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Forage Yield and Quality Characteristics of Winter Legume / Grass Mixtures Harvested at Different Phenological Stages

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Abstract: Winter forage crop mixtures are important sources of quality forage as well as the benefits they bring to the soil and the main crop. In regions with year-round production, leaving the soil fallow during the winter months and preferring maize-maize or cotton-cotton production model may negatively affect the nutrient balance in the soil. For this purpose, forage yield and quality characteristics of 12 different winter forage crop applications (4 pure and 8 mixtures) were investigated between 2014-2016 in Büyük Menderes basin (Aydın / Türkiye). The experiment was designed according to the split-plot in randomized blocks and harvest were carried out in 2 different phenological periods with 4 replications. Herbage yield (kg da^{-1}), ADF (%), NDF (%), ADL (%), crude protein ratio (%), crude protein yield (kg da^{-1}) and relative feed value averages were measured. According to the results obtained from the experiment, there are differences between crude protein yield and relative feed values. When analyzed in line with the production purpose, 75% forage pea + 25% oat mixtures stand out in terms of crude protein yield, while 55% forage pea + 45% annual ryegrass stands out in terms of relative feed value. Due to the changes in harvest times depending on the characteristics, it is thought that it would be ideal to perform harvest operations between 50%-100% flowering.

Farklı Fenolojik Dönemlerde Biçilen Kışlık Baklagil / Buğdaygil Karışımlarının Yem Verim ve Kalite Özellikleri

Anahtar Kelimeler
Kışlık ara ürün yem bitkileri, Tek yıllık yem bitkileri karışımları, Yem kalitesi, biçim zamanı

Öz: Kışlık yem bitkileri karışımları kaliteli kaba yem kaynağı olmaları yanı sıra toprağa ve ana ürüne kazandırdıkları ile önemli kaynaklardır. Yıl boyu üretim yapılan bölgelerde kış aylarında toprağın nadasa bırakılıp mısır-mısır ya da pamuk-pamuk üretim modelinin tercih edilmesi toprakta bitki besin elementi dengesini olumsuz yönde etkileyebilmektedir. Bu amaçla Büyük Menderes havzasında (Aydın / Türkiye) 2014-2016 yılları arasında 4 saf ve 8 karışım olmak üzere 12 farklı kışlık ara ürün yem bitkileri uygulamasının yem verim ve kalite özellikleri incelenmiştir. Deneme tesadüf bloklarında bölünmüş parsellere göre 4 tekerrürlü olarak tasarlanmış ve 2 farklı fenolojik dönemde biçim işlemleri gerçekleştirilmiştir. Denemede kuru ot verimi (kg da^{-1}), ADF(%), NDF(%), ADL(%), ham protein oranı (%) değerleri ölçülürken ham protein verimi (kg da^{-1}) ve nispi yem değeri ortalamaları hesaplanmıştır. Denemeden elde edilen neticelere göre ham protein verimi ve nispi yem değerleri arasında farklılıklar söz konusudur. Üretim amacı doğrultusunda incelendiğinde ham protein verimi açısından %75 yem bezelyesi + %25 yulaf karışımları öne çıkarken nispi yem değeri açısından %55 yem bezelyesi + %45 tek yıllık çim öne çıkmaktadır. Biçim zamanlarında özelliklere bağlı olarak yaşanan değişimlerden dolayı %50-%100 çiçeklenme arasında biçim işlemlerinin gerçekleştirilmesinin ideal olacağı düşünülmektedir.

1. INTRODUCTION

Food products derived from livestock have become very important in meeting the increasing demand for food worldwide. With the increasing population in developing countries, meat demand has increased by 5-6% and dairy demand by 3.4-3.8% compared to the end of the 20th century [1]. As a result of these data, it is clear that feeding livestock will be one of the main issues of our future with the increasing human population. One of the main objectives of livestock feeding management is to provide the forage of sufficient quality, high efficiency and at the same time cheap. While species selection is a factor that directly affects feed quality, the effect of area selection on feed quality by itself is low [2]. In addition to being included in the cropping pattern, forage crops, which contain the nutrients necessary for the gastric microflora of livestock in a sufficient and balanced ratio, contain the nutrients necessary for microorganisms that help the digestive systems of livestock to function more regularly [3]. In addition to being a source of quality roughage, legume forage crops make elemental nitrogen useful to the plant thanks to *Rhizobium* bacteria [4].

In Türkiye, forage production is quite inadequate according to the livestock population [5] and our dry forage production, which can be qualified as high quality, is around 4 million tons. Field cultivation of forage crops share was 1.6% in the early 2000s, to 8.2% in 2010 with the impact of subsidies and In 2019, it increased to 13%. Although the Ministry of Agriculture and Forestry of the Republic of Türkiye provides subsidies for forage crops today, production has not reached the desired point. It is clear that the subsides have increased the cultivation areas of forage crops [6]. Although there are approximately 17.220.903 livestock in Türkiye in 2018, we are in a very insufficient situation in terms of animal product production. In today's conditions, it is not possible to close the forage deficit by growing traditional forage crops as the main crop and utilizing rangeland-pasture areas.

In Aegean region, wheat and cotton are the traditional main crops in the field. In the form of cotton-cotton cultivation, the field can remain empty for 5-6 months in winter and in wheat-wheat cultivation, the field can remain empty for 4-5 months in summer [7]. Also, in terms of land use efficiency, especially in countries bordering the Mediterranean Sea, mixed cultivation of annual legume and cereal forage crops has been adopted more than growing them separately today [8]. In this cropping pattern, cropping areas are better utilized, and the legumes in the mixture play a role in increasing soil fertility in a way to meets the needs of the cereals with the nitrogen they release into the soil. Due to this effect of legumes, the use of pesticides and chemical fertilizers is reduced and the damage caused by agriculture to the environment is minimized. In addition to reducing diseases and pests, intercropping increases forage quality and yield as it increases crude protein yield [3,9,10].

One of the most important legumes that can be used as a winter forage crop in mixtures is common vetch (*Vicia sativa* L.), which we are at the forefront of the world in terms of production and is an important forage crop in Türkiye. Another one is forage pea (*Pisum sativum* ssp. *arvense* (L.) Asch.), whose production and importance are increasing today. The species that can be used in mixtures are oat (*Avena sativa* L.), which can provide good yield and is easy to produce, and annual ryegrass (*Lolium multiflorum* Lam.), which is known to protect the soil with its dense root structure as well as its ability to dry quickly due to its thin stems. These crops can be planted in mixtures and have high yield potential [35].

This study was conducted to determine the most suitable forage crop mixtures for winter cover crop in Aydın province and similar ecologies. It is thought that the findings obtained from the study can be an example for agricultural enterprises in this region and agricultural organizations and researchers working on the subject can benefit from these results.

2. MATERIAL AND METHOD

2.1. Material

Forage pea (*Pisum sativum* ssp. *arvense* (L.) Asch. cv. Ürünlü), common vetch (*Vicia sativa* L. cv. Alper), oat (*Avena sativa* L. cv. Sarı) and annual ryegrass *(Lolium multiflorum* Lam. cv. Caramba) were used as mixture materials.

2.2. Method

The research was conducted in the experimental fields of Aydın Adnan Menderes University, Faculty of Agriculture (37º 45' 51" N, 27º 45' 32" E, 27 m altitude) during the 2014-2016 winter production seasons. Soil analysis of the experimental field was carried out in the laboratories of the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Aydın Adnan Menderes University. Soil texture was determined by Bouyoucos hydrometer method [11]. Soil pH was measured by pH meter according to Richards [12]. Phosphorus content was calculated calorimetrically. Potassium content was measured by flame photometer method as described by Richards [12]. Organic matter content was determined by wet burning and organic carbon value was multiplied by Van Benmelen factor [13].

According to the results of the soil sample analysis, the soil with high sand content had a loamy texture and showed an alkaline characteristic with a pH of 8.10. Phosphorus, which is a macronutrient element, was high in the soils, which were also observed to be low in terms of organic matter. When analyzed in terms of micronutrients, it is seen that K, Na, Fe, are high, Mg is very high, but Ca mineral are sufficient (Table 1.).

Table 1 Analysis results of the experimental area soil (0-30 cm)

P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Fe ppm	pH	Total Salt (%)	Organic Matter (%)
19	903	2740	1164	46	8.32	8.16	0.0093	1.20

According to the averages of climate data of the experiment area during the production period and long-term climate data, the first-year temperature values are similar to the long-term data except December. However, in the second year temperatures were fluctuating and higher than the first year (Figure 1). According to the precipitation data, the higher and irregular precipitation of the first year compared to the long-term data caused negative effects on the plants during the development period. In the second year, there was no precipitation data collected in December, but the precipitation during the vegetation period did not cause any obvious negative effects on development. The fact that differences were observed in both years compared to the long-term data was seen as a sign of how the mixtures may react under extreme conditions.

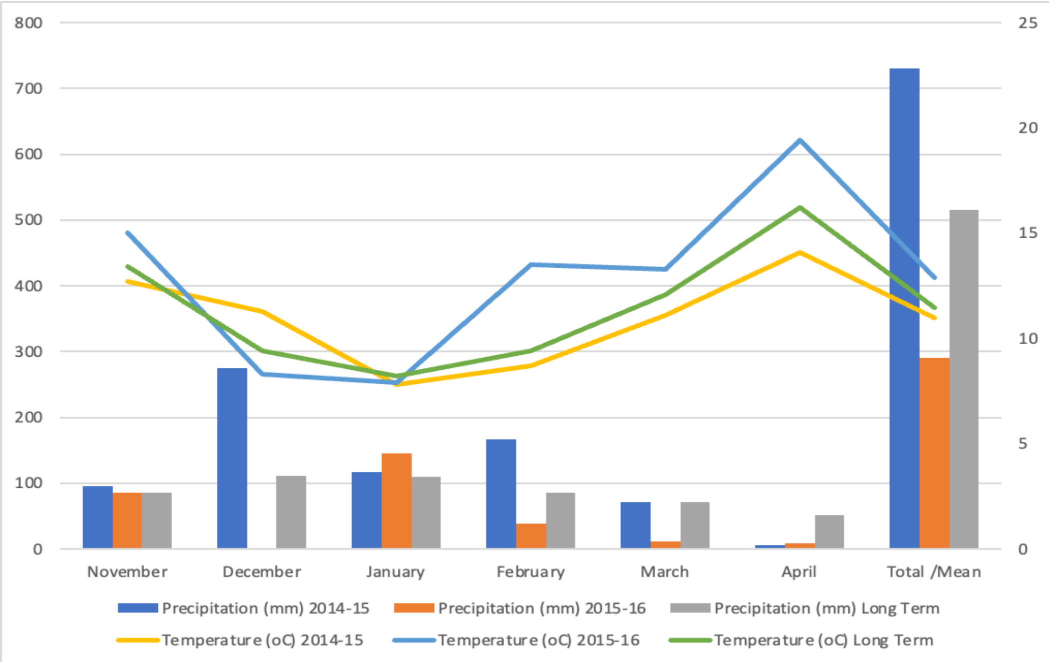

Figure 1. Climatic data during the experiment and according to long-term averages

Sowing was done manually with lines in November in both years. In the experiment, a plot of 2m × 5m = 10 m^2 was planted in 10 rows with 4 replications. In order to control weeds, a gap of 2 meters was left between blocks and 50 cm between plots. There were 12 treatments as 4 pure (100% forage pea, 100% common vetch, 100% oat, 100% annual ryegrass) and 8 legume:grass mixtures (75:25, 55:45). The research was designed according to the randomized block design with split plots. In pure plots, 12 kg da^{-1} forage pea, 12 kg da^{-1} common vetch, 18 kg da^{-1} oat, and 2 kg da^{-1} annual ryegrass were used [14,15]. Before sowing, legumes were inoculated with *Rhizobium* bacteria in the evening hours in an environment without high light, and according to the results of soil analysis, 3 kg da^{-1} pure nitrogen and 7 kg da^{-1} pure phosphorus were applied as base fertilizer. Forage pea, common vetch and their mixtures were harvested at 50% and 100% flowering time of legumes. During this period, oats were in Zadoks 73 (milking season) and annual ryegrass was in full flowering. Herbage yield was measured after both harvest times.

Herbage yield (kg da^{-1}) was measured by fan drying oven (Mikrotest, MST, Ankara, Türkiye) at 70°C until the weight was fixed [16]. The dried samples were ground in a mill passed through a 1 mm screen. The crude protein ratio (%) of the samples taken from the experiment was measured with Kjehdahl method according to AOAC [17]; NDF, ADF and ADL contents (%) were measured (ANKOM A200, Macedon, NY, USA) according to Van Soest et al. [18]. The crude protein yield (kg da^{-1}) and relative feed value were calculated by the obtained data by following the procedures of Horrocks and Vallentine [4].

To compare values, the analysis of variance was performed with the LSD multiple comparison method using the 'agricolae' package [19] in R Studio (V4.1.2). Correlogram was created in R Studio using the 'metan' package [20]. Heat map was made in R Studio using the heatmap.2 command within the ‘gplots’ package [21].

3. RESULTS AND DISCUSSION

According to the data obtained from the experiment, statistically significant treatments and interactions are presented in Table 2. According to these data, while the mixture treatments have statistically significant differences in terms of all the examined traits, the same is also seen in the interaction with harvest time.

Table 2. Sources of variation in the experiment and statistical significance levels of interactions between each other

	HY	ADF	NDF	ADL	CPR	CPY	RFV
Year (Y)	**	**	**	ns	ns	**	**
Mixtures (Mx)	**	**	**	**	**	**	**
Harvest (Har)	**	ns	**	ns	ns	**	**
Y x Mx	**	**	*	**	ns	**	**
Y x Har	**	ns	**	ns	**	**	**
Mx x Har	**	**	**	**	**	**	**
Y x Mx x Har	**	*	**	**	**	**	**
	*: P≤0.05 **: P≤0.01 ns: non-significant						

HY: Herbage yield; CPR: Crude Protein Ratio; CPY: Crude Protein Yield; RFV: Relative Feed Value;

While there was a difference between the two years in terms of herbage yield values, the average of the values obtained in the second year was higher with 469.64 kg da^{-1}. In terms of harvesting time, it was observed that the harvest at 100% flowering period was in the lead with 437.71 kg da^{-1}. Among the treatments, the application with the highest herbage yield was found in pure oat application with 578.89 kg da^{-1}, while 75% common vetch + 25% annual ryegrass had the highest value among the mixtures (Appendix A,B). Kocer and Albayrak [22] reported that in a mixture trial with forage pea, oat and barley, oat had the highest yield in terms of herbage yield and in mixtures, forage pea - oat mixtures had high values. In some studies, it was stated that pure sowing had higher values than mixtures [23,24], while in some others mixtures had higher values [25,26]. In addition, Aşçı et. al. [27] stated that harvesting in phenologically later periods according to the harvest time will provide yield increase. The reason for the change between the years and the increase in yield in the second year is thought to be the ponding of water in the area where cultivation was carried out in the first year due to irregular precipitation during the vegetation period. Especially in legumes, waterlogging can reduce photosynthesis, plant growth, grain yield, the formation, function and survival of nodules, biological nitrogen fixation, and cause plant death during or some weeks after the end of waterlogging [28].

When ADF averages were analyzed, statistically significant differences were found between years. In the first year of the experiment, higher ADF value was obtained with 36.49%. There was no difference between harvest times. Among the treatments, the highest values were observed in %55CV %45AR, %55CV %45O, %75FP %25AR, and pure common vetch treatments, while the lowest value was obtained from pure annual ryegrass (Table 3).

Table 3. Mean values of Herbage yield and ADF parameters for year, time of harvest and mixture values

	Herbage yield (kg da^{-1})			ADF (%)		
	1st Harvest	2nd Harvest	Mean	1st Harvest	2nd Harvest	Mean
Years						
2015	346.22 B			36.49 A		
2016	469.64 A			34.87 B		
Mixtures						
%100 FP	362.70	320.49	341.60 F	34.87	35.84	35.36 CF
%100 CV	345.25	382.26	363.76 EF	37.58	36.30	36.94 AC
%100 Oat	598.16	559.61	578.89 A	33.54	35.99	34.76 EF
%100 AR	389.68	507.69	448.69 C	32.53	33.11	32.82 G
%75FP %25O	534.22	533.68	533.95 B	34.10	36.06	35.08 DF
%55FP %45O	226.95	336.53	281.74 G	32.27	35.42	33.85 FG
%75FP %25AR	279.20	365.45	322.33 FG	36.60	36.74	36.67 AC
%55FP %45AR	315.40	391.13	353.27 EF	33.68	34.50	34.09 FG
%75CV %25O	371.23	461.84	416.54 CD	34.09	38.68	36.38 BD
%55CV %45O	360.15	422.70	391.43 DE	37.06	38.43	37.74 AB
%75CV %25AR	437.50	459.23	448.37 C	37.02	35.50	36.26 BE
%55CV %45AR	317.47	511.86	414.67 CD	41.91	34.42	38.16 A
Mean	378.16 B	437.71 A		35.44	35.92	

Table 4. shows that there were significant differences between harvest time and mixture treatments according to NDF averages. 46.89% value was lower in the first harvest time with 50% flowering than in the second harvest time. There was also a change in NDF values between years. Among all forage crops and mixtures, the highest NDF was found in pure oat with 53.95%. The lowest values were in %100 FP, %55FP, %45AR, %75CV, %25AR treatments. While it was concluded that the time of harvest and years did not cause significant differences in terms of ADL, the lowest value among the treatments was observed in pure oat treatment. Alatürk et. al. [29] stated that legumes were lower in terms of ADF, NDF and ADL. It was stated that legumes should be lower because they contain less fiber than grass. Although these were the expected results, in terms of ADF and ADL contents, legume and grass values were similar to each other. In terms of NDF, legumes were lower. This situation was thought to be due to competition between plant species. Sohail et. al. [30] similarly obtained lower values for legumes and their mixtures.

Table 4. Mean values of NDF and ADL parameters for year, time of harvest and mixture

	NDF (%)			ADL (%)		
	1st Harvest	2nd Harvest	Mean	1st Harvest	2nd Harvest	Mean
Years						
2015	49.84 A			7.63		
2016	45.61 B			7.86		
Mixtures						
%100 FP	44.85	43.45	44.15 F	9.76	8.81	9.28 A
%100 CV	39.72	41.07	40.39 G	7.44	9.26	8.35 BC
%100 Oat	52.67	55.22	53.95 A	5.79	5.69	5.74 F
%100 AR	49.50	46.85	48.18 CE	4.73	4.44	4.59 G
%75FP %25O	48.15	52.24	50.20 C	10.51	7.82	9.17 A
%55FP %45O	48.05	52.44	50.25 BC	6.75	6.49	6.62 E
%75FP %25AR	47.43	47.45	47.44 DE	8.95	8.28	8.62 AB
%55FP %45AR	42.60	47.81	45.20 F	8.49	8.08	8.28 BC
%75CV %25O	49.96	49.26	49.61 CD	6.62	7.69	7.15 DE
%55CV %45O	49.31	55.56	52.43 AB	6.44	8.91	7.67 CD
%75CV %25AR	41.23	48.24	44.73 F	8.44	9.22	8.83 AB
%55CV %45AR	49.17	43.22	46.19 EF	8.74	8.55	8.64 AB
Mean	46.89 B	48.57 A		7.72	7.77	

The mean values of crude protein ratio and crude protein yield, which are important quality criteria in forage crops, are given in Table 5. It is seen that harvest time and years did not cause significant differences among forage crops mixtures in crude protein ratio averages. The highest values among the treatments were found in the mixtures including common vetch and common vetch. In terms of crude protein yield, it was observed that there were statistically significant differences between the years and the time of harvest depending on the herbage yield, while an increase in crude protein ratio could be detected as the phenological period progressed. The highest value among the mixture treatments was obtained from 75% common vetch + 25% oat with 103.74 kg da^{-1}. In terms of protein values, legumes have higher values than grasses. This has also been reported in some studies [31,32].

Table 5. Mean values of crude protein ratio and crude protein yield parameters for year, harvest time and mixture

	Crude Protein Ratio (%)			Crude Protein Yield (kg da^{-1})		
	1st Harvest	2nd Harvest	Mean	1st Harvest	2nd Harvest	Mean
Years						
2015	18.35			60.42 B		
2016	18.32			85.68 A		
Mixtures						
%100 FP	19.83	18.53	19.18 C	71.91	57.57	64.74 CD
%100 CV	24.16	24.51	24.34 A	78.44	96.84	87.64 B
%100 Oat	11.84	12.09	11.96 F	68.43	68.60	68.52 CD
%100 AR	14.96	12.46	13.71 E	57.60	66.19	61.89 D
%75FP %25O	18.83	20.03	19.4 C	105.79	101.70	103.74 A
%55FP %45O	16.09	16.51	16.30 D	38.13	52.23	45.18 E
%75FP %25AR	19.64	20.44	20.04 BC	50.76	79.31	65.04 CD
%55FP %45AR	18.85	21.61	20.23 BC	63.66	79.42	71.54 C
%75CV %25O	17.16	16.60	16.88 D	69.04	68.37	68.70 CD
%55CV %45O	16.70	16.45	16.57 D	65.70	62.34	64.02 D
%75CV %25AR	21.35	19.23	20.29 BC	87.66	88.56	88.11 B
%55CV %45AR	20.78	21.40	21.09 B	63.08	111.91	87.49 B
Mean	18.42	18.25		68.35 B	77.75 A	

When the relative feed value averages were examined, significant differences were found in terms of harvest time, and it was observed that the first harvest time had a higher relative feed value. Among the treatments, pure common vetch stood out, while the mixtures with the highest values were %55FP-%45AR and %75CV-%25AR.

Table 6. Averages of years, harvest time and mixture values of relative feed value

	Relative Feed Value		
	1st Harvest	2nd Harvest	Mean
Years			
2015	114.41 B		
2016	127.90 A		
Mixtures			
%100 FP	129.57	130.60	130.08 B
%100 CV	147.31	137.53	142.42 A
%100 Oat	111.22	102.85	107.03 F
%100 AR	119.70	125.73	122.72 CD
%75FP %25O	121.61	108.73	115.17 E

%55FP %45O	124.34	109.28	116.81 DE
%75FP %25AR	118.90	118.66	118.78 DE
%55FP %45AR	139.36	120.82	130.09 B
%75CV %25O	118.07	111.48	114.77 E
%55CV %45O	113.95	98.93	106.44 F
%75CV %25AR	139.77	119.07	129.42 BC
%55CV %45AR	106.59	133.66	120.12 DE
Mean	124.20 A	118.11 B	

According to Pearson's correlation in Figure 2., the highest positive significance was found in Herbage yeild and crude protein yield, while the most significant negative correlation was found between relative feed value and %NDF. Rizvi et. al [33] and Zaeem et. al. [34] obtained similar results, while Rizvi et. al [33] stated that ADF and NDF had a negative correlation with RFV. Zaeem et. al. [34] stated that NDF has a negative correlation with total digestible nutrient affecting RFV.

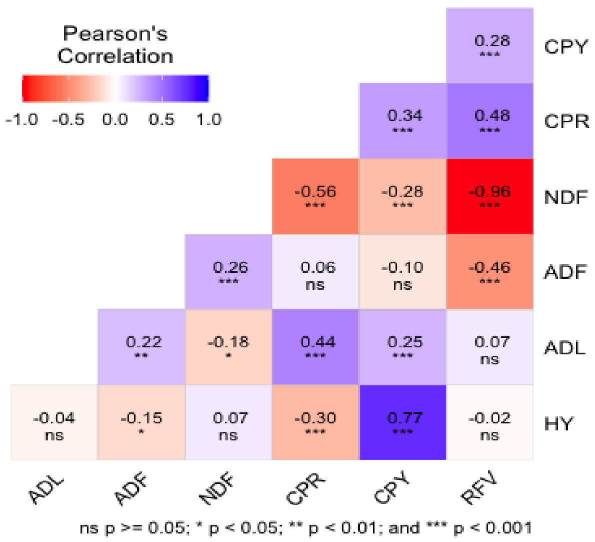

Figure 2. Pearson's correlation between features

4. CONCLUSION

In order to ensure sustainability in agricultural production, crop rotation practices are inevitable in year-round production. Mixtures including legumes can both protect the soil and enrich the soil in terms of nitrogen. At the same time, these mixtures are very important in terms of being a source of quality roughage. The results revealed that legumes and the mixtures in which they were included had higher values in terms of quality, while there were fluctuations in some traits between the harvests, but in general, it was concluded that harvesting between at 50%- 100% flowering periods could be preferred. The mixture of 75% forage pea + 25% oat produced better crude protein yield, and the mixtures of 55% forage pea + 45% annual ryegrass and 75% common vetch + 25% annual ryegrass had higher relative feed values.

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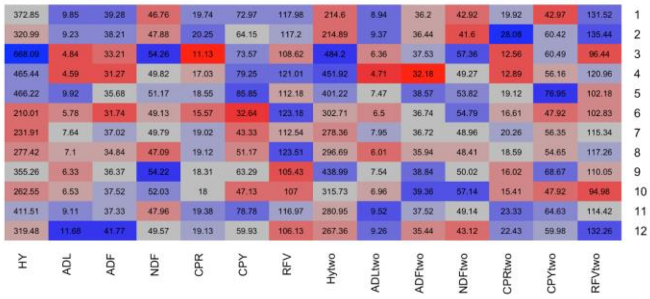
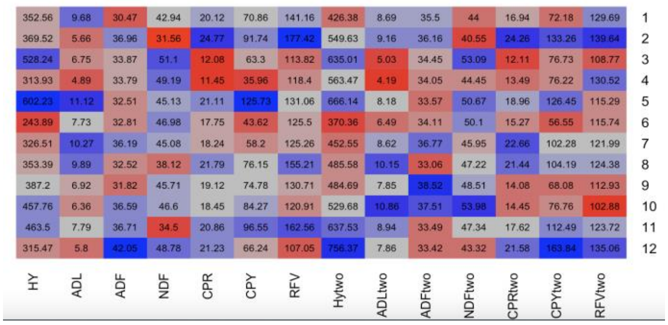
Appendices



Appendix A. Heatmap values of the traits examined at two harvest times with mixture treatments in 2014-2015



Appendix B. Heatmap values of the traits examined at two harvest times with mixture treatments in 2015-2016

(1: %100 Forage Pea; 2: %100 Common vetch; 3: %100 Oat; 5: %75FP %25O; 6: %55FP %45O; 7: %75FP %25AR; 8: %55FP %45AR; 9: %75CV %25O; 10: %55CV %45O; 11: %75CV %25AR; 12: %55CV %45AR – HY: Herbage yield; CPR: Crude Protein Ratio; CPY: Crude Protein Yield; RFV: Relative Feed Value; HYtwo: Herbage yield-Second Harvest)