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Nutritional and anti-nutritional characterization of six Tunisian local forage legumes species

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Abstract. Nutritional and anti-nutritional value of six local forage legume species accessions (*Medicago truncatula*, *Medicago ciliaris*, *Hedysarum coronarium* (cultivar Bikra 21), *H. carnosum*, *Lathyrus cicera* and *Scorpiurus muricatus* ssp *muricatus* (cultivar haffouz) was investigated. Dried samples of each accession were analyzed for NDF, organic matter, crude protein (CP), soluble nitrogen contents in pepsin, non-protein nitrogen, soluble nitrogen in the buffer and non-digestible nitrogen (N-ADF), total phenols, total and condensed tannins. Biological parameters were determined using *in vitro* gas production technique. Except from *Hedysarum coronarium*, all species had a CP content ranging between 200 and 230 g kg^{-1} DM. The proteins of all species are of good quality as suggested by low proportion of fiber-bounded and soluble nitrogen. Secondary compounds had no negative effect on nutritive value of the six species, even for the case of *Hedysarum coronarium* which had relatively high level of condensed tannins (37 g kg^{-1} DM). High gas production registered during fermentation and real degradability (superior to 70%) suggest that studied species present an excellent forage quality potential.

Key words. Local forage legume – Proteins quality – Phenols – Tannins – Gas production – Real degradability.

Rendement, caractérisation nutritionnelle et anti-nutritionnelle de six légumineuses fourragères locales tunisiennes

Résumé. *Le rendement et la valeur nutritionnelle et anti-nutritionnelle de six accessions d'espèces de légumineuses fourragères locales (*Medicago truncatula, Medicago ciliaris, Hedysarum coronarium *(cultivar Bikra 21),* H. carnosum, Lathyrus cicera *et* Scorpiurus muricatus *ssp* muricatus *(variété Haffouz) ont été étudiés. Les échantillons séchés et broyés étaient analysés pour leurs teneurs en fibres, matière organique, matières azotées totales (MAT), la qualité des protéines, et les teneurs en phénols totaux et tannins totaux et condensés. Les paramètres biologiques étaient déterminés en utilisant la technique* in vitro *de production de gaz. À part* Hedysarum coronarium*, toutes les espèces ont montré une teneur en CP comprise entre 20 et 23%. La proportion d'azote lié aux fibres est faible pour toutes les espèces ce qui traduit une bonne qualité des protéines de toutes les espèces. Les composés secondaires n'ont pas montré un effet négatif sur la valeur nutritive des six espèces, même dans le cas de* Hedysarum coronarium *qui a montré un niveau relativement élevé de tannins condensés (37 g kg^{-1} DM). La production de gaz enregistrée pendant la fermentation et la dégradabilité réelle (supérieure à 70%) suggèrent que les espèces étudiées présentent une excellente qualité fourragère.*

Mots clés. *Légumineuses – Qualité des protéines – Phénols – Tannins – Production de gaz – Dégradabilité réelle.*

I – Introduction

Forage legumes of Mediterranean pastures play a considerable role as protein source in ruminant diets. They provide grazable material in spring and early summer or silage, hay straw or seeds in period of forage shortage such autumn and winter, while ensuring soil fertility

maintenance through symbiotic nitrogen fixation (Cocks and Bennett, 1999; Chatterton and Chatterton, 1996). Tunisia is known by an important phytogenetic diversity of native forage and pasture legumes (Chakroun and Zouaghi, 2000). Several varieties and cultivars have been released by INRAT (National Institute of Agricultural Research) originating from local legume species such as *Medicago* sp., *Hedysarum* sp. and others. However, they still underutilized because of a failing seed production system to provide quality seeds to farmers (Hassen *et al*., 2013). Moreover, few studies investigated the nutritional and anti-nutritional value of released cultivars (Gasmi-Boubaker *et al*., 2012). The objective of this work is to determine the nutritive value of some new released forage legume cultivars according to their chemical composition and *in vitro* gas production.

II – Material and Methods

The trial was carried out in Tunis at the experimental station of the National Institute of Agricultural Research of Tunisia (INRAT). Six local forage legume species accessions (*Medicago truncatula*, *Medicago ciliaris*, *Hedysarum coronarium* (cultivar Bikra 21), *Hedysarum carnosum*, *Lathyrus cicera* and *Scorpiurus muricatus* ssp *muricatus* (cultivar Haffouze) were sown in plots of 8 m² according to completely randomized block design.

Three 1 m² quadrates of each plot were cut at 50% flowering stage, then weighed and a subsample of 0.5 kg is dried at 60°C during 72 h and then milled to pass through a 1mm screen. Ground sampled of each of the six legumes separately were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), Ca, P according to AOAC (1990) and crude fiber (NDF) according to Van Soest *et al.* (1991). Nitrogen profile of the six legumes was investigated (Soluble CP (pepsin), Non-protein nitrogen, Soluble CP (buffer) and N-ADF according to the methods developed by Licitra *et al*., 1996). They were also analyzed for total phenols, tannins and condensed tannins according to Makkar (2003). A 72 h *in vitro* gas production technique was carried out to determine the extent and rate of gas production as affected by different substrates (Menke and Steingass, 1988). Ruminal juice was collected from ewes fed oaten hay and commercial concentrate. Gas volumes were recorded after 2, 4, 6, 8, 12, 24, 48 and 72 h of incubation. Metabolizable energy was estimated according to equation developed by Menke and Steingass (1988) using the gas produced in 24 h and CP content. Gas production parameters (a, b and c) were generated using the procedure NLIN of SAS software (SAS, 1994).

Data were subjected to an analysis of variance using GLM procedure (SAS, 1994). The LSMEANS procedure was used to compare means of each variable as affected by the species.

III – Results and discussion

Lathyrus cicera has the highest OM (88.4 %) while *Scorpiurus muricatus* (73.1 %) the lowest one (Table 1). There was no significant difference in the CP content of the tested species which was high, expect *Hedysarum carnosum* NDF content was high (Table 1) and without significant difference in the tested species expect *Scorpiurus muricatus*.

Ca content varied significantly among species, and the highest content was noted in *Hedysarum carnosum accession.* There was no significant difference in the P content among the tested species. P content was relatively low which resulted in a high Ca /P ratio. This imbalance could affect negatively, animal growth, feeds digestibility and ruminal fermentation (Underwood and Suttle, 1999). Overall, total phenols and tannins content of the six legumes was relatively low (Table 1). *Hedysarum coronarium* had the significant higher content of these secondary compounds compared to other tested species. According to Makkar (2003), these concentrations of tannins do not have negative effect on the digestion and animal performance. Moreover, tannins can improve performance in sheep through protecting the protein of legume

against ruminal degradation and therefore induce an increase in the flow of alimentary proteins that are found in the intestine where they are transformed into amino acids Barry *et al.*, (2001).

Table1. Chemical composition of six local forage legume species accessions

Accession	DM %	OM	CP	NDF	Total phenols	Total tannins	Conden-sed tannins	Ca	P
Medicago truncatula	11.0	86.3 a	22.2 a	68.2 a	6.20 b	3.6 c	1.02 b	1.85 bc	0.32
Lathyrus cicera	13.3	88.4 a	21.5 a	64.1 a	11.22 b	7.48 bc	1.4 b	1.92 bc	0.35
Medicago ciliaris	13.4	85.9 a	22.9 a	66.9 a	10.28 b	7.51 bc	1.42 b	1.72 c	0.34
Hedysarum carnosum	9.7	73.6 b	15.1 b	62.8 ab	10.54 b	7.99 bc	8.04 b	4.79 a	0.15
Hedysarum coronarium	12.3	85.1 a	21.3 a	67.6 a	19.69 a	15.59 a	37.49 a	2.36 bc	0.28
Scorpiurus muricatus	9.5	73.1 b	20.5 a	57.5 b	11.7 b	9.31 b	11.77 b	3.83 ab	0.38
SE	0.9	2.1	0.5	0.9	1.085	0.916	2.247	0.35	0.22
Pr	0.2297	0.0345	0.0004	0.0103	0.0091	0.0048	0.0002	0.016	0.58

SE and Pr are Standard errors and probability of significant difference, respectively.

Nitrogen quality of the six species was investigated on the basis of soluble nitrogen contents in pepsin, non-protein nitrogen, soluble nitrogen in the buffer and non-digestible nitrogen (N-ADF) (Table 2). The proportion of soluble nitrogen in pepsin ranged from 13 to 51% of CP. This proportion varied significantly among species (Pr = 0.0002) with a deviation of 6.87 % between the maximum value observed in *Lathyrus cicera* (20.29 % DM) and the minimum value observed in *Hedysarum carnosum* (13.42 % DM). The proportion of non-protein nitrogen ranged from 3.6 to 5 % of total nitrogen with the minimum observed in both *Scorpiurus muricatus and Hedysarum carnosum*. This type of nitrogen is considered totally degradable in the rumen. All legumes Buffer soluble nitrogen showed no significant differences among species, except *Hedysarum carnosum*. N-ADF, which represents non digestible nitrogen fraction, was low in all species (inferior to 4%).

Table 2. Nitrogen profile of legume species

	Soluble CP (pepsin)	Non-protein nitrogen	Soluble CP (buffer)	N-ADF
Medicago truncatula	19.4 ab	4.95 b	17.8 a	1.36 a
Lathyrus cicera	20.3 ab	4.43 bc	17.03 a	1.3 ab
Medicago ciliaris	21.5 a	5.87 a	17.9 a	3.5 a
Hedysarum carnosum	13.4 c	3.667 c	11.5 b	0.95 b
Hedysarum coronarium	18.5 b	4.82 b	15.7 a	1.45 a
Scorpiurus muricatus	19.0 ab	3.93 c	16.8 a	1.09 ab
SE	0.46	0.16	0.39	0.07
Pr	0.0002	0.0011	0.0002	0.0441

SE and Pr are Standard errors and probability of significant difference, respectively

Gas production parameters obtained through modeling gas volumes and estimated real degradability and metabolizable energy are given in Table 3. According to results, all of the produced gas came from the insoluble fraction potentially degradable. Indeed, the value of 'b' varied between 92 and 100 %. Gas production rate 'c' is considered high for all species. *Medicago ciliaris* and *Hedysarum carnosum* were distinguished from other species by the highest rate 'c' (P = 0.0218). This rate is an indicative of how fast the DM is fermented in the rumen. Estimated real degradability had not significant difference among the species with a range of 72.7 to 80.1 %. However, metabolizable energy (ME) varied significantly between species. ME content seems to be dependent on DM, OM, CP and Fiber contents and to nitrogen quality. The maximum gas production took place during the first 24 hours of incubation,

which suggests that nutrients of studied material including nitrogen and energy are easily used by ruminal micro flora. The relatively high levels of CP and the good quality of nitrogen associated with the high content of ME could explain high gas production rates.

Table 3. Gas production parameters, real degradability and estimated metabolizable energy

Species	a (%)	b (%)	c (/h)	Real degradability (%)	ME (MJ/kg DM)
Medicago truncatula	0.6	93.6	0.08 b	73.9	17.7 ab
Lathyrus cicera	0.5	92.7	0.09 ab	72.7	17.4 ab
Medicago ciliaris	0	97.5	0.10 a	74.5	18.5 a
Hedysarum. carnosum	2.3	95.0	0.10 a	80.1	13.8 c
Hedysarum coronarium	0	100	0.08 b	80.0	18.6 ab
Scorpiurus muricatus	0	100	0.076 b	78.8	16.3 b
SE	0.58	1.75	0.003	1.39	0.3
Pr	0.5648	0.1144	0.0218	0.1649	0.0005

SE and Pr are Standard errors and probability of significant difference, respectively.

IV – Conclusion

This study confirms the high nutritional quality of local forage species which were rich in CP and energy. The high content of proteins and the good degradability of dry matter exclude any negative effect of tannins. Despite their presence in a relatively important amount in *Hedysarum coronarium*, tannins seem to have no effect on its nutritive value.

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