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Evolution of production and forage quality in sown meadows of a mountain area inside Parmesan cheese consortium

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Abstract. Sown meadows, encompassing alfalfa and grass-legume mixtures, are the forage crops on which is based Parmesan cheese production system in the mountain area of the Apennines (central Italy). These grassland types experience, during their development, deep changes in terms of production potentiality, botanical composition and forage quality, thus these meadows have to be periodically renewed to guarantee adequate productive and qualitative performances. To have an accurate assessment of this evolution along time, a survey was performed in different mountain farms inside the Parmesan cheese consortium, analysing alfalfa meadows and grass-legumes mixtures of different ages. Grasslands were monitored during 2019, performing three samplings during growing season. Aboveground biomass production, botanical composition and crude protein content were collected during the survey. Results permitted to evaluate the level of production decrease along years, the evolution of analysed parameters among cutting dates and the dependence of productive and qualitative features on botanical composition and presence of sown species in the swards. Results were useful to hypothesize the composition of future mixtures, to improve management issues and to delineate the possible duration of sown meadows for the area with respect to different purposes in terms of desired productive or qualitative objectives.

Key words: alfalfa, botanical composition, forage, grass-legume mixtures, mowing.

INTRODUCTION

Parmesan cheese is one of the food excellences of Italy and is exported all over the world (Lovarelli et al., 2019). Its production district is limited to a very restricted area almost completely occurring in Emilia-Romagna region (central Italy) that encompasses farms rearing mainly dairy cattle of Holstein Friesian breed. Most of these dairy farms are located in the plane, but about 100 of them are in mountain and less favoured areas (Mancini et al., 2019). In this territory, main forage resources employed are artificial crops cut for hay production of high quality (Tabacco et al., 2020). Occurrence of wide surfaces devoted to grasslands inside a specific territory allows also the provision of specific ecosystem services that go beyond the primary productive function, such as soil protection, preservation of landscape heterogeneity, C-footprint reduction, biodiversity conservation and reduced surplus of nutrients (Giustini et al., 2007; Argenti et al., 2011;

Hao et al., 2017; Pulina et al., 2017; Bengtsson et al., 2019; Viira et al., 2020). Furthermore, the quality of hay and the maximization of their inclusion in the animal diet represent the basis of dairy cow nutrition, for the following milk and dairy products quality but they also play a role in the improving of animal health and welfare (Mordenti et al., 2017).

Among sown meadows inside the studied area, alfalfa (*Medicago sativa*) is one of the most spread mainly for its adaptation to local environmental condition, the high nutritive value and the remarkable potential productivity (Pacchioli & Fattori, 2014). Employment of this species in artificial meadows permits to provide forages with high concentrations of protein, reducing external inputs, economic costs and environmental impacts (Tabacco et al., 2018; Kic, 2019). Other forage resources occurring in the area are represented by mixtures based on grasses and legumes. Species belonging to these two botanical families, chosen according to peculiar pedo-climatic conditions (Movedi et al., 2019), are combined in many different ways, from very simple, based only on two species, a grass and a legume, such as *L. perenne* mixed with *Medicago sativa*, *Trifolium repens* or *Lotus corniculatus*, as reported by Høgh-Jensen et al. (2006), to very complex, encompassing many different species with the aim to establish a forage resource able to became more resilient to adverse weather conditions, to yield at higher rates with respect to monocultures and to provide a more balanced forage quality (Lüscher et al., 2014). Species used for these complex mixtures are represented by productive grasses (such as *Lolium perenne*, *Dactylis glomerata*, *Festuca arundinacea*, *Phleum pratense*) and by already cited legumes, and in some cases also by species belonging to other botanical families, such as *Cichorium intybus* (Skinner, 2008; Sanderson, 2010; Roca-Fernández et al., 2016). Advantages from mixtures in comparison to monocultures are attributed to the presence of species with a very high different behaviour that in turn will result in optimized resource utilization through niche complementarity (Lüscher et al., 2014). In both above mentioned cases, a careful utilization is essential for a proper management of grasslands and to maintain them in an efficient way (Argenti et al., 2020).

Assessment of botanical composition of sown grasslands and its evolution is of extreme importance as it is closely related to yield and forage quality (Chataigner et al., 2010; Targetti et al., 2018). Floristic composition can change remarkably in terms of ground cover of sown species and presence of native species (Sanderson et al., 2005; Ponzetta et al., 2010). Assessment of this development can determine the more appropriate moment for reseeding (Mocanu et al., 2017), when productive and qualitative features fall under specific thresholds. The main aims of this study are: i) to assess the development of botanical composition in sown grasslands along time; ii) to evaluate evolution of main productive characteristics in meadows and how they are affected by botanical composition; iii) to provide technical recommendations for a proper management of artificial forage crops for the studied territory.

MATERIALS AND METHODS

The investigated area is inside the Consortium ‘Terre di Montagna’, that gathers about 100 farms producing Parmesan cheese in Apennines mountain areas, at an altitude over 600 m a.s.l. and comprising some municipalities inside the provinces of Bologna and Modena (Emilia-Romagna Region, central Italy). Soil types mainly developed on sandstone, limestone, and marl, with a very different range of pH (from acidic to

calcareous). Climate of meteorological station of Montese, roughly in the centre of the studied area (44,268844N, 10,942369E), is characterized by a mean annual temperature of 10.1 °C and by an average precipitation of 930 mm, generally without drought during summer (Regione Emilia-Romagna, 2020).

Inside the territory belonging to the Consortium, 6 sites of artificial meadows devoted to hay production were chosen. Sites were selected to be representative of the main forage resources occurring in the area. They differed for environmental location, sown crop (4 monocultures of *Medicago sativa* and 2 grasses-legumes mixtures) and age of meadows, ranging from 2 to 12 years since sowing (Table 1).

Table 1. Main characteristics of investigated sites

Site	Altitude (m a.s.l.)	Average slope (%)	Forage crop	Years since sowing
1A	740	15	Alfalfa	2
2A	870	12	Alfalfa	4
3A	750	8	Alfalfa	8
4A	690	4	Alfalfa	12
5M	780	5	Mixture	2
6M	740	14	Mixture	4

Inside each site, a survey was conducted during 2019, with 3 sampling dates along growing season: in spring, summer and late summer, namely 30−31 May, 15−16 July, and 28−29 August. During each sampling date, three replications inside each experimental area were randomly chosen. Plots were represented by a square of 0.5×0.5 m, according to Mikhailova et al. (2000), and in each plot above ground biomass was harvested using battery-operated grass clippers, stored in sealable bags and then took back to laboratory and oven-dried at 80 °C for 48 hours to constant weight, in order to obtain dry matter production (Wang et al., 2019). On the same sample, chemical analyses were conducted to obtain percentage of crude protein (CP) content (AOAC, 2012) as a main drivers of forage quality (Berauer et al., 2020).

Botanical composition was assessed inside the same sample plots for each cutting. Proportion of each occurring species was estimated visually as percentage contribution to the herbage mass according to Boob et al. (2019). In following elaboration, herbaceous species were grouped in grasses, legumes and other forbs as usually performed in forage research (Wilson et al., 2020). Moreover, knowledge of introduced species, *i.e.* alfalfa or those present in original mixtures, permitted the discrimination of sown species from weeds deriving from natural recolonization in following elaboration.

Results were utilized to compare areas seeded by different crops by means of ANOVA, eventual means separation was performed adopting Tukey test. Moreover, obtained results were used to find out possible relationships among investigated variables and to evaluate evolution of different forage characteristics along time. All analyses were performed using statistical software SPSS (release 26, IBM, 2019).

RESULTS AND DISCUSSION

Productive trends in studied meadows

The trend in production capacity along the season is the same for all investigated sites as the first cut is always the most productive in comparison to the others, with a range from just over 2 tons per hectare of dry matter (oldest sites 3A and 4A) to about 5, in the case of the two-year alfalfa of site 1A (Fig. 1). The decrease in production between the first and second cutting is considerable, but it is much less in the cases of

the most recent meadows, regardless of whether they are alfalfa or mixtures (sites 5M and 1A). Occurrence of sown species, more productive, stable, and more represented in the first years since sowing, produced a sort of stabilization effect on the loss of productivity as the growing season progresses. The last cut is the least productive (in some cases around a ton per hectare) and this condition characterizes the oldest alfalfa meadows, referable to sites 3A and 4A of the trial.

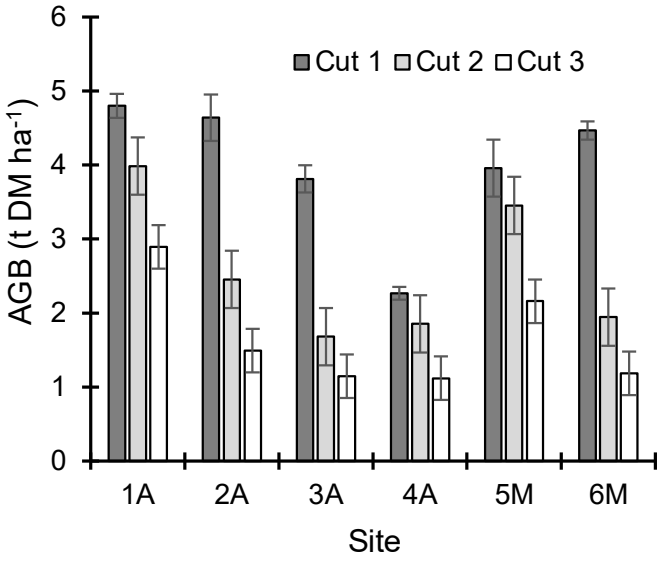

Figure 1. Above ground dry matter biomass (ABG, t ha^{-1}) for each cut in the investigated sites. Bars on each column represent standard errors.

Results give evidence of the importance of management in meadows and its impact on their evolution, especially if located at reduced altitudes (such as in our study) as in higher altitude forage resources characteristics are more forced by environmental factors (Pierik et al., 2017). Findings are consistent with those reported in the research of D'Ottavio & Ziliotto (2003), in which first cut represented almost the 50% of total annual dry matter production. Similar results are reported by Borreani et al. (2005) for grasslands in North Italy at altitude similar to ours, in which the first cut represents a percentage of 40−60% of the total annual harvested biomass. Decline of production along years depicted in our research is coherent with results of Bélanger et al. (2017) that provided evidence of a stable production for at least the first 5 years since seeding.

Botanical evolution in studied meadows

Proportion of legumes is constantly increasing in all sites along the growing season, accounting a remarkable presence also in mixtures (site 5M and 6M), in many cases not significantly different from what observed in pure stands of alfalfa, and roughly ranging between 75−90% in second and third cut (Table 2). The complete list of species observed along all botanical samples is reported in Table S1 (Supplementary materials). Main legumes occurring in the samples are *Medicago sativa* (largely the predominant legume) followed by *Trifolium repens* and *Trifolium pratense*. Presence of forbs (mainly represented by *Taraxacum officinale*, *Geranium dissectum*, *Plantago lanceolata*, *Ranunculus arvensis* and *Rumex obtusifolius*) is very limited with exception of oldest sites, and this is especially true for the 12-years old meadow (4A), with a maximum value of 32.3 at second cut. Higher proportion of grasses (such as *Dactylis glomerata*, *Arrhenatherum elatius* and *Lolium* sp.) in the swards at the first cut is due to their general fast growth after winter at reduced temperatures, roughly 4−6 °C as reported by Brum et al. (2009), and this is true also for alfalfa stands, in which ground cover of grasses is higher than legumes at first cut, except for the most recent meadow in site 1A. To

confirm this, Pacchioli & Ligabue (2013) consider the first cut of alfalfa a sort of forage mixture due to high presence of grasses. Infestation of alfalfa is a generalized issue, and our data confirms previous research. Mseddi et al. (2017) found a constant increase of spontaneous species in alfalfa pure stands and in fourth year since sowing the density of weeds was higher than the sown species, with a corresponding decrease in productivity and forage quality. Also, environmental conditions play a crucial role in determining potential productivity of alfalfa crops, as on poor soil crop decline begins after 3−4 years (Lazarev & Starodubtseva, 2015).

Relationships among productive and qualitative characteristics and botanical composition and age of meadows

Evolution of crude protein content along different cuts is clearly linked to production and botanical composition already analysed (Fig. 2). In almost all investigated meadows there is a remarkable effect of sampling dates on this parameter, with a very reduced value for old alfalfa meadows and for forage mixtures at first sampling date. Second and third cut presented a remarkable increase in protein content, with average values always over 20%. In sites 1A and 2A, represented by recently sown pure stand of *Medicago sativa*, CP content is more constant among cuts, especially in the youngest one.

The narrow relationship between presence of legumes and forage quality in studied meadows is testified by the relationship among occurrence of legumes in the swards

Table 2. Percentage presence grouped for grasses, legumes, and forbs in different sites for each cutting period. Values are shown as average ± standard error. Values, within the same column followed by the same letters are not significantly different at $P < 0.05$ according to Tukey test

Cut 1			
Site	Grasses	Legumes	Forbs
1A	16.3 ± 0.3^{c}	78.4 ± 4.4^{a}	5.3 ± 0.7^{b}
2A	51.3 ± 5.0^{b}	35.7 ± 2.9^{b}	13.0 ± 2.6^{ab}
3A	77.3 ± 5.5^{a}	10.7 ± 3.5^{c}	12.0 ± 2.1^{ab}
4A	66.3 ± 9.2^{ab}	15.0 ± 6.1^{c}	18.7 ± 4.3^{a}
5M	45.3 ± 0.7^{b}	45.0 ± 1.7^{b}	9.7 ± 1.2^{ab}
6M	80.3 ± 1.4^{a}	11.7 ± 1.6^{c}	8.0 ± 2.0^{ab}
Cut 2			
Site	Grasses	Legumes	Forbs
1A	11.0 ± 4.0^{c}	79.0 ± 3.7^{a}	10.0 ± 2.5^{bc}
2A	4.7 ± 0.8^{c}	90.7 ± 2.9^{a}	4.7 ± 2.0^{c}
3A	36.7 ± 4.9^{ab}	48.7 ± 3.6^{b}	14.7 ± 2.4^{b}
4A	42.0 ± 8.9^{a}	25.7 ± 8.4^{b}	32.3 ± 1.9^{a}
5M	14.7 ± 5.5^{bc}	81.7 ± 6.3^{a}	3.7 ± 1.4^{c}
6M	15.7 ± 2.9^{bc}	79.3 ± 3.2^{a}	5.0 ± 0.6^{c}
Cut 3			
Site	Grasses	Legumes	Forbs
1A	1.3 ± 0.9^{d}	90.0 ± 1.7^{a}	8.7 ± 1.7^{c}
2A	0.7 ± 0.3^{d}	93.7 ± 2.0^{a}	5.7 ± 1.7^{c}
3A	40.7 ± 3.5^{a}	42.0 ± 3.7^{c}	17.3 ± 0.3^{b}
4A	38.3 ± 6.0^{a}	33.3 ± 4.4^{c}	28.3 ± 3.3^{a}
5M	7.3 ± 1.3^{c}	84.0 ± 2.5^{a}	8.7 ± 1.2^{c}
6M	18.7 ± 5.7^{b}	74.7 ± 3.0^{b}	6.7 ± 2.8^{c}

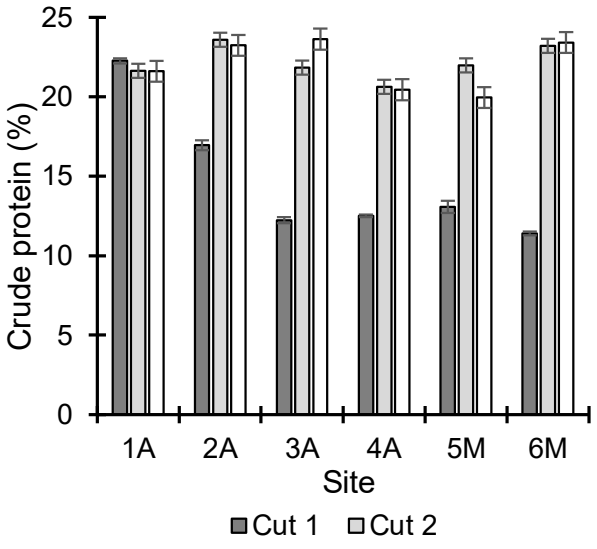

Figure 2. Crude protein content in above ground biomass (% on dry matter) for each cut in the investigated sites. Bars on each column represent standard errors.

(mainly alfalfa) and crude protein content in each cut (Fig. 3). At increasing level of this botanical family in the sward there is a corresponding increase of percentage of protein in forage biomass, even if no significant increase of protein content is observed at high percentage presence of legumes due to the logarithmic form of the regression line.

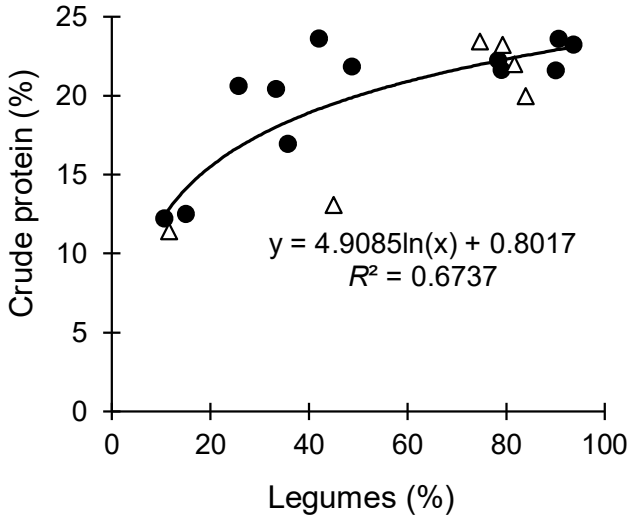

Figure 3. Regression between presence of legumes in the sward (%) and crude protein content in above ground biomass (%). Alfalfa meadows: solid circles; mixtures: triangles.

Evolution of forage quality along season observed in the study is in relation to climatic conditions that strongly affect grassland botanical composition in mountain areas (Dibari et al., 2015), with a higher presence of legumes in summer and, in turn, a higher forage value in this period (Cervasio et al., 2016). This evolution toward a richer presence of legumes is of extremely importance in mountain areas as pointed out by D'Ottavio & Ziliotto (2003), whose results are similar to ours, with a remarkable increase of crude protein of forage biomass along growing season. Results are consistent also with data of Borreani et al. (2005) that observed an increase of protein content from 4% to 8% from first to third cut along the vegetative season in mountain grasslands. Presence of legumes in semi-natural or sown grasslands can be considered an elementary driver of quality and a key factor in the diet of animals in order to improve their performances (Lüscher et al., 2014); this is especially true for dairy cows considering both the increase of individual production and the protein quote of milk composition that occurred in the last years (Mordenti et al., 2017). In a mixture, percentage of legumes in the sward ranging between 40−60% can be considered optimal to achieve effective benefits in terms of forage quality, higher productivity and reduced cost of feed (Phelan et al., 2015). Importance of botanical composition of meadows for crude protein content has already pointed out in many works. For instance, Reiné et al. (2020) found a narrow relationship between percentage of legumes in natural meadows in Spain and crude protein content in forage biomass. Moreover, Deak et al (2007) reported that legume proportion in some grasslands is able to explain a high amount of variability of crude protein in forage samples ($r^2 = 0.85$). Similar results were found by Wróbel & Zielewicz (2018) for Polish artificial meadows in which crude protein content of hay increased with the proportion of sown legumes occurring in the sward (represented in these cases by *Lotus corniculatus* and *Trifolium pratense*) in a very highly significantly way.

Aboveground biomass collected yearly was in relation with both years since sowing (Fig. 4) and occurrence of sown species collected during samplings (Fig. 5).

Reduction in productive potentiality is well correlated with age of meadows, and after some years since sowing (in both cases of monocultures and mixtures) annual dry matter production is sensibly reduced. In our case, acceptable annual productions (for

instance, setting a threshold of 8 t ha^{-1}) are provided by meadows with at maximum 5 or 6 years since sowing (Fig. 4). This evolution towards grasslands characterized by reduced productivity should be attributed to development in the canopy of native species, that are characterized by reduced productivity and low forage quality. In fact, in our case, less productive meadows are those with a very low proportion of sown species (roughly 20−30%) represented by old grasslands (Fig. 5). On the other hand, recent meadows with a very high occurrence of sown species (at least 70−75%) have remarkable yield. The importance of presence of sown species on productive parameters in artificial forage resources has been already highlighted previously (e.g. Cavallero & Talamucci, 2002). Even if the persistency of seeded plants can reach notable duration in restored meadows (for instance 20 years, Švamberková et al., 2019), the efficient productive role can deplete in few years in sown resources that should be adequately renewed periodically with appropriate species in oder order to reduce loss of quality and potential yield and, in turn, of animal performances (Eriksen et al., 2014; Jing et al., 2017). Penetration of native species in sown grasslands is a natural process that affects all types of forage resources, monocultures and mixtures, even if the former is considered generally more sensible to weed invasion (Helgadóttir et al., 2018). Our data is consistent with previous research. For instance, Sturludóttir et al. (2013) report a constant decrease of productivity along years in an analysis conducted on artificial temperate grasslands. Persistency of sown species, with a higher presence in youngest meadows, had a stronger influence on productive performances, as reported by Adamovics et al. (2017) for different Latvian sown grasslands. Also Bélanger et al. (2017), analysing different binary mixtures in Canada for some years after sowing, highlighted the importance of sown species on forage features, and especially how

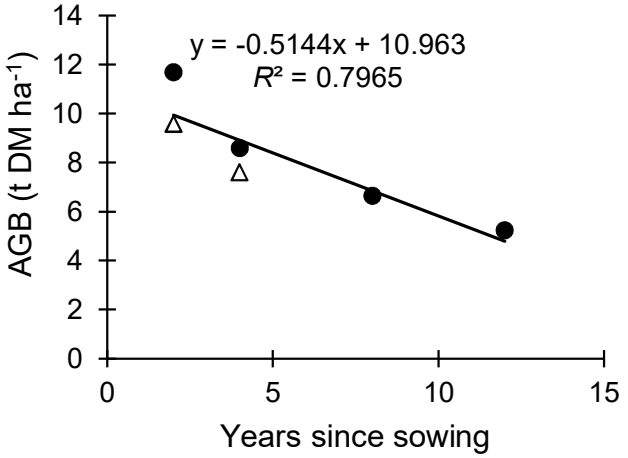

Figure 4. Regression between number of years since sowing and annual above ground biomass (t DM ha^{-1}). Alfalfa meadows: solid circles; mixtures: triangles.

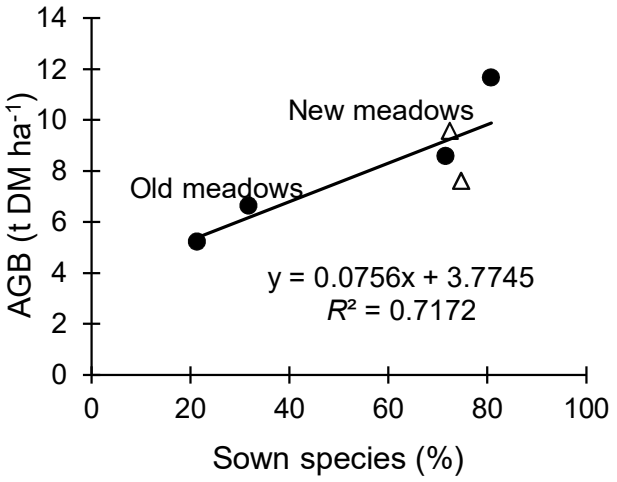

Figure 5. Regression between occurrence of sown species in the sward (%) and annual above ground biomass (t DM ha^{-1}). Alfalfa meadows: solid circles; mixtures: triangles.

legumes proportion, generally decreased along time, affects qualitative characteristics of harvested biomass.

CONCLUSIONS

The research permitted a proper and accurate characterization of forage resources employed in the investigated area, in terms of evolution along years and during the growing season. Even if they are based on different types (monocultures and mixtures) it was possible to depict the general development of main forage features, such as productivity and quality and how these characteristics are closely related to botanical composition.

The results allowed not only to describe possible development along years but, from a technical point of view, they were useful to foresee hypothetical management of meadows for the area in relation to specific objective. For instance, the productive decreasing resulted highly correlated to age of meadows, and to achieve an average dry matter yield of at least 8 t ha^{-1} per year, the appropriate duration can be scheduled to be about 5−6 years. The quality of forage is closely linked to a specific component of botanical composition (presence of legumes) and is conceivable that quality reduction is faster than loss of productivity, as legumes are generally characterized by reduced persistency compared to grasses. For these reasons an appropriate duration, having as key guideline forage quality, can be foreseen in 3−4 years since sowing, in order to obtain at least 20% of crude protein content as yearly average. Concerning mixtures, data collected permitted also to assess potential composition of meadows. Among legumes, *Medicago sativa* confirmed its high adaptability to the area, even if also some clovers could be useful employed to enhance legumes presence in the long term, mainly *Trifolium repens*. Among grasses, *Dactylis glomerata* was the species more suitable to be utilized, followed by *Arrhenaterum elatius*, *Festuca arundinacea* and *Lolium* sp.

As resulted by previous literature analysis, the appropriate duration of a sown meadow and its composition along time are the most important issues in artificial forage crops and they have to be adequately considered to achieve high performances, under a quantitative and qualitative point of view, for Parmesan cheese production.

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