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ORIGINAL ARTICLE

Conversion of *Posidonia oceanica* wastes into alternative feed for ruminants by treatment with microwaves and exogenous fibrolytic enzymes produced by fermentation of *Trichoderma longibrachiatum*

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Abstract
Posidonia oceanica wastes is abundant biomass in Mediterranean coasts characterized by a high content of dietary fiber and polyphenolic compounds and pose a serious pollution problem. The aim of this study was to valorize these seagrasses into suitable alternative feed for ruminants through conversion approach with microwaves treatment at a power of 750 W for 240 s and exogenous fibrolytic enzymes (EFE) produced by fermentation of *Trichoderma longibrachiatum* at a rate of 4 µl/g dry matter for 24 h.
The results showed that microwave treatment improved the amount of rumen fermentation and digestibility of cell wall polysaccharides and dry matter without altering the fermentation rate. The EFE treatments stimulated the rate of rumen fermentation especially in the initial phase and reduced the half-time of fermentation. The effect of EFE became less pronounced with increasing incubation time, as well as did not affect amount of rumen fermentation and digestibility. Only microwave treatment followed by EFE converted some of their cellulose and hemicellulose to reducing sugars. This modification provides a suitable substrate for proliferation of rumen protozoa, accelerates rumen fermentation process, shortens time of onset of rumen fermentation, and increases extent of rumen fermentation. As a result, their digestibility and net energy available for lactation are improved. In addition, this approach stimulates rumen microbiota to produce short-chain fatty acids and to bio-convert rumen ammonia nitrogen to microbial protein. This environmentally friendly process could be used to convert these seagrass wastes into suitable ruminant feeds.

Keywords Conversion · *Posidonia oceanica wastes* · Exogenous fibrolytic enzymes · Microwaves · Net energy available for lactation

1 Introduction

Posidonia oceanica is a seagrass endemic to the Mediterranean sea that forms extensive meadows extending from the surface to 40 m depth, occupying about 2% of the Mediterranean seabed and 3.5 to 3.7 million hectares [1]. These fast-growing plants have an annual growth cycle characterized by the tearing off of their leaves, which are transported to the coastal areas as banquettes, reaching a thickness of up to 2.5 m and carrying about 5 to 50 million tons annually [2, 3]. Their accumulation and decomposition pose a serious ecological, economic, social, and sanitary problem [4].

Recent studies have shown that these seagrass wastes can be used as fiber feed for dairy goats as an alternative to barley straw up to 450 g/day, which increases the efficiency of product cost and milk fat, decreases the number of somatic cells in milk, and reduces the risk of oxidative stress without changing their body weight, milk production, and metabolic status [5]. In addition, these seagrass wastes can serve as a forage source for dairy ewes and provide an alternative to barley straw (up to 75 g/day) without affecting their tissue function, feed consumption, nitrogen balance, final live weight, and metabolic status, but it has negative effects on nutrient digestion of diet [6]. In situ studies over 72 h in the rumen of adult ewes have shown that the rumen degradability of *Posidonia oceanica* is very low. In fact, more than 70% of their organic matter, crude protein and neutral detergent

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fibers are not degradable in the rumen [7]. In order to better utilize these fiber biomass wastes and better incorporate them into the diets of ruminants in intensive production systems, it is recommended to upgrading their rumen digestion.

Our previous in vitro studies have shown that pretreatment of various fibrous feeds such as crude olive cake, sesame seed coats, and peanut hulls with exogenous fibrolytic enzymes (EFE) produced by fermentation of *Trichoderma longibrachiatum* or mixed cultures of *Aspergillus* strains and *Neurospora intermedia* is a promising biological strategy that dissolves dietary fiber and leads to an increase in rumen enzymes and rumen microbiota, which in turn improves rumen fermentation patterns, digestibility in the rumen, energy utilization, production of short-chain fatty acids, and crude protein by the rumen microbiota [8–10]. In addition, a recent in vitro study proved that ensiled fiber feeds such as date palm leaves with an EFE preparation containing xylanase, cellulase, and β-glucanase were a promising strategy to improve the degradability of their neutral detergent fibers and the production of short-chain fatty acids and to sustainably improve environmental conditions by reducing methane and carbon dioxide emissions produced by ruminants compared to date palm leaves ensiled without EFE [11]. Moreover, our previous in vivo study showed that pretreatment of olive cake with EFE preparations produced by fermentation of *Trichoderma longibrachiatum* improved feed intake and digestibility of dietary fiber as well as growth performance of lambs without negative effects on their health [12]. Recently, an in vivo study in lactating dairy ewes demonstrated that supplementing their diet with an EFE preparation produced by fermentation of *Aspergillus niger* improved fiber digestibility, lactation performance, and feed conversion and provided a clear economic benefit to the animals birding [13]. However, the effects of EFE treatment on the nutritional value of the feed vary depending on the type of feed [9].

Recent research has shown that microwave treatment, a non-ionizing electromagnetic physical heating technique, is an environmentally friendly and efficient technology that causes destruction of plant cell walls of fibrous feeds such as alfalfa hay [14] and could improve the feed value of some low-quality feeds such as alfalfa hay, wheat hay, and canola hay by increasing in vitro pepsin cellulase dry matter and organic matter digestibility [14, 15]. An in situ study over 96 h in the rumen of yak cows showed that pretreated wheat straw with microwaves improved their dry matter degradability, organic matter degradability, and acid detergent fibers degradability [16]. The animal response study also showed that pretreated lucerne hay with microwave improved the growth performance of wethers [15]. However, the effect of microwave treatment on the nutritional value of the feed varied depending on the type of feed [14].

To our knowledge, the effect of biological or physical treatment to improve the nutritional value of *Posidonia oceanica* wastes has not been studied yet. With this in mind, it can be hypothesized that the microwave and EFE treatments alone or in combination will improve the nutritional value of *Posidonia oceanica* wastes. Therefore, the objective of the current study was to investigate the effect of these treatments on chemical composition, rumen fermentation patent, rumen digestibility, net energy available for lactation, and short-chain fatty acids of *Posidonia oceanica* wastes.

2 Materials and methods

2.1 Collection of *Posidonia oceanica wastes* and treatment with microwaves and exogenous fibrolytic enzymes

Samples of fresh *Posidonia oceanica* wastes were randomly collected from different coastal sections of the Sfax region (in southeastern Tunisia with a semi-arid bioclimate) and immediately transported to the laboratory. These samples were treated with microwaves or EFE or microwaves flowed by EFE. Microwave treatment was performed in microwave oven with a power of 750 W and a frequency of 2450 MHz for 240 s according to the protocols of Dong et al. [13]. EFE treatment was performed with EFE produced by fermentation of *Trichoderma longibrachiatum* (50% xylanase plus and 50% cellulase plus, Dyadic International Inc. Jupiter, FL, USA) at a concentration of 4 µl/g dry matter for 24 h at 39 °C room temperature according to the protocol of Abid et al. [10]. The enzyme activity of the EFE preparation was measured at 39 °C and pH 6.6. Xylanase activity was determined according to the protocol of Baiely et al. [17], and endoglucanase and exoglucanase activities were determined as described by Wood and Bhat [18]. This preparation has an average xylanase activity of 2267 international units/mL, an endoglucanase activity of 1161 international units/mL, and an exoglucanase activity of 113 international units/mL. The treated or untreated samples were ground through a 1 mm sieve using a Retsch mill (Retsch ZM200, Retsch GmbH, Haan, Germany) to analyze their chemical composition and in vitro rumen fermentation.

2.2 Chemical composition

Samples of ground wastes of *Posidonia oceanica*, untreated or treated, were used for determination of crude protein (N × 6.25; method 968.06), ether extract (method 920.30), and ash (method 923.03) according to the standard method of the Association of Official Chemists Analytical Chemists [19]. Concentrations of neutral detergent fiber, acid detergent

fiber, and acid detergent lignin were determined according to the method described by Van Soest et al. [20] using an ANKOM220 fiber analyzer (ANKOM technology, Macedon, NY, USA). Reducing sugars were determined by the 3,5-dinitrosalicylic acid method using spectrophotometry at 540 nm absorbance [21]. Total polyphenols were analyzed by the Folin-Ciocalteu colorimetric method at an absorbance of 750 nm. Gallic acid was used as a reference standard, and the result was expressed in µg gallic acid equivalent/g sample [22].

2.3 In vitro rumen incubation

The evaluation of in vitro rumen fermentation with the gas production technique on the samples of *Posidonia oceanica* wastes, untreated or treated with the different treatments, was performed according to the methodology described by Theodorou et al. [23] and repeated at three times (3 runs).

Rumen fluid content was sampled at different locations of the rumen of three slaughtered Holstein cows (age ~6 years and ~700 kg body weight) from a local slaughterhouse in Tunis (Tunisia). Prior to slaughter, cows were fed 7 kg of oat hay and 7 kg of commercial concentrate and had free access to water. The collected rumen was rapidly transported to the laboratory in thermos flasks preheated to 39 °C and flushed with CO_2. In the laboratory, the rumen liquor was flushed with a constant flow of CO_2 at 39 °C, sieved through four layers of cheesecloth to remove feed particles, and mixed at the same volume ratio. An artificial buffer solution was also prepared from macromineral, micromineral, and resazurin solutions, buffer, and distilled water according to the method described by Menke and Steingass [24]. Rumen inoculum was mixed with an artificial buffer solution (1:2 v/v) under a constant CO_2 flow at 39 °C.

Samples of 0.2 g of *Posidonia oceanica* wastes were transferred to sterile amber glass serum bottles of 120 mL prewarmed to 39 °C and incubated in triplicate with 30 mL of the buffered rumen inoculum. Three amber glass serum bottles containing 30 mL of the buffered rumen inoculum without feed sample were also used as negative controls to correct for gas production from the buffered rumen inoculum. All bottles were immediately capped with a rubber cap and an aluminum crimp cap and incubated in a shaking water bath at a constant temperature of 39 °C and an oscillation speed of 120 rpm. The gas pressure in each incubated bottle was recorded 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. [25]:

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

The net gas production data were fitted with the exponential model proposed by France et al. [26] (Eq. 2) using the Marquardt method and the nonlinear package from SAS Institute Inc. [27]

$$GP_{(t)} = A\left(1 - e^{\left(-C\ (t-lag)\right)}\right) \quad (2)$$

where GP is the net gas production (mL/g DM); t is the incubation time (h); A is the amount of rumen fermentation (mL/g DM) and C is the rate of fermentation (mL/h); and lag is the time of the onset of fermentation (h).

The half-time of amount of rumen fermentation ($T_{1/2}$) was the time during which half of the amount of rumen fermentation was produced and was calculated according to Eq. 3:

$$T_{1/2} = \frac{Ln\ 2}{C} + \text{lag} \quad (3)$$

where $T_{1/2}$ is half-time of amount of rumen fermentation (h); C is the rate of fermentation (h); and lag is time of the onset of fermentation (h).

The average fermentation rate (AFR) was defined as the average gas production rate between the start of the incubation and $T_{1/2}$ and was calculated according of Eq. 4:

$$AFR = \frac{A \times C}{2\left[\ln 2 + C \times Lag\right]} \quad (4)$$

where AFR is the average fermentation rate (mL /h); C is the rate of fermentation (h); and Lag is the time of the onset of fermentation (h).

At the end of fermentation, rumen pH was immediately measured using a pH meter (Jenway Ltd Felsted, model 3020, England). The contents of each serum bottle were filtered using filter paper disks (Whatman 541). The residues were collected and their dry matter and neutral detergent fiber were determined according to the Association of Official Chemists Analytical Chemists [19] protocols (Method 934.01) and Van Soest et al. [20], respectively. The in vitro digestibility of dry matter and neutral detergent fiber were determined according to Eq. 5 and Eq. 6, respectively.

$$\text{Dry matter digestibility (\%)} = \frac{\text{initial dry matter} - \text{residual dry matter}}{\text{initial dry matter}} \times 100 \quad (5)$$

$$\text{Neutral detergent fiber digestibility (\%)} = \frac{\text{initial neutral detergent fiber} - \text{residual neutral detergent fiber}}{\text{initial neutral detergent fiber}} \times 100 \quad (6)$$

Table 1 Influence of pretreatment of *Posidonia oceanica* wastes with microwave and exogenous fibrolytic enzymes on chemical composition

	Control	EFE	Microwave	Microwave + EFE	SEM	*p* value
Crude protein (g/kg dry matter)	86	87	86	86	3	NS
Ether extract (g/kg dry matter)	12	12	11	13	2	NS
Neutral detergent fiber (g/kg dry matter)	713[a]	700[a]	715[a]	662[b]	8	**
Acid detergent fiber (g/kg dry matter)	489[a]	483[a]	492[a]	451[b]	6	**
Acid detergent lignin (g/kg dry matter)	95	94	94	93	3	NS
Cellulose (g/kg dry matter)	394[a]	389[a]	398[a]	358[b]	5	**
Hemicellulose (g/kg dry matter)	224[a]	217[a]	223[a]	211[b]	6	**
Ash (g/kg dry matter)	137	135	136	136	4	NS
Reducing sugars (g/kg dry matter)	4.2[b]	4.6[b]	4.3[b]	8.0[a]	0.9	***
Total polyphenol (µg gallic acid equivalent/g)	712	710	708	706	12	NS

[a,b]Means value flowed by different superscript in same line differed at $p < 0.05$ (Tukey test); *SEM*, standard error of means; *EFE*, exogenous fibrolytic enzymes; ***p value <0.001, **p value < 0.01;*p value < 0.05; NS, p value >0.05.

Samples of 1 mL of supernatant fluid from each bottle were mixed with 1 mL of physiological methyl green formalin solution to ensure counting of rumen protozoa using light microscopy and the Levy-Hausser counting chamber (Husser Scientific, Horsham, PA) according to the protocol of Dehority [28]. Samples of 4 mL of supernatant fluid from each bottle were preserved by adding 2 mL of 1 N H_2SO_4 to determine rumen ammonia nitrogen by the phenol-hypochlorite method at 630 nm absorbance, as described by Broderick and Kang [29]. Additional samples of 6 mL supernatant fluid from each bottle were mixed with 1 mL carbon tetrachloride and 1 mL lysozyme. The mixture was incubated at 40 °C for 3 h and then sonicated at 4 °C. This was followed by centrifugation at 24,000×g for 20 min at 4 °C according to the protocol of Patra et al. [30]. The supernatant was used to determine xylanase activity according to Baiely et al. [17], and endoglucanase and exoglucanase activities were determined as described by Wood and Bhat [18].

Net energy available for lactation, microbial crude protein, and total short-chain fatty acids were determined according to the regression Eqs. 7, 8, and 9 of Menke and Steingass [24], Blümmel et al. [31], and Getachew et al. [32], respectively.

$$\text{Net energy available for lactation} = 0.101 \times GP_{24} + 0.051 \times \text{crude protein} + 0.112 \times \text{ether extract} \quad (7)$$

$$\text{Microbial crude protein} = \text{amount of digestible dry matter} - 2.2 \times GP_{24} \quad (8)$$

$$\text{Total short} - \text{chain fatty acids} = -0.00425 + 0.0222 \times GP_{24} \quad (9)$$

where net energy available for lactation in MJ/kg dry matter, microbial crude protein in mg/g dry matter, total short-chain fatty acids in mmol/200 mg dry matter, GP24 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.

2.4 Statistical analysis

All collected data were analyzed using the GLM procedure of SAS Institute Inc [27], flowing the static model:

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

where Y_{ij} is the individual observation, µ is the overall mean, treatment $_i$ is the effect of the i^{th} treatment, and ε_{ij} is the residual experimental error associated with the observation. In addition, the difference between the means of the treatments was compared using the Tukey multiple range test, and the differences were considered significant if the p value was less than 0.05%.

3 Results and discussion

3.1 Chemical composition

It is important to study the chemical composition of unconventional biomass in order to use it as a low-cost alternative raw feed for ruminants. Few studies investigate the possibility of using marine plants as alternative feed for ruminants. Table 1 illustrates the chemical composition of *Posidonia oceanica* wastes and the effects of their treatment with EFE and microwaves. Our study showed that the untreated Tunisian *Posidonia* oceanica wastes are an important source of fiber in terms of neutral detergent fiber (713 g/kg), acid detergent fiber (489 g/kg), and acid detergent lignin (95 g/kg) content, which is comparable to the results obtained by Castillo et al [6] for Spanish *Posidonia oceanica* wastes and

higher than the values obtained by Torbatinejad et al. [33] for Australian *Posidonia australis* wastes. These differences may be attributed to the diversity of *Posidonia* cultivars. The acid detergent fiber content of these seagrass wastes is well above to the lower limit (210 g/kg) recommended by the National Research Council [34] for diets of dairy cows to ensure proper rumen function. As well as, *Posidonia oceanica* wastes can be used as great unconventional roughage for ruminants that consume large amounts of rapidly fermenting feed. The crude protein detected in these marine plant wastes (86 g/kg) is higher than the minimum required for normal rumen microbiota function and growth (80 g/kg) [35]. It is better than the crude protein detected in the wastes of Australian *Posidonia australis* [33] and Spanish *Posidonia oceanica* [6]. This difference is related to many factors, including the difference in maturity stage, harvest time, edaphoclimatic conditions, and *Posidonia* cultivar. Compared to oat hay and wheat straw, which are most commonly used as roughage for ruminants in Tunisia, these marine biomass wastes had better crude protein content and comparable fiber composition in terms of neutral detergent fiber and acid detergent fiber to wheat straw. Nevertheless, their acid detergent lignin compounds are higher than acid detergent lignin compounds of oat hay and wheat straw [36, 37]. Based to fiber and protein composition, these seagrass wastes can be used as unconventional roughage for ruminants. However, their mineral content (137 g/kg) is very high compared to oat hay and wheat straw [36, 38], as well as their utilization requires a change in mineral supplementation of ruminant diets. On the other hand, these seagrass wastes are rich in phenolic compounds (712 µg gallic acid equivalent/g), which is due to the self-defense mechanisms. These bioactive compounds can improve animal health, animal reproduction, and animal products quality [39–41].

Treatment of these marine plant wastes with microwaves did not alter their chemical composition; a similar result was demonstrated for wheat straw treated with the same microwave power, frequency, and duration [16]. In contrast, Yu et al. [42] proved the ability of this treatment to remove some of the lignin and hemicellulose from wheat straw. The lack of effect in our study was related to several factors, such as the different treatment duration, microwave frequency and power, and the substrate used. Accordingly, treatment of these seagrass wastes with EFE had no significant effect on their chemical composition. A similar result was found for barley straw treated with xylanase enzyme produced by *Aspergillus niger* [43]. In contrast, Jabri et al. [36] demonstrated that the same level EFE preparation can increase the content of reducing sugars in wheat straw. The lack of effect in our study might be related to the highly acid detergent lignin compounds in *Posidonia oceanica* wastes compared to cereal straw, which block the effect of EFE. However, the addition of EFE after microwave treatment greatly reduces the fiber content (neutral detergent fiber, acid detergent fiber, hemicellulose, and cellulose) of *Posidonia oceanica* and significantly increases their content of reducing sugars. These synergistic effects between these two treatments are explained by the elimination of the wax layer on the outer surface and the destruction of the molecular structure of the plant cells by the microwave treatment [14, 44], as well as the EFE can optimally penetrate into the interior of the plant cells, allowing part of the cellulose and hemicellulose to be converted into reducing sugars.

3.2 Nutritional value

The in vitro rumen fermentation is a widely used, as rapid and effective method for screening the nutritional value of feeds under simulated rumen conditions [8–11]. The nutritional value of untreated or treated *Posidonia oceanica* wastes with microwaved and EFE is shown in Table 2. Our study showed that untreated *Posidonia oceanica* wastes are characterized by low rumen fermentation and a large portion of their dry matter and fiber bypasses the digestive tract without being utilized. This result is consistent with previous in situ studies on Spanish *Posidonia oceanica* wastes [7]. Their low fermentability and digestibility is due to their high content of cell wall tissue, which is difficult to metabolize by rumen microbes, and their high content of acid detergent lignin, which is not degraded in the rumen environment and forms a physical barrier for the rumen microbiota and digestive enzymes to penetrate to other components [35]. In addition, their high polyphenol content may interact with the rumen microbiota, which has a toxic effect on some rumen microbiota, alters their enzyme activity, and impairs rumen fermentation processes and rumen degradability [45]. Compared to agro-industrial wastes, the rumen fermentation kinetics of *Posidonia oceanica* wastes is comparable to that of olive tree leaves [46]. Although microwave treatment does not change their chemical composition, it increases their amount of fermentation and pansal digestibility of both the fiber and dry matter fractions. A similar effect was demonstrated in the in sacco degradability of the dry matter and acid detergent fiber fractions of wheat straw treated with the same power, frequency, and duration of microwave treatment [16]. This improvement could be due to the cleavage of lignin-hemicellulose bonds, disruption of cell microstructure, reduction of fiber stability, and alteration of their fiber structures, thus providing better access to the rumen microbiota and its associated enzymes to the substrate during rumen fermentation [14–16].

The EFE preparation mainly stimulates the initial fermentation rate and shortens the half-time of rumen fermentation. However, the effect of EFE decreasing with increasing incubation time as well the amount of rumen fermentation and ruminal digestibility are not change. A similar effect was

Table 2 Influence of pretreatment of *Posidonia oceanica* wastes with microwave and exogenous fibrolytic enzymes on their nutritional value.

	Control	EFE	Microwaves	Microwaves + EFE	SEM	*p* value
Amount of rumen fermentation (mL/g dry matter)	158.9^{c}	160.3^{c}	174.4^{b}	200.7^{a}	6.7	***
Rate of rumen fermentation (mL/h)	0.039^{c}	0.046^{b}	0.040^{c}	0.053^{a}	0.004	***
Time of the onset of rumen fermentation (h)	1.11^{a}	0.95^{a}	1.13^{a}	0.35^{b}	0.14	**
Half-time of amount of rumen fermentation (h)	19.11^{a}	16.02^{b}	18.46^{a}	13.43^{c}	1.11	***
Average fermentation rate (mL/h)	4.16^{c}	5.00^{b}	4.72^{bc}	7.47^{a}	0.42	***
Rumen pH	6.86^{a}	6.83^{b}	6.85^{a}	6.82^{a}	0.09	NS
Rumen ammonia nitrogen (mg/L)	238^{a}	238^{a}	233^{a}	209^{b}	15	**
Dry matter digestibility (mg/g)	383^{c}	388^{c}	418^{b}	453^{a}	9.1	***
Neutral detergent fiber digestibility (mg/g)	321^{c}	325^{c}	351^{b}	378^{a}	6.4	***
Net energy available for lactation (MJ/kg dry matter)	2.45^{b}	2.69^{b}	2.67^{b}	3.48^{a}	0.19	**
Total short-chain fatty acids (mmol/200 mg dry matter)	0.41^{b}	0.46^{b}	0.46^{b}	0.63^{a}	0.03	**
Microbial crude protein (mg/g dry matter)	342^{b}	342^{b}	354^{b}	390^{a}	22	**
Total protozoa (10^5 cells/ mL)	6.4^{b}	6.6^{b}	6.5^{b}	7.4^{a}	0.3	**
Ruminal xylanase activity (unites/mL)	1.44^{b}	1.45^{b}	1.48^{b}	1.94^{a}	0.18	**
Ruminal endoglucanase activity (unites/mL)	6.66^{b}	6.67^{b}	6.71^{b}	8.11^{a}	0.41	**
Ruminal exoglycanase activity (unites/mL)	35.6^{b}	36.0^{b}	35.8^{b}	40.3^{a}	1.2	**

^{a,b,c}Means value flowed by different superscript in same line differed at $p < 0.05$ (Tukey test); *SEM*, standard error of means; *EFE*, exogenous fibrolytic enzymes; ***p value <0.001, **, p value < 0.01;*p value < 0.05; NS, p value >0.05.

found by Díaz et al. [47] in tropical forages treated with an EFE preparation of *Trichoderma longibrachiatum* and by Ranilla et al. [48] in alfalfa hay, grass hay, and barley straw. The lack of effect at the end of fermentation could be due to the hydrolysis of this EFE by rumen protease and/or the competition between endogenous and exogenous enzymes for the available binding sites in the substrate, which are saturated at the end of fermentation [47, 49].

The treatment of *Posidonia oceanica* wastes with microwaves flowed with EFE shortened the delay phase of the onset of rumen formation. This result is due to the increase in reduction sugar in these treated substrates, which attracts the rumen microbiota to the substrate [50]. In addition, the bioconversion of fiber to reducing sugars enhances the proliferation of rumen protozoa, which bind oxygen and promotes the development of anaerobic rumen bacteria and fungi [51]. As a result, the activity of rumen enzymes increases. This improvement increase the rate and amount of rumen fermentation by 42 mL/g dry matter, dry matter digestibility by 7 %, cell wall polysaccharides digestibility by 5.6 %, and energy available for milk production by 1.03 MJ/ kg dry matter. According to Oba and Allen [52] this improvement on digestibility of cell wall polysaccharides can increase the feed intake by 0.17 kg/ unit improvement of cell wall polysaccharides digestibility and 4% fat corrected milk production of dairy cows by 0.25 kg/unit improvement of cell wall polysaccharide digestibility. On the other hand, this approach provides a suitable substrate that can be used by the rumen microbiota for the production of short-chain fatty acids and for the bioconversion of rumen ammonia nitrogen into microbial crude protein without disturbing rumen pH.

4 Conclusions

Posidonia oceanica wastes are an abundant biomass on the Mediterranean coast and pose a serious environmental, economic, social, and sanitary problem. Pretreatment with microwaves at 750 W for 240 s followed by treatment with EFE produced by *Trichoderma longibrachiatum* at a concentration of 4 µl/g dry matter for 24 h is a simple and promising environmentally friendly strategy to convert it into suitable alternative feed for ruminants. This approach converts some of the dietary fiber into reducing sugars and promotes the proliferation of rumen protozoa. It also increases the rumen fermentation process and the rumen digestibility of dry matter and fiber. This improvement provides more energy that can be used by ruminants for milk production and a suitable substrate for the rumen microbiota to produce short-chain fatty acids and bio-convert rumen ammonia nitrogen into microbial crude protein.

Abbreviations *A*: Amount of rumen fermentation; *AFR*: Average fermentation rate; *C*: Rate of fermentation; *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 means; *t*: Incubation time; $T_{1/2}$: Half-time of amount of rumen fermentation

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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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