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Genetic and environmental influences on *in vitro* digestibility of alfalfa

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ABSTRACT: To study the relationships between *in vitro* NDF or true dry matter digestibility (NDFD and IVTDMD) and forage fibre content, 95 alfalfa samples from 5 cultivars grown in 3 different locations and from different cuts were analysed for fibre fractions and evaluated for NDFD and IVTDMD. NDFD was mainly influenced by order of cut and age of the fields. The multifoliate cultivar controlled did not appear to differ for fibre composition and NDFD from the other ones. Fibre fractions contents explained a large part of the difference in NDFD and IVTDMD, and no significant differences in this relationships appeared for first-cut vs. aftermath forage. The best predictive equation of NDFD from fibre factions was: NDFD = 73.61 -0.62 * NDF (% DM) – 56.33 * ADL/NDF (R^2=0.39). Variations in fibre content and quality do not completely explain differences in NDFD suggesting the interference of other factors that are worth to be better studied.

Key words: Alfalfa, *In vitro* NDF digestibility, Cultivars.

INTRODUCTION – Forage plays a pivotal role in ruminant feeding, representing a relevant, and sometimes unique, component of the diet and variation in its quality has direct effects on digestive functionality, concentrate supplementation, animal performance and health. The main factors influencing forage quality are forage species, stage of maturity at harvest and, eventually, harvest and storage conditions, while environmental parameters are generally considered of secondary importance (Van Soest, 1994; Buxton and Fales, 1994). In legumes the quality worsening is mainly attributable to stems, whereas changes in leaf quality are less relevant, and the forage quality is mainly related to its leafiness (Van Soest, 1994). Temperature can modify plant morphology and forages grown at temperature higher than optimal are reported to be smaller and with thinner but more lignified stems (Buxton and Fales, 1994). The main variations in forage digestibility are attributable to changes in fibre digestibility and the NRC (2001) equation for forage energy calculation suggests to evaluate NDF digestibility by *in vitro* procedure or from ADL content. Genetic variation among cultivars for fibre digestibility contents has been reported, as well as a negative relationship between digestibility and forage yield (Julier and Huyghe, 1997). Aim of the present work was to evaluate *in vitro* dry matter and NDF digestibility of alfalfa forage obtained from different cultivars grown in three environments and from different cuts, as well as their relationship with forage fibre fractions.

MATERIAL AND METHODS – The trial was carried out on 90 forage samples taken during the 2004 growth season, within a crop evaluation program, from fields seeded in 2003 and 2004. They were from 5 alfalfa cultivars, representing old (Garisenda and Prosementi) and more recently released (Azzurra and Minerva) traditional cultivars and a multileaf one (Hystory). The forages were from three different location, two in the Po Valley and one on the border hills, where all the five cultivars were contemporary grown. No regular watering was given to the crops, and plants were harvested at their early flowering stage. As a consequence, the intervals between the cuts were quite variable and different among locations and fields planted in the two years. First to fourth cuts were considered in the plane but only two cuts were obtained from the hills station. Forage samples were taken at harvest, dried at 55°C in a ventilated oven and ground to pass through a 1 mm sieve using a Thomas-Wiley mill (Thomas Scientific, Hoboken, NJ). The fibre factions (NDF, ADF and ADL) were measured according to the ASPA guidelines adapted to the ANKOM Fiber Analyzer. Ash free NDF were measured using sodium sulfite. *In vitro* 48h NDF (NDFD) and true dry matter (IVTDMD) digestibility was measured, in triplicate, using the Ankom Daisy^{II}

Incubator. Rumen fluid from two dairy heifers fed at maintenance a 90 % forage diet was taken before morning meal via rumen cannula, filtered through a cheese cloth and added to a buffer solution in a 1:4 ratio. Statistical evaluation was carried out by ANOVA (GLM procedure of SAS, version 8 TS M0) using a model including as main factors: cultivar, location, year of seeding, order of cut and interaction of the former factor with the other one's. Statistical significance of the difference was declared at P<0.05. The CORR procedure of SAS were used to evaluate the correlations among the parameters considered and the REG procedure (Stepwise option) was used to estimate a predictive equation of NDFD from the forage chemical composition.

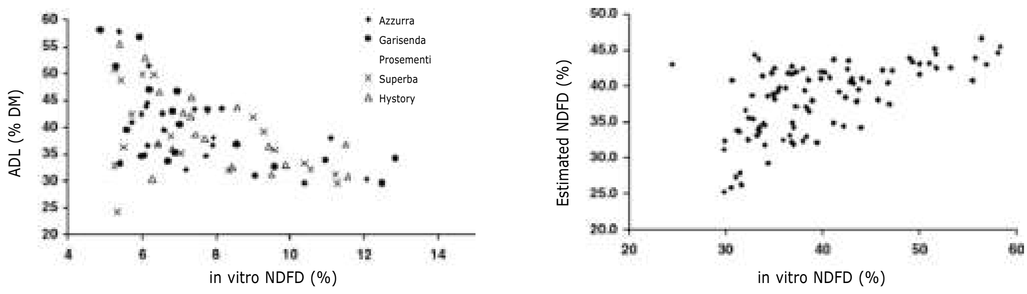
RESULTS AND CONCLUSIONS – The forages covered a wide range of fibre content (Table 1). NDF ranged from 27.55 to 54.41% DM, and its degree of lignification (ADL/NDF) from 0.14 to 0.28. Difference in fibre contents were numerically small and no significant among the cultivars. The year of seeding and the order of forage cut significantly affected the fibre contents and the ADL/NDF: forage from the 2nd year fields (seeded in 2003) had higher and more lignified fibre (NDF=43.18 *vs* 32.30 %DM; ADL=8.44 *vs*. 5.12 %DM; ADL/NDF=0.194 *vs* 0.168). The fibre content declined from the 1st to the 4th cut, and differences among locations were detected for ADL/NDF ratio, that was higher for the plane location 1, with a clay rich soil.

Table 1. Fibre fractions, NDFD and IVTDMD (LS means) of forages from five alfalfa cultivars (DFE=38).

Cultivar	NDF (% DM)	ADF (% DM)	ADL (% DM)	ADL/NDF	NDFD (%)	IVTDMD (%)
Prosementi	38.58	29.13	6.83	0.18	43.99	78.37
Garisenda	37.30	27.63	7.02	0.19	44.05	79.18
Superba	38.25	28.19	6.84	0.17	41.01	77.60
Azzurra	36.11	27.71	6.19	0.18	43.12	79.65
Hystory	38.45	28.17	7.18	0.19	41.71	77.80
SEM	2.7284	2.0448	0.8294	0.0011	2.3949	2.4911

Digestibility of NDF ranged from 24.4 to 58.23 %. No significant differences were detected among cultivars, but Superba and History showed the numerically lowest values. NDFD and IVTDMD increased from the 1st to the 4th cut and they were highly (*P<0.01*) and negatively correlated with all the fibre fractions. For NDFD similar correlations (r) were found with NDF (-0.56), ADF (-0.51) and ADL (-0.58) but weaker with ADL/NDF (-0.38). Within the cultivars, a lower correlation between NDFD and ADL was found for Superba (-0.45, *P=0.06*) *vs* the other ones (r=-0.60, *P<0.01* for Azzurra, Garisenda and Hystory; r=-0.75 for Prosementi, *P<0.01*), suggesting possible differences in the interaction of lignin with the other fibre components. The correlations between NDFD and fibre were higher for the forages seeded in 2003 (with ADL/NDF r=-0.74, *P<0.01*) than for the 1st year crop samples (with ADL/NDF r=-0.22, *NS*). IVTDMD ranged from 59.04 to 87.12 % and it appeared influenced by all the non genetic factors taken into account, but no significant difference were pointed out among cultivars. The best multiple regression calculated among NDFD and fibre factions was: NDFD=73.61-0.62*NDF(%DM)–56.33*ADL/NDF(R^2=0.39).

Figure 1. Relationships between measured in vitro NDF digestibility (NDFD, %) with ADL (% DM; r=0.58) or estimated NDFD (r=0.61).



No significant difference was found in this relationship for the forages seeded in 2003 *vs* those seeded in 2004 or among the cultivars, so that a unique equation can be used. The negative relationship between lignin concentration and digestibility of forages has been known for many years, although measuring lignin concentration is not very easy as all methods are empirical. In addition, lignin value for predicting digestibility is considered more important when forages cover a large maturity range, but the lignin/digestibility relationship appears weaker for forages of a single species and of similar maturity (Van Soest, 1994). Van Soest *et al.* (2005) reported a good relationship between lignin and NDFD when measured after prolonged incubation, but correlation similar to those found in the present research were reported by Allen and Oba (1998) for alfalfa and even lower by Bani *et al.* (2003) for mixed hays. Bani *et al.* (2003) and Spanghero *et al.* (2003) found almost identical correlation (r=-0.73) between NDFD and NDF for mixed hays from different areas of Alps.
When NDFD is evaluated after a 48h in vitro incubation, large difference exist among alfalfa forage from different cuts and from field of different age. Differences among alfalfa cultivars, that could be taken into account in alfalfa breeding programmes are worth to be better studied. NDFD appeared well correlated with all the Van Soest fibre fractions and a unique equation can be used to predict NDFD for alfalfa forages of different origin. NDFD variations is well but not completely explained by the forage fibre content, suggesting the interference of other factors, that are worth to be better studied, but also the importance to directly measure this nutritional parameter.

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