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Original Article

The effect of seasonal variation on macromineral contents of sainfoin genotypes and assessments with biplot analysis

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Abstract. *Background and aim*: The use of quality forages in animal nutrition makes great contribution to increase in animal products both in quantity and quality. Macromineral content is an important factor affecting the quality of forages, and the contents in forages can vary due to many factors. Seasonal variation is one of the among the factors, and the study was held to reveal the effect of spring and autumn seasons cuttings on macromineral contents of sainfoin (*Onobrychis viciifolia* Scop.) which is one of the important and mostly cultivated perennial legume forage species in Turkey. *Methods:* For this reason; a field experiment was conducted according to randomized blocks design with three replications in the research area of GAP International Agricultural Research and Training Center (GAP IARTC) Diyarbakir, Turkey. *Results:* The study results showed that there were highly significant differences ($P<0.01$) between the forage harvesting seasons in terms all of the examined traits, and calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) contents forages of sainfoin genotypes in autumn season were found lower than those of in the spring season cutting. Furthermore, the following ranges determined in the examined traits; Ca content 10.22–12.46 g kg^{-1} DM, K content 25.41– 37.16 g kg^{-1} DM, Mg content 1.00–1.61 g kg^{-1} DM, P content 1.99–3.22 DM g kg^{-1} DM, Ca/P ratio 3.59–5.79 and K/(Ca+Mg) (tetany) ratio 1.98–2.86. On the other hand, both biplot and correlation analysis showed that there was highly significant and positive relationship among Ca, Mg contents, Ca/P ratio traits. However, there was negative relationship between the three traits and P content, K/(Ca+Mg) (tetany) ratio traits. In addition, there was highly significant ($P<0.01$) but negative relationship between P content and K content traits in sainfoin genotypes. *Conclusions*: By taking into to all of the examined traits together, biplot analysis revealed that Lütfibey and Peschanjy-1251 cultivars came to fore in spring season cutting, while CTC Population and Ozerbey-03 cultivars came to fore in autumn season cutting. Also, sainfoin forage obtained in spring season cutting was found richer in terms of Ca, Mg, P and K contents, but due to higher K/(Ca+Mg) (tetany) ratio of the spring season, animal feed with the spring season sainfoin forage may be more susceptible to catching grass tetany incidence especially for dairy cows. To avoid risk of the incidence, the feeds having more Mg content and lower K content should be taken place in the animal rations.

Key words: biplot analysis, correlation analysis, forage macro nutrients content, sainfoin (*Onobrychis viciifolia* Scop.), Ca/P ratio, K/(Ca+Mg) (tetany) ratio

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.), a perennial cool season forage legume species, owing to its wide adaptability, is grown in many part of World, having very different climates (1). It can well perform even at low phosphorus doses (2), and high lime content in soil conditions (3). Furthermore, the sainfoin plant thanks to well developed its root system, it is famous at drought resistance (4). As a legume plant, sainfoin has the ability to fixate free atmospheric nitrogen to soil. In this way, it enriches the soil both in terms of nitrogen content and organic matter content. Moreover, it leaves a favorable soil conditions for the next crops and increasing the crops yields (5). Because of these and similar superior features, sainfoin has taken part in traditional culture of world throughout the history (6).

Shortage of quality roughage is one of the important problem of animal husbandry in Turkey. Due the deficit, grain stubble, straw and expensive concentrate feedstuffs were used in feeding of livestock in the country (7). Undoubtedly, increasing in forage crops cultivation in arable lands of the country is one of the most effective way to meet this quality roughage deficit (8). When harvested in the appropriate stage, sainfoin has high quality, palatability and nutritious forage, due to its high crude protein and nutrients contents (9). In the same time, sainfoin forage can consume by livestock freshly, without risk of swelling due to its tannin content, so it is a well rangeland plant, suitable for grazing (10,11). Also, it can be grown mixed with perennial grasses species for regulate nutritive values of the forages both for hay and for silage purposes (12). For all the reasons stated, sainfoin has been one of the most widely grown forage crops in Turkey. According to the recent official data, the total size of sainfoin cultivation area in Turkey is 174.494,90 ha, and 1.934.697 tons fresh forage is produced in this area (13). With this production, sainfoin has an important contribution to closing the roughage deficit in the country.

There are many factors affecting forage quality such as plant species and variety, protein content and digestibility. Mineral elements in forages are of great importance in forage quality as well (14). Minerals according to their concentration in the living body classified into two main groups; macro (major) and micro (trace) minerals. Minerals are necessary almost for all vital processes of the living body, despite different effectiveness on body functions (15). For example, calcium (Ca) and phosphorus (P) take part in the formation of the basic structure of bones and teeth. Potassium (K) is important in osmotic pressure regulation and acid-base and water balances, nerve impulse transmission, muscle contractions and certain enzyme reactions (16). Magnesium (Mg) involved in the activation of more than 300 enzymes (17). According to Spears (15) minerals optimize rumen microbial activity and enhance forage utilization. Hence, adequately supplying of these minerals is vital for growth, health and reproduction of livestock (18,19).

Minerals aren't synthesized by livestock body, so they should be supplied from outside. And most of the minerals enter the animal body through feeds (20). Forages are the most important feed ingredient in animal nutritions. They have crucial role in meeting the minerals requirement of livestock (21). Therefore, knowing minerals content of the forages has importance in animal feeding. Accordingly, until now many researches have been made on determinig mineral content of forages. Genarally, the researches focused on soil nutrient contents, plant genotypic structure, fertilization treatments and plant maturity stages. However, seasonal variation effect on forage mineral content rarely has been studied. Accordingly, the study was held to determine effect of seasonal variation on Ca, K, Mg and P contents and Ca/P and K/(Ca+Ma) ratios of sainfoin genotypes. The results of the research were also examined with the biplot analysis method, and the results were presented with understandable biplot graphs.

Materials and methods

The used plant materials and features of the experimental area

A field experiment was conducted in the research area of GAP International Agricultural Research and Training Centre (GAP IARTC), Diyarbakır, Turkey (37°56'43.0"N, 40°15'15.0"E and altitude 601 m) for three consecutive years (2009-2010-2011). In the study, five sainfoin (*Onobrychis viciifolia*) genotypes, Ozerbey, Lutfibey and Peschanjy-1251 sainfoin cultivars and two sainfoin populations, were used as genetic materials. One of the used sainfoin population was INV Population, collected from natural vegetation of Idil district, Sırnak, Turkey, the other one was the CTC population, supplied from Cim Teknik Seed Company, Ankara, Turkey.

The results of the soil analysis made on soil samples taken from 30 cm depths of the experiment area are submitted in the Table 1. The soil analysis results indicated that the experiment area soil, which is in red-brown color, clay loamy texture and slightly alka-

Table 1. Soil properties of the research area

Depth	Color	pH	Saturation (%)	Organic Matter (%)	$CaCo_3$ (%)	P_2O_5 (kg ha^{-1})	K_20 (kg ha^{-1})	Texture
0-30 cm	Red-brown	7.76	63	1.43	13.72	28	482	Clay-loamy

line character, is rich in terms of potassium and calcium content, and poor in terms of useful phosphorus and organic matter content (Table 1).

Climatic data of the experimental area during the research years and long-term average are submitted in the Table 2. The trial location has a typical continental climate features. Namely, its summers are very hot and dry, and its winters are cold and rainy. Therefore, there is not much chance of herbaceous plants to survive in the region without watering in summer. For this reason, the trial area was regularly irrigated through the sprinkling method throughout the summer months.

The methods used in the establishing and running of the experiment

The experiment was established according to Randomized Complete Block Design (RCBD) method with three replications. Each plot of the experiment consisted of 6 rows in 6 m in length, and rows were spaced 30 cm apart. In this way, size of each plot in the experiment was 10.8 m^2. Diammonium phosphate fertilizer (DAP 18-46-00) was used with the trial sowing at the 150 kg ha^{-1} amount. The sowing of the experiment was done by manually leaving the seeds on the drawings opened with the marker beforehand in spring of 2009. The seeding rate was applied as 100 kg seeds per hectare (22). In the experiment was carried out in the irrigated conditions, and the plots forage harvestings were made during the 10% flowering period of the plants. Accordingly, forage harvesting in the spring season was made in the first half of May, while forage harvesting in autumn was made in the last half of September. Fresh forage and dry matter yields of the plots were determined according to Official Technical Instruction (22) Crude Protein, ADF and NDF contents of the sainfoin cultivars forages were determined with the Foss XDS NIRS (Near Infrared Reflectance Spectroscopy) analysis device using C-0904FE-Hay and Fresh Forage calibration (23-26) in the Eastern Mediterranean Agricultural Research Institute, Adana, Turkey.

The statistical analyses

In the study, the statistical analyses of data were made by using the JMP 5.0.1 statistical software pack-

Table 2. Monthly Climatic data of the experimental area during the research years and averages of long years.

	J	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	
Years					**Total Precipitation (mm)**								**Total**
2009	12.4	70.0	63.9	43.9	9.1	25.8	1.6	0.0	25.2	62.4	55.6	87.2	457.1
2010	113.4	40.2	68.7	22.4	31.6	11.2	0.0	0.0	0.4	63.0	0.0	48.0	398.9
2011	40.0	49.9	46.6	209.0	80.1	13.6	0.6	0.0	9.2	11.8	73.0	40.2	574.0
Long years	62.8	67.8	67.3	67.7	39.6	9.0	0.4	0.4	4.3	32.1	51.1	67.4	469.9
					Mean air temperature (°C)								**Mean**
2009	1.4	5.6	7.9	11.8	18.2	25.9	29.5	28.6	22.9	18.5	9.8	7.1	15.6
2010	5.4	6.6	11.1	14.2	20.4	27.2	32.3	32.0	27.0	18.1	11.1	6.5	17.7
2011	3.5	4.7	9.0	13.0	17.7	25.5	31.4	30.7	25.0	16.4	6.4	2.3	15.5
Long years	1.6	3.6	8.6	13.8	19.2	26.3	31.2	30.3	24.7	17.1	9.0	3.7	15.8
					Mean relative humidity (%)								**Mean**
2009	73.3	82.5	73.8	71.3	51.8	32.2	26.1	19.8	33.0	42.0	83.5	80.9	55.9
2010	80.9	79.9	66.6	60.4	49.3	29.1	19.6	17.5	27.4	56.0	41.1	68.9	49.7
2011	73.4	69.5	56.4	75.7	67.6	38.0	22.5	21.7	30.2	41.6	58.8	73.9	52.4
Long years	75.1	70.8	65.5	64.4	56.4	37.4	28.1	27.7	33.5	51.2	66.4	74.7	54.3

age (27), and the least significant difference (LSD) test at the 0.05 probability level (28) was used for determining the differences between means. On the other hand, biplot analysis and its graphics were formed by using GENSTAT statistical package program (29). And interpretations of the graphics were made as specified by Yan and Kang (30).

Results

Variance analysis showed that there were highly significant differences between cutting seasons, genotypes and genotype × cutting season interaction in terms of calcium (Ca) content trait in sainfoin forage dry matters (DM) (Table 3). As seen in the Table 3, Ca contents determined in the spring cutting from sainfoin genotypes were found to be higher than those of autumn cutting. Accordingly, the spring cutting average was recorded as 11.87 g kg^{-1} of DM, while autumn cutting was 11.42 g kg^{-1} of DM. Moreover, according to the genotype average of the seasons except for Peschanjy-1251 (11.21 g kg^{-1} of DM), cultivar the other sainfoin genotypes had similar Ca content, with sharing the same statistical group. Meanwhile, as in Ca content, cutting seasons, genotypes and genotype × cutting season interactions were found to be highly significant ($P<0.01$) for potassium (K) content trait in sainfoin forage dry matters as well.

Means of potassium (K) content of the genotypes were ranged from 26.41 g kg^{-1} DM to 36.17 g kg^{-1} DM between the cutting seasons. K contents determined in the autumn cuttings were found lower than those of determined in the spring cuttings for all of the genotypes. Besides, by taken into consideration genotypes average, the highest K content was determined in Ozerbey-03 (32.33 g kg^{-1} of DM) and CTC Population (32.78 g kg^{-1} DM) sainfoin genotypes, while the lowest K content determined in INV Population (30.77 g kg^{-1} DM) (Table 3).

The results of the study revealed that there were significant differences between the cutting seasons means at 0.01 level and among the genotypes means at 0.05 level. However, genotype × cutting season interaction was found non-significant ($P>0.05$) for magnesium (Mg) content of sainfoin forages. The spring cutting mean (1.47 g kg^{-1} DM) of Mg content was found quite higher than that of autumn cutting mean (1.18 g kg^{-1} DM) (Table 4). Also, according to the two seasons average, Mg content of Lütfibey (1.41 g kg^{-1} DM), INV Population (1.33 g kg^{-1} DM) and CTC Population (1.39 g kg^{-1} DM) were found higher than Mg contents of Ozerbey-03 (1.28 g kg^{-1} DM) and Peschanjy-1251 (1.21 g kg^{-1} DM) cultivars. By the way; the differences between cutting seasons and

Table 3. The means of calcium and potassium contents of sainfoin genotypes in spring and autumn cuttings+

Genotypes	Calcium (Ca) content (g kg^{-1} DM)			Potassium (K) content (g kg^{-1} DM)		
	Spring cutting	Autumn cutting	Mean	Spring cutting	Autumn cutting	Mean
1- Lütfibey	11.65b-c	12.05a-b	11.85a	36.03b	26.41f	31.22b-c
2- INV Population	11.95b-c	11.53c	11.74a	34.39c	27.15e	30.77c
3- Peschanjy-1251	11.56c	10.22d	10.89b	37.16a	25.41g	31.28b
4- Ozerbey-03	11.71b-c	11.78b-c	11.75a	36.90a	27.75e	32.33a
5- CTC Population	12.46a	11.52c	11.99a	34.78c	30.78d	32.78a
Mean	11.87A	11.42B		35.84A	27.49B	
CV (%)		2.23			1.19	
LSD (0.05)						
Cutting seasons		0.09**			0.29**	
Genotypes		0.31**			0.45**	
Genotype × cutting season		0.43**			0.64**	

+ Means with different letters in the same column are significantly different ($P< 0.05$)
Significant at *: $P<0.05$; **: $P<0.01$, ns: Non-significant

among genotypes mean were found significant at 0.01 statistical level, genotype × cutting season interaction was found significant at 0.05 statistical level in terms of phosphorus (P) content in sainfoin (Table 4). Additionally, in all of the examined sainfoin genotypes, P content of spring season cuttings were found higher than autumn season cuttings. The means of P content in sainfoin forages were determined as 3.02 g kg^{-1} DM and 2.05 g kg^{-1} DM for spring season cutting and autumn season cutting respectively. Furthermore, genotypes averages showed that except for CTC Population (2.51 g kg^{-1} DM), having lowest P content, there wasn't statically any differences (P>0.05) among P content of the other sainfoin genotypes (Table 4).

There were highly significant (P<0.01) differences between the cutting seasons and genotypes in terms of Ca/P ratio trait in sainfoin forages, even though there was found to be significant (P<0.05) for genotype ×

Table 4. The means of magnesium and phosphorus contents of sainfoin genotypes in spring and autumn cuttings+

Genotypes	Magnesium (Mg) content (g kg^{-1} DM)			Phosphorus (P) content (g kg^{-1} DM)		
	Spring cutting	Autumn cutting	Mean	Spring cutting	Autumn cutting	Mean
1- Lütfibey	1.53a-b	1.29c-e	1.41a	3.11b	2.11d	2.61a
2- INV Population	1.42b-c	1.23d-e	1.33a-c	3.17a-b	2.02e	2.60a
3- Peschanjy-1251	1.43a-c	1.00f	1.21c	3.22a	2.06d-e	2.64a
4- Ozerbey-03	1.38b-d	1.18e	1.28b-c	3.11b	2.06d-e	2.58a
5- CTC Population	1.61a	1.18e	1.39a-b	3.02c	1.99e	2.51b
Mean	1.47A	1.18B		3.13A	2.05B	
CV (%)		3.57			1.55	
LSD (0.05)						
Cutting seasons		0.07**			0.03**	
Genotypes		0.12*			0.05**	
Genotype × cutting season		ns			0.08*	

+ Means with different letters in the same column are significantly different (P< 0.05)
Significant at *: *P*<0.05; **: *P*<0.01, ns: Non-significant

Table 5. The means of Ca/P and K/(Ca+Mg) ratios of sainfoin genotypes forages in spring and autumn cuttings+

Genotypes	Ca/P			K/(Ca+Mg)		
	Spring cutting	Autumn cutting	Mean	Spring cutting	Autumn cutting	Mean
1- Lütfibey	3.75d	5.71a	4.73b	2.73b	1.98g	2.36c
2- INV Population	3.77d	5.69a	4.73b	2.57c	2.13f	2.35c
3- Peschanjy-1251	3.59d	4.97b	4.28c	2.86a	2.27e	2.57a
4- Ozerbey-03	3.76d	5.73a	4.75b	2.82a-b	2.14f	2.48b
5- CTC Population	4.13c	5.79a	4.96a	2.47c-d	2.42d	2.45b
Mean	3.80B	5.58A		2.69A	2.19B	
CV (%)		3.41			2.45	
LSD (0.05)						
Cutting seasons		0.12**			0.05**	
Genotypes		0.19**			0.08**	
Genotype × cutting season		0.27*			0.11**	

+ Means with different letters in the same column are significantly different (P< 0.05)
Significant at *: *P*<0.05; **: *P*<0.01. ns: Non-significant

cutting season interaction. Means of Ca/P ratio trait were ranged from 3.59 to 5.79 between the cutting seasons in sainfoin genotypes. Contrary to what seen in all examined traits of the study, Ca/P ratio means of the genotypes determined in spring season cuttings were found lower than those of determined in autumn season cuttings. The average Ca/P ratios for spring season and autumn season recorded as 3.80 and 5.58 respectively. According to the genotypes average, the highest Ca/P ratio was determined CTC Population (4.96). It was followed by Lütfibey (4.73) and INV Population (4.73). In contrary, the lowest Ca/P ratio was determined in Peschanjy-1251 sainfoin cultivar. In the meantime, there were highly significant differences (P<0.01) between cutting seasons, the genotypes and genotype × cutting season interaction in terms of K/(Ca+Mg) or grass tetany ratio trait in sainfoin forages. The K/(Ca+Mg) ratio means in sainfoin genotypes varied from 2.86 to 1.98 among the cutting seasons. K/(Ca+Mg) ratio means determined in sainfoin genotypes in spring season were found higher than those of determined in the autumn season. For spring and autumn seasons average K/(Ca+Mg) ratios were recorded as 2.69 and 2.19 respectively. The genotypes averages indicated that the K/(Ca+Mg) ratio mean was belong to Peschanjy-1251 (2.57) cultivar, while K/(Ca+Mg) ratio mean of Lütfibey (2.36) and INV Population (2.35) genotypes were found lower than the other sainfoin genotypes (Table 5).

Assessments with biplot analysis:

According to results of combined the two cutting seasons, the biplot graph indicated that PC1 (the first Principal Component) and PC2 (the second Principal Component) accounted for 67.09% and 27.93% of the total variation respectively, so the total PC score was found to be as 95.02% (Figure 1). Additionally, PC1, PC2 and total PC scores of biplot graph submitted on the Figure 2 were 69.35%, 30.65% and 100% respectively. Both biplot and correlation analysis indicated that there was highly significant (P<0.01) and positive relationship between Ca, Mg contents and Ca/P ratio in sainfoin genotypes. However, there was a negative relationship between these three traits with P content and K/(Ca+Mg) (tetany) ratio traits. Moreover, there was highly significant and similar, negative, relationship between P and K contents in sainfoin genotypes (Figure 1, Table 6). Additionally, relationship among the sainfoin genotypes and cutting seasons was submitted in the

Figure 1. When the Figure 1 examined, with regard to Ca content and Ca/P ratio traits CTC Population and Ozerbey-03 cultivars, with regard to Mg

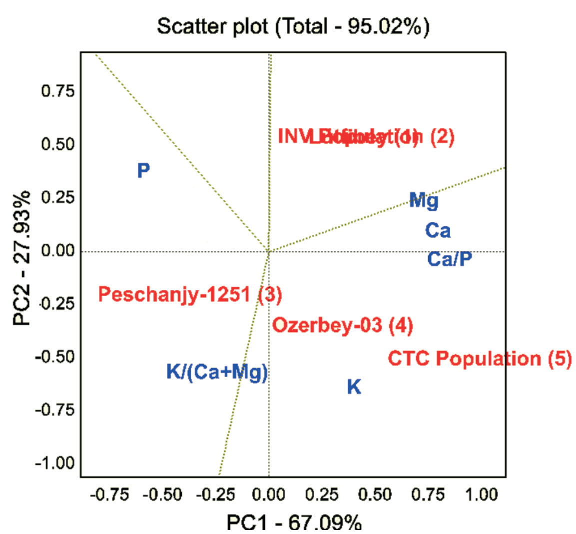

Figure 1. Scatter plot biplot graph showing relationship among the investigated traits and the sainfoin genotypes

Table 6. Correlation coefficients indicated relations among the examined traits in sainfoin genotypes

	Ca	K	Mg	P	Ca/P	K/(Ca+Mg)
Ca	-					
K	0.348^{ns}	-				
Mg	0.762**	0.061 ^{ns}	-			
P	-0.574*	-0.718**	-0.280 ^{ns}	-		
Ca/P	0.959**	0.509*	0.631*	-0.765**	-	
K/(Ca+Mg)	-0.758**	0.340 ^{ns}	-0.769**	0.066 ^{ns}	-0.600*	-

Significant at *: *P*<0.05; **: *P*<0.01, ns: Non-significant

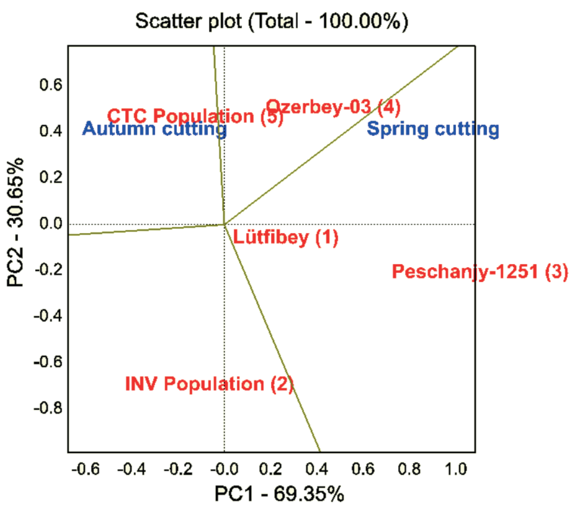

Figure 2. Scatter plot biplot graph showing relationship among the sainfoin genotypes and cutting seasons

content Lütfibey and INV Population genotypes, in terms of P content and K/(Ca+Mg) (tetany) ratio Peschanjy-1251 cultivar came to the fore by considering the two seasons means of the examined traits (Figure 1). On the other hand, by taking into to all of the examined traits together, Lütfibey and Peschanjy-1251 cultivars associated with spring season cutting, while CTC Population and Ozerbey-03 cultivars associated with autumn season cutting. However, in terms of the examined traits INV Population genotypes didn't associate with both of the seasons cuttings (Figure 2).

Discussion

The results showed that genotype × cutting season interaction were found as highly significant (P<0.01) for all of the examined traits. This indicated that the different cutting seasons led to change in ranking of sainfoin genotypes in terms of all of the examined traits (31). Furthermore, Ca, K, Mg and P contents determined in the study from the sainfoin genotypes, in spring cutting were found higher than those of determined in autumn season cutting. The decreasing in the minerals in the forage concentration with the advancing time can be attributed to use of the plants the mineral to their growth. Consistent with our findings, McDowell (32) and Spears (15) reported that macromineral contents of forages were high in early cuttings, whereas with progress in the plant growth period reduce plant macromineral contents because of using the nutrients in plant growth.

Calcium (Ca) is of importance in animal health. Almost 99% of the Ca element takes part in the structure of bones and teeth, the remaining 1% is involved in the fulfillment of important tasks in the animal body such as enzyme activity, blood coagulation, membrane permeability, secretion of certain hormones and nerves stimulation (15, 32). It was reported that there should be at least 3.00 g kg^{-1} Ca content in dry matter of forages in order to avoid any Ca deficiency in livestock feeding (14, 16, 33). In addition, comply with our research result, Underwood (34) and Spears (15) emphasized that Ca content of legume species higher than grasses and other family plants. Also, Khan et al. (35) reported that temperate forages generally contain more Ca content than those grown in the tropics. Accordingly, determined Ca contents (10.22 g kg^{-1} DM to 12.46 g kg^{-1} DM) in spring and autumn seasons in sainfoin, belong to legume family and cool season plant, were found easily enough for livestock requirements (Table 3). Additionally, consist with our Ca content findings, Ayan et al (36) reported that Ca content of forages were ranged from 5.40 g kg^{-1} DM to 12.90 g kg^{-1} DM. On the other hand, Kidambi et al (37) reported that Ca content in sainfoin forage was 6.5 g kg^{-1} DM. The cited Ca content was found lower than our findings. As a reason the differences between the Ca findings can be attributed to the differences between ecological and genotypic differences between the studies. By the way, according to Sabah and Celik (38), Basbag et al. (39) and Sayar (40) Ca deficiency leads to bone softening in young animal and bones deformations in the elderly ones. Additionally, they noted that it leads to the eggs to be thin-shelled in poultry. Moreover, McDowell (32) and Spears (15) cited that Ca deficiency causes reducing of growth and milk production in livestock.

Potassium (K) is an essential element in animal nutrition. However, it is an ignored element in livestock nutrition in the world owing to its abundant in the feeds of animals, especially in forages. In fact, the study result confirmed that among the examined minerals, the most abundant one by far was K element in forage content of sainfoin. Potassium works together with the sodium and chlorine elements in order to supply adjustment of the acid-base balance. By the way, the National Research Council (16) recommended that K content of forages shouldn't be under the 6.50 g kg^{-1} DM. Also Tajeda et al. (41) reported that for livestock requirements K content of forages should be at least 8.00 g kg^{-1} of DM. In Fact, K contents (25.41 g kg^{-1} DM to 37.16 g kg^{-1} DM) determined in the forages of the sainfoin genotypes were found to be quite above the references data, and the K contents found to be sufficient to meet potassium requirements of livestock (Table 3). Vuskovic et al (42) reported that K content of sainfoin populations in Serbia ranged from 14.89 g kg^{-1} DM to 20.91 g kg^{-1} DM. Although these K contents were found to be sufficient for livestock needs, they found considerably lower than our study the K contents detected in sainfoin forages. The reason for this difference between the K contents can be specified with the differences between the soils, especially in K contents of the soils. Indeed, our study research area soils having very high level K content and alkaline character (Table 1), whereas Serbia soils having lower amount K content and generally in acidic character (43). On the other hand, Underwood (34) and Spears (15) reported that signs potassium deficiency are rather nonspecific and include pica, eating or craving of things that are not food or feed, and reduced feed intake, growth, and milk production. Hence, potassium (K) supplementation is critical in dairy cattle during early or peak lactation for obtaining high levels milk producing and milk protein content.

Magnesium is called as "anti-stress mineral", and it helps to calm down in livestock, by reducing the hypersensitivity of the nervous system (44). Furthermore, it takes part in the mobilization of enzymes and the transformation sugar into energy in the blood. Additionally, it was reported that Mg supplementation in feeds not only eliminates the adverse effects of Mg deficiency in livestock but also increases intake and digestibility of feeds (15,45,46). In order to well perform these vital functions, Mg content of the feeds to be consumed by animal should be sufficient. It was reported that in order to prevent the abnormalities caused by Mg deficiency in animals, the Mg content in the feeds to be used in animal nutrition should be at least 1.00 g kg^{-1} of DM (14-16,19,47). In fact, Mg content determined in sainfoin genotypes in spring and autumn seasons were found just sufficient for livestock needs and a little above the indicated value (Table 4). Moreover, Vuskovic et al (42) reported that Mg contents of sainfoin genotypes ranged from 1.49 g kg^{-1} DM to 2.67 g kg^{-1} DM. The reported Mg contents were found to be partially compatible with our Mg contents, and the partially was found to be higher them. The slight differences between the findings can be attributed to the differences between the soils Mg contents and genotypic structure of the used genotypes.

Phosphorus (P) is an important element for living organisms. Since these two elements take place together in the structure of bones, P is generally evaluated together with Ca. In the same time, approximately 80% of the P element in the body takes place in the structure of bones and teeth, the remaining part is involved in the formation of vital tasks for living organisms. Indeed, the following important P involved tasks can be accounted; it takes part in cell growth, in the structure of DNA, RNA, ATP, ADP and AMP, in the formation of phospholipid and in the regulation of acid-base and osmotic pressure (15,34). It was reported in many literatures that 2.50 g kg^{-1} P content in forage dry matter is sufficient for the livestock needs (14, 16, 19). Accordingly, P contents determined in sainfoin genotypes in spring season (3.02 g kg^{-1} DM to 3.22 g kg^{-1} DM) were found to be sufficient for animal P requirements. However, P contents determined in sainfoin genotypes in autumn season (1.99 g kg^{-1} DM to 2.06 g kg^{-1} DM) were found lower than the specified P content for animal needs. Nonetheless, upon taking account the two seasons cuttings average, all of the tested sainfoin genotypes had enough P contents to meet livestock needs. In great agreement with our P findings detected in sainfoin genotypes in different seasons, Vuskovic et al (42) reported that P contents of sainfoin genotypes were ranged from 2.00 g kg^{-1} DM to 3.45 g kg^{-1} DM. P deficiency occurs when animals are fed with low-phosphorus feeds. According to Underwood (34) and Spears (15) signs of P deficiency in livestock as follows; decreasing in growth, milk production and efficiency of feeds, loss of appetite, impaired in reproduction and fragility and weakening of bones. With P supplementation, these undesirable disorders caused by P deficiency can be eliminated.

Existence of mineral contents in animal feeds in certain ratios are of great importance in terms of animal health (48,49). And, Ca/P ratio is one of the important ratio to be taken into account in livestock diets for the animal health. Generally, the desired Ca/P ratio in livestock feeds is from 1:1 to 2:1 (34). However, this ratio up to 10:1 can be tolerated without any adverse effect, provided there is sufficient P intake in livestock feeds (50,51). According to Acıkgoz (52) and Basaran et al. (49), if the Ca/P ratio is higher than 2:0, it probably causes *Milk Fewer* or *Hypocalcaemia* disorder in livestock. In the study, Ca/P ratios determined in the sainfoin genotypes in spring season cutting was found rather lower than those of in the autumn season cutting. Due to the fact that forage P contents of the sainfoin genotypes in spring cutting were higher than the reference data, so there is no condition that will cause Milk Fewer incidence in dairy cows. However, P contents of forages of sainfoin genotypes determined in autumn season cutting were found lower than the reference P content (2.5 g kg^{-1} DM). Hence, it should be caution against Milk fewer incidence, when livestock feed with autumn season forages. It was recommended that P supplementation in feeds and appropriate proportions of phosphorus fertilization application would assist to avoid risk of Milk Fewer incidence (49).

K/(Ca+Mg) or *Grass Tetany* ratio was firstly cited by Kemp and Hart (53). They reported that there is a significant increase in the incidence of the *Grass Tetany* or *Hypomagnesaemia* incidence. Afterwards, the K/(Ca+Mg) equation was accepted as *Tetany Ratio* by researchers and they emphasized that this ratio shouldn't exceed 2.2 in livestock feeds in order to avoid risk of the incidence (51,54-56). The incidence is one of the most important disorder seen in livestock (36). The disorder is caused by low Mg content in the blood, and its symptoms appear when this ailment is combined with Hypocalcemia (Milk Fewer) (57). According to Ensminger et al. (44), spasm of the legs and head backwards removal symptoms are seen in the livestock, when they suffer from grass tetany incidence. It has been reported that Mg deficiency caused by grass tetany mostly derived from imbalance of mineral elements (36,49,51). Result of the study showed that except for Peschanjy-1251 and CTC Population, the other sainfoin genotypes had lower K/(Ca+Mg) (tetany) ratios than the critical tetany value (2.2) in the autumn season cutting. However, K/(Ca+Mg) (tetany) ratios of determined in the sainfoin genotypes in the spring season cutting were found to be higher than the critical tetany value (2.2). In consistent with our results, Dahlen and Stoltenow (58) reported that with advancing plant maturity stage, grass tetany incidence is declining. Since the available Mg content in forages increases with progress of the maturation process. Additionally, it was reported that grass tetany illness generally is seen in livestock grazing on pastures having intensively lush green forage from grass species (40,51,52). In the end, to avoid risk of grass tetany incidence, Mg-rich supplementary feeds can be given to animals and K content of forages should be reduced.

Since the first time biplot analysis referenced by Gabriel (59), it has been used by researchers in many different disciplines (60). It has been used by agricultural researchers in various crops as well (61- 64). Among the reasons for the widespread use of the biplot analysis method in agriculture studies is that it is the ability to easily reveal the relationships of the investigated traits with each other and with genotypes via a simple graph (65). The total PC score ratio of a biplot graph has importance for a reliable interpretation, and many researchers reported that the total PC score of biplot graph should be at least 50% (66-69). In fact, the total scores of the biplot analysis graphs were found as 95.02% and 100% for Figure 1 and Figure 2 respectively. This indicated that the results reflected on the graphs had sufficient reliability for interpretation. In addition, it was reported that there is a significant ($P<0.05$) and positive relationship between traits or environments when their vectors located in close to each other on the biplot scatter plot graph. In other words, when there is an angle between the vectors less than 90°, this indicates the relationship (30,60,69,70). Accordingly, there was highly significant ($P<0.01$) positive relationship between Mg, Ca contents and Ca/P ratio traits, since the three traits were located in the same direction and close each other with lower than 90° angels among them on the biplot graph axis (Figure 1, Table 6). In fully agreement with the study findings, Basbag et al (71) reported that there was a highly significant and positive relationship among Ca, Mg contents and Ca/P ratio in animal feeds as results of the biplot analysis method. Also, similar relationship cited by Greaves (72) and Kidambi et al (37) for Ca and Mg contents of forages. On the other hand, it was cited that in the biplot analysis method graphs showing relationships among the environments or the examined traits, it indicates that there is a negative relationship between the environments or the examined traits located in opposite directions on the biplot graph axis (60,63,69-71). Accordingly, it was determined that there was a negative relationship between Ca Mg contents, Ca/P ratio and P content, K/(Ca+Mg) (tetany) ratio traits. Furthermore, a similar negative but highly significant relationship was also detected in sainfoin genotypes between the P and K contents traits located in opposite directions on the biplot graph axis (Figure 1). The results confirmed with the correlation analysis results in Table 6. By the way, relationship among the sainfoin genotypes and cutting seasons was submitted in the Figure 1. When the Figure 1 examined, with regard to Ca content and Ca/P ratio traits CTC Population and Ozerbey-03 cultivars, with regard to Mg content Lütfibey and INV Population genotypes, in terms of P content and K/(Ca+Mg) (tetany) ratio Peschanjy-1251 cultivar came to the fore by considering the two seasons means of the examined traits (Figure 1). Meanwhile, the examine traits of the study greatly influenced with seasonal variation between spring and autumn cuttings genotypes, this led to different rankings of the sainfoin genotypes in terms of the examined traits. Accordingly, by taking into to all of the examined traits together, Lütfibey and Peschanjy-1251 cultivars associated with spring season cutting, while CTC Population and Ozerbey-03 cultivars associated with autumn season cutting. However, in terms of the examined traits INV Population genotypes didn't associate with both of the seasons cuttings (Figure 2).

Conclusions

The study results showed that there were highly significant differences (P<0.01) between the cutting seasons in terms all of the examined traits. Biplot analysis revealed that by taking into to all of the examined traits together, Lütfibey and Peschanjy-1251 cultivars came to fore in spring season cutting, while CTC Population and Ozerbey-03 cultivars came to fore in autumn season cutting. In addition, the study results showed that Ca, Mg, P and K contents determined in the autumn season cutting were found lower than those of in the spring season cutting, but K/(Ca+Mg) (tetany) ratio of the spring cutting was found higher in sainfoin genotypes. Accordingly, sainfoin forage obtained in spring season cutting was found richer in terms of Ca, Mg, P and K contents, but due to higher K/(Ca+Mg) (tetany) ratio of the spring season, animal feed with the spring season sainfoin forage may be more susceptible to catching grass tetany incidence especially for dairy cows. To avoid risk of the incidence, the feeds having more Mg content and lower K content should be taken place in the animal rations.

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