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In vitro Digestibility of Some Local Feeds Resources in Southern Tunisia: Comparison Between Two Inoculums Sources

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Abstract

Face to the climatic factors variability and in order to manage seasonal and prolonged droughts, the breeders developed several strategies in southern Tunisia. To ensure their animal feed, they benefit from the favourable seasons to stock reserves by harvesting and drying some local feed such as: spontaneous plant species as natural hay locally known as “khortane”, the olive by-product and the collecte of *Stipa tencissima*. This work aims to estimate the *in vitro* digestibility of some local feed resources used in southern Tunisia by two sources of inoculum: faecal fluid (LF) and ruminal fluid (LR). The results showed that the best *in vitro* digestibility values of dry matter (IVDMD) using both inoculums were recorded for dried olive leaves (65.5 ± 0.25 and 65.4 ± 0.24%, respectively, for LR and LF). The lowest IVDMD value was recorded for *Stipa tenacissima* (29.64 ± 0.52 and 29.80 ± 0.80% respectively for LR and LF). The IVDMD was similar in the Khortane (grass hay) and oat hay (52.07 ± 0.79 and 51.57 ± 0.16%, respectively, for the LR and LF). These results led to the conclusion that IVDMD values of these local feed resources are close by using both sources of inoculum. Fecal liquor can without loss of effectiveness of replace the rumen liquor to estimate feed digestibility (R2 = 0.999).

Keywords

in vitro Digestibility, Arid Region, Local Feed Resources, Inoculum

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1. Introduction

In Tunisia's arid regions, the feeding of small ruminants is based on natural resources, spontaneous vegetation in rangelands and the rest of agriculture. The availability of these resources is uncertain [1]. Dry regions are marked by a long dry season (6-9 months) and pasture is only available for a short period in the spring (3-4 months) [2]. When rangeland resources cannot meet livestock maintenance and growth needs; pastoralists in arid land have developed several strategies to meet the nutritional needs of their livestock. During the good years, pastoral species can provide excellent food for animals from autumn to spring. These species are used either green by direct grazing during grass growth or harvested to be dried and preserved as natural hay called "khortane" and *Stipa tenacissima* and used during dry periods [3-5]. The use of natural resources is a common practice in southern Tunisia, in summer and in times of drought, khortane and gueddim are very important forage resources for small ruminants as well as equines and camels [6]. The appreciation of the nutritional value of these feeds is strongly linked to the determination of their digestibility. Measurements on animals require complex facilities and large quantities of test feeds, so they are very expensive. To eliminate these difficulties, the *in vitro* digestibility estimation technique was used using two sources of inoculum

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(fecal liquor (FL) and rumen liquor (RL)) to obtain a correct estimate of the digestibility of the animals. in this context, the objective of this work was to determine *in vitro* digestibility (by two sources of inoculum: rumen juice and faecal liquor) of some local feeds resources in the southern Tunisia, in order to estimate the digestibility of rations distributed and to evaluate the possibility of their use as a food constituent.

2. Materials and Methods

2.1. Study Area and Sampling

The study was carried out in southeastern of Tunisia in livestock and wildlife laboratory. In this zone, the average annual precipitation is of the order of 100 mm. Rainfall is characterized by its intra- and inter-annual irregularity, the region is marked by a long dry season (6 to 9 months) and the dominance of drying "Sirocco" winds, which considerably increases the potential evapo-transpiration and consequently increases the water deficit.

Sampling was carried out in the governorate of Medenine. Khortane was purchased from the Medenine market and was composed of 20 annual and perennial species harvested during the spring season; the specific contribution of khortane was 21% *Chrysanthemum coronarium*, 18% *Malva aegyptiacae* and other species. *Stipa tenacissima* was harvested from the natural ranges of the Benikadeche (From Medenine) Mountains during the end of growth period (April), to be dried in the open air and stored in a dry place. The dried olive leaves were provided from private farmers to the research institute's neighbors. Oat hay was purchased from the market.

2.2. *In vitro* Digestibility

To determine *in vitro* digestibility, the first step of Tilly and Terry's [7] method was applied. Two male goats (mean weight = 25kg and mean age = 3years) of the local population were served for the collection of ruminal fluid and feces. The latter were adapted to a diet for 15 days (1 Kg of oat hay divided into two meals: morning and evening), 500g of concentrate in the evening and the water is given once / day.

Both sources of inoculum were collected in the morning before meal distribution; ruminal fluid collected by oral esophageal rumen recovery by using a soft, flexible rubber and a vacuum pump (Alcatel, Annecy, France) from adult goat. The ruminal liquid (RL) was collected in a flask placed in an isothermal container containing warm water (39°C). The juice was filtered using six layers of compressed fabric.

Feces were collected manually from rectum of same goat used for rumen juice sampling. 50g of faeces collected was ground in a mortar and then mixed with 8 volumes of artificial saliva (2.35 g NaCl, 2.25 g KCl, 0.5013 MgCl $2H_2O$, 0.275g $CaCl_2$, 46.2 g $NaHCO_3$; 2g Na_2HPO_4, 7.5g $FeSO_4$ $7H_2O$, 0.2 g $CuSO_4$ $5H_2O$, 0.01 $ZnSO_4$ $2H_2$, 0.1 g Methylene blue, 0.625 g cysteine-HCl and 4 ml NaOH) and the contents are filtered to get the fecal liquid. Incubation was conducted in 100 mL flasks, adding into each 0.5 g of each sample of local feed resources (ground to 1 mm), 40 m of artificial saliva (mix of 6 solutions) and 10 mL of rumen fluid (RL) or fecal liquid (FL). Flasks were then transferred to a water bath oven at 39°C. Simultaneously, a series of blank flasks (without rumen fluid or fecal liquid) were prepared. All samples were incubated for 48 h then saturated with CO_2; the pH was adjusted to 6.8. After incubation, samples were filtered; the residue obtained was dried at 105°C, then was ashed at 550°C and weighted. *In vitro* digestibility was calculated as follow:

$$IVDMD\ (\%) = (I\text{-}F1)*100/I$$

$$IVOMD\ (\%) = (I\text{-}F2)*100/I$$

IVDMD: *in vitro* dry matter digestibility; *IVOMD*: *in vitro* organic matter digestibility; *I:* sample % DM: the intake; *F1*: residual dry weight (105°C); and *F2*: residual ash weight (550°C).

2.3. Statistical Analysis

The effect of the digestibility method on feed was analyzed by single-factor ANOVA using SPSS software (20.). The means and standard deviations are calculated, the significant difference between means was determined by the Duncan test ($P < 0.05$).

3. Results and Discussion

3.1. *In vitro* Digestibility Estimation by Rumen Fluid (RL)

The analysis of variance shows a significant effect between the digestibilities of the different feeds studied (Table 1). *Stipa tenacissma* has the lowest digestibility (29.64 ± 0.50%) due to its high cell wall content (NDF = 74.5 DM).

In vitro dry matter digestibility (*IVDMD*) of oat hay was 51.57 ± 0.20%, comparable to that reported (52%) by Kayouli [8] for oat hay and that of alfalfa hay made in reference in the livestock laboratory and wildlife. *IVDMD* and *IVOMD* of Khortane (Table 1) were close to that (51%) reported by Ayeb et *al.* [9]. digestibilities of khortane were relatively good since the khortane is composed of annual species and perennial herbaceous plants that are easily digestible. Similarly, our results are close to the average value reported by Longo-Hammouda *et al.* [10] for herbs

from Algerian rangelands (50.2% DM). The *IVDMD* of the dried olive leaves was 65.48%. In general, digestibility is influenced by several factors such as climatic conditions, harvesting and storage conditions and the interactions between these factors [11]. Other sources of variation may also affect in vitro digestibility such as fineness of grinding, incubation time, proportion of rumen / saliva juice, and in some cases the quality of rumen juice [12].

Table 1. Variation in *In vitro* digestibility (%) of Feeds according to inoculum source.

Feeds	Ruminal liquid		Fecal liquid	
	IVDMD	***IVOMD***	***IVDMD***	***IVOMD***
Khortane (grass hay)	52.07 ± 0.80^{b}	60.25 ± 3.70^{b}	52.08 ± 0.50^{b}	60.08 ± 2.50^{b}
Oat hay	51.57 ± 0.20^{b}	55.20 ± 0.10^{b}	51.56 ± 0.20^{b}	55.43 ± 0.30^{c}
Dried olive leaves	65.48 ± 0.20^{a}	68.47 ± 0.20^{a}	65.38 ± 0.20^{a}	68.78 ± 0.20^{a}
Stipa tenacissima	29.64 ± 0.50^{c}	36.88 ± 1.80^{c}	29.79 ± 0.80^{c}	32.60 ± 1.90^{d}

a, b; Values on the same column with different letters were significantly different ($P < 0.05$).

3.2. *In vitro* Digestibility Estimation by Fecal Liquid (FL)

By using fecal fluid as a source of inoculums, the best *IVDMD* values are recorded for dry olive leaves (Table 1) The lowest value was obtained in *Stipa tenacissima* (29.79 ± 0.8 0%), because of its richness in fiber (74.5% DM). The mean value of oat hay (51.56%) was lower than that reported (54.37%) by Borba et al. [13]. The results obtained for Khortane (52.08 ± 0.50%) are comparable to the result (51.90%) reported by Borba et al. [13] for *Lolium perenne*. However, this value is lower than that (58.9%) mentioned by Laudadio et al. [14] for *Salicornia arabica*.

3.3. Comparison of Digestibilitty According to Inoculum Source

A comparison of *in vitro* digestibility of local feed resources using two sources of inoculum (RL and FL) was investigated with the aim of having a more simple and available alternative for determining digestibility without recourse to sampling stressful rumen fluid for the well being of the animal. Examples, for Khortane, the IVDMD values were 52.07 and 52.08% respectively for RL and FL. These values are in agreement with the study of Vander Baan [15] who obtained at *Atriplex numularia* a highly significant correlation of digestibility which was 39.5% and 38.5% respectively using RL and FL. Comparable results were obtained by Mir et al. [16] for *Lolium* (IVDMD were 48.8 and 46.6% respectively for RL and FL).

These results in this study have shown that FL can, without loss of effectiveness, be used as a substitute for RL for the measurement of *in vitro* dry matter digestibility. To better exploit these results, it is possible to combine these two sources to finally obtain a single regression line that can be applicable to all animal species. The regression equations obtained between dry matter digestibility and rumen fluid organic matter (reference method) and those obtained using faeces are shown in figure 1. The highly significant positive correlation between the values obtained by the reference method (rumen fluid) is highly correlated (R2 = 0.999 for dMS and R2 = 0.976 for BMD) positively with values obtained using faecal fluid.

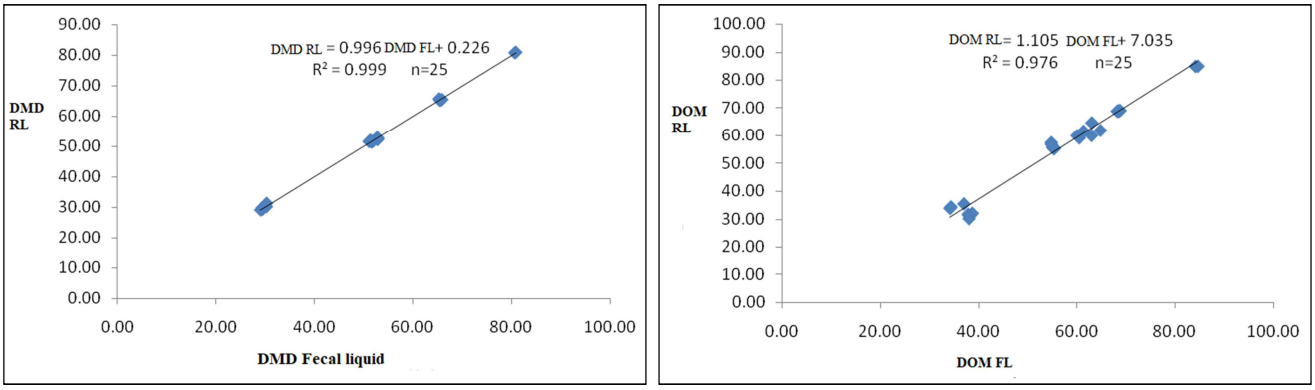

Figure 1. Regression equation between DMDRL-DMDFL and DOMRL-DOMRL.

4. Conclusion

The results obtained in the present study allow to conclude that a highly significant positive correlation between the values obtained by the reference method (rumianl fluid) and that using faecal fluid as inoculum source was observed (R^2 = 0.999 and R^2 = 0.976 respectively for DMD and OMD) and that the use of FL as a source of inoculum has the advantage of respecting the welfare of animals by avoiding delicate

manipulations for the extraction of rumen juice. It is simpler to perform and avoids the use of certain equipment (vacuum pump, hot water and insulated container) and avoids the stress of the animal during oesphogeal collection.

References

[1] Ayeb N., Hammadi M., Addis M., Khorchani T. 2014. Chemical composition and fatty acid profiles of some local forage resources in southern Tunisia. *Options Méditerranéennes* 109, 115-118.

[2] Le Houérou, H. 1962. The natural pastures of arid and desert Tunisia. 110 p.

[3] Ayeb N., Ouled belgacem A., Debbebi S., Khorchani T. 2011. Variation of the floristic composition of a local forage resource "khortane" according to the region in the south-east of Tunisia. Review of the Faculty of Sciences Bizerte. pp 53-57.

[4] Génin D. 2005. Animal production and natural resources. utilization in the Mediterranean mountain areas. EAAP publication N° 115, 2005, Ioannima, Greece, pp: 614- 619.

[5] Visser M., Morand F., Mahdhi H., 2002. Left of Stipa lagascae and rehabilitation of private lands in Saharan Tunisia. Cahiers Agricultures. Volume 11, Number 6, 377-83.

[6] Ayeb N., Ouled Belgacem A, Debbabi S, Khorchani T. (2015). Traditional Method of preparation and use of local forage resources "the khortane" in southern. Tunisia. *Journal of Material and Environment Science*, 6: 1-09.

[7] Tilley JMA, Terry RA (1963) A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18, 104–111.

[8] Kayouli C. 2000. Profile fourrager. Tunisie. Country pasture profiles. FAO.

[9] Ayeb N. Ouled Belgacem A. Khorchani T. 2009.

[10] Longo Hammouda F. Siboukheur O. E. Chehma A. 2007. Aspects nutritionnels des pâturages les plus appréciés par *Camelus.*

[11] Fournier, P. 2003. New tool to more accurately assess value forage energy. Info-fodder Nuro 1. CQPF hay day (Council Quebecois of Forage Plants) pp 1-12.

[12] Chenost. 1970. Utilisation de la technique de digestibilité in vitro pour prévoir la valeur alimentaire des fourrages. In «Annual Zootechnie». pp: 243-253.

[13] Borba A. E. S. Correia P. J. A. Fernandes J. M. M. Borba A. F. R. S. 2001. Comparison of three sources of inocula for predicting apparent digestibility of ruminant feedstuffs. Anim. Res. 50 (2001) 265–273.

[14] Laudadio V., Lacalandra G. M., Monaco D., Khorchani T., Hammadi M., Tufarelli V. 2009. Faecal liquor as alternative microbial inoculum source for in vitro (DaisyII) technique to estimate the digestibility of feeds for camels Journal of Camelid Science 2 (2009) 01-07.

[15] Van der Baan A. van Niekerk W. A. Rethman N. F. G. Coertze R. J. 2004. The determination of digestibility of Atriplex nummularia cv. De Kock (Oldman's Saltbush) using different in vitro techniques. South African Journal of Animal Science 2004. 34. 95-97.

[16] Mir. Z. Acharya. S. N. Mir. P. S. Taylor. W. G. Zaman. M. S. Mears. G. J. and Goonewardene. L. A. 1997. Nutrient composition. in vitro gas production and digestibility of fenugreek (Trigonella foenum-graecum) and alfalfa forages. Can. J. Anim. Sci. 77: 119–124.