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# Pasture plants of central Italy and possible traceable compounds in sheep cheese

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**SUMMARY** – Italian food is known for its taste and quality, but often too expensive to compete on the market. However, the greater quality and better taste of Italian food products, compared to food products from conventional agriculture, attract considerable interest from customers, and as a consequence certified organic, biodynamic and typical food products show interesting added value on the market. One of the problems with these food products is the traceability of the product from the point of origin to the customers. A research has been conducted about pasture plants that might be traced down to the final sheep caciotta cheese "Pecorino Toscano". Three farms have been chosen in central Italy, each with different levels of pasture complexity, one farm on a flat area (simple botanical composition), one on a hill (medium specific diversity), and one in the mountains (highly diverse pastures). The botanic composition of all the pastures of the three farms was analysed using a linear analysis, and a list of common species was extrapolated, the most preferred species were *Bromus sterilis*, *Lolium perenne* and *L. multiflorum*. Finally, a list of species is given with some data about possible traceable compounds. Analyses of macro- and micro-elements by optical emission spectrometer (ICP-OES) and elemental analyzer (CHN), and of flavonoids and terpenes (HPLC) are now in progress.

**Keywords:** Linear analysis, weighted specific contribution, contribution to livestock diet, traceable compounds.

**RESUME** – "Plantes des pâturages d'Italie Centrale et possibles substances traçables dans le fromage de brebis". Les spécialités alimentaires italiennes sont connues pour leur qualité et leur goût, mais elles sont souvent trop coûteuses pour être compétitives dans les marchés. Toutefois, leur qualité supérieure et leur meilleur goût en comparaison des produits de l'agriculture conventionnelle maintiennent un intérêt considérable du côté des consommateurs ; en conséquence, les produits biologiques certifiés, biodynamiques et traditionnels gardent une intéressante valeur ajoutée dans les marchés. L'un des problèmes majeurs qui concernent ces produits, est la traçabilité de l'origine jusqu'à la vente au détail. Une recherche a été conduite au sujet des plantes de pâturage qui peuvent être tracées jusqu'au fromage de brebis "Pecorino Toscano". Trois fermes ont été choisies dans l'Italie centrale, chacune avec un différent niveau de complexité des pâturages : une ferme dans région plate (composition botanique simple), une de colline (diversité spécifique moyenne), une de montagne (pâturages avec haute diversité). La composition botanique de tous les pâturages des trois fermes a été analysée par analyse linéaire, et une liste d'espèces communes a été extrapolée. Les plus utilisées ont été *Bromus sterilis*, *Lolium perenne* et *L. multiflorum*. Finalement, une liste d'espèces qui contiennent de possibles substances traçables, avec quelques données au sujet de ces substances, est donnée. Des analyses de macro et micro-éléments par spectromètre à émission optique (ICP-OES) et analyseur élémentaire (CHN), et de flavonoïdes et terpènes (HPLC), sont aussi en cours.

**Mots-clés :** Analyse linéaire, contribution spécifique pondérale, contribution à l'alimentation du bétail, composés traçables.

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## Introduction

Italian natural or naturalised pastures comprise several tens of species, most of these are good forages. Many within these species contain chemical traceable compounds that are useful to identify the origin of milk and cheese (Prache *et al.*, 2005; Buccioni *et al.*, 2006). The livestock do not eat all the species as palatability and animals preferences for each plant species is relative and depends on the overall botanic composition and hanger of the livestock. It is possible that traceable compounds contained in plants normally eaten are not ingested in sufficient quantity to become detectable in the final product, this suggests to investigate not only the botanical composition as also the actual specific contribution of different plants to animal diet.

A new method that we propose to calculate the specific utilization takes a start from the methodology of linear analysis, already used for the analysis of botanical composition (Warren, 1959; Daget and Poissonet, 1971). The specific contribution to the diet of the livestock is calculated as percentage of the plant species that have bites within 5 cm of distance from the identification points on the lines, this percentage can be multiplied by the biomass (measured) and we calculate the ponderal specific contribution to livestock diet (Pardini, 2005).

Chemical compounds that are contained in the grazed species and that are traceable are not well known yet, especially because each plant species might have many such compounds whose actual presence and quantities are very much influenced by the environment. As this study is the first carried out in the three areas, we got a preliminary list of compounds from literature.

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## Materials and methods

We choose 3 pastures in Tuscany (Central Italy) with different location in altitude and each with different complexity due to the number of species (from a preliminary investigation we identified 39 species in the pasture of flat area, 65 species in the mountain pasture, 78 species in the hill pasture, this data has been used to chose the pasture in which to start the trial).

Measurements were repeated the central day of each season (15 May for spring, 15 August for summer, 15 October for autumn, 15 January for winter), concerning the botanical composition, utilization and chemical compounds. In this article we present only the results of the springtime as this is the only season when livestock can stay full time on the pastures of the chosen areas without integrations.

The measurements started in Winter 2006 and went on until Autumn 2007. Measurements were:

- (i) Biomass of the season: cuts inside exclusion cages of 1 m<sup>2</sup>, 5 cages per pasture.
- (ii) Botanical composition: linear analysis, 5 lines per pasture, each 20 m long with identification points every 20 cm of distance. All lines were marked with metal blocks deeply stucked in the soil (metal stems hammered at 50 cm depth) in order to repeat later analysis exactly at the same identification points.
- (iii) Grazed species: these measurements were done after grazing periods of 5 days. We identified all species whose leaves were bitten in a circle of 5 cm around the identification points on the lines of botanical analysis. The quantity of bites counted per species, were converted to percentage of specific utilization. The specific percentage of utilization was related to the quantity of available biomass and used to calculate the specific contribution to the livestock intake. In this article we limit the discussion to the 15 species with higher specific contribution per pasture.
- (iv) Traceable compounds: the main traceable compounds contained in the species that were eaten in our pastures were seek on literature as this is a preliminary investigation to limit chemical analysis that will be carried out later.

## Results and discussion

### Biomass

The higher yearly production (Table 1) was obtained in the mountain pasture and in the flat area. The higher productivity in the mountain (5.984 t ha<sup>-1</sup> DM) is due to the best soils in comparison to the other two sites and to some summer rains; the high productivity of the flat pasture is due to the longer growing season in comparison to the other two sites. Summer was actually the most productive season (2.304 t ha<sup>-1</sup> DM in the average of the three areas) because the climate of the mountain and the hill is warm only in that season, moreover the flat area received some unexpected rains during the dry season. The total production of the three pastures was not high and can surely be increased with mineral fertilization.

Table 1. Available biomass in the four seasons of 2006-2007

Pasture location	Dry matter ( t ha <sup>-1</sup> )				
	Winter 2006	Spring 2007	Summer 2007	Autumn 2007	Year
Flat area	0.817 a	1.547 ab	2.093 ab	1.227 a	5.684 a
Hill	0.374 b	0.962 b	1.692 b	0.692 b	3.720 b
Mountain	0.153 c	1.815 a	3.128 a	0.888 ab	5.984 a
Average	0.448	1.441	2.304	0.936	

Values with different letters in columns differ at P=0.05.

### Botanical composition and specific utilization

The pastures included some good forages (Table 2) like *Bromus* spp., *Dactylis glomerata*, *Festuca pratensis*, *Lolium* spp., *Medicago* spp., *Phleum pratense*, *Trifolium* spp., *Vicia sativa*. The specific contribution of these species was different in the three areas, however some of these are present in all the areas. The most grazed in the average of the areas were *Bromus sterilis* (20.83%), *Lolium perenne* (12.50%) and *L. multiflorum* (10.31%). Unfortunately these three grasses do not contain much of traceable compounds, probably because they express competitively by rapid growth and density of the plant.

Table 2. Specific contribution (SC) of the 10 most frequent species per pasture in spring (15 May) and specific utilization of the ten most grazed species (average % of the three areas in the same period, the sum of these percentages is less than 100 because only the 10 most grazed are reported)

Species	Flat SC (%)	Hill SC (%)	Mountain SC (%)	% utilization of the 10 most grazed species (average)
<i>Anagallis arvensis</i>	2.62	-	-	
<i>Anthoxanthum odoratum</i>	-	4.24	-	
<i>Arrhenaterum elatius</i>	-	-	7.60	
<i>Avena sativa</i>	-	5.32	-	
<i>Bromus erectus</i>	-	-	12.07	1.56
<i>Bromus sterilis</i>	-	8.35	-	20.83
<i>Dactylis glomerata</i>	-	-	5.76	4.26
<i>Festuca pratensis</i>	-	-	3.45	
<i>Galium verum</i>	-	-	6.34	
<i>Holcus lanatus</i>	-	5.13	-	2.60
<i>Lathyrus montanus</i>	-	4.32	-	1.14
<i>Lolium multiflorum</i>	31.17	-	-	10.31
<i>Lolium perenne</i>	14.95	10.63	-	12.50
<i>Medicago arabica</i>	-	3.94	-	
<i>Medicago sativa</i>	11.83	-	-	
<i>Papaver rhoeas</i>	3.67	-	-	
<i>Phalaris coerulescens</i>	3.33	-	-	
<i>Phleum pratense</i>	-	-	4.04	
<i>Plantago lanceolata</i>	-	-	11.34	4.69
<i>Potentilla erecta</i>	-	5.65	-	
<i>Poterium sanguisorba</i>	-	-	3.53	
<i>Ranunculus repens</i>	-	3.98	-	
<i>Sonchus oleraceus</i>	3.85	-	-	
<i>Taraxacum officinalis</i>	-	-	3.22	1.56
<i>Trifolium campestre</i>	2.07	-	-	
<i>Trifolium pratense</i>	-	7.68	-	
<i>Trifolium repens</i>	-	-	6.06	3.31
<i>Vicia sativa</i>	7.95	-	-	
<i>Vulpia myuros</i>	7.68	-	-	

## Traceable compounds

Some of the grazed plants contain traceable compounds (Table 3), like *Plantago lanceolata*, *Taraxacum officinalis*, *Trifolium repens*. Several kinds of alkaloids, flavonoles, flavonoids, fitoestrogens, isoflavones, phenolic acids, glycosides, terpenes, steroids, most of which are useful to the plant as defences from stresses are normally found and they can be useful traceable. As an example the fitoestrogens Daizein and Genistein contained in *T. pratense* have effect on livestock reproduction and consequently they are useful to the plant to control the number of predators, these compounds are found in the meat of the animals and might be found also in milk.

Moreover, in our trial we found other species (not reported in the tables) that have only little frequency but contain several traceable compounds (*Achillea millefolium*, *Cynodon dactylon*, *Lotus corniculatus*, *Thymus vulgaris*, *Verbena officinalis*), these species would be useful for traceability, but they are short and less competitive than tall grasses, consequently fertilization should be kept at low levels and animal stocking rate be controlled frequently in order to conserve them.

Table 3. Most grazed species and some possible traceable contained (Prache *et al.*, 2005)

Species	Frequent probable traceable compounds
<i>Galium verum</i>	Flavonoles, Monoterpens, Iridoid glycosides
<i>Medicago sativa</i>	Isoflavons, Alkaloids
<i>Plantago lanceolata</i>	Flavonoids, Iridoid glycosides, Tannins
<i>Poterium sanguisorba</i>	Tannins, Flavonoids, Triterpenes, Steroids, Saponines
<i>Taraxacum officinalis</i>	Flavonoids, Triterpenies, Steroids
<i>Trifolium pratense</i>	Isoflavonoids, Isoflavones, Flavonoles, Cyanogenic glycosides, Phenolic acids, Fitoestrogens

## Conclusions

Many good forage plants are present, some of the most frequent species are also effectively grazed by livestock and some of the grazed species contain compounds that might be useful traceable for milk and cheese. However many of the species found that contain traceable (*Plantago* spp., *Taraxacum officinalis*, *Trifolium* spp., including some of those not very much grazed like *Lathyrus montanus*, *Medicago arabica*, *Papaver rhoeas*, *Potentilla erecta*) are short plants, consequently it is possible that excessive fertilization or too little animal stocking rate favour their replacement by more vigorous and competitive grasses that are more productive (like *Avena sativa*, *Bromus erectus*, *Lolium multiflorum*) but have little or none utility for traceability.

The productivity of the three areas was not very high and, even if fertilization could increase the biomass, it would be preferable to maintain fertilization at a low level in order to conserve biodiversity, this, in turn, will be useful also for traceability.

The next step of this research is to verify the presence of some compounds in the plants and in the final product be it milk or cheese, this is being done by chemical analysis with Optical Emission Spectrometer, Elemental Analyzer, and High Performance Liquid Chromatography.

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