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Milk Production and Nutrient Utilization Efficiency in Dairy Ewes Fed Alfalfa Hay, Sulla Hay or Sulla Haylage Under Pasture-Based Conditions

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Abstract

This study evaluated the effects of replacing alfalfa hay with sulla [*Sulla coronaria* (L.) B.H. Choi & H. Ohashi] hay or sulla haylage in pasture-based diets for lactating Sarda ewes on milk production, milk composition, and nutrient utilization efficiency. Seventy-two mid-lactation ewes were assigned to three dietary treatments for 56 days—alfalfa hay (AH), sulla hay (SH), or sulla haylage (SHL)—all combined with 3 h/day grazing and a fixed amount of supplemental concentrate. The diets were formulated to be theoretically isoenergetic and isoproteic. Pasture nutritive value was generally comparable among the groups throughout the experimental period. Milk yield did not differ among treatments; however, fat- and protein-corrected milk (FPCM) and milk fat and protein concentrations were higher in AH compared with the sulla-based diets. Actual nutrient intake differed among treatments, with nitrogen intake (NI) being greatest in AH, consistent with the higher milk urea concentration observed in this group. Estimated apparent energy utilization efficiency (FPCM/UFL intake) showed treatment-associated differences in exploratory analyses, with lower values observed in SH compared with AH and SHL. Similarly, exploratory estimates of apparent nitrogen utilization efficiency (Milk N/NI) were highest in SHL, intermediate in SH, and lowest in AH ($p < 0.001$). Condensed tannins were not detected in the conserved sulla forages under the analytical conditions adopted in this study. Therefore, the observed responses were unlikely to be directly associated with detectable condensed tannin activity. Overall, the results suggest that forage conservation method may influence milk composition and estimated apparent nutrient utilization indices, with sulla haylage showing higher exploratory estimates of apparent nitrogen utilization efficiency without negative effects on milk yield under the conditions of the present study.

Keywords: dairy ewes; *Sulla coronaria*; forage conservation; haylage; nutrient utilization; pasture-based systems

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1. Introduction

Legume-based forages are increasingly recognized as important components of sustainable small ruminant production systems, particularly in Mediterranean environments, owing to their high nutritive value, biological nitrogen fixation capacity, and positive effects on animal performance and product quality [1,2]. Among these species, *Sulla coronaria* (L.)

B.H. Choi & H. Ohashi (sulla) is a short-lived forage legume well adapted to the calcareous and clayey soils of southern Europe and widely used in sheep production systems [3]. Several studies have reported increased milk yield and improved milk quality in dairy sheep grazing sulla-based pastures compared with grass-dominated swards, including positive effects on milk protein content and fatty acid profile [4–6].

The favourable nutritional properties of sulla have often been associated with its balanced chemical composition and moderate concentrations of condensed tannins (CTs), generally ranging between 20 and 40 g/kg dry matter [7,8]. Under Mediterranean conditions, CT concentrations may vary considerably (8–50 g/kg DM) depending on phenological stage, genotype, and environmental factors [2,9,10]. At moderate concentrations, CTs do not generally impair feed intake, digestibility, or animal performance and may contribute to improved nitrogen utilization by reducing ruminal protein degradation and increasing the flow of undegraded dietary protein to the small intestine [11,12].

In addition, condensed tannins may protect polyunsaturated fatty acids from ruminal biohydrogenation, thereby increasing their transfer to milk and dairy products [5,6]. They may also exert antioxidant activity, potentially improving animal health and the oxidative stability of dairy products [13]. Beyond their nutritional role, CT-containing legumes have also been associated with reduced enteric methane emissions and improved resilience to gastrointestinal parasites, contributing to the environmental sustainability of small ruminant systems [14,15].

The nutritional advantages of sulla are generally greater when consumed fresh rather than as conserved forage [16]. However, in Mediterranean sheep farming systems, the utilization of sulla under grazing conditions is largely confined to spring, leading to underexploitation of its productive potential. Consequently, there is growing interest in conservation strategies aimed at extending its availability beyond the grazing season. Traditional haymaking may result in substantial losses of biomass, nutrients, and polyphenolic compounds, particularly under unstable weather conditions [17]. Alternative conservation techniques, such as dehydration or ensiling in wrapped bales (haylage), may provide greater flexibility in harvest timing and potentially improved preservation of nutrients and CTs [18].

Despite the extensive literature on fresh sulla utilization in dairy sheep nutrition, information regarding the effects of conserved sulla forages, particularly wrapped haylage, on animal performance and milk quality remains limited.

Therefore, the objective of the present study was to compare the effects of three theoretically isoenergetic and isoproteic diets differing in forage type and conservation method (alfalfa hay, sulla hay, and wrapped sulla haylage) on milk production and composition, as well as on estimated intake and apparent nutrient utilization indices under group-based grazing conditions, in lactating dairy sheep.

2. Materials and Methods

2.1. Experimental Site and Ethical Approval

The experiment was conducted at the Bonassai Experimental Farm of the Agricultural Research Agency of Sardinia (AGRIS Sardegna), located in north-western Sardinia, Italy (40°40′16.2″ N, 8°22′0.4″ E; 32 m a. s. l.). All experimental procedures complied with European Union legislation concerning the protection of animals used for scientific purposes. Animal handling, management, and data collection were performed by trained personnel and were approved by the institutional ethical committees of AGRIS Sardegna and the University of Sassari (authorization n° 52682, 6 May 2021).

2.2. Animals, Management, and Experimental Design

The experiment lasted 56 days and was carried out during the spring season of 2025 (mid-April to mid-June). During the experimental period, mean air temperature and relative humidity recorded during grazing hours were 18.2 ± 3.4 °C and 68.5 ± 7.2%, respectively. A total of 72 multiparous lactating Sarda ewes were selected and allocated to three experimental groups (n = 24 ewes per group) balanced for physiological and productive characteristics. Each experimental group was assigned to a dedicated paddock and received a specific dietary treatment throughout the trial. Therefore, dietary treatments were applied at the group level under grazing conditions rather than to individually replicated animals. At the beginning of the trial, ewes averaged 3.5 ± 0.8 years of age, lambing date 17 December 2024 ± 2.5 days, 102 ± 2.5 days in milk, daily milk yield 2.23 ± 0.04 kg/day, live weight (LW) 51.6 ± 0.6 kg, and body condition score (BCS) 2.5 ± 0.03.

Throughout the experimental period, the ewes were machine-milked twice daily (morning and evening) and managed under a pasture-based feeding system. All the animals grazed Italian ryegrass pasture for 3 h/day (from 08:30 to 11:30 h) and received conserved forage indoors immediately after grazing, together with concentrate supplementation provided during milking. Following a 10-day adaptation period, during which all animals received the same diet consisting of 4 h/day grazing on Italian ryegrass pasture plus ryegrass hay and concentrate supplementation, the ewes were assigned to one of the following dietary treatments, expressed on a fresh matter (FM) basis:

- Alfalfa hay (AH): A total of 3 h/day grazing on Italian ryegrass pasture, plus alfalfa hay (1.35 kg/head per day) and concentrates consisting of maize grain (0.350 kg/head per day at morning milking) and commercial concentrate (0.350 kg/head per day at evening milking);
- Sulla hay (SH): A total of 3 h/day grazing on Italian ryegrass pasture, plus sulla hay (1.70 kg/head per day) and the same concentrate supplementation as the AH group;
- Sulla haylage (SHL): A total of 3 h/day grazing on Italian ryegrass pasture, plus sulla haylage (3.50 kg/head per day) and the same concentrate supplementation as the other groups.

The alfalfa and sulla forages were harvested at the early flowering stage. Conserved forages were stored under sheltered conditions and used within approximately 3 months of harvest. No visible spoilage or mould growth was observed during the storage period.

The diets were formulated and evaluated using the Small Ruminant Nutrition System (SRNS; [19]) and were designed to be theoretically isoenergetic and isoproteic.

2.3. Pasture Management

The grazing area was divided into three paddocks, each allocated to one experimental group of sheep. Each paddock was further subdivided into sub-paddocks (approximately 1500 m^2, 63 m^2/ewe), which were grazed for seven consecutive days according to a rotational grazing system.

The animals remained in the same experimental group throughout the trial and were not rotated among the paddocks. Therefore, pasture intake, nutrient intake, and intake-derived variables were obtained under a group-based grazing design in which treatments were confounded with paddock. Consequently, these variables should be interpreted as exploratory estimates describing treatment-associated responses under the conditions of the present study rather than as fully replicated treatment effects.

2.4. Feed Sampling and Chemical Analyses

Pasture biomass availability and botanical and chemical composition were assessed at the beginning and end of each grazing rotation. In each sub-paddock, four herbage samples were collected by cutting 0.5 m^2 quadrats randomly distributed across the grazed area. In total, 192 pasture samples were collected during the study (4 pre-grazing cuts + 4 post-grazing cuts × 3 sub-paddocks × 8 grazing rotations). Samples of offered herbage and post-grazing residues were oven-dried at 65 °C for 48 h to determine dry matter (DM) concentration according to AOAC procedures [20].

For the characterization of the herbage offered, the four pre-grazing samples collected within each sub-paddock at each rotation were pooled into two composite samples prior to chemical analyses.

Climatic parameters, including air temperature, relative humidity, rainfall, wind speed, and wind direction, were automatically recorded hourly using an on-site weather station. The temperature–humidity index (THI) was calculated according to the procedure described by NRC [21].

Samples of maize grain and commercial concentrate were collected on three occasions during the experimental period and composited before analysis because no relevant variation in feed appearance or batch composition was expected. Samples of conserved forages were collected at the beginning, midpoint and end of the experimental period. In addition, hand-plucked herbage samples representative of the grazed pasture were collected fortnightly for each experimental group. All feed samples were oven-dried at 65 °C for 48 h and ground to pass through a 1 mm screen prior to analysis.

The chemical composition of the offered herbage, hand-plucked herbage, and conserved forages was determined using near-infrared reflectance spectroscopy (NIRS 5000, FOSS NIRSystems, Silver Spring, MD, USA). Calibration and validation equations were continuously updated using a database including more than 5000 forage spectra, from which approximately 400 representative samples were selected for calibration.

The calibration database included pasture herbage and conserved forages representative of Mediterranean sheep production systems, including matrices comparable to those evaluated in the present study. Cross-validation statistics indicated satisfactory predictive performance, with R^2/SECV values of 0.99/0.77, 0.93/2.86, 0.93/2.07, and 0.87/4.04 for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and in vitro dry matter digestibility (IVDMD), respectively. Concentrates and maize grain were analyzed by conventional wet chemistry methods according to AOAC [20]. Fibre fractions, including amylase-treated neutral detergent fibre (aNDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), were determined according to Van Soest et al. [22]. Non-structural carbohydrates (NSC) were calculated as:

$$\text{NSC} = 100 - (\text{CP} + \text{EE} + \text{ash} + \text{NDF})$$

In vitro dry matter digestibility (IVDMD) was determined using the pepsin–cellulase method described by Aufrère and Demarquilly [23]. Freeze-dried forage subsamples were analyzed for total phenols, non-tannic phenols and tannic phenols using the Folin–Ciocalteu method [24], whereas condensed tannins were determined using the butanol–HCl assay and expressed as leucocyanidin equivalents [25]. The analytical procedure quantified extractable condensed tannins and did not specifically assess protein-bound or fibre-bound tannin fractions. The detection limit of the butanol–HCl assay under the analytical conditions adopted in this study was 1.0 g/kg DM. Samples with condensed tannin concentrations below this threshold were considered non-detectable.

2.5. Animal Measurements, Intake Estimation, and Milk Analysis

Live weight and body condition score (BCS) were recorded monthly according to Russel [26]. The ewes were weighed immediately after the morning milking using an electronic livestock scale with ±0.1 kg accuracy. Individual milk yield was recorded weekly at both morning and evening milking. Individual concentrate intake was measured daily, whereas hay intake was recorded at group level as the difference between forage offered and refusals. Herbage dry matter intake at pasture was estimated individually using the predictive model proposed by Molle et al. [27] based on animal performance, pasture composition and access time. Therefore, pasture intake, total dry matter intake, and nutrient intake variables are presented as estimated individual intake measures derived from predictive and group-level measurements rather than as directly measured individual intakes.

The net energy content (UFL/kg DM) and metabolizable protein content (MP, g/kg DM) of the diets were calculated according to Cannas [28].

Milk samples were collected weekly from each ewe during milk recording and analyzed for fat, true protein (N × 6.38), lactose, urea, and somatic cell count (SCC) using an infrared analyzer (Milkoscan, FOSS, Hillerød, Denmark). Milk fat, protein, and lactose concentrations are expressed as percentages (%). Fat- and protein-corrected milk yield (FPCM; 6.5% fat and 5.8% protein) was calculated according to Pulina and Nudda [29].

Energy utilization efficiency (EUE) was calculated as the ratio between FPCM and net energy intake expressed as UFL intake (UFLI). Nitrogen utilization efficiency (NUE) was calculated as the ratio between milk nitrogen output (MilkN) and nitrogen intake (NI). Because intake variables were partly derived from predictive estimates and group-level measurements, EUE and NUE are hereafter referred to as estimated apparent EUE and estimated apparent NUE and should be interpreted as indicative indices rather than direct measures of physiological efficiency.

2.6. Statistical Analysis

The biomass availability, botanical composition, and chemical composition of the herbage at the beginning and end of the grazing period were analyzed using linear mixed models including experimental group, grazing phase, and their interaction as fixed effects, with week included as a repeated factor when appropriate. Differences among least-squares means (LSMeans) were assessed using Tukey's adjustment. Because pasture-related measurements were obtained from repeated subsamples collected within a single paddock assigned to each treatment, statistical comparisons should be interpreted as descriptive estimates characterizing the treatment paddocks rather than as fully replicated treatment effects.

The chemical composition and nutritive value of conserved forages were analyzed using linear mixed models including forage type as fixed effects. Least-squares means were compared using Tukey's adjustment.

Live weight and BCS were analyzed using mixed linear models including experimental group, sampling month, and their interaction as fixed effects, with ewe included as a random effect.

Milk yield and milk composition variables were analyzed using mixed linear models for repeated measures including experimental group, sampling week, and their interaction as fixed effects, with ewe included as a random intercept. Repeated observations over time were modelled using a first-order autoregressive covariance structure [AR (1)]. Models were fitted using restricted maximum likelihood (REML), and denominator degrees of freedom were estimated using the Kenward–Roger method.

Initial milk yield was included as a covariate in the analysis of milk production traits, whereas initial LW and BCS were included as covariates in the respective models.

Intake variables and intake-derived efficiency indices (EUE and NUE) were analyzed using mixed models for repeated measures including experimental group, sampling week, and their interaction as fixed effects. However, because part of the intake data was based on group-level measurements and predictive estimates of pasture intake, statistical inference for intake-related variables should be interpreted as exploratory rather than confirmatory analyses under the group-based grazing conditions of the present study. When significant interactions were detected, pairwise comparisons among treatments within each week were performed using Tukey–Kramer-adjusted LSMeans.

Differences were considered statistically significant at $p \leq 0.05$. Model assumptions were evaluated by visual inspection of residual plots to assess normality and homoscedasticity. No major deviations from model assumptions were detected.

All statistical analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) [30].

3. Results

3.1. Pasture and Climatic Conditions

No animals were removed from the experiment during the experimental period, and all 72 ewes completed the study.

At grazing entry, total herbage mass (3.12 ± 0.17 t DM/ha), herbage allowance per animal per day (0.94 ± 0.05 kg DM) and herbage allowance per animal per hour (0.235 ± 0.013 kg DM) did not differ among the experimental groups (Table 1, $p > 0.05$). Italian ryegrass was the dominant species in all paddocks. Small but significant differences in botanical composition were observed among the treatment paddocks. The SH paddock showed a higher proportion of Italian ryegrass compared with the SHL paddock ($p < 0.05$), whereas the proportion of other species showed the opposite trend (Table 1). Residual herbage mass differed among treatment paddocks ($p \leq 0.05$), despite similar herbage availability at grazing entry. Residual herbage allowance expressed on both a daily and hourly basis also differed among treatment paddocks ($p \leq 0.04$). Pairwise comparisons indicated higher residual herbage mass in SHL compared with AH, whereas SH showed intermediate values.

Table 1. The herbage availability, botanical composition and residual biomass measured at grazing entry and after grazing in the three experimental groups (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value
Total herbage mass (t DM/ha)	3.06 ± 0.32	3.07 ± 0.30	3.23 ± 0.29	0.50
Italian ryegrass (%)	88.0 ± 2.7 ^{ab}	91.3 ± 2.3 ^{a}	85.7 ± 2.8 ^{b}	0.04
Other species (%)	12.0 ± 2.7 ^{ab}	8.7 ± 2.3 ^{b}	14.3 ± 2.8 ^{a}	0.04
Herbage allowance (kg DM/head/day)	0.94 ± 0.10	0.92 ± 0.09	0.96 ± 0.09	0.70
Herbage allowance (kg DM/head/hour)	0.24 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.70
Residual herbage mass (t DM/ha)	1.19 ± 0.25 ^{b}	1.21 ± 0.25 ^{ab}	1.43 ± 0.25 ^{a}	0.02
Residual herbage allowance (kg DM/head/day)	0.368 ± 0.08	0.366 ± 0.08	0.430 ± 0.08	0.04
Residual herbage allowance (kg DM/head/hour)	0.092 ± 0.02	0.092 ± 0.02	0.108 ± 0.02	0.04

Different superscript letters within rows indicate significant differences ($p \leq 0.05$). Measurements were obtained from repeated subsamples collected within a single paddock assigned to each treatment and should therefore be interpreted as descriptive estimates.

The chemical composition of herbage on offer was generally comparable among groups (Table 2). Crude protein (CP) and in vitro dry matter digestibility (IVDMD) did not differ significantly among treatment paddocks ($p > 0.05$). Small differences were detected for fibre fractions, with slightly lower neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations in AH and SH compared with SHL ($p < 0.05$).

Table 2. The chemical composition and nutritive value of the pasture herbage on offer in the three experimental groups. The values are expressed on a dry matter basis as least-squares means (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value
CP [1] (% DM)	9.18 ± 0.41	9.33 ± 0.29	8.37 ± 0.30	0.27
NDF [2] (% DM)	57.66 ± 0.60 [b]	56.95 ± 0.59 [ab]	58.99 ± 0.49 [a]	0.04
ADF [3] (% DM)	32.63 ± 0.50 [b]	32.47 ± 0.48 [ab]	33.67 ± 0.39 [a]	0.03
IVDMD [4] (% DM)	62.90 ± 0.63	63.07 ± 0.60	61.39 ± 0.62	0.26

Different superscript letters within rows indicate significant differences among the treatment paddocks ($p \leq 0.05$). [1] CP = crude protein; [2] NDF = neutral detergent fibre; [3] ADF = acid detergent fibre; [4] IVDMD = in vitro dry matter digestibility. Because measurements were obtained from repeated subsamples collected within a single paddock assigned to each treatment, comparisons should be interpreted as descriptive estimates rather than fully replicated treatment effects.

Marked changes in pasture nutritive value were observed between grazing entry and post-grazing residues (Table 3). Residual herbage showed lower CP and IVDMD, together with higher NDF and ADF concentrations compared with herbage on offer ($p \leq 0.001$). No significant group × grazing phase interaction was detected for any variable ($p > 0.05$), indicating a consistent pattern of change among treatments. Because pasture measurements were obtained from repeated subsamples collected within a single paddock assigned to each treatment, comparisons among treatments should be interpreted as descriptive estimates characterizing the treatment paddocks rather than as fully replicated treatment effects.

Table 3. Changes in the nutritive value of pasture herbage between entry (herbage on offer) and post-grazing residues. The values are expressed on a dry matter basis as least-squares means (LSMeans ± SE), averaged across the experimental groups.

	Herbage on Offer	Residual Herbage	*p*-Value
CP [1] (% DM)	9.00 ± 0.24	6.73 ± 0.17	<0.001
NDF [2] (% DM)	57.87 ± 0.49	66.25 ± 0.45	<0.001
ADF [3] (% DM)	32.92 ± 0.32	38.78 ± 0.33	<0.001
IVDMD [4] (% DM)	62.45 ± 0.62	54.03 ± 0.49	<0.001

[1] CP = crude protein; [2] NDF = neutral detergent fibre; [3] ADF = acid detergent fibre; [4] IVDMD = in vitro dry matter digestibility.

Air temperature recorded during grazing hours progressively increased throughout the experimental period, reflecting the seasonal transition from mid-spring to early summer typical of Mediterranean environments. Mean weekly air temperature increased from approximately 14–15 °C in the initial weeks to 21–22 °C in the final phase of the trial (Figure 1a). Relative humidity showed moderate temporal variability, with higher values recorded at the beginning of the experimental period, lower values during the intermediate weeks, and a slight increase toward the end of the trial (Figure 1a). Wind speed remained generally low throughout the experimental period, with only a transient increase around week 6, whereas rainfall events were sporadic and limited (Figure 1b).

As a consequence of the combined temperature and humidity patterns, the temperature–humidity index (THI) recorded during grazing sessions (08:30–11:30) progressively increased across weeks (Figure 2). THI values remained mostly below 65 during the early phase of the experiment and gradually approached or exceeded this threshold during the final weeks. Values within the range $68 \leq \text{THI} < 72$, indicative of potential thermal discomfort [31], were observed mainly during the last part of the experimental period.

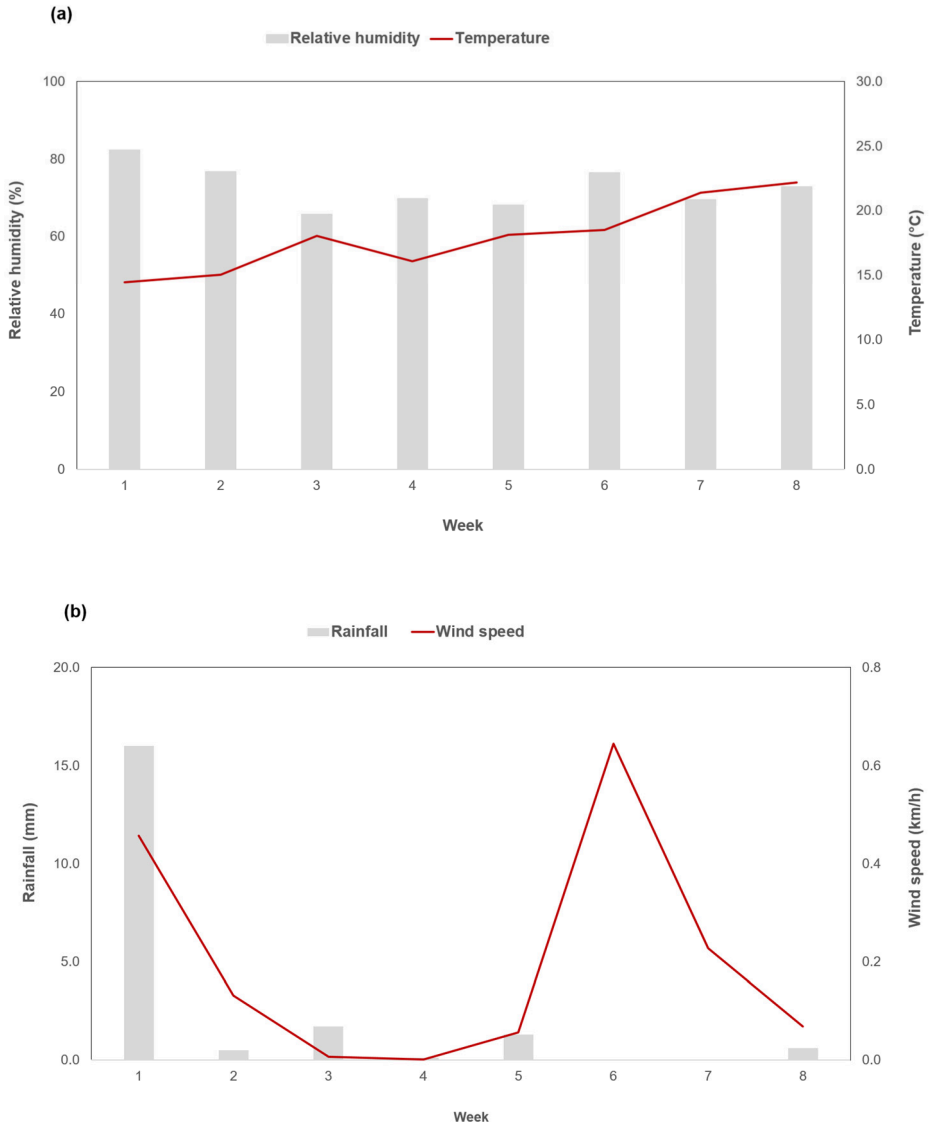


Figure 1. The weekly mean air temperature and relative humidity (**a**), and wind speed and rainfall (**b**) recorded during the grazing sessions (08:30–11:30 h) throughout the experimental period.

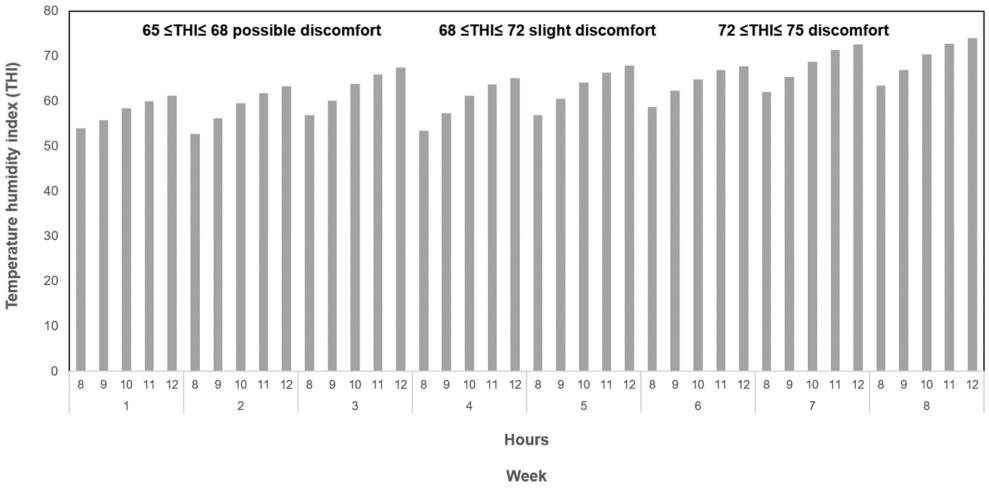


Figure 2. The hourly and weekly trends of the temperature–humidity index (THI) recorded during the grazing sessions (08:30–11:30 h) over the experimental period.

3.2. Nutritive Values of Experimental Diets

The chemical composition and nutritive value of concentrates used during the experiment are reported in Table 4, whereas the composition of conserved forages offered to the animals is presented in Table 5. Crude protein (CP) concentration was higher in alfalfa hay compared with both sulla-based forages ($p \leq 0.001$). Ether extract (EE) showed minor differences among forage types ($p \leq 0.05$). Significant differences were observed for fibre fractions: neutral detergent fibre (NDF) concentration was higher in SH compared with AH and SHL ($p = 0.01$), whereas acid detergent fibre (ADF) was higher in SHL compared with AH ($p = 0.05$). No significant differences were detected for acid detergent lignin (ADL).

Table 4. The chemical composition and nutritive value of the concentrates used during the experimental period (on a dry matter basis).

	DM [1]	OM [2]	CP [3]	EE [4]	NSC [5]	aNDF [6]	ADF [7]	ADL [8]	IVDMD [9]	UFL [10]	MP [11]
Maize grain	88.0	98.4	9.4	5.2	70.7	13.1	6.4	0.6	94.6	1.31	89.7
Concentrate	88.6	89.2	18.0	2.6	39.7	28.9	15.1	3.3	81.8	0.96	165.3

[1] DM = dry matter; [2] OM = organic matter; [3] CP = crude protein; [4] EE = ether extract; [5] NSC = non-structural carbohydrates; [6] aNDF = amylase-treated neutral detergent fibre; [7] ADF = acid detergent fibre; [8] ADL = acid detergent lignin; [9] IVDMD = in vitro dry matter digestibility; [10] UFL = estimated feed unit for milk production; [11] MP = estimated metabolizable protein. The values are based on composited samples collected at the beginning, midpoint, and end of the experimental period.

Table 5. The chemical composition and nutritive value of the conserved forages offered during the experimental period (LSMeans ± SE; on a dry matter basis unless otherwise specified).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value
DM [1] (%)	85.42 ± 2.26 [a]	80.06 ± 2.26 [a]	35.14 ± 2.26 [b]	<0.001
OM [2] (% DM)	83.44 ± 1.22	88.10 ± 1.22	85.52 ± 1.22	0.09
CP [3] (% DM)	19.86 ± 0.95 [a]	10.91 ± 0.95 [b]	12.00 ± 0.95 [b]	<0.001
EE [4] (% DM)	2.83 ± 0.08 [a]	2.43 ± 0.08 [b]	2.59 ± 0.08 [ab]	0.05
NSC [5] (% DM)	11.56 ± 1.34 [b]	17.65 ± 1.34 [b]	21.47 ± 1.34 [a]	0.05
NDF [6] (% DM)	49.19 ± 1.05 [b]	57.11 ± 1.05 [a]	49.47 ± 1.05 [b]	0.01
ADF [7] (% DM)	35.19 ± 0.93 [b]	39.09 ± 0.93 [ab]	40.35 ± 0.93 [a]	0.05
ADL [8] (% DM)	6.56 ± 0.52	6.01 ± 0.52	7.15 ± 0.52	0.36
Total Phenols (% DM)	0.86 ± 0.02 [b]	0.86 ± 0.02 [b]	1.28 ± 0.02 [a]	0.02
Tannic Phenols (% DM)	0.20 ± 0.04 [b]	0.19 ± 0.04 [b]	0.44 ± 0.04 [a]	0.01
IVDMD [9] (% DM)	63.98 ± 1.42	59.55 ± 1.42	63.78 ± 1.42	0.12
UFL [10] (unit/kg DM)	0.642 ± 0.02	0.650 ± 0.02	0.675 ± 0.02	0.58
MP [11] (g/kg DM)	174.5 ± 8.0 [a]	99.1 ± 8.0 [b]	109.3 ± 8.0 [b]	0.01

Different superscript letters within rows indicate significant differences among treatments. [1] DM = dry matter; [2] OM = organic matter; [3] CP = crude protein; [4] EE = ether extract; [5] NSC = non-structural carbohydrates; [6] NDF = neutral detergent fibre; [7] ADF = acid detergent fibre; [8] ADL = acid detergent lignin; [9] IVDMD = in vitro dry matter digestibility; [10] UFL = estimated feed unit for milk production; [11] MP = estimated metabolizable protein. The values are based on forage samples collected at the beginning, midpoint, and end of the experimental period.

Non-structural carbohydrate (NSC) concentration was higher in SHL compared with the other conserved forages ($p \leq 0.05$). Total phenols and tannic phenols were detected at low concentrations in all conserved forages but were higher in SHL ($p \leq 0.02$). Condensed tannins were not detected in any of the conserved forages under the analytical conditions adopted in the present study. No significant differences among forage types were observed for organic matter (OM), in vitro dry matter digestibility (IVDMD), or net energy concentration expressed as UFL ($p > 0.05$). Metabolizable protein (MP) concentration was higher in AH compared with both sulla-based forages ($p \leq 0.01$).

The chemical composition and nutritive value of grazed herbage estimated from hand-plucked samples are presented in Table 6. No significant effects of experimental group

were detected for any analyzed variable, including DM, CP, EE, fibre fractions, IVDMD, UFL, or MP ($p > 0.05$).

Table 6. The effects of experimental group (G), sampling week (W), and their interaction (G × W) on the chemical composition and nutritive value of grazed herbage estimated from hand-plucked samples. The values are expressed as least-squares means (LSMeans ± SE) on a dry matter basis unless otherwise specified.

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value		
				G	W	G × W
DM [1] (%)	32.92 ± 1.3	32.36 ± 1.3	34.41 ± 1.3	0.90	<0.001	0.90
OM [2] (% DM)	89.79 ± 0.5	89.66 ± 0.5	89.68 ± 0.5	0.30	0.90	0.30
CP [3] (% DM)	9.23 ± 0.8	8.80 ± 0.8	9.08 ± 0.8	0.70	0.80	0.60
EE [4] (% DM)	3.05 ± 0.2	2.97 ± 0.2	3.03 ± 0.2	0.80	0.08	0.60
NSC [5] (% DM)	16.84 ± 1.9	17.18 ± 1.9	16.42 ± 1.9	0.90	0.50	0.90
NDF [6] (% DM)	60.66 ± 2.8	60.69 ± 2.8	61.15 ± 2.8	0.90	0.80	0.80
ADF [7] (% DM)	34.18 ± 1.6	34.20 ± 1.6	34.40 ± 1.6	0.80	0.30	0.80
ADL [8] (% DM)	2.27 ± 0.4	2.03 ± 0.4	2.17 ± 0.4	0.90	0.20	0.90
IVDMD [9] (% DM)	55.68 ± 2.6	55.32 ± 2.6	54.71 ± 2.6	0.80	0.90	0.70
UFL [10] (unit/kg DM)	0.63 ± 0.04	0.62 ± 0.04	0.61 ± 0.04	0.90	0.80	0.80
MP [11] (g/kg DM)	84.38 ± 7.0	80.65 ± 7.0	83.03 ± 7.0	0.80	0.80	0.60

[1] DM = dry matter; [2] OM = organic matter; [3] CP = crude protein; [4] EE = ether extract; [5] NSC = non-structural carbohydrates; [6] NDF = neutral detergent fibre; [7] ADF = acid detergent fibre; [8] ADL = acid detergent lignin; [9] IVDMD = in vitro dry matter digestibility; [10] UFL = estimated feed unit for milk production; [11] MP = estimated metabolizable protein. The values are based on hand-plucked samples collected fortnightly from each experimental paddock throughout the experimental period. Because the samples were collected within paddocks assigned to each treatment, comparisons among groups should be interpreted as descriptive estimates rather than fully replicated treatment effects.

Sampling week significantly affected dry matter concentration only ($p \leq 0.001$), reflecting the progressive maturation of the pasture over the experimental period. No significant group × week interactions were detected for any variable, indicating a similar temporal pattern among treatments. Because hand-plucked samples were collected within paddocks assigned to each experimental group, comparisons among groups should be interpreted as descriptive estimates characterizing the grazed herbage available to the treatment paddocks rather than as fully replicated treatment effects.

The effects of experimental group, sampling week, and their interaction on estimated feed intake variables are reported in Table 7. Exploratory differences among experimental groups were observed for estimated herbage dry matter intake (HDMI) ($p \leq 0.05$), with higher estimated values observed in AH compared with SHL, whereas SH showed intermediate values. Both sampling week and the group × week interaction significantly affected HDMI ($p \leq 0.001$).

Differences among the experimental groups were observed for estimated total dry matter intake (TDMI), total crude protein intake (TCPI), total net energy intake (TUFLI), and total metabolizable protein intake (TMPI) ($p \leq 0.001$ for all variables).

The SH group showed the highest estimated TDMI and TUFLI, whereas SHL showed the lowest values for all intake-related variables. AH showed intermediate TDMI and TUFLI values but the highest estimated TCPI and TMPI.

Forage refusal rate was descriptively higher in the SHL group, averaging approximately 31.5% of the offered forage dry matter, compared with 11.2% and 7.4% observed in SH and AH, respectively. Because refusals were recorded at the group level, these values should be interpreted descriptively rather than as fully replicated treatment responses.

Table 7. Effects of experimental group (G), sampling week (W) and their interaction (G × W) on estimated feed intake of Sarda dairy ewes. Values are expressed as least-squares means (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value		
				G	W	G × W
HDMI [1] (kg/head/day)	0.690 ± 0.02 [a]	0.648 ± 0.02 [ab]	0.614 ± 0.02 [b]	0.05	<0.001	<0.001
TDMI [2] (kg/head/day)	2.598 ± 0.02 [b]	2.702 ± 0.02 [a]	1.970 ± 0.02 [c]	<0.001	<0.001	<0.001
TCPI [3] (g/head/day)	405 ± 1.5 [a]	300 ± 1.5 [b]	231 ± 1.5 [c]	<0.001	<0.001	<0.001
TUFLI [4] (unit/head/day)	1.97 ± 0.01 [b]	2.04 ± 0.01 [a]	1.58 ± 0.01 [c]	<0.001	<0.001	<0.001
TMPI [5] (g/head/day)	363 ± 1.3 [a]	274 ± 1.3 [b]	212 ± 1.3 [c]	<0.001	<0.001	<0.001

Different superscript letters within rows indicate exploratory statistical differences among experimental groups ($p \leq 0.05$). [1] HDMI = estimated herbage dry matter intake; [2] TDMI = estimated total dry matter intake; [3] TCPI = estimated total crude protein intake; [4] TUFLI = estimated total feed unit for milk production intake; [5] TMPI = estimated total metabolizable protein intake. Herbage intake was estimated using a predictive model [27], whereas hay intake was measured at group level. Consequently, TDMI, TCPI, TUFLI, and TMPI represent estimated intake variables derived from predictive and group-level measurements. Statistical comparisons reported in this table should therefore be regarded as exploratory rather than confirmatory and should not be interpreted as directly measured individual intake responses.

Sampling week and the group × week interaction significantly affected all intake-related variables ($p = 0.001$), indicating temporal variation in treatment differences throughout the experimental period.

Because pasture intake was estimated using a predictive model and hay intake was measured at the group level, intake-related variables should be interpreted as exploratory estimates derived from predictive and group-level measurements under the grazing conditions of the present study.

3.3. Live Weight, Body Condition Score, Milk Yield, and Milk Composition

The effects of experimental group, sampling month, and their interaction on live weight (LW) and body condition score (BCS) are reported in Table 8. No significant treatment effects were detected for either LW or BCS ($p > 0.05$). In contrast, sampling month significantly affected both variables ($p \leq 0.01$), whereas no significant group × month interaction was found.

Table 8. Effects of experimental group (G), sampling month (M) and their interaction (G × M) on live weight (LW) and body condition score (BCS) in Sarda dairy ewes. Values are expressed as least-squares means (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value		
				G	M	G × M
LW [1] (kg)	51.54 ± 0.31	52.06 ± 0.31	51.60 ± 0.31	0.44	<0.001	0.52
BCS [2]	2.59 ± 0.03	2.61 ± 0.03	2.57 ± 0.03	0.79	0.01	0.69

[1] LW = live weight; [2] BCS = body condition score.

Live weight increased slightly throughout the experimental period across all treatments, with higher values recorded in June compared with April and May ($p \leq 0.001$). A similar temporal pattern was observed for BCS, which progressively increased during the trial, irrespective of dietary treatment ($p < 0.01$).

The effects of experimental group, sampling week, and their interaction on milk yield and milk composition are presented in Table 9.

Table 9. Effects of experimental group (G), sampling week (W) and their interaction (G × W) on milk yield and milk composition of Sarda dairy ewes. Values are expressed as least-squares means (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value		
				G	W	G × W
Milk (kg/head/day)	1.47 ± 0.05	1.45 ± 0.05	1.37 ± 0.05	0.35	<0.001	0.59
FPCM [1] (kg/head/day)	1.41 ± 0.04 [a]	1.33 ± 0.04 [b]	1.21 ± 0.04 [b]	0.01	<0.001	0.98
Fat (%)	6.25 ± 0.09 [a]	5.65 ± 0.09 [b]	5.36 ± 0.09 [c]	<0.001	<0.001	<0.001
Protein (%)	5.57 ± 0.06 [a]	5.33 ± 0.06 [b]	5.02 ± 0.06 [c]	<0.001	<0.001	<0.001
Lactose (%)	4.52 ± 0.03 [a]	4.26 ± 0.03 [b]	4.13 ± 0.03 [c]	<0.001	<0.001	<0.001
Urea (mg/dL)	61.83 ± 0.6 [a]	46.55 ± 0.6 [b]	45.88 ± 0.6 [b]	<0.001	<0.001	<0.001
SCCS [2]	5.71 ± 0.08 [a]	5.74 ± 0.08 [a]	5.50 ± 0.08 [b]	0.05	0.24	<0.001

Different superscript letters within rows indicate significant differences among experimental groups ($p \leq 0.05$). [1] FPCM = fat- and protein-corrected milk; [2] SCCS = somatic cell count score (log10 somatic cell count).

Milk yield did not differ among experimental groups ($p = 0.35$), whereas sampling week significantly affected milk production ($p \leq 0.001$). No significant group × week interaction was detected for milk yield. In contrast, fat- and protein-corrected milk (FPCM) differed among the experimental groups ($p \leq 0.01$), with higher values observed in AH compared with both sulla-based treatments.

Significant treatment effects were also detected for milk fat, protein, and lactose concentrations ($p \leq 0.001$). Milk fat, protein, and lactose concentrations progressively decreased from AH to SH and SHL. Sampling week and the group × week interaction also significantly affected these variables. Although the overall ranking among treatments remained generally consistent throughout the experimental period, the magnitude of the differences varied across weeks, as reflected by the significant group × week interactions.

Milk urea concentration was higher in AH compared with both sulla-based treatments ($p \leq 0.001$). Sampling week and the group × week interaction also significantly affected milk urea concentration ($p \leq 0.001$). Weekly variation in the magnitude of treatment differences was also observed for milk urea concentration, although AH consistently showed higher values than the sulla-based treatments. Somatic cell count score (SCCS; log10 SCC) differed among treatments ($p \leq 0.05$), with lower values observed in SHL compared with AH and SH. No significant effect of sampling week was detected for SCCS ($p = 0.24$), although the group × week interaction was significant ($p \leq 0.001$), indicating that treatment differences were not constant across weeks, despite the absence of a significant overall week effect. Because dietary treatments were applied to three experimental groups managed in separate paddocks, milk responses should be interpreted as responses observed under the specific group-based grazing conditions of the present study.

3.4. Estimated Apparent Energy and Nitrogen Utilization Efficiency Indices

Exploratory estimates of the effects of experimental group, sampling week, and their interaction on estimated apparent energy and nitrogen utilization efficiency indices are presented in Table 10 and Figure 3. Estimated apparent EUE values showed exploratory differences among the experimental groups ($p \leq 0.001$) and were significantly affected by sampling week ($p \leq 0.001$), with a significant group × week interaction ($p \leq 0.001$). Overall LSMeans indicated that SHL did not differ from AH, whereas SH showed lower estimated EUE values compared with both AH and SHL ($p \leq 0.001$). Weekly patterns indicated that differences among the experimental groups were not constant throughout the experimental period, as reflected by the significant group × week interactions (Figure 3a).

Table 10. Exploratory estimates of experimental group (G), sampling week (W), and their interaction (G × W) on estimated apparent energy and nitrogen utilization efficiency indices of Sarda dairy ewes. Values are expressed as least-squares means (LSMeans ± SE).

	Alfalfa Hay	Sulla Hay	Sulla Haylage	*p*-Value		
				G	W	G × W
EUE [1] (FPCM/UFLI)	0.734 ± 0.02 [a]	0.640 ± 0.02 [b]	0.776 ± 0.02 [a]	<0.001	<0.001	<0.001
NUE [2] (MilkN/NI)	0.197 ± 0.01 [c]	0.249 ± 0.01 [b]	0.299 ± 0.01 [a]	<0.001	<0.001	<0.001

Different superscript letters within rows indicate exploratory statistical differences among experimental groups ($p \leq 0.05$) and should not be interpreted as confirmatory treatment effects. [1] EUE = estimated apparent energy utilization efficiency; [2] NUE = estimated apparent nitrogen utilization efficiency. Because estimated apparent EUE and NUE were derived from intake variables partly based on predictive estimates of pasture intake and group-level forage intake measurements, these comparisons should be interpreted as exploratory rather than confirmatory evidence of treatment-associated responses.

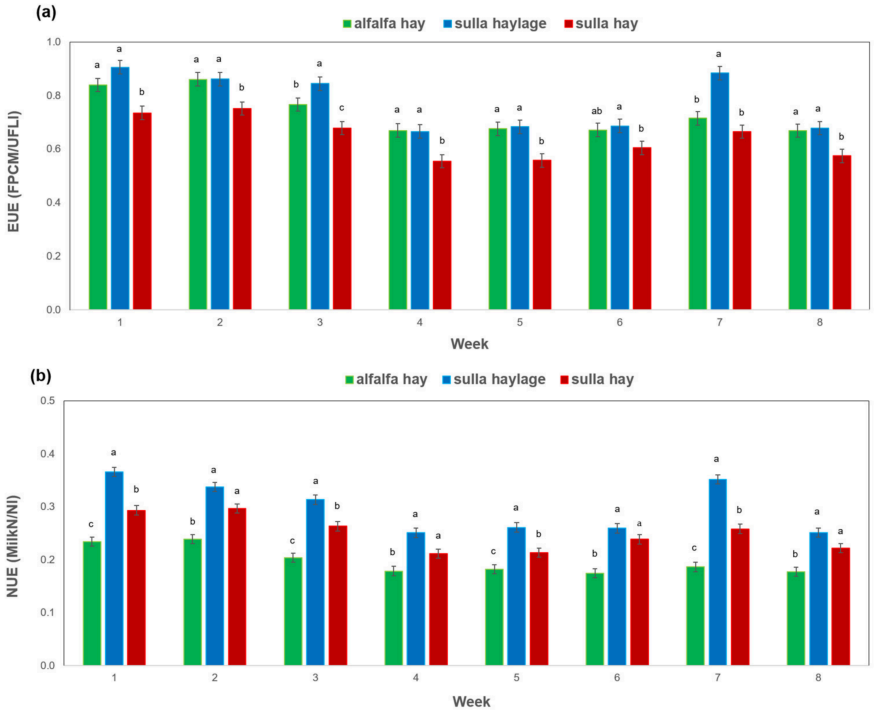


Figure 3. The least-squares means (±SE) of estimated apparent energy utilization efficiency (EUE; FPCM/UFLI) (**a**) and estimated apparent nitrogen utilization efficiency (NUE; MilkN/NI) (**b**) in the three experimental groups. Different letters indicate exploratory statistical differences among the experimental groups within each week (Tukey–Kramer-adjusted $p \leq 0.05$) and should not be interpreted as confirmatory treatment effects. Because both indices were derived from intake variables partly based on predictive estimates of pasture intake and group-level forage intake measurements, the reported comparisons should be regarded as exploratory evidence of treatment-associated responses under the grazing conditions of the present study.

The estimated apparent NUE values showed exploratory differences among the experimental groups ($p \leq 0.001$) and were significantly affected by sampling week ($p \leq 0.001$), with a significant group × week interaction ($p \leq 0.001$). SHL showed the highest estimated NUE values, followed by SH and AH, with exploratory pairwise comparisons indicating differences among all the experimental groups (Tukey–Kramer-adjusted $p \leq 0.001$). Nevertheless, SHL generally maintained higher estimated apparent NUE values than AH across most sampling weeks (Figure 3b).

Because both estimated apparent EUE and estimated apparent NUE were derived from intake variables based on predictive estimates and group-level forage measurements,

these indices should be regarded as exploratory indicators describing treatment-associated responses rather than as direct measures of physiological nutrient utilization efficiency.

4. Discussion

Overall, the nutritive value of the pasture offered to the three experimental groups was comparable throughout the grazing period, with only minor differences in nutritive value observed in the SHL group (Table 2), which coincided with the lower estimated pasture intake observed in this group (Table 7). The differences detected between grazing entry and post-grazing residues reflected a consistent selective grazing process rather than treatment-specific effects (Tables 1 and 3). Therefore, the observed differences in milk composition and estimated apparent nutrient utilization indices were more likely associated with the type and conservation method of the supplemented forages than with major differences in pasture quality.

The progressive increase in air temperature and temperature–humidity index (THI) reflected the expected seasonal transition under Mediterranean conditions (Figures 1 and 2). Although THI values increased toward the end of the experimental period, they remained mostly within the thermoneutral or mild heat load range reported for dairy sheep. Consequently, environmental heat load was unlikely to have been a major factor affecting animal performance during the study, although modest temporal effects cannot be excluded.

The differences in chemical composition among the conserved forages were consistent with both forage species and conservation method. Alfalfa hay provided higher crude protein and metabolizable protein concentrations than the sulla-based forages, confirming its established role as a forage with high crude protein concentration in dairy sheep nutrition. In contrast, SHL showed slightly higher ADF and phenolic concentrations, likely reflecting fermentation-related modifications and reduced leaf losses during conservation (Table 5).

Fresh sulla (*Sulla coronaria* L.) is known to contain moderate concentrations of condensed tannins, although tannin concentration may vary substantially according to genotype, phenological stage, and environmental conditions [2,7]. Condensed tannins have frequently been associated with reduced ruminal protein degradation and improved nitrogen utilization efficiency [8]. However, forage conservation processes such as drying, wilting, and fermentation have been reported to alter tannin extractability and biological activity [32–35], although fresh sulla was not analyzed in the present study and therefore direct conservation effects could not be assessed.

In the present study, condensed tannins were not detectable in the conserved forages under the analytical conditions adopted, suggesting that tannin concentration and/or extractability may have been below biologically relevant thresholds. Therefore, the production responses observed in the present experiment should be interpreted cautiously and are more likely related to differences in nutrient composition, forage physical characteristics, intake regulation, and protein–energy balance than to direct tannin-mediated effects.

The relatively high refusal rate observed for SHL (approximately 32% of offered forage DM) suggests a lower acceptance of haylage compared with the other conserved forages. However, because haylage conservation quality was not assessed through measurements of pH, fermentation profile, ammonia-N, or dry matter losses, the factors underlying this response cannot be conclusively identified. The elevated moisture content and bulk density of haylage may have contributed to the observed intake pattern, although other conservation-related factors cannot be excluded.

Milk yield was maintained across the dietary treatments, indicating that the inclusion of conserved sulla forages in place of alfalfa hay did not negatively affect overall milk production under the conditions of the present study (Table 9). Although milk yield and milk composition were measured at the individual animal level, the group-based application

of dietary treatments under grazing conditions should be considered when extrapolating treatment effects beyond the present study. Nevertheless, differences were observed in milk composition and estimated apparent nutrient utilization indices (Tables 9 and 10). The higher milk urea concentration detected in the AH group is consistent with a greater nitrogen surplus and lower apparent nitrogen utilization efficiency, as milk urea concentration is commonly considered an indirect indicator of inefficient nitrogen utilization [36]. This effect could be mediated mainly by differences in estimated nitrogen intake, although the ruminally degradable protein supply might also have played an important role. Therefore, under the conditions of the present study, differences in estimated nitrogen intake were likely an important contributor to the observed variation in milk urea concentration and apparent NUE. It is also important to distinguish between milk protein concentration and nitrogen utilization efficiency, because higher milk protein concentration does not necessarily imply improved efficiency of nitrogen use when nitrogen intake is proportionally increased.

Estimated apparent energy utilization efficiency showed exploratory differences among the experimental groups and across sampling weeks (Table 10). Although the SH group showed greater estimated energy intake, this was not associated with a proportional increase in milk energy output. However, because both energy intake and EUE were partly derived from predictive estimates and group-level measurements, the observed differences should be interpreted cautiously. The present data do not allow discrimination between true biological differences and uncertainty associated with intake estimation, and no mechanistic interpretation can be made in the absence of direct metabolic or behavioural measurements.

Estimated apparent nitrogen utilization efficiency (NUE) showed exploratory differences among the experimental groups, with SHL showing the highest estimated apparent NUE values (Table 10). One possible explanation is that differences in the balance between protein and energy supply may have influenced nitrogen utilization under the conditions of the present study [37]. However, because nitrogen intake was partly derived from estimated intake variables and no measurements of rumen fermentation, microbial protein synthesis, or nitrogen metabolism were performed, this interpretation remains speculative. Therefore, the mechanisms underlying the observed differences in apparent NUE cannot be established from the present data.

The potential contribution of phenolic compounds cannot be excluded, although their biological role could not be assessed in the absence of direct measurements [38].

Taken together, the present results suggest that forage species and conservation method influenced nutrient intake patterns, milk composition, and apparent nutrient utilization efficiency mainly through differences in nutrient composition, forage physical characteristics, and feeding dynamics rather than through detectable condensed tannin activity. These findings suggest that the nutritional functionality of conserved sulla forage may reflect both botanical characteristics and changes associated with forage conservation. However, because fresh sulla was not analyzed before conservation, the present study cannot directly demonstrate the effects of conservation on condensed tannin concentration or activity.

5. Conclusions

This study suggests that conserved sulla-based forages can be used as alternatives to alfalfa hay in pasture-based diets for lactating dairy ewes during a 56-day experimental period without detrimental effects on milk yield, live weight, or body condition score. Although overall milk production was maintained across the treatments, differences in milk composition and estimated apparent nutrient utilization indices suggest that both

forage species and conservation method influenced animal responses under the conditions of the present study.

Alfalfa hay supported higher milk fat and protein concentrations but was associated with higher milk urea concentration and lower estimated apparent nitrogen utilization efficiency, suggesting a greater apparent nitrogen surplus. In contrast, sulla haylage (SHL) showed higher exploratory estimates of apparent nitrogen utilization efficiency while maintaining comparable estimated apparent energy utilization efficiency. Despite higher estimated intake levels, the sulla hay treatment showed lower estimated apparent energy utilization efficiency than the other treatments.

Condensed tannins were not detected in the conserved sulla forages under the analytical conditions adopted in this study. Because the fresh sulla was not analyzed before conservation, no direct conclusions can be drawn regarding the effects of forage conservation on tannin concentration, extractability, or biological activity. Under the conditions of the present study, the observed responses appeared to be more closely related to differences in nutrient composition and intake patterns than to detectable condensed tannin activity.

Overall, sulla haylage was associated with higher exploratory estimates of apparent nitrogen utilization efficiency under the conditions of this exploratory pasture-based study, without negative effects on milk yield. However, because several intake-related variables were partly derived from predictive estimates and group-level measurements, and because the treatments were applied to single experimental groups grazing separate paddocks, these findings should be interpreted with caution. Further studies including direct measurements of feed intake, rumen fermentation, and nutrient utilization are needed to confirm the mechanisms underlying these responses.

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Abbreviations

The following abbreviations are used in this manuscript:

AH	Alfalfa hay
SH	Sulla hay
SHL	Sulla haylage
LW	Live weight
BCS	Body condition score
DM	Dry matter

OM	Organic matter
CP	Crude protein
EE	Ether extract
NSC	Non-structural carbohydrates
NDF	Neutral detergent fibre
aNDF	Amylase-treated neutral detergent fibre
ADF	Acid detergent fibre
ADL	Acid detergent lignin
IVDMD	In vitro dry matter digestibility
CT	Condensed tannin
UFL	Feed unit for milk production
MP	Metabolizable protein
FPCM	Fat- and protein-corrected milk
SCC	Somatic cell count
SCCS	Somatic cell count score (log10 SCC)
N	Nitrogen
EUE	Estimated apparent energy utilization efficiency
NUE	Estimated apparent nitrogen utilization efficiency

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