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Effect of Different Dietary Levels of *Sargassum muticum* Powder and/or Extract on Broilers Performance and Blood Biochemistry

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Abstract

A total number of 270 chicks were used to determine the effect of using dietary Sargassum muticum and artificial antioxidants as broiler's feed supplements for enhancing growth efficiency: blood chemistry and lipid oxidation of broiler's meat Thiobarbituric Acid-Reactive Substances (TBARS). All chicks were brooded together at the first 2 days. On the third day, 15 chicks of each replicate (45 for each group)were randomly distributed into 6 experimental treatments:T_1:(Control+) received (23% crude protein) and (19% crude protein). T_2: (control$^-$): a starter diet (20% crude protein) and grower & finisher diet (16% crude protein).; T_3:T_2 + Butylated hydroxytoluene (BHT) 150 *ppm*in drinking water; T_4: T_2 + 0.5% Sargassum powder (50 g/kg feed); T_5:T_2 + 10 mL Sargassum extract/kg feed in drinking water. The results showed that highest ($P \leq 0.05$) final body weight (2515.78 g) was recorded withT_4 group (T_2 + 0.5% Sargassum powder (50 g/kg feed). Also, The group fed T_4 diet recorded the highest ($P \leq 0.05$) total body weight gain (TG) (2445.02g), and the lowest total feed intake (6373.94g) when compared with control. The best ratio of feed conversion was recorded with birds fed T_4 diet followed by those fed T_5 and T_6 being 2.7; 2.74 and 2.79, respectively. It can be concluded that the addition of dietary 0.5 % Sargassum powder/kg feed or Sargassum extract up to 15 mL Sargassum extract/kg feed.in drinking water to broiler diets has a positive effect on growth performance, lipid metabolism, immune status, blood chemistry and carcass shelf life.

Keywords: Sargassum muticum algae, broilers, feed additives, Performance, blood biochemistry.

Introduction

Because Poultry feedstuffs are costly, the poultry industry's expansion in the tropics is constrained. The majority of developing nations are located in tropical regions, and the funds needed to import the ingredients needed to feed human and livestock is lacking. To sustain poultry enterprises and increase profit margins by reducing the reliance on conventional protein sources, Research for possibilities of novel locally produced feed sources, including leaf diet, for productive animals, is warranted given the severe shortage of animal protein that currently exists in developing countries., is warranted given the acute shortage of animal protein that exists currently in developing countries (**Nworgu and Fasogbon 2007; Atawodi et al., 2008**).

In recent years, consumers have increased awareness of a healthy nutritional diet, demanding more healthy and nutritious foods with functional properties **(Granato et al., 2020).** Marine microorganisms are a very good source of a wide variety of bio compounds like proteins, carotenoids, polysaccharides, phenolic compounds, omega-3 fatty acids, minerals, and vitamins **(Agregán et al., 2017; Cikoš et al., 2018)**. These plant nutrients are responsible for many bioactivities and health benefits attributed to marine algae, such as antibacterial, antivirals, anti-inflammatory, antioxidants, antihypertensives, neuroprotective antihyperlipidemic, anticoagulants, anticancer properties and prebiotic **(Rodrigues et al., 2015; Wang et al., 2016; Gullón et al., 2020).**

Seaweed is rich in minerals and carbohydrates, while protein content varies between species **(Bjarnadóttir *et al.*, 2018; Ramine *et al.*, 2019).** *Sargassum* is rich in carbohydrates and some essential aminoacids like arginine, tryptophan, and phenylalanine and is also known for its low anti-nutrient content, i.e., the presence of substances that do not contribute nutrients to the diets of animals **(Gupta and Abu-Ghannam, 2011; Peng et al., 2012)**

It is also rich in beta-carotene and vitamins and has a high mineral content. In addition, research has shown that its inclusion in diets increased digestibility and allowed large amounts of algal nutrients to be readily available to animals on the Sargassum seaweed diet **(Gojon et al., 1998).**

Agriculture contributes 60% of the anthropogenic methane emission, with the leading source being enteric fermentation which contributes 33% (80 million tons) of total (245 million tons) agricultural emissions. Further, the production of red meat was 150% more greenhouse gas intensive than other meats **(Weber and Matthews 2008).** Therefore, Sargassum has important implications for the development of a more sustainable climate.

Some studies have described the chemical components of the Egyptian *Sargassum spp.*, which were collected from the coasts of the Red Sea, and stated that *Sargassum spp.* could be considered promising natural sources of anti-inflammatory and antioxidant compounds **(Fawzy et al., 2017; Fouda et al., 2019)**. *Sargassum muticum (S. muticum)* is brown edible algae. *S. muticum* extract (SME). It has various biological activities that include antioxidant, antimicrobial and anti-inflammatory properties **(Ad Lordan et al., 2011; Erum et al., 2017)**.

Furthermore, seaweed was used in poultry to boost their immune systems to reduce the microbial load in the digestive tract and have a beneficial effect on the quality of poultry eggs and meat **(Erum *et al.*, 2017).** So, the current study was conducted to ascertain the effect of using dietary *Sargassum muticum*and artificial antioxidants as feed supplements to enhance broiler blood chemistry, immunological condition, lipid metabolism, growth performance, lipid metabolism, immune status, blood chemistryand blood chemistryof broilers.

Materials and methods

The ELRAHMA Poultry Company farms in KOM OSHEEM, Fayoum Governorate, Egypt, were the study implementation site.All experimental procedures were carried out according to the local Experimental Animal Care Committee and approved by the ethics of the institutional committee of Environmental Studies and Research Institute (ESRI), University of Sadat City. In October 2020, the experiment was started, while November 2020 marked its conclusion.

Collection and preparation of seaweed (*Sargassum muticum)*

Sargassum muticum was collected from MarsaAllam, Red Sea, Egypt (Fig. 1), and carried straight to the lab in plastic bags with water to prevent deterioration. According to **(Azzazy et al., 2019)**, the identified algae was washed thoroughly with sterilized seawater to remove extraneous materials. The sample was shade-dried until a constant weight was obtained and ground into powder using a blender. The powdered samples were stored in airtight containers and kept in the refrigerator for later usage.

Fig. 1. *Sargassum muticum* proliferates in tropical waters. In Red Sea, *Marsa Allam,* Egypt.

Preparation of algal extracts

Seaweed powder was soaked in polar solvents, such as methanol, in a 1:3 w/v ratio and kept for 48 hrs., then, the methanolic extract was prepared. Initially, Whatman No. 1 filter paper was used to filter the extract through a Buchner funnel. Next, the extracts using water were prepared by the same method and were filtrated and then dried under pressure using a rotary vacuum evaporator at 50°C. Next, the crude extracts were weighed, and 7.5 g/100 g from methanol solvent 5.6 g/100 g of aqueous solution. Finally,the extracts were tested against antibacterial and antifungal activities (**Yuvraj et al., 2016**). Twenty grams of leaves from the plant were individually extracted with 600 ml of 90% methanol, ethanol, and diethyl ether (separately) in an ultrasonic device at room temperature, The extracts were filtered and the residues were re-percolated three times and eliminated from the solvent using a rotary evaporator and sample was preserved at 4°C until used.

Birds, Treatments, and Experimental design

A total number of 270 chicks were used from a commercial flock from DesokyCompany broiler breeder (IR Strain) at 32 weeks (wks.) of age. All one – day old body weight (37.37 ± 0.38) chicks were brooded together at the first two days. On the third day, 15 chicks of each replicate (45 for each group), were used to measure their growth performance up to 42 days of age. Birds were given unrestricted access to food and drink during the fattening period. The birds were kept in 18 floor pens (3 m2), which were covered with wood shavings as litter material. Each pen was equipped with two hanging feeders and one drinker. The lighting cycle was kept 24 hrs. perday. Chicks were vaccinated against infectious Bronchitis disease. The vaccination program was applied during the growing period, however, programmed multivitamin AD_3E were given at a dose of 1ml/L of water for a 3rd day each week.

The chicks received a starter diet till 10 d of age, a grower diet from 11 to 24 d of age, and a finisher diet from 25 to 42 d of age. The experimental diets (starter, grower, and finisher) were formulated to meet nutrient requirement of broiler chicks from 1 to 42 d according to breeder's company guideline Indiana River (2018) and experimental conditions. Tables 2 and 3 show the ingredients, chemical composition, chemical analysis of experimental and basal diets (starter, grower, and finisher). The chicks received standard broiler starter, grower, and finisher diet without the addition of antioxidants, coccidiostatics, except in group 1 (positive control). The experimental diets were as follows:

T_1:(Control+):obtained a commercial diet starter (23% crude protein) and grower &finisher diet (19% crude protein). .

T_2: (Control-): obtained a tested diet starter period(20% crude protein) and grower &finisher diet (16% crude protein).

T_3:obtained**T_2**diet+ BHT 150 *ppm* in drinking water.

T_4:obtained**T_2**diet+ 0.5% *Sargassum* powder (50 g/kg feed).

T_5:obtained**T_2**diet+ *10 ml Sargassum* algae extract /kg feedin drinking water.**,**

T_6:obtained**T_2**diet+ *15 ml Sargassum* algae extract/kg feed.in drinking water.

All experimental birds were kept under the same managerial conditions.

Data collection

Growth performance

The growth performance of broilers was evaluated by recording Initial body weight, body weight gain (BWG), live body weight (LBW), Feed intake (FI) at 1, 7, 14, 21, 28 and 35 day of age, and feed conversion ratio (FCR), and mortality rate (MR) were calculated, Feed conversion ratio (FCR), and Mortality rate (MR) were calculated for each .

Table 1. Ingredients and chemical composition of commercial diets control (+).

Ingredients	**Starter**	**Grower**	**Finisher**
Ground yellow corn (8.5%).	57.260	62.220	69.055
Soybean meal (44.0%).	33.700	24.800	18.90
Corn gluten meal	3.750	6.000	6.00
Sun flower oil.	0.600	1.300	1.200
Lime stone	1.850	1.800	1.500
Mono calcium phosphate	1.400	1.200	1.100
L- lysine	0.320	1.800	1.500
Vitamin&Mineral premix**	0.300	0.300	0.300
Sodium chloride (NaCl).	0.300	0.300	0.299
DL-Methionine	0.235	0.220	0.223
Sodium bicarbonate	0.220	0.220	0.218
L-Theronine	0.065	0.070	0.114
Total (kg)	**100.0**	**100.0**	**100.0**
Calculated diet composition:* **			
Crude protein %.	22.440	20.47	18.450
Metabolizable energy (Kcal ME/Kg).	3003	3152	3222
Lysine %.	1.280	1.200	1.060
Methionine %.	0.580	0.560	0.540
Methionine + Cysteine%.	0.960	0.910	0.870
Calcium %.	1.050	0.980	0.840
Available phosphorus %.	0.500	0.450	0.420

According to IR broiler performance guide requirements (2014) and **NRC (1994).**
** Pre-mix each 3 kg of vitamin and mixture contains: Vitamin D3 2000 I.U., Vitamin A 12000 I.U., Vit. K 34 mg, Vitamin E 40 mg, Vitamin B6 4 mg, Vitamin B2 6 mg, Vitamin B12 0.03 mg, Biotin 0.088 mg,, Niacin 30 mg, Folic acid 1.5 mg, Pantothenic acid 12 mg , Cu 10 mg, Mn 80 mg, Fe 40 mg, Se. 0.2 mg, Co . 0.25mg, Zn 70 mg, Vitamin B l 3 mg and Choline chloride 700 mg.*** Calculated according to **A.O.A.C. (2012).**

Results and Discussions

Proximate analysis of*Sargassum muticum*algae

The chemical composition of the studied algae and plants is presented in Table 2. The chemical composition showed that*Sargassum muticum*has CP; CF and ash (8.4; 17.9 and 28.1) %respectively**. Marín et al. (2009)** found thatthe chemical composition of*Sargassum* was7.7; 6.4; 0.45, and 33.3% for CP; CF; EEand ash, respectively. **Azad and Teo Zhi Xiang (2012)** found that *Sargassum sp.* contains 13.85 ;7.58;0.48;24.88, and 53.21% for CP; CF; Lipid; ash and NFE, respectively. **Muraguri (2016)** found that *Sargassum oligocystum* contains 5.64;9.40;0.46, and 13.08% for CP; CF; lipid and ash, respectively. **Arguelles et al. (2019)** also found that *Sargassum vulgare* contains 7.69;22.59; 34.18, and 27.09% for CP; CF; Carbohydrate and ash, respectively.*Sargassum* are poor in protein but is a good source of carbohydrates and of readily available minerals (**Gojon-Baez et al., 1998 and Marín et al., 2009**). *Sargassum* is rich in beta-carotene and vitamins and does not containant- nutritional factors (**Casas-Valdez et al., 2006**).

Table 2. Proximate analysis of *Sargassum muticum* powder algae.

Ingredient	DM%	Organic Matter %	Crude Protein %	Crude Fiber %	Ether Extract %	N.F.E.%	Ash%	D.E.* Kcal/kg	M.E.** Kcal/kg
Sargassum muticum (SM)	87.2	59.1	8.4	17.9	3.1	29.7	28.1	2366	2140

* Digestible Energy (DE) wascalculated according to **Cheeke (1978)**
** Metabolizable Energy (ME) calculated according to **Campbell *et al* (1983)**.

According to the information in Table 3, Sargassum muticum contains 8.7% crude protein, 11.12% crude fiber, good amounts of NDF (28.4%), ADF (20.73%), and only small amounts of lignin (4.5%) and fats (1.62%). Additionally, Sargassum muticum has high levels of complex carbohydrates and polysaccharides, as evidenced by its high Gross energy content (8.55MJ/kg). According to **(Evans and Critchley, 2014),** brown algae contain laminarin, sulphatedfucose-containing polymers, and alginates.

Table 3. Chemical Composition of *Sargassum muticum.*

Analysis	*Sargassum spp.*[(1)]	*Sargassummuticum*
Crude protein (%)	8.5 ± 1.8 (10)	8.7±1.31
Crude fiber (%)	10.1 ± 2.4 (9)	11.12±2.11
NDF (%)	29.5 ± 3.4 (5)	28.4±2.71
ADF (%)	21.3 ± 4.4 (4)	20.73±2.85
Lignin (%)	4.5 (1.0–7.9)	3.97±1.89
Ether extract (%)	1.2 ± 0.9 (9)	1.62±1.1
Ash (%)	35.9 ± 12.8 (9)	34.81±7.33
Gross energy (MJ/kg)	9.1 (8.9–9.2)	8.55±1.15

[(1)]According **to Makkar et al., (2016)**.

According to **Abu Hafsa et al. (2021),** freshwater algae have a lower mineral content than marine algae. Thus, marine algae may be a source of trace elements such as Zn, Ca, I, Co, Mg, Mn, Cd, Fe, Se and K, all of which are essential to maintain animal health. Because marine algae contain large amounts of minerals, they can be used as a mineral substitute in farm animal diets **(Evans and Critchley, 2014)**.

Makkar et al. (2016) concluded that seaweeds, especially brown algae, contain high levels of iodine and may result in iodine poisoning when consumed over extended periods of time. Additionally, they stated that although brown algae only contain minor levels of lipids (1-5% DM), most of these are polyunsaturated n-3 and n-6 fatty acids. Red and Brown seaweeds' lipids are primarily composed of 20:5 n-3 (EPA) and 20:4 n-6 (arachidonic acid). Green seaweed U. lectica and brown seaweed Darville Antarctica had lipids that were 73% and 52% unsaturated fatty acids, respectively **(Misurcová, 2012),** while red seaweed Gracillariid Changi had lipids that were 75% polyunsaturated fatty acids (33% EPA) and 26% palmitic acid **(Norziah and Ching, 2000).**

Productive performanceand carcass traits

Live body weight (g)

Results in Table 4 showed LBW values (g) as effected by dietary treatments. The initial body weight was almost equal within all groups with average of 71.49g (ranging from 71.13g to 72.2g). When compared to the control and other experimental diets, the data on growth performance at 2 and 4 weeks of age showed that the chicks given diet (T6) had the significantly greatest values (P≤0.01). For final body weight (g) the results showed that the best body weight value (2515.78 g) was notably noted using T_4. Meanwhile,T_2 group achievedthe worst body weight (1770.89g). Similar results had been observed by **Opoola et al. (2019)**. Also, **Cheong et al. (2016)** observed that the use of microalgae as a supplement has been recommended to benefit poultry involving growth, survival, feed utilization and carcass quality .Algae imply the presence of more than one group of growth–prompting substances such as (auxins, cytokinins and gibberellins) which might positively be influenced the body growth **(Michalak et al., 2016).** These results also agree with the findings obtained by **Erum et al. (2017)** who stated that birds given Sargassum muticum eventually gained more final body weight than birds fed commercial feed. They also reported that the higher the percentage of *Sargassum muticum* in the diet of birds, the greater the weight gain for the birds because *Sargassum* contains antioxidants, antimicrobials and anti-inflammatory properties.

These properties improved the performance of birds fed on *Sargassum muticum* compared to birds fed on pure commercial feed. **El-Deek et al. (2011)** concluded that broilers fed untreated *Sargassum spp.* had higher body weight compared to broilers fed *Sargassum SPP* boiled and autoclaved or treated with enzymes, but it recorded less weight than the group that fed the control ration.On the other hand, **Rossi et al. (2020)** mentioned that the final weight of the rabbit fed on control diet did not differ from that of rabbits fed on a diet containing different levels (0.3% and 0.6%) of brown seaweed and plant polyphenols even if the average daily gain tended to be higher in those groups.

Table 4. Broilers body weight as impacted by supplemented dietary levels of*Sargassum muticum* and synthetic antioxidant (Means ±SE).

Item	Control^{+}T$_1$	Control $^{-}$T$_2$	Trt.$_3$	Trt.$_4$	Trt.$_5$	Trt.$_6$	Sig
I LBW	71.13±0.72	71.44±0.72	71.58±0.72	70.76±0.72	72.2±0.72	71.80±0.72	NS
LBW W_2	410.22^{a} ±7.44	322.11^{d} ±7.44	363.33^{c} ±7.44	386.00^{b} ±7.44	411.11^{a} ±7.44	423.33^{a} ±7.44	*
LBW W_4	828.89^{cd}±14.80	839.56^{c}±14.80	886.22^{ab}±14.80	1726.9^{a}±14.80	858.33^{bc}±14.80	919.22^{a}±14.80	*
LBW W_6	2216.00^{b}±47.14	1770.89^{c}±47.14	2157.00^{b}±47.14	2515.78^{a}±47.14	2500.56^{a}±47.14	2472.56^{a}±47.14	*

*a, b, c and d = means within a row that have distinct superscripts exhibit a significant difference ($P \leq 0.05$). SE= standard error NS = not significant, ****T$_1$: (positive control):** received a commercial diet starter (23% crude protein) and grower &finisher diet (19% crude protein). ;**T$_2$: (negative control):** received a tested diet starter (20% crude protein) and grower &finisher diet (16% crude protein); **T$_3$:**received **T$_2$** diet+ BHT 150 *ppm* in drinking water.;**T$_4$:** received **T$_2$** diet+ 0.5% *Sargassum* powder (50 g/kg feed);**T$_5$**: received **T$_2$** diet+ *10 ml Sargassum* algae extract /kg feedin drinking water.**;T$_6$:** received **T$_2$** diet+ *15 ml Sargassum* algae extract/kg feed.in drinking water, *** ILBW: Initial live body Weight; LBW: Live body weight; W2, W4and W6 = meansweeks.

Also, Abaza et al. (2021) found that the final body weight was not significant differed among different experimental groups at the end of experiment. Algae are considered advantageous because they are an abundant natural supply of several nutrients, including vitamins, minerals, vital fatty acids, amino acids, and other nutrients that might accelerate growth **(Gershwin and Belay, 2008).**

Live body weight gain.

Data concern broilers live body weight gain (g) as impacted by supplemented dietary levels of *Sargassum muticum* (powder or extract) and synthetic antioxidant are illustrated in Table 5. At the second week, T_6 groups ignificantly ($P \leq 0.05$) recorded the highest increase in live body weight gain (351.53g), followed by control and T_5 groups (339.09 and 338.91 g) . At the fourth week, T_3 groupappeared similar ($P \leq 0.05$) with T_6 for achieving highest increase in live body weight (522.89g) and (495.89g)), compared with control and other tested treatments.

At the sixth week, T_4 group significantly ($P \leq 0.05$) recorded the highest increase in live body weight gain (1721.67g) compared with control, T_2,T_3 and T_5 groups being (2144.87, 1699.44,2085.42 and 2428.36g , respectively).

Results of total gain (TG) (Table 5) showed that the T_4 group significantly ($P \leq 0.05$) recorded highest increase in total live body weight gain (TG) (2445.02g), and T_6 group which significantly ($P \leq 0.05$) recorded more total live body weight gain (2400.76g) than control and other groups. This increase in liveweight and body weight gain may be due to the algae containing some polysaccharides, antioxidants and minerals that improve the environment of the gut, which improves the efficiency of digestion and boosts health of broilers.

These findings agree with what was mentioned by both **El-Banna (2003); El-Banna et al. (2005)** who reported that adding macro algae to rabbit diets increases feed efficiency through improved nutrient absorption, gut integrity, and infection resistance. Consequently, growing rabbits perform more productively in terms of body weight gain. Also, **Vidanarachchi et al. (2009)** stated that Polysaccharides found in macro algae can serve as prebiotics, enhancing animal growth and general health by having positive impacts on their digestive systems. In the same way, **Sweeney et al. (2012)** demonstrated that macro algal extracts can improve gut health and growth performance by changing the gut architecture, improving nutrient absorption and digestibility, modifying immunological function, and strengthening the gut barrier.

Table 5. Live body weight gain of broilers as affected bysupplemented dietary levels of *Sargassum muticum* (powder or extract) and synthetic antioxidant (Means ±SE).

Item	T_1	T_2	T_3	T_4	T_5	T_6	sig
G2	339.09^{a}±6.50	250.67^{d}±6.37	291.76^{c}±5.87	315.24^{b}±7.11	338.91^{a}±9.48	351.53^{a}±7.47	*
G4	418.67^{bc}±12.21	517.44^{a}±11.03	522.89^{a}±11.93	408.11^{c}±11.52	447.22^{b}±15.17	495.89^{a}±9.33	*
G6	1387.11^{c}±47.47	931.33^{d}±56.64	1270.78^{c}±40.98	1721.67^{a}±43.96	1642.22^{ab}±38.63	1553.33^{b}±56.28	*
TG	2144.87^{b}±50.36	1699.44^{c}±57.88	2085.42^{b}±38.27	2445.02^{a}±42.39	2428.36^{a}±36.67	2400.76^{a}±53.02	*

a, b, c and d = means within a row that have distinct superscripts exhibit a significant difference ($P \leq 0.05$). SE= standard error NS = not significant, ****T_1: (positive control):** received a commercial diet starter (23% crude protein) and grower &finisher diet (19% crude protein). ;**T_2: (negative control):** received a tested diet starter (20% crude protein) and grower &finisher diet (16% crude protein); **T_3:**received **T_2** diet+ BHT 150 *ppm* in drinking water.;**T_4:** received **T_2** diet+ 0.5% *Sargassum* powder (50 g/kg feed);**T_5**: received **T_2** diet+ *10 ml Sargassum* algae extract /kg feedin drinking water.**;T_6:** received **T_2** diet+ *15 ml Sargassum* algae extract/kg feed.in drinking water, ***G1, G2and G8 = meansperformance index -TG = meanstotalgain.

El-Deek et al. (2011) reported that broiler chickens fed on untreated *Sargassum SPP* with enzymes had a higher in body weight gain compared to broiler chickens fed on treated *Sargassum SPP*. **Also, Erum*et al.* (2017)** found that *Sargassum muticum* improved the final weight gain of birds and *Sargassum muticum* is preferred as an alternative to broiler feed for up to 10% and reported that birds given Sargassum muticum had greater ultimate body weights than birds fed commercial diets.

Abu Hafsa et al. (2021) found that daily weight gain was improved significantly in rabbits group fed on a diet containing 4% *Ulvalactuca* algae compared with the control group.Also data obtained are inconsistent with those stated by **El-Deek and Brikaa (2009)** who found that adding seaweed with levels of 0, 4, 8, 12% did not affect duck performance.Also **Fan et al. (2021)** found that all groups fed on the *Sargassum* meal had a numerically lower body weight gain than the control group.

Feed intake (FI).

Data of feed intake (FI) illustrated in Table 6cleared that, the T_6 group, significantlyrecordedthe lowest FI at 2;4 or 6 weeks of age compared to the control and other groups. By presenting a basal diet supplemented with BHT 150 *ppm* in drinking water), the total feed intake was greatly decreased and recorded at it's the lowest value (6373.94g), but it was observed insignificant differences between control and T_4,T_5 and T6 among total feed intake being (6660.23, 6534.38, 6610.77 and 6586.01g, respectively). These outcomes concur with the conclusions drawn by **Al-Banna et al. (2005)** who reported that control group consumed more feed than the rabbits fed on *ulvalactuca* and the group fed on *Entromorpha intestinalis*. Also, **Rossi et al. (2020)** found that average daily feed intake was lower in the group fedon diet supplemented with 0.3% of brown seaweed and plant polyphenols than the other groups.

El-Deek et al. (2012) fed brown Sargassum species from the Red Sea shore to laying hens during 20–30 weeks at 1–12% dietary level and detected it had no deleterious effect on body weight, egg weight, egg production, feed conversion ratio and egg quality. Also, **El-Deek et al. (2012)** fed Sargassum dentifolium as raw, boiled, or autoclaved at levels of 3% or 6% and observed an improvement in egg quality with decreased yolk cholesterol, triglycerides, and n-6 fatty acids but increased carotene, lutein, and zeaxanthin contents. Boiling improved high-density lipoprotein, which is a desirable trait for human health.On the other hand, obtained results are inconsistent with those mentioned by **El-Deek et al. (2011)** whoindicated that using different levels of algae in broiler diet increased feed intake comparing to control treatment. Also, **Erumet al. (2017)** found that substituting 5% Sargassum muticum increased feed consumption of birds.

Table 6. Feed intake(g)/2wksof broilers as affected bysupplemented dietary levels of Sargassum muticum (powder or extract) and synthetic antioxidant (Means ±SE).

Item	T_1	T_2	T_3	T_4	T_5	T_6	Sig
FI2	368.338^{a}±2.9	352.47^{c}±2.9	361.72^{ab}±2.9	367.60^{a}±2.9	362.90^{ab}±2.9	356.56^{bc}±2.9	*
FI4	1889.56^{a}±22.83	1661.33^{b}±22.83	1818.89^{a}±22.83	1872.33^{a}±22.83	1850.53^{a}±22.83	1866.0^{a}±22.83	*
FI6	4402.33^{a}±62.76	4033.44^{c}±62.76	4193.33^{bc}±62.76	4294.44^{ab}±62.76	4397.33^{a}±62.76	4363.4^{ab} ±62.76	*
TFI	6660.23^{a}±69.04	6047.25^{c}±69.04	6373.94^{b}±69.04	6534.38^{ab}±69.04	6610.77^{a}±69.04	6586.01^{a}±69.04	*

*a, b, c and d = means within a row that have distinct superscripts exhibit a significant difference (P≤0.05), SE= standard error NS = not significant, ****T_1: (positive control):** received a commercial diet starter (23% crude protein) and grower &finisher diet (19% crude protein). ;**T_2: (negative control):** received a tested diet starter (20% crude protein) and grower &finisher diet (16% crude protein); **T_3:**received **T_2** diet+ BHT 150 *ppm* in drinking water.;**T_4:** received **T_2** diet+ 0.5% *Sargassum* powder (50 g/kg feed);**T_5**: received **T_2** diet+ *10 ml Sargassum* algae extract /kg feedin drinking water.**;T_6:** received **T_2** diet+ *15 ml Sargassum* algae extract/kg feed.in drinking water.
***W0, W1and W8 = meansweeks - TFI= means total feed intake.

With the same trend, **Singh et al. (2017)** detected that feed intake of nutrients was similar in the control group and cow groups fed on 20% *Sargassum* in their concentrated diet. **Fan et al. (2021)** found that daily feed intake was not affected by isocaloriciso nitrogenous diets in chickens, including groups fed a ration containing *Sargassum* .Also, **Abu Hafsa et al. (2021)** reported that there were no differences in dry matter intake and feed intake was not affected when rabbits fed on a ration containing 4% algae Ulvalactuca.

Feed conversion ratio (FCR)

Data presented in Table 7 found that group T6 had significantly the best FCR values. Meanwhile,there were no significant differences among FCR values within T_4; T_5 or and T_6 groups at 6 weeks of age (2.57; 2.74 and 2.96, respectively) and when compared with other groups and control (2.70; 2.74 and 2.79, respectivily).

Based on the results of Table 7, T4 had the higher total feed conversion (g feed/g gain), followed by T5 and T6, who had respective values of 2.7, 2.74, and 2.79, while control group (T_1) achieved the worst total feed conversion ratio (3.82).

Table 7. Feed conversion ratio (FCR)of broilers as impacted by dietary supplements of Sargassum muticum and synthetic antioxidant (Means ±SE).

Object	T1	T2	T3	T4	T5	T6	sig
FCR w2	1.11^{cd}±0.04	1.46^{a}±0.04	1.27^{b}±0.04	1.19^{bc}±0.04	1.13^{cd}±0.04	1.03^{d}±0.04	*
FCR w4	4.74^{a}±0.20	3.27^{b}±0.20	3.56^{b}±0.20	4.79^{a}±0.20	4.59^{a}±0.20	3.83^{b}±0.20	*
FCR w6	3.36^{bc}±0.17	5.10^{a}±0.17	3.49^{b}±0.17	2.57^{d}±0.17	2.74^{d}±0.17	2.96^{cd}±0.17	*
TFCR	3.82^{a}±0.08	3.19^{b}±0.08	3.10^{b}±0.08	2.70^{c}±0.08	2.74^{c}±0.08	2.79^{c}±0.08	*

*a, b, c and d = means within a row that have distinct superscripts exhibit a significant difference (P≤0.05). SE= standard error NS = not significant, ****T_1:** (positive control): received a commercial diet starter (23% crude protein) and grower &finisher diet (19% crude protein). ;T_2: (**negative control**):received a tested diet starter (20% crude protein) and grower &finisher diet (16% crude protein); **T_3**:received **T_2** diet+ BHT 150 *ppm* in drinking water.;**T_4:** received **T_2** diet+ 0.5% *Sargassum* powder (50 g/kg feed);**T_5**: received **T_2** diet+ *10 ml Sargassum* algae extract /kg feedin drinking water.**;T_6:** received **T_2** diet+ *15 ml Sargassum* algae extract/kg feed.in drinking water.
***W2, W4and W6 = meansweeks -TFCR= meanstotalfeed conversion ratio.

The increased body weight gain and decreased feed intake that resulted from better conditions in the broiler's digestive tract fed natural antioxidant substances in sargassum powder may have contributed to this rise in feed conversion for these treatments (50 g/kg feed or *10 ml Sargassum* algae extract /kg feedin drinking water or *15 ml Sargassum* algae extract /kg feed.in drinking water**.**

These results agree with those obtained by **El-Banna (2003)** and **El-Banna et al. (2005)** who reported that adding seaweed supplementation to the diet of growing rabbits improved the feed conversion ratio. This improvement may have resulted from the addition of macroalgae to the diet, which enhances gut integrity, nutrient absorption, and infection resistance, all of which improve the growing rabbits' productive performance. **Rossi et al. (2020)** also reported that feed conversion ratio was positively affected (P≤0.001) in groups fed on brown seaweed and plant polyphenols with (0.3% and 0.6%). Also, **Abu Hafsa et al. (2021)** found that rabbits fed the *Ulvalactuca* diet had higherFCR may be because of the adequate amounts of Zn in the feed. On the other hand, **Fan et al. (2021)** found that the feed conversion ratio (feed intake /

egg mass) decreased in the group that was fed 5% *Sargassum* compared to the rest of the groups. **El-Deek et al. (2011)** indicated that using different levels of diet contain of *Sargassum spp.*in broiler finisher diet did not improved the FCR comparing to control treatment.

Performance index (PI %)

Results in Table 8 showed that, T_6 grouprecorded the highest performance index (PI) (42.52) at the second week, in contrast to the T1 and T4 control groups, which had the lowest performance index (PI) (38.40 and 33.77) respectively. At the fourth week, T_2 group recorded the highest PI (26.60) compared to the control and other groups.

Table 8. Performance index (PI) of broilers as impacted by dietary supplements of Sargassum muticum and synthetic antioxidant (Means ±SE**).**

Item	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**Sig**
PI2	38.40^{b}±1.34	23.45^{e}±1.34	29.81^{d}±1.34	33.77^{c}±1.34	39.44^{ab}±1.34	42.52^{a}±1.34	*
PI4	18.97^{c}±0.98	26.60^{a}±0.98	25.86^{a}±0.98	17.81^{ab}±0.98	21.95^{c}±0.98	24.82^{a}±0.98	*
PI6	72.54^{c} ±4.28	44.28^{d} ±4.28	67.11^{c} ±4.28	104.92^{a}±4.28	94.93^{ab}±4.28	91.53^{b}±4.28	*
TPI	73.46^{b} ±3.34	51.93^{c} ±3.34	71.67^{b} ±3.34	95.99^{a} ±3.34	93.09^{a} ±3.34	92.23^{a} ±3.34	*

a, b, c and d = means within a row that have distinct superscripts exhibit a significant difference (P≤0.05). SE= standard error NS = not significant ****T_1 :(positive control) :**(commercial) received a dietary 23% CP starter and 19% CP Grower and finisher without additives.**; T_2: (negative control):** received a basal diet 20% CP starter and 16% CP Grower and finisher (according to the breed requirements) without additives**; T_3 : (** received basal diet 20% CP starter and 16% CP Grower and finisher + BHT 150 *ppm* in drinking water; **T_4 :** received basal diet 20% CP starter and 16% CP Grower and finisher + 0.5% *Sargassum* powder (50 g/kg feed).; **T_5** : received basal diet 20% CP starter and 16% CP Grower and finisher + *10 ml Sargassum* algae extract /kg feedin drinking water and **T_6 :** received basal diet 20% CP starter and 16%CP in Grower and finisher + *15 ml Sargassum* algae extract /kg feed.in drinking water**,** ***PI2, PI4and PI6 = meansperformance index -TPI= meanstotalperformance index.

At the sixth week of age,T_4 group recorded the highest PI (104.92%) compared to the other groups and control. Total PI results indicated that the highest value was significant (P≤0.05) recorded for T_4,T_5 and T_6 groups (95.99, 93.09 and 92.23) compared with T_2 groupwhich recorded the lowest value (51.93). This increase possibly as a result of the feed conversion improvingas well as higher live body weight of those groups led to an increase in PI values.

Abo-Eid et al. (2019) reported that there were no significant differenceswithin all groups in performance indexand the T3 group recorded the greatest score (77.77%), while the T2 group reported the lowest value (70.16%). Additionally, the enhanced feed conversion could be the cause of the PI increase in the T3 group.

Effect of dietary levels of*Sargassum muticum*on Blood Biochemical Indices

Data of total protein (TP), albumin (Alb), globulin (Glo) and A/G ratio and levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broiler blood plasma at 6weeks of age as affected by different dietary levels Sargassum muticum are shown in Table (9). Results of TP indicated that T_6 group had significantly ($P \leq 0.05$) highest TP value (5.85 m/dl), Meanwhile,T_3 group had significantly ($P \leq 0.05$) lowest TP value (4.22 m/dl) compared to other tested groups and control but, As a result, when compared to the other tested groups and control, the birds in group (T6) had the best immunity (Table 9). Results of A/G ratio showed that (15 ml *Sargassum muticum* extract /kg diet) achieved insignificantly the lowest percent (1.21) which mean that this group had more disease resistance and immune response.

These findings concur with the findings published by **Granato et al., (2020)** who stated that globulin level has been used as an indicator of immune responses and source of antibody production and reported that bird's high globulin level and low A/G ratio indicated more disease resistance and immune response.

In the current trial, total lipid profile (Total cholesterol,) showed that dietary supplemented of Sargassum muticum extract significantly decreased total cholesterol of broilersand group fed (T_6) diet that achieved significantly ($P \leq 0.05$) the best value (99.16 mg/dl).

Kidney functions (Creatinine and Urea) and liver functions (ALT and AST) were significantly affected by both dietary supplements of *Sargassum muticum* powder or extract. Results showed that the experimental groups with (T_5) and (T_6) significantly ($P \leq 0.05$) improved kidney functions (0.40,0.51mg/dl and 3.38,3.10 mg/dl) (Creatinine and Urea compared to control and other tested groups (Table 9). Also, the same trend was observed among data of liver functions (ALT and AST) which showed that groups fed (T_4) and (T_5) diets recorded considerably ($P \leq 0.05$) lower values than the control group or other examined groups.

TBA-Reactive Substances (TBARS) of broiler's meat:Lipid oxidation:

The thigh muscle TBA-Reactive Substances (TBARS) of broilers fed the dietary treatments shown in (Table 10) (g kg-1 malonaldehyde). After 7 days of refrigeration, the results showed that there were no differences in the level of lipid oxidation (TBA number) in the meat from the thighs across all treatments. Nevertheless, following 90 days of freezer storage, the malonaldehyde concentration differed in the carcasses of the birds of (T4), (T5), and (T6), which had lower TBA numbers, whereas the birds fed the control group had the highest TBA numbers. These findings are consistent with studies **(Webb et al., 1972; Bartov& Bornstein, 1977 and Strange et al., 1977)** that found that poultry meat was more stable when treated with synthetic antioxidants. Furthermore, BHT additions to broiler meals at levels of 125 (mg/kg) for 60 days improved the oxidative stability of the fat around the chicken abdomen **(Bartov and Bornstein, 1981).**

Table 9. Blood chemistry; lipids profile; kidney functions and liver functions of broilers as affected by different levels of dietary Sargassum muticum (Means ± SE).

Item	Treatments**						Sig.
	Control T_1 (+)	T_2 (-)	T_3	T_4	T_5	T_6	
Total Protein	5.29^{ab}±0.665	5.40^{ab}±0.69	4.22^{b} ± 0.150	4.61^{ab} ±0.44	5.08ab±0.27	5.85a±0.35	*
Albumin (A)	3.29±0.51	2.986±0.62	2.35 ±0.17	2.62 ±0.409	2.99 ±0.34	2.96 ±0.72	NS
Globulin	1.99 ±0.57	2.41±0.29	1.87±0.23	1.99 ±0.14	2.08±0.11	2.89 ±0.58	NS
A/G ratio	1.97±0.70	1.26±0.279	1.32±0.27	1.32±0.21	1.45 ±0.22	1.21±0.52	NS
Total cholesterol mg/dl	116.47^{b} ±1.785	190.61^{a}±4.57	186.72^{a}± 3.43	112.07^{b}±1.34	116.45^{b}±3.23	99.16^{c} ± 5.65	*
Triglycerides mg/dl	65.33^{ab}±1.218	68.126^{ab}±2.179	71.70^{a}±4.14	59.31^{bc}±2.60	52.01^{cd}±4.09	47.50^{d}±1.83	*
Creatinine mg/dl	0.70^{a} ±0.04	0.56^{ab}±0.02	0.71^{a}±0.09	0.69^{a} ±0.08	0.40^{b}±0.02	0.51^{ab} ±0.09	*
Ureamg/dl	5.34^{a} ± 0.62	4.99^{ab}± 0.58	3.83b^{c} ± 0.18	3.64^{c}± 0.27	3.38^{c}± 0.16	3.10c ± 0.45	*
AST U/L	193.28^{a} ±2.26	166.52^{bc} ±5.239	181.25^{ab} ±4.11	150.15^{d} ±7.48	151.06^{cd} ±2.905	154.20^{cd}±5.51	*
ALT U/L	57.05^{a} ± 0.21	60.36^{a}±2.316	63.5^{a} ±0.56	57.19^{a} ± 1.15	54.30^{a}±6.52	42.75^{b}±0.97	*
Total antioxidant capacity	0.672^{c}±0.00	0.55^{d}±0.01	0.673^{c}±0.004	0.741^{b}±0.027	0.76^{b}±0.01	0.86a±0.04	*

a, b, c…. means within a row that have distinct superscripts exhibit a significant difference ($P \leq 0.05$). SE= standard error NS = not significant **T_1 T_1: (positive control): commercial diet 23% CP starter and 19% CP grower and finisher without additives; T_2: (negative control): basal diet 20% CP starter and 16% CP grower and finisher (according to strain requirements) without additives; T_3: basal diet 20% CP starter and 16% CP grower and finisher + BHT 150ppm in drinking water; T_4: basal diet 20% CP starter and 16% CPgrower and finisher + 0.5% *Sargassum* powder (5 g/kg feed); T_5: basal diet 20% CP starter and 16% CP grower and finisher + 10 ml *Sargassum* algae extract/kg feedin drinking water and T_6: basal diet 20% CP starter and 16%CP grower and finisher + 15 ml *Sargassum* algae extract/kg feedin drinking water.

Also, Phenolic antioxidant is less well known but improved stability of vegetable oils under storage **(Pinkowski et al., 1986). Mohdaly et al. (2010)** reported that sugar beet pulp (ethanolic extract) is apowerful natural antioxidants source concluded that predominant acids (ferulic, gentisic and p-coumaric acid) explored inhibited oxidation of storage vegetable oils. In this regard **(Cinzia Castelluccio et al., 1996)** reported that ferulic acid is a more effective antioxidant against LDL oxidation than the hydrophilic antioxidant ascorbic acid**.)**

Lin et al. (1989) used BHA/BHT 12.5 mg/chick/day from the 3rd week till last 5 days before slaughter and chickens had a major weight increase over the control group. The meat cooled up to 9 days at 4 ºC and then frozen for up to 6 months at -20 ºC. Data showed a better oxidative stability in cooled (4 ºC) and frozen meat (<-18 ºC), in addition to a significant increase in weight gain compared to chicks fed with the control diet with a slight content of TBARS.

Table 10. Broiler meat Reactive Substances (TBARS) (g kg-1 malonaldehyde) as affected by different levels of dietary *Sargassum muticum* (Means ± SE).

Item		Treatment						Sig.
		Control+ T1	Control⁻ T2	T3	T4	T5	T6	
Days after storage	7	0.48±0.02	0.470±0.215	0.455±0.015	0.453±0.04	0.470±0.08	0.456±0.01	NS
	90	0.80^{a}±0.03	0.71^{ab}±0.232	0.649^{b}±0.049	0.509^{c}±0.031	0.473^{c}±0.03	0.436^{c}±0.03	*

a, b,c means within a row that have distinct superscripts exhibit a significant difference (P≤0.05). NS = not significant, ****T₁**: (positive control): commercial diet 23% CP starter and 19% CP grower and finisher without additives; **T_2**: (negative control): basal diet 20% CP starter and 16% CP grower and finisher (according to strain requirements) without additives; **T_3**: basal diet 20% CP starter and 16% CP grower and finisher + BHT 150ppm in drinking water; **T_4**: basal diet 20% CP starter and 16% CPgrower and finisher + 0.5% *Sargassum* powder (5 g/kg feed); **T_5**: basal diet 20% CP starter and 16% CP grower and finisher + 10 ml *Sargassum* algae extract/kg feedin drinking water and **T_6**: basal diet 20% CP starter and 16%CP grower and finisher + 15 ml *Sargassum* algae extract/kg feedin drinking water**.**

Conclusions

It can be concluded that the addition ofdietary 0.5 % Sargassum powder in feedor Sargassum extract up to 15 mlperkg feed.in drinking waterof broiler chickens diets had positive effects on growth performance, lipid metabolism, immune status, blood chemistryand carcass meat shelf life.

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