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The Quality of Silage and Yield in Hungarian Vetch and Forage Crops and Rye Intercropping System

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HIGHLIGHTS

- The intercropping is a widespread cultivation system based on land use efficiency.
- The silages from same ratios in legume/cereal intercropping were prepared and analysed for quality.
- 80%Rye+20%Pea treatment was in both with high yield before ensiling and silage quality.
- Consequently, H. vetch and F. pea with rye silage combinations were in complementary.
- Features and chemical contents defined in silages confirm that sole treatments are not profitable for silage.

Abstract

The aim of the study was to determine silage yield and quality of Hungarian vetch/forage pea (V/P) with rye (R) mixed in an intercropping system that provides maximum level of faulting from the field. The field experiment was conducted in 2021-2022 to examine the effects of different binary sowing ratio (20:80%R/P, 40:60%R/P, 60:40%R/P; 80:20%R/P; 20:80%R/V, 40:60%R/V, 60:40%R/V; 80:20%R/V, 100%R, 100%P, 100%V) in 3 replications. The treatments were harvested in July 2022 for silage, and hay yield and fresh yield were determined in the intercropping treatments before ensiling. On the same day, silages were filled by the same mix ratios in plastic cans. After fermentation, the dry matter ratio, pH, sucrose, crude protein, ADF, NDF, mineral matters and organic acid were defined in silages. Before ensiling the highest fresh and dry yield were obtained from 80:20%R/P. Yield values were decreased by increase of rye ratio in the mix. The dry matter, crude protein, lactic acid formation, the inhibition of undesirable micro-organisms and nutritional quality has been improved in rye silages prepared with H. vetch and F. pea contribute. Consequently, the combinations of the H. vetch and F. pea contribute to rye silage are complementary, and the intercropping of the binary combination made profitable forage yield and silage quality, according to sole treatments. The positive effects in the investigated parameters are in all mixed ratios, but, 80:20R/P, 60:40R/P and 80:20R/V silages were more superior to the others. Intercropping system, mix silage, rye, legumes, silage quality

Keywords: Intercropping System, Mix Silage, Rye, Legumes, Silage Quality

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1. Introduction

Intercropping of annual legumes with winter cereals is a very common practice for forage production in many countries. Legumes are highly nutritious forage crops produced both as grass and seeds due to their high protein content (30-35%), inorganic minerals, calcium and phosphorus, and vitamins. In addition, leguminous proteins are characterized as proteins of high biological value, making them an essential component of animal feed in addition to grains. Especially, forage peas and vetch crops have a high potential in intercropping due to their widespread cultivation in Turkey and their high protein content. Rye, on the other hand, is a grain whose use as a fodder is inactive, has low crude protein (CP) and is rich in fiber. Its forage quality can be improved by mixing with legumes rich in CP. According to some research, legumes-cereals intercropping ensure stable biomass yield and feed quality (Lithourgidis et al., 2007; Galanopoulou et al. 2019). Especially in dry agriculture, many studies have been carried out with legumes-cereals intercropping by aimed at silage or forage grazing (Crusciol et al. 2013; Pariz et al. 2016) and thus the intercropping system has proven to be a way to improve the quality of silage (Lima et al. 2010; Amado et al. 2012; Gulumser et al. 2021a). Besides all these, the intercropping system stands out as an opportunity to reduce the environmental impact of animal production due to the reduction in rumen methanogenesis and increased protein supply for animals (Melesse et al. 2017).

The quality of the silage material is a fundamental consideration in any forage production system. High quality forage should have high intake, digestibility and efficiency in use (Costa et al. 2014). It is known that quality silages have higher dry matter, protein and nutrient content and high-quality fiber content, low pH. Neutral detergent fiber (NDF) is a measure of the total cell wall fraction, and acid detergent fiber (ADF) forms the indigestible portion of the forage mix. Lactic acid in silage is the dominant fermentation product, another important evaluation index of silage quality. Organic acids present in properly fermented silage are energy sources that affect the performance of ruminants (Daniel et al. 2013). The most important of these organic acids, lactic acids, are produced by lactic acid bacteria (LAB), which is naturally present in the plant. Epiphytic LAB convert water-soluble carbohydrates (WSC) into lactic acid (LA) under anaerobic conditions. There is great variation by species in LAB numbers in crops (Broberg et al. 2007; Comino et al. 2014).

Because legumes have low WSC, silage quality is low, but DM and protein content are high. On the other hand, rye is just the opposite. Moreover, some undesirable properties such as low dry matter, nutritional value, protein and quality fiber content, including intense alcoholic fermentation, when cereals are ensiled in pure form, limit the use of silage (Lopes and Evangelista, 2010; Pedroso et al. 2005; Rezende et al. 2011; Siqueira, et al. 2012). The less and slow lactic acid is formed, the more acetic and butyric acid are increased (Guo et al. 2018). High concentration of acetic acid, the main metabolite of Acetobacter, always leads to a higher pH, which benefits the unwanted microorganism Clostridia (Zheng et al. 2017). This reduces the quality of silage by causing a decrease in protein content and nutritional value, and silage deteriorates. To prevent these problems, additives such as urea, calcium oxide, virgin lime, limestone, sodium chloride and LAB are used (Ribeiro et al. 2010; Balieiro Neto et al. 2007; Amaral et al. 2009; Rezende et al. 2011). However, additives can increase production costs and adversely affect the environment. The best sustainable and cost-effective alternative to improve silage quality is the legume-cereal intercropping system. In this way, both the efficiency of land use is increased and cheap and high-quality roughage is obtained.

Intercropping forage pea (*Pisum sativum* spp arvense L.) or Hungarian Vetch (*Vicia pannonica* CRANTZ.) with rye (*Secale cereale* L.) is an alternative way of cropping to improve forage yield and quality for hay production compared to mono-cropping. However, it has been determined that the water, nutrient and light competition of cultivated plants generally reduces the yield of the mixture compared to monoculture (Lithourgidis et al. 2011, Lithourgidis and Dordas 2010). Therefore, it is important to choose the intercropping system with the appropriate sowing rate. In this context, binary mixes of H. vetch and F. pea with rye were grown at four different ratios and silages were prepared at the same ratios. As a result of the study, the effect of sowing rates on the chemical composition and yield of silages was investigated.

2. Materials and Methods

2.1. Field experiment

The field experiment in 2021–2022 years was conducted at the Agriculture Production and Research Centres at Yozgat Bozok University (39° 38′ 17″ N, 34° 28′ 1″E), Turkey. According to the data of the Turkey meteorology general directorate, the monthly minimum, maximum temperature and total precipitation of this field are 11.6°C, 22.7°C and 174 mm, respectively. The soil samples taken from the three sites at a depth of 0–30 cm were analyzed by the University-Industry-Public Cooperation Development Application and Research Center (USKIM) laboratory. According to this analysis result, the soil at the field is a clay loam texture with moderate organic matter, lightly salted and highly phosphorus.

Table 1. Sowing method of mix plots and seeding rate by weight and component crop seeding ratio of legumes–rye intercrops.

Treatments	Component crop seeding ratio (%)	Seeding rate by weight (kg da^{-1})	Sowing method of mix plots (o=rye, ж= intercrop legume species)
Rye	100	22	o ж o ж o ж
H. Vetch	100	12	o ж o ж o ж
F. Pea	100	12	o ж o ж o ж
20:80 R/P	20:80	4.4:9.6	o ж o ж o ж
40:60 R/P	40:60	8.8:7.2	o ж o ж o ж
60:40 R/P	60:40	13.2:4.8	o ж o ж o ж
80:20 R/P	80:20	17.6:2.4	o ж o ж o ж
20:80 R/V	20:80	4.4:9.6	o ж o ж o ж
40:60 R/V	40:60	8.8:7.2	o ж o ж o ж
60:40 R/V	60:40	13.2:4.8	o ж o ж o ж
80:20 R/V	80:20	17.6:2.4	- 30cm - 30cm - 30cm - 30cm - 30cm -

o: rye, ж: legumes

For each legume, binary mixtures of forage pea (*Pisum sativum* ssp. *arvense* L., "P") and Hungarian vetch (*Vicia pannonica* CRANTZ.; "V") and rye (*Secale cereale* L.; "R") four different ratios were used in the experiment. The field trial was in a randomized block experiment design with four replications. Seeding rate (kg da^{-1}) and mixture ratios in the intercropping system are given in Table 1. Sowing was done in October 2021 into 6 rows on 6 meter long plots with a distance of 30 cm. The mixture plots were in the form of one row of rye and one row of legume (Table 1). The plots were harvested on 18 July 2022 for silage, in account the development period of rye. The fresh yield (kg da^{-1}) was determined by weighing the plants that were separated by hand to each species and in 1 m2 area located homogeneous of the plots (3 repeat). Hay yield (kg da^{-1}) was calculated by weighing after drying at 65 ^{0}C. The Materials and Methods should be described with sufficient details to allow others to replicate and build on the published results. Please note that the publication of your manuscript implies that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose any restrictions on the availability of materials or information at the submission stage. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

2.2. Silage experiments

After harvest, plants were chopped in 2 cm size (Gulumser et al., 2021b) with same mix ratios and filled in 1.0 kg plastic cans with 3 replications. The silages, which were sealed in an airtight way, were left for fermentation at 25±2 ^{0}C for 45 days. When the cans were opened after fermentation, the tops of 3-4 cm were discarded. The dry matter ratio was calculated by weighing after dried 100 g samples from each silage at 105 ^{0}C. To determine the sucrose and pH of the silages, 20 g of sample was mixed homogeneously with 100 ml of distilled water in a blender and filtered into 50 ml eppendorf tubes with filter paper (Basaran et al. 2018). The pH was determined with the HANNA Edge digital pH meter. The sucrose content was determined with a refractometer (HANNA HI 96801 Digital Refractometer 0.85% Brix) device.

For chemical analysis of silage, the 100 g silage sample was dried at 65 0C until the constant weight was ground (<1 mm). ADF (acid detergent fiber), NDF (neutral detergent fiber), crude protein (CP), Ca, Mg, K and P ratios were determined by Foss NIR Systems Model 6500 Win ISI II v1.5 device with IC-0904-FE calibration program. The organic acids (lactic, acetic and butyric acids) were determined on HPLC (Shimadzu, Kyoto, Japan) auto sampler system model LC - 20AT equipped with four pumps and an SPDM20A diode array detector (DAD) at YOBU Science and Technology Application and Research Center. However, in the result analysis, butyric acid was not detected.

2.3. Statistical analyzes

All of the data were analyzed using the SPSS 20.0 (SPSSInc., Chicago, IL, USA) Duncan test was performed to determine the significant differences among treatments and those with a p value less than 0.05 were considered significant differences.

3. Results

The fresh and hay yields of legume-rye intercropping were in significant differences (p<0.01) among the treatment combinations (Figure 1). Since high yield is directly related to high biomass, both yields peaked in sole rye, as expected. Also 80:20 R/P and 80:20 R/V mixing ratios are in the same group with sole rye. The lowest value was obtained from the sole H. vetch and F. pea plots.

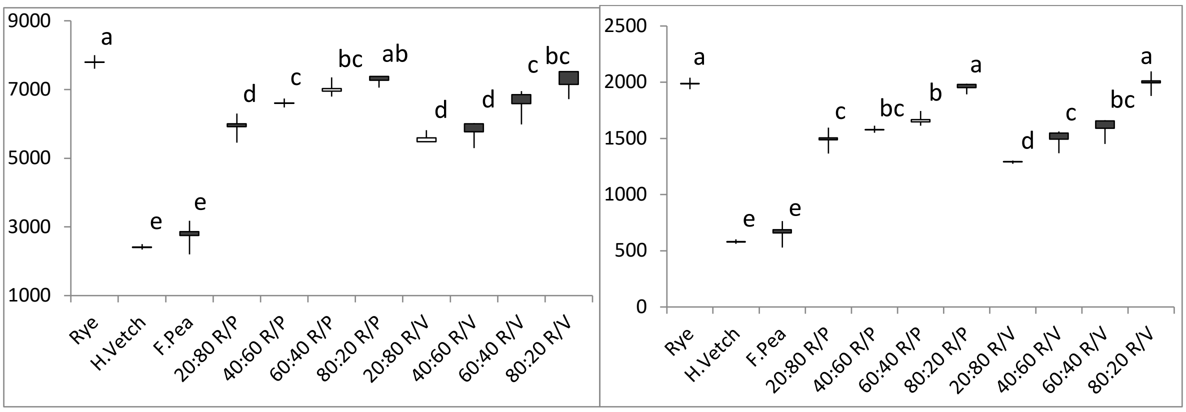

Figure 1. Effects of the legumes-rye binary mixture ratio on fresh** and hay yield** (There is no difference between the means shown in the same letter (p<0.05). **:P<0.01)

Table 2. Chemical composition (%) of silages.

Treatments	pH**	Sucrose **	DM**	CP**	ADF**	NDF**
Rye	4.74 bc	4.50 a	37.76 a	6.41 h	39.76 a	70.72 a
H. Vetch	4.98 a	2.27 b	23.83 e	18.81 b	33.46 bc	46.78 g
F. Pea	4.78 b	4.40 ab	22.75 e	22.82 a	22.82 e	39.78 h
20:80 R/P	4.71 cd	4.53 a	28.07 d	14.70 d	34.24 b	53.90 ef
40:60 R/P	4.68 de	4.50 a	30.13 cd	13.26 d	35.74 b	58.22 de
60:40 R/P	4.62 fg	4.50 a	33.67 b	10.00 ef	39.15 a	65.59 bc
80:20 R/P	4.58 g	4.50 a	36.86 a	8.61 fg	40.48 a	68.39 ab
20:80 R/V	4.72 cd	2.30 b	28.89 d	16.51 c	29.05 d	51.54 f
40:60 R/V	4.62 fg	2.37 b	31.60 bc	13.93 d	30.99 cd	56.22 ef
60:40 R/V	4.62 fg	3.80 ab	33.99 b	11.13 e	34.75 b	61.43 cd
80:20 R/V	4.65 ef	4.60 a	37.32 a	7.50 gh	40.06 a	70.47 a
Means	4.70	3.85	31.35	13.06	34.59	58.46

**:p<0.01There is no difference between the means shown in the same letter (p<0.05).

The chemical components presented in Table 2 were significantly affected by legume-rye mix silages. The highest pH was determined in H. vetch (4.98), while the lowest was in 80:20 R/P (4.58). The pH of mixture silages were dramatically reduced when rye ratio was increased. The best results of sucrose were determined in all applications except sole H. vetch, 20:80 R/V and 40:60 R/V. The sucrose content of silages was affected more positively by F. pea compared to H. vetch contribution. The DM was much higher in the 80% rye combinations and sole rye than in the other mixed arrangements. The lowest values were in sole H. vetch (23.83%) and F. pea (22.75%). Therefore, the effect of both legumes on rye silage DM was similar. An increased legume contribution in the mixture increased the rye silage CP. However, among intercrops, F. pea affected the CP of rye silages much more than the H. vetch. The ADF content of the silages varied between 22.82 (sole F. pea) and 40.48% (80:20 R/P), while NDF ranged from 39.78% (sole F. pea) to 70.72% (sole rye).

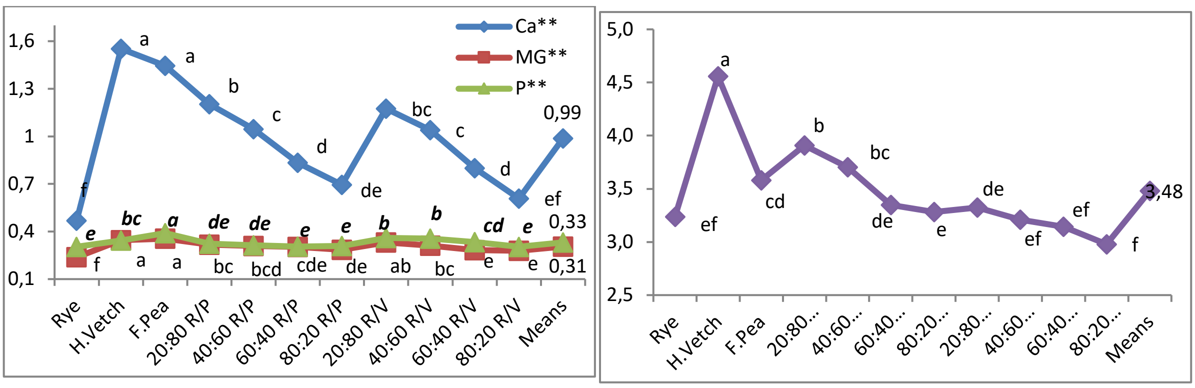

Figure 2. Effects of the legumes-rye binary mixture ratio on lactic** and acetic acid** ratio of silages *(There is no difference between the means shown in the same letter (p<0.05). **:P<0.01)*

The mineral content of the silage samples was significantly affected by the mixing ratios (Figure 2). Mineral content of sole legume silages was higher than rye silage. Therefore, in parallel with the increase in the legumes ratio in the mixture, Ca, P, Mg and K of the silages increased. Forage pea contribution to Ca and K contents of rye silage was more effective than the cowpea contribution. In terms of Mg content, both legumes were equally effective, while H. vetch was more effective in P content. Organic acids were primarily influenced by the effects of sainfoin with maize and sorghum binary mix ratios (Figure 3). The highest lactic acid (LA) was found in sole rye (3.05%), 80:20 R/P (3.10%), 60:40 R/V (3.12%) and 80:20 R/V (3.08%). In other words, unlike the mineral matter, lactic acid increased in parallel with the rye ratio. The lowest LA was in sole forage pea (2.08%). The acetic acid (AA), which is directly related to the spoilage of silages, ranged from 0.31 (80:20 R/V) to 0.53% (20:80 R/P). Due to the low water-soluble carbohydrates of legumes, the lactic acid level has increased with increasing rye ratio, but acetic acid level decreased. The best result of AA content was defined in 80:20 R/V. As a last observation, the effect of H. vetch added silages on organic acids was greater than that of F. pea.

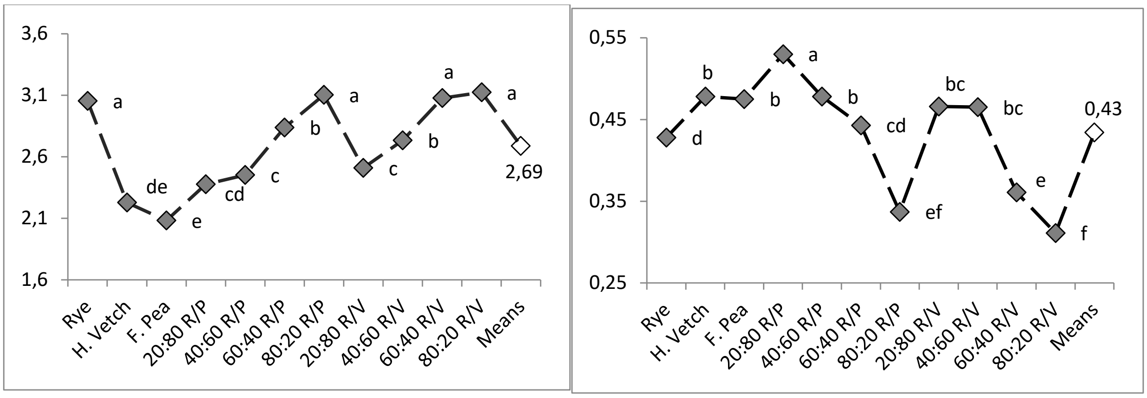

Figure 3. Effects of the legumes-rye binary mixture ratio on lactic** and acetic acid** ratio of silages (*There is no difference between the means shown in the same letter (p<0.05). **:P<0.01*

4. Discussion

Intercropping legumes with cereals is an alternative way to improve forage yield and quality for hay production compared to mono-cropping. The fresh and dry yield accumulate of silage plants is should be sufficient to produce the best quality profitable of silage, before ensiling (Jeroch et al. 1999).The yield parameters examined for this purpose were obtained from the highest 80:20R/P mixture, except sole sowing. Also, the decrease in the rate of rye with high biomass in the mixtures caused a decrease in yield. Many researches (Gianoli et al. 2006; Basaran et al. 2017; Lienhard et al. 2020; Igbal et al. 2021) support these results.

For quality animal production, DM in silages should be 25-40% (Panyasak and Tumwasorn 2013). The DM was found suitable in mixed silages, but it was low in sole legumes. Low DM in the silages increased pH (sole H. vetch) and decreased sucrose (sole H. vetch, 20:80 R/V and 40:60 R/V). The all mix silages yielded a pH within the range of 3.8 to 4.8, which is considered ideal for good quality silage (Fiyla 2001). Moreover, sucrose decreased with increased percentage of H. vetch silage in the mixtures possibly due to sole H. vetch having a low WSC content suitable for lactic acid production. This situation can be explained that if the DM is going out of ideal limits, anaerobic bacteria can grow and decompose sucrose and protein into butyric acid and ammonium. In addition, increasing the unwanted epiphytic microorganisms reduces the fermentation process and quality of the silage (Jeroch et. al. 1999).

Intercropping legumes with cereals in silage is mainly aimed at increasing the silage CP content, since grasses such as rye have low CP levels (Neres et al. 2012). Minson (2012) reported that minimum protein level in the feed should be 7% CP for ruminal fermentation. The CP content of other silages except sole rye was found above this value. Thus, including 20% legumes in rye silage provided the minimum CP content for good ruminal functioning. The increase in CP of mix silages is explained by the legumes' high CP content (Amaefule et al. 2011). ADF and NDF of silages are factors affecting energy intake and milk yield of dairy cows (Ferraretto et al. 2015; Tharangani et al. 2020). NRC (2001) reported that quality silages are typically expected to have 25-35% ADF and 40-50% NDF. It has been observed that the ADF content of the mixtures with high legume contribution is between these limits. The decrease in these contents may be due to the plant species, the harvest period as well as the competition from high mixed sowing density. Similar results were found in some studies in which the CP, ADF and NDF contents of legume-grass silages were determined (Baležentienė and Mikulionienė 2006; Seydoşoğlu 2019; Mut et. al. 2020; Gulumser et al. 2021a).

Successfully fermented silages generally have a higher nutrient content (Kung et al. 2018). The mineral substances examined in silages are the elements that must be met from the feed in order for the animals to perform their functions in a healthy way (Yogeshpriya and Selvara 2018; Ahemad et al. 2009; Trailokya et al. 2017; Arnoud 2008). Kidambi et al. (1993) and Tekeli and Ateş (2005) concluded that at least 0.8% K, 0.21 P, 0.3 Ca and 0.1 Mg in forage for balanced nutrition of animals are reported. In addition, legumes have a richer nutritional content than grass (Dumlu Gul and Tan 2018). All silages in the study had minerals above these values. Also, mineral content rye silage has been increased with the legumes contributing and this result is similar to Gulumser et al. (2021b).

Organic acids present in properly fermented silage are energy sources that affect the performance of ruminants (Daniel et al. 2013). Lactic acid in silage is the dominant fermentation product, another important evaluation index of silage quality. Acetic acid and Butyric acid are indicators of increased unwanted organisms and unsuccessful fermentation in silages. In all of the silages, the existence of high LA, low AA and lack of butyric acid determined and it indicate the quality fermentation, which is an indicator of well preservation (Auerbach et al. 2013). Also, lactic acid should be above 2% and acetic acid should be below 0.8% in quality silage (Alcicek and Özkan 1997), and our results were found to be within these limits. However, the highest LA and lowest AA were determined in 80:20 R/P and 80:20 R/P mix silages. These results show that the addition of legumes to high carbohydrate rye silage is successful.

With the ratio H. vench and F. pea contributed to rye silages, the pH and acetic acid were decreased, lactic acid formation was increased, and better-quality silage was obtained compared to the lean ones. The effect of H. vetch added silages on organic acids was greater than that of F. pea. This finding was consistent with previous data and Gulumser et al. (2021b) stated that can increase lactic acid content with mixtures, while Basaran et al. (2018) reported that levels of different ratios of mixed have varying effects (Li et al. 2022). However, some studies (Li et al. 2016; König et al. 2019) have shown different effects. These differences in silage quality may be due to plant species, cultivation, climatic conditions, soil fertility, growing period and harvest time.

5. Conclusions

In this study, the quality of silages obtained from different mixtures of legume-rye grown by making maximum use of the unit area was examined. The main purpose of this study was to reduce DM loss; support lactic acid formation, the inhibition of undesirable micro-organisms and the improvement of nutritional quality in rye silages prepared with H. vetch and F. pea contribute. Consequently, the combinations of the H. vetch and F. pea contribute to rye silage are complementary, and the intercropping of the binary combination made profitable forage yield and silage quality. The forage yield of the mixtures before ensiling was higher than the sole legumes, and the highest yield was determined in 80:20R/P. Positive indices such as CP, lactic acid and chemical contents defined in H. vetch and F. pea rye mix silages confirm that sole rye is not profitable for silage. We have determined that the positive effects in the investigated parameters are in all mixed ratios. However, 80:20R/P, 60:40R/P and 80:20R/V silages were more superior to the others.

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