Research Article

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Effects of *Posidonia oceanica* banquettes on intake, digestibility, nitrogen balance and metabolic profiles in sheep

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Abstract

BACKGROUND: The marine plant *Posidonia oceanica* (L.) (PO) has been demonstrated in goats to be a source of fibre. The aim of the present study was to assess the effects of introducing this marine plant as a substitute for barley straw in the feed of mature ewes, assessing the effects of its addition on intake, digestibility and ruminal fermentation and on the ewes' metabolic profiles (energy and protein). PO was used at 75 g day^{-1} per ewe (15% of the total forage), 150 g day^{-1} per ewe (30% of the total forage) and 300 g day^{-1} per ewe (60% of the total forage).

RESULTS: Substitution of 15% of the forage with PO has no negative consequences on dry matter intake, final live weight and metabolic status in mature ewes; in addition, PO may improve the animal's nitrogen utilisation. The upper limit of substitution was 30%, where only few changes were noted without metabolic consequences. Substitution of 60% impaired performance and affects tissue functions in the animal's body.

CONCLUSION: Moderate quantities of barley straw (between 75 and 150 g day^{-1} per ewe) can be replaced by PO in feed rations for mature ewes.

Keywords: ewes; *Posidonia oceanica*; forage; metabolites; nutrition

INTRODUCTION

In the Mediterranean coasts, natural pasture and crop residues such as barley straw during the long dry season or other residues are the main nutrient fonts in small ruminant production. In the case of forage, the straw is increasingly being used to obtain biofuel,[1] contributing to the scarcity of this raw material or increasing its price, which is closely connected with farm productivity. In the search for a sustainable livestock system that balances natural resources and economic benefits, considerable efforts have been made to find new sources such as marine plants.

Posidonia oceanica (L. Delile) (PO) is a marine flowering plant widely distributed along Mediterranean coastlines. It forms extensive meadows that are highly important to the environmental conservation of several Mediterranean regions. The plant has an annual growth cycle characterised by the development, growth and loss of leaves. When these leaves cease to perform photosynthesis, they lose their original green colour and acquire a brown colour until they break down and are carried to the coastline, appearing as banquettes. These banquettes are currently considered a nuisance by citizens and bathers. Removal of tons of them is a common practice on Mediterranean shores to allow for the recreational use of beaches, with great economic cost to the Councils involved.[2,3]

In recent studies, we have demonstrated that these banquettes could play a role as a fibre source substituting for barley straw in the feed of goats, which could help to optimise production costs without detrimental effects on milk production and health.[4]

Based on our previous results on the chemical composition of *P. oceanica*,[5] the main objective of the present study is to assess the effects of introducing this marine plant as a substitute for barley straw in the feed of ewes, first assessing the effects of its addition on intake, digestibility and ruminal fermentation and secondly assessing these effects on metabolic profiles (energy and protein balances).

METHODS

The animals used in these experiments were managed according to protocols approved by the University of León Institutional Care

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and Use Committee and according to Spanish regulations under EU Directive 2010/63/EU for animal experiments.

Plant material

The collection and management of the dried marine plant residues that were used as feedstuff was fully described in a previous report,[4] as well as the chemical analysis of the product.[5] Briefly, after permission for sampling from the local government, samples of banquettes of *P. oceanica* from six different points of the selected area were randomly collected on the same day directly from the beach above the water line, washed in a warehouse with distilled water and sun dried for 48 h. Approximately 500 g of each sample of plant material was chopped and two subsamples of 200 g each were placed in airtight plastic containers and sent to the laboratory for mineral and chemical analysis. The chemical analyses show that the mean dry matter (DM) content of *P. oceanica* was 16.4% and the values of other components (based on DM values) were 155 g kg^{-1} DM of ash, 42 g kg^{-1} DM of crude protein (CP), 13 g kg^{-1} DM of ether extract (EE), 760 g kg^{-1} DM of neutral detergent fibre (NDF), 533 g kg^{-1} DM of acid detergent fibre (ADF) and 116 g kg^{-1} DM of acid detergent lignin (ADL).

Treatments and design

In this experiment, sheep were used to examine effects of PO banquettes on feed intake, digestibility and urinary excretion of nitrogen (N). With this purpose, 40 mature Merino ewes (63.7 ± 1.6 kg) were housed in individual pens and fed *ad libitum* a total mixed ration (TMR) consisting of (g kg^{-1} DM) barley straw (500 g), barley flour (150 g), rapeseed (100 g), maize flour (100 g), sunflower meal (50 g), corn distillers dried grains with solubles (DDGS, 30 g), palm oil (20 g), molasses (15 g) and vitamin mineral premix (35 g). The ration was prepared in a single batch and was consumed by all animals during an adaptation period of 7 days. After this period, ewes were randomly distributed into four treatment groups of ten each. The control group (CTRL) was fed the previous TMR, while each of the three experimental groups was fed an altered ration in which the barley straw was substituted by the marine plant in a different proportion: (1) 15%: 425 g of straw and 75 g of PO (PO-15% group), (2) 30%: 350 g of straw and 150 g of PO (PO-30% group) and (3) 60%: 200 g of straw and 300 g of PO (PO-60% group). Food was distributed to animals twice daily at approximately 09:00 and 18:00 as two equal meals. Fresh water and food were always available.

The chemical composition of each ration is shown in Table 1. The ash and N contents were analysed in *P. oceanica* and barley straw following AOAC methods 942.05 and 984.13 respectively.[6] NDF was analysed following the procedure of Van Soest.[7] Samples were weighed in F57 Ankom filter bags (Ankom Technology Corp., Macedon, NY, USA) and washed at 100 °C for 1 h with neutral detergent in an Ankom fibre analyser. Sodium sulfite and a heat-stable amylase were used in the analysis of NDF, which was expressed inclusive of residual ash. ADF and ADL were determined in the bags containing residual NDF in an Ankom fibre analyser according to AOAC method 973.18.[6] The determination of total energy in the ration (gross energy) was obtained from the complete combustion of organic materials using bomb calorimetry.[8]

Those parameters connected to intake, feed selection indices, nutrient digestibility and N balance were monitored daily and divided into three stages after an adaptation period of 4 days to the new rations: (1) from day 4 to day 9 (sampling 1), from day 10 to day 16 (sampling 2) and from day 17 to day 23 (sampling 3). A similar schedule was used for blood venipuncture, as will be described later.

Table 1. Ingredients (g kg^{-1} DM) and energy content of rations

Item	Groups[a]			
	CTRL	PO-15%	PO-30%	PO-60%
Dry matter (DM)	983.5	982.1	985.9	981.2
Crude protein (CP)	91.4	90.2	83.6	65.8
Neutral detergent fibre (NDF)	506.4	487.5	494.5	521.8
Acid detergent fibre (ADF)	288.2	290.8	317.1	357.1
Acid detergent lignin (ADL)	32.7	37.0	48.1	62.8
Ash	73.7	97.2	170.1	192.1
Gross energy (MJ kg^{-1} DM)	17552.17	16953.15	15610.08	14962.40

[a] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.

The intake trial was monitored by weighing the ration offered daily. Daily orts were preserved and a pooled sample by group among the different samplings was created at the end of the experiment for DM intake averages, nutrient intake parameters and preference or feed selection indices (proportion of consumed/available) per group. This parameter was estimated for the different rations with the assumption that the time spent on a treatment reflects the proportion of *P. oceanica* in the ration and the time spent feeding it, according to previous studies that evaluate the addition of different forage sources. The calculation was done as follows: feed selection index = proportion of ration consumed/proportion of ration offered = time spent feeding on the ration. Thus increasing the percentage of PO added to the ration will increase the average feeding time.[9,10]

Afterwards, the three heaviest sheep of each group were placed in metabolic cages at room temperature with *ad libitum* access to water and monitored on the same rations during the whole experiment. Orts were weighed and stored; a pooled sample was collected (per group and sampling period) for chemical analysis of organic matter (OM), CP, NDF, ADF, the amount of N associated with ADF (N-ADF) and ADL in order to gather information about digestibility. Cages were used for physical separation of urine, and total outputs were collected from each group during the three samplings. Urine was collected in buckets where a known quantity (50 mL day^{-1}) of 1 mol L^{-1} sulfuric acid had been added. Urine was collected twice daily and filtered through glass fibre. Daily urine output was weighed and a sample of 0.1 g g^{-1} total output was frozen according to the method described by López *et al.*[11] At the end of the experiment, a pooled sample for each sheep among samplings was collected for analysis of N balance. Finally, the sheep were weighed three times: at the beginning of the experiment, when they were moved to the metabolic cages and at the end of the experiment.

Serum parameters

Blood samples were collected by jugular venipuncture (Vacutainer® tubes without EDTA) on days 0, 7, 14 and 21 between 08:30 and 09:00, corresponding to baseline values prior to the addition of PO, and between samplings 1, 2 and 3 of the ruminal trials. Blood samples were centrifuged (2000 × *g*, 20 min) and the serum was immediately frozen (−20 °C) for subsequent analysis. Serum glucose, β-hydroxybutyrate (BHB), serum urea nitrogen (SUN), creatinine, total serum proteins (TSP), aspartate

Table 2. Mean values (mean ± standard error of mean) of feed and nutrient intake in four groups during study period

Item[a]	Groups[b] CTRL[a]	PO-15%	PO-30%	PO-60%	SL[c]
Dry matter intake (g day^{-1})					
Days 4–9	1615 ± 89c	1376 ± 125bc	1082 ± 26b	398 ± 45a	<0.001
Days 10–16	1449 ± 142b	1518 ± 105b	1217 ± 60b	417 ± 51a	<0.001
Days 17–23	1240 ± 165b	1327 ± 90b	1105 ± 81b	436 ± 36a	<0.001
Nutrient intake (g day^{-1})					
OM	1267 ± 73c	1250 ± 79c	945 ± 42b	331 ± 36a	<0.001
CP	138 ± 8c	140 ± 9c	106 ± 4b	31 ± 3a	<0.001
NDF	633 ± 40b	628 ± 40b	533 ± 31b	189 ± 26a	<0.001
ADF	353 ± 23b	369 ± 24b	334 ± 19b	125 ± 18a	<0.001
N-ADF	87 ± 3d	63 ± 4c	22 ± 2b	7 ± 3a	<0.001
ADL	3a	4a	8b	3a	<0.001

[a] OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; N-ADF, amount of ADF associated with N; ADL, acid detergent lignin.
[b] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[c] SL, significance level; means in a row with different letters differ significantly ($P < 0.05$).

Table 3. Feed selection indices (mean ± standard error of mean) in different groups

Item[a]	Groups[b] CTRL	PO-15%	PO-30%	PO-60%	SL[c]
CP	1.1 ± 0.01a	1.12 ± 0.01a	1.11 ± 0.01a	1.19 ± 0.03b	0.011
N-ADF	1.27 ± 0.04c	1.04 ± 0.02c	0.71 ± 0.05b	0.27 ± 0.19a	< 0.001
NDF	0.91 ± 0.01ab	0.93 ± 0.004b	0.94 ± 0.01b	0.88 ± 0.02a	0.042
ADF	0.89 ± 0.01ab	0.92 ± 0.004b	0.92 ± 0.01b	0.84 ± 0.03a	0.033
ADL	0.07 ± 0.002a	0.09 ± 0.005a	0.15 ± 0.006b	0.1 ± 0.03a	0.011

[a] CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; N-ADF, amount of ADF associated with N; ADL, acid detergent lignin.
[b] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[c] SL, significance level; means in a row with different letters differ significantly ($P < 0.05$).

aminotransferase (AST) and γ-glutamyl transferase (GGT) were measured using standardised diagnostic kits from RAL (RAL Técnica para el Laboratorio SA, Barcelona, Spain). Non-esterified fatty acids (NEFA) were assayed using a kit from Wako Chemicals GmbH (Neuss, Germany). In all cases, appropriate controls were used. Physiological and pathological control sera were analysed alongside the samples for two-point quality control.

Statistical procedures

All variables measured in relation to intake, selection indices, weight, digestibility or N balance were subjected to one-way analysis of variance (ANOVA) according to the statistical model

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} represents each individual observation, μ is the overall mean, T_i is the effect of treatment (i = CTRL, PO-15%, PO-30% and PO-60%) and ε_{ij} is error. The level of statistical significance was established using Dunnett's test ($P < 0{\cdot}05$).

On the other hand, blood data were tested for normal distribution using the Shapiro–Wilk test and subjected to ANOVA with 'group' as the fixed main effect and 'sampling date' as a repeated measure effect; thus the model considered the effects of treatment (TR), time (T) and the T × TR interaction. Bonferroni corrections were included for *post hoc* analysis. Significant differences were declared at $P < 0{\cdot}05$.

RESULTS

In vivo trials

Table 2 presents the dry matter intake (DMI) and nutrient intake in the four periods in those ewes that remained in the experimental pens.

In relation to DMI, those ewes fed 15% of PO in their ration were statistically similar to CTRL, in contrast to PO-30% and PO-60%, during the first days (days 4–9). Nevertheless, after this period, PO-15% and PO-30% showed similar values to CTRL over time. On the contrary, the administration of 60% of the marine plant clearly reduced intake from beginning to end of the experiment, suggesting a lack of adaptation to this new diet throughout the study period.

From a nutritive point of view, no differences were observed between CTRL and PO-15% ewes, except the amount of N-ADF, which was lower in the experimental group. The addition of 30% of PO significantly affected OM, CP, N-ADF and ADL; this proportion of the marine plant gives less OM and

Table 4. Effects of feeding different proportions of dry leaves of *Posidonia oceanica* on body weight, body condition score (BCS) and average daily gain (ADG) (mean ± standard error of mean)

	Groups[a]				
Item	CTRL	PO-15%	PO-30%	PO-60%	SL[b]
Initial weight (kg)	63.6 ± 1.5	64.4 ± 1.6	64 ± 1.5	63.4 ± 1.6	0.974
Initial BCS (1–5)	2.69 ± 0.21	2.75 ± 0.18	2.38 ± 0.25	2.53 ± 0.31	0.711
ADG (g day^{-1})	159 ± 23c	100 ± 8c	39 ± 4b	−211 ± 31a	< 0.001
Final weight (kg)	67.2 ± 0.5c	67 ± 0.2c	64.5 ± 0.1b	58.5 ± 0.7a	< 0.001

[a] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[b] SL, significance level; means in a row with different letters differ significantly ($P < 0.05$).

Table 5. Nutrient digestibility (%) coefficients (mean ± standard error of mean) for ewes fed different diets in metabolic cages

	Groups[b]				
Item[a]	CTRL	PO-15%	PO-30%	PO-60%	SL[c]
DM	67.8 ± 1.55d	60.2 ± 0.74c	52.5 ± 0.99b	41.2 ± 4.12a	<0.001
OM	69.3 ± 1.54c	63.2 ± 0.76b	54.2 ± 0.96a	50.0 ± 2.90a	<0.001
CP	65.3 ± 2.76c	63.8 ± 1.36c	58.0 ± 1.43b	47.5 ± 2.27a	<0.001
NDF	56.9 ± 1.99c	47.9 ± 1.22b	45.6 ± 0.62ab	38.7 ± 4.41a	<0.001
ADF	53.5 ± 1.93c	41.7 ± 1.21b	41.7 ± 0.76b	32.1 ± 5.07a	<0.001

[a] DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre.
[b] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[c] SL, significance level; means in a row with different letters differ significantly ($P < 0.05$).

proteins and a greater amount of indigestible fibre (due to the ADL content).

Those parameters connected to the preference indices are shown in Table 3.

Chemical composition played a great role in diet selection in our study. Taking into account our results, no differences were found between CTRL and PO-15%, indicating that both groups ate their respective rations equally well. A similar observation was made in PO-30%, except the values of ADL and N-ADF. Finally, the PO-60% group behaved differently in comparison with the others, showing a significant increase in CP (mainly associated with the consumption of concentrate and the lowest preference for forage). Certainly, the results obtained in this group should be taken with caution, because these animals never fed a real 60% treatment.

The initial and final weights and ADG for the ewes in the metabolic cages are shown in Table 4.

The results in this trial closely correspond with the above-mentioned findings. The addition of any quantity of the marine plant above 15% seems to lead to a lesser weight gain, as can be seen in those animals included in the PO-30% and PO-60% groups. Indeed, CTRL and PO-15% finished the study with statistically similar ADG and final weights. PO-30% constitutes an intermediate group between PO-15% and PO-60%, suggesting that 30% should be the maximum level for PO as a forage source for ewes.

Table 5 exposes the digestibility coefficients in the caged groups. As expected, the best results were obtained for CTRL ewes that were fed the traditional ration. For PO-15%, significant lower digestibilities were observed for DM, OM, NDF and ADF, but not for CP. It is evident that the addition of the marine plant decreased the digestibility of the ration as the proportion of PO increased.

Finally, Table 6 shows those values connected with N balance. The statistical analysis of the results showed the lack of differences between CTRL and PO-15%; PO-15%, however, showed a numerically higher value, with lower N retention in PO-30%. The balances between N retention and digestion (NR/ND) and between N retention and ingestion (NR/NI) show that those ewes that were fed 15 or 30% of the marine plant were statistically similar to CTRL.

Serum parameters

Table 7 represents the evolution of those parameters related to the energy metabolism of the animal and enzymes during the study period. Glucose concentrations fluctuated throughout the experiment in the four groups, although without significant relevance in CTRL and PO-15% ewes. In PO-30%, a significant decrease was observed in the second sampling (day 7), with a recovery in their concentrations after this period, when they became statistically similar to CTRL and PO-15%. These findings were the consequence of the decrease in DMI in the same stage. Finally, the group that received 60% of the marine plant as a forage source was different throughout the study in parallel to their lowest DMI, upholding the idea that 60% is not suitable to apply in ewe's ration.

NEFA values were steady in the CTRL group during the entire experiment, suggesting a sufficient energy intake of the designed TMR. In the PO-15% group, a significant increase occurred on day 7, in parallel with a decrease in the DMI, followed by a later recovery and final similar values to the CTRL ewes.

The BHB concentrations were in line with the findings described for glucose and NEFA parameters. It is clear that the first days of adaptation were crucial from a metabolic point of view, with a significant increase in those groups that were fed the marine plant. Nevertheless, after day 7, the BHB values were statistically

Table 6. Mean values (mean ± standard error of mean) of N retention, balance between N retention and digested N (NR/ND) and balance between N retention and ingested N (NR/NI)

Item	Groups[a]				SL[b]
	CTRL	PO-15%	PO-30%	PO-60%	
N retention (g day^{-1})	7.62 ± 0.60c	9.07 ± 0.75c	5.72 ± 0.65b	0.67 ± 0.36a	<0.001
NR/ND (%)	53.57 ± 3.63b	64.47 ± 4.65b	58.34 ± 6.15b	26.49 ± 15.07a	0.027
NR/NI (%)	34.72 ± 2.44b	40.91 ± 2.69b	33.99 ± 3.79b	11.55 ± 7.2a	0.001

[a] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[b] SL, significance level; means in a row with different letters differ significantly ($P < 0.05$).

Table 7. Mean values (mean ± standard deviation of mean) of serum glucose, NEFA and BHB concentrations and AST and GGT enzymes

Item[a]	Sampling	Groups[b]				*P* value[c]		
		CTRL	PO-15%	PO-30%	PO-60%	T	TR	T × TR
Glucose (mmol L^{-1})	Day 0	3.48 ± 0.26a	3.85 ± 0.30a	3.66 ± 0.21a	3.67 ± 0.34a	0.001	<0.001	0.002
	Day 7	3.81 ± 0.27a	3.47 ± 0.15a	3.30 ± 0.34b	3.12 ± 0.44b			
	Day 14	3.64 ± 0.19a	3.39 ± 0.38a	3.52 ± 0.12a	3.07 ± 0.24b			
	Day 21	3.61 ± 0.16a	3.36 ± 0.44a	3.54 ± 0.47a	3.12 ± 0.22b			
NEFA (mmol L^{-1})	Day 0	0.25 ± 0.08a	0.24 ± 0.14a	0.31 ± 0.10a	0.31 ± 0.16a	<0.001	<0.001	<0.001
	Day 7	0.20 ± 0.17a	1.11 ± 0.34b	1.70 ± 0.49b	1.95 ± 0.67b			
	Day 14	0.16 ± 0.07a	0.38 ± 0.19a	0.65 ± 0.23b	1.43 ± 0.61b			
	Day 21	0.22 ± 0.20a	0.20 ± 0.11a	0.60 ± 0.21b	1.36 ± 0.27b			
BHB (mmol L^{-1})	Day 0	0.26 ± 0.06a	0.27 ± 0.05a	0.31 ± 0.05a	0.26 ± 0.03a	<0.001	<0.001	<0.001
	Day 7	0.27 ± 0.08a	0.43 ± 0.04b	0.62 ± 0.06b	0.90 ± 0.10b			
	Day 14	0.31 ± 0.09a	0.27 ± 0.04a	0.29 ± 0.04a	0.62 ± 0.10b			
	Day 21	0.34 ± 0.11a	0.26 ± 0.03a	0.33 ± 0.07a	0.62 ± 0.02b			
AST (IU L^{-1})	Day 0	104.8 ± 17.0	100.7 ± 14.3	103.8 ± 27.5	88.2 ± 15.1	0.492	<0.001	0.153
	Day 7	106.3 ± 12.8	89.3 ± 12.2	88.8 ± 16.1	93.9 ± 18.5			
	Day 14	100.1 ± 12.3	90.9 ± 16.7	97.8 ± 20.3	81.3 ± 16.6			
	Day 21	121.1 ± 25.7	87.3 ± 22.4	96.5 ± 20.7	75.7 ± 12.7			
GGT (IU L^{-1})	Day 0	47.1 ± 8.1	59.9 ± 16.7	53.2 ± 15.6	66.0 ± 6.3	0.605	<0.001	0.100
	Day 7	51.0 ± 10.5	61.9 ± 3.8	48.5 ± 8.2	53.5 ± 3.8			
	Day 14	51.5 ± 11.3	63.1 ± 4.9	57.8 ± 15.2	47.8 ± 9.1			
	Day 21	49.2 ± 12.6	65.4 ± 9.5	57.9 ± 15.3	56.0 ± 13.3			

[a] NEFA, non-esterified fatty acids; BHB, β-hydroxybutyrate; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.
[b] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[c] *P* value, significant variation among groups; T, effect of sampling date; TR, effect of PO added in the ration; T × TR, interaction between T and TR. Means in a row with different letters differ significantly ($P < 0.05$).

similar among CTRL, PO-15% and PO-30%. Finally, no significant T × TR interaction was observed in those parameters connected to hepatic function (AST and GGT).

In spite of the fluctuations found in the different groups during the study, except for albumin, no significant T × TR effects were detected in those parameters closely connected with protein metabolism such as TSP, SUN or creatinine, as seen in Table 8. The significance found in albumin concentrations was shown only on day 7 in the PO-30% group.

DISCUSSION

Effects of *P. oceanica* on intake, digestibility and nitrogen balance

The chemical and nutrient properties of *P. oceanica*[5] showed that this marine plant contains more ash and lignin fraction (ADL) than other traditional forage sources such as cereal straw or rye grass hay. Before starting the analysis of the different applied treatments and as we pointed out in the results section, we should discard the PO-60% group. Clearly, this percentage does not constitute a viable alternative to apply in the ration of sheep, given the low consumption of the marine plant and the negative consequences appreciated in digestion- and gain-related values and energy-related parameters (these animals developed a lipomobilisation status compatible with a state of ketosis).[12]

In the current study, supplemented ewes received a ration with higher ADF, ADL and ash contents than the non-supplemented ewes. On the other hand, the contents of CP were reduced compared with CTRL when PO was increased in the ration PO-30%.

The experimental ewes needed an adaptation period not longer than 9 days for both PO-15% and PO-30%. Sheep are well known

Table 8. Mean values (mean ± standard deviation of mean) of serum total protein, albumin, urea nitrogen and creatinine concentrations

Item[a]	Sampling	Groups[b]				*P* value[c]		
		CTRL	PO-15%	PO-30%	PO-60%	T	TR	T × TR
TSP (g dL^{-1})	Day 0	8.10 ± 0.50	7.63 ± 0.41	8.10 ± 0.48	8.01 ± 0.60	<0.001	0.014	0.991
	Day 7	7.93 ± 0.65	7.63 ± 0.40	7.91 ± 0.34	7.91 ± 0.45			
	Day 14	7.58 ± 0.23	7.30 ± 0.27	7.37 ± 0.47	7.51 ± 0.58			
	Day 21	7.80 ± 0.42	7.43 ± 0.24	7.60 ± 0.42	7.58 ± 0.46			
Albumin (g dL^{-1})	Day 0	3.79 ± 0.09	3.60 ± 0.14	3.63 ± 0.40	3.46 ± 0.56	0.527	0.001	0.022
	Day 7	3.70 ± 0.16	3.69 ± 0.10	4.0 ± 0.16	3.91 ± 0.26			
	Day 14	3.63 ± 0.16	3.66 ± 0.14	3.56 ± 0.16	3.6 ± 0.09			
	Day 21	3.80 ± 0.15	3.68 ± 0.15	3.63 ± 0.07	3.72 ± 0.16			
SUN (mmol L^{-1})	Day 0	4.42 ± 0.8	4.00 ± 0.8	3.81 ± 0.5	4.25 ± 0.6	0.004	<0.001	0.121
	Day 7	5.30 ± 0.9	4.16 ± 0.6	3.80 ± 0.6	3.90 ± 1.0			
	Day 14	5.24 ± 0.5	3.44 ± 0.7	4.28 ± 0.41	3.75 ± 0.97			
	Day 21	5.40 ± 0.5	4.45 ± 0.7	4.63 ± 0.9	4.55 ± 0.97			
Creatinine (mmol L^{-1})	Day 0	0.097 ± 0.007	0.100 ± 0.008	0.099 ± 0.007	0.99 ± 0.006	<0.008	<0.001	0.509
	Day 7	0.098 ± 0.008	0.108 ± 0.008	0.110 ± 0.007	0.109 ± 0.013			
	Day 14	0.097 ± 0.007	0.103 ± 0.009	0.106 ± 0.008	0.107 ± 0.008			
	Day 21	0.095 ± 0.008	0.099 ± 0.012	0.101 ± 0.011	0.110 ± 0.006			

[a] TSP, total serum proteins; SUN, serum urea nitrogen.
[b] CTRL, control group (non-supplemented); PO-15%, ewes offered 75 g of *P. oceanica*; PO-30%, ewes offered 150 g of *P. oceanica*; PO-60%, ewes offered 300 g of *P. oceanica*.
[c] *P* value, significant variation among groups: T, effect of sampling date; TR, effect of PO added in the ration; T × TR, interaction between T and TR. Means in a row with different letters differ significantly ($P < 0.05$).

for feeding on a wide spectrum of plants and are said to possess some degree of 'nutritional wisdom' which enables them to select foods that meet their nutritional needs and avoid those that are harmful or useless for them.[9] The introduction of a new forage source with higher lignin content not only brings changes in the digestion process due to changes in the cellulolytic bacterial species and their fibrolytic enzyme activities[13] but is also negatively correlated with DMI, since fibre ferments slowly and is retained in the rumen longer than other feed components.[14] When rumen fill is increasing with low digestible material, animals increase the time spent ruminating per kg of ingested feed.[15] This would explain the reduced feed consumption observed during the first days of adaptation in the experimental groups. The addition of the marine plant affected nutrient intake and digestibility, although PO-15% was not different from CTRL in most of the studied parameters. Plant maturity stage is one of the main factors affecting the nutritive value of the forage.[11] In our study, the collected *P. oceanica* dry leaves have reached their maximum maturity and therefore contained a large amount of highly lignified cell walls.

The lack of statistical differences between CTRL and PO-15% groups indicates that ewes were not reluctant to eat this experimental ration. The PO-30% ration led to a lower index for N-ADF and ADL; therefore this percentage of PO can be considered the upper limit for its inclusion into the ration. Although these results showed consequences in the ADG and final weights of caged animals, there were no statistical differences between CTRL and PO-15%.

The parameters connected with N balance suggest that PO-15% and PO-30% were in several aspects similar to CTRL. Degradability of N-containing feed components is closely linked with ruminants' productivity; CP degradation also plays a major role in the amount of energy that is used for blood ammonia (NH_3) transformation into urea.[16]

A recent review[17] points out that low N content of feed is a precondition for an efficient use of this system, i.e. utilisation of endogenous urea, which recycles the metabolic end product as a nutrient. According to this idea, the addition of PO banquettes, although decreasing the CP content of the ration, did not affect N retention for PO-15% and PO-30%, probably owing to the improvement of the use of the endogenous urea offered by the 'rumino-hepatic cycle'.

Serum parameters

The metabolic profiles make it possible to identify nutritional constraints before they impair the productivity of the herd.[18] Taking into account the parameters connected with the energy balance, glucose concentrations were within the physiological ranges for all groups throughout the study.[12,19] The lack of statistical differences in CTRL and PO-15% showed that the decrease in DMI in the first days did not affect glucose balance in this group, unlike the remaining groups.

In this scenario, the level of NEFA is a sensitive indicator of energy balance; it is useful for monitoring the energy status of ewes when changes in the ration may not be detectable from changes in the body condition score.[20] The physiological NEFA level ranges between 0.6 and 0.7 mmol L^{-1}.[12] In PO-15%, except on day 7, NEFA concentrations were under these values, suggesting that this ration supplied the animals with sufficient energy during the study. For ewes, a subclinical/clinical ketosis status is established in a range of BHB between 0.7 and 1.1 mmol L^{-1}.[21] Neither CTRL nor PO-15%, and even PO-30%, reached this risk throughout the study time.

Clinical enzymology helps to clarify if the addition of any component of the diet affects tissue functions in the animal's body. The activities of AST and GGT are considered together, as they are usually used to assess liver function and may be relevant when

new dietary supplements are given to ruminants.[12] Both enzymes remained within the physiological range of variations for ewes, indicating that supplementation with *P. oceanica* had no detrimental effects on this organ.

No differences were observed among groups for TSP values remaining within physiological ranges,[12,19] although they fluctuated throughout the study without a clear pattern. Serum albumin is related to the protein status of the animal;[12] for this reason, we regard the fluctuations found on day 7 in the PO-30% group as not clinically relevant, because all animals maintained their concentrations within physiological ranges.

SUN has long been known to be an indicator of protein status. The SUN concentrations in CTRL were always numerically higher than in the supplemented groups, although all animals were within physiological ranges.[19,22] This may indicate less efficient N utilisation in CTRL. An excess of rumen degradable protein results in an increase in the concentration of rumen NH_3, which is absorbed through the rumen wall and transported to the liver, where it is converted to urea.[21,22] Excessive urea will be excreted with urine and faeces and will not contribute to N retention.[16] Our results suggest that the addition of *P. oceanica* in an adequate proportion may support N retention in the body. In fact, PO-15% did not show significant differences from CTRL in all studied parameters. Also, the results obtained for the PO-30% group revealed similarity with CTRL and PO-15% for the N balances. Minimising N excretion is regarded as an environmental necessity in livestock, because CP degradability plays a major role in the amount of energy that is wasted for blood NH_3 transformation into urea and in environmental pollution resulting from NH_3, both of urine and faecal origin.[16,17] Taking into account digestion- and gain-related and metabolic parameters, the potential ability of *P. oceanica* to reduce N loss is an interesting finding that should be assessed in further studies.

CONCLUSION

Posidonia oceanica can serve as a forage source for sheep. Our results demonstrate that the substitution of barley straw with this marine plant (15% of the total forage) has no negative consequences on the performance or metabolic status of mature ewes and can improve N utilisation of the animals.

According to our observations, the upper limit of barley straw substitution by *P. oceanica* would be 30%, a limit for which there were only few performance changes and no metabolic consequences. An exchange of 60% barley straw by *P. oceanica* negatively affects the performance and energy status of the animals.

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