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Morphological and Chemical Characterization of a Variety of *Medicago* (Triad) in Flowering Stage and *in Vivo* Digestibility of its Hay

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Abstract

Nutrient value of sustainable *Medicago* imported in Algeria, used of dual-purpose green and hay in feeding ruminants. Chemical composition of *Medicago* in budding stage gives a weak ash content, varying between 6,547 and 7,011% DM. Content in TNM found at the triad of this stage varies between 15,34% MS to 20,75% DM. As for fibers contents NDF it is of 44%. Analysis of parameters variance studied of the three sections show a difference very highly significant ($P < 0.0001$) except for DM and MM. The Principal Components Analysis (PCA) shows that diameter of stems is opposing to phosphorus and lignin quantity. The two last parameters have contributed to locate the first section in this axis. Germination density (number of plant /m²), yield in dry and in green and average height of stems locate the second section in the same axis. The DWC of OM and of hay *Medicago* TNM at budding stage is relatively weak for nitrogen, respectively of 69% and 55.5%. Different tests on the same fodder according to years and regions of harvest are necessary in order to have weaker and more accurate averages values.

Keywords: *Medicago*; Triad; Chemical composition; Biometric parameters; Food value

Abbreviations:

DM: Dry Matter; GM: Green Matter; OM: Organic Matter; TNM: Total Nitrogenous Matter; MM: Mineral Matter; NDF: Natural Detergent Fiber

Introduction

Cultivated plants intended to fodder production are particularly coming from two botanic families: grasses and leguminous. The grasses family is one of the most important which is about 10,000 species spreading throughout the world. It constitutes the base of natural herbaceous flora from which feed wild and domestic herbivores. The leguminous for their part constitute, a family of about 13,000 species belonging of order of Rosales.

The Papilionaceae with some 10,000 species, form the leguminous family the most important and the most interesting in agricultural production notably fodder. Whether they are leguminous or grasses, their zoo-technical value depends mainly of cultivated varieties. For a species and for given variety, it depends of vegetative stage and of harvest cycle and also of conditions and spots of cultures. A good control of forage crops is absolutely essential in livestock development policy, notably dairy.

In Algeria, fodder crops are not much diversified and are often occupying reduced areas. The annual fodder associations (vetch-oat, vetch-barley and pea-barley) constitute the main cultures whose production is intended doing reserves (hay and silage) used in off-peaks periods. These annual Poaceae are dominant fodder cultures. Pulses also constitute a resource enough important in winter, and in spring, clover often forms the single green fodder resource for the dairy cattle.

In summer it is relayed by sustainable alfalfa and sorghum know for a long time from Mediterranean agriculture, already cultivated in the same time for its hay and for its green, the common alfalfa (*Medicago sativa*) although it remained one of pulses the most interesting, as well as by quantity as quantity of its fodder (consumed in green, ensiled or in hay) where its culture is not properly spread in livestock regions of Algeria.

Target of this work is to accurately predict biometrical parameters at phenological bud stage (considered as optimum stage which offers the best nitrogen value) of *Medicago* introduced (Triad), to know its chemical composition, and finally measure digestibility of its hay in local sheep. This work is an introduction of a similar work which will be realized on local population of *Medicago El-Menea*.

Material and Methods

Experimental sites

Essay of *Medicago* stands is performed in experimental farm of Oued Esmar. This farm is characterized by the following geographical coordinates longitude: 30°08 E; latitude: 36°43 North; altitude: 24 m. Experiment on animals was conducted in farm of application and research of National High School of Agronomy El harrach.

The climate of the region is of Mediterranean type, in bioclimatic sub-humid stage, characterized by a rainy season from average September until May, and by a sunny summer, [1].

Cumulative rainfall during experimental period of seeding (November 2010) at the last mowing (June 2012) was of 1217.5 mm. With average of 72 mm and the higher value recorded on February 2012 (238.3 mm).

The sum of average temperatures during experimental period was of 27.5°C with the hottest month August 2011 (30.52°C).

Nutritional part

The foods

The used *Medicago* hay comes from the Farm. It has been sown to dose of 10 kg/ha in March 2010, brought in dry and without manuring input it has been harvested at bud stage of the fourth cycle.

The ration distributed comprises hay of *Medicago* distributed at will and a dose of sheep concentrate of 200 g by animal and by day. The used concentrate is composed of barley, maize, *Medicago* hay, bran, soya and minerals for wilting April 3, 2014.

The animals

Digestibility is measured according to method of reference, by total collection of faeces (Charlet-Levy, 1969), on 4 adults sheep's of breed non-castrated Ouled Djellal race, aged about 36 months and weighing alive between 50 and 60 kg. Animals are dewormed by IVOMEC injection and spread in lot with homogenous weights.

Sequence of experiment

Experience is divided in two phases, the first one of adaptation followed by another step of digestibility measure. The first phase lasts 21 days from weighing animals in fasting. They are put in individual boxes of about 1 m² and they are fed by daily ration (8h 30 am and 4 pm). Drink water is renewed once a day and let a will (Figure 1).

At the end of this adaptation phase animals are weighed in fasting state for a second time before to be placed in metabolism cages for 10 days of digestibility measure (Figure 2). They receive their daily rations at the same schedules.

The weighing of distributed, (hay and concentrate), refusal and faeces are daily done throughout period measure [Table 1].

A representative sample of each component is then collected, dried at 150°C in oven until having a constant weight to determine the dry matter, then cumulated for each animal

and for all period. Upon completion of the measuring process, samples are ground and stored for chemical analyses.



Figure 1: Adaptation in animals in individual boxes.



Figure 2: Animals in metabolism crates.

The amount of dry matter intake is calculated over the entire measurement period and is expressed by the difference between the daily distributed and turn down (Quantity refused (g)).

DMI (g) = MQD - DMQR (MQD: dry matter quantity dispensed (in g); MSR: dry matter Quantity refused (g).

The apparent digestibility of the diet components (DM, OM, and TNM) between ingested (I) and excreted (E) is determined using the following equation:

$$\text{Coefficient of apparent digestible use (\%)} = \frac{\text{ingested (g)} - \text{excrete (g)}}{\text{Ingested (g)}} \times 100$$

Table 1: Quantity of organic matter intake.

Animals	P ⁰ , 75	Organic matter intake (g)			Concentrated in the total ration (%)	Ingested total organic matter /kg P ⁰ , 75
		Hay	concentrated	Total		
Animal 1	23,69	1826,80	172,32	1999,12	8,62	84,39
		± 138,70	± 3,53	± 140,92	± 0,61	
Animal 2	20,24	1275,49	172,32	1447,81	11,90	71,53
		± 221,42	± 3,53	± 221,51	± 3,79	
Animal 3	20,62	1358,10	172,32	1530,42	11,26	74,22
		± 216,40	± 3,53	± 215,38	± 1,97	
Batch medium	21,52	1486,80	172,32	1659,12	10,59	76,71
	± 1,89	± 297,33	± 0,00	± 297,33	± 1,74	± 6,80

Expressed in grams per day, the total amount of OM ingested by the animals 1659.12 g per day per animal. By relating this value to metabolic weight of the animals, it gives moyenne 76.71 g per kg P⁰,75.

The quantities of total nitrogen-containing material ingested by the animals during the experiment are shown in Table 2.

Table 2: Quantities of nitrogenous matter intake for the four lots.

Animals	P ⁰ ,75	MAT ingested (g)			Concentrated in the total ration (%)	Total nitrogen containing material ingested/kg P ⁰ ,75
		Hay	concentrated	Total		
Animal 1	23,69	258,96 ± 21,58	15,83 ± 0,44	274,80 ± 21,78	5,76 ± 0,42	11,60
Animal 2	20,24	186,06 ± 31,77	15,83 ± 0,44	201,89 ± 32,02	7,84 ± 1,70	9,98
Animal 3	20,62	192,96 ± 33,83	15,83 ± 0,44	208,79 ± 34,07	7,58 ± 1,09	10,13
Batch medium	21,52 ± 1,89	212,66 ± 40,25	15,83 ± 0,00	228,49 ± 40,25	7,06 ± 1,13	10,57 ± 0,90

Part of morphological characterization

The sampling

The used samples of *Medicago* come from farm of Oued-Smar and managed as previously announced. Sampling for fodder analyses in green is carried out in second and third cycle on the same culture of *Medicago*.

That is why we have delimited to studied stages (floral buds: FB) four micro plots of square meter (Figure 3) at different places of the *Medicago* stands. One of the micro plot is intended to the determination of report of leaves on stems.

The three other micro-plots have been the subject of measuring biometric parameters that are related to the nutritional value of forage and yield rating after mowing.

**Figure 3:** Delimitation of a square meter in the alfalfa.

Biometric parameters

The biometric parameters studied are components of yield in relation with variation of chemical composition of fodder which are, number of plants by square meter, number of stems by square meter, stems diameter, the report in dry and in green on stems, length of stems, and yield in green and in dry.

Chemical analyses

The focused on dry matter (DM) by passing in oven of 3 g of the ground matter at 150°C; parietal constituents (NDF, ADF, ADL) by method of Van Soest; the total nitrogen matters (TNM) by Kjeldhal method; the raw cellulose by method of Weende (CBw) and so mineral matter (MM) by incineration in calibrated into muffle furnace set at 550°C. Calcium and phosphorus are determined by atomic absorption.

Statistical analyses

The results obtained made subject of statistical analyses with calculation software stat view and statistica 1987.

Results and Discussion

Yield depends of the plant growth, but also of the number of plants by surface unit and number of shoots [2]. And so the number by square meter found in triad variety at flowing ud is an average of 11.78 plants by m².which corresponds an average of stems number of the three sections of 142.12 stems by m² (Table 3).

The factor which determines growth of the plant is the average height of stems. At the bud stage, it is of 69,45cm (Table 3). Average yield of the three sections by square meter,

at this height and at this stage, is of 1795,556 g/m² against 447,83g /m² de DM with a report leaves on stems of 0.95 in green and of 0.79 in dry (Table 3).

Diameter of the stem at adult age is a varietal character. Stems' fineness is sought after for *Medicago* intended to wither. In the same plant, evolution of diameter is according to phenological stage.

At budding stage, the average diameter of the three sections is of 0.215 cm (Table 3). The chemical composition given by Table 3, shows content in TNM of the triad at flowering bud (FB) between 15.5% and 17% of the DM.

This value is low with result given by Mauries [2] which can vary of 14 and 29% of DM according to stage and cycle of the section. This weak content may be explained by cycle number of the plant (3RDcycle) since according to the same author, at the second cycle content decreases with age of shoots but more slowly than in the first cycle .

The quantity in ash of triad *Medicago* varies between 6.547 and 7.011% DM (Table 4). These values are low to those given by Meschy and Gueguen [3] which constitute 8 to 15% of dry matter of fodder with a general manner.

As for this chemical element, it seems having opposition between minerals accumulation and the full growth of vegetative organs which is followed by an active polymerisation of carbohydrate.

Thus, age influences on quantity of minerals found in the plant. Furthermore, *Medicago* includes a combination particularly interesting of minerals and oligo elements.

Professor Van Belle, of University of Louvain in Belgium reports experimentation on sheep in which addition of calcined *Medicago*, which that means containing only minerals of *Medicago*, allowed to double microbial synthesis in the rumen [4].

Table 3: Biometric parameters of triad *Medicago*.

Samples	Total number/m ²	Plants	Stems number/m ²	Stems length(m)	GM g/m ²	DM g/m ²	Diameter of fresh stems (en cm)	fresh F/T	dry F/T
ITGC First cut BF	11,333		3,06,000	71,527	15,06,667	4,00,667	0,065	0,843	0,965
ITGC Second cut BF	12,667		65,787	77,527	28,06,667	7,57,073	0,347	0,803	0,4
ITGC Third cut BF	11,333		54,573	59,293	10,73,333	185,74	0,233	1,19	1,03
Average	11,778		1,42,120	69,45	17,95,556	4,47,827	0,215	0,946	0,798
Distance type	0,77		1,42,035	9,293	9,02,055	2,88,574	0,1417	0,2126	0,346

GM : green matter, DM :dry matter, fresh F/T :Green leaves on stems, Dry F/T : Dry leaves on stems, BF: flower buds, ITGC: variety Triad

Table 4: Chemical composition (in % of DM) of the Triad variety.

Samples	DM%	In % of DM								
		TNM	RC	Ca	MM	Phosphorus	NDF	ADF	HC	ADL
ITGC First section FB	92,77	15,48	25,03	1,525	6,547	0,328	42,100	19,746	13,991	8,552
ITGC Second section FB	92,64	17,04	27,15	1,593	7,108	0,324	44,529	30,784	13,744	10,165
ITGC Third section FB	92,71	16,26	26,09	1,559	6,827	0,326	43,314	25,265	13,868	9,359
Average	92,71	16,26	26,09	1,559	6,827	0,326	43,314	25,265	13,868	9,359
Distance type	0,063	0,781	1,059	0,034	0,281	0,0018	1,214	5,518	0,123	0,806

DM : Dry matter, TNM: total nitrogenous matter, RC: crude fiber, ca: calcium, MM: mineral matter, NDF: natural detergent fiber, ADF: acid detergent fiber, HC: hemicelluloses, Ln: lignin, ITGC: variety Triad

Contents recorded in calcium at the triad of the same stage vary between 1.52 and 1.59% of DM. Content given by Daccord et al. [5] in leguminous is of $1.51 \pm 0.25\%$ MS. These last ones increase with age advancement. However, we record contents in phosphorus varying between 0.324 and 0.328% of DM (Table 4), which are identical values to those given by Daccord et al. [5] comprise between 0.34 and 0.36 of DM. Content in minerals of the plant is reflecting of the soil which produces it [6]. Phosphoric liquid fertilizers seem provoking a more substantial increase of content in P of fodder [3].

According to Chadja [7], phosphate fertilizers have a positive effect on accumulation rate of total nitrogen in plants, and so on rate of totals proteins. In our *Medicago* plot, no report of P has been done. Results of cellulose found give an average for the three sections made of 26.087% of DM (Table 4). These values agreed with results of some authors (INRF, 1998; ITEBO) on a *Medicago* harvested at the same stage. Average content in NDF given by the three sections is of 43.31% of DM (Table 4), which are identical values with those of Piva et al. [8] at stage 3 and Corsi et al. [9]. As for content in ADF, it is of 25.26% of DM (Table 4) and similar to result of Torricelli et al. [10]. The chemical element, lignin, as determinant for digestibility in ruminants is an average of 9.36% DM for the three sections at budding stage (Table 4). Variance analysis of parameters studied at bud stage (details in Annex 1) shows a difference highly significant ($P < 0.0001$), to significant ($P < 0.05$), between the three sections on all parameters measured except for mineral (MM) of *Medicago* of the three sections which shows no significant difference that high distance decrease lignin rate of the plant.

The Principal Component Analysis (PCA) of parameters studied allows us to say that criterion of Kaiser let us to retain an axis. Projection of parameters studied (Annex 2) shows that all studied elements are located at the level of circle periphery. We can conclude that all parameters are highly correlated with

parameters constituting this plan (1 and 2). This factorial axis gives 100% of information ($79.07\% + 20.93\% = 100\%$), the axis according to which is preserved by projection the maximum of spreading of cloud's points. The maximum angle between two variables of a same plan is low at 90° . That suggests that all variable are positively correlated between them. Sampling is made in third circle of *Medicago* stands' life.

The first section is done on 19/04/2012. Projection of factor studied number of section at bud stage (Annex 2) show that the most chemical parameters (ADF, NDF, RB, TNM, CB) and diameter of stems are opposing to phosphorous and lignin quantity. The last two parameters have contributed to locate the first section in this axis. Germination density (number of plant m^2), yield in dry and in green and average stems' height locate the second section made on 7/05/2012 in the same axis. The quoted factors are opposing parameters leaves report on stems in dry and in green and the last ones locate the third section made on 28/05/2012 in this axis.

The report showed from the first section of the spring beginning that we can vary number of stem by m^2 by increasing seeding density or space between plant to decrease rate of lignin in stems (both parameters are negatively correlated). However, seeding density depends closely of germinal capacity of the variety. The yield variation in green and in dry depends of number of plant by square meter and average height of the stems at this stage.

The number of plant by square meter decreases with age of *Medicago* regardless of seeding dose and space between lines. So the second section in spring at this stands *Medicago* age depends on installation conditions of *Medicago* stands. At spring end and at summer beginning, among all parameters studied, those are reports leaves on stems in green and in dry which justify value of the third section. The chemical composition of *Medicago* hay is characterized by contents in DM and in OM respectively of 90.6% and of 87.93% (Table 5).

These values are close of those obtained by Kamalak et al. [11] with respectively 93% and 88.9% on average of 14 different varieties of *Medicago* hay. Content in mineral matter is enough

high (12%; Table 5) according to results obtained on the green triad at the same stage. That may be explained by presence of ground in hay after its baling.

Table 5: Chemical composition of food distributed to sheep.

Chemical Components	DM%	In % of DM		
		MM	OM	TNM
Food				
Hay	90,58 ± 0,35	12,07 ± 1,20	87,93 ± 0,85	12,56 ± 0,11
Concentrated feed	92,02 ± 0,49	7,98 ± 1,13	83,67 ± 1,48	8,60 ± 0,21

Content in TNM obtained in hay is of 12.6%, it is weaker compared to 16.8% obtained by Chibani [12]. Presence of weeds, drying conditions, lost of leaves during gathering of hay; stage and number of plant cycle are other factors of variations which can explain this difference. Furthermore, although contents in nitrogen in green found vary between 15 and 20% of DM, it is logical that content in hay nitrogen be low to 15% of DM.

Results concerning the coefficient of digestibility (DWC) in dry matter obtained during measure phase with triad hay at budding period are of 61.4% (Table 6). It is acknowledged that digestibility decreases with increase of fodder's content in dry matter [13]. This increases with growth of physiological stage of the plant.

Table 6: Apparent digestive working coefficient of DM, OM and of TNM (in %).

Animals	DWC DM	DWC OM	DWC NTM
Animal 1	62,79 ± 6,37	68,3 ± 4,82	57,58 ± 6,93
Animal 2	58,35 ± 13,38	71,62 ± 11,44	51,74 ± 12,21
Animal 3	62,96 ± 5,20	66,82 ± 5,40	57,1 ± 7,51
Average of lot	61,37 ± 2,61	68,91 ± 2,45	55,48 ± 3,24

The triad hay organic matter of budding stage offers a DWC of 69% (Table 6), this digestibility is inversely proportional to lining content, [14] cells contents are most digestive. As for hay TNM of the triad, we recorded weak digestibility with an average on animal lot of 55.5%. The apparent digestibility is weaker compared to real digestibility of fodder's nitrogen matter. In effect, faeces contain substances endogenous and microbial whose quantity is proportional to the quantity of dry matter ingested [15].

Conclusion

The chemical composition of *Medicago* studied shows that content in ash is weak that is perhaps because of absence of soil fertilization. As for content in TNM, it varies between 15.5 and 17.04% of DM which is low to values usually given (INRA, 2007) but that can be explained by number of cycle growth of the plant. Contents in fibers, raw cellulose, ADF and NDF agree with those given by authors which did not modify digestibility of the species.

However, the chosen season of sampling does not mark influence of these parameters in PCA. The determinant chemical component for digestibility, lignin offers us an average of 9.36% of DM.

This content can be decreased by genetic handling and would increase then DWC of the plant. The average of

chemical factors studied, of the three sections of the Triad *Medicago* stands, in spring of its third cycle, corresponds to average value of the third section. The yield in green and in dry offers an average respectively of 1795,56 g/m² et 447,83 g/m². So it is more logical to measure fodder dry than in green to quantify the dry synthesized matter and non water quantity retained to a given stage since this one evolves with plant age. For the most parameters studied analysis of variance is either significant or highly significant. This shows there is a variation from a section to another within parameters studies in the plant, except MM which a parameter linked much more to the soil than to the plant.

Several correlations are recorded between parameters studied, which is more striking is that $r = 1$ between both minerals (Ca, P) and NDF and so between number of plant and content of lignin. This observation will allow to the far the immediate action on seeding dose and space between lines to decrease rate of lignin in the plant. The principal components analysis, separates the three sections of the same stage in different axes of the single plan formed in this analyse. The chemical composition of Triad hay in budding stage does not show notable differences compared to *Medicago* hays already studied outside of content in TNM which is slightly weaker. The Digestive Working Coefficient of OM and TNM is respectively of 69% and 55.5%, which is relatively weak for TNM. Finally, multiplication of essays on the same fodder according to years

and harvest regions is necessary in order to have more reliable and accurate average values.

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