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Influence of maturity stage of forage grasses and leguminous on their chemical composition and *in vitro* dry matter digestibility

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Abstract. In this study we investigated the effect of maturity stage on chemical composition, *in vitro* dry matter digestibility (IVD) and volume of gas produced at 24 h (G24) of forages from *Avena sativa* L. (oats), *Trifolium alexandrinum* (berseem), *Vicia sativa* L. (vetch), *Avena sativa-Vicia sativa (OV)* and *Avena sativa-Trifolium alexandrinum (OB)* mixtures collected at progressive morphological stages (S1, S2, S3, S4) from the emergence to the beginning milky maturation of the grain and the pod stage. Lowest CP content (56 g/kg DM) was recorded for *A. sativa-T. alexandrinum* at the mature stage (S4), whilst *V. sativa* collected at young stage (S1) had the highest content (274 g/kg DM). NDF content varied from 423.8 g/kg DM to 760.9 g/kg DM, and ADF content varied between 291.5 g/kg DM (vetch-oat) and 462 g/kg DM (vetch collected in S3). For all studied species the highest IVD coefficient (>0.800) corresponded to forage sampled at young maturity stage (S1) and the lowest values (0.500) to mature stage (S4) of Gramineae and mixtures. The same trend was followed by G24. It is pertinent to mention that for all studied species with increasing maturity CP content, IVD and G24 declined linearly while NDF and ADF contents followed an opposite trend. It was concluded that CP, IVD and G24 of Gramineae (oat) can be enhanced by its association with leguminous specially vetch, the most common combination in Tunisian agricultural systems.

Keywords. Gramineae – Leguminous – Chemical composition – *In vitro* digestibility.

Influence du stade de maturité sur la composition chimique et la digestibilité in vitro de la matière sèche de certaines graminées et légumineuses

Résumé. *Dans cette étude on a détermine l'effet du stade de maturité sur la composition chimique, la digestibilité* in vitro *(DIV) et le volume de gaz produit a 24 h d'incubation des fourrages de l'*Avena sativa *L.,* Trifolium alexandrinum *(B),* Vesce sativa *L., vesce-avoine (VA) et avoine-trifolium (AB) collectés dans des stades morphologiques différents, de la montaison (S1) jusqu'au stade pâteux laiteux (S4). Les contenus les plus faibles en protéine brute (PB) ont été enregistres dans l'association AB collecte au stade S4 (56 g/kg MS), alors que les contenus les plus élevés (274 g/kg MS) ont été trouvés dans la vesce en debut de vegetation (S1). Les teneurs en FND varient entre de 423,8 et 761 g/kg MS et les contenus en FAD oscillent dans entre 291 et 462 g/kg MS. Pour toutes les espèces étudiées, les coefficients de DIV ont été élevés (>0,800) au début du stade végétatif et faibles (0,500) vers la fin de croissance végétative (S4) des graminées et des asscociations. De même G24 a suivie la même allure. Il convient de mentionner que pour toutes les espèces étudiées et en fonction du grade de maturité du fourrage, PB, DIV et G24 ont marqué une chute décroissante à l'opposé de FND et ADF. On a conclu que les teneurs en PB, DIV et G24 de l'avoine peuvent être améliorés suite à son association avec les légumineuses en particulier avec la vesce, technique souvent appliquée en Tunisie dans les systèmes d`élevage.*

Mots-clés. *Graminée – Légumineuse – Composition chimique – Digestibilité* in vitro.

I – Introduction

In Mediterranean countries, agricultural policies are enhancing the increase of biodiversity in all the ecosystems included in pastures. In general, leguminous are a low input energy and efficient crops that improve soil fertility, and their importance is rising with the increase of public

interest in sustainable agriculture. Furthermore, forage legumes occupy a significant economic position in the animal feed market and deserve a particular attention in arid and semi-arid Mediterranean areas (Kokten *et al*., 2009). However, grasses are rich in carbohydrates and highly competitive in contrast with legumes. Within this scenario, biodiversity in sward composition is an attractive target, and forage legume-grass mixtures are potentially advantageous strategies in animal production systems. In Tunisia, ruminant nutrition is based on the use of grass and legume species either as monocultures or in associations. Even though, there is still a lack of information to accurately evaluate their nutritive values, and when necessary, data from similar forages cultivated in other Mediterranean countries are used as approximated values. The aim of the present trial was to bring-up appropriate data on the chemical composition and the *in vitro* digestibility of some Tunisian gramineous and leguminous forages (monoculture and mixture) mainly used in ruminant nutrition. Effect of maturity stage on the studied parameters was also assessed.

II – Material and methods

1. Vegetal material

Five fresh forages made of three monocultures from forage species namely vetch (*Vicia sativa* L.), berseem clover (*Trifolium alexandrinum*), oats (*Avena sativa* L.) and two intercropped grass-legumes forages (50%, 50%) of oat + berseem (OB) and oat + vetch (OV) harvested at the Ecole Supérieure d'Agriculture de Mateur in Tunisia were used. The study area is located in the north of Tunisia at an altitude of 65 m above sea level. The total annual precipitation is 533 mm. Each of the studied forage species was sown in three fields each of 600 m². Each field was divided into four plots of 150 m², which were harvested at different growth stages: S1= emergence, S2 = beginning of ear emergence, S3 = ear emergence, S4 = beginning of the milky maturation of the grain and pod formation. There was no application of fertilizers or irrigation in any of the experimental plots. At each cutting stage, samples of herbage from each forage species or forage combination were collected, oven-dried at 60°C for 48 h and subsequently ground to pass a 1-mm screen.

2. Chemical analysis

Forages collected were submitted to the analysis of their dry matter (DM, method ID 934.01), ash (method ID 942.05) and crude protein (CP, method ID 984.13) contents following the methods of AOAC (1999). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined with the ANKOM fibre analyzer (Ammar *et al*., 1999) using the reagents described by Van Soest *et al.* (1991).

3. *In vitro* digestibility

The procedure followed in this trial simulated general conditions described in the standard *in vitro* fermentation method (Goering and Van Soest, 1970) with the modifications proposed by the ANKOM-DAISY procedure (Ammar *et al*., 1999). Samples (250 mg) were weighed out into four polyester bags which were sealed with a heater and placed in incubation jars (22 bags/jar). Buffered rumen fluid was prepared according to Goering and Van Soest (1970) and transferred (2 l) into the jars containing the bags. The jars were then placed in an incubator (DAISY, ANKOM) at 39ºC, with continuous rotation. After 48 h of incubation in buffered rumen fluid, bags were gently rinsed under cold tap water and then again in a washing machine (short washing cycle –10 min, with cold water) and dried at 60ºC for 48 h. After washing out in a neutral detergent solution (100ºC, 1 h), the residue was dried and weighed out and considered as the truly indigestible matter to calculate the *in vitro* DM digestibility (IVD) (Goering and Van Soest, 1970). Incubations were performed in two runs carried out in two consecutive weeks.

4. *In vitro* gas production

The method used for the gas production measurements was described by Theodorou *et al*. (1994). About 500 mg of each sample were weighed out into 120 ml serum bottles kept at approximately 39ºC and flushed with CO_2. Each substrate was incubated (2 replications) in the bottles anaerobically dispensed with 50 ml of buffered rumen fluid prepared as described before. All the bottles were crimped and placed in the incubator at 39ºC. Volume of gas produced in each bottle was recorded at 24 h (G24) after inoculation time, using a pressure transducer (Theodorou *et al*., 1994). Two runs in two separated weeks were carried out.

III – Results and discussion

Chemical composition of the samples of the grass, legume and grass-legume associations at each sampling time is presented in Table 1. Berseem harvested at different maturity stage and young foliage of vetch (S1) had particularly high ash contents (173-194 g/kg DM). Therefore, as compared with shrub species, conventional forages, particularly legume species, seem to be rich in minerals (Ammar *et al.*, 2004). The CP contents varied widely between species and with maturity stage. The lowest CP content (56 g/kg DM) was recorded in the foliage of OB association harvested at S4 and the highest (274 g/kg DM) in the young foliage (S1) of vetch.

Table 1. Effect of maturity stage on the chemical composition (% DM), true *in vitro* dry matter digestibility (IVD) coefficients and volume of gas produced (ml/g DM) at 24 h (G24) incubation time of the studied forages

Forage specie	Maturity stage	MM	CP	NDF	ADF	IVD	G24
Oat	S1	115	137	601.3	324.3	0.808	170
	S2	103	90	611.5	354.6	0.765	173
	S3	92	65	697.6	388.6	0.646	170
	S4	91	74	760.9	417.6	0.502	120
Vetch	S1	188	274	423.8	292.3	0.823	163
	S2	106	106	580.5	349.5	0.755	154
	S3	134	160	600.7	462.0	0.684	131
	S4	101	158	667.0	418.4	0.642	152
Berseem	S1	194	203	460.7	297.0	0.759	138
	S2	184	192	515.1	331.5	0.718	108
	S3	173	188	535.9	384.2	0.728	97
	S4	173	164	588.1	379.8	0.684	158
Vetch-oat (VO)	S1	131	157	507.3	291.5	0.855	178
	S2	94	146	565.3	402.4	0.611	140
	S3	97	77	634.4	379.0	0.693	151
	S4	85	65	728.2	406.4	0.508	136
Berseem-oat (BO)	S1	128	153	552.7	316.5	0.832	187
	S2	105	98	648.9	375.1	0.688	135
	S3	94	63	690.8	405.7	0.650	157
	S4	91	56	734.0	447.7	0.501	118

With the exception of berseem, CP in all the studied species followed a drastic decrease (>50%) as the crop matured and the most pronounced decrease was observed in OB association (63%) and vetch (62%). Similar results were reported earlier on fresh forages (Razec *et al.*, 2002), silage (Wallsten and Martinsson, 2008) and hay (Turgut *et al*., 2008) of other grass and legume species. It is pertinent to mention that oat growing with legumes (vetch and berseem) contained more CP than forage from oat monoculture. Consistently with the results of Sleugh *et al*. (2000), legumes in monoculture or in binary mixtures with oat had lower NDF and ADF contents than grasses grown in monoculture (Table 1). Both NDF and ADF contents followed generally linear increase with advancing maturity. Similar results were reported by Turgut *et al*. (2008). As plants mature, photosynthetic products are more rapidly converted to structural components, thus having the effect of decreasing protein and soluble carbohydrate and increasing the structural cell wall components (Ammar *et al.*, 2004).

In agreement with results reported by Sleugh *et al.* (2000), binary mixtures harvested at the young maturity stage (S1) had higher IVD than legumes and grass monocultures (Table 1). This can be explained by better utilization of symbiotically fixed nitrogen, enhanced light interception and allelopathic interactions. The digestibility of all studied forages decreased with advancing maturity in an approximately linear fashion; this is typically related to the declining leaf:stem ratio and increasing fibre contents. Similar results were reported earlier (Wallsten and Martensson, 2008). A slight decrease of IVD was observed in berseem (11%) and vetch (22%), whereas the most drastic decrease (40%) was primarily in grass-legume mixtures and pure oat herbage (38%). With respect to G24, the highest was recorded in OB associations harvested at S1 (187 ml/g DM) and the lowest values (97 ml/g DM) in berseem collected at S3. This was an indication that legumes had a positive influence on their companion grasses and that most of the nutrients especially crude protein would be more balanced for the animals.

IV – Conclusion

Our study confirmed that, as compared with pure oat, oat/legume mixtures have generally better nutritional value in terms of chemical composition and digestibility. Moreover, with advancing maturity all the forage quality attributes investigated have been shown to decrease and the most pronounced decrease was observed in oat than in mixtures and legumes. Our results suggest that the oat component may well benefit from the N fixed by legumes, especially vetch, when grown together in binary combinations.

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