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Effects of Fermented and Non-fermented Green Seaweed Supplementation on Performance, Caecal Bacterial Population, and Blood Constituents of Japanese Quails

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ABSTRACT

Green Seaweeds are a valuable feedstock that can be utilized in poultry feed. Due to their recalcitrant polysaccharide structure, their use is still limited in poultry farming. This structure can be broken by a biotechnological approach such as solid-state fermentation (SSF) such as *Trichoderma reesei*, which simultaneously increases the nutritional value of the biomass. The current study aimed to investigate the effect of supplementation of fermented and non-fermented green seaweed (*Ulva lactuca*) on growth performance, nutrient digestibility, carcass characteristics, caecal microbiota, serum biochemistry, and antioxidant status in growing Japanese quails. Japanese quails (n = 375; one day old) were divided into five groups, with three replicates per group (25 quails in each replication). The quails were fed with five experimental diets, namely a control diet (basal diet without supplement), a basal diet supplemented with 1% and 2% green seaweed (GS) as well as 1% and 2% fermented green seaweed (FGS) for 42 days. The results showed that the groups fed FGS had a greater body weight gain and better feed conversion ratio than the other groups. The FGS groups showed the highest digestibility of crude protein and crude fiber, followed by the GS groups. FGS supplementation decreased abdominal fat percentage while increasing the bursa of Fabricius weight. The count of *Lactobacillus* was significantly increased in quails fed either GS or FGS, while *Clostridium perfringens* and *Escherichia coli* were decreased. The green seaweed-fed groups had significantly greater total protein, albumin, and globulin levels than the control group. Total lipids, triglycerides, cholesterol, HDL, and LDL were decreased in quails-fed diets containing 1% and 2% FGS. The quails in FGS diet groups had higher levels of total antioxidant capacity, catalase, and superoxide dismutase than the other groups, but lower levels of MDA. In conclusion, adding up to 2% fermented *Ulva lactuca* to the basal diet of Japanese quail promotes the growth and health of quails.

Keywords: Antioxidant status, Caecal microbiota, Fermentation, Green seaweed, Performance, Lipid profile, Japanese quail

INTRODUCTION

There is currently a great interest in using seaweed in poultry feed. Seaweed, as a rich source of bioactive components, can improve poultry health and performance while it increases the quality of poultry products (meat and eggs) when incorporated into feed (Holdt and Kraan 2011; Michalak and Mahrose, 2020). Seaweeds offer a lot of

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promise as mineral feed additives, especially Ca, Cu, Fe, I, Mg, Mn, P, Se, and Zn (Abu Hafsa et al., 2019; 2021). Seaweed-chelated micrometals are more readily available to animals (including poultry) than inorganic compounds (Evans and Critchley, 2014). Green seaweed species, such as *Ulva lactuca*, contain a variety of compounds that are not found in terrestrial plants, such as ulvan, alginate, and fucoidan, that have various biological functions, including the ability to modulate gut health due to their prebiotic, antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (Li et al., 2018; Cañedo-Castro et al., 2019). *Ulva* sp. may have higher levels of protein (15-20%), essential amino acids, fiber (290-670 g/kg), with both soluble and insoluble fibers (Øverland et al., 2018), polysaccharides, minerals, vitamins (A, B12, C, D, E, and K), lipids, polyunsaturated fatty acids including omega-3 fatty acid, pigments, and many antioxidant compounds, including polyphenols (Holdt and Kraan, 2011; Li et al., 2018; Øverland et al., 2018). El Basuini et al., (2023) reported that incorporating Arthrospira or Chlorella in the diet of rabbits improved performance, nutrient utilization, intestinal efficacy, and antioxidants. Fermentation of feed with microorganisms can result in functional feeds formulated to enhance gut microbiome, performance, and health, with the potential to improve feed quality for poultry and animals by increasing the availability of fiber and nutrients (Al-Harthi and El-Deek, 2012) and elongating the storage period (Choi et al., 2014; Dewi et al., 2019). Fermentation is an environmentally friendly safe technique that has various advantages, including reducing the risk of microbial contamination during storage and allowing obtaining a wide variety of bioactive compounds (Missotten et al., 2013; Xie et al., 2016), improving the anticoagulant, and anti-inflammatory properties of seaweed, as well as increasing the antioxidants and bioactive potential (Dordević et al., 2010). Thus, fermentation techniques, primarily those involving fungus, are increasingly being used to apply particular enzymes using materials such as substrates. According to a review of the literature, *Trichoderma reesei* (*T. reesie*) is one of the most well-known organisms capable of producing high amounts of enzymes, including cellulases, hemicellulases, and xylanases. Furthermore, beneficial microorganisms utilized in microbial fermentation can improve poultry performance by acting as probiotics. Thus, fermentation of seaweed by microorganisms can lead to synergistic effects (Lin et al., 2016). The findings obtained by Choi et al. (2014) suggested that feeding broilers 0.5% fermented seaweed improved body weight gain, feed conversion ratio (FCR), and immunological status, compared to the control group. It has been reported that fermented feeds improved animal performance and gut morphology (Chiang et al., 2010; Sun et al., 2013), enhance the immunity of animals (Gao et al., 2009; Sugiharto and Ranjitkar, 2018), and modulate the gut microbiota, partially by the selective inhibition of intestinal pathogens (Canibe and Jensen, 2012; Missotten et al., 2015), and improve nutrient digestibility and neutralization of anti-nutritional agents in the feed (Missotten et al., 2015; Wang et al., 2019). *Ulva* sp. has been intensively studied as a feed ingredient for broiler chickens. Abudabos et al. (2013) reported incorporating 3.0% *Ulva lactuca* into broiler diets improved breast muscle yield and dressing percentage, as well as lowered abdominal fat and mortality. These improvements were due to the availability of soluble fibers and sulfur-containing essential amino acids, such as methionine and cysteine. Cañedo-Castro et al. (2019) found that increasing the length of the intestinal villi led to an increase in the intestinal surface area as well as higher brush-border enzyme activity, resulting in a larger surface area for absorption and digestion capacity. In addition, *Ulva* treatments had significantly lower serum triglycerides and total cholesterol levels than the control (Abudabos et al., 2013; Cañedo-Castro et al., 2019). Obviously, whilst promising, further work in this field of application is required. Recently, feed additives pre-fermented with probiotic organisms have attracted attention for their ability to enhance growth performance and gut health in poultry production. The objective of this study was to investigate the effects of supplementation of fermented and non-fermented green seaweed byproducts on growth performance, nutrient digestibility, carcass traits, caecal bacterial population, and serum biochemistry and antioxidants status parameters in growing Japanese quail.

MATERIALS AND METHODS

Ethical approval

All experimental procedures of the study were performed according to the Animal Care and Use Committees and approved by the ethics committee of the City of Scientific Research and Technological Applications (Protocol No. 33-2B-0621), Alexandria, Egypt.

Collection and preparation of green seaweed (*Ulva lactuca*)

Green seaweed (*Ulva lactuca*) was handpicked from submerged rocks in Abu Qir Bay, on the Mediterranean

Sea coast of Alexandria, Egypt. The collected *Ulva lactuca* was washed and rinsed three times in fresh water to remove sand and debris before being sun-dried for three days. The dried samples were ground into powder and placed in airtight bags for further chemical analyses. The chemical analysis of *Ulva lactuca* powder as a feed additive is presented in Table 1.

Table 1. The chemical composition of non-fermented and fermented green seaweed

Items	Non-fermented green seaweed	Fermented green seaweed
Chemical analysis (Percentage on a dry matter basis)		
Organic matter	81.67	78.59
Crude protein	21.05	31.77
Crude fiber	9.88	7.69
Ether extract	3.18	2.87
Nitrogen free extract	47.56	36.6
Ash	18.33	21.41
Neutral detergent fiber	38.44	35.52
Acid detergent fiber	24.28	22.06
Acid detergent lignin	7.36	7.02
Hemicellulose	14.16	13.46
Cellulose	16.92	15.04
Neutral detergent fiber nitrogen	34.26	30.11
Non-fiber carbohydrate	23.18	13.84
Mineral composition (mg/kg)		
Sodium	193.8	211.7
Potassium	96.9	102.3
Calcium	72.4	78.6
Magnesium	200.1	210.5
Phosphorus	306.4	311.9
Iodine	188.9	174.6
Lead	0.052	0.047
Cadmium	0.029	0.021
Iron	2.06	2.11
Cobalt	0.10	0.04
Manganese	0.08	0.04
Selenium	1.11	0.99
Zinc	0.84	0.91

Fermentation of green seaweed with *Trichoderma reesei*

In this study, the fermentation process was a solid-state fermentation type (SSF). Fermentation of green seaweed (*Ulva lactuca*) was performed using *T. reesie* (ATCC 28217) for 96 h at 37°C and under aerobic conditions. The *T. reesie* was obtained from the Microbiology Research Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. The total substrate consisted of *Ulva lactuca* seaweed powder, 4% molasses, 0.4% urea (46.5% N), 0.2% KH2PO4, 0.03 MgSo4 (7H2O), and water; the ratio of green seaweed powder to water was 1:2 from the total volume of the substrate. The fermentation endpoint was determined from total sugar, reduced sugar, and pH according to Abd Ellatif et al., (2019). After fermentation, green seaweed was dried in an oven and then ground into a powder in a blender. The chemical composition analysis was performed for FGS to compare GS byproducts before and after fermentation (Table 1). Fermentation conditions were handled according to the optimum growth conditions in Abd Ellatif et al (2019). The fermented green seaweed byproduct was stored at –20°C until supplementation.

Experimental design, diets, and laboratory analyses

A total of 375 unsexed one-day-old Japanese quail quails (average weight of 8.53 ± 0.34) were distributed randomly into five equal experimental treatments (75 quails each) and three replicates (25 quails each). Dietary treatments included the control (basal diet without any supplementation), while the second and the third treatments received the basal diet containing green seaweed (GS) at 1% and 2% of the diet respectively. The fourth and fifth treatment groups received the basal diet containing fermented green seaweed (FGS) at 1% and 2% of the diet respectively. All quails were reared in wire battery cages in a well-ventilated room and kept under the same managerial, hygienic, and environmental conditions. In the first 4 days, the brooding temperature was 33°C. Then, it was lowered to 30°C until the end of the first week, and it was reduced to 28°C in the second week. From the third week, the ambient temperature was maintained at around 25°C and the relative humidity was between 60 and 70%, with 23 hours/day light throughout the experimental period. Feed and fresh water were available *ad libitum* throughout the entire 42-day experimental period. The experimental diets were formulated according to the NRC (1994) and presented in Table 2.

Table 2. Ingredients and chemical composition of the basal diet of growing Japapnese quail.

Ingredients (%)	Starter phase (0-3 weeks)	Finisher phase (4-6 weeks)
Yellow corn	54.1	60.4
Soybean meal (44% CP)	28.5	22.3
Protein concentrate*	10.00	9.1
Wheat bran	6.00	6.8
Vegetable oil	0.50	0.50
Dicalcium phosphate	0.20	0.20
Vitamin and mineral premix**	0.30	0.30
L-lysine	0.15	0.15
Dl- methionine	0.25	0.25
Total	100	100
Proximate chemical analysis (%)		
Crude protein	23.07	20.46
Crude fiber	3.42	3.71
Ether extract	4.73	4.94
Calculated nutritional values		
Metabolizable energy (MJ /kg)	14.712	14.80
Calcium (%)	0.83	0.79
Available phosphorus (%)	0.33	0.29

*Protein concentrate contained: 52% Crude protein, 2.03% Crude fiber, 6.17% Ether extract, ME 2080 (Kcal/Kg), 1.50 % Methionine, 2.00% Methionine and Cystine, 3.0% Lysine, 7.00% Calcium, 2.93% available Phosphorus, and 2.5% Nacl. **Each 3 kg of vitamin and mineral premix contains (per ton of feed), Vit. A 12000000 IU, Vit. D3 2000000 IU, Vit. E I0g, Vit. K3 1000 mg, Vit. B1 1000 mg, Vit. B2 5g, Vit. B6 1.5g, Vit. B12 10 mg, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50 mg, Iron 30g, Manganese 60g, Choline chlorite 10g, Iodine 300 mg, Copper 4g, Zinc 50g, and Selenium 100 mg.

Growth performance

Individual quail weights were recorded weekly to determine the final body weight (FBW), and the feeder in each cage was recorded daily to determine daily feed intake (DFI). Body weight gain (BWG) is the final body weight (g) - initial body weight (g), and feed conversion ratio (g feed/g gain) was calculated. The mortality rate was recorded daily, and at the end of the experiment, the percentage was recorded for each group.

Nutrient digestibility

During the final week of the trial, 8 quails (4 males and 4 females) were chosen from each replicate/treatment and placed in individual battery cages for the digestibility study. Quails were allowed to acclimate for 2 days, then the feed intake was recorded and faeces samples were gathered daily from each cage before feeding in the morning for 5 consecutive days. All the fresh samples were stored at -20°C for further analysis. Before being pulverised for chemical analysis the feed and excreta samples were oven-dried for 48 h at 70°C.

Carcass characteristics and caecal microbes

At the end of the experimental period (at 42 days of age), 24 quails (12 males and 12 females) per treatment were randomly chosen and the quails were weighed and slaughtered using the Islamic Halal method after 12 hours of feed deprivation. Carcass traits were assessed after complete bleeding, and the weights of abdominal fat, gizzard, and bursa of Fabricius were weighed and calculated as a percentage of the live body weight. Dressing percentage was computed as carcass weight relative to slaughter weight. The lengths (cm) of the intestine and caecum were determined. To determine caecal bacterial counts, another three quails from each replicate/treatment were euthanized by cervical dislocation. Caeca were quickly removed and their contents were collected in sterile tubes. Tenfold dilutions of 1 g of each sample were serially prepared in phosphate buffer solution and poured directly onto Petri dishes containing culture media. *Lactobacillus sp*. and anaerobic bacteria were cultured in de Man–Rogosa–Sharpe (MRS) agar and incubated in anaerobic conditions for 48 hours at 37°C. Total aerobic and *Escherichia coli* bacteria were plated on MacConkey agar and incubated aerobically for 24 h at 37°C. *Clostridium perfringens* was plated on a Perfringens agar base (Oxoid) mixed with 400 mg of D-cycloserine/liter and incubated under anaerobic conditions for 48 h at 37°C according to (Abu Hafsa and Hassan, 2022). Bacterial colony-forming units (CFU) in the Petri dishes were counted using a range of 30-300 cfu/g, depending on the growth characteristics of the bacterial species. The counts were expressed as log cfu/g.

Chemical analyses

Green seaweed, feed, feces, and meat samples were finely pulverized in a Cyclotec mill (Cyclotec 1093; Foss, Germany) and stored before chemical analysis. Moisture was determined in oven-dried samples at 70°C to constant weight. The content of CP (N 6.25) was determined according to Kjeldahl's method (Method No. 978.04, AOAC, 2005). The ether extract was determined according to the Soxhlet extract method using petroleum ether as an extracting agent (40-60°C, AOAC, 2005). Ash content was determined by incinerating the samples in a muffle furnace at 550°C. The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using a Tecator Fibretic System, according to the method of Van Soest et al. (1991). The content of nitrogen free extract (NFE) was calculated as NFE (%) = 100 % – (EE perncetage + CP perncetage + Ash perncetage + CF perncetage). Cellulose was measured as Cellulose (%) = ADF percentage - ADL percentage and hemicellulose was calculated as hemicellulose (%) = NDF percentage - ADF percentage. Mineral elements such as calcium, cadmium, cobalt, iron, lead, magnesium, manganese, phosphorus, potassium, sodium, and zinc were analyzed after samples were dissolved in 1 $MHNO_3$ and H_2O_2 and microwaved. The elements' concentrations in dried seaweed samples were determined with an atomic absorption spectrophotometer (Unicam 919; Unicam Ltd., Cambridge, UK), whereas phosphorus was colorimetrically determined using molybdovanadate reagent. Nitschke and Stengel (2015) method was used to determine Iodine. Selenium was determined by inductively coupled plasma–mass spectrometry (ICP-MS, Lavu et al., 2013).

Blood biochemical parameters and antioxidant status

At the end of the experiment, 24 quails from each treatment were slaughtered by severing the jugular vein to collect blood samples into sterile tubes. Subsequently, blood samples were immediately centrifuged for 15 minutes at 2000 × g by a centrifuge (T32c; Janetzki, Wallhausen, Germany), and stored at -20°C for further biochemical analysis. Total protein, albumin, glucose, total lipids, triglyceride, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), commercial kits from the biodiagnostic company (Giza, Egypt) were determined calorimetrically using standard kits supplied by the biodiagnostic company (Cairo, Egypt), according to the manufacturer's instructions. The total globulin fraction is generally determined by subtracting the albumin from the total protein. Total antioxidant capacity (TAC), catalase, superoxide dismutase (SOD), and malondialdehyde (MDA) were determined using commercial kits from Biodiagnostic Company (Giza, Egypt) and a spectrophotometer (Shimadzu, Japan) according to the manufacturer's instructions.

Statistical analyses

Data were subjected to statistical analyses in a randomized complete block design using the general linear model procedures of SAS/STAT (Statistical Analysis System, version 9.3, SAS Institute Inc., Cary, NC, USA) (2011). The obtained data were tested by an analysis of variance with a one-way design to test the treatment at each sampling, according to the following formula:

$Yij = \mu + Ti + \mathcal{E}ij$

Where Yij denotes the represented observation in the j^{th} non-fermented or fermented green seaweed, μ is the overall mean effect, Ti signifies the effect of the j^{th} non-fermented or fermented green seaweed, and Ɛij refers to the random error associated with the j^{th} birds assigned to the i^{th} non-fermented or fermented green seaweed treatment. The means were compared using Duncan's Multiple Range Test (Duncan, 1955). Significant differences among means were considered to be significant at the $p < 0.05$. All results are presented as the least squares mean.

RESULTS

Chemical composition of fermented and non-fermented green seaweed

The chemical and mineral compositions of non-fermented green seaweed (GS) and fermented green seaweed (FGS) are shown in Table 1. Contents of organic matter, crude fiber, ether extract, NFE, ADF, ADL, NDFn, NFC, cellulose, and hemicellulose were decreased after fermenting green seaweed with *T. reesie*. Fermenting green seaweed with *T. reesie* increased crude protein ash content. An appreciable increase in mineral content was observed in green seaweed when fermented with *T. reesie* compared to non-fermented seaweed.

Growth performance

Table 3 shows that quails fed the control diet and those provided a diet supplemented with GS and FGS had significant differences ($p < 0.05$) in final body weight, body weight gain, and FCR. Final body weight, body weight gain, and FCR were highest ($p < 0.05$) in quails fed a diet supplemented with 1% and 2% FGS, followed by those fed a diet supplemented with 1% and 2% GS. However, there were no significant variations in DFI between the treatment groups ($p > 0.05$). The increase in the birds' general health is shown in the lower mortality rate of birds fed the diet with GS or FGS compared with the control diet.

Nutrient digestibility

The nutrient digestibility of the experimental diets provided to Japanese quail is shown in Table 4. In general, dry matter, organic matter, crude protein, and crude fiber digestibility values increased significantly ($p < 0.05$) with quail-fed diets supplemented with GS or FGS compared with the control group, except NFE, which did not differ significantly ($p > 0.05$). Quails fed 1% and 2% FGS had the best CP and CF digestibility, followed by GS-fed quails.

Carcass characteristics and caecal microbes

Adding green seaweed, either non-fermented or fermented, to the basal diet significantly increased carcass yield, intestinal and cecum lengths, and bursa of Fabricius weight ($p < 0.05$, Table 5). Quails fed the 1% and 2% FGS diet had the longest ($p < 0.05$) intestinal and cecum lengths and the highest ($p < 0.05$) weight of bursa of Fabricius, followed by those fed 1% and 2% GS. Quails fed the 1% and 2% FGS diet had the lowest ($p < 0.05$) abdominal fat percentage followed by those fed 1% and 2% GS compared to the control diet. The experimental treatments did not affect the weights of the liver and gizzard. Quails fed either GS or FGS had reduced meat moisture compared with the control group ($p < 0.05$, Table 5). Quails fed 1% and 2% FGS diets had the highest CP content but the lowest EE content, followed by those fed 1% and 2% GS. The meat ash content was higher in quails fed either GS or FGS than those in the control ($p < 0.05$).

In groups fed 1% or 2% of GS or FGS, total anaerobic bacteria counts increased, but total aerobic bacteria count decreased significantly ($p < 0.05$, Table 5). In quails fed with either 1% or 2% of GS or FGS, the *lactobacillus* count increased dramatically, whereas the *Escherichia coli* and *Clostridium perfringens* counts declined significantly ($p < 0.05$). Quails fed 1% and 2% FGS had the highest *Lactobacillus* count but the lowest *Escherichia coli* and *Clostridium perfringens* counts.

Blood biochemistry and antioxidant status

The effect of the experimental diets on serum biochemical parameters and antioxidant status is shown in (Table 6). Total protein, albumin, and globulin in the serum were significantly greater in the green seaweed treatment groups than in the control group ($p < 0.05$). However, quails fed either the GS or FGS diet had significantly lower AG ratio, total lipids, triglycerides, cholesterol, HDL-c, and LDL-c concentrations than those on the control diet ($p < 0.05$). Quails fed the 1% and 2% FGS diet exhibited the lowest reduction in total lipids, triglycerides, cholesterol, HDL-c, and LDL-c levels compared with the control group, while there were no variations in glucose levels between treatment groups. Quails fed 1% and 2% FGS diets had the highest ($p < 0.05$) levels of TAC, catalase, and SOD but the lowest level of MDA, followed by those fed 1% and 2% GS ($p < 0.05$, Table 6). However, no significant differences were observed between quails fed 2% GS and quails fed 1% GS, or between those fed 1% and 2% FGS ($p > 0.05$).

Table 3. Effects of dietary supplementation of fermented or non-fermented green seaweed on growth performance of Japanese quail

Parameters \ Treatments	Control	GS 1 %	GS 2 %	FGS 1 %	FGS 2 %	SD	P-value
Initial body weight (g)	8.52	8.44	8.61	8.58	8.49	0.34	0.873
Final body weight (g)	198.34^{c}	209.52^{b}	211.61^{b}	215.73^{a}	218.54^{a}	2.17	0.024
Body weight gain (g)	4.52^{c}	4.79^{b}	4.83^{b}	4.93^{a}	5.00^{a}	0.05	0.001
Daily feed intake (g)	14.45	14.33	14.36	14.44	14.39	0.42	0.788
Feed conversion ratio	3.20^{a}	2.99^{b}	2.97^{bc}	2.93^{c}	2.88^{c}	0.05	0.002
Mortality rate (%)	4.00^{a}	1.33^{b}	1.33^{b}	2.67^{b}	1.33^{b}	1.14	0.016

$^{a-c}$ Different superscript letters in the same row differed significantly at $p < 0.05$. GS: Green seaweed. FGS: Fermented green seaweed. SD: Standard deviation

Table 4. Effects of dietary supplementation of fermented or non-fermented green seaweed on the nutrient digestibility of Japanese quail

Parameters / Treatments	Control	GS 1 %	GS 2 %	FGS 1 %	FGS 2 %	SD	P-value
Dry matter (%)	75.39 b	78.35 a	78.42 a	79.33 a	79.39a	1.45	0.029
Organic matter (%)	76.41 b	77.95 a	78.05 a	78.88 a	78.82 a	0.78	0.016
Crude protein (%)	73.26 c	74.53 b	74.66 b	75.84 a	75.97 a	0.36	0.001
Crude fiber (%)	45.18 c	49.07 b	50.16 b	52.58 a	52.85 a	1.12	0.011
Nitrogen free extract (%)	78.02b	90.29a	90.26a	90.83a	90.77a	2.68	0.721

a-c Different superscript letters in the same row differed significantly at $p < 0.05$. GS: Green seaweed. FGS: Fermented green seaweed, SD: Standard deviation

Table 5. Effects of dietary supplementation of non-fermented or fermented green seaweed on carcass traits and caecal bacterial population of Japanese quail

Parameters / Treatments	Control	GS 1 %	GS 2 %	FGS 1 %	FGS 2 %	SD	*P*-value
Carcass yield (%)	69.27b	70.73a	70.66a	70.83a	70.88a	0.32	0.026
Liver weight (%)	2.21	2.15	2.09	2.13	2.06	0.18	0.726
Abdominal fat (%)	1.42a	1.26b	1.19b	1.12c	1.06c	0.05	0.031
Gizzard weight (%)	2.57	2.61	2.48	2.55	2.64	0.22	0.835
Intestinal length (cm)	57.34c	60.44b	60.16b	62.32a	63.06a	0.74	0.001
Cecum length (cm)	9.11c	9.86b	9.88b	10.21a	10.22a	0.11	0.006
Bursa of fabricius (%)	0.08 c	0.11 b	0.11 b	0.14 a	0.15 a	0.01	0.001
Chemical composition of meat (%)							
Moisture	72.39a	71.06b	70.97b	70.83b	70.99b	0.27	0.021
Crude protein	20.05b	20.88a	20.82a	20.94a	21.01a	0.22	0.033
Ether extract	4.29a	3.96b	3.84b	3.91b	3.81b	0.25	0.007
Ash	1.45	1.51	1.55	1.53	1.56	0.13	0.578
Caecal bacterial count (log cfu/g)							
Total anaerobic bacteria	5.87 c	6.43 b	6.59 ab	6.77 a	6.89 a	0.21	0.026
Total aerobic bacteria	5.73 a	5.24 b	5.04 cb	4.92 c	4.75 c	0.17	0.018
Lactobacillus	6.41 c	6.92 b	7.19 b	7.47 a	7.58 a	0.10	0.001
Escherichia coli	4.55 a	4.16 b	4.08 b	3.89 c	3.77 c	0.13	0.001
Clostridium perfringens	2.67 a	2.22 b	2.15 b	1.88 c	1.76 c	0.11	0.001

a-c Different superscript letters in the same row differed significantly at $p < 0.05$. GS: Green seaweed. FGS: Fermented green seaweed, SD: Standard deviation

Table 6. Effects of dietary supplementation of non-fermented or fermented green seaweed on serum biochemical parameters and antioxidant status of Japanese quail.

Parameters / Treatments	Control	GS 1 %	GS 2 %	FGS 1 %	FGS 2 %	SD	P-value
Biochemical parameters							
Total protein (g/dl)	3.37 b	3.81 a	3.87 a	3.89 a	3.92a	0.06	0.017
Albumin (g/dl)	1.86 b	2.04 a	2.07 a	2.06 a	2.09 a	0.07	0.024
Globulin (g/dl)	1.51 b	1.77 a	1.80 a	1.89 a	1.84 a	0.09	0.031
A/G ratio	1.23 a	1.15 b	1.15 b	1.09 b	1.14 b	0.04	0.012
Glucose (mg/dl)	108.76	111.05	109.76	110.35	110.74	2.73	0.853
Total lipids (mg/dl)	389.37 a	278.46 b	289.04 b	244.61 c	248.36 c	24.93	0.027
Triglycerides (mg/dl)	96.83 a	89.66 b	88.04 b	68.85 c	66.77 c	2.06	0.007
Cholesterol (mg/dl)	143.29 a	129.73 b	121.76 b	99.97 c	94.06 c	12.04	0.004
HDL-c (mg/dl)	84.71 a	76.26 b	75.33 b	66.16 c	63.05 c	1.52	0.001
LDL-c (mg/dl)	39.21 a	35.54 ab	28.82 b	20.04 c	17.66 c	7.77	0.004
Antioxidant status							
TAC (U/L)	10.46 c	14.64 b	15.83 ab	16.44 a	17.89 a	1.78	0.002
Catalase (U/L)	104.53 c	140.86 b	145.06 ab	149.55 a	154.42 a	5.05	0.039
SOD (U/L)	28.58 c	36.26 b	38.27 ab	40.07 a	43.66 a	3.16	0.004
MDA (U/L)	5.73 a	4.02 b	3.94 bc	3.78 c	3.58 c	0.29	0.001

a-c Different superscript letters in the same row differed significantly at $p < 0.05$. GS: Green seaweed; FGS: Fermented green seaweed. HDL-c: High-density lipoprotein concentration, LDL-c: Low-density lipoprotein concentration, TAC: Total antioxidant capacity, SOD: Superoxide dismutase activity, MDA: Malondialdehyde

DISCUSSION

The results showed that the organic matter, crude fiber, NFE, NDF, ADF, ADL, cellulose, and hemicellulose contents of the FGS with *T. reesie* were lower than the GS. This result may be due to the fact that *T. reesie* consumes carbohydrates particularly soluble carbohydrates and crude fiber and its fraction as carbon sources to produce CO_2 and energy and then uses this energy to proliferate and convert nitrogen sources in the media to microbial protein. Moreover, crude protein and ash contents of FGS were higher than GS. The biological treatment increased crude protein from 21.05 to 31.77% for GS and FGS, respectively. The enhancement in the content of CP could be due to fungus growth (El-Ashry et al., 2002). El-Menniawy (2008) reported that treatment of sugar cane bagasse by *T. viride* resulted in a decrease in NFE. Abdel-Azim et al. (2011) found that treated rice straw and corn stalks with *T. viride* had higher CP, ash, and EE content than the untreated substrate. These results might be attributed to the breakdown of lignocellulose bonds where the cellulose can be hydrolyzed by fungi (El-Ashry et al., 2002; Bilal, 2008; Abdel-Azim et al., 2011). The result of the present study demonstrates the potential use of solid-state fermentation (SSF) to biovalorize the nutritional value of seaweeds and produce high-value carbohydrates (data not shown). Especially in the green seaweed *U. lactuca*, *T. reesei* successfully produced enzymes that modified the structure of the seaweed, resulting in increased protein. Therefore, SSF is an environmentally friendly, economical and sustainable biovalorization approach that may lead to nutritionally suitable seaweed for poultry feed.

In this study, green seaweed (*Ulva lactuca*) affected growth performance. The nutritive value of *Ulva lactuca*, which is an enriched natural source of various components including protein, fatty acids, polysaccharides, minerals, and nearly all essential vitamins, has favorable effects due to its palatability and high nutrient content, which can stimulate body metabolism and enhance digestion and nutrient absorption, improved the body weight gain of groups treated with GS or FGS compared with the control group. The considerable enhancement in growth performance found in quails given the green seaweed diet is thought to be due to an increase in necessary amino acids, particularly sulfur-containing amino acids (Burtin, 2003); green seaweed offers remarkable nutritional qualities and could, thus, serve as a feasible alternative source of nutrients for poultry (Al-Harthi and El-Deek, 2012). Furthermore, macroalgae such as green seaweed are an excellent source of iodine, and the results in Table 1 show that *Ulva lactuca* can accumulate large amounts of iodine, which is necessary for thyroid function and health (Burtin, 2003). *Ulva rigida* was utilized in broiler diets at 2%, 4%, and 6% as a prebiotic enriched with microelements cobalt, copper, chromium, manganese, and zinc to improve broilers growth performance (Cañedo-Castro et al., 2019). Erum et al. (2017) reported that adding 5%, 10%, or 15% *Sargassum muticum* as a feed supplement to broiler feeds improved final body weight, average daily gain, FI, and FCR. Because of their prebiotic properties, seaweed polysaccharides can help increase poultry performance and improve general gut health (Kulshreshtha et al., 2014). However, Abu Hafsa and Hassan (2022) reported that the *Sargassum siliquastrum*-supplemented diet did not influence final body weight, average body gain, or average feed intake, but it improved FCR and resulted in a lower mortality rate. Similarly, Bai et al. (2019) reported that supplementing broilers' diet with 1% *L. japonica* enhanced FCR compared with the control. The findings of this study are in agreement with those of previous studies on laying quails (Abu Hafsa et al., 2019) and laying hens (Rizk et al., 2017) and demonstrated that the supplementation of seaweed enhanced their productive performance. A study by Abu Hafsa et al. (2021) showed that 4% of seaweed supplements improved rabbit growth performance. Rabbit diets with Arthrospira at 500 mg/kg and Chlorella at 300 and 500 mg/kg had the highest feed conversion ratio and improved nutrient utilization, according to El Basuini et al. (2023). Sweeney et al. (2012) found that extracts of macroalgae can improve growth performance and health by altering the gut structure, increasing nutrient digestion and absorption, gut microbiota, and/or modulating immune function, thereby enhancing the gut barrier function. Polysaccharides found in macroalgae can act as prebiotics, promoting animal growth and overall health through positive effects on the digestive tract (Vidanarachchi et al., 2009).

In the present study, the digestibility of a diet containing green seaweed was improved using biological treatment, except for NFE. Biological treatments of seaweed and agro-industrial byproducts have been reported to improve palatability and digestibility in several species (Fayed et al., 2009; Okab et al., 2013; Choi et al., 2014). The enhancement upon pretreatment of green seaweed by-product could also be attributed to the production of enzymes by *T. reesie* during fermentation, which plays a significant role in the efficacy of nutrient breakdown and digestion in the birds' digestive system (Singh et al., 2019). The significant differences between the control group and treatment groups on quail growth performance further confirm the efficacy of the pretreatment of green seaweed to improve the utility of the green seaweed. Abu Hafsa and Hassan (2022) reported that *Sargassum siliquastrum* supplementation improved the digestibility of nutrients in Japanese quails. El Basuini et al., (2023) reported that incorporating Arthrospira or Chlorella in the diet of rabbits improved performance and nutrient utilization.

In this study, a significant increase in carcass yield and a significant decrease in abdominal fat were associated with feeding *Ulva lactuca*. These results are in agreement with those of Abudabos et al. (2013) who found that birds given 3% *Ulva lactuca* had a higher dressing percentage but less abdominal fat than the control group. Wang et al. (2013) found that adding 2%, 3%, or 4% dry green algae enhanced the quality of the breast meat: the fat content was dramatically reduced, and the abdominal fat rate was reduced. Erum et al. (2017) found that supplementation of broiler diets with *Sargassum muticum* improved carcass traits and increased the dressing percentage; however, it decreased the fat content of the carcass. The presence of soluble fibre in *Ulva lactuca*, which was reported to have 21.3% of soluble fibre (Lahaye and Jegou, 1993); could also be responsible for the decreased abdominal fat percentage observed. This is supported by the present findings of serum total lipids and cholesterol analysis. The hypocholesterolemic impact is associated with various properties characteristic of soluble fibre such as fermentability, viscosity, and bile salt binding capacity (Davidson and McDonald, 1998). Another reason contributing to the low content of fat is that *Ulva lactuca* has a high composition of polyunsaturated fatty acids, especially in terms of omega 3 and 6 fatty acids (Wahbeh, 1997). The intestine and cecum lengths of the S. siliquastrum-fed quails were much longer than those of the control group (Abu Hafsa and Hassan, 2022). In general, seaweed has a good impact on meat composition, which is usually improved as a result of fat reduction. Broiler chickens fed a diet containing seaweed had higher protein content but a lower fat content than the control birds (Zahid et al., 2001). *Spirulina* supplementation may improve meat quality by enhancing the integrity of muscle fibers and, as a result, the ability of muscles to retain water (Dal Bosco et al., 2014).

Green seaweed (*Ulva lactuca*) supplementation increased *Lactobacillus* abundance while reducing *Escherichia coli* and *Clostridium perfringens* counts in the quail caecum. Seaweed polysaccharides could be used as prebiotic ingredients in animal health applications, promoting the growth and/or activity of beneficial gut microbiota like *Lactobacillus* sp., which, in turn, helps the host by reducing pathogen invasion and disease (O'Sullivan et al., 2010; Ford et al., 2020). *Lactobacillus* bacteria produce lactic and acetic acids, which decrease the gut pH and render it unsuitable for pathogen growth. *Lactobacillus* bacteria also boost immunity by upregulating the synthesis of intestinal mucins, which limit pathogen adherence to the intestinal epithelium and so prevent pathogen translocation (Gibson and Roberfroid (1995) and Dhama et al. (2008). The observed improvements in the microbial community could be due to a rise in *Lactobacillus* bacteria count, which leads to the establishment of resistance to *Escherichia coli* and *Clostridium perfringens* colonization through a competitive exclusion mechanism. Marine seaweeds contain a wide range of active components and proteins (Øverland et al., 2018); phlorotannins (Gupta and Abu-Ghannam, 2011); and pigments, such as carotenoids (O'Sullivan et al., 2011), which function as prebiotics to promote the growth of beneficial bacteria while preventing the growth of pathogenic bacteria, hence, improving overall health (Vidanarachchi et al., 2009).

Total protein intake has a significant impact on blood protein profile. As a result, the higher total serum protein level of quails fed GS or FGS in the diet could be an indication of high protein content. An alteration in normal systemic protein utilization is usually indicated by abnormal serum albumin. Furthermore, a low albumin/globulin ratio in GS or FGS groups showed higher disease resistance and immunological response in growing quails, implying that GS or FGS groups' immunity was improved over the control group. Rizk et al. (2017) found that adding dried green seaweed to the diet of Sinai hens (0.1 and 0.2 %) reduced total lipids, triglycerides, cholesterol, and LDL levels when compared to the control group. Al-Harthi and El-Deek (2012) stated that *Sargassum* utilized as a feed additive in different forms (sun-dried, autoclaved, or boiled) at a dose of 3% or 6% significantly lowered triglycerides, cholesterol, and HDL levels when compared with the control hens. El Basuini et al., (2023) found that arthrospira- or chlorella-containing algae groups had higher serum total protein levels and lower total cholesterol. Ulvan contains a significant amount of sulfate, which has the ability to deconstruct cholesterol and could be used as an antihyperlipidemic medication, contributing to the reduction in cholesterol content (Qi and Sheng, 2015). Cañedo-Castro et al. (2019) suggested that dietary seaweed can be an alternative for improving intestinal integrity and lowering serum cholesterol concentrations.

A bird's antioxidant status is important for its resistance to infection, health maintenance, and productivity (Surai, 2002). Abdel-Daim et al. (2015) found that increases in TAC, catalase, and SOD and decreases in MDA, were indicators of decreased levels of lipid peroxidation. The most essential antioxidant enzyme, superoxide dismutase (SOD), is required for the elimination of superoxide in animals (Vijayavel et al., 2007). The significant rise in SOD values in *Ulva lactuca*-supplemented groups could imply a strong association between seaweed supplementation and better antioxidant capacity, as reported by Droge (2002). Seaweed is a promising source of bioactive peptides with numerous beneficial properties, including antioxidant potential (Chandini et al., 2008; Fan et al., 014). Li et al. (2018) reported that the concentrations of TAC and SOD were considerably greater in the groups supplemented with 0.5% to 1% ulvan than in the control group; nevertheless, MDA concentration was lower. Abu Hafsa and Hassan (2022) reported that quail treated with *Sargassum siliquastrum* had higher TAC and SOD than the control group. El Basuini et al., (2023) found that the groups fed an algal diet including either Arthrospira or Chlorella had the best GPx, whereas excellent SOD and CAT efficiency occurred at 500 mg/kg of Arthrospira and 300 and 500 mg/kg of Chlorella. Sulphfated polysaccharides, specific to marine algae, are structurally analogous to animal glycosaminoglycans, which explains their high reactivity and specific biological activities when administered to animals (Suarez, 2019). Sulfated polysaccharides have an excellent antioxidant effect in vitro, including the ability to scavenge radicals (Wang et al., 2016).

CONCLUSION

Supplementation of up to 2% fermented green seaweed to the basal diet of Japanese quail promotes the growth and health of quail. *T. reesei* successfully produced enzymes that modified the structure of the seaweed, resulting in increased protein, especially in green seaweed. This finding led to an increase in growth performance, the beneficial caecal bacterial population, immunity, and antioxidant status, while lowering triglycerides, cholesterol, and pathogenic bacteria and improving the intestinal health of treated birds. These findings suggested that SSF has a promising future in biovalorizing the nutritional value of green seaweed and producing high-value carbohydrates.

DECLARATIONS

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This study was conducted at the Noubaria experimental station, Animal Production Research Institute, and the Agricultural Research Centre. All experimental procedures of the study were performed according to the Animal Care and Use Committees and approved by the ethics committee of the City of Scientific Research and Technological Applications (Protocol No. 33-2B-0621), Alexandria, Egypt.

Authors' contributions

Abu Hafsa and Hassan created the idea and designed the study, collected data, wrote the paper, and performed the statistical analysis. Formal analysis and methodology carried on by Abu Hafsa, Abd-Ellatif, Abdel Razik, and Hassan. Abu Hafsa drafted the manuscript and approved the final manuscript. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

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Competing interests

The authors declared that they have no competing interests.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/submission, and redundancy) have been checked by the authors before the submission. The final results of the statistical analysis have been also checked and confirmed by all authors.

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