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Sulla (*Sulla coronaria* L.) nutritive value in calcareous soil pastures of Mediterranean area

Anass EL Yemlahi[1,*], Abdelhay Arakrak[1], Mounir Hassani Zerrouk[1], Amin Laglaoui[1], Mohammed Ayadi[2], Mohammed Bakkali[1]

[1] *Biotechnology and Biomolecular Engineering Research Team, FSTT, Abdelmalek Essadi University Tetouan, Morocco.*
[2] *Animal Production Research Unit, National Institute of Agricultural Research, Tangier, Morocco.*

[*] **Corresponding author:** elyemlahi@hotmail.fr

Original Research

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Abstract:

In this study investigated the nutritive value of Sulla harvested from two calcareous soil sites containing different concentrations of $CaCO_3$, i.e. Kariat Benaouda (10.7%) and Mosmoda (26.0%) located in the northwest of Morocco. Small differences emerged among samples as a result of the sampling site's effect regarding the crude protein and fiber content. However, a significant variation was observed concerning the mineral composition, particularly for iron (Fe) content. Comparatively, Sulla at the Mosmoda site had a higher Fe concentration (875 mg/kg Dray Matter (DM) at the vegetative stage and 194 mg/kg DM at the flowering stage) than at Kariat Benaouda (139 and 15.1 mg/kg DM, respectively). The results of measured secondary metabolites show a high concentration of total phenolics and oxalate content during the flowering stage at the Mosmoda site (4.88% DM and 27.87 mg/100g, respectively) as compared with the Kariat Benaouda site (3.37% DM and 16.07 mg/100g). Overall, the plant could maintain a very high value of organic matter digestibility, at the vegetative stage at both harvesting sites (71.11% OM at the Mosmoda site and 84.16% OM at Kariat Benaouda). These findings indicate the efficiency of Sulla for animal nutrition, to improve calcareous soil pastures indigenous to the Mediterranean area.

Keywords: Sulla; Chemical composition; Antinutritional components; Calcareous soil

Introduction

Efficient use of pastoral genetic resources implies preliminary evaluation of environmental conditions that could affect plant yield and quality. Among others, calcareous soil is one of many factors affecting nutrient uptake and plant growth, especially in arid and semi-arid regions where precipitation is scarce (Hopkins and Ellsworth, 2005; FAO, 2016). Plants in calcareous soils, where the pH is alkaline, suffer from low availability of nutrients, especially phosphorous, potassium, and iron (Farhat et al., 2015). Generally, chlorotic symptoms and restricted biomass production arise in plants that grow in calcareous soils, including forage legumes (Farhat et al., 2015). Under such conditions, it is hard to reach the desired results.

In this context, Sulla species, tribe Hedysareae and family Fabaceae constitute important phytogenetic patrimony able to promote pastoral production. Originating from the Mediterranean region, these species are notable as some of the most valuable phytogenetic assets recognized for their specific ability to tolerate stressful environmental conditions (Annichiarico et al., 2008), for instance, drought stress and calcareous soil conditions (Farhat et al., 2015; Tounsi-Hammami et al., 2016). This genus consists of a variety of annual or perennial species, either diploid or tetraploid, characterized by their morphology, reproductive mechanism, and nutritive value (Issolah et al., 2014; El Yemlahi et al., 2019; El Yemlahi et al., 2024).

Within the Sulla species, *Sulla coronaria* [L.] Medik is also known as *Hedysarum coronarium* [L.] or Sulla is a short-lived pastoral legume ideally suited for agricultural systems with minimal inputs and rainwater (Ruisi et al., 2011). The plant is recognized for its high genetic diversity (Marghali et al., 2012), and forage quality and quantity (De Koning et al., 2010; Issolah et al., 2014). It contains a relatively high proportion of non-structural to structural carbohydrates (Bonanno et al., 2011) and an adequate amount of condensed tannins (Amato et al., 2005), which enhances animal intake and efficiency (Bonanno et al., 2011).

However, the occurrence of stressful conditions such as calcareous soil could potentially elevate the levels of antinutritional compounds to harmful levels (Rahman et al., 2013). Due to their biochemical properties, compounds like oxalates play a crucial role in various physiological functions such as photosynthesis, pH regulation, and detoxification of heavy metals (Li et al., 2022). Nevertheless, given their nature as antinutrients, oxalates limit the bioavailability of certain nutrients by binding to minerals, diminishing thereby their absorption and utilization (Natesh et al., 2017). On the other hand, *S. coronaria* has been well known for its peculiarity in accumulating Ca^{2+} from calcareous soil as insoluble crystals of calcium oxalate (Tola et al., 2009). Such accumulation could potentially impact both plant growth and quality.

In this regard, the nutritional value of wild *S. coronaria* was investigated from two sampling sites containing different concentrations of calcium carbonate ($CaCO_3$) collected across the Northwest region of Morocco. The findings could provide valuable information regarding the use of legume plants for the management of calcareous soil pastures and to enhance soil fertility and crop productivity.

Materials and methods

Plant sampling and soil characterization

Plant samples (stems and leaves) of *S. coronaria* from three square plots (1 m^2) set up at every field location were collected during January and June of 2014 across the North of Morocco. Samples were harvested at the two growth stages i.e., early vegetative and flowering stages. Collected samples were oven-dried at 60 °C, then crushed using POLYMIX® PX-MFC 90 D Hammer mill. Additionally, soil samples were collected in three replicates from each harvesting site. These samples were air-dried, crushed, homogenized, and finally sieved for analysis. The densimetry method was employed to ascertain soil particle size (Bouyoucous, 1962). Organic matter was assessed using the Walkley and Black method (Walkley and Black, 1934). Total calcium carbonate ($CaCO_3$) was quantified using Bernard's calcimeter. Total nitrogen content was measured using the Kjeldahl technique (ISO, 1995). The Olsen method was utilized to ascertain the availability of phosphorus (Olsen et al., 1954). Exchangeable potassium was measured using a flame photometer as described by Bower et al. (1952).

Morphological characteristics

Specimens from natural populations of *S. coronaria* from the North of Morocco where the plant grew spontaneously in calcareous soil pastures were randomly collected at the flowering stage for the examination of various phenological and morphological characteristics such as plant height, leaf number, and number and Length of inflorescences as described by Flores et al. (1997). Leaf area was determined using multiple regression analysis performed by ImageJ software (version 1.46r, National Institutes of Health, USA) and the following formula:

$$LA = -8.53 + (1.27 \times L) + (4.41 \times W)$$

where;
LA: Leaf area (cm^2);
L: length (cm), and
W: width (cm).

Chemical analysis:

Samples were examined for crude protein (CP) content using the routine Kjeldahl nitrogen technique and for ether extract (EE) following the Soxhlet method ((AOAC, 1997): method 920.39). Total Ash content was determined according to ((AOAC, 1997): method 942.05), and acid insoluble ash (AIA) using 2 N acid-insoluble ash methods (Bergero et al., 2009).

The determination of water-soluble carbohydrates was conducted utilizing the phenol-sulfuric acid protocol, as described by Dubois et al. (1956). Crude fiber (CF) was determined using the ANKOM fiber analyzer (Ankom Technology Co.) according to the Weende method as described by ((AOAC, 1997): method 978.10). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were assessed using sodium sulfite and thermo-stable α-amylase as delineated by Van Soest et al. (1991).

$$HEM = NDF - ADF$$
$$CEL = ADF - ADL$$
$$NFC = 100\% - (EE + CP + Ash + CF)$$

where;
HEM = Hemicellulose, CEL = Cellulose, NFC = Non fiber carbohydrate, CP = Crude protein, EE = ether extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, CF = Crude fiber.

Phenolic compounds and condensed tannins analysis

Plant specimens (200 mg), previously dried at 50 °C following Makkar (2000)'s guidelines, were analyzed for total extractable phenolics (TEP) and tannins (TET) utilizing the Folin Ciocalteu method, and for soluble condensed tannins (ECT) using the butanol-HCl-Fe^{3+} procedure as outlined by Makkar (2000). The solid residue remaining from the extraction was used to estimate the fraction of condensed tannins bound to proteins (PCT) and those to the fibers (FCT) according to the protocol described by (Terrill et al., 1992).

Antinutritional constituents

The concentration of phytate in the samples was determined following the methodology outlined by Vaintraub and N.A. (1988), which employed Wade's reagent (0.03% of $FeCl_3$ and 0.3% of sulfosalicylic acid) and sodium phytate (Sigma-Aldrich, St. Louis, MO), serving as the standard. To assess total oxalate content, samples were extracted with hydrochloric acid (6N) for 1 hour in a boiling water bath. The extracted oxalates are measured after oxidation with potassium permanganate (0.09N) in a hot sulfuric acid (1:4 v/v) medium following the methodology outlined by AOAC (1990). The nitrate concentrations were ascertained using the salicylic acid method, following the procedure detailed by Cataldo et al. (1975), with (KNO_3) utilized as a standard.

Fermentation kinetics and digestibility

Dried samples (300 mg) were subjected to in vitro incubation with rumen fluid in a glass piston syringe, following the protocol established by Menke and Steingass (1988).

The rumen fluid, flushed with CO_2, was added (1:3 v/v) to a buffered mineral solution maintained in a thermostatically controlled water bath at 39 °C. The gas production measurements were taken at 0, 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours of incubation. All samples were incubated in

triplicate, and three syringes filled solely with the incubation medium were included as blanks. Cumulative gas production data were adjusted to adhere to the (Orskov and Mcdonald, 1979) model:
$y = a + b\,(1 - e^{-ct})$
where;
y: the gas production (mL);
a: the gas production (mL) from the soluble fraction;
b: the gas production (mL) from the insoluble fraction;
c: the gas production rate (h^{-1}) constant, and t: the incubation time (h).
The metabolizable energy (ME) was evaluated by applying the formula proposed by Menke et al. (1979), as follows:
$ME\ (MJ/Kg\ DM) = 2.2 + 0.1357\ GP + 0.0057\ CP + 0.0002859\ CF^2$
where;
GP: gas volume (mL/0.2g DM) at 24 h of incubation,
CP: crude protein (g/kg DM) and
CF: crude fat (g/kg DM) in the sample.
Microbial biomass production (MBP), and the partitioning factor (PF) were calculated according to the equation of

Table 1. Chemical soil analysis of the different harvesting sites.

Soil characteristic	Unit	Site	
		Mosmoda	**Kariat Benaouda**
Water content	%	4.18	4.14
pH (acidity)		8.8	9.1
N,	%	0.21	0.24
P_2O_5	ppm	17.8	8.4
K_2O	ppm	268	389
Ca	%	12.75	3.83
OM	%	2.3	1.6
$CaCO_3$	%	**26.0**	**10.7**
Clay	%	20.8	21.1
Fine silt	%	20.8	31.6
Coarse silt	%	22.4	29.6
Fine sand	%	5.2	7.1
Coarse sand	%	4.7	0.0

Table 2. Agro-morphological characteristics of *S. coronaria* at two harvesting sites.

Characteristics name	Unit	Kariat Benaouda	Mosmoda	*P*-value
Morphological				
Plant height	cm	162±10.6	19.2±6.71	0.00
Central leaf area[a]	cm	13.3±0.72	2.94±1.32	0.00
Leaf number	No.	132±38.1	37.3±6.43	0.01
Number of leaflets per leaf	No.	9.00±0.00	7.00±0.00	0.00
Stem number per plant	No.	13.7±5.03	7.00±2.64	0.11
Diameter of the collet	mm	0.85±0.21	0.40±0.10	0.01
Length of leaf peduncle	cm	19.0±1.00	11.0±0.50	0.00
Internode number[a]	No.	11.0±2.00	7.50±1.50	0.07
Number of inflorescences	No.	60.7±3.05	23.0±6.08	0.00
Length of inflorescences	cm	12.5±1.80	6.17±0.76	0.00
Biomass production				
Vegetative (Year 2013)	$Kg.ha^{-1}$	783±41.0	667±28.9	0.00
Flowering (Year 2013)	$Kg.ha^{-1}$	3554±61.5	962±29.2	0.00
Vegetative (Year 2014)	$Kg.ha^{-1}$	588±30.8	454±40.6	0.00
Flowering (Year 2014)	$Kg.ha^{-1}$	2668±46.2	645±63.6	0.00

[a]: Internode number of principal stem.

Blümmel et al. (1997):
PBM = TDDM − (GV72×FS)
$PF\ (mg/mL) = \frac{TDDM\ (mg)}{GV72}$
where;
TDDM: truly degraded dry matter (mg/gOM),
GV_{72} (mL): gas volume at 72 h of incubation, and
SF: the stoichiometrical factor.

Protein degradability

In vitro, crude protein degradability was assessed according to the procedure outlined by Aufrere et al. (1989). Dried samples (500 mg) were incubated in a buffered protease enzyme solution (Streptomyces griseus Type XIV, Sigma, St Louis, MO) for 1 h at 40 °C. The mixture was centrifuged at 845 × g at 4 °C for 5 min and the supernatant was evaluated for nitrogen content using the Kjeldahl method. Protein degradability was estimated as the proportion of nitrogen disappearing using the following formula:
$IVCPD\ (g/g\ DM) = \frac{DN(\%DM)}{TN(\%DM)}$
where,
DN: degradable nitrogen after digestion with protease enzyme solution, and
TN: total nitrogen in the sample.

Mineral composition

Grinding and compressing samples (into a pellet of 32 mm diameter) were essayed for mineral contents (i.e., Ca, P, S, Mg, Na, K, Fe, and I) using the Axios X-ray fluorescence spectrometry method according to procedure outline by National Center of Scientific and Technical Research in Rabat, Morocco.

Numerical analysis

The data from the two maturity stages, from each site were statistically analyzed using (Proc GLM) using (SAS) software. The parameters of gas production were calculated using (Proc NLIN) of SAS.

Results

Soil properties in the study area

Two field sites from North-west Morocco where *S. coronaria* a short perennial pasture legume grown spontaneously were selected for analysis. The findings from diverse physico-chemical analyses of distinct soil samples are outlined in Table 1. The soil characteristics at both harvesting sites indicated the presence of *S. coronaria* in alkaline clay soil, rich in potassium and nitrogen, and poor in phosphorus. In addition, mineral analysis of the collection sites shows noticeable variation in calcium carbonate ($CaCO_3$) concentration (Table 1). The lowest value of $CaCO_3$ was obtained in the Kariat Benaouda site (10.7%). While the highest value was registered at the Mosmoda site (26.0%), exceeding the critical level of 15% defined for soil to be classified as calcisols (FAO, 2016).

Morphological characteristics and biomass production

The pheno-morphological attributes of *S. coronaria* populations (Table 2) exhibited considerable variability among the harvesting site, particularly the plant height, length of leaf peduncle, central leaf area of the main stem, and length and number of inflorescences ($P < 0.001$). The leaf number and diameter of the main stem displayed some degree of phenotypic variation ($P > 0.01$), while no significant differences were observed for the stem number and internode number of the main stem. Furthermore, the field investigation results (Table 2) indicated a notable difference ($P < 0.05$), specifically during the flowering stage, among the evaluated sites regarding the biomass produced by *S. coronaria* over two successive years of growth (2013 and 2014). The highest value was obtained at the Kariat Benaouda site in 2013 with 783 Kg DM/ha at the vegetative stage and 3554 Kg DM/ha at the flowering stage in comparison with Mosmoda site (667 Kg DM/ha at the vegetative and 962 Kg DM/ha at the flowering stage). The lowest value was recorded at the Mosmoda site (454 Kg DM/ha at the vegetative stage and 645 Kg DM/ha at the flowering stage) in 2014 as compared with Kariat Benaouda site (588 and 2668 Kg DM/ha, respectively).

Chemical composition

The chemical composition of *S.coronaria* is presented in Table 3. Remarkably, small differences have arisen among samples, especially at the flowering stage, as a result of the harvesting site effect regarding their average Ash content of 11.2% and 15.5% at Kariat Benaouda and Mosmoda sites, respectively. In addition, a high concentration of crude protein (CP) was recorded at the Mosmoda site ($P < 0.05$) at both growth stages (21.3% and 17.3% at the vegetative and the flowering stage, respectively, as compared with that obtained at the Kariat Benaouda site (16.5% and 13.2%), respectively. Similarly, higher fat content, denoted as ether extract, was registered in samples from the Mosmoda site, notably at the flowering stage (3.35%). An acute decrease was observed at the Kariat Benaouda site as the plant progressed from the vegetative stage to the flowering stage (1.68%). Furthermore, the results of the evaluated fibers revealed no significant difference among samples according to the harvesting site (Table 3). The highest levels of neutral and acid detergent fiber (NDF and ADF) were recorded at the Mosmoda site during the flowering stage (NDF 43.9% and ADL 34.8%) in comparison with Kariat Benaouda (NDF 41.0% and ADL 32.8%). By contrast, no significant differences were noted for ADL content.

Total phenolics and condensed tannins analysis:

Regarding phenolic content (Table 4), results disclose significant fluctuations ($P < 0.05$) in total extractable phenols (TEP) and tannins (TET). The main concentration was noted at the Mosmoda site, especially at the flowering stage (4.88% DM and 3.53% DM, respectively) in comparison with the Kariat Benaouda site (TEP 3.37% DM and TET 1.80% DM). By the same token, the amount of extractable condensed tannin (CT) was significantly ($P < 0.05$) higher at the Mosmoda site than in Kariat Benaouda, notably at the flowering stage (2.23% DM at and 0.20% DM, respectively). The total bound CT fraction was similarly greater at the Mosmoda site, at the flowering phase (1.74% DM) compared to Kariat Benaouda (0.70% DM). By contrast, a significant decrease in the proportion of CT bound to fiber

Table 3. Chemical composition (% DM) of *S. coronaria* at two harvesting sites.

Chemical composition	Abbrev.	Kariat Benaouda		Mosmoda		Effect (*P*-value)		
		Vegetative	Flowering	Vegetative	Flowering	S	M	S×M
Dry matter	DM	14.1±0.84	18.2±0.37	11.6±0.73	17.2±0.40	0.01	0.00	0.14
Total ash	Ash	17.6±0.13	11.2±0.08	16.5±0.00	15.5±0.00	0.00	0.00	0.00
Organic matter	OM	82.4±0.13	88.7±0.08	83.5±0.00	84.5±0.00	0.00	0.00	0.00
Crude protein	CP	16.5±0.30	13.2±0.32	21.3±0.54	17.3±0.19	0.00	0.00	0.24
Crud fiber	CF	16.8±0.83	20.8±0.15	17.6±0.90	22.6±0.92	0.06	0.00	0.37
ether extract	EE	4.16±0.01	1.68±0.08	4.09±0.14	3.35±0.03	0.00	0.00	0.00
Water-soluble carbohydrate	WSC[a]	35.4±1.78	54.6±1.40	45.4±1.57	53.1±1.01	0.00	0.00	0.00
Acid insoluble ash	AIA	2.00±0.01	0.60±0.06	3.04±0.90	0.98±0.09	0.50	0.03	0.97
Neutral detergent fiber	NDF	34.8±0.21	41.0±0.67	34.9±0.57	43.9±0.68	0.02	0.00	0.03
Acid detergent fiber	ADF	28.6±0.26	32.8±0.42	28.9±0.96	34.8±0.71	0.03	0.00	0.08
Acid detergent lignin	ADL	14.1±0.65	16.9±0.21	9.40±0.21	20.2±1.14	0.21	0.00	0.00
Hemicellulose	HEM	6.32±0.21	8.17±1.09	6.60±0.77	9.01±0.98	0.38	0.01	0.65
Cellulose	CEL	14.3±0.64	15.9±0.21	19.1±0.41	14.7±0.43	0.00	0.00	0.00
Non-fibre carbohydrates	NFC	27.0±0.62	32.8±0.52	23.7±0.62	20.0±0.45	0.00	0.05	0.00

Data represented as means ± SD (n=3). S: site; M: maturity stage. [a] expressed as eq-mg glucose/g DM.

was observed at the Mosmoda site as the goes from vegetative (6.99% DM) to the flowering stage (4.50% DM). However, it is higher than those at the Kariat Benaouda site (2.94% DM at the vegetative stage and 3.43% DM at the flowering stage).

Antinutritional substances:

The levels of measured anti-nutritional components revealed significant variation ($P < 0.05$) among the harvesting sites (Table 5). The highest values (mg/100g DM) of nitrate and phytate were recorded at the Kariat Benaouda site typically during the flowering stage (3.46 and 30.70, respectively) as compared with the Mosmoda site over a similar period (3.43 and 21.30, respectively). Likewise, a high concentration of total oxalate (mg/100g DM) content was obtained at the Mosmoda site (18.70 at the vegetative stage and 27.87 at the flowering stage) in comparison with the Kariat Benaouda site (12.47 at the vegetative stage and 16.07 at the flowering stage).

Mineral analysis

Mineral analysis (Table 6) demonstrated notable differences ($P < 0.05$) among the collected sites of *S. coronaria* generally higher at the Mosmoda site than in Kariat Benaouda, except for chloride. Thus, a high concentration of Ca (28.2 g/Kg DM) and K (23.5 g/Kg DM) was detected in samples harvested at the Mosmoda site at the vegetative stage compared with Kariat Benaouda (Ca 13.9 g/Kg and K 8.30 g/Kg). Likewise, the content of Fe was very high at the Mosmoda site at both growth stages (875 mg/Kg at the vegetative stage and 194 mg/Kg at the flowering stage) as compared with Kariat Benaouda (139 mg/Kg DM at and 15.1 mg/Kg DM). The sulfur (S) content was similarly high at the Mosmoda site, particularly during the flowering stage (8.07 g/kg DM), compared to the Kariat Benaouda site over the same growth stage (3.71 g/Kg DM). By contrast, a notable decline in Cl contents was recorded in *S. coronaria* collected at the Mosmoda site (42.5 g/Kg DM at the vegetative stage and 31.8 g/Kg DM at the flowering stage) than in Kariat Benaouda (26.1 g/Kg DM and 19.3 g/Kg DM at vegetative and flowering stage, respectively).

In vitro gas production and digestibility

Significant differences ($P < 0.05$) were observed in gas production parameters between the two sites (Table 7). Overall, the gas production was slightly higher at the Kariat Be-

Table 4. Polyphenols and condensed tannin (% DM) in the browse *S. coronaria*.

Composition name	Abbrev.	Kariat Benaouda		Mosmoda		(*P*-value)		
		Vegetative	Flowering	Vegetative	Flowering	S	M	S×M
Total extractable phenolics	TEP[a]	3.03±0.10	3.37±0.17	4.41±0.13	4.88±0.08	0.00	0.00	0.83
Total extractable tannins	TET[a]	2.21±0.05	1.80±0.22	2.72±0.39	3.53±0.20	0.00	0.71	0.00
Soluble condensed tannin	ECT[b]	0.17±0.00	0.20±0.02	0.11±0.00	2.23±0.06	0.00	0.00	0.00
Protein-bound condensed tannin	PCT[b]	0.39±0.03	0.70±0.05	0.40±0.04	1.74±0.06	0.00	0.00	0.00
Fibre-bound condensed tannin	FCT[b]	2.94±0.33	3.43±0.72	6.99±0.07	4.50±0.52	0.00	0.00	0.00

Data represented as means ± SD (n=3). S: site; M: maturity stage. [a] expressed as eq-mg leucocyanidin/gDM. [b] expressed as eq-mg tannic acid/gDM.

Table 5. Anti-nutritional substances (mg/100g DM) of *S. coronaria* at two harvesting sites.

Anti-nutritional	Kariat Benaouda		Mosmoda		*P*-value		
substances	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**	**S**	**M**	**S×M**
Oxalate	12.5±0.00	16.1±0.00	18.7±0.00	27.9±0.00	0.00	0.00	0.00
Phytate	16.5±1.87	21.3±2.40	20.6±0.15	30.7±0.22	0.00	0.00	0.00
Nitrate	2.66±0.12	3.43±0.15	2.33±0.05	3.46±0.07	0.04	0.00	0.02

Data represented as means ± SD (n=3). S: site; M: maturity stage.

naouda site, especially during the flowering stage, in comparison to the Mosmoda site.

There was a marked variation ($P < 0.05$) in both dry and organic matter digestibility (IVDMD and IVOMD) across the harvesting sites (Table 8). The highest values were recorded during the vegetative stage at the Kariat Benaouda site (IVDMD 81.07% DM and IVOMD 87.34% OM), while the lowest values were documented during the flowering stage at the Mosmoda site (IVDMD 66.57% DM and IVOMD 66.69% OM). By the same token, the *in vitro* crude protein degradability (IVCPD) was relatively higher at the Mosmoda site at the vegetative stage (32.70% DM) as compared with Kariat Benaouda (25.56% DM). On the contrary, a significant reduction in IVCPD was observed at the Mosmoda site as the plant progressed to the flowering stage (7.27% DM) compared to Kariat Benaouda site, where the IVCPD decreased by 18.36% DM (Table 8).

Discussion

The soils in Mediterranean countries, including various regions in Morocco, frequently display increased levels of both total and active calcium carbonate FAO (2016). Such a situation could potentially have an effect on plant mineral nutrition and growth as the presence of $CaCO_3$ can adversely affect water holding capacity and the absorption of essential mineral elements. Such a situation requires the use of acidifying materials to achieve the desired yield and quality (Alghamdi et al., 2023). On the other hand, bioremediation involves plants with a high capacity to bioconcentrate Ca from soil, which appears as a good and inexpensive method.

S. coronaria, a forage legume, is one of the indigenous floras adapted to Mediterranean calcareous soil conditions. In this study, the values of measured soil calcium carbonate ($CaCO_3$) revealed a higher concentration at the Mosmoda site, than those defined by FAO (2016) at which the soil could be called calcareous ($> 15\%$).

Such ability has been also attributed to rhizosphere-buffering features of the plant and to its capacity to accumulate intracellularly Ca^{2+} from the soil as crystalline salts in a specific organ called "shovels" localized in their roots system, resulting in a localized reduction of $CaCO_3$ from the soil (Tola et al., 2009). In this study, a significant increase of Ca content was observed in the upper part of *S. coronaria* collected at the Mosmoda site, in concordance with those proposed by Gasmi-Boubaker et al. (2012) and with El Yemlahi et al. (2019) for *Sulla flexuosa* L. harvested at different $CaCO_3$ soil concentrations. Excessive calcium usually results in its precipitation as calcium salts like calcium oxalate (Franceschi and Nakata, 2005). The function of calcium oxalate is likely to vary i.e. roles in maintaining ion balance, enhancing tissue rigidity, and facilitating the detoxification of heavy metals (Franceschi and Nakata, 2005). However, this accumulation of calcium oxalateis often associated with health complications in grazing animals (Rahman et al., 2013).

Equally important, P and Fe content was relatively high at the Mosmoda site suggesting capacity of *S. coronaria* for phosphate and iron-solubilizing can become highly efficient under calcareous soil conditions (Tola et al., 2009). In concordance with the present findings, the results of measured Mg content show high concentration at the Mosmoda site. This suggests that excessive calcium was not the primary factor leading to Mg deficiency in *S. coronaria* in calcareous soils (Farhat et al., 2015).

Table 6. Minerals composition (g/kg DM) of *S. coronaria*.

Minerals	**Abbrev.**	Kariat Benaouda		Mosmoda		*P*-value		
		Vegetative	**Flowering**	**Vegetative**	**Flowering**	**S**	**MS**	**S×M**
Calcium	**Ca**	13.9±0.35	11.2±0.49	28.2±1.77	21.3±1.84	0.00	0.00	0.08
Potassium	**K**	8.30±0.07	14.7±1.06	23.5±0.52	19.9±1.20	0.00	0.04	0.00
Magnesium	**Mg**	1.60±0.15	1.38±0.08	2.40±0.14	2.30±0.03	0.00	0.12	0.47
Phosphorus	**P**	1.40±0.05	1.32±0.18	2.35±0.52	2.56±0.03	0.00	0.77	0.49
Sulfur	**S**	4.93±0.49	3.71±0.11	5.33±0.97	8.07±0.26	0.00	0.15	0.01
Sodium	**Na**	10.2±0.92	5.17±0.78	9.12±0.22	9.23±0.94	0.05	0.01	0.01
chloride	**Cl**	42.5±0.79	31.8±0.95	26.1±0.78	19.3±0.99	0.00	0.00	0.03
Iron	**Fe**[1]	139±1.24	15.1±0.11	875±1.58	194±1.77	0.00	0.00	0.00

Data represented as means ± SD (n=3). S: site; M: maturity stage. [1] expressed as mg/kg DM.

Table 7. *In vitro* gas production (mL/200 mg DM) and estimated parameters of *S. coronaria*.

Incubation time (hour)	Kariat Benaouda		Mosmoda		*P*-value		
	Vegetative	Flowering	Vegetative	Flowering	S	M	S×M
2	39.7±0.26	32.9±0.16	39.4±0.10	25.5±0.82	0.00	0.00	0.00
4	68.6±0.89	59.1±0.88	64.7±0.79	48.6±0.79	0.00	0.00	0.00
6	92.0±0.85	92.1±0.64	91.9±0.23	72.1±0.88	0.00	0.00	0.00
8	105±0.87	99.1±0.26	98.4±0.25	85.8±0.61	0.00	0.00	0.00
10	125±1.02	119±0.93	126±0.79	105±0.86	0.00	0.00	0.00
12	145±1.00	138±0.88	150±0.79	119±0.91	0.00	0.00	0.00
24	211±0.82	185±0.64	209±0.78	178±0.92	0.00	0.00	0.01
48	238±0.81	204±0.64	229±0.51	190±0.94	0.00	0.00	0.01
72	244±0.81	211±0.93	242±1.01	198±0.66	0.00	0.00	0.00
Estimate parameters							
a, mL/gDM	0.93±0.23	1.34±0.24	0.54±0.02	-4.14±0.49	0.00	0.00	0.00
b, mL/gDM	206±2.95	203±2.05	246±1.27	206±1.91	0.00	0.00	0.00
c, h^{-1}	0.09±0.00	0.06±0.00	0.07±0.00	0.07±0.00	0.26	0.00	0.00
a+b, mL	207±3.09	204±1.81	247±1.30	202±2.40	0.00	0.00	0.00
ME, MJ/kg DM	30.7±0.24	26.5±0.24	33.3±0.36	28.2±0.19	0.00	0.00	0.06
MBM, mg	474±8.74	318±4.01	300±8.33	328±2.59	0.00	0.00	0.00
PF, mg/mL	3.43±0.04	2.59±0.02	2.24±0.03	2.80±0.01	0.00	0.00	0.00

Data represented as means ± SD (n=3). a: the gas production from the soluble fraction; b: the gas production from the insoluble fraction; c: the gas production rate constant; a+b: potential gas production; ME: metabolizable energy; MBP: microbial biomass production; PF: partitioning factor. S: site; M: maturity stage.

Remarkably, plants grown in the Kariat Benaouda site were distinguished by elevation in reach (100 – 115 cm) in correlation with high yield biomass at the flowering stage when compared with those from the Mosmoda site where the plant height was characterized by length shortened (7 – 20 cm at the flowering stage), much lower to some natural populations of *S. coronaria* collected across Algeria (Zirmi-Zembri and Kadi, 2020) and Italy (Ruisi et al., 2011). However, no sign of leaf chlorosis was detected in both harvesting sites. In optimal circumstances regarding seed density, temperature, and altitude, *S. coronaria* can yield approximately 10 t DM/ha in the initial year and 20 t DM/ha in the subsequent year (Patane, 2000; De Koning et al., 2010).

Considering the quality aspect, the crud protein was generally high at the Mosmoda site and similar to those reported by Issolah et al. (2014), and with *Medicago truncatula* L. and *Vicia sativa* L. (Gasmi-Boubaker et al., 2012), and above the optimal rumen function and nutritional requirements necessary for ruminant lactation and growth (McDonald et al., 2002). Coupled with this, the results of phenolic contents unveiled significant variation ($P < 0.05$) of soluble and bond condensed tannins (CT), generally high at the Mosmoda site. Similarly, results were obtained by Rossi et al. (2023) who reported an increase of both soluble (extractable) and bound CT levels of *S. coronaria* under drought stress conditions. Such results have been attributed to calcium concentration, permitting the establishment of CT-protein complexes (Carnovale et al., 2015). However, the values obtained at the Mosmoda site where the concentration of soil $CaCO_3$ is high, remained acceptable, enhancing protein conservation against decomposition in the rumen and boosting nitrogen provision to ruminants fed with Sulla (Bonanno et al., 2011).

The fiber content, expressed as neutral detergent fiber (NDF), was typically lower than the range of 60 – 65% recommended as the crucial threshold, and exceeding the recommended minimum level of dietary fiber (NRC, 2001). The acid detergent fiber (ADF) was similarly low and

Table 8. Digestibility value as (% DM) of *S. coronaria*.

Digestibility value	Abbrev.	Kariat Benaouda		Mosmoda		*P*-value		
		Vegetative	Flowering	Vegetative	Flowering	S	M	S×M
in vitro DM digestibility	**IVDMD**	80.42±0.62	74.74±0.75	70.77±0.13	68.30±0.20	0.00	0.00	0.00
in vitro OM digestibility	**IVOMD**	84.16±0.85	80.33±0.70	71.11±0.29	66.43±0.22	0.00	0.00	0.30
in vitro CP degradability	**IVCPD**	25.56±0.54	18.36±0.70	32.70±0.46	7.27±0.49	0.00	0.00	0.00

Data represented as means ± SD (n=3). S: site; M: maturity stage.

reached 34.8% at the flowering stage. However, above the recommended values of $17-21\%$ for ruminant diets (NRC, 2001). The content of lignin (represented by ADL fiber fraction) was within the range obtained by Issolah et al. (2014). The fraction of nonfibrous carbohydrates (NFC), an estimate of the rapidly available carbohydrates, was fairly below the threshold values of $36-44\%$ set by (NRC, 2001). Regarding the anti-nutritional factors, the values obtained from oxalate, nitrate and phytate inspection were very low in comparison with those reported (mg of oxalate per 100 DM) for some leguminous browse plants such as *Acacia auriculata* (58.79), *Caesalpina pulcharina* (104.15) and *Bauhinia thoningii* (223.16), commonly consumed by ruminant animals (Njidda, 2010; Atiku et al., 2016); however, they are similar to those recorded for others Sulla species, for instance, *S. spinosissima* and *S. pallida*, located in the arid region of the North of Morocco (El Yemlahi et al., 2024). These findings generally appear to fall below the toxic levels, and incredibly to have any detrimental impacts on the animals' performance (Rahman et al., 2013).

Conclusion

From an applied perspective, results revealed the considerable potential of *S. coronaria* grown naturally in calcareous soil concerning the mean crud protein content (19.3% DM), fiber concentrations (NDF 39.4% and ADF 31.8% DM), andtotal oxalate (23.3), phytate (25.6) and nitrate (2.89 mg/100g) anti-nutritional factors. This research marks the inaugural investigation aimed at assessing the effect of calcareous soil on antinational factors of *S. coronaria*. Accordantly, the use of *S. coronaria* appears as a good forage species for revegetation and soil-remediation of calcareous pastures and to fulfill the dietary needs of small ruminants. However, it is essential to conduct *in vivo* analysis on animal feed with Sulla to substantiate these results.

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Authors Contributions
All authors have contributed equally to prepare the paper.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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

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