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The effect of brown seaweed and polyphenol supplementation in male rabbits on the blood profile and antioxidant markers

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Abstract: Currently, in animal nutrition, the replacement of synthetic substances with natural ones was expected to improve animal health. The aim of the present study was to evaluate the effects of a dietary brown seaweed and plant polyphenol mixture in adult male rabbits on the haematological profile and antioxidant markers. Twenty-four adult male rabbits were divided into three experimental groups receiving a control diet (C) or diets supplemented with 0.3% (T1) and 0.6% (T2) of a feed additive containing brown seaweed (*Laminaria* spp.) and plant extracts of seaweed origin. The trial lasted for 90 days. A lower potassium concentration was observed at 30 days in the T2 group, compared with the T1 and C groups. An increase in the antioxidant status was observed ($P < 0.05$) from day 60 of the trial in the rabbits fed diets with an algae-polyphenolic supplement (T1 and T2 groups). Concluding, the diet supplementation of brown seaweed and polyphenol stimulates the antioxidant status of the blood, however, it does not affect the haematological profile.

Keywords: algae; antioxidants; biochemical analysis; haematology; rabbit

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Nowadays, animal welfare is key issue for a sustainable livestock production system. In fact, the authorities have asked for the lowered use of antimicrobials for problems related to antibiotic resistance, and trace elements for problems related to the residue in the environment (Singer et al. 2016).

Therefore, the use of natural additives has great potential in replacing synthetic substances in feeding animals. In literature, a search for dietary sustainable additives was started to find the ones with antimicrobial, antioxidant, anti-inflammatory, immunostimulant and prebiotic functions. It is important also to identify the optimal dosage of the dietary supplementation of the natural extract to avoid problems related to the feed palatability or the overconsumption that leads to "antioxidative stress", with negative effects on the organism (Corino and Rossi 2021), several studies on natural extracts from plants in animal feed have been performed in order to find an alternative to the synthetic substances able to sustain the growth performance, animal health and product quality (Dalle Zotte et al. 2016; Mahfuz et al. 2021; Tsiplakou et al. 2021).

A natural extract mixture was used to support the reproductive performances, enhancing the antioxidant status in does and to sustain the growth performance and meat quality parameters in growing rabbits (Rossi et al. 2020; Vizzarri et al. 2020). The type of natural substances and dosage should be carefully considered in order to avoid problems with some parameters (Zbynovska et al. 2016; Kovacikova et al. 2019). Moreover, it is important to test the natural extract dosage in male rabbits that are fundamental for the reproductive traits, influencing the fertility and prolificacy (Vizzarri et al. 2019). Algae contain a high amount of polyphenols that act as an antioxidant, antimicrobial, anti-inflammatory and immunostimulant (Valenzuela-Grijalva et al. 2017).

Particularly, brown seaweed is characterised by a high content of sulfated polysaccharides, phlorotannins, diterpenes, minerals and vitamins (Corino et al. 2019). The extract of these plants, containing prebiotic polysaccharides from brown seaweed (*Laminaria* spp.) plus phenolic acid, hydroxycinnamic acids, tannins, and flavonoids from plant extracts, was successfully used in the nutrition of rabbit does and broilers (Rossi et al. 2020; Vizzarri et al. 2020), which also improved the antioxidant status of the semen in male rabbits (Vizzarri et al. 2021).

Considering these data, it could be hypothesised that dietary supplementation with a brown seaweed and polyphenol mixture should synergistically influence the blood profile and antioxidant markers.

MATERIAL AND METHODS

Animals and design of the experiment

All the experimental procedures were conducted in accordance with European Community guidelines No. 86/609/EEC regarding the protection of animals for experimental purpose.

All the experimental procedures involving animals were approved by the National Agricultural and Food Centre ethical committee (No. NPPC 18-10-2016).

Adult New Zealand male rabbits (n = 24) were provided by the National Agricultural and Food Centre, Nitra (Slovak Republic). The animals were placed in separate cages that were equipped by a feeder and automatic watering system. The entire experiment lasted for 90 days. The environmental conditions in the rabbitry were 16 h of light and 8 h of dark per day (maximal intensity being 80 lux), an air temperature of 20–24 °C and 65% humidity.

The rabbits were randomly selected and divided into three groups homogeneous for age (18.5 ± 1.5 months) and body weight (4.90 ± 0.87 kg), and then they received a control diet (C) or diets supplemented with 0.3% (T1) and 0.6% (T2) of the feed additive containing prebiotic polysaccharides from brown seaweed (*Laminaria* spp.) and plant extracts containing phenolic acid, hydroxycinnamic acids, tannins, and flavonoids originating from brown seaweed. The dosage of feed additive was chosen based on a previous experiment (Vizzarri et al. 2019; Knizatova et al. 2021; Vizzarri et al. 2021).

The feed additive was included in the mashed diets, then the diets were pelleted. The rabbits were fed *ad libitum*. There was no adaptation period set for the rabbits in the experiment, the sample collections were performed throughout the entire experiment since the start of the supplementation.

The ingredients and the chemical composition of the experimental diets are reported in Table 1. All the analyses on the experimental diets were performed in accordance with the methods of the Association of Analytical Chemists (AOAC 2000).

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Table 1. Ingredients and chemical composition of the experimental diets (g/kg)

Ingredients	Experimental diet		
	C	T1	T2
Maize	282	279	276
Alfalfa hay	305	305	305
Sunflower meal	135	135	135
Palm seed oil	8	8	8
Soybean oil	7	7	7
Wheat	80	80	80
Cane molasses	20	20	20
Carob bean meal	90	90	90
Oat	53	53	53
Calcium carbonate	7	7	7
Sodium chloride	3	3	3
Dicalcium phosphate	2	2	2
DL-Methionine (99%)	2.5	2.5	2.5
L-Lysine HCl (78.5%)	1.6	1.6	1.6
Choline (75%)	1.4	1.4	1.4
Vitamin and mineral premix*	2.5	2.5	2.5
Dietary supplement	0	3	6
Chemical composition[1]			
Crude protein	184	183.6	183.5
Ether extract	35.7	35.5	35.5
Crude fibre	187	186.8	187
Ash	86	85.7	85.8
Nitrogen free extract	507	507.1	506.9
NDF	302.1	301.5	301.7
ADF	195.8	195.4	195.3
ADL	39.9	39.5	39.5

*Supplied per kg diet: 13 500 IU vitamin A (*trans*-retinyl acetate); 800 IU vitamin D3 (cholecalciferol); 35 mg vitamin E (α-tocopherol min 91%), 35 mg copper (cupric sulfate pentahydrate); [1]Analyses determined in triplicate
ADF = acid detergent fiber; ADL = acid detergent lignin; C = control group; NDF = neutral detergent fiber; T1 = group supplemented with 0.3% of brown seaweed and plant polyphenols; T2 = group supplemented with 0.6% of brown seaweed and plant polyphenols

The extract used in this experiment is based on the addition of natural substances into a standard feed and fulfils the nutrition requirements of an organism with the purpose of improving the physiological functions and natural immunity response of an organism (Vizzarri et al. 2019).

Table 2. Composition of the feed additive

Compounds	Dry weight (mg/kg)
Phenolic acids	
Dihydroxybenzoic acid	≤ LOD
Syringic acid	1 059.79 ± 62.82
Hydroxycinnamic acids	
Neochlorogenic acid	7 979.23 ± 468.11
Rosmarinic acid	126.54 ± 8.67
trans-sinapic acid	105.54 ± 8.09
Chlorogenic acid	21.45 ± 3.65
Tannins	
Ellagic acid	2 440.88 ± 148.29
Rutin	272.37 ± 20.82
Flavonoids	
Myricetin	53.88 ± 5.68
Kaempferol	≤ LOD

LOD = limit of detection

High-performance liquid chromatography (HPLC) with a diode-array detector (DAD) – HPLC-DAD was used to identify and quantify the natural compounds of the dietary supplement (Russo et al. 2019; Table 2).

Four main phyto-derivate families were identified, such as phenolic acid, with syringic acid as the most represented; a hydroxycinnamic acid group, with neochlorogenic acid as the most abundant; a tannin class, with ellagic acid as the most present; and a flavonoid group, with rutin as the most represented.

Blood sampling

Blood samples were collected into two separate tubes (one with heparin to prevent the blood from coagulating and a second one for the serum) from all the rabbits at day 0 (D0), day 30 (D30), day 60 (D60) and day 90 (D90) of the experimental trial from the ear marginal vein using a common (animal fixation) blood sampling technique (Parasuraman et al. 2010).

The samples were mixed and placed into a thermobox, and afterwards transported to the laboratory where the blood was centrifuged at 1 006 × *g* for 15 min, and the blood serum was stored at –80 °C.

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Analysis of the haematological profile

A fully automatic Abacus Vet5 (Diatron Mi Ltd., Budapest, Hungary) haematological analyser was used to measure the haematological profile. The following haematological variables were analysed: total leukocyte count (WBC, 10^9/l), total lymphocyte count (LYM, 10^9/l), total granulocyte count (GRA, 10^9/l), lymphocyte percent (LYM, %), total monocyte count (MON, 10^9/l), granulocyte percent (GRA, %), total erythrocyte count (RBC, 10^{12}/l), haemoglobin (HGB, g/l), haematocrit (HCT, %), average erythrocyte volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/l), red cell distribution width (RDWc, %) (Massanyi et al. 2020).

Analysis of the blood serum and antioxidant markers

Determined serum variables: magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na), potassium (K), chlorides (Cl), total proteins, urea, cholesterol, triacylglycerols (TAG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).

The urea, uric acid, albumins, Ca, P, Mg, AST, ALT, cholesterol, and TAG were measured using DiaSys commercial kits (Diagnostic Systems GmbH, Holzheim, Germany) on a Randox RX Monza analyser (Crumlin, United Kingdom) and an EasyLyte Plus analyser (Medica Corporation, Bedford, MA, USA). Selected antioxidant markers and additional variables, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), ferric ion reducing antioxidant power (FRAP), total oxidative status (TOS), and albumins, were analysed.

The SOD activity was assessed using a Randox RANSOD commercial kit (Randox Laboratories, Crumlin, Great Britain) employing xanthine and xanthine oxidase (XO) to generate the superoxide radicals, which will react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was subsequently measured by the inhibition degree of the reaction at 505 nm using a Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The results are expressed as IU/mg protein. The glutathione peroxidase (GPx) activity was evaluated using a Randox Ransel commercial kit (Randox Laboratories, Crumlin, Great Britain), applying the method of Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase (Gr) and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidised glutathione is subsequently converted to a reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in the absorbance was measured using a Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 340 nm. The GPx activity is expressed as IU/mg protein.

The protein concentration was assessed using a DiaSys Total Protein (DiaSys, Holzheim, Germany) commercial kit and a semi-automated Microlab 300 Clinical Chemistry Photometric Analyser (Merck, Darmstadt, Germany). The measurement is based on the biuret method, according to which copper sulfate reacts with proteins to form a violet blue colour complex in an alkaline solution, and the intensity of the colour is directly proportional to the protein concentration when measured at 540 nm (Tvrda et al. 2016).

Analysis for FRAP was performed according to the method proposed by (Benzie and Strain 1996). The test determines the total antioxidant power, based on the reduction of a ferric-tripyridyl triazine complex to its ferrous coloured form in the presence of antioxidants. The FRAP reagent contains 10 mmol/l of a TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mmol/l of HCl (Centralchem, Bratislava, Slovak Republic) plus 5 ml of 20 mmol/l of $FeCl_3$ (Centralchem, Bratislava, Slovak Republic) and 50 ml of a 0.3 mol/l acetate buffer (pH = 3.6; Centralchem, Bratislava, Slovak Republic). Aliquots of a 100 µl sample were mixed with 3 ml of the FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm for 4 min using a Genesys 10 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The MDA analysis was based on the reaction between the MDA and thiobarbituric acid (TBA) and executed using an enzyme-linked immunosorbent assay (ELISA) kit for MDA detection (Bevan et al. 2003).

Statistical analysis

A statistical analysis was carried out using the program GraphPad Prism v6.1 (for Windows; GraphPad Software, La Jolla, CA, USA; www.graph-

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pad.com). After assessing whether the frequency distribution assumed normality with the Shapiro-Wilk test, the data on the biochemical markers and antioxidant status were submitted to a repeated-measures analysis of variance (ANOVA) to assess the main effects of the treatment and time and their interaction. The rabbit was considered the experimental unit for all the measured variables. The data were reported as means ± pooled SEM. The differences were considered statistically significant at a level of $P < 0.05$.

RESULTS

Analysis of the haematological profile

The red blood cells indices are reported in Table 3. All the considered variables, such as the RBC, HGB, HCT, MCV, MCH, MCHC and RDWc did not present significant differences ($P > 0.05$) in relation to the dietary treatment and sampling time. All the obtained results are in line with the normal range of values for the rabbit species.

All the white blood cells indices, such as the WBC, GRA, LYM, GRA (%), LYM (%), and MON did not show any statistical changes ($P > 0.05$) among the groups after the dietary treatment during the whole experiment. All the obtained results are in line with the normal range of values for the rabbit species (Melillo 2007; Pavlik et al. 2008; Leineweber et al. 2018) (Table 4).

The other haematological variables on the platelets [total platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc)] did not show any influence ($P > 0.05$) due to the dietary treatment with the natural extract mixtures and sampling time (Table 5).

Analysis of the serum profile

A significant decrease ($P < 0.05$) in the potassium level was detected in the T2 group than the T1 group at 30 days of the experiment. However, this difference was not observed for the other sampling time. The other analysed variables, such as the calcium, magnesium, phosphorus, sodium and chlorides did not show any differences ($P > 0.05$) among the experimental groups (Table 6).

Table 3. Red blood cell variables in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Total erythrocyte count; RBC (10^{12}/l)							
D0	6.82	7.22	7.05	0.199	ns	ns	ns
D30	6.57	7.38	7.18	0.190			
D60	6.79	7.21	7.16	0.201			
D90	7.09	7.22	7.34	0.193			
Haemoglobin; HGB (g/l)							
D0	127.10	133.30	128.80	2.713	ns	ns	ns
D30	122.20	134.70	131.00	2.866			
D60	126.10	129.50	129.20	2.194			
D90	129.90	130.80	132.30	2.174			
Haematocrit; HCT (%)							
D0	41.24	42.65	40.62	0.492	–	–	–
D30	39.95	43.70	43.78	0.993	–	–	–
D60	41.06	42.14	42.62	0.835	–	–	–
D90	42.84	42.78	44.13	0.561	ns	ns	ns
Average erythrocyte volume; MCV (fl)							
D0	60.62	59.13	57.65	1.264	ns	ns	ns
D30	60.77	59.30	61.05	1.313			
D60	60.41	58.61	59.69	0.983			
D90	60.51	59.32	60.21	1.184			
Mean corpuscular haemoglobin; MCH (pg)							
D0	18.69	18.47	18.27	0.193	ns	ns	ns
D30	18.60	18.25	18.27	0.340			
D60	18.56	18.00	18.08	0.407			
D90	18.34	18.13	18.07	0.348			
Mean corpuscular haemoglobin concentration; MCHC (g/l)							
D0	308.40	312.50	317.00	2.443	ns	ns	ns
D30	306.40	308.00	299.40	3.651			
D60	307.10	307.20	303.00	1.106			
D90	303.00	305.80	300.00	4.828			
Red cell distribution width; RDWc (%)							
D0	13.93	13.83	13.89	0.185	–	–	–
D30	14.05	14.53	13.70	0.209			
D60	14.10	14.08	13.47	0.166			
D90	14.83	14.75	13.91	0.249			

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

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Table 4. White blood cell variables in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Total leukocyte count; WBC (10^9/l)							
D0	11.00	10.21	10.62	1.007	ns	ns	ns
D30	10.72	9.90	9.17	1.142			
D60	8.74	9.28	8.69	0.416			
D90	10.19	10.58	9.08	1.173			
Total granulocyte count; GRA (10^9/l)							
D0	7.80	5.95	5.21	1.052	ns	ns	ns
D30	6.90	4.88	4.02	0.954			
D60	5.59	4.47	3.26	1.288			
D90	7.20	7.80	4.99	0.992			
Total lymphocyte count; LYM (10^9/l)							
D0	2.32	5.66	3.40	0.606	ns	ns	ns
D30	3.47	4.37	4.52	0.566			
D60	2.84	4.21	5.03	0.611			
D90	2.24	2.07	4.38	0.527			
Granulocyte percentage; GRA (%)							
D0	68.71	54.40	50.61	5.147	ns	ns	ns
D30	59.77	48.61	41.73	5.430			
D60	61.56	46.53	57.44	7.774			
D90	78.49	66.21	60.12	5.946			
Lymphocyte percentage; LYM (%)							
D0	43.81	57.37	45.73	6.415	ns	ns	ns
D30	36.72	44.61	51.49	5.093			
D60	46.61	46.60	57.87	6.559			
D90	44.35	47.07	52.38	5.134			
Total monocyte count; MON (10^9/l)							
D0	0.88	0.66	0.57	0.093	ns	ns	ns
D30	0.35	0.65	0.63	0.091			
D60	0.31	0.60	0.41	0.104			
D90	0.75	0.71	0.71	0.088			

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

Table 5. Platelet variables in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Total platelet count; PLT (10^9/l)							
D0	418.20	224.20	286.50	66.58	ns	ns	ns
D30	307.00	289.00	239.90	51.40			
D60	296.60	373.30	344.80	63.01			
D90	365.20	276.20	263.90	61.88			
Platelet percentage; PCT (%)							
D0	0.25	0.14	0.17	0.031	ns	ns	ns
D30	0.18	0.18	0.14	0.031			
D60	0.19	0.23	0.20	0.024			
D90	0.22	0.17	0.15	0.036			
Mean platelet volume; MPV (fl)							
D0	5.99	6.14	6.11	0.164	ns	ns	ns
D30	5.89	6.15	5.99	0.094			
D60	6.50	6.09	5.84	0.081			
D90	6.07	6.16	5.86	0.073			
Platelet distribution width; PDWc (%)							
D0	31.71	31.36	31.21	0.214	ns	ns	ns
D30	31.91	32.48	32.08	0.489			
D60	32.30	33.05	31.15	0.374			
D90	32.03	31.71	31.15	0.516			

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

The dietary treatments with the different dosages of the natural extract mixture did not affect ($P < 0.05$) variables mentioned in Table 4. No difference was observed in relation to the sampling time ($P > 0.05$). The total protein, urea, uric acid and albumins did not show any significant change ($P > 0.05$) between the experimental groups. No time effect was observed either with regards to the previous variables ($P > 0.05$) (Table 8). All the obtained results are consistent with the normal range of values for the rabbit species.

Analysis of the antioxidant markers

The values revealed an increase in the FRAP values in both experimental groups (T1 and T2) in comparison with the control group after 90 days of the dietary supplementation. In addition, a time

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Table 6. Blood mineral profile in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Calcium (mmol/l)							
D0	3.08	3.25	3.25	0.115	ns	ns	ns
D30	3.01	3.15	2.38	0.181			
D60	2.95	3.03	3.03	0.036			
D90	3.02	3.07	2.98	0.057			
Magnesium (mmol/l)							
D0	1.39	1.20	1.16	0.039	ns	ns	ns
D30	1.24	1.09	1.11	0.040			
D60	1.25	1.08	1.20	0.063			
D90	1.28	1.02	1.21	0.096			
Phosphorus (mmol/l)							
D0	1.74	1.64	1.91	0.311	ns	ns	ns
D30	1.63	1.74	1.87	0.364			
D60	1.48	1.31	2.52	0.694			
D90	1.44	1.23	1.31	0.112			
Sodium (mmol/l)							
D0	137.80	141.60	137.60	0.753	ns	ns	ns
D30	137.10	137.50	141.60	0.958			
D60	145.80	143.90	150.10	0.746			
D90	140.40	137.10	139.50	0.751			
Potassium (mmol/l)							
D0	4.40	4.36	3.96	0.202	< 0.05	ns	ns
D30	4.32[1]	4.38[1]	3.78[2]	0.105			
D60	4.55	4.26	4.33	0.047			
D90	4.04	3.95	3.94	0.132			
Chlorides (mmol/l)							
D0	111.30	109.20	109.50	0.602	ns	ns	ns
D30	109.70	109.20	109.60	0.707			
D60	116.90	114.80	112.50	0.613			
D90	111.00	109.30	109.80	0.593			

[1,2]Within the same row, means with different numbers differ significantly ($P < 0.05$)

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

effect between the first and the last samplings was observed in the C and T2 groups (Table 9).

Table 7. Blood glucose and hepatic profile in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Glucose (mmol/l)							
D0	4.86	5.18	5.96	0.428	ns	ns	ns
D30	5.24	5.55	5.86	0.345			
D60	5.25	5.18	5.92	0.200			
D90	5.27	5.22	5.60	0.238			
Triglycerides (mmol/l)							
D0	0.83	0.71	0.88	0.063	ns	ns	ns
D30	0.76	0.54	0.65	0.057			
D60	0.84	0.64	0.89	0.050			
D90	0.81	0.91	1.01	0.029			
Cholesterol (mmol/l)							
D0	1.10	1.03	0.69	0.118	ns	ns	ns
D30	1.01	1.06	0.61	0.127			
D60	1.08	0.93	0.86	0.121			
D90	1.24	0.85	0.84	0.207			
Aspartate aminotransferase; AST (µkat/l)							
D0	0.19	0.19	0.23	0.029	ns	ns	ns
D30	0.19	0.23	0.26	0.034			
D60	0.26	0.21	0.21	0.025			
D90	0.28	0.24	0.29	0.045			
Alkaline phosphatase; ALP (µkat/l)							
D0	0.67	0.72	0.80	0.109	ns	ns	ns
D30	0.54	0.73	0.68	0.179			
D60	0.49	0.96	0.86	0.117			
D90	0.54	0.88	0.61	0.121			
Alanine aminotransferase; ALT (µkat/l)							
D0	0.24	0.18	0.23	0.014	ns	ns	ns
D30	0.31	0.30	0.35	0.081			
D60	0.36	0.33	0.27	0.013			
D90	0.41	0.34	0.38	0.072			

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

At day 60, a lower TOS ($P < 0.05$) was observed in the T2 group than the T1 and control groups. At the end of the trial, this variable was lower ($P < 0.05$) in both experimental groups (T1 and T2)

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Table 8. Nitrogen profile in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Total proteins (g/l)							
D0	51.82	58.33	55.14	1.026	ns	ns	ns
D30	53.33	59.00	56.29	1.030			
D60	53.30	58.06	55.20	1.216			
D90	53.09	58.54	54.88	1.078			
Urea (mmol/l)							
D0	7.33	6.55	5.67	0.953	ns	ns	ns
D30	5.40	4.71	3.73	0.513			
D60	5.14	5.81	4.72	0.303			
D90	5.56	4.53	4.55	0.878			
Uric acid (µmol/l)							
D0	60.07	68.4	49.37	0.109	ns	ns	ns
D30	51.75	70.19	43.42	0.093			
D60	52.94	46.39	55.91	0.181			
D90	48.18	41.04	36.28	0.047			
Albumins (g/l)							
D0	37.7	38.5	35.0	0.086	ns	ns	ns
D30	37.6	39.2	39.6	0.109			
D60	35.2	38.7	37.3	0.094			
D90	35.2	37.7	39.0	0.103			

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

Table 9. Antioxidant markers in relation to the dietary treatments and sampling time

Item	Group			SEM	*P*-value		
	C	T1	T2		G	T	G × T
Ferric ion reducing antioxidant power; FRAP (mmol Fe^{2+})							
D0	233.40^{a}	224.20	238.70^{a}	13.46	< 0.05	< 0.05	ns
D30	230.10	218.70	230.30	23.60			
D60	203.40	234.50	215.50^{b}	15.75			
D90	185.80^{1b}	238.10^{2b}	218.40^{2b}	11.88			
Total oxidative status; TOS (mmol H_2O_2)							
D0	3.94	4.09^{a}	3.61^{a}	0.264	< 0.05	< 0.05	ns
D30	3.23	3.85	3.19	0.126			
D60	2.92^{1}	2.63^{1b}	2.40^{2b}	0.210			
D90	2.69^{1}	1.68^{2b}	2.03^{2b}	0.109			
Malondialdehyde; MDA (mmol MDA)							
D0	57.11	63.07^{a}	67.42^{a}	6.496	< 0.05	< 0.05	ns
D30	56.21	56.26	61.55	4.647			
D60	73.14^{1}	49.32^{2}	63.52^{1}	6.085			
D90	66.35^{1}	44.36^{2b}	48.21^{2b}	5.127			
Superoxide dismutase; SOD (IU/ml)							
D0	1.84	1.80	1.64	0.120	ns	ns	ns
D30	1.94	1.83	1.66	0.110			
D60	1.92	2.03	2.14	0.200			
D90	1.80	1.95	1.84	0.174			
Glutathione peroxidase; GPx (U/l)							
D0	0.21	0.21	0.21	0.013	ns	ns	ns
D30	0.21	0.23	0.22	0.009			
D60	0.22	0.23	0.22	0.004			
D90	0.19	0.23	0.19	0.001			

[1,2]Within the same row, means with different numbers differ significantly ($P < 0.05$); [a,b]Within the same column, means with different letters differ significantly ($P < 0.05$)

C = rabbits fed by commercial feed; G = fixed effect of the dietary supplementation; G × T = interaction of the dietary supplementation × time; ns = not significant; T = fixed effect of time; T1 = rabbits fed by feed supplemented with 0.3% of an algae-polyphenolic extract mixture; T2 = rabbits fed by feed supplemented with 0.6% of an algae-polyphenolic extract mixture

than the control group. A time effect ($P < 0.05$) was reported in both experimental groups, between the first and the last samplings. The MDA values were lower ($P < 0.05$) in both experimental groups after 90 days of the dietary supplementation. At 60 days, a lower value ($P < 0.05$) was observed in the T1 group than the T2 and C groups. A time effect ($P < 0.05$) in both experimental groups, between the first and the last samplings, was reported.

During the experimental trial, the SOD and GPx activity showed the same level in all the experimental groups, without any appreciable changes.

The brown seaweed extract did not affect the haematological profile, blood glucose, hepatic, and nitrogen profile ($P > 0.05$).

DISCUSSION

Many studies are reported in the literature on natural substances, medicinal herbs and plant extracts and their effect on animals' health by analysing changes in their blood profile, as they are consid-

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ered as good marker of animal welfare (Pozzo et al. 2015; El-Nomeary et al. 2016; Kovacs et al. 2016).

In the present study, we evaluate the effect of an algae-polyphenolic supplement in a male rabbit diet on the blood haematological profile, as well as the nitrogenous, hepatic, mineral profile, and antioxidant markers. The resulting values of all the analysed blood profile variables are in line with the ranges for healthy rabbit species (Ozkan et al. 2012). Even though previous studies on rabbits have reported that dietary supplementation with phytogenic additives reduced the blood lipid parameters, our presented data show that dietary supplementation with natural mixture had no adverse effects of blood variables. Variables remained in the normal range without any proof of toxicity or side effects. (Abdelnour et al. 2018; Ismail et al. 2019).

A similar study in rabbit does, using the same dosage of the algae-polyphenolic supplement, reported an improvement in the blood lipid parameters (Vizzarri et al. 2020). Moreover, a recent study reported that the dietary seaweed decreased the blood lipid and cholesterol levels (Abu Hafsa et al. 2021). The lack of effects of the dietary supplement on the variables observed in the present study could be probably related to the high weight of the male rabbits, as an increased weight correlates with the concentrations of the total lipids and cholesterol, which can cause deviations in the results, especially in variables, such as the glucose and nitrogen profile.

Okab et al. (2013) did not notice any pathological changes in the hepatic enzymes concentration after a dietary supplementation with brown seaweed, using a similar dosage.

Other experimental studies reported that a rabbit dietary phytogenic supplementation did not affect the nitrogen profile (Dalle Zotte et al. 2016; Kovitvadhi et al. 2016). In the present study, similar results were observed with the hepatic and nitrogen profile. Therefore, we can state that algae-polyphenolic based supplement can be safely supplemented in a male rabbit diet.

The dietary treatment with seaweed and polyphenol mixture improved the antioxidant status of the blood serum after 60 days of supplementation. In fact, an increase in the FRAP and a decrease in the TOS and MDA was observed in the T2 group. In compliance with the present data, an increase in the antioxidant status was previously reported in rabbit does fed the same supplement (Vizzarri et al. 2020) and in rabbits fed bay laurel leaves (Casamassima et al. 2017) or selenium-enriched olive leaves (Mat-tioli et al. 2020). In these studies, an increase in the FRAP and a decrease in the MDA production was observed.

These results are probably due to the action of the antioxidant bioactive compounds contained in the feed additive. In fact, the antioxidant compounds from the plant extract have a chemical structure that makes them able to directly scavenge the reactive oxygen and nitrogen species and also has the ability to interact with several redox signalling pathways modulating the redox enzyme activity (Hunyadi et al. 2019).

In fact, bioactive compounds with antioxidant, antiviral and antimicrobial activities have been detected in brown, red and green seaweed (Cox et al. 2010). The antioxidant molecules in seaweed are different, such as carotenoids and vitamin E (α-tocopherol), as a fat-soluble fraction, whereas water-soluble vitamins (B1, B2, B3 and C), sulfates, polysaccharides and polyphenols are powerful water-soluble antioxidants (Kovacikova et al. 2019). Brown seaweeds, such as *Laminaria* spp., *Ascophyllum nodosum* and *Fucus* spp., showed a high content of vitamins E and C (Dominguez 2013). Data on natural extracts, essential oils, and by-products from plants highlighted that they contain bioactive compounds that are strong natural antioxidants. Considering that oxidative stress is relevant in livestock, polyphenols might be the most promising antioxidant due to their recognised antioxidative and gene regulatory properties (Gessner et al. 2017).

In summary, the antioxidant profile of a male rabbit can be improved by a mixture of brown seaweed and polyphenols after 90 days of dietary supplementation, however, without affecting the blood profile. Therefore, brown seaweed and a mixture of plant polyphenols are safe for animals and seem like a good way to increase their antioxidant status. Considering the present data, the supplement in the tested levels can be considered safe in male rabbits. From the data, we can conclude that a brown seaweed and plant polyphenol mixture is a valid approach to boost male rabbits' antioxidant status.

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Conflict of interest

The authors declare no conflict of interest.

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