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Digestibility, nitrogen balance and weight gain in sheep fed with diets supplemented with different seaweeds

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Abstract
Three completely randomised design experiments examined the effects of the inclusion of three seaweed species (*Ruppia* sp., *Ulva* sp. or *Chaetomorpha* sp.) into the diet on digestibility and nitrogen balance in Barbarine sheep. Diets were composed of oat hay ad libitum supplemented with 600 g of concentrate. Seaweeds were incorporated into the concentrate at increasing levels (0, 200, 300 or 400 g seaweed kg^{-1} concentrate, dry matter (DM) basis) in replacement of other ingredients. Feed intake and water consumption were increased ($P < 0.01$) linearly with *Ruppia* supplementation. As the proportion of *Ruppia* was increased in the diet, the digestibility of organic matter decreased linearly ($P < 0.01$) from 0.698 (no seaweed) to 0.642 (400 g seaweed kg^{-1} concentrate). Increasing the level of inclusion of *Chaetomorpha* up to 300 g kg^{-1} did not affect the intake of concentrate. Organic matter digestibility decreased linearly ($P < 0.001$) from 0.685 with the control diet to 0.622 with the diet containing 400 g *Chaetomorpha* kg^{-1} concentrate. The level of inclusion of *Ulva* did not affect feed intake or water consumption, but decreased linearly ($P > 0.001$) organic matter digestibility from 0.637 with the control diet to 0.599 with the diet containing 400 g *Ulva* kg^{-1} concentrate. In all the experiments, nitrogen balance was positive and there were no differences among levels of seaweed supplementation in N retention or daily weight gain. These results suggest that seaweeds such as *Ruppia*, *Ulva* or *Chaetomorpha* can be incorporated into sheep concentrates up to 30% (DM basis) without adverse effects on feed digestibility or growth performance.

Keywords Seaweed · Sheep · Feeding · Digestibility · Feed

Introduction

The severe fluctuations in environmental conditions in arid and semi-arid regions (e.g. many areas of northern Africa) limit the quantity of herbage available to animals from natural grazing. In most of these regions, oat hay is the most available forage used to feed ruminants and, for different reasons, its nutritive value is generally limited. During the dry season, this resource is scarce and ruminant feeding often depends on low-quality crop residues and the availability of concentrate feeds. To increase animal productivity in these dry regions and in countries with scarce feed resources, it is crucial to identify every local plant resource with potential to be used economically as a feed in animal rations.

Seaweeds and aquatic plants are abundant along the coasts, with a high regeneration rate after harvest and a suitable chemical composition (Mabeau and Fleurence 1993; Fleurence 1999; Casas-Valdez et al. 2003), with a promising potential to be used in animal nutrition (Evans and Critchley 2014). It is very important to demonstrate this issue further and to seek the possibility to incorporate this resource in animal feeding.

Palatability of seaweeds and algae may be a limiting factor in their use as forage (dried or ensiled) for ruminants (Woodward 1951; Burt et al. 1954; Hentges and Salveson 1970; Linn et al. 1975; Altomonte et al. 2018; Lamminen et al. 2019). However, Arieli et al. (1993) reported no feed refusals when the species *Ulva lactuca* was included at 20% in the concentrate fed to growing rams, concluding that this seaweed is suitable for use in diets with high energy content. The

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same species was studied for goats showing that its protein digestibility and energy content are similar to those of medium-quality lucerne hay, but with a greater protein content (Ventura and Castanon 1998).

Two other species, namely *Chaetomorpha linum* and *Ruppia maritima*, have also been tested as feed for sheep, suggesting that they can be incorporated into concentrate mixtures for fattening lambs up to a level of 20% ration dry matter (DM) without adverse effects on growth performance (Rjiba-Ktita et al. 2010). It seems timely to test whether or not the incorporation level of these seaweeds into concentrate mixtures can be further increased. This is the purpose of the present study in which the animal response (voluntary feed intake, nutrient digestibility and nitrogen balance) to increasing levels of incorporation of three seaweed species (*Ruppia* sp., *Chaetomorpha* sp. and *Ulva* sp.) in lamb diets was examined.

Materials and methods

Seaweeds and concentrate preparation

Seaweeds (*Ruppia* sp., *Chaetomorpha* sp. and *Ulva* sp.) were manually collected from the lagoon of Ghar El Melh in Bizerte (North East of Tunisia). The collected material was washed with fresh water in order to remove sand, detritus and excess of salts. The material collected was then air-dried, ground with a vegetable crusher and preserved in plastic bags for approximately 7 days before the beginning of each experiment.

Considering their chemical composition (Table 1), each species was mixed at increasing levels (200, 300 and 400 g seaweed kg^{-1} concentrate, on DM basis) with barley and soybean meal in order to have isonitrogenous concentrates (Table 2). Commercial mineral and vitamin mixtures were added (5 g premix kg^{-1}) to these concentrates. A control concentrate was prepared with the same ingredients (barley, soybean meal and 25 g mineral and vitamin mix kg^{-1}) but without any seaweed material. All concentrate mixtures were pelleted in a livestock feed mill.

Animals and feed management

Three distinct and sequential experiments to test each of the three seaweed species were carried out. Twenty Barbarine male lambs (33 ± 3.2 kg initial body weight; 8 months old), treated for internal parasites were used. In each experiment, lambs were randomly allocated to four dietary treatments (five sheep per treatment) according to a completely randomised design. Only one seaweed was tested in each experiment, each with four levels of addition to the concentrate (0, 200, 300 or 400 g seaweed kg^{-1}). *Ruppia* sp. was tested in the first experiment, followed by *Chaetomorpha* sp. in the second experiment and *Ulva* sp. in the last experiment. In each experiment, animals were first housed for 17 days (adaptation period) in individual boxes provided with feed and water troughs and subsequently, placed in metabolism cages for 10 days (collection period). During each experiment, each animal received 600 g day^{-1} of the corresponding experimental concentrate distributed twice daily (at 09:00 am and 13:00 pm) and fed oat hay ad libitum once daily (11:00 am) so that orts of hay represented 20% of the offered quantity. A period of 15 days was left between each seaweed species trial, during which all the animals received the control concentrate. At the beginning of each trial, lambs were reallocated to the four experimental treatments on the basis of their body weights. Water was freely available all the time and renewed every day. In each experiment, animals were weighed the first and last day of the collection period using an electronic balance.

Balance trials

Metabolic cages were used in the digestibility and balance trials for total and individual collection of faeces and urine. Feed orts, water refusals and faecal and urinary outputs were collected quantitatively and weighed daily just before the morning feed distribution. The cages were equipped with devices specifically designed for the separate collection of faeces and urine. Nitrogen loss from urine by volatilisation was prevented by adding 100 mL of a sulphuric acid solution (100 mL L^{-1}) into the urine collection containers. Representative aliquots of the total faecal and urine outputs were collected every day, and then all the samples for the same sheep were pooled at the end of the collection period. Feed orts were collected in plastic bags and stored in a dry place. After each collection period, samples of seaweed material, feeds, diets, orts and faeces were dried at 50 °C until constant weight and ground through a 1-mm screen, whereas urine samples were preserved at 4 °C for subsequent analyses.

Chemical analysis

Samples of seaweed material, feeds, diets, orts and faeces were analysed for ash (AOAC 942.05) and N (Kjeldahl-N, AOAC 984.13) according to standard AOAC methods (AOAC 1984), and for the ash-free neutral detergent fibre (NDF) as described by Van Soest et al. (1991), except that α-amylase and sodium sulphite were not included in the analytical solution. Crude protein (CP) was calculated as 6.25 × N. Nitrogen concentration in urine was determined by the same AOAC (984.13) method.

Statistical analysis

Data obtained in each of digestion and balance trial were subjected to analysis of variance using general linear model (GLM) procedures (SAS 1987). For each seaweed, the level

Table 1 Chemical composition of concentrate ingredients and oat hay (all in g kg^{-1} dry matter)

	Seaweed species (air-dried matter)			Feed		
	Ruppia sp.	*Chaetomorpha* sp.	*Ulva* sp.	Soybean meal	Barley	Oat hay
Ash	200	320	180	66	45	70
Crude protein	120	125	118	435	100	47
Neutral detergent fibre	397	319	415	132	330	545
Ca	21.3	52.6	13.7	2.4	0.7	3.8
Na	86.2	64.3	56.1	6.4	1.7	2.5
K	51.7	94.6	55.0	4.4	2.1	3.6

of inclusion was considered as the main fixed effect in the model, with five replicates (sheep) per level. The differences among multiple treatment means (levels of seaweed inclusion) were established using the Tukey test. Orthogonal polynomial contrasts with unequally spaced factor levels were performed to assess linear and quadratic effects of the level inclusion of each seaweed species in the concentrate, and to compare each level of inclusion of each seaweed to the control diet (no seaweed addition to the concentrate). Differences were considered statistically significant when $P < 0.05$.

Results

Chemical composition of seaweeds and concentrate mixtures are presented in Tables 1 and 2. Ash contents of the three species varied from 180 g kg^{-1} DM (*Ulva*) to 320 g kg^{-1} DM (*Chaetomorpha*). However, their CP level averaged 121 g kg^{-1} DM. In comparison with *Ulva* and *Ruppia*, *Chaetomorpha* had the lowest NDF content (415 and 397 vs. 319 g kg^{-1} DM).

Crude protein content of the concentrates averaged 196 g kg^{-1} DM, whereas the fibre fraction (NDF) varied from 215 g kg^{-1} DM (control, *Chaetomorpha*- and *Ulva*-based concentrates) to 251 g kg^{-1} DM (*Ruppia*-based concentrates). Despite the small quantities of mineral and vitamin mixtures (5 g kg^{-1}) added to the concentrates, the seaweed-supplemented concentrates contained more ash than the control (Table 2). At the same level of seaweed inclusion, the concentrates supplemented with *Chaetomorpha* showed the highest ash contents (210 g ash kg^{-1} DM in the concentrate containing 400 g of this species).

Table 2 Ingredients and chemical composition of experimental concentrates (all in g kg^{-1} dry matter unless otherwise stated)

		Seaweed species								
		Ruppia sp.			*Chaetomorpha* sp.			*Ulva* sp.		
Inclusion level (g seaweed DM kg^{-1} concentrate DM)	0	200	300	400	200	300	400	200	300	400
Ingredients										
Barley	800	585	542	445	590	549	455	625	520	415
Soybean meal	175	210	153	150	205	146	140	170	175	180
Ruppia sp.	0	200	300	400	0	0	0	0	0	0
Chaetomorpha sp.	0	0	0	0	200	300	400	0	0	0
Ulva sp.	0	0	0	0	0	0	0	200	300	400
Vitamin-mineral premix[1]	25	5	5	5	5	5	5	5	5	5
Chemical composition										
Dry matter (g kg^{-1})	940	935	934	933	922	877	873	900	900	890
Ash	100	113	127	151	155	150	210	120	110	130
Crude protein	195	200	190	190	210	203	195	200	190	195
Neutral detergent fibre	213	227	259	266	223	217	219	205	217	217
Ca	12	14	16	16	21	24	35	11	11	13
Na	27	53	63	70	84	90	124	34	44	44
K	41	55	59	63	75	80	95	33	40	42

[1] Commercial mineral and vitamin premix (declared composition per kg: 600 g calcium carbonate, 300 g sodium chloride, 50 g trace minerals and 50 g vitamins)

The effect of *Ruppia* concentrate mixtures on feed and water intakes, digestibility and nitrogen balance of the lambs is shown in Table 3. Similar intakes of concentrate were recorded in all the groups (40 g DM kg^{-1} BW$^{0.75}$), whereas a linear increase ($P < 0.01$) was observed on DM hay intake (from 29 to 48 g DM kg^{-1} BW$^{0.75}$) from the control diet to the diet containing 400 g *Ruppia* kg^{-1} concentrate. Daily water consumption increased linearly ($P < 0.001$) from 144 mL kg^{-1} BW$^{0.75}$ (control diet) to 234 mL kg^{-1} BW$^{0.75}$ (diet with 400 g *Ruppia* kg^{-1} concentrate). There was a linear decrease in OM ($P < 0.01$) digestibility as the level of inclusion of *Ruppia* was increased (Table 3). With the inclusion of *Ruppia* in the concentrate, fibre digestibility was increased ($P < 0.05$) from 0.386 (control) to 0.452 g g^{-1} (*Ruppia* concentrates). All the diets resulted in a positive N balance, with no differences among diets in nitrogen retention. Daily weight gain averaged 124.5 g with no differences ($P > 0.05$) among diets.

Effects of *Chaetomorpha* supplementation are presented in Table 4. Concentrate and total DM and OM intakes decreased when *Chaetomorpha* inclusion in the concentrate mixture reached 400 g kg^{-1}, although no differences with the control diet were observed up to 300 g kg^{-1}. Water consumption was not affected by *Chaetomorpha* inclusion. Digestibility (DM, OM and CP) decreased linearly ($P < 0.01$) with the level of *Chaetomorpha* inclusion. However, the digestibility of NDF (on average 0.444) was unaffected by the level incorporation of this algal species. Nitrogen balance was positive with all the diets, with no diet effects on nitrogen retention. Animals of all the tested diets grew at a similar rate with an average daily weight gain of 162 g day^{-1}.

No differences were observed in feed intake or water consumption when the proportion of *Ulva* included into the concentrate was increased (Table 5). The control diet was ($P < 0.01$) more digestible than the diet with the concentrate containing 400 g *Ulva* kg^{-1}, although the difference was significant only for OM digestibility (Table 5). The level of inclusion of this species decreased linearly the digestibility of DM, OM and CP. Nitrogen balance was positive with all the diets and not affected by the level of *Ulva* in the concentrate. Sheep fed with *Ulva* concentrates showed a growth performance similar to those fed with the control diet, with an average daily weight gain across all the groups of 170 g day^{-1}.

Digestibility of each seaweed was estimated by regression of apparent digestibility against level of supplementation

Table 3 Intake, digestibility and nitrogen balance in Barbarine sheep fed with *Ruppia* sp. supplemented diets

	Inclusion level (g *Ruppia* DM kg^{-1}concentrate DM)				SEM[1]	*P* value	Orthogonal contrasts		
	0	200	300	400			Seaweed addition[2]	Linear	Quadratic
Feed intake									
Hay (g DM day^{-1})	426^{b}	617^{ab}	606^{ab}	672^{a}	62.2	0.048	0.008	0.009	0.457
Concentrate (g DM day^{-1})	564	531	555	560	16.7	0.447	0.415	0.889	0.170
DM intake (g day^{-1})	990	1148	1161	1232	82.3	0.218	0.058	0.044	0.786
DM intake (g kg^{-1}BW$^{0.75}$)	69.8^{b}	82.2^{ab}	83.0^{ab}	89.0^{a}	4.96	0.072	0.017	0.011	0.757
OM intake (g day^{-1})	948	1047	1049	1101	82.3	0.535	0.192	0.162	0.889
Water consumption									
(mL day^{-1})	2034^{c}	2428^{bc}	2976^{ab}	3237^{a}	177.3	< 0.001	< 0.001	< 0.001	0.505
(mL kg^{-1} BW$^{0.75}$)	144^{c}	173^{bc}	214^{ab}	234^{a}	13.1	< 0.001	< 0.001	< 0.001	0.479
Digestibility (g digested g^{-1} ingested)									
DM	0.649	0.655	0.634	0.623	0.0127	0.304	0.437	0.140	0.296
OM	0.698^{a}	0.679^{ab}	0.648^{ab}	0.642^{b}	0.0133	0.029	0.016	0.005	0.832
CP	0.663	0.624	0.612	0.615	0.0139	0.070	0.012	0.015	0.314
NDF	0.386^{b}	0.480^{a}	0.430^{ab}	0.446^{ab}	0.0212	0.045	0.016	0.086	0.074
Nitrogen balance									
N retained (g day^{-1})	5.73	4.81	4.50	5.25	0.592	0.498	0.216	0.394	0.239
N retention (g kg^{-1} N ingested)	327	244	238	254	33.4	0.244	0.050	0.097	0.240
N retention (g kg^{-1} N absorbed)	492	388	381	412	45.9	0.331	0.082	0.165	0.228
Initial body weight (kg)	33.4	33.1	32.8	32.4	1.18	0.938	0.627	0.541	0.909
Average daily weight gain (g day^{-1})	123	113	126	135	30.2	0.964	0.961	0.763	0.692

DM dry matter, *OM* organic matter, *CP* crude protein, *NDF* neutral detergent fibre, *N* nitrogen, *BW* body weight

[1] Standard error of the mean

[2] "Seaweed addition" contrast: concentrate with no seaweed vs. concentrates including seaweed

[a,b,c] Means with different superscripts within a row are significantly different ($P < 0.05$)

Table 4 Intake, digestibility and nitrogen balance in Barbarine sheep fed with *Chaetomorpha* sp. supplemented diets

	Inclusion level (g *Chaetomorpha* DM kg^{-1} concentrate DM)				SEM[1]	*P* value	Orthogonal contrasts		
	0	200	300	400			Seaweed addition[2]	Linear	Quadratic
Feed intake									
Oat hay (g DM day^{-1})	699	729	710	724	16.7	0.592	0.274	0.388	0.600
Concentrate (g DM day^{-1})	564[a]	553[a]	522[a]	340[b]	18.4	< 0.001	< 0.001	< 0.001	< 0.001
DM intake (g day^{-1})	1263[a]	1282[a]	1232[a]	1064[b]	26.1	< 0.001	0.033	< 0.001	< 0.001
DM intake (g kg^{-1} $BW^{0.75}$)	80.5[a]	82.9[a]	78.0[a]	68.9[b]	2.22	0.002	0.149	0.003	0.004
OM intake (g day^{-1})	1150[a]	1135[a]	1199[a]	932[b]	21.7	< 0.001	0.002	< 0.001	< 0.001
Water consumption									
(mL day^{-1})	2301	2967	2903	2586	202.1	0.114	0.042	0.209	0.036
(mL kg^{-1} $BW^{0.75}$)	146	192	183	167	12.1	0.082	0.027	0.165	0.028
Digestibility (g digested g^{-1} ingested)									
DM	0.664[a]	0.637[ab]	0.617[ab]	0.604[b]	0.0129	0.026	0.009	0.003	0.933
OM	0.685[a]	0.654[ab]	0.640[ab]	0.622[b]	0.0117	0.011	0.003	0.001	0.941
CP	0.618[a]	0.624[a]	0.559[ab]	0.499[b]	0.0175	< 0.001	0.012	< 0.001	0.009
NDF	0.454	0.450	0.416	0.456	0.0243	0.624	0.644	0.742	0.526
Nitrogen balance									
N retained (g day^{-1})	6.12	6.78	5.89	4.73	0.475	0.075	0.572	0.078	0.036
N retention (g kg^{-1} N ingested)	283	291	294	299	23.1	0.974	0.683	0.649	0.991
N retention (g kg^{-1} N absorbed)	456	465	529	571	31.7	0.085	0.098	0.020	0.290
Initial body weight (kg)	38.4	37.7	38.7	37.7	1.42	0.944	0.835	0.855	0.973
Average daily weight gain (g day^{-1})	170	167	163	147	37.7	0.972	0.802	0.692	0.817

DM dry matter, *OM* organic matter, *CP* crude protein, *NDF* neutral detergent fibre, *N* nitrogen, *BW* body weight

[1] Standard error of the mean

[2] "Seaweed addition" contrast: concentrate with no seaweed vs. concentrates including seaweed

[a,b] Means with different superscripts within a row are significantly different ($P < 0.05$)

(Dhanoa et al. 2008). Using this approach, the estimated OM digestibility coefficients for *Ruppia*, *Chaetomorpha* and *Ulva* were 0.540, 0.497 and 0.535, respectively.

Discussion

Chemical composition of the three seaweeds is within the ranges reported in the literature (Wong and Leung 1979; Mabeau and Fleurence 1993; Rjiba-Ktita et al. 2010, 2017; Zitouni et al. 2014; Makkar et al. 2016). In any case, a large variability was observed throughout studies that can be related to geographical origin, season, and other environmental, physiological and growth conditions (Fleurence 1999; Zitouni et al. 2014).

In comparison with conventional feeds, such as dried grasses or grains, seaweeds contain greater amounts of ash, especially the species *Chaetomorpha*. Marine macroalgae concentrate minerals from seawater and contain 10 to 20 times more ash than land plants, so they can be an important source of valuable minerals for animal nutrition (Misurcová 2012; Moreda-Pineiro et al. 2012; Cabrita et al. 2016). They can be also considered as fibrous resources, rich in specific polysaccharides such as alginate, laminarin and fucoidin, which can be degraded by ruminal microbiota (Williams et al. 2013).

The protein content of the three species (around 12% DM) is slightly greater than the average value for barley grain and relatively higher than the maintenance requirement of lambs. These seaweeds may partly contribute to replace, in concentrate mixtures, the protein and energy supplied by barley and soybean meal, which are largely imported at high costs. The protein concentration in the concentrate mixtures was about 196 g kg^{-1} DM, as recommended for growing lambs (INRA 1988). Seaweeds, such as those of the genus *Ulva*, may have a promising essential amino acids profile (Gaillard et al. 2018), in particular methionine and cysteine (Fleurence 1999; Yaich et al. 2011; Makkar et al. 2016). These sulphur-containing amino acids are substantially higher in feed material obtained from *Ulva* than in soybean meal (Makkar et al. 2016), which makes this species as a potential supplement for wool-producing animals. It has been reported that algal proteins are degraded at slow rates in the rumen and can be considered

Table 5 Intake, digestibility and nitrogen balance in Barbarine sheep fed with *Ulva* sp. supplemented diets

	Inclusion level (g *Ulva* DM kg^{-1} concentrate DM)				SEM[1]	*P* value	Orthogonal contrasts		
	0	200	300	400			Seaweed addition[2]	Linear	Quadratic
Feed intake									
Oat hay (g DM day^{-1})	802	754	812	780	18.4	0.167	0.355	0.722	0.415
Concentrate (g DM day^{-1})	562	540	541	536	12.3	0.861	0.875	0.775	0.869
DM intake (g day^{-1})	1364	1294	1352	1316	17.7	0.057	0.058	0.183	0.254
DM intake (g kg^{-1} $BW^{0.75}$)	80.4	77.4	79.1	77.4	2.18	0.722	0.355	0.413	0.782
OM intake (g day^{-1})	1246[a]	1170[b]	1233[ab]	1184[ab]	17.5	0.020	0.023	0.071	0.299
Water consumption									
(mL day^{-1})	2831	2245	3061	3066	196.7	0.542	0.216	0.421	0.306
(mL kg^{-1} $BW^{0.75}$)	166	194	179	180	11.4	0.411	0.189	0.448	0.225
Digestibility (g digested g^{-1} ingested)									
DM	0.664	0.653	0.638	0.634	0.0081	0.059	0.027	0.009	0.833
OM	0.637[a]	0.621[ab]	0.608[ab]	0.599[b]	0.0087	0.035	0.014	0.005	0.815
CP	0.629	0.617	0.602	0.596	0.0099	0.122	0.053	0.021	0.824
NDF	0.521	0.484	0.511	0.508	0.0123	0.215	0.176	0.573	0.125
Nitrogen balance									
N retained (g day^{-1})	7.54	6.67	6.52	6.40	0.591	0.528	0.157	0.165	0.681
N retention (g kg^{-1} N ingested)	336	310	296	294	27.6	0.697	0.278	0.255	0.833
N retention (g kg^{-1} N absorbed)	533	499	487	492	37.3	0.825	0.371	0.396	0.724
Initial body weight (kg)	42.5	41.0	42.8	42.8	1.65	0.852	0.874	0.829	0.545
Average daily weight gain (g day^{-1})	191	134	180	177	28.8	0.534	0.418	0.824	0.276

DM dry matter, *OM* organic matter, *CP* crude protein, *NDF* neutral detergent fibre, *N* nitrogen, *BW* body weight

[1] Standard error of the mean

[2] "Seaweed addition" contrast: concentrate with no seaweed vs. concentrates including seaweed

[a,b] Means with different superscripts within a row are significantly different ($P < 0.05$)

as a source of bypass protein to be digested in the small intestine (Mora et al. 2009; Zitouni et al. 2014; Tayyab et al. 2016).

Few studies have investigated the effect of seaweeds on feed intake and nutrient digestibility. Our results are in agreement with other studies testing seaweed-supplemented diets (Al-Shorepy et al. 2001; Carvalho et al. 2009; Godard et al. 2009). Concentrate intake was not affected by the inclusion of *Ruppia* or *Ulva* up to 400 g kg^{-1}, but it was decreased when the green alga *Chaetomorpha* was added to the concentrate at 400 g kg^{-1}. A previous study with *Chaetomorpha* showed that, when introduced at 200 g kg^{-1} into the concentrate mixture, it has no effect on intake (Rjiba-Ktita et al. 2010). However, the effect on concentrate and total DM intake observed in the current study with the highest level of inclusion of this species could be attributed in part to its palatability. Previous researchers (Woodward 1951; Burt et al. 1954; Hentges and Salveson 1970; Linn et al. 1975; Altomonte et al. 2018; Lamminen et al. 2019) have reported decreased feed intake attributed to limited palatability when other seaweeds and algae were evaluated. In contrast, *Ulva* would be a palatable feedstuff, and no refusals have been recorded when *U. lactuca* was included into sheep diets at 200 g kg^{-1} (Arieli et al. 1993).

Water consumption was increased when *Ruppia* sp. was included in the concentrate due to the relatively high content of mineral salts existing in the seaweed, which rises with the level inclusion into concentrate mixtures. Ruminants tolerate high consumption of minerals in their diets adjusting water intake to regulate the osmotic balance in the intestinal tract (Underwood and Suttle 1999).

Seaweeds are known for their richness in dietary fibre and particularly in soluble fibre (Darcy-Vrillon 1993; Mabeau and Fleurence 1993). Al-Shorepy et al. (2001) suggested that seaweeds may have a laxative effect when added to sheep diets as water content of daily faecal output is consistently increased, indicating that the passage rate of digesta through the tract could be increased reducing the digestibility of the diet. The increasing water content of the faeces (data not shown) could be attributed to the water retention capacity of algal species and especially its soluble fibre fraction. Kuda et al. (2005) reported that dried marine algae can swell to about 20 times their volume when watered. The water retention capacity and the gelling or binding capacity of *Ulva* may have their effect in the gastrointestinal tract, where they form viscous gels (Renn 1990; Jiménez-Escrig and Sanchez-Muniz 2000), resulting in increased volume of softer faeces. Increased faecal output in sheep fed with *Ruppia*-supplemented concentrates can be attributed to a higher ingestion of insoluble fibre (Baghurst et al. 1996; Potty 1996) derived from both the seaweed and the increased hay intake.

The negative effect observed on OM digestibility when any of the three seaweeds were incorporated to the concentrate at 400 g kg^{-1} has been noted in goats and sheep fed with different algal species (Al-Shorepy et al. 2001; Cabrita et al. 2017). Material from *Ruppia* spp. contains high amounts of sulphated polysaccharides, which are absent in terrestrial and freshwater plants (Aquino et al. 2005). Most of these polysaccharides are indigestible by humans (Fleurence 1999), poultry (Ventura et al. 1994) or ruminants (Makkar et al. 2016). Unexpectedly, the digestibility of the NDF was enhanced by this species, maybe due to the nature of the different cell wall constituents and the effects on the ruminal microbiota.

Wong and Leung (1979) studied the seaweed *Ulva* sp. as a supplementary feed for chicks and noted a reduction in animal appetite and digestibility. They attributed this effect to the increasing ash provided with *Ulva*. In our case, this could explain the results with the species *Chaetomorpha* but not with *Ulva*. This green alga, also named sea lettuce, has been studied in human nutrition for its polysaccharide composition and physiological benefits. Studies conducted with rats (Carvalho et al. 2009) and hamsters (Godard et al. 2009) led to similar observations concerning the soluble polysaccharides of *Ulva* spp., which are rather resistant to bacterial fermentation. This fact could explain the observed decrease in OM digestibility when the level of *Ulva* into the concentrate mixture is raised up to 400 g kg^{-1}. The NDF digestibility was similar in all the diets, in agreement with Arieli et al. (1993) in sheep fed with a diet (oaten hay and concentrate) with a level of incorporation of *Ulva* of 20%. As seaweed material was incorporated to the concentrates at increasing inclusion rates, OM digestibility of the seaweeds could be estimated by regression. The OM digestibility estimates for *Ruppia* sp., *Chaetomorpha* sp. and *Ulva* sp. were 0.540, 0.497 and 0.535, respectively. Digestibility of the seaweeds has been measured in vivo only in a very limited number of studies (Cabrita et al. 2017) because this material is not intended to be fed as the only feedstuff, but instead as a potential supplement for ruminant diets. Therefore, studies available in the literature report digestibility for seaweed-supplemented diets, but not for the seaweed material as such. Cabrita et al. (2017) estimated OM digestibility by difference for a red (*Gracilaria vermiculophylla*) and a green (*Ulva rigida*) seaweed, feeding sheep alfalfa hay-supplemented diets. The digestibility coefficients were comparable to those observed in the present study.

Increasing the level of inclusion up to 400 g seaweed kg^{-1} concentrate had no effects on nitrogen balance or average daily gains. Protein digestibility was not affected with either *Ruppia* or *Ulva*, but was reduced when *Chaetomorpha* was added to 400 g kg^{-1} concentrate. Nitrogen excreted in faeces could correspond to the N compounds bound to indigestible cell walls and thus resistant to ruminal degradation. Some algal proteins may be closely linked to polysaccharide cell walls (Jordan and Vilter 1991; Fleurence et al. 1995) that could decrease the protein digestibility of *Chaetomorpha*-supplemented diets. Nevertheless, N retention and average daily gain were not significantly affected by any of the three seaweeds, suggesting that the dietary inclusion of *Ruppia*, *Ulva* or *Chaetomorpha* did not have an effect on growth performance. A similar outcome has been observed with other seaweed species like *Ascophyllum nodosum* (Hopkins et al. 2014).

Conclusions

Seaweeds, which are abundantly available along the Mediterranean coasts, can be used in animal nutrition. Material collected from *Ruppia* sp., *Chaetomorpha* sp. and *Ulva* sp. was studied as potential feeds to be incorporated into growing lamb diets. Different concentrate mixtures based on increasing levels of these species were tested. Inclusion of the seaweeds did not affect feed intake, except when the concentrate was supplemented with 400 g *Chaetomorpha* kg^{-1}. Generally, OM digestibility decreases linearly with the increasing level of these seaweeds into the concentrate, although inclusion rates up to 300 g seaweed kg^{-1} concentrate has no effects of feed digestibility. Nitrogen balance was positive with the three seaweed species at all levels of inclusion. The inclusion of seaweeds in sheep concentrates up to 400 g kg^{-1} has no adverse effects on lamb growth performance.

The feeding value of seaweeds for ruminants varies widely, depending on the species, their chemical composition and the level of incorporation in the diet. They seem to be promising alternative feed ingredients that can be used to replace conventional energy and protein feed sources in ruminant diets to achieve a similar growth performance.

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Compliance with ethical standards

The experiments were carried out at the Small Ruminants Research Unit of the Institut National de la Recherche Agronomique de Tunisie (INRAT) in strict accordance with good animal practices as defined by national authorities and European Union Directive 2010/63/EU. The experimental animal procedures complied with the institutional guidelines of INRAT and were conducted by trained specialised personnel to ensure animal welfare.

Conflict of interest The authors declare that there are no conflicts of interest.

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