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FORAGE YIELD AND QUALITY OF INTERCROPPED ANNUAL RYEGRASS WITH BERSEEM CLOVER SOWN AT DIFFERENT SEED MIXTURE RATIOS UNDER RAINFALL CONDITIONS OF MEDITERRANEAN CLIMATE

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ABSTRACT

This research was carried out under rainfall conditions of the Mediterranean climate to study the effects of different seed mixture ratios (Berseem clover (BC100), annual ryegrass (AR100), BC80-AR20, BC60-AR40, BC40-AR60, BC20-AR80) on forage yield and quality characteristics of mixture. The experiments were conducted in a randomized complete block design with three replications during the growing season of 2020-2021 and 2021-2022. Annual ryegrass sown as pure produced higher green forage yield than berseem clover sown as pure and the mixtures. In the mixture of berseem clover at 20% seed rate and pure annual ryegrass, higher hay yields were determined compared to the other mixtures. In terms of total LER, BC80-AR20 and BC20-AR80 mixtures were the two most advantageous mixtures, while the aggressivity value of annual ryegrass showed a positive value and became the dominant species. Pure sown berseem clover had lower ADF, NDF and higher crude protein content than pure sown annual ryegrass and other mixtures tested. According to these results, it can be said that berseem clover can be included in the mixture at low seed rates for high forage yield under rainfall conditions, while berseem clover can be grown as pure for high forage quality.

Keywords: Legume-grass mixture, seed mixture rate, yield and quality

INTRODUCTION

Intercropping systems, known as low-cost production systems, not only produce more biomass, but also provide a more balanced nutrient supply to ruminants than pure legume or grass sowings (Tahir et al., 2022; De Silva et al., 2023). This system, which is widely applied in Mediterranean countries (Salama, 2020; Ertekin and Yilmaz, 2022), serves the ecosystem by reducing the use of chemicals (pesticides, herbicides, fertilizers) and ensures the sustainability of agriculture due to climate change (Lithourgidis et al., 2011; Brooker et al., 2015; Erkovan, 2022). The fact that legume-grass species within this system have different responses to diseases, pests, soil and climatic conditions and different abilities to use limited resources minimises the risks in terms of crop production (Atis et al., 2012). Indeed, in legume-grass mixtures, legumes reduce the need for nitrogen fertiliser by fixing nitrogen (Giambalvo et al., 2011; Raza et al., 2023) and increase the quality of the mixture forage with high protein content (Solomon et al., 2011; Rajab et al., 2021). On the other hand, grasses reduce legume-induced risks such as bloat (Vasilakoglou and Dhima, 2008), bone disorders (Hall et al., 1991), provide structural support and also minimize harvest losses (Salama and Badry, 2015). In addition, it should not be forgotten that the intercropping system will reduce the grazing pressure on meadow-pasture areas, which are dependent on natural rainfall, especially in countries such as Turkey, which is located in the arid zone, and will meet the quality roughage needs of livestock by contributing to the utilisation of lands left vacant in the winter period in barren areas (Kavut et al., 2014).

Although intercropping systems have many advantages mentioned above, it is quite difficult to establish mixtures due to the difference in agricultural practices of the species included in the mixture compared to pure sowing. Sowing times, fertiliser and water requirements, phenology and harvesting times may be different for species grown in mixed cropping (Droushiotis, 1989). In addition, species in the mixture may compete with each other in terms of light, water and nutrients, leading to yield losses (Chen et al., 2004). In this context, it is necessary to select legume and grass species suitable for the ecological conditions in which the crop will be established and to adjust the seed rates of these species correctly in order to obtain forage with high yield and quality and to ensure the sustainability of the mixed crop (Osman and Nersoyan, 1986). Indeed, Caballero et al. (1995) reported that oat had a competitive advantage over vetch, and that oat sown pure gave higher herbage yield than vetch-oat mixtures. Hatipoglu et al. (2005) reported that in mixtures of Persian clover and annual ryegrass at different seeding rates, pure Persian clover and different mixture treatments gave higher quality herbage yields than pure annual ryegrass. El-Karamany et al. (2014) reported that in mixtures of berseem clover and barley at different seeding rates, pure sown berseem clover gave higher forage yield than the mixtures, but had lower dry matter ratio than the other mixtures. Salama (2020) studied the forage yield of berseem clover and mixtures of some grass species at different seeding rates and found that the highest forage yield was recorded in the mixture of berseem clover and triticale (75%:25%).

The aim of this study was to determine the effect of seed mixture ratios on the forage yield and quality of a mixture of berseem clover and annual ryegrass, which can be grown as an alternative crop for the crop rotation of wheat under rainfall conditions.

MATERIALS AND METHODS

Site Description

This study was conducted for two years (2020-2021 and 2021-2022) at the research and application field of Kozan Vocational School of Cukurova University (37°27'57"N, 35°48'12"E, altitude: 151 m). According to the results of the analysis of the soil samples taken from the experimental area, the soil had a pH of 7.50, a high lime content (26.9%), a low phosphorus content (47.7 kg ha^{-1}), a high potassium content (922.7 kg ha^{-1}) and a clayey texture. The daily average precipitation and temperature values of Kozan district, where the research was conducted, obtained from the 6th Regional Directorate of Meteorology, and the cumulative precipitation values for the growing seasons and long years are shown in Figure 1. As seen in Figure 1, the cumulative rainfall realised in 2020/2021 (390.4 mm) and 2021/2022 (492.0 mm) was below the long-term average cumulative rainfall (542.6 mm). In addition, the average monthly temperature in the 2020-2021 and 2021-2022 growing seasons was 13.8 °C and 12.2 °C, respectively.

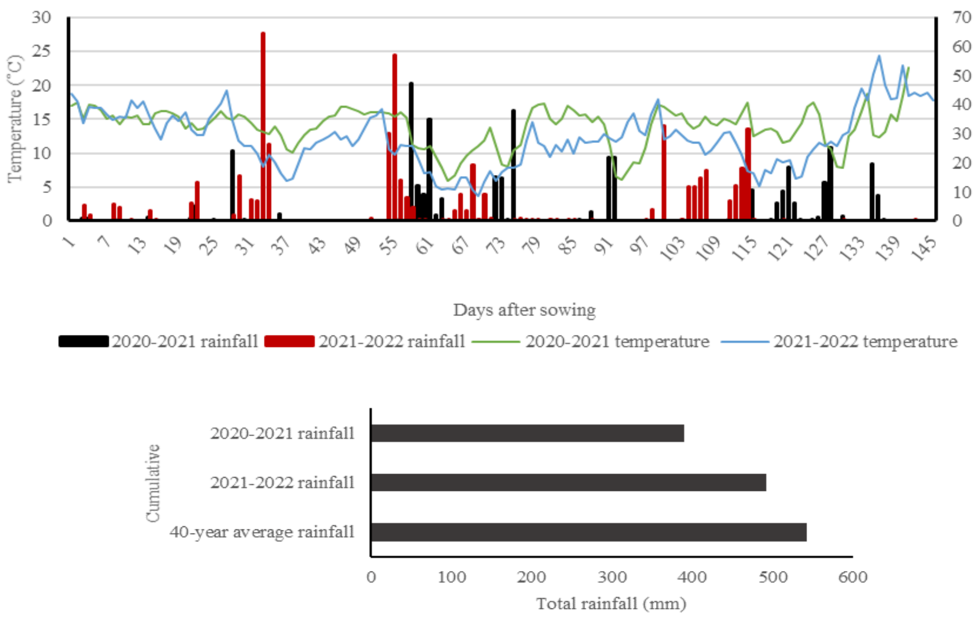

Figure 1. Some climatical values of experimental area for the experiment periods and long term averages

Experimental Design and Treatments

The experiment was conducted in a complete randomized block design with three replications. Treatments were; berseem clover (*Trifolium alexandrinum* L. cv Derya) in pure stand (BC); annual ryegrass (*Lolium multiflorum* Lam. cv Elif) in pure stand (AR); and their mixture with different seeding ratios (BC80-AR20; BC60-AR40; BC40-AR60; BC20-AR80). Pure stands were sown at a sowing ratio of 25 kg ha^{-1} for berseem clover and 20 kg ha^{-1} for annual ryegrass (Acikgoz, 2001).

Agricultural Practices

In both years of the study, the experimental plots were left fallow during the summer and prepared for sowing in early autumn after ploughing and levelling. In the experiment, each plot consisted of 6 rows of 5 m length. The row spacing was 20 cm. In both growing seasons, mixed and pure stands were sown by hand on November 18. The seeds of the species in the mixture were mixed and sown in the same row. Diammonium phosphate (18-46-0) fertilizer (100 kg ha^{-1}) was applied to the area where the experiment was conducted. There was no irrigation during the study years. No herbicide treatments were used; all plots were kept free of weeds by hand-weeding.

Sampling and Measurements

In both years of the study, harvesting was carried out on 07.04.2021 and 12.04.2022, during the full flowering period of berseem clover (Yucel et al., 2018a). At harvest time, three 60x60 cm wooden frames were randomly placed in each plot and clipped with sickle at a stubble height of 5 cm. In each plot, green forage weight of the plot was calculated by taking the average of the green weights determined in three sampling areas, and green forage yield per hectare was calculated by making the necessary conversions. The mixture species harvested from the three sampling sites were manually separated and each species was placed in different paper bags and dried in dry oven at 70 °C for 48 hours. The dried samples were allowed to reach room temperature and the dry weights of each species were determined. The dry weights of berseem clover and annual ryegrass were collected for each sampling area and the dry weight of the plot was determined by averaging the total dry weights of the species in the three sampling areas. Then, hay yield per hectare was calculated by making the necessary conversions. The dry weight of berseem clover determined in each sampling area was proportioned to the total dry weight and the proportion of berseem clover in the botanical composition by weight was calculated by taking the average of the values determined from the three sampling areas. The dried samples of each species were ground to pass through a 1 mm screen. Crude protein was determined by the Kjeldahl procedure (N concentration x 6.25) (Kacar and Inal, 2010). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were sequentially determined as described by Van Soest et al. (1991) using the semiautomatic ANKOM^{220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Relative feed value (RFV) was calculated using the equations described by Sheaffer et al. (1995);

Digestible dry matter (DDM, % of body weight) = 88.9 - (0.779 x ADF, % of DM) (Eq. 1)

Dry matter intake (DMI, % of DM) = 120 / NDF, % of DM (Eq. 2)

Relative feed value (RFV) = DDM (% of body weight) x DMI (% of DM) / 1.29 (Eq. 3)

The land equivalent ratio (LER) was used as index for mixed stand advantage for both legume (berseem clover) and grass (annual ryegrass) (Willey, 1979). LER values were calculated as follow;

$$LER = LER_{annual\ ryegrass} + LER_{berseem\ clover}, \quad (Eq.\ 4)$$

$$LER_{annual\ ryegrass} = \frac{Y_{ab}}{Y_a}, \quad LER_{berseem\ clover} = \frac{Y_{ba}}{Y_b} \quad (Eq.\ 5)$$

Where Y_b and Y_a were the yields of berseem clover and annual ryegrass as sole crop, respectively, and Y_{ab} and Y_{ba} were yields of berseem clover and annual ryegrass in the mixture, respectively.

Aggressivity (A) was calculated using the equations described by McGilchrist (1965);

$$A_{annual\ ryegrass} = \frac{Y_{ab}}{Y_a \times Z_{ab}} - \frac{Y_{ba}}{Y_b \times Z_{ba}} \quad (Eq.\ 6)$$

$$A_{berseem\ clover} = \frac{Y_{ba}}{Y_b \times Z_{ba}} - \frac{Y_{ab}}{Y_a \times Z_{ab}} \quad (Eq.\ 7)$$

Where, Z_{ba} and Z_{ab} were the seed rates of berseem clover and annual ryegrass in the seed mixture.

Competitive ratio (CR) is another index used to determine the competition between species in a mixture. The CR index was formulated as follows (Atis et al., 2012);

$$CR_{annual\ ryegrass} = \frac{LER_{annual\ ryegrass}}{LER_{berseem\ clover}} \times \frac{Z_{ab}}{Z_{ba}} \quad (Eq.\ 8)$$

$$CR_{berseem\ clover} = \frac{LER_{berseem\ clover}}{LER_{annual\ ryegrass}} \times \frac{Z_{ba}}{Z_{ab}} \quad (Eq.\ 9)$$

Statistical Analyses

The yield and quality values obtained from the research were analysed by analysis of variance (ANOVA) in the MSTAT-C (Michigan State University V. 2.10) statistical package program. As a result of the analysis of variance, treatment means for the statistically significant were compared using the Duncan test at a significance level of $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS

Green Forage Yield

In the research carried out for two years, year and mixture factors and year x mixture interaction made a statistically significant ($P \leq 0.01$) difference in green forage yield (Table 1).

Table 1. Mean squares for traits in different mixture treatments

Measurements	Year (Y)	Mixture (M)	Y x M
Green forage yield (t ha^{-1})	116.10**	70.36**	21.71**
Hay yield (t ha^{-1})	16.30**	19.55**	2.98**
Berseem clover ratio (%)	1142.39**	2612.00**	12.59ns
LER value of berseem clover	0.01ns	0.36**	0.04**
LER value of annual ryegrass	0.01ns	0.32**	0.01ns
Total LER value	0.03ns	0.03*	0.03ns
Aggressivity value of berseem clover	0.09ns	0.58**	0.23*
Aggressivity value of annual ryegrass	0.09ns	0.58**	0.23*
Competitive ratio of berseem clover	0.13*	0.50**	0.24**
Competitive ratio of annual ryegrass	0.02ns	0.99**	0.22ns
NDF (DM%)	109.69**	35.40*	20.59ns
ADF (DM%)	136.45**	24.32*	15.17ns
Crude protein (DM%)	61.91**	52.57**	13.05**
Relative feed value	871.74**	60.46ns	136.03ns
Degrees of Freedom (df)	1	5^{+}	5^{+}

*:P≤0.05, **: P≤0.01, ns: not significant, $^{+}$3 for Berseem clover ratio, LER values, Aggressivity values and Competitive ratios

In the first year of the research, the averaged green forage yield was 17.0 t ha^{-1}, while in the second year of the research; the averaged green forage yield was significantly higher than the first year of the research and was determined as 20.6 t ha^{-1}. According to the two-year mean values, the average green forage yield ranged from 15.6 t ha^{-1} (BC80-AR20) to 24.2 t ha^{-1} (AR100) in different mixture treatments (Table 2).

Table 2. Means of green forage yield, hay yield and berseem clover ratio determined in different mixture treatments.

Mixtures	Green forage yield (t ha^{-1})			Hay yield (t ha^{-1})			Berseem clover ratio (%)		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC100	10.8 h*	21.5 c	16.2 d	1.6 f	3.8 cd	2.7 d	-	-	-
BC80-AR20	13.6 g	17.6 e	15.6 d	2.6 e	3.7 d	3.2 c	57.3	67.6	62.5 a
BC60-AR40	14.7 f	17.8 e	16.2 d	2.7 e	3.9 cd	3.3 c	22.8	39.2	31.0 b
BC40-AR60	19.7 d	19.3 d	19.5 c	3.5 d	4.3 bc	3.9 b	15.0	28.1	21.5 c
BC20-AR80	20.3 d	21.5 c	20.9 b	3.9 cd	4.6 ab	4.3 a	7.5	23.3	15.4 d
AR100	22.7 b	25.8 a	24.2 a	4.3 bc	4.9 a	4.6 a	-	-	-
Mean	17.0 b[1]	20.6 a		3.1 b	4.2 a		25.7 b	39.6 a	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

The fact that the year x mixture interaction was found to be significant indicates that the effects of the years on green forage yield differed significantly depending on the mixture treatments. As a matter of fact, while the average green forage yield did not differ significantly in BC40-AR60 mixture, the average green herbage yield in the other mixtures tested showed a significantly higher value in the second year compared to the first year (Table 2).

Hay Yield

The results of variance analysis revealed that year (P≤0.01), mixture (P≤0.01) and year x mixture (P≤0.01) interactions had a statistically significant effect on hay yield (Table 1).

In parallel with the green forage yield, the averaged hay yield was significantly higher in the second year (4.2 t ha^{-1}) compared to the first year (3.1 t ha^{-1}) of the study (Table 2). According to the two-year averages, the mean hay yield varied between 2.7 t ha^{-1} (BC100) and 4.6 t ha^{-1} (AR100) in different mixture treatments (Table 2).

In the 2020-2021 growing season, the average hay yield was between 1.6 t ha^{-1} (BC100) and 4.3 t ha^{-1} (AR100), while in the 2021-2022 growing season, the average hay yield was between 3.8 t ha^{-1} (BC100) and 4.9 t ha^{-1} (AR100) in different mixture treatments (Table 2).

Berseem Clover Ratio

According to the results of the analysis of variance, the effect of year (P≤0.01) and mixture (P≤0.01) factors on the rate of berseem clover was statistically significant, while the year x mixture interaction did not make a statistically significant difference on the rate of berseem clover (Table 1).

In the first year of the research, averaged berseem clover ratio was 25.7%, while in the second year of the research, averaged berseem clover ratio showed a statistically significant higher value and was determined as 39.6%. According to the two-year average values, the ratio of berseem clover in different mixture treatments varied between 15.4% and 62.5%. The highest proportion of berseem clover was found in BC80-AR20 mixture, while the lowest proportion of berseem clover was found in BC20-AR80 mixture (Table 2).

Land Equivalent Ratio (LER)

According to the results of variance analysis, the mixture factor significantly affected the LER value of berseem clover, the LER value of annual ryegrass and total LER value. Also, it was found that year x mixture interaction made a significant difference in the LER value of berseem clover (Table 1).

According to the two-year average values, the averaged LER value of berseem clover ranged between 0.24 and 0.80, and the average LER value of annual ryegrass ranged between 0.25 and 0.78. The highest total LER value average was found in the mixture of BC80-AR20 (1.05), while the mixtures of BC20-AR80 (1.02) and BC40-AR60 (0.98) were in the same statistical group in terms of LER value average (Table 3).

Table 3. Means of LER value of species in mixtures and total LER value determined in different mixture treatments.

Mixtures	LER value of berseem clover			LER value of annual ryegrass			Total LER value		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC80-AR20	0.92 a*	0.67 b	0.80 a	0.26	0.25	0.25 d	1.18	0.91	1.05 a
BC60-AR40	0.39 cd	0.41 c	0.40 b	0.48	0.49	0.48 c	0.86	0.90	0.88 b
BC40-AR60	0.32 cd	0.32 cd	0.32 c	0.68	0.63	0.66 b	1.00	0.95	0.98 ab
BC20-AR80	0.18 e	0.29 d	0.24 d	0.84	0.73	0.78 a	1.02	1.02	1.02 a
Mean	0.45	0.42		0.56	0.52		1.02	0.95	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.

In the study, berseem clover LER value did not show a statistically significant difference in BC60-AR40 and BC40-AR60 mixtures according to growing seasons, whereas in BC80-AR20 mixture, berseem clover LER value showed a significantly lower value in the second year compared to the first year. The LER value of berseem clover in the BC20-AR80 mixture showed a significantly higher value in the second year compared to the first year (Table 3).

Aggressivity (A)

The results of the analysis of variance showed that the effect of the year factor on the aggressivity value was statistically insignificant, while the mixture factor and year x mixture interaction made a significant difference on the aggressivity values of the species in the mixture (Table 1).

According to the two-year average values, the mean of berseem clover aggressivity value varied between -0.54 (BC60-AR40) and 0.20 (BC20-AR80) in different mixture treatments (Table 4).

Table 4. Means of aggressivity value and competitive ratio of species determined in different mixture treatments.

Mixtures	Aggressivity value of berseem clover			Aggressivity value of annual ryegrass			Competitive ratio of berseem clover			Competitive ratio of annual ryegrass		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC80-AR20	-0.14 b*	-0.40 b	-0.27 b	0.14 a	0.40 a	0.27 a	0.92 b	0.70 bc	0.81 b	1.13	1.53	1.33 b
BC60-AR40	-0.55 b	-0.54 b	-0.54 b	0.55 a	0.54 a	0.54 a	0.54 c	0.57 c	0.56 c	1.90	1.88	1.89 a
BC40-AR60	-0.33 b	-0.25 b	-0.29 b	0.33 a	0.25 a	0.29 a	0.72 bc	0.78 bc	0.75 bc	1.46	1.38	1.42 b
BC20-AR80	-0.13 b	0.54 a	0.20 a	0.13 a	-0.54 b	-0.20 b	0.88 b	1.60 a	1.24 a	1.16	0.64	0.90 c
Mean	-0.29	-0.16		0.29	0.16		0.77 b[1]	0.91 a		1.41	1.36	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

Effects of the year on the aggressivity values of berseem clover did significantly change depending on the mixture. In the second year, agressivity value of berseem clover in the mixture of BC20-AR80 was significantly higher than that in the first year while it did not significantly vary in the other mixtures depending on the year (Table 4).

Competitive Ratio (CR)

The results of variance analysis revealed that the effects of year (P≤0.05), mixture (P≤0.01) and year x mixture interaction (P≤0.01) on berseem clover competition rate were statistically significant, while the effect of mixture treatments on annual ryegrass competition rate was significant at 1% level (Table 1).

In the first year of the study, the average competition ratio of berseem clover was 0.77, while in the second year of the study, the average competition ratio of berseem clover showed a significantly higher value compared to the first year of the study and was determined as 0.91. Berseem clover competition ratio varied between 0.56 (BC60-AR40) and 1.24 (BC20-AR80), while annual ryegrass competition ratio varied between 0.90 (BC20-AR80) and 1.89 (BC60-AR40) in different mixture treatments (Table 4).

In the study, the significant effect of year x mixture interaction on the competition rate of berseem clover showed that the effect of the years on the competition rate of berseem clover differed significantly depending on the mixtures. In fact, while the competition rate of berseem clover in the BC20-AR80 mixture showed a significantly higher value in the second year compared to the first year, the competition rate of berseem clover in the other mixtures tested did not differ significantly between years (Table 4).

NDF Ratio

The results of the analysis of variance showed that the effect of year (P≤0.01) and mixtures (P≤0.05) on the NDF ratio made a statistically significant difference (Table 1).

In the first year of the research, the average NDF ratio was determined as 61.9%, while in the second year of the research, the average NDF ratio was significantly lower than the first year of the research and was determined as 58.4%. According to the two-year average values, the average NDF ratio varied between 56.6% (BC100) and 63.3% (BC20-AR80) in different mixture treatments (Table 5).

Table 5. Means of NDF, ADF, crude protein ratio and relative feed value determined in different mixture treatments.

Mixtures	NDF Ratio (DM%)			ADF Ratio (DM%)			Crude protein ratio (DM%)			RFV		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC100	55.0	58.2	56.6 c*	37.4	33.7	35.5 b	22.1 a	19.7 b	20.9 a	98.0	90.5	94.2
BC80-AR20	62.1	55.2	58.7 bc	40.2	35.3	37.8 ab	16.7 de	19.5 bc	18.1 b	83.5	103.5	93.5
BC60-AR40	65.1	59.5	62.3 ab	37.3	34.0	35.7 b	12.7 f	18.2 b-d	15.5 c	81.5	96.6	89.0
BC40-AR60	61.3	58.8	60.0 a-c	41.0	35.0	38.0 ab	12.0 f	17.7 c-e	14.8 c	90.9	98.8	94.9
BC20-AR80	66.3	60.4	63.3 a	42.6	35.2	38.9 ab	13.2 f	16.0 e	14.6 c	81.1	95.0	88.0
AR100	61.8	58.5	60.1 a-c	39.9	41.8	40.8 a	11.9 f	13.3 f	12.6 d	90.7	100.3	95.5
Mean	61.9 a[1]	58.4 b		39.7 a	35.8 b		14.8 b	17.4 a		87.6 b	97.5 a	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

ADF Ratio

The results of the analysis of variance showed that the factors year (P≤0.01) and mixture (P≤0.05) had a statistically significant difference on the ADF ratio (Table 1).

While the average ADF rate was 39.7% in the first year of the study, the average ADF rate in the second year of the study was significantly lower than the first year of the study and was determined as 35.8%. In different mixture treatments of berseem clover and annual ryegrass, the lowest ADF ratio average was 35.5% in the pure sowing of berseem clover, while the highest ADF ratio average was 40.8% in the pure sowing of annual ryegrass (Table 5).

Crude Protein Ratio

According to the results of variance analysis, the effect of year (P≤0.01) and mixture (P≤0.01) factors and year x mixture interaction (P≤0.01) on crude protein ratio was statistically significant (Table 1).

While the average crude protein ratio was 14.8% in the first year of the research, the average crude protein ratio in the second year of the research was significantly higher than the first year of the research and was determined as 17.4%. According to the two-year averages, the mean crude protein ratio was significantly higher in pure sown berseem clover (20.9%) than in the other mixture treatments tested, while the mean crude protein ratio was significantly lower in pure sown annual ryegrass (12.6%) than in the other mixture treatments tested (Table 5).

In the study, the significant effect of the year x mixture interaction on crude protein ratio revealed that the effect of years on crude protein ratio differed significantly depending on the mixtures. As a matter of fact, while the average crude protein ratio of pure sown berseem clover showed a significantly lower value in the second year compared to the first year, the average crude protein ratio of pure sown annual ryegrass did not show significantly different values depending on the years. On the other hand, the average crude protein ratio of the mixture treatments including both species showed a significantly higher value in the second year compared to the first year.

Relative Feed Value (RFV)

The results of the analysis of variance showed that the effect of years (P≤0.01) on RFV was statistically significant, while the effect of mixture and the interaction of year x mixture was not statistically significant (Table 1).

In the first year of the study, RFV was 87.6, while in the second year of the study, RFV was significantly higher than the first year of the study and was found to be 97.5 (Table 5).

DISCUSSION

This study showed that pure sowings and mixtures of annual ryegrass and berseem clover at different seeding rates significantly affected forage yield and some quality characteristics under rainfall conditions. However, the yield values (green forage yield, hay yield) obtained from pure sowings and mixtures of annual ryegrass and berseem clover, and the beseem clover ratio differed depending on the years in which the trials were conducted (2020-2021, 2021-2022). This can be explained by the fact that the first year of the study (2020-2021) was warmer and drier than the second year of the study (2021-2022), which was conducted under rainfall conditions without irrigation (Figure 1). Osman and Nersoyan (1986) reported that the effect of drought on the species in the mixture varied and the herbage yield in the mixture showed lower values in drought years. However, the authors stated that the legume species in the mixture were less affected by drought than the grass species and the reason for this was that the ground water was high in the experimental area where the research was carried out, and the legumes benefited more from this water thanks to their deep roots. In our study, contrary to the researchers, the fact that our experimental area was not in the bottom land and that the legumes could not fully benefit from the limited rainfall in the first experimental year can be considered as the reasons for the lower rate of legume mixture participation in the yield in the first year compared to the second year. (Yucel et al., 2018b) reported that in legume-grass mixtures, the drought resistance of legumes was lower than that of grass and the rate of legume participation in the mixture yield decreased in the drought year. Pedraza et al. (2017) reported that yield losses in legume species in annual forage mixtures grown under different environmental conditions in the dry season (<300 mm) and in grass species in the wet season (>630 mm). On the other hand, the quality of the mixed diets (NDF, ADF, crude protein ratio, RFV) varied according to the year of the study. The higher proportion of berseem clover, an annual legume forage crop, in the mixture yield in the second year compared to the first year and the difference in climatic conditions between years can be considered as reasons for the higher quality of the mixture herbage in the second year compared to the first year. Solomon et al. (2011) reported that the use of legumes alone or in mixtures had a positive effect on crude protein ratio compared to grass forage crops, Thompson et al. (1992) reported that herbage quality increased in mixtures with legumes. In another study, Negesse et al. (2010) reported that low rainfall and high temperature accelerated plant maturation, causing excessive accumulation of structural carbohydrates in the plant and decreased herbage quality.

In addition to the significant effect of years on the forage yield and some quality values obtained from the research, the effect of pure sowing of berseem clover and annual ryegrass and their mixtures at different seed rates were also significant. As a matter of fact, according to the two-year average values, pure sown annual ryegrass gave higher forage yield and hay yield than the mixtures of the two species at different seed rates (except of BC20-AR80) and pure sown berseem clover. This shows that the species in the mixture compete with each other in terms of light, water and nutrients, while intra-specific competition is lower in pure sowings (Caballero et al., 1995). Although many researchers (Osman and Nersoyan, 1986; Giambalvo et al., 2011; Rajab et al., 2021; Meza et al., 2022) reported that legume-grass mixtures gave higher herbage yields than pure sowing of species, Pedraza et al. (2017) reported that under rainfall conditions, there was no significant difference in yield between pure sowing and mixtures of species depending on soil structure, rainfall and frost event during the year. Similarly, Vasilakoglou and Dhima (2008) reported that there was no difference in herbage yield between pure berseem clover and berseem clover-barley mixtures. Lithourgidis et al. (2006) reported that the herbage yields of legume-grass mixtures were lower than those of the pure sowings of the species due to the competition between the mixture species. On the other hand, there was a linear increase in herbage yield as the proportion of annual ryegrass seed in the mixture increased (Ross et al., 2003), while the proportion of berseem clover in the botanical composition decreased linearly. Yucel et al. (2018a) reported that legume species are less competitive than grass species and herbage yield increased as the seed rate of legumes in the mixture decreased.

The results of the study revealed that the mixtures of berseem clover and annual ryegrass at different seed rates had both complementary and competitive effects. Different indexes are used to determine the effect of competition between species in the mixture and the advantage of the mixture (Anil et al., 1998). One of these indexes, LER, shows the efficiency of mixed sowings of species in utilising environmental resources in comparison to pure sowings of species (Mead and Willey, 1980). According to the two-year average values in our study, as the proportion of berseem clover in the botanical composition decreased, the LER value of annual ryegrass increased, while the LER value of beseem clover decreased (Dhima et al., 2007). In general, in different mixtures, the LER value of berseem clover was less than 0.5, while the LER value of annual ryegrass was greater than 0.5. In this case, it shows that the intercropping system is an advantage for annual grass and a disadvantage for berseem clover. In terms of total LER, BC80-AR20 (1.05) and BC20-AR80 (1.02) were the two most advantageous mixtures (Table 3). This result indicates that between 2 and 5 per cent more land is needed to obtain the same yields from the mixture species than from the pure sowing (Ofori and Stern, 1987). Another index used to determine the advantage of a mixture is the aggressivity value, which expresses how much the relative yield increase of one species in the mixture exceeds that of the other species in the mixture (McGilchrist, 1965). In the study, except for the BC20-AR80 mixture, the aggressivity value of annual ryegrass in the mixtures showed a positive value and it was the dominant species. Saia et al. (2016) reported that in legume-grass mixtures, grasses generally had higher aggressivity values and grasses were more dominant species than legumes. On the other hand, the competition ratio is another way of assessing the competition between different species in the mixture. In the study, except for the mixture BC20-AR80, the competitive species in the mixtures was annual ryegrass (Table 4).

In the study, lower NDF, ADF ratio and higher crude protein ratio were determined in pure sown berseem clover compared to pure sown annual ryegrass and different mixture treatments. In addition, there was a linear decrease in the crude protein ratio as the seed rate of berseem clover in the mixture decreased (Salama and Badry, 2015). De Santis et al. (2004) reported that berseem clover leaves were soft textured and had high digestible carbohydrates and protein. In another study, Solomon et al. (2011) reported that the use of legumes in pure or mixed cropping had a positive effect on crude protein ratio compared to grasses. El-Karamany et al. (2014) reported that pure sown legume species gave higher crude protein than grass species and legume-grass species and legume-wheatgrass mixtures. Salama (2020) reported that while the inclusion of berseem clover in the mixtures increased the crude protein content of the mixtures as compared to the pure grasses, there was a lower crude protein content in the mixtures compared to the pure berseem clover.

In the light of these results, it can be said that in regions with low rainfall, it would be more economical for growers who prefer high-yielding forage as an alternative crop for the crop rotation of wheat to establish pure annual ryegrass or to include a low proportion of berseem clover in the mixture. On the other hand, if high quality forage is preferred, either a low proportion of annual ryegrass should be included in the mixture or berseem clover should be established as pure. However, it should be kept in mind that pure berseem clover may cause bloating in animals and may cause bone disorders in animals due to Ca and P imbalance.

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LITERATURE CITED

Acikgoz, E. 2001. Forage Crops. Bursa Uludag University Empowerment Foundation Publication No:182.

Anil, L., J. Park, R.H. Phipps and F.A. Miller. 1998. Temperate intercropping of cereals for forage: A review of the potential for growth and utilization with particular reference to the UK. Grass and Forage Sci. 53(4):301-317.

Atis, I., K. Kokten, R. Hatipoglu, S. Yilmaz, M. Atak and E. Can. 2012. Plant density and mixture ratio effects on the competition between common vetch and wheat. Australian Journal of Crop Sci. 21(4): 975-985.

Brooker, R.W., A.E. Bennett, W.F. Cong, T.J. Daniell, T.S. George, P.D. Hallett, C. Hawes, P.P.M. Iannetta, H.G. Jones, A.J. Karley, L. Li, B.M. Mckenzie, R.J. Pakeman, E. Paterson, C. Schöb, J. Shen, G. Squire, C.A. Watson, C. Zhang, F. Zhang, J. Zhang and P.J. White. 2015. Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. New Phytologist 206(1):107-117.

Caballero, R., E.L. Goicoechea and P.J. Hernaiz. 1995. Forage yields and quality of common vetch and oat sown at varying seeding ratios and seeding rates of vetch. Field Crops Research 41(2):135-140.

Chen, C., M. Westcott, K. Neill, D. Wichman and M. Knox. 2004. Row configuration and nitrogen applicant for barley-pea intercropping in Montana. Agronomy Journal 96(6):1730-1738.

De Santis, G., A. Iannucci, D. Dantone and E. Chiaravalle. 2004. Changes during growth in the nutritive value of components of berseem clover (*Trifolium alexandrinum* L.) under different cutting treatments in a Mediterranean region. Grass and Forage Science 59(4):378-388.

De Silva, C., P. Rathor, H.P. Poudel and M.S. Thilakarathna. 2023. Effects of drought stress on red clover-grass mixed stands compared to grass monoculture stands in nitrogen-deficient systems. Nitrogen (Switzerland) 4(4):382-396.

Dhima, K.V., A.S. Lithourgidis, I.B. Vasilakoglou and C.A. Dordas. 2007. Competition indices of common vetch and cereal intercrops in two seeding ratio. Field Crops Research 100(2-3):249-256.

Droushiotis, D.N. 1989. Mixtures of annual legumes and small-grained cereals for forage production under low rainfall. Journal of Agricultural Science 113:249-253.

El-Karamany, M.F., B.A. Bakry and T.A.E-F. Elewa. 2014. Integrated action of mixture rates and nitrogen levels on quantity and quality of forage mixture from Egyptian clover and barley in sandy soil. Agricultural Sciences 5:1539-1546.

Erkovan, S. 2022. Row seeding configuration regulates yield, quality and competition in common vetch (*Vicia sativa* L.)-Sudangrass (*Sorgum sudanense* (Piper.) Stapf.) mixture. Turkish Journal of Field Crops 27(1):87-94.

Ertekin, I. and Yilmaz, S. 2022. The effects of sowing designs on forage yield and quality of sweet sorghum and mung bean mixtures under Mediterranean conditions. Turkish Journal of Field Crops 27(2):188-199.

Giambalvo, D., P. Ruisi, G. Di Miceli, A.S. Frenda and G. Amato. 2011. Forage production, N uptake, N_2 fixation, and N recovery of berseem clover grown in pure stand and in mixture with annual ryegrass under different managements. Plant and Soil 342(1-2):379-391.

Hall, D.D., G.L. Cromwell and T.S. Stahly. 1991. Effects of dietary calcium, phosphorus, calcium: phosphorus ratio and vitamin K on performance, bone strength and blood clotting status of pigs. Journal of animal science 69(2):646-655.

Hatipoglu, R., K. Kokten, I. Atis and B. Kutluay. 2005. Effects of mixture rate on the hay yield and hay quality of the mixture of Persian clover (*Trifolium resupinatum* L.) and annual ryegrass (*Lolium multiflorum* Lam.) under dry land condıtıons of Cukurova. Turkey VI. Field Crops Congress, Antalya, 803–808.

Kacar, B. and A. Inal. 2010. Plant Analysis. Ankara, Nobel Publication Distribution.

Kavut, Y.T., H. Geren, H. Soya, R. Avcioglu and B. Kir. 2014. Effects of rate of mixture and time of harvest on the winter second crop performances of mixtures of some annual legumes and Italian ryegrass. Journal of Ege University Faculty of Agriculture 51(3):279-288.

Lithourgidis, A.S., I.B. Vasilakoglou, K.V. Dhima, C.A. Dordas and M.D. Yiakoulaki. 2006. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding

ratios. Field Crops Research 99(2–3):106-113.
Lithourgidis, A.S., C.A. Dordas, C.A. Damalas and D.N. Vlachostergios. 2011. Annual intercops: an alternative pathway for sustainable agriculture. Australian Journal of Crop Sci. 5(4):396-410.
McGilchrist, C.A. 1965. Analysis of competition experiments. Biometrics 21(4):975-985.
Mead, R. and R.W. Willey. 1980. The concept of a 'land equivalent ratio' and advantages in yields from intercropping. Experimental Agriculture 16(3):217-228.
Meza, K., S.J. Vanek, Y. Sueldo, E. Olivera, R. Ccanto, M. Scurrah and S.J. Fonte. 2022. Grass–legume mixtures show potential to increase above- and belowground biomass production for Andean forage-based fallows. Agronomy 12(142):1-20.
Negesse, T., S. Funte, and A. Tolera. 2010. Forage Dry Matter yield and nutritive value of the natural pasture of Umbulo Wacho Watershed, Southern Ethiopia. East African Journal of Sci. 4(1):1-10.
Ofori, F. and W.R. Stern. 1987. Cereal–legume intercropping systems. Advances in Agronomy 41(C):41-90.
Osman, A.E. and N. Nersoyan. 1986. Effect of the proportion of species on the yield and quality of forage mixtures, and on the yield of barley in the following year. Experimental Agriculture 22(4):345-351.
Pedraza, V., F. Perea, M. Saavedra, M. Foetes and C. Alcantara. 2017. View of *Vicia narbonensis-Avena strigosa* mixture, a viable alternative in rainfed cropping systems under Mediterranean conditions. Spanish Journal of Agricultural Research 15(4):1-13.
Rajab, M.N., W.M.E. Mousa and A.M.A. Abd El-Monem. 2021. Yield and quality of Egyptian clover and ryegrass mixtures under nitrogen levels and bio-fertilizer. Journal of American Science 17(5):74-88.
Raza, M.A., W. Zhiqi, H.S. Yasin, H. Gul, R. Qin, S.U. Rehman, A. Mahmood, Z. Iqbal, Z. Ahmed, S. Luo, C. Juan, X. Liang, H. Gitari, M.H. Bin Khalid, Y. Feng and M. Zhongming. 2023. Effect of crop combination on yield performance, nutrient uptake, and land use advantage of cereal/legume intercropping systems. Field Crops Research 304: 109144.
Ross, S.M., J.R. King, J.T. O'Donovan and R.C. Izaurralde. 2003. Seeding rate effects in oat-berseem clover intercrops. Canadian Journal of Plant Science 83(4):769-778.
Saia, S., V. Urso, G. Amato, A.S. Frenda, D. Giambalvo, P. Ruisi and G. Di Miceli. 2016. Mediterranean forage legumes grown alone or in mixture with annual ryegrass: biomass production, N_2 fixation, and indices of intercrop efficiency. Plant and Soil 402(1-2):395-407.
Salama, H.S.A. and H.H. Badry. 2015. Influence of variable mixing rates and nitrogen fertilization levels on the fodder quality of Egyptian clover (*Trifolium alexandrinum* L.) and annual ryegrass (*Lolium multiflorum* Lam.). African Journal of Agricultural Research 10(53):4858-4864.
Salama, H.S.A. 2020. Mixture cropping of berseem clover with cereals to improve forage yield and quality under irrigated conditions of the Mediterranean basin. Annals of Agricultural Sciences 65(2):159-167.
Sheaffer, C.C., M.A. Peterson, M. Mccalin, J.J. Volene, J.H. Cherney, K.D. Johnson, W.T. Woodward and D.R. Vinads. 1995. Acid detergent fiber, neutral detergent fiber concentration, and relative feed value, https://www.naaic.org/stdtests/acidfiber.pdf, (Accessed October 22, 2023)
Solomon, J.K.Q., B. Macoon, D.J. Lang, J.A. Parish and R.C. Vann. 2011. A novel approach to grass-legume management. Crop Science 51(4):1865-1876.
Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2. ed. New York: McGraw-Hill Book Company.
Tahir, M., C. Li, T. Zeng, Y. Xin, C. Chen, H.H. Javed, W. Yang and Y. Yan. 2022. Mixture composition influenced the biomass yield and nutritional quality of legume–grass pastures. Agronomy 12:1449.
Thompson, D.J., D.G. Stout, Z. Mir and T. Moore. 1992. Yield and quality of forage from intercrops of barley and annual ryegrass. Canadian Journal of Plant Science 72(1):163-172.
Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583-3597.
Vasilakoglou, I. And K. Dhima. 2008. Forage yield and competition indices of berseem clover intercropped with barley. Agronomy Journal 100(6):1749-1756.
Willey, R.W. 1979. Intercropping-its importance and research needs, Part1: Competition and yield advantages. Field Crop Abstracts 32:1-10.
Yucel, C., M. Avci, H. Yucel, U. Sevilmis and R. Hatipoglu. 2018a. Effects of seed mixture ratio and harvest time on forage yield and silage quality of intercropped berseem clover with triticale. Fresenius Environmental Bulletin 27:5312-5322.
Yucel, C., I. Inal, D. Yucel and R. Hatipoglu. 2018. Effects of mixture ratio and cutting time on forage yield and silage quality of intercropped berseem clover and Italian ryegrass. Legume Research 41(6):846-853.