DOI: <https://doi.org/10.5327/fst.116122>

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# **Marine macroalgae as an alternative in the feeding of broiler quails in an environment of thermal stress**

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## **Abstract**

The objective of this research was to evaluate the productive performance, carcass quality, and intestinal biometry of European quails (*Coturnix coturnix* coturnix), kept under cyclic heat stress (air temperature of 32.5°C±0.5) receiving a ration with different concentrations (0, 2.5, 5, and 7.5%) of seaweed meal of *Sargassum* sp. A total of 240 quails were used, distributed in a completely randomized design with 4 levels of bran inclusion, 6 replications, and 10 birds per experimental unit. An analysis of variance (ANOVA) was performed, and the means were compared by Tukey's test with 5% probability and subjected to regression by PROC REG of SAS® (2002). No similarity was observed in water and feed consumption between treatments. There was a significant difference in most of the variables studied, with a significant difference only occurring in the dorsum, gizzard, and large intestine. Therefore, it is recommended to include up to 7.5% of sargassum bran in the diet of European quails kept under a 12-h heat cycle (32°C±0.5).

**Keywords:** *Coturnix coturnix* coturnix; animal environment; alternative feeding; seaweed.

**Practical Application:** In the quail breed as a means of supplement replacing antibiotics.

# **1. Introduction**

Coturniculture is an activity that presents a quick economic return, where quails have high productivity, low food consumption, fast growth, double productive aptitude, and need little space for their creation (Guimarães et al., 2014), food being one of the highest costs of production in the activity.

One of the alternatives to maintaining production and profitability in poultry production is the use of alternative foods (Ferreira et al., 2019; Nnadi et al., 2022) and among these foods, seaweed stands out (Øverland et al., 2019; Michalak & Mahrose, 2020), such as green (Abudabos et al., 2013; Matshogo et al., 2020), red (Kulshreshtha et al., 2014), brown (Hafsa et al., 2019), green, Sargassum (Hafsa & Hassan, 2022), and Spirulina (Abouelezz, 2017), estimating that about 187 species of seaweed can be used for animal consumption, including 45 green, 35 brown, and 107 red algae (Shabaka, 2018).

The use of natural extracts and algae-based products in diets can improve the growth, reproduction, and immunity of birds and may be an alternative to probiotics and antibiotics (Arif et al., 2022). The genus *Sargassum* is a brown seaweed of tropical and subtropical origin, comprising 150 species (Olabarria et al., 2009), exhibiting good antioxidant activities (Idu & Seenivasan, 2013), and may contribute to reducing the cholesterol content of quail eggs and improving yolk color (Carrillo et al., 2012).

Seaweeds usually originate from natural sources of biomass found in various marine environments, have a good amount of nutrients in their composition, and can be used as additives or part of the composition of the animal diet (Hafsa et al., 2019; Alfaia et al., 2021), improving the health and performance of birds in addition to increasing the quality of meat and eggs (Hajati & Zaghari, 2019), as they are rich sources of bioactive compounds (Michalak & Mahrose, 2020), contributing to the reduction of thermal stress in quails kept in environments with high temperatures (Hajati et al., 2020).

High temperatures, as they occur in arid and semi-arid regions, especially during the day, can cause metabolic, endocrine, and behavioral changes, such as increased water consumption and reduced food consumption (Furtado et al., 2022), as a way to reduce endogenous heat production due to the thermogenic

*Received 16 Nov., 2022.*

*Accepted 20 Feb., 2023.*

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effect of food, which can negatively affect weight gain and carcass yield (Bonfim and Melo, 2015), with consequent commercial devaluation (El-kholy et al., 2017).

The objective of this research was to evaluate the productive performance, length, and weight of the large and small intestines of European quails (*Coturnix coturnix* cotrunix) in a thermal stress environment (32°C±0.5), receiving feed with different concentrations (0, 2.5, 5, and 7.5%) of marine macroalgae bran of the species *Sargassum* sp.

# **2. Materials and Methods**

#### *2.1. Experiment location*

The present study was carried out at the Laboratory of Rural Constructions and Ambience – LaCRA (7°13'51" South, 35°52'54" West), at the Universidade Federal de Campina Grande, Paraíba, Brazil, in a masonry shed with three windows and vents that were closed during the night and open during the day under natural conditions, with dimensions of 7.40×3.70×3.0 m in length, width, and height, respectively.

#### *2.2. Ethics committee and animals*

The procedures performed in this study were approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil, Protocol CEP No. 03/2021.

A total of 240 European quail (*Coturnix coturnix* coturnix) chicks, with an initial age of one day and an average weight of 8±0.50 g, acquired from a commercial hatchery, vaccinated, dewormed, and not sexed, were used. The experimental period started in the first 42 days of the birds' lives.

In the first 14 days of life, the quails were weighed and distributed in four closed protection circles with materials like MDF (medium-density fiberboard), sawdust bedding (shavings), an average of 5 cm in height, and artificially heated with four 60-W incandescent bulbs. In each circle, 60 quails were housed and provided with feeders and drinkers. At 15 days of age, the birds were housed in galvanized wire cages with dimensions of front  $100 \times$  side  $50 \times$  height 15 cm, arranged in five floors, with each floor divided into three parts: front  $33.3 \times$  side  $50 \times$  height 15 cm, totaling an area of 0.167 m<sup>2</sup>, where 10 quails were housed per cage, for a density of 60 birds per m², where they stayed until the end of the experimental period, at 42 days.

## *2.3. Feed preparation*

Seaweeds of the *Sargassum* genus, Sargassaceae family, and *Sargassum* sp. species were used to make the seaweed meal, and the collection was carried out by using seaweeds released by the action of sea waves, called "algae aribadas," without harm to the environment or compromise of fauna and flora. The algae were stored in permeable bags with natural ventilation for transport to LACRA, where they were removed and placed in a ventilated environment, and then the cleaning process began.

The cleaning process consisted of the removal of probable epiphytic "contaminants" and fauna associated with the algae and was carried out in three stages: washing with fresh running water, draining the sand and salt from the seawater, and thoroughly verifying the presence of pollutants and anthropic substances such as plastics, hair, remains of marine fauna, etc. After the screening and washing process, the raw material was placed in a thin layer on solvent paper to absorb excess water and dried in the shade for 7 days, then weighed and placed to dry in a forced ventilation oven at 65°C, remaining at this temperature until reaching a constant dry mass.

After removal from the oven, the material was placed to cool naturally and ground in forage until it reached the granulometry of soybean and corn bran. Bran samples were sent for laboratory analysis of micro- and macronutrients and material toxicity. The cytotoxicity analysis followed the methodology of Meyer et al. (1982) using the *Artemia salina* bioassay method.

## *2.4. Experimental design and experimental procedures*

The experiment was arranged in a completely randomized design, with four treatments (four levels of algal inclusion: 0, 2.5, 5, and 7.5%) with six replications in each experimental plot.

Feed and water were provided ad libitum. Seaweed meal (*Sargassum* sp.) was included in the diet at increasing levels (0.0, 2.5, 5.0, and 7.5%). During the entire experimental period, the birds were weighed at intervals of 7 days, totaling six weighing during the experimental period. The lighting program was continuous, with 24 h of uninterrupted daily light (12 h natural and 12 h artificial) throughout the experimental period. The food (Table 1) was composed according to the composition indicated by the NRC (2007).

#### *2.5. Environmental variables*

The environmental variables air temperature (TA), relative air humidity (RH), and black globe temperature (TNG) were obtained using a KR42 datalogger, recording at 5-min intervals throughout the experimental period, and the lighting program used was 24 h of artificial lighting.

The values of temperature and relative humidity of the average air during the period of thermal stress of 32.2°C and 69.76%, respectively, were registered during the experiment. In the other hours, an average temperature of 27.16°C and a relative humidity of 71.02% were recorded. With the temperature and relative humidity data, the temperature and humidity index (ITU) were calculated according to the formula proposed by Bunffington et al. (1977) and the Black Globe Temperature and Humidity Index (ITGU). Mean values of UTI and ITGU were obtained during heat stress of 31.56 and 83.22, respectively. In the other hours, mean UTI and UGTIs of 26.33 and 76.11 were obtained, respectively (Figures 1 and 2).

#### *2.6. Performance evaluation and carcass yield*

The live weight and weight gain of the birds were evaluated per treatment and obtained weekly in grams by directly weighing the birds using a precision analytical balance (0.1 g resolution). Feed and water consumption were calculated weekly by the ratio of the difference between the amounts offered and the leftovers

Phase I (1-21 days)											
	Sargassum meal inclusion levels (%)										
Ingredients (%)	2.50 0		5.00	7.50							
Corn	51.82	47.79	43.76	39.72							
Soybean meal	42.00	41.97	41.94	41.91							
Sargassum bran	0.00	2.50	5.00	7.50							
Dicalcium phosphate	0.14	0.14	0.14	0.14							
Soybean oil	1.04	2.61	4.17	5.73							
Mineral supplement <sup>1</sup>	5.00	5.00 5.00		5.00							
<b>Composition calculated</b>											
Met energy poultry (kcal/kg)	2,900	2,900	2,900	2,900							
Crude protein (%)	25.00	25.00	25.00	25.00							
Total limestone (%)	1.10	1.25	1.41	1.57							
Available phosphorus (%)	0.38	0.38	0.38	0.38							
Crude fiber (%)	2.96	3.17	3.37	3.56							
Sodium (%)	0.22	0.23	0.23	0.24							
Arginine (%)	1.52	1.51	1.49	1.47							
Threonine (%)	0.80	0.79	0.78	0.77							
Isoleucine (%)	0.95	0.93	0.92	0.912							
Tryptophan (%)	0.28	0.28	0.27	0.271							
Valine (%)	1.02	1.00	0.99	0.973							
Leucine (%)	1.85	1.81	1.77	1.726							
Lysine $(\%)$	1.33	1.32	1.31	1.303							
Methionine (%)	0.38	0.37	0.36	0.355							
Methionine + cystine (%)	0.71	0.70	0.68	0.67							
	Phase II (22-42 days)										
Ingredients (%)		Sargassum meal inclusion levels (%)									
	0	2.50	5.00	7.50							
Corn	58.37	54.34	50.31	46.28							
Soybean meal	34.30	34.27	34.24	34.21							
Sargassum bran	0.00	2.50	5.00	7.50							
Dicalcium phosphate	0.00	0.00	0.00	0.00							
Soybean oil	2.33	3.89	5.45	7.01							
Mineral supplement <sup>1</sup>	5.00	5.00	5.00	5.00							
	<b>Composition calculated</b>										
Met energy poultry (kcal/kg)	3,050.00	3,050.00	3,050.00	3,050.00							
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							
Crude fiber (%)	2.96	3.17	3.37	3.56							
Sodium (%)	0.21	0.22	0.23	0.24							
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							

**Table 1**. Ingredients and nutritional composition used in the formulation of feed for European quail in two phases.

<sup>1</sup>Calcium (min) 196.5 g/kg (19.65%), calcium (max) 210 g/kg (21%), phosphorous (min) 27.5 g/kg (2.75%), sodium (min) 48.5 g/kg, methionine (min) 39.6 g/kg, lysine (min) 15.2 g/kg, 6 phytase (min) 12500 FTU/kg, vitamin A (min) 150000 UI/kg, vitamin D3 (min) 48000 UI/kg, vitamin E (min) 450 UI/kg, vitamin K3 (min) 37.5 mg/kg, vitamin B1 (min) 37.5 mg/kg, vitamin B2 (min) 97.5 mg/kg, vitamin B3 (min) 600 mg/kg, vitamin B5 (min) 150 mg/kg, vitamin B6 (min) 60 mg/kg, vitamin H (vitamin B7) (min) 1.44 mg/ kg, vitamin B9 (min) 15 mg/kg, vitamin B12 (min) 375 mcg/kg, Colina (min) 3540 mg/ kg, Cobre (min) 180 mg/kg, Ferro (min) 700 mg/kg, and Iodo.



**Figure 1**. Averages of temperature and relative air humidity.



**Figure 2**. Means of temperature and humidity index (THI) and black globe temperature and humidity index (BGTHI).

divided by the number of animals, which was rectified according to the mortality of the birds. Feed conversion was calculated as the ratio of feed intake per bird divided by weight gain.

The birds were fasted for 12 h before slaughter, with only water available at will, and, after this period, the slaughter was carried out, stunning and bleeding, plucking in boiling water, removing the feathers, feet, head, and viscera, and obtaining the weight of the cleaned and eviscerated carcass. The carcass yield (CY%) was calculated by relating the carcass weight to the liver (HL), and gizzard (HG), which were weighed using an analytical balance with a precision of ±0.1. To evaluate intestinal biometry, intestinal length was weighed and measured; the small and large intestines were weighed on a precision scale, and the length was determined with the aid of a tape measure.

#### *2.7. Statistical analysis*

The data were evaluated employing analysis of variance (ANOVA) and the means compared by the Tukey test at 5% probability through the GLM procedure (General Linear Model), and the data were submitted to regression by the PROC REG of SAS® (2002).

#### **3. Results**

In the first phase  $(1-21 \text{ days})$ , feed intake  $(p=0.004)$ , water ( $p<0.001$ ), and weight gain ( $p=0.0052$ ) showed a significant difference with the inclusion of sargassum meal (Table 2), where





\*Different letters on the rows differ from each other by Tukey's test; SEM: standard error means; 'Y=760.72-5.38x (R²=0.58); <sup>2</sup>Y=135.15-1.32x (R²=0.57); 3Y=1027.33-32.14x+3.30x²  $(R^2=0.84)$ ;  $Y=1785.39-34.31x+2.87x^2 (R^2=0.67)$ ;  $Y=198.89-1.58x (R^2=0.58)$ .

feed intake was similar between the 5% inclusion levels and the control group. At levels of 2.5 and 7.5% sargassum meal inclusion, feed intake was lower. There was a reduction of 6.46 and 4.26% in feed intake compared to the control group at levels 2.5 and 7% of bran inclusion, respectively, and there was an increase of 2.6% in feed intake in the treatment with inclusion level 5%.

Water consumption and weight gain showed a decreasing linear regressive effect with the inclusion of sargassum, with water consumption 4.65, 1.01, and 7.07% higher in the control group compared to levels 2.5, 5.0, and 7.5%, respectively. Weight gain was higher in the control group and the lowest at the 2.5 and 7.5% levels, and comparing the control group with the 5% level, an increase in feed intake of 2.6% is observed and a reduction in water consumption of 1.01% (Table 2). Feed conversion was similar between treatments. In the second phase (22–42 days), feed intake, weight gain, and feed conversion were similar between treatments, with water consumption increasing (p<0.0001) in the control group and at the 5% level of inclusion of sargassum, with the lowest value at the 2.5% level and the highest in the control group, and water consumption showing a quadratic regressive effect with a maximum point of 4.87% of inclusion of seaweed (Table 2). In the total period, water consumption showed a significant difference  $(p<0.0001)$ between the levels of algae inclusion, being higher in the control group and lower in the 7.5% level. Feed consumption, weight gain, and feed conversion did not show significant differences by the Tukey test (p>0.0001; Table 2).

In Table 3, the results show that the inclusion of sargassum significantly affected two of the nine evaluated parameters. The dorsum and gizzard parameters showed significant differences (p<0.05) regarding the inclusion of sargassum and based on relative weight. So for the back, the control diet and the inclusion levels of 2.5 and 5% did not obtain a significant difference between them and showed a significant difference in relation to the inclusion level of 7.5%. For the gizzard, a significant

difference was found in relation to the 5% inclusion level, which presented the highest relative weight, and the control diet, which presented the lowest relative weight of the gizzard.

Regarding the parameters evaluated in the intestinal biometry of the six, only one showed a significant difference. For the weight of the large intestine (p=0.0475), it showed a significant difference depending on the inclusion of seaweed in the diet (Table 4), where the levels of 0, 2.5, and 5.0% of inclusion of sargassum bran did not show a significant difference with each other. At a level of 7.5%, it presented the lowest value of the large intestine weight.

# **4. Discussion**

The lower consumption of feed in phase I is related to the greater amount of fiber in the feed with the inclusion of bran, where the birds need to adapt their physiology and the bacteria to digest this type of food, and, as quails are rustic animals and can adapt to the most diverse food sources, such as seaweed, there was no significant difference in this consumption in phases II and in the final phase, with this consumption being within the average of 25 g/bird/day observed by Silva et al. (2012).

Water consumption was higher in the control diet with 5% inclusion of algae, which may be associated with the amount of nutrients available at this addition level, causing the quails to have the same behavior as the control group. Hafsa and Hassan (2022), in a study with seaweed of the *Sargassum siliquastrum* species enriching in two levels of inclusion (1 and 2%), the feed of Japanese quails aged 1–42 days, did not observe a difference in the consumption of water and feed. Hafsa et al. (2019) did not identify any similarity between the water and feed consumption variables in the final phase.

Water consumption in relation to food consumption was 2.66 for the control group and 2.55, 2.54, and 2.56 for levels 2.5,

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**Table 3**. Absolute and relative weights of carcass, cuts and edible viscera of European quails at 42 days of age\*.

\*Different letters on the rows differ from each other by Tukey's test; SEM: standard error means.

**Table 4**. Intestinal biometry of beef quails at 42 days of age, fed with sargassum bran\*.

<b>Variables</b>	Sargassum bran inclusion levels (%)					p-value		
	0.0	2.5	5.0	7.5	<b>SEM</b>	p-value	Linear	Ouadratic
Intestine slender (cm)	56.83a	62.17a	55.83a	54.83a	7.99	0.4112	0.4102	0.3468
Intestine slender $(g)$	5.09a	5.80a	5.24a	4.48a	1.03	0.2073	0.2292	0.0911
Intestine large (cm)	13.83a	9.17a	12.17a	11.50a	2.93	0.0798	0.5092	0.1367
Intestine large $(g)$	1.53ab	1.88a	1.53ab	1.18 <sub>b</sub>	0.39	0.0475	0.0859	0.0510
Total length (cm)	71.17a	68.00a	68.00a	66.33a	9.99	0.8638	0.4148	0.8527
Total weight	6.62a	7.68a	6.78a	5.65	1.30	0.0936	0.1443	0.0509

\*Different letters on the rows differ from each other by Tukey's test; SEM: standard error means.

5, and 7.5% of algae inclusion for the total period, respectively. The results are within the average for the species, even with the birds kept in hot environments, which demonstrates a high adaptability of these birds to hot environments. Under heat stress, birds can increase their intake frequency (Santos et al., 2017) without significant changes in their total intake. Furtado et al. (2022) cited that the increase in water consumption in response to thermal stress can be offset by the reduction in water consumption at night in milder thermal conditions.

Slaughter weight, carcass weight, and yield with the inclusion of bran were within the average for the species, demonstrating the adaptability of birds to hot weather and that the offer of levels of up to 7.5% of sargassum bran does not affect these variables, with the adaptability of birds to this type of food. Similar results were found by Matshogo et al. (2020), working with inclusions of 2, 2.5, 3, and 3.5% of seaweed in the diet of Cobb broiler chickens that did not identify impairment of carcass traits. Carlos et al. (2011), working with calcareous seaweed flour replacing calcitic limestone and cyclic thermal stress reaching a maximum of 32°C, also found no significant difference in weight gain and feed conversion of Japanese quails.

As for the back weight variable, the development of the birds is guided by the variables weight at slaughter of the birds and carcass weight, with symmetry between the three in each treatment. Similar results were found by Santos et al. (2014), in which the back weight was proportional to the weight of the birds and the carcass. These results are for different breeding environments, as the relationship is with the development of the bird and not with the influence of the environment on the bird.

Therefore, even with the birds being raised in environments with temperatures of up to 32°C, this was not enough to change this variable. With the addition of sargassum bran levels, there was an increase in fiber levels in the diet, but this was not enough to provide an increase in the liver. However, the gizzard

underwent changes in relation to the control group, this fact being related to the sensitivity of the development of this organ when there is an increase in the concentration of fiber in the feed. Even under thermal stress, the weight of the organs was within normal limits. Furtado et al. (2022), by keeping laying quails at stress temperature (32°C), did not observe a reduction in feed intake and heart, liver, and gizzard weight compared to birds kept in thermal comfort (24°C).

Intestinal biometry can be altered with the greater addition of fiber, which slows down the passage of food through the gastro-intestinal tract with greater retention in the large intestine so that the fiber can be digested by the bacteria present in the cecum and colon, but the addition of up to 7.5% of sargassum meal was not enough to change the weight and length of the intestines of the birds.

Abudabos et al. (2013), working with broiler chickens fed with green algae, found a greater weight of edible internal organs with the addition of 3% green algae meal in the feed. Hafsa and Hassan (2022), working with the seaweed *Sargassum siliquastrum*, found an increase in the width and height of intestinal villi and increased antioxidant properties, concluding that it may provide beneficial effects on performance, cecal fermentation, populations of beneficial bacteria, and the immune response and can be considered as an alternative feed additive in poultry production.

Similar results were found by Cañedo-Castro et al. (2019), where they observed the growth of intestinal villi and decreased serum concentrations of total cholesterol and triglycerides. This can be treated as a probiotic that can improve the health of chickens. Consequently, the obtained results show an increase in the weight of the large and small intestines due to the increase in fibers in the ideal concentration for the development of birds and intestines.

# **5. Conclusion**

The inclusion of up to 7.5% of the macroalgae *Sargassum* sp. In European quail diets had no impact on the performance and intestinal biometry of the quails during the entire experimental period. In general, our data validate the feasibility of using *Sargassum* sp. At a high level of incorporation in diets for meat quails without impairing the growth performance of the birds and with added value in nutritional quality. The inclusion of high percentages of macroalgae in poultry diets could contribute to reducing the dependency of poultry farming on soybean meal.

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