Journal of Elementology ISSN 1644-2296

Perżyło, Ł. and Chwastowska-Siwiecka, I. (2026)
'Effect of dietary supplementation with Ascophyllum nodosum on selected growth performance indicators and blood parameters in sheep',
Journal of Elementology, 31(2), 401-416,
available: https://doi.org/10.5601/jelem.2026.31.1.3845

RECEIVED: 18 March 2026
ACCEPTED: 20 April 2026

ORIGINAL PAPER

Effect of dietary supplementation with *Ascophyllum nodosum* on selected growth performance indicators and blood parameters in sheep*

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Abstract

The aim of this study was to determine the effect of dietary supplementation with brown alga (*Ascophyllum nodosum*) meal on selected growth performance indicators, hematological parameters, and blood iodine concentration in rams. The experimental material consisted of 18 rams of the White-headed Mutton Sheep (*Polish: Białogłowa Owca Mięsna*, BOM) aged 16 months. Before the experiment, the animals were divided into three groups with comparable initial body weight: a control group (K) and two experimental groups (D1 and D2), with six animals per group. Control group rams received a standard diet, whereas the diets of animals in the experimental groups were supplemented with 5 g (D1) or 15 g (D2) of brown alga meal mixed with 500 g of ground carrots as a carrier. The seaweed supplement was administered individually once daily in the morning. In each group, animals were weighed individually at the beginning (day 0) and at the end of the experimental period (day 42) to determine body weight gain and the relative growth rate (RGR). Whole blood and serum samples were collected at the same time points to assess hematological parameters and serum iodine concentration. A significant interaction between dietary treatment and the duration of seaweed supplementation was observed for the following parameters: WBC, Ht, MCHC, and PLT. Seaweed supplementation at 5 g and 15 g resulted in higher WBC and PLT counts in group D2 and lower mean MCHC values in groups D1 and D2. At the end of the supplementation period (day 42), WBC and MCHC values decreased but remained within the normal reference ranges. A significant increase in thrombocyte production and an increase in erythrocyte parameters (RBC, Ht, and Hb) were also noted at the end of seaweed supplementation. An analysis of serum iodine concentration in the experimental groups revealed that both dietary supplementation with 5 g and 15 g of seaweed and intake duration contributed to a significant increase in this parameter.

Keywords: White-headed Mutton Sheep, nutrition, brown alga (*Ascophyllum nodosum*), hematological parameters, serum iodine concentration

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* This work was financed from the statutory research fund of the Department of Sheep and Goat Breeding University of Warmia and Mazury in Olsztyn.

INTRODUCTION

Macroalgae are an alternative source of biomass for the production of protein with high biological value, intended for use in the food and feed industries. In Europe, seaweed has been used as animal feed since Roman times. In Iceland, France, and Norway, livestock were traditionally fed macroalgae to enhance the nutritional value of their diets. In 2004, 1% of globally produced seaweed was used in animal feed, with *Ascophyllum nodosum* being the dominant species (Chojnacka et al. 2012). Macroalgae are characterized by more rapid growth and reproduction as well as higher productivity and photosynthetic efficiency than terrestrial plants (Noga et al. 2024). Norway, France, and Ireland are the largest producers of macroalgae in Europe. Seaweed is also produced on a smaller scale in Iceland, Russia, Portugal, Italy, Estonia, Denmark, and Bulgaria (Rahikainen, Yang 2020).

Algae are generally classified into two groups: microalgae and macroalgae (seaweed), which differ in nutritional value and harvesting methods. The most widely utilized macroalgae include brown algae – *Phaeophyta*, red algae – *Rhodophyta*, and green algae – *Chlorophyta* (Frąc et al. 2009, Chojnacka et al. 2012). Marine algae play an important role in agriculture, particularly in coastal regions, where they have been traditionally used as organic fertilizers or compost. Brown algae biomass is most commonly applied to arable land to improve soil properties and as an alternative to conventional fertilizers (WSH 2016, Michalak et al. 2016, Oluwadare et al. 2020). In addition, algae act as biostimulants that promote plant growth and enhance their resilience by improving nutrient uptake and utilization efficiency. In modern agriculture and horticulture, aqueous seaweed extracts are widely applied as foliar sprays or soil amendments (Oluwadare et al. 2020, Mzibra et al. 2021, Kisvarga et al. 2022). In aquatic ecosystems, algae perform key ecological functions by assimilating dissolved nutrients into biomass, thereby contributing to water purification and oxygen production necessary for aerobic organisms, including aerobic bacteria (Chojnacka et al. 2012). Algae are used more frequently as feed additives than as components of the human diet. Numerous nutritional and toxicological evaluations have confirmed that algal biomass is a valuable feed supplement (Becker 2007). Algae are extensively applied in animal nutrition due to their diverse chemical composition, which includes essential nutrients such as proteins (5-8%), amino acids, lipids (2-4%), vitamins, minerals, polysaccharides (45-60%), and polyamides. The micronutrient content of algal biomass exceeds that of conventional grains such as oats, wheat, and maize, often by several orders of magnitude (Garcia-Vaquero 2018).

Knotted wrack (*A. nodosum*) is one of the most widely studied macroalgae with potential applications in the agri-food sector. This species belongs to brown algae, which are rich in iodine (up to 64 mg 100 g^{-1} dry matter, DM) and bioactive polysaccharides such as alginic acid and fucoidan. Fucoidan enhances cell-mediated immunity, promotes the growth of gut microbiota, and inhibits the classical complement pathway, a key component of humoral immunity (Jękot et al. 2016, Muszyńska et al. 2016). In addition, *A. nodosum* exhibits antiviral and antibacterial properties and stimulates phagocytic activity. Dietary supplementation with this seaweed has been found to reduce intestinal *Escherichia coli* populations in steers and lambs and improve oral health in dogs and cats (Gawor, Jank 2023). Research has also shown that the supplementation of poultry diets with seaweed extracts may reduce susceptibility to stress, including under exposure to chronic heat stress. Although no consistent effects on growth performance or feed conversion have been observed, seaweed extracts have the potential to improve bird welfare in periods of elevated temperature and to reduce corticosterone levels (Archer 2023). Seaweed contains many bioactive compounds and minerals that serve as natural sources of feed additives, representing a potential alternative to antibiotic use in livestock production, particularly in pigs, and a strategy aimed at reducing antibiotic resistance (Morais et al. 2020). Many marine algae, including brown seaweed, exhibit prebiotic effects when their dietary inclusion levels are low (up to 5%). However, the inclusion of macroalgae in animal diets, especially for ruminants, is limited mainly due to their high iodine content. Supplementation levels with various macroalgal species generally depend on the concentrations of essential and potentially toxic elements, as excessive intake of these elements may have adverse effects (Cabrita et al. 2016). According to Hansen et al. (2003) and Makkar et al. (2016), Orkney sheep on the North Ronaldsay Island consume brown algae, including *Laminaria digitata*, *Laminaria hyperborea*, and *Saccharina latissima*, for most of the year, and these species account for up to 90% of their summer diet. These sheep also consume other seaweed species, including *Alaria esculenta*, *A. nodosum*, *Fucus* spp. (brown algae), *Palmaria palmata* (red algae), and certain green algae. This feeding strategy largely meets the animals' nutritional requirements (13% of protein), but it may lead to excessive mineral intake. Seaweeds of the order Fucales, including *Fucus vesiculosus* and *A. nodosum*, have strong antimethanogenic properties, which may contribute to mitigating climate change and improving the environmental sustainability of animal production (Campbell 2021). According to Heidarpour et al. (2011), supplementation of calf diets with *Spirulina platensis* microalgae at 2, 6, and 25 g per day for eight weeks led to a significant reduction in plasma cholesterol and LDL (low-density lipoprotein) and HDL (high-density lipoprotein) lipoprotein concentrations, compared with control animals. In the cited studies, *Spirulina* supplementation had no effect on blood urea nitrogen, albumin, or globulin levels. In the context of feed utilization, Holman and Malau-Aduli (2013) observed that ruminants have a greater capacity to digest high-fiber diets than non-ruminant species, which results in the highest efficiency of protein utilization from algal sources.

Seaweed is also a valuable dietary supplement due to its naturally high iodine content. Iodine is an essential trace element that is routinely added to animal feeds (Grabež et al. 2022). However, brown algae are able to bioaccumulate iodine from seawater. Both iodine deficiency and excess pose health risks to consumers, and iodine levels in feed and food are strictly regulated. Food safety authorities, including EFSA and the FDA, have established maximum limits for iodine content in animal feed and animal-derived products. To ensure animal safety, iodine intake in ewes and lambs should not exceed 10 mg per animal per day (Lind et al. 2023). Dietary iodine is highly bioavailable, and approximately 70-90% of iodine is absorbed in the gastrointestinal tract, beginning from the proventriculus (rumen, reticulum, and omasum) and continuing in the small intestine (NRC 2007). The absorbed iodine is transported by the portal vein to the liver and subsequently enters the bloodstream, where it circulates loosely bound to plasma proteins before reaching the thyroid gland. The thyroid gland acts as the main iodine reservoir in the body, storing up to 80% of the total iodine content. Iodine is essential for the biosynthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) – Paulíková et al. (2002). These hormones play a key role in regulating the metabolic rate, and they stimulate energy production and cellular respiration. Consequently, they affect energy metabolism, muscle function, growth processes, circulation, immune responses, and reproductive cycles (Huszenicza et al. 2002). Some seaweeds, such as *A. nodosum*, may contain high concentrations of iodine (up to 700 μg g^{-1} DM). Özkan Gülzari et al. (2019) reported that sheep diets containing *S. latissima* were characterized by elevated iodine levels (180 mg kg^{-1} DM), which could explain why they were unwillingly consumed by some of the animals even when the iodine concentration in the biomass was reduced through processing to 1.2 g kg^{-1} DM. Tolerance to excess iodine varies among species, and sheep appear to be more resistant to iodine oversupply than cattle (Pauliková et al. 2002). Iodine concentrations in milk and urine are reliable indicators of the body's iodine status. Iodine excretion via milk and urine correlates with plasma iodine levels and dietary intake. In non-lactating animals, approximately 90% of excess iodine is eliminated with urine (Anke 2004).

The aim of the present study was to evaluate the effect of dietary supplementation with brown alga (*A. nodosum*) meal on selected growth performance indicators, hematological parameters, and blood iodine concentration in rams.

MATERIALS AND METHODS

The study was approved by the Local Ethics Committee for Animal Experiments (Resolution No. 72/2025 of 10 September 2025). The experimental material consisted of 18 males (rams) of the White-headed Mutton Sheep (Polish: Białogłowa Owca Mięsna, BOM) aged 16 months, maintained at the Animal Research Laboratory of the Department of Sheep and Goat Breeding, University of Warmia and Mazury in Olsztyn, Poland. During the 42-day experiment, the animals were housed under conditions meeting welfare requirements for this category of livestock. They had continuous access to drinking water in bowl drinkers and a mineral lick. Before the experiment, rams were divided into three groups with comparable initial body weight: a control group (K) and two experimental groups (D1 and D2), with six animals per group. Control group animals received a standard diet composed of meadow hay, crushed oat grain, and grated carrots. The diet of the experimental groups was additionally supplemented with brown alga (*A. nodosum*) meal with particle size of 1.4 mm (Figure 1), purchased from

Fig. 1. Brown alga *(Ascophyllum nodosum*) meal. Source: I. Chwastowska-Siwiecka

a domestic supplier. In groups D1 and D2, seaweed meal was mixed with 500 g of ground carrots as a carrier. After washing and peeling, the carrots were ground in a laboratory colloid mill. Subsequently, 500 g portions of the obtained pulp were weighed and placed into separate containers. Each container with carrot pulp was supplemented with 5 g of algae in experimental group D1 and 15 g in experimental group D2. To ensure homogeneity, the carrot pulp with the added algae was mixed using a mixer. The mixture was then supplied individually to each ram in a feeder. The seaweed supplement and ground carrots were administered individually to each animal once daily in the morning. According to the manufacturer's specifications, seaweed meal had the following chemical composition: 12% water, 22% crude ash, 6% crude protein, 2% crude fat, 6% crude fiber, and 52% carbohydrates. The average content of selected minerals and trace elements (mg kg^{-1}) was as follows: Ca - 20,000, S - 13,000, P - 2,000, Mg- 7,000, Na - 3,000, K - 2,500, Fe - 600, I - 500, Si - 160, B -90, Zn - 65, Ba - 35, Mn - 13, Cu - 10, Co - 5, Se - 4, Ti - 4, V - 4, and Mo - 0.6. The vitamin content (mg kg^{-1}) was as follows: β-carotene - 45, B1 - 3.5, B2 - 8, B3 - 20, B12 - 0.004, C - 1000, D - 4, E - 225, K - 10, biotin - 0.3, folic acid - 0.3, and folinic acid - 0.3.

The proximate composition of feed components and brown alga (*A. nodosum*) meal – Table 1 used in ram diets was determined in the laboratory of the Department of Animal Nutrition, Feed Science and Cattle Breeding, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, using standard methods (AOAC 2005).

Table 1

Proximate chemical composition of feed components used in ram diets

Specification	Meadow hay	Crushed oat grain	Brown alga (*Ascophyllum nodosum*) meal
Dry matter (%)	86.99	86.63	90.58
Crude ash (%)	9.28	2.75	26.00
Total protein (%)	11.44	8.69	8.06
Crude fat (%)	1.23	3.87	2.31
Crude fiber (%)	27.52	9.75	3.86
Acid detergent fiber (% DM)	33.95	13.92	37.52
Neutral detergent fiber (% DM)	55.68	20.80	54.76
Acid detergent lignin (% DM)	4.41	4.01	23.74
Gross energy (MJ kg^{-1} DM)	15.31	16.35	12.44

The animals from each group were weighed individually before the experiment (day 0) and after its completion (day 42) to determine body weight gain during the entire feeding trial. Feed refusals were also recorded. The relative growth rate (RGR) of the rams during the supplementation period was calculated according to the following formula (Milewski 2009):

$$\text{RGR} = \frac{\text{final body weight} - \text{initial body weight}}{0.5 \times (\text{final body weight} + \text{initial body weight})} \times 100\%.$$

Hematological analyses

Blood samples (4 mL) were collected before the experiment (day 0) and after its completion (day 42) from the jugular vein into disposable tubes (Improve Medicals) containing K_2EDTA as an anticoagulant. The following hematological parameters were determined in whole blood: red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cell count (WBC), and red blood cell distribution width (RDW-CV). The analyses were performed using flow cytometry based on laser light scattering with a hematology analyzer (ADVIA 2120, Siemens Healthcare Diagnostics, Tarrytown, USA).

Biochemical analyses

Serum iodine concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS) in a diagnostic laboratory (Labokin Polska Sp. z o.o., Warsaw, Poland). Blood samples (5 mL) were collected twice (day 0 and day 42) from the jugular vein into disposable tubes without an anticoagulant (improve medicals). Within 20 min of collection, the samples were centrifuged for 5 min at 4000 rpm and cooled. The serum obtained was transferred to clean tubes for analysis.

Statistical analysis

The data were subjected to statistical analysis. The results were presented as means ($\bar{x}$) and standard deviations (±SD). The significance of differences between the mean values of the analyzed traits across the study groups was determined using one-way and two-way analysis of variance (ANOVA) and Duncan's multiple range test at significance levels of $p \leq 0.05$ and $p \leq 0.01$. All statistical analyses were conducted in Statistica v. 13.3 software (StatSoft Inc. 2017).

RESULTS AND DISCUSSION

Selected growth performance indicators in the studied groups of rams are presented in Table 2. At the beginning of the experiment (day 0), initial body weights were similar across groups, ranging from 42.85 kg to 44.40 kg. At the end of the experiment (day 42), final body weights did not differ significantly between the control group (K) and the experimental groups (D1 and D2), and ranged from 45.83 to 46.42 kg. Total weight gain during the experiment (days 0-42) was highest in the control group (2.98 kg) and lower in the experimental groups (D1 and D2), and the differences were statistically significant ($p \leq 0.01$). The RGR was highest in the control group

Table 2

Effect of dietary supplementation with brown alga (*Ascophyllum nodosum*) on selected growth performance indicators in rams ($\bar{x}^1 \pm SD$)

Specification	Dietary treatment group			*P-value*
	K	D1	D2	
Body weight on day 0 (kg)	42.85±1.35	43.80±1.34	44.40±1.42	0.450
Body weight on day 42 (kg)	45.83±1.15	46.15±1.17	46.42±0.93	0.739
Weight gain between day 0 and day 42 (kg)	2.98^A±0.28	2.35^B±0.23	2.02^B±0.30	0.001
Relative growth rate (RGR, %) between day 0 and day 42	6.76^A±0.39	5.22^B±0.51	4.45^C±0.51	0.001

$^{A\text{-}C}$ mean values in rows marked with different superscript letters differ significantly at $p \leq 0.01$,
^{1} N=6.

(6.76%), and it was significantly lower, by 1.54% and 2.54%, in the groups receiving brown seaweed supplementation, which corresponded with the lower weight gain in the experimental groups.

The study demonstrated that dietary supplementation with brown alga (*A. nodosum*) meal did not significantly affect the final body weights of rams, which is consistent with the findings of other authors. The literature suggests that marine algae used as feed additives exhibit mainly functional properties, such as improvements in animal health and antioxidant status, whereas their influence on fattening performance, including final body weight and weight gain, tends to be neutral or marginal. Grabež et al. (2023) reported that in female Norwegian White lambs with an average body weight of 37.3 kg, dietary supplementation with 2.5% and 5% of brown seaweed (*S. latissima*) for 35 days had no significant effect on average daily gain, compared with control group animals. Lind et al. (2023) found that the inclusion of 6% brown seaweed (*L. hyperborea*) in the diet of four-month-old male Norwegian White lambs with an initial body weight of 37.8 kg resulted in lower final body weight (52.2 kg) relative to the control group (56.2 kg). The cited authors also reported significantly lower average daily gain in lambs receiving the seaweed supplement (167.9 g/day) than in animals fed the control diet (203.8 g/day), which resulted in higher final body weight in the latter. According to Belanche et al. (2016), brown algae have a high content of antinutritional compounds, including phlorotannins and complex polysaccharides. These compounds may inhibit rumen microbial activity and form complexes with proteins, which reduces nitrogen digestibility by up to 24%. In consequence, despite adequate feed intake, animals are unable to efficiently convert feed into muscle tissue, which may explain the lower weight gain and lower RGR in the present study. In a study by Rossi et al. (2020), New Zealand White rabbits were fed 42 days a control diet and two experimental diets, which were supplemented with 0.3% and 0.6% of feed additive consisting of prebiotic polysaccharides from brown seaweeds (*L. digitate* and *hyperborea*, ratio 1:1) plus phenolic acid, hydroxycinnamic acids, tannins, and flavonoids from plant extracts. The authors noted that, in terms of the productive performance of growing rabbits, the live weight was improved at 21 days and tended to be higher at 42 days in rabbit fed a lower dosage of the dietary supplement (0.3% of algae) than the other two groups. Therefore, the average daily gain (0-42 days) tended to be higher in this group (47.4 vs. 43.5 and 46.1 g/day). The feed conversion ratio was also improved and amounted to (3.35 kg kg^{-1}) in rabbits fed with a diet containing 0.3% of the natural extract mixture. The average daily feed intake (ADFI) was lower in the group receiving 0.6% brown seaweed than in control groups in the first period of the trial (0-21 days). Considering the ADFI in the first period of the trial, it is possible that the high dosage of bioactive compounds of the supplement negatively affected diet palatability and consequently growth, as previously observed in rat fed high dosage of ellagic acid.

The data presented in Table 3 indicate that selected hematological

Table 3

Effect of dietary supplementation with brown alga (*Ascophyllum nodosum*) meal on hematological parameters in rams ($\bar{x}$ ± SD)

Specification	Dietary treatment group (A)			Duration of seaweed supplementation (B)		Significance of main effects and their interaction *(P-value)*		
	K	D1	D2	0	42	A	B	A x B
WBC (m mm^{-3})	6.90^{b}±0,47	6.46^{c}±0.84	7.59^{a}±0.52	7.32^{x}±0.51	6.64^{y}±0.85	0.001	0.001	0.019
RBC (M mm^{-3})	10.98±0.65	11.10±0.62	10.94±0.87	10.68^{y}±0.65	11.33^{x}±0.62	0.819	0.005	0.234
Ht (%)	28.90±0.87	28.68±1.14	28.78±1.66	27.88^{y}±0.79	29.69^{x}±0.88	0.788	0.001	0.022
Hb (g/dL)	11.24±0.69	11.38±0.91	11.18±0.95	10.97^{y}±0.81	11.57^{x}±0.77	0.807	0.031	0.195
MCV (fL)	26.33±0.65	25.92±0.79	26.25±0.87	26.22±0.73	26.11±0.83	0.421	0.683	0.958
MCH (pg)	10.28±0.45	10.23±0.54	10.20±0.30	10.30±0.43	10.17±0.44	0.899	0.405	0.810
MCHC (g L^{-1})	39.20^{a}±0.88	38.43^{b}±1.20	38.59^{b}±0.68	39.39^{x}±0.71	38.09^{y}±0.75	0.013	0.001	0.047
RDW-CV (%)	24.93±0.46	24.38±0.86	24.98±0.74	24.75±0.86	24.78±0.62	0.098	0.901	0.444
PLT (m mm^{-3})	236.83^{b}±0.71	213.75^{c}±0.44	256.42^{a}±0.55	227.67^{y}±0.92	243.67^{x}±0.84	0.001	0.001	0.001

$^{a-c\ i\ x-y}$ – mean values in rows marked with different superscript letters differ significantly at $p \leq 0.05$, A – dietary treatment group, B – duration of seaweed supplementation, A × B – dietary treatment group × duration of seaweed supplementation, WBC – white blood cell count, RBC – red blood cell count, Ht – hematocrit, Hb – hemoglobin concentration, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, RDW-CV – red blood cell distribution width, PLT – platelet count,

parameters, including WBC, Ht, MCHC, and PLT, were significantly influenced by the interaction between dietary treatment and the duration of seaweed supplementation. Significant differences ($p \leq 0.01$) in the total WBC count of rams were found between the dietary treatment groups. This parameter was highest in group D2 (7.59 m mm^{-3}) and lowest in group D1 (6.46 m mm^{-3}). This observation could suggest that dietary supplementation with 15 g of seaweed modulated the immune response differently than in the control group and group D1. An analysis of leukocyte parameters revealed that the duration of seaweed supplementation (42 days) contributed to a significant decrease (by 0.68 m mm^{-3}, $p \leq 0.01$) in total WBC count. In contrast, dietary treatments induced no significant ($p \leq 0.05$) differences in erythrocyte parameters (RBC, Ht, Hb, MCV, MCH, RDW-CV) among groups (Table 3). However, after 42 days of seaweed supplementation, a significant ($p \leq 0.05$) increase in RBC, Ht, and Hb was observed, accompanied by a decrease in MCHC values. An analysis of platelet parameters indicated a significant increase in thrombocyte count in group D2 (256.42 m mm^{-3}), compared with the control group and group D1 ($p \leq 0.01$). The duration of seaweed supplementation also had a significant ($p \leq 0.01$) effect on PLT count, which increased by 16.00 m mm^{-3} relative to the initial value measured on day 0 (227.67 m mm^{-3}).

Research results indicate that *A. nodosum* may effectively modulate the immune system in small ruminants when included in the diet at appropriate levels. The increase in leukocyte counts in group D2 is consistent with the findings of Saker et al. (2004) and Archer et al. (2007), who described the immunostimulatory properties of macroalgae. Brown seaweed is a rich source of bioactive polysaccharides, including fucoidans, laminarin, and alginates, which may activate macrophages and lymphocytes, leading to physiological leukocytosis. These processes may support immune function and enhance resistance to infection in livestock herds.

In the present study, the dietary inclusion level of *A. nodosum* did not affect erythrocyte parameters, including RBC, Hb, Ht, MCV, MCH, and RDW-CV. Similar observations were made by Antunović et al. (2012), who found that in young ruminants, these parameters are more strongly correlated with age and physiological development than with dietary modifications alone. The aim of the study of Karatzia et al. (2012) was to investigate the effect of *A. nodosum*, an edible brown macroalga, on some hematologic parameters of dairy cows. Nineteen clinically healthy Holstein cows, an average 4.3 years old, were divided into two groups for 49 days. Ten cows received control diet (roughages and concentrate) while the concentrate fed to 9 cows was additionally supplemented with 80 g powdered *A. nodosum*/cow/day. Average daily milk production (controls 39.6 kg/cow; *A. nodosum* 40.2 kg/cow), milk protein and fat were not affected by the alga supplementation. Glucose, sorbitol dehydrogenase, hemoglobin, hematocrit and white/red blood cells were evaluated in weekly blood samples. It was seen that *A. nodosum* increased blood glucose and decreased sorbitol dehydrogenase compared to controls, without any adverse effects on the other examined parameters, like hemoglobin (Hb), packed cell volume (PCV), erythrocyte count (TEC), total leukocyte count (TLC). The authors cited consider that, *A. nodosum* may be suggested as a functional ingredient in dairy cow nutrition, improving energy utilization and expressing hepatoprotective effect.

Therefore, the increase in hematocrit and hemoglobin values observed on day 42 in all groups may reflect normal maturation of the hematopoietic system and metabolic adaptation of the animals. Although *A. nodosum* contains hematopoietically relevant micronutrients such as iron and copper, the basal diet in the control group probably met the animals' maintenance needs, which may explain the absence of additional erythropoietic effects of seaweed supplementation. According to Tissot et al. (2003) and Muszyńska et al. (2016), brown algae exhibit anticoagulant and anti-inflammatory activity due to the presence of polysaccharides such as laminarin and fucoidan. Branched sulfated polysaccharides with sulfate residues at the 4-O position, derived from species such as *A. nodosum* and *F. vesiculosus*, exhibit stronger anticoagulant activity than linear polysaccharides. Branched fucoidans limit thrombin activity, whereas linear fucoidans inhibit thrombin in the presence of antithrombin or heparin cofactor II. Despite the increase in PLT in the group supplemented with the higher seaweed dose (15 g/day), PLT values remained within the lower limit of the reference range, which may reflect platelet utilization associated with thrombin activity. However, further research is needed to rule out proinflammatory states, although no clinical signs of pathological conditions were observed in animals during the experiment.

Changes in serum iodine concentration in dietary treatment groups between day 0 and day 42 of seaweed supplementation are presented in Figure 2. The interaction between these two factors (dietary treatment and

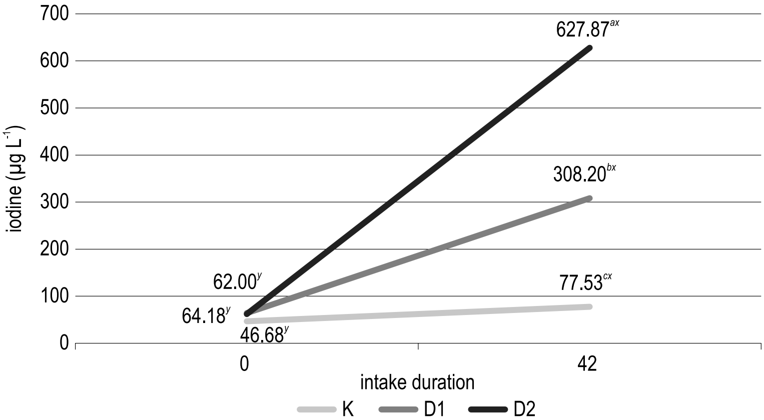

Fig. 2. Effect of dietary supplementation with brown alga (*Ascophyllum nodosum*) meal and intake duration on serum iodine concentration in rams ($\bar{x} \pm SD$): $^{a\text{-}c,\ x\text{-}y}$ mean values marked with different superscript letters differ significantly at $p \leq 0.05$, K – control group, D1 – experimental group, D2 – experimental group

the duration of seaweed supplementation) significantly affected serum iodine levels in rams. At the beginning of the experiment (day 0), serum iodine concentration was low in all groups, ranging from 46.68 μg L^{-1} in the control group (K) to 64.18 μg L^{-1} in group D1. At the end of the supplementation period (day 42), a significant increase in this parameter was observed in the experimental groups ($p \leq 0.05$). Serum iodine levels increased to 308.20 μg L^{-1} in the group receiving 5 g of seaweed (D1) and to 627.87 μg L^{-1} in the group receiving 15 g of seaweed (D2). In contrast, serum iodine concentration remained low and stable (77.53 μg L^{-1}) in the control group (K) throughout the feeding period. The differences between dietary treatment groups were statistically significant ($p \leq 0.01$) on day 42.

The results clearly indicate that *A. nodosum* is an exceptionally rich and bioavailable source of iodine for sheep. The significant increase in serum iodine levels in the experimental groups (nearly fivefold in group D1 and tenfold in group D2) was a direct consequence of the high iodine content of brown seaweed biomass. Seaweed (*A. nodosum*) inclusion in dairy Holstein cow diets at a level of 330 g/day for a period of 9 weeks did not affect productivity and feed efficiency, or measured hematological parameters, but increased the milk concentrations of Mg, P, and I. These effects may be explained by the higher intakes when corn meal was substituted with seaweed in dairy cows' diets, as transfer efficiencies from feed to milk were similar (in the case of I) or lower (in the case of Mg and P) – Newton et al. (2023). According to Sorge et al. (2016) and Antaya et al. (2015), *A. nodosum* macroalgae are recognized as a naturally rich source of iodine. The strong physiological response in the analyzed rams indicates that iodine present in seaweed in organically bound form is highly bioavailable. Iodine is an essential trace element that is crucial for proper thyroid function and metabolic regulation, but excessive iodine levels may raise concerns regarding the safe limits of dietary supplementation. According to the literature, ruminants possess effective homeostatic mechanisms for excreting excess iodine with urine and milk (Lind et al. 2023). Nevertheless, when livestock diets are supplemented with seaweed at 15 g/animal/day for prolonged periods, thyroid function should be monitored to avoid excess iodine intake or excessive iodine transfer to milk in ewes (Paulíková et al. 2002, EFSA 2013). On the other hand, in regions characterized by endemic iodine deficiency, even a lower dose of 5 g/animal/day could be an effective natural strategy for preventing iodine deficiency and could provide an alternative to synthetic mineral supplements (Rey-Crespo et al. 2014). According to EFSA (2013) guidelines, the maximum permitted iodine concentration in complete feed for small ruminants used for milk production should not exceed 2 mg kg^{-1} feed. Paulíková et al. (2002) found that the toxic dose of iodine for sheep ranges from 94 to 785 mg when administered for three weeks in the form of ethylenediamine dihydroiodide (EDDI) or potassium iodide. McCauley et al. (1973) observed clinical signs of iodine toxicity with a fatal outcome in lambs with an average body weight of 30 kg following the ingestion of at least 562 mg of iodine as EDDI or 393 mg as potassium iodide over three weeks. These doses correspond to 18.7 and 13.1 mg iodine kg^{-1} body weight/day, respectively. In the current study, despite a marked increase in serum iodine concentration by day 42, the recommended intake threshold of 10 mg iodine/animal/day was not exceeded.

CONCLUSIONS

This study demonstrated that dietary supplementation with *A. nodosum* did not increase body weight gain or the RGR in rams during the experiment. Both weight gain and the RGR were lower in the experimental groups than in the control group. A significant interaction between dietary treatment and the duration of seaweed supplementation was observed for the following parameters WBC, Ht, MCHC, and PLT. Seaweed supplementation at 5 g and 15 g resulted in higher WBC and PLT counts in group D2 and lower mean MCHC values in groups D1 and D2. At the end of the supplementation period (day 42), WBC and MCHC values decreased but remained within the normal reference ranges. Thrombocyte production increased significantly on day 42 of supplementation, which is relevant for blood coagulation. Erythrocyte parameters (RBC, Ht, and Hb) also increased significantly by the end of the supplementation period. An analysis of serum iodine concentration in the experimental groups revealed that both dietary supplementation with 5 g and 15 g of seaweed and intake duration contributed to a significant increase in this parameter. This observation suggests that brown seaweed supplementation may be an effective strategy for improving the iodine status of sheep under conditions of iodine deficiency. However, further research is needed to determine the optimal dietary inclusion level of brown algae, duration of supplementation, and iodine excretion in urine. Such research would provide valuable insights into sheep production practices.

Author contributions

I.Ch. and Ł.P. – conceptualization., I.Ch . – methodology, Ł.P. and I.Ch. – validation, Ł.P. – formal analysis, Ł.P. and I.Ch – investigation, Ł.P. – resources, data curation, software, writing – original draft preparation, I.Ch.– writing – review and editing, Ł.P. – visualization, I.Ch. – supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This research was funded by the Minister of Science under the “Regional Initiative of Excellence Program”.

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