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The influence of nitrogen fertilization and legume species on the productivity of multi-species swards in four production years

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Abstract. The aim of current research was to investigate forage yield and crude protein content of legume-grass mixtures during four years of sward use, applying three nitrogen (N) fertilisation rates: N0, N60, and N120. Three perennial legume species - red clover *Trifolium pratense* (Tp), lucerne *Medicago sativa* (Ms) and fodder galega *Galega orientalis* (Go) were tested in mixtures with grasses (G). Mixtures were composed of 50% of legumes and 50% of grasses. Lucerne and fodder galega are introduced forage legume species in Latvia, and are raising an ever-growing interest in Latvia. The studies of persistency, quality and yielding ability of these legume species in Latvian agroclimatic condition in comparison with traditionally used red clover are actual. Significant differences in dry matter (DM) yields were found between successive production years, mixtures, and N fertilization rates. The highest average DM yield was found for the mixture with lucerne (Ms+G). The decline in productivity between the first and fourth production years was more expressed for red clover mixture, but a more stable productivity was demonstrated by swards with galega (Go+G). The N rate increase contributed to a significant DM yield increase for all mixtures. The positive effect of the increased N rates on DM yield increase was better expressed for red clover mixture (Tp+G). Red clover mixture (Tp+G) had the lowest average crude protein (CP) content. Mixture with galega had a higher CP content in the third and fourth production years. The CP content of red clover mixtures increased by nitrogen rate.

Key words: grass-legume mixture, persistency, crude protein, nitrogen.

INTRODUCTION

The increasing role of sustainable grassland-based livestock agriculture in Europe highlights the use of multi-species swards and the need for comprehensive studies on its productivity and persistence, especially in different local conditions (Helgadóttir et al., 2018). Legume including in grass mixtures contributes to the improvement of forage quality (Meripõld et al., 2022) and the reduction in N fertilisation, keeping balance between yield, quality, and N efficiency (Thers et al., 2022). Legume-grass mixtures using low to moderate N fertilization rates sustain a high multifunctionality for the sustainable intensification of agriculture (Suter et al., 2021). Persistency and nutritive value of legumes are important factors for including them in mixtures. Red clover has good forage quality; however, its persistency is limited (Carswell et al., 2022). Due to its high forage quality and good persistency, lucerne is reported a promising legume in

the northern part of Europe (Bender, 2022; Tang et al., 2022). Fodder galega is suggested as an alternative to lucerne under more harsh environmental conditions (Ignaczak et al., 2022), with very good persistency and a high yield potential (Meripõld et al., 2018). It has also been noted that further research is required to determine the suitability of fodder galega for use in the mixtures of grasses (Żarczyński et al., 2021).

Field trials were carried out with the aim to investigate forage yield and crude protein content of legume-grass mixtures during four years of sward use, applying three nitrogen (N0, N60, N120 kg ha^{-1}) fertilization rates. Red clover, lucerne and fodder galega were tested in mixtures with grasses.

MATERIALS AND METHODS

Field trials were established at the 'Peterlauki' Study and Research Farm of the Latvia University of Life Sciences and Technologies (56°53' N, 23°71' E) on sod-calcareous soil (pH_{KCl} 6.7, containing available P - 60 mg kg^{-1}, K - 144 mg kg^{-1}, organic matter content - 24–28 g kg^{-1} of soil) without a cover crop, in three replications, and with a 10 m^2 plot size. Sowing rate was 30 kg pure live seeds ha^{-1}. The following grass combination was used in mixtures: festulolium (×*Festulolium loliaceum* (Huds.) P.Fourn.), tall fescue (*Festuca arundinacea* Schreb.), hybrid ryegrass (*Lolium boucheanum* Kunth) in equal parts (G). Mixtures were composed of 50% of red clover (*Trifolium pratense* L.) and 50% of grasses (Tp+G); 50% of lucerne (*Medicago sativa* L.) and 50% of grasses (Ms+G); 50% of fodder galega (*Galega orientalis* Lam.) and 50% of grasses (Go+G). At the sowing year and every year of sward use the plots were fertilised with phosphorus (P) 78 kg ha^{-1} and potassium (K) 90 kg ha^{-1} before spring regrowth. Every year of sward use three N fertilization rates (N0, $N60_{(30+30)}$, and $N120_{(60+60)}$ kg ha^{-1}) were used. Split applications of N were used, with first - before spring regrowth, second - after first cut. Swards were cut three times during the vegetation season with 'Hege212' at the grass tillering end/ear emergence stage. DM yield was evaluated over the sum of three cuts every year. Meteorological data during the years of sward use are summarized in Table 1.

Table 1. Monthly average air temperature and total amount of precipitation (the data of the 'Peterlauki' Study and Research Farm meteorological station)

Month	Air temperature, °C				Precipitation, mm			
	Year of sward use				Year of sward use			
	First	Second	Third	Fourth	First	Second	Third	Fourth
April	6.7	6.4	4.8	9.0	38	65	29	31
May	10.8	14.2	11.5	16.1	42	52	15	21
June	14.5	16.6	15.1	16.8	23	98	41	15
July	16.7	18.3	16.6	20.8	99	71	87	34
August	18.7	16.8	16.8	19.4	17	68	31	28
September	13.7	14.0	13.0	14.9	65	18	80	26
October	5.9	4.9	8.0	8.5	4	56	80	11

The crude protein (CP) content of dry matter (DM) yield was determined by Improved Kjeldahl Method 46-10.01. The data were statistically analysed using a three-way analysis of variance, with mixture type (MT), nitrogen fertiliser rate (N) and

year of sward use (Y) as factors, difference among means was detected by LSD at the $P < 0.05$ probability level (Excel for Windows, 2003). To ascertain how much the variation of traits in the investigation period was related to the influence of the factors 'mixture (MT)', 'year of sward use (Y)', and 'nitrogen fertilizer rate (N)' and their interaction, the relative proportion (after the three-factor analysis of variance) of these factors (η, %) in total variance was determined.

RESULTS AND DISCUSSION

Lucerne mixtures (Ms+G) provided a significantly higher DM yield compared to the red clover (Tp+G) and galega (Go+G) mixtures (Table 2). Lucerne showed fast development and good competitiveness with grasses during the first three production years. The average DM yields of the Go+G and Tp+G mixture were not significantly different in the four production years.

Sward aging could cause a significant decline of multicomponent mixture productivity (Adamovics & Gutmane, 2020). Forage legumes are characterized by different productive longevities, which influences changes in productivity between successive production years. Red clover is the most common legume in the sown grasslands in Latvia. Its development is fast in the first production year, but productive longevity is limited. The decrease in legume content in Tp+G swards contributed to a more expressed decline in productivity between production years. Already in the second year of the harvest, an average decline in productivity by 7.13 Mg ha^{-1} or 34% was detected. Galega is characterised by slow establishment and development in the first production year; however, it significantly surpassed other forage legumes regarding productive longevity. For the Go+G sward, the decline in productivity between the first and second production years made only 1.03 Mg ha^{-1} or 7%, and between the third and fourth production years - 1.34 Mg ha^{-1} or 13%. Lucerne is characterised by a better longevity longevity in comparison with red clover. For the Ms+G sward, no significant differences between the yields of the first and second production years were found, whereas productivity decline between the second and fourth production years was 6.76 Mg ha^{-1}

Table 2. Dry matter yield, Mg ha^{-1}, during four production years

Mixtures (MT)	Year of sward use (Y)	N rate, kg ha^{-1} (N)			
		N0	N60	N120	Mean
Tp+G	First	20.31	20.72	21.49	20.84 ^{a}
	Second	11.58	14.02	15.52	13.71 ^{b}
	Third	6.29	8.82	10.22	8.44 ^{c}
	Fourth	4.51	6.60	8.56	6.56 ^{c}
	Mean	10.67 ^{a}	12.54 ^{b}	13.95 ^{b}	12.39 ^{A}
Ms+G	First	18.73	19.83	20.67	19.74 ^{a}
	Second	19.64	20.56	22.04	20.75 ^{a}
	Third	13.41	14.02	14.54	13.99 ^{b}
	Fourth	8.01	9.28	10.59	9.29 ^{c}
	Mean	14.95 ^{a}	15.92 ^{ab}	16.96 ^{b}	15.94 ^{B}
Go+G	First	12.65	14.73	17.06	14.81 ^{a}
	Second	13.14	13.70	14.51	13.78 ^{a}
	Third	9.25	10.65	11.56	10.49 ^{b}
	Fourth	7.87	8.85	10.73	9.15 ^{b}
	Mean	10.73 ^{a}	11.98 ^{ab}	13.47 ^{b}	12.06 ^{A}
$LSD_{0.05}$ MT= 1.51; N= 1.51; Y=1.74; MT/Y= 3.02					

Within rows means with different lowercase letters differ significantly ($P < 0.05$) between fertilization rate treatment (N); Within columns means with different lowercase letters differ significantly between year of sward use (Y) and with different uppercase letters differ significantly ($P < 0.05$) between mixture type (MT).

or 33%. The cool spring with good precipitation in first production contributed to the good grass development. The highest legume content was observed in the second year of sward use (Fig. 1). This contributed to the preservation of Ms+G and Go+G sward yield levels, without significant yield decrease between first and second production years. The proportion of legumes in sward at the third production year contributed to legume longevity. The highest average proportion of legumes at the third production year stated in Go+G sward (57%), but lowest in Tp+G swards (29%). Proportion of legumes in Ms+G sward at third production year was 44%. Considerable lucerne and galega content decrease in the fourth production year could be explained by hot and dry summer and increasing forbs content in sward.

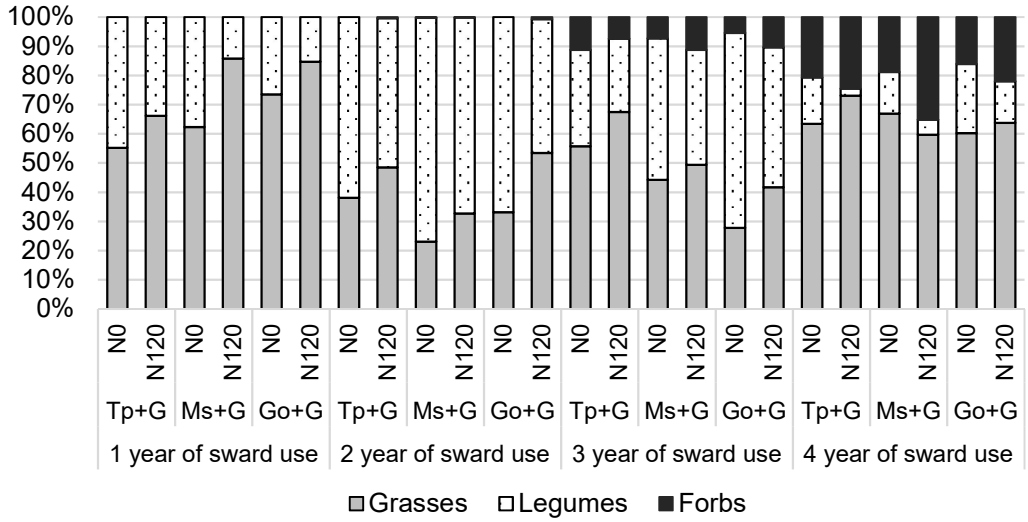

Figure 1. Proportions of the groups of herbage species in the sward (%).

The N rate increase from 0 to 120 kg ha^{-1} contributed to a significant average DM yield increase for all mixtures. The positive effect of N fertilisation on DM yield was closely connected with legume species and the proportion of grasses and legumes in sward. In the first production year, Go+G swards demonstrated the highest proportion of grasses and the highest DM yield increase (by 4.41 Mg ha^{-1} or 35%) compared to other mixtures. In the fourth production year, Tp+G swards had the highest grass proportion and the highest DM yield increase (by 4.05 Mg ha^{-1} or 90%). The N rate increase from 0 to 60 kg ha^{-1} resulted in a significant average DM yield increase of 1.87 Mg ha^{-1} or 17% only for the Tp+G mixture. The N rate increase from 60 to 120 kg ha^{-1} do not result to significant average DM yield increase.

Nitrogen fertilization of mixed grass-legume swards can suppress legume content and reduce the yield response. For all mixtures, N application negatively affected the proportion of legumes in the sward in all years of sward use. This is consistent with studies reporting that clover proportion is generally decreasing with increased N fertilizer application (Thers et al., 2022). On average over four production years the application of N fertiliser contributed to a legume content decrease in Tp+G swards (by 11%), in Ms+G swards (by 13%) and Go+G swards (by 15%).

Red clover mixture had a significantly lower CP content than the Ms+G and Go+G mixtures (Table 3). The average values of CP content determined in our trials for Ms+G and Go+G mixtures were not significantly different. Galega is reported a highly valuable legume with a higher proportion of leaves but a lower nutritive value of stems in comparison with lucerne (Ignaczak et al., 2022). The low CP content for the Go+G mixture in the first year of sward use could be explained by the large grass (74%) proportion in swards.

A successive CP content decrease with sward aging was not observed. The significant CP differences between the years of sward use can be explained not only by different contents of legumes but also by different meteorological conditions - the dry summer in the first and fourth production years.

Increasing the N fertiliser application rate from 0 to 120 kg ha^{-1} contributed to a significant CP content increase only for the Tp+G mixture. CP content did not show an increasing tendency for the Ms+G and Go+G mixtures with an increasing N rate.

The year of sward use as a factor provided the greatest differences between the DM yields. The high influence of the year factor could be explained not only by sward aging but also by differences in meteorological conditions. Legume species used in mixtures were an important factor and significantly ($P < 0.05$) influenced the DM yield. The influence of the nitrogen factor proved to be the lowest (Table 4). The greatest variations in the data of CP content were provided by the interaction between MT and N factors. The influence of interaction effect was higher than the influence of separate factors. This suggests that CP content is closely connected with the proportion of grasses and legumes in sward and the response of species to N rate increase which is in agreement with Adamovics & Gutmane (2020).

Table 3. CP content in DM yield, g kg^{-1}, during four production years

Mixtures (MT)	Year of sward use (Y)	N rate, kg ha^{-1} (N)			
		N0	N60	N120	Mean
Tp+G	First	92.8	107.5	122.2	107.5 ^{a}
	Second	92.7	102.7	115.0	103.5 ^{a}
	Third	98.9	91.8	87.1	92.6 ^{a}
	Fourth	82.7	93.9	106.9	94.5 ^{a}
	Mean	91.8 ^{a}	99.0 ^{a}	107.8 ^{b}	99.5 ^{A}
Ms+G	First	102.2	107.2	112.2	107.2 ^{a}
	Second	159.8	140.6	142.4	147.6 ^{b}
	Third	131.0	128.1	123.0	127.3 ^{c}
	Fourth	86.9	98.0	109.0	98.0 ^{a}
	Mean	120.0 ^{a}	118.5 ^{a}	121.6 ^{a}	120.0 ^{B}
Go+G	First	75.6	91.1	106.6	91.1 ^{a}
	Second	132.7	131.1	131.2	131.6 ^{bc}
	Third	155.8	135.8	137.6	143.1 ^{b}
	Fourth	104.6	115.6	130.6	116.9 ^{c}
	Mean	117.2 ^{a}	118.4 ^{a}	126.5 ^{a}	120.7 ^{B}

$LSD_{0.05}$ MT = 12.91; N = 12.91; Y = 14.90; MT/N = 22.36

Within rows means with different lowercase letters differ significantly (P <0 .05) between fertilization rate treatment (N); Within columns means with different lowercase letters differ significantly between year of sward use (Y) and with different uppercase letters differ significantly ($P < 0.05$) between mixture type (MT).

Table 4. Influence of the mixture type, nitrogen fertilisation, and year of sward use factors on average dry matter yield (DM) and crude protein (CP) content in DM yield (η, %)

Factors	DM	CP
Mixture (MT)	10.5*	11.1*
N rate, kg ha^{-1} (N)	4.1*	11.4*
Year of sward use (Y)	55.5*	4.7*
Interaction (MT ×x N)	0.3	44.0*
Interaction (MT × Y)	9.4*	5.4
Interaction (N × Y)	0.2	4.4
Interaction (MT × N × Y)	0.8	2.3

* Influence of the factor is significant ($P < 0.05$).

CONCLUSIONS

On average for four production years, the lucerne-containing mixture surpassed red clover and galega mixtures in terms of productivity. Red clover mixture had a significantly lower average CP content in comparison with the Ms+G and Go+G mixtures. All mixtures had a significant decline in productivity between the first and fourth production years. Productivity changes between successive production years were influenced by legume species used in a mixture. The Tp+G swards showed a more expressed decline in productivity. Galega and lucerne-containing swards demonstrated a more stable productivity. The nitrogen fertiliser rate increase from 0 to 120 kg ha^{-1} provided a significant increase in average DM yields for all mixtures. The increase in N application did not provide higher values of CP content for lucerne and galega-containing mixtures.

Three production years are maximal length of sward use for mixture containing red clover. Mixtures containing lucerne are appropriate for four years of sward use in Latvia. Longer (five or more) year use of Ms+G sward in three cutting management is not recommended due to significant productivity decline. There is limited amount of information about fodder galega use in the multi-species swards in Latvia. Mixtures containing fodder galega could be recommended for more than four-year sward use. However, long-term experimental data is also required to evaluate galega-grass sward productive longevity. More detailed studies of N rate and sward aging influence on CP content changes would be useful.

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