

Effects of *Gracilaria birdiae* supplementation on physiological parameters and carcass quality of laying quails in different thermal environments

Dietas à base de *Gracilaria birdiae* para *Coturnix coturnix japonica* na fase de postura em diferentes ambientes: parâmetros fisiológicos e qualidade de carcaça

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Highlights

Algae improves heat dissipation and well-being in quails under thermal stress.

Heat stress increased respiratory rate by 8.58%, indicating metabolic adaptation.

Gracilaria birdiae does not affect the productive performance of quails under heat stress.

Algal inclusion supports organ health even under extreme thermal conditions.

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Abstract

This study evaluated the physiological responses and carcass traits of Japanese quails (*Coturnix coturnix japonica*) fed diets supplemented with the macroalga *Gracilaria birdiae* across different thermal environments. A completely randomized 4×3 factorial design tested four macroalgae inclusion levels (0, 3, 6, and 9%) and three temperatures (25, 29, and 33 °C). Cloacal temperature (CT), respiratory rate (RR), and surface temperature (ST) were not significantly influenced by algal inclusion ($P = 0.1413$ and $P = 0.9616$, respectively). On the other hand, RR decreased significantly in quails receiving macroalgae ($P = 0.0002$), indicating improved heat dissipation. No interaction between dietary algae levels and temperature was detected for absolute or relative organ weights ($P > 0.05$). Inclusion of 9% *Gracilaria birdiae* in the diet of female Japanese quails was safe and effective at 29 °C, maintaining live weight, carcass quality, and organ development. At 33 °C, however, breast yield decreased significantly, highlighting the importance of heat-stress management strategies.

Key words: Environmental chamber. Liver. Macroalgae. Cloacal temperature.

Resumo

Este estudo teve como objetivo avaliar os parâmetros fisiológicos e a qualidade da carcaça de codornas japonesas (*Coturnix coturnix japonica*) alimentadas com dietas contendo a macroalga *Gracilaria birdiae* sob diferentes condições térmicas. O experimento foi conduzido em um delineamento inteiramente casualizado, em arranjo fatorial 4×3 , testando quatro níveis de inclusão da macroalga (0, 3, 6 e 9%) e três temperaturas (25, 29 e 33 °C). Parâmetros fisiológicos, como temperatura retal (TR), frequência respiratória (FR) e temperatura da superfície (TS) das codornas, foram avaliados. O peso corporal, o consumo de ração, a temperatura cloacal, a temperatura da superfície e a frequência respiratória foram significativamente influenciados pela temperatura ambiente ($P < 0,05$), com valores mais elevados observados a 33 °C. O desempenho produtivo, o rendimento da carcaça e os pesos dos órgãos (por exemplo, fígado e coração) não apresentaram diferenças significativas devido à inclusão da macroalga ($P > 0,05$). Mesmo no nível mais alto de inclusão (9%), o peso relativo da carcaça foi maior sob condições de estresse térmico, sugerindo um ajuste metabólico por parte das aves. Além disso, a macroalga contribuiu para a redução da temperatura da superfície, possivelmente devido às suas propriedades bioativas que auxiliam na dissipação de calor. Conclui-se que a inclusão de até 9% de *Gracilaria birdiae* na dieta de codornas japonesas é uma alternativa viável e sustentável, sem impactos negativos no desempenho produtivo, mesmo sob condições de estresse térmico.

Palavras-chave: Câmara climática. Fígado. Macroalgas. Temperatura da cloaca.

Introduction

Quail farming is expanding due to the species' easy management, rapid growth, low production costs, and efficient feed utilization. Their high feed conversion

efficiency and strong performance in both carcass yield and egg production make quail production a viable source of income for producers of varying scales (Ferronato et al., 2020; Valentim et al., 2022).

Producers in tropical and subtropical regions face challenges such as intense solar radiation, high temperatures, and elevated humidity for most of the year. These conditions often induce thermal discomfort, reduce productive performance, and represent a major constraint to quail farming (Porto & Fontenele, 2020). Another difficulty in poultry farming is the reduced carcass quality of birds after the laying period, which affects their market value.

These issues are exacerbated in hot climates, where heat stress further compromises bird health. When ambient temperatures exceed the thermoneutral zone, quails may experience severe physiological disturbances due to increased energy expenditure for thermoregulation and reduced feed intake (Onagbesan et al., 2023). Heat-induced reductions in feed consumption can prevent birds from meeting their nutritional requirements (He et al., 2018), particularly essential minerals such as calcium and phosphorus (Ribeiro et al., 2025).

Given these constraints, dietary additives with antioxidant activity or the capacity to reduce metabolic heat production are a promising strategy to mitigate heat stress in Japanese quails. These additives can enhance animal welfare and, consequently, help maintain productive performance and carcass quality (Renaudeau et al., 2010; El-Hack et al., 2017).

Marine macroalgae are promising alternative feed ingredients for poultry. These photosynthetic microorganisms require minimal nutrients and provide biomass rich in bioactive compounds for human and animal consumption (Andrade & Costa, 2008). *Gracilaria birdiae*, in particular,

is notable for its mineral and bioactive composition, offering potential benefits to quail production. It contains compounds with recognized anti-inflammatory, antioxidant, anticoagulant, and antimicrobial activities (Fidelis et al., 2014).

Alternative feeds have been widely investigated in quail nutrition, with positive outcomes for feed efficiency, growth, and egg production (Ferronato et al., 2020). Although some studies have explored macroalgae supplementation in quail diets, research specifically examining the effects of *Gracilaria birdiae* in Japanese quails (*Coturnix japonica*) remains limited.

This study, therefore, aimed to assess the physiological responses and carcass quality of 49-112-day-old laying Japanese quails (*Coturnix coturnix japonica*) fed diets with increasing levels of *Gracilaria birdiae* (0, 3, 6, 9%) under different thermal environments.

Material and Methods

All experimental procedures were approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil (Protocol No. 11/2024).

The study was conducted at the Laboratory of Rural Constructions and Ambience (LaCRA) of the Federal University of Campina Grande, using three controlled-environmental chambers ($3.07 \times 2.77 \times 2.50$ m in width, length, and height, respectively). The chambers remained closed from 7 a.m. to 7 p.m. and were programmed to maintain constant temperatures of 25, 29, and 33 °C (Figure 1).

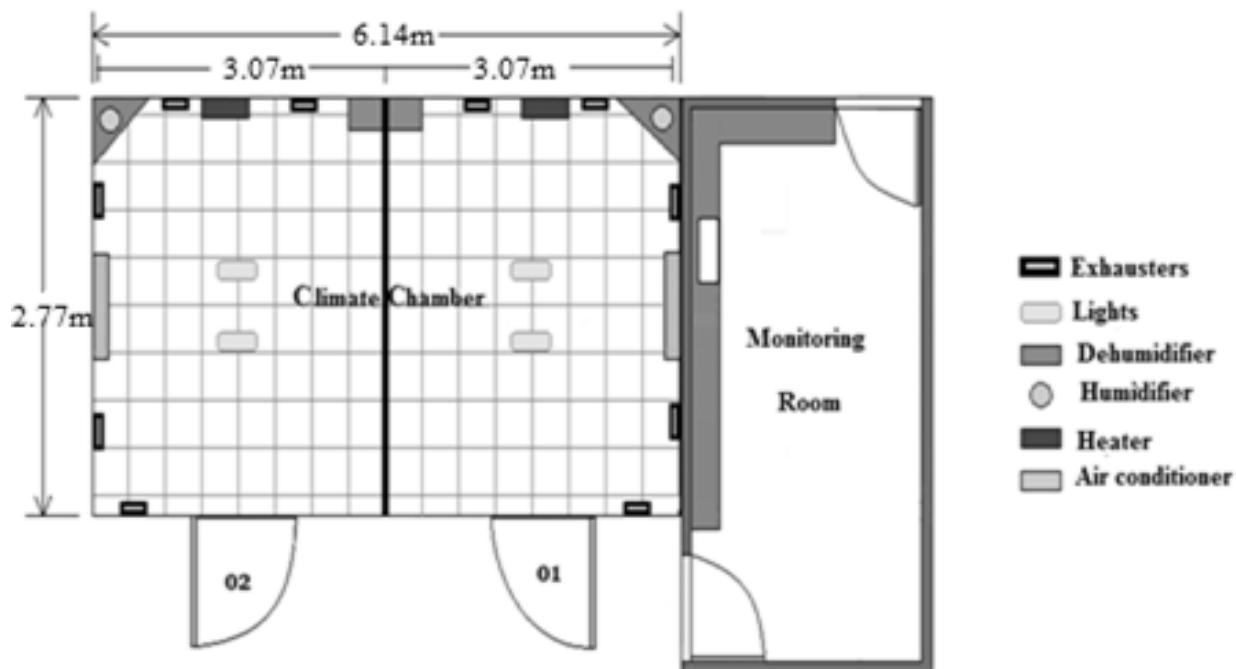


Figure 1. Schematic layout of the environmental chamber and adjacent monitoring room.

A total of 576 female Japanese quails, 7 weeks old with an average body weight of 140 ± 0.50 g, were used. The birds were acquired from the FUJIKURA farm (Suzano, São Paulo) and certified as pure lineage. The experiment followed a completely randomized 4×3 factorial design with four dietary treatments (0, 3, 6, and 9% *Gracilaria* *birdiae* meal) and three temperatures (25, 29, and 33 °C) with six replicates per treatment.

During the first week, quails were vaccinated, dewormed, weighed, and allocated into four experimental groups. They were housed in brooding circles containing

wood-shaving litter and continuously heated for 12 days using 60-W incandescent lamps. Water and feed were provided ad libitum, and diets already contained the designated levels (0, 3, 6, and 9%) of *Gracilaria* *birdiae*.

The macroalgae, dried and milled in Santa Catarina state, Brazil, were analyzed for mineral composition and bioactive compounds at the Federal University of Campina Grande's Laboratory for Storage and Processing of Agricultural Products and the Laboratory for Food Engineering. All analyses were performed in triplicate, and mean values are presented in Table 1.

Table 1

Ingredient composition and calculated nutrient profile of diets containing increasing levels of *Gracilaria birdiae*

Ingredient (%)	Inclusion levels of <i>Gracilaria birdiae</i> (%)			
	0	3	6	9
Corn	58.37	54.34	50.30	46.28
Soybean meal	34.16	34.13	34.16	34.21
<i>Gracilaria birdiae</i>	0.00	3.00	6.00	9.00
Dicalcium phosphate	0.14	0.14	0.14	0.14
Soybean oil	2.33	3.89	4.40	5.37
Nucleus	5	5	5	5
Total	100.00	100.00	100.00	100.00
Calculated nutrient composition				
Metabolizable energy (kcal/kg)	3050.00	3050.00	3050.00	3050.00
Crude protein (%)	22.00	22.00	22.00	22.00
Total limestone (%)	1.04	1.19	1.35	1.51
Available phosphorus (%)	0.34	0.34	0.34	0.34
Sodium (%)	0.219	0.226	0.234	0.242
Arginine (%)	1.30	1.29	1.27	1.25
Threonine (%)	0.70	0.69	0.68	0.67
Isoleucine (%)	0.81	0.80	0.79	0.78
Tryptophan (%)	0.24	0.24	0.23	0.23
Valine (%)	0.89	0.87	0.86	0.84
Leucine (%)	1.66	1.62	1.58	1.54
Lysine (%)	1.14	1.14	1.13	1.12
Methionine (%)	0.34	0.34	0.33	0.32
Methionine+cystine (%)	0.64	0.63	0.62	0.60

In the third week, the quails were evenly assigned to thermal environments of 25, 29, and 33 °C. Physiological and microclimatic data were collected in week seven.

Feed ingredient composition and nutrient values were determined following Rostagno (2011) and the NRC (2007) (Table 2). Water and feed were provided manually twice daily at 8:00 a.m. and 4:00 p.m., ad libitum, ensuring continuous availability.

Table 2

Mineral composition, bioactive compounds, and chemical profile of *Gracilaria birdiae* used in the experimental diets

Minerals	mg/100g	Minerals	mg/100g	Bioactive	mg/100g	Chemical composition.	%
Na	128.14	Ca	4211.39	CF	170.16	Protein	14.47
Mg	335.43	Mn	3.85	FLV	44.46	Moisture	3.47
Al	207.01	Fe	126.06	ATCN	14.21	Total lipid	5.49
Si	519.55	Cu	0.47	CRF	1.98	Ash	45.8
K	38.00	Zn	0.10	CTN	51.54	CT	49.3

Na- Sodium. Mg- Magnesium. Al- Aluminum. Si- Silicon. K- Potassium. Ca- Calcium. Mn- Manganese. Fe- Iron. Cu- Copper. Zn- Zinc. CF- Phenolic compounds. FLV- Flavonoids. ATCN- Anthocyanins. CRF- Total chlorophylls. CTR- Total carotenoids. CT - Total carbohydrates.

Climatological variables (relative humidity (RH) and air temperature (AT) were recorded every 30 minutes using a HOBO U12-012 ONSET Comp® datalogger. The

temperature-humidity index (THI) was then calculated using the formula proposed by Lallo et al. (2018) for laying hens (Figures 2, 3, and 4).

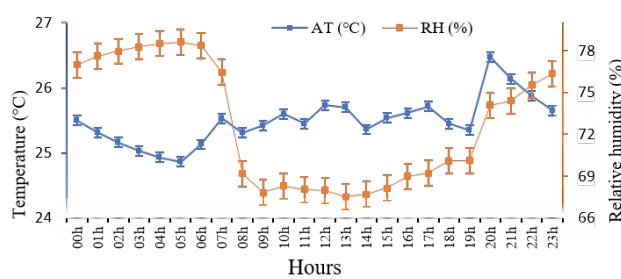


Figure 2. Microclimatic conditions recorded in the environmental chamber under thermal comfort (25°C).

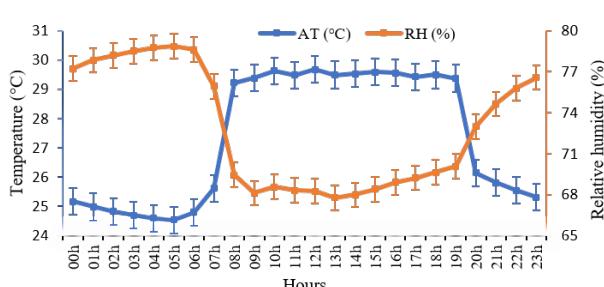
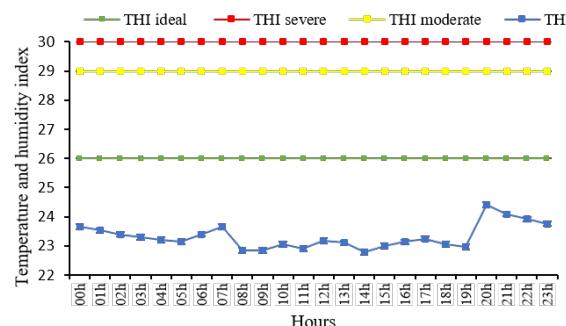
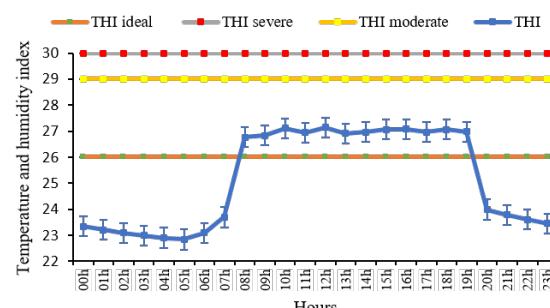


Figure 3. Microclimatic conditions recorded in the environmental chamber under moderate stress (29°C).



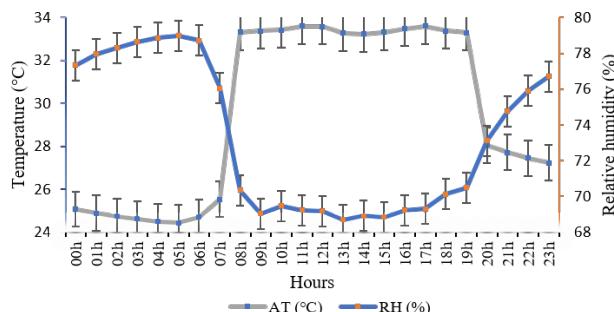


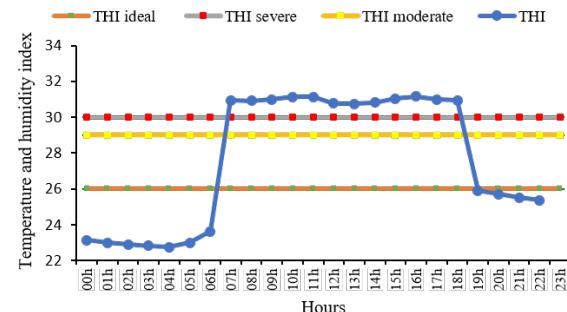
Figure 4. Microclimatic conditions recorded in the environmental chamber under severe stress (33°C).

Quails were subjected to the thermal treatments and different dietary inclusion levels of *Gracilaria birdiae*. From each replicate ($n=8$ birds), four birds were randomly selected for physiological measurements, taken between 11:00 a.m. and 1:00 p.m., and included cloacal temperature (CT), surface temperature (ST), and respiratory rate (RR).

Cloacal temperature was measured using a Bioland® T102 digital thermometer (rigid stem; ± 0.1 °C). The probe was inserted into the cloaca until an audible signal indicated that stabilization had occurred. Respiratory rate was recorded by counting chest movements for 15 s after the bird's breathing had stabilized. and multiplying by four to obtain movements min^{-1} .

Average surface temperature (AST) was calculated from measurements at the head, back, foot, and wing using an infrared digital thermometer (accuracy ± 0.5 °C), placed approximately 10 cm from the bird. AST was computed using the equation proposed by Richards (1971).

Before slaughter, birds were fasted for 12 hours with *ad libitum* access to water. Slaughter involved stunning, bleeding, scalding in boiling water, and removal of



feathers, feet, head, and viscera to obtain the eviscerated carcass weight.

Carcass yield (CY%) was calculated as the ratio of carcass weight (CW) to live weight (LW) multiplied by 100. Carcass components (thighs, breast, wings, and back) were separated using appropriate knives. Organ yield was determined by weighing the heart, liver, and gizzard on an analytical balance (± 0.1 g accuracy).

Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a 5% significance level via the GLM (General Linear Model) procedure in SAS OnDemand (2024).

Results

Recorded values of air temperature, average relative humidity, and temperature-humidity index (Figures 2, - 4) in the environmental chambers remained close to the programmed levels of 25, 29, and 33 °C ± 0.8 . Based on average, minimum, and maximum THI values, quails housed at 29 °C and 33 °C experienced heat stress during chamber operation, whereas conditions approached thermal comfort when the

chambers were off. At 25 °C, birds remained within the thermal comfort zone throughout the experiment.

CT ($P = 0.1413$) and ST ($P = 0.9616$) did not differ significantly with dietary algae inclusion (Table 3). However, RR was significantly reduced ($P = 0.0002$) with algae supplementation.

Table 3

Effects of increasing dietary levels of *Gracilaria birdiae* on the physiological parameters of laying Japanese quails subjected to cyclic heat stress

Inclusion levels of <i>Gracilaria birdiae</i> – LI (%)	Physiological variables		
	CT ¹	ST	RR
0	87.34a	41.35a	34.29a
3	87.64a	41.35a	34.19ab
6	88.07a	41.33a	33.99bc
9	88.73a	41.35a	33.83c
Air temperature – AT (°C)			
25	84.17c	41.01c	32.38c
29	87.58b	41.43b	34.21b
33	92.07a	41.59a	35.64a
SEM	1.86	0.16	0.31
	<i>P</i> -value		
LI	0.1413	0.9616	0.0002
AT	<.0001	<.0001	<.0001
LI*AT	0.0062	0.0125	0.7229

Different letters in the same column differ according to Tukey's test at 5% probability; SEM= standard error of the mean. CT = Cloacal temperature, ST = Surface temperature, RR = Respiratory rate.

Physiological parameters were significantly affected ($P<.0001$) by air temperature (Table 3). RR increased by 8.58% with rising temperature, reflecting a typical heat-stress response in the birds, aimed at enhancing heat dissipation by increasing pulmonary ventilation to minimize the harmful effects of excessive heat. A significant interaction between algae inclusion and temperature was observed for CT and ST ($P < 0.05$).

No interactions were detected between algae levels and air temperature for absolute or relative organ weights ($P>0.05$), and algae inclusion alone did not significantly affect these traits ($P>0.05$) (Table 4).

Liver weight (absolute and relative) was higher at 33 °C (7.24 g) than at 25 °C (6.11 g) ($P=0.0038$ and $P=0.0102$, respectively) (Table 4). Conversely, gizzard weight (absolute and relative) was higher at 25 °C (3.48 g and 3.74 g, respectively) and lower at 33 °C (3.23 g and 3.5 g, respectively) ($P=0.0370$ and $P=0.0346$) (Table 4).

Table 4
Effects of *Gracilaria birdiae* supplementation on performance, carcass traits, and organ weights of laying Japanese quails subjected to cyclical heat stress

Variable	Inclusion level of <i>Gracilaria birdiae</i> - L/ (%)			Air temperature - AT °C			SEM	P-value		
	0	3	6	9	25	29		LI	AT	LI*AT
Weight (grams)										
Live	155.33a	156.89a	153.19a	157.53a	153.81a	157.31a	156.08a	13.19	0.6497	0.7646
Carcass	94.18a	92.77a	95.22a	94.00a	93.46a	94.87a	93.81a	8.26	0.8272	0.8478
Thigh	20.34a	19.48a	19.82a	22.04a	20.07a	19.55a	21.65a	3.15	0.0623	0.0815
Breast	32.23a	31.35a	31.73a	32.34a	30.84a	31.99a	32.92a	3.64	0.1484	0.8353
Back	33.95a	34.17a	35.80a	31.89a	34.87a	35.69a	31.30a	6.83	0.0687	0.4021
Wing	7.28a	7.46a	7.47a	7.48a	7.31a	7.23a	7.73a	0.96	0.1662	0.9072
Heart	1.18a	1.28a	1.18a	1.16a	1.32a	1.13a	1.16a	0.43	0.2739	0.8239
Liver	6.56a	6.36a	6.32a	6.96a	6.11b	6.31b	7.24a	1.18	0.3543	0.0038
Gizzard	3.33a	3.32a	3.25a	3.47a	3.48a	3.32ab	3.23b	0.33	0.2483	0.0370
Intestine	7.79a	6.93a	7.46a	7.81a	7.08a	7.52a	7.90a	1.81	0.2985	0.4267
Relative Weight (%)										
Carcass	60.77a	59.28a	62.38a	59.74a	60.92a	60.48a	60.20a	4.38	0.8493	0.1634
Thigh	21.67a	21.03a	20.89a	23.51a	21.57a	20.63a	23.13a	3.13	0.0561	0.8260
Breast	34.28a	33.77a	33.36a	34.40a	33.04a	33.73a	35.10a	2.87	0.6792	0.5478
Back	35.87a	36.79a	37.44a	33.80a	37.10a	37.54a	33.28a	5.56	0.2331	0.5184
Wing	7.77a	8.06a	7.87a	8.00a	7.86a	7.65a	8.26a	1.10	0.1645	0.8612
Heart	1.25a	1.37a	1.24a	1.23a	1.41a	1.18a	1.23a	0.43	0.1837	0.7610
Liver	7.06a	6.84a	6.66a	7.45a	6.60b	6.66b	7.75a	20.33	0.3870	0.0102
Gizzard	3.55a	3.60a	3.70a	3.43a	3.74a	3.51ab	3.45b	0.39	0.2183	0.0346
Intestine	8.33a	7.45a	7.89 a	8.34a	7.65a	7.94a	8.42a	2.06	0.4285	0.5127

Different letters in the same column differ according to Tukey's test; SEM= standard error of the mean

Discussion

Thermal comfort for quails occurs between 25.6 and 26.7 °C, while temperatures from 30.4 to 33.2 °C constitute moderate-to-severe heat stress (Matshogo et al. 2020). The inherent adaptability of these birds to elevated temperatures is supported by Michalak et al. (2022), who observed no significant differences in the performance of broiler quails at 29 °C. In the present study, dietary algae inclusion mitigated the heat-induced increase in RR, suggesting a protective effect. This likely reflects the nutritional and antioxidant properties of microalgae, which help regulate the birds' physiological responses (Moraleco et al., 2024). Our findings align with those of Oliveira et al. (2023), who noted that supplementing quail diets with *Sargassum* sp did not compromise respiratory function. Additionally, the scientific literature indicates that macroalgae enhance immune function, antioxidant capacity, and intestinal integrity in poultry, thereby improving tolerance to heat stress and more stable productive performance.

The CT of the quails ranged between 41.33 °C and 41.35 °C, consistent with the established normal thermal range for the species (41-41.5 °C) under thermoneutral conditions (Marchini et al., 2007). Despite this minor variation, the upward trend in CT with rising air temperature, even within the normal range, confirms the impact of heat stress. This slight elevation in body temperature demonstrates the activation of thermoregulation mechanisms to maintain homeothermy under warmer conditions. CT is a critical indicator of the

bird's thermal state, and this physiological response serves as a defense mechanism to preserve homeothermy under challenging environmental conditions.

The fact that CT remained within the species' normal limits across all temperatures in this study suggests that the potential benefits of *Gracilaria birdiae* may be temperature-dependent. This may reflect variations in heat dissipation influenced by dietary compositions. According to Castilho et al. (2015), CT values within the normal physiological range indicate the absence of overt thermal discomfort.

High air temperatures markedly impair enzymatic activity in birds (Hu et al., 2023). Heat stress increases metabolic rate and free radical production (Ribeiro et al., 2024). The phenolic compounds in *Gracilaria birdiae* likely scavenged these free radicals, reducing the physiological effort required to maintain acid-base balance, which may explain the lower surface temperatures observed at higher inclusion levels.

The stable, productive performance across temperatures and algae inclusion levels is encouraging. The absence of changes in organ weight (liver, heart, gizzard, and intestines) indicates that *Gracilaria birdiae* did not disrupt metabolic function or pose adverse health effects. This aligns with prior studies reporting no negative impacts from macroalgae supplementation in poultry diets (Marareni et al., 2023).

Bioactive compounds in *Gracilaria birdiae*, including antioxidants, vitamins, and fiber, likely contributed to maintaining physiological stability under heat stress. These components may enhance gut health,

support beneficial microbiota, and improve nutrient absorption, collectively helping birds cope with oxidative stress and maintain homeostasis.

The observed increase in liver weight at 33 °C likely reflects adaptive metabolic activity in response to heat stress, given the liver's central role in detoxification and nutrient metabolism. The lack of changes in liver weight driven by algae suggests that supplementation did not interfere with these adaptive processes.

Differences between the present findings and those of SuBakir et al. (2020), who observed reduced live weight in chickens fed *Sargassum polycystum*, may stem from variations among macroalgal species, inclusion levels, and experimental conditions. Distinct chemical composition and bioactive profiles among macroalgal species can markedly influence their physiological effects in poultry.

This study demonstrates the potential of *Gracilaria birdiae* as a functional dietary ingredient for Japanese quails, since its inclusion did not compromise productive performance, even under thermal stress. Bioactive compounds within the algae may have supported bird health and well-being by enhancing intestinal function and reducing oxidative stress.

Further research is needed to elucidate the mechanisms by which microalgal bioactives exert physiological effects and to evaluate responses of different algal species across multiple avian developmental phases.

Notably, quails exhibited stable performance under severe thermal stress,

with a non-significant trend toward higher live weight at elevated temperatures. This response may reflect both the beneficial effects of *G. birdiae* and a possible compensatory feeding behavior during cooler periods when the environmental chambers were off.

The consistent, productive performance across algae inclusion levels underscores the potential of macroalgae as a sustainable poultry feed ingredient that supports bird health and aligns with broader sustainability goals in quail production.

By contrast, Subakir et al. (2020) reported reduced live weight in broiler chickens fed 100 g kg⁻¹ of brown algae (*Sargassum polycystum*), which may be attributed to the macroalgal species used in the research, highlighting the positive effects of *Gracilaria birdiae* in laying quails.

The scientific literature suggests that incorporating macroalgae into poultry diets can affect the development of internal organs, particularly the intestinal tract. While studies using various bird species and algae types have indicated no significant changes in liver, gizzard, or muscle weights, even at high inclusion levels (El-Deek & Brikaa, 2009; Abu Hafsa et al., 2022; Mararen et al., 2023), others show significant increases in small and large intestine weights when brown algae were used, possible as an adaptive response to higher dietary fiber (Abu Hafsa & Hassan, 2022). These findings emphasize that the physiological effects of macroalgae are species- and ingredient-dependent, reinforcing the need to better understand their digestibility and nutrient utilization pathways.

Conclusions

A dietary inclusion level of 9% *Gracilaria birdiae* is recommended for female Japanese quails, as it maintained live weight, carcass quality, and organ development at 29 °C. However, exposure to 33 °C significantly reduced breast yield, emphasizing the importance of adopting management strategies (ventilation, shading, or dietary additives) to mitigate the adverse effects of heat stress. Temperature was the primary factor influencing physiological responses, while algae inclusion had no significant effect on these parameters.

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