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BEHAVIOUR OF FODDER MIXTURES WITH ALFALFA IN NORTH-EASTERN ROMANIA

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ABSTRACT

One of the most effective measures that contribute to increasing the amount of fodder is the establishment of temporary grasslands. This paper presents the behavior of perennial grasses and legumes mixtures, fertilized with different fertilizers, in terms of dry matter production, vegetation development, fodder quality and nutritional status of plants. The area that hosted the experiment is characterized by a dry climate, and uneven distribution of rainfall during the vegetation period. Due to its biological and physiological peculiarities, alfalfa managed to produce high yields regardless of the climatic conditions, reason for which it was used in all the mixtures that we have studied. We tested five mixtures consisting of *Medicago sativa* L., *Dactylis glomerata* L., *Festuca pratensis* L., *Poa pratensis* L. and *Lolium perenne* L. in different combinations and proportions of participation. The plots were fertilized with mineral, organic fertilizers and with vinassa (a by-product obtained after the evaporation of wastewater from bakers' yeast factories. Based on experimental data, we recommend the use of those mixtures in which alfalfa has a participation rate of almost 60 to 70%, and application of chemical fertilizers.

Key words: mixtures, grasses, legumes, fertilizer, fodder quality, nutritional status.

INTRODUCTION

Permanent grasslands are an important forage resource in Romania, but irrational management systems during the last period have led to their present state of degradation (Vintu et al., 2011). For this raison some of this grassland could be transformed in temporary grasslands.

In Romania, the conditions are very different, and therefore it is necessary to develop specific technologies to establish temporary grasslands that meet these conditions. The idea of approaching the behaviour of temporary grasslands established on the basis of mixtures of perennial grasses and legumes on various agricultural fields was imposed by the need to supplement the production of fodder obtained from permanent grasslands. The choice of species for the preparation of these mixtures depends upon several criteria: adaptation to external environmental conditions, specific biological characteristics, the very usage and duration of use of any grassland (Charles et al., 1993; Lemaire et al., 1985; Moga et al., 1996, 2007; Morand et al., 1996; Orr et al., 1990; Mosimann, 2002, 2006; Mosimann et al., 2003; Schitea et al., 2007; Schwinning et al., 1996). Alfalfa is widely used in feed mixtures, because it is a plant that yields high quality crops, even in less favorable climatic conditions (Charrier et al., 1993; Cruz et al., 1986; Haynes, 1988; Moga et al., 2005; Mosimann et al., 1995; Petcu et al., 2008; Rochon et al., 2004; Schitea et al., 2007). Water and heat stress lead to a decrease in production and a destabilization of the balance between the main groups of plants (Mauriere and Paillat, 1997; Petcu et al., 2006, 2009). Under these conditions, we witness a decrease in grasses and an increase in leguminous plants. Taking into account these considerations, we took into consideration the use of fodder mixtures in which the participation of alfalfa is mandatory.

For economic and ecological reasons, at the same time with a view to better acclimatize alfalfa to these climatic conditions, we have tested three different types of fertilization with mineral fertilizers, organic and vinassa. This paper presents the

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behaviour of mixtures of perennial grasses and legumes, fertilized with different fertilizers, in terms of dry matter production, the development of vegetation, fodder quality and nutritional status of plants.

MATERIAL AND METHODS

The experiment set up on the Ezareni farm in the spring of 2006. It was located on a field with a slope angle of 10^o, at an altitude of 107 m, on cambic chernozem, with a pH value ranging between 6.7 and 6.8 and 2.73 to 2.93% content in humus, 21-25 ppm P_{AL}, 226-232 ppm K_{AL} and 112-139 ppm CaO. The climatic conditions during the experiment were characterized by an average annual temperature of 9.5^o C and annual rainfall of 552.4 mm. It should be noted that in all experimental years, the distribution of rainfall during the vegetation period was extremely irregular, which is why we have encountered problems, concerning the grass growth and recovery after harvest.

Experimental treatments

The experiment was bifactorial, arranged in split plot design, in four repetitions, with a plot size of 3 m x 5m. The main experimental factor was represented by fertilization, whereas the second factor was represented by the type of fodder mixture.

Factor 1: The fertilization

Fertilization was performed with three different types of fertilizers, chemicals, organic and vinassa, with doses considered optimal for each type of fertilizer. Thus, we tested the following doses of fertilizers:

a_1 – control variant – unfertilised;

a_2 - $N_{100}P_{50}$ kg ha^{-1};

a_3 - 5 t ha^{-1} vinassa + P_{50};

a_4 - 30 t ha^{-1} manure applied annually. At a rate of 1000 kg manure, the chemical composition was of 5 kg N, 3 kg P_2O_5 and 7 kg K_2O.

Manure, phosphorus-based fertilizer, and vinassa were administered in the autumn, whereas nitrogen-based fertilizers were used twice, in the spring, before the start of vegetation, and after the first mowing. Vinassa is a by-product obtained after the evaporation of wastewater from factories producing bakers' yeast. In Romania, vinassa is produced by the yeast factory in Pascani, Iasi County, which has a production capacity of 20,000 t $year^{-1}$ fresh yeast and 9-10 thousand tons $year^{-1}$ vinassa. Vinassa has a complex chemical composition, being rich in total nitrogen (3.0%), very rich in potassium (6%), but low in phosphorus content (0.4%).

Factor 2: Fodder mixtures

The composition of perennial grasses and legumes mixtures, as well as the participation rates of these species, is presented in Table 1. Grasses that complemented alfalfa in the tested mixtures were: *Dactylis glomerata* L., *Festuca pratensis* L., *Poa pratensis* L., *Lolium perenne* L. to varying degrees of participation.

Table 1. Structure of tested mixtures (%)

Species	Variety	Mixture structure				
		b_1	b_2	b_3	b_4	b_5
Medicago sativa L.	Selena	30	40	50	60	70
Dactylis glomerata L.	Regent	25	20	20	10	10
Festuca pratensis L.	Transilvan	15	15	10	10	10
Poa pratensis L.	Balin	15	15	10	10	5
Lolium perenne L.	Marta	15	10	10	10	5
Total (%)		100	100	100	100	100

Experimental protocol

1) Production and structure of vegetal canopy

The sampling performed depended on the type of mixture. Thus, the b_1, b_2 and b_3 mixtures were harvested during the heading phase of grasses, whereas the b_4 and b_5 mixture were harvested during the flowering stage of the alfalfa. Depending on the climatic conditions, the type of mixture and the type of fertilization, the number of mowing obtained was between 3 and 5 per year. The yields obtained were weighed and biomass samples were harvested in order to determine the dry matter and to perform laboratory chemical analyses.

The changes in the structure of the vegetal canopy were determined in the spring, during the first harvest, using the gravimetric method. The gravimetric method consisted of a botanical analysis of an average sample of biomass weighing 1,000 g. The average sample to be analyzed was weighed with an accuracy of 0.1 g, after which the following groups were separated: grasses, legumes, and species from other botanical families. Each plant group was weighed with the same accuracy of 0.1 g and the result was expressed in percentages.

2) Fodder quality

In order to determine the chemical composition of the fodder the following methods of analysis were used: the Kjeldahl method using ISO 13325/1995 determined the total nitrogen; the method EN ISO 6865/2000 Wende determined the crude fiber content; phosphorus was determined with the UV-VIS spectrometry; potassium and calcium through dosage assisted by the atomic absorption spectrophotometer, using SR EN ISO 6869/2002 (Sara and Odagiu, 2005).

3) Analyses and their statistical interpretation

The nitrogen nutrition index was determined with the formula of Lemaire et al. (1989):

INN = 100 x N / 4.8 x (SU) -0.32,
where: N – Nitrogen content of plant (%) ;
SU – Dry matter production (t ha^{-1}).

The nitrogen nutrition status was considered normal when INN varies between 80 to 100 per cent. Lower values show that nitrogen is insufficient, and higher values indicate a nutritional excess.

The index of phosphorus and potassium nutrition was calculated with the following formulae of Thélier-Huché L. et al., 1992:

IP = 1000 [P - (0.15 + 0.065 x N)]

KI = 100 [K - (1.6 + 0.525 x N)]

where: N, P, K – the content of these elements in plants (%).

Nutrition is considered satisfactory when the IP and IK values vary between -50 and +50. The data on yield and structure of the vegetal canopy was analysed by ANOVA and by comparison with the Least Significant Differences.

RESULTS AND DISCUSSION

Influences upon the biomass yield

Temporary grassland productivity is influenced by climatic conditions, application of fertilizers, especially nitrogen -based fertilizers, as it is also influenced by the mixtures of perennial plants that make it up. (Golinski, 2008; Smit et al., 2008; Puia et al., 1975; Thumm, 2008).

Obtaining high forage production depends to a great extent on the intensity of the forage mixtures fertilization. Analysing the average yields achieved during the four years it appears that fertilization led to higher average yields for all the tested mixtures (Table 2). Yield averages ranged between 7.9 t ha^{-1} DM and 9.2 t ha^{-1} DM for a_1, 9.7 t ha^{-1} DM and 12.0 t ha^{-1} DM for a_2, 9.4 t ha^{-1} DM and 11.0 t ha^{-1} DM for a_3, and 9.5 t ha^{-1} DM and 11.1 t ha^{-1} DM for a_4.

Table 2. Dry matter yield and the average number of harvests during 2007-2010

Mixture	Fertilization variant							
	a_1		a_2		a_3		a_4	
	t ha^{-1}	Average number of harvests	t ha^{-1}	Average number of harvests	t ha^{-1}	Average number of harvests	t ha^{-1}	Average number of harvests
b_1	7.9	3.0	9.7***	3.3	9.4**	3.3	9.5***	3.3
b_2	8.2 NS	3.3	10.5***	3.8	9.8***	3.5	10.1***	3.5
b_3	8.4*	3.5	10.9***	3.8	10.4***	3.5	10.6***	3.5
b_4	8.9**	3.8	11.2***	4.3	10.7***	4.0	10.8***	4.0
b_5	9.2**	4.0	12.0***	4.5	11.0***	4.3	11.1***	4.3
LSD 5 %= 0.5 t ha^{-1}; LSD 1 % = 0.9 t ha^{-1}; LSD 0.1% = 1.6 t ha^{-1}								

LSD = Least Significant Difference.
*P < 0.05, **P < 0.01, ***P < 0.001, NS = P > 0.05.

If we compare the yields obtained by the same type of mixture, we notice that, in the case of the b_1 mixture, it varied between 7.9 t ha^{-1} DM and 9.7 t ha^{-1} DM, b_2 varied between 8.2 t ha^{-1} DM and 10.5 t ha^{-1} DM, b_3 varied from 8.4 t ha^{-1} DM and 10.9 t ha^{-1} DM, b_4 from 8.9 t ha^{-1} DM and 11.2 t ha^{-1} DM, and the b_5 mixture ranged between 9.2 t ha^{-1} DM and 12.0 t ha^{-1} DM.

The number mowings is greatly influenced by the nitrogen fertilization, and the type of mixture (Emmenegger, 1985). The number of mowing varied between 3.0 and 3.3 at b_1, 3.3 and 3.8 at b_2, 3.5 to 3.8 at b_3, 3.8 and 4.3 at b_4 and between 4.0 and 4.5 at b_5, respectively. At a_1, the number of mowings ranged between 3.0 and 4.0, between 3.3 and 4.5 at a_2, between 3.3 and 4.3 at a_3, and between 3.3 and 4.3 at a_4.

All four mixtures displayed positive significant correlations, between the percentage of participation of alfalfa and the final dry matter yield (Figure 1).

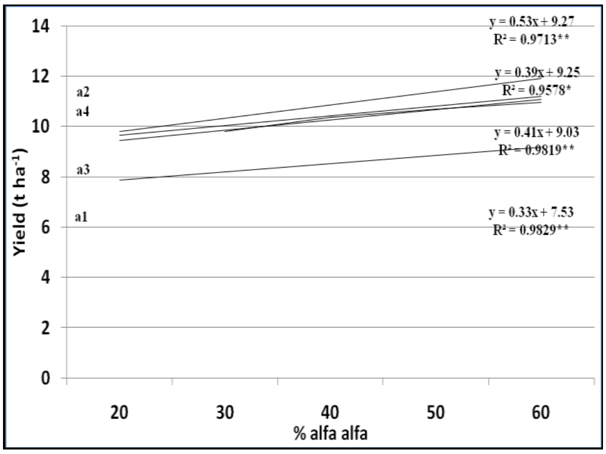

Figure 1. Correlations between the percentage of *Medicago sativa* participation in the mixtures and the average fodder yield, at all fertilization variants

The dry matter outputs obtained for each kilogram of applied N equivalent are presented in Table 3. Of all four fodder mixtures, the highest outputs for each kilogram of applied N equivalent was recorded at the a_2 fertilization variant, followed by the a3, and a4.

The fertilization with $N_{100}P_{50}$ kg ha^{-1} can be recommended as a solution that better capitalizes the applied nitrogen.

Due to their high yields, temporary pastures are large mineral consumers, and this makes the fertilization of these pastures a necessity (Thomas, 1992).

Table 3. Production output / kg N

Mixture	Fertili-zation variant	Applied N equivalent kg ha^{-1}	2007-2010 t DM ha^{-1}	Kg DM/ Applied N equivalent kg
b_1	a_1	0	7.9	-
	a_2	100	9.7	18
	a_3	150	9.4	10
	a_4	150	9.5	11
b_2	a_1	0	8.2	-
	a_2	100	10.5	23
	a_3	150	9.8	11
	a_4	150	10.1	13
b_3	a_1	0	8.4	-
	a_2	100	10.9	25
	a_3	150	10.4	13
	a_4	150	10.6	15
b_4	a_1	0	8.9	-
	a_2	100	11.2	23
	a_3	150	10.7	12
	a_4	150	10.8	13
b_5	a_1	0	9.2	-
	a_2	100	12	28
	a_3	150	11	12
	a_4	150	11.1	13

The structure of the vegetal canopy in 2010

A balanced fertilization improves the floristic composition, either by increasing the proportion of legumes, or that of valuable grasses at the expense of less valuable species, depending on the fertilization process and type of mixture. Organic and mineral fertilizers cause significant changes in the structure of the vegetal canopy of temporary grasslands (Nyfeler et al., 2008).

In order to determine the influence of fertilization upon the structure of the vegetal canopy, we compared the average percentage of participation of each species in the vegetal canopy, in 2010 and 2007, for each of the five mixtures and the four types of fertilization (Table 4).

The unfertilised variant (a_1) in all five mixtures, witnesses an invariable percentage of grass, keeping the values close to 2007, a decrease in the percentage of alfalfa plants and the appearance of forbs (herbaceous flowering plants). The a_2 and a_3 fertilization variants witness an increase in the percentage of participation of grasses, the appearance of forbs, and a decrease in the percentage of alfalfa.

Vinassa, which is much richer in nitrogen than manure, substantially influences the floristic structure of the vegetal canopy, stimulates the growth of grasses and inhibits the growth of legumes. At the same time, nitrogen fertilizers favor the growth of grasses and inhibit the growth of legumes, and the mixture of nitrogen fertilizers with phosphor-rus and potassium ones reduces this process to some extent, without being able to stop it.

Table 4. The structure of the vegetal canopy (%) in 2007 and 2010

Mixture	2007		Fertilization variant											
			a_1			a_2			a_3			a_4		
	Grass	Legumes	Grass	Legumes	Forbs	Grass	Legumes	Forbs	Grass	Legumes	Forbs	Grass	Legumes	Forbs
b_1	70	30	68	24	8	73	21	6	68	24	8	53	26	17
b_2	60	40	62	31	7	68	28	4	63	29	8	52	32	16
b_3	50	50	56	37	7	62	34	5	54	37	7	47	38	15
b_4	40	60	47	46	7	46	48	6	45	48	7	44	54	12
b_5	30	70	33	62	5	40	56	4	37	65	9	24	64	12

The chemical composition of fodder

The quality of feed produced from temporary grassland species was influenced by the components, the proportion of their participation in the mix, the type and level of fertilization (Table 5). Total nitrogen content of feed increased with increasing the percentage of participation of alfalfa, especially combined with fertilizer application.

Table 5. The chemical composition of feed (2010)

Mixture	Fertilization variant	Nt (g kg $^{-1}$)	Crude protein (g kg $^{-1}$)	Crude fibre (g kg $^{-1}$)	P (g kg $^{-1}$)	K (g kg $^{-1}$)	Ca (g kg $^{-1}$)	Ca/P	Crude protein production (kg ha^{-1})
b_1	a_1	19.8	123.8	241.7	3.2	26.9	6.5	1.91	978
	a_2	21.8	136.3	250.1	3.5	26.7	6.4	1.88	1322
	a_3	22.2	138.8	245.1	3.4	26.8	6.3	1.85	1304
	a_4	22.5	140.1	249.0	3.5	26.6	6.3	1.80	1331
b_2	a_1	22.4	140.0	240.2	3.4	26.6	6.6	1.83	1148
	a_2	22.6	141.3	248.1	3.6	27.3	6.5	1.81	1484
	a_3	22.8	142.5	238.4	3.5	27.5	6.6	1.89	1397
	a_4	23.1	144.4	242.0	3.6	27.9	6.6	1.83	1458
b_3	a_1	23.1	144.4	232.0	3.3	26.9	6.9	1.97	1230
	a_2	23.5	146.9	239.1	3.6	26.9	6.8	1.89	1601
	a_3	23.6	147.5	236.5	3.6	26.9	6.9	1.92	1354
	a_4	23.8	148.8	237.2	3.4	27.4	6.9	2.03	1577
b_4	a_1	23.3	145.6	230.2	3.2	27.9	7.3	2.15	1296
	a_2	23.5	146.9	238.1	3.4	27.5	7.4	2.18	1645
	a_3	23.6	147.5	236.0	3.3	26.8	7.3	2.21	1578
	a_4	24.2	151.3	235.4	3.3	27.1	7.3	2.21	1634
b_5	a_1	24.6	153.8	222.5	3.1	27.6	7.6	2.38	1449
	a_2	24.5	153.1	233.7	3.4	26.2	7.4	2.18	1837
	a_3	24.8	155.0	234.6	3.3	26.4	7.5	2.27	1705
	a_4	25.2	157.5	232.8	3.3	26.6	7.7	2.33	1748

Nt – nitrogen from forage; P – phosphorus; K – potassium; Ca – Calcium

The crude fibre content of the fodder increased, when the percentage of participation of alfalfa in the fodder mixture decreased. The phosphorus content of the fodder slightly increased with the decrease of the participation percentage of alfalfa and with the application of fertilizers. Phosphorus nutrition improved at all fodder mixtures, for all the fertilization variants.

The potassium content of the fodder decreased in almost all fodder mixtures with the administration of nitrogen and phosphorus fertilizers, but increased when vinassa and manure was applied. The calcium content was good in all the mixtures, a fact determined by the relatively high percentage of legumes in the structure of the vegetal canopy. The Ca/P ratio was good for all fodder mixtures, and all four types of fertilization, and did not drop below the minimum threshold of 1.50.

Temporary pastures give high-quality fodders, because their composition includes the most valuable forage grasses and legumes (Koukoura et al., 2009). The special quality of the fodder is given by the high protein content and high content in minerals and vitamins (Cardasol et al., 1988; Scehovic, 1991). Because of these properties, the fodder obtained from the temporary pastures is good to be consumed both fresh as well as hay, and it is characterized by a high digestibility degree.

Nutritional status

The analysis the nutritive value of the fodder is a useful practical tool assisting us to better manage the fertilization with nitrogen, phosphorus and potassium of temporary pastures (Farruggia et al., 2000).

The nitrogen nutritional status was good, a situation explained by the symbiotic activity of the legumes in the fodder mixtures, and by fertilization (Table 6). The variant that had not been fertilized, recorded an INN value of 82 for b_1, 95 b_2, 99 for b_3, 101 for b_4, and 107 for b_5.

The data presented show that the nitrogen nutritional status in all five mixtures, all types of fertilization was within normal values. The level of phosphorus nutrition ranged between 10 and 63, a fact that is considered normal, explained as it is by the chemical composition of alfalfa, as well as by the intake of phosphorus through fertilization.

Table 6. Indices of nitrogen nutrition (INN), phosphorus (IP) and of potassium (IK) in 2010

Mixture	Fertilization variant	INN	IP	IK
b_1	a_1	82	41	5
	a_2	93	58	-7
	a_3	95	46	-9
	a_4	96	54	-12
b_2	a_1	95	44	-12
	a_2	98	63	-6
	a_3	99	52	-5
	a_4	101	60	-2
b_3	a_1	99	30	-12
	a_2	102	57	-14
	a_3	104	57	-15
	a_4	106	35	-11
b_4	a_1	101	19	-3
	a_2	103	37	-8
	a_3	105	27	-16
	a_4	108	23	-16
b_5	a_1	107	10	-13
	a_2	109	31	-27
	a_3	111	19	-26
	a_4	113	16	-26

The potassium level of nutrition can be considered within normal values, ranging between -26 and 5. These lower values in potassium can be accounted for by the low potassium intake through fertilization, even under the conditions in which the soil recorded normal values of this element. The nutritional status of plants with N, P and K significantly improved through fertilization, applied to all kinds of fodder mixtures, recording the best results when organic fertilizers (a_2) were used.

Export of nitrogen and phosphorus and the coefficients of their use efficiency

The export of nitrogen and phosphorus fertilizers, as well as the coefficients of their use efficiency was influenced by the fodder mixtures and the type of fertilizer used (Table 7).

Table 7. Export of nitrogen and phosphorus and the coefficients of use efficiency

Mixture	Fertilization variant	Applied N kg ha^{-1}	Total exported N, kg ha^{-1}	Use efficiency of applied N, %	Applied P, kg ha^{-1}	Total exported N, kg ha^{-1}	Use efficiency of applied P, %
b_1	a_1	0	156	-	0	25	-
	a_2	100	211	55	50	34	18
	a_3	150	209	35	70	32	10
	a_4	150	214	39	90	33	9
b_2	a_1	0	184	-	0	28	-
	a_2	100	237	53	50	38	20
	a_3	150	223	26	70	34	9
	a_4	150	233	33	90	36	9
b_3	a_1	0	194	-	0	28	-
	a_2	100	256	62	50	39	22
	a_3	150	245	34	70	37	13
	a_4	150	252	39	90	36	9
b_4	a_1	0	207	-	0	28	-
	a_2	100	263	56	50	38	20
	a_3	150	253	31	70	35	10
	a_4	150	261	36	90	36	9
b_5	a_1	0	226	-	0	29	-
	a_2	100	294	68	50	41	24
	a_3	150	273	31	70	36	10
	a_4	150	280	36	90	37	9

There was an increase in the amount of nitrogen exported, and also an increase of the coefficient of utilization with an increased percentage of alfalfa participation in the fodder mixture. Of all the five mixtures tested, the largest amount of nitrogen exported and the largest coefficient of nitrogen use was obtained by the a_2, a_3 variants, followed by the a_4 variant (Figure 2).

A slight increase in the amount of exported phosphorus, and use efficiency was observed alongside with the increased percentage of alfalfa participation in the fodder mixture.

The highest amount of phosphorus exported and the highest coefficient of phosphorus use, was recorded by the same a_2 variant, followed by the a_3 and a_4 (Figure 3).

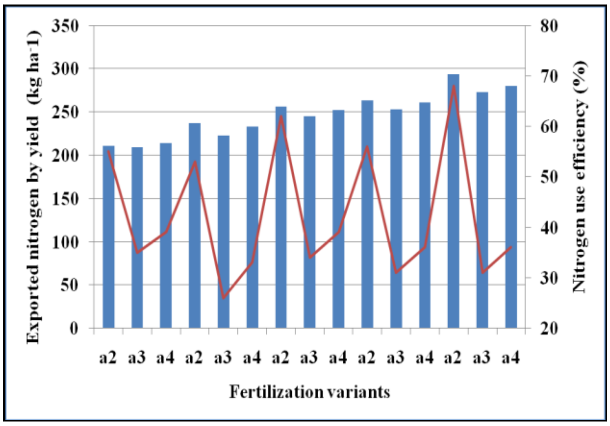

Figure 2. Nitrogen export and use efficiency

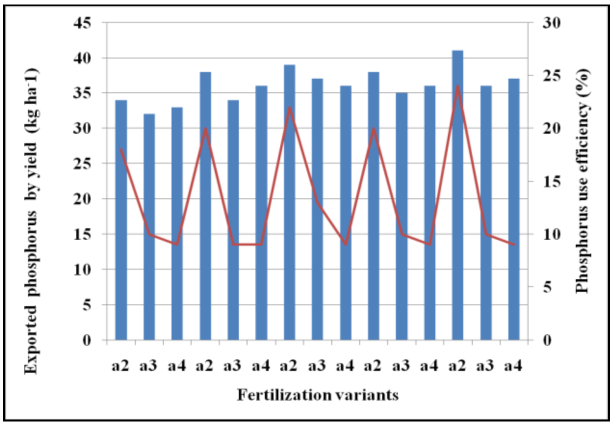

Figure 3. Phosphorus export and use efficiency

Phosphorus had lower values compared to nitrogen, both in terms of the quantity exported and as well as of the use efficiency.

According to the size of N and P export coefficients and apparent use of fertilizers, the application of mineral fertilizers (a_2)

accounted for the most effective way of fertilization.

CONCLUSIONS

The highest average yields achieved in the four years were obtained by fertilizing all five mixtures with mineral fertilizers (a_2).

Regardless of the applied fertilizer, the highest yields were obtained by the fodder mixture in which alfalfa was the dominant participant species (b_5).

Changes in the vegetal canopy were determined by the participation of mixed species and by the nature and doses of applied fertilizers.

The state of nutrition with nitrogen, phosphorus and potassium was good, a fact explained on the one hand by the symbiotic activity of the alfalfa within the fodder mixtures, and on the other hand, by fertilization.

The quality of the fodder produced from temporary pastures was influenced by the proportion of participation of the various plant species in the mix, and by the type and level of fertilization.

The export of nitrogen and phosphorus and the coefficients of using them from fertilizers were influenced by the type of the mixture and the type of fertilizer used. The largest amount of nitrogen exported and the largest coefficient of nitrogen use was obtained by the a_2, and a_3 variants, followed by the a_4 variant.

Based on our experimental data, we recommend the use of fodder mixtures, where alfalfa accounts for 70% of the mixture.

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