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ORIGINAL ARTICLE

Bioconversion of green algae *Ulva lactuca* biomass with *Saccharomyces cerevisiae* yeast and exogenous fibrolytic enzymes into suitable ruminant feed

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Abstract
Ulva lactuca algae is a fast-growing aquatic biomass that poses a serious problem to marine ecosystem and ecology. It is characterized by a high content of protein, fiber, and important antioxidant activity. However, its low fiber digestibility (22%) and net energy available for lactation (2.2 MJ/kg dry matter) are the main obstacle for large-scale integration in ruminant diets. Therefore, this research was conducted to improve their nutritional value by pretreated them for 24 h with exogenous fibrolytic enzymes (EFE) and *Saccharomyces cerevisiae* yeast (SCY) at two doses (0 and 4 mg/g dry matter) in a 2 × 2 factorial arrangement. Pretreatment with EFE bioconverted some fiber to simple sugars and enhanced rumen microbiota, resulting in increase in ruminal fermentation, fiber digestibility by 11%, and net energy available for lactation by 17%, and depress ruminal pH. Pretreatment with SCY lowers its polyphenol and improves rumen bacteria, resulting in increase in rumen fermentation, fiber digestibility by 6%, and net energy available for lactation by 12% without altering rumen pH. A synergistic interaction between EFE and SCY was observed, resulting in increase in fiber digestibility by 16%, net energy available for lactation by 32%, and conversion of ammonia–nitrogen to microbial crude protein without altering rumen pH. Ultimately, pretreatment of *Ulva lactuca* with EFE + SCY is an eco-friendly strategy to convert this abundant biomass into a cost-effective and suitable ruminant feed that does not compete with human demand for fertile farmland and freshwater resources. This strategy can be explored on a large-scale in future to evaluate its impact on ruminant performance and product quality.

Keywords Bio-conversion · *Ulva lactuca* · Exogenous fibrolytic enzymes · *Saccharomyces cerevisiae* yeast · Ruminal digestibility · Net-energy available for lactation

1 Introduction

Ulva lactuca, commonly known as sea lettuce, is classified as a macroalgae in the phylum Chlorophyta [1]. This aquatic plant grows worldwide in various ecosystems such as the intertidal zone of brackish or marine environments and in sheltered harbors with calm waters and is characterized by a high growth rate, biomass yield, and productivity [2]. Under suitable environmental conditions, it causes *Ulva* blooms that damage marine ecosystems by reducing biodiversity and even smothering other algal species by covering the water surface [3]. Their accumulation in coastal areas and biodegradation produces acidic vapors that lead to the death of animals and possibly humans [1, 4]. No effective method has been exposed to control the *Ulva* blooms [1, 5]. Therefore many countries worldwide apply costly procedures for disposal this abundant biomass [4, 5].

Recent studies have shown that the natural components of this green macroalgae biomass can be used as a raw material to treat various fungal and bacterial infections [6, 7]. It can be used as a promising sustainable rumen feed alternative to alfalfa hay if it constitutes 5% of the total diet [8]. However, its incorporate in high amounts in ruminants diets (20%, 30%, and 40% of concentrate) has negative effects on rumen digestibility and growth performance [9]. An *in situ* study

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has shown that more than 65% of their organic matter, 90% of their crude protein, and 94% of their cell wall polysaccharides are not degraded in the rumen [10]. In order to better utilize these abundant biomasses in ruminant diets on a large scale in intensive ruminant systems, it is necessary to improve their ruminal digestibility.

In recent years, *Saccharomyces cerevisiae* yeast (SCY) has been used as a promising probiotic to improve rumen fermentation patent [11] and nutrient digestibility [12, 13] by stimulating the growth of beneficial microbiota in the rumen [14] and the development of the rumen epithelium [13], leading to improved growth performance [12, 13, 15], slaughter performance [13, 15], milk yield [12, 16, 17], milk composition [16], and feed conversion [11, 13, 16, 17], and provide a clear economic advantage for ruminant farming [12, 16]. It also reduces digestive problems [18] and stimulates immune responses of ruminants [19]. However, the effects of adding SCY to ruminant diets are variable and attributed to several interacting factors such as animal species, dose, and feed [20].

Our previous studies have also demonstrated that exogenous fibrolytic enzymes (EFE) are a useful and safe strategy to stimulate rumen fermentation patent and nutrient digestibility of different low-quality feeds such as sesame seed coats and peanut hulls by releasing sugars during the hydrolysis of cell wall polysaccharides at the preincubation period and enhancing rumen enzyme activity [21, 22]. In addition, it removes barriers that restrict microbial attachment to subtract and increase the rumen microbiota [23, 24]. Consequently, it improves average daily gain [25, 26], lactation performance of ruminants [27], and provides a cost-effective return on investment for ruminant breeding [28]. However, the results of EFE pretreatment are variable and highly dependent on the feeds treated, the dosage of the EFE preparation, the enzyme complex, and the animal species [29].

To our knowledge, this is the first study to examine the effects of treating macroalgae with EFE and SCY and to investigate the interaction between the SCY and EFE extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia*. With this in mind, we hypothesize that pretreatment of this aquatic macroalgae biomass with these biological additives will improve its nutritional value and a synergistic interaction between these two additives could be established to bioconvert this abundant aquatic biomass into a suitable feed for ruminants that does not compete with human demand for fertile farmland and freshwater resources. Therefore, the objective of the current study was to investigate the effects of pretreating *Ulva lactuca* biomass with EFE and SCY alone or in combination on its chemical composition, rumen microbiota, rumen fermentation patent, nutrient digestibility, and net energy available for lactation.

2 Materials and methods

2.1 Collection and preparation of *green algae Ulva lactuca* biomass

Samples of fresh *Ulva lactuca* algae biomass were collected in the winter season from the south of Lake of Tunis, Tunisia. They were washed with lake water at the collection site to remove impurities and immediately transported to the laboratory. The washed biomass was oven dried at 40 °C until constant weight. Then, the dried biomass was ground through a 1-mm sieve and stored until use.

2.2 Experimental treatments

The collected *Ulva lactuca* algae biomass was pretreated with two feed additives for 24 h at 39 °C. The first feed additive is an industrial SCY preparation called Yea-Sacc1026, manufactured by Alltech Inc., Nicholasville, KY, USA. According to the industry, it contains 5×10^{10} CFU of *Saccharomyces cerevisiae* per g dry weight. The second feed additive is an industrial EFE preparation called MAXFIBER-I® produced by SHAU-MANN GmbH, Wahlstedt, Germany, by fermentation of a mixture of *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus oryzae*, *Aspergillus sojae*, and *Neurospora intermedia*. The enzyme activity of this EFE preparation was measured at 39 °C and pH 6.6 according to the protocols of Baiely et al. [30] and Wood and Bhat [31]. This EFE preparation has an average xylanase activity of 1180 international units/mg, an endoglucanase activity of 750 international units/mg, and an exoglucanase activity of 440 international units/mg.

These biological additives were diluted with distilled water at 0 mg of EFE + 0 mg of SCY/20-ml distilled water, 40 mg of EFE + 0 mg of SCY/20-ml distilled water, 0 mg of EFE + 40 mg of SCY/20-ml distilled water, and 40 mg of EFE + 40 mg of SCY/20-ml distilled water. Each of these 4 preparations were added to 10 g dry matter ground *Ulva lactuca* biomass. The final concentration of these additives was 0 (control), 4 mg EFE, 4 mg SCY, and 4 mg EFE + 4 mg SCY/g dry matter *Ulva lactuca* biomass.

2.3 Chemical characterization

Crude protein ($N \times 6.25$; method 968.06), ether extract (method 920.30), and ash (method 923.03) of ground untreated or treated *Ulva lactuca* biomass were determined according to the method described by the Association of Official Analytical Chemists [32]. Neutral detergent fiber, acid detergent fiber, and acid detergent lignin were quantified according to the method described by Van Soest et al. [33] using an ANKOM fiber analyzer (ANKOM technology,

Macedon, NY, USA). Hemicellulose and cellulose were calculated by the difference between neutral detergent fiber and acid detergent fiber and between acid detergent fiber and acid detergent lignin, respectively [33]. Reducing sugars were determined by the 3,5-dinitrosalicylic acid method at 540-nm absorbance [34].

Ulva lactuca extract was performed according to the protocol of Abd El-Baky et al. [35]. Briefly, samples of 15 g of the green algae *Ulva lactuca* biomass were mixed with 100 ml of dichloromethane and methanol (1 : 1, v/v) and incubated in a shaking water bath at 25 °C and 150 rpm for 48 h. The mixture was then filtered, and the solvent evaporated at 40 °C *in vacuo*. The dried residual extract was used for the analysis of antioxidant activity and total phenolic compounds. Total polyphenols were analyzed by the Folin–Ciocalteu colorimetric method at 750-nm absorbance, using gallic acid as a reference standard [36]. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scaring method at 517-nm absorbance [37].

2.4 Rumen incubation

The *in vitro* rumen fermentation procedure was performed according to the methodology reported by Theodorou et al. [38] in three runs with three replicates per treatment. In this study, no animals were used directly, and rumen fluid samples were collected from three humanely slaughtered adult Holstein cows from a local slaughterhouse in Tunis (Tunisia) following the protocol of Palangi et al. [39]. These cows weighed 600 kg and were fed 7 kg of concentrate and 7 kg of oat hay ten days before slaughter. The collected rumen fluid was quickly transported to the laboratory in thermos flasks preheated to 39 °C and flushed with CO_2. In the laboratory, the rumen fluid was filtered through four layers of cheesecloth. The filtered rumen fluid was mixed with a buffer solution as described by Menke and Steingass [40] at a ratio of 1 : 2. Prior to incubation, 200 mg of ground untreated or treated green algae *Ulva lactuca* biomass was weighed into sterile amber glass serum bottles of 120 ml and incubated with 30 ml of the buffered rumen inoculum. Amber glass serum bottles containing 30 ml of buffered rumen inoculum without subtraction were also used as negative controls. These bottles were immediately closed with a rubber stopper and metal ring and incubated in a shaking water bath at 39 °C and 120 rpm [41]. The entire preparation was operated under anaerobic conditions by continuous influx of CO_2 at 39 °C in a water bath. The gas pressure in each bottle was measured after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation using a pressure transducer connected to a data logger and converted to volume using Eq. (1) described by Mauricio et al. [42]:

$$\text{Gas volume (ml)} = \left[\text{gas pressure (psi)} \times 4.8843\right] + 3.1296 \quad (1)$$

At the end of incubation, rumen pH was immediately measured using a pH meter (Jenway Ltd. Felsted, model 3020, England). The contents of each bottle were filtered through filter paper disks (Whatman 541). The dry matter and the natural detergent fiber of the unfermented solid residue from each bottle were determined according to the Method 934.01of Association of Official Analytical Chemists [32] and Van Soest et al. [33], respectively, and used to determine the digestibility of dry matter and natural detergent fiber. Samples of the filtered rumen fluid were taken from each bottle and used to determine rumen ammonia nitrogen according to the protocol of Broderick and Kang [43]. Second portion of rumen fluid was immediately fixed with 10% formalin salt solution and used for direct total counts of rumen protozoa and rumen bacteria using a hemocytometer (Boeco, Hamburg, Germany) under a microscope according to the protocols of Galyean et al. [44]. The net energy available for lactation, total short-chain fatty acids, and microbial crude protein were determined from gas production after 24 h of incubation and chemical composition analysis according to regression Eqs. (2), (3), and (4) of Menke and Steingass [40], Getachew et al. [45], and Blümmel et al. [46], respectively.

$$\text{Net energy available for lactation} = 0.101 \times \text{GP}_{24} + 0.051 \times \text{crude protein} + 0.112 \times \text{ether extract} \quad (2)$$

$$\text{total short} - \text{chain fatty acids} = -0.0425 + 0.0222 \times \text{GP}_{24} \quad (3)$$

$$\text{Microbial crude protein} = \text{amount of digestible dry matter} - 2.2 \times \text{GP}_{24} \quad (4)$$

where net energy available for lactation in MJ/kg dry matter, total short-chain fatty acids in mmol/200 mg dry matter, microbial crude protein in mg/ g dry matter, G_{P24} is net gas production in ml per 200 mg dry matter after 24 h of incubation, crude protein in percent of dry matter, ether extract in percent of dry matter, and the amount of digestible dry matter in mg/ g at the end of incubation.

2.5 Statistical analysis

The dynamics of the cumulative gas value was fitted to the exponential model proposed by France et al. [47] (Eq. (5)):

$$\text{GP}_{(t)} = A\left(1 - e^{\left(-C\ (t-\text{lag})\right.}\right) \quad (5)$$

Table 1 Chemical composition of green alga *Ulva lactuca* biomass, untreated or treated with *Saccharomyces cerevisiae* yeast and exogenous fibrolytic enzymes

	Control	EFE	SCY	EFE+ SCY	SEM	*P* value
Crude protein (g/kg dry matter)	218	220	221	221	5	NS
Neutral detergent fiber (g/kg dry matter)	329[a]	281[b]	321[a]	272[b]	11	**
Acid detergent fiber (g/kg dry matter)	131[a]	111[a]	125[a]	106[b]	8	**
Acid detergent lignin (g/kg dry matter)	16	15	16	15	2	NS
Cellulose (g/kg dry matter)	115[a]	96[b]	109[a]	91[b]	5	*
Hemicellulose (g/kg dry matter)	198[a]	170[a]	196[a]	166[b]	6	***
Ash (g/kg dry matter)	181	180	180	181	4	NS
Ether extract (g/kg dry matter)	21	22	20	21	2	NS
Reducing sugars (g/kg dry matter)	142[b]	177[a]	145[b]	179[a]	4	***
Total polyphenol (g/kg dry matter)	6.0[a]	5.9[a]	5.4[b]	5.4[b]	0.2	**
DPPH radical scavenging activity (%)	81.1[a]	80.2[a]	74.9[b]	75.2[b]	2.1	***

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

SEM, standard error of the mean; *EFE*, exogenous fibrolytic enzymes; *SCY*, *Saccharomyces cerevisiae* yeast

***P value < 0.001

**P value < 0.01

*P value < 0.05

NS, P value > 0.05; *DPPH*, 2,2-di-phenyl-1-picrylhydrazyl

where GP is the net gas production (ml/g dry matter), t is the incubation time (h), A is the amount of rumen fermentation (ml/g dry matter), C is the rumen fermentation rate (ml/h), and lag is the time of the onset of fermentation (h). A, C, and lag were determined using the Marquardt method and the nonlinear package from SAS Institute Inc [48].

All data were statically analyzed with the general linear model procedure in SAS Institute Inc. [48], using the fitted static model:

$$Y_{ij} = \mu + \text{treatment}_i + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the overall mean, treatment_i is the effect of the i^{th} treatment, and ε_{ij} is the residual experimental error. In addition, the difference between the means of the treatments was compared using the Tukey multiple range test. Significance was declared at a P value < 0.05.

3 Results and discussion

Ulva lactuca is the most abundant aquatic macroalgae in the south of Lake Tunis, Tunisia [49]. However, no study has assessed the nutritional value of this abundant macroalgae biomass in this lake through chemical analysis and in *in vitro* rumen fermentation method. Its chemical composition, antioxidant activity, and effects of pretreatment with EFE and SCY are shown in Table 1. The untreated *Ulva lactuca* macroalgae biomass collected in the winter season from the south of Lake of Tunis, Tunisia, is characterized by a high crude protein content (212 g/ kg dry weight). Compared to terrestrial feedstocks used in animal nutrition, it has comparable crude protein to Fabaceae seeds [50]. According to Yaich et al. [51], all essential amino acids are present in high amounts in this macroalgae biomass (42% of total acids). Comparable crude protein content was found in the same macroalgae species cultured in experimental containers (211 g/ kg dry weight) [10] and collected from the Buyukcekmece coast of the sea of Marmara, Turkey (225 g/kg dry weight) [52]. However, the same macroalgae species collected in Annaba, Algeria, in the same season had a low crude protein content (153 g/ kg dry weight) [53]. This variability is closely related to the aquatic ecosystem and the ability of this macroalgae to fix dissolved ammonia [54, 55]. In addition, this macroalgal biomass was found to have a high content of cell wall polysaccharides (NDF = 329 g/ kg dry weight), so it is more interesting to incorporate on the diet of ruminant animals than in the diet of monogastric animals. Compared to biomass widely incorporated into ruminant diets, this macroalgae has a cell wall polysaccharide content comparable to that of orange pulp (NDF = 308 g/ kg dry weight) [56]. Due to its crude protein and cell wall polysaccharide content, this green macroalgae biomass can be used as a potential ingredient for the formulation of concentrate mixture for ruminants, allowing to increase their fiber content as well as reduce the risk of acidosis in ruminants consuming highly concentrated diets. Analysis of the cell wall polysaccharide composition of this macroalgae biomass showed that hemicellulose was the most abundant fraction

Table 2 Influence of pretreatment of green alga *Ulva lactuca* biomass with *Saccharomyces cerevisiae* yeast and exogenous fibrolytic enzymes on its nutritional value

	Control	EFE	SCY	EFE+SCY	SEM	*P* value
Amount of rumen fermentation (ml/g dry matter)	99.6^{c}	128.3^{b}	126.8^{b}	146.9^{a}	5.2	***
Rate of rumen fermentation (ml/h)	0.024^{c}	0.033^{b}	0.031^{b}	0.040^{a}	0.003	***
Time of the onset of rumen fermentation (h)	2.11^{a}	1.25^{b}	2.03^{a}	0.91^{c}	0.14	***
Rumen pH	6.66^{a}	6.53^{b}	6.65^{a}	6.63^{a}	0.02	***
Rumen ammonia nitrogen (mg/l)	314^{a}	301^{ab}	303^{ab}	289^{b}	6.9	*
Dry matter digestibility (mg/g)	393^{c}	425^{b}	421^{b}	459^{a}	9.1	***
Neutral detergent fiber digestibility (mg/g)	220^{c}	245^{b}	239^{b}	255^{a}	7.2	***
Net energy available for lactation (MJ/kg dry matter)	2.33^{c}	2.72^{b}	2.61^{b}	3.09^{a}	0.19	**
Total short-chain fatty acids (mmol/200 mg dry matter)	0.213^{c}	0.294^{b}	0.272^{b}	0.376^{a}	0.039	**
Microbial crude protein (mg/g dry matter)	371^{c}	395^{b}	394^{b}	411^{a}	22	**
Total protozoa (10^5 cells/ml)	3.4^{b}	4.3^{a}	3.6^{b}	4.5^{a}	0.22	***
Total bacteria (10^9 cells/ml)	2.1^{c}	2.8^{b}	2.9^{b}	3.2^{a}	0.18	***

^{a,b,c}Means with different superscript within in same line are significantly different ($P < 0.05$)

SEM, standard error of the mean; *EFE*, exogenous fibrolytic enzymes; *SCY*, *Saccharomyces cerevisiae* yeast; *NS*, P- value >0.05

***P value < 0.001

**P value < 0.01

*P value < 0.05

(198 g/ kg dry weight), followed by cellulose (115 g/ kg dry weight) and lignin (16 g/ kg dry weight); a comparable fiber composition was found in the same species collected during the summer season in the coastal area of Monastir, Tunisia [51]. Moreover, this green macroalgae biomass has a high mineral compound (181 g/kg dry weight) due to its saline habitat and ability to absorb minerals [55]. Also, its inclusion in ruminant diets requires a change in mineral supplementation. Comparable mineral content was found on various *Ulva* sp. collected from different Mediterranean countries [9, 51, 55] and Mexico [57]. Furthermore, this macroalgae biomass has a high content of reducing sugars (142 g/kg dry weight) and a low lipid content (21 g/ kg), similar to green algae *Ulva prolifera* biomass collected from India [58]. On the other hand, this abundant biomass has a high polyphenolic compound and antioxidant activity due to their exposure to a variety of environmental stresses such as rapid temperature changes and osmotic stress. Recent researches have shown that the admixture of different phenolic feeds to the diet of ruminants is an interesting strategy to protect them from chronic diseases related to oxidative stress [59], improve their reproduction [60], and increase the quality of their products [61]. Compared to previous studies, this macroalgae collected from the south of Lake of Tunis, Tunisia, has comparable antioxidant activity to the same species collected in Tamilnadu, India, [6] and better polyphenolic compounds and antioxidant activity than the same species collected from Marsa-Matroh government, Egypt [35]. This variability attributed to many factors such as growing conditions, salinity of water, temperature, and maturity of macroalgae.

Pretreatment of *Ulva lactuca* biomass with EFE extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia* containing a xylanase, endoglucanase, and exoglucanase, increased their reducing sugars content from 142 to 177 g/kg dry weight and reduced their cell wall polysaccharide content from 329 g/kg dry weight to 281 g/kg dry without altering their bioactive compounds and antioxidant activity. These findings are in line with those in peanut hulls containing about twice the cell wall polysaccharides of *Ulva lactuca* biomass, pretreated with the same EFE preparation [22]. Pretreatment of this macroalgae biomass with SCY decrease their polyphenolic compounds by 10%. A similar effect was demonstrated in previous studies with cherry wines treated with different *Saccharomyces cerevisiae* strains [62] and apple ciders treated with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains [63]. This result due to the adsorption of polyphenols on yeast cell walls and reaction with cell wall protein compounds [62]. Also, pretreatment of this macroalgae with SCY reduces their antioxidant activity; this reduction is explained by reduction of their polyphenol content which is a strongly positive correlated with antioxidant activity [64].

The *in vitro* rumen fermentation method, based on the production of gas, is widely used to efficiently predict the nutritional value of feeds and the efficacy of feed additives. The nutritional value of this macroalgae biomass and the effect of pretreatment with EFE and SCY are shown

in Table 2. Although this macroalgae biomass collected in the winter season from the south of Lake of Tunis, Tunisia, has cell wall polysaccharides comparable to those of orange pulp [56] and has high content of reducing sugars, its pansal fermentation is very low and slow with a longer latency, and a high amount of its dry matter and cell wall polysaccharides bypass the digestive tract without being utilized. A comparable dry matter digestibility was obtained on *Ulva lactuca* collected at the same season from Annaba, Algeria [53]. This low ruminal fermentation and ruminal digestibility of *Ulva lactuca* biomass can be explained by its high crystalline cellulose content, which prevents access of rumen hydrolytic enzymes and rumen microbiota to various compounds [65], and by its high polyphenol content, which interacts with the rumen microbiota and alters its enzyme activity [66].

Pretreatment of *Ulva lactuca* with EFE extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia* containing xylanase, endoglucanase, and exoglucanase at 39 °C for 24 h promoted the proliferation of rumen bacteria and protozoa. Similar Zhang et al. [67] proved on *in vivo* experience that pretreatment with EFE containing mixture of cellulase, xylanase, pectinase, and laccase of diets of Holstein bulls improve the total amount of rumen bacteria, fungi, and protozoa. The increase in ruminal microbiota by the addition of EFE is attributed to the bioconversion of the same cell wall polysaccharides of this macroalgae into simple sugars, which stimulate microbial colonization of feed particles in the rumen and promote their prefoliation [68]. This increase in rumen microbial biomass stimulates rumen fermentation by shortening the delay phase of the onset of rumen formation, improving the rate of rumen fermentation, and increasing the amounts of rumen fermentation. Likewise, our previous study proved that pretreated peanut hulls with the same EFE preparation at 26 °C for 24 h improved ruminal fermentation [22]. This feed additive also improved the digestibility of dry matter and cell-wall polysaccharides of this macroalga biomass. A similar result was obtained in an *in situ* and *in vitro* experiences with whole plant faba bean silage pretreated with EFE containing mixture of cellulase and xylanase produced by fermentation of *Trichoderma reesei*, [27]. According to Oba and Allen [69], this improvement in the digestibility of cell wall polysaccharides indirectly leads to an increase in feed intake by 0.17 kg/unit improvement in the digestibility of cell wall polysaccharides. On the other hand, this approach provides a suitable substrate that can be used by the rumen microbiota to increase the production of short-chain fatty acids. Similarly, Zhang et al. [67] demonstrated that the pretreated of diets for Holstein bulls with EFE preparation containing a mixture of cellulase, xylanase, pectinase, and laccase increased concentration of short-chain fatty acids, acetate, propionate, and isovalerate. The increase in rumen fermentation and short-chain fatty acid production leads to a decrease in rumen pH, which may cause the risk of rumen acidosis in dairy cows. A similar result was obtained in an *in vivo* experiment when an EFE preparation containing a mixture of cellulase, xylanase, pectinase, and laccase was added to diets of Holstein bulls [67]. The pretreatment of this macroalgae with EFE had not effect on rumen ammonia–nitrogen and microbial protein synthesis. A similar result was found in previous study of *Posidonia oceanica* wastes, corn silage, and bermudagrass silage pretreated with EFE produced by fermentation of *Trichoderma longibrachiatum* [23, 70].

Pretreatment of *Ulva lactuca* macroalgae with SCY also increased the ruminal bacteria. Similarly, Jiang et al. [14] proved *in vivo* experience that the addition of SCY to the dairy cows diets increased amylolytic and cellulolytic rumen bacteria in the solid and liquid fractions of the rumen. This improvement is due to the ability of yeast to reduce antinutritional compounds (such as total polyphenol) during the pre-treatment period; their capacity to produce stimulatory factors such as organic acids, vitamins, and cofactors; and their capacity to scavenge oxygen and provides better anaerobic conditions in the rumen [20]. This increase in the rumen bacteria increased rumen fermentation and nutrient digestion of *Ulva lactuca* biomass. Similarly, Wang et al. [13] reported in an *in vivo* study that the addition of SCY to sheep feed at 20 and 40 g/day improved apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber. This improvement provide more net energy that can be used by rumen for milk production and provide a suitable substrate for rumen microbiota to produce of short-chain fatty acids. A similar beneficial effect was reported in an *in vivo* study when SCY was added to the buffaloes feed at 14 g/day [12]. Although this feed additive increased rumen fermentation and short-chain fatty acids, it did not alter rumen pH. These findings are consistent with previous *in vivo* studies in which SCY was added to diets of Holstein calves at 28 g/day [11], buffaloes at 14 g/day [12], and sheep at 20 and 40 g/day [13]. The lack of difference in the rumen pH is explained by the ability of SCY to modulate rumen pH by regulating both lactate generating and lactate utilizing bacteria [20]. This feed additive did not modify ruminal ammonia–nitrogen or microbial protein synthesis. Similarly, Wang et al. [13] proved that SCY supplementation in diets of sheep at 20 and 40 g/day cannot modify microbial protein.

The effects of the combination of EFE and SCY on rumen nutrition are rarely investigated. To our knowledge, this is the first study to investigate the interaction between SCY and EFE extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia*. The pretreatment of *Ulva lactuca* with complex of SCY and EFE has better improved rumen bacteria compared to their pretreatment with SCY or

EFE alone. Therefore, this complex improves rumen fermentation and rumen digestion and provides more net energy from this macroalgae that can be used by ruminants for milk production better than use SCY or EFE alone. This complex also improved the ability of the rumen microbiota to convert rumen ammonia–nitrogen to microbial protein. According Fonty et al. [71] the improvement of microbial crude protein synthesis is interesting strategy to protect the environment by the reduce the emission of nitrous oxide (greenhouse gas) and ammonia produced by ruminants. Similarly, a synergy interaction between SCY and EFE containing a cellulase and xylanase activity added to straw have been reported on growth performance buffalo calves [72] and rumen fermentation of goats [73]. However, Kholif et al. [74] reported that there was no significant interaction between SCY and EFE preparations on feed intake, rumen fermentation, rumen nutrient digestibility, and lactation performance of goats. This variability could be due to the different substrates treated, the dose of EFE and SCY and the animals used.

4 Conclusions

Ulva lactuca is an abundant biomass representing serious ecological and environmental problems worldwide. This aquatic biomass is characterized by a high proteins and cell wall polysaccharide compounds and important phenolic and antioxidant activity. Its valorization as an unconventional alternative protein feed for ruminants is very important from both economic and environmental points of view. However, their low rumen fermentation, nutrient digestibility, and net energy available for lactation are the main obstacle for large-scale integration in ruminant feeding especially in intensive ruminant systems. Pretreatment with EFE and SCY for 24 h is a simple and promising strategy to bio convert some of these cell wall polysaccharides to simple sugars and reduce their phenolic compounds. This simple strategy increases the rumen microbiota biomass; also, it improves the rumen fermentation process and nutrient digestion and provides more net energy that can be used by ruminants for milk production. It also increases the capacity of rumen microbiota to convert ammonia–nitrogen to microbial crude protein and produce short-chain fatty acids. This strategy can bioconvert this macroalgae biomass considered as wastes in many countries into suitable feed for ruminants not compete with human demand for cropland and freshwater consumption. We can only demonstrate the synergistic interaction between SCY at a dosage of 4 mg/g dry weight and EFE enzymes extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia* at a dosage of 4 mg/g dry weight, but a dose–response study of these two additives might be able to prove or disprove the hypothesis and determine the optimal dose. Future large-scale studies should be conducted to investigate the effects of inclusion of this aquatic biomass treated with EFE and SCY on meat and milk production and quality and ruminant health.

Abbreviations *A*: amount of rumen fermentation; *C*: rumen fermentation rate; *DPPH*: 2,2-diphenyl-1-picrylhydrazyl; *EFE*: exogenous fibrolytic enzymes; *GP*: net gas production; G_{P24}: net gas production after 24 h of incubation; *Lag*: time of the onset of fermentation; *SEM*: standard error of mean; *SCY* : *Saccharomyces cerevisiae* yeast; *t*: incubation time

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Data availability The datasets and materials used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The article does not contain any studies with human participants. It also does not perform experiments directly on animals. So, this experience does not need ethics statement.

Consent to participate All the authors of this article are consented to participate.

Competing interests The authors declare no competing interests.

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