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Performances of Some Perennial Legume and Grass Mixtures under Rainfed Conditions of a Continental Climate Region

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Forage yield and the quality of the rangelands in Türkiye, especially in Central Anatolia, where the continental climate is dominant, are very low, and their vegetation is largely degraded due to mismanagement practices. The inadequacy of the forage crop production in agricultural land and the low yield and quality of forage are among the main problems of animal husbandry. The present study aimed to determine perennial legume and grass mixtures with high forage yield and quality under rainfed conditions of the continental climate region in Türkiye. The experimental design was randomized blocks with three replications. In the experiment, smooth brome (SB), intermediate wheatgrass (IW), alfalfa (A), sainfoin (S), and lesser burnet (LB) were sown as sole and mixed in different ratios. Two years averaged values of dry matter yield and crude protein, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) contents of dry matter varied between 2613 and 6268 kg ha^{-1};118 and 205 g kg^{-1}; 249 and 424 g kg^{-1}, 416 and 558 g kg^{-1}, respectively. Higher dry matter yields were obtained from A+S+IW+SB, S+LB+IW+SB, A+LB+IW+SB, S+IW, and A+IW+SB mixtures. The sole sowings of the SB, IW, and LB gave lower dry matter yields than the mixtures. Crude protein contents of alfalfa and sainfoin were higher than other sole sowings and mixtures. The ADF and NDF contents of mixtures were higher than those of sole sown of alfalfa, sainfoin, and lesser burnet, they were lower than those of IW and SB. In terms of dry matter yield, crude protein yield, ADF and NDF content, A+S+IW+SB, A+LB+IW+SB, and S+LB+IW+SB mixtures were superior to other mixtures and sole sowings. Alfalfa may be predominant in mixtures over time, and animal health problems may occur under grazing conditions. Therefore, the mixtures of A+S +IW+SB and A+LB+IW+SB can be recommended for mowing, while the S+LB+IW+SB mixture can be recommended for grazing.

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1. Introduction

As in the world, Türkiye's primary quality roughage sources are natural grazing lands and forage crop production in agricultural cultivated areas. Pasture-based livestock systems must meet the increasing demand for meat and milk by the increasing demand for meat and milk by increasing production volume with fewer resources (Lüscher et al., 2014). However, the yield of the rangelands in Türkiye, especially in Central Anatolia, where the continental climate is dominant, is very low, and their vegetation is largely degraded. Because of the high palatability of the legume species, and uncontrolled grazing, the rangelands in Central Anatolia are very poor in perennial forage legume species (Anonymous, 2012). With the current situation of the rangelands, the inadequacy of the growing areas of forage crops and the low yield and quality of forage are among the main problems of animal husbandry. High-quality roughage produced in Türkiye meets 37.6% of the forage needs of livestock, and the resulting forage deficit is trying to be completed using cereal straw and other crop residues (Yavuz et al., 2020). Fibrous forages, especially cereal straw, and stubble, have particular importance in the diet of ruminants in the Mediterranean production systems. (Bruno-Soares et al., 2000). As a result of the intensive use of cereal straw in animal feeding, the quality and quantity of animal products are naturally adversely affected. To meet the quality forage deficit of animal husbandry in Türkiye, growing areas of forage crops in agricultural land must be increased, and established pastures with mixtures of perennial legumes and grasses must be extended, especially in marginal areas. Legumes, especially the mixtures containing 30-50% legumes, have great potential to achieve this goal (Altın et al., 2021; Lüscher et al., 2014).

The benefits of mixtures of legumes and grasses are well known, but the water requirement for growing legumes limits their use in semi-arid and arid areas (Cui et al., 2013). Mixtures of grasses and legumes produce more biomass compared to sole sowings of grasses and legumes (Foster et al., 2014; Gökkuş et al., 1999; Sanderson et al., 2013; Serajchi et al., 2018) because they adapt better to changing environmental conditions during the growing season (Cox et al., 2017; Helgadóttir et al., 2018). The introduce of legumes into the mixtures with the grasses positively affects productivity (Barneze et al., 2020). Also, the primary benefit of legumes in the mixtures is to improve forage quality rather than yield (Bork et al., 2017). Nitrogen transfer from legumes to grasses is very important in low-input roughage production systems and the efficiency of N transfer can be enhanced by selecting compatible species or varieties (McElroy et al., 2016). Legumes provide many advantages by improving soil fertility with nitrogen fixation as well as the quantity and quality of the forage (Unathi et al., 2018). The functions of legumes reveal their importance in the development of roughage production systems (Malisch et al., 2017).

Before extensively using the mixtures of perennial legumes and grasses to solve the problem of quality roughage, first of all, it is necessary to determine the proper mixtures with high hay yield and quality for the ecological conditions of the region. Otherwise, it will not be possible to benefit from the advantages of legume and grass mixtures fully.

The present study aimed to determine the suitable mixtures of perennial legumes and grasses with high yield and quality for cutting under rainfed conditions of a continental climate region in Türkiye. The study investigated the forage yield and quality parameters of alfalfa, sainfoin, smooth brome, intermediate wheatgrass and lesser burnet species in sole sowings and their mixtures.

2. Materials and Methods

The study was carried out at the research and application fields of Kırşehir Ahi Evran University (39° 08' N, 34° 06' E, and 1084 m elevation) under rainfed conditions between 2013 and 2015. The Carlton cultivar of smooth brome (*Bromus inermis* Leyss.), Victoria cultivar of alfalfa (*Medicago sativa*), Bünyan 80 cultivar of lesser burnet (*Sanguisorba minor* Scop.), and local populations of intermediate wheatgrass (*Agropyron intermedium* (Host) Beauv.), and sainfoin (*Onobrychis sativa* Lam.) were used as plant materials (Table 1).

Table 1. Species and mixtures were tested in the study.

Species	Pure sowing ratios
Intermediate Wheatgrass	20
Smoot Brome (SB)	20
Alfalfa (A)	20
Sainfoin (S)	100
Lesser Burnet (LB)	30
Mixtures	**Seed Mixture ratios**
A+ IW	30% + 70%
A+SB	30% + 70%
A+IW + SB	30% +35% + 35%
A+S+IW+SB	15%+15%+35% + 35%
A+LB+IW+SB	15%+15%+35% + 35%
S+IW	30% + 70%
S+SB	30% + 70%
S+ IW + SB	30% +35% + 35%
S+LB+IW+SB	15%+15%+35% + 35%
LB+IW	30% + 70%
LB+SB	30% + 70%
LB+IW+SB	30% +35% + 35%

According to the results of the soil samples taken from the experimental area, the soil of the study area had a loamy texture at a depth of 0-30 cm, was slightly alkaline (pH 7.59), poor in organic matter (18.1 g kg^{-1}), and rich in available phosphorus (21.4 kg ha^{-1}), potassium (666.2 kg ha-1) and calcium (279 kg ha^{-1}).

The average temperatures in 2014 except September, November, and December were higher than the same months of 2015 (Table 2). Monthly average temperatures in 2014 were higher than the average long-term average. Total precipitation (471.4 mm) in 2015 was higher than those of 2013, 2014, and the long-term averages (254.7, 379.0, and 388.2 mm, respectively). The total precipitation in 2015, especially in June, was approximately four times higher than that of June 2014. Also, the total precipitation in 2013 and 2014 was lower than the long-term average total precipitation (Table 2).

Table 2. The monthly temperature, precipitation, and relative humidity values for the study area. *.

Months	Average Temperature (°C)				Total Precipitation (mm)				Relative Humidity (%)			
	2013	2014	2015	Long-term	2013	2014	2015	Long-term	2013	2014	2015	Long-term
January	1.4	1.9	1.2	-0.1	29.1	46.2	35.2	44.3	83.8	85.8	85.6	79.0
February	4.7	4.4	3.5	1.3	39.4	23.4	38.3	31.6	74.7	64.0	77.6	74.1
March	7.0	7.4	7.0	5.6	14.2	52.2	89.0	36.7	63.2	64.4	76.2	67.3
April	11.8	13.2	8.8	10.9	46.2	20.0	26.8	42.4	63.8	54.8	66.2	63.3
May	18.0	16.3	16.0	15.4	15.1	46.6	39.2	45.6	50.9	61.3	58.1	61.3
June	21.1	19.9	18.4	19.7	1.0	36.0	161.4	36.4	42.0	54.1	66.9	55.5
July	22.7	25.5	23.0	23.3	6.6	13.0	20.6	8.9	41.5	39.2	47.0	48.9
August	23.2	25.9	24.8	23.4	0.2	17.0	11.8	8.8	39.6	39.7	47.5	48.1
September	17.1	19.9	23.0	19.1	32.0	29.8	1.0	14.5	50.0	50.9	40.8	51.6
October	10.5	13.7	14.5	13.1	20.5	37.2	30.8	30.4	53.3	67.0	63.3	62.7
November	7.8	6.5	7.5	6.3	40.0	28.4	8.2	41.6	66.7	73.8	58.1	72.4
December	-2.1	5.9	-1.1	2.0	10.4	29.2	9.1	47.1	75.1	88.2	80.5	79.0
Total/Average	12.0	13.4	12.3	11.7	254.7	379.0	471.4	388.2	58.6	61.9	63.9	63.6

*Meteorological Service (1980-2020). Minister of Environment, Urbanization, and Climate Change Retrieved from https://mevbis.mgm.gov.tr/mevbis/ui/index.html#/Workspace

The experimental field was plowed with a moldboard plow in the fall and prepared for sowing with rotary tillers in the spring. The experimental layout was randomized blocks with three replications. Species and mixtures were sown on April 13, 2013. The experimental plots were 2.8 × 6 m (16.8 m^2) with a 1 m buffer between each plot. Each plot consisted of 8 rows with row spacing of 35 cm. The seeds of species in the mixtures were manually sown in the same row and pressed with a roller. Before planting, 50 kg N ha^{-1} and 70 kg P ha^{-1} were applied with diammonium phosphate fertilizer. Ammonium sulfate fertilizer (40 kg N ha^{-1}) was applied to the experimental plots as the top fertilizer on April 5 in 2014 and 2015. The data of the study were not obtained in 2013 when the field experiment was established, general conditions of the experiment were monitored, and weed control and cleaning mowing were carried out. The data collection and observations were carried out in the 2nd and 3rd years of the study. The harvesting was carried out at the beginning of flowering in sole sowings and 10% flowering period of legumes in mixtures (Aponte et al., 2019). The mowing was done on May 14 for sainfoin + grass and lesser burnet + grass mixtures and on May 28 for alfalfa + grass mixtures in 2014. In the research, sole sowings and mixtures reached harvest maturity twice in 2015. The first mowing was done on May 12 for sainfoin + grass and lesser burnet + grass mixtures and on May 25 for alfalfa + grass mixtures in 2015. The second mowing of all sole sowings and mixtures was done on July 5.

Four quadrats (each 0.3 m^2) from each plot were harvested using shears at a height of 5 cm and weighed to determine the green forage yield. 500 g of fresh samples from each quadrat were taken and dried at 60 °C until reaching a constant weight and the dry matter (DM) yield for each plot was calculated (Biligetu et al., 2014). Contributions of the legumes and lesser burnet in the hay yield of the mixtures were determined as explained by Castillo et al. (2015). The dried forage samples from each plot were ground to 1 mm in a mill for quality analysis. The nitrogen content of the forage was determined by the Kjeldahl method, and the crude protein (CP) content was calculated by multiplying the total nitrogen value with a coefficient of 6.25 (AOAC, 1990). CP yields were calculated by multiplying the CP contents with the DM yields. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) contents were determined using the ANKOM200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA), which was developed according to the method suggested by Van Soest et al. (1991). Acid Detergent Lignin (ADL) content was determined according to ANKOM (2005).

Two years of data from the experiment were combined and subjected to analysis of variance (ANOVA) according to the randomized block designed in an arrangement of split plots in time defined by Steel and Torrie (1980) using the MSTAT C 1.2v. software. Duncan's multiple comparison test at $P \leq 0.05$ was used post hoc to determine the differences among the mean values of treatments for statistically significant characters.

3. Results

The average DM yield in 2014 was significantly lower than that in 2015 (Table 3). The averaged DM yields over two years varied between 2613 and 6286 kg ha^{-1} depending on the treatments, and this variation was statistically significant ($p \leq 0.05$). The mixture of A+S+IW+SB gave significantly higher DM yield than all of the sole sowings as well as all of the other mixtures with the exceptions of A+LB+IW+SB, A+IW+SB, S+LB+IW+SB and S+IW. Sole sowing of SB provided significantly lower DM yield than all of the mixtures as well as all of the sole sowings with the exceptions of IW and LB. On the other hand, mixtures with the exceptions of binary and triplet mixtures of LB provided significantly higher DM yield than all of the sole sowings. DM yields from binary and ternary mixtures of LB were not significant different from that of sole sowing alfalfa (Table 3). The interactions of years by treatments (sole sowings and mixtures) in DM yield were insignificant.

The contribution of A to the DM yields of mixtures was significantly ($p \leq 0.05$) influenced by the years. In the second year, A contribution increased in all mixtures. The average A contribution in 2015 was significantly higher than 2014. (Table 3). The lowest A contribution was determined from the A+S+IW+SB mixture, and the highest contribution was obtained from the A+IW mixture. It was determined that the contribution of A in mixtures with IW and SB was relatively higher compared to those in the other mixtures. The contribution of A to the DM yields of the mixtures was generally related to the sowing ration of A in mixture. It can be point out that the botanical compositions of A+S+IW+SB and A+LB+IW+SB mixtures are more balanced than other A mixtures (Table 3). The interactions of years by mixtures in the contribution of A to the DM yield of the mixture were insignificant.

In the study, the average S proportion in the second year was slightly higher than that in the first year but the difference between the years in that perspective was not statistically significant (Table 3). According to the two-year averaged results, significant ($p \leq 0.05$) differences were determined among the contribution percentage of S in the different mixtures (Table 3). The lowest S proportion was obtained from the A+S+IW+SB mixture and the highest from the S+SB mixture. As in A, increasing the sowing ratio of S in the mixture increased its contribution to the DM yields of the mixtures. The interactions of years by mixtures in the contribution of S to the DM yield of the mixture were insignificant.

The averaged proportion of the LB in the DM yields of the mixtures was not influenced significantly by the year. The proportion of the LB in the different mixtures was significantly different. This difference was due to the seeding ratio of LB in the mixture. Therefore, the proportion of LB in the DM yield of the quaternary mixture, S+LB+IW+SB, was significantly lower than those in the other mixtures with the LB. (Table 3). The interactions of years by mixtures in the contribution of LB to the DM yield of the mixture were insignificant.

The CP content was significantly ($p \leq 0.05$) influenced by the years. Average CP content in 2014 was significantly lower than that in 2015 (Table 4). As average for two years, CP content varied between 118 g kg^{-1} and 205 g kg^{-1} depending on the treatment and this variation was statistically significant (Table 4). The mean CP content of sole sown A was significantly higher than that of all other sole sowings and mixtures. Sole sown grasses, IW, and SB showed significantly lower CP content than the other sole sowings and mixtures. The mixtures with A had higher CP content than those without A and increasing the proportion of A in the mixture increased the CP content of hay.

CP content of sole sown S was significantly higher than those of the sole sown grasses and LB as well as all of the mixtures. Binary or ternary mixtures of LB with grasses showed higher CP content than sole sown grasses (Table 4). The interactions of years by treatments in the CP ratio were not statistically significant.

Table 3. Dry matter yields (DM) of pure sowings and mixtures as well as contributions of alfalfa, sainfoin, and lesser burnet to the DM yields of the mixtures in the experimental years**.**

Species and Mixtures	DM (kg ha^{-1})			Contribution of A (%)			Contribution of S (%)			Contribution of LB (%)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
IW$^{\pm}$	2727 f*	3077 d*	2902 e*									
SB	2486 f	2739 d	2613 e									
A	4144 de	4530 c	4337 cd									
S	4574 cd	4763 c	4669 c									
LB	2927 f	3201 d	3064 e									
A+ IW	5124 bc	5674 b	5399 b	60.3 a	65.2 a	62.8 a*						
A+SB	5262 abc	5572 b	5417 b	58.6 a	61.4 ab	60.0 ab						
A+IW+SB	5356 ab	6085 ab	5721 ab	48.3 ab	55.3 abc	51.8 bc						
A+S+IW+SB	5937 a	6598 a	6268 a	36.1 b	42.4 c	39.3 d	21.6 b	23.6 b	22.6 c*			
A+LB+IW+SB	5485 ab	6180 ab	5832 ab	41.6 b	48.8 bc	45.2 cd				22.2 b	19.1 c	20.7 b*
S+IW	5267 abc	6057 ab	5662 ab				38.8 a	41.9 a	40.4 ab			
S+SB	5172 abc	5847 ab	5509 b				39.9 a	46.1 a	43.0 a			
S+ IW+SB	5466 ab	5740 b	5603 b				36.8 a	40.6 a	38.7 ab			
S+LB+IW+SB	5731 ab	5997 ab	5864 ab				34.6 a	38.4 b	36.5 b	22.9 b	25.6 bc	24.3 b
LB+IW	3825 e	4125 c	3975 d							33.5 a	38.5 a	36.0 a
LB+SB	3741 e	4174 c	3958 d							31.7 ab	33.9 ab	32.8 a
LB+IW+SB	3845 e	4245 c	4045 d							34.4 a	35.1 ab	34.7 a
Mean	4534 B^{+}	4977 A	4755	49.0 B^{+}	54.6 A	51.8	34.3	38.1	36.2	28.9	30.4	29.7
CV (%)	9.00	8.28	6.49	14.56	12.10	12.25	13.71	12.03	18.29	17.80	16.30	12.71

$^{\pm}$) IW: Intermediate Wheatgrass, SB: Smoot Brome, A: Alfalfa, S: Sainfoin, LB: Lesser Burnet. *) Mean values indicated with the same lower-case letter in a column are not statistically significantly different from each other according to the Duncan test at p ≤0.05. $^{+}$) Mean values with the same upper-case letter for a characteristic are not statistically significantly different from each other (p ≤0.05).

Years and treatments did significantly (p ≤0.05) affect the CP yield. The average CP yield in 2015 was significantly higher than that in 2014 (Table 4). Two years averaged values of CP yield varied between 324 kg ha^{-1} and 1074 kg ha^{-1} depending on the treatments, and this variation was statistically significant (Table 4). The quaternary mixture of A+S+IW+SB with the highest DM yield among the treatments gave a statistically significant higher CP yield than the other mixtures and all of the sole sowings. Sole sowings of grasses provided significantly lower CP yield than sole sowings of legumes and all of the mixtures. CP yield of sole sowing S was not statistically significant than that of A. The higher DM yield of S, but not statistically significantly higher than that of A, resulted in the CP yield of S being not significantly different from that of A while the CP content of A was significantly higher than that of S (Table 4). CP yields of binary and ternary mixtures of S, A, and LB with grasses were not significantly different than those of their sole sowings. The interactions of years by treatments in CP yield were insignificant.

NDF content was significantly (p ≤0.05) influenced by the years and treatments. The average value of NDF in 2014 was significantly higher than that in 2015 (Table 5). NDF content of DM was also significantly changed by the treatments, and its two-year average values varied between 416 g kg^{-1} and 558 g kg^{-1} depending on the treatments. Sole sowing of LB showed significantly lower NDF content than all of the other sole sowings and mixtures. The highest NDF content among the treatments was determined in IW, and it was significantly higher than those of all of the other sole sowings and mixtures. The mixtures showed lower NDF content than sole sowing grasses but higher than sole sowing legumes and LB. The interactions of years by treatments in NDF content were not significant.

Years, treatments, and their interactions significantly (p ≤0.05) affect the ADF content of DM from sole sowings and mixtures of the tested perennial legume and grass species. The average ADF content in 2014 was significantly higher than that in 2015 (Table 5). According to the two-year average of ADF content, it varied between 280 g kg^{-1} and 424 g kg^{-1} depending on the treatments (Table 5). ADF content of sole sowing IW was statistically significantly higher than the other sole sowings and all of the mixtures. Low leafiness and higher stem/leaf ratio in IW may be the reason for its higher ADF content. The lowest ADF ratio was recorded in the sole sown LB.

Table 4. Crude protein ratios (CP), and crude protein yields (CPY) of pure sowings and mixtures in the experimental years.

Species and Mixtures	CP ($g\ kg^{-1}$)			CPY ($kg\ ha^{-1}$)		
	2014	2015	Mean	2014	2015	Mean
IW±	115 j*	120 h*	118 ı*	315 d*	372 fg*	344 e*
SB	123 ıj	125 h	124 ı	304 d	343 g	324 e
A	203 a	207 a	205 a	843 b	940 bcd	892 bc
S	184 b	188 b	186 b	843 b	895 cd	869 bc
LB	151 g	154 f	152 g	444 c	493 ef	468 d
A+ IW	174 bc	183 bc	178 bc	890 ab	1036 abc	963 bc
A+SB	172 cd	181 bc	177 c	905 ab	1007 abcd	956 bc
A+IW + SB	161 defg	173 cd	167 de	862 b	1051 ab	957 bc
A+S+IW+SB	171 cde	172 cd	171 cd	1014 a	1133 a	1074 a
A+LB+IW+SB	164 cdef	167 de	166 de	897 ab	1033 abc	965 b
S+IW	159 efg	163 def	161 ef	840 b	986 bcd	913 bc
S+SB	157 fg	163 def	160 efg	814 b	951 bcd	883 bc
S+ IW + SB	151 g	152 f	152 g	825 b	875 d	850 c
S+LB+IW+SB	155 fg	157 ef	156 fg	888 ab	939 bcd	914 bc
LB+IW	135 h	137 g	136 h	512 c	564 e	538 d
LB+SB	134 hı	136 g	135 h	501 c	570 e	536 d
LB+IW+SB	134 hı	136 g	135 h	515 c	578 e	546 d
Mean	155 B+	160 A	158	718 B+	810 A	764
CV (%)	4.21	3.95	4.07	10.04	9.37	8.16

±) IW: Intermediate Wheatgrass, SB: Smoot Brome, A: Alfalfa, S: Sainfoin, LB: Lesser Burnet. *) Mean values indicated with the same lower-case letter in a column are not statistically significant different from each other according to the Duncan test at $p \leq 0.05$. +) Mean values with the same upper-case letter for a characteristic are not statistically significant different from each other ($p \leq 0.05$).

Years by treatment interaction for ADF was significant ($p \leq 0.05$). The ADF contents of IW and SB in 2014 were not significantly different from each other while the ADF content of IW was significantly higher than that of SB in 2015 (Table 5). This result may be because of the different responses of these two grass species to ecological conditions due to the decreasing temperature and increased precipitation in 2015, especially the increase in stem ratios at different levels (Barnes et al., 2003). Due to the same reasons, binary mixtures of A+SB, S+SB, and L+SB in 2015 showed lower ADF contents than their ternary mixtures while their ADF contents were not statistically significant from each other in 2014.

ADL content was significantly ($p \leq 0.05$) influenced by the years and treatments. As for ADF, the average ADL content in 2014 was also significantly higher than that in 2015 (Table 5). According to the two-year averaged values, the ADL content varied between 59 g kg^{-1} and 99 g kg^{-1} depending on the treatments (Table 5). ADL content of sole sowing S was significantly higher than those of other sole sowings and the mixtures. Sole sown SB had significantly lower ADL than the other sole sowings and the mixtures. ADL contents of sole sowing legumes and LB were significantly higher than those of sole sowing grasses and mixtures.

The effect of the years on the ADL content significantly ($p \leq 0.05$) changed depending on the treatments. ADL content of sole sowing LB in 2014 was significantly lower than the sole sowing legumes but significantly higher than the sole sowing grasses and the mixtures. In 2015, ADL content of LB was not significantly different from those of binary and ternary mixtures of grasses + legumes as well as quaternary mixtures of grasses + legumes + LB.

Table 5. The ADF, ADL, and NDF ratios of pure sowings and mixtures in two experimental years.

Species and Mixtures	ADF (g kg^{-1} DM)			ADL (g kg^{-1} DM)			NDF (g kg^{-1} DM)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
IW±	435 a*	412 a*	424 a*	76 g*	61 ı*	68 h*	574	542	558 a*
SB	426 a	390 b	408 b	65 h	53 j	59 ı	560	530	545 b
A	287 g	272 j	280 k	98 b	86 b	92 b	458	414	436 j
S	332 f	307 i	319 j	103 a	95 a	99 a	482	456	469 ı
LB	255 h	243 k	249 l	95 c	73 cde	84 c	431	401	416 k
A+ IW	363 de	334 fg	349 g	85 def	75 cde	80 de	507	454	480 ghı
A+SB	354 e	325 gh	340 hı	85 def	75 cde	80 de	503	459	481 ghı
A+IW + SB	351 e	342 ef	346 gh	86 def	76 cd	81 d	509	471	490 fg
A+S+IW+SB	334 f	334 fg	334 ı	85 def	77 c	81 d	506	461	484 gh
A+LB+IW+SB	327 f	315 hı	321 j	86 de	76 cd	81 d	491	451	471 hı
S+IW	396 b	364 c	380 c	88 d	77 c	82 cd	536	502	519 cd
S+SB	397 b	345 def	371 d	82 f	72 de	77 f	524	495	509 cde
S+ IW + SB	396 b	378 b	387 c	83 ef	71 ef	77 ef	531	509	520 c
S+LB+IW+SB	354 e	342 ef	348 gh	86 de	75 cde	80 d	517	479	498 ef
LB+IW	369 cd	350 de	360 ef	85 def	68 fg	76 f	523	491	507 de
LB+SB	374 cd	333 fg	354 fg	77 g	63 hı	70 gh	518	481	500 ef
LB+IW+SB	377 c	357 cd	367 de	77 g	65 gh	71 g	530	496	513 cd
Mean	360 A+	338 B	349	85 A+	73 B	79	512 A+	476 B	494
CV (%)	2.07	2.19	2.20	2.45	3.20	2.80	2.04	2.07	2.08

±) IW: Intermediate Wheatgrass, SB: Smoot Brome, A: Alfalfa, S: Sainfoin, LB: Lesser Burnet. *) Mean values indicated with the same lower-case letter in a column are not statistically significantly different from each other according to the Duncan test at p ≤0.05. +) Mean values with the same upper-case letter for a characteristic are not statistically significantly different from each other (p ≤0.05).

4. Discussion

Higher total precipitation and lower mean temperature in the year 2015 compared to the year 2014 resulted in higher DM yield and CP content of DM. The plant grown under higher temperature condition produced less leaf and more stem compared to grown under cooler condition. Consequently, produced forage contains higher fibrous and less CP content (Barnes et al., 2003). Climate conditions in the second year, plants produced much more leaf, causing an increase in CP ratios and a decrease in ADF and NDF ratios. In addition, as the maturation stage progresses, the fiber and lignin content in the plant increases, and the forage quality decreases (Grev et al., 2017; Suryanah et al., 2018).

In the study, the mixtures were superior to the sole sowings of mixture component species in the DM yield. The superiority of mixtures to sole sown species has also been reported in previous studies (Albayrak & Türk, 2013; Annicchiarico et al., 2019; Dhakal & Islam, 2018; Meza et al., 2022; Serajchi et al., 2018; Yavuz & Karadağ, 2016). Also, Sanderson et al. (2005) reported that the yields obtained from multiple legume-grass mixtures were higher than those of the simple mixtures.

The increased contribution of the legumes and LB to the DM yields of the mixtures in 2015 resulted in higher CP content as compared to that in 2014. Due to the effect of A in the mixture, the CP ratio of the mixture of LB with A and IW (A+LB+IW+SB) was higher than those of sole sown LB. The effect of legumes in the mixture on yield and quality was higher than that of grasses (Elgersma & Søegaard, 2016).

The results of the study revealed that the CP ratios of sole sown A and S were higher than those of sole sown grasses and their mixtures, and the CP ratios of the mixtures were higher than those of sole sown grasses. Due to symbiotic nitrogen fixation of A and S legume species, their higher CP content is an expected result. This result was in line with the findings reported by Tessema and Feleke (2018), who reported that sole-sown legumes and legume-grass mixtures have higher CP and lower fiber contents than sole sown grasses. Growing legumes with grasses increases the CP content of the mixtures compared to sole sown grasses (Sturludóttir et al., 2014). Similarly, the other researchers have also reported that CP ratios of sole sown legumes are higher than those of sole sown grasses and their mixtures (Ćupina et al., 2017; McDonald et al., 2021; Yavuz & Karadağ, 2016).

Higher averaged DM yield and higher averaged CP content in the year 2015 resulted in higher CP yield as compared to the year 2014. The CP yield increased with the increase in DM yield and CP ratio of species and mixtures. Solati et al. (2018) emphasized a linear relationship between forage yield and protein yield.

The mixtures showed lower NDF content than sole sowing grasses but higher than sole sowing legumes and LB. The mixtures were expected to have lower NDF contents than sole grasses and higher NDF contents than legumes. The legumes in the mixture caused a decrease in the NDF concentration, whereas the grasses increased (Brink et al., 2015). Tessema and Baars (2006) emphasized that sole sown legumes, and legumes-grass mixtures had higher CP and lower fiber contents compared to sole sown grasses. Also, grasses have higher NDF content than legumes (Hoffman et al., 2001), and the addition of legumes to grasses in mixtures decreases the ADF and NDF ratios whereas it increases the CP ratio (Yüksel & Balabanli, 2021). The contribution of legumes in mixtures is significant because they increase dry matter intake and milk production (Johansen et al., 2018), and cattle prefer legumes rather than grasses under free grazing conditions (Villalba et al., 2015). Baron et al. (2000) indicated that the differences in nutritional values among the species were related to the leaf/stem ratio rather than the mass and morphology of the grass. Therefore, despite the high CP content of S as a legume, sainfoin-grass mixtures had a higher NDF ratio than alfalfa-grass mixtures. The results are in line with the findings of Albayrak et al. (2011) who reported that binary and ternary alfalfa-grasses mixtures had lower NDF ratios than sainfoin-grass mixtures.

Cooler temperatures and higher precipitation in 2015 may have increased leaf proportion of forages and this situation caused lower fiber content (Barnes et al., 2003) as compared to that in 2014. Low leafiness and increasing stem/leaf ratio by maturation in IW may be the reason for its higher ADF content. The lowest ADF ratio was obtained in the sole sown LB. The high leaf ratio of LB may be a reason for its low ADF content (Açıkgöz, 2021; Kaplan et al., 2014). Mülayim et al. (2009) have reported that the CP ratio of LB was higher than that of the grasses and similar to legumes whereas the crude fiber ratio was much lower than that of other forage crops. Elgersma et al. (2014) also obtained the lowest ADF and NDF values in pure sown LB. The grasses in the mixtures cause an increase in the ADF ratios of the mixtures whereas the legumes cause a decrease. The nutritional quality of mixtures is mainly related to the legumes (Gierus et al., 2012). The increase of the A ratio in the mixture positively affects the quality parameters including ADF and NDF (Yüksel & Balabanli, 2021).

Cinar and Hatipoglu (2015) reported that the ADF ratios in sole sown alfalfa, dallis grass, Bermuda grass, Rhodes grass, and alfalfa-grasses mixtures varied between 26.7%, and 40.2% and Zemenchik et al. (2002) reported the ADF ratios ranged between 25.5 and 26.9% for smooth brome + Caucasian clover and between 26.9 and 28.5% for orchardgrass + Caucasian clover mixtures. Jeranyama and Garcia (2004) determined the mean ADF ratio for alfalfa + grasses mixtures as 39%, and 49% for smooth brome at the heading stage. The differences in harvest time, mixture ratio, and climatic factors such as temperature and precipitation may have led to differences in ADF content among the experiments. Bhattarai et al. (2016) emphasized that the nutritional value of sainfoin varies not only with the maturity stage but also with different experimental conditions and locations during the same growing period.

ADL contents of sole sown legumes and LB were significantly higher than those of sole sown grasses and the mixtures. Legume species contain more ADL than the grasses (Lardy, 2018). Due to the lower ADL content of the grasses, the mixtures of legumes and grasses contain lower ADL than the pure sown legumes but higher ADL than the pure sown grasses. The ADL ratios in alfalfa-grass silage were reported as 5.1%, and the ADL ratios in the hay of cool-climate grasses mixture as 3.8% (Mandebvu et al., 2001). The ADL value was also reported between 5.12 and 8.44% for five different alfalfa varieties (Bani et al., 2007), 10.87% for alfalfa, and 11.87% for sainfoin (Canbolat & Karaman, 2009), and 5.45% for intermediate wheatgrass (Gürsoy et al., 2021). The differences in ADL ratios between the aforementioned studies and our results were probably originated form the differences in plant materials and the ecological conditions of study areas.

5. Conclusions

The results of the study revealed that the yield and quality performances of legume-grass mixtures were higher compared to the performances of sole legume or grass sowings. In an overall evaluation of DM yield, CP yield, ADF, and NDF ratios, which are important indicators of forage quality, the A+S+IW+SB, A+LB+IW+SB, and S+LB+IW+SB mixtures were superior to other mixtures and sole sowings. These mixtures can be used in similar ecological conditions, as in Central Anatolia, in producing high-quality hay or establishing artificial pastures. However, as the research findings emphasize, A may become dominant in A+S+IW+SB and A+LB+IW+SB mixtures over time, which may cause animal bloating problems; therefore, these mixtures should be evaluated by mowing or care should be taken if the mixtures in question are to be grazed. On the other hand, the S+LB+IW+SB mixture can be preferred for quality roughage production in both grazing and mowing conditions.

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