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# *In vitro* digestibility of a pastoral ecotypes collection of *Melilotus officinalis*

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**Abstract.** The aim of this study was to determinate the *in vitro* enzymatic digestibility of twenty ecotypes of yellow sweet clover (*Melilotus officinalis*) which were collected from the North-West Moroccan pasture. This collection was cultivated in the INRA experimental station in Tangier. The samples were collected during three growth stages of vegetative development. From the vegetative to the flowering stage, the fermentation parameters have also experienced a clear decrease. Indeed, we recorded a very high significant drop (P<0.001) for production of microbial biomass "PMB" (330.84 to 119.17 mg), for partition factor "PF" (4.07 to 2.77 mg/ml), for the speed coefficient of gaz production "c" (0.11 to 0.09 $h^{-1}$) and for protein digestibility "dE" (49.54% to 26.41% of crude protein "CP"). The gaz production decreased from 216.33 to 179 ml/g in the budding stage and then increased highly to 185.33ml/mg in the flowering stage. The best ecotypes have recoded values of 340.04 mg for PMB, 4.15 mg/ml for PF, 0.11 $h^{-1}$ for c, 64.86% CP for dE. This study showed that yellow Melilot presents a wide genetic diversity of nutritional parameters that can be exhibited in the field of animal nutrition, essentially in the vegetative stage, which presented interesting nutritional parameters.

**Keywords**. *Melilotus officinalis* – Ecotype – Harvest stage – Digestibility – Enzymatic.

***Digestibilité* in vitro *d'une collection d'écotypes pastoraux de Melilotus officinalis***

***Résumé.*** *Vingt écotypes de Mélilot* (Mélilotus officinalis) *des pâturages du Nord-Ouest du Maroc ont été installés dans la station expérimentale de l'INRA pour la détermination de leur digestibilité in-vitro par production de gaz et enzymatique. Des fauches ont été réalisées au stade végétatif, bourgeonnement et à la floraison. En allant du stade végétatif à la floraison, les paramètres de fermentation diminuent aussi très significativement, de 330,84 mg à 119,17 mg pour la production de la biomasse microbienne PBM, de 4,07 mg/ml à 2,77 mg/ml pour le facteur de partition (FP), de 0,11 à 0,09 $h^{-1}$ de la vitesse de production de gaz, de 49,54% à 26,41% pour la digestibilité des protéines (dE). La production de gaz a diminué de 216,33 à 179 ml/g au bourgeonnement puis connait une faible hausse à 185,33ml/g à la floraison. Selon les écotypes, les paramètres variaient entre 340,04 et 115,25 mg pour PBM, 4,15 et 2,77 mg/ml pour FP, 0,11 et 0,09 $h^{-1}$ pour c, 64,86 et 22,78% MAT pour dE. Cette étude a montré que le mélilot présente une variabilité significative, cependant, les écotypes E34 et E23 se présentent comme un fourrage prometteur dans l'alimentation caprine.*

***Mots-clés.*** Melilotus officinalis – *Ecotype – Stade de coupe – Digestibilité – Digestibilité enzymatique.*

## I – Introduction

In Morocco, goat's production is confronted to pressures of technical, economic and environmental order, which can be limited by improving the food value of the basic feed ration (a good digestibility and a wealth of nutrients) and also by a valorization of the feeding resources locally available not exploited. *Mélilotus officinalis* is a species which pushes naturally in the North-West of Morocco. This leguminous plant can reveal an important agro-economic and nutritional interest considering its availability in the area and his highly content of proteins, phosphorus and calcium (Urness and Al, 1975). The aim of the study was to better know the effect of ecotypes and the stage of plant growth on *in vitro* digestibility and the estimated fermentation parameters.

## II – Materials and methods

The seeds of 20 ecotypes (E1, E2, ....., E23) of *Melilotus officinalis* (the yellow sweet clover) collected last year (2017) from various ecological areas of the North-West of Morocco were sown in the Experimental Field of Boukhalef in November 13, 2017. See Lahkim Bennani *et al*. (in this volume) for more information about the collection sites, climate and soil. Each ecotype was installed on a plot of 10x2 m with 1m separation from each other. The plot was subdivided in 5 lines with a line space of 40 cm. Each line is sowing with an amount of 10 g which makes a density of 25 kg/ha. Fermentation kinetics and *in vitro* digestibility were estimated by the *in vitro* method of Menke and Steingass (1989). The rumen fluid used for incubation was taken from three slaughtered goats grazing on forest pasture. The inoculum was prepared as described by Goering and Van Soest (1975). The volume of gas was recorded at 0, 2, 4, 8, 12, 24, 48, 62 and 72 hours of incubation using 100 ml gradual glass syringe plunger. At the end of the incubation, contents of each syringe were used to estimate the potential in vitro dry matter (DM) and organic matter (OM) disappearance (IVDMD and IVOMD, respectively) The potential of gas's production of the soluble and insoluble fraction and also the speed of production of gas were calculated by using the exponential model of Ørskov and Mc Donald (1979). The production of microbial biomass (PBM) and the factor of partition (FP) were estimated by using the formulas of Blümmel (1997). The digestibility of proteins was determined by the protease method (Aufrère and Cartailler., 1988). The effect of the ecotype on the studied parameters was analyzed by the statistical software of analysis SAS (version 9, 2004) by using GLM's procedure. The multiple comparison of average was carried out using test LSD.

## III – Results and discussion

The quantity of microbial biomass produced in mg informed on the forage ability to provide essential nutrients (protein and energy) for the proliferation of rumen microorganisms. Indeed, E21, E22, E23 were characterized by an important produced microbial biomass (>306.36 mg). The organic matter degraded (mg) relative to the quantity of gas produced during incubation (FP) was affected significantly by the variability of the ecotypes ($P<0.001$). The higher values were recorded for E21, E22 and E23 with 4.15, 4.08 and 4.07 mg/ml respectively (Table 1). The production of gas coming from the fraction potentially degradable (a) depended very significantly on the origin of ecotypes($P<0.001$). E1 and E2a recorded the higher values with 3.39 and 3.69 ml/g DM respectively.The quantity of gas released by the insoluble fraction potentially degradable (b) depended very significantly on the source of the ecotypes ($P<0.001$). E34 and E13 recorded the higher values with 221.66 and 212.90 ml/g DM respectively. The origin of the ecotypes affected the speed of gas's production of the insoluble fraction (c) with $P<0.001$. The higher values were recorded for E3 and E17 with 0.11 $h^{-1}$.The variability of ecotypes affected the protein's digestibility ($P<0.001$). The highest value was recorded for E23 with 64.86% DM whereas E1 recorded the minimal value with 22.78% DM (Table 1).

The PBM decreased very significantly while passing from the vegetative stage (330.84 mg) to the flowering's one (119.17 mg) ($P<0.001$). The quantity of average microbial biomass was about 222.52 mg. The factor of partition decreased from the vegetative stage (4.24 mg/ml) to the flowering stage (2.77 mg/ml; $P<0.001$). The factor of partition's average was about 3,44 mg/ml. The production of gas coming from the fraction potentially degradable depended on the development stage ($P<0.01$). The highest value was recorded at the vegetative stage with 2.54 ml/g DM with an average of 0.62 ml/g DM. The quantity of gas by the insoluble fraction depended on plant's development. The maximum value recorded was about 208.87 ml/g DM at the vegetative stage and the average was at 189.18 ml/g DM. The speed of production of gas fell from the vegetative stage with 0.11 $h^{-1}$ to the flowering stage with 0.09 $h^{-1}$. The average speed was about 0.10 $h^{-1}$ (Table 2). The digestibility of proteins decreased with the advance of the stages. The highest value was recorded during the vegetative stage with 49.54% CP with an average value of 37.86% DM (Table 2).

**Table 1. Fermentation parameters of *Melilotus officinalis* ecotypes**

| Ecotype | PBM (mg) | FP (mg/ml) | a (ml/g DM) | b (ml/g DM) | c '($h^{-1}$) | dE |
|---|---|---|---|---|---|---|
| E1 | 219.20±60.13 | 3.56±0.46 | 3.39±1.24 | 191.47±12.67 | 0.10±0.00 | 22.78±4.13 |
| E2a | 160.16±30.65 | 3.09±0.22 | 3.69±1.68 | 203.53±11.18 | 0.09±0.00 | 28.78±4.09 |
| E2 | 153.60±18.19 | 2.98±0.11 | 0.59±0.10 | 183.43±5.67 | 0.10±0.00 | 29.78±2.18 |
| E3 | 115.26±21.88 | 2.77±0.12 | 1.75±0.43 | 188.33±8.53 | 0.11±0.00 | 32.65±2.43 |
| E34 | 166.68±8.06 | 3.07±0.11 | 1.56±0.46 | 221.66±1.26 | 0.10±0.01 | 27.23±2.34 |
| E9 | 249.74±21.56 | 3.61±0.17 | 1.63±0.75 | 183.33$^{f}$±8.89 | 0.09±0.00 | 36.60±1.72 |
| E10 | 285.10±42.05 | 3.81±0.31 | 0.15±0.01 | 171.73±3.88 | 0.10±0.00 | 38.90±2.05 |
| E11 | 251.99±39.43 | 3.65±0.26 | 3.13±1.59 | 187.43±9.16 | 0.10±0.01 | 30.48±3.06 |
| E12HN | 164.14±47.23 | 3.05±0.27 | 3.20±1.10 | 198.73±6.11 | 0.10±0.00 | 38.72±5.07 |
| E13 | 123.52±40.45 | 2.81±0.21 | 2.35±1.01 | 212.90±7.52 | 0.10±0.00 | 43.67±3.73 |
| E14 | 262.73±55.99 | 3.69±0.33 | 1.06±0.36 | 186.60±7.00 | 0.09±0.00 | 40.30±4.11 |
| E15 | 170.40±46.05 | 3.06±0.26 | 1.79±0.41 | 201.13±10.19 | 0.10±0.00 | 41.03±3.28 |
| E16 | 243.17±32.33 | 3.49±0.24 | 1.64±0.72 | 203.37±6.52 | 0.10±0.00 | 43.41±4.89 |
| E17 | 169.89±42.14 | 3.13±0.26 | 1.74±0.40 | 190.67±9.70 | 0.11±0.00 | 32.14±2.89 |
| E18 | 290.10±37.59 | 3.98±0.32 | 1.10±0.52 | 182.90±14.84 | 0.09±0.00 | 46.10±4.73 |
| E19 | 273.16±35.64 | 3.41±0.35 | 2.37±0.92 | 187.40±2.21 | 0.10±0.00 | 43.46±2.61 |
| E20 | 197.16±23.24 | 3.32±0.16 | 0.94±0.41 | 193.63±12.57 | 0.10±0.01 | 33.83±5.86 |
| E21 | 340.04±12.86 | 4.15±0.05 | 0.10±0.03 | 162.93±9.60 | 0.10±0.00 | 34.78±4.32 |
| E22 | 308.11±5.57 | 4.08±0.15 | 0.44±0.18 | 167.97±9.54 | 0.10±0.00 | 47.83±3.61 |
| E23 | 306.36±22.32 | 4.07±0.22 | 1.29±0.61 | 164.37±10.48 | 0.09±0.01 | 64.86±2.11 |
| P | <0.001 | <0.001 | 0.0092 | <0.001 | 0.0034 | <0.001 |
| Significance | *** | *** | ** | *** | ** | *** |

**Table 2. Estimated fermentation parameters with the stage of plant growth**

| Stade | PBM (mg) | FP (mg/ml) | a (ml/g DM) | b (ml/g DM) | c '($h^{-1}$) | dE |
|---|---|---|---|---|---|---|
| Vegetative | 330.84$^{a}$±10.15 | 4.24$^{a}$±0.08 | 2.54$^{a}$±0.33 | 208.87$^{a}$±3.38 | 0.11±0.00 | 49.54a±1.33 |
| Budding | 217.57$^{b}$±11.14 | 3.30$^{b}$±0.59 | 1.21$^{b}$±0.35 | 177.60$^{b}$±2.74 | 0.10±0.00 | 37.65b±1.14 |
| Flowering | 119.17$^{c}$±10.95 | 2.77$^{c}$±0.06 | 1.33$^{b}$±0.19 | 181.06$^{b}$±3.73 | 0.09±0.00 | 26.41c±1.39 |
| Average | 222.52±8.94 | 3.44±0.06 | 0.62±0.18 | 189.18±2.17 | 0.10±0.00 | 37.86±1.02 |
| Pro. | <0.0001 | <0.0001 | 0.0035 | <0.0001 | 0.3377 | <0.0001 |
| Sign. | *** | *** | ** | *** | n.s | *** |

Concerning digestibility, Gasmi *et al.*, 2012 reported that OMD was 78.1% DM for *Vicia sativa,* 74.6% DM for *H. coronarium*, the values were similar to those found by *Mélilotus officinalis* with an average of 74.84% DM. However digestibility during the vegetative stage was higher for *Melilotus officinalis* with 82.25% than sulla with 77.61% (Errasi, 2016) and to the sainfoin 71.2% (Aufrère *et al.*, 2008). The relation between PBM and OMD is strong (r=0.89), the PBM depended on the source of the ecotypes and the stage of development; the highest values were recorded for E21, E22 and E23 with 340.04, 308.11 and 306.36 mg respectively. The vegetative stage recorded the highest PBM value with 330.84 mg, which is much higher than those found by Errassi *et al.,* 2016 for Sulla with a production of 238.09 mg. The reduction in the microbial production could be the complexation of the tannins with the nutrients that decreases the degree of their availability by the attack of the micro-organisms (Makkar, 2003). The speed of gas's production (c) varied significantly according to the stages; E3 and E17 recorded the highest values with 0.11 $h^{-1}$. The speed of gas's production decreased from 0.11 $h^{-1}$, recorded in the vegetative stage to 0.09 $h^{-1}$ recorded in the flowering stage. Values are practically similar to those found by Errassi *et al.,* 2016.

The crude protein digestibility is higher at the vegetative stage, which can explain the high value recorded at this stage with 49.54% DM, whereas E23 recorded the important value with 64.86% DM.

## IV – Conclusions

The difference in fermentation's parameters revealed an heterogeneity within the ecotypes which can be related to the chemical composition of the plant. The performances of fermentation and digestibility can be improved by using ecotypes collected at an early stage (vegetative). For this stage, E23 followed by E10 and E14 recorded the highest OMD.

The genotypes of Melilot collected from the pastures of the North-West of Morocco appear as a promising forage resources of goat production, particularly the E23 ecotype which is characterized by a ODM relatively higher for the three stages of harvest, and an interesting profile of fermentation.

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