Dietary tropical seaweed supplementation modulates antioxidant status and immune response in adult Labrador Dogs

Kiran Shinde Kiran
ICAR-Indian Veterinary Research Institute
Asit Das Asit
ICAR-Indian Veterinary Research Institute
Sunil E Jadhav Sunil
ICAR-Indian Veterinary Research Institute
Suhana P.Muquit Suhana
ICAR-Indian Veterinary Research Institute
Hrishikesh B. Gudaghe Hrish
ICAR-Indian Veterinary Research Institute
P Bagavan Reddy Bagavan
ICAR-Indian Veterinary Research Institute
V. B. Chaturvedi V.B.chaturvedi
ICAR-Indian Veterinary Research Institute
Anju Kala
anjukalavet2002@gmail.com

ICAR-Indian Veterinary Research Institute

Research Article

Keywords: Gracilaria Salicornia, Turbinaria Conoides, Adult Labrador Dogs, Antioxidant, Immunity

Posted Date: August 14th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10541139/v1

Additional Declarations: No competing interests reported.

Title: Dietary tropical seaweed supplementation modulates antioxidant status and immune response in adult Labrador Dogs.

[1]Kiran Shinde., [1]Asit Das., [1]Sunil E. Jadhav., [2]Suhana P. Muquit., [1]Hrishikesh B. Gudaghe., [1]P Bagavan Reddy., [1]V.B. Chaturvedi, Anju Kala*

[1]Division of Animal Nutrition, [2]Division of Physiology & Climatology

ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, India

*Corresponding Author: **Anju Kala** – anjukalavet2002@gmail.com

Abstract:

The present study evaluated the effects of dietary supplementation with the tropical seaweeds *Gracilaria salicornia* and *Turbinaria conoides* on blood biochemical parameters, antioxidant status, and immune responses in adult Labrador dogs, using *Cichorium intybus* (chicory) as a reference functional feed ingredient. A 4 × 4 Latin square design was employed using four adult Labrador Retriever dogs (~7 years; BW 16.48 ± 2.63 kg), four dietary treatments, and four experimental periods of 21 days. Dogs in the control group (CON) received a basal diet(NRC 2006), while groups CH, TC, and GS were supplemented with chicory, *T. conoides*, and *G. salicornia*, respectively, at 3% dietary dry matter. Hematological and serum biochemical parameters remained within normal physiological ranges and were not significantly affected by dietary treatments ($P > 0.05$). Serum T3 and T4 concentrations were comparable among treatments, whereas serum cortisol concentrations were significantly lower ($P < 0.05$) in the GS and TC groups than in the control. Catalase and superoxide dismutase activities were unaffected ($P > 0.05$); however, supplementation with *G. salicornia* and *T. conoides* significantly increased ($P < 0.05$) glutathione, glutathione peroxidase, ferric reducing antioxidant power,serum IgG, serum IgA, and cell-mediated immune response, while significantly reducing malondialdehyde concentrations. Among the treatments, *G. salicornia* produced the most consistent improvements in antioxidant and immune indices, indicating its potential as a functional dietary ingredient for adult dogs.

Key words: *Gracilaria Salicornia*, *Turbinaria Conoides*, Adult Labrador Dogs, Antioxidant, Immunity.

1.Introduction

Seaweeds, the marine macroalga possess high biomass productivity and can be cultivated sustainably. India, a tropical country with a coastline of about 7,500 km, produces approximately 9.7 million metric tons of tropical marine macroalgae annually (Reddy et al. 2024). Seaweeds are broadly classified into three groups: red, brown, and green algae. *Gracilaria salicornia* is one of the most important red seaweeds cultivated in India (Ganesan et al. 2019), whereas *Turbinaria conoides* is an important brown seaweed species (Shah et al. 2023). These seaweeds possess appreciable nutritional value; *G. salicornia* contains around 17% protein (Srinivas et al. 2024), while *T. conoides* contains approximately 15% protein (Rasyid et al. 2025), along with a favorable essential amino acid profile. In addition, they are rich sources of vitamins, minerals, and other essential nutrients that contribute to the overall health and physiological functions of animals. Beyond their nutritional value, seaweeds are rich in various bioactive compounds such as polyphenols(Touhamia et al. 2025) pigments (Chakraborty et al. 2020), sulfated polysaccharides(Rajauria *et al.* 2021) soluble dietary fibers(Luo et al. 2020), polyunsaturated fatty acids (PUFAs)(Ginneken et al. 2011).These compounds are known to exert several biological activities including antioxidant (Michalak et al. 2022), immunomodulatory activity(Paul et al. 2021), prebiotic(Cui et al. 2020), antidiabetic (Ouahabi et al. 2023), hypolipidemic (Alagan et al. 2020), antimicrobial(Palaniyappan et al. 2023) and oral health(Gawor et al. 2018), Among these functional properties, the antioxidant and immunomodulatory activities of seaweed-derived bioactive compounds have gained considerable attention due to their ability to mitigate oxidative stress and enhance immune responses in animals. Oxidative stress occurs due to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, which can adversely affect cellular integrity, metabolic processes, and immune functions. Natural antioxidants derived from marine resources have been widely explored as potential dietary supplements to improve antioxidant status and immune competence in animals. Seaweeds, particularly red and brown macroalgae, are rich in phenolic compounds, sulfated polysaccharides, and pigments such as carotenoids and phycobiliproteins that exhibit strong antioxidant and immunomodulatory properties (Michalak et al. 2022;Paul et al. 2021). Although several studies have reported the

beneficial effects of seaweed supplementation in livestock and aquaculture species, limited information is available regarding their functional effects in companion animals, particularly dogs. Srinivas et al. (2024) reported that dietary supplementation of tropical seaweeds improved antioxidant status and gut health in dogs. Similarly, Cabrita et al. (2023) demonstrated that dietary inclusion of selected microalgae improved gut health status and feed acceptability in dogs without negatively affecting nutrient digestibility. However, systematic studies evaluating the antioxidant and immunomodulatory effects of tropical macroalgae in canine nutrition remain scarce. Dogs are susceptible to oxidative stress associated with ageing, environmental challenges and metabolic activity. *Gracilaria salicornia* and *Turbinaria conoides* contain various bioactive constituents such as sulfated polysaccharides, polyphenols, and other functional metabolites that may contribute to antioxidant defense and immune regulation. Considering the rich bioactive composition of these seaweeds, it was hypothesized that their dietary supplementation could enhance antioxidant status and modulate immune responses in dogs. Therefore, the present study was undertaken to evaluate the effects of dietary supplementation with *Gracilaria salicornia* and *Turbinaria conoides* on antioxidant status and immune responses in adult Labrador dogs.

2.Material and method:

The study was conducted at the experimental kennel facility of the Division of Animal Nutrition, ICAR–Indian Veterinary Research Institute (ICAR-IVRI), Izatnagar, Uttar Pradesh, India, where the experimental dogs were maintained under standard management practices and routine veterinary care. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-IVRI (Approval No. V-137(21)/4/2024-CPCSEA-DADF) and were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Upon completion of the experiment, all dogs were retained at the institutional kennel under routine management and veterinary supervision.

2.1 Animal, Experimental design

The study was conducted by using a 4 x 4 Latin square design, involving a four adult Labrador Retriever dogs ,(7 yr and BW. 16.48 ± 2.63 kg). The design consisted of four dietary treatments (T1= only basal diet, T2 = basal diet + 3% DM Chicory, T3=basal diet + 3%DM

T. Conoides, T4=basal diet + 3% DM *G .Salicornia*) and four 21-day periods, with the final four days of each period allocated for sample collection. A 15 days adaptation period is also provided before the start of experiment and 7-day washout period is allotted after each treatment period.

2.2 Dogs housing and their management

The dogs involved in the study were housed in individual kennels to ensure precise monitoring and control over dietary intake and health parameters. Each kennel was equipped with adequate ventilation, temperature control, and comfortable bedding to ensure the well-being of the dogs. Prior to the start of the experiment, all dogs went through a comprehensive veterinary examination, including deworming and vaccination against common canine diseases. Fresh water was provided ad libitum to each dog in clean, easily accessible bowls, ensuring they remained hydrated throughout the study . The housing and management practices were designed to minimize stress and ensure reliable data collection throughout the study.

2.3 Feeding management

The basal diet was formed according to the recommendation of NRC 2006 (18% CP and 4000 kcal ME) . The dog's daily food requirements were calculated based on their maintenance energy needs (130 x $BW^{0.75}$ kcal).The diet includes rice, crushed wheat, soybean meal, Bengal gram, and soybean oil, all of which are cooked under 15 lbs. of pressure for 20 minutes in a pressure cooker. In addition, each dog is given 220 mL of boiled whole milk, one boiled egg, and 2 g of a mineral mixture daily to meet their nutritional needs (Table 1).The dogs are fed twice a day, with their daily food and supplements requirements divided into two equal portions. After cooking, the supplements are mixed into the food before feeding. The chemical composition of the basal diet(table 2) is near to the standard recommended maintenance diet of adult dogs. seaweed powders (*G. salicornia* and *T. conoides*) procured from the Aquagri Processing Pvt Ltd, New Delhi, India. Powder was prepared by drying fresh seaweed and then grinded using a hammer mill, while chicory powder was sourced from R. A. contractors' chicory processors Etah (UP) India and was prepared from sun-dried chicory, which was then ground using a grinder.

2.4 Analysis of bioactive compounds and antioxidant potential of supplements

Total phenolic content (TPC) was determined using a two-step procedure involving extraction of phenolic compounds with boiling 95% ethanol as described by Saggu et al. (2014), followed by quantification using the Folin–Ciocalteu assay (Arabshahi-Delouee and Urooj, 2007), with absorbance recorded at 760 nm and results expressed as gallic acid equivalents (GAE).Total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method according to Woisky and Salatino (1998), where the extract was mixed with methanol and 5% $AlCl_3$ solution, incubated for 30 min at room temperature, absorbance was measured at 425 nm, and the results were expressed as quercetin equivalents (QE).The antioxidant potential of the supplements was evaluated using ABTS and FRAP assays. In the ABTS assay, the ABTS radical cation was generated by reacting ABTS with potassium persulfate and incubating the mixture in the dark for 12 h, and antioxidant activity was determined by measuring the decrease in absorbance at 744 nm and expressed as percentage inhibition of ABTS radicals. Ferric reducing antioxidant power (FRAP) was determined according to the method of Thaipong et al. (2006), based on the reduction of the Fe^{3+}–TPTZ complex to Fe^{2+}–TPTZ under acidic conditions, with absorbance measured at 593 nm.

2.5 Serum hormones

Serum hormones T3 and T4 was determined using the BIOGENIX. INC.PVT. LTD ELISA test kit. This kit test works on a competitive, one-step incubation principle. T3 and T4 in the sample competes with HRP-conjugated T3 and T4 antigen for binding to anti-T3 and T4 antibodies pre-coated in the wells respectively . After washing chromogen is added, and the resulting color intensity is measured at 450nm.Serum Cortisol levels were measured using the GENLISATM ELISA kit (KRISHIGEN Biosystems).

2.6 Antioxidant and Immune response Analysis

At the end of each experimental period, approximately 2 mL of blood was aseptically collected from the cephalic vein of each dog into Eppendorf tubes containing acid citrate dextrose (ACD) as an anticoagulant for erythrocyte antioxidant assays. The samples were processed immediately by centrifugation to separate erythrocytes. The packed erythrocytes were washed

three times with isotonic saline, and hemolysates were prepared for the estimation of antioxidant parameters. Reduced glutathione (GSH) concentration in erythrocytes was determined using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) following the method of Prins and Loos (1969). Lipid peroxidation was estimated in erythrocyte hemolysates as thiobarbituric acid reactive substances (TBARS) according to Placer et al. (1966), while catalase (CAT) activity was measured spectrophotometrically following the method of Aebi (1983). Glutathione peroxidase (GSH-Px) activity was determined following the method of Rotruck et al. (1973).For serum antioxidant assays, blood was collected separately into clot activator tubes, allowed to clot at room temperature, and centrifuged to obtain serum, which was stored at −20°C until analysis. Superoxide dismutase (SOD) activity was estimated using a commercial assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Ferric reducing antioxidant power (FRAP) was determined following the method of Thaipong et al. (2006).Cell-mediated immune response (CMI) was evaluated using phytohemagglutinin-P (PHA-P) as described by Pawar et al. (2017). Briefly, 50 µg of PHA-P was injected intradermally into the inguinal region on the final day of each experimental period, and skin thickness was measured using a digital vernier caliper at 0, 6, 12, 24, 48, and 72 h post-injection. The increase in skin thickness relative to the baseline (0 h) measurement was considered the index of cell-mediated immune response. Humoral immune response was assessed by estimating serum immunoglobulin G (IgG) and immunoglobulin A (IgA) concentrations using canine-specific ELISA kits (KRISHGEN BioSystems, Mumbai, India) according to the manufacturer's instructions. Serum cortisol concentration was also determined using a canine cortisol ELISA kit (KRISHGEN BioSystems, Mumbai, India) according to the manufacturer's instructions.

2.7 Statistical analysis

The data were analyzed using the General Linear Model procedure of SPSS (version 26.0; SPSS Inc., USA), following the standard statistical methods outlined by Snedecor and Cochran (1994). A univariate ANOVA was performed, with treatment means separated by Tukey’s Honestly Significant Difference (HSD) test. Results were considered significant at $P<0.05$, and trends were noted for mean differences at $P<0.10$. Pearson’s correlation coefficient (r) relationships assessed by using SPSS Statistics version 26.0.

3.Results

3.1 Functional properties and antioxidant potential of basal diet and supplements.

The nutrient composition of the basal diet and the supplements (*Cichorium intybus*, *Turbinaria conoides*, and *Gracilaria salicornia*) is presented in Table 2, showing variations in proximate composition among the diets. Among the supplements, bioactive components differed markedly. *Gracilaria salicornia* exhibited the highest levels of total phenolics (55.4 mg GAE g^{-1} DM) and flavonoids (3.64 mg QE g^{-1} DM), followed by *Turbinaria conoides*, while *Cichorium intybus* and the basal diet showed comparatively lower values(Fig.1). Consistent with these findings, antioxidant activity measured through ABTS inhibition and FRAP assays was also highest in *G. salicornia*, followed by *T. conoide*s (Fig.2).

3.2 DMI ,body weight , palatability, digestibility

Feed intake and apparent total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), total carbohydrates (TCHO), ether extract (EE), and nitrogen-free extract (NFE) were comparable among the experimental groups ($P > 0.05$). Similarly, palatability scores and body mass change did not differ significantly among treatments ($P > 0.05$).However, the daily intake of total phenolic content (TPC) and flavonoids differed significantly among treatments ($P < 0.001$). The intake was significantly higher in GS, followed by TC and CH, compared to the CON group(Table 3).

3.3 Antioxidant and Immunity

Data pertaining to the antioxidant and immune status of the experimental dogs are presented in Table.4 The activities of catalase (CAT) and superoxide dismutase (SOD) did not differ significantly among the dietary groups ($P > 0.05$). In contrast, serum reduced glutathione (GSH), ferric reducing antioxidant power (FRAP), and glutathione peroxidase (GSH-Px) activity increased significantly ($P < 0.001$), whereas erythrocytic malondialdehyde (MDA) concentration decreased significantly ($P < 0.001$) in the supplemented groups. Among the dietary treatments, dogs supplemented with Gracilaria salicornia (GS) exhibited the highest GSH, FRAP, and GSH-Px values, while both seaweed-supplemented groups (TC and GS) showed significantly lower MDA concentrations than the control and chicory (CH) groups, indicating improved antioxidant status (Fig. 3).

Humoral immune response was significantly influenced by dietary supplementation. Serum immunoglobulin G (IgG) concentration was higher ($P < 0.001$) in all supplemented groups than in the control, with the highest value observed in the GS group. Similarly, serum immunoglobulin A (IgA) concentration was significantly greater ($P = 0.021$) in the TC and GS groups than in the CON and CH groups (Fig.4). Cell-mediated immune (CMI) response, measured as absolute skin induration and relative (% increase over the baseline, 0 h) skin thickness following phytohemagglutinin-P (PHA-P) injection, was significantly higher in the TC and GS groups than in the CON group. Although the CH group showed a numerical increase compared with the control, the difference was not significant. Maximum skin induration was observed 12 h after PHA-P administration, followed by a gradual decline over the subsequent observation period (Fig. 5).Regression analysis of the total daily phenolic intake from the dietary supplements revealed significant positive associations with serum GSH concentration ($P = 0.0074$) and skin thickness ($P = 0.0467$), and a negative association with erythrocytic MDA concentration ($P = 0.0327$) (Fig. 7). Similarly, flavonoid intake was positively associated with serum GSH concentration ($P = 0.0350$) and cell-mediated immunity ($P = 0.0018$), while exhibiting a negative association with erythrocytic MDA concentration ($P = 0.0060$) (Fig. 8).

3.3 Serum hormone profile

Data pertaining to serum concentrations of T3, T4 and cortisol presented in Table.5 showed that T3 and T4 levels were not influenced by dietary supplementation ($P > 0.05$). However, serum cortisol concentration was significantly higher in the control (CON) group compared to the supplemented groups ($P < 0.001$) (Fig. 6).

4.Discussion:

4.1 Functional Phytochemicals and antioxidant potential of supplements

Phenolic compounds and flavonoids are important functional constituents that contribute significantly to the antioxidant potential of dietary supplements. Although chicory is widely recognized for its prebiotic properties, it also contains appreciable amounts of phenolic and flavonoid compounds that contribute to its antioxidant and immunomodulatory activities, as evidenced by ABTS inhibition and FRAP assays (Hassan et al. 2010; Lepczyński et al. 2021).

However, seaweeds such as *Gracilaria salicornia* and *Turbinaria conoides* generally possess higher concentrations of these bioactive compounds than chicory. Previous studies on Gracilaria species have reported phenolic contents ranging from 7.34 to 400 mg/100 g DM (Kim et al. 2023; Ramezanpour et al. 2021) and flavonoid contents between 2.12 and 18.97 mg/g DM (Smita et al. 2022; Chan et al. 2015). Similarly, in Turbinaria species, phenolic content has been reported to range from 1.61 to 35.1 mg/100 g DM, while flavonoid levels vary between 0.3 and 2.3 mg/g DM (Hans et al. 2023; El-Shenody et al. 2019). The phenolic and flavonoid contents observed in the present study are consistent with these earlier findings, demonstrating higher levels in *Gracilaria salicornia* and *Turbinaria conoides* compared with chicory and the control diet. These results suggest that supplementation with GS and TC may provide greater antioxidant potential than chicory.

4.2 Antioxidant Profile

The serum ferric reducing antioxidant power (FRAP) assay demonstrated a significant improvement in the overall antioxidant capacity of dogs supplemented with functional feed additives, with the highest values observed in the Gracilaria salicornia (GS) group, followed by the Turbinaria conoides (TC) and chicory (CH) groups compared with the control. FRAP reflects the cumulative reducing capacity of plasma antioxidants by measuring their ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions and is therefore considered an indicator of systemic antioxidant potential. The higher FRAP values in the GS and TC groups indicate that these seaweeds enhanced systemic antioxidant defenses, corroborating previous reports of improved antioxidant capacity following supplementation with chicory (Lepczyński et al. 2021; Shang et al. 2018), Turbinaria species (Wang et al. 2010; Chattopadhyay et al. 2010; Vijayabaskar et al. 2012), and Gracilaria species (Chan et al. 2015; Di et al. 2017).

The superior antioxidant response observed in the GS and TC groups is attributable to their greater intrinsic antioxidant potential, as evidenced by the higher total phenolic content, ferric reducing antioxidant power, and ABTS radical-scavenging activity of the seaweeds compared with chicory. Unlike chicory, whose antioxidant effects are largely mediated indirectly through modulation of gut microbiota and increased short-chain fatty acid production (Jurgoński et al. 2012), Gracilaria salicornia and Turbinaria conoides are rich sources of phenolics, flavonoids, carotenoids and sulfated polysaccharides that directly scavenge reactive

oxygen species, donate hydrogen atoms or electrons, chelate transition metals and reinforce endogenous antioxidant defenses (Shahidi and Wanasundara, 1992; Wang et al. 2010; Wu et al. 2017; Banjarnahor and Artanti, 2014). These mechanisms are further supported by the regression analysis, which demonstrated positive associations between dietary phenolic and flavonoid intake and serum GSH concentrations, together with negative associations with serum MDA concentrations (Fig. 6 and 7).

Consistent with the FRAP results, dogs supplemented with GS and TC exhibited significantly higher serum reduced glutathione (GSH) concentrations than those fed the control diet, whereas the CH group showed an intermediate response. Reduced glutathione is the principal intracellular non-enzymatic antioxidant that directly scavenges reactive oxygen species while serving as the major reducing substrate for glutathione-dependent antioxidant enzymes, thereby maintaining cellular redox homeostasis (McMichael et al. 2007). Consequently, elevated serum GSH concentrations indicate an improved antioxidant defense system and greater capacity to counter oxidative stress. Similar increases in GSH have been reported in Labrador dogs supplemented with Gracilaria salicornia (Srinivas et al. 2024), Wistar rats receiving carrageenan, which also exhibited enhanced glutathione-S-transferase activity and reduced lipid peroxidation (Sanjivkumar et al. 2020), and experimental models supplemented with Turbinaria polysaccharides (Bhardwaj et al. 2021; Chale-Dzul et al. 2015). Comparable improvements have also been observed following chicory or inulin supplementation in broiler chickens and laying hens (Fathi et al. 2024; Shang et al. 2018), indicating that both seaweed- and chicory-derived bioactive compounds can strengthen endogenous antioxidant defenses.

Consistent with the increased GSH concentrations, serum GSH-Px activity was also significantly elevated in dogs supplemented with GS and TC, with the GS group exhibiting the highest enzyme activity ($P<0.05$). Glutathione peroxidase is a selenium-dependent antioxidant enzyme that catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides into water and corresponding alcohols using GSH as an electron donor, thereby preventing oxidative damage to cellular membranes (Rotruck et al. 1973; Surai, 2016). The concurrent increase in both GSH concentration and GSH-Px activity suggest coordinated enhancement of the glutathione antioxidant system. Increased GSH availability provides the reducing equivalents required for efficient GSH-Px activity, facilitating rapid

297 detoxification of reactive oxygen species and lipid hydroperoxides before they initiate lipid
298 peroxidation. This response is likely mediated by the synergistic action of seaweed-derived
299 phenolics, flavonoids, carotenoids and sulfated polysaccharides, which directly neutralize
300 reactive oxygen species and reduce oxidative burden. In addition, marine macroalgae can
301 accumulate selenium, an essential cofactor required for the synthesis and catalytic activity of
302 glutathione peroxidase (Belda-Antolí et al. 2025), while seaweed-derived polyphenols and
303 sulfated polysaccharides have been reported to activate the nuclear factor erythroid 2-related
304 factor 2 (Nrf2)-antioxidant response element (ARE) signaling pathway, thereby promoting
305 glutathione biosynthesis and antioxidant enzyme expression (Chen et al. 2024; Wang et al.
306 2013). Together, these mechanisms may have contributed to the enhanced glutathione-
307 dependent antioxidant response observed in the present study. Similar improvements in
308 glutathione-dependent antioxidant enzymes have been reported following supplementation
309 with seaweed polysaccharides (Bhardwaj et al. 2021) and chicory extract, which increased
310 GSH-Px activity together with catalase and superoxide dismutase in broiler chickens (Abo et
311 al. 2023). Collectively, these findings indicate that Gracilaria salicornia, owing to its richer
312 profile of antioxidant phytochemicals, provides the greatest enhancement of GSH-Px activity,
313 with comparable benefits to Turbinaria conoides for GSH and MDA responses.

314 The enhancement of the glutathione antioxidant system was accompanied by a significant
315 reduction in serum malondialdehyde (MDA) concentrations in dogs supplemented with
316 Gracilaria salicornia and Turbinaria conoides, with both groups showing comparably low
317 values that did not differ significantly from each other. Malondialdehyde, a stable end product
318 of polyunsaturated fatty acid peroxidation, is widely recognized as a reliable biomarker of
319 oxidative stress and membrane damage (Saleh et al. 2022). Therefore, the lower MDA
320 concentrations indicate that supplementation with seaweed-derived bioactive compounds
321 effectively attenuated lipid peroxidation and protected cellular membranes against oxidative
322 injury.

323 The reduction in MDA is likely attributable to the coordinated enhancement of both enzymatic
324 and non-enzymatic antioxidant defenses. Increased serum GSH concentrations together with
325 elevated GSH-Px activity would facilitate rapid detoxification of hydrogen peroxide and lipid
326 hydroperoxides before they initiate membrane lipid oxidation. Moreover, the phenolics,

flavonoids and sulfated polysaccharides present in Gracilaria salicornia and Turbinaria conoides possess potent radical-scavenging and metal-chelating activities that further suppress oxidative chain reactions (Shahidi and Wanasundara, 1992; Wang et al. 2010; Wu et al. 2017). This mechanism is further supported by the significant negative association between dietary phenolic and flavonoid intake and serum MDA concentrations observed in the regression analysis, indicating that higher dietary antioxidant intake was associated with lower oxidative damage.

The present findings are consistent with previous studies demonstrating the antioxidant potential of seaweed supplementation. Srinivas et al. (2024) reported reduced serum MDA concentrations in Labrador dogs supplemented with Gracilaria salicornia, while Zhang et al. (2023) observed decreased lipid peroxidation in kittens receiving enzymatically processed seaweed powder. Likewise, polysaccharide-rich extracts from Gracilaria lemaneiformis (Jiang et al. 2023) and sulfated polysaccharides from Turbinaria ornata (Bhardwaj et al. 2021) significantly reduced MDA concentrations in broiler chickens and rats, respectively. Similar reductions have also been reported following chicory supplementation in broiler chickens (Fathi et al. 2024), although Gazwi et al. (2022) observed no significant effect, suggesting that the antioxidant response may vary according to the source, processing method and dietary inclusion level of the functional ingredient.

Unlike GSH and GSH-Px, serum superoxide dismutase (SOD) and catalase (CAT) activities were not significantly affected by dietary treatments. These enzymes constitute the primary enzymatic defense against superoxide radicals and hydrogen peroxide and are generally upregulated under conditions of substantial oxidative stress rather than in response to dietary antioxidants alone (Surai, 2016; Halliwell and Gutteridge, 2015). The absence of significant changes therefore suggests that supplementation with GS, TC and chicory maintained redox homeostasis without requiring additional induction of these enzymes. Instead, the enhanced plasma antioxidant capacity, reflected by increased FRAP values together with elevated GSH concentrations and GSH-Px activity, appears to have provided sufficient protection against oxidative stress.

Comparable findings have been reported in previous studies. Srinivas et al. (2024) likewise observed no significant changes in serum SOD or CAT activities in Labrador dogs

supplemented with Gracilaria salicornia, while Gracilaria viridae supplementation did not alter tissue SOD activity in rats (Barros-Gomes et al. 2018). Similarly, Azizi et al. (2023) found no significant effect of brown or green seaweed supplementation on SOD or CAT activities in broiler chickens, and Wang et al. (2019) and Juśkiewicz et al. (2011) reported comparable observations following inulin or chicory polyphenol supplementation. Collectively, the superior antioxidant response observed in dogs supplemented with Gracilaria salicornia is attributable to its richer profile of phenolics, flavonoids, carotenoids and sulfated polysaccharides, which collectively enhance free radical scavenging, reinforce glutathione-dependent antioxidant defenses and limit lipid peroxidation. These complementary mechanisms explain the greater increases in FRAP and GSH-Px activity, together with the marked reduction in MDA shared with Turbinaria conoides, compared with chicory. Overall, these findings identify *G. salicornia* as the most effective functional feed ingredient for enhancing antioxidant status and oxidative resilience in adult dogs, supporting its potential application in companion animal nutrition.

4.4 Immunity

Supplementation with Gracilaria salicornia (GS) and Turbinaria conoides (TC) at 3% DM significantly enhanced both humoral and cell-mediated immunity in dogs, as evidenced by higher serum IgA and IgG concentrations and increased delayed-type hypersensitivity skin thickness compared with the chicory and control groups. Serum IgA and IgG are complementary components of humoral immunity, with IgA providing the first line of protection at mucosal surfaces and IgG mediating systemic immune defense through pathogen neutralization, opsonization and complement activation (Brandtzaeg, 2013; Schroeder and Cavacini, 2010). Regression analysis further demonstrated positive associations between dietary phenolic and flavonoid intake and immune responses. These effects are likely mediated by the combined action of seaweed-derived phenolics, flavonoids and sulfated polysaccharides, together with increased production of short-chain fatty acids (SCFAs). SCFAs promote intestinal epithelial integrity, support leukocyte development and regulate immune function through activation of G protein-coupled receptors (FFAR2, FFAR3, GPR109A and Olfr78) and modulation of histone deacetylases and hypoxia-inducible factor signalling (Corrêa-Oliveira et al. 2016), while seaweed polysaccharides regulate cytokine

production, including IL-6 and TNF-α, thereby promoting antibody production and immune homeostasis (Ren et al. 2017).

The present findings are consistent with previous studies demonstrating the immunomodulatory potential of seaweeds. Srinivas et al. (2024) reported enhanced cell-mediated immunity in Labrador dogs supplemented with Gracilaria salicornia. Similarly, polysaccharides from Gracilaria lemaneiformis increased serum IgA and IgG concentrations in broiler chickens (Jiang et al. 2023), whereas sulfated polysaccharides from Turbinaria ornata reduced pro-inflammatory mediators, including IL-6, IL-1β, COX-2 and TNF-α (Bhardwaj et al. 2021). Comparable improvements in humoral immunity have also been reported following supplementation with Kappaphycus alvarezii (Leonard et al. 2010) and chicory, which increased circulating IgG in swine and IgA and IgG titres in broiler chicks (Zhang et al. 2021; Asadi et al. 2014).

Collectively, the superior immune response observed in dogs supplemented with *Gracilaria salicornia* and *Turbinaria conoides* is attributable to their richer content of phenolics, flavonoids and sulfated polysaccharides, together with enhanced SCFA production, which synergistically promoted antibody production and cell-mediated immunity. These complementary mechanisms explain the greater increases in serum IgA, serum IgG and delayed-type hypersensitivity responses, identifying *G. salicornia* as the most effective functional ingredient for improving immune competence in companion animals.

4.3 Serum hormones

The serum concentrations of T3 and T4 were not affected by any of the supplements in this study. Similarly, previous reports have shown that tropical seaweed-based formulations did not influence serum T3 and T4 levels in lactating buffaloes (Maheswari et al. 2021; Dahiphale et al. 2024). Consistent with this finding similar results were reported by Noahsen et al. (2020) in humans, where serum T4 levels remained unchanged in euthyroid individuals supplemented with Japanese and Greenlandic seaweed salads. In contrast, seaweed supplementation has been shown to improve serum T3 and T4 concentrations in certain species, such as chickens (Fan et al. 2020) and juvenile black sea bream (Yu et al. 2020). Additionally, the inclusion of mannan oligosaccharides (MOS) in the basal diet of rabbits

similarly showed no adverse impact on serum concentrations of these hormones (Basha et al. 2023).

Furthermore, the improvement in antioxidant status and immune function observed in dogs supplemented with *Gracilaria salicornia* (GS) and *Turbinaria conoides* (TC) appeared to positively influence the reduction in serum cortisol levels. By enhancing cellular defenses and supporting immune resilience, these compounds likely helped stabilize the internal environment, reducing the body's need to produce cortisol as a stress response. This was evidenced by significantly lower serum cortisol levels in the GS and TC groups compared to the chicory (CH) and control (CON) groups.

Regression analysis further highlighted that the phenolic and flavonoid content of these seaweed supplements had a negative correlation with serum cortisol levels (Fig 7 and Fig 8). Similar findings have been reported in rats supplemented with *Sargassum hystrix* powder, a functional food for stress management, where reduced serum cortisol levels were observed. These results are consistent with studies by Maheswari et al. (2021) and Dahiphale et al. (2024), which demonstrated that tropical seaweed-based formulations reduced serum cortisol levels in lactating buffaloes. Likewise, a significant decrease in serum cortisol was noted in weaned piglets supplemented with brown seaweed (*Laminaria digitata*) (Ribeiro et al. 2023).In contrast, Anderson et al. (2023) reported no effect on serum cortisol in crossbred calves supplemented with byproducts of three tropical red seaweeds. However, supplementation of *Cichorium intybus* was shown to reduce cortisol levels in stress-induced albino rats (Pasha et al. 2016), and similar results were observed in hybrid catfish supplemented with inulin (Do et al. 2023).These findings underline the potential of GS and TC as effective stress-modulating supplements, with possible applications across various species and stress-related conditions.

Conclusion:

In conclusion, the present study demonstrates that tropical seaweeds, *particularly Gracilaria salicornia* and *Turbinaria conoides*, are promising functional feed additives capable of enhancing antioxidant status and immune responses in adult Labrador dogs without adversely affecting nutrient intake or nutrient utilization. Among the tested ingredients, *G. salicornia* produced the most consistent improvements across antioxidant and immune parameters, while

T. conoides showed comparable benefits for glutathione concentration and lipid peroxidation, suggesting that both seaweeds share overlapping bioactive mechanisms. These findings provide preliminary evidence supporting the potential use of tropical seaweeds, and *G. salicornia* in particular, as natural functional feed ingredients for promoting antioxidant and immune health in adult dogs. Further studies with larger sample sizes and direct mechanistic assays are warranted to confirm these observations and clarify the pathways underlying their functional properties.

Acknowledgments

The authors express their sincere gratitude to Director, ICAR–IVRI, Izatnagar, Bareilly (U.P.), India for providing the institutional support and facilities necessary for conducting this research. The authors also thank R. A. Contractors' Chicory Processors, Etah (U.P.), India and Aquagri Processing Pvt. Ltd., Madurai, Tamil Nadu, India for supplying chicory and seaweed samples, respectively.

Abbreviations

1.FRAP – Ferric Reducing Antioxidant Power
2.TPC – Total Phenolic Content
3.TFC – Total Flavonoid Content
4.ABTS – 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
5.PHA-P – Phyto-haemagglutinin-P
6.DTNB- 5,5′-dithiobis-2-nitrobenzoic acid

Disclosure statement

Authors declare that there are no financial or personal conflicts of interest that could have influenced the results or interpretation of this study.

Funding: No funding information to disclose

CRediT authorship contribution statement

Asit Das: Conceptualization, resources, and supervision, **Kiran Shinde:** Research conduction and writing-original draft preparation, **Anju Kala:** Formal analysis and review and editing of the draft, **Sunil. E. Jadhav:** Investigation and Technical guidance, **Suhana.P.Muquit:** Data analysis, software support and draft writing, **Hrishikesh B Gudaghe:** Draft writing, **P**

Bagavan Reddy: Data Analysis and Draft writing, , **V.B.Chaturvedi:** Data Curation and Draft writing.

Consent for publication:

All Authors have reviewed and approved the final version of the manuscript and consent to its publication.

Ethical approval:

All Animal procedures were approved by the Institutional Animal Ethics Committee (IAEC), ICAR-IVRI, Izatnagar, Bareilly, and conducted in accordance with CPCSEA guidelines (Approval No. V-137(21)/4/2024-CPCSEA-DADF).

Data Availability:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References:

Aebi H (1983) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Vol 3. Verlag Chemie, Weinheim, pp 273–277.

Anderson P, Malik R, Ojha L, Adjei-Mensah B, Naliyapara HB (2023) Investigations on modulating effect of three tropical red seaweed by-products on growth performance, immune response, antioxidant status and endocrine variables in crossbred calves. J Appl Phycol 35(1):445–457.

Arabshahi-Delouee S, Urooj A (2007) Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. Food Chem 102(4):1233–1240.

Asadi M, Mohammadi M, Roostaei Alimehr M (2014) Effect of alcoholic Cichorium intybus L. extract on performance and immune response of broilers. Res Anim Prod 5(9):36–49.

Azizi MN, Loh TC, Foo HL, Akit H, Izuddin WI, Shazali N, Teik Chung EL, Samsudin AA (2023) Brown and green seaweed antioxidant properties and effects on blood plasma antioxidant enzyme activities, hepatic antioxidant genes expression, blood plasma lipid profile, and meat quality in broiler chickens. Animals 13(10):1582.

Bajury DM, Rawi MH, Sazali IH, Abdullah A, Sarbini SR (2017) Prebiotic evaluation of red seaweed (Kappaphycus alvarezii) using in vitro colon model. Int J Food Sci Nutr 68(7):821–828.

Banjarnahor SD, Artanti N (2014) Antioxidant properties of flavonoids. Med J Indones 23(4):239–244.

Barros-Gomes JAC, Nascimento DLA, Silveira ACR, Silva RK, Gomes DL, Melo KRT, Almeida-Lima J, Camara RBG, Silva NB, Rocha HAO (2018) In vivo evaluation of the antioxidant activity and protective action of the seaweed Gracilaria birdiae. Oxid Med Cell Longev 2018:9354296.

Basha SK, Reddy LV, Sivakumar A, Suresh Babu DS, Naik B, Reddy GB, Chakravarthi MK (2023) Effect of supplementation of feed additives on haematology and thyroid hormones in New Zealand white rabbits. Indian J Anim Prod Manag 37(3):233–237.

Bhardwaj M, Mani S, Malarvizhi R, Sali VK, Vasanthi HR (2021) Immunomodulatory activity of brown algae Turbinaria ornata-derived sulfated polysaccharide on LPS-induced systemic inflammation. Phytomedicine 89:153615.

Cabrita AR, Guilherme-Fernandes J, Spínola M, Maia MR, Yergaliyev T, Camarinha-Silva A, Fonseca AJ (2023) Effects of microalgae as dietary supplement on palatability, digestibility, fecal metabolites, and microbiota in healthy dogs. Front Vet Sci 10:1245790.

Chakraborty K, Dhara S (2020) First report of substituted 2H-pyranoids from brown seaweed Turbinaria conoides with antioxidant and anti-inflammatory activities. Nat Prod Res 34(24):3451–3461.

Chale-Dzul J, Moo-Puc R, Robledo D et al (2015) Hepatoprotective effect of the fucoidan from the brown seaweed Turbinaria tricostata. J Appl Phycol 27(5):2123–2135.

Chattopadhyay N, Ghosh T, Sinha S, Chattopadhyay K, Karmakar P, Ray B (2010) Polysaccharides from Turbinaria conoides: Structural features and antioxidant capacity. Food Chem 118(3):823–829.

Chen N, Hu M, Jiang T, Xiao P, Duan JA (2024) Insights into the molecular mechanisms, structure–activity relationships and application prospects of polysaccharides by regulating Nrf2-mediated antioxidant response. Carbohydr Polym 333:122003.

Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR (2016) Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunol 5(4):e73.

Crnogaj M, Cerón JJ, Šmit I, Kiš I, Gotić J, Brkljačić M, Matijatko V, Rubio CP, Kučer N, Mrljak V (2017) Relation of antioxidant status at admission and disease severity and outcome in dogs naturally infected with Babesia canis canis. BMC Vet Res 13(1):114.

Cui M, Zhou R, Wang Y, Zhang M, Liu K, Ma C (2020) Beneficial effects of sulfated polysaccharides from the red seaweed Gelidium pacificum Okamura on mice with antibiotic-associated diarrhea. Food Funct 11(5):4625–4637.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179(4073):588–590.

Dahiphale GB, Das A, Reddy PB, Kumar S, Tyagi N, Tyagi AK (2024) Beneficial effects of dietary supplementation of tropical seaweeds on rumen fermentation, antioxidant status, immunity, and milk yield of lactating Murrah buffaloes. J Appl Phycol 36(6):3697–3715.

Do Nascimento Veiga PT, Rodrigues TAR, Fantini-Hoag L, Rodrigues RA, Pilarski F, Owatari MS, Martins ML, de Campos CM (2024) Inulin dietary supplementation attenuates the stress induced by pursuit/capture/atmospheric exposure and improves innate immune response in hybrid catfish (Pseudoplatystoma reticulatum♀ × Leiarius marmoratus♂) after exposure to Aeromonas hydrophila. Aquac Int 32(2):1771–1784.

El-Shenody RA, Ashour M, Ghobara MME (2019) Evaluating the chemical composition and antioxidant activity of three Egyptian seaweeds: Dictyota dichotoma, Turbinaria decurrens, and Laurencia obtusa. Braz J Food Technol 22:e2018203.

Fan GJ, Shih BL, Lin HC, Lee TT, Lee CF, Lin YF (2020) Effect of dietary supplementation of Sargassum meal on laying performance and egg quality of Leghorn layers. Anim Biosci 34(3):449.

Fathi M, Hoseini M, Alizadeh S, Zandi R, Rahmati S, Saeidian S, Fard MS, Rezaee V, Zarrinkavyani K, Mardani P (2024) Effects of chicory (Cichorium intybus L.) distillation wastewater on antioxidant status, immune response, cecal microbial population, growth performance, and meat quality in broiler chickens. Livest Sci 282:105442.

Ganesan M, Trivedi N, Gupta V, Madhav SV, Reddy CRR, Levine IA (2019) Seaweed resources in India—Current status of diversity and cultivation: Prospects and challenges. Bot Mar 62(5):463–482.

Gawor J, Jank M, Jodkowska K, Klim E, Svensson UK (2018) Effects of edible treats containing Ascophyllum nodosum on the oral health of dogs: A double-blind, randomized, placebo-controlled single-center study. Front Vet Sci 5:168.

Gazwi HS, Mahmoud ME, Toson EM (2022) Analysis of the phytochemicals of Coriandrum sativum and Cichorium intybus aqueous extracts and their biological effects on broiler chickens. Sci Rep 12(1):6399.

Hans N, Pattnaik F, Malik A, Naik S (2023) Comparison of different green extraction techniques and their influence on chemical characteristics of sulfated polysaccharide (fucoidan) from Padina tetrastromatica and Turbinaria conoides. Algal Res 74:103199.

Hassan HA, Yousef MI (2010) Ameliorating effect of chicory (Cichorium intybus L.)-supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats. Food Chem Toxicol 48(8–9):2163–2169.

Jiang S, Yang C, Xiao Y, Zheng S, Jiang Q, Chen J (2023) Effects of polysaccharides-rich extract from Gracilaria lemaneiformis on growth performance, antioxidant capacity, immune function, and meat quality in broiler chickens. J Poult Sci 60(2):18–30.

Jurgoński A, Juśkiewicz J, Zduńczyk Z, Król B (2012) Caffeoylquinic acid-rich extract from chicory seeds improves glycemia, atherogenic index, and antioxidant status in rats. Nutrition 28(3):300–306.

Juśkiewicz J, Zduńczyk Z, Żary-Sikorska E, Król B, Milala J, Jurgoński A (2011) Effect of the dietary polyphenolic fraction of chicory root, peel, seed and leaf extracts on caecal

585 fermentation and blood parameters in rats fed diets containing prebiotic fructans. Br J Nutr
586 105(5):710–720.

587 Kim JS, Choi C, Lee HH (2023) Changes in the biological activities of Gracilaria verrucosa
588 extracted using different extraction solvents. Appl Sci 13(22):12314.

589 Leonard SG, Sweeney T, Bahar B, Lynch BP, O'Doherty JV (2010) Effect of maternal fish oil
590 and seaweed extract supplementation on colostrum and milk composition, humoral immune
591 response, and performance of suckled piglets. J Anim Sci 88(9):2988–2997.

592 Lepczyński A, Herosimczyk A, Barszcz M, Ożgo M, Michałek K, Grabowska M, Tuśnio A,
593 Szczerbińska D, Skomiał J (2021) Diet supplemented either with dried chicory root or chicory
594 inulin significantly influences kidney and liver mineral content and antioxidative capacity in
595 growing pigs. Animal 15(2):100129.

596 Liu J, Kandasamy S, Zhang J, Kirby CW, Karakach T, Hafting J, Critchley AT, Evans F,
597 Prithiviraj B (2015) Prebiotic effects of diet supplemented with the cultivated red seaweed
598 Chondrus crispus or with fructo-oligo-saccharide on host immunity, colonic microbiota and
599 gut microbial metabolites. BMC Complement Altern Med 15(1):279.

600 Luo M, Hu K, Zeng Q, Yang X, Wang Y, Dong L, Huang F, Zhang R, Su D (2020)
601 Comparative analysis of the morphological property and chemical composition of soluble and
602 insoluble dietary fiber with bound phenolic compounds from different algae. J Food Sci
603 85(11):3843–3851.

604 Maheswari M, Das A, Datta M, Tyagi AK (2021) Supplementation of tropical seaweed-based
605 formulations improves antioxidant status, immunity and milk production in lactating Murrah
606 buffaloes. J Appl Phycol 33(4):2629–2643.

607 McMichael MA (2007) Oxidative stress, antioxidants, and assessment of oxidative stress in
608 dogs and cats. J Am Vet Med Assoc 231(5):714–720.

609 Michalak I, Tiwari R, Dhawan M, Alagawany M, Farag MR, Sharun K, Dhama K (2022)
610 Antioxidant effects of seaweeds and their active compounds on animal health and production
611 – A review. Vet Q 42(1):48–67.

612 Noahsen P, Kleist I, Larsen HM et al (2020) Intake of seaweed as part of a single sushi meal,
613 iodine excretion and thyroid function in euthyroid subjects: A randomized dinner study. J
614 Endocrinol Invest 43(4):431–438.

615 Ouahabi S, Daoudi NE, Loukili EH, Asmae H, Merzouki M, Bnouham M, Challioui A,
616 Hammouti B, Fauconnier ML, Rhazi L, Ayerdi Gotor A (2024) Investigation into the
617 phytochemical composition, antioxidant properties, and in vitro anti-diabetic efficacy of Ulva
618 lactuca extracts. Mar Drugs 22(6):240.

619 Ouahabi S, Loukili EH, Daoudi NE, Chebaibi M, Ramdani M, Rahhou I, Bnouham M,
620 Fauconnier ML, Hammouti B, Rhazi L, Ayerdi Gotor A (2023) Study of the phytochemical
621 composition, antioxidant properties, and in vitro anti-diabetic efficacy of Gracilaria bursa-
622 pastoris extracts. Mar Drugs 21(7):372.

623 Palaniyappan S, Sridhar A, Kari ZA, Téllez-Isaías G, Ramasamy T (2023) Evaluation of
624 phytochemical screening, pigment content, in vitro antioxidant, antibacterial potential and
625 GC–MS metabolite profiling of green seaweed Caulerpa racemosa. Mar Drugs 21(5):278.

626 Pasha S, Mahurkar N, Jayaveera KN (2016) Protective effect of Vitis vinifera and Cichorium
627 intybus on adrenocortical activity in stress-induced albino rats. Res J Pharm Technol
628 9(5):521–526.

629 Paul SS, Vantharam Venkata HGR, Raju MV, Rama Rao SV, Nori SS, Suryanarayan S, Kumar
630 V, Perveen Z, Prasad CS (2021) Dietary supplementation of extracts of red seaweed
631 (Kappaphycus alvarezii) improves growth, intestinal morphology, expression of intestinal
632 genes and immune responses in broiler chickens. J Sci Food Agric 101(3):997–1008.

633 Pawar MM, Pattanaik AK, Sinha DK, Goswami TK, Sharma K (2017) Effect of dietary
634 mannanoligosaccharide supplementation on nutrient digestibility, hindgut fermentation,
635 immune response and antioxidant indices in dogs. J Anim Sci Technol 59(1):11.

636 Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation
637 (malonyl dialdehyde) in biochemical systems. Anal Biochem 16(2):359–364.

638 Prins HK, Loos JA (1969) Glutathione. In: Yunis JJ (ed) Biochemical methods in red cell
639 genetics. Academic Press, New York, pp 115–137.

640 Rajauria G, Ravindran R, Garcia-Vaquero M, Rai DK, Sweeney T, O'Doherty J (2021)
641 Molecular characteristics and antioxidant activity of laminarin extracted from the seaweed
642 species Laminaria hyperborea, using hydrothermal-assisted extraction and a multi-step
643 purification procedure. Food Hydrocoll 112:106323.

644 Ramezanpour Z, Ghanbari Pirbasti F, Rasouli Dogaheh S (2021) Bioactivity potential of
645 Gracilaria salicornia, Padina boergesenii, Polycladia myrica: Antibacterial, antioxidant, and
646 total phenol assays. Plant Algae Environ 5(1):597–615.

647 Rasyid A, Rachman F, Handayani T, Kotta R (2025) Nutritional potential of dried seaweed
648 Turbinaria decurrens: Biochemical insights and health benefits. J Teknol (Sci Eng) 87(4):785–
649 792.

650 Reddy PB, Das A, Verma AK (2024) Seaweed as a functional feed supplement in animal diet –
651 A review. Indian J Anim Sci 94(4):291–300.

652 Ren Y, Zheng G, You L, Wen L, Li C, Fu X, Zhou L (2017) Structural characterization and
653 macrophage immunomodulatory activity of a polysaccharide isolated from Gracilaria
654 lemaneiformis. J Funct Foods 33:286–296.

655 Ribeiro DM, Pinto RM, Lopes PA, Pestana JM, Alfaia CM, Costa MM, Carvalho DF, Mourato
656 MP, de Almeida AM, Freire JP, Prates JA (2023) Effect of Laminaria digitata dietary
657 inclusion and CAZyme supplementation on blood cells, serum metabolites and hepatic lipids
658 and minerals of weaned piglets. Sci Rep 13(1):6598.

659 Saggu S, Sakeran MI, Zidan N, Tousson E, Mohan A, Rehman H (2014) Ameliorating effect of
660 chicory (Cichorium intybus L.) fruit extract against 4-tert-octylphenol-induced liver injury
661 and oxidative stress in male rats. Food Chem Toxicol 72:138–146.

662 Sanjivkumar M, Chandran MN, Suganya AM, Immanuel G (2020) Investigation on bio-
663 properties and in vivo antioxidant potential of carrageenans against alloxan-induced oxidative
664 stress in Wistar albino rats. Int J Biol Macromol 151:650–662.

665 Shah Y, Mantri VA (2023) Rediscovery of brown alga Turbinaria indica P. Gopalakrishnan ex
666 V. Krishnamurthy from Mandvi Kutch, India after 50 years. J Bombay Nat Hist Soc 120:2.

667 Shang HM, Zhou HZ, Yang JY, Li R, Song H, Wu HX (2018) In vitro and in vivo antioxidant
668 activities of inulin. PLoS One 13(2):e0192273.

669 Smita KM, Stanley Abraham L, Sunitha S, Vasantharaja R, Thirugnanasambandam R (2022)
670 Extraction and correlation of total phenolic and flavonoid content in seaweeds. Indian J Geo-
671 Mar Sci 51(5):432–438.

672 Srinivas KY, Das A, Reddy PB, Verma AK (2024) Supplementation of tropical red seaweeds
673 improved gut health indices, antioxidant status and immunity in adult dogs. J Appl Phycol
674 36(4):2183–2198.

675 Surai PF (2016) Antioxidant systems in poultry biology: Superoxide dismutase. J Anim Res
676 Nutr 1(1):8.

677 Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006) Comparison of
678 ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit
679 extracts. J Food Compos Anal 19(6–7):669–675.

680 Thavasi Alagan V, Nakulan Vatsala R, Sagadevan I, Subbiah V, Ragothaman V (2020) Effect
681 of dietary supplementation of seaweed (Ulva lactuca) and Azolla on growth performance,
682 haematological and serum biochemical parameters of Aseel chicken. Beni Suef Univ J Basic
683 Appl Sci 9(1):58.

684 Touhamia Y, Aamiri A, Patel M, Adnan M, Rezzoum NE, Pereira L, Allouche N, Salah HB,
685 Lahcen TOB (2025) In vitro and in silico assessment of antioxidant potential of crude sulfated
686 polysaccharide and phenolic extracts from Gracilaria gracilis macroalgae harvested from
687 Atlantic and Mediterranean coasts: A comparative study. Algal Res 104229.

688 Van Ginneken VJ, Helsper JP, de Visser W, van Keulen H, Brandenburg WA (2011)
689 Polyunsaturated fatty acids in various macroalgal species from North Atlantic and tropical
690 seas. Lipids Health Dis 10(1):104.

691 Belda-Antolí M, Ros Bernal FR, Vicente-Mampel J (2025) From sea to relief: The therapeutic
692 potential of marine algal antioxidants in pain alleviation. Mar Drugs 23:270.

693 Wang J, Zhang Q, Zhang Z, Song H, Li P (2010) Potential antioxidant and anticoagulant
694 capacity of low molecular weight fucoidan fractions extracted from Laminaria japonica. Int J
695 Biol Macromol 46(1):6–12.

696 Wang R, Paul VJ, Luesch H (2013) Seaweed extracts and unsaturated fatty acid constituents
697 from the green alga Ulva lactuca as activators of the cytoprotective Nrf2–ARE pathway. Free
698 Radic Biol Med 57:141–153.

699 Wang W, Chen D, Yu B, Huang Z, Luo Y, Zheng P, Mao X, Yu J, Luo J, He J (2019) Effect of
700 dietary inulin supplementation on growth performance, carcass traits, and meat quality in
701 growing-finishing pigs. Animals 9(10):840.

702 Woisky RG, Salatino A (1998) Analysis of propolis: Some parameters and procedures for
703 chemical quality control. J Apic Res 37(2):99–105.

704 Yu C, Liu G, Yu J, Lin F, Wen X (2020) Dietary Saccharina japonica is a natural and effective
705 tool to fortify marine teleost black sea bream fillets with iodine: Effects on growth, flesh
706 quality, and serum thyroid hormones. J Appl Phycol 32(5):3447–3456.

707 Zhang T, Hu BW, Duan YH, Deng JP, Yin YL, Kong XF (2021) Dietary chicory powder
708 supplementation affects growth performance, carcass traits, and muscular profiles of amino
709 acids and fatty acids in growing-finishing Xiangcun black pigs. J Appl Anim Res 49(1):46–

710

711

712

713

714

715

716

717

718

719 Tables

720 Table no 1. Ingredients of basal diet

721

722

Ingredients	% in diet
Rice	30
Wheat	23
Soyabean meal	14
Bengal gram	25
Soya oil	6
Mineral and vitamin mix.	2

730

731 [α]Per kg Composition: Vit A - 7,00,000 IU, Vit D_3:70,000 IU, Vit E: 250 mg, Zn:9600 mg, Mg: 6000 mg, Mn:1500 mg,
732 Fe:1500 mg, Cu:1200 mg, I:325 mg, Methionine:1000 mg, Co:150 mg, K:100 mg, Na:5.9 mg, Ca:25.5%, P: 12.75%, S:0.72%

733

734

735

736

737

738

739

740

741

742

743

744

745

746

747

Table no 2. Nutrients composition and functional properties of basal diet and supplements.

Parameter	Basal diet	Cichorium intybus	Turbinaria conoides	Gracilaria salicornia
Organic matter (%)	98.6	93.5	64.4	72.6
Crude protein (%)	18.6	12.5	4.87	17.4
Ether extract (%)	8.55	1.86	0.75	0.31
Crude fibre(%)	2.29	5.96	4.95	9.00
Ash (%)	1.45	6.50	35.6	27.4
Nitrogen free extract(%)	69.1	73.2	53.9	46.0
Total carbohydrates(%)	67.6	79.1	58.8	55.0
$^{\beta}$ME(kcal/kg)	3970	3157.6	2120.7	2245.4
Total phenolic mg GAE g^{-1} DM	16.1	34.6	41.7	55.4
Total flavonoids mg QE g^{-1} DM	0.13	1.97	2.21	3.64
ABTS inhibition%	12.3	31	51.3	53.8
FRAP mg AAE (100 g^{-1} DM)	126.5	408.3	682.3	884.9
$^{\beta}$ME(kcal/kg)	3970	3157.6	2120.7	2245.4

$^{\beta}$Calculated using modified Atwater factors: (3.50 × CP + 8.50 × EE + 3.50 × NFE) NRC (2006).

779

780 **Table no 3. Nutrient and functional bioactive compound intake, digestibility, and body**
781 **weight changes in dogs fed different dietary treatments.**

	Dietary groups[α]				SEM[¥]	P value
Parameter	**CON**	**CH**	**TC**	**GS**		
Palatability	1.25±0.25	1.25±0.25	1.25±0.25	1.25±0.25	0.112	1
Nutrients intake g day^{-1}						
DM	274.01±4.66	271.13±6.86	276.5±7.65	277.8±7.87	3.14	0.901
OM	259.9±8.91	255.9±14.7	260.8±19.9	260.8±17.4	7.07	0.995
CP	40.0±1.9	39.3±2.8	40.2±3.8	40.1±3.24	5.4	0.996
EE	18.25±0.36	17.93±1.04	18.32±1.19	18.28±1.22	0.45	0.992
CF	7.94±0.59	8.23±0.74	8.14±0.91	8.5±0.41	0.313	0.949
NFE	193.52±6.89	190.52±10.17	193.99±14.38	194.14±13.1	5.16	0.995
TCHO	201.79±6.83	198.71±11.09	202.29±15.01	202.31±13.08	5.34	0.996
SDF	4.4^{a}±0.3	5.8^{c}±0.6	5.5^{b}±0.7	6.4^{d}±0.9	0.25	0.013
Apparent total tract digestibility %						
DM	84±1.54	80.8±1.68	81.6±2.26	81.1±0.98	0.822	0.528
OM	82.5±2.18	81.10±2.1	81.5±1.91	81.2±0.97	0.84	0.943
CP	85.3±0.51	83.9±0.84	84.1±1.1	84.2±0.45	0.371	0.203
EE	93.9±1.14	87.4±1.67	93.3±1.56	91.5±2.98	1.09	0.133
CF	40.7±0.539	41.2±1.178	42.1±1.70	41.8±0.715	0.522	0.828
NFE	84.7±2.13	81.2±2.16	81.6±2.60	81±0.89	0.993	0.564
TCHO	82.9±1.94	79.5±2.07	80±2.64	79.4±0.98	0.963	0.595
Body weight (kg)						
0 week	17.2±1.06	17.1±0.95	17.4±0.66	17.5±1.24	0.45	0.992
3 weeks	17.3±1.09	17.2±0.94	17.5±0.66	17.6±1.22	0.447	0.990
Net change	0.07±0.03	0.08±0.06	0.06±0.04	0.14±0.05	0.014	0.210
BCS	5.0±0.08	5.1±0.09	5.0±0.08	5.1±0.08	0.04	0.275

782 [abcd]Means with different superscript within a row differs significantly.

[α]Animal with **CON**: Feeding of basal diet alone,**CH**,**TC** and **GS** supplemented with *Cichorium intybus*, *G.salicornia* and *T.Conoides* each at the 3%DM of feed respectively. [¥]Standard error of the mean

Table no 4. Effects of supplements on the antioxidant profile and immunity status of dogs.

Parameter	Dietary groups[α]				SEM[ψ]	P value
	CON	CH	TC	GS		
Antioxidant parameter						
Hemoglobin (g dL^{-1})	8.77±0.15	8.61±0.29	10.8±0.98	9.27±0.39	0.330	0.058
GSH(nmol mg^{-1} Hb)	6.37±0.12[a]	9.71±0.32[b]	12.8±0.46[c]	14.5±1.43[c]	0.846	0.001
MDA(nmol mg^{-1} Hb)	12.1±0.23[c]	9.7±0.20[b]	5.95±0.79[a]	5.49±0.27[a]	0.731	0.001
Catalase U g^{-1}Hb	11.9±1.47	13.2±2.85	14.2±1.07	16.2±1.57	0.922	0.471
SOD % inhibition	67.3±5.81	52.1±5.60	55.5±5.70	53.3±6.20	2.820	0.210
FRAP Fe^{2+}µmolL^{-1}	442.2±4.92[a]	547.3±6.21[b]	641.5±3.76[c]	684.2±4.83[d]	24.10	0.001
GSH-Px(U mL^{-1})	210.3±3.46[a]	223.71±3.77[ab]	239.37±3.71[b]	245.51±3.92[c]	7.430	0.001
Immunity						
Serum IgG (mg dL^{-1})	1558.8±10.5[a]	1665.9±8.19[b]	1684.7±10.5[b]	1728.8±14.6[c]	16.50	0.001
Serum IgA (mg dL^{-1}	195.5±8.41[a]	203.8±8.50[a]	241.9±15.2[b]	262.4±8.20[b]	8.50	0.021
Cell mediated immunity						
Absolute skin induration (mm)	4.81[a]	5.13[ab]	5.36[bc]	5.56[c]	0.212	0.008
Relative skin induration (% h value)	134.9[a]	144.7[ab]	149.7[b]	153.3[b]	1.79	0.0014

[abcd]Means with different superscript within a row differs significantly.

[α]Animal with CON: Feeding of basal diet alone,CH,TC and GS supplemented with *Cichorium intybus, G.salicornia* and *T.Conoide*s each at the 3%DM of feed respectively.

[¥]Standard error of the mean.

Table no 5. Effect of tropical seaweed supplementation on serum T3, T4 and cortisol concentrations in adult Labrador dogs.

Parameter	Dietary groups[α]				SEM[ψ]	P value
	CON	CH	TC	GS		
Serum hormones ($ngdL^{-1}$)						
T3	10.9±1.77	12.9±1.53	14±3.09	16.6±2.5	0.99	0.235
T4	15.9±1.82	18.5±6.03	20.2±1.55	23.8±1.57	1.64	0.420
Cortisol ($ngmL^{-1}$)	48.5^{c}±2.63	38^{b}±1.65	28.6^{a}±1.45	24.1^{a}±0.16	2.52	<0.001

[abcd]Means with different superscript within a row differs significantly.

[α]Animal with **CON**: Feeding of basal diet alone,**CH**,**TC** and **GS** supplemented with *Cichorium intybus*, *G.salicornia* and *T.Conoides* each at the 3%DM of feed respectively. [¥]Standard error of the mean.

812

813

814

815 **List of Figures:**

816

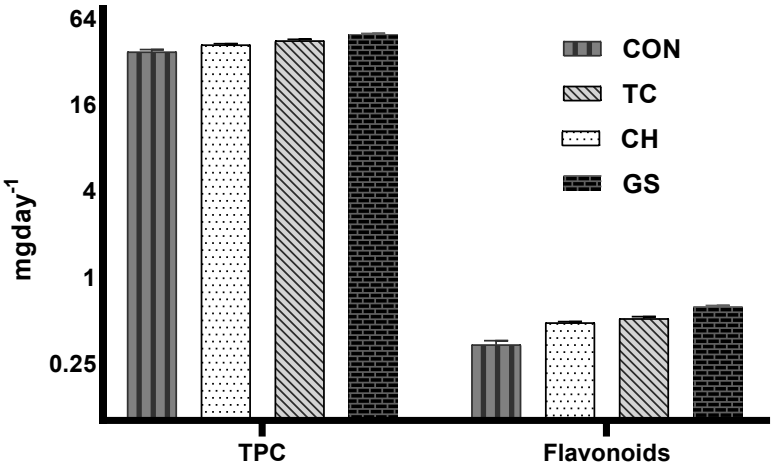
817

818

819

820

821



822

823 Fig 1. Total phenolic and flavonoid intake per day across dietary treatments.

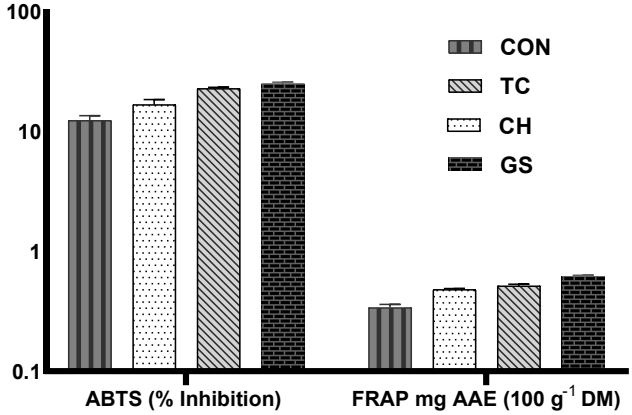
824

825

826

827

828



829

Fig 2. Comparative antioxidant potential of basal diet and supplements.

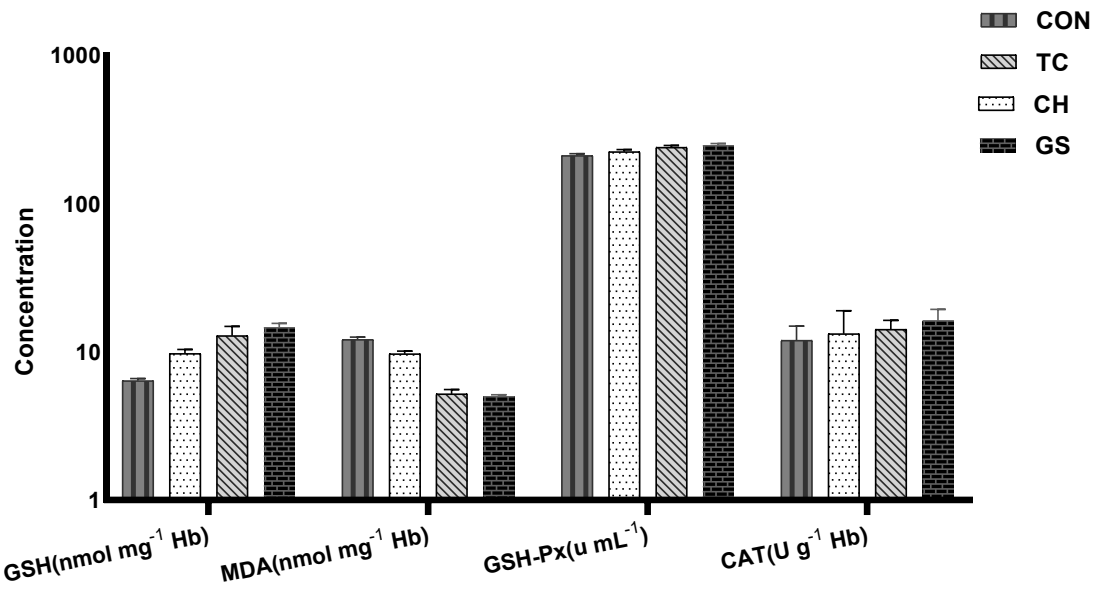


Fig 3. Effect of seaweed supplementation on antioxidant and oxidative stress biomarkers.

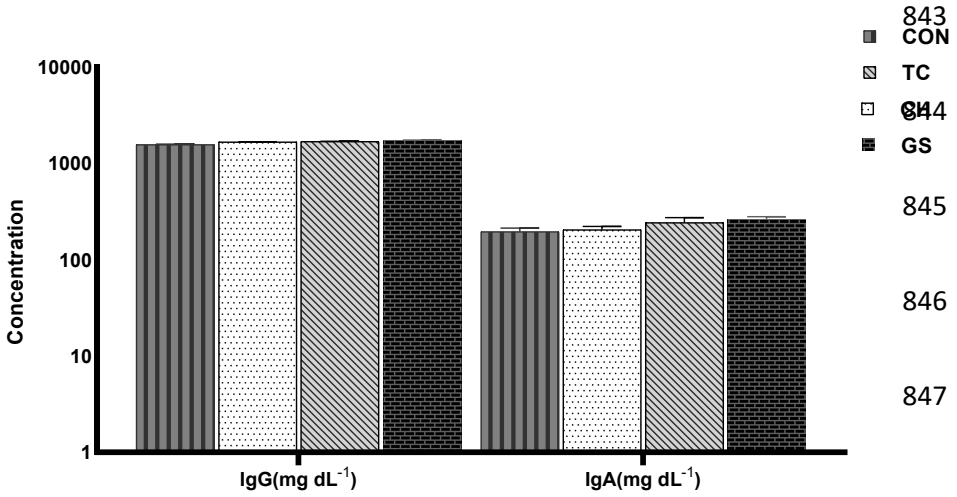


Fig:4 Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) concentrations in adult Labrador dogs fed the control diet (CON), chicory (CH), *Turbinaria conoides* (TC), and *Gracilaria salicornia* (GS).

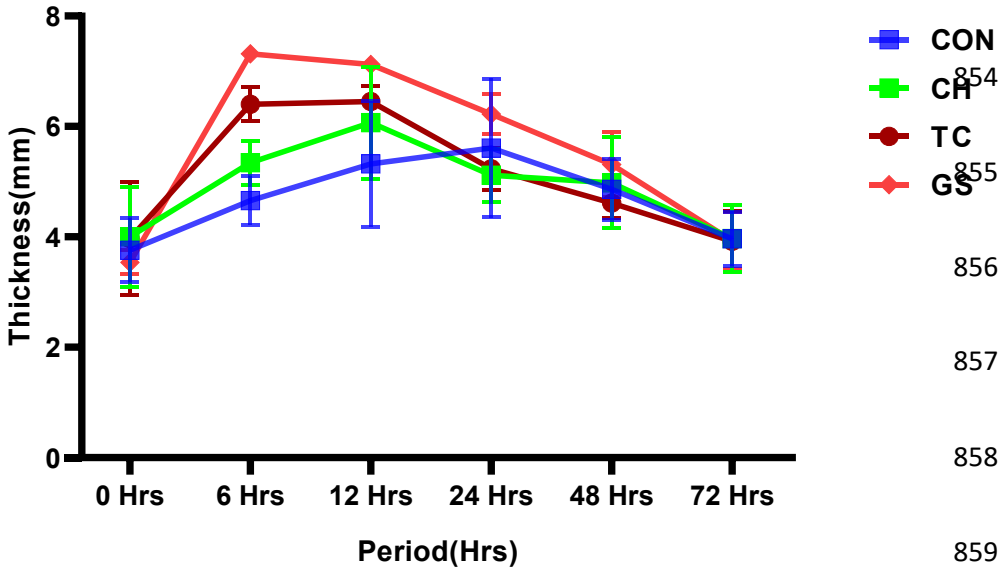


Fig.5 Seaweed supplementation modulates cell-mediated immune response.

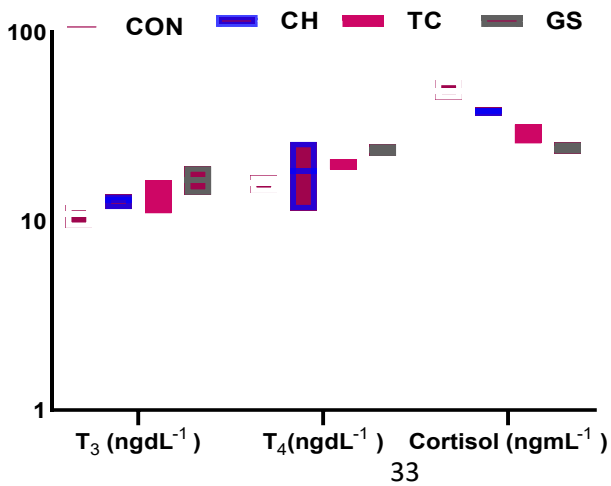


Fig.6 Serum thyroid hormones (T3,T4) and cortisol response to dietary seaweed supplementation.

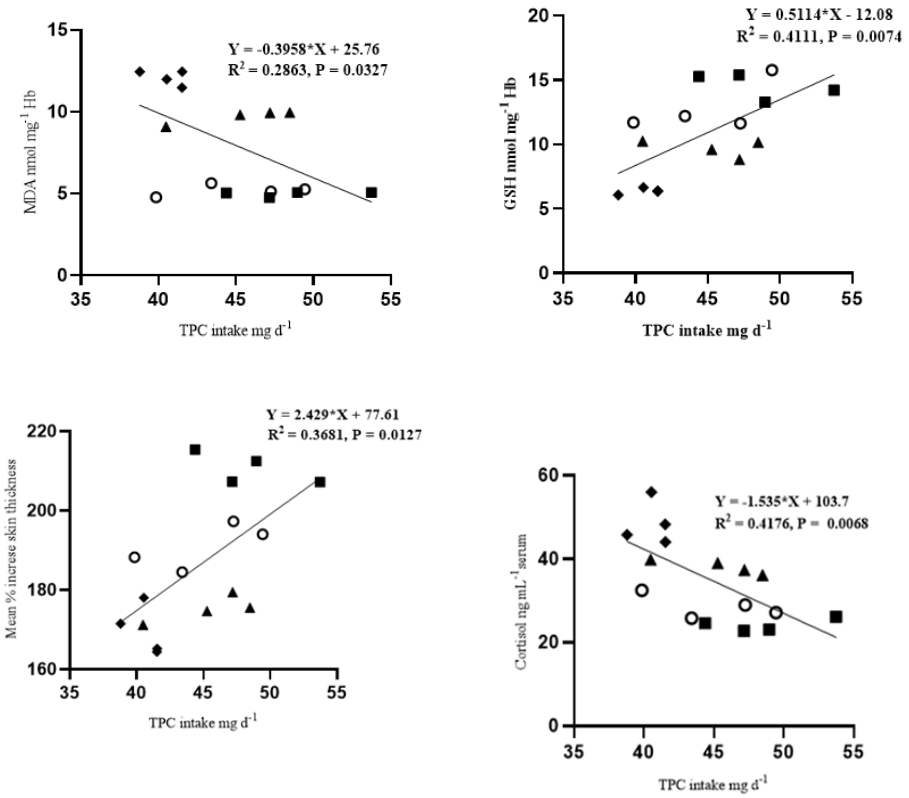


Fig.7 Regression analysis between total phenolic compound intake (mg GAE day^{-1}) and malondialdehyde (MDA, nmol mg^{-1} Hb), reduced glutathione (GSH, nmol mg^{-1} Hb), cell-mediated immunity response (mean % increase skin thickness) and serum cortisol (ng mL^{-1}).[■ GS (*Gracilaria salicornia*), ○ TC (*Turbinaria conoides*), ▲ CH (*Cichorium intybus*), ◆ Control]

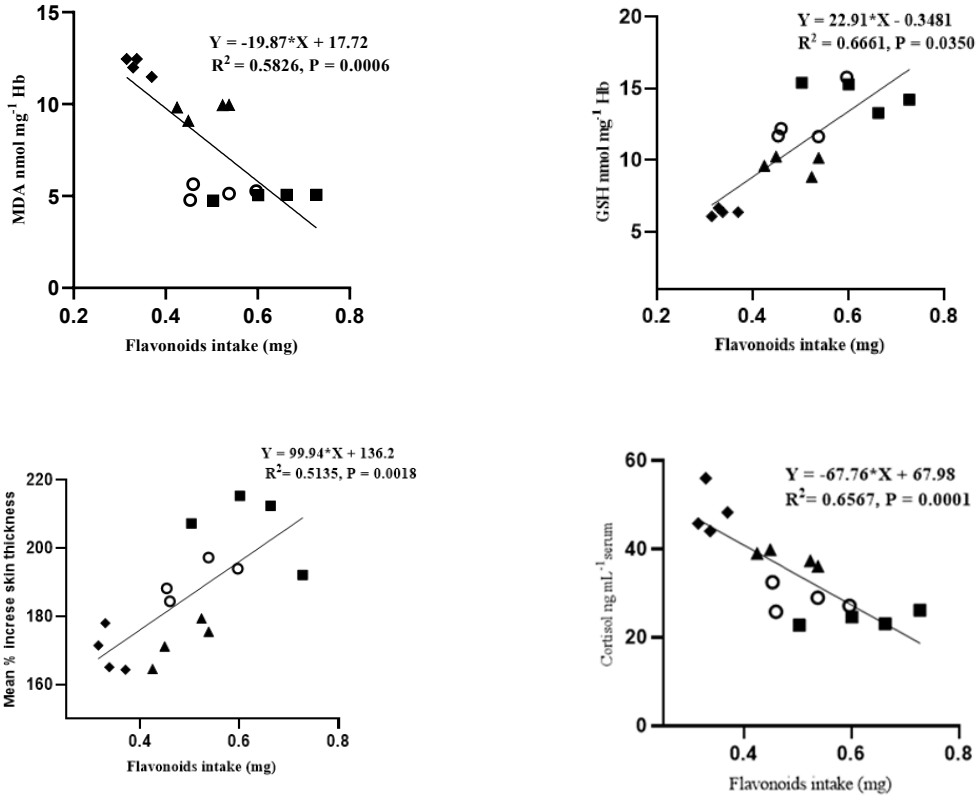


Fig.8 Relationship between total flavonoid intake (mg QE day^{-1}) and oxidative stress, antioxidant and immune markers: malondialdehyde (MDA, nmol mg^{-1} Hb), reduced glutathione (GSH, nmol mg^{-1} Hb), cell-mediated immunity (% increase in skin thickness) and serum cortisol (ng mL^{-1}).$^{-1}$).[■ GS (*Gracilaria salicornia*), ○ TC (*Turbinaria conoides*), ▲ CH (*Cichorium intybus*), ◆ Control]

906

907 **List of figures:**

908 **Fig 1.** Total phenolic and flavonoid intake per day across dietary treatments.

909 **Fig 2**. Comparative antioxidant potential of basal diet and supplements.

910 **Fig 3.** Effect of seaweed supplementation on antioxidant and oxidative stress biomarkers.

911 **Fig 4.** Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) concentrations in adult
912 Labrador dogs fed the control diet (CON), chicory (CH), *Turbinaria conoides* (TC), and
913 *Gracilaria salicornia* (GS).

914 **Fig.5** Seaweed supplementation modulates cell-mediated immune response.

915 **Fig.6** Serum thyroid hormones (T3,T4) and cortisol response to dietary seaweed
916 supplementation.

917 **Fig.7** Regression analysis between total phenolic compound intake (mg GAE day^{-1}) and
918 malondialdehyde (MDA, nmol mg^{-1} Hb), reduced glutathione (GSH, nmol mg^{-1} Hb), cell-
919 mediated immunity response (mean % increase skin thickness) and serum cortisol (ng mL^{-1}).[■
920 GS (*Gracilaria salicornia*), ○ TC (*Turbinaria conoides*), ▲ CH (*Cichorium intybus*), ◆ Control]

921 **Fig.8** Relationship between total flavonoid intake (mg QE day^{-1}) and oxidative stress,
922 antioxidant and immune markers: malondialdehyde (MDA, nmol mg^{-1} Hb), reduced glutathione
923 (GSH, nmol mg^{-1} Hb), cell-mediated immunity (% increase in skin thickness) and serum cortisol
924 (ng mL^{-1}).$^{-1}$).[■ GS (*Gracilaria salicornia*), ○ TC (*Turbinaria conoides*), ▲ CH (*Cichorium*
925 *intybus*), ◆ Control]

926 **TABLE LEGENDS:**

927 Table no 1. Ingredients of basal diet

928 Table no 2. Nutrients composition and functional properties of basal diet and supplements.