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Full Length Article

Seagrass Debris as Source of Fiber and Bioactive Compounds in Feed for Dairy Goats

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Abstract

Climate change has severely affected rainfall regularity, limiting grass growth and thus fodder availability. Finding unconventional resources has become a challenge for small and medium breeders to feed their livestock. The objective of this study is to determine whether the use of marine plant debris, *Posidonia oceanica*, as total or partial replacement of oat hay in alpine goat diets will affect or not milk yield and quality. The sea grass was characterized according to the sampling site. Chemical and phytochemical analysis revealed significantly higher levels of dry matter, minerals, total polyphenols and carotenoids in *P. oceanica* on banquettes than in floating Posidonia. The condensed tannin content was almost the same for both sites. Partial or total replacement of oat hay by *P. oceanica* in alpine goat rations significantly increased milk production and milk fat content, while somatic cell count decreased significantly. In addition, *P. oceanica* significantly enhanced milk biochemical composition by increasing flavonoid and total phenol contents. However, protein, lactose and freezing point parameters were not affected. Sensory analysis revealed substantial improvement in the organoleptic quality of *P. oceanica* fed goats' milk compared to the other group, which was highly appreciated by most of the panelists. The seagrass *P. ocanica* could therefore be a safe solution to improve fodder availability for small and medium breeders, reduce production costs and increase milk market value.

Keywords: Alternative food; Bioactive compounds; Dairy goat; Goat milk quality; *Posidonia oceanica*

Introduction

Posidonia is a marine plant of the phanerogam family, present in several regions of the world, characterized by a fairly long lifespan (4 to 30 years) with leaves living between 70 and 350 days and highly productive biomass (Gobert *et al.* 2006). The species *Posidonia oceanica* (*P. oceanica*) forms dense meadows occupying about 2% of the Mediterranean seabed, *i.e.*, 3.5 to 3.7 million hectares (Boudouresque *et al.* 2006). In Australia's subtropical zone, another very similar species, *P. australis* is found. These two species have almost the same chemical and biochemical composition (Augier *et al.* 1982).

As soon as the Posidonia leaves stop photosynthesis, they lose their original green colour and become brown until detached then transported to coast as litter or banquettes (Ambrosio and Segovia 2000). After deposition on the sand, Posidonia debris decomposes, causing serious environmental problems. Thus, the removal of tons of *P. oceanica* debris is a common practice in the Mediterranean shores to allow the recreational use of the beaches (Falco *et al.* 2008). On southern Australian beaches, hundreds of tons of sea grass, *P. australis* are yearly massed by wave action, causing various environmental problems, such as mosquito proliferation and even navigation and fishing problems (Torbatinejad *et al.* 2007). According to Cocozza *et al.* (2011), the removal of one ton of Posidonia debris costs the municipality about 56 Euros. Generally, the main destination of the removed banquettes is either landfill or incineration (Castaldi and Melis 2002). Historically, *P. oceanica* has been used for a wide variety of purposes. It has been used not only as livestock bedding, but also as packing material for transporting fragile objects or even fresh fish from the coast to the cities (Cocozza *et al.* 2011). Posidonia litter has also been used as filling material for mattresses and cushions, especially for people with respiratory allergy problems (Cocozza *et al.* 2011).

Feeding livestock is an on-going problem for small and medium-scale breeders in Mediterranean region. During the dry season, pasture availability and quality, decrease significantly, negatively affecting body weight as well as animal performance and health (Almeida and Cardoso 2008). During these periods breeders often use cereal and grain concentrates for animal feed (Eldik *et al.* 2017). To help solve this food problem, some research studies have tried to utilize Posidonia in animal feed for different species in order to exploit this very important natural biomass. In this context, and after estimating the annual production of this sea grass at approximately 5–50 million tons, Dural *et al.* (2011) affirmed that the Mediterranean Sea could be a potential source of cheap raw materials for animal feed for all countries along the shore. Torbatinejad and Sherlock (2008) argued that *P. australis* species could be one of the most important unconventional resources that should be seriously considered worldwide, and particularly in Australia for animal feed. Eldik *et al.* (2017) reported that the high fibre content of Posidonia makes it suitable for use as a substitute for straw in ruminant rations as it can be fermented in the rumen. Furthermore, Calsamiglia *et al.* (2004) demonstrated that the potential digestibility of *P. oceanica* was similar to other fibrous feeds such as cereal straw, confirming its use in ruminant feeds. In a trial carried out on Murciano-Granadina goats, Castillo *et al.* (2015) found that the incorporation of *P. oceanica* in the diet had no negative effects on body weight, milk production and metabolism, but on the contrary, it improved fat content while reducing somatic cell count. In addition, these goats also had a lower risk of oxidative stress. After complete replacement of barley straw by *P. oceanica* in Murciano-Granadina goats' ration, no significant difference was found in the main physico-chemical and sensory parameters of the resulting milk and cheese (Eldik *et al.* 2017). Similarly, Castillo *et al.* (2018) observed no negative effects on dry matter intake, final body weight and metabolic status of adult Merino ewes when 15% of the barley straw was replaced by *P. oceanica*. However, they did notice an improvement in nitrogen utilization.

This work aimed to explore the possibility of using debris from *P. oceanica*, stranded in very large quantities on the Mediterranean costs in dairy goat feed, as a partial or total substitute for oat hay in an attempt to improve fodder availability among small and medium breeders and thus ensure sustainable ruminant production in these regions. To this direction, the study explored the chemical and phytochemical composition of *P. oceanica* debris and explored the effects of its integration in goats' ration on body weight, milk production and composition as well as its organoleptic quality.

Materials and Methods

Posidonia collection and drying

Posidonia oceanica was collected during February, 2020 in Monastir's beach-Tunisia (Google maps coordinates: 35°40'59.4"N 10°52'17.1"E). The collected quantities were taken directly above the waterline (Fig. 1). As soon as they reached the farm of the professional agricultural training center in Monastir Tunisia, where the trial was conducted, *P. oceanica* quantities were properly rinsed with fresh water and cleaned of impurities then spread out on the ground in a large yard for drying during 48 h in direct sunlight (Fig. 2). In order to estimate yield of the final product derived after fresh *P. oceanica* was washed and dried, an amount was weighed before and after the washing and drying steps. Yield was calculated according to the following formula:

$$Yld = \left(\frac{\mathrm{Wwd}}{\mathrm{Wr}}\right) \times 100$$

Y_{ld}: Yield of dry *P. oceanica*
W_{wd}: Weight of washed and dried *P. oceanica*
W_r: Weight of rudimentary *P. oceanica*

Chemical composition

P. oceanica samples were analyzed in triplicate for dry matter and minerals using the AOAC method (2005), and for total nitrogen using the Kjeldahl method (AOAC 1996). Cell wall fractions (NDF, ADF and ADL) were determined in triplicate according to AOAC (2003) procedure.

Phytochemical composition

The extract used for antioxidant compounds determination in fresh goat's milk was produced according to the method described by Li *et al.* (2007) with some modifications. In 60 mL dark glass bottles, 1 mL of fresh milk is added to 10 mL of a normal solution of HCl (1N)/95% ethanol (v/v, 15/85) and stirred at 350 rpm for two hours at 30°C. The resulting mixture was then centrifuged at 7800 × g at 4°C for 20 min. The supernatant was stored in darkness at -20°C until analysis of DPPH radical scavenging activity and total polyphenols content.

Milk biochemical composition was evaluated twice a week during the four-month trial period for total polyphenols, total flavonoids and antioxidant activity by the DPPH scavenging system. All analyses were performed in triplicate.

Total polyphenols contents were determined in triplicate by a colorimetric method using the Folin-Ciocalteu reagent according to the protocol adopted by Agrawal *et al.* (2011) with some modifications. Briefly, to 200 μL of the extract, 1.5 mL of reagent (diluted 10 times) was added and the mixture was allowed to stand for five min at 22°C. Subsequently, 1.5 mL of sodium bicarbonate solution was added and the mixture was incubated for 30 min at 40°C. The optical density was then measured at a wavelength of 765 nm using T60 UV-Visible Spectrophotometer (PG-instruments UK).

Total flavonoids were assessed in triplicate by a colorimetric method described by Zhishen *et al.* (1999). Briefly, a 250 μL dose of the diluted extract was mixed with 1.25 mL of distilled water. At time 0, 75 μL of $NaNO_2$ (5%) was added. After 6 min rest, 150 μL $AlCl_3$ was added. Freshly prepared H_2O (10%) was added to the mixture. Six minutes later, 0.5 mL NaOH (1 *M*) was added. Finally, 250 μL distilled water was added. After mixing, the sample

Fig. 1: *P. oceanica* collection site

Fig. 2: Sun drying of *P. oceanica*

absorbance was measured at 510 nm using T60 UV-Visible Spectrophotometer (PG-instruments UK).

The evaluation of antioxidant activity by the DPPH scavenging system was carried out in triplicate according to the protocol described by Brand *et al.* (1995) with some modifications. Briefly, to prepare the base solution, 40 mg of DPPH powder was dissolved in 100 mL of methanol. One milliliter of each sample extract with 1 mL of DPPH methanolic solution was prepared and stored in darkness for 30 min. Absorbance was measured by a T60 UV-Visible Spectrophotometer (PG-instruments UK) at 517 nm.

DPPH scavenging activity (%) = ((A blank–A sample) / A blank) × 100

P. oceanica tannin content was measured in triplicate using a colorimetric test according to the method described by Julkunen-Tiitto (1985). Briefly, aliquots of crude extract (0.1 – 0.5 mL) and standard solution of (+)-catechin (Sigma–Aldrich Chemicals Co., St. Louis, MO, USA) were placed in screw-capped tubes with 3 mL of 4% (w/v) vanillin (Merck KGaA Germany) in methanol and 1.5 mL of concentrated HCl then vigorously mixed. The absorbance was read at 500 nm using T60 UV-Visible Spectrophotometer (PG-instruments UK), after being left to stand for 20 min at room temperature. Tannin content was calculated in triplicate as catechin equivalent mg/g of dry plant weight, using a catechin calibration curve.

P. oceanica chlorophyll and carotenoids were quantified in triplicate according to the methods described by Lichtenthaler and Buschmann (2001). To 150 mg of fresh *P. oceanica* leaves, 150 mg of $MgCO_3$ and 3 mL of acetone (100%) were added. These ingredients are manually crushed using a pestle. The resulting turbid extract is transferred to a 5 mL graduated centrifuge tube. The grinding device is rinsed with 1.5 mL of additional solvent and this rinsing solution is added to the crude extract. Tubes are then centrifuged for 5 min at 500 × g at 10°C. The supernatant is recovered using a micropipette and its absorbance is measured using T60 UV-Visible Spectrophotometer (PG-instruments UK) at 662 nm and 470 nm respectively for chlorophyll and carotenoids determination.

Mercury content determination

In order to ensure that *P. oceanica* is free of harmful levels of mercury, a quantitative analysis was carried out using the Direct Mercury Analyzer (DMA 80) according to the AOAC (1990) procedure. Samples were analyzed in raw form of *P. oceanica* without any prior chemical treatment.

Goat allocation and rations composition

Twenty-four Alpine goats undergoing their third and fourth kidding were selected from a herd of 47 goats born and bred in Tunisia, with the aim of having the closest initial weight and lactation number. All selected goats were in the first month of lactation. They were randomly divided into three groups of eight animals each and were individually fed. The same amount of commercial concentrate feed (1.7 kg/goat. day^{-1}) was given to all goats, the composition of which is presented in Table 1. Similarly, each type of roughage was fed at a rate of 1.7 kg per goat per day. Concentrate and roughage were presented simultaneously in the same container. Control lot 1 (*P. oceanica*-0%) was given concentrate and oat hay. Lot 2 (*P. oceanica*-50%) received concentrate and a mixture (50/50) of *P. oceanica* and oat hay, whereas Lot 3 (*P. oceanica*-100%) was fed concentrate and *P. oceanica* only. Before initiating the sampling, all animals went through an adaptation period of 18 days. Feed was offered twice a day (8 h and 16 h) with free access to water.

Forage refusals (oat hay/or *P. oceanica*) were weighed daily before the distribution of fresh feed to determine the average daily feed intake. No concentrate refusal was observed during the four-month trial period (February, March, April and May). A weekly fasting weighing of all goats was carried out during the trial period in order to monitor weight changes for each batch. Commercial concentrate and oat hay proximate compositions are in Table 1.

Milk production and composition

Goats were mechanically milked once a day in the morning and at the same time. Individual milk production was evaluated daily by weighing. Individual milk composition was assessed twice a week throughout the four-month trial period. Chemical composition (fat, protein and lactose) and somatic cell count were performed in triplicate using the Milko-Scan FT 120 (Foss-Electric, DK) and the Cell Counter 5000 (Foss-Electric, DK).

Sensory analysis

In order to assess the impact of *P. oceanica* on milk flavor, sensory attributes of milk samples (color, odor, taste and overall appreciation) were evaluated on a 0 (lowest) to 5 (highest) points scale by a volunteer group of 48 unqualified tasters, including professors, students, administrative staff and workers. Fresh milk samples were coded and simultaneously presented to panelists. During evaluation, participants were required to rinse their mouths with mineral water after each milk tasting. Spontaneous attributes were used to describe the perceived differences between milks. Scores for each sample were obtained by averaging 48 panelists' scores.

Statistical analysis

Analysis of variance (ANOVA) was conducted using the general linear model procedure of XLSTAT 2016-0.2, to determine the effect of *P. oceanica* on feed intake, body weight, milk yield, milk composition and sensory quality. Somatic cell counts were normalized by $\log_{10}$ transformation. All statements of significance were based on 5% probability. A significant difference between means was identified by Duncan test.

Results

Chemical and biochemical composition of Posidonia according to its location on shore

Table 2 summarizes changes in *P. oceanica*'s chemical and biochemical composition according to its location on shore. The chemical and biochemical composition of *P. oceanica* was significantly different ($P < 0.05$) between samples collected on the banquette and those immersed in seawater. Compared to the submerged *P. oceanica*, the banquette *P. oceanica* showed the highest rates ($P < 0.05$) in both dry matter (18.54 ± 1.9% *vs.* 17.85 ± 2.0%) and mineral content (30.34 ± 1.2% *vs.* 19.49 ± 1.6%). But submerged *P. oceanica* showed the highest levels ($P < 0.05$) of total nitrogen (4.34 ± 0.09% *vs.* 3.56 ± 0.07%), polyphenols (295.24 ± 3.1 mg EAG/g DM *vs.* 280.53 ± 5.7 mg EAG/g DM), flavonoids (46. 81 ± 1.9 mg EQ/g DM *vs.* 40.99 ± 2.3 mg EQ/g DM), total chlorophyll (2.91 ± 0.02 *vs.* 1.94 ± 0.01 μg/mL) and carotenoids (1.65 ± 0.03 μg/mL *vs.* 1.37 ± 0.02 μg/mL). In addition, antioxidant activity was higher in submerged *P. oceanica* than in banquette *P. oceanica* (22.04 ± 0.18% and 20.3 ± 0.28% respectively). No significant difference was observed between *P. oceanica* in banquettes and submerged *P. oceanica* for cell wall fractions, tannin and mercury levels (Table 2).

Dry *P. oceanica* yield

Following a preliminary step of washing and sun drying for 48 h, the amount of dried plant biomass produced from 50 kg of raw *P. oceanica* was about 12.4 kg, corresponding to a yield of 24.8%.

Ration effect on production parameters

Effect on body weight

Goats' body weight was not significantly affected for all the 3 lots. Average weights were 47.59 ± 7.91 kg, 48.56 ± 8.12 kg and 47.06 ± 6.90 kg respectively for Posidonia-0%, Posidonia 50% and Posidonia 100% lots (Table 3).

Effect on roughage intake

During the three-month trial period, no remaining concentrate was observed. The refusal was only observed in roughage. An important decrease of average daily roughage intake was observed in the Posidonia-100% lot compared to the Posidonia-0% lot (1.35 ± 0.03 *vs.* 1.60 ± 0.07 kg/d) ($P < 0.05$), whilst no significant difference was observed in the average daily roughage intake between the Posidonia-50% lot and the Posidonia-0% lot (1.59 ± 0.05 *vs.* 1.60 ± 0.07 kg/d) (Table 3).

Effect on milk production

Ration type significantly affected goats' milk production in all three lots ($P < 0.05$). The highest average milk production was recorded in goats receiving Posidonia-50% ration (1.79 ± 0.04 kg/d), followed by Posidonia-0% (1.68 ± 0.06 kg/d) and Posidonia-100% (1.64 ± 0.02 kg/d) (Table 3). Total replacement of oat hay with *P. oceanica* in Posidonia-100% lot did not affect milk production when compared to Posidonia-0% control lot (1.64 ± 0.02 kg/d *vs.* 1.68 ± 0.06 kg/d). However, the partial substitution of oat hay with sea grass in Posidonia-50% ration resulted in significant increase in milk production compared to the control lot Posidonia-0% (1.79 ± 0.04 *vs.* 1.68 ± 0.06 kg/d) ($P < 0.05$).

Table 1: Chemical composition of oat hay and commercial concentrate feed (g/kg DM)

	DM (%)	CP	Ash	NDF	ADF	ADL
Concentrate	89 ± 12.5	162 ± 21.4	81.5 ± 10.9	344.4 ± 38.5	195.8 ± 28.7	-
Oat hay	87.1 ± 16.2	47.5 ± 3.4	55.4 ± 12.1	745.8 ± 44.3	427.6 ± 52.7	89.5 ± 27.4

DM = dry matter; CP=crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin

Table 2: Chemical and biochemical composition of *P. oceanica* according to sampling site

	P. oceanica banquette	*P. oceanica* submerged
Dry matter (%)	$18.54^{a} \pm 1.9$	$17.85^{b} \pm 2.0$
Ash (%)	$30.34^{a} \pm 1.2$	$19.49^{b} \pm 1.6$
Crude protein (%)	$3.56^{b} \pm 0.07$	$4.34^{a} \pm 0.09$
NDF (%)	78.74 ± 1.18	79.01 ± 1.22
ADF (%)	48.03 ± 1.74	47.67 ± 1.57
ADL (%)	13.09 ± 0.84	12.84 ± 0.99
Total Polyphenols (mg EAG/g DM)	$280.53^{b} \pm 5.7$	$295.24^{a} \pm 3.1$
Flavonoids (mg EQ/g DM)	$40.99^{b} \pm 2.3$	$46.81^{a} \pm 1.9$
Tannins (mg EC/g DM)	10.12 ± 0.09	9.68 ± 0.04
Total Chlorophyll (μg/mL)	$1.94^{b} \pm 0.01$	$2.91^{a} \pm 0.02$
Carotenoids (μg/mL)	$1.37^{b} \pm 0.02$	$1.65^{a} \pm 0.03$
DPPH (%)	$20.3^{b} \pm 0.28$	$22.04^{a} \pm 0.18$
Mercury content (ppm)	0.0228 ± 0.0014	0.0221 ± 0.0012

[ab] Values assigned with different letters on the same line differ significantly (*P < 0.05*)

Table 3: Mean values of weight, daily roughage intake and daily milk yield

	Weight (kg)	DRI (kg DM)	Milk yield (kg)
Posidonia-0%	47.59 ± 7.91	$1.60^{a} \pm 0.07$	$1.68^{b} \pm 0.06$
Posidonia-50%	48.56 ± 8.12	$1.59^{a} \pm 0.05$	$1.79^{a} \pm 0.04$
Posidonia-100%	47.06 ± 6.90	$1.35^{b} \pm 0.03$	$1.64^{b} \pm 0.02$

DRI = daily roughage intake; DM = dry matter
[a,b] Means assigned with different letters on the same column differ significantly (*P < 0.05*)

Effect on milk composition

Table 4 illustrates the variation in milk composition according to ration. Diet affected milk fat levels significantly (*P < 0.05*). The highest fat content (FC) was obtained by Posidonia-100% ration (5.03 ± 1.20%) followed by Posidonia-50% ration (4.82 ± 0.84%), while the lowest fat content was recorded for the control ration Posidonia-0% (4.36 ± 0.85%). Hence, using *P. oceanica* as partial or total substitute for oat hay significantly improved fat content in goat's milk (*P < 0.05*) but didn't affect milk protein, lactose or freezing point (Table 4).

Effect on milk biochemical composition

Table 5 describes the variation in biochemical composition of goat's milk according to the diet type. The incorporation of *P. oceanica* in Alpine goat's ration significantly (*P < 0.05*) affected milk biochemical composition. The highest levels of flavonoids, total phenols and antioxidant activity were recorded in milk produced by Posidonia-100% ration feed (871.31 ± 49.69 μg/mL, 319.59 ± 18.93 μg/mL and 78.76 ± 0, 40% respectively) followed by milk produced by Posidonia-50% diet (645.33 ± 6.35 μg/mL, 165.62 ± 5.25 μg/mL and 72.06 ± 2.49% respectively) and finally milk from the control diet Posidonia-0% (457.67 ± 5.86 μg/mL, 102.91 ± 13.92 μg/mL and 49.60 ± 0.65% respectively).

Effect on milk somatic cell count

The somatic cell counts (SCC) of milk from goat groups in this experiment are shown in Table 4. Total or partial substitution of oat hay with *P. oceanica* significantly affected milk SCC (*P < 0.05*). Goats receiving Posidonia-100% ration showed the lowest SCC (561 ± 22 cells/mL), followed by those fed Posidonia-50% ration (579 ± 19 cells/mL), while the highest SCC (684 ± 26 cells/mL) was recorded in the control lot Posidonia-0%.

Effect on milk organoleptic quality

Sensory attributes of fresh goat's milk are detailed in Table 6. Integration of Posidonia in Alpine goat diet significantly affected the milk color, odor and taste (*P < 0.05*). The most appreciated milk was the one produced by goats fed *P. oceanica* in their rations. The highest average final score was attributed, first, to the milk obtained by Posidonia-100% ration (4.81 ± 0.9) second, milk produced by Posidonia-50% ration (4.59 ± 0.8) and finally control diet milk, Posidonia-0% (4.14 ± 1.0).

Discussion

The chemical and biochemical composition of *P. oceanica* collected on banquette was significantly different from that

Table 4: Effect of feeding *P. oceanica* on milk composition

Ration type	Fat (g/L)	Protein (g/L)	Lactose (g/L)	FP (°C)	SCC Log_{10}
Posidonia-0%	$43.6^c \pm 1.5$	33.4 ± 1.4	46.3 ± 2.1	$-0{,}536 \pm 0.025$	$5.83^a \pm 0.3$
Posidonia-50%	$48.2^b \pm 1.4$	33.1 ± 1.5	45.9 ± 2.3	$-0{,}534 \pm 0.031$	$5.76^b \pm 0.1$
Posidonia-100%	$50.3^a \pm 1.2$	33.3 ± 1.6	46.1 ± 2.5	$-0{,}538 \pm 0.036$	$5.74^b \pm 0.2$

^{a,b} Means assigned with different letters on the same column differ significantly (*P < 0.05*)
FP= freezing point; SCC = somatic cell count

Table 5: Effect of feeding *P. oceanica* on milk biochemical composition

	Flavonoids (µg EQ/mL)	Phenols (µg EAG/mL)	DPPH (%)
Posidonia-0%	$457.67^c \pm 5.86$	$102.91^c \pm 13.92$	$49.60^c \pm 0.65$
Posidonia-50%	$645.33^b \pm 6.35$	$165.62^b \pm 5.25$	$72.06^b \pm 2.49$
Posidonia-100%	$871.31^a \pm 49.69$	$319.59^a \pm 18.93$	$78.76^a \pm 0.40$

^{a,b,c} Means assigned with different letters in the same column differ significantly (*P < 0.05*)
Ration 1: 100% Oat hay; Ration 2: *P. oceanica* (50%)-Oat hay (50%); Ration 3: 100% *P. oceanica*

Table 6: Average scores for sensory evaluation of goat's milk according to ration type

	Color	Odor	Taste	Final score
Posidonia-0%	$3.00^b \pm 1.2$	$3.32^b \pm 1.4$	$4.19^c \pm 0.9$	$4.14^c \pm 1.0$
Posidonia-50%	$3.63^a \pm 0.9$	$3.39^b \pm 1.2$	$4.66^b \pm 1.0$	$4.59^b \pm 0.8$
Posidonia-100%	$3.70^a \pm 1.1$	$3.95^a \pm 1.6$	$4.94^a \pm 0.7$	$4.81^a \pm 0.9$

^{a,b,c} Means assigned with different letters in the same column differ significantly (*P < 0.05*)

submerged in seawater. Antioxidant activity was higher in submerged than in banked *P. oceanica*, while no significant differences were observed in cell wall fractions, tannin and mercury levels. Similar dry matter values were reported by Castillo *et al.* (2014) and Mateo *et al.* (2003), 16.4 and 19%, respectively, for debris from banquettes, long time exposed to the sun on the shore. Lower mineral contents of around 15.6% were mentioned by Castillo *et al.* (2014). Thelin *et al.* (1982) reported much lower mineral contents ranging between 1 and 4%. Kesraoui *et al.* (2011) found slightly higher values than ours for polyphenols (328 mg EAG/g DM) and flavonoids (44.8 mg EC/g DM). According to Hammami *et al.* (2013), Posidonia is generally rich in secondary metabolites mainly phenolic compounds and flavonoids regardless which collection site was involved. Regarding heavy metals, Ancora *et al.* (2004) showed that mercury, cadmium and lead concentrations measured in different parts of the plant were within the ranges considered to be a low risk for heavy metal contamination.

Partial or total substitution of oat hay by *P. oceanica* didn't affect goats' body weight. Similar results were reported by Castillo *et al.* (2015), who mentioned that the substitution of barley straw by *P. oceanica* did not show any significant effects on Murciano-Granadina goats' body weight. Similarly, Castillo *et al.* (2019) mentioned that dry residues of *P. oceanica* could be used as a source of fiber in animal feed without affecting production or health status. Torbatinejad and Sherlock (2008) stated that *P. australis* mixed with alfalfa can compete with straw in terms of sheep weight gain.

An important decrease of average daily roughage intake was observed in the Posidonia-100% lot compared to the Posidonia-0% lot. This significant decrease can probably be attributed to a difference in palatability between forage types and goat specificity. In this context, Gomes *et al.* (2012) reported that feeding *P. oceanica* resulted in a slight decrease in the dry matter intake due to lower rates of ruminal degradation of this marine plant and to slower rates of passage and digestion, resulting in greater rumen filling. Castillo *et al.* (2015) mentioned that goats preferred straw rather than *P. oceanica* at equal amounts, which is attributed to a selective natural effect for this species against a new feed. Likewise, Abijaoude *et al.* (2003) observed that goats adapt their feeding behavior to the diet they receive and suggested mixing unpalatable rough feeds with concentrate to avoid decrease in feed intake. However, Castillo *et al.* (2015) found that goats were able to consume 450 g/day of dry *P. oceanica* without any adverse effects on milk production. Castillo *et al.* (2018) introduced *P. oceanica* into Merino sheep rations in limited quantities ranging from 75 to 150 g d^{-1} without experiencing any problems. They recommended a maximum incorporation rate of 30% *P. oceanica* to prevent performance alteration, as well as a gradual adaptation period long enough for animals, to accept this marine plant. Leleux (2019) emphasized that palatability of a new feed is difficult to dissociate from selective animal behavior. Therefore, he proposed to introduce *P. oceanica* at a young age to ensure that it is properly accepted by animals.

Total replacement of oat hay with *P. oceanica* in Posidonia-100% lot didn't affect milk production when compared to Posidonia-0% control lot. However, the partial substitution of oat hay with sea grass in Posidonia-50% ration resulted in significant increase in milk production compared to the control lot Posidonia-0%. According to Eldik *et al.* (2017), complete substitution of barley straw by *P. oceanica* showed no significant effect on milk production in Murciano-Granadina goats. Similar results were reported by Castillo *et al.* (2015, 2019), demonstrating that Murciano-Granadina goats can be fed dried *P. oceanica* as a source of fiber at levels up to 450 g/d without affecting milk production.

Using *P. oceanica* as partial or total substitute for oat hay significantly improved fat content in goat's milk. Similar results were reported by Castillo *et al.* (2015, 2019) where higher levels of fat were observed in the milk of Murciano-Granadina goats that were fed diets containing 50 and 100% *P. oceanica* compared to the control lot receiving only barley straw as a source of fiber. They attributed the increased fat content in milk to high levels of flavonoids characterizing the sea grass *P. oceanica*, which did not only improve milk fat quantity but also its quality. According to Vázquez-Añón *et al.* (2008) the richness in antioxidant agents in certain plants can contribute to lipid metabolism improvement and thus increase fat production. However, by substituting all the barley straw with *P. oceanica* in Murciano-Granadina goats' rations, Eldik *et al.* (2017) found no significant variation in milk's fat content.

Milk protein and lactose levels as well as freezing point were not affected by incorporating *P. oceanica* into goats' diets. Castillo *et al.* (2015) observed similar results in milk from goats receiving diets containing 50 and 100% *P. oceanica* as substitute for barley straw, compared to the control lot. However, Eldik *et al.* (2017) reported a significant increase in protein content yet a significant decrease in lactose content in goat's milk when completely substituting barley straw with *P. oceanica*.

The highest levels of flavonoids, total phenols and antioxidant activity were recorded in milk produced by goats fed Posidonia-100% ration. Therefore, this milk might have an added value as a source of antioxidants for human nutrition. These results are confirmed by Castillo *et al.* (2019) who attributed milk's high level of antioxidant agents to *P. oceanica*'s wealth of these elements, secreted in the milk of animals that consumed this marine plant and gave it a very high dietary added value. Hilario *et al.* (2010) proved that goats fed polyphenol-rich pasture grass, had a significant increase in bioactive polyphenolic compounds in their milk. Feo *et al.* (2006) attributed antioxidant richness of goat's milk to the consumption of fodder with high levels of flavonoids, such as quercetin and rutin. According to Castellano *et al*. (2012) high content of flavonoids and polyphenols in plants is closely related to high concentration of antioxidant agents that can be found in animal products.

Total or partial substitution of oat hay with *P. oceanica* significantly reduced milk SCC. Goats receiving Posidonia-100% ration showed the lowest SCC. Indeed, it appears that incorporation of this seagrass as an alternative fiber source to oat hay improved goat mammary health status. Similar findings were reported by Castillo *et al.* (2015, 2019) who observed significant decreases in SCC in Murciano-Granadina goats fed *P. oceanica*. These authors explained this phenomenon by the high content of secondary metabolites in *P. oceanica*, mainly phenolic compounds and flavonoids, known for their defensive and antioxidant properties, which can significantly reduce the occurrence of udder infections. In addition to its richness in antioxidant agents, *P. oceanica* contains antibacterial agents proved to be very active against Gram+, Gram-, dermatophytes and yeast that can be exploited to fight infections (Haznedaroglu and Zeybek 2007; Abdelmohsen *et al.* 2016). Castillo *et al.* (2017) confirmed that the incorporation of such natural products rich in antioxidant and antibacterial agents in livestock rations could reduce the use of antibiotics.

Using *P. oceanica* in Alpine goats feed as a partial or total substitute for oat hay significantly improved milk organoleptic quality. Eldik *et al.* (2017) reported that the use of *P. oceanica* as a substitute for barley straw in feeding Murciano-Granadina goats, allowed the production of milk with the same sensory characteristics as the one produced by the control diet. However, this milk was distinguished by a less intense goat smell, a milder and more flavorful taste, but without any significant difference from the control milk. A sensory study conducted by Sotillo *et al.* (2015) on Posidonia-fed Murciano-Granadina goat's milk and cheese showed no significant effect on milk organoleptic quality but nevertheless significantly improved cheese taste.

Conclusion

This study revealed that it is possible to safely exploit debris from a marine plant, stranded in very large amounts onto Mediterranean beaches, in dairy goats' feed as a partial or total substitute for oat hay. In addition to improving the availability of fodder reserves among small and medium breeders, the advantage of incorporating *P. oceanica* lies in improving milk yield and animal health status, leading to lower production costs and higher profit margins, especially since the produced milk is richer in fats and bioactive compounds. This makes it possible to attribute an added value to this product while giving it a particular specificity that can increase its marketability.

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Author Contributions

Yasser Hachana proposed the topic, designed the experimental protocol, performed data analysis and results interpretation, and wrote the article. Amal Jebbari carried out experimental work, laboratory analyses and participated in data interpretation. Habib El Mejdoub contributed to experimental herd selection, trial monitoring and data interpretation. Wafa Yousfi participated in data interpretation and article revision. Riccardo Fortina participated in data organization and interpretation, layout and review of the final document. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data presented in this study are available on request from the corresponding author.

Ethics Approval

Not applicable in this paper

References

Abdelmohsen M, HD Hassanein, RA Hassan, AC Abreu, M Simões (2016). Phytochemical analysis, *in vitro* evaluation of antioxidant and antimicrobial activities of phenolic extracts from *Posidonia oceanica* (L.) Delile leaves. *J Chem Pharm Res* 8:449–457

Abijaoude JA, P Morand-Fehr, J Tessier, P Schmidely, D Sauvant (2003). Diet effect on the daily feeding behavior, frequency and characteristics of meals in dairy goats. *Livest Prod Sci* 64:29–37

Agrawal S, GT Kulkarni, VN Sharma (2011). A comparative study of the antioxidant activity of methanolic extracts of *Terminalia paniculata* and *Madhuca longifolia*. *Free Rad Antiox* 1:62–68

Almeida AM, LA Cardoso (2008). Animal production and genetic resources in Guinea Bissau, I–Northern Cacheu Province. *Trop Anim Health Prod* 40:529–536

Ambrosio LD, E Segovia (2000). *Las praderas de Posidonia, importancia y conservación*, WWF/Adena, Madrid

Ancora S, N Bianchi, A Butini, MC Buia, MC Gambi, C Leonzio (2004). *Posidonia oceanica* as a biomonitor of trace elements in the gulf of Naples, temporal trends by lepidochronology. *Environ Toxicol Chem* 23:1093–1099

Augier H, H Calvert, E Wollaston, M Santimone (1982). A comparison of the c, h, n, protein and amino acid composition of *Posidonia australis* with that of *Posidonia oceanica* (L.) Delile and several other marine phanerogams. *Aquat Bot* 12:69–80

AOAC (2005). Water/dry matter (Moisture) in animal feed, Gr

AOAC (2003). Fiber contents in feed (NDF, ADF and ADL)

AOAC (1996). 954.01-1954. Protein (Crude) in animal feed and pet f

Boudouresque CF, G Bernard, P Bonhomme, E Charbonnel, G Diviacco, A Meinesz, G Pergent, C Pergent-Martini, S Ruitton, L Tunesi (2006). *Préservation et conservation des herbiers à Posidonia oceanica*. RAMOGEBrand W, ME Cuvelier, C Berset (1995). Use of free radical method to evaluate antioxidant activity. *Food Sci Technol* 28:25–30

Calsamiglia S, A Ferret, A Bach (2004). *Tablas FEDNA de valor nutritivo de Forrajes y Subproductos fibrosos húmedos*, pp:105-109. Fundacion para el Desarrollo de la Nutricion Animal, Madrid, Spain

Castaldi P, P Melis (2002). Composting of Postdonia oceanica and its use in agriculture. *In: Microbiology of Composting*, pp: 425–434. Insam H, N Riddech, S Klammer (Eds). Springer-Verlag, Berlin

Castellano G, J Tena, F Torrens (2012). Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia oceanica* (L.) Delile. *Environment* 67:231–250

Castillo C, J Benedito, J Hernández (2019). Use of seagrasses as natural forage source for small ruminants, the example of the marine plant *Posidonia oceanica*. *Mod Conc Dev Agron* 3:1–4

Castillo C, J Hernández, J Sotillo, C Gutiérrez, AM Montes (2018). Effects of *Posidonia oceanica* banquettes on intake, digestibility, nitrogen balance and metabolic profiles in sheep. *J Sci Food Agric* 98:2658–2664

Castillo C, J Hernández, JS Mesanza, C Gutiérrez, AM Montes, AR Mantecónc (2017). Effects of *Posidonia oceanica* banquettes on intake, digestibility, nitrogen balance and metabolic profiles in sheep. *Adv Anim Sci Zool* 7:43–64

Castillo C, AR Mantecón, J Sotillo, C Gutiérrez, A Abuelo, J Hernández (2015). *Posidonia oceanica* banquettes as a substitute for straw in dairy goat rations, Metabolic and productive effects. *J Sci Food Agric* 96:602–609

Castillo C, A Mantecón, J Sotillo, J Benedito, A Abuelo, C Gutiérrez, J Hernández (2014). The use of banquettes of *Posidonia oceanica* as a source of fiber and minerals in ruminant nutrition. An observational study. *Animal* 8:1663–1666

Cocozza C, A Parente, C Zaccone, C Mininni, P Santamaria, T Miano (2011). Chemical, physical and spectroscopic characterization of *Posidonia oceanica* (L.) delile. residues and their possible recycle. *Biomass Bioener* 35:799–807

Dural MU, L Cavas, SK Papageorgiou, FK Katsaros (2011). Methylene blue absorption on activated carbon prepared from *Posidonia oceanica* (L.) dead leaves, kinetics and equilibrium studies. *Chem Eng J* 168:77–85

Eldik AV, MI Hortelano, D Álvarez, J Sotillo, C Gutiérrez, V Garcia, M Bacchiocchi, BL López (2017). Influence of feeding Murciano-Granadina Goats with *Posidonia oceanica* banquettes on the resulting milk and cheese. *J Food Nutr Res* 5:54–62

Falco GD, S Simeone, M Baroli (2008). Management of Beach-Cast Posidonia oceanica Seagrass on the lsland of Sardinia (ltaly, Western Mediterranean). *J Coast Res* 4:69–75

Feo VD, E Quaranta, V Fedele, S Claps, R Rubino, C Pizza (2006). Flavonoids and terpenoids in goat milk in relation to forage intake. *Ital J Food Sci* 1:85–92

Gobert S, ML Cambridge, B Velimirov, G Pergent, CP Martini, DI Walker (2006). Biology of Posidonia. *In: Biology, Ecology and Conservation Netherland Springer,* pp: 387–408. Anthony RJ, WD Larkum (Eds). Seagrasses, Netherland

Gomes LM, A Nunes de Medeiroso, AM Vieira, G Garcia, D Araújo, AC Chacon, JL de Albuquerque, A Rodrigues (2012). Growth performance of native goats fed diets containing different levels of energy. *Rev Bras Zoot* 41:675–682

Hammami S, AB Salem, ML Ashour, J Cheriaa, G Graziano, Z Mighri (2013). A novel methylated sesquiterpene from seagrass *Posidonia oceanica* (L.) Delile. *Nat Prod Res* 27:1265–1270

Haznedaroglu MZ, U Zeybek (2007). HPLC determination of chicoric acid in leaves of *Posidonia oceanica*. *Pharm Biol* 45:745–748

Hilario MC, CD Puga, AN Ocana, FPG Romo (2010). Antioxidant activity, bioactive polyphenols in Mexican goats' milk cheeses on summer grazing. *J Dairy Res* 77:20–26

Julkunen-Tiitto R (1985). Phenolic constituents in the leaves of Northern Willows, methods for the analysis of certain phenolics. *J Agric Food Chem* 33:213–217

Kesraoui O, MN Marwouki, T Maugard, F Limam (2011). *In vitro* evaluation of antioxidant activities of free and bound phenolic compounds from *Posidonia oceanica*. *Afr J Biotechnol* 10:3176–3185

Leleux P (2019). *Étude de l'impact d'une modification de l'alimentation des chèvres laitières de la ferme de la Baillerie (Belgique) dans une optique d'autonomie alimentaire*. Faculté des bio-ingénieurs, Université catholique de Louvain

Li W, MD Pickard, T Beta (2007). Effect of thermal processing on antioxidant properties of purple wheat bran. *Food Chem* 104:1080–1086

Lichtenthaler HK, C Buschmann (2001). Chlorophylls and carotenoids, measurement and characterization by UV-VIS Spectroscopy, pp: 431–438. Current Protocols in Food Analytical Chemistry, Wiley, UK

Mateo MA, JL Sanchez-Lizaso, J Romero (2003). *Posidonia oceanica* banquettes, a preliminary assessment of the relevance for meadow carbon and nutrients budget. *Estuar Coast Shelf Sci* 56:85–90

Sotillo J, C Gutiérrez, AM Montes, J Hernández, C Castillo, A Abuelo, AR Mantecón, BO López, MD Ruiz, V García (2015). Influence of feeding Murciano-Granadina goats with *Posidonia oceanica* banquettes in milk. *In: Proceedings of of XXII International Congress FMESPRUM, Sassary, Italy*

Thelin I, AL Bedhomme, CF Boudouresque, AJ De Grissac (1982). *Sur la calcification des feuilles de Posidonia oceanica (LINNAEUS) Delile*, pp:27–32. Thalasso-graphica

Torbatinejad N, RG Sherlock (2008). Comparison of feeding value of a treated sea plant, *Posidonia australis*, with lucerne, pasture and wheat. *Intl J Plant Prod* 2:47–56

Torbatinejad N, G Annison, K Rutherfurd-Markwick, JR Sabine (2007). Structural constituents of the seagrass *Posidonia australis*. *J Agric Food Chem* 55:4021–4026

Vázquez-Añón J, G Bowman, T Hampton, C Atwell, P Vazquez, T Jenkins (2008). Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. *J Dairy Sci* 90:4361–4367

Zhishen J, T Mengcheng, W Jianming (1999). The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559