

Potential contribution of *Sulla spinosissima* (L.) and *Sulla pallida* (Desf.) as a forage crop for arid pastures rehabilitation

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Abstract

Mediterranean climate is reported to harbor a wide range of forage legume species. However, little information is available on the potential use of these forages as fodder. We, therefore, conducted a study to evaluate the chemical composition, mineral content, and *in vitro* dry and organic matter digestibility of two forage legume species from arid pastures located in the Northeast of Morocco: *Sulla spinosissima* subsp. *capitata* and *Sulla pallida* Desf. To our knowledge, this is the first report about the nutritive value of these two species. Our results showed a wide variation between selected *Sulla* species; the phenological stage had a significant effect ($p < 0.05$) on most evaluated chemical traits and digestibility parameters. Comparatively, *Sulla spinosissima* L. had higher organic matter digestibility (72.43%OM) at the vegetative stage in correlation with lower acid detergent fiber (ADF) (24.23%DM), lower condensed tannin, (CT) (0.27%DM), and higher crude protein (24.01%DM). Conversely, *Sulla pallida* Desf. plants with a considerable amount of ADF (40.34%DM) and CT (4.50 %DM) had lower organic matter digestibility (56.09%OM). Both species are mineral-rich and contain satisfactory mean (gram per Kilogram Dry Matter) levels of calcium: 23.25 in *Sulla spinosissima* L. and 13.75 in *Sulla pallida* Desf.; phosphorus 1.01 in *Sulla spinosissima* L. and 1.53 in *Sulla pallida* Desf. The results of the present study suggest the suitability of both *Sulla* species for domestication to enhance pasture productivity and to ensure animal nutrition of small ruminants in arid pastures of Mediterranean area.

Introduction

In Morocco, small ruminant husbandry is one of the most important sectors in the livestock production system, which plays a vital agronomic and socioeconomic role, especially in northeastern Morocco (Dahan et al. 2012; Acherkouk 2013), home to a wide diversity of species (Chambouleyron 2020). This part of Morocco is mostly marked by an arid and/or semi-arid climate with a high risk of drought (Bechchari et al. 2014; Jilali et al. 2018), which therefore constitutes an acute challenge for smallholders searching to provide feedstuffs for ruminants. The aptitude of local ruminants to meet their nutritional needs mainly depends on the quality of forage available from natural pastures, resulting in greater pressure on pastoral lands (Dahan et al. 2012). The study conducted by Bechchari et al. (2014) revealed a decreasing trend in pastoral dry matter production estimated to be 2.8 kg per hectare. This situation urges local researchers to search for new resources that can support sustainable livestock production in the region.

Thus, the use of forage legumes such as *Hedysarum* sp. is increasingly becoming crucial. Originating from the western Mediterranean region as well as North Africa, this genus is one of the most interesting local resources. In Morocco, nine different *Hedysarum* spp. native to the region have been localized and identified (Fennane et al. 2007). Earlier studies of *Hedysarum* genus have only taken into account its geographical and ecological distribution, its morphology, and its root-nodule bacteria (Abdelguerfi-Berrekia et al. 1991; Kishinevsky et al. 2003; Salis et al. 2010; Ezzakkioui et al. 2015). However, its nutritive value and its potential use as fodder have not been sufficiently investigated. On the other hand, studies on certain *Hedysarum* species such as *Sulla flexuosa* L., and *Sulla coronaria* L., have shown that

they have good forage value (Martiniello et al. 2000; Kadi et al. 2011; Elyemlahi et al. 2019), resulting in high animal performance (Burke et al. 2002; Bonanno et al. 2011; Kadi et al. 2011). Hence, they have become established as a forage crop in several countries (Casella et al. 1984; Mitchell et al. 1999).

Given this situation of pastoral land degradation, in particular, the herbaceous layer, combined with the climatic impacts, and to introduce new forage species into new pastoral regions, it is important to have a good knowledge of their forage characteristics. Therefore, the present study aimed to assess the chemical composition, mineral content, and *in vitro* dry and organic matter digestibility of two indigenous *Sulla* species from the arid pastures of Morocco, namely *Sulla spinosissima* subsp. *capitata* and *Sulla pallida* Desf. This is the first time a study has been conducted to determine the nutritive value of these two selected *Sulla* species. Species with high potential nutritive value can be domesticated to restore overgrazed lands.

Materials And Methods

Plant sample collection and storage

A random sampling method was used to collect above-ground samples of *Sulla spinosissima* subsp. *capitata* and *Sulla pallida* Desf. at late vegetative and flowering stages in the Taourirt and Touissit regions, respectively, both located in the Northeast area of Morocco, near Oujda city (Fig. 1). The ecological characteristics of these herbs' natural habitats are shown in Table 1. Samples were hand-clipped, chopped, and wilted in the field. Later, grab samples were oven-dried at 70°C to constant weight and milled using POLYMIX® PX-MFC 90 D Hammer mill. Finally, ground samples were stored at 4°C until analysis.

Chemical analyses

Samples were analyzed for crude ash and organic matter (OM) by incinerating them overnight at 550°C (AOAC 1997), and for acid insoluble ash (AIA) using 2 N acid-insoluble ash methods (Bergero et al. 2009). Crude protein (CP) was determined using the Kjeldahl method with a conversion factor of 6.25. Crude fat, also known as ether extract (EE), was determined using the reflux Soxhlet extraction method with diethyl ether as extracting solvent (AOAC 1997). Structural fibers, i.e., neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were measured, including residual ash, sequentially with heat-stable alpha-amylase and sodium sulfite, as described by Van Soest et al. (1991), using an automated fiber analyzer (ANKOM²⁰⁰ Fiber Analyzer). Other chemical components were calculated as follows:

Hemicellulose, HEM = NDF-ADF;

Cellulose, CEL = ADF-ADL;

Non fiber carbohydrate, NFC = 100% – (EE+ CP+ Ash + CF).

Extraction and determination of total extractable condensed tannins

Plants samples (200 mg DM), pre-dried at 50°C and sieved through a 0.5 mm mesh screen, were extracted in 10 ml of aqueous acetone (7:3, v/v) in an ultrasonic water bath for 20 min at room temperature, then subjected to centrifugation for 10 min at 3000g at 4°C. The supernatant was collected and the pellet was subject to the same extraction as described above. Finally, the supernatants were combined and assayed for total extractable condensed tannins (ECT) by the butanol-HCl-Fe³⁺ method as described by Porter et al. (1986).

Dry and organic matter digestibility

In vitro dry matter digestibility analysis was completed using the *in vitro* gas production technique following the procedures of Menke and Steingass (1988). 0.200g dry weight were incubated in triplicate in a water bath at 39°C in prewarmed calibrated glass syringes, lubricated with vaseline pistons, and fitted with 30 ml of buffered strained rumen liquor taken before the morning feeding from three goats grazing from natural pastures and mixed (1:3 v/v) with buffered mineral solution (Goering and Van Soest 1970) under continuous flushing of CO₂. The syringes were gently shaken every 2 h, and cumulative gas production data were recorded at different incubation times. Finally, the residue remaining at end of the incubation period was then filtered, oven-dried at 60°C for 48 h, weighed, then incinerated at 550°C for 12 h to determine *in vitro* dry (IVDMD) and organic matter digestibility (IVOMD).

Fermentation kinetics and estimated parameters

The volume of gas produced was corrected for blank syringes (containing only incubation medium), and the fermentation kinetics were determined according to the exponential equation of Ørskov and McDonald (1979) as follows:

$$GP = a + b(1 - e^{-ct})$$

Where, GP (mL) is the gas produced at time t; a = the gas production from the soluble fraction; b = the gas production from the insoluble fraction; c = the gas production rate constant, and t = incubation time (h).

The amount of metabolizable energy (ME) was estimated using the equation of Menke et al. (1979), as follows:

$$ME \text{ (MJ/kgDM)} = 2.20 + 0.136 \times GP_{24} + 0.0574 \times CP.$$

Where, GP₂₄ (mL/0.2 gDM) = gas volume at 24 h of incubation and CP (g/kgDM) = crude protein in the sample.

In addition, microbial biomass production (MBP) and the partitioning factor (PF) as an indicator of fermentation efficiency were calculated following the equation of Blümmel et al. (1997):

$$\text{MBP (mg/gDM)} = \text{TDDM} - (\text{GV}_{72} \times \text{SF});$$

$$\text{PF (mg/mL)} = \text{TDDM} / \text{GV}_{72}.$$

Where, TDDM (mg/gOM) = truly degraded dry matter; GV_{72} (mL) = gas volume at 72 h of incubation, and SF = the stoichiometrical factor = 2.25.

Mineral content

Major mineral element content was determined using an Axios X-ray fluorescence spectrometer with 1 kW wave-length dispersion at the Unit of Technical Support for Scientific Research (UATRS) of the National Centre for Scientific and Technical Research (CNRST) in Rabat (Morocco).

Energy estimate

The energy value of the analyzed fodder species was calculated according to the French nutrition system (UFL-UFV) based on chemical composition, using the approach and the equations outlined by Vérité and Peyraud (1988); Vermorel (1988); Richard et al. (1990) and Baumont et al. (2010).

Statistical analysis

All data obtained were analyzed via completely randomized design (CRD) using the General Linear Model procedure (Proc GLM) of the Statistical Analysis System (SAS) (version 9.1.3 SAS Institute Inc., Cary, NC, USA). The model used for data analysis included the main effects of species, the harvesting stage, and their interaction during the time of harvest, following the model equation:

$$Y_{ijkl} = \mu + h_j + s_k + (h \times s)_{jk} + e_{ijkl}$$

where: Y_{ijkl} , the observed quality trait; μ , the overall mean; h_j , the effect of the phenological stage; s_k , the effect of species type, and e_{ijkl} random error.

Significant differences between individual means were identified using Duncan's multiple range tests. The *in vitro* gas production parameters (a, b, and c) were estimated using Proc NLIN. Finally, a simple correlation analysis was used to establish the relationship between chemical composition, *in vitro* gas production, and crude protein degradability using the CORR procedure of SAS.

Results

Primary results show that these plants grow predominantly in coarse-grained silty soils (Table 1) with a climate dominated by dry summers and low rainfall, characteristic of the arid and semi-arid climate of northeastern Morocco (Jilali et al. 2018).

The results of the chemical composition of tested *Hedysarum* species are summarized in Table 2. Results show an increasing trend in fiber contents (NDF, ADF and ADL), while, a decreasing pattern in ether extract (EE) and crude protein (CP) content was observed. Thus, the dry matter yield was significantly ($P <$

0.05) higher in *Sulla pallida* Desf. and attained (41%FM) at the flowering stage compared to *Sulla spinosissima* L. (25.28 %FM) at the same stage of growth (Table 2). The mineral composition of the present *Sulla* species is presented in Table 3. Their contents seem to be species-dependent ($p < 0.05$), and lie generally above the critical requirement for the metabolic processes of ruminants (McDowell 1985). Similarly, the amount of crude proteins (CP) is highly sufficient (Table 2) and appears to be suitable to meet the optimal (7-8%DM) rumen function (Van Soest 1994; Annison and Bryden 1998) and requirement needs (15%DM) for ruminant lactation and growth (McDonald et al. 2002). Another important quality parameter for forages is the concentration of fiber cell wall contents. The results of measured fibers indicated significant differences ($P < 0.05$) among studied species according to phenological stages (Table 2). Thus, the lowest value of NDF was found in *Sulla spinosissima* L. (38.51%DM at the vegetative stage), while *Sulla pallida* Desf. recorded the highest value: up to 55.69 %DM at the flowering stage. A similar result was observed for ADF concentration, which showed an increasing trend (24-28%DM) during the growth stages of *Sulla spinosissima* L., whereas, *Sulla pallida* Desf. has revealed excellent ADF content (40-43%DM).

Table 4 presents the cumulative gas production from 72 h of ruminal dry matter incubation, a widely used method for the determination of the nutritive value of forages. The results show that the species and the phenological stage had a significant ($P < 0.05$) effect on the gas production rate and estimated parameters. On average, a higher gas production rate (0.10 h^{-1}) was recorded in *Sulla spinosissima* L. in comparison with *Sulla pallida* Desf. (0.06 h^{-1}). Such results could have a great effect on plant digestibility and nutrient availability. Markedly, a significant ($P < 0.05$) decline of *in vitro* organic matter digestibility (IVOMD) was observed in *Sulla spinosissima* L. and *Sulla pallida* Desf. over a similar period (flowering stage): up to 69.00%OM and 48.23%OM, respectively (Table 5). Such a decrease is most likely due to the increase in fiber content (Table 6), but also to the presence of condensed tannin (Hatew et al. 2015). In this study, the CT concentrations ranged between 0.27-4.5%DM, in compliance with those obtained by Amato et al. (2005) and Stienezen et al. (1996) for related species (0.8 to 5% of whole plant dry matter). Finally, the energy-feeding values (Table 5) of evaluated *Hedysarum* spp. estimated by the French system were found to be highest for *Sulla spinosissima* L. (UFL-UFV 0.94-0.86 at the vegetative stage and 0.87-0.79 at the flowering stage). However, they decreased drastically from vegetative (UFL-UFV 0.67-0.55) to flowering stage (UFL-UFV 0.54-0.42) in *Sulla pallida* Desf., as a result of low organic matter digestibility (Richard et al. 1990).

Discussion

Forage legumes pertaining to *Hedysarum* genera are important components of many natural pasture regions of North Africa, including Morocco. Information about their nutritional value as fodder species remains nevertheless scant. Hence, screening of *Hedysarum* species with high nutritive value is undeniably important to determine their potential use in ruminant diets. Within this framework, two *Hedysarum* species at two maturity stages, namely *Sulla spinosissima* subsp. *capitata* and *Sulla*

pallida Desf., synonym of *Hedysarum spinosissimum* subsp. *capitatum* and *Hedysarum pallidum* Desf. respectively (Choi and Ohashi 2003), were harvested for analysis.

Results show a broad variation within the analyzed species, which could be mainly due to genotypic factors that control the synthesis and accumulation of nutrients (Koutsoukis et al. 2013). Furthermore, agronomic and environmental factors such as grass-legume intercropping (Mantino et al. 2016), irrigation treatments (Martiniello et al. 2012), the effect of arbuscular mycorrhiza symbiosis (Sabia et al. 2015), soil type (Snyman and Joubert 1995) and salinity (Kapulnik and Heuer 1991) and altitude (Mountousis et al. 2006), could have a strong effect upon plant yield and quality. Notably, *Sulla spinosissima* L. at 397 m tends to bloom earlier and produce less forage compared to *Sulla pallida* Desf. harvesting at 1113 m, which tends to bloom later and could therefore support long-term regrowth of the forage. Altitude tends to affect plant chemical composition through the interrelation of light intensity, carbon dioxide, and temperature. Such an effect has been suspected to be responsible for the elongation cycle, morphological types, blooming, and fructification of *Sulla* (Abdelguerfi-Berrekia et al. 1991; Issolah and Khalfallah 2007) and thus of nutrient compounds. Forages are generally satisfactory sources of protein and mineral elements for grazing livestock, especially when they contain leguminous species (Underwood and Suttle 1999).

Particularly, our results show that both species have higher average Ca and sublevel of P, comparable to those advanced by Haddi et al. (2009); Gasmi-Boubaker et al. (2012) and Laamouri et al. (2015). In addition, chemical analysis of the selected *Hedysarum* species shows that both species contain high amounts of crude protein. Therefore, the two *Hedysarum* species under study could be classified as good (>19%) protein sources (Kazemi et al. 2012), which has been related to the high performance of ruminants fed with *Sulla* (Bonanno et al. 2011). Moreover, a particular result was observed for fiber content, one of the most important chemical parameters determining the forage quality (Van Soest 1995). The NDF content was generally lower than the range of 60-65% suggested as the critical limit (Muia 2000; Hoffman et al. 2001). On the other hand, a significant increase was registered for the ADF content, particularly for *Sulla pallida* Desf. Therefore, it can be categorized as a low-quality (ADF>31%) forage legume (Kellems and Church 1998; Kazemi et al. 2012) as ADF and IVOMD are negatively correlated (-0.92**, Table 6). To emphasize, it has been reported that forage digestibility is mainly attributed to both lignin concentration and composition (Van Soest 1994; Zhong et al. 2021). Therefore, the reduction of lignin content in *Sulla* plants could have an advantage in enhancing their forage quality (Gallego-Giraldo et al. 2014).

A comparison of gas production characteristics and estimated parameters indicated some significant differences among the studied *Sulla* species (Table 4). The difference is likely owed to the content of rapidly fermentable fractions (10.26 mL/gDM and 7.23 mL/gDM respectively) such as crude protein (CP) and soluble carbohydrates.

Furthermore, forages such as *Sulla pallida* Desf. containing high dry matter (DM) content is associated with adequate well-preserved silage (Niezen et al. 1998) and high feed intake of *Sulla* (Terrill et al. 1992;

Douglas et al. 1999; Molle et al. 2003). While, moderate quantities of condensed tannin (2-5%DM), could have beneficial results, protecting plant protein from degradation in the rumen and increasing protein supply to ruminants fed with Sulla (Aerts et al. 1999; Barry and McNabb 1999; Burke et al. 2002; Bonanno et al. 2011; Di Trana et al. 2015).

Conclusion

To evaluate the autochthonous phytopastoral genetic resources of Morocco, an analysis was undertaken in different pastoral regions of North Morocco, to determine the nutritive value of two species belonging to the genus *Hedysarum*, i.e., *Sulla spinosissima subsp. capitata* and *Sulla pallida* Desf. Results show that both species have good nutritive value regarding their average crude protein content (21.30%DM), neutral detergent fiber (47.31%DM), and *in vitro* organic matter digestibility (61.41%OM) even in the late harvesting stage. Therefore, the use of both species of Sulla as forage crops may have good potential to meet the needs of small ruminant nutrition and rehabilitation of natural pastures in arid Mediterranean regions.

Declarations

Competing interests All the authors declare no competing interests.

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Author contributions MDPI

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Tables

Tables 1 to 6 are available in the Supplementary Files section

Figures

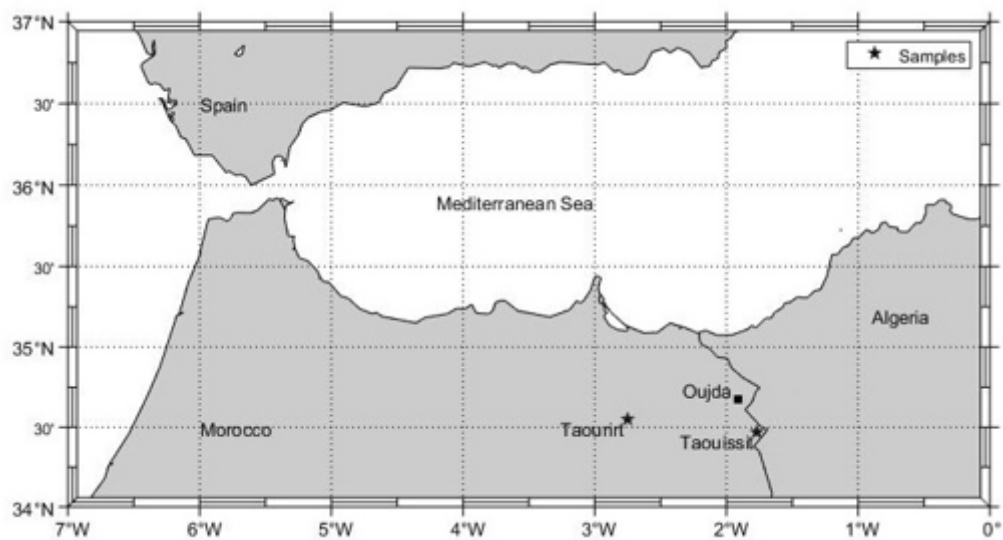


Figure 1

Location of sampling sites

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