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Growth Performance, Apparent Ileal Digestibility, and Nutrient Transporter Gene Expressions of Broilers Fed Seaweed-Supplemented Diets

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ABSTRACT

Seaweed provides macro-, micro-nutrients, and biological bioactive components that may improve broiler production. The study aimed to evaluate the effects of various levels of brown seaweed (BS) and green seaweed (GS) on growth performance, carcass characteristics, apparent ileal digestibility (AID), and hepatic growth and nutrient transporter gene expressions. The study followed a completely randomized design (CRD) (twelve treatments, six replicates, and seven birds per replicate). The dietary treatments contained: basal diet [negative control (NC)], basal diet + vitamin E (100 mg/kg feed) [positive control (PC)], basal diet + 0.25%, 0.50%, 0.75%, 1.0%, and 1.25% BS and GS, respectively. The data were analyzed using the General Linear Model (GLM) of the statistical analysis system (SAS 9.4) by one-way ANOVA. Duncan's Multiple Range Test was used to assess the significant differences between treatment groups at p<0.05. Various levels of BS and GS (p<0.05) improved body weight (BW), body weight gain (BWG), and feed intake (FI) at the starter phase. No significant effects were observed in the carcass characteristics. The AID of crude protein (CP), organic matter (OM), and dry matter (DM) during the starter phase were significantly improved. The hepatic growth hormone receptor (GHR) gene had increased expression in birds fed 0.50% and 0.75% of GS-contained diets. Similarly, birds fed 0.50% of BS and 0.25%, 0.50%, and 0.75% of GS had higher (p<0.05) expression of the hepatic Insulin-like growth factor-1 (IGF-1) gene. Furthermore, there were no significant effects on the intestinal nutrient transporters genes, including aminopeptidase (APN), glucose transporter (SGLT5), and oligopeptide transporter (PepT1) at the jejunum tissue. It was therefore concluded that different levels of BS and GS in the broiler chickens' diet improved the starter period growth performance and nutrient digestibility.

Keywords: broiler chicken; brown seaweed; green seaweed; growth performance; apparent ileal digestibility

INTRODUCTION

The aquatic environment provides several useful food and feed components, such as seaweed (Bonos *et al.*, 2017). Seaweed is a type of marine, non-flowering, photosynthetic macroalgae found in the streaming sections of seas, oceans, and rivers (Rao *et al.*, 2018). Seaweed is divided into three groups: brown seaweed, green seaweed, and red seaweed, which are scientifically distinguished based on their colors (Hayes, 2012). Marine algae have recently gained popularity as a source of nutrients and bioactive components (Cherry *et al.*, 2019). Seaweed has a variety of biological bioactive components, including essential fatty acids, vitamins, polyphenols, carotenoids, phenolics, sterols, alkaloids, dietary fibers, and proteins (Garcia-Vaquero & Hayes, 2016; Diyana *et al.*, 2019; Corino *et al.*, 2019). However, the composition of seaweed varies from species to species. Furthermore, processing methods and environmental parameters may also significantly affect seaweed's chemical composition (Azizi *et al.*, 2021a). In a previous study, the vitamin E and C contents of four seaweed species including *Porphyra umbilicalis*, *Laminaria* spp., *Palmaria palmata,* and *Himanthalia elongata* ranged from 0.17 to 2.24 mg/100 g and 0.61 to 46.66 mg/100 g, respectively. Among the examined seaweed species, *Laminaria* spp. recorded the highest fucoxanthin content, and *Himanthalia elongata* recorded the highest polyphenolic content (Ferraces-Casais *et al.*, 2012). In another study, the fucoidan, mannitol, and laminarin contents *of Ascophyllum nodosum* were 11.6 g/100 g, 7.5 g/100 g, and 4.5 g/100 g on a dry weight basis, respectively (MacArtain *et al.,* 2007). In terms of the proximate composition, the BS and GS contained 59.8% and 55.88% CP, 5.78% and 5.19% crude fiber (CF), 1.28% and 0.30% ether extract (EE), 9.7% and 9.14% ash contents, and 29.19% and 34.68% carbohydrate, respectively (Azizi *et al.,* 2021a).

Seaweed or its components have been offered to animals in order to enhance their growth performances (Kim, 2011). Innovative extraction technology, sustainable supply, effective drying and processing techniques, and safe usage make seaweed a valuable agricultural product for livestock nutrition (Garcia-Vaquero & Hayes, 2016; Azizi *et al.,* 2023). Literature shows that seaweed can improve poultry growth performances (Andri *et al.,* 2020). The growth-promoting effects of seaweed might be attributed to the availability of soluble fibers and essential sulfur-containing amino acids such as methionine and cysteine (Abudabos *et al.,* 2013; Kulshreshtha *et al.,* 2020). The improvement in growth performance might also be due to the feed supplemented with seaweed providing amounts of fatty acids and minerals necessary for birds' growth compared to the basic feed (Sadh *et al.,* 2018). The poultry industry also may consider using dietary seaweed supplements as a feed addition for broilers (Balasubramanian *et al.,* 2021). Generally, broiler chicken feed is formulated based on nutrient digestibility and absorption (Loh, 2017). However, utmost seaweed species have little digestible protein for being a suitable protein source for livestock (Øverland *et al.,* 2019).

It has been extensively documented that seaweed contains various bioactive compounds such as carotenoids, phenolics, sterols, alkaloids, and polysaccharides that have been investigated for their health and growth-promoting benefits (Matanjun *et al.,* 2008; El-Deek *et al.,* 2011; Peng *et al.,* 2011; Øverland *et al.,* 2019; Kidgell *et al.,* 2019; Corino *et al.,* 2019). In contrast, there is still a lack of published data to describe the effects of seaweed on broiler growth and production performance. Thus, the objectives of this study were to investigate the effects of different levels of brown and green seaweed on growth performance, hepatic growth gene expression, carcass characteristics, apparent ileal digestibility, and nutrient transporter gene expression in broiler chickens.

MATERIALS AND METHODS

This manuscript is part of a wider research using the same animal husbandry and dietary treatments, whereas a part of this research has already been published (Azizi *et al.,* 2023).

Animal Ethics

The research was conducted at the Poultry Unit, Department of Animal Science, Universiti Putra Malaysia (UPM), according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) (UPM/IACUC/AUP-R093/2019).

Animals and Husbandry

A total of 504 one-day-old male broiler chickens (Cobb 500) were obtained from a local hatchery, individually labeled, weighed, and randomly assigned into twelve treatment groups. Each group had six replicates, while each replicate had seven birds.

The rearing conditions followed commercial recommendations for Cobb 500. Birds were raised in a commercial closed house equipped with a penning cage system (120 × 120 cm in length × width) with plastic mesh flooring. The house temperature was set at 32 ± 1 °C on day 1. Afterward, the temperature gradually reduced to about 24 ± 1 °C until 10 and was maintained until day 42. The Newcastle disease and infectious bronchitis disease (ND-IB) vaccination was performed with eye drops on 7 and 21 days. Meanwhile, the infectious bursal disease (IBD) vaccine was administered on day 14 with eye drops.

Dietary Treatments

The seaweed was provided by Promise Earth (M) Sdn. Bhd., a biotechnology company (Selangor 42600, Malaysia). The dietary treatments were as follows; PC= positive control (basal diet + vitamin E, 100 mg/kg feed), NC= negative control (basal diet), BS 0.25= basal diet + 0.25% BS, BS 0.50= basal diet + 0.50% BS, BS 0.75= basal diet + 0.75% BS, BS 1= basal diet + 1% BS, BS 1.25= basal diet + 1.25% BS, GS 0.25= basal diet + 0.25% GS, GS 0.50= basal diet + 0.50% GS, GS 0.75= basal diet + 0.75% GS, GS 1= basal diet + 1% GS, GS 1.25= basal diet + 1.25% GS. As stated earlier, this manuscript is part of a wider research in which the PC group was considered to study the antioxidants-related parameters (Azizi *et al.,* 2023). The birds were fed the diets for the starter period (Table 1) and finisher period (Table 2) from days 0 through 21 and 22 through 42, respectively. Diets were formulated based on the Cobb 500 nutritional requirements (NRC, 1994) using the FeedLIVE software (FeedLIVE 1.60, Mueang Nonthaburi, Thailand).

Performance Measurement and Sampling

The initial body weight (IBW) of chicks was recorded on the first day of the feeding trial. Afterwards, the individual body weight (BW) of birds, feed offered, and refusal per replicate (pen) were recorded weekly for the determination of feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). The production efficiency was measured through the European broiler index (EBI) (Marcu *et al.,* 2013). The BWG, FI, FCR, and EBI were calculated as follows:

BWG= current week's weight - former week's weight

FI / bird= [(weight of given feed - weight of excess feed)] / number of birds

FCR= total feed intake / total weight gained

EBI= [average daily gain (ADG) (g) × survival rate (%)] / (FCR × 10)

At week three, six birds were randomly selected from each treatment (one bird per replicate) for the ileal digesta collection. At the end of the feeding trial, six birds were randomly selected from each treatment (one bird

Table 1. Ingredient composition of the starter period (days 1-22) diet of broiler chickens

Ingredients (%)	Dietary treatments[1]											
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25
Corn	46.0	46.0	46.0	46.0	46.0	46.0	46.0	46.0	46.0	46.0	46.0	46.0
Soybean meal	40.0	40.0	39.8	39.5	39.3	39.0	38.8	39.8	39.5	39.3	39.0	38.8
Wheat pollard	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Palm oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
L-Lysine[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine[3]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DCP[4]	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
Calcium carbonate	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral mix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin mix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Antioxidants	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Seaweed	-	-	0.25	0.50	0.75	1.00	1.25	0.25	0.50	0.75	1.00	1.25
Vitamin E	-	0.01	-	-	-	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis[7]												
ME (kcal/kg)[8]	3040.16	3039.86	3041.02	3041.88	3042.74	3043.60	3044.46	3040.74	3041.31	3041.89	3042.48	3043.04
Protein	21.95	21.95	21.94	21.91	21.90	21.89	21.87	21.93	21.90	21.87	21.85	21.82
Fat	5.98	5.98	5.98	5.98	5.98	5.98	5.98	5.98	5.98	5.98	5.97	5.97
Fiber	4.34	4.34	4.33	4.31	4.31	4.29	4.28	4.32	4.31	4.30	4.29	4.28
Calcium	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Total phosphorous	1.01	1.01	1.01	1.01	1.01	1.00	1.00	1.01	1.01	1.00	1.00	1.00
Available phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Note: [1]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [2]L-Lysine 78.8% (minimum). [3]DL-Methionine 99%. [4]Dicalcium phosphate. [5]Mineral mix provided per kilogram of product (Mineral mix): Selenium 0.20 g; iron 80.0 g; manganese 100.0 g; zinc 80.0 g; copper 15.0 g; potassium 4.0 g; sodium 1.50 g; iodine 1.0 g and cobalt 0.25 g. [6]Vitamin premix provided per kilogram of product (Vitamin premix): Vitamin A 35.0 MIU; vitamin D3 9.0 MIU; vitamin E 90.0 g; vitamin K3 6.0 g; vitamin B1 7.0 g; vitamin B2 22.0 g; vitamin B6 12.0 g; vitamin B12 0.070 g; pantothenic acid 35.0 g; nicotinic acid 120.0 g; folic acid 3.0 g; biotin 300.000 mg; phytase 25000.0 FTU cobalamin 0.05 mg; thiamine 1.43 mg; riboflavin 3.44 mg; folic acid 0.56 mg; biotin 0.05 mg; pantothenic acid 6.46 mg; niacin 40.17 mg and pyridoxine 2.29 mg. [7]The diets were formulated using FeedLIVE software. [8]Metabolizable energy.

per replication) for carcass characteristics and another six birds for collecting ileal digesta, liver tissue, and jejunum tissue. Birds were euthanized by cervical dislocation and the ileal digesta was collected from Meckel's diverticulum at 1 cm before the ileocecal junction for the apparent ileal digestibility (AID) analysis. In addition, tissue samples from the liver and jejunum were taken, frozen in liquid nitrogen, and kept at -80 °C for gene expression analysis.

Carcass Characteristics and Internal Organs Weight

After bleeding, the birds (one bird per replication) were scalded, de-feathered, and weighed as hot carcasses for the carcass characteristics. The carcass was cut into different parts and weighed accordingly. The carcass was cut with breast, thigh, drumstick, and back. The internal organs were removed and evaluated. The carcass parts and viscera weights were presented as a percentage based on the following equation:

Carcass yield (%)= (carcass weight / live body weight) × 100

Apparent Ileal Digestibility of Nutrients

The titanium dioxide (TiO_2) at 0.3% level was added to the feed during the end of the starter period (17 to 21 days) and finisher period (38 to 42 days) as an indigestible marker to calculate the AID of nutrients. Proximate analysis of feed and digesta was performed as described in the Association of Official Analytical Chemistry (AOAC, 1995).

The TiO_2 was determined based on the method described by Short *et al.* (1996). The samples were ashed at 580 °C for 13 h, and the ash was then digested in 7.4 M sulfuric acid and topped up to 100 mL with distilled water. Standard solutions of TiO_2 were prepared, and the absorbance of samples and standards were measured using a spectrophotometer at 410 nm wavelength (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, USA).

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction

About 30 mg of finely powdered tissue samples were used for the RNA extraction. The RNA was extracted following the manufacturer's instructions using the Nucleo-Spin® RNA Plus kit (MACHEREY-NAGEL, Allentown, USA). First, the gDNA was removed through the lysate filtration using a NucleoSpin® gDNA Removal Column (MACHEREY-NAGEL, Allentown, USA). Next, the RNA was purified using a Nucle-oSpin® RNA Plus

Table 2. Ingredient composition of the finisher period (days 22-42) diet of broiler chickens

Ingredients (%)	Dietary treatments[1]											
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25
Corn	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52.0
Soybean meal	32.0	32.0	31.8	31.5	31.3	31.0	30.8	31.8	31.5	31.3	31.0	30.8
Wheat pollard	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Palm oil	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10
L-Lysine[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DCP[4]	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40
Calcium carbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral mix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin mix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Antioxidants	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Seaweed	-	-	0.25	0.50	0.75	1.00	1.25	0.25	0.50	0.75	1.00	1.25
Vitamin E	-	0.01	-	-	-	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated analysis[7]												
ME (kcal/kg)[8]	3149.82	3149.50	3150.68	3151.54	3152.40	3153.26	3154.12	3150.39	3150.97	3151.55	3152.13	3152.70
Protein	19.06	19.06	19.05	19.03	19.01	19.00	18.98	19.04	19.01	18.98	18.96	18.93
Fat	7.19	7.19	7.19	7.19	7.19	7.19	7.19	7.19	7.19	7.18	7.18	7.18
Fiber	4.00	4.00	3.99	3.98	3.97	3.96	3.95	3.99	3.98	3.97	3.96	3.94
Calcium	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Total phosphorus	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Available phosphorus	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47

Note: [1]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [2]L-Lysine 78.8% (minimum). [3]DL-Methionine 99%. [4]Dicalcium phosphate. [5]Mineral mix provided per kilogram of product (Mineral mix): Selenium 0.20 g; iron 80.0 g; manganese 100.0 g; zinc 80.0 g; copper 15.0 g; potassium 4.0 g; sodium 1.50 g; iodine 1.0 g and cobalt 0.25 g. [6]Vitamin premix provided per kilogram of product (Vitamin premix): Vitamin A 35.0 MIU; vitamin D3 9.0 MIU; vitamin E 90.0 g; vitamin K3 6.0 g; vitamin B1 7.0 g; vitamin B2 22.0 g; vitamin B6 12.0 g; vitamin B12 0.070 g; pantothenic acid 35.0 g; nicotinic acid 120.0 g; folic acid 3.0 g; biotin 300.000 mg; phytase 25000.0 FTU cobalamin 0.05 mg; thiamine 1.43 mg; riboflavin 3.44 mg; folic acid 0.56 mg; biotin 0.05 mg; pantothenic acid 6.46 mg; niacin 40.17 mg and pyridoxine 2.29 mg. [7]The diets were formulated using FeedLIVE software. [8]Metabolizable energy.

Column (MACHEREY-NAGEL, Allentown, USA) based on the instructions of the manufacturer. The ultraviolet-visible spectroscopy (absorbance 260/280) was used to determine the concentration and purity of RNA by using a spectrophotometer (Multiskan GO, Thermo Scientific, USA). Finally, the purified RNA was converted into complementary DNA (cDNA) using a cDNA synthesis kit (Biotechrab-bit, Hennigsdorf, Germany), following the manufacturer's protocol.

Real-time PCR was conducted using a LightCycler® 480 qPCR system (Roche Molecular Systems, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to standardize the target genes. A qPCR master mix (20 µL) was prepared using a CAPITAL™ qPCR Green Mix, 4x (Biotechrabbit, Hennigsdorf, Germany). The master mix contained 5 µL of SYBR Green Master Mix, 1 µL of each 200 nM forward and reverse primers, 1 µL of template cDNA, and 12 µL of RNase-free water.

The qPCR cycling condition is programmed as initial denaturation temperature at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 13 sec, annealing for 30 s at 60 °C, and final extension for melt analysis based on the instrument instruction, LightCycler® 480 qPCR system (Roche Molecular Systems, USA). Melting curve analysis was performed at the end of the amplification cycle to confirm the specificity of the amplification. The amplification efficiency of target and housekeeping genes was analyzed based on the standard curve of 5-fold serial diluted cDNA. In addition, the relative gene expression based on the housekeeping gene was quantified following the recommendation of Livak & Schmittgen (2001). The sequences of the housekeeping and targeted gene primers are presented in Table 3.

Statistical Analysis

Statistical analysis was performed using the General Linear Model (GLM) of the statistical analysis system (SAS 9.4) by one-way ANOVA. Duncan's Multiple Range Test was used to assess the significant differences between treatment groups at $p<0.05$. The orthogonal polynomial contrast of SAS was used to determine the linear and quadratic effects of dietary increasing brown and green seaweed inclusion levels. The negative control group was considered the 0.0% seaweed inclusion. The positive control treatment was not considered in the contrast analysis. The statistical model was $Y_{ijk} = \mu + T_{ij} + E_{ijk}$. Where Y_{ijk} is the dependent variable, µ is the general mean, T_{ij} is the effect of dietary treatment, and E_{ijk} is the experimental error.

Table 3. The primer sequences of target genes

Target genes	Primer sequences 5′ - 3′		Product size (bp)	Accession No.
GHR	F- AACACAGATACCCAACAGCC	R- AGAAGTCAGTGTTTGTCAGGG	145	NM_001001293.1
IGF-1	F- CACCTAAATCTGCACGCT	R- CTTGTGGATGGCATGATCT	140	NM_001004384.2
APN	F- AATACGCGCTCGAGAAAACC	R- AGCGGGTACGCCGTGTT	70	NM_204861.1
SGLT5	F- ATACCCAAGGTAATAGTCCCAAAC	R- TGGGTCCCTGAACAAATGAAA	75	XM_040678521.1
PepT1	F- CTGTCTGCGTGACCCTTCTA	R- TGTCCAAGTTCCTGCTATGTG	151	NM_204365.1
GAPDH	F- CTGGCAAAGTCCAAGTGGTG	R- AGCACCACCCTTCAGATGAG	275	NM_204305.1

Note: F= Forward, R= Reverse. bp (base pair)= Product size. GHR= Growth hormone receptor, IGF-1= Insulin-like growth factor 1, AP = Aminopeptidase N, SGLT5= Glucose transporter, PepT1= Oligopeptide transporter, GAPDH= Glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Growth Performance

Different levels of BS and GS significantly affected the body weight (BW) of chickens in the starter period (Table 4). All GS group birds had significantly higher BW in the starter period than the control birds. The highest BW of the starter period was recorded at 0.50% and 1.25% GS groups, which were also higher (p<0.05) than the BS groups. Meanwhile, the 1.25% BS group had a significantly higher BW than the NC during the starter period. The BS and GS had no significant effects on broiler BW in the finisher period and on the final body weight (FBW). The starter period BWG of all GS groups was higher (p<0.05) than the NC and PC groups. At the same time, the 1.25% BS had significantly higher BWG compared to the NC and PC groups during the starter period. The BWG of the finisher period and the final body weight gain (FBWG) were not affected (p>0.05) by the BS and GS supplementations. The chickens fed with GS groups had higher (p<0.05) FI at the starter and finisher periods than the NC and PC groups. The final feed intake (FFI) was also significantly higher for the GS groups than for the NC and PC groups. The 1.25% BS treatment had higher (p<0.05) FI in the starter period than the NC and PC groups, while no significant difference was found in the finisher period FI for the BS groups compared to the NC and PC groups. In contrast, the 1% BS group recorded a significantly higher FFI than the NC and PC groups. No differences (p>0.05) were found in the FCR, IBW, ADG, EBI, and mortality among the dietary treatment groups.

Carcass Characteristics and Internal Organs Weight

The results (Table 5) showed that supplementation with BS and GS had no effects (p>0.05) on plucked, carcass, breast, thigh, wing, and back yields. Conversely, there was a quadratic improvement in the drumstick yield for the 1.25% GS group compared to the NC group. No significant difference was observed among the dietary groups in the internal organs of broiler chickens fed the BS and GS.

Apparent Ileal Digestibility of Nutrient

The findings showed (Table 6) that the DM digestibility of 0.50% and 1.25% BS, and 0.50%, 0.75%, and 1.25% GS groups were higher (p<0.05) than the NC group in the starter period. In contrast, the DM digestibility for all BS and GS groups (except for the 0.25% BS and GS groups) was lower (p<0.05) compared to the NC and PC groups in the finisher period. Regarding the AID of OM, the 0.25% BS group had higher (p<0.05) OM digestibility in the starter period. In contrast, the OM digestibility was lower (p<0.05) in all GS and BS groups (except for the 0.25% BS) as compared with the NC and PC groups in the finisher period.

The AID of crude protein CP was significantly higher in birds fed 0.25%, 0.50%, and 1.25% BS and GS groups than in the NC group during the starter period. No difference (p>0.05) was observed for the AID of CP in the BS and GS groups compared to the NC group in the finisher period. The results showed that the birds fed a 0.25% BS-supplemented diet had significantly higher ash digestibility than the NC group during the starter period. The digestibility of ash was significantly decreased linearly and quadratically in birds fed 0.25%, 0.50%, and 0.75% BS compared to the NC group during the finisher period. Meanwhile, no difference (p>0.05) was observed in ash digestibility for the GS groups compared to the NC group during the finisher period.

Hepatic Growth mRNA Expression

The effects of various brown and green seaweed on the hepatic growth hormone receptor (GHR) and Insulin-like growth factor-1 (IGF-1) mRNA expression are presented in Table 7. The mRNA expression of the GHR gene was higher (p<0.05) for broiler fed 0.50% and 0.75% GS compared to the NC group. Furthermore, birds fed 0.50% BS and 0.25%, 0.50%, and 0.75% GS had significantly higher hepatic IGF-1 mRNA expression.

Intestinal Nutrient Transporter mRNA Expressions

The effects of brown and green seaweed on aminopeptidase (APN), glucose transporter (SGLT5), and oligopeptide transporter (PepT1) mRNA expression in broiler chickens are presented in Table 8. The result showed that supplementation with various brown and green seaweed levels did not affect (p>0.05) the APN, SGLT5, and PepT1 mRNA expression in jejunum tissue.

DISCUSSION

Growth Performance

To confront the expanding population issue in certain nations and lower diet expenses, it is important to continue seeking natural alternatives to conventional

Table 4. Growth performances of broiler chickens fed with various levels of brown and green seaweed

Growth performance[1]	Dietary treatments[2]												SEM[3]	p-values	Contrast p-values[4]	
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25			Line.	Quad.
0-3 weeks (Starter period)																
IBW, g	43.04	44.83	43.02	44.39	44.38	44.33	43.81	43.06	43.7	43.36	43.66	43.87	0.55	0.313	0.422	0.239
BW, g	736.37^{d}	750.75^{cd}	777.33^{bcd}	779.85^{bcd}	765.95^{cd}	751.24^{cd}	807.43^{abc}	834.48^{ab}	844.95^{a}	800.1^{abc}	825.42^{ab}	843.24^{a}	18.19	<.0001	0.049	0.738
BWG, g	693.8^{e}	705.61^{de}	734.3^{bcde}	744.5^{abcde}	721.6^{cde}	706.74^{de}	763.6^{abc}	791.41^{a}	801.25^{a}	756.6^{abcd}	781.78^{ab}	799.37^{a}	17.99	<.0001	0.048	0.775
FI, g	1037.5^{de}	1042.6^{ed}	1072.5^{cd}	1037.26^{de}	1031.55^{e}	1011.67^{e}	1096.67^{bc}	1132.9^{a}	1135.36^{a}	1071.31^{cd}	1125.44^{ab}	1107.0^{abc}	11.3	<.0001	0.245	0.337
FCR, (g/g)	1.53	1.46	1.48	1.41	1.54	1.5	1.46	1.49	1.47	1.47	1.45	1.44	0.04	0.600	0.249	0.688
4-6 weeks (Finisher period)																
BW, g	2837.31	2909.31	2954.9	2999.52	2970.26	2867.64	2917.8	3004.71	3048.5	3002.36	2967.46	3011.97	54.83	0.245	0.325	0.670
BWG, g	2102.31	2190.43	2180.03	2207.79	2173.37	2093.14	2118.27	2194.85	2201.57	2207.86	2108.31	2168.1	42.52	0.445	0.517	0.922
FI, g	3352.42^{d}	3392.81^{cd}	3476.6b^{c}	3411.8^{cd}	3423.82^{cd}	3317.01^{d}	3423.75^{cd}	3551.5^{ab}	3552.48^{ab}	3544.6^{ab}	3537.38^{ab}	3591.07^{a}	32.79	0.000	0.475	0.948
FCR, (g/g)	1.62	1.61	1.69	1.66	1.60	1.61	1.57	1.59	1.62	1.63	1.63	1.63	0.03	0.539	0.488	0.764
0-6 weeks (Overall)																
FBW, g	2837.3	2909.31	2954.9	2999.52	2970.26	2867.64	2917.8	3004.71	3048.5	3002.36	2967.46	3011.97	54.83	0.245	0.325	0.670
FBWG, g	2826.9	2863.8	2911.76	2955.16	2925.49	2823.42	2873.9	2997.7	3005.05	2958.97	2923.55	2994.84	53.02	0.193	0.464	0.960
FFI, g	4392.5^{d}	4401.47^{d}	4554.8^{abcd}	4456.3^{cd}	4465.9^{cd}	4569.5^{abc}	4520.4^{bcd}	4688.8^{a}	4692.1^{a}	4616.8^{abc}	4666.17^{ab}	4695.85^{a}	47.66	<.0001	0.353	0.839
FFCR, (g/g)	1.62	1.56	1.58	1.55	1.54	1.66	1.59	1.60	1.58	1.56	1.64	1.60	0.03	0.368	0.9	0.731
ADG, g	67.31	68.73	69.33	70.36	69.65	67.22	68.43	71.37	71.55	70.45	69.61	71.31	1.49	0.223	0.464	0.961
EBI	407.78	462.08	422.86	429.42	406.65	405.33	431.01	440.34	441.58	446.42	404.18	452.73	16.18	0.176	0.487	0.942
Mortality	2/42	1/42	1/42	1/42	3/42	0/42	0/42	1/42	1/42	0/42	3/42	0/42	-	0.916	-	-

Note: [1]Growth performance: IBW = initial body weight, BW = body weight, BWG = body weight gain, FI = feed intake, FCR = feed conversion ratio, FBW = final body weight, FBWG = final body weight gain, FFI = final feed intake, FFCR = final feed conversion ratio, ADG = average daily gain, EBI = European broiler index. [2]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [3]SEM = standard error of the mean. 4Contrast p-values = orthogonal polynomial contrasts of dietary increasing brown and green seaweed inclusion levels (0.0 to 1.25%). $^{a-e}$ Means with different superscripts in the same row indicates a significant difference ($p<0.05$).

Table 5. Characteristics and relative weights of internal organs of broiler chickens fed with various levels of brown and green seaweed

Variables	Dietary treatments[1]												SEM[2]	p-values	Contrast p-values[3]	
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25			Line.	Quad.
Carcass																
Plucked, g	2761.0	2903.2	2697.6	2864.0	2673.3	2845.0	2674.3	2897.0	3060.8	2832.3	2675.7	2797.7	81.09	0.1106	0.0542	0.0599
Carcass, g	2209.0	2293.2	2185.6	2280.7	2138.0	2274.0	2130.3	2356.0	2411.6	2316.7	2169.3	2257.0	69.38	0.1753	0.1636	0.1238
Dressing, %	73.89	71.73	73.49	72.26	72.63	72.55	72.39	73.88	74.09	74.24	73.58	73.16	0.87	0.7771	0.5349	0.1332
Breast, %	28.17	25.99	27.33	26.7	28.44	27.15	26.84	29.13	29.36	27.65	27.83	27.75	0.82	0.2192	0.3053	0.0587
Drumstick, %	8.79	9.23	9.29	9.45	9.24	9.30	9.33	9.11	8.84	9.58	9.36	9.86	0.27	0.4790	0.3453	0.0248
Thigh, %	10.52	10.42	10.67	9.93	10.49	10.70	10.69	11.13	9.79	10.08	10.23	10.16	0.36	0.5882	0.3516	0.4165
Wing, %	8.09	7.59	7.74	7.40	7.57	7.54	7.55	7.41	7.85	7.57	7.89	7.55	0.23	0.8129	0.2711	0.0674
Back, %	18.32	18.42	18.42	18.02	16.25	18.76	18.59	18.01	17.90	18.49	17.84	18.06	0.61	0.4461	0.6482	0.5841
Internal organs, %																
Proventriculus	0.45	0.45	0.42	0.45	0.36	0.43	0.48	0.46	0.38	0.36	0.42	0.40	0.04	0.7366	0.7400	0.8128
Gizzard	2.34	2.36	2.16	1.87	2.15	2.03	2.33	2.37	2.03	2.12	1.83	2.14	0.15	0.3250	0.6474	0.3122
Liver	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	0.10	0.6783	0.8983	0.8983
Spleen	0.10	0.06	0.11	0.09	0.10	0.08	0.11	0.09	0.11	0.06	0.10	0.10	0.01	0.5316	0.4610	1.0000
Heart	0.40	0.34	0.43	0.40	0.37	0.43	0.46	0.41	0.4	0.39	0.43	0.41	0.02	0.0825	0.4258	0.7157
Intestine	4.18	4.41	4.12	4.44	4.10	4.75	4.69	3.86	4.24	4.13	4.49	4.19	0.19	0.1074	0.7145	0.5198
Abdominal fat	0.95	1.05	1.14	1.01	1.04	1.33	1.25	0.95	0.96	1.13	0.79	1.48	0.19	0.6294	0.9263	0.4408

Note: [1]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [2]SEM = standard error of means. [3]Contrast p-values = orthogonal polynomial contrasts of dietary increasing brown and green seaweed inclusion levels (0.0 to 1.25%).

Table 6. Apparent ileal digestibility of nutrients in broiler chickens fed different brown and green seaweed levels

Nutrients[1]	Dietary treatments[2]												SEM[3]	p-values	Contrast p-values[4]	
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25			Line.	Quad.
0-3 weeks (Starter period)																
DM	54.8^{e}	59.78^{abc}	57.38^{bcde}	58.48^{bcd}	56.04^{de}	55.66^{de}	59.94^{ab}	56.95^{cde}	61.81^{a}	62.58^{a}	55.45^{e}	61.5^{a}	0.84	<.0001	0.064	0.374
OM	47.59^{bc}	48.03^{bc}	50.56^{a}	45.7^{cd}	49.55^{ab}	44.64^{d}	46.56^{cd}	47.59^{bc}	47.96^{bc}	50.12^{ab}	47.5^{bc}	46.34^{cd}	0.78	0.001	0.061	0.093
CP	73.7^{f}	76.58^{abcd}	76.98^{abc}	77.62^{a}	74.87^{def}	75.38^{cdef}	76.86^{abc}	75.56^{bcde}	77.15^{abc}	74.63^{ef}	74.17^{ef}	77.35^{ab}	0.51	0.000	<.0001	0.009
Ash	37.25^{bc}	38.55^{abc}	41.95^{a}	35.94^{c}	38.49^{abc}	35.5^{c}	38.59^{abc}	38.72^{abc}	38.91^{abc}	40.87^{ab}	38.03^{bc}	35.86^{c}	1.04	0.013	0.751	0.805
4-6 weeks (Finisher period)																
DM	69.56^{ab}	70.83^{a}	69.95^{a}	64.68c	64.21^{c}	64.75^{c}	65.35^{c}	66.13^{bc}	63.65^{c}	62.53^{c}	63.18^{c}	64.28^{c}	1.13	<.0001	0.331	0.333
OM	70.84^{a}	72.07^{a}	71.87^{a}	64.34^{b}	63.36^{bc}	65.11^{b}	65.61^{b}	65.69^{b}	63.93^{bc}	60.11^{c}	62.76^{bc}	63.1^{bc}	1.22	<.0001	0.352	0.103
CP	80.08^{ab}	81.89^{a}	81.99^{a}	78.11^{b}	79.28^{ab}	79.79^{ab}	78.89^{ab}	77.99^{b}	78.98^{ab}	77.96^{b}	77.67^{b}	78.09^{b}	0.91	0.021	0.925	0.689
Ash	36.21^{ab}	33.77^{abc}	28.42^{de}	26.3^{e}	29.91^{cde}	35.02^{abc}	31.7^{bcd}	33.66^{abc}	34.13^{abc}	38.25^{a}	36.11^{ab}	33.31^{abcd}	1.57	0.000	0.046	0.001

Note: $^{a-f}$Means with different superscripts in the same row indicate a significant difference (p<0.05). [1]Nutrients: DM = dry matter, OM = organic matter, CP = crude protein. [2]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [3]SEM = standard error of means. [4]Contrast p-values = orthogonal polynomial contrasts of dietary increasing brown and green seaweed inclusion levels (0.0 to 1.25%).

Table 7. GHR and IGF-1 mRNA expressions in broiler chickens fed with various levels of brown and green seaweed

Variables[1] (mRNA fold change)	Dietary treatments[2]												SEM[3]	P-values	Contrast p-values[4]	
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25			Line.	Quad.
GHR	1^{cd}	0.924^{cd}	0.929^{cd}	0.691^{d}	1.210^{ac}	0.865^{cd}	0.850^{cd}	1.126^{bcd}	1.487^{a}	1.557^{a}	0.802^{bcd}	0.816^{bcd}	0.054	0.004	0.887	0.033
IGF-1	1^{c}	1.048^{bc}	0.955^{c}	0.873^{c}	1.686^{a}	1.077^{bc}	0.779^{c}	1.792^{a}	1.469^{ab}	1.660^{a}	1.089^{bc}	0.979^{c}	0.066	0.000	0.224	0.011

Note: [1]Variables: GHR = Growth hormone receptor, IGF-1 = Insulin-like growth factor 1. [2]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [3]SEM = standard error of means. [4]Contrast p-values = orthogonal polynomial contrasts of dietary increasing brown and green seaweed inclusion levels (0.0 to 1.25%). $^{a-d}$ Means with different superscripts in the same row indicate a significant difference (p<0.05).

Table 8. mRNA APN, SGLT5 and PepT1 expressions in broiler chickens fed with various levels of brown and green seaweed

Variables[1] (mRNA fold change)	Dietary treatments[2]												SEM[3]	p-values	Contrast p-values[4]	
	NC	PC	BS 0.25	BS 0.50	BS 0.75	BS 1	BS 1.25	GS 0.25	GS 0.50	GS 0.75	GS 1	GS 1.25			Line.	Quad.
APN	1	1.031	0.77	1.015	1.304	1.128	0.778	0.726	1.049	0.741	0.689	0.843	0.049	0.210	0.341	0.891
SGLT5	1	1.091	1.163	1.504	1.327	1.444	0.878	1.099	1.364	0.926	1.250	1.331	0.05	0.068	0.597	0.573
PepT1	1	1.035	1.045	1.250	1.029	1.259	1.721	0.788	1.133	1.103	1.027	0.931	0.061	0.329	0.185	0.241

Note: [1]Variables: APN = Aminopeptidase N, SGLT5 = Glucose transporter, PepT1 = Oligopeptide transporter. [2]Dietary treatments: NC (negative control) = basal diet, PC (positive control) = basal diet + vitamin E (100 mg/kg feed), BS 0.25 = basal diet + 0.25% brown seaweed, BS 0.50 = basal diet + 0.50% brown seaweed, BS 0.75 = basal diet + 0.75% brown seaweed, BS 1 = basal diet + 1% brown seaweed, BS 1.25 = basal diet + 1.25% brown seaweed, GS 0.25 = basal diet + 0.25% green seaweed, GS 0.50 = basal diet + 0.50% green seaweed, GS 0.75 = basal diet + 0.75% green seaweed, GS 1 = basal diet + 1% green seaweed, GS 1.25 = basal diet + 1.25% green seaweed. [3]SEM = standard error of means. [4]Contrast p-values = orthogonal polynomial contrasts of dietary increasing brown and green seaweed inclusion levels (0.0 to 1.25%).

poultry feedstuffs (El-Sabrout *et al.*, 2023; Khalifah *et al.*, 2023). Seaweed, as a natural feed additive, is a source of macro and micronutrients, containing many biological bioactive components that may impact the growth of broiler chickens (El-Deek *et al.*, 2011; Garcia-Vaquero & Hayes, 2016; Corino *et al.*, 2019). In the current study, the 1.25% BS and different GS levels (0.25%, 0.50%, 0.75%, 1%, and 1.25%) significantly increased the BW of broiler chickens during the starter period compared to the NC and PC groups. These findings are consistent with the previous reports that 0.50% BS and GS improved the BW of broiler chickens (Choi *et al.*, 2014; Mohammadigheisar *et al.*, 2020). The positive effects of seaweed on broiler BW may be attributed to the prebiotic effects of polysaccharides present in seaweed (Corino *et al.*, 2019). In addition, seaweed polysaccharides might improve the immune status of birds by reducing the pathogenic microbial load in the digestive tract, which may influence body metabolism and increase feed conversion rate (ShuBai *et al.*, 2013; Rizk *et al.*, 2017).

The findings of this study showed that all GS groups' starter period BWG was significantly higher than the NC and PC groups. At the same time, the BWG of 0.25%, 0.50%, and 1.25% GS treatments were also significantly higher compared to the BS groups. The better performance of GS in BWG may be accredited to the presence of Ulvan polysaccharides in GS. Ulvan has various biological activities such as immunomodulation, anti-viral, antioxidant, and anti-hyperlipidemic (Bhatia *et al.*, 2013; Kidgell *et al.*, 2019).

The result showed that birds fed different levels of GS had higher FI than the NC and PC groups in both starter and finisher periods. In addition, there were no significant differences in the FI at the finisher period for 0.25%, 0.50%, 0.75%, and 1% BS treatments compared to the NC and PC groups. Earlier studies also reported similar findings as captured in a study reported by Choi *et al.* (2014), who reported that 0.50% BS in broiler feed had no significant effect on the FI of birds. In this study, the highest BS level (BS 1.25%) increased the FI at the starter phase. This finding agrees with the previous submission that the inclusion of BS in broiler feed at a high level can increase the FI (El-Deek *et al.*, 2011). No significant difference was found in the FCR among various levels of BS and GS treatment groups. Earlier studies also reported similar findings. Abudabos *et al.* (2013) stated that the FCR of broiler chickens was not affected when fed 1% and 3% GS-supplemented feed. Bonos *et al.* (2017) determined no significant difference in FCR when broiler chickens were fed 0.50%, 1%, and 2% BS in their diet.

Carcass Characteristics and Internal Organs Weight

Carcass yield and carcass cut weights are essential because they are used to grade meat products and directly impact market pricing. The inclusion of various levels of BS and GS in broiler chicken diets did not affect the carcass characteristics and internal organs' weight. The absence of dietary seaweed influence on carcass characteristics and internal organs' weight supports the findings of various prior studies. For instance, Abudabos *et al.* (2013) reported that 1% and 3% GS *Ulva Lactuca* supplemented feed did not affect broiler thigh yields. Moreover, Choi *et al*. (2014) reported that a 0.50% inclusion of BS by-product had no effects on broiler breast meat yield. Regarding the internal organ relative weight, our findings are consistent with Choi *et al.* (2014), who reported that 0.50% inclusion of BS by-product had no significant effects on broiler spleen and abdominal fat relative weights. These findings indicate that dietary seaweeds may have minimal anti-nutritional factors, potentially causing harm to the carcass and visceral organs in broiler chickens.

Apparent Ileal Digestibility of Nutrient

The results showed that the DM digestibility of the starter period in 0.50% and 1.25% BS, and 0.50%, 0.75%, and 1.25% GS, were significantly higher than the NC group. Furthermore, the 0.25% BS had higher ash digestibility during the starter period. On the other hand, various BS and GS inclusion levels decreased the digestibility of DM, OM, and ash contents during the finisher period. Nutrient digestibility is an imperative factor for feed formulation. In this study, the improvements in the starter period growth performance of birds fed various BS and GS levels were associated with improvement in AID of nutrients.

Earlier studies have reported that seaweed has low digestibility and utilization in animals (Choi *et al.*, 2014). Besides the health benefits of many compounds, seaweed also has content that may reduce nutrient digestibility (Kim, 2011). In addition, algae contain different amounts of polysaccharides (Lahaye & Robic, 2007; Øverland *et al.*, 2019), affecting the digestibility of nutrients (Holdt & Kraan, 2011). Furthermore, the soluble fiber in the diet increases the ingesta passage speed, decreasing the nutrient digestibility in monogastric animals (Montagne *et al.*, 2003; Azizi *et al.*, 2021b). Regarding the nutrient digestibility in the finisher period, results are inconsistent with studies that reported that seaweed might increase animal nutrient digestibility (Holdt & Kraan, 2011; Kim, 2011; Choi *et al.*, 2014). The inconsistency with previous research can be explained by the animals' differences, basal feeds, housing conditions, and production systems employed in various trials.

Hepatic Growth mRNA Expression

IGF-1 is a primary mediator of growth hormone (GH) effects. IGF-1 is a hormone linked to skeletal growth (Yan *et al.*, 2016). Hepatic IGF-1 is an essential growth hormone that stimulates muscle protein synthesis (Soumeh *et al.*, 2019). The GH stimulates the production of hepatic IGF-1. The presence of GH in the body leads to the synthesis and release of IGF-I through the GHR pathway (Del Vesco *et al.*, 2013). The animal's nutritional status modulated the ability of hepatic tissue to respond to the GH (Beckman, 2011). In this study, the mRNA expression of the hepatic IGF-1 gene was upregulated for birds fed 0.50% BS and 0.25%, 0.50%, and 0.75% GS compared to the NC group.

Additionally, birds fed 0.50% and 0.75% of GS also have higher hepatic GHR mRNA expression. Literature shows that including seaweed and its extracts in the broiler feeding diet may positively affect birds' growth performance (Abudabos *et al.,* 2013; Choi *et al.,* 2014; Sweeney *et al.,* 2016). The growth-promoting effects of seaweed might be associated with the IGF-1 and GHR growth metabolic pathways.

Seaweed contains abundant unique bioactive compounds such as alginate, ulvan, laminarin, fucoidan, and fucoxanthin that might promote the growth of beneficial gut microbes (Andri *et al.,* 2020). Furthermore, research showed that a higher population of beneficial bacteria might contribute to the upregulation of IGF-1 and GHR gene expression (Humam *et al.,* 2019).

Intestinal Nutrient Transporters mRNA Expression

The result showed that various brown and green seaweed supplement levels did not affect the intestinal nutrient transporter mRNA expression in the jejunum tissues. Furthermore, the findings reported by Sweeney *et al.* (2017) showed that laminarin and fucoidan extracts from seaweed did not affect the intestinal nutrient transporter genes. The inclusion of 300 parts per million (ppm) laminarin and 240 ppm fucoidan, either individually or combinedly in pigs' diet, did not affect the expression of intestinal nutrient transporter genes such as PepT1 and SGLT5 (Heim *et al.,* 2014).

CONCLUSION

It is concluded that 1.25% of BS and various levels of GS, including 0.25%, 0.50%, 0,75%, 1%, and 1.25%, in broiler chickens' diet can be recommended to promote bird growth in the starter phase. Furthermore, various BS and GS supplements increased the mRNA expression of hepatic GHR and IGF-1 genes. However, seaweed did not affect intestinal nutrient transporter gene expression, including APN, SGLT5, and PepT1. The current research findings are useful for further studies investigating the mechanisms and components responsible for higher growth performance and nutrient digestibility during the starter period of broiler chickens.

CONFLICT OF INTEREST

T. C. Loh serves as an editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. The authors also declare no conflicts of interest.

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REFERENCES

Abudabos, A. M., A. B. Okab, R. S. Aljumaah, E. M. Samara, K. A. Abdoun, & A. A. Al-Haidary. 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 12:177–181. https://doi.org/10.4081/ijas.2013.e28

Andri, F., N. D. Dono, H. Sasongko, & Z. Zuprizal. 2020. The effects of dietary seaweed inclusion on growth performance of broiler chickens: A systematic review and meta-analysis. F1000Res. 9:1087. https://doi.org/10.12688/f1000research.25726.1

Association Official Analytical Chemistry. 1995. Association of Official Analytical Chemistry: Official Methods of Analysis. VA, USA.

Azizi, M. N., T. C. Loh, H. L. Foo, H. Akit, W. I. Izuddin, N. Shazali, E. L. T. Chung, & A. A. Samsudin. 2021a. Chemical compositions of brown and green seaweed, and effects on nutrient digestibility in broiler chickens. Animals 11:2147. https://doi.org/10.3390/ani11072147

Azizi, M. N., T. C. Loh, H. L. Foo, & E. L. T. Chung. 2021b. Is palm kernel cake a suitable alternative feed ingredient for poultry? Animals 11:1–15. https://doi.org/10.3390/ani11020338

Azizi, M. N., T. C. Loh, H. L. Foo, H. Akit, W. I. Izuddin, & D. Yohanna. 2023. Brown and green seaweed antioxidant properties and effects on blood plasma antioxidant enzyme activities, hepatic antioxidant genes expression, blood plasma lipid profile, and meat quality in broiler chickens. Animals 13:1582. https://doi.org/10.3390/ani13101582

Balasubramanian, B., S. Shanmugam, S. Park, N. Recharla, J. S. Koo, I. Andretta, & I. H. Kim. 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, fecal gas emission, and microbial counts in broilers. Animals 11:1244. https://doi.org/10.3390/ani11051244

Beckman, B. R. 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. Gen. Comp. Endocrinol. 170:233–252. https://doi.org/10.1016/j.ygcen.2010.08.009

Bhatia, S., P. Rathee, K. Sharma, B. B. Chaugule, N. Kar, & T. Bera. 2013. Immuno-modulation effect of sulphated polysaccharide (porphyran) from *Porphyra vietnamensis*. Int. J. Biol. Macromol. 57:50–56. https://doi.org/10.1016/j.ijbiomac.2013.03.012

Bonos, E., A. Kargopoulos, I. Nikolakakis, P. Florou Paneri, & E. Christaki. 2017. The seaweed *Ascophyllum nodosum* as a potential functional ingredient in chicken nutrition. Journal Oceanography Marine Research 04:1–5. https://doi.org/10.4172/2572-3103.1000140

Cherry, P., C. O'hara, P. J. Magee, E. M. Mcsorley, & P. J. Allsopp. 2019. Risks and benefits of consuming edible seaweeds. Nutr. Rev. 77:307–329. https://doi.org/10.1093/nutrit/nuy066

Choi, Y. J., S. R. Lee, & J. W. Oh. 2014. Effects of dietary fermented seaweed and seaweed *fusiforme* on growth performance, carcass parameters and immunoglobulin concentration in broiler chicks. Asian-Australas. J. Anim. Sci. 27:862–870. https://doi.org/10.5713/ajas.2014.14015

Corino, C., S. C. Modina, A. Di Giancamillo, S. Chiapparini, & R. Rossi. 2019. Seaweeds in pig nutrition. Animals 9:1–26 Available at https://www.mdpi.com/2076-2615/9/12/1126. https://doi.org/10.3390/ani9121126

Del Vesco, A. P., E. Gasparino, A. R. Oliveira Neto, S. E. F. Guimarães, S. M. M. Marcato, & D. M. Voltolini. 2013. Dietary methionine effects on IGF-I and GHR mRNA expression in broilers. Genet. Mol. Res. 12:6414–6423. https://doi.org/10.4238/2013.December.10.2

Diyana, S., A. Aziz, N. U. R. F. Jafarah, S. Sabri, M. As, W. A. D. Abdul, Z. Norhana, & B. Yusof. 2019. Antifungal activity of dichloromethane and hexane extracts of four Malaysian seaweed species against *Ganoderma boninense*. Malays. Appl. Biol. 48:189–196.

El-Deek, A. A., M. A. Al-Harthi, A. A. Abdalla, & M. M. Elbanoby. 2011. The use of brown algae meal in finisher broiler. Egypt. Poult. Sci 31:767–781.

El-Sabrout, K., M. R. T. Dantas, & J. B. F. Souza-Junior. 2023. Herbal and bee products as nutraceuticals for improving poultry health and production. Worlds Poul. Sci. J. 79:223–242. https://doi.org/10.1080/00439339.2021.1960238

Ferraces-Casais, P., M. A. Lage-Yusty, A. R. B. de Quirós, & J. López-Hernández. 2012. Evaluation of Bioactive compounds in fresh edible seaweeds. Food Anal. Methods 5:828–834. https://doi.org/10.1007/s12161-011-9321-2

Garcia-Vaquero, M., & M. Hayes. 2016. Red and green macroalgae for fish and animal feed and human functional food development. Food Reviews International 32:15–45. https://doi.org/10.1080/87559129.2015.1041184

Hayes, M. 2012. Marine Bioactive Compounds: Sources, Characterization and Applications (M Hayes, Ed.). 1st ed. Springer US, Dublin 15, Ireland. https://doi.org/10.1007/978-1-4614-1247-2

Heim, G., A. M. Walsh, T. Sweeney, D. N. Doyle, C. J. O'Shea, M. T. Ryan, & J. V. O'Doherty. 2014. Effect of seaweed-derived laminarin and fucoidan and zinc oxide on gut morphology, nutrient transporters, nutrient digestibility, growth performance and selected microbial populations in weaned pigs. Br. J. Nutr. 111:1577–1585. https://doi.org/10.1017/S0007114513004224

Holdt, S. L. & S. Kraan. 2011. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 23:543–597. https://doi.org/10.1007/s10811-010-9632-5

Humam, A. M., T. C. Loh, H. L. Foo, A. A. Samsudin, N. M. Mustapha, I. Zulkifli, & W. I. Izuddin. 2019. Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals 9:644. https://doi.org/10.3390/ani9090644

Khalifah, A., S. Abdalla, M. Rageb, L. Maruccio, F. Ciani, & K. El-Sabrout. 2023. Could insect products provide a safe and sustainable feed alternative for the poultry industry? A comprehensive review. Animals 13:1534. https://doi.org/10.3390/ani13091534

Kidgell, J. T., M. Magnusson, R. de Nys, & C. R. K. Glasson. 2019. Ulvan: A systematic review of extraction, composition and function. Algal Res. 39:101–422. https://doi.org/10.1016/j.algal.2019.101422

Kim, S. K. 2011. Handbook of Marine Macroalgae: Biotechnology and Applied Phycology. Wiley-Blackwall publishing, Oxford. https://doi.org/10.1002/9781119977087

Kulshreshtha, G., M. T. Hincke, B. Prithiviraj, & A. Critchley. 2020. A review of the varied uses of macroalgae as dietary supplements in selected poultry with special reference to laying hen and broiler chickens. J. Mar. Sci. Eng. 8:536–564. https://doi.org/10.3390/jmse8070536

Lahaye, M. & A. Robic. 2007. Structure and function properties of Ulvan, a polysaccharide from green seaweeds. Biomacromolecules 8:1765–1774. https://doi.org/10.1021/bm061185q

Livak, K. J. & T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Loh, T. C. 2017. Animal Feed the way forward. University Putra Malaysia, Selangor. Inaugural lecture. http://psasir.upm.edu.my/id/eprint/66849

MacArtain, P., C. I. R. Gill, M. Brooks, R. Campbell, & I. R. Rowland. 2007. Nutritional value of edible seaweeds. Nutr. Rev. 65:535–543. https://doi.org/10.1111/j.1753-4887.2007.tb00278.x

Marcu, A., I. Vacaru-opri, G. Dumitrescu, L. Petculescu, A. Marcu, M. Nicula, I. Pe, D. Dronca, B. Kelciov, & C. Mari. 2013. The influence of genetics on economic efficiency of broiler chickens growth. Anim. Sci. Biotechnol. 46:339–346.

Matanjun, P., S. Mohamed, N. M. Mustapha, K. Muhammad, & C. H. Ming. 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J. Appl. Phycol. 20:367–373. https://doi.org/10.1007/s10811-007-9264-6

Mohammadigheisar, M., V. L. Shouldice, J. S. Sands, D. Lepp, M. S. Diarra, & E. G. Kiarie. 2020. Growth performance, breast yield, gastrointestinal ecology and plasma biochemical profile in broiler chickens fed multiple doses of a blend of red, brown and green seaweeds. Br. Poult. Sci. 61:590–598. https://doi.org/10.1080/00071668.2020.1774512

Montagne, L., J. R. Pluske, & D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95–117. https://doi.org/10.1016/S0377-8401(03)00163-9

NRC. 1994. Nutrient Requirements of Poultry. 9th rev ed. Washington (DC): National Academy Press.

Øverland, M., L. T. Mydland, & A. Skrede. 2019. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. J. Sci. Food Agric. 99:13–24. https://doi.org/10.1002/jsfa.9143

Peng, J., J. P. Yuan, C. F. Wu, & J. H. Wang. 2011. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. Mar. Drugs 9:1806–1828. https://doi.org/10.3390/md9101806

Rao, P. V. S., C. Periyasamy, K. S. Kumar, A. S. R. And, & P. Anantharaman. 2018. Seaweeds: Distribution, Production and Uses. Pages 59–78 in Bioprospecting of Algae. M. M. Noor, S. K. B. and S. K. S., ed. Society for Plant Research.

Rizk, Y. S., I. I. Ismail, S. H. A. Hafsa, A. A. Eshera, & F. A. Tawfeek. 2017. Effect of dietary green tea and dried seaweed on productive and physiological performance of laying hens during late phase of production. Egypt. Poult. Sci. J. 37:685–706. https://doi.org/10.21608/epsj.2017.7534

Sadh, P. K., S. Duhan, & J. S. Duhan. 2018. Agro-industrial wastes and their utilization using solid state fermentation: A review. Bioresour. Bioprocess. 5:1–15. https://doi.org/10.1186/s40643-017-0187-z

Short, F. J., P. Gorton, J. Wiseman, & K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221. https://doi.org/10.1016/0377-8401(95)00916-7

ShuBai, W., J. YuHui, W. LiHua, Z. FengHua, & L. YingTing. 2013. *Enteromorpha prolifera* supplemental level: Effects on laying performance, egg quality, immune function and microflora in feces of laying hens. Chinese Journal Animal Nutrition 25:1346–1352.

Soumeh, E. A., H. Mohebodini, M. Toghyani, A. Shabani, A. Ashayerizadeh, & V. Jazi. 2019. Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. Poult. Sci. 98:6797–6807. https://doi.org/10.3382/ps/pez409

Sweeney, T., H. Meredith, M. T. Ryan, V. Gath, K. Thornton, & J. V. O'Doherty. 2016. Effects of *Ascophyllum nodosum* supplementation on *Campylobacter jejuni* colonization, performance and gut health following an experimental challenge in 10 day old chicks. Innov. Food Sci. Emerg. Technol. 37:247–252. https://doi.org/10.1016/j.ifset.2016.03.016

Sweeney, T., H. Meredith, S. Vigors, M. J. McDonnell, M. Ryan, K. Thornton, & J. V. O'Doherty. 2017. Extracts of laminarin and laminarin/fucoidan from the marine macroalgal species *Laminaria digitata* improved growth rate and intestinal structure in young chicks, but does not influence *Campylobacter jejuni* colonization. Anim. Feed Sci. Technol. 232:71–79. https://doi.org/10.1016/j.anifeedsci.2017.08.001

Yan, J., J. W. Herzog, K. Tsang, C. A. Brennan, M. A. Bower, W. S. Garrett, B. R. Sartor, A. O. Aliprantis, & J. F. Charles. 2016. Gut microbiota induce IGF-1 and promote bone formation and growth. Proc. Natl. Acad. Sci. U. S. A. 113:E7554–E7563. https://doi.org/10.1073/pnas.1607235113