)} \times 100$. The dimensions of the eggs, including width and length, were assessed with a Vernier Caliper to the nearest tenth of a millimeter (Youssef et al., 2023). The yolk index (YI) was determined by the formula: $\text{Height} / \text{Diameter (mm)} \times 100$. The diameter was assessed using a Vernier Caliper to the nearest millimeter, while the height was quantified with a tripod micrometer, accurate to 0.01 cm (Youssef et al., 2024).

To quantify the albumen height, the egg was broken onto a smooth, level area and the highest point of the albumen was measured, positioned at the center of the thick albumen, distant from the chalaza. Both the yolk and albumen heights were evaluated using a tripod micrometer. The thickness of the shell was evaluated with an Ames shell thickness gauge, accurate to 0.001 millimeters. For this study, the average shell thickness was determined by taking measurements at both the wide and slim ends of the egg, in addition to along the equatorial plane.

Digestibility trial

At the conclusion of the trial laying phase (at 5 months of age), nine digestibility traits were assessed to determine the digestibility of the trial diets.

Digestibility trials technique

For each digestibility study, five quails were housed independently in metabolic cages. To monitor weight stability, the birds were weighed both pre and post the sampling period. The metabolic cages, measuring $50 \times 50 \times 70$ cm, were made of metallic wires and wooden frames.

To hold feed and fresh water, two bins with adjustable height were used, and a polyethylene sheet for garbage collection was placed in each cage. A small compartment ($50 \times 30 \times 30$ cm) located under the feeding container was designed to collect spilled food. The initial phase spanned five days and was designed to reduce leftover feed by modifying the birds' feed intake. The collection phase was prolonged to four days to collect manure samples suitable for chemical analysis. The mean feed consumption during this period was utilized to determine the daily feed portions. On the initial day of collection, the portions were weighed and placed in cardboard containers for storage. Any leftover feed after the trial was weighed and subtracted from the total feed provided. Manure collection started 24 hours into the collection phase, and feathers or other external material were eliminated. The droppings were collected using a spatula and placed in a dish ($20 \times 10 \times 3$ cm), then dried at 65°C for 24 hours. After drying, the samples were left in the open for a few hours, bulked for each bird, crumbled, and stored in glassware bottles. The daily dry droppings were then weighed to the nearest 0.1 g. Urinary nitrogen was calculated by subtracting fecal nitrogen from total nitrogen (excreta N - fecal N). The equation suggested by Abou Raya and Galal (1971) was used to determine urinary organic matter, calculated by

multiplying urine N x 2.62. To calculate the nitrogen-free extract (NFE) percentage, the proportion of urinary organic matter in the feces was combined to the sum of the other components (% fecal CP + % EE + % CF + % ash). The formula for NFE% is $100 - (CP + EE + CF + \text{urine OM} + \text{ash})$. The dry matter intake was assessed to establish the digestion coefficient for each nutrient, which was then compared to the excreta, followed by a percentage analysis of each component.

Calculation of nutritional value

Metabolizable energy (ME) and total digestible nutrients (TDN) were used to determine the nutritional values. TDN was calculated by applying the following factors: 1 for crude fiber (CF and NFE), 2.25 for EE, and 1 for CP. As per the recommendation of Titus and Fritz (1971), metabolizable energy was estimated at 4.2 kcal per gram of TDN.

Chemical analysis

The basic composition of the test substance, feed, and excreta was conducted in triplicate for each sample, subsequent the guidelines of the Association of Official Analytical Chemists (AOAC, 2006).

Economic efficiency

Economic efficiency (EE) was assessed using an input-output analysis that considered the differences in egg production and feed costs, as outlined in the method by Saliu et al. (2016).

Seasonal changes (SV) between winter and summer on studied parameters of laying Japanese quail

The study examined the effects of seasonal changes on several parameters, FCR, egg mass, egg quality, digestibility, and nutritional profile in laying Japanese quail. These seasonal changes were quantified by evaluating the gap between the winter and summer parameters, presented as a percentage of the winter values and multiply by 100, a process referred to as "seasonal variation." Negative seasonal variation values show that growth rates were higher in warmth than in cold, potentially due to the various levels of protein and energy used in the trial.

Statistical analysis

The analysis of the data was performed using a totally random design and an ANOVA test with general linear model (GLM) techniques in SPSS 26 software (SPSS, 2019) Variations in means ($P < 0.05$) were detected by Duncan's New Multiple Range

Test (Duncan, 1955). Unless specified differently, a p-value below 0.05 was considered to indicate significant differences in the findings. The statistical model used was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} = the perceived value of the treatment in question, μ = overall means observed for the treatment in question, T_i = treatment impact and e_{ij} = error related to an individual observation.

RESULTS

Live body weight (LBW)

Table 2 indicates how varying dietary protein levels and metabolizable energy affect initial body weight (IBW) at 8 weeks and final body weight (FBW) at 20 weeks in the winter and summer seasons. Variance analysis showed that the treatments had no notable differences in IBW and FBW.

Egg production traits

Egg weight (EW)

The impact of shifting dietary protein levels and metabolizable energy on egg weight (EW) at various points during the winter and summer experiments is depicted in Table 3. Variance analysis found no significant changes between the treatments in EW overall times of experimentation.

Egg number (EN)

Table 4 displays how varied dietary protein levels and metabolizable energy affect egg number (EN) at different experimental points in winter and summer. Variance analysis showed a notable difference ($P < 0.05$) related to the treatments in EN. Throughout the time frame from 8-12 Wks. of age in the winter season, the highest average of EN was in the T4, T5 and T6 groups, with an energy level of 2900 Kcal ME/kg diet and CP level of 18, 20, and 22%, respectively. Also, during the period from 12-16 Wks. of age in the winter, the highest average of EN was in the T4 and T6 groups, while during the summer, the highest average was in the T3 and T9 groups. During the period from 16-20 Wks. of age in the winter season, the highest average of EN was in the T4, T5 and T6 groups, with an energy level of 2900 Kcal ME/kg diet and CP level of 18, 20, and 22%, respectively. In the summer, its highest rate was in the T7 group (18% CP and 3000 Kcal ME/kg diet). Moreover, during the total period of the experiment (8-20 Wks.), the highest average of EN during the winter in the T4 and T6 groups, while during the summer, the T3 (22% CP and 2800 Kcal ME/kg diet) group was the highest.

Table 2. Seasonal variation of initial and final body weight of laying Japanese quail during the different experimental periods as affected by different dietary protein levels and metabolizable energy

Item	Live body weight (g)					
	8 wks. of age			20 wks. of age		
	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	197.5	204.4	-3.49	184.3	195.6	-6.13
T2	199.5	206.1	-3.31	188.1	191.9	-2.02
T3	199.9	208.2	-4.15	201.8	184.7	8.47
T4	197.4	217.4	-10.13	190.2	198.6	-4.42
T5	196.6	213.6	-8.65	186.0	212.3	-14.14
T6	196.7	200.9	-2.14	187.4	198.1	-5.71
T7	198.3	193.1	2.62	187.3	193.9	-3.52
T8	190.9	209.2	-9.59	192.5	194.1	-0.83
T9	196.2	204.3	-4.13	195.4	208.7	-6.81
SEM	1.24	1.12	1.84	2.07	2.10	2.75
P value	0.523	0.421	0.845	0.414	0.445	0.654

S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

Table 3. Seasonal variation of egg weight of laying Japanese quail during the different experimental periods as affected by different dietary protein levels and metabolizable energy

Item	Egg weight (EW) (g)											
	8-12 wks. of age			12 – 16 wks. of age			16 – 20 wks. of age			8 – 20 wks. of age		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	11.5	11.6	-0.87	11.8	11.4	3.39	12.0	11.4	5.00	11.8	11.5	2.54
T2	11.4	11.4	0.00	11.8	11.2	5.08	11.6	11.7	-0.86	11.7	11.5	1.71
T3	11.6	11.2	3.45	11.9	11.1	6.72	12.2	11.8	3.28	11.9	11.4	4.20
T4	11.7	11.8	-0.85	12.0	11.7	2.50	11.9	12.1	-1.68	11.9	11.9	0.00
T5	11.8	11.3	4.24	12.1	11.9	1.65	12.2	12.1	0.82	12.1	11.9	1.65
T6	11.9	10.7	10.08	12.6	11.2	11.11	12.5	11.8	5.60	12.3	11.2	8.94
T7	12.0	11.5	4.17	12.1	11.4	5.79	12.2	11.9	2.46	12.1	11.5	4.96
T8	11.7	11.8	-0.85	12.2	11.6	4.92	12.1	12.2	-0.83	12.0	11.8	1.67
T9	11.8	11.0	6.78	12.4	11.5	7.26	12.2	11.9	2.46	12.1	11.5	4.96
SEM	0.02	0.04	1.27	0.10	0.16	1.33	0.47	0.26	1.19	0.07	0.03	1.56
P value	0.856	0.663	0.552	0.844	0.221	0.563	0.669	0.556	0.896	0.478	0.486	0.326

S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

Egg mass (g egg/bird/day)

The impact of each dietary component on egg mass during both the cold and warm seasons is demonstrated in Table 5 ($P < 0.05$). It was observed that egg mass was lower in the cold season compared to the warm season, with values of 8.426 g/bird/day for the 22% CP diet containing 2800 kcal ME/kg feed (T3), versus 8.193 g/bird/day. Although no significant effect was found on egg mass during the cold

months (9.030 - 9.050), a seasonal decline of 3.23% in protein content during the warm season resulted in a gradual decrease in egg mass (from 8.426 to 6.828 g/hen/day). Periodic differences of 31.88% and 12.83% were observed for the low and medium CP diets, respectively. According to the findings, a diet containing 22% CP and 2800 kcal ME/kg is essential for achieving the highest egg mass in Japanese quail during the warm months. In contrast, the other

Table 4. Seasonal variation of egg number of laying Japanese quail during the different experimental periods as affected by different dietary protein levels and metabolizable energy

Item	Egg Number (EN) (bird/day)											
	8-12 wks. of age			12 – 16 wks. of age			16 – 20 wks. of age			8 – 20 wks. of age		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	0.77 ^c	0.60	22.08	0.81 ^c	0.61 ^d	24.69	0.75 ^b	0.58 ^d	22.67	0.78 ^c	0.60 ^c	23.08
T2	0.74 ^c	0.67	9.46	0.81 ^c	0.73 ^b	9.88	0.77 ^b	0.65 ^c	15.58	0.77 ^c	0.68 ^b	11.69
T3	0.76 ^c	0.66	13.16	0.73 ^d	0.79 ^a	-8.22	0.63 ^c	0.70 ^b	-11.11	0.71 ^d	0.72 ^a	-1.41
T4	0.85 ^a	0.60	29.41	0.89 ^a	0.70 ^{bc}	21.35	0.82 ^a	0.70 ^b	14.63	0.85 ^a	0.67 ^b	21.18
T5	0.85 ^a	0.69	18.82	0.82 ^c	0.71 ^{bc}	13.41	0.83 ^a	0.70 ^b	15.66	0.83 ^{ab}	0.70 ^{ab}	15.66
T6	0.85 ^a	0.60	29.41	0.88 ^a	0.62 ^d	29.55	0.83 ^a	0.59 ^d	28.92	0.85 ^a	0.60 ^c	29.41
T7	0.75 ^c	0.63	16.00	0.85 ^b	0.66 ^c	22.35	0.78 ^b	0.74 ^a	5.13	0.80 ^b	0.68 ^b	15.00
T8	0.83 ^b	0.64	22.89	0.84 ^b	0.74 ^b	11.90	0.81 ^a	0.70 ^b	13.58	0.83 ^{ab}	0.70 ^{ab}	15.66
T9	0.85 ^a	0.67	21.18	0.80 ^c	0.78 ^a	2.50	0.76 ^b	0.64 ^c	15.79	0.80 ^b	0.70 ^{ab}	12.50
SEM	1.32	1.47	1.92	0.98	1.10	1.84	1.09	1.17	1.77	1.38	1.42	1.89
P value	0.025	0.885	0.365	0.035	0.041	0.864	0.015	0.041	0.964	0.022	0.036	0.563

^{a, b, c} means within the same column with different letters are significantly different ($P < 0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

three diets, which averaged 2900 kcal ME/kg in energy and had reduced CP levels (22–18%), resulted in higher egg mass (10.338 g/hen/day) during the cold months. The seasonal variance in egg mass for the ongoing average energy diets between the cold and warm seasons ranged from 19.48% to 42.0%, suggesting that a diet with 22% CP and 2900 kcal ME/kg is needed to maximize egg mass in Japanese quail during the winter. The 22% CP diet with 2900 kcal ME/kg showed an improvement of 13.48% - 20.65% over the previous three higher-energy diets (3000 kcal ME/kg) with the earlier CP levels (22% to 18%). Furthermore, seasonal differences in egg mass revealed that a diet containing 18–20% CP and energy levels of 3000–2800 kcal ME/kg is unsuitable for Japanese quail laying in the winter.

Feed conversion ratio (FCR)

Table 6 presents the seasonal fluctuations in FCR for laying Japanese quail during various trial phases, influenced by distinct dietary protein levels and metabolizable energy. The findings indicated important differences ($P < 0.05$) in FCR across all trial periods. During the first 8 to 12 weeks of laying, the feed conversion ratio in cold months ranged from 2.323 to 3.193, while in warm months, it varied from 2.591 to 3.231, which is 1.38 to 16.18% lower than in the cold season. The warm season showed a higher feed conversion ratio in the group receiving the lower ME level of 2800 kcal ME/kg diet compared to the cold

season group that received a higher ME level of 3000 kcal ME/kg diet with 22% CP (T9), which had poorer feed conversion rates (2.323 - 2.792). In the second laying phase (12–16 weeks), the winter FCR ranged from 2.658 to 4.048, while the summer FCR ranged from 3.514 to 4.734, showing an increase of 20.42% to 30.87% compared to winter. The research found that quails fed of 22% CP diet with 2900 kcal ME/kg (T6) during the cold periods were more efficient in their diet utilization than those who fed 20% or 18% CP diets. Similarly, quails consuming a 22% CP diet with 2800 kcal ME/kg (T3) throughout the warm also showed better feed utilization compared to those on 20% or 18% CP diets. Furthermore, in the last laying cycle (16–20 weeks), summer feed utilization ranged from 3.967 to 5.014, which was 10.47% to 36.64% higher than the winter feed usage, which spread from 3.072 to 5.141. It was noted that birds on a 22% CP diet with 2900 kcal ME/kg in winter utilized their feed with greater efficacy in the previous period, and those on a 20% CP diet with 3000 kcal ME/kg throughout warm had better feed efficiency than those on either 22% or 18% CP diets (Table 6). During the entire laying period (8–20 weeks), amount feed use ranged from 2.797 to 4.046 in winter and from 3.643 to 4.770 in summer, indicating a seasonal variation of 12.33% to 32.99%. A diet with 22% CP and 2900 kcal ME/kg (T6) in winter is more appropriate for laying Japanese quail than diets with 20% or 18% CP, while a diet of 20%

Table 5. Seasonal variation of egg mass and economic efficiency of laying Japanese quail during the different experimental periods as affected by different dietary protein levels and metabolizable energy

Item	Egg Mass (EM) (g egg/bird/day)																											
	8-12 wks. of age						12 – 16 wks. of age						16 – 20 wks. of age						8 – 20 wks. of age						Economic efficiency (\$)			
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.				
T1	8.94 ^c	7.04	31.96 ^c	9.67 ^b	7.15	35.24 ^b	8.84 ^{bc}	6.59 ^d	34.24 ^b	9.00 ^b	6.82 ^c	31.88 ^b	4.07 ^{ab}	3.57 ^c	14.13 ^b													
T2	8.51 ^d	7.98	46.78 ^a	9.64 ^b	8.69	10.94 ^e	8.95 ^b	7.74 ^{bc}	15.65 ^c	9.03 ^b	8.00 ^{ab}	12.83 ^d	4.10 ^{ab}	4.12 ^b	-0.43 ^e													
T3	8.88 ^c	8.03	23.91 ^d	8.77 ^c	9.57	-8.30 ^j	7.69 ^c	8.57 ^{ab}	-10.21 ^e	8.15 ^c	8.42 ^a	-3.23 ^e	3.74 ^b	4.31 ^b	-13.16 ^e													
T4	9.95 ^{ab}	7.16	40.80 ^b	10.74 ^{ab}	8.34	28.80 ^c	9.76 ^b	8.57 ^{ab}	13.85 ^c	10.04 ^a	8.02 ^{ab}	25.20 ^c	4.64 ^a	4.48 ^a	10.89 ^b													
T5	10.08 ^a	8.21	23.12 ^d	9.98 ^b	8.60	16.08 ^d	10.07 ^a	8.89 ^a	13.27 ^c	10.00 ^a	8.37 ^a	19.48 ^c	4.47 ^{ab}	4.25 ^b	5.21 ^c													
T6	10.09 ^a	7.06	45.16 ^a	11.16 ^a	7.64	45.94 ^a	10.35 ^a	7.12 ^c	45.45 ^a	10.33 ^a	7.28 ^b	42.00 ^a	4.55 ^a	3.51 ^c	29.64 ^a													
T7	9.02 ^b	7.33	15.81 ^e	10.47 ^{ab}	7.62	37.27 ^b	9.47 ^b	8.71 ^a	8.68 ^d	9.38 ^b	7.78 ^b	20.65 ^c	4.38 ^{ab}	4.20 ^b	4.20 ^c													
T8	9.72 ^b	7.75	17.14 ^e	10.34 ^{ab}	8.79	17.56 ^d	7.79 ^c	8.65 ^a	13.13 ^c	9.63 ^b	8.20 ^{ab}	17.46 ^d	4.65 ^a	4.20 ^b	10.69 ^b													
T9	10.03 ^a	8.32	23.98 ^d	9.92 ^b	9.68	2.47 ^f	9.15 ^b	8.00 ^b	14.38 ^c	9.50 ^b	8.37 ^a	13.48 ^d	4.09 ^{ab}	4.12 ^b	0.77 ^d													
SEM	1.83	1.24	1.44	1.51	1.68	1.66	1.22	1.86	1.09	1.10	1.43	1.82	0.97	0.67	1.05													
<i>P</i> value	0.025	0.566	0.021	0.033	0.546	0.041	0.031	0.022	0.041	0.043	0.030	0.024	0.042	0.032	0.033													

a, b, c means within the same column with different letters are significantly different ($P < 0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

Table 6. Seasonal variation of feed conversion ratio (FCR) of laying Japanese quail during the different experimental periods as affected by different levels of dietary protein and metabolizable energy

Item	Feed conversion ratio (g feed / g egg)															
	8-12 wks. of age				12 – 16 wks. of age				16 – 20 wks. of age				8 – 20 wks. of age			
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	
T1	3.07 ^a	3.23 ^a	-4.83 ^c	3.42 ^b	4.50 ^{ab}	-24.09 ^b	4.29 ^b	5.01 ^a	-14.35 ^c	4.05 ^a	4.77 ^a	-15.17 ^b				
T2	2.93 ^{ab}	2.97 ^{ab}	-1.38 ^b	3.36 ^b	4.73 ^a	-28.85 ^c	4.04 ^c	4.88 ^a	-17.21 ^{cd}	3.69 ^{ab}	4.36 ^a	-15.31 ^b				
T3	2.75 ^b	2.93 ^b	6.23 ^a	4.04 ^a	3.51 ^d	15.19 ^a	5.14 ^a	4.22 ^b	-15.09 ^c	3.96 ^a	3.78 ^c	4.73 ^a				
T4	3.19 ^a	3.23 ^a	-1.21 ^b	2.75 ^c	3.92 ^c	-29.95 ^c	3.37 ^{de}	3.96 ^c	-28.86 ^d	2.92 ^{cd}	3.73 ^c	-21.75 ^c				
T5	2.51 ^c	2.73 ^c	-8.05 ^d	3.17 ^{bc}	4.16 ^b	-23.74 ^b	3.15 ^c	4.43 ^{ab}	-36.64 ^e	3.50 ^b	4.00 ^b	-12.33 ^b				
T6	2.95 ^{ab}	3.18 ^a	-7.15 ^d	2.65 ^d	4.10 ^b	-35.17 ^d	3.07 ^c	4.84 ^a	-10.47 ^b	2.80 ^d	4.18 ^{ab}	-32.99 ^d				
T7	2.79 ^b	3.01 ^{ab}	-7.11 ^d	2.98 ^c	4.32 ^b	-30.87 ^c	3.62 ^d	4.05 ^b	-10.47 ^b	3.15 ^c	3.92 ^b	-19.54 ^{bc}				
T8	2.51 ^c	2.99 ^{ab}	-16.18 ^f	3.02 ^c	3.94 ^c	-23.32 ^b	3.39 ^{de}	3.96 ^c	-14.34 ^c	2.96 ^{cd}	3.64 ^d	-18.85 ^{bc}				
T9	2.32 ^d	2.59 ^d	-10.34 ^e	3.28 ^b	4.13 ^b	-20.42 ^b	3.69 ^d	4.67 ^{ab}	20.91 ^a	3.14 ^c	4.07 ^{ab}	-23.02 ^c				
SEM	1.04	1.07	0.98	1.11	1.08	1.03	1.04	1.12	1.01	0.94	0.83	0.90				
P value	0.001	0.021	0.032	0.021	0.035	0.032	0.002	0.049	0.032	0.001	0.033	0.023				

a, b, c means within the same column with different letters are significantly different ($P < 0.05$). S. V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

CP and 3000 kcal ME/kg in warm periods is more suitable than diets with 22% or 18% CP.

Economic Efficiency (EE)

Table 5 illustrates how varying amount of dietary protein and metabolizable energy over different testing periods influenced the seasonal differences in EE of laying Japanese quail. In cold periods, a diet consisting of 20% CP and 3000 kcal ME/kg yielded the highest EE, while the lowest was recorded at 3.748 for the 22% CP diet with 2800 kcal ME/kg. Notably, wintertime economic comparison data indicate that the optimal diet for Japanese quail layers aged 8 to 20 weeks is an 18% CP diet with 2900 kcal ME/kg. During warm months, at the same 18% CP diet levels with 2900 kcal ME/kg, the EE ranged from a maximum of 4.48 to a minimum of 3.51. Feeding winter-laying quails an 18% CP diet with 2900 kcal ME/kg, followed by a diet with 22% CP and the same energy content, resulting in a seasonal EE variation of 4.20% to 29.64%, highlighting the greater efficiency of winter-laying birds compared to those in summer. Economically, data comparing seasonal and dietary treatments reveal that the greatest diets were those with 18% CP in winter and 22% CP at the same energy level, while summer diets were most effective with 22% CP. The 18% CP diet with 2900 kcal ME/kg, followed by a 20% CP diet, was identified as optimal (Table 3).

Egg components and quality

Adjusting the concentrations of dietary protein and energy had no notable influence on the percentages of albumen, yolk, or shell in the eggs, as demonstrated by the data in Table 7. However, Table 8 shows that dietary protein and energy levels had a significant impact ($P<0.05$) on the egg shape index (ESI) during both winter and summer seasons. The seasonal variance in ESI ranged from 5.13 to 1.27%, with values of 0.79 to 0.76 in winter and 0.78 to 0.74 in summer. Moreover, as indicated in Table 8, summer dietary protein and energy values had a strong effect on the yolk index (YI), while there was little impact observed in winter. During summer, YI fluctuated between 0.32 and 0.18. Additionally, Table 8 reveals that albumen height in winter was notably influenced by dietary protein and energy levels, whereas treatment groups had little influence in summer. The seasonal variation in albumen height ranged from 13.46% to 51.48% in winter, measuring between 4.65 and 5.22 mm. The seasonal fluctuation in shell thickness ranged from 17.50% - 28.79%.

Digestibility of nutrients

The digestibility of ether extract (EE) and dry matter (DM) during both warm and cold months was significantly affected ($P<0.05$) by the protein and energy levels in the diet, as indicated by the information in Table 9. Conversely, no important variations were

Table 7. Seasonal variation of egg components of laying Japanese quail as affected by different levels of dietary protein and metabolizable energy

Item	Egg components (%)								
	Albumen			Yolk			Shell		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	58.80	53.10	1.32	31.10	31.50	-1.26	15.00	14.30	4.89 ^d
T2	55.80	53.10	5.08	30.00	32.80	-8.53	14.20	14.00	1.42 ^e
T3	55.10	52.30	5.35	29.90	33.80	-11.53	14.80	13.80	7.24 ^c
T4	54.30	52.80	2.84	29.50	32.60	-9.50	16.20	14.50	11.72 ^a
T5	55.10	54.70	0.73	30.10	32.10	-6.23	14.80	13.10	11.48 ^a
T6	57.00	55.50	2.70	30.90	31.30	-1.27	13.00	12.70	2.36 ^d
T7	54.50	53.10	2.06	30.90	32.80	-5.79	14.60	13.70	6.56 ^c
T8	54.10	53.70	0.74	30.70	31.90	-3.76	15.20	14.01	7.04 ^c
T9	55.60	53.80	3.34	29.70	32.90	-9.72	14.60	13.30	9.77 ^b
SEM	2.05	1.95	2.11	1.57	1.33	1.64	0.92	1.01	1.10
P value	0.653	0.686	0.945	0.458	0.321	0.322	0.333	0.451	0.022

a, b, c means within the same column with different letters are significantly different ($P<0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

Table 8. Seasonal variation of egg quality of laying Japanese quail as affected by different levels of dietary protein and metabolizable energy

Item	Egg quality											
	Egg shape index			Yolk index			Albumin height (mm)			Shell thickness (mm)		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	0.76 ^c	0.74 ^c	2.63 ^b	0.21	0.22 ^b	-0.37 ^d	5.16 ^a	3.69	39.83 ^b	0.22 ^c	0.18 ^b	18.18 ^c
T2	0.77 ^b	0.75 ^b	2.60 ^b	0.21	0.18 ^c	0.20 ^c	4.65 ^c	3.92	18.62 ^d	0.24 ^{ab}	0.18 ^b	24.47 ^{ab}
T3	0.79 ^a	0.77 ^a	2.53 ^{bc}	0.20	0.21 ^b	-0.10 ^c	5.02 ^{ab}	3.73	34.58 ^{bc}	0.26 ^a	0.18 ^b	28.79 ^a
T4	0.76 ^c	0.74 ^c	2.63 ^b	0.21	0.32 ^a	-0.37 ^d	5.04 ^{ab}	3.55	41.97 ^b	0.23 ^b	0.19 ^a	19.13 ^b
T5	0.79 ^a	0.78 ^a	1.27 ^d	0.20	0.20 ^b	0.20 ^c	5.11 ^a	4.08	25.24 ^c	0.24 ^{ab}	0.19 ^a	20.42 ^b
T6	0.77 ^b	0.75 ^b	2.60 ^b	0.21	0.20 ^b	0.82 ^a	4.72 ^b	4.16	13.46 ^c	0.23 ^b	0.18 ^b	22.70 ^{ab}
T7	0.78 ^{ab}	0.74 ^c	5.13 ^a	0.21	0.21 ^b	0.19 ^c	5.22 ^a	4.20	24.28 ^c	0.22 ^c	0.18 ^b	17.50 ^c
T8	0.78 ^{ab}	0.76 ^{ab}	2.56 ^c	0.21	0.22 ^b	-0.37 ^d	5.09 ^{ab}	3.36	51.48 ^a	0.23 ^b	0.19 ^a	20.25 ^b
T9	0.77 ^b	0.76 ^{ab}	1.30 ^d	0.21	0.20 ^b	0.40 ^b	4.65 ^c	3.88	20.77 ^d	0.23 ^b	0.18 ^b	20.96 ^b
SEM	1.08	0.85	0.69	0.76	0.55	0.93	2.04	1.93	1.87	0.84	0.68	1.05
P value	0.021	0.031	0.023	0.656	0.036	0.042	0.023	0.589	0.024	0.032	0.021	0.043

a, b, c means within the same column with different letters are significantly different ($P < 0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

Table 9. Seasonal variation of nutrient digestibility of laying Japanese quail as affected by different levels of dietary protein and metabolizable energy

Item	Digestibility coefficients (%)											
	Dry matter			Crude protein			Ether extract			Crude fiber		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	73.70 ^a	77.60 ^a	-5.02	84.60	84.60	0.00	79.40 ^b	71.20 ^{bc}	11.51 ^{ab}	36.20	37.60	-3.72
T2	71.20 ^{ab}	75.80 ^{ab}	-6.07	81.40	85.60	-4.90	77.90 ^c	70.50 ^c	10.49 ^{ab}	37.60	38.10	-1.31
T3	73.30 ^a	75.10 ^{ab}	-2.39	84.30	86.00	-1.97	75.60 ^d	70.40 ^c	7.38 ^b	34.20	35.70	-4.20
T4	73.70 ^a	74.40 ^b	-3.53	84.60	83.50	1.31	76.40 ^c	70.40 ^c	8.52 ^b	35.20	37.90	-7.12
T5	71.90 ^{ab}	74.30 ^b	-3.23	83.00	84.70	-2.00	84.80 ^a	68.40 ^d	19.34 ^a	35.90	36.70	-2.18
T6	71.30 ^{ab}	75.20 ^{ab}	-5.18	84.50	84.50	0.00	78.50 ^b	75.30 ^{ab}	4.25 ^c	33.80	36.90	-8.40
T7	72.20 ^{ab}	75.00 ^{ab}	-3.73	82.20	83.60	-1.55	79.70 ^b	73.70 ^b	8.14 ^b	36.80	39.40	-6.69
T8	70.80 ^b	77.80 ^a	-8.99	83.20	85.80	-3.03	75.60 ^d	79.50 ^a	-4.91 ^c	37.40	37.50	-0.27
T9	70.90 ^b	73.10 ^c	-3.01	85.10	85.20	-0.12	80.30 ^{ab}	76.00 ^{ab}	5.52 ^c	35.60	38.10	-6.56
SEM	1.08	0.89	0.96	1.10	1.06	0.94	0.88	0.73	0.81	1.25	1.55	1.60
P value	0.021	0.035	0.545	0.865	0.542	0.421	0.042	0.031	0.034	0.546	0.865	0.512

a, b, c means within the same column with different letters are significantly different ($P < 0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

recorded in crude protein (CP), crude fiber (CF), nitrogen-free extract (NFE), and organic matter (OM) among the treatments in both seasons. In winter, DM values ranged from 73.70 - 70.80, while in summer, they varied from 77.80 - 73.10. The statistical variance (SV) values indicated that DM was higher in summer than in winter, attributable to the differing protein and energy levels utilized in the study.

Similarly, EE values ranged from 84.80 - 75.60 in winter and from 79.50 - 70.40 in summer. The SV values showed that EE was greater in winter than in summer due to the varying protein and energy levels applied in the experiment. It is noteworthy that, aside from EE digestibility, nutrient digestibility was generally higher in summer compared to winter. Throughout both winter and summer, birds

on an 18% CP diet with 2800 kcal ME/kg achieved the highest digestibility coefficient for OM. As the energy level increased across the three protein levels, OM digestibility also decreased, as shown in Table 9.

The feeding values

The various treatments significantly influenced ($P<0.01$) the dietary criteria, measured as TDN and ME (Table 10). Higher nutritive values (TDN and ME) were achieved when energy intake was increased from 2800 to 3000 kcal ME/kg and protein levels were raised from 18% to 22%. Furthermore, Table 10 shows that TDN levels were higher in summer than in winter for the same experimental diets. Of all the tested levels, the diet with 3000 kcal ME/kg and 20% CP provided the highest values for both TDN and ME.

DISCUSSION

Providing Japanese quail with different levels of protein and energy during winter and summer improves performance, especially FCR and EM results. High ME diets improve feed efficiency, consistent with previous research (Ratriyanto et al., 2017; Ashour et al., 2024). No changes were observed in LBW and EW, consistent with the findings of Agboola et al. (2016) and Hijab and Albaddy (2022). High crude protein (CP) levels contribute to increased egg production, egg weight, egg mass, and FCR (El-Hindawy et al., 2021; Jesuyon et al., 2021; Salih et al., 2021). High energy diets improved laying

efficiency, aided estrogen synthesis and supported productivity (Gunawardana et al., 2008). Based on our results, a winter diet of 2900 kcal ME/kg with 22% CP and a summer diet of 3000 kcal ME/kg with 20% CP are recommended for optimal performance. However, high energy diets may reduce nutrient intake suggesting combining high CP and high energy density diets (Da Silva Fonseca et al., 2021). Energy also plays a crucial role in improving protein digestibility (Barszcz et al., 2024). A protein level of 20% or more is optimal for egg quality, although excess protein ($> 22\%$) may result in lighter eggs (Arulnathan et al., 2024). On the other hand, low protein affects egg weight and yolk quality, while diets of 20% CP and 2,900 kcal ME/kg produce larger and higher quality eggs (Gao et al., 2021; Ashour et al., 2024). Higher energy levels improve dry matter digestibility, as birds on high-energy diets consume less feed but utilize nutrients more efficiently due to a slower feed transition, known as the “extra-caloric effect” (Baião and Lara, 2005). Furthermore, oil supplementation improves crude fiber (CF) digestibility (Dänicke et al., 2020) and optimal protein levels during summer support gut flora and overall digestibility (Singh and Kim, 2021). Maintaining an adequate protein-energy balance is particularly critical in hot weather conditions, where heat stress reduces digestive efficiency. Energy ensures the efficient use of nutrients, preventing the diversion of metabolic resources away from production processes, which in turn improves digestibility coefficients and heat

Table 10. Seasonal variation of nutrient digestibility and feeding values of laying Japanese quail as affected by different levels of dietary protein and metabolizable energy

Item	Digestibility coefficients (%)						Nutritive values (as fed)					
	N free extract			Organic matter			TDN (%)			ME (kcal / kg)		
	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.	Winter	Summer	S.V.
T1	81.50	84.50	-3.55	76.30	79.30	-3.78	66.14 ^b	68.49 ^{ab}	-3.43 ^c	2777.90 ^c	2876.80 ^b	-3.43
T2	80.60	85.50	-5.73	72.60	76.80	-5.47	64.46 ^c	65.81 ^b	-2.05 ^d	2707.30 ^d	2763.90 ^c	-2.05
T3	81.70	81.60	0.12	74.90	76.60	-2.22	65.29 ^c	65.24 ^b	0.08 ^{ab}	2742.20 ^c	2740.10 ^c	0.08
T4	82.10	85.00	-3.41	75.10	78.40	-4.21	68.89 ^{ab}	68.89 ^{ab}	0.00 ^{ab}	2891.80 ^b	2893.70 ^b	-0.07
T5	81.50	84.10	-3.09	73.10	77.30	-5.43	69.05 ^{ab}	69.13 ^{ab}	-0.12 ^b	2900.10 ^{ab}	2903.50 ^{ab}	-0.12
T6	80.40	83.90	-4.17	72.10	76.00	-5.13	68.46 ^{ab}	68.85 ^{ab}	-0.57 ^b	2875.20 ^b	2891.80 ^b	-0.57
T7	83.10	84.80	-2.00	75.00	77.30	-2.97	71.42 ^a	71.22 ^a	0.28 ^a	2999.60 ^a	2991.40 ^{ab}	0.27
T8	82.80	85.70	-3.38	72.20	79.30	-8.95	71.41 ^a	72.74 ^a	-1.82 ^c	2994.20 ^a	3055.20 ^a	-1.99
T9	83.00	84.80	-2.12	71.00	75.40	-5.83	71.29 ^a	71.34 ^a	-0.07 ^b	2988.00 ^a	2996.60 ^a	-0.29
SEM	1.30	1.26	1.19	0.85	1.10	1.34	1.96	1.55	1.48	1.22	1.35	1.41
P value	0.445	0.858	0.512	0.544	0.542	0.845	0.001	0.001	0.021	0.024	0.001	0.865

^{a, b, c} means within the same column with different letters are significantly different ($P<0.05$). S.V.: Seasonal Variation. SEM: Standard error of the means. P value: Probability value

stress management (Day et al., 2022; Mottet and Assouma, 2024).

CONCLUSIONS

Nutritional recommendations for Japanese quail in laying should be carefully adapted to the season and local climate conditions to ensure optimal performance, egg quality, and maximum nutrient digestibility. Therefore, it is essential to consider regional specificities and husbandry practices when defining the ideal levels of crude protein (CP) and metabolizable energy (ME) in the diet. The results of this study suggest that during the summer season, a diet containing approximately 20% CP and 3000 kcal ME/kg is optimal, while in winter, a diet with approximately 22% CP and 2900 kcal ME/kg was more effective. However, in regions with extremely cold climates, it may be appropriate to further increase the energy level, while in areas with mild winters, a moderate adaptation may be sufficient. It is therefore

essential to fine-tune the feeding recommendations based on the specific environmental and management context, in order to maximize production efficiency and ensure the welfare of quail.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTIONS

Conceptualization and supervision, E.A.A., A.K.A. and I.M.Y.; Methodology and investigation, A.I.E. and Y.A.A.; Original draft writing, F.B., N.F.A. and R.A.A.; Writing–review and editing: E.A.A., A.K.A., I.M.Y., A.I.E., Y.A.A., F.B. and N.F.A., R.A.A. All authors read and approved of the final manuscript.

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