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Feeding behaviour, oxidative status, and milk production of dairy ewes fed fresh or dehydrated sulla (*Sulla coronaria* (L.) Medik.) forage

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ABSTRACT

This investigation aimed to explore utilisation of sulla as fresh or stored forage, and the improvement of forage stocks by dehydration as alternative to haymaking for reducing biomass and nutrients losses and preserving properties of bioactive components, mainly condensed tannins. In two experiments carried out in spring and autumn, the responses of Valle del Belìce ewes to different diets were evaluated for their feeding behaviour, oxidative status and milk production. In spring, 12 ewes were divided into two groups fed diets consisting of fresh forage of SULLA or BARLEY *ad libitum* according to a 2 × 2 Latin square design (LSD). In autumn, pellets of dehydrated sulla forage (DSF) cut in April or May were compared with sulla hay (SH); a 3 × 3 LSD involved 9 ewes, divided into three groups fed the diets: A-DSF, 2 kg/day of April pellets and SH *ad libitum*; M-DSF, 2 kg/day of May pellets and SH *ad libitum*; SHL, SH *ad libitum*. Concentrate feed was provided at 600 (spring) or 800 g/day (autumn). Ewes fed SULLA significantly improved milk yield, milk casein, efficiency of DM and energy conversion to milk and antioxidant balance than BARLEY ewes. Both DSF diets significantly increased feed intake, DM and energy digestibility, and milk yield, whereas A-DSF induced a higher plasma free polyphenols level. These results confirm the potential of sulla as fresh or dehydrated forage in small ruminants' feeding, and evidence how dehydration can represent an alternative to haymaking, ensuring ewes' health and dairy production in periods of unavailable fresh forage.

HIGHLIGHTS

- Feeding fresh sulla forage containing condensed tannins confirmed its positive impact on ewes' oxidative status and dairy production.
- Dehydration represents a valid alternative to haymaking in preserving nutrients and bioactive molecules of fresh sulla.
- Ewes fed dehydrated sulla forage showed higher feeding efficiency and milk yield than ewes fed hay.

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Dehydrated pellets; condensed tannins; feed intake; digestibility; oxidative balance

Introduction

The use of high-quality forages for feeding is a key element in promoting health, welfare, and production performance of livestock. The forage of *Sulla coronaria* (L.) Medik., also known as *Hedysarum coronarium* (L.), is an example of a resource recognised for its positive impact on ruminants' performance and the quality of their meat (Priolo et al. 2005; Bonanno et al. 2011) and dairy products (Bonanno et al. 2016; Ponte et al. 2022).

Sulla is a biennial forage legume native to Mediterranean regions, where is commonly included in cereal-based rotations. This forage species is widely established in areas where climatic and soil conditions favour its adaptability and cultivation, such as central and southern Italy, Iberian Peninsula, North America, Australia and New Zealand. Botanically, sulla develops an upright habit with substantial above-ground biomass and a deep, vigorous root system, traits that confer drought resilience and enable effective symbiotic nitrogen fixation that can improve soil fertility (Ruisi et al. 2011; Piccirillo et al. 2025). In Mediterranean environments, sulla can provide a substantial biomass, ranging from 5 to 12 t/ha of dry

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matter (DM) in relation to year of crop and plants phenological phase (Amato et al. 2016). In the same areas, especially south of Italy, sulla is highly exploited for its advantageous agronomic and compositional features, as well as for its suitability to multiple forage uses, such as grazing, hay or dehydration, and silage; however, it is commonly utilised as hay obtained by forage mowed in spring, and as fresh forage by grazing its regrowths in autumn and winter (Piccirillo et al. 2025).

The nutritional value of this legume species for ruminants depends on its phenological phase and is largely due to its good protein content (ranging from 25% to 12% DM), relative low fibre level (neutral detergent fibre (NDF) ranging from 23% to 48% DM) and favourable balance between non-structural and structural carbohydrates (Burke et al. 2004; Gannuscio et al. 2022). Moreover, the benefits of feeding ruminants fresh sulla forage (FSF) are linked to its content of molecules with antioxidant properties, such as carotenoids, tocopherols, and polyphenols (Ponte et al. 2022; Rufino-Moya et al. 2022), which may sustain the antioxidant defences of animals and, being partially transferred and deposited in animal tissues and milk, enhance the oxidative stability of their products (Di Trana et al. 2015; Soldado et al. 2021).

Among polyphenols of sulla forage, the most represented are the condensed tannins (CT), mainly contained in leaf blades and flowers (Tibe et al. 2011; Piluzza et al. 2014; Tava et al. 2021); being present in the whole plant DM at moderate concentrations ($<$ 55 g/kg DM) (Min et al. 2003), the CT from sulla forage do not exhibit antinutritional effects but result beneficial for animals, their products and the environment. In dairy ruminants, CT intake seems to support animal health, improve their milk yield, the quality of products, other than the environmental sustainability of the production process (Mueller-Harvey et al. 2019).

In this regard, the CT from FSF have been shown to reduce the presence of nematodes responsible for gastrointestinal parasitic infestations in small ruminants (Hoste et al. 2006). Moreover, due to the antioxidant capacity of compounds derived from their rumen degradation, that are moved to various tissues by crossing the intestinal barrier (Soldado et al. 2021), the CT contribute to improving plasma oxidative status, immune defences and thermo-tolerance of animals (Gladine et al. 2007; Di Trana et al. 2015; Soldado et al. 2021) and, once their metabolites are transferred into the milk, enhancing the derived dairy products concerning their technological traits, antioxidant capacity, oxidative stability, and health benefits for consumers (Di Trana et al. 2015; Ponte et al. 2022).

Moreover, the ability of CT to modulate ruminal fermentations contributes to reducing methanogenesis, methane emissions, and thus the environmental impact of ruminant livestocks (Piluzza et al. 2014; Mueller-Harvey et al. 2019; Vasta et al. 2019). In the rumen, CT form complexes with dietary proteins, limiting their degradation and increasing the intestinal absorption of amino acids (Makkar 2003; Min et al. 2003; Piluzza et al. 2014) thereby contributing to enhancing the milk casein synthesis (Gannuscio et al. 2022) and reducing the nitrogen excretion into the environment (Addis et al. 2005; Molle et al. 2009; Mueller-Harvey et al. 2019). The CT also protect dietary polyunsaturated fatty acids (PUFA) from ruminal biohydrogenation, favouring their transfer into dairy products that improve their nutritional and health properties (Frutos et al. 2020; Ponte et al. 2022).

Although the potential benefits of FSF are already known, the role of its phenological stage and storage method in preserving the presence of nutrients and bioactive molecules in forage stocks and related dairy products remains underexplored. In a recent investigation (Gannuscio et al. 2022), dehydration and pelleting of FSF proved to be a promising alternative to haymaking for obtaining dried forages with higher nutritional value than hay, as the process resulted in smaller reductions in the content of protein, PUFA, vitamin E, and polyphenols than those observed in the hay.

Thus, following previous preliminary investigations (Gannuscio et al. 2022; Ponte et al. 2022), this study aimed to further evaluate the effects of pelleted dehydrated sulla forage (DSF) included as an alternative to hay in the diet of dairy ewes, introducing as new determinant factor the effect of phenological stage when the FSF was cut before processing.

Two experiments were conducted in two different seasons. The first experiment was carried out in spring, when FSF is widely available and commonly exploited by ewes through grazing; it aimed to compare the physiological and productive responses of dairy ewes fed FSF or with another green resource available in the same season, such as fresh barley forage. The second experiment was conducted in autumn when, due to the limited availability of green forage, the main forage component of the ewes' diet generally consists of hay as stored resource; it aimed to compare diets based on dried sulla forage, either processed as hay or obtained through dehydration in two successive phenological phases of the plants. In both

experiments, the responses of dairy ewes were evaluated in terms of feed intake, digestibility, oxidative balance, milk yield, and quality, with the aim to confirm the positive effects of feeding FSF and explore the implications of cutting time on DSF nutritive value and the related ewes' productive efficiency.

The effects of the experimental diets on microbiological, nutritional and sensory properties of manufactured sheep cheeses, including their fatty acid profile, are the subject of another article (Ponte et al. 2026).

The overall results could support the sulla crop management, in terms of cutting time and drying process, and contribute to a larger and better exploit of sulla forage in usual or new cultivation environments.

Materials and methods

Animals, experimental design and feeding treatments

Two experiments were carried out during different seasons, in spring (April-May) and autumn (November-December) at a sheep farm located in Santa Margherita di Belìce (Agrigento, Sicily, Italy). Ewes of the Valle del Belìce breed with a parity higher than two lambings were confined indoors into single-wide straw-bedded pens equipped with feeders and water troughs.

Spring and autumn experiments were conducted for 40 and 55 days, respectively, using a Latin square experimental design.

The experimental protocols were approved by the Animal Welfare Body (OPBA) of the University of Palermo (2021–UNPA CLE-0059470), which found the deemed requirements established by the Italian Legislative Decree No. 26/2014, implementing the Directive 2010/63/EU, as not applicable.

In spring, 12 ewes at 70 days in milk were homogeneously divided into two groups based on live weight (LW) (53.6 ± 5.9 kg) and milk yield (2213 ± 277 g/day). After 10 days of adaptation to the new housing condition, the experimental groups were fed diets based on FSF of the Avorio population (SULLA) or fresh barley forage (BARLEY), both provided *ad libitum*, according to a 2 × 2 Latin square (two groups of 6 ewes and two experimental phases of 15 days). Fresh forages were mowed daily in the morning from close crops and, after being roughly cut, supplied in the feeder in two meals provided in the morning and afternoon in amounts ensuring sufficient refusals. Both diets were integrated with 600 g/day per head of a commercial concentrate feed divided into two meals supplied after morning (7:00) and afternoon (15:00) milking.

In the autumn experiment, sulla hay (SH) was compared with DSF in pellets obtained from FSF of the Avorio population cut and sampled at two different times, April and May, corresponding to the phenological phases of partial and full flowering, in order to differentiate their botanical and chemical composition.

To produce the DSF pellets, dehydration was carried out using a small pilot-plant consisting of a container into which hot air at a temperature always below 55 °C, generated by a diesel-powered system, was conveyed. After harvesting, the FSF was immediately cut into 3–4 cm pieces; then, for each drying turn, a forage amount of about 400 kg was placed on mesh platforms mounted on trolleys to be introduced into the container where the mass was dried for 18–24 h, until reaching approximately 15% humidity. Successively, the dehydrated forage was ground and passed through a small pelleting machine.

In the autumn experiment, nine ewes at 60 days in milk were divided into three homogeneous groups for live weight (52.3 ± 3.4 kg) and milk yield (1812 ± 223 g/day). After a 10-day period of adaptation, the ewes were fed, according to a 3 × 3 Latin square design (three groups of 3 ewes and three experimental phases of 15 days), with the three following diets: A-DSF, 2 kg/day per ewe of April pellets and SH *ad libitum*; M-DSF, 2 kg/day per ewe of May pellets and SH *ad libitum*; SHL, SH *ad libitum*.

The diets were supplemented with 800 g/day per head of commercial concentrate feed. The daily amounts of DSF, SH, and concentrate feed were divided into two meals offered after morning (7:00) and afternoon milking (15:00), ensuring enough refusals of SH, being provided *ad libitum*.

Measurements and sampling

In both experiments, measurements and sampling related to feeding resources and ewes were carried out during the last 5 days of each 15-day experimental phase, after an initial 10-day period of adaptation to the new diets.

Ewes were monitored for their LW and body condition score (BCS) at the start and end of each experimental phase. Offered feeds and the corresponding refused amounts were weighed daily for each ewe to calculate feed intake, and sampled twice during each phase to be analysed and estimate the nutrients intake. Fresh forages were frozen at −20 °C to be lyophilised before analyses.

Individual milk yield was recorded daily during morning and afternoon hand milking, and sampled

twice in the last two days of each phase to be stored at 4 °C until analyses.

Blood and faeces were collected before the morning feeding from each fasting ewe at the end of each experimental phase. To assess the ewes' oxidative status, blood was sampled by jugular venipuncture filling two 9 mL vacutainer tubes, one containing lithium heparin to obtain plasma, and the other without anticoagulant for serum. Samples to obtain plasma were immediately placed on ice and then centrifuged at 3000 rpm for 10 min at 4 °C, while samples to obtain serum were kept at room temperature until clotting, and then centrifuged at 3000 rpm for 7 min. Both plasma and serum samples were stored at −80 °C until analyses. To estimate nutrients digestibility, individual ewes' faeces samples (greater than 100 g) were collected directly from the rectum, and frozen at −20 °C to be freeze-dried for subsequent analyses.

Analyses of feeding resources and faeces

Collected samples of pre dehydration FSF, offered and refused forages, concentrate feeds and freeze-dried faeces were analysed according to AOAC methods (2005) for DM (method 934.01), crude protein (CP, N x 6.25) (method 2001.11), ether extract (EE, method 920.39) and ash (method 942. 05). The methods of AOAC International (2005) and Van Soest et al. (1991) were used to determine the fibrous fractions, such as neutral detergent fibre using thermostable amylase and excluding residual ash (aNDFom) (AOAC method 2002.04), ash-free acid detergent fibre (ADFom) (AOAC method 973.18), acid detergent lignin (ADL) (AOAC method 973.18), and calculate lignin acid insoluble ash (AIA), cellulose and hemicellulose contents. The percentage of non-structural carbohydrates (NSC) was obtained as follows: NSC % = 100 – CP% – EE% – ash% – aNDFom%. Gross energy (GE, kcal/kg DM) of faeces, GE and net energy for lactation (NEL, kcal/kg DM) of diets components were estimated according to INRA (2018).

Vitamin E (α-tocopherol) in dried or freeze-dried forage samples was determined in duplicate following the extraction and reversed-phase HPLC methods reported by Panfili et al. (1994) and Manzi et al. (1996) with modifications described by Gannuscio et al. (2022).

Extracts of the feeding resources were prepared according to Gannuscio et al. (2022) to determine in duplicate the content in total polyphenols (g gallic acid equivalent (GAE)/kg DM) using the Folin-Ciocalteau colorimetric method (ISO (International Organization for Standardization) 2005), CT (g delphinidin equivalent (DE)/kg DM) (Tava et al. 2021), using the butanol-HCl assay (Porter et al. 1985), and TEAC (Trolox equivalent antioxidant capacity) (mmol trolox/kg DM) according to Re et al. (1999); the absorbance of the samples was read using a HACH DR3900 spectrophotometer (Hach, Loveland, CO, USA) at 725, 550 and 734 nm for polyphenols, CT and TEAC, respectively.

The fatty acid (FA) profile of freeze-dried forages, hay and concentrate samples (50 mg) was determined using the one-step extraction and transesterification procedure; each FA was identified by comparing its retention time to those of a solution of FAME in hexane (Nu Check-Prep, Elysian, MN, USA), quantified using C23:0 (Sigma-Aldrich, Milan, Italy) as internal standard at a concentration of 0.4 mg/g sample, and expressed as g/kg DM.

Analyses for determining DM, CP, aNDFom, ADL and AIA were performed on freeze-dried faeces samples as described for feeding resources; to estimate the nutrients digestibility the AIA content in the faeces as an internal marker were used, according to Sunvold and Cochran (1991). The nitrogen retained (%) was estimated on the basis of the nitrogen eliminated with faeces and excreted with milk, as follows: (Nitrogen intake − (Nitrogen faeces + Nitrogen milk))/nitrogen intake × 100.

Analyses of plasma

Plasma oxidants were measured as reactive oxygen metabolites (ROMs, expressed as Unit Carr), which especially consisted of hydroperoxides generated by the oxidation of biomolecules. Antioxidant capacity was evaluated as biological antioxidant potential (BAP, mmol/L reduced iron equivalent), measuring the capacity of plasma to reduce iron from ferric (Fe^{3+}) to ferrous (Fe^{2+}) form through specific kits from Diacron (Grosseto, Italy), and using the ferric ion reducing antioxidant power (FRAP) assay (Benzie and Strain 1996).

The oxidative stress index (OSI) was calculated as the ratio ROMs/BAP proposed by ranade et al. (2014), as well as the ratio ROMs/FRAP. Plasma total polyphenols (PTP) and free polyphenols (PFreeP), both expressed as g GAE/mL, were determined using the Folin-Ciocalteau colorimetric method (ISO (International Organization for Standardization) 2005) after extraction performed following the procedure described by Serafini et al. (1998) for PTP, and by Santiago-Arteche et al. (2012) for PFreeP. Serum non-

esterified fatty acids (NEFA) were analysed using the commercial kit FA 115 (Randox Laboratories) according to the manufacturer's instructions.

Milk chemical composition and physical traits

During each experimental phase, samples of daily ewes' individual milk were collected, stored at 4 °C and analysed within 24 h after sampling for lactose, fat, urea and somatic cell count (SCC) by infra-red spectroscopy (Combi-foss 6000, Foss Electric, Hillerød, Denmark), pH by pH-meter HI 9025 (Hanna Instruments Inc., MI, USA), and titratable acidity by Soxhlet-Henkel method (°SH/50 mL). Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined following the standard FIL-IDF procedures (FIL-IDF 29 International Standard 1964; FIL-IDF 20B International Standard 1993) and used to calculate total protein (TN × 6.38), casein [TN-(NCN × 0.994) × 6.38] and whey protein [(NCN-NPN) × 6.38]. Fat- and protein-corrected milk yield (FPCM, to 6.5% fat and 5.8% protein) was calculated according to Pulina and Nudda (2004) as follows: FPCM g/day = milk yield g/day × (0.25 + 0.085%fat + 0.035%protein).

Individual milk samples were also evaluated for their clotting ability by a Formagraph instrument (Foss Electric), measuring coagulation time (r, min), curd-firming time (k_{20}, min), and curd firmness at 30 min (a_{30}, mm) on 10 mL of milk heated to 35 °C after addition of 0.2 mL of a diluted solution (1.6:100) of rennet (1:15,000; Chr. Hansen, Parma, Italy).

Statistical analysis

The SAS 9.2 software (SAS (Statistical Analysis Systems Institute) 2010) was used to statistically analyse the individual ewes' parameters according to a MIXED model in which experimental phase (2 levels in Spring and 3 levels in Autumn) and diet (2 levels in Spring = SULLA and BARLEY; 3 levels in Autumn = A-DSF, M-DSF, and SHL) were treated as fixed factors, and the ewe was considered a random factor and used as error term; an unstructured covariance was assumed on the basis of the lowest Akaike Information Criterion (AIC). Before analysis, somatic cell count (SCC) values were transformed into logarithmic form (log10). The Tukey-Kramer multiple comparison test with adjusted P-values was used for means comparisons. Significance was declared for $p \leq 0.05$, and tendency was declared until $p \leq 0.10$. In both experiments, the Latin square design provided adequate statistical power assessed for the main variables by a post-hoc power analysis using the G*Power software.

Results and discussion

Feeding resources

Table 1 reports the botanical disaggregation and the chemical and FA composition of the entire fresh plant of sulla and barley forages and concentrate feed offered to ewes in the spring experiment, as well as those of sulla leaves and flowers. Compared to fresh barley forage, the plants of FSF were lower in DM and higher in protein, especially due to the good incidence (30.9% DM) of the high-protein leaves (27.8% DM), whereas they were lower in fibre (aNDFom), although this fraction resulted in higher ADL. Regarding antioxidant compounds, FSF was richer in polyphenols, mostly represented by CT, as expected; consequently, it recorded a higher antioxidant capacity (TEAC) than barley forage, although this latter was higher in vitamin E, in line with the superior levels generally detected in grasses than in legumes (Danielsson et al. 2008).

The FA profile of FSF was characterised by major levels of α-linolenic acid (ALA, C18:3 n-3) and stearic acid (C18:0), and lower amounts of oleic acid (OLA, C18:1 *c9*) and linoleic acid (LA, C18:2 n-6). Analogous differences were observed by Bonanno et al. (2016) when compared grazing forage of sulla with that of another graminaceous species such as annual ryegrass.

Table 2 shows the chemical composition and FA profile of the feeding resources used in the autumn experiment, such as April and May pelleted DSF (A-DSF and M-DSF), SH, and the concentrate feed. Table 2 also includes the composition of FSF obtained from the April and May cuts, which was used to produce the corresponding A-DSF and M-DSF pellets, to assess the changes induced by the dehydration process.

The comparison between the pre- and post-treated sulla forages reveals how dehydration led to a reduction of protein content by about 1% in both fresh forage cuts, an increase in aNDFom of 1% in A-DSF pellets and 5% in M-DSF pellets, and an increase in ash in both pellets. These changes can mainly be associated with the inevitable loss of leaves during dehydration process, from forage harvesting to pelleting. The vitamin E increased in both pellets compared to the original forage, presumably due to the higher incidence of flowers, which are richer in α-tocopherol (Maxin et al. 2020), as consequence of the leaves' loss;

Table 1. Botanical components (% DM), chemical composition (% DM) and fatty acid profile (g/kg DM) of forages and concentrate feed received by ewes in spring experiment.

	Fresh sulla forage (FSF)				
	entire plant	leaves	flowers	Fresh barley forage	Concentrate feed
Flowers/ears	14.82			31.66	
Leaves	30.85			13.04	
Stems/culms	46.21			44.01	
Dry parts and weeds	8.12			11.30	
DM, %	15.32	17.40	18.10	32.88	90.89
Crude protein	16.82	27.80	21.44	8.63	20.94
Ether extract	2.43	3.95	2.95	2.04	4.40
Ash	11.11	14.07	8.12	8.28	7.34
aNDFom	40.45	22.50	32.12	58.06	20.35
ADFom	37.02	21.15	27.58	35.87	8.95
ADL	7.32	6.00	4.79	4.05	0.81
AIA	0.368	0.458	0.288	2.24	1.26
Cellulose	29.70	15.15	22.79	31.82	8.14
Hemicellulose	3.67	1.50	4.77	21.12	10.57
Non-structural carbohydrates	28.58	31.07	34.85	21.81	46.55
NEL, kcal/kg DM	1197	1863	1662	1312	2013
Vitamin E, mg/kg DM	11.91			17.53	–
Condensed tannins, g DE/kg DM	15.22	26.77	27.73	1.50	2.60
Polyphenols, g GAE/kg DM	20.16	37.33	35.96	7.22	10.85
TEAC, mmol trolox/kg DM	98.73	148.77	153.77	47.13	76.71
C12:0	0.153			0.081	0.061
C14:0	0.166			0.303	0.232
C16:0	4.88			4.12	12.78
C18:0	0.959			0.578	1.47
C18:1 c9	0.797			1.56	13.77
C18:2 n-6	1.91			3.46	11.03
C18:3 n-3	7.58			5.61	0.507

Abbreviations: ADFom = ash-free acid detergent fibre; ADL = acid detergent lignin; AIA = lignin acid insoluble ash; aNDFom = neutral detergent fibre using thermostable amylase and excluding residual ash; DE = delphinidin equivalent; DM = dry matter; GAE = gallic acid equivalent; NEL = net energy for lactation according to INRA (2018); TEAC = trolox equivalent antioxidant capacity.

Table 2. Chemical composition (% DM) and fatty acid profile (g/kg DM) of forages and concentrate feed received by ewes in autumn experiment.

	April pelleted dehydrated sulla forage		May pelleted dehydrated sulla forage			
	Pre dehydration	Post dehydration and pelleting	Pre dehydration	Post dehydration and pelleting	Sulla hay	Concentrate feed
DM, %	14.51	86.65	19.50	87.76	90.24	89.94
Crude protein	16.17	15.07	12.03	11.26	11.79	22.40
Ether extract	2.45	2.32	2.50	2.65	1.39	4.30
Ash	10.89	14.45	10.21	12.38	11.52	7.93
aNDFom	36.63	37.45	46.28	50.99	53.80	17.89
ADFom	33.33	30.38	34.96	40.05	42.11	10.08
ADL	5.26	5.31	5.25	7.17	7.88	0.86
AIA	0.244	1.61	0.115	1.22	0.820	1.64
Cellulose	28.07	25.06	29.72	32.88	34.23	9.22
Hemicellulose	3.47	7.51	11.70	11.25	11.86	7.70
Non-structural carbohydrates	33.45	28.67	28.49	21.20	20.52	45.95
NEL, kcal/kg DM	1370	1310	1308	1009	941	1992
Vitamin E, mg/kg DM	11.84	14.73	16.95	20.36	11.65	–
Condensed tannins, g DE/kg DM	15.80	7.17	9.20	4.83	4.60	2.56
Polyphenols, g GAE/kg DM	18.19	13.57	11.62	10.03	9.72	10.86
TEAC, mmol trolox/kg DM	104.94	95.74	84.88	77.13	61.69	78.20
C12:0	0.103	0.115	0.000	0.036	0.084	0.061
C14:0	0.208	0.178	0.183	0.211	0.195	0.232
C16:0	5.79	5.60	4.41	4.70	3.65	12.78
C18:0	0.790	0.733	0.751	0.794	0.677	1.469
C18:1 c9	1.03	0.945	1.69	1.91	0.804	13.77
C18:2 n-6	3.75	2.99	4.78	4.05	1.48	11.03
C18:3 n-3	11.40	10.52	6.89	6.77	0.968	0.507

Abbreviations: ADFom = ash-free acid detergent fibre; ADL = acid detergent lignin; AIA = lignin acid insoluble ash; aNDFom = neutral detergent fibre using thermostable amylase and excluding residual ash; DE = delphinidin equivalent; DM = dry matter; GAE = gallic acid equivalent; NEL = net energy for lactation according to INRA (2018); TEAC = trolox equivalent antioxidant capacity.

Table 3. Effect of diet on oxidative status biomarkers, plasma polyphenols and NEFA in ewes.

	Spring experiment				Autumn experiment				
	Spring diets				Autumn diets				
	SULLA[1]	BARLEY[2]	SEM	*P*-value	A-DSF[3]	M-DSF[4]	SHL[5]	SEM	*P*-value
ROMs, Unit Carr	131.57	195.87	10.14	0.0004	150.82	136.50	123.53	10.01	0.1859
BAP, µmol/L	3329	3566	105.83	0.1211	3972	3810	3709	93.11	0.1579
OSI, ROMs/BAP*100	4.09	5.56	0.344	0.0041	3.80	3.60	3.36	0.265	0.5251
FRAP, µmol/L	309.32	253.49	14.25	0.0081	292.72	308.65	317.87	9.97	0.2238
OSI, ROMs/FRAP*10	4.38	7.89	0.440	<0.0001	5.65	4.50	3.90	0.499	0.0576
PTP, µg GAE/mL	22.08	21.32	1.50	0.7208	25.94	21.85	19.26	3.21	0.0773
PFreeP, µg GAE/mL	11.03	8.48	0.356	<0.0001	13.43[a]	13.02[ab]	11.46[b]	0.795	0.0429
PCP, µg GAE/mL	10.98	12.83	1.55	0.4022	13.91	6.06	7.14	3.51	0.2836
NEFA, mmol/L	0.054	0.091	0.011	0.0225	0.069	0.062	0.081	0.015	0.6458

Abbreviations: BAP = biological antioxidant potential; FRAP = ferric reducing antioxidant power; GAE = gallic acid equivalent; NEFA = non-esterified fatty acids; OSI = oxidative stress index; PCP = plasma conjugated polyphenols; PFreeP = plasma free polyphenols; PTP = plasma total polyphenols; ROMs = reactive oxigen metabolites; SEM = standard error of mean.
[1]Fresh sulla forage.
[2]Fresh barley forage.
[3]2 kg/day dehydrated sulla forage from April cut.
[4]2 kg/day dehydrated sulla forage from May cut.
[5]Sulla hay ad libitum.
[a,b]Values within a row with different superscripts differ significantly at $p < 0.05$.

however, this trend highlights the lower susceptibility of vitamin E to the heat temperatures of dehydration and pelleting, confirming the previous finding (Gannuscio et al. 2022). Moreover, the vitamin E showed an evident increase in May forage compared to April, linked to the advanced development stage of plants, as also observed in alfalfa and sainfoin (Maxin et al. 2020).

In contrast, as a consequence of dehydration and pelleting, CT were greatly reduced, practically halved, in both cuts. At the same time, the reduction in total polyphenols and antioxidant capacity (TEAC) was less consistent. In this regard, recent studies (Nitasha Thakur et al. 2020) have highlighted dehydration's ability to increase polyphenols' bio-accessibility. Based on this, it could be hypothesised that the DSF contains transformation products of CT that are more bioavailable and analytically counted among polyphenols. Additionally, the LA and ALA content of FSF forage showed only negligible variations attributable to the dehydration process, as also observed by Gannuscio et al. (2022).

The chemical composition of SH used in the autumn experiment was comparable to that of the pellets produced from the forage of May cut. Both hay and pellets from May DSF exhibited higher fibre content and lower levels of protein and polyphenolic molecules compared to the earlier April DSF pellets. Moreover, vitamin E, LA and ALA in SH were lower than in both pellets, probably due to the higher loss of both leaves and flowers, rich in α-tocopherol and PUFA, occurring during haymaking, in line with Rufino-Moya et al. (2022).

Overall, these results suggest that dehydration maintains almost unchanged protein and fibre levels in the forage and preserves the presence of its nutritional and bioactive components, such as polyphenols, vitamin E and PUFA, that are important for animals' productivity and the health properties of their dairy products. Therefore, the results support the effectiveness of dehydrating FSF, especially when it is harvested at an earlier development stage, which ensures the highest concentration of nutrients.

Oxidative status of ewes

Table 3 shows the effects of the diets on plasma parameters linked to the nutrition, welfare, and health status of ewes, such as oxidative biomarkers, plasma polyphenols, and NEFA.

Overall, compared to the BARLEY diet, the SULLA diet increased the antioxidant defences of ewes, potentially contributing to their enhanced welfare and health conditions. Indeed, the effect of SULLA was evident in the reduction by 33% ($p = 0.0004$) of plasma oxidant metabolite level, expressed as ROMs, as well as in the increase by 22% of antioxidant capacity when measured as antioxidant power (FRAP, $p = 0.0081$).

This result could be related to the higher levels of plasma free polyphenols (Table 3), likely derived from the metabolic action exerted by the rumen microflora on the ingested amounts of total polyphenols and CT present in sulla (Tables 1 and 2), recognised for their antioxidant activity (Tava et al. 2021). The action of the rumen and intestinal microbiota influences the bioavailability of polyphenols (Bravo 1998). It is known that the bacterial population modulates the ruminal degradation and intestinal absorption of metabolites

Table 4. Effect of diet on live weight variation, feed intake, and digestibility.

	Spring experiment				Autumn experiment				
	Spring diets				Autumn diets				
	SULLA[1]	BARLEY[2]	SEM	*P*-value	A-DSF[3]	M-DSF[4]	SHL[5]	SEM	*P*-value
Initial LW, kg	55.16	55.10	1.78	0.8827	53.73	53.59	54.06	1.14	0.6716
Final LW, kg	53.72	52.81	1.76	0.0062	54.91	55.47	54.89	1.12	0.3992
Weight gain, kg	−1.44	−2.29	0.355	0.1212	1.18	1.88	0.83	0.489	0.3346
Initial BCS	3.56	3.54	0.060	0.7342	3.25	3.22	3.22	0.163	0.9412
Final BCS	3.42	3.38	0.088	0.7134	3.44	3.36	3.42	0.170	0.6680
BCS variation	−0.146	−0.167	0.087	0.8690	0.194	0.139	0.194	0.090	0.8819
Total DM intake, g/day	2416	2439	78.85	0.5533	3591^{a}	3596^{a}	2963^{b}	88.17	<0.0001
Total DM intake, g/day per kg LW$^{0.75}$	122.86	124.67	4.67	0.3869	178.40^{a}	177.46^{a}	147.32^{b}	5.20	<0.0001
Forage DM intake, g/day	1870	1894	78.85	0.5533	2872^{a}	2876^{a}	2244^{b}	88.17	<0.0001
Concentrate, % total DM intake	22.74	24.46	0.957	0.0010	20.28^{b}	20.54^{b}	24.89^{a}	0.678	<0.0001
Total crude protein intake, g/day	486.66	289.91	9.41	<0.0001	626.54^{a}	562.19^{b}	495.02^{c}	10.27	<0.0001
Total aNDFom intake, g/day	734.53	1199.42	60.96	<0.0001	1272^{b}	1539^{a}	1240^{b}	56.02	<0.0001
Total energy intake, kcal NEL/day	3702	3606	113.43	0.0002	5105^{a}	4494^{b}	3844^{c}	86.78	<0.0001
Condensed tannins intake, g DE/day	36.15	4.18	0.150	<0.0001	19.69^{a}	17.65^{b}	17.30^{b}	0.793	0.0032
Polyphenols intake, g GAE/day	51.39	20.16	0.848	<0.0001	43.31^{a}	39.29^{b}	34.55^{c}	0.996	<0.0001
DM digestibility, %	74.03	68.30	0.954	<0.0001	75.27^{a}	69.60^{b}	67.44^{c}	0.713	<0.0001
Organic matter digestibility, %	75.42	69.56	0.979	<0.0001	76.89^{a}	70.37 a^{b}	68.81^{b}	0.687	<0.0001
Crude protein digestibility, %	71.92	66.29	0.980	<0.0001	74.35^{a}	70.65^{b}	67.69^{c}	1.16	<0.0001
Nitrogen retained, % nitrogen intake	53.06	44.14	1.02	<0.0001	59.08^{a}	54.78^{b}	52.30^{c}	1.34	<0.0001
aNDFom digestibility, %	56.99	57.81	1.30	0.3738	65.41^{a}	58.86^{b}	53.77^{c}	1.49	<0.0001
Energy digestibility, %	74.67	68.46	1.01	<0.0001	76.26^{a}	69.81^{b}	68.00^{c}	0.707	<0.0001

Abbreviations: aNDFom = neutral detergent fibre using thermostable amylase and excluding residual ash; BCS = body condition score; DE = delphinidin equivalent; DM = dry matter; GAE = gallic acid equivalent; LW = live weight; NEL = net energy for lactation according to INRA (2018); SEM = standard error of mean.
[1]Fresh sulla forage.
[2] Fresh barley forage.
[3]2 kg/day dehydrated sulla forage from April cut.
[4]2 kg/day dehydrated sulla forage from May cut.
[5]Sulla hay ad libitum.
[a,b,c]Values within a row with different superscripts differ significantly at $p < 0.05$.

derived from dietary phenolic compounds (Tufarelli et al. 2017; Olagaray and Bradford 2019). In this regard, the contribution of the antioxidant power of vitamin E has to be excluded since its level was higher in barley than in sulla forage (Table 1). These trends led to a favourable significant decrease in OSI for the ewes receiving SULLA compared to those fed BARLEY. Additionally, the SULLA diet contributed to a significant reduction in the plasma level of NEFA, indicating a lower lipomobilization, and thus a better nutritional status in ewes fed SULLA.

In autumn, plasma analyses revealed that the diet did not significantly influence NEFA, as well as the ewes' oxidative status in terms of ROMs, BAP and FRAP. While the A-DSF diet led to a tending increase in OSI ($p = 0.0576$) expressed as ROMs/FRAP ratio, and enhanced the plasma free polyphenols ($p = 0.0429$), that also contributed to a tendency for higher total polyphenols ($p < 0.0773$). Nevertheless, these major levels did not result in a significant improvement in antioxidant capacity (BAP and FRAP) or a reduction in OSI in ewes fed A-DSF; this condition can mainly be attributed to the formation of ROMs that, although not significantly higher, were probably linked to the metabolic stress due to higher milk yield, which was not fully balanced by the antioxidant protection provided by the higher intake of polyphenols and vitamin E. On the other hand, both pellet-based diets tended to increase the OSI obtained using FRAP ($p = 0.0576$), as a measure of the antioxidant capacity. This result contrasts with that of Gannuscio et al. (2022), who found a lower OSI in ewes receiving 2 kg/day of DSF pellets than in ewes fed hay, attributed to the effective antioxidant action of vitamin E and CT metabolites present in higher significant levels in DSF than in hay; the higher content of vitamin E recorded in the hay used in this experiment compared to that reported by Gannuscio et al. (2022) (11.6 vs 5.0 mg/kg DM) could explain this opposite trend.

Feed utilisation

Table 4 reports the results related to the effect of diets on ewes' LW, BCS, feed intake, and digestibility.

During spring experiment, both groups reduced their LW, with a lesser extent in ewes of the SULLA group, which showed a significantly higher final LW ($p = 0.0062$) than those of the BARLEY group; however, the slight difference (+1 kg) did not correspond to a significantly improved BCS.

Table 5. Effect of diet on individual milk yield and composition.

	Spring experiment				Autumn experiment				
	Spring diets				Autumn diets				
	SULLA[1]	BARLEY[2]	SEM	*P*-value	A-DSF[3]	M-DSF[4]	SHL[5]	SEM	*P*-value
Milk yield, g/day	1777	1317	52.23	<0.0001	1667[a]	1557[b]	1308[c]	56.92	<0.0001
FPCM, g/day	1612	1232	41.02	<0.0001	1709[a]	1669[a]	1436[b]	65.80	<0.0001
FPCM/DM intake, kg/kg	0.670	0.523	0.017	<0.0001	0.478	0.472	0.496	0.017	0.3356
FPCM/NEL intake, kg/Mcal	437.91	372.22	11.85	<0.0001	334.52[b]	375.48[a]	378.67[a]	11.78	<0.0001
Milk nitrogen/nitrogen intake	0.189	0.221	0.006	<0.0001	0.153	0.159	0.154	0.004	0.3029
Somatic cells count, log_{10} n/mL	5.75	5.93	0.169	0.2296	5.23	5.17	5.08	0.152	0.4667
Lactose, %	4.58	4.59	0.048	0.7028	4.62[a]	4.61[a]	4.54[b]	0.054	0.0011
Fat, %	5.62	6.09	0.173	<0.0001	6.72[b]	7.38[a]	7.63[a]	0.224	<0.0001
Protein, %	5.27	5.02	0.105	0.0007	5.90	5.91	5.99	0.112	0.5880
Casein, %	4.00	3.82	0.095	0.0020	4.59	4.66	4.55	0.107	0.6285
Whey protein, %	1.03	0.945	0.042	0.0880	1.06[b]	1.01[b]	1.19[a]	0.042	0.0004
Non-protein nitrogen, %	0.042	0.038	0.001	0.0013	0.040	0.039	0.040	0.001	0.7899
Urea, mg/dL	33.37	27.91	1.24	<0.0001	29.57[c]	32.09[b]	38.75[a]	2.23	<0.0001
pH	6.68	6.65	0.014	0.1475	6.60	6.60	6.63	0.018	0.2660
Titratable acidity, °SH/50 mL	5.03	4.96	0.102	0.4964	5.88	5.77	5.52	0.165	0.0516
Coagulation time (r), min	24.61	22.68	1.51	0.3551	20.32	21.91	18.91	1.70	0.2407
Curd firming time (k_{20}), min	1.86	1.70	0.114	0.2848	1.81	1.52	1.66	0.115	0.0876
Curd firmness (a_{30}), mm	47.40	51.30	3.26	0.3575	52.46	53.41	56.21	1.95	0.1893

Abbreviations: DM = dry matter; FPCM = fat- and protein-corrected milk yield to 6.5% fat and 5.8% protein (Pulina and Nudda, 2004); NEL = net energy for lactation according to INRA (2018); SEM = standard error of mean.
[1]Fresh sulla forage.
[2]Fresh barley forage.
[3]2 kg/day dehydrated sulla forage from April cut.
[4]2 kg/day dehydrated sulla forage from May cut.
[5]Sulla hay ad libitum.
[a,b,c]Values within a row with different superscripts differ significantly at $p < 0.05$.

Despite the analogous forage (g/day) and total DM intake (g/day and g/day per kg $LW^{0.75}$) recorded in the groups, ewes fed the SULLA diet ingested more ($p < 0.0001$) protein, energy, polyphenols and CT, while consuming less aNDFom, as a consequence of the different composition of the forages; they also exhibited higher ($p < 0.0001$) digestibility levels of DM, organic matter (OM), CP and energy. The nitrogen retained, net of nitrogen excreted with faeces and secreted in milk, was superior ($p < 0.0001$) for ewes fed SULLA diet, explaining their more moderate LW loss.

In autumn, no change was observed in ewes' LW and BCS due to the effect of diet. The DSF-based diets were responsible for significantly higher forage intakes ($p < 0.0001$), which can be attributed to the good palatability and the limited encumbrance of pellets, as also observed by Gannuscio et al. (2022). Consequently, ewes fed A-DSF and M-DSF diets showed higher DM and energy intake and lower incidence of concentrate than ewes fed SHL ($p < 0.0001$). Passing from SHL to M-DSF and A-DSF, there was a significant increase ($p < 0.0001$) in protein, energy and polyphenols intake, whereas the ingestion of CT increased only with A-DSF diet ($p = 0.0032$). Similar improvements from SHL to A-DSF diets were observed for DM, OM, CP, aNDFom and energy digestibility and the incidence of nitrogen retained ($p < 0.0001$); however, this latter did not induce an analogous positive trend in ewes' LW and BCS, being presumably more linked to the increasing CP intake.

Milk production

Table 5 describes the effects of the diet on individual milk yield and quality and the efficiency of feeding conversion to milk.

Milk yield and FPCM were higher ($p < 0.0001$) with the SULLA diet in comparison with the BARLEY diet, by 460 and 380 g/day respectively. Also, the efficiency of DM and energy conversion to FPCM resulted more favourable ($p < 0.0001$) with SULLA diet.

Moreover, the CT from FSF contributed to significantly improving protein ($p = 0.0007$) and casein ($p = 0.0020$) levels in milk, confirming the findings of Bonanno et al. (2016) and Gannuscio et al. (2022). Indeed, CT bind to dietary proteins, thereby protecting them from degradation in the rumen and favouring intestinal digestibility and absorption of amino acids used to synthesise casein in the mammary gland (Makkar 2003; Min et al. 2003; Piluzza et al. 2014). Nevertheless, SULLA diet induced a lower efficiency of nitrogen utilisation for milk ($p < 0.0001$), attributable to the proportionally higher nitrogen intake. Such a condition is consistent with the significant increase of non-protein nitrogen ($p = 0.0013$), and especially milk

urea ($p < 0.0001$) with SULLA diet, in accordance with Bonanno et al. (2011); indeed, high urea levels, denoting superior nitrogen excretion, are recognised to be associated to high CP intake. However, the reached value of urea (33.4 mg/dL), close to the average value of the Valle del Belìce sheep (Todaro et al. 2023), resulted in the range indicating an efficient use of dietary protein, and lower than the threshold (56 mg/dL) identified to ensure the reproductive function in the ewes.

In relation to milk quality, the lower fat ($p < 0.0001$) in milk from SULLA ewes can be linked to the well-known negative relationship with milk yield, other than to the lower intake of degradable fibre, as cellulose and emicellulose. In this regard, cellulose and emicellulose are responsible of milk fat content since they represent the substrate of fermentations to produce acetic acid, precursor of de novo synthesis of short- and medium-chain FA in the udder tissues. Somatic cell count (SCC), pH, titratable acidity and coagulation parameters of individual milk did not show significant differences between the two spring diets and are in line with the average values of the Valle del Belìce sheep (Todaro et al. 2023).

In autumn, both DSF pellets resulted in higher milk yield and FPCM ($p < 0.0001$), by about 350 and 270 g/day with A-DSF and 250 and 230 g/day with M-DSF compared to the SHL diet, without negatively affecting milk protein and casein levels. Whereas the efficiency of DM conversion to FPCM and that of nitrogen utilisation for milk did not differ among diets, the energy conversion to FPCM resulted less favourable ($p < 0.0001$) for A-DSF group, indicating how the higher NEL intake recorded with A-DSF diet (Table 4), mainly due to the superior NEL values of A-DSF pellet (Table 2), did not result in a further proportional increase in milk secretion; the major metabolic exigencies to sustain the more intense milk synthesis could explain this result.

The maintenance of milk protein and casein contents with both DSF-based diets, despite increased produced amounts, confirms the findings of Gannuscio et al. (2022); also in this case, this result can be referred to the action of CT in the rumen, which is reflected in terms of casein synthesis in the mammary tissue, as previously observed. Also the reduction in whey protein ($p = 0.0004$) and urea ($p < 0.0001$) in both DSF groups is presumably linked to the effect of CT on ruminal microflora. However, the lowest urea content recorded in the milk from ewes fed the A-DSF diet denotes a better utilisation of dietary protein.

Instead, milk fat content decreased significantly with A-DSF ($p < 0.0001$), an effect of its inverse relationship with milk yield. Furthermore, a slight significant decrease in lactose emerged in SHL milk ($p = 0.0011$); since this variation has not been accompanied by an increase of somatic cells count, that was within a moderate range and comparable among groups, it cannot be referred to the mammary health, as found by Leitner et al. (2004). Probably, the significance of this difference in lactose is mainly a consequence of the known low variability of this component. However, such differences for lactose did not occur comparing the corresponding bulk milk used for cheese- making (4.58%, 4.57% and 4.51% in bulk milk from A-DSF, M-DSF and SHL groups, respectively; $p = 0.8320$), as reported in Ponte et al. (2026). No significant difference was observed for somatic cell count (SCC), pH, acidity or coagulation parameters of individual milk samples due to the diet.

Conclusions

The results of this study confirmed the superiority of a diet in which fresh sulla forage was offered as unique forage source, linked to its CT moderate content that, improving the efficiency of nutrients utilisation and digestibility (+5.7%), contributed to enhancing the oxidative status of ewes and increasing milk yield (+460 g/day) and casein content in comparison with ewes fed fresh barley forage.

The results further confirmed the validity of dehydration as an alternative to haymaking when grazing resources are insufficient or climate conditions are unfavourable for hay production. The results also proved the potential advantages of dehydration to preserve the nutrients content of sulla forage, particularly protein and PUFA, as well as and bioactive molecules such as polyphenols and vitamin E, which benefit animal welfare and productivity. Indeed, compared to hay, dehydrated pellets from the earlier sulla forage improved digestibility (+7.8%) and milk yield (+350 g/day) maintaining the level of milk casein, while at plasma level, the increased polyphenols slightly improved the oxidative status of ewes.

In perspective, it is hoped that the findings of this study could have promising practical implications in favouring the production and utilisation of dehydrated forage pellets of high-nutritional-quality that, replacing hay, ensure good levels of sustainability, animal welfare and productivity, and product quality comparable to that obtained with green sulla forage.

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Author contributions

CRediT: **Marialetizia Ponte**: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing; **Marianna Pipi**: Data curation, Investigation, Methodology, Visualization, Writing – original draft; **Riccardo Gannuscio**: Data curation, Methodology, Visualization; **Adriana Di Trana**: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing; **Massimo Todaro**: Investigation, Methodology, Resources, Visualization; **Antonino Di Grigoli**: Conceptualization, Data curation, Investigation, Supervision, Validation, Visualization, Writing – original draft; **Adriana Bonanno**: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Data availability statement

Data will be made available on request.

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