Nutritional potential of marine and freshwater algae as dietary supplements for growing rabbits

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ABSTRACT
The aim of this study was to investigate the effect of including marine and freshwater algae in rabbit diets on their performance, digestibility, carcass characteristics, and blood metabolites. One hundred growing male rabbits, aged 30 d and weighing 783.5 ± 2.01 g initial body weight, were randomly assigned to five treatment groups (\(n = 20\) rabbits). Five comparable dietary groups were formulated as follows: the control diet was a basal diet without aquatic plants inclusion, while the diets for the other four treatments included the basal diet plus 4\% of marine algae (\textit{U. lactuca} and \textit{P. capillacea}) or freshwater algae (\textit{S. polyrrhiza} and \textit{C. aegagropila}). Compared with the control group, final body weight, daily weight gain, and feed conversion ratio improved significantly in \textit{UL} group; followed by those in \textit{PC} and \textit{SP} groups (\(p < 0.05\)). Total digestible nutrients and digestible crude protein increased significantly in \textit{UL}, \textit{PC} and \textit{SP} groups. Rabbits in the \textit{CA} group had lower nitrogen intake, N digested, and N balance decreased than the other groups (\(p < 0.05\)). The \textit{UL} group had dressing weight and meat protein significant increases, but the \textit{SP} and \textit{CA} groups had significant increases in liver, kidney, and spleen weights (\(p < 0.05\)). Including marine and freshwater algae reduced total lipids, total bilirubin, triglycerides, and cholesterol especially in the \textit{UL} and \textit{PC} groups, and similarly, HDL and LDL levels were lower in the \textit{UL}, \textit{PC}, and \textit{SP} groups compared to the \textit{CA} and control groups. In conclusion, the responses in performance, digestibility, and blood metabolites suggest that aquatic plants (with the exception of \textit{CA}) have the potential to be a sustainable feedstock for growing rabbits’ diets.

HIGHLIGHTS
- \textit{Ulva lactuca}, \textit{Pterocladia capillacea} and \textit{Spirodela polyrhiza} improve rabbits’ performance.
- Marine and freshwater algae inclusion showed no signs of disease.
- Marine and freshwater algae reduced total lipids, triglycerides, and cholesterol.

Introduction
Aquatic plants have spread through Egypt’s coastal areas and are considered an environmental hazard. Marine algae inclusions have recently been used as an unconventional source of animal nutrition to address feed shortages in developing countries, and are considered a promising sustainable alternative to traditional animal feed supplies (Verland et al. 2019). Using algae for protein production has many advantages over using conventional high-protein crops in terms of both efficiency and nutritional value. Algae has a higher protein yield per unit area (2.5–7.5 tons/ha/year) when compared to terrestrial crops, like soybean, legumes, and wheat (0.6–1.2, 1–2, and 1.1 tons/ha/year, respectively) (Van Krimpen et al. 2013). In this context, macroalgae are nutritionally interesting from multiple perspectives, with proteins, sulfur-containing amino acids, vitamins A, B, and C, thiamine and Riboflavin; \(\beta\)-carotene, tocopherols (Burtin 2003), and mineral contents up to 30–39\% of DM (Balboa et al. 2015). They are also natural sources of long-chain polyunsaturated essential fatty acids, particularly \textit{n}3 and \textit{n}6, which can enhance the productivity of livestock (Madeira et al. 2017) and poultry (Abdelnour et al. 2019). Microalgae may make an important contribution to animal nutrition due to their high content of...
bioactive molecules (Makkar et al. 2016). Duckweed is high in macro and microelements, which a protein content of 6.8–45% DM and low fibre content (Cheng and Stomp 2009). Freshwater Cladophora species and their use as a natural source of nutrients have been the subject of a few studies. Cladophora glomerata (L.) Kütz contains a variety of primary metabolites, including protein, carbohydrates, glycosides, vitamins (A, C, E, B1, and B2), minerals (Khalid et al. 2012) and unsaturated and saturated fatty acids (Horincar et al. 2014), as well as secondary metabolites, such as carotenoids (Khuantrairong and Traichaiyaporn 2012), phenolic compounds (Soltani et al. 2011), which make these algae a very useful raw material for the nutritional application. In addition, including microalgae in animal feeds can enhance the growth performance, immunity, antioxidant indices, and meat quality of poultry (Madeira et al. 2017; Abdelnour et al. 2019), as well as the efficient performance of growing rabbits (El-Banna 2003; El-Banna et al. 2005). Furthermore, including microalgae in rabbits’ diet does not cause pathological alterations in blood albumin and haemoglobin (Okab et al. 2013). In light of recent findings, there has been renewed interest in macroalgae biomass as a potentially sustainable feedstock for the production of monogastric livestock feed ingredients. The aim of this study was to compare the performance, digestion, and carcass and blood biochemistry characteristics of growing NZW rabbits after incorporating different types of marine and freshwater algae to their feed rations.

Materials and methods

The present study was performed at the Rabbit Research Laboratory, Noubaria experimental station, Animal Production Research Institute, the Agricultural Research Centre, in accordance with the guidelines approved by the Institutional Care and Use Committees (Protocol No. 26-IU-0321) of the city of Scientific Research and Technological Applications, Alexandria, Egypt.

Collection and preparation of aquatic plants

In this study, four types of microalgae were used, two marine algae Ulva lactuca and Pterocladia capillacea, and two freshwater algae Spirodela polyrrhiza and Cladophora aegagropila. Marine algae were hand-picked from submerged rocks in Abu Qir Bay, the Mediterranean Sea coast of Alexandria, Egypt, freshwater algae Spirodela polyrrhiza and Cladophora aegagropila were collected from irrigation canals in Al-Noubaria Province, Egypt (approx. 60 km from Alexandria City). Immediately after collection, the macroalgae were washed and rinsed three times in freshwater to remove sand, debris and other extraneous matter attached to the thalli before being sundried for 2 to 3 d. The dried samples were ground into a fine powder and placed in airtight bags for further chemical analyses.

Animals, feeding, and management

One hundred growing male New Zealand White (NZW) rabbits, aged 30 d and weighing 783.5 ± 2.01 g initial body weight, were randomly assigned to five treatment groups (n = 20 rabbits), each group subdivided into five replicates of four rabbits each. Five comparable dietary treatment groups were formulated as follows: the control diet was a basal diet without aquatic plants inclusion, while the diets for the other four treatments included the basal diet plus 4% of marine macroalgae (U. lactuca and P. capillacea) or freshwater algae (S. polyrrhiza and C. aegagropila) per kg diet. Rabbits were housed in double flat galvanised wire batteries (50 × 50 × 40 cm) and maintained under the same managerial, hygienic, and environmental conditions in rooms with standard air conditioning, with an ambient temperature 23 ± 2 °C, humidity 55–65% and a photoperiod of 16 L:8 D. All cages were equipped with feeding hoppers and drinking nipples. Feed and freshwater were available at all times throughout the experiment period that lasted 90 d. A conventional concentrate rabbit ration with a crude protein (CP) content of 17% was formulated to cover the nutrient requirements of the rabbits according to (De Blas and Mateos 2010). The mineral and chemical compositions of the four aquatic plants are listed in Table 1. The formulation and chemical composition of the experimental diets are presented in Table 2.

Growth performance

During the experiment, body weight (BW) was recorded weekly, while dry matter intake (DMI) was recorded daily. The daily weight gain (DWG) and feed conversion ratio (FCR) were calculated, and the mortality rate was recorded as the number of dead animals in each group.

Digestibility trials

A digestibility trial was performed to determine the nutrient digestibility coefficients of the experimental
rations according to (Perez et al. 1995). Five rabbits from each treatment were housed individually in metabolic cages and fed the experimental rations for 7 d (preliminary period) for adaptation. Then faeces were collected every 24 h prior to feeding in the morning for 5 consecutive days (collection period).

### Table 1. Chemical composition and mineral content of the aquatic plants.

|               | U. lactuca | P. capillacea | S. polyrrhiza | C. aegagropila |
|---------------|------------|---------------|---------------|---------------|
| Organic matter | 81.670     | 77.460        | 74.290        | 73.420        |
| Crude protein  | 21.050     | 17.660        | 18.380        | 10.440        |
| Crude fibre    | 9.880      | 16.870        | 9.550         | 23.110        |
| Ether extract  | 3.180      | 2.780         | 2.320         | 2.010         |
| NFE            | 47.560     | 40.150        | 44.040        | 37.860        |
| Ash            | 18.330     | 22.540        | 25.710        | 26.580        |
| NDF            | 38.440     | 40.330        | 40.740        | 43.640        |
| ADF            | 24.280     | 25.950        | 29.680        | 32.880        |
| ADL            | 7.360      | 7.930         | 7.880         | 8.540         |
| Hemicellulose  | 14.160     | 14.380        | 11.060        | 10.760        |
| Cellulose      | 16.920     | 18.020        | 21.800        | 24.340        |
| Minerals composition, mg/kg | | | | |
| Sodium         | 193.800    | 203.900       | 96.770        | 93.570        |
| Potassium      | 96.900     | 92.100        | 69.660        | 61.870        |
| Calcium        | 72.400     | 68.300        | 43.650        | 37.620        |
| Magnesium      | 200.100    | 190.300       | 88.940        | 84.960        |
| Major Anions, mg/kg | | | | |
| Phosphorus     | 306.400    | 292.900       | 218.660       | 188.680       |
| Iodine         | 188.900    | 162.700       | 79.300        | 56.650        |
| Minor Cations, mg/kg | | | | |
| Lead           | 0.052      | 0.090         | 0.100         | 0.100         |
| Cadmium        | 0.029      | 0.041         | 0.075         | 0.08          |
| Iron           | 2.060      | 2.430         | 1.970         | 1.990         |
| Cobalt         | 0.100      | 0.140         | 0.150         | 0.170         |
| Manganese      | 0.080      | 0.090         | 0.100         | 0.100         |
| Selenium       | 1.110      | 1.020         | 1.000         | 1.010         |
| Zinc           | 0.840      | 0.580         | 0.660         | 0.620         |

NFE: nitrogen free extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

### Table 2. Formulation and chemical proximate composition of the experimental diets.

| Ingredients, % | Control | UL | PC | SP | CA |
|----------------|---------|----|----|----|----|
| Alfalfa hay    | 33.950  | 31.950 | 31.950 | 31.950 | 30.950 |
| Aquatic plants | 0.000   | 4.000  | 4.000 | 4.000 | 4.000 |
| Corn           | 13.000  | 12.500 | 12.000 | 12.000 | 11.000 |
| Barley grain   | 20.000  | 20.000 | 20.000 | 20.000 | 20.000 |
| Wheat bran     | 7.000   | 7.000  | 7.000 | 7.000 | 7.000 |
| Soybean meal   | 20.000  | 18.500 | 19.000 | 19.000 | 21.000 |
| Molasses       | 3.000   | 3.000  | 3.000 | 3.000 | 3.000 |
| Lime stone     | 1.500   | 1.500  | 1.500 | 1.500 | 1.500 |
| Calcium diphosphate | 0.700 | 0.700  | 0.700 | 0.700 | 0.700 |
| Sodium chloride | 0.500  | 0.500  | 0.500 | 0.500 | 0.500 |
| DL-methionine  | 0.050   | 0.050  | 0.050 | 0.050 | 0.050 |
| Premix\(^*\)   | 0.300   | 0.300  | 0.300 | 0.300 | 0.300 |
| Total          | 100     | 100    | 100  | 100  | 100  |

Chemical analysis, % as DM

|               | Control | UL | PC | SP | CA |
|---------------|---------|----|----|----|----|
| Dry matter    | 89.450  | 87.880 | 87.690 | 87.560 | 87.240 |
| Organic matter| 92.270  | 91.880 | 91.630 | 89.470 | 87.260 |
| Crude protein | 17.160  | 17.080 | 17.100 | 17.220 | 17.250 |
| Crude fibre   | 14.460  | 13.960 | 14.260 | 13.310 | 14.520 |
| Ether extract | 3.210   | 3.110  | 3.050  | 3.100  | 2.990  |
| NFE           | 57.440  | 57.730 | 57.220 | 55.840 | 52.500 |
| DE ( MJ/kg)   | 11.080  | 11.470 | 11.330 | 10.940 | 10.260 |
| Ash           | 7.730   | 8.120  | 8.370  | 10.530 | 14.740 |
| NDF fibre     | 36.750  | 37.960 | 38.370 | 36.470 | 40.620 |
| ADF           | 18.950  | 19.640 | 20.940 | 20.880 | 22.330 |
| ADL           | 5.040   | 5.180  | 5.340  | 5.060  | 5.950  |

UL: U. lactuca; PC: P. capillacea; SP: S. polyrrhiza; CA: C. aegagropila.

\(^*\)Each kg contained: VA 6,000,000 IU; VD3 1,250,000 IU; VE 15,000 mg; VB1 1000 mg; VB6 1000 mg; VB12 6 mg; nicotinic acid 15,000 mg; pantothenic acid 5000 mg; biotin 50 mg; folic acid 500 mg and choline chloride 500 mg; Mn 35 mg; Fe 40 mg; Cu 3.5 mg; Zn 25 mg; iodine 0.25 mg; selenium 0.075 mg; cobalt 0.10 mg; CaCO3 1000.

NFE: Nitrogen free extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: Acid detergent lignin. NFE % = 100 %: (%EE + %CP + %Ash + %CF).
Daily faecal samples were collected from each rabbit, oven-dried at 70 °C for 48 h, then was ground and stored for proximate chemical analysis. Samples of feed and dried faeces were stored for routine analyses according to Association of Official Analytical Chemists (AOAC) (2005). In addition, 10% of the urine collected daily from each animal was preserved for nitrogen determination.

**Carcass characteristics and meat composition**
At the end of the feeding trial, five rabbits per treatment were euthanized by cervical dislocation after fasting for 12 h. The dressing percentage (slaughter weight/body weight) was calculated, while the total edible offal (giblets) was weighed, including the liver, heart, kidneys, and spleen.

**Chemical analyses**
Samples of the feed, faeces and meat were finely ground through a 1-mm screen in a Cyclotec mill (Cyclotec 1093; Foss, Germany) and stored prior to chemical analysis. Moisture content was determined in dried samples in an oven at 70 °C to a constant weight. The CP content (N 6.25) was determined according to Kjeldahl’s method (Method No. 978.04) (AOAC 2005). Ether extract was determined according to the Soxhlet extract method using petroleum ether as an extracting agent (40-60 °C) (Method No. 930.09) (Association of Official Analytical Chemists (AOAC) 2005). Ash content was assayed by incinerating the samples in a muffle furnace at 550 °C (Method No. 905.01) (Association of Official Analytical Chemists (AOAC) 2005). Hemicellulose (HC) and cellulose (C) were calculated according to the following equations:

\[
HC(\%) = NDF(\%) - ADF(\%)
\]

and

\[
C(\%) = ADF(\%) - ADL(\%),
\]

The nutritive values expressed in terms of total digestible nutrient (TDN) and digestible crude protein (DCP) in the experimental ratios were calculated according to Cheeke et al. (1982). Mineral elements such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), lead (Pb), cadmium (Cd), iron (Fe), cobalt (Co), manganese (Mn), and zinc (Zn) were determined by dissolving samples in 1 M HNO₃ and H₂O₂ before being digested by a microwave. The concentrations of the elements in the dried seaweed samples were determined using an atomic absorption spectrophotometer (Unicam 919; Unicam Ltd., Cambridge, UK) while P was determined colorimetrically using molybdovanadate reagent. Iodine (I) was determined according to (Nitschke and Stengel 2015). Selenium (Se) was determined by an inductively coupled plasma–mass spectrometer (ICP-MS) (Lavu et al. 2013).

**Hematological and biochemical analyses**
At the end of the experiment, blood samples collected from 5 rabbits were equally allocated and dispersed into heparinised and non-heparinised tubes for hematological and biochemical analyses. The heparinised blood samples were used to determine haemoglobin (Hb), white blood cells (WBCs) and red blood cells (RBCs). The non-heparinised blood samples were immediately centrifuged at 3000 rpm for 15 min and the plasma was separated and preserved at −20 °C for biochemical analyses to determine total protein, albumin, glucose, total lipids, triglyceride, total bilirubin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein HDL, very low-density lipoprotein (VLDL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Blood bio-chemicals were determined calorimetrically using standard kits supplied by (Bio-diagnostic, Cairo, Egypt) based on the procedure outlined by the manufacturer.

**Statistical analysis**
Statistical analysis was carried out using the General Linear Models Procedure by (SAS 2005) and simple one-way analysis of variance. Duncan’s new multiple range test (Duncan, 1955) was applied to separated differences among treatment means. The following model used was:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]

where \(Y_{ij}\) refers to the observed dependent variable, \(\mu\) refers to the overall mean, \(T_i\) is the main effect of \(i^{th}\) different types of algae, and \(e_{ij}\) is the random residual error. Comparisons with \(p < .05\) were considered significant, and all statements of statistical differences were based on this level unless otherwise noted.
Results

Aquatic plant composition

The chemical and mineral compositions of the four aquatic plant species are shown Table 1. The marine algae *U. lactuca* and *P. capillacea* had a similar dry biomass content, which differed from that of the freshwater algae *S. polyrrhiza* and *C. aegagropila*. *U. lactuca* had the highest content of CP and EE, but low content of CF and ash. *C. aegagropila* was found to have the highest ash content. The two marine algae had higher mineral contents than the freshwater algae. Furthermore, *U. lactuca* biomass had the highest concentrations of K, Ca, Mg, P, Se, Zn, and I despite having low levels of Co and Mn.

Growth performance

Rabbits fed the UL diet had the highest final body weight (FBW) and daily weight gain (DWG), followed by those fed the PC and SP diets (*p* < .05) (Table 3). Rabbits fed the CA diet had the lowest FBW and DWG. Rabbits that received CA diet consumed less feed, while DMI did not vary significantly between the UL, PC, SP, and control groups. The FCR was the best in rabbits fed the UL diet, followed by those fed the PC and SP diets. No mortality rate was found in the UL and PC groups.

Digestibility coefficients

The nutrient digestibility of rabbits fed UL ration was higher (*p* < .05) than that in other groups, with no significant differences between the UL and PC groups in DM, OM, NFE, NDF, and ADF digestibilities (Table 4). There were no significant differences in nutrient digestibility of DM, CP, CF, EE, and NDF between the PC, SP and control groups. While, the lowest nutrient digestibilities were found for rabbits fed CA ration, except for NFE. The UL group had the highest TDN and DCP contents, while the CA group had the lowest TDN and DCP contents (*p* < .05). Rabbits fed the CA ration had significantly lower (*p* < .05) N intake, N digested, and N balance than the other treatments.

Table 3. Growth performance of growing rabbits fed on the experimental diets.

| Item                      | Experimental groups | SEM | p Value |
|---------------------------|---------------------|-----|---------|
| Item                      | Control             | UL  | PC     | SP      | CA     | SEM | p Value |
| Initial body weight, g    | 782.3               | 782.3| 783.0  | 785.0   | 784.3  | 16.27 | .846    |
| Final body weight, g      | 2758.800           | 3313.700| 3033.700| 2900.300| 2520.300| 42.660 | .001    |
| Daily weight gain, g      | 21.960              | 28.130| 25.010  | 23.500  | 19.290  | 0.350 | .001    |
| Dry matter intake, g      | 91.470              | 89.890| 90.210  | 88.470  | 81.470  | 4.190 | .004    |
| Feed conversion ratio     | 4.170               | 3.200 | 3.610   | 3.760   | 4.220   | 0.210 | .017    |
| Mortality, %              | 0.000               | 0.000| 0.000   | 5.000   | 5.000   | 0.730 | .006    |

The least square mean values with different superscript letters in the same row are significantly different at *p* < .05. SEM: standard error of mean.

UL: *U. lactuca*; PC – *P. capillacea*; SP: *S. polyrrhiza*; CA: *C. aegagropila*.

Table 4. Digestibility coefficients, nutritive values, and nitrogen balance of growing rabbits fed on the experimental diets.

| Item                      | Experimental groups | SEM | p Value |
|---------------------------|---------------------|-----|---------|
| Digestibility coefficients, % | Control             | UL  | PC     | SP      | CA     | SEM | p Value |
| Dry matter                | 62.160              | 63.890| 63.310  | 62.530  | 60.460  | 0.510 | .017    |
| Organic matter            | 62.820              | 64.090| 63.540  | 62.670  | 60.520  | 0.680 | .014    |
| Crude protein             | 63.380              | 64.660| 63.450  | 62.990  | 59.160  | 0.730 | .003    |
| Crude fibre               | 46.920              | 49.970| 46.680  | 46.260  | 41.070  | 0.670 | .001    |
| Ether extract             | 71.030              | 73.780| 70.610  | 71.230  | 65.900  | 1.410 | .011    |
| NFE                       | 65.070              | 67.030| 67.310  | 65.970  | 66.080  | 0.310 | .026    |
| NDF                       | 56.850              | 58.930| 57.440  | 56.870  | 52.250  | 1.570 | .001    |
| ADF                       | 49.720              | 51.660| 50.830  | 49.680  | 44.150  | 0.830 | .001    |
| Nutritive value, %        | 59.750              | 61.860| 61.090  | 58.980  | 55.340  | 0.830 | .031    |
| DCP                       | 16.650              | 11.010| 10.830  | 10.840  | 10.170  | 0.220 | .029    |
| Nitrogen utilisation:     |                     |     |         |         |         |      |         |
| Nf                        | 4.420               | 4.450| 4.430   | 4.430   | 3.920   | 0.040 | .001    |
| ND                        | 2.720               | 2.880| 2.820   | 2.800   | 2.270   | 0.120 | .017    |
| NB                        | 1.870               | 2.080| 2.030   | 1.970   | 1.440   | 0.050 | .001    |

The least square mean values with different superscript letters in the same row are significantly different at *p* < .05. SEM: standard error of mean.

UL: *U. lactuca*; PC – *P. capillacea*; SP: *S. polyrrhiza*; CA: *C. aegagropila*; NFE: nitrogen free extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; TDN: total digestible nutrients; DCP: digestible crude protein; Nf: nitrogen intake; ND: nitrogen digested; NB: nitrogen balance.
Although there were no significant differences between the UL, PC, and SP, and control groups.

**Carcass characteristics and meat composition**

Carcass weight was similar between the UL, PC, and control treatments \((p > .05)\), with those treatments had the highest carcass weights compared to those fed with CA ration that being characterised the lowest carcass weight \((p < .05)\). While dressing percentage weight was significantly higher \((p < .05)\) for rabbits in the UL group than those in the other treatments. The UL group had the lowest \((p < .05)\) relative weights of the liver, kidney, and spleen, while the CA group had the highest \((p < .05)\) relative weights of the liver, kidney, and spleen. There were no significant differences in relative heart weight among the treatment groups.

Rabbits fed CA ration had higher \((p < .05)\) moisture, EE, and ash contents than the other groups. The group UL had the highest \((p < .05)\) CP content but the lowest EE content.

**Hematological and biochemical parameters**

No significant differences were detected in WBCs between the treated groups and the control, while the UL and PC groups had the lowest \((p < .05)\) WBCs counts. The CA group had the lowest \((p < .05)\) RBCs counts and Hb concentration than in the other groups. Total protein and albumin levels did not differ between the UL, PC, SP and control treatments, with those treatments had the highest \((p < .05)\) levels of total protein and albumin compared to those fed with CA ration. The concentrations of glucose,

### Table 5. Effect of experimental diets on growing rabbit carcass characteristics and body composition.

| Item              | Control     | Marine algae | Freshwater algae | SEM  | p Value |
|-------------------|-------------|--------------|------------------|------|---------|
| Carcass, g        | 1760.500a   | 1781.500a    | 1769.750a        | 1674.250b | 1530.500c | 61.060 | .019 |
| Dressing, %       | 69.300b     | 70.400a      | 69.950b          | 66.680b | 61.700d  | 0.210  | .001 |
| Edible giblets    |             |              |                  |      |         |        |      |
| Liver, g          | 63.530c     | 61.760d      | 63.690c          | 66.850b | 74.820a  | 0.580  | .010 |
| Kidney, g         | 22.640c     | 22.320d      | 22.780c          | 24.610b | 26.470a  | 0.360  | .010 |
| Heart, g          | 16.110      | 15.570       | 15.980           | 16.320 | 16.770   | 0.620  | .834 |
| Spleen, g         | 1.110a      | 1.030b       | 1.100            | 1.180b  | 1.230    | 0.020  | .001 |
| Chemical composition of meat | | | | | | |
| Moisture          | 70.150b     | 70.780b      | 69.980b          | 70.350b | 72.860a  | 1.060  | .015 |
| Crude protein     | 5.210c      | 4.410d       | 4.920            | 5.180b  | 5.510a   | 0.090  | .021 |
| Ash               | 1.490a      | 1.520b       | 1.570            | 1.710b  | 1.880a   | 0.030  | .001 |

The least square mean values with different superscript letters in the same row are significantly different at \(p < .05\). SEM: standard error of mean.

UL: *U. lactuca*; PC: *P. capillacea*; SP: *S. polyrrhiza*; CA: *C. aegagropila*.

### Table 6. Hematological and biochemical parameters of growing rabbits fed on the experimental diets.

| Item                | Control     | Marine algae | Freshwater algae | SEM  | p Value |
|---------------------|-------------|--------------|------------------|------|---------|
| WBCs, \(\times 10^6/\text{mm}^3\) | 3.260b      | 3.010b       | 3.080b           | 3.390ab | 3.490a   | 0.230  | .031 |
| RBCs, \(\times 10^6/\text{mm}^3\) | 5.410b      | 5.740a       | 5.710a           | 5.610b  | 5.170c   | 0.140  | .025 |
| Hb, mg/dl           | 13.630b     | 14.220a      | 14.200a          | 13.840b  | 12.590d  | 0.340  | .019 |
| Total protein, g/dl | 7.420a      | 7.890a       | 7.550a           | 7.440a   | 6.820b   | 0.490  | .026 |
| Albumin, g/dl       | 4.610a      | 4.640a       | 4.550b           | 4.490a   | 4.170b   | 0.120  | .021 |
| Glucose, mg/dl      | 78.310b     | 73.140c      | 74.070c          | 78.530b  | 80.330a  | 0.740  | .001 |
| Total lipids (g/dl) | 3.370b      | 3.120b       | 3.310c           | 3.640b   | 3.910a   | 0.210  | .017 |
| Total bilirubin, mg/dl | 0.710b | 0.640c       | 0.670c           | 0.750b   | 0.890a   | 0.030  | .006 |
| Triglyceride, mg/dl | 75.550b     | 72.740c      | 73.060a          | 75.750b  | 79.740a  | 0.490  | .001 |
| Cholesterol, mg/dl  | 84.410b     | 78.330c      | 79.880c          | 84.240b  | 86.520c  | 0.740  | .001 |
| LDL, mg/dl          | 23.440a     | 21.380c      | 21.740c          | 22.990b  | 23.410b  | 0.250  | .019 |
| vLDL, mg/dl         | 144.850c    | 193.630a     | 189.600b         | 175.520b  | 156.100c  | 2.480  | .009 |
| AST, IU/L           | 26.490      | 27.880       | 27.190           | 26.890   | 28.270   | 1.780  | .839 |
| ALT, IU/L           | 30.680      | 31.440       | 30.880           | 31.020   | 31.870   | 1.270  | .765 |

The least square mean values with different superscript letters in the same row are significantly different at \(p < .05\). SEM: standard error of mean.

UL: *U. lactuca*; PC: *P. capillacea*; SP: *S. polyrrhiza*; CA: *C. aegagropila*.

WBCs: white blood cells; RBCs: red blood cells; Hb: haemoglobin; HDL: high density lipoprotein; LDL: low density lipoprotein; vLDL: very low density lipoprotein; AST: aspartate amino transferase; ALT and alanine amino transferase.
bilirubin, triglyceride, and cholesterol in the UL and PC groups were significantly lower ($p < .05$) than in the SP, CA and control groups. Although total lipids levels were higher ($p < .05$) in SP and CA groups than in the other groups. The concentration of HDL and LDL decreased ($p < .05$), while vLDL concentration increased ($p < .05$) in UL, PC and SP groups. There were no significant differences observed in AST and ALT activities due to the dietary treatments.

Discussion

The present results revealed that marine algae have a higher mineral content as compared to freshwater algae. This suggests that macroalgae may be a source of trace elements like Ca, Zn, I, Co, Mg, Mn, Cd, Se, Fe and K, all of which are necessary for the maintenance of animal health. Because of their high mineral content, marine macroalgae have traditionally been used as a mineral substitute for farm animals (Evans and Critchley 2014). Despite the fact that macroalgae are high in nutrients including I, Ca, K, P, Mg, Fe, and Zn, less is known regarding their bioavailability (Evans and Critchley 2014; Makkar et al. 2016; Wells et al. 2017). Large amounts of heavy metals can accumulate in macroalgae, and high levels of Pb, Cd, and other heavy metals in some species limit their use in animal feeds. The bioavailability of these metals is critical in evaluating the toxicity risk (Almela et al. 2006; Smith et al. 2010), and the levels of available heavy metals in many macroalgal species are naturally below the food and feed safety limits (Holdt and Kraan 2011). A further important consideration is that the low bioavailability of an undesirable component means high levels will be excreted in manure, which in turn will be applied to field crops. Mineral content varies greatly among macroalgae species, and many other factors, such as season and environmental conditions, can have an effect (Holdt and Kraan 2011; Wells et al. 2017). We believe that an increase in essential amino acids, especially sulfur-containing amino acids, is responsible for the significant improvement in growth performance observed in NZW rabbits fed the UL ration. Macroalgae have notable nutritional values due to their high levels of diverse proteins and sulfur-containing amino acids (Burtin 2003), and thus could serve as a possible alternative source of nutrients for poultry (Al-Harthi and El-Deek 2012). Furthermore, marine algae are a good source of iodine, and the results in Table 1 indicated that *U. lactuca* and *P. capillacea* have the ability to accumulate high levels of iodine, which is an essential element for thyroid function and health (Circuncisão et al. 2018). The positive effects of *U. lactuca* and *P. capillacea* on rabbit FBW, BWG, and FCR may be due to the adequate amounts of Zn and I in the feed. Zinc is necessary for animal growth because it affects a variety of functions, including gene expression, DNA and protein synthesis, the synthesis of structural proteins such as collagen and keratin, the development of bone and tissue, reproduction, resistance to oxidative stress, and immune system function (Sun et al. 2011; Sloup et al. 2017). Macroalgae also contain bioactive amino acids and peptides such as taurine, carnosine, and glutathione (Holdt and Kraan 2011), that could be used as functional health-promoting constituents in animal feeds (Garcia-Vaquero and Hayes 2016). It has also been suggested that macroalgae bioactive components could function as alternatives to antibiotics in feed because of their health and growth-promoting properties (Lee et al. 2017; Seedevi et al. 2017). The poor growth performance of rabbits fed the CA ration could be attributed to a decrease in nutrient digestibility associated with the high level of CF (23.11%) in this ration compared to the UL (9.88%), PC (16.87%), and SP (9.55%) rations as shown in Table 4. It has been reported that including macroalgae in rabbit diets improves feed efficiency by enhancing gut integrity, nutrient absorption, and resistance to infections, which consequently improves the productive performance of growing rabbits in terms of BWG, FCR, and diet digestibility (El-Banna 2003; El-Banna et al. 2005). The present results are consistent with those obtained for laying hens (Rizk et al. 2017) and laying quails (Abu Hafsa et al. 2019), in that both previous studies reported an improvement in productive performance associated with dietary inclusion of marine seaweeds. The improved FCR of rabbits fed diets containing the algae *U. lactuca, P. capillacea, and S. polyrrhiza*, which is reflected in the increased growth rate of the rabbits, maybe due to the improvements in rabbit nutrient digestibility observed in the current study. According to Sweeney et al. (2012), macroalgal extracts can enhance growth performance and gut health by altering gut architecture, increasing nutrient digestibility and absorption, and altering the gut microbiota and/or modulating immune function, and thus strengthening gut barrier function . Polysaccharides found in macroalgae can function as prebiotics, promoting growth and improving overall health in animals through beneficial effects on their digestive tracts (Vidanarachchi et al. 2009). Macroalgae carbohydrates, in the form of polysaccharides, have a high digestibility, so their use in dried feed is unrestricted (Becker
El-Banna et al. (2005) found that adding 1% *U. lactuca* as a feed additive to the diets of growing Baladi rabbits improved diet digestibility. The relative weights of the liver, kidney, and spleen all decreased when *U. lactuca* was including the diet. This result may be due to variations in the amounts of oligosaccharides among the different treatments, as oligosaccharide-rich seaweed enhances immune status, growth performance, and gut microflora (O’Sullivan et al. 2010). Furthermore, Chermiti et al. (2009) observed a decline in carcass adiposity when up to 30% dried *Ulva* species was included in rabbit diets. However, the rabbits’ liver, kidney, and spleen relative weights were significantly higher in the CA group than the other groups. An increase in the liver relative weight could be explained in terms of fat synthesis (Carew et al. 2003). The low-fat percentage recorded in the meat of UL and PC rabbits may be due to the presence of the soluble fibre fraction, which is consistent with the results of total blood lipid and cholesterol analyses (Table 6). This result is also in line with findings of Carvallo et al. (2009) in rats, who observed that the soluble fraction of *U. fasciata* fibre was responsible for the hypocholesterolemic effect observed. A further reason for the low fat content is that *U. lactuca* has a high composition of polyunsaturated fatty acids, particularly omega 3 and omega 6 fatty acids (Wahbeh 1997). The results of hematological analyses revealed significant increases in the number of RBCs and Hb concentration, while WBCs decreased significantly when rabbits were fed the UL and PC diets, followed by the SP diet. The increases in RBCs and Hb concentration were related to an increase in O2 carrying capacity accompanied by an increase in respiratory rate. Notable hematological changes play an important role in modifying animal functions, particularly during the growth stage. Kang et al. (2013) showed that broilers fed 1% fresh liquid *Chlorella* had a significantly higher number of WBCs than those fed 1% *Chlorella* powder. However, incorporating *U. lactuca* into the diet of rabbits has been found to be unrelated to any pathological alterations in haemoglobin concentration (Okab et al. 2013). Recent studies have shown that seaweed possesses health-promoting nutrients and phytochemicals with good antioxidant and cholesterol-lowering properties (Patarra et al. 2011). In the present study, the consumption of macroalgae decreased blood lipid and cholesterol levels. It appears that the ability of macroalgae polysaccharides to lower serum cholesterol levels is due to their ability to disperse in water, retain cholesterol and other physiologically active compounds, and inhibit lipid absorption in the gastrointestinal tract (Jiménez-Escrig and Sánchez-Muniz 2000). The present results are in line with those of Abudabos et al. (2013), who found that serum total lipid and cholesterol concentrations were significantly lower in a group that received *U. lactuca* compared to the control group. The incorporation of different levels of brown algae (*Sargassum spp.*) in broiler finisher diets was found to have a significant effect on HDL and LDL values (El-Deek et al. 2011). This mechanism is most likely connected to the activity of the seaweed polysaccharide fucoidan, which decreases cholesterol absorption and increases its excretion while also modulating reverse cholesterol transport-related protein expression (Yang et al. 2019). Vizzarri et al. (2017) observed a cholesterol-lowering effect in rabbit-fed plant extract containing polyphenols. In the present study, the activity of liver enzymes AST and ALT did not alter pathologically in response to macroalgae inclusion. Abudabos et al. (2013) reported that ALT activity was significantly lower in the groups treated with 1% and 3% of *U. lactuca* compared to those in the control group. Therefore, a 4% proportion of aquatic plants could be safely incorporated into the diet of growing rabbits, with the potential to control the lipid metabolism cycle as well. Sulphated polysaccharides, which are specific to marine algae and not in freshwater algae (Suárez 2019), are structurally analogous to animal glycosaminoglycans, like heparin, which explains their high reactivity and specific biological activities when fed to animals. In vitro, Sulphated polysaccharides have an excellent antioxidant function, including both radical-scavenging capacity and metal-chelating abilities (Wang et al. 2016).

**Conclusions**

Growing rabbits fed a diet containing 4.0% of marine algae *U. lactuca* and *P. capillacea* as well as freshwater algae *S. polyrrhiza* perform better. These results suggest that these macroalgae can be used as unconventional dietary components to improve rabbit production and health without affecting their performance or blood characteristics. However, further research is required to determine the most effective algal sources and the optimum inclusion level for feeding rabbits.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
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