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Dietary Green Seaweed Compromises Overall Feed Conversion Efficiency but not Blood Parameters and Meat Quality and Stability in Broiler Chickens

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Abstract: Using seaweeds as sources of nutrients and beneficial bioactive compounds can promote sustainable production of functional poultry products. This study investigated the physiological and meat quality responses of Cobb 500 broiler chickens to graded levels of green seaweed (*Ulva sp*.) meal (SWM). Three hundred, two-week-old male chicks (159.3 ± 11.76 g live-weight) were randomly assigned to five diets formulated by diluting a standard broiler diet with SWM at 0 (SW0), 20 (SW20), 25 (SW25), 30 (SW30) and 35 g/kg (SW35). There were neither linear nor quadratic trends ($p > 0.05$) for overall feed intake, overall growth performance and carcass and meat quality traits. Overall feed conversion efficiency ($R^2 = 0.192$, $p = 0.018$) and spleen weights ($R^2 = 0.182$; $p = 0.020$) linearly declined as SWM levels increased. Linear and quadratic responses ($p > 0.05$) were observed for lymphocytes. There were linear effects for meat pH except on day 7 of storage. Meat lightness (L^*) linearly increased whereas meat redness (a^*) quadratically responded to SWM levels (day 3 of storage). While an optimum inclusion level could not be established for seaweed based on growth performance, improvements in some meat shelf life indicators were observed in the broilers reared on seaweed-containing diets.

Keywords: chicken; growth performance; hematology; meat quality; seaweed; serum biochemistry

1. Introduction

The Cobb 500 broiler is a modern commercial chicken hybrid known for high feed efficiency, fast growth rates and competitive breast meat yields at various processing stages when compared to other commercial hybrids currently produced around the world [1]. Under intensive poultry production systems, feed costs contribute close to 70% of the total production cost, which is due to increases in global feed prices. Consequently, the use of inexpensive unconventional feed ingredients has received worldwide interest [2]. Additional challenges include the ban of in-feed antibiotic growth promoters in several dozen countries, which results in the uncontrolled proliferation of pathogenic bacteria to the detriment of growth performance and product quality [3]. The presence of antibiotic residues in poultry products is undesirable for consumers who continue to demand high quality and safe foods with functional properties [4]. This has led to the search for alternative feed additives with growth-boosting and product-enhancing properties such as seaweeds. Indeed, these

marine macroalgae have been reported to exhibit growth-stimulating, antioxidant, antimicrobial and meat quality-boosting properties in an indigenous chicken strain [5].

In South Africa, about 2884 tons (wet weight) of seaweed per year [6] are produced for use as a feed supplement for abalone farms [7]. Seaweeds contain fucoidan and laminarin polysaccharides that can be utilized as novel sources of beneficial bioactive compounds and alternatives to antibiotics due to their wide range of biological effects [8]. Indeed, seaweeds have been used in animal diets as sources of protein, essential amino acids, omega-3 fatty acids, minerals, carotenoids pigments, vitamins, phenolics, phlorotannins and prebiotic substances [9,10]. Most of these phytonutrients exhibit antimicrobial, anticoagulant, anti-inflammatory, antioxidant and prebiotic properties [11]. In other studies, the inclusion of seaweeds in animal diets increases the shelf life and the keeping quality of meat products during processing and storage [12,13].

The beneficial bioactivities of seaweed phytonutrients suggest that seaweed-based diets could help prevent subclinical and clinical infections and thus improve animal performance [14]. However, for chickens, the presence of non-starch polysaccharides such as cellulose and hemicellulose in seaweeds [10] may limit their utility as feed ingredients. At high levels, cellulose and hemicellulose impair digestibility in chickens, which may interfere with the bioavailability of beneficial bioactive compounds and consequently reduce growth performance and meat quality and stability. Consequently, it is imperative that maximum tolerance levels of seaweed (*Ulva sp*.) meal (SWM) are established for each broiler strain so as not to compromise growth and meat quality traits. This study was, therefore, designed to investigate the effect of graded levels of SWM on growth performance, blood parameters, internal organs, carcass characteristics and breast meat quality and stability of Cobb 500 broiler chickens. The experiment explored the hypothesis that inclusion of SWM in broiler diets would improve feed intake, physiological responses and meat quality and stability parameters.

2. Materials and Methods

2.1. Study Site and Ingredient Sources

The study was carried out during winter season at the North-West University Farm (26°41′36″ S, 27°05′35″ E) in Mafikeng, South Africa. The seaweed was collected from Aquinion Abalone Farm (34°34′58″ S, 19°21′8″ E) in Gansbaai (Western Cape, South Africa) as described by Nhlane et al. [5]. Nutroteq (Gauteng, South Africa) supplied all the other feed ingredients used in the study.

2.2. Diet Formulation and Analyses

Five experimental diets, in mash form, were formulated using a nutritional software by Nutroteq (Guateng, South Africa) to be isonitrogenous and isoenergetic by diluting a standard broiler diet for grower and finisher phases with SWM at 0, 20, 25, 30 and 35 g/kg, as shown in Table 1.

Table 1. Gross ingredient composition (g/kg, as fed basis) of experimental diets in grower and finisher phases.

Ingredients	Grower (14–28 d)					Finisher (29–49 d)				
	SW0	**SW20**	**SW25**	**SW30**	**SW35**	**SW0**	**SW20**	**SW25**	**SW30**	**SW35**
Seaweed (*Ulva sp.*)	0	20.0	25.0	30.0	35.0	0	20.0	25.0	30.0	35.0
Yellow maize 8.0%	630.3	643.7	647.3	648.1	648.8	636.3	633.2	635.7	637.4	646.3
Extruded full fat soya	120.0	81.10	61.49	46.59	31.88	120.0	120.0	120.0	120.0	34.57
Oil Crude Soya 47%	176.6	192.8	203.0	207.5	211.9	150.6	163.4	160.0	156.8	218.1
Oil Crude Sunflower 36%	30.00	30.00	31.64	36.60	41.51	30.0	30.0	30.0	30.0	30.0
Limestone	11.94	9.66	9.07	8.48	7.89	11.16	8.82	8.26	7.70	7.05
Monocalcium phosphate	7.80	8.05	8.14	8.21	8.28	5.65	5.66	5.70	5.75	5.99
Salt-fine	2.56	0.56	0.06	0	0	2.55	0.57	0.07	0	0
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	2.82	2.88	2.88	2.90	2.92	2.08	2.27	2.29	2.32	2.29
L-Threonine	0.67	0.84	0.88	0.93	0.98	0.31	0.47	0.52	0.57	0.58

Lysine HCL	2.72	3.01	3.12	3.26	3.40	1.81	1.59	1.69	1.80	1.85
Crude soya oil mixer	7.16	0	0	0	0	15	7.18	3.827	0.76	11.39
Lignobond	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Grower premix	2.5	2.5	2.5	2.5	2.5	0	0	0	0	0
Finisher premix	0	0	0	0	0	2.0	2.0	2.0	2.0	2.0
AxtraPhy10000	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salinomycin 12%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Prime gluten 60	0	0	0	0	0	17.62	0	0	0	0
Zinc Bacitracin	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

SW0 = a standard grower or finisher diet with no seaweed meal; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet with 25 g/kg of seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal.

The experimental diets were analyzed (Table 2) for dry matter, crude protein, amino acids (AP lysine, methionine and threonine), ash, crude fiber, crude fat, metabolizable energy and minerals (calcium, phosphorus, chloride and sodium) as described by Nhlane et al. [5].

Table 2. Chemical composition (g/kg, unless stated otherwise) of experimental diets in grower and finisher phases.

	Grower (14–28 d)					**Finisher (29–49 d)**				
	SW0	**SW20**	**SW25**	**SW30**	**SW35**	**SW0**	**SW20**	**SW25**	**SW30**	**SW35**
Dry Matter	884.8	882.5	882.1	881.8	881.6	885.0	883.0	882.3	881.7	882.3
[1] ME (MJ/kg)	12.92	12.92	12.92	12.92	12.92	13.26	13.26	13.26	13.26	13.26
Crude protein	192.2	192.2	192.2	192.2	192.2	189.5	189.5	189.5	189.5	189.5
AP Lysine	10.65	10.65	10.65	10.65	10.65	9.5	9.5	9.5	9.5	9.5
AP Methionine	5.6	5.6	5.6	5.6	5.6	4.98	4.98	4.98	4.98	4.98
AP Threonine	6.9	6.9	6.9	6.9	6.9	6.5	6.5	6.5	6.5	6.5
Crude fat	56.16	43.35	40.23	37.87	35.54	64.17	56.7	53.62	50.78	46.87
Crude fiber	35.45	44.0	46.23	49.05	51.87	34.83	44.5	46.8	49.09	49.88
Ash	25.12	30.92	32.39	33.91	35.44	24	30.87	32.32	33.77	35.16
Avail. phosphorus	4.2	4.2	4.2	4.2	4.2	3.8	3.8	3.8	3.8	3.8
Calcium	8.4	8.4	8.4	8.4	8.4	7.6	7.6	7.6	7.6	7.6
Chloride	2.4	2.71	2.79	3.15	3.54	2.26	2.49	2.58	2.92	3.29
Sodium	1.8	1.8	1.8	1.97	2.16	1.8	1.8	1.8	1.96	2.16
Total phosphorus	5.48	5.46	5.46	5.46	5.47	4.95	4.97	4.97	4.97	4.94

SW0 = a standard grower or finisher diet with no seaweed meal; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet with 25 g/kg of seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal; [1] ME = metabolizable energy.

2.3. Ethics Approval Statement and Experimental Design

The experimental procedures used to rear and slaughter the chickens were reviewed and approved (NWU-00356-19-A5) by the Animal Production Research Ethics Committee of the North-West University. A total of 300-day-old male Cobb 500 broiler chicks were purchased from Montshego Chicken Farm (25°03′49″ S, 26°15′64″ E) in Zeerust, South Africa. The chicks were offered vitamins and electrolytes (stress pack) through drinking water for the first three days and were reared using a standard commercial starter diet until they were 10 days old. At day 11 of age, the birds were weighed and randomly and evenly distributed to 30 replicate pens (experimental units) measuring 3.5 m × 1.0 m × 1.85 m (L × W × H) each with sunflower husk-covered floors. The five experimental diets were randomly allocated to the 30 experimental units. The chicks were adapted to the dietary treatments until they were 13 days old. Measurements were taken from day 14 to 28 and day 29–49 for the grower and finisher phases, respectively. For the first two weeks, temperature was maintained at 35 °C using infrared electric bulbs. For the entire duration of the feeding trial, the birds had

unlimited access to the diets and clean, fresh water. Rearing was conducted under natural lighting (12 h interval).

2.4. Feed Intake and Growth Performance

Feed intake was measured daily and calculated as the difference between the feed offered and refusals collected in the morning before feeding. All the birds were weighed weekly using a weighing scale (ADAM scale, readability 0.5 g to 2 g, Adam Equipment S.A. PTY, Johannesburg, South Africa) to determine average weekly body weight gain (ABWG). Feed conversion efficiency (FCE) was calculated as weight gain divided by feed intake.

2.5. Blood Collection and Analysis

At day 47 of age, blood samples were collected from the brachial vein of two birds randomly selected from each pen using 23-gauge needles and 5 mL syringes and immediately transferred into serum and whole blood tubes. An automated IDEXX LaserCyte Hematology Analyzer (IDEXX Laboratories Inc., Gauteng, South Africa) was used to determine hematocrit, white cell count (WCC), platelets, heterophils, lymphocytes and monocytes. An automated IDEXX Vet Test Chemistry Analyzer (IDEXX Laboratories Inc., Gauteng, South Africa) was used to analyze glucose, symmetric dimethylarginine (SDMA), creatinine, albumin, lipase, blood urea nitrogen to creatinine ratio (BUN/CREA), phosphorus, calcium, total protein, gamma-glutamyl transferase (GGT) and amylase.

2.6. Slaughter Procedures, Carcass Traits and Internal Organ Weights

At day 49 of age, after fasting for 12 h to ensure emptying of the crop, all the birds were weighed to determine final body weight. After weighing, all the birds were electrically stunned and slaughtered by cutting the jugular vein with a sharp knife in a locally registered abattoir. After bleeding, the plucker machine was used to remove feathers then manually eviscerated. Carcasses per experimental unit were identified using woolen fiber of different colors tied to the drumstick. Hot carcass weight (HCW) was recorded immediately after slaughter whereas cold carcass weight (CCW) was measured 24 h post-mortem after chilling in a cold room (16 °C). Carcass yield was determined as the proportion of HCW on final body weight (BW). Weights of breast, drumstick, wing, thigh, gizzard, proventriculus, spleen, liver, duodenum, jejunum and ileum) and large intestines including caeca were measured using a weighing scale (ADAM scale, readability 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa).

2.7. Meat pH, Color and Shelf Life Determination

Breast meat pH was recorded 24 h post-mortem using a portable meat pH meter (HI98163, Hanna Instruments, Woonsocket, RI, USA) fitted with a spear-type electrode in the inner surface of the pectoralis major muscle. After every 10 measurements, the pH meter was calibrated with pH 4, pH 7 and pH 10 standard solutions provided by the supplier. Color coordinates (L^* = lightness, a^* = redness and b^* = yellowness) were determined in triplicate on the surface of the breast muscle using a Minolta color-guide (BYK-Gardener GmbH, Geretsried, Germany) that had a 20 mm diameter measurement area (aperture size) and an illuminant D65-day light. Measurements were taken using a 10° observation angle. The color guide was set and calibrated following the manufacturer prescription. For shelf life, breast meat samples from each replicate pen were used to determine stability of the meat at room temperature using pH and L^*, a^* and b^* as indicators. The samples were placed in labelled foil trays and stored for a duration of seven days and measurements were taken daily.

2.8. Cooking Loss and Meat Tenderness

Breast meat samples were pre-weighed (ADAM scale, readability 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa) and then placed in a foil plate and oven-broiled at

140 °C for 20 minutes as described by Kumanda at al. [15]. The samples were then removed from the oven and left to cool for 20 minutes. The samples were then re-weighed to obtain the cooked weight. Cooking loss was calculated as the difference between the weight of raw meat and cooked meat in proportion to the weight of the raw meat. Thereafter, the cooked breast samples were sheared using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture Analyzer (TA XT plus, Stable Micro Systems, Surrey, UK) to determine shear force (N), a measure of meat tenderness.

2.9. Water Holding Capacity and Drip Loss

Water holding capacity (WHC) was determined in duplicate breast meat samples following the filter-paper press method developed by Grau and Hamm [16]. Drip loss was determined in triplicates using breast meat sample following the method by Zhang et al. [17].

2.10. Statistical Analysis

All tested parameters were assessed for linear and quadratic effects using polynomial contrasts. To determine the optimum inclusion level of SWM, a response surface regression analysis [18] was employed. Repeatedly measured (average weekly feed intake, average body weight gain, average feed conversion efficiency and meat stability) data were analyzed using the repeated measures analysis procedure of SAS [18] to determine the interaction effect between dietary treatment and time. In a completely randomized design, overall feed intake, blood parameters, growth performance, carcass characteristics, internal organ weights and meat quality and stability data were analyzed using one-way ANOVA (GLM Proc; SAS [18]) where diet was the only factor. For all statistical tests, significance was declared at $p < 0.05$.

3. Results

3.1. Feed Intake and Physiological Responses

Repeated measures analysis revealed no week (chicken age) × diet interaction effect on average weekly feed intake (AWFI), average body weight gain (ABWG) and average feed conversion efficiency (FCE). Table 3 shows that there were neither linear nor quadratic trends ($p > 0.05$) for overall feed intake (FI), initial and final body weight as well as overall body weight gain in response to dietary SWM levels. Overall FCE linearly decreased ($y = 0.533$ (±0.014) − 0.002 (±0.0018)x; $R^2 = 0.192$, $p = 0.019$) with increasing levels of dietary SWM.

Table 3. Overall feed intake and overall growth performance of Cobb 500 broiler chickens offered seaweed meal-containing diets.

	[1] Diets					[2] SEM	*p* Value	
	CON	**SW20**	**SW25**	**SW30**	**SW35**		**Linear**	**Quadratic**
Initial BW (g/bird)	157.0	160.8	157.2	159.1	162.3	5.18	0.475	0.863
Final BW (g/bird)	1390.3	1379.1	1301.5	1339.2	1287.4	61.29	0.274	0.721
Overall FI (g/bird)	2311.8	2424.6	2313.8	2397.8	2286.0	74.75	0.846	0.277
Overall BWG (g/bird)	1233.3	1218.2	1144.3	1180.1	1125.1	61.11	0.249	0.710
Overall FCE (g:g)	0.533	0.502	0.493	0.490	0.490	0.014	0.019	0.586

[1] Diets: SW0 = a standard grower or finisher diet with no seaweed meal; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet with 25 g/kg of seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal; [2] SEM = standard error of the mean.

There were significant linear and quadratic effects for lymphocytes ($y = 0.030$ (±0.014) $x^2 - 1.329$ (±0.489) $x + 32.8$ (±3.85); $R^2 = 0.123$, $p = 0.042$) in response to dietary SWM levels (Table 4). However, neither linear nor quadratic trends ($p > 0.05$) were observed for all the other hematological and serum biochemical parameters with dietary SWM levels.

Table 4. The effects of seaweed meal-containing diets on hematological and serum biochemical parameters of Cobb 500 broiler chickens.

[3] Blood indices	[1] Diets					[2] SEM	*p* Value	
	SW0	SW20	SW25	SW30	SW35		Linear	Quadratic
Hematology								
Hematocrits (%)	32.02	32.17	31.67	31.42	33.0	0.884	0.781	0.481
Heterophils (%)	7.43	11.97	12.37	10.45	14.16	3.252	0.181	0.872
WCC (×10⁹/L)	11.03	16.27	16.75	13.66	18.36	2.898	0.124	0.759
Platelets (×10⁹/L)	7.87	10.15	10.05	8.24	11.06	2.040	0.401	0.891
Lymphocytes (×10⁹/L)	3.29	2.19	2.91	2.97	2.46	0.438	0.028	0.042
Monocytes (×10⁹/L)	1.02	1.02	0.81	0.81	0.96	0.332	0.077	0.601
Serum Biochemistry								
Glucose (mmol/L)	6.34	7.28	4.43	6.438	5.89	0.742	0.839	0.549
SDMA (μg/dL)	3.92	4.92	6.93	4.63	5.52	1.523	0.707	0.409
Creatinine (μmol/L)	24.5	31.49	33.77	24.75	54.83	11.89	0.177	0.064
Albumin (g/L)	32.42	43.3	54.58	37.25	53.8	9.836	0.866	0.954
Lipase (U/L)	3.56	5.46	4.29	3.75	5.59	0.664	0.921	0.053
BUN/CREA	27.63	14.33	23.71	27.33	24.19	5.988	0.987	0.376
Phosphorus (mmol/L)	4.13	2.55	4.37	4.13	3.62	0.485	0.681	0.359
Calcium (mmol/L)	76.82	117.4	82.58	104.5	92.58	11.56	0.132	0.484
Total protein (g/L)	97.5	58.08	94.67	83.08	88.92	10.85	0.499	0.141
GGT (U/L)	320.5	206.6	295.8	330.1	328.8	61.53	0.837	0.640
Amylase (U/L)	303.2	441.3	471.0	331.5	346.2	0.254	0.492	0.838

[1] Diets: SW0 = a standard grower or finisher diet with no seaweed meal; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet in with 25 g/kg of seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal; [2] SEM = standard error of the mean; [3] Blood indices: WCC = white cell count; SDMA = symmetric dimethylarginine; BUN/CREA = blood urea nitrogen/creatinine ratio; GGT = gamma-glutamyl transferase.

3.2. Carcass Traits and Internal Organ Weights

Table 5 shows the effect of graded levels of SWM on carcass characteristics and internal organ weights of Cobb 500 broiler chickens. There were no significant linear and quadratic effects for all carcass traits and internal organ weights with the exception of spleen weights, which linearly decreased (y = 0.24 (±0.016) − 0.000 (±0.002) x; R^2 = 0.182; p = 0.020) with SWM levels.

Table 5. The effects of seaweed meal-containing diets on carcass characteristics and internal organ weights (% HCW, unless stated otherwise) of Cobb 500 broiler chickens.

	[1] Diets					[2] SEM	*p* Value	
	SW0	SW20	SW25	SW30	SW35		Linear	Quadratic
Carcass yield (%)	69.48	71.22	70.17	69.21	71.18	1.745	0.693	0.869
[3] HCW (g)	967.3	976.9	912.4	929.3	917.9	47.79	0.378	0.748
[4] CCW (g)	952.4	957.7	895.3	913.3	889.7	47.65	0.306	0.675
Breast	21.13	23.19	20.43	21.69	23.92	1.064	0.268	0.531
Drumstick	6.41	6.62	6.13	6.43	6.87	0.270	0.514	0.353
Wing	6.12	5.81	5.61	6.11	6.27	0.252	0.879	0.067
Thigh	7.47	7.04	6.82	7.39	7.56	0.371	0.959	0.135
Gizzard	2.64	2.64	2.567	2.76	2.61	0.111	0.895	0.947
Proventriculus	0.67	0.65	0.65	0.9	0.76	0.101	0.307	0.469
Spleen	0.24	0.21	0.22	0.21	0.17	0.016	0.020	0.464
Liver	3.57	3.39	3.48	3.58	3.59	0.148	0.894	0.265
Duodenum	1.97	1.71	2.02	2.13	2.01	0.269	0.736	0.539
Jejunum	3.35	2.39	3.41	3.65	3.39	0.872	0.827	0.504
Ileum	3.75	3.08	2.14	2.97	3.12	0.825	0.419	0.444
Large intestine	0.65	1.17	0.88	0.84	0.49	0.249	0.914	0.052
Caeca	1.04	1.05	1.15	1.16	1.12	0.054	0.141	0.928

[1] Diets: SW0 = a standard grower or finisher diet with no seaweed meal; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet with 25 g/kg of

seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal; [2] SEM = standard error of the mean; [3] HCW = hot carcass weight; [4] CCW = cold carcass weight.

3.3. Meat Quality and Stability

Table 6 indicates that there were no linear and quadratic ($p > 0.05$) trends for meat pH, color, cooking loss, shear force, drip loss and water holding capacity (WHC) in response to increasing levels of dietary SWM.

Table 6. The effects of seaweed meal-containing diets on meat quality parameters of Cobb 500 broiler chickens.

	[1] Diets					[2] SEM	*p* Value	
	SW0	**SW20**	**SW25**	**SW30**	**SW35**		**Linear**	**Quadratic**
pH	6.22	6.09	6.36	6.25	6.33	0.091	0.361	0.349
*L** (lightness)	51.90	55.04	51.39	53.13	51.45	1.100	0.906	0.105
*a** (redness)	1.03	0.98	0.92	0.95	1.16	0.153	0.849	0.336
*b** (yellowness)	10.08	9.41	10.11	9.92	10.28	0.505	0.839	0.313
Cooking loss (%)	25.40	24.45	23.54	25.11	24.67	1.171	0.621	0.469
Shear force (N)	9.33	9.22	9.63	9.03	7.55	1.060	0.388	0.286
Drip loss (%)	7.87	7.45	7.93	7.58	8.55	0.667	0.671	0.342
[3] WHC (%)	12.22	12.33	16.66	12.05	13.10	0.919	0.451	0.278

[1] Diets: SW0 = a standard grower or finisher diet with no seaweed meal inclusion; SW20 = a standard grower or finisher diet with 20 g/kg of seaweed meal; SW25 = a standard grower or finisher diet with 25 g/kg of seaweed meal; SW30 = a standard grower or finisher diet with 30 g/kg of seaweed meal; and SW35 = a standard grower or finisher diet with 35 g/kg of seaweed meal; [2] SEM: Standard error of the mean; [3] WHC: Water holding capacity.

For shelf life indicators, repeated measures analysis revealed significant diet and time interaction effects on meat pH (5.43–9.18), *L** (24.4–49.7) and *a** (0.81–5.49) but not ($p > 0.05$) on *b** (8.64–17.81). Meat *L** linearly increased on day 1 ($R^2 = 0.162$, $p = 0.049$), day 2 ($R^2 = 0.184$, $p = 0.020$), day 3 ($R^2 = 0.186$, $p = 0.014$) and day 7 ($R^2 = 0.131$; $p = 0.049$) of storage in response to dietary SWM levels. There were significant linear and quadratic effects for *a** on day 3 ($R^2 = 0.125$, $p = 0.043$) in response to increasing levels of SWM. Neither linear nor quadratic trends ($p > 0.05$) were observed for *b** for the entire storage period. Linear effects ($p < 0.05$) of SWM were observed for meat pH on all days except day 7 of storage. Meat pH linearly declined ($p < 0.05$) on day 1, 2, 4 and 6 of storage but linearly increased ($p < 0.05$) on day 3 and 5 of storage in response to dietary SWM levels. Significant quadratic trends were only observed for pH on days 4, 5 and 6 of storage.

4. Discussion

4.1. Feed Intake and Physiological Responses

Seaweeds are valuable functional feed ingredients that possess several nutraceutical and growth-stimulating properties [19] that may be exploited to improve the efficiency of feed utilization and performance of broiler chickens when included in their diets. Sohail et al. [20] stated that feed intake is directly proportional to an animal's age. In this study, repeated measures analysis revealed that there was no significant interaction effect between diet and week (bird age) on average weekly feed intake, body weight gain and feed conversion efficiency, which means that experimental diet-induced variation in feed utilization and growth performance did not depend on the age of the birds. Inclusion of graded levels of dietary SWM in Cobb 500 broiler diets had no linear or quadratic effects on overall feed intake and overall body weight gain. Similar findings were reported by Abudabos et al. [21] who found that the inclusion of *Ulva lactuca* up to 30 g/kg in broiler chicken diets had no significant effect on cumulative feed intake and body weight gain. El-Deek and Brikaa [22] also

reported a lack of dietary effects on growth traits and feed intake when seaweed was included at 30 g/kg in duck diets. However, the inclusion of green seaweed showed a significant linear decrease on FCE of the chickens, which shows that the inclusion of seaweeds compromised the conversion of feed into body mass. This effect could be due to the presence of non-starch polysaccharides such as hemicellulose and cellulose in seaweeds [10]. Indeed, the crude fiber levels of the diets tended to increase as dietary levels of SWM increased. This suggests a need for pre-treatment of seaweeds using exogenous fibrolytic enzymes to improve its utilization by broiler chickens. It is important to determine the maximum inclusion level of SWM that can be incorporated in broiler diets so as not to compromise growth performance and their health status. According to Verheyen et al. [23], blood parameters are useful and effective diagnostic tools for assessing any pathophysiological changes and nutritional status of animals. Thus, to monitor the effect of seaweed inclusion on the wellbeing of the birds, hematological and serum biochemical indices were determined and used as health indicators. No significant linear and quadratic effects were observed for all the blood parameters except for lymphocytes. Nonetheless, all the blood values obtained in this study fell within the normal ranges for healthy broiler chickens as reported by several authors [24,25]. Similarly, Kulshreshtha et al. [26] reported a lack of dietary effects on blood serum concentrations of laying hens when SWM was supplemented into their diets. This illustrates that SWM did not induce any adverse effects on health and nutritional status of the chickens.

4.2. Carcass Characteristics and Internal Organ Weights

Carcass yield and weights of carcass cuts are important traits because are used when grading meat products and have a direct bearing on market prices. The inclusion of SWM in the diet of Cobb 500 broiler chickens did not compromise the measured carcass traits. The lack of dietary effect on carcass traits and dressing percentage corroborate the findings of several scholars [27,28] who reported similar effects in broiler chickens. After storage, cold carcass yield is said to be a good indicator of total edible meat, indicating that the inclusion of SWM did not reduce the amount of edible meat. With regards to internal organ weights, no changes were recorded except for spleen weights, which showed a linear decrease in response to SWM levels. It is not clear why higher inclusion levels of SWM resulted in lower spleen weights. However, the spleen weights and the size of all other visceral organs, fell within the normal range for healthy birds [15,24]. Indeed, the lack of dietary influences on the relative weights of visceral organs were in agreement with the reports by Brenes et al. [29] as well as Kulshreshtha et al. [26] who observed no variation between a control diet and diets-containing natural plants products on organ weights of broiler chickens. These results suggest that dietary seaweeds have low anti-nutritional factors that may negatively affect the gastro-intestinal tract and ancillary organs in broiler chickens.

4.3. Meat Quality and Stability

Seaweeds contain a wide range of bioactive compounds [8] that can be exploited to produce functional poultry products with health benefits for consumers and to act as natural preservatives that enhance shelf life. The quality of meat and meat products is normally associated with attributes such as pH, cooking loss, shear force, WHC, drip loss and shelf life, which intimately interacts to influence meat tenderness [30]. Results from this study revealed that SWM inclusion had no effects on meat quality parameters of the birds, which was consistent with the findings of Nhlane et al. [5], who reported a lack of dietary seaweed influences on drip loss, cooking loss and shear force values in indigenous chickens. Meat color values measured 24 h post-mortem were normal when considering the color classification guidelines by Barbut [31], further demonstrating that dietary inclusion of SWM did not affect the freshness and quality of the final product. According to Muchenje et al. [30] the ultimate pH of the meat provides an accurate indication of the extent of pH decline 24 h post-slaughter. This is said to be influenced by the amount of glycogen in meat muscle before slaughter and how fast the remaining glycogen is converted to lactic acid post-slaughter [32]. Thus, the lack of dietary effects on pH could be an indication that dietary SWM did not interfere with the

glycogen levels of the birds. The pH values found in this study were in line with the ultimate meat pH values (5.5–6.5) reported for broilers by Barbut [31]. Seaweeds have antimicrobial properties that can be exploited to prevent microbial growth and delay oxidation reactions in meat products [33]. Indeed, the inclusion of seaweed compounds in animal diets has been reported to increase shelf life during processing and storage [12]. Repeated measures analysis showed significant diet and time interaction effects on pH, L^* and a^*, indicating that dietary effects on shelf life indicators depended on length of storage time. Results showed that dietary SWM has the potential to alter lightness and redness of meat products at room temperature but not meat yellowness. This requires further research to fully understand the effect of SWM-containing diets on meat stability of broiler chickens, as color indicators play an important role on consumer perception and preference when buying meat products [30]. The pH values significantly increased after day 3 of storage, which could indicate that green seaweeds supplementation failed to maintain normal meat pH beyond three days of storage at room temperature.

5. Conclusions

We concluded that the inclusion of green seaweed in diets of broiler chickens can reduce overall feed conversion efficiency without any negative effects on blood parameters and meat quality and stability traits. An optimum inclusion level of the seaweed could not be determined but a negative linear relationship with FCE suggests that high inclusion levels of seaweed may suppress feed utilization. Therefore, pre-treatment of seaweed with exogenous fibrolytic enzymes to improve the feed value of seaweeds should be explored for broiler chickens.

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