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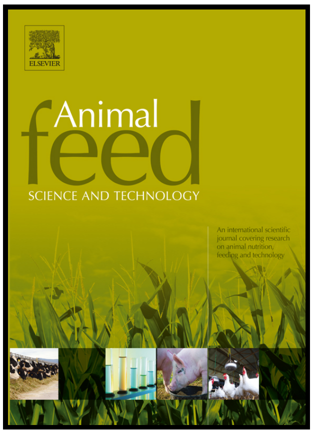

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Effects of feeding prickly pear by-product silage as a partial replacement of concentrate on dairy ewes: Milk characteristics, nutrient utilisation and *in vitro* ruminal fermentation

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Highlights

- Feeding prickly pear peel (PPP) silage affects ruminal fermentation.
- PPP silage-based diet increased casein levels.
- PPP silage-based diet reduced milk urea nitrogen levels.
- PPP silage-based diet increased dry matter and organic matter degradability.
- PPP silage-based diet increased *in vitro* fermentation rate and gas production.

Abstract

Prickly pear fruit processing industries generate a substantial amount of fibrous by-products as waste rich in bioactive compounds, including polyphenols and tannins, and that contain considerable minerals and water-soluble carbohydrates. This study investigated the potential of prickly pear by-product silage as feed in the diet of Valle del Belice ewes and its effects on body weight, milk yield and composition, nutrient utilisation and degradability and *in vitro* ruminal fermentation characteristics. A total of 12 ewes (60 d in lactation) were selected and randomly divided into three experimental groups, homogeneous for parity, live weight and milk yield. Each group was fed for 14 d (9 d for diet adaptation + 5 d for sampling), with one of the three experimental diets based on a Latin square design. The diets with the same crude protein and NDF were: 1) control (CTR) diet with hay and concentrate; 2) prickly pear peels (PPP) diet with PPP silage, hay and concentrate; and 3) pulp, peels and seeds (PPS) diet with PPS silage, hay and concentrate. Nutrient intake varied between diets, with total DM intake being greater in the CTR and PPS ($p<0.01$) diets than in the PPP diet. Daily milk yield tended to be lower in ewes fed the PPP and PPS diets than in those fed the CTR diet, whereas no differences were found for fat- and protein-corrected milk between diets. Protein and casein ($p\leq0.05$) levels were higher in the milk of ewes fed the PPP diet. Compared with the milk urea concentration of CTR-fed ewes, that of PPP-fed ewes was 15% lower. The *in vivo* nutrient degradability, *in vitro* fermentation rate and volatile FAs were greater ($p<0.01$) in the PPP diet than in the PPS diet. These results suggest that PPP silage can be partially incorporated into dairy ewe diets to reduce feeding costs and improve milk nitrogen efficiency.

Abbreviations

AIBPs, agro-industrial by-products; ADIA, acid detergent-insoluble ash; ADF, acid detergent fibre; AOAC, Association of Official Analytical Chemists; CP, crude protein; DM, dry matter; DMI, dry matter intake; EE, ether extract; FPCM, fat- and protein-corrected milk; PPP, prickly pear peel; PPS, prickly pear peel + pulp + seed; PPB, prickly pear by-product; DMD, dry matter degradability; OMD, organic matter degradability; NDFD, neutral detergent fibre degradability; VFA, volatile fatty acid; BCFA, branched chain fatty acid; OMCV, cumulative volume of gas related to incubated organic matter; Tmax, time to reach the maximum fermentation rate; Rmax, maximum fermentation rate; SEM, standard error of mean.

Keywords
prickly pear by-products; silages; milk casein; nutrient degradability; ewe

1. Introduction

Agro-industrial by-product (AIBP) utilisation in livestock feeding, particularly in ruminants, has gained renewed interest because of its potential to replace conventional feeding resources, reduce production costs, compete with human food resources and mitigate environmental impacts (Vastolo et al., 2022a, Georganas et al., 2023). In addition, incorporating AIBPs into ruminant diets aligns with circular economy principles and fosters sustainability in livestock production systems (Sun et al., 2024). The cultivation of prickly pear (*Opuntia ficus-indica* L.) in the Sicilian region (Italy) has increased the demand for fresh edible fruits or juice for human consumption in recent years. Consequently, a substantial amount of prickly pear by-products (PPBs) comprising peel, pulp and seeds following juice extraction from fruits is produced as waste (Todaro et al., 2020), often remains underutilised, is improperly disposed of in landfills and leads to environmental pollution (Kilama et al., 2023). Italy is the world's third-largest producer of prickly pear after Mexico and the United States, with Sicily contributing 156,641 tonnes/year of fresh fruit and 97.72% of the national output (ISTAT, 2024). The fractionation of PPBs showed that they consist of approximately 28% peel + pulp and 72% seeds on a dry matter (DM) basis (Todaro et al., 2020). Incorporating these by-products, including PPBs, as either a complete replacement or partial supplementation into small ruminant diets offers a practical and eco-friendly approach to feeding (Boudalia et al., 2024).

Within Europe's small ruminant livestock sector, Italy is a significant contributor, maintaining a substantial population of approximately 5.9 million sheep and 1 million goats (Di Marco Lo Presti et al., 2024). Sicily has the second-largest sheep population in Italy, with 662,305 sheep, representing 11.3% of the national total. However, sheep and goat breeding is predominantly semi-extensive in this region, involving shared pastures and supplementary feeding, particularly during the dry season (Di Marco Lo Presti et al., 2024). Therefore, seasonal fluctuations in forage production, characterised by spring abundance and summer–autumn scarcity, are due to high temperatures and aridity (Todaro et al., 2015), leading to a recurring deficit in fodder quality and quantity (Porqueddu et al., 2016). To address these challenges, it is essential to explore alternative feeding resources and sustainable feeding practices to ensure the long-term viability of ruminant livestock systems in the region (Boudalia et al., 2024). Recently, AIBPs, including PPBs, have emerged as promising alternative feeding resources for small ruminants in European Mediterranean countries (Ku-Vera et al., 2020, Vastolo et al., 2020, Vastolo et al., 2022a). Interestingly, PPBs are available at the end of summer when fresh forage is scarce (Morshedy et al., 2020). Therefore, silage has significant potential as a feeding resource, providing ruminants with a source of digestible fibre and water (de Sá et al., 2024). However, the exclusive use of PPB silage has certain constraints, including low crude protein (CP) and fibre contents and high moisture and mineral contents, leading to nutritional disorders, low dry matter intake (DMI) and subsequent body weight (BW) loss (Silva et al., 2021). To address these constraints, it is recommended to ensile PPBs along with other forage or by-products (i.e. wheat straw or bran). This approach partially absorbs excess moisture, balances water-soluble carbohydrate (WSC) and nitrogen contents and improves DM content (Vastolo et al., 2020, Gannuscio et al., 2024a). Consequently, the overall nutritional value of ruminant diets can be improved (Silva et al., 2022).

In our previous study (Gannuscio et al., 2024b), 12% wheat bran was added to PPBs during ensiling to enhance their nutritional profile and silage quality. Previous studies have mainly focused on improving the nutritive value of PPB silages, whereas the direct use of these by-products in dairy ewe diets to investigate their potential impact on performance has been rarely explored. Therefore, the aim of this study was to assess the impact of feeding silages of two PPBs ensiled with 12% wheat bran on the performance of dairy ewes (i.e. BW, milk yield and composition). The *in vivo* nutrient degradability and *in vitro* ruminal fermentation characteristics were also studied to improve our knowledge of the nutritional differences of prickly pear by-product (PPBs) substitution in ewe diets.

2. Materials and methods

The experimental trial was conducted on a commercial farm in Menfi, located in Sicily's Agrigento Province, Italy. The farm housed 500 Valle del Belice breed ewes that were raised in a semi-extensive system. The trial followed the ethical principles of animal experimentation adopted by the Animal Welfare Commission of the University of Palermo (protocol number: UNPA-CLE 201954 – 12/12/2023).

2.1. Raw material collection and ensiling

In September 2023, 600 kg of fresh PPPs was obtained after peeling and processing prickly pear fruits using an automatic dyeing machine (AGRIMAT s.r.l.), which separated the peels, seeds, pulp and juice. The PPPs were immediately transported to the sheep farm and ensiled with 12% wheat bran (based on raw weight) for 50 d in hermetically sealed plastic containers equipped with a degassing valve. In addition, a local juice extraction company

(Agres s.r.l., Carini, Palermo) supplied 600 kg of a mix of prickly pear PPS obtained after juice extraction (earthworm press) from whole fruits. The leftovers were loaded into trucks and, after 24 h, transported to a sheep farm and ensiled with 12% wheat bran (based on raw weight) for 50 d in hermetically sealed plastic containers equipped with a degassing valve. The ensiling time was evaluated based on previous ensiling experiences of PPP (Gannuscio et al., 2024a) and PPS (Vastolo et al., 2020) by-products.

2.2. Animals, experimental design and feeding treatments

Twelve Valle del Belice breed ewes were selected from a farm group at 90 d of lactation and randomly divided into three experimental groups that were homogeneous for parity (3rd-6th lambings), live weight (53.66 ± 6.57 kg) and milk yield (1.038 ± 0.144 kg/d). The selected ewes were treated for internal and external parasites with 2-mL ivermectin (Ivomec®, Merial, France) 14 d before the start of the trial. The ewes were housed in a farm building containing individual straw-bedded pens, each equipped with a feeder and a drinker. Initially, the ewe groups underwent a 2-week adaptation period to their new housing conditions and diet. During this period, the ewes were fed a preliminary diet comprising sulla hay *ad libitum* and commercial concentrate (800 g/head/d) to ensure adequate coverage of their daily nutritional requirements (INRA, 2018). After the adaptation period, each ewe group was randomly assigned to one of three experimental diets using a Latin square design (3 × 3) with three phases, each of which lasted for 14 d, 9 d for adaptation to diets and 5 d for sampling (Gannuscio et. al, 2022). The diets were formulated as follows: 1) control (CTR) diet: 900 g/d per head of commercial concentrate + 3,000 g/d per head of sulla hay; 2) prickly pear peels (PPP) diet: 1,500 g/d per head of PPP silage + 500 g/d per head of commercial concentrate + 2,700 g/d per head of sulla hay; and 3) pulp, peels and seeds (PPS) diet: 1,000 g/d per head of PPS silage + 500 g/d per head of commercial concentrate + 2,300 g/d per head of sulla hay (Table 1). Diets were developed to ensure the same fibre and CP contents. The experimental groups were provided with PPP and PPS silage at 9 a.m. and concentrate at 4 p.m. daily. Hay was administered daily in the morning and was left available for the entire day. The control group was given a daily intake of concentrate in two feeds: 50% (450 g) of the assigned feed administered in the morning and the other 50% (450 g) administered in the evening.

Table 1. Diets ingredients (g/d) offered to lactating ewes.

Ingredients	**Diets**		
	CTR	**PPP**	**PPS**
Sulla hay	3,000	2,700	2,300
Silage	-	1,500	1,000
Concentrate	900	500	500

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels+pulp+seeds

2.3. Data recording and sampling

The live BW and body condition score (BCS) of the ewes were recorded at the start and end of each phase (experimental period). The live BW was measured by using the electronic balance (Albert Kerbl GmbH, Germany). BCS were measured by palpation of the lumbar vertebrae and associated soft tissue using a scale of one (thin) to five (fat) scale. The remaining data were recorded and samples were collected within the last 5 d of each phase. The offered diet and each pen’s refusal diet (PPP and PPS silages, hay and concentrate) were weighed daily and sampled three times on 2, 3 and 4 d of the sampling period to determine the quantity and composition of dietary DMI. The silage samples (offered and refused) were stored at −20°C and freeze-dried before analysis. During the sampling period of each phase, individual milk yields were recorded daily by using electronic balance (Sinergica Soluzioni S.r.l., Milan) in the morning (7:00 a.m.) and evening (4:00 p.m.) and sampled three times on 2, 3 and 4 d of the sampling period. The daily milk yield was obtained by adding the morning and evening milkings.

2.4. Milk yield, milk composition and microbiological analysis

Individual milk samples were collected on the third day of the sampling period during the morning and evening milkings (Gannuscio et. al, 2022) and analysed for fat, protein, casein, lactose, urea, somatic cell count (SCC) and differential somatic cell count (DSCC) using an infrared method (Combi-Foss 6000, Foss Electric, Hillerød, Denmark). For the chemical parameters, as well as for the SSC and DSSC after logarithmic transformation, the mean between the morning and evening data was calculated before statistical analysis. After routine cleaning and disinfection of the udder and discarding the first streaks of milk, samples from both mammary halves (volume: 50 mL) were aseptically collected during morning milking. Every milk sample, without preservatives, underwent rapid cooling and was transported on the same day in iceboxes directly to the Experimental Zooprophylactic Institute of Sicily 'A. Mirri' to determine mastitis agents. The protocol of Adkins and Middleton (2018) was followed to isolate bacteria. Specifically, 10 µL of milk from each sample was seeded onto blood and mannitol salt agar plates and then incubated in an inverted position at 37°C for 24–48 h under either aerobic or microaerophilic conditions. Subsequently, the suspected colonies (evaluated based on morphological aspects) were subcultured onto brain heart infusion slants. The final identification was conducted using Gram staining and a series of biochemical tests, such as catalase, oxidase and coagulase tests, and a commercial miniaturised biochemical identification system (bioMérieux's API identification products), specifically API Staph.

2.5. *In vivo* nutrient degradation measurements

To assess dietary nutrient degradability, individual faecal samples were collected daily in the morning (10:00) and evening (05:00). Samples were collected by stimulating defaecation or directly from the rectum of the animal. A single composite sample was obtained by combining the morning and evening samples and frozen at −20°C until laboratory analysis. Freeze-dried faecal samples were ground using a Wiley mill with a 1-mm screen (Thomas Scientific). Feed (offered and refused) and faecal samples were analysed for acid detergent-insoluble ash (ADIA) according to Van Keulen and Young (1977) and for DM, aNDFom, ADFom and ash, as described below for the feed samples. ADIA was used as an internal marker to estimate faecal DM output. Dry matter degradability (DMD), organic matter degradability (OMD) and neutral detergent fibre degradability (NDFD) were calculated using the following equations.

$$\text{DMD (\%)} = 100 - \left[100 \times \left(\frac{\%\ \text{ADIA in DM consumed}}{\%\ \text{ADIA in faeces}}\right)\right]$$

$$\text{OMD or NDFD (\%)} = 100 - \left[100 \times \left(\frac{\%\ \text{ADIA in DM consumed}}{\%\ \text{ADIA in faeces}}\right) \times \left(\frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in consumed DM}}\right)\right]$$

2.6. Chemical analyses

Samples of offered and refused feed (PPP silage, PPS silage, hay and concentrate) were collected, transferred to the Department of SAAF laboratory at the University of Palermo and stored at −20°C. The silage samples were then freeze-dried. Freeze-dried hay, concentrate and silage were ground in a Willey mill (Thomas Scientific, Swedesboro, NJ, USA) using a 1.0-mm sieve (AOAC, 2005; Method 934.01) for further analyses. These samples were analysed in triplicate for DM (934.01), ether extract (EE, 920.39), CP (2001.11) and ash (942.05) according to the AOAC method described by Lee (1995). Neutral detergent fibre (aNDFom, 2002.04) was determined following the protocol of Van Soest et al. (1991) using a modified method by adding heat-stable α-amylase and sodium sulphite to express it as residual ash-free NDF, as described by Detmann et al. (2012). Acid detergent fibre (ADFom, 973.18) and acid detergent lignin (ADL, 973.18) were determined following the methods described by Van Soest et al. (1991).

To calculate the net energy for lactation (NE_L) of the diets, based on the INRA (2018) approach, a pepsin-cellulase method was used to evaluate the organic matter's apparent digestibility (OMd) (Aufrère and Michalet-Doreau, 1988; Aufrère et al., 2007). Samples were oven-dried at 60°C and ground through a 1.0-mm screen. Approximately 500 mg of each sample was then weighed into a glass crucible. The first phase of the method consisted of incubation for 24 h in a water bath at 40°C with a hydrochloric solution (0.1 N) containing pepsin (2 g/l). This phase was followed by a 30-min wash step at 80°C to remove starch. The second phase consisted of incubation for 24 h in a water bath at 40°C with a cellulase solution (1 g/l) buffered in sodium acetate. The cellulase used was Onozuka R10 derived from Trichoderma viride (Merck KGaA, Darmstadt, Germany). After incubation, the crucibles were filtered on a filtering plate, washed with distilled water and oven-dried to determine the residue. To estimate the energy content of the samples, the INRA (2018) approach was used by applying the equations reported in Table 24.14, which allowed for the prediction of gross energy (GE, kcal/kg OM) from specific chemical parameters (i.e. ash, EE, CP and CF, expressed in g/kg OM). The following equation was used:

$$GE = 4{,}134 + 1.473 \times CP + 5.239 \times EE + 0.925 \times CF - 4.44 \times Ash.$$

Subsequently, equation 3.11 of INRA (2018) was applied to estimate the digestible energy as follows:

$$DE = GE\ x\ 0.01\ x\ Ed$$

where Ed is the digestibility of energy (%) predicted from OMd (%) (Table 24.15 INRA 2018). In particular, the equation adopted was suggested for grass and legume silages (i.e. Ed = −5.723 + 1.0263 × OMd). Then, the metabolisable energy (kcal/kg DM) was calculated using equations 3.12 and 3.13 of INRA (2018). Finally, the net milk energy (kcal/kg DM) was calculated using the following equation:

$$NEL = ME\ x\ kls$$

where kls for milk production and maintenance is 0.65 + 0.247 × (q − 0.63), with q = concentration of feed in ME = ME/GE, as reported in Table 24.1 (INRA, 2018).

2.7. *In vitro* ruminal fermentation characteristics

In *vitro* ruminal fermentation characteristics were studied according to the method reported by Vastolo et al. (2022b). Briefly, substrates (experimental and control diets) were incubated at 39°C under anaerobic conditions with an inoculum consisting of a pool of ruminal fluid. Ruminal fluid was collected from three adult ewes in a slaughterhouse authorised according to EU regulations (EC Regulation 882/2004). All procedures involving animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 2019/0013729 of 08/02/2019). Donor animals were fed a standard diet of hay and concentrate after 12 h of fasting before rumen fluid collection. The collected material was immediately placed inside preheated thermos to avoid exposure to air and quickly transferred to the Feed Analysis Laboratory, Department of Veterinary Medicine and Animal Production, University of Naples, Federico II. The ruminal inoculum was filtered through a two-layer cheesecloth, insufflated with CO_2 and added to each bottle (10 mL) containing a buffer solution (medium) to obtain a 1:7.5 inoculum–medium ratio. Specifically, one run was performed to process a large batch of bottles in a single incubation, which has been proven not to affect the rate or extent of gas production with respect to higher inoculum proportions (Amanzougarene and Fondevila, 2020). In one run, for each diet (CTR, PPP and PPS) and each phase (1, 2 and 3), substrates were incubated in six replicates (n = 18, 1.0244 g ±

0.020 on a DM basis) in 120-mL serum bottles to which medium (75 mL) and a reducing solution (4 mL) were added. The medium used consisted of a bicarbonate-phosphate buffer, reducing agent, macro-mineral solution, micro-mineral solution and resazurin as a redox indicator (Theodorou et al., 1994). During 120 h of incubation, the gas produced was measured 24 times using a manual system consisting of a pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA) connected to a three-way valve with a graduated syringe. The cumulative volume of gas produced after 120 h of incubation is related to incubated organic matter (OM) (OMCV, mL/g). After 120 h of incubation, the bottles were opened, and the pH of the fermentation liquor was measured using a pH metre (Thermo Orion 720 A+, Fort Collins, CO, USA). The contents of the bottles were filtered (sintered glass crucibles; Schott Duran, Mainz, Germany, porosity # 2), and the residue was burned at 550°C for 3 h. OMD was calculated as the weight difference between incubated and untreated OM. In accordance with the protocol, three bottles were incubated without substrate (blank) to correct for OMD and OMCV. After incubation, the fermentation liquor was centrifuged at 12,000 × g for 10 min at 4°C (Universal 32R centrifuge, Hettich FurnTech Division DIY, Melle-Neuenkirchen, Germany), and 1 mL of the supernatant was collected and acidified with 1 mL of oxalic acid (0.06 mol) to analyse volatile fatty acids (VFAs). The VFAs were determined using gas chromatography (ThermoQuest 8000top Italia SpA, Rodano, Milan, Italy) equipped with a fused silica capillary column (30 m, 0.25 mm ID, 0.25 m film thickness), as reported by Vastolo et al. (2023). The VFA production (mmol/l) was previously corrected for blank; subsequently, the corrected amount of each VFA was calculated as mmol/g iOM.

For each bottle, the gas production profiles were processed using a sigmoid model, as described by Groot et al. (1996):

$$G = \frac{A}{1 + \left(\frac{B}{t}\right)^{c}}$$

where G represents the total gas produced (mL/g of OM) at a specific time t (h), A is the asymptotic gas production (mL/g of OM), B (h) denotes the time required to reach half of the asymptote and C is the switching characteristic of the curve.

The maximum fermentation rate (Rmax, mL/h) and corresponding time (Tmax, h) were determined using the following equations (Bauer et al., 2001):

$$\mathrm{R}_{\mathrm{max}} = \frac{A * B^{C} * C * T_{max}{}^{(-C-1)}}{(1 + B^{C} * T_{max}{}^{-C})^{2}}$$

$$T_{max} = B * (\frac{C - 1}{C + 1})^{\frac{1}{C}}$$

2.8.1 Statistical analysis

Data on silage chemical composition were analysed using a one-way analysis of variance (ANOVA) model (SAS 9.2 software, 2010). The following statistical model was applied:

$$Y_{ik} = \mu + F_{i} + \epsilon_{ik}$$

where Y_{ik} is the dependent variable, μ is the general average, F_i denotes the fixed effect of the i feed (i = PPP and PPS silages) and ε_{ik} is the residual error.

The live BW, BCS, nutrients intake, *in vivo* degradability, milk yield and milk composition of ewes were analysed using a mixed model (SAS 9.2 software, 2010). The following statistical model was applied:

$$Y_{ijlklm} = \mu + P_{i} + D_{j} + S_{l} + E(S)_{kl} + \epsilon_{ijlkm}$$

where Y_{ijlkm} is the dependent variable, μ is the general average, P_i denotes the fixed effect of phase *i* (i = 1, …, 3), D_j is the fixed effect of the *j* diet (j = CTR, PPP and PPS), S_l is the fixed effect of the square, $E(S)_{kl}$ is the random effect of the k^{th} ewe (k = 1 − 12) within square, and ε_{ijlkm} is the residual error.

The *in vitro* ruminal fermentation characteristics were analysed using a two-way ANOVA model (SAS 9. software, 2010). The following statistical model was applied:

$$Y_{ijk} = \mu + P_i + D_j + \epsilon_{ijk}$$

where Y_{ijk} is the dependent variable. μ is the general average, P_i denotes the fixed effect of phase i (i = 1, …, 3), D_j is the fixed effect of the j diet (j = CTR, PPP and PPS) and ε_{ijk} is the residual error. The least-squares means were compared using P-values adjusted according to the Tukey–Kramer multiple comparison test.

3 Results

3.1 Chemical composition of dietary ingredients

The fractioning of PPS showed that it included 26% of peel and pulp and 74% of seeds on a DM basis. The chemical composition and organic acids of silages are presented in Table 2. The DM and fibre content (aNDFom, ADFom and ADL) of PPS silage were higher ($p \leq 0.01$) than those of PPP silage. In contrast, CP, non-fibrous carbohydrate and ash contents were significantly higher in PPP silage than in PPS silage. The pH value of PPP silage was significant lower ($p \leq 0.01$) than PPS silage.

The level of lactic acid detected in PPP silage was 15 times higher ($p \leq 0.01$) than those of PPS silage, while acetic and propionic acids were detected at the same concentration. Other significant differences between silages were found in the concentration of butyric acid, which was higher in PPP silage than in PPS silage.

Net energy for lactation of PPP silage was more than double that of PPS silage ($p \leq 0.01$).

Table 2. Chemical composition of the dietary ingredients (means; n=3) and silages characteristics (least square means; n=3)

Items		Concentrate	Hay	PPP	PPS	SEM	p-value
DM	g/kg FM	882.9	900.9	200.3	413.7	0.62	0.01
CP	g/kg DM	247.4	71.5	120.2	95.5	3.51	0.01
EE	g/kg DM	40.2	14.4	61.5	37.8	7.22	0.08
aNDFom	g/kg DM	265.8	796.8	253.1	666.6	10.22	0.01
ADFom	g/kg DM	97.0	523.0	113.8	500.3	2.19	0.01
ADL	g/kg DM	15.9	83.6	21.8	267.7	2.60	0.01
NFC	g/kg DM	369.8	49.2	450.1	119.2	10.20	0.01
Ash	g/kg DM	76.8	68.2	115.2	80.8	3.70	0.01
pH				3.77	4.07	0.05	0.01
NH_3-N	g/kg TN			12.77	15.25	2.04	0.41
Lactic acid	g/kg DM			20.02	1.26	1.17	0.01
Acetic acid	g/kg DM			1.96	1.92	0.15	0.85
Propionic acid	g/kg DM			0.48	0.24	0.17	0.33
Butyric acid	g/kg DM			0.70	0.02	0.05	0.01
NE_L	(MJ/kg DM)			7.82	3.52	0.20	0.01

PPP silage: prickly pears peels + 12% wheat bran; PPS silage: prickly pears peels, pulp, seeds + 12% wheat bran. DM: dry matter; CP: crude protein; EE: ether extract; aNDFom: neutral detergent; ADFom: acid detergent fiber; ADL: Acid Detergent Lignin; SEM: standard error of mean. NFC: non-fibrous carbohydrates = 100 - (CP + ether extract + ash + aNDFom). NH_3-N = ammonia-N; TN = total nitrogen. NE_L: net energy for lactation.

3.2 Dietary ingredients and nutrient offered and intake by ewes

The nutrients contained in the feeds offered to the three experimental groups are reported in table 3. Despite the different dry matter content offered to the ewes on the three diets, the amount of fibre (aNDFom), crude protein and their ratios were similar.

Table 3. Diets nutrients offered to lactating ewes (g/d/head).

Ingredients	Diets		
	CTR	**PPP**	**PPS**
DM	3,500	3,170	2,930
aNDFom	2,360	2,130	2,040
CP	390	320	300
aNDFom/CP	6.07	6.68	6.89
NFC	430	420	338

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels+pulp+seeds

The ingredients and nutrient intake of the ewes varied between diets (Table 4). Although the ewes fed the three diets were offered different amounts of hay, the amount they ingested show statistically significant differences (p<0.05) between CTR and PPP diets. The lowest DMI was observed in ewes that received the PPP diet (p<0.01) compared with those fed the CTR and PPS diets, which showed similar voluntary feed intake. Furthermore, CP intake was significantly different between diets (p<0.01), with the significant highest intake observed in CTR-fed ewes.

The ewes of PPS diet showed highest fibre ingestion (NDF, ADF and ADL) among all diets. Consequently, NFC intake was the lowest with the PPS diet (p<0.01). The carbohydrate/CP ratio of ingesta was affected by the diet; when the PPS diet was administered, the ratio was significantly higher (p<0.01) compared with the other two diets.

Voluntary feed intake by lactating ewes resulted in the highest NE_L value with the CTR diet compared with the other two diets, while PPP ewes ingested higher NE_L (p<0.05) than PPS ewes.

Table 4. Ingredients and nutrients intake of dairy ewes.

Items	Diet			SEM	Diet
	CTR	PPP	PPS		p-value
Ingredients intake (g of DM/d/head)					
Hay	1499^{a}	1269^{b}	1403^{ab}	178	0.05
Silage	-	307^{b}	410^{a}	2.63	0.01
Concentrate	795^{a}	442^{b}	442^{b}	0.78	0.01
Nutrients intake (g/d/head)					
DM	2307^{a}	2031^{b}	2267^{a}	175	0.01
CP	322^{a}	261^{b}	270^{b}	3.54	0.01
EE	55.6	56.8	55.5	1.73	0.69
aNDFom	1384^{a}	1176^{b}	1474^{a}	144	0.01
ADFom	794^{a}	669^{b}	909^{a}	102	0.01
ADL	123^{b}	104^{b}	218^{a}	19.2	0.01
NFC	396^{a}	397^{a}	321^{b}	4.86	0.01
(aNDFom + NFC) / CP	5.52^{c}	6.11^{b}	6.71^{a}	0.378	0.01
NE_L (MJ intake/d/head)	11.13^{a}	10.40^{b}	9.85^{b}	0.292	0.01

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels+pulp+seeds. DM: dry matter; CP: crude protein; EE: ether extract; aNDFom: neutral detergent; ADFom: acid detergent fiber; ADL: Acid Detergent Lignin; SEM: standard error of mean. NFC: non-fibrous carbohydrates = 100 - (CP + ether extract + ash + aNDFom). NE_L: net energy for lactation. In the row, values with different superscript letters are significant.

3.3 Ewe's body weight, body condition score and milk parameters

The effects of diet on BW, BCS, milk yield and composition are presented in Table 5. The BW change (∆BW) of ewes was significantly influenced by the diets. Ewes fed the CTR diet experienced BW loss compared with ewes fed the other diets, whereas ewes fed the PPS diet gained BW, significant differences (p<0.05) were found only between CTR and PPS diets. All ewes showed improved BCS during the experiment, but no significant differences were found between diets.

Table 5. Body weight, body condition score, and milk parameters of dairy ewes

	Diet			SEM	Diet
	CTR	**PPP**	**PPS**		**p-value**
Body weight (BW; kg)	44.94	45.77	46.15	3.63	0.68
∆ BW (kg)	-0.99[b]	+0.08[ab]	+0.43[a]	0.415	0.05
Body condition score (BCS)	3.26	3.39	3.41	0.085	0.20
∆ BCS	+0.104	+0.313	+0.125	0.084	0.17
Milk yield and constituents					
Daily milk yield (kg/d)	0.81[a]	0.67[b]	0.69[b]	0.07	0.03
FPCM (kg/d)*	1.01	0.91	0.92	0.12	0.14
Fat (g/kg)	92.9	99.9	97.9	8.01	0.09
Protein (g/kg)	59.8[b]	64.1[a]	61.6[ab]	2.48	0.05
Casein (g/kg)	45.7[b]	49.8[a]	47.4[ab]	2.11	0.05
Urea (mg/dl)	64.4[a]	54.1[b]	56.4[b]	7.31	0.01
Lactose (g/kg)	43.0	41.5	42.3	1.06	0.64
Somatic cell count (Log_{10})	5.10	5.28	5.19	0.094	0.39
Differential somatic cell count (Log_{10})	4.69	4.66	4.52	0.140	0.66

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels+pulp+seeds.

*Fat and Protein Corrected Milk (FPCM; 6.5% fat; 5.8% protein) calculated according to Pulina et al. (2005).

In the row, values with different superscript letters are significant.

Although ewes fed the PPP and PPS diets exhibited lower (*p*<0.05) milk yields than those fed the CTR diet, no statistical differences were found when milk production was calculated using the fat- and protein-corrected milk (FPCM) (Table 5). Compared with ewes fed the CTR diet, those fed the PPP diet produced milk with higher protein (*p*=0.05) and casein content (*p*=0.05) and lower urea content (*p*<0.01).

No significant differences in SCC and DSCC were observed among the ewe milk samples from the three diets. Coagulase-negative staphylococci (CNS) were detected in samples from three sheep (always the same animals in all three phases).

3.4 *In vivo* nutrient degradability and *in vitro* ruminal fermentation characteristics

The *in vivo* and *in vitro* nutrients degradability of the diets is presented in Table 6. The *in vivo* degradability of DM, OM and NDF was influenced by the diet. The degradability coefficients of the PPS diet were significantly lower (*p*<0.01) than those of the other two diets. The *in vitro* degradability of DM and OM was significantly lower in the PPS diet.

Table 6. *In vivo* and *in vitro* nutrients degradability of diets.

Items	Diet			SEM	Diet
	CTR	PPP	PPS		p-value
In vivo parameters					
DMD (g/kg)	841[a]	858[a]	736[b]	68.9	0.01
OMD (g/kg)	777[a]	790[a]	689[b]	61.5	0.01
NDFD (g/kg)	862[a]	899[a]	776[b]	68.9	0.01
In vitro parameters					
OMD (g/kg)	648[a]	674[a]	528[b]	14.7	0.01
NDFD (g/kg)	705[ab]	761[a]	625[b]	33.2	0.01
OMCV, mL/g	218[b]	229[a]	196[c]	2.90	0.01

T_{max}, h	11.0^{a}	7.38^{b}	10.4^{ab}	1.08	0.01
R_{max}, mL/h	4.92^{ab}	5.52^{a}	4.18^{b}	0.40	0.01

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels + pulp + seeds; DMD: dry matter degradability; OMD: organic matter degradability; NDFD: neutral detergent fiber degradability. OMCV: cumulative volume related to incubated organic matter; T_{max}: time to reach the maximum fermentation rate; R_{max}: maximum fermentation rate; SEM, standard error of mean. In the row, values with different superscript letters are significant.

The OMCV produced by the PPP diet was consistently greater (p<0.01) than that produced by the other two diets. The PPP diet showed higher fermentability with a shorter time to peak (T_{max}) than CTR diet (p<0.01), and a higher maximum fermentation rate (R_{max}) than the other two diets.

Table 7. Volatile fatty acids (VFAs) produced by diets during the *in vitro* trials.

Items	**Diet**			**SEM**	**Diet**
	CTR	**PPP**	**PPS**		**p-value**
pH	6.40^{b}	6.41^{ab}	6.45^{a}	0.02	0.04
VFA, mmol/g iOM					
Acetate	34.8^{b}	41.7^{a}	34.1^{b}	0.56	0.01
Propionate	12.4^{b}	14.0^{a}	12.7^{b}	0.37	0.01
Butyrate	6.53^{b}	9.98^{a}	6.74^{b}	0.40	0.01
Iso-Butyrate	0.99^{b}	1.18^{a}	0.94^{b}	0.42	0.01
Valerate	1.29^{c}	1.95^{a}	1.57^{b}	0.73	0.01
Iso-Valerate	1.52^{b}	1.88^{a}	1.64^{b}	0.83	0.01
Acetate/Propionate	3.34^{ab}	3.54^{a}	3.19^{b}	0.72	0.01
Total VFA	57.5^{b}	69.0^{a}	58.8^{b}	0.97	0.01

CTR: control; PPP: prickly pear peels; PPS: prickly pear peels + pulp + seeds; VFA: volatile fatty acids; iOM: incubated organic matter; SEM: standard error of means. In the row, values with different superscript letters are significant.

VFAs produced *in vitro* varied according to diet (Table 7). Incubation with the PPP diet produced significantly higher total VFAs (p<0.01) than those with the other diets. All VFAs detected were significantly higher values (p<0.01) in the PPP diet than in the other diets. The acetate–propionate ratio showed significant differences only between the PPP and PPS diets.

4 Discussion

A key factor in the ensiling process is the DM content of the material to be ensiled because it influences the type of fermentation that occurs inside the silo (Perazzo et al., 2020). PPBs typically have high-moisture levels owing to the presence of mucilage (a substance composed of glycoproteins and organic acids), which enables them to retain water (Du Toit et al., 2019) and low DM content (Todaro et al., 2020, Vastolo et al., 2020, Gannuscio et al., 2024a). To address this challenge, wheat bran (12%) was added to the substrates as a moisture sequester. The addition of wheat bran increased the DM content of both PPP and PPS silages (200 g/kg and 414 g/kg, respectively) compared with the raw materials PPP and PPS (150 g/kg and 385 g/kg, respectively), as reported by Gannuscio et al. (2024b).

The main advantage of mixed silage production is the enhanced CP content of the ensiled material. This advantageous effect was also observed in these silages, in which 12% wheat bran was added to PPBs in mixed silages, resulting in a higher CP content in PPP and PPS silages (CP: 120.2 g/kg and 95.5 g/kg DM, respectively). Thus, the CP content of the PPP and PPS diets (128 vs 119 g/kg DM) comprising PPP and PPS silages exceeded the minimum threshold required for optimal rumen fermentation without compromising the efficient utilisation of fibrous carbohydrates. According to the NRC (2001), feeding small ruminants a diet containing less than 70 g/kg CP and limited nitrogen availability can hinder fibre digestion and reduce feed intake because of slower feed movement through the rumen. Adequate CP levels in the diet may stimulate the proliferation of rumen microflora and enhance fermentation, leading to an increased passage of nitrogen-containing compounds into the small intestine (Matias et al., 2020). The NDF and ADF contents in PPP silage remained below the maximum recommended thresholds for small ruminant diets, which were 600 g/kg and 400 g/kg, respectively, as suggested by Van Soest (1994), whereas PPS silage exhibited higher values due to the high presence of seeds, similarly to what reported by Todaro et al. (2020). Therefore, a higher concentration of seeds in the ensiled mass makes this type of by-product (as was the case with PPS, where seeds accounted for 74% of the ensiled material on a DM basis) more suitable for other preservation and utilization technologies compared to ensiling, as it enhances seed utilization by ruminant animals (Bryszak et al., 2019).

The pH detected in both silages showed values that remained below 4.5, considered the threshold value for good quality (Collins et al., 2017); similar values were found in PPBs silages (Vastolo et al., 2020; Gannuscio et al., 2024a). This high level of fermentation quality was likely due to the WSC content of the PPBs being higher in the PPP than in the PPS (Gannuscio et al., 2024a).

The percentage of ammonia in the total nitrogen (TN) was found to be low for both silages, similar values were previously found for the PPP silage (Vastolo et al., 2020), while values around 15% of the TN were reported for the PPS silage (Todaro et al., 2020). The well-preserved silages should contain less than 10% of the TN in the form of ammonia-N (Collins et al., 2017).

The higher content of lactic acid detected in the PPP silage was probably due to the content of WSC and the sugar detected in raw materials and used by LAB for their fermentation

(Gannuscio et al., 2024b). Moreover, the high fermentative power of the prickly pear peel has already been highlighted in vitro fermentation trials by Gannuscio et al. (2024a). Acetic and propionic acids were detected in the prickly pear silages at low concentrations, and no differences were found between them; furthermore, the concentrations detected in these silages were lower than in other studies on prickly pear silages (Vastolo et al., 2020; Gannuscio et al., 2024a). The concentration of butyric acid detected in the PPP silage was significantly higher than in the PPS silage, but the levels achieved indicate that the silage did not undergo clostridial fermentation, which is one of the poorest fermentations of silages (Kung and Shaver, 2001).

NE_L was significantly higher in PPP silage, probably due to the higher digestibility of DM and OM compared to PPS silage; this fact is also due to the high seed content present in the latter silage.

Individual DMI is an important factor that can provide information on animal feed efficiency. The total DMI observed in this trial was between 4.4% and 5.1% of the BW of the sheep, in agreement with previous studies on lactating Valle del Belice ewes (Todaro et al., 2017; Gannuscio et al., 2022) but significantly higher than that reported for ewes of the Sarda breed (Carta et al., 2020; Lunesu et al., 2021). The PPP-fed ewes showed a significantly lower DMI than ewes that were fed the other diets, despite still having the possibility of ingesting hay as evidenced by the amount of DM offered (table 3). The aNDFom intake was also statistically lower in the PPP-fed ewes despite the sheep having been offered the same amount, this is therefore due to the free choice made by the animals. This marked and unexpected contraction of voluntary intake of DM and aNDFom is probably likely due to the soluble carbohydrates intake associated with this diet, resulting from the feeding with PPP silage. In fact this silage presents a very high WSC content, approximately three times higher than that in PPS silage (Gannuscio et al., 2024b). Thus, it could be hypothesised that the sheep fed PPP silage had a lower appetite due to an increase in postprandial glycaemic levels. In addition, Morshedy et al. (2020) observed a reduction in feed intake among ewes fed diets supplemented with a higher level of dried PPP (10 g/head/d), which could be due to its high organic acid content, resulting in an acidic smell that may have negatively affected the voluntary intake of hay.

The lower DMI observed in ewes fed the PPP and PPS diets, which contained low-protein silage, likely resulted in lower CP intake. The ewes that were fed the PPS diet also had a lower CP intake than those that were fed the CTR diet, despite their similar DMI. This result was attributed to the replacement of the same amount of 247 g/kg CP concentrate with PPS silage at 95 g/kg CP. The DMI and CP intake of ewes fed the CTR diet resulted in a significantly higher NE_L intake than that of the other diets, which was found to be adequate as calculated by the INRA (2018). Despite this, ewes fed the CTR diet experienced BW loss, likely due to the partitioning of nutrients for milk production instead of body reserves.

Nevertheless, the increased milk production observed in ewes fed the CTR diet can be attributed to the higher consumption of dietary DM and CP, which enhance metabolic protein and allocate more energy to lactation (Daniel et al., 2016). In contrast, ewes fed the PPP and PPS diets exhibited increased BW but decreased daily milk production compared with those fed the CTR diet. The lower milk yields observed in ewes fed the PPP and PPS diets can also be attributed, in addition to the lower intake of CP and NE_L, to the effects of silage. In fact, Lahr et al. (1983) reported that cows fed high-moisture diets characterised by low pH or high levels of certain silage fermentation products could negatively influence milk yield.

Although ewes fed the CTR diet produced higher daily milk yields than those fed silage-based diets, the latter produced milk that was richer in nutrients. Consequently, the production of FPCM was not statistically different across the three diets in the present study.

The chemical composition of the milk showed slightly higher fat and protein percentages than the average values reported for the Valle del Belice breed (Todaro et al., 2023); however, this variation is likely attributable to the low milk production recorded in our study relative to the average production of this breed (Cappio-Borlino et al., 1997), although it could be linked to the distortions of the estimates due to the statistical model used. However, feeding the PPP silage-based diet improved the ewes' milk constituents compared with the CTR diet. The observed increase in milk protein (+4.3 g/kg) and casein (+4.1 g/kg) percentages may be due to the higher ratio of carbohydrates to dietary protein, (aNDFom + NFC) / CP, intake and probably also to bioactive compounds in PPP, particularly tannins (Tahir et al., 2019, Morshedy et al., 2020), which resulted in more efficient dietary protein utilisation (Lin et al., 2024). This effect is mainly attributed to the formation of protein complexes, which may have reduced the ruminal degradation of proteins and ultimately increased the supply of rumen-undegraded proteins to the small intestine (Natalello et al., 2020), which could promote an increase in milk protein yield (Grazziotin et al., 2020). This effect was also found in the milk of ewes fed the PPS diet; however, the effects were smaller, and the differences compared with the milk from ewes fed the CTR diet were not significant.

Therefore, it is possible to hypothesise that PPBs added to the diet of lactating ewes may increase milk nitrogen efficiency, which refers to the efficiency of conversion of dietary N into milk protein, thereby reducing the public pressure for sustainable livestock production (Huhtanen & Hristov, 2009).

A positive correlation exists between dietary CP intake and milk urea nitrogen (MUN) concentrations. Studies have shown that MUN linearly increases with higher dietary CP levels (Cannas et al., 1998). Consistent with these findings, the higher dietary CP intake observed with the CTR diet resulted in higher urea levels. Conversely, ewes fed the PPP and PPS diets, characterised by a significantly higher ingested fibre/CP ratio, demonstrated lower urea levels (an indirect indication of higher N utilisation efficiency) compared with those fed the CTR diet. This finding is consistent with studies in which prickly pear cladodes were administered to cows (de Albuquerque Saraiva et al., 2020, Maniaci et al., 2024).

Individual sheep milk samples were analysed for SCC and DSCC, which are important indicators of udder health. The absence of statistical differences between the milk samples of the ewes fed the three diets suggests the lack of effect of the diet. However, the mean SCC and DSCC values were lower than those reported in previous studies on individual samples of the Valle del Belice breed (Todaro et al., 2023, Tolone et al., 2023). Various factors, including diet, breed, lactation stage and management practices, can influence SCC (Tvarožková et al., 2019). In the present study, CNS were detected only in three samples, all of which were obtained from the same sheep throughout the three phases; notably, the CNS detected were *Staphylococcus hyicus* and *Staphylococcus epidermidis*, thereby suggesting no dietary impact.

The *in vivo* degradability of DM, OM and NDF varied between the diets, but significant differences were found between the PPS diet and the other two diets, with lower percentages. Similarly, the *in vitro* degradability of NDF and OM was lower in the PPS diet. The lower OM degradability of the PPS diet observed in the *in vivo* and *in vitro* trials was consistent with that reported by Vastolo et al. (2020) and probably due to the high abundance of seeds in the PPS by-product, which are only partially digestible. The indigestible fibre fraction (ADL = 267.7 g/kg DM) was higher in PPS silage than in other feeds used as diet ingredients. In contrast, the OM degradability of the PPP diet (estimated *in vivo* and *in vitro*) was 14%–27% higher than that of the PPS diet, with percentages comparable to those reported by Morshedy et al. (2020) using ewe diets enriched with 5% and 10% dried prickly pear peels (PPPs).

The high percentage of NFC detected in PPP silage may lead to increased rates of *in vitro* fermentation and higher gas production (OMCV) in the PPP diet, which is consistent with the findings of Nayel et al. (2023) and Adebayo et al. (2017). In our previous studies, PPP silage produced a higher OMCV than PPS silage (Vastolo et al., 2020, Gannuscio et al., 2024a), confirming that PPPs contain highly fermentable carbohydrates. The higher OMCV levels were detected in the PPP diet during the first 14 and 30 h of the *in vitro* fermentation test compared with the CTR and PPS diets, respectively. The PPP diet achieved the fastest fermentation rate after 8 h and a significantly higher maximum fermentation rate than the PPS diet. The fermentation characteristics of PPP silage were consistent with the *in vitro* results reported by Gannuscio et al. (2024a) and were probably due to the higher WSC content in this diet. Higher lactic acid (LA) content in PPP silage, approximately 15-fold higher than that in PPS silage (Gannuscio et al., 2024b), may have influenced microorganism activity and, consequently, the Rmax value of the PPP diet. In a previous study, LA infused into the sheep rumen at a single dose of 20–50 g was rapidly metabolised, with a half-life of approximately 25 min (Chamberlain et al., 1983).

WSCs can significantly influence ruminal fermentation and nutrient utilisation, whereas the WSC concentration in silage can be affected by various factors, including temperature, moisture levels, DM content, use of additives and duration of the ensiling or storage process (Ali and Tahir, 2021). Borges et al. (2023) reported that higher fibre content can decrease WSC and NFC, which is consistent with the current findings for the PPS diet. In fact, the PPS diet exhibited a lower NFC than the PPP diet, and probably also a lower WSC, due to the lower WSC content in PPS silage (11.0 g/kg) than in PPP silage (33.6 g/kg) (Gannuscio et al., 2024b).

In the gas production test, the higher molar proportion of VFAs in the PPP diet was probably determined by the higher contents of WSC and NFC in PPP silage, which are easily fermentable carbohydrates (Ben Salem et al., 1996, Wang et al., 2020). Furthermore, the reduction in ruminal N-NH_3 concentration in ovine diets supplemented with PPP was correlated with higher ruminal total VFA content, indicating increased microbial protein synthesis (Kholif et al., 2014). The observed increases in total VFA and acetate concentrations may also be attributed to increased cellulolytic bacterial activity in rumen (Morsy et al., 2015). In the present study, the significantly higher proportion of propionic acid in the PPP diet during the *in vitro* trial were likely influenced by the higher LA content in PPP silage (Gannuscio et al., 2024b). A previous study by Mackie et al. (1984) indicated that the succinate pathway is quantitatively more important for the conversion of LA to propionate. Similarly, Jaakkola and Huhtanen (1992) reported that propionate is the main end-product of LA fermentation in the rumen on a grass silage-based diet. Jaakkola calculated the metabolic fate of infused LA on a molar basis: 0.52 of LA was converted to propionate, 0.27 to butyrate and 0.21 to acetate (Jaakkola and Huhtanen, 1992).

Conclusion

In conclusion, PPP silage can be added to the diet of lactating ewes without any negative effects. The inclusion of 15.2% PPP silage on the DM basis in the diet of lactating ewes may have reduced DMI and CP intake, with a negative effect on daily milk yield but not on milk composition, which was improved, as evidenced by the non-different FPCM. In addition, the PPP silage-based diet increased the *in vitro* and *in vivo* OM degradability by up to 4% and the total VFA production by up to 11.5% compared with the CTR diet.

PPP silage can be used as an alternative feed for lactating ewes to reduce feeding costs, enhance sustainability in animal production and improve milk composition via modification of ruminal fermentation. On the contrary, the use of the PPS, for the high seed content, limits the possibility of preparing good-quality silage for ruminant feeding.

Further herd-level studies are recommended to exploit the most appropriate dose of PPP silage and the potential in sheep feed, including the possible enrichment of polyphenols and nutrients in milk and cheese.

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CRediT authorship contribution statement

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CRediT authorship contribution statement

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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Massimo Todaro reports financial support was provided by University of Tuscia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.