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Study *in-Vitro* Digestibility of Vicia Narbonensis L. and Vicia Sativa L. Seeds Cultivated for Ruminants

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Abstract: Vicia narbonensis L. and Vicia sativa L. seeds are legumes rich in protein and energy, commonly used for ruminant feeding. Their integration into feed rations requires an assessment of their digestibility, a crucial factor for their nutritional value. This study aims to determine the *in-vitro* organic matter digestibility of these seeds, obviously while taking into account the influence of the semi-arid climatic conditions of Sétif on their chemical composition. The experiment involved two species of the genus Vicia, with ten ecotypes of Vicia narbonensis L. from ICARDA and two ecotypes of Vicia sativa L. from Algeria, the latter serving as controls due to their wide use by Algerian farmers. The data were subjected to variance analysis followed by a Fisher test (LSD) at significance threshold at 5%. The results show a significant variability ($P < 0.05$) in digestibility between the ecotypes of the two species. The most digestible ecotypes are for Vicia narbonensis L.; ecotypes N-2392 (dMO = 77%) and N-2464 (dMO = 75%), and for Vicia sativa L.; ecotype S-BBA (dMO = 74%). Thus, the Vicia narbonensis and Vicia sativa seeds grown in Sétif can be an excellent source feed for ruminants. Their digestibility is generally high but can be optimized by technological processes such as soaking or cooking. It is important to take into account local specificities to determine the best way to incorporate these seeds into animal rations.

Keywords: Vetch, Digestibility, Ruminants, Nutritive value, Agricultural engineering

Introduction

Although fodder legumes are less demanding than many other crops and play a key role in production systems, especially by contributing to crop rotation through their cleansing effect and by enriching the soil with nitrogen and organic matter while serving as a food source for livestock, they have not always received the attention they deserve in Algeria. In particular, research on species of the *Vicia* genus, specifically *Vicia narbonensis* L., remains scarce in our country.

Forage legumes occupy a significant economic position in the animal feed market and deserve particular attention in arid and semi-arid Mediterranean areas (Kokten et al., 2009), and due to the diversity of breeding conditions and the nature, level and composition of products, a targeted protein diet for ruminants must be mastered (Andrieu & Demarquilly, 1992).

It would therefore be beneficial to further encourage the cultivation of fodder legumes. Development strategies should be tailored to the specific characteristics and potential of the semi-arid agro-ecological zone, ensuring a balance between the availability of fodder resources and the nutritional needs of livestock. In other words, it is essential to establish a compromise between fodder supply and herd requirements in this region to achieve food self-sufficiency and, ultimately, to implement a strategy that fosters complementarity between different zones.

Method

Objectif

This study aims to determine the organic matter (OM) content and assess the *in vitro* digestibility of ten ecotypes of *Vicia narbonensis* L., compared to two ecotypes of *Vicia sativa* L., under the characteristic rainfed conditions of the semi-arid region of Sétif. The goal is to estimate their nutritional value for ruminant feeding. The pepsin-cellulase enzymatic method, developed by Aufrère (1982), is widely used to predict organic matter digestibility in forages. This method provides a significantly more accurate *in vivo* digestibility prediction than crude fiber measurement alone. In fact, incorporating nitrogenous matter content alongside crude fiber does not enhance prediction accuracy compared to using crude fiber alone.

Presentation of the Study Region

The trials were conducted on plots located at the FERHAT Abbas-Sétif1 University Campus (36°12’ N; 5°21’ E) in the Sétif region under rainfed conditions. This region experiences a continental climate, characterized by significant annual and daily temperature variations, along with two major constraints: frost and the sirocco. Situated in the semi-arid bioclimatic zone at an altitude of 1,025 meters, winter temperatures can drop below 0 °C, while summer temperatures occasionally exceed 40 °C. Additionally, the temperature differences between day and night—reaching up to 20 °C in winter and spring, promote frost episodes that pose significant challenges to plant growth (Bouzerzour & Benmahammed, 1994).

The soils in the experimental area belong to the steppe soil group (Perrier & Soyer, 1970) and are characterized by a silty-clayey texture, a lumpy structure, a basic pH (7.81), a total limestone content of 17.7%, and an organic matter content ranging between 2.0% and 3.0%. The region receives an average annual rainfall of approximately 450 mm (Seltzer, 1947), with an average of 373.8 mm recorded between 2006 and 2017 (ONM, 2017), although significant intra- and inter-annual variations have been observed. The experiment was conducted over three consecutive growing seasons (2017-2018, 2018-2019 and 2019-2020). Rainfall showed significant variation from one year to the next, with 469.05 mm recorded during the first season, 321.20 mm during the second, and 384.56 mm during the third. On the other hand, maximum and minimum temperatures remained relatively stable throughout the three growing seasons.

Presentation of Plant Material

The study focused on two species of the *Vicia* genus, including ten ecotypes of *Vicia narbonensis* L. from various origins (provided by ICARDA) and two ecotypes of *Vicia sativa* L. from Algeria. The latter were chosen as controls, as they are well known to Algerian farmers (Table 1).

Table 1. Origins of the Vicia narbonensis ecotypes and Vicia sativa L. studied

Species	Ecotypes	Code	Origin
Vicia narbonensis L.	1	N-2380	Libanon
	2	N-2383	Libanon
	3	N-2390	Libanon
	4	N-2392	Libanon
	5	N-2393	Syria
	6	N-2461	Turqey
	7	N-2464	Turqey
	8	N-2466	Turqey
	9	N-2468	Libanon
	10	N-2561	Syria
Vicia sativa L.	11	S-174	Algéria (Sétif)
	12	S-BBA	Algéria (BBA)

Presentation of the Test

Sowing was performed using a single seed lot over three growing seasons (January 2018, December 2019, and December 2020). The ecotypes were manually sown in randomized blocks on plots previously cultivated with durum wheat. Each plot consisted of four rows, each 4 meters long and spaced 30 cm apart, with approximately 336 seeds sown (70 seeds/m²). The cultivation process included deep plowing, two cover crop passes to control weeds and prepare the seedbed, and continuous manual weeding without fertilizer application. Harvesting was conducted manually at specific times for each season.

In Vitro Digestibility of Organic Matter (dOM)

The pepsin-cellulase enzymatic method, also known as the Aufrère method (1982), is used to estimate the digestibility of organic matter in feed for ruminants. It replicates ruminal digestion by first exposing the sample to pepsin, followed by cellulase. The amount of undigested organic matter is then measured through gravimetric weighing.

Procedure **:**

- Weigh 0.5 g of the sample (denoted as *Ps*) and place it in a porous bag (porosity 2).
- Place the bag in an Erlenmeyer flask and add 40 mL of preheated (40 °C) pepsin-HCl 0.1 solution.
- Cover the Erlenmeyer flask with parafilm and place it in a water bath at 40 °C for 24 hours, ensuring the water level fully submerges the reagent (mechanical agitation is maintained).
- After 24 hours, rinse the residue and the bottom of the Erlenmeyer flask with hot distilled water.
- Add 40 mL of preheated (40 °C) cellulase solution, cover again with parafilm, and return to the water bath at 40 °C for another 24 hours.
- Rinse the residue and the bottom of the Erlenmeyer flask again with hot distilled water.
- Place the bag in a porcelain crucible and dry it at 105 °C for 24 hours.
- Weigh the cooled crucible after placing it in a desiccator (P_0).
- Ash the crucible at 550 °C for 5 hours, then weigh it again after cooling in a desiccator (*Pa*).

Calculations **:**

- E(OM) = (Ps × % OM) / 100
- % DcellOM = [E(OM) – ((P0 – Pa) / E(OM))] × 100 (Aufrère et al., 2005)
- % dOM = 0.699 × DcellOM + 22.6 (Baumont et al., 2007)

E(OM) corresponds to the test intake and DcellOM represents the cellulosic digestibility of the organic matter

Statistical Processing

The data were analyzed using SPSS 20 software, applying a one-way analysis of variance (ANOVA) to evaluate differences among the ten ecotypes of *Vicia narbonensis* L. Mean comparisons were subsequently conducted using Tukey's test (homogeneous groups) with a significance threshold of 5%.

Results and Discussion

Chemical Composition and In Vitro Digestibility of Organic Matter

Significant differences (P <0.05) were observed in the chemical composition and *in vitro* digestibility of seed organic matter, highlighting variability both between and within species (Table 2).

Table 2. Variance in chemical composition and in vitro digestibility of seed organic matter from 12 vetch ecotypes

Sources of variation	df	DM (%)	TA (g/kg DM)	OM (g/kg DM)	CP (g/kg MS)	Fat (g/kg MS)	CF (g/kg MS)	dOM (%)
Total	35	0.31	11.87	11.87	3738.17	51.15	1215.26	85.87
Ecotype	11	0.27*	10.28*	10.28*	3726.22*	46.47*	1020.41*	80.65*
Average		89.27	37.21	962.79	386.37	37.41	124.79	69.46
Standarddeviation		0.33	2.08	2.08	34.34	4.22	21.31	5.38
CV (%)		0.37	5.58	0.22	8.89	11.28	17.08	7.74

* : significant à 5% ; df : degree of freedom ; DM : dry matter ; TA : Totales ashes ; OM : Organic matter ; CP : Crudes Proteins ; MG : Fat ; CF : Crude fiber ; dOM : Digestibility *in vitro* of organic matter ; CV : Coefficient of variation.

Total Ash and Organic Matter Contents

Significant differences (P <0.05) were found among vetch ecotypes in total ash and organic matter contents (Table 3). On average, *Vicia sativa* L. exhibited a slightly higher total ash content (37.57 g/kg DM) compared to *Vicia narbonensis* L. (37.14 g/kg DM). Among the *Vicia narbonensis* L. ecotypes, the highest total ash contents were observed in N-2380 (41.48 g/kg DM), N-2466 (38.82 g/kg DM), and N-2392 (38.33 g/kg DM). Conversely, ecotype N-2461 had the lowest total ash content, measuring 34.28 g/kg DM (Figure 1).

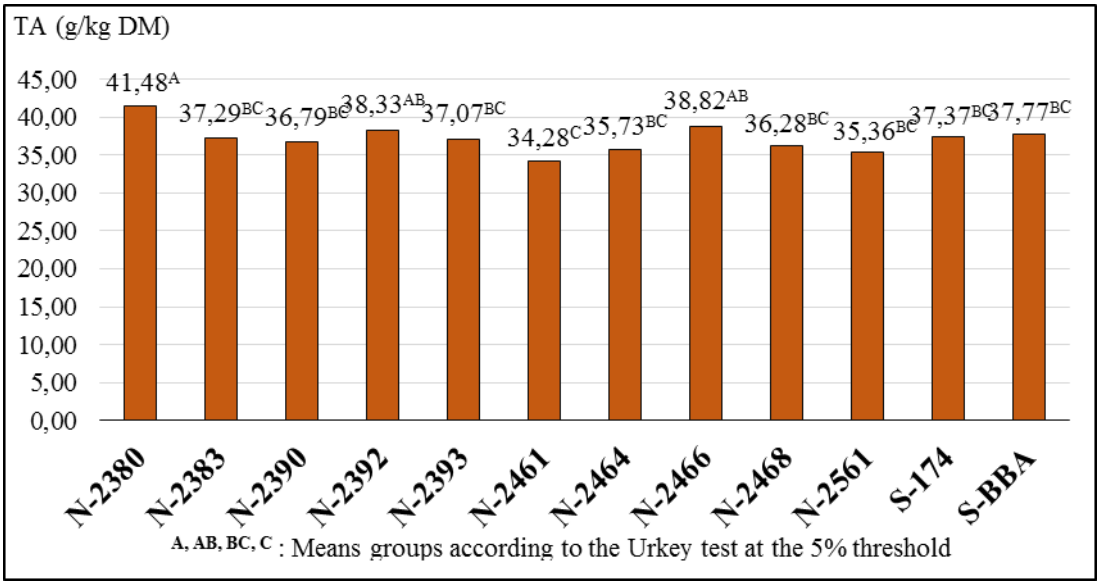

Figure 1. Total ash content of ecotypes Vicia narbonensis L. and Vicia sativa L.

In terms of organic matter content, *Vicia narbonensis* L. averages 962.86 g/kg DM, closely followed by *Vicia sativa* L. with 962.43 g/kg DM. The highest organic matter contents were recorded in ecotypes N-2461 (965.72

g/kg DM), N-2561 (964.64 g/kg DM), and N-2464 (964.27 g/kg DM). In contrast, ecotype N-2380 exhibited the lowest organic matter content, at 958.52 g/kg DM (Figure 2).

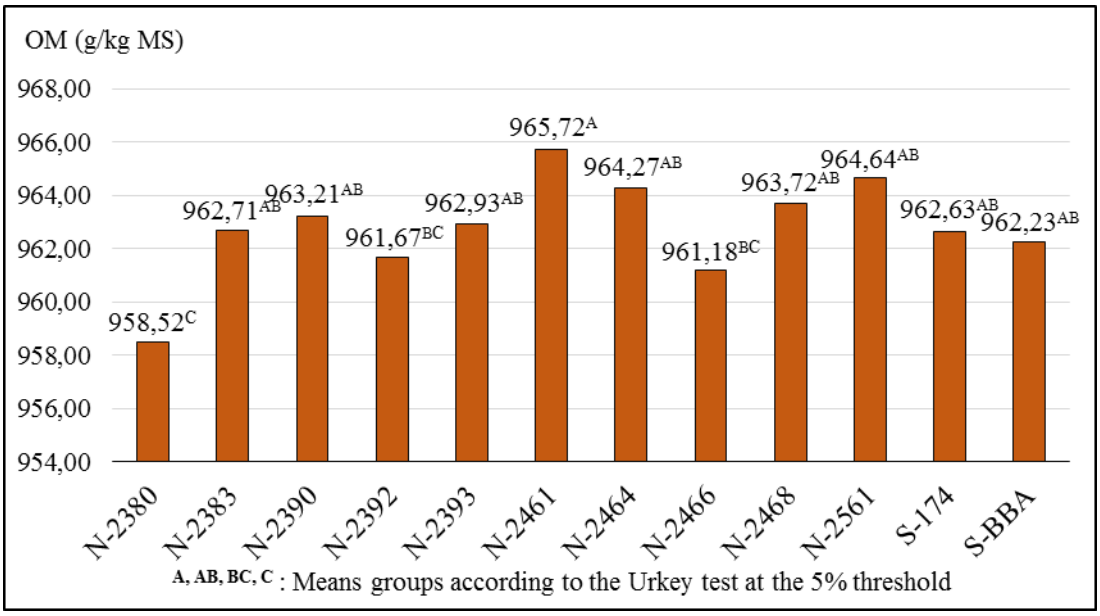

Figure 2. Organic matter content of Vicia narbonensis L. and Vicia sativa ecotypes

Guedes and Dias da Silva (1996) reported total ash contents in *Vicia narbonensis* and *Vicia sativa* seeds that align with our findings. According to Hadjipanayiotou (2000), *Vicia narbonensis* seeds contain 45.0 g/kg DM of total ash and 955.0 g/kg DM of organic matter. In contrast, soybeans have approximately 54 g/kg DM of total ash, a higher value than those observed in our study (Batterham & Egan, 1986). As noted by Jarrige et al. (1995) and Tobia et al. (2008), factors such as soil quality, climatic conditions, and root system development play a key role in determining mineral matter content.

In Vitro Digestibility of Organic Matter

Significant differences ($P < 0.05$) were found in the *in vitro* digestibility of organic matter (dOM) among *Vicia narbonensis* L. and *Vicia sativa* L. ecotypes. On average, *Vicia narbonensis* L. had a slightly higher dOM (69.56%) compared to *Vicia sativa* L. (68.97%). Among the ecotypes studied, N-2392 exhibited the highest digestibility at 76.65%, followed by N-2464 at 74.81% (both from *Vicia narbonensis* L.), and S-BBA at 73.76% (*Vicia sativa* L.). Conversely, ecotype N-2390 had the lowest digestibility, at 61.37% (Figure 3).

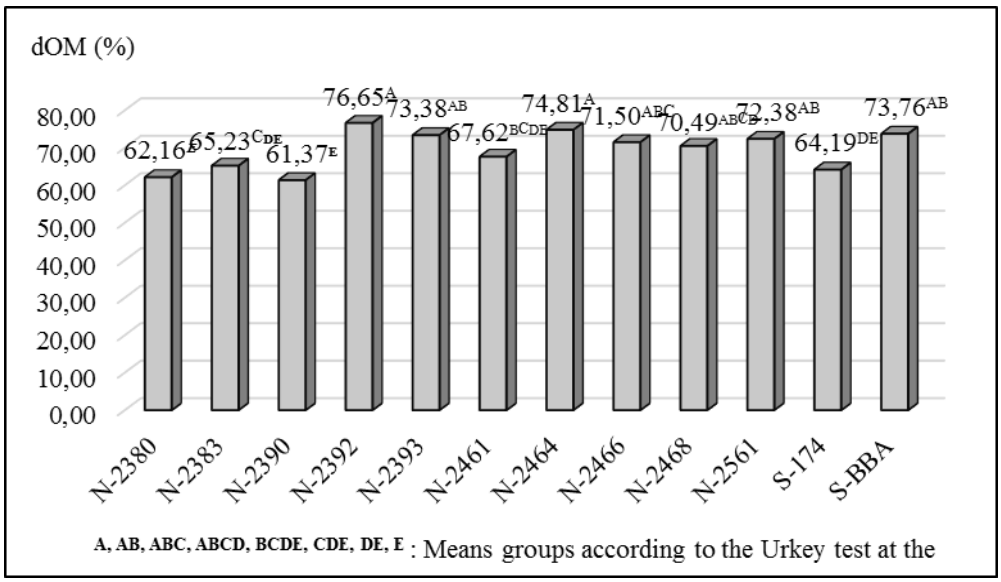

Figure 3. In vitro digestibility of organic matter of Vicia narbonensis L. and Vicia sativa L. ecotypes

In this study, Narbonne vetch (*Vicia narbonensis* L.) demonstrated slightly higher *in vitro* digestibility of organic matter compared to common vetch (*Vicia sativa* L.). This difference was attributed to the lower crude fiber content in *Vicia narbonensis* L. relative to *Vicia sativa* L. The *in vitro* digestibility values observed for *Vicia narbonensis* L. and *Vicia sativa* L. seeds were lower than those reported by Hadjipanayiotou (2000), who recorded a digestibility of 79.9% for *Vicia narbonensis* seeds. This result is in contradiction with those reported by Khériji (1999). In addition, the digestibility values for common vetch hay reported by Cheng et al. (2024) are also higher than current results. It was established that the variety significantly influenced the in vitro digestibility of common vetch, Narbonne vetch, and pea (Larbi et al., 2010).

According to Baumont et al. (2008), the digestibility of organic matter in forage plants is primarily determined by their cell wall content and its digestibility. While intracellular components such as sugars and fructans are almost entirely digestible, cell wall digestibility ranges from 40% to 90%, depending on lignin content. For a given sample, the pepsin-cellulase enzymatic method provides a more accurate estimation of *in vivo* digestibility than crude fiber measurement alone. Moreover, incorporating nitrogenous matter content into crude fiber analysis does not significantly enhance the precision of digestibility prediction compared to crude fiber alone (Aufrère, 1982). Whereas more optimal growth conditions were linked to increased fiber content and reduced digestibility (Piltz et al., 2021).

Certain plant species, particularly woody plants, exhibit lower digestibility, with enzymatic digestibility values ranging from 20% to 36% of dry matter. This reduction is primarily due to the presence of anti-nutritional compounds such as tannins, lignins, and polyphenols, which can bind to proteins and digestive enzymes, thereby hindering the digestion process.

Moreover, a study by Kahlaoui et al. (2021) found that *Vicia narbonensis* and *Vicia sativa* exhibit higher *in vitro* digestibility of organic matter compared to *Vicia villosa*, making them more nutritionally valuable for animal feed. Finally, as forage mature, there is a point at which the accumulation of digestible DM declines despite increasing forage DM yields (Gezahagn et al., 2014). in the same perspective, significant differences ($p < 0.001$) were also observed among the four common vetch in rapidly degradable DMfraction and effective DM degradability of straw; however, no difference was observed in other DMdegradation parameters and neutral detergent fiber degradation parameters (Yafeng et al., 2019).

Conclusion

The findings of this study enhance our understanding of the nutritional potential of *Vicia narbonensis* L. as a ruminant feed, offering essential insights for optimizing their diet.

Recommendations

Encouraging the integration of *Vicia narbonensis* L. into ruminant feeding systems would be beneficial, with larger-scale trials needed to assess its performance under real farming conditions. Additionally, further research on optimizing its cultivation and evaluating its overall impact on animal productivity could facilitate its adoption by livestock farmers.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

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