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Quality characteristics of forage in the sowing year of a standard perennial pasture established with annual ryegrass, barley, and berseem clover as companion crops

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This study was conducted to determine the effect of different seed sowing rates (25%, 50%, 75%, 100% of the recommended pure stand of the species) and harvesting stages (early harvest, late harvest) of annual ryegrass, barley, and berseem clover used as companion crops on the forage quality in the sowing year of standard perennial pasture mixture [alfalfa (15%) + white clover (15%) + perennial ryegrass (20%) + orchard grass (20%) + tall fescue (30%)]. The experiments (Experiment I:2019, Experiment II:2020) were conducted in randomised complete blocks with a split-plot arrangement in three replications. The treatments with berseem clover as a companion crop had higher herbage quality than the treatments with barley and annual ryegrass as companion crops and gave forage quality equivalent to the control treatment without companion crop. Although a significant decrease was observed in the forage quality of the mixture with the advancement of the harvesting stage of the companion crop, the sowing ratio of the companion crop did not significantly affect the forage quality of the mixture. It was concluded that berseem clover can be used as companion crop in the establishment of the pasture under conditions of the Mediterranean climate in terms of forage quality and that the harvesting stage should not be delayed.

Keywords: companion crop, harvest stage, pasture establishment, quality characteristics

Introduction

Natural pasture areas have been the most important source of cheap, high-quality roughage for livestock for centuries in Turkey, which has been home to many civilisations. However, pastures in Turkey, which have an area of 14.6 million ha (BUGEM 2022), have approached a critical level of vegetation cover loss due to uncontrolled grazing and unsuitable ecological conditions, and they have decreased in quality and productivity in terms of forage yield (Altin et al. 2005). In these areas, rapidly improving pasture yield and plant cover by increasing inputs such as fertilisers is not feasible. Therefore, it is necessary to remove the existing vegetation by cultivation in areas where soil and topographical characteristics are suitable for tillage and to revegetate with perennial forage legume and grass mixtures suitable for the ecological conditions of the area. Furthermore, the establishment of cultivated pastures comprising mixtures of perennial grass and legume crops that are well-suited to the ecological conditions of the region is of paramount importance. These pastures can be integrated into short-term crop rotations on arable land, thereby reducing grazing pressure on natural pastures while also quickly providing abundant, high-quality forage (Bakir 1985; Genckan 1985).

In the establishment of cultivated pastures, the combination of perennial forage crops selected to match local ecological conditions and soil characteristics tends to yield the best results in terms of nutrient balance and forage productivity (Tahir et al. 2022). Legumes, with their high protein content, complement the carbohydrate-rich grasses, resulting in a balanced and productive pasture ecosystem (Cinar and Hatipoglu 2015). In addition, grasses utilise the nitrogen fixed by legumes in the soil (Salama 2020), and can also reduce bloating issues in animals caused by certain legumes (Majak et al. 2003). In the initial phase of establishment, perennial forage crops utilised in cultivated pastures may not exhibit a competitive advantage in terms of light, water and nutrient uptake over fast-growing broad-leaved weeds. This is mainly because broad-leaved weeds tend to develop deeper subsoil roots that can cause damage to young seedlings and their eventual withdrawal from vegetation. To improve establishment success, prevent broadleaf weed infestations, and ensure high yield and quality of forage during the establishment year, it is advisable to sow perennial forage crops in association with fast-growing annual crops, often referred to as protective, nurse or companion crops (Kilcher and Heinrichs 1960; Davis 1962). Barley, wheat, triticale, oats, annual ryegrass and berseem clover, which exhibit rapid growth and produce quality forage, are often employed as companion crops (Tan and Serin 2004; Asci 2009). This practice is supported by research (Sowiński 2014; Coulman et al. 2019; St-Pierre-LEpage et al. 2023) and can significantly contribute to the effectiveness of pasture establishment efforts.

Although companion crops provide many benefits in the establishment of cultivated pastures, they can also compete with perennial forage crops in the mixture, particularly in terms of light, water and nutrient availability. It is possible that this competition may result in a reduction in the yield and quality of perennial pasture mixes during the establishment year (Tossell and Fulkerson 1960; Hoy et al. 2002). It has been reported that under arid conditions, the establishment of perennial forage crops with certain companion crops significantly reduces forage yield due to increased competition for limited soil moisture (Jefferson et al. 2005). In a study by Lanini et al. (1991), it was found that when alfalfa was established with a companion crop, as the seeding rate of the companion crop in the mixture increased, forage yield increased at the first harvest. However, this was followed by a decline in alfalfa yield in subsequent harvests. Chapko et al. (1991) observed that the forage quality of alfalfa, when established with different companion crops, varied according to the companion crop used. Furthermore, they found that early harvesting improved forage quality, provided that the seeding rate of the companion crop was not increased excessively. Malhi and Foster (2011) found that, in the establishment of cultivated pastures with various companion crops, control plots without the presence of companion crops had a higher forage quality (high crude protein, low neutral detergent fibre) compared to plots with companion crops. Additionally, it was observed that the influence of the seeding rate of the companion crop on the quality of the forage mixture was relatively significant. In this context, it is of the utmost importance to select the companion crop species with great care, paying attention to the seeding rates within the mixture and determining the optimum time for the harvest of the companion crop. These measures are essential to maximise establishment success and provide high forage yield and quality in cultivated pasture systems.

This study was carried out over a period of two years under irrigated conditions in a Mediterranean climate and consisted of two separate experiments. The aim was to evaluate the effects of various companion crop species, different seeding rates and harvesting stages on the hay quality of the pasture mixture during the establishment year. An article has already been published with data on forage yield and other characteristics from the same research (Donmez and Hatipoglu 2023).

Material and methods

Experimental site and plant material

As part of this study, two field experiments were established in November 2019 and 2020 at the research area of the Field Crops Department of Agricultural Faculty, Cukurova University, Adana (located at 37° 01' 05" N, 35° 21' 25" E, altitude 33 m). In the study, alfalfa (cv. Nimet), berseem clover (cv. Derya), and spring barley (cv. Ay) from the Eastern Mediterranean Agricultural Research Institute, tall fescue (cv. Nilufer) and annual ryegrass (cv. Elif) from the Aegean Agricultural Research Institute, white clover (cv. Rivendel), orchard grass (cv. Lidacta) and perennial ryegrass (cv. Temprano) from Maro Seed Company were used as plant material.

Soil and climate characteristics

According to the results of the soil analysis (Burt 2004), the soils of the experimental area were found to have a clay soil texture, low organic matter (1.30%), slightly alkaline soil pH (7.30), moderate lime (12.76%), low levels of available P_2O_5 (18.9 kg ha^{-1}), and high levels of available K_2O (789 kg ha^{-1}).

Climatic data for the study period and the long-term average obtained from Adana Meteorology Regional Directorate are shown in Figure 1. The average temperature values for the 2019/20 (20.5 °C) were slightly lower than that of the 2020/21 (20.6 °C) growing season. In the 2019/20 growing season, the lowest temperature was at 93 days after sowing (DAS93) (−3.1 °C) and the highest temperature was at DAS298 (45.1 °C). In the 2020/21 growing season, the lowest temperature was at DAS72 (-0.1 °C) and the highest temperature was at DAS260 (41 °C). The total annual rainfall during year 1, at 883 mm was higher than the long-term average (670 mm), while it was lower in year 2 (362 mm). Approximately 31% (272 mm) of the total rainfall in the 2019/20 growing season occurred over a period of 4 days.

Experimental design and field treatment

The experimental design was a randomised complete block design with split plots and three replications. In the experiment, the main plots were defined by two different harvest stages of the companion crop species. The sub-plots were established with a simple perennial forage mixture without companion crops and with four different companion crop seeding rates (25%, 50%, 75%, and 100% of the recommended pure seeding rate) (Table 1).

After the triticale harvest in mid-June prior to the establishment of both experiments, the experimental fields, which were left empty during the summer period, were made ready for sowing after tillage and levelling operations in early fall. In the study, alfalfa, white clover, perennial ryegrass, orchard grass, tall fescue, barley, annual ryegrass and berseem clover species were prepared for sowing by considering recommended pure seeding rates of 20, 10, 28, 28, 18, 160, 20, 20 kg ha^{-1}, respectively (Table 1). The row spacing was 20 cm (6 rows). Sub-plot size was set as 1.2 m × 5 m = 6 m^2 and each trial consisted of 78 sub-plots in total. The total area of each trial was 78 sub-plots × 6 m^2 = 468 m^2. Experiments I and II were sown in different fields at the same location on 11.11.2019 and 11.11.2020, respectively. Before sowing, 100 kg ha^{-1} Nand 100 kg ha^{-1} P were applied to the experimental area in a fertiliser composed of 20:20:0 N:P:K (Cinar and Hatipoglu 2014). Sprinkler irrigation was performed in the experimental area depending on soil moisture (field capacity) and rainfall (Abraha et al. 2015).

Sampling and measurement

In the study, there were 7 harvests in the 2019/20 growing season and 6 harvests in the 2020/21 growing season. Harvesting was carried out at a height of 10 cm in the first cutting, taking into account the development stage of the companion crop species as shown in Table 1, and at a height of 5 cm, taking into account 10% alfalfa in the pasture mixture, in the second and subsequent cuttings. Harvesting was carried out by randomly placing a 50 × 50 cm wooden frame with three replications in each sub-plot. After the harvest, to determine the contribution of species to the sward, forage samples from each wooden frame were separated into companion crop, perennial mixture species and weeds. From

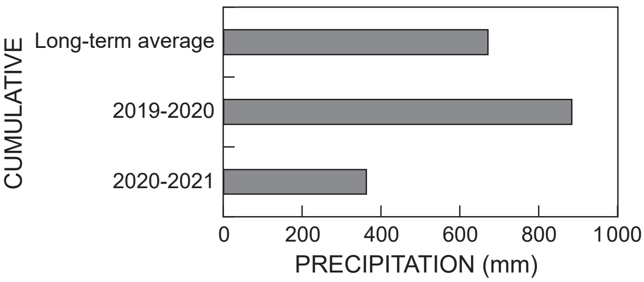

Figure 1: Long term and actual climatic data during the study period

the second harvest onwards, only perennial mixture species and weeds were separated out. The separated samples were dried in a forced-air oven (70 °C for 48 hours) and weighed to determine dry weights of each component. Utilising the dry weights, total dry matter yield and contribution of each component to the total yield was calculated. These samples were then ground to pass through a 1 mm screen and the crude protein content (%), acid detergent fibre (ADF((%) and neutral detergent fibre (NDF) (%) contents of the species (except for weeds) were determined by The Foss XDS NIRS (Near Infrared Reflectance Spectroscopy) analyser using calibration C-0904FE-Hay and Fresh Forage (at least three repetitions). Crude protein (%), ADF (%) and NDF (%) contents in the dry matter of each species were determined by proportioning the obtained values with the dry matter ratios of each species (Kacar and Inal 2010). The crude protein, ADF and NDF contents of the dry matter from each plot were determined with the help of the values determined from the mixture components in the plot and the equation given below (Donmez 2022):

$$(Q_1 \times P_1) + (Q_2 \times P_2) + \ldots \qquad (1)$$

where: Q_1: Quality parameter of species 1 (crude protein, ADF or NDF content), Q_2: Quality parameter of species 2, P_1: Proportion of species 1 in the mixture dry matter yield, P_2: Proportion of species 2 in the mixture dry matter yield.

Using ADF and NDF results, relative feed value was calculated using the following equations described by Sheaffer et al. (1995).

$$\text{digestible dry matter } (DDM) = 88.9 - (0.779 \times ADF\%)$$

$$\text{dry matter intake } (DMI) = \frac{120}{NDF\%} \qquad (2)$$

$$\text{relative feed value } (RFV) = \frac{DDM \times DMI}{1.29}$$

Crude protein yield and digestible dry matter yield were calculated by multiplying the dry matter yield of the plot by the crude protein and the digestible dry matter contents, respectively.

Statistical analysis

The data obtained from the study were analysed using the MSTAT-C (V. 2.10, Michigan State University, USA) statistical package program in accordance with the split-plot experimental design (Steel and Torrie 1980), and the means of the experimental variants for statistically significant

Table 1: Combination of pure sowing perennial mixture and perennial mixture with companion crops

Mixture applications and seeding rates of species within mixture	Early harvest	Late harvest	Abbreviation
Perennial pasture mixture (PM) [Alfalfa: 3 kg ha^{-1} (15%) + white clover: 1.5 kg ha^{-1} (15%) + perennial ryegrass: 5.6 kg ha^{-1} (20%) + orchard grass: 5.6 kg ha^{-1} (20%) + tall fescue: 5.4 kg ha^{-1} (30%)]	When mixture species reach 30 cm in height	At the beginning of flowering of legumes	PM
PM + 25% Barley: 40 kg ha^{-1}	Heading stage of barley	Milk stage of barley	PMB25
PM + 50% Barley: 80 kg ha^{-1}	Heading stage of barley	Milk stage of barley	PMB50
PM + 75% Barley: 120 kg ha^{-1}	Heading stage of barley	Milk stage of barley	PMB75
PM + 100% Barley:160 kg ha^{-1}	Heading stage of barley	Milk stage of barley	PMB100
PM + 25% Annual ryegrass: 5 kg ha^{-1}	Heading stage of annual ryegrass	Milk stage of annual ryegrass	PMAR25
PM + 50% Annual ryegrass: 10 kg ha^{-1}	Heading stage of annual ryegrass	Milk stage of annual ryegrass	PMAR50
PM + 75% Annual ryegrass: 15 kg ha^{-1}	Heading stage of annual ryegrass	Milk stage of annual ryegrass	PMAR75
PM + 100% Annual ryegrass: 20 kg ha^{-1}	Heading stage of annual ryegrass	Milk stage of annual ryegrass	PMAR100
PM + 25% Berseem clover: 5 kg ha^{-1}	Bud stage of berseem clover	Full flowering of berseem clover	PMBC25
PM + 50% Berseem clover: 10 kg ha^{-1}	Bud stage of berseem clover	Full flowering of berseem clover	PMBC50
PM + 75% Berseem clover: 15 kg ha^{-1}	Bud stage of berseem clover	Full flowering of berseem clover	PMBC75
PM + 100% Berseem clover: 20 kg ha^{-1}	Bud stage of berseem clover	Full flowering of berseem clover	PMBC100

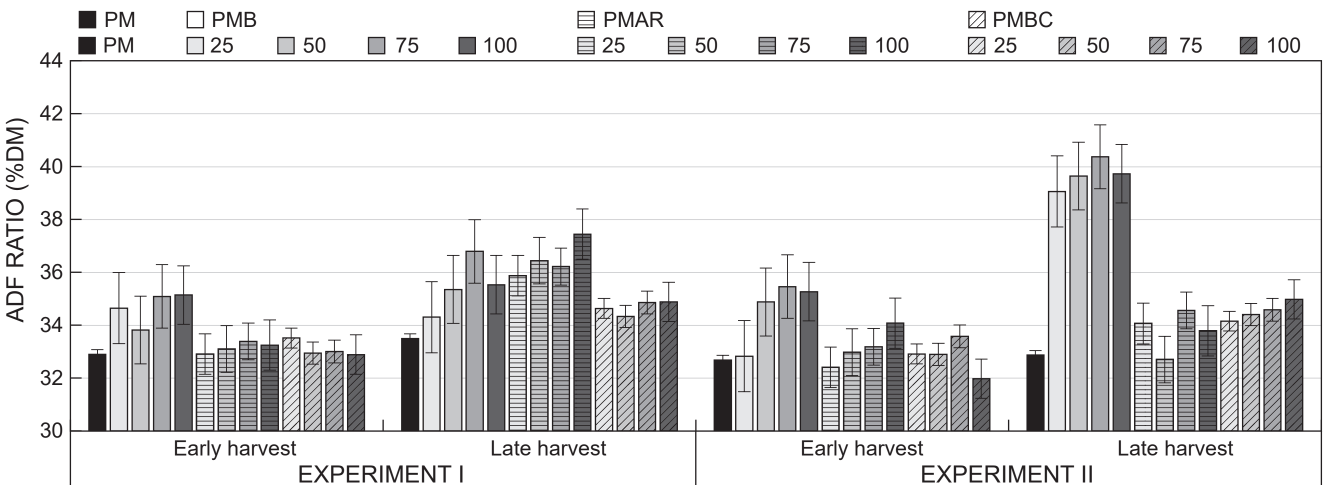

Figure 2: Changes in the average ADF content of perennial pasture mixtures sown at different seeding rates of the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

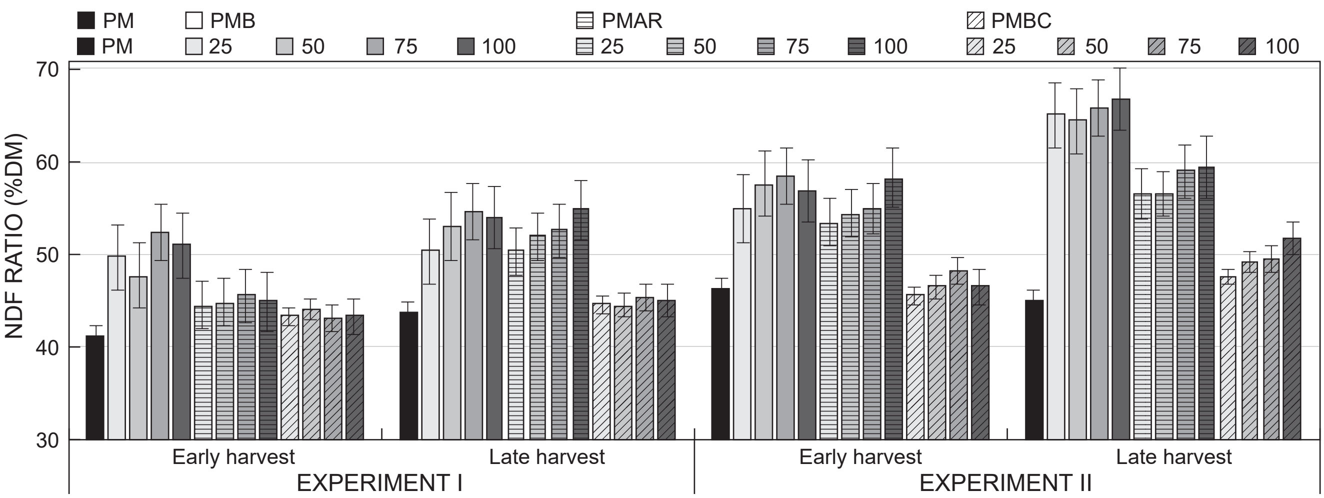

Figure 3: Changes in the average NDF content of perennial pasture mixtures sown at different seeding rates for the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

characteristics were compared using Duncan's multiple range test ($p \leq 0.05$). Graphics were created using Excel 2016.

Results

Acid detergent fibre content

According to the results of variance analysis, the effects of harvest stage ($p \leq 0.01$) and companion crop ($p \leq 0.01$) factors was well as the interactions of experiment × companion crop ($p \leq 0.01$), experiment × harvest stage × companion crop ($p \leq 0.01$) on ADF content were statistically significant (Table 2).

In the study, while the average ADF content was 33.5% at the early harvest stage, it showed a significantly higher value of 35.6% at the late harvest stage (Table 3). The mean ADF content was significantly lower in the pasture mixture without companion crop treatment (33.0%) than those in the mixtures with companion crop treatments of barley with all seeding rates and annual ryegrass with 75% and 100% seeding rates. The ADF contents of PMBC50 (33.7%), PMBC100 (33.7%), PMBC25 (33.8%), PMAR25 (33.8%), and PMAR50 (33.8%) treatments were not statistically significant different from that of the pasture mixture without companion crop treatment. The highest average ADF was found for the PMB75 (36.9%) treatment but the PMB100 (36.4%) and PMB50 (35.9%) treatments were not statistically significant ($p < 0.01$) different from it.

In the first experiment, the mean ADF content varied between 32.9% and 37.4% depending on the harvesting stages, while in the second experiment, the mean ADF content varied between 32.0% and 40.4% depending on the harvesting stages (Figure 2).

Neutral detergent fibre content

The results of the analysis of variance showed that experiment, harvest stage and companion crop factors and binary and triple interactions made a significant difference in the

Table 2: Results of variance analysis for different quality characteristics

Measurements	Experiment (E)	Harvest stage (HS)	Companion crop (CC)	E × HS	E × CC	HS × CC	E × HS × CC	CV (%)
ADF (DM%)	ns	**	**	ns	**	ns	**	3.88
NDF (DM%)	**	**	**	ns	**	**	**	4.20
CP (DM%)	**	**	**	ns	**	**	**	4.51
RFV	**	**	**	ns	*	ns	**	5.04
CPY (kg ha^{-1})	*	**	**	ns	**	**	**	14.60
DDMY (kg ha^{-1})	**	**	**	ns	**	**	**	12.93

CP = crude protein content CV = coefficient of variation RFV = relative feed value CPY = crude protein yield
DDMY = digestible dry matter yield * $p \leq 0.05$, ** $p \leq 0.01$, ns = nonsignificant

Table 3: Averages of ADF, NDF, crude protein content (CP), relative feed value (RFV), crude protein yield (CPY) and digestible dry matter yield (DDMY) of a perennial pasture mixture sown at different seeding rates for different companion crops and when harvested at two maturity stages of companion crops

	ADF (DM %)	NDF (DM %)	CP (DM %)	RFV	CPY (kg ha^{-1})	DDMY (kg ha^{-1})
Experiment (E)						
Experiment I	34.5	47.7^{b1}	18.5^{a}	122.1^{a}	2 288^{a}	7 738^{b}
Experiment II	34.6	54.6^{a}	15.7^{b}	107.6^{b}	2 127^{b}	8 861^{a}
Harvesting stage (HS)						
Early harvest	33.5^{b}	49.2^{b1}	17.8^{a}	120.4^{a}	2 016^{b}	7 198^{b}
Late harvest	35.6^{a}	53.1^{a}	16.3^{b}	109.3^{b}	2 399^{a}	9 401^{a}
Companion crop (CC)						
PM	33.0^{e}	44.1^{g}	20.7^{a}	133.8^{a}	1 688^{e}	5 124^{h}
PMB25	35.2^{bc}	55.0^{c}	15.1^{bc}	105.6^{de}	2 184^{cd}	9 141$^{a–c}$
PMB50	35.9^{ab}	55.6^{bc}	15.1^{bc}	103.5^{ef}	2 147^{cd}	8 884$^{b–d}$
PMB75	36.9^{a}	57.8^{a}	14.4^{d}	98.0^{g}	2 257^{bc}	9 720^{ab}
PMB100	36.4^{a}	57.1^{ab}	14.5^{cd}	100.0^{fg}	2 308^{bc}	9 969^{a}
PMAR25	33.8^{de}	51.2^{e}	15.7^{b}	114.8^{c}	2 095^{cd}	8 503$^{c–f}$
PMAR50	33.8^{de}	51.9^{e}	15.7^{b}	113.0^{c}	2 147^{cd}	8 706$^{c–e}$
PMAR75	34.3^{cd}	53.0^{de}	14.7^{cd}	110.2^{cd}	1 936^{d}	8 406$^{c–f}$
PMAR100	34.6^{cd}	54.3^{cd}	14.5^{cd}	107.6^{de}	2 043^{cd}	8 965$^{b–d}$
PMBC25	33.8^{de}	45.3^{fg}	20.6^{a}	128.9^{b}	2 265^{bc}	6 891^{g}
PMBC50	33.7^{de}	46.1^{f}	20.3^{a}	126.9^{b}	2 526^{ab}	7 805$^{e–g}$
PMBC75	34.0^{de}	46.6^{f}	20.2^{a}	125.1^{b}	2 474^{ab}	7 645^{fg}
PMBC100	33.7^{de}	46.7^{f}	20.2^{a}	125.6^{b}	2 628^{a}	8 136$^{d–f}$
Average	34.6	51.1	17.1	114.8	2 208	8 300

Averages with different letters are statistically significantly different; there is no statistically significant difference between the averages shown with similar letters in the same column according to Duncan's test ($p \leq 0.05$)

NDF content at the 1% level, except for the experiment × harvest stage interaction (Table 2).

Averaged over all the treatments, the NDF content was 47.7% in the first year and 54.6% in the second year, which was significantly higher than that in the first experiment. The NDF content was significantly higher ($p < 0.01$) for the late harvest stage (53.1%) than the early harvest stage (49.2%).

The average NDF content in different companion crop treatments varied between 44.1% and 57.8% and this variation was found to be statistically significant (Table 3). In the PM treatment, the average NDF (44.1%) was significantly lower than in the other companion crop treatments, except for the companion crop treatment in which berseem clover was included at 25% seeding rate (45.3%). The average NDF content was found to be significantly higher in the companion crop treatment with 75% seeding rate of barley (57.8%) than in other companion crop treatments where barley was included at a 100% seeding rate (57.1%).

The lowest NDF content was for the PM treatment during both years, although for the early harvest and late harvest stage during years 1 and 2, respectively. The lowest NDF ratios were reported for PMAR100 and PMB100 at the late harvest stage during year 1 and year 2, respectively (Figure 3).

Crude protein content

The results of the analysis of variance showed that experiment, harvest stage and companion crop factors as well as binary and triple interactions made significant differences in the crude protein content at the 1% level except for the experiment × harvest stage interaction (Table 2).

The average crude protein content, which was determined as 18.5% in the first experiment, showed a significantly lower value in the second experiment where it was determined as 15.7%. The average crude protein content of companion crop treatments was statistically significantly lower at the late harvest stage (16.3%) than the early harvest stage (17.8%) (Table 3). In the study, the average crude protein content

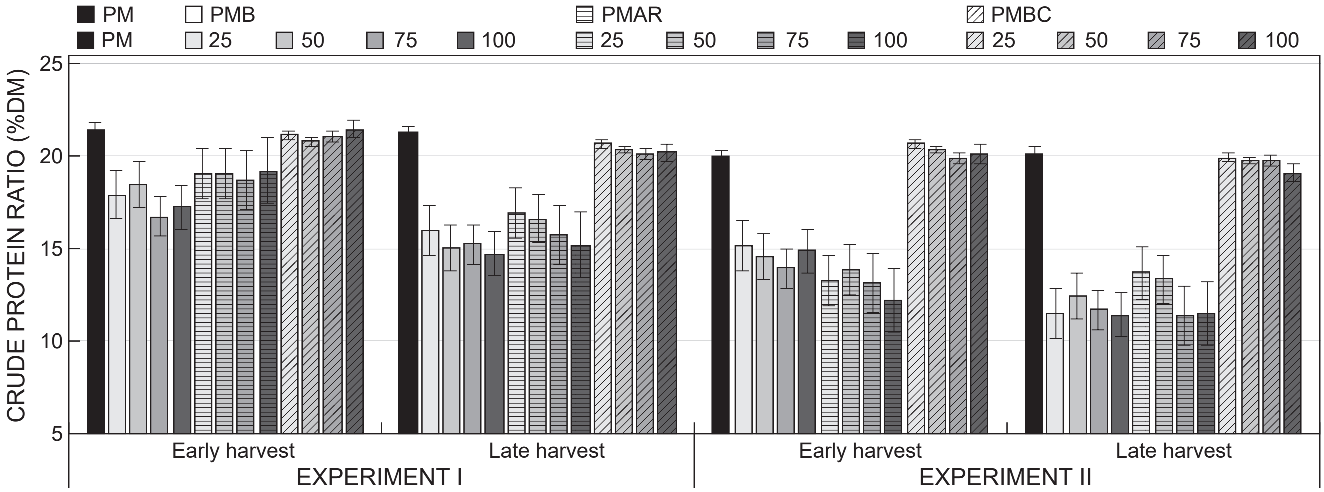

Figure 4: Changes in the average crude protein content of perennial pasture mixtures sown at different seeding rates for the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

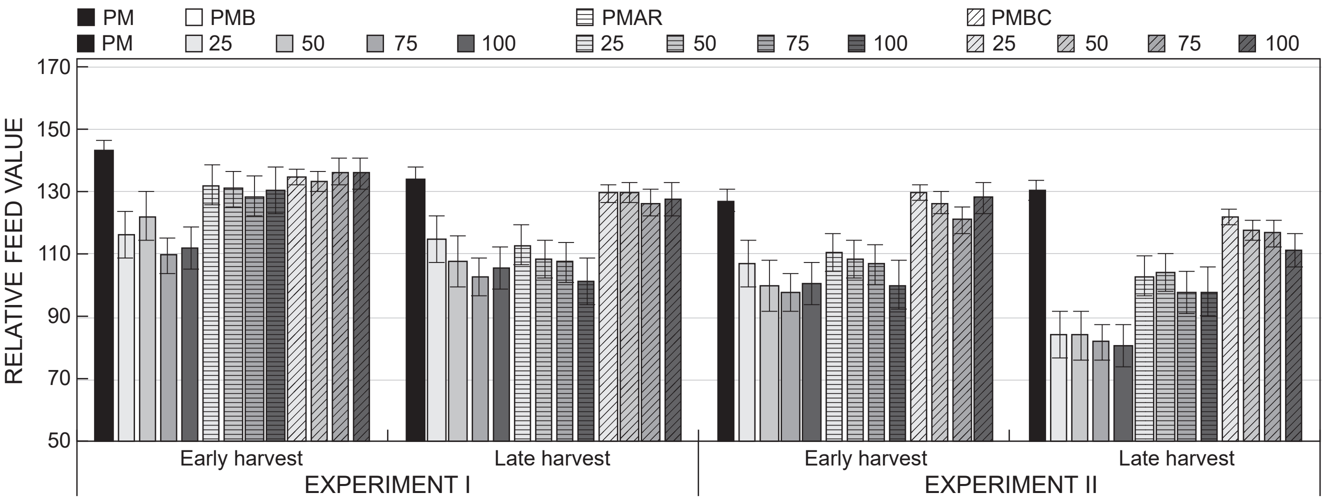

Figure 5: Changes in the average relative feed value of perennial pasture mixtures sown at different seeding rates for the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

varied between 14.4% and 20.7% in different companion crop treatments and this variation was found to be statistically significant (Table 3).

In the first experiment, the average crude protein content varied between 14.7% and 21.4% in different companion crop treatments depending on the harvesting stages, while in the second experiment, the average crude protein content ranged between 11.4% and 20.6% in different companion crop treatments depending on the harvesting stages (Figure 4).

Relative feed value

The results of variance analysis showed that the factors of experiment ($p \leq 0.01$), harvest stage ($p \leq 0.01$), companion crop ($p \leq 0.01$) and the interactions of experiment × companion crop ($p \leq 0.05$), experiment × harvesting stage × companion crop ($p \leq 0.01$) made a significant difference in relative feed value (Table 2).

The average relative feed value, which was 122 in the first experiment, showed a significantly lower value in the second experiment compared to the first experiment and was determined as 108. In the study, the average relative feed value as the average of different companion crop treatments was 120 at the early harvest stage while it was 109 at the late harvest stage, which was significantly lower than that at the early harvest stage. The average of the relative feed value varied between 98 (PMB75) and 134 (PM) in different com- panion crop treatments and this change was found to be statistically significant (Table 3).

In the first experiment, the average of relative feed value in the companion crop treatments with 50% seeding rate of barley, 75% seeding rate of berseem clover and all seeding rates of annual ryegrass was statistically significantly lower at the late harvest stage compared to the early harvest stage, whereas the average of relative feed value in the other companion crop treatments did not differ significantly with the harvest stages. On the other hand, in the second trial, the

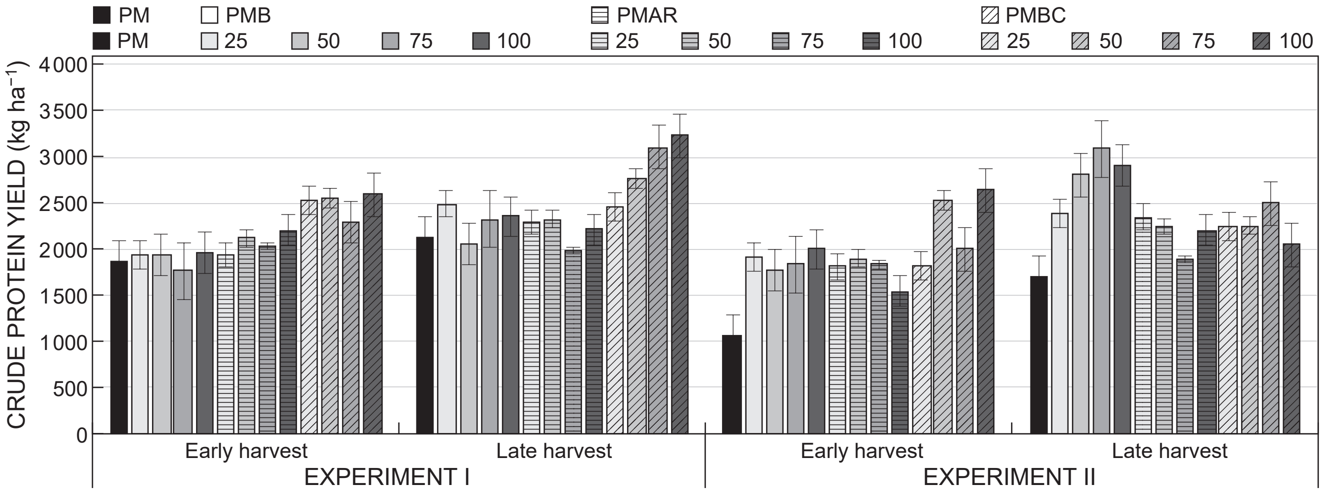

Figure 6: Changes in the average crude protein yield of perennial pasture mixtures sown with different seeding rates for the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

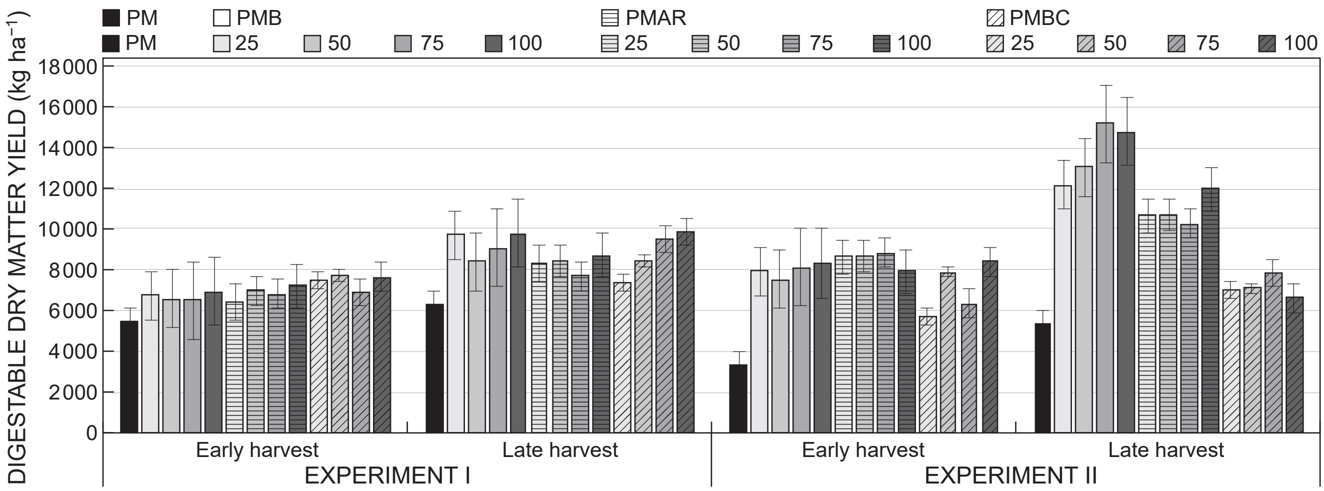

Figure 7: Changes in the average digestible dry matter yield of perennial pasture mixtures sown at different seeding rates for the different companion crop species and harvested at two different maturity stages of companion crops (I: standard error of mean)

average of relative feed value in the companion crop treatments with 100% seeding rate of berseem clover and all seeding ratios of barley was significantly lower in late harvest compared to early harvest, whereas the average of relative feed value in the other companion crop treatments did not show a significant difference depending on the harvest stages (Figure 5).

Crude protein yield

The results of variance analysis showed that the factors of experiment ($p \leq 0.05$), harvest stage ($p \leq 0.01$), companion crop ($p \leq 0.01$) and the interactions of experiment × companion crop ($p \leq 0.01$), harvesting stage × companion crop ($p \leq 0.01$), and experiment × harvesting stage × companion crop ($p \leq 0.05$) made a significant difference in relative feed value (Table 2).

The average crude protein yield was significantly lower in the second year (2 121 kg ha^{-1}) compared to the first year (2 288 kg ha^{-1}). In the study, the average crude protein yield, which showed a value of 2 016 kg ha^{-1} at the early harvest stage, was higher at the late harvest stage compared to the early harvest stage and was determined as 2 399 kg ha^{-1}. The average crude protein yield varied between 1 688 kg ha^{-1} (PM) and 2 628 kg ha^{-1} (PMBC100) in different companion crop treatments and this variation was found to be statistically significant (Table 3).

In the first experiment, the average crude protein yield ranged from 1 770 kg ha^{-1} (PMB75) to 3 229 kg ha^{-1} (PMBC100) in different companion crop treatments depending on harvest stages, while in the second experiment, the average crude protein yield ranged from 1 055 kg ha^{-1} (PM) to 3 093 kg ha^{-1} (PMB75) in different companion crop treatments depending on harvest stages (Figure 6).

Digestible dry matter yield

The results of the analysis of variance showed that the effect of experiment ($p \leq 0.01$), harvest stage ($p \leq 0.01$), companion crop treatment ($p \leq 0.01$) and the interaction of experiment ×

companion crop ($p \leq 0.01$), harvest stage × companion crop ($p \leq 0.01$) and experiment × harvest stage × companion crop ($p \leq 0.01$) on digestible dry matter yield were statistically significant (Table 2).

In the study, the average digestible dry matter yield was significantly higher in the second experiment (8 861 kg ha^{-1}) compared to the first experiment (7 738 kg ha^{-1}) (Table 3). The average digestible dry matter yield, which was 7 198 kg ha^{-1} at the early harvest stage, increased significantly as the harvest stage progressed and was determined as 9 401 kg ha^{-1} (Table 3).

In the study, while the digestible dry matter yield of 5 124 kg ha^{-1} in the pure sown pasture mixture (PM) was significantly lower than the results in the tested companion crop treatments, the digestible dry matter yield of the companion crop treatment, in which barley was included at 100% seeding rate (PMB100), was significantly higher than in the other companion crop treatments tested, except for the companion crop treatments of the barley with seeding rates at 75% and 25% (Table 3).

In the first trial, the average digestible dry matter yield ranged from 5 520 kg ha^{-1} (PM) to 9 856 kg ha^{-1} (PMBC100) in different mixture treatments depending on the harvesting stages, while in the second trial, the average digestible dry matter yield ranged from 3 348 kg ha^{-1} (PM) to 15 204 kg ha^{-1} in different mixture treatments depending on the harvesting stages (Figure 7).

Discussion

This research revealed that including the right species of companion crop at the optimum seeding rate in the pasture mixture and harvesting the companion crop at the appropriate time greatly improved the quality of the forage in the first year of establishment. However, the values related to the quality characteristics examined in the research (NDF, CP, RFV, CPY, DDMY) showed that the effect of the experiment carried out in two different years on the digestibility and quality of the mixed herbage is significant. Due to frost damage in February of the first year and the fact that 31% of the total rainfall occurred in 4 days during the growing season (Figure 1), the companion crop species were damaged during the establishment phase. In addition, due to the increased temperatures in the early period, the companion crop species entered the reproductive phase and could not show sufficient vegetative development (Chen et al. 2019). In the second experimental year, cooler weather (Figure 1), normal rainfall distribution and regular irrigation increased the biomass production of companion crop species. The temperatures were more suitable between December and March for the development of barley and annual ryegrass in the second year compared to the first year, and therefore a higher contribution of the companion species to the sward resulted in differences in NDF, CP, CPY and DDMY values between years. In addition, the fact that the relative feed value, which is used to determine the quality and price of the forage calculated with ADF and NDF contents (Ball et al. 2001; Cinar and Hatipoglu 2015), varied depending on the years of the experiment can be explained by the difference in NDF content in the different years. Temperature has a significant effect on the quality of forage, as it has been reported that cell wall materials are less lignified at low temperatures, while lignin synthesis increases at high temperatures (Nelson and Moser 1994). In addition, Buxton (1995) reported that high temperatures accelerated plant maturation and stem growth, decreased the leaf/stem ratio, and with the decrease in this ratio, the crude protein content of the plant decreased. In another study, Mueller and Orloff (1994) reported that the effect of rainfall on forage quality varied depending on the amount, intensity and duration of rainfall. With excessive rainfall, soluble nutrients were leached out and as the amount and duration of rainfall increased, the digestibility of the forage decreased and the fibre concentration increased. Sulc et al. (1993) reported that the amount and seasonal distribution of rainfall had a great effect on the herbage quality of the plant in alfalfa established with a companion crop.

The results of the study revealed that besides the significant effect of the different years on the forage quality, the harvest stages determined according to the maturation period of the companion crop also had a significant effect. Some quality parameters (ADF, NDF, CPY, DDMY) increased while CP and RFV values decreased as the harvest stage of the companion crops progressed. Indeed, it is an expected result that ADF, NDF, CPY and DDMY values showed significantly higher values at the late harvest stage compared to the early harvest stage, while CP and RFV values showed higher values at the early harvest stage compared to the late harvest stage. The ADF content expresses the amount of cellulose and lignin in the cell walls of plant material. Cellulose is the basic building block of plant cells and affects the digestibility of plant materials. Lignin, on the other hand, is a substance that provides the hardness and resistance of plant cells and reduces digestibility (Stokes and Prostko 1998; Yavuz et al. 2009). As forage crops mature, the amount of lignin and cellulose in the cell wall increases and the digestibility of the forage decreases (Putnam and Orloff 2016; Asci and Acar 2018). In addition, delaying the harvest of plants increases the NDF content and as this occurs the feed intake potential for the animal decreases (Ball et al. 2001). Brink and Marten (1986) reported that the NDF content increased with the progression of the harvest stage of the companion crop in the establishment of different companion crop applications with alfalfa. Asci and Acar (2018) reported that as a plant develops, its dry matter content increases, but the nitrogen content in plant tissues becomes more diluted, leading to a decrease in crude protein content. Klebesadel and Smith (1960) reported that protein yield decreased linearly with the progression of the harvesting stage of the companion crop in the establishment of alfalfa with a companion crop of oats.

In the study, the ADF content in the pasture mixture established with barley and annual ryegrass (except for PMAR25 and PMAR50) showed higher values than the pasture mixture without companion crops, while the ADF content in the pasture mixture established with berseem clover showed similar values with the pasture mixture without companion crops. In our study, lower NDF content, CPY, DDMY value and higher RFV value were determined in pasture mixture without companion crop application compared to pasture mixtures with companion crop application. The CP content showed similar values in the pasture mixture which did not contain any companion crop application and in the pasture mixture in which berseem clover was included. It has been reported that barley and annual ryegrass are fast-growing species and that these species contain higher levels of lignin and cellulose in their cell walls than do legumes (Buxton and Fales 1994; Mertens and Grant 2007), while their CP contents are lower than those found in legumes (Linn and Martin 1989). In addition, the competitive advantage of barley and annual ryegrass species over perennial species in the pasture mixture due to their high tillering tendency and the increase in weed rate due to the poor development of the species in the mixture after removing the companion crops at the first cutting (Cosgrove and Barrett 1987) can be shown as the reasons for the higher ADF and NDF contents in the companion crop treatments including barley and annual ryegrass. Hall et al. (1995) reported that the degree of reduction in herbage quality of what depends on the growth stage of the weed component and the amount of weed at the harvest stage. Wiersma et al. (1999) reported that there was a negative correlation between the proportion of legume in the mixture and ADF and NDF content, while there was a positive correlation with CP; the correlations stated above make this fact clear. Malhi and Foster (2011) reported that the CP rate was higher in pasture mixtures with no companion crop application than in mixtures with companion crop application due to the high proportion of alfalfa in the mixture. Chapko et al. (1991) reported that in alfalfa establishment with different companion crops, the NDF content of the forage differed according to the companion crops. Cicek et al. (2020) reported that the legume-legume mixture contained higher CP and lower NDF than the legume-grain mixture, and that the proportion of legumes in the mixture decreased due to the fact that cereals were more competitive, and the quality of the forage decreased.

In the study, while the effect of companion crop species on the quality of the forage was significant, it was found that the seeding rates of the companion crop did not make a significant difference on the quality of the forage. This situation can be explained by the differences in stem and leaf ratios of perennial forage crops and companion crop species in the pasture mixture due to intraspecific and interspecific competition depending on the seeding rates of the companion crop species in the pasture mixture. Putnam and Orloff (2016) reported that leaf ratio, CP, ADF and lignin values were not affected by the seeding rates of the species in the establishment; the number of stems in the root crown decreased significantly at high plant density, while the stems thickened at low plant density. In addition, Bakir (1985) reports that the amount of seed to be sown in the pasture establishment depends on the seed quality, seed size, growth force of the seedling and the competition index of other species with which it grows.t As the number of seeds sown per square metre increases, the number of seedlings increases but the number of stems decreases, and as the number of seeds sown per square metre decreases, the weed rate increases, the plant stems become coarse, and they are not consumed by animals.

Conclusion

According to the results obtained from the study, climatic variation over years, including rainfall and temperature, had a significant impact on forage quality of all treatments. In addition, it was found that the forage quality was higher in the companion crop treatments with berseem clover than with annual ryegrass and barley. However, the forage quality of the berseem clover treatments did not differ significantly from that of the control pasture treatment without companion crops. Although a significant decrease in the forage quality of the mixture was observed with the advancement of the harvest stage of the companion crop, the seeding rate of the companion crop did not make significant differences in the forage quality of the mixture. In the light of these results, it was concluded that berseem clover can be included in cultivated pasture establishment under Mediterranean climatic conditions in terms of high forage quality, and the harvesting stage should be at the bud stage of berseem clover.

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