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Effects of compost organic amendments on chemical composition and *in vitro* digestibility of alfalfa (*Medicago sativa* L.)

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ABSTRACT

The following fertiliser treatments were compared during the years 2002 and 2003 on alfalfa forage (*Medicago sativa* L.): compost obtained from the organic fraction of the Municipal Solid Waste (MSW); olive pomace compost (OPC); mineral fertiliser (Min). All the treatments allowed a distribution of 75kg ha^{-1} of P_2O_5. Three cuttings occurred: at 168, 206 and 351 days after compost application (DAA) in 2002; 119, 152 and 320 DAA in 2003. Cumulative biomass and dry matter yields were measured during each experimental year. Furthermore, chemical composition and *in vitro* digestibility of dry matter (DMd), organic matter (OMd), crude protein (CPd) and NDF (NDFd) were determined. MSW treatment showed a significantly ($P<0.01$) higher content of ADL than OPC and Min (77.0, 66.0 and 65.0g kg^{-1} DM, respectively). Fertiliser treatments also affected ($P<0.01$) digestibility parameters. In fact, DMd and OMd values showed the same trend with lower percentages in MSW treatment than in the OPC and Min ones. The NDFd differed in all treatments having the highest value in OPC (40.1%). The results indicated that the soil distribution of organic materials offer the possibility to reduce the application of mineral fertilisers and production costs without decreasing alfalfa yield, forage chemical composition and *in vitro* digestibility.

Key words: Alfalfa forage, Mineral and organic P fertiliser, Chemical parameters, *In vitro* digestibility.

RIASSUNTO

EFFETTI DI DIVERSI FERTILIZZANTI ORGANICI SULLA COMPOSIZIONE CHIMICA E SULLA DIGERIBILITÀ *IN VITRO* DELL'ERBA MEDICA (*MEDICAGO SATIVA L.*)

*In una ricerca biennale (2002 e 2003) sull'erba medica (*Medicago sativa L.*) sono stati confrontati i seguenti trattamenti sperimentali: compost ottenuto dalla frazione organica dei Rifiuti Solidi Urbani (RSU); compost da sanse di olive (SOC); fertilizzante minerale (Min). Tutti i trattamenti sono stati effettuati in* *modo da ottenere una distribuzione di P_2O_5 pari a 75kg ha^{-1}. Per ogni annata agraria sono stati effettuati tre tagli: a 168, 206 e 351 giorni dopo l'applicazione del compost (DAC) nel 2002; 119, 152 e 320 DAC nel 2003. In entrambi gli anni, oltre alle produzioni cumulative di biomassa e di sostanza secca, sono state determinate la composizione chimica e la digeribilità in vitro della sostanza secca (SSd), sostanza organica (SOd), proteine grezze (PGd) e NDF (NDFd). Il trattamento con compost da RSU ha mostrato nell'erba medica contenuti di ADL significativamente (P<0,01) più elevati rispetto ai trattamenti con SOC e Min (77,0, 66,0 e 65,0g kg^{-1} SS, rispettivamente). I trattamenti fertilizzanti hanno anche influenzato significativamente (P<0,01) i parametri di digeribilità. Infatti, i valori della SSd e della SOd hanno mostrato lo stesso andamento, con le percentuali più basse nel caso del trattamento con RSU, rispetto a quelli con SOC e Min. La NDFd è risultata differente tra i vari trattamenti, mostrando la percentuale più elevata con il SOC (40,1%). I risultati indicano che la distribuzione di sostanza organica sul suolo rappresenta una possibile via per ridurre l'impiego di fertilizzanti inorganici e i costi di produzione, senza variazioni della produzione, della composizione chimica e della digeribilità in vitro del foraggio.*

Parole chiave: *Erba medica, Fertilizzazione fosfatica organica e minerale, Composizione chimica, Digeribilità* in vitro.

Introduction

Alfalfa (*Medicago sativa* L.) is one of the main fodder crops in many countries as its yield, protein content and palatability are greater than other forage legumes. The soil application of mineral or organic fertilisers, and in particular phosphorus (P), has been shown to significantly increase alfalfa yield (Malhi *et al.*, 2001). The use of organic fertiliser to provide P for plant growth has long been of interest, especially in Mediterranean conditions in which the mineralization process is great because of high temperatures, which means the soil needs more organic matter (Montemurro *et al.*, 2004). Moreover, growing concerns relating to land degradation, the inappropriate use of inorganic fertilisers, atmospheric pollution, soil fertility and biodiversity have rekindled global interest in organic recycling practices. In this matter, composting Municipal Solid Waste (MSW), which represents a possible alternative to incineration for recycling organic material, has recently been studied as a soil amendment or as an organic fertiliser in different crops (Montemurro *et al.*, 2005). Also the utilisation of agro-industrial residues increased and, among these, the olive pomace (OP, residue of pressed olives) is very interesting because it arranges the recycling of organic substances with both soil and plant benefits (Montemurro *et al.*, 2004). Few studies have addressed the impact of herbage fertilisation on ruminant nutrition. Fodder quality is ultimately only defined by animal productivity, that is measuring milk yield or live weight changes. However, these direct animal performance trials may be substituted by more short-term, indirect animal performance measurements, such as digestibility. The aim of our research was to evaluate the effects of two organic amendments (MSW compost and OP compost), in comparison with mineral fertiliser, on yield, chemical composition and *in vitro* digestibility of alfalfa forage.

Material and methods

Site and experimental setup

The field research was conducted at the "Agostinielli" Experimental Farm in Rutigliano - Italy (41°01' lat. N, 4°39' long. E., 112m a.s.l.) during the years 2002 and 2003. In the trial area the climate is "accentuated thermomediterranean" (UNESCO-FAO, 1963), with winter temperatures that can fall below 0°C, summer temperatures which can rise above 40°C and rains unevenly distributed during the year, being concentrated mainly in the winter months. In a poly annual rain-fed meadow of alfalfa (*Medicago sativa* L., cv. Garisenda), the following three P fertiliser treatments were compared in a completely randomized experimental design with three replications: compost obtained from the organic fraction of the Municipal Solid Waste (MSW), allowing a distribution of 75kg ha^{-1} of P_2O_5; olive pomace compost (OPC), allowing a distribution of 75kg ha^{-1} of P_2O_5; mineral fertiliser (Min), equal to 75kg ha^{-1} of P_2O_5, as mineral superphosphate. The 75kg ha^{-1} of P_2O_5 is the normal rate of mineral fertiliser used by farmers in the experimental area. Each experimental plot consisted of 7m^2 (3.5 x 2m). The alfalfa crop was sown in a single row with a row-spacing of 20cm and a seeding rate of 30kg ha^{-1}. The composition of organic fertilisers (Table 1), the composting processes and the main chemical characteristics of MSW compost and OPC are described in Montemurro *et al*. (2006). The organic materials (MSW compost and OPC) and the mineral fertiliser were broadcast approximately one month before sowing in the first year (2002) and in January in the second year (2003). Three cuttings occurred: at 168, 206 and 351 days after compost application (DAA) in 2002; 119, 152 and 320 DAA in 2003. In both years, shoots of the first two cuttings were harvested when 50% of the plants reached the flowering stage (height of about 20-30cm), while the last cutting occurred before the winter period without considering the crop stage. Shoots were harvested from the entire experimental plot at 5cm from the soil level to minimize soil contamination and then fresh weight and dry weight (oven-dried at 70°C till a constant weight) were determined.

Table 1. Chemical characteristics of Municipal Solid Waste and Olive Pomace composts used in the field experiments.

		Municipal Solid Waste compost	Olive Pomace compost
pH		6.32	5.97
Total N	g kg^{-1}	1.47	1.40
Available P	mg kg^{-1}	33.6	210.1
Exchangeable K	"	95.8	506.0
Total carbon	g kg^{-1}	13.7	50.5
C/N		9.55	36.07
Zn	mg kg^{-1}	751.0	40.0
Cu	"	330.0	17.2
Ni	"	217.0	0.05
Pb	"	670.0	16.2
Cr	"	112.7	<0.01
Mn	"	163.8	11.23
Co	"	20.8	10.9
Cd	"	1.3	0.01

Analytical procedures

Dried samples were ground to pass a 1 mm screen (Brabender Wiley mill, Brabender OHG Duisburg, Germany) for chemical analyses and *in vitro* digestibility determinations. Dry matter (DM), ash, crude protein (CP), ether extract (EE), were determined according to AOAC (2000) (method numbers: 945.18, 973.18, 2002.04) procedures. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the ANKOM fiberteck method. Sodium sulfite was not included in NDF determination. Hemicellulose (HC) and cellulose were estimated by the difference of NDF minus ADF, and ADF minus ADL, respectively. Non-Structural Carbohydrates (NSC) were estimated as NSC=1000-(CP+Ash+EE+NDF) (NRC, 2001), where nutrient contents are expressed in g kg^{-1}DM. *In vitro* digestibility of DM (DMd), organic matter (OMd), CP (CPd) and NDF (NDFd) were determined using the DAISY^{II} Ankom incubator**.** Nine replications of each substrate (3 for DMd and OMd, 3 for CPd and 3 for NDFd) were weighed (about 0.5g) into ANKOM F57 filter bags (ANKOM Technology, Macedon, New York, USA). For each determination (DM/OM, CP and NDF) ten bags were incubated without substrate and used as "blank" to correct the results. The high number of blanks was made to make full the ANKOM flasks. All bags were heat sealed using a 200mm Parker IS/7300H impulse sealer (Packaging Aids Limited, 103Wantz Road, Dagenham, Essex, UK) 0.5mm from the edge of the bags and, therefore, the bags were placed into the flasks (24 bags per flask). The total of 192 bags (18 samples x 9 replications, plus 30 blanks) were distributed in two consecutively trials. The bags for DMd and OMd determinations were divided between the two trials. For each trial, the flasks have been filled up with 1600 ml of anaerobic medium D and placed into the DAISY^{II} incubator at 39°C. Faeces were collected directly from rectum of four lactating cows consuming a diet consisting, on dry matter basis, in: corn silage (30%), oat hay (11%), wheat straw (11%) and a commercial concentrate (48%). Faeces were collected after the second daily milking (at 16.00h), placed in a pre-warmed thermos and transported as soon as possible to the laboratory (about 1h). In the laboratory, 500ml of pooled faeces were diluted with 1000ml of anaerobic medium, stirred for 5 minutes and strained through six layers of gauze under CO_2. The retained solids were then mixed with 1000ml of medium and homogenised in a blender for 20s under CO_2. The homogenate was then re-strained through six layers of gauze; the resulting liquid was combined with the other strained fluid and held at 39°C under CO_2 until use (final dilution 4:1 medium:faeces). The time taken to prepare faecal inoculum was around 30min. The 400ml of inoculum were introduced in each flask and the flasks were placed into DAISY^{II} ANKOM system. After 48h of incubation, filter bags were removed from the flasks and washed with distilled water. For each substrate, three bags were dried to a constant weight at 103°C, and OMd was obtained by difference following ashing (5h at 550°C); three bags were used to determine residue N and, finally, three bags were used to determine residue NDF. For each parameter (P) *in vitro* digestibility was calculated as: $dP\%=(P_{BD}-P_{AD})/P_{BD}x100$, where P_{BD} is the percentage of parameter in the samples before the digestion and P_{AD} the percentage of the parameter after the digestion.

Statistical analysis

The data were analysed by ANOVA using the General Linear Model (GLM) procedure of SAS (SAS, 2000) considering the fertiliser treatment as a fixed effect, the year as a random effect and the cutting time as a repeated measure. The interaction between year and fertiliser treatment was not significant, therefore, it was removed from the model. The logarithm transformation of data was used to evaluate statistical differences among variables expressed as percentage numbers, so having a normal distribution of data. Pearson correlation coefficients were used to correlate digestibility parameters.

Results

Higher cumulative biomass and dry matter yields were found in the first (11.09 and 2.51 t ha^{-1} for biomass and dry matter yields, respectively) and second (9.82 and 2.51t ha^{-1} for biomass and dry matter yields respectively) cuttings compared with the third one (6.47 and 1.50t ha^{-1} for biomass and dry matter yields, respectively), probably due to the different alfalfa cropping stages at harvesting, while no significant difference was found among the fertiliser treatments.

Table 2 shows the chemical composition of alfalfa forage according to the effects of the year, cutting time and fertiliser treatment. All chemical parameters varied significantly between 2002 and 2003, with the exception of NDF and ADL. The DM, CP, ADF and cellulose contents were significantly higher in 2002 than in 2003. The OM, EE, HC and NSC content showed the opposite trend. Cutting time significantly affected all chemical parameters. The second cutting showed the highest DM content, while the other two cuttings had similar values. Otherwise, in the third cutting, alfalfa had the lowest OM content, increasing the values in the second and first cuttings. The CP and EE levels had the same trend, showing lower values in the second cutting than in the remaining two ones. There was also the same effect of cutting time on NDF, ADF and cellulose contents, showing the first two cuttings

Table 2. Effects of year, cutting time and fertiliser treatment on chemical composition (g kg^{-1} DM) of fresh alfalfa forage.

	DM	OM	CP	EE	NDF	ADF	ADL	HC	Cellulose	NSC
Years:										
2002	279.5^{A}	858.4^{B}	219.7^{A}	19.1^{B}	379.5	330.9^{A}	72.3	48.6b	257.3^{a}	240.0^{B}
2003	226.8^{B}	871.9^{A}	193.1^{B}	22.4^{A}	369.4	310.0^{B}	73.8	59.4^{a}	237.9^{b}	286.9^{A}
Cuttings:										
First	230.5^{B}	869.8^{a}	209.4^{a}	21.8^{A}	386.9^{A}	324.5^{A}	70.7^{B}	62.3^{a}	258.1^{A}	251.7^{b}
Second	293.6^{A}	865.7^{ab}	199.2^{b}	17.1^{B}	386.7^{A}	334.4^{A}	77.0^{A}	52.3^{b}	267.2^{A}	262.7^{ab}
Third	235.5^{B}	860.0^{b}	210.8^{a}	23.5^{A}	349.8^{B}	302.5^{B}	71.1^{B}	47.2^{b}	217.5^{B}	275.9^{a}
Treatments:										
MSW	244.3	868.1	212.5	20.9	368.8	316.0	77.0^{A}	52.9	241.7^{b}	265.8
OPC	250.0	864.6	201.7	21.2	374.9	321.3	66.0^{B}	53.6	252.0^{ab}	266.8
Min	263.6	864.7	204.1	20.4	381.4	328.5	65.0^{B}	52.9	258.8^{a}	258.8
MSE	146.3	11.3	24.0	1.00	48.8	48.5	7.10	17.3	59.9	69.8

Within year, cutting time and fertiliser treatment the values in each column followed by a different letter are significantly different.

^{a,b}: P<0.05; ^{A,B}: P<0.01.

MSE=Mean Square Error.

higher values than those of the third cutting. The ADL content, however, was higher in the second cutting than in the other two ones, while, inversely, HC content was higher in the first cutting. Finally, NSC content increased with the following harvests. Fertiliser treatment affected ADL and cellulose contents. Among treatments, the MSW showed a significantly ($P<0.01$) higher content of ADL than OPC and Min. Conversely, cellulose content was significantly lower in MSW compared with the Min. However, the level of cellulose in OPC did not statistically differ from that of the other treatments.

The effects of year, cutting time and fertiliser treatment on CPd, DMd, OMd and NDFd are presented in Table 3. Significant differences ($P<0.01$) were detected between 2002 and 2003 for CP and NDF digestibility values. CPd was higher in 2002 than in 2003, whereas for NDFd there was the opposite. There was also a cutting time effect on DMd and OMd. The DMd was significantly ($P<0.01$) lower in the second cutting than in the remaining ones. On the other hand, OMd significantly ($P<0.01$) differed in the three cuttings, showing the highest value in the third cutting, an intermediate value in the first one and the lowest value in the second one. Fertiliser treatment also affected ($P<0.01$) DMd, OMd and NDFd. The DMd and OMd values showed the same trend, with lower percentages in MSW treatment compared with the OPC and Min ones. The NDFd differed in all treatments having the highest value in OPC.

The Pearson correlation coefficients amongst ADL (%DM), DMd, OMd, CPd and NDFd are reported in Table 4. As expected, ADL was negatively correlated with DMd ($P<0.01$), OMd ($P<0.01$), CPd ($P<0.05$) and NDFd ($P<0.01$). The DMd was positively cor-

Table 3. Effects of year, cutting time and fertiliser treatment on crude protein (CPd), dry matter (DMd), organic matter (OMd) and neutral detergent fibre (NDFd) digestibilities (%) of fresh alfalfa forage.

	DMd	OMd	CPd	NDFd
Years:				
2002	54.38	58.37	56.90^{A}	34.65^{B}
2003	55.10	58.20	49.90^{B}	36.43^{A}
Cuttings:				
First	54.90^{A}	58.11^{B}	54.41	36.68
Second	53.39^{B}	56.72^{C}	53.55	34.74
Third	55.95^{A}	60.04^{A}	52.22	35.22
Treatments:				
MSW	53.76^{B}	56.94^{B}	54.13	33.62^{C}
OPC	56.11^{A}	59.85^{A}	54.88	40.10^{A}
Min	56.10^{A}	59.99^{A}	52.41	36.95^{B}
MSE	3.99	4.48	24.94	6.03

Within year, cutting time and fertiliser treatment the values in each column followed by a different letter are significantly different.
^{A,B}: $P<0.01$.
MSE=Mean Square Error.

related (P<0.01) with OMd, CPd and NDFd. Finally, the OMd was positively correlated (P<0.01) with CPd and NDFd.

Discussion

Malhi *et al*. (2001) found that the application of P fertiliser in alfalfa crops increased yield and quality, but the response varied with the level of extractable P in the soil. The results of our research indicated no different effects of mineral or organic P applications, thus showing the possible utilisation of organic materials. Furthermore, the low difference of yield among treatments could also be explained considering that N was not limiting factors in alfalfa because it is a N fixing crop.

The second cutting showed the significantly highest percentage of DM (230.5, 293.6 and 235.5g kg^{-1} DM for first, second and third cutting, respectively) as a consequence of the higher mean temperature recorded during this period, compared with both initial and final periods of the cropping cycles. Among treatments MSW showed a significantly (P<0.01) higher content of ADL than OPC and Min. Considering that the three treatments supplied the same quantity of phosphorous, the results might probably be related to the presence of heavy metals in MSW (Table 1). In fact, heavy metals can be toxic, beyond entering the food chain as contaminated water with a subsequent effect on human health, also for the plants (Carlson *et al*., 1975). In particular, heavy metals can be absorbed by plants and form complexes with organic macromolecules. Several authors indicated that plant fibres have a remarkable ability to bind heavy metals, in particular hemicellulose and pectin, less cellulose and lignin and the binding ability varies with the source of origin of relevant fractions (Davidson and McDonald, 1998; Sangnark and Noomhorm, 2003). Unfortunately, it was not possible to analyze the heavy metal level in the roughages, but our hypothesis is that the higher level of heavy metals with MSW induce the formation of fibre complexes, increasing the indigestible proportion of fibre. The DMd and OMd (Table 3) are slightly lower than the data reported in the published literature. Moreover, data reported by other authors vary in a wide range due both to the cutting stage of the fodder crop and

Table 4. Pearson correlation coefficients amongst ADL (%DM), dry matter digestibility (DMd), organic matter digestibility (OMd), crude protein digestibility (CPd) and NDF digestibility (NDFd).

	ADL	DMd	OMd	CPd
DMd	-0.7124 **	-	-	-
OMd	-0.7006 **	0.9633 **	-	-
CPd	-0.4440 *	0.5909 **	0.7346 **	-
NDFd	-0.5829 **	0.7722 **	0.6481 **	0.1587

**, **: Significant at the P<0.05 and 0.01 levels, respectively.*

the analytical method. In particular, Aufrère *et al*. (2000) found an *in situ* digestibility of DM of fresh forage from alfalfa of 61.7%, using rumen fluid of sheep; Adesogan (2005) found an *in vitro* DM digestibility of alfalfa hay, determined with Daisy ANKOM system and using bovine rumen fluid, of 59.8%. With the same method, Holden (1999) found an average DM digestibility of alfalfa hay of 58.9%. Our different results are due to the use of faeces instead of rumen liquor as inoculum for *in vitro* studies.

Both DMd and OMd were significantly (P<0.01) higher in the third cutting, probably due to decreases in NDF and ADF. Regarding CPd, the average values reported in Table 3 are also lower than data reported in literature. In particular, Hristov (1998) using the *in sacco* method on three steers, found an effective digestibility of CP of fresh alfalfa of 66.5% after 72h of incubation. In the same way, Repetto *et al*. (2000) using an *in sacco* method on wethers within 72h of incubation found a percentage of protein degradability of 82%. Our values have to take into account the low incubation time (48h) and the use of faecal inoculum. Also NDFd (average 35.5%) was very low compared to the values reported in literature (from 54.5 to 62.9%; Oba and Allen, 1999). The results are due to both the shorter incubation time and the use of faeces as source of inoculum.

Incidences of lower *in vitro* digestibilities obtained using faecal liquor, when compared to rumen inocula is expected on the basis of previous studies. In fact, endpoint measures, such as digestibility after 24h or more, from faecal inocula are lower with respect to estimates using rumen fluid inocula (Zhao and Chen, 2004). If the diet of the donor animal is highly fibrous, such that the microbial activity of the rumen is low, then differences between rumen fluid and faeces are much smaller (Mauricio *et al*., 1999), but when characterising feeds that will be fed to highly productive animals (as is the case of the present trial), such inocula are of limited value. Microbial activity of faecal inocula is generally much lower than that of rumen fluid inocula (Rymer *et al*., 2005), and apart from the drastic changes to the diet as in the work of Mauricio *et al*. (1999), it is not clear how activity of faecal inocula might be increased (Rymer *et al*., 2005).

As expected, due to different ADL contents (Table 2) and the significant (P<0.01) negative correlation between ADL content and NDFd (Table 4), fertiliser treatment significantly (P<0.01) affected NDFd and, in particular, OPC showed the highest value. Among the determined digestibilities, CPd was the lowest. In fact, the variability of the results was high (MSE=25.0, Table 3) and the average values were little correlated with ADL content of the samples and not correlated with NDFd. The DMd and OMd were significantly (P<0.01) and positively correlated both with CPd and NDFd.

Conclusions

The results of this study pointed out no significant difference for cumulative biomass and dry matter yields among fertiliser treatments in both years, indicating that it is possible to substitute mineral P fertiliser with organic ones, without a decrease in the alfalfa production. Since no significant decrease was found on digestibility of fresh alfalfa forage in OPC compost, which is characterised by a low heavy metal content, it could be important to give more attention to soil contamination and quality than to the risk of plant or animal toxicity, which could only be present after repeated compost application for a large number of years.

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