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The Seaweed *Ascophyllum nodosum* as a Potential Functional Ingredient in Chicken Nutrition

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Abstract

The marine environment is a source of many valuable food and feed ingredients such as seaweeds or macroalgae. In recent years, consumers' demands have increased the research on the production of functional foods, especially through the inclusion of bioactive ingredients in the animal feeds. One candidate ingredient is *Ascophyllum nodosum*, a brown seaweed, containing polysaccharides, proteins, polyunsaturated fatty acids, pigments and antioxidants. Aim of this study was to examine the effects of dietary *A. nodosum* supplementation on broiler chicken performance parameters, meat fatty acid composition and meat resistance to oxidation during refrigerated storage. One hundred sixty 1-day-old broiler chickens were randomly assigned to four treatment groups with four replications of ten birds each. Birds were housed in floor pens with litter and were offered appropriate commercial diets with the addition of 0 g/kg (Control), 5 g/kg (Asc-5), 10 g/kg (Asc-10) or 20 g/kg (Asc-20) dried *A. nodosum*. There was no difference ($P > 0.05$) in the average body weight, feed consumption and feed conversion ratio of the birds between the four treatment groups until the end of the experiment (42 of age). In addition, feed consumption and feed conversion ratio did not differ ($P > 0.05$) between the four groups. No significant differences ($P > 0.05$) were noted for total saturated, monounsaturated and polyunsaturated fatty acids in the breast or the thigh meat, although some individual polyunsaturated fatty acids were modified. Lipid oxidation determined as thiobarbituric acid reacting substances (TBARS) on air packed skinless breast and thigh samples stored at 4°C for 5 days, did not differ ($P > 0.05$) between the four groups. Dietary *A. nodosum* could be utilized in chicken diets up to 2%, without any adverse effects on performance, meat fatty acid profile and lipid oxidation. Additional investigation is needed in order to evaluate the possible benefits of *A. nodosum* as a potential functional ingredient in chicken nutrition.

Keywords: *Ascophyllum nodosum*; Broilers; Performance; Meat fatty acid profile; Meat oxidative stability

Introduction

During mankind's history, the marine environment has been a unlimited source of diverse valuable food and feed ingredients [1]. Marine animals and plants have been used traditionally as main or supplementary dietary ingredients for humans and their domesticated animals [2].

Nowadays, research interest on algae (macroalgae or seaweeds, as well as microalgae and cyanobacteria) has been renewed, because they are considered to be promising resources of functional ingredients in the development of novel products [1,3,4]. The reason is that consumers are increasingly interested in the possible benefits of functional foods, since this trend is in relation to nutritional genomics (nutrigenomics and nutrigenetics) of functional foods and aims to utilize their health-promoting or disease preventing properties [5,6]. Functional foods can be produced by the addition of new ingredients or modification of the quantities of existing ingredients [7,8]. Algae due to the their abundant availability in the aquatic ecosystem have the potential to become excellent sources of essential nutrients and new high biological value compounds, with health benefits, such as antioxidants, unsaturated fatty acids, vitamins and pigments.

One important novel marine ingredient is *Ascophyllum nodosum*, edible seaweed belonging to the brown algae (Phaeophyceae) [9]. A. nodosum is naturally found in the northern Atlantic Ocean from the north-western coasts of Europe to the north-eastern coasts of North America [10]. It has a high content of total polysaccharides (42-70% of dry weight) [11], such as alginic acid, fucoidan, laminarin and mannitol [11,12]. Many of the *A. nodosum* polysaccharides can reach the lower gastrointestinal tract largely undigested and they can act as a substrate of bacterial fermentation, acting as prebiotic compounds and beneficially modifying the gut microflora [9,13]. *A. nodosum* protein which content varies between 3-15% and has different structure and activities from those found in terrestrial plants [9]. In addition, *A. nodosum* has lipid content about 2-7% (of dry weight), with sufficient amounts of polyunsaturated fatty acids, which are important of the heart health [9,14]. *A. nodosum* is alternative source of vitamins (A, C, D and E), minerals (Ca, P, Na and K) [15], and contains polyphenols such as phlorotannins (up to 15% of dry weight) with antioxidant and antimicrobial effects [11], as well pigments such as chlorophyll and fucoxanthine with antioxidant capacity [16].

The inclusion of *A. nodosum* in animal diets might be a simple and convenient method to introduce its beneficial bioactive ingredients in the meat, milk, or eggs, due to the strong demand of the consumers for natural eco-friendly and renewable products. There are recent studies on *A. nodosum* (meals and extracts) [1], which is examined in the diets of ruminants [9,17,18] and monogastric animals such as pigs [19] and poultry [13]. Possible benefits of dietary *A. nodosum* are the

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improvement of animal health and performance, as well as the quality of the animal products. Review of available literature has revealed that the information on the effect of *A. nodosum* supplementation in chicken diets is very limited, while the evaluation of the lipid oxidation and the profile of fatty acids in chicken meat are missing. For this reason this study was conducted to examine the effect of dietary *A. nodosum* on the growth performance and some parameters of meat quality of broiler chickens.

Materials and Method

The experiment was carried out at the School of Agriculture Technology, Food Technology and Nutrition, Department of Agricultural Technology, Technological Educational Institution of Western Macedonia, Florina, Greece.

For this experiment, one hundred sixty 1-day-old chicken broilers as hatched were assigned randomly to four treatment groups with four replications of ten birds, of equivalent average body weight. Each replication was housed for a period of 42 day, in floor cages with litter. Conventional breeding and management procedures were applied throughout the trial period, according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

The birds of the Control group were fed with maize and soybean meal commercial diets: starter (1–14 days), grower (15–28 days) and finisher (29–42 days), based on the guidelines of NRC [20]. The birds of groups Asc-5, Asc-10 and Asc-20 were offered the same feeds with extra addition of dried A. nodosum at 5 g/kg, 10 g/kg and 20 g/kg, respectively.

Ingredient composition and the proximate chemical analysis - dry matter, crude protein, crude fat, crude fiber and ash [21] of the three diets is presented in (Table 1). Calcium, total phosphorus, lysine, methionine plus cystine and metabolisable energy content were calculated from the composition of the feed ingredients, based on Novus [22] and NRC [20] recommendations.

Feed consumption and mortality were recorded on daily basis and all birds were individually weighted at weekly intervals. At the end of the feeding trial, body weight gain and feed conversion ratio were calculated.

At day 42, two birds from each replication (1 male, 1 female) were randomly selected, and were slaughtered under commercial conditions. Skinless breast (m. pectoralis superficialis) and thigh (m. biceps femoris) samples were prepared for the determination of lipid oxidation during refrigerated storage at 4°C for five days. Skinless samples were used as they are more homogenous than muscles with their skin on and they represent the type of poultry meat that is preferentially consumed in Europe [23]. Samples were vacuum packaged and placed at -45°C pending analysis. Prior to analyses, the stored samples were thawed at 4°C overnight.

The fatty acid composition of the samples was determined by gas chromatography. Fatty acids methyl esters were obtained from the frozen samples using the protocol described by O'Fallon et al. [24]. Then, the separation and quantification of the methyl esters was carried out with a gas chromatographic system (TraceGC model K07332, ThermoFinnigan, ThermoQuest, Milan, Italy) equipped with a flame ionization detector, a model CSW 1.7 chromatography station (CSW, DataApex Ltd, Prague, Czech Republic) and a fused silica capillary column, 30 m × 0.25 mm i.d., coated with cyanopropyl polysiloxane (phase type SP-2380) with a film thickness of 0.20 μm (Supelco, Bellefonte, PA, USA). Fatty acids were quantified by peak area measurement and the results are expressed as percentage (%) of the total peak areas for all quantified acids.

Determination of the lipid oxidation of the samples was performed using a modified version of the method of Vyncke [25], as described by Kasapidou et al. [26]. The previously frozen samples were placed in plastic trays, overwrapped with transparent air-permeable polyethylene (cling) film as usual for retail sales and stored in a refrigerated cabinet at 4°C for 5 days. On the second and the fifth refrigeration day, for each sample breast muscles (m. pectoralis superficialis) and thigh muscles (m. biceps femoris) were separated from the bones and skin, were trimmed of external / adjacent fat and connective tissue and blended in a food processor. Subsamples (5 g) were homogenized in 25 ml of 7.5% trichloroacetic acid (w/v) containing 0.1% (w/v) of both n-propyl gallate and ethylenediaminetetraacetic acid disodium salt, using a Polytron (Kinematica AG, Littau, Switzerland model PT-MR 3000). Samples were left for approximately 15 to 20 min to allow the extraction of the thiobarbituric acid reacting substances (TBARS), the resulting slurry was filtered, and 5 ml of the filtrate was mixed with 5 ml of 0.02 M thiobarbituric acid. A blank sample containing 5 ml of the trichloroacetic acid solution and 5ml of the thiobarbituric acid solution was prepared. All samples were left in the dark overnight and on the following day absorbance were read at 532 nm against the blank sample using an UV–VIS spectrophotometer (U-2800 Double Beam Spectrophotometer, Hitachi, Tokyo, Japan). TBARS were calculated using 1,1,3,3 tetraethoxypropane (5-20 nM) as standard and expressed as mg of malondialdehyde (MDA) per kg muscle. Each sample was

	Diets		
	Starter	**Grower**	**Finisher**
Ingredients (g/kg)	1 d – 14 d	15 d – 28 d	29 d – 42 d
Maize	505.5	560	637.3
Soybean meal	339	342	283
Herring meal	46.5	-	-
Soybean oil	68	63	52
Dicalcium phosphate	15.8	20	21
Sodium bicarbonate	12.1	8	0.7
Methionine	3.5	1	-
Salt	6.6	3	3
Vitamin and mineral premix *	3	3	3
Total	1000	1000	1000
Chemical analysis			
Dry matter	931.6	906.1	907.7
Crude protein	261.5	182.1	181.9
Crude fibre	32.4	36.6	35.3
Crude fat	65.6	63.3	31.5
Ash	63	42.2	45.2
Calculated analysis			
Metabolisable energy (MJ/kg)	13.3	13.3	13.3
Lysine	12.6	10.3	8.9
Methionine + Cystine	10.5	7.2	5.7
Ca	9.9	7.3	7
P (total)	8	8	8

* Supplying per kg feed: vit. A 13,000 IU, vit. D_3 5,000 IU, vit. E 30, vit. K 3 mg, thiamin 1 mg, riboflavin 5 mg, pyridoxine 3 mg, vit. B_{12} 0.02 mg, niacin 10 mg, pantothenic acid 15 mg, folic acid 0.8 mg, biotin 0.05, vit. C 10 mg, choline chloride 480 mg, Zn 100 mg, Mn 120 mg, Fe 20 mg, Cu 15 mg, Co 0.2 mg, I 1 mg, Se 0.4 mg

Table 1: Ingredients and chemical analysis of the experimental diets.

analysed in duplicate and the average value of the measurements was used.

The statistical analysis was performed using the IBM SPSS Statistics 20 statistical package (SPSS Inc., Chigaco, IL, USA). Each individual replication (cage) was regarded as the experimental unit. The one-way analysis of variance (ANOVA) was performed, using the groups as fixed factors. Post-hoc analysis was undertaken using Tukey's test at $P < 0.050$ [27]. The homogeneity of the measurements was examined with Levene's test [28].

Results and Discussion

The effect of A. nodosum supplementation in broiler performance parameters are presented in (Table 2). Bird live weight did not differ ($P > 0.05$) in the middle (21 d) and the end (42 d) of the trial. Moreover, feed conversion ratio and mortality did not differ ($P > 0.05$) between the groups. Other researchers [13] that examined the dietary use of dried *A. nodosum* reported that inclusion levels from 0.5% to 3.0% increased the body weight, as well as the feed consumption, compared to the control group. In another trial [29] that examined *A. nodosum* extract supplementation in broilers' water (1 ml and 2 ml per 5 L of water), it was found that it increased body weight at day 45, compared to the control treatment group. It is possible that these contradictory results can be explained by the different basal feeds, housing conditions and production systems employed in the different trials. It has been hypothesized that *A. nodosum* compounds can act as prebiotics comparable to inulin [1], beneficially modifying the gut microflora and improving animal health and performance [9,13,29], especially under stressful or unhygienic housing conditions.

(Tables 3 and 4) present the effect of dietary *A. nodosum* on chicken breast and thigh meat fatty acid profile respectively. It was found that Asc-20 group had significantly ($P = 0.001$) higher amounts of gamma-linolenic fatty acid (C18:3n6), compared to the control group in chicken breast meat. Also, Asc-20 group had significantly ($P = 0.019$) lower eicosenoic fatty acid (C20:1n9), compared to the control group in chicken thigh meat. No significant differences ($P > 0.05$) were noticed for total saturated, monounsaturated and polyunsaturated fatty acids in the breast or the thigh meat. These findings cannot be compared with other research of broilers, since similar reports have not been found in recently published literature, to the best of our knowledge. Different fat sources in broiler diets directly affect the total amount and the percentages of individual fatty acids in the meat and the subcutaneous fat, thus it is possible to increase the polyunsaturated fatty acids percentage through dietary means [30,31]. Due to the fact that polyunsaturated fatty acids cannot be synthesized by humans, they should be included in their daily diet [32]. Diets in western societies are often deficient for these fatty acids and their consumption can protect from numerous chronic diseases [32-34].

Figure 1 presents the effect of *A. nodosum* supplementation on chicken breast meat lipid oxidation after two and five days of refrigerated storage. Moreover, Figure 2 shows the effect of *A. nodosum* supplementation on chicken thigh meat lipid oxidation after two and five days of refrigerated storage. The four groups did not differ ($P > 0.05$) on any the measured TBARS values. Lipid oxidation increased as the refrigeration period was extended in both examined muscles, as expected. The increased TBARS values in thigh muscles compared to the breast muscles, can be attributed to the high haem iron and myoglobin contents of these muscles [35,36]. Lipid oxidation was far below the reported threshold values for the detection of rancidity by expert taste panellists. Different values for the detection of rancidity in sensory evaluation tests have been reported: Melton [37] and Fernandez et al. [38] reported that oxidized flavours were detectable at TBARS numbers in the range of 1.0 or 2.0 mg malondialdehyde / kg tissue in chicken. Furthermore, O'Neil et al. [39] stated that TBARS value higher than 0.8 mg/kg meat can considered as an indication of perceptible rancidity in poultry meat.

Conclusion

Dietary supplementation of *A. nodosum* at levels up to 20 g/kg feed in chicken diets did not affect the performance parameters and the

	Control	Asc-5	Asc-10	Asc-20	SEM	P
Live weight at 21 d (kg)	0.774	0.773	0.76	0.755	0.008	N.S.
Final live weight at 42 d (kg)	2.458	2.517	2.401	2.335	0.036	N.S.
Feed conversion ratio	2.07	2.027	2.066	2.174	0.019	N.S.
Mortality (%)	2.5	0	2.5	2.5	1.083	N.S.

Control: 0 g *A. nodosum* / kg feed; Asc-5: 5 g *A. nodosum* / kg feed; Asc-10: 10 g *A. nodosum* / kg feed; Asc-20: 20 g *A. nodosum* / kg feed.
N.S. = Not Significant ($P > 0.05$)

Table 2: Effect of dietary *A. nodosum* on broiler performance parameters.

Fatty acid	Controls	Asc-5	Asc-10	Asc-20	SEM	P
C12:0	0.021	0.036	0.027	0.029	0.002	N.S.
C14:0	0.336	0.286	0.319	0.338	0.01	N.S.
C14:1	0.032	0.039	0.039	0.056	0.003	N.S.
C16:0	16.708	15.763	16.445	16.331	0.202	N.S.
C16:1	1.588	1.803	1.752	2.211	0.106	N.S.
C18:0	9.184	8.647	8.183	7.31	0.327	N.S.
C18:1n9t	0.324	0.219	0.212	0.34	0.046	N.S.
C18:1n9c	26.948	28.075	28.313	29.178	0.616	N.S.
C18:2n6t	0.044	0.044	0.038	0.046	0.002	N.S.
C18:2n6c	26.412	26.716	29.878	29.6	0.737	N.S.
C18:3n6	0.160 [a]	0.203 [ab]	0.269 [b]	0.266 [b]	0.01	0.001
C20:0	0.204	0.182	0.167	0.172	0.006	N.S.
C18:3n3	1.72	1.724	2.133	2.164	0.106	N.S.
C20:1n9	0.257	0.285	0.275	0.258	0.01	N.S.
C21:0	0.022	0.043	0.034	0.063	0.006	N.S.
C20:2	0.601	0.565	0.565	0.466	0.042	N.S.
C20:3n3	0.838	0.824	0.555	0.691	0.06	N.S.
C20:4n6	5.573	5.792	4.217	3.788	0.454	N.S.
C22:1n9	0.035	0.037	0.02	0.017	0.006	N.S.
C20:5n3 EPA	0.232	0.279	0.18	0.192	0.019	N.S.
C24:0	1.237	1.357	0.995	0.902	0.105	N.S.
C22:5n3 DPA	0.874	0.917	0.713	0.599	0.073	N.S.
C22:6n3 DHA	0.859	0.922	0.674	0.641	0.078	N.S.
Σ SFA	28.418	27.002	26.663	25.588	0.499	N.S.
Σ MUFA	29.775	31.094	31.049	32.512	0.638	N.S.
Σ PUFA	37.314	37.984	39.223	38.451	0.391	N.S.

Control: 0 g *A. nodosum* / kg feed; Asc-5: 5 g *A. nodosum* / kg feed; Asc-10: 10 g *A. nodosum* / kg feed; Asc-20: 20 g *A. nodosum* / kg feed. Values in rows with no common superscript differ significantly ($P < 0.05$). N.S. = Not Significant ($P > 0.05$).
EPA: Eicosapentaenoic F.A.; DPA: Docosapentaenoic F.A.; DHA: Docosahexaenoic F.A.
SFA: Saturated F.A.; MUFA: Monounsaturated F.A.; PUFA: Polyunsaturated F.A.

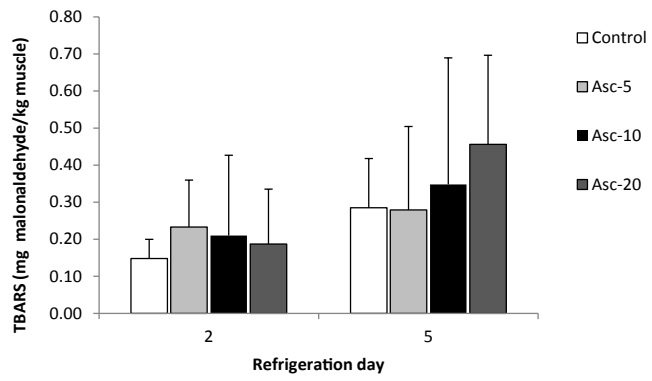
Table 3: Effect of dietary *A. nodosum* on breast meat fatty acid composition (% of total fatty acids).

oxidative stability of their meat. The total saturated, monounsaturated and polyunsaturated fatty acids were not significantly affected, although meat breast and thigh fatty acid profile was modified for some individual polyunsaturated fatty acids. Additional research would be

Fatty acid	Controls	Asc-5	Asc-10	Asc-20	SEM	P
C12:0	0.021	0.015	0.028	0.034	0.005	N.S.
C14:0	0.347	0.322	0.354	0.368	0.011	N.S.
C14:1	0.047	0.057	0.051	0.06	0.003	N.S.
C16:0	17.229	16.912	17.53	17.417	0.178	N.S.
C16:1	1.938	1.751	1.5	1.869	0.104	N.S.
C18:0	8.117	8.988	9.49	8.614	0.347	N.S.
C18:1n9t	0.323	0.241	0.299	0.275	0.038	N.S.
C18:1n9c	29.24	25.135	23.895	25.848	0.721	N.S.
C18:2n6t	0.048	0.045	0.062	0.049	0.004	N.S.
C18:2n6c	28.928	27.704	28.188	28.563	0.642	N.S.
C18:3n6	0.248	0.237	0.248	0.251	0.008	N.S.
C20:0	0.147	0.116	0.108	0.122	0.007	N.S.
C18:3n3	2.004	1.571	1.528	1.718	0.111	N.S.
C20:1n9	0.287 [b]	0.213 [ab]	0.214 [ab]	0.181 [a]	0.011	0.019
C21:0	0.057	0.031	0.04	0.029	0.007	N.S.
C20:2	0.523	0.595	0.781	0.555	0.044	N.S.
C20:3n3	0.729	0.989	1.016	0.902	0.066	N.S.
C20:4n6	3.515	5.781	5.367	4.724	0.41	N.S.
C22:1n9	0.01	0.02	0.015	0.005	0.003	N.S.
C20:5n3 EPA	0.126	0.225	0.163	0.22	0.016	N.S.
C24:0	0.795	1.218	1.28	1.08	0.091	N.S.
C22:5n3 DPA	0.633	0.961	0.976	0.797	0.074	N.S.
C22:6n3 DHA	0.692	1.019	0.952	0.887	0.085	N.S.
Σ SFA	27.159	28.196	29.393	28.259	0.558	N.S.
Σ MUFA	32.242	28.07	26.632	28.861	0.753	N.S.
Σ PUFA	37.447	39.126	39.281	38.667	0.348	N.S.

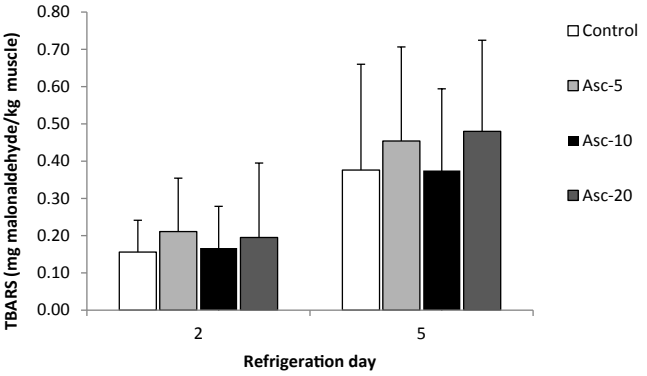
Control: 0 g *A. nodosum* / kg feed; Asc-5: 5 g *A. nodosum* / kg feed; Asc-10: 10 g *A. nodosum* / kg feed; Asc-20: 20 g *A. nodosum* / kg feed. Values in rows with no common superscript differ significantly (P < 0.05). N.S. = Not Significant (P > 0.05).
EPA: Eicosapentaenoic F.A.; DPA: Docosapentaenoic F.A.; DHA: Docosahexaenoic F.A.
SFA: Saturated F.A.; MUFA: Monounsaturated F.A.; PUFA: Polyunsaturated F.A.

Table 4: Effect of dietary *A. nodosum* on thigh meat fatty acid composition (% of total fatty acids).



No significant differences were found (P > 0.05)

Figure 1: Effect of dietary *A. nodosum* on breast muscle lipid oxidation (TBARS, mg malonaldehyde / kg muscle ± SD) after 2 and 5 days of refrigeration.



No significant differences were found (P > 0.05)

Figure 2: Effect of dietary *A. nodosum* on thigh muscle lipid oxidation (TBARS, mg malonaldehyde / kg muscle ± SD) after 2 and 5 days of refrigeration.

recommended to examine all the possible benefits of the seaweed *A. nodosum* as a natural innovative ingredient in poultry nutrition.

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