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REGULAR ARTICLE

Forage yield and quality of simple and complex grass-legumes mixtures under Mediterranean conditions

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ABSTRACT

In the experiments conducted in 2013/2014 at the National Institute for Agricultural and Veterinary Research (INIAV) experimental station in Elvas - Portugal, the agronomic and forage quality traits of various species (Triticale (X *Triticosecale* wittmack), Oat (*Avena sativa* L.), Grasspea (*Lathyrus sativus* L.), Red vetchling (*Lathyrus cicera* L.). Hairy vetch (*Vicia villosa*, Roth), Common vetch (*Vicia sativa* L.) and Fiel pea (*Pisum sativum* L.)) in pure stands as well as in mixtures were evaluated. The objective was to identify interesting species or fodder mixture able to produce early interesting biomass, both in quantity and quality, for the periods when there is scarcity of pasture. A randomized complete block design with three replications was used in the experiment. The studied parameters were: dry matter yield, crude protein content, neutral detergent fiber content (NDF) and in vitro digestibility. Analysis of variance revealed highly significant differences ($P < 0.05$) among treatments for all agronomic and forage quality parameters. Lowest dry matter production was recorded in the combination on red vetchling in the first cut (133 kg ha^{-1}) and with grasspea in the second cut (1266 kg ha^{-1}), but these treatments are characterized by the highest values of protein contents (16-23%), the highest digestibility (66-81%) and the lowest NDF content (24-39%).

Keywords: Dry Mediterranean climate; Fodder mixtures; Forages species

INTRODUCTION

During the past three decades, the livestock sector is growing due to the global expansion in production and consumption of animal products (FAO, 2016). The cattle sector production costs are important, mainly as a result of feed costs, which represent more than 50 percent of the total cost (Sulas et al., 2012). This makes necessary to find solutions to provide high quality animal feed using sustainable production methods. The dominating aspect of Mediterranean climate on agriculture is the scarcity in rainfall and the erratic distribution along the year, showing a coincidence of drought and high temperatures during the summer season. Furthermore, it is also expected that in winter, temperature increase in the Mediterranean region, +1 to +2°C based in the majority of models (Dumont et al, 2011). It is believed these climate changes will affect plant growth and development along with crop yield, Increased inter-annual variability of rainfall and temperature may be another significant aspect of climate change, and this is of high ecological relevance. For this region a slight reduction of precipitation ranging between 0 an 10% in winter and 10 and 20% in summer is predicted (Leliévre et al., 2010). Thus, herbage fodder mixtures suitable to grow in these conditions and with high nutritional value in late winter/early spring (time of greatest need of fodder) should be found. Forage nutritional value depends on many factors and it has a direct effect on forage value, animal performance, and, ultimately on farm profits (Ball et al., 2001). The most important factors affecting the forage nutritional value are forage species and growth stage in the moment of harvest. Among the factors affecting forage quality, the objective of this study was to select species and fodder mixtures for high dry matter content and for high quality potential (crude protein, digestibility and NDF).

MATERIALS AND METHODS

The experiment was conducted during 2013/2014 at the National Institute for Agrarian and Veterinarian Research (INIAV) experimental station in Elvas, Portugal (38°53' N, 7°08' W, 220 m above sea level), under vertic-calcaric-chromic Cambisol soil. Portugal is mainly characterized

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by Mediterranean climate (warm to hot dry summers and mild to cool wet winter; rainfall in this area has a strongly seasonal pattern). Two winter cereals (Triticale (X *Triticosecale* wittmack), Oat (*Avena sativa* L.)) and five legumes (Grasspea (*Lathyrus sativus* L.), Red vetchling (*Lathyrus cicera* L.). Hairy vetch (*Vicia villosa* Roth), Common vetch (*Vicia sativa* L.), Field pea (*Pisum sativum* L.)) in pure stands as well as is mixtures were evaluated (Table 1). INIAV-Portugal cultivars were chosen to this study.

A randomized complete block design with three replications was used in the experiment. Each plot consisted of 8 rows with 5 m length. The space between rows was 25 cm. Sowing was done by hand on November 14th, 2013. Dry matter yield was evaluated by two sequential cuts throughout the growing season. The plant samples were taken from a randomly selected 0,438 m^2 (1,75 m x 0,25m) area of each plot. The first harvest time was based on (i) the occurrence of flowering (at 10% of plants) in legumes pure stands and (ii) at appearance of the first node (Zadoks stage 31) (Zadoks et al., 1974) for cereal pure stands and for all mixtures. Plots were clipped for a second time, in the end of April at cereal grain watery ripe - GS71 according to Zadoks's scale - Zadoks et al., 1974). Plant sub-samples were taken from each plot, dried in a forced-air oven at 50°C and weighted to obtain dry matter yield. The crude protein content was measured by Kjeldahl method (ISO 5983-1: 2005) and the conventional factor of N x 6.25 was used. The NDF content (neutral detergent fiber) was measured according to ISO 16472.2005 with lower modifications. The *in vitro* digestibility was estimated according to the determination *in vitro* of the dry matter digestibility of samples that was performed by the two stage pepsin-cellulase enzymatic method (Jones and Hayward, 1975). Data were analyzed using SPSS version 17.0 software (SPSS Inc., Chicago, IL). The differences between means were separated by Tukey multiple range test ($P \leq 0.05$).

Table 1: Species, mixtures, varieties, and seed density used in the experiment

Treatments		Seed density
T1	Triticale (Fronteira)	160 kg ha^{-1}
T2	Oat (S^{ta} Eulália)	130 kg ha^{-1}
T3	Grasspea A	160 kg ha^{-1}
T4	Grasspea B	80 kg ha^{-1}
T5	Red vetchling A (Grão da Gramicha)	130 kg ha^{-1}
T6	Red vetchling B (Grão da Gramicha)	65 kg ha^{-1}
T7	Hairy Vetch A (Amoreiras)	30 kg ha^{-1}
T8	Hairy Vetch B (Amoreiras)	15 kg ha^{-1}
T9	Field pea A (Pixel)	160 kg ha^{-1}
T10	Common vetch (Graveza)	80 kg ha^{-1}
T11	Triticale+Grasspea	60 kg ha^{-1}+80 kg ha^{-1}
T12	Triticale+Red vetchling	60 kg ha^{-1}+65 kg ha^{-1}
T13	Triticale+Hairy vetch	60 kg ha^{-1}+20 kg ha^{-1}
T14	Triticale+Field pea	60 kg ha^{-1}+80 kg ha^{-1}
T15	Triticale+Common vetch	60 kg ha^{-1}+50 kg ha^{-1}
T16	Oat+Grasspea	45 kg ha^{-1}+80 kg ha^{-1}
T17	Oat+Red vetchling	45 kg ha^{-1}+65 kg ha^{-2}
T18	Oat+Hairy vetch	45 kg ha^{-1}+20 kg ha^{-3}
T19	Oat+Field pea	45 kg ha^{-1}+80 kg ha^{-4}
T20	Oat+Common vetch	45 kg ha^{-1}+50 kg ha^{-4}
T21	Triticale+Grasspea+Hairy vetch	60 kg ha^{-1}+60 kg ha^{-1}+10 kg ha^{-1}
T22	Triticale+Grasspea+Field pea	60 kg ha^{-1}+60 kg ha^{-1}+60 kg ha^{-1}
T23	Triticale+Red vetchling+Hairy vetch	60 kg ha^{-1}+45 kg ha^{-1}+10 kg ha^{-1}
T24	Triticale+Red vetchling+Field pea	60 kg ha^{-1}+45 kg ha^{-1}+60 kg ha^{-1}
T25	Oat+Grasspea+Hairy vetch	45 kg ha^{-1}+60 kg ha^{-1}+10 kg ha^{-1}
T26	Oat+Red vetchling+Hairy vetch	45 kg ha^{-1}+45 kg ha^{-1}+10 kg ha^{-2}

RESULTS AND DISCUSSION

The annual precipitation for this agricultural year was 775 mm, and the mean monthly temperature varied between 8.9°C in December and 24.9°C in July. After sowing date, a very dry period of 1.5 months (rainfall below 15 mm per month) was reported. Therefore, emergence was low and in every plot the distribution of plants was irregular.

Significant differences were observed among treatments in dry matter yield, crude protein content, neutral detergent fiber content and digestibility, indicating considerable genetic and agronomic variability for these parameters.

Dry matter yield

The dry matter yield recorded at two different growth stages of pure stands and forage mixtures is presented in Table 2.

Average dry matter (DM) content increased with delaying cutting date from first to second cutting date. DM varied from 581 kg ha^{-1} to 3327 kg ha^{-1}. From Table 2 it can be seen that, at cut I, the highest dry matter was obtained by the common vetch (T10) that was not significantly different from T8 compared to almost all the other treatments. For for both cuts and unlike another studies (Ansar et al., 2010; Alami et al., 2015), no significant differences were recorded between binary and ternary mixtures. This occurrence is not in agreement with the conclusions of Papadopoulos et al. (2012), who reported increased forage yields when mixture complexity increased. Likewise, Picasso et al. (2011) asserted that choosing a single well-adapted species for maximum productivity could not be the best alternative over the long term, and that high levels of species diversity should be

Table 2: Dry matter yield (DM) of 26 treatments in both cutting times

Treatments	DM (kg ha^{-1})		Treatments	DM (kg ha^{-1})	
	Cut I	Cut II		Cut I	Cut II
T1	658bc	3520abc	T14	661bc	3276abc
T2	434bc	6430a	T15	409bc	4849abc
T3	594bc	1266c	T16	517bc	5366ab
T4	351bc	1374c	T17	571bc	3760abc
T5	133c	1332c	T18	530bc	4617abc
T6	223c	2055bc	T19	574bc	2721abc
T7	447bc	1949bc	T20	501bc	3904abc
T8	1153ab	-	T21	374bc	4777abc
T9	918bc	2795abc	T22	650bc	3045abc
T10	2014a	-	T23	463bc	2644abc
T11	594bc	2808abc	T24	497bc	3458abc
T12	336,0bc	2091bc	T25	382bc	4971abc
T13	632bc	2727abc	T26	501bc	3922abc
Mean$_{(T1-T26)}$	581	3327	Mean$_{(T1-T26)}$	581	3327
Level of significance	***	***	Level of significance	***	***

Means followed by the same letter are not significantly different at P<0.05

Table 3: Crude protein (CP) content of 26 treatments in first cut time

Treatments	CP (%)	Treatments	CP (%)
	Cut I		Cut I
T1	12.3ef	T14	12.60ef
T2	12.10f	T15	16.93abcdef
T3	22.51ab	T16	16.87abcdef
T4	23.00a	T17	16.30cdef
T5	20.33abcd	T18	14.60def
T6	21.80abc	T19	12.60ef
T7	17.70abcdef	T20	15.17def
T8	17.80abcdef	T21	17.70abcdef
T9	14.70def	T22	15.67cdef
T10	17.10abcdef	T23	18.37abcde
T11	16.43bcdef	T24	16.47bcdef
T12	18.23abcdef	T25	13.88ef
T13	16.53bcdef	T26	16.19cdef
Mean$_{(T1-T26)}$	16.69	Mean$_{(T1-T26)}$	16.69
Level of significance	***	Level of significance	***

Means followed by the same letter (s) are not significantly different at P < 0.05.

included in the design of productive and ecologically sound agricultural systems. At the present study, in pure stand, there were significant differences among some treatments and between cutting time (Cut I and Cut II). The highest dry matter yield was obtained in oat (Cut II (cut at the end of spring without winter use) - 6430 kg ha^{-1}), which has typically a highest-yielding forage, and in common vetch (Cut I - 2014 kg ha^{-1}). Common vetch and hairy vetch are late flowering species, when compared with the other legumes. So, the total accumulated dry matters until occurrence of flowering (Cut I) were bigger. On the other hand the lowest dry matter yield was observed in red vetchling (Cut I – 133 kg ha^{-1}) and grasspea (Cut II – 1266 kg ha^{-1}).

Crude protein content

Forage protein content is often considered a good index of quality. The crude protein content of different treatments at different growth stages are shown in Tables 3 and 4. The average crude protein contents declined by increasing crops maturity (Mean Cut I: 16.7%; Mean Cut II: 13.0%). Crude protein content was always lower for triticale and oat pure stand and for binary mixture cereal + field pea (T1, T2 and T14) compared with *Lathyrus* pure stand treatments (T3, T4 and T5). One of main qualities of these forages crops (grasspea and red vetchling) consist of its high protein content (Polignano, 2007). The results of this study, show higher protein content than observed by Foster et al. (2014) and similar than Van Saun (2016) results. This is because, there were selected for this study various forage legumes varieties which generally produce high quality forage.

Digestibility

Concerning to the forage quality, digestible energy (digestibility) is the most common limiting factor. However, there are times when protein and minerals are the nutrients that limit animal performance, especially in grazing situations when supplementation is impractical. (Ball et al, 2001). Maturity stage at harvest is the most important factor that determines forage quality of a given species, including digestibility. According to Ball et al (2001), digestibility declines by 1⁄3 to 1⁄2 percentage units per day until it reaches a level below 50% (it may happen after the blooming period). In the first cut, digestibility values ranged in this study from 61.8% (T8-Hairy vetch) to 84.7% (T2-Oat) (Table 5). The beginning of flowering for vetch species (date of first cut) happened late in relation to all the other species in this study. Hence, this is related to the lowest values of their digestibility, which are significantly lower than those obtained from other treatments.

As it is known, digestibility varies greatly. This parameter decreases as the plant matures. So, as expected, the value for this characteristic is lower in the second cut (average variation between cuts: 62% (Cut II) – 78% (Cut I). The lowest value in the second cut was obtained in T1-Triticale (49.3%) and the highest in the T9- field pea (70.7%).

Neutral detergent fiber (NDF) content

Legumes usually have less neutral fibre content (NDF) than grasses which promote a higher intake of the former family by animals. Neutral Detergent Fibre content is important because they reflect the amount of forage consumed by the animal. The higher the NDF percent, the lower the dry matter intake. Favourable average NDF were recorded in

Table 4: Crude protein (CP) content of 26 treatments in second cut tim

Treatments	CP (%) Cut II	Treatments	CP (%) Cut II
T1	8.50d	T14	9.82bcd
T2	7.95d	T15	12.43abcd
T3	17.78a	T16	13.17abcd
T4	17.93a	T17	10.43bcd
T5	16.93a	T18	13.90abcd
T6	15.60abc	T19	9.34cd
T7	16.17ab	T20	14.97abc
T8	-	T21	13.37abcd
T9	12.30abcd	T22	12.77abcd
T10	-	T23	12.53abcd
T11	14.20abcd	T24	12.00abcd
T12	11.75abcd	T25	12.60abcd
T13	14.10abcd	T26	11.66abcd
$Mean_{(T1-T26)}$	13.01	$Mean_{(T1-T26)}$	13.01
Level of significance	***	Level of significance	***

Means followed by the same letter (s) are not significantly different at P<0.05

treatments with grasspea (T3, T4) and red vetchling (T5, T6) on pure stand in both cutting times (Fig. 1). On the other hand, the highest average NDF content (62.63%) was determined in triticale harvested at cereal grain watery ripe - GS71 of Zadoks's scale (Zadoks et al., 1974). These results were comparable to values reported by Lithourgidis et al. (2006) and Kocer and Albayrak (2012). Neutral Detergent Fibre contents of all treatments increased by delaying harvesting; this was as expected because of the maturation progress of each species.

CONCLUSIONS

This study showed that for combined positive productivity and quality it is essential include legumes on fodder mixtures, especially those with higher contribution to increase the protein content and forage digestibility, as well as to decrease NDF content, such as, the genus *Lathyrus*.

Table 5: Digestibility (Digest) content of 26 treatments in two cutting times

Treatments	Digest (%) I	Digest (%) II	Treatments	Digest (%) I	Digest (%) II
T1	81,83ab	49,25c	T14	79,87abcd	56,20bc
T2	84,70a	59,30abc	T15	79,73abcd	56,20bc
T3	75,03cd	66,73ab	T16	80,93abc	64,57ab
T4	76,47bcd	66,70ab	T17	81,07ab	63,03ab
T5	80,37abcd	66,00ab	T18	77,12bcd	63,17ab
T6	81,77ab	66,20ab	T19	81,93ab	63,07ab
T7	68,00e	60,57abc	T20	81,50ab	67,13ab
T8	61,83f	-	T21	76,83bcd	59,37abc
T9	80,43abcd	70,67a	T22	80,13abcd	62,97ab
T10	68,00e	-	T23	76,23bcd	61,40ab
T11	81,33ab	58,67bc	T24	79,80abcd	61,75ab
T12	80,20abcd	58,05bc	T25	80,70abc	60,37abc
T13	74,50d	59,45abc	T26	78,37bcd	61,30ab
$Mean_{(T1-T26)}$	78,03	61,76	$Mean_{(T1-T26)}$	78,03	61,76
Level of significance	***	***	Level of significance	***	***

Means followed by the same letter (s) are not significantly different at P<0.05

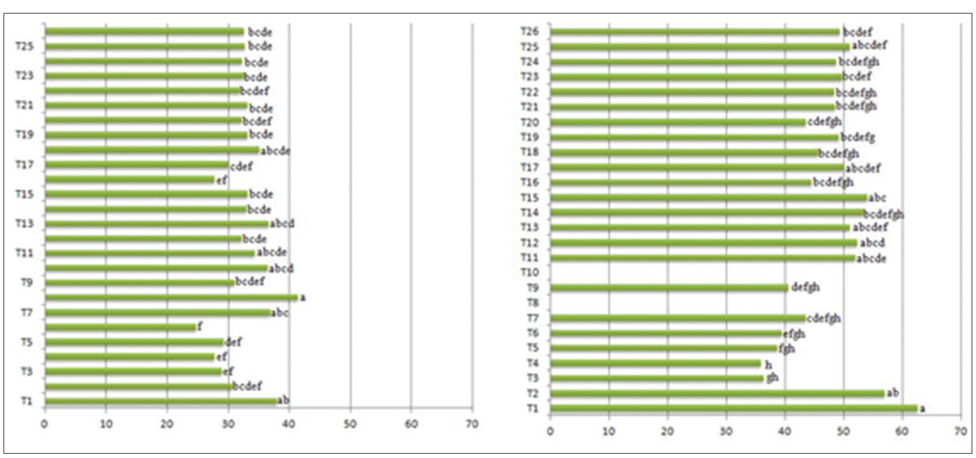

Fig 1. Neutral detergent fibre values of the 26 treatments in cut I (left) and in Cut II (right) cutting times (means followed by the same letter(s) are not significantly different at P<0.05 according to Tukey analysis).

It has also been shown that increasing the complexity of the mixture had slightly different effects on the forage production and forage quality.

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