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Bioactivators and nitrogen fertilization applied to durum wheat: Effects on the chemical composition and *in vitro* digestibility of straw

D. De Giorgio*, A. Lestingi, F. Bovera*** and G. Convertini***
*CRA - Research Unit for Agriculture in Dry Environments,
Via Celso Ulpiani, no. 5, 70125 Bari, Italy
**Dipartimento di Sanità e Benessere Animale, Sezione di Scienze Zootecniche, University of Bari,
Str. Prov. Casamassima km 3, Valenzano, Bari, Italy
***Department of Scienze Zootecniche e Ispezione degli Alimenti, University of Napoli Federico II,
Via F. Delpino 1, Napoli, Italy

SUMMARY – The objective of this research was to study the effects of three nitrogen fertilisation levels and bio-activators on the chemical composition and dry and organic matter digestibilities of durum wheat straw. The following applications of bio-activators were compared; mycorrhizal, *Azospirillum* and mycorrhizal plus *Azospirillum* inoculation and in addition a control with no application. These treatments were in factorial combination with three levels of N fertilization (0, 50, 100 kg/ha of N). The following parameters were determined: dry matter, crude protein, ether extract, ash, neutral detergent fibre, acid detergent fibre and acid detergent lignin. Hemicellulose and cellulose were estimated. The highest dose of nitrogen fertilizer increased neutral and acid detergent fibre and cellulose straw percentages. The two bio-activators had different effects on the quality of the straw: the mycorrhizal bio-activator increased neutral and acid detergent fibre and the cellulose contents of the straw, the *Azospirillum* also showed an increase in the same parameters. Neither treatment affected dry and organic matter digestibilities. The results of this research show that high levels of nitrogen can have a negative effect on the quality of straw and it is therefore recommended that a quantity of 50 kg/ha of N be used. Mycorrhizal, even in small doses, reduced fibre and increased cellulose content.

Key words: Wheat straw, chemical composition, bio-activators, nitrogen fertilisation.

RESUME – *"Bioactivateurs et fertilisation azotée appliquée au blé dur : effets sur la composition chimique et la digestibilité* in vitro *de la paille". L'objectif de cette recherche était d'étudier les effets de trois niveaux de fertilisation azotée et de bioactivateurs sur la composition chimique et sur la digestibilité de la matière organique de la paille de blé dur. Les applications suivantes des bioactivateurs ont été comparées : inoculation de mycorhize, d'Azospirillum, de combinaison de mycorhize avec Azospirillum et un témoin sans application. Ces traitements sont mis en combinaison factorielle avec trois niveaux de fertilisation azotée (0, 50, 100 kg ha^{-1} de N). Les paramètres suivants ont été déterminés : matière sèche, protéine brute, extrait d'éther, cendre, fibre au détergent neutre, fibre au détergent acide et lignine au détergent acide. Par contre l'hémicellulose et la cellulose ont été estimées. La dose la plus élevée d'azote a augmenté les pourcentages de fibre au détergent neutre, de fibre au détergent acide et de cellulose de la paille. Les deux bioactivateurs ont influencé de manière diverse la qualité de la paille, la mycorhize a diminué la teneur en fibre au détergent neutre, de fibre au détergent acide et de cellulose de la paille, par contre l'Azospirillum a déterminé un accroissement de ces mêmes paramètres. Ces traitements n'ont pas affecté la digestibilité de la matière sèche et organique. Les résultats de cette recherche ont montré que de hauts apports azotés influencent d'une manière négative la qualité de la paille, par conséquent il est conseillé l'utilisation de 50 kg ha^{-1} de N. La mycorhize, même à faible dose, a réduit la teneur en fibre et a augmenté celle de la cellulose.*

Mots-clés : *Paille de blé, composition chimique, bioactivateurs, fertilisation azotée.*

Introduction

The need to eliminate cultivation waste from the land involves costs that weigh heavily on a farm's budget. Some of these products, such as wheat straw, can be utilized in livestock feeding and may even be of considerable use in meeting feeding requirements during periods of low fodder (Doyle *et al.*, 1986, Xing *et al*. 1993). There is also the growing need to integrate animal feed with products that have a high content of dry matter, are easily transportable, storable and low in cost. Due to its qualitative characteristics wheat straw could once again play an integral part in the dietary rations of animals (Tan *et al*., 1995). In order to formulate a correct diet for animals it is necessary to have a good knowledge of their composition and how this may vary according to agronomic techniques and the zone of cultivation (Russel *et al*., 1992; Ferri *et al*., 2004). The need to find wholesome products that use low-impact environmental production methods has led to a review of the type of farming techniques that include the use of synthetic products. Although on one hand nitrogen fertilization has led to increased productivity, on the other its excessive use has been shown to have a negative effect on vegetal quality and cause environmental pollution issues.

The Agricultural Policy of the European Community, together with increased concern about the effects of intensive production systems in the nitrate content of water resources and on the emission of nitrogenous gases into the atmosphere, is likely to lead to a reduction in the use of fertilizer N in crops. The use of bio-activators needs to be evaluated to see if they could be an alternative to reach these goals, above all in areas low in rainfall (Plassard *et al*., 2000; Yegorenkova *et al*., 2001; Lestingi *et al*., 2007). The mycorrhiza and *Azospirillum* bacteria are known to benefit the uptake of plant nutrients. In the majority of cases enhanced phosphorus uptake and improved phosphorus nutrition are the primary causes of growth and yield increases in mycorrhizal plants. This improvement in growth may itself lead to a more rapid uptake of other mineral nutrients. Several studies have shown the significance of inoculation for improved N uptake from soil sources and the role of *Azospirillum* as an associative nitrogen-fixing soil bacterium. The long-term benefit of using these bio-activators in the field could be an alternative strategy for plant nutrition, which would reduce agrochemical applications. These effects were found on seeds, but not in the durum wheat straw.

Materials and methods

This research was carried out in an area of the sub-apennine Dauno hills near Foggia (southern Italy), an area with a typical Mediterranean environment ("accentuated thermo Mediterranean" classified by UNESCO-FAO). The field trial was conducted over 2 years (2002-2003) on *Triticum durum* (Desf.), Simeto cultivar. Annual rainfall in this period was of 670 and 570 mm respectively, slightly higher than the average of the preceding 50 years (549 mm). During the second year rainfall in the period February-March was extremely low and often broken up into small precipitations. The average monthly temperatures were within the norm in the first year and slightly higher in the second, in particular between May and August. The soil has a clay-loam textural class and is classified by Soil Taxonomy-USDA as a fine, mesic, Typic Chromoxererts. A completely randomized block design with three replications was performed. Durum wheat seeds were thoroughly mixed with soil samples containing the nitrogen-fixing soil with the bio-activator bacteria *Azospirillum brasilense* and/or spores and mycelia of endo-mycorrhizal fungi of the genus *Glomus*. Four treatments were compared: no bio-activators (test); treatment of bio-activator with Mycorrhiza (M); treatment of bio-activator with *Azospirillum* (A); treatment of bio-activators with *Azospirillum* plus Mycorrhiza (A+M). These treatments were in factorial combination with three levels of N fertilizer: test without N (N0), N 50 (N50) and N 100 (N100) kg ha^{-1}. The sowing was carried out in November. Samples of 1 kg of straw were dried to a constant weight at 65℃ in a forced-air oven to determine dry matter (DM) and chemical parameters. Ash was determined overnight by combustion at 550℃; crude protein (CP) using the Kjeldahl method (N x 6.25); ether extract (EE) was determined with a Soxhlet apparatus; crude fibre (CF) was analyzed with a Fibertec apparatus (Tecator). Cell walls were analyzed using a Tecator apparatus: neutral detergent fibre (NDF) was assayed with a heat-stable amylase and expressed exclusive of residual ash; acid detergent fibre (ADF) expressed exclusive of residual ash and acid detergent lignin (ADL) was determined by solubilization of cellulose with 72% H2SO4 and expressed exclusive of residual ash. The *in vitro* digestibility of dry matter (dDM) and organic matter (OM) was studied using the DAISY^{II} Ankom incubator. Three replications of each substrate were weighed (about 0.5 g) into ANKOM F57 filter bags (ANKOM Technology, Macedon, New York, USA).

The statistical analysis was conducted using SAS procedures (SAS, 1990). The effect of the treatments was evaluated through the general linear model, considering the cutting time as a random effect and mineral N fertilization levels and bio-activators as fixed effects. The differences amongst least square means of the treatments were evaluated using the Student-Neumann-Keuls (SNK) test at P=0.05.

Results and discussion

As the two trial years did not show any considerable variances between themselves, examination of the results has been made on an average of this two-year period. Nitrogen fertilization led to increased straw yield in proportion to Nitrogen levels with significant differences between the three levels (Fig. 1). The bio-activators, singularly or in combination didn't have strong results on straw yield.

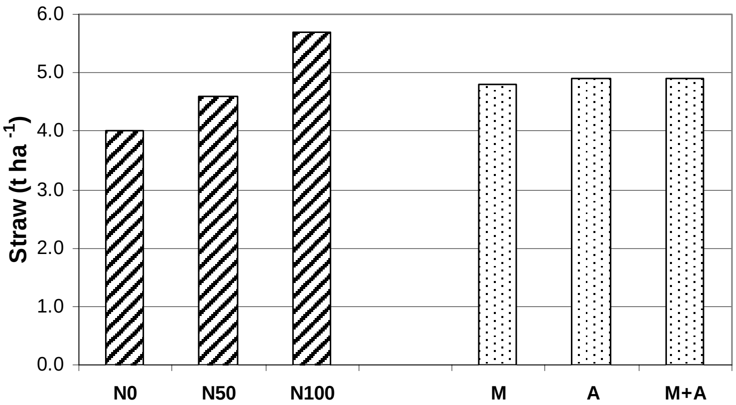

Fig. 1. Effects of nitrogen fertilization rate and bio-activators on straw yield.

Few qualitative parameters were shown to be influenced by the treatments. Considerable differences were noted for the effect of nitrogen fertilization on crude protein and neutral and acid detergent fibre while bio-activator effected on neutral and acid detergent fibre and cellulose.

Among the three levels of nitrogen fertilization only the highest level of 100 kg ha^{-1} showed significant effects on CP, NDF and ADF (Table 1). Several studies carried out in the same trial area have demonstrated that levels of 100 kg ha^{-1} of N, particularly in years of low rainfall, do not lead to an increased yield in grain when compared to doses of 50 kg ha^{-1} of N (De Giorgio and Fornaro, 2004).

Table 1. Effects of nitrogen fertilization and bio-activators on chemical composition (%DM) of straw of durum wheat

Parameter	Level of N (kg ha^{-1}) fertilization			Bio-activator			
	0	50	100	Test	Mycorrhiza (M)	*Azospirillum*(A)	M+A
DM	91.55*	91.83	91.5	91.51	91.87	91.58	91.55
OM	89.71	90.28	89.98	90.32	89.23	90.17	90.22
CP	2.50 b	2.46 b	3.03a	2.66	2.71	2.58	2.7
EE	0.79	0.79	0.79	0.80 a	0.81 a	0.74 b	0.73 b
NDF	73.11 b	73.49 b	74.93 a	73.88 a	72.25 b	74.38 a	74.85 a
ADF	64.62 b	65.39 ab	66.54 a	65.40ab	63.71 b	66.16 a	66.79 a
ADL	8.25 b	8.43 b	9..6 a	8.69	8.39	8.74	8.5
Hemi	8.48	8.1	8.39	8.49	8.53	8.22	8.06
Cell	56.37	56.96	57.48	56.71 ab	55.32 b	57.42 a	58.30 a
dSS	34.24	34.42	35.58	34.7	34.54	35.69	34.05
dOM	35.5	35.71	36.62	35.75	36.05	36.07	35.3

*The value in each row with different letters are significantly different at $P<0.05$.

A dosage of 100 kg ha^{-1} of N added to wheat leads to increased fibre content and straw lignin; two parameters that make it less digestible and therefore of lower quality.

The 2 bio-activators only affected a limited number of parameters and ways that opposed each other. The mycorrizal resulted in a reduction of the NDF, ADF and cellulose values, both in comparison with the *Azospirillum* and with the test. The most interesting variation is with increased cellulose which improves digestibility. In the combination of the two bio-activators the *Azospirillum* appears to have played a dominant role over the mycorrizal leading to results that show a similar effect to the application of just *Azospirillum*: a decrease in EE and an increase in NDF and ADF when compared with the test and the mycorrizal and an increase in cellulose content when compared only with the mycorrizal. Treatments did not affect dry matter and organic matter digestibility. However, recorded values for DM digestibility agree with the results that Tejada *et al.* (1979) found using a Tilley and Terry (1969) method.

Conclusion

Nitrogen fertilization showed increases in yield proportional to the doses applied to durum wheat. With regard to qualitative parameters, the effects were not great and were limited to increases in fibre content and lignin making the straw less digestible. Fibre content was shown to reduce with the administration of mycorrizal, making the straw more digestible while it increased with the treatments of *Azospirillum* and mycorrizal and *Azospirillum* combined making it less digestible. Cellulose content increased with the application of *Azospirillum*, both on its own and in combination with the mycorrizal. All in all we can see that the experimental treatments had very little effect on qualitative characteristics. The limited effect of the bio-activators can be attributed to the fairly good rainfall in the two-year trial period as their effect is more marked during years of drought.

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