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AN ASSESSMENT OF THE EFFICIENCY OF UNDERSOWING PERMANENT MEADOW WITH RED CLOVER AT VARIOUS WAYS OF FERTILISATION

Summary

Studies were carried out at the Experimental Farm in Falenty on the permanent productive meadow, situated on mineral soil (proper dry ground habitat) at the four consecutive years (2011-2014). The aim of the study was to assess the effect of undersowing permanent meadow with the red clover (Trifolium pratense L.) at various ways of fertilisation. Plots has been selected randomly and fertilised with mineral (phosphorus and potassium) or natural fertilisers (manure and liquid manure). In spring 2011, part of selected plots has been undersown with the tetraploid Bona variety of red clover, in the amount of 8 kg·ha^{-1}. Botanical composition of the meadow sward, yields and protein content in the sward has been assessed in each of consecutive years. Nutritive components content in the sward have been determined in the years 2013-2014. Undersowing with red clover was the most effective on the manure fertilised plot and the least of all on the plot fertilised with the liquid manure, where the contribution of red clover was 2-fold lower, than in the other plots. Increased share of red clover in the meadow sward enabled obtaining by 20% higher dry matter yields and by 1000 kg·ha^{-1} greater protein yields on the manure fertilised plot. The presence of red clover improved the nutritive value of obtained green forage, through an increased protein content, but on the other hand its usefulness as a raw material for ensilage has been slightly decreased.
Keywords*: type of fertilisation, grasslands, Trifolium pretense* L.*, dry matter yield, protein production*

OCENA EFEKTYWNOŚCI PODSIEWU ŁĄKI TRWAŁEJ KONICZYNĄ ŁĄKOWĄ PRZY RÓŻNYCH SPOSOBACH NAWOŻENIA

Streszczenie

Badania przeprowadzono w Zakładzie Doświadczalnym w Falentach na trwałej łące produkcyjnej, położonej na glebie mineralnej (w siedlisku grądowym) w czterech kolejnych latach (2011-2014). Celem badań była ocena wpływu podsiewu łąki koniczyną łąkową (Trifolium pratense L.*) na tle różnych sposobów nawożenia. Wybrane losowo łany były nawożone nawozami mineralnymi (fosfor i potas) lub nawozami naturalnymi (obornikiem i gnojówką). Wiosną 2011 roku połowa każdego łanu została podsiana tetraploidalną odmianą koniczyny łąkowej Bona w ilości 8 kg·ha^{-1}. Corocznie oceniano skład botaniczny runi łąkowej, plony oraz zawartość białka ogólnego w runi. Zawartość składników pokarmowych określano w latach 2013-2014. Podsiew łąki koniczyną łąkową był najskuteczniejszy na obiekcie nawożonym obornikiem, a najmniej na łące nawożonej gnojówką, gdzie udział koniczyny łąkowej w runi był 2-krotnie mniejszy niż na innych obiektach. Zwiększony udział koniczyny łąkowej w runi na łące nawożonej obornikiem spowodował wzrost plonów suchej masy o 20% i pozwolił na zebranie o 1000 kg·ha^{-1} większych plonów białka. Obecność koniczyny łąkowej poprawiła też wartość pokarmową uzyskanej zielonki, dzięki zwiększonej zawartości białka, ale z drugiej strony nieznacznie pogorszyła się jej przydatność jako surowca do zakiszania.*
Słowa kluczowe*: rodzaj nawożenia, użytek zielony, Trifolium pratense* L.*, plon suchej masy, produkcja białka*

1. Introduction

The share of legumes in the sward of permanent meadows is usually small. However, legumes have been linked with various benefits associated to the presence i.e.: they have quite a remarkable nutrition profile and are a rich source of healthy fibers and protein [8, 13, 15]. Undersowing the old sward with legume species is an inexpensive way of complementing botanical composition with legume plants [4]. Increasing the share of legume plants in the sward results in an increased yield and protein content in plant forage [3, 20, 22] and improves its mineral content [7, 18, 27]. The symbiosis of legumes with N-fixing bacteria of the genera *Rhizobium*, *Bradyrhizobium* and *Sinorhizobium* [6, 12] enables a limiting N fertilisation, which may decrease the costs of plant production [9]. *Trifolium pratense* L. is the most important legume plant on meadows [1, 5]. It is popular among farmers for its high protein content, high biomass production and good re-growth capability after mowing. For this reason it is widely used for forage or cut and conserved as silage. Widespread interest in this species stems from the fact that red clover contains high levels of the enzyme polyphenol oxidase, that has beneficial effects in improving nitrogen utilization in ruminants [14] and in protecting lipids from degradation, both ‘in silo’ as well as in the rumen, leading to a higher output of polyunsaturated fatty acids (PUFA) in ruminant products (meat and milk) [24].

The aim of the study was to assess the effect of undersowing permanent meadow with the red clover (*Trifolium pratense* L.) at various ways of fertilisation.

2. Material and methods
2.1 Experimental design

Studies were carried out in the years 2011-2014 in the ITP Experimental Farm in Falenty (52°12N, 20°91E) on a permanent meadow in dry-ground habitat. The soil was a loamy to heavy sand with 1.5% organic matter, 0.15 g·kg^{-1}

K, 0.26-0.41 g·kg^{-1} P, 0.16-0.24 g·kg^{-1} Mg and an average pH of 5.5-6.0. Dominating species in the sward were the tussock grass (*Poa pratensis* L.) and meadow foxtail (*Alopecurus pratensis* L.); legumes constituted from 1% to 4% of the sward. Separation on each combination (according to earlier scheduled methodology: fertilizer - undersowing) from the one main plot has been established in spring 2011. For that purpose, a rectangular plot (100 m x 180 m) has been separated on the meadow, and divided into three smaller rectangular plots, with an area of 60 m x 100 m each. The plots sizes were adjusted to the size of the machinery used for fertilisation. Each of the three plots has been assigned to the one of the fertilisation methods: the 1st plot (PK) was fertilised with phosphorus and potassium, the 2nd – with solid manure (M) and the 3rd– with liquid manure (LM). At the next stage, each of the three plots has been divided into two subplots. A half of each plot has been undersown with the tetraploid Bona variety of the red clover (*Trifolium pratense* L.) (Table 1).

2.2. Fertilisers and application of fertilizers

PK plot was fertilised with 30 kg P·ha^{-1} in a form of ground phosphate rock and with 60 kg K·ha^{-1} in a form of potassium sulphate. Manure (20% DM) was applied every year in autumn, after 6 months long storage on manure slab, at a rate of 18 - 22 t·ha^{-1}. Liquid manure (4% DM), at a rate of 20 - 25 m^3·ha^{-1}, was applied in two equal doses: in spring and after the 1st cut. Nitrogen content and the equivalent of its utilisation were considered when estimating the doses of natural fertilisers. The equivalent value was 0.5 for manure and 0.8 for liquid manure when both were applied to the soil. The equivalent of phosphorus utilisation was 1 for both fertilisers [16, 17].

2.3. Undersowing with red clover

For undersowing with red clover, in early spring 2011 one half of the area of each plot has been harrowed 3- times with a heavy harrow and seeds of red clover were sown in the amount of 8 kg·ha^{-1} (50% of the norm). After undersowing the meadow surface was rolled.

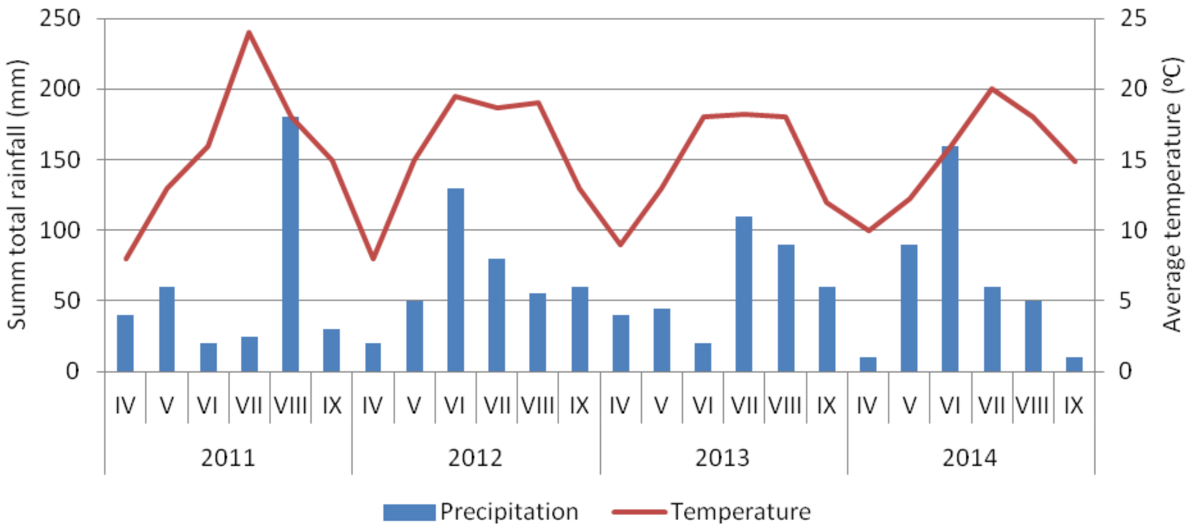
2.4 Weather data

In 2011, the weather was warm during June-August. June and July were dry, while in the August the precipitation was above average. In the following years, the vegetation period had normal temperatures (from 5 to 20°C) and the precipitations were close to average (Fig. 1).

Table 1. Scheme of experiment
Tab. 1. Schemat doświadczenia

Experimental treatment	Fertilisation dose	Form of fertiliser
PK	30 kg P·ha^{-1} + 60 kg K·ha^{-1}	ground phosphate rock and potassium phosphate
PK+U	as above + symbiotically fixed N	P, K – as above with undersown red clover
M	60 kg N·ha^{-1}, 30 kg P·ha^{-1}, 60 kg K·ha^{-1}	solid manure covered the requirements for N, P and K
M+U	as above + symbiotically fixed N	solid manure as above with undersown red clover
LM	60 kg N·ha^{-1}, 30 kg P·ha^{-1}, 60 kg K·ha^{-1}	liquid manure covered the requirements for N and K, P was supplemented with ground phosphate rock
LM+U	as above + symbiotically fixed N	liquid manure as above with undersown red clover

Source: own study / Źródło: opracowanie własne



Source: own study / Źródło: opracowanie własne

Fig. 1. Average temperature and sum of monthly precipitation during years 2011-2014 for the place where the experiment has been conducted (Falenty, Poland)
Rys. 1. Średnia temperatura i suma miesięcznych opadów w latach 2011-2014 dla miejsca, w którym przeprowadzono eksperyment (Falenty, Polska)

2.5. Plant material sampling

In subsequent years, three times in a vegetation season during the flowering phase of red clover, the meadow was mown and harvested green mass was intended for forage. Botanical composition of the sward on each combination was analyzed every year, before the first cut by the Klapp's method [11]. Annual yields of dry mass were also determined. For performing this within each of main plot five subplots (area 25 m^2) in fixed points were marked. From each subplot a strip of herbage was cut. Dry matter yields per hectare were calculated after drying the samples at 105ºC. At each harvest, herbage samples were collected for chemical analyses. Nitrogen content in harvested sward was determined by the method of Sapek [21] further being recalculated to total protein. Based on the amount of yield and total protein content, the increments of protein production on each fertilisation object were calculated. In the years 2013 and 2014 (the greatest share of red clover in the sward) an additional analysis of the content of nutritive components was performed by the NIRS method using NIRFlex N-500 instrument calibrated by the INGOT firm for bulk forage.

2.6. Statistical analyses

Data from the individual plots concerning dry matter, protein yields and nutritive components in meadow sward obtained in the experiment conducted in the following years were analysed using standard ANOVA based on Randomized Complete Design (RCD) model. The first factor was fertilization (PK, M and LM) and the second factor was sward undersowing (not undersown and undersown). Main effect of fertilisation and of undersowing treatment and interactions among fertilisation and undersowing treatment were analysed using the following model:

$Y_{ijkl} = \mu + (\text{fertilisation})_i + (\text{undersowing})_j + (\text{fertilisation x undersowing})_{ij} + (\text{error})_{ijk}$

The data sets for yields of meadow sward, yields of protein and content of nutrients were analysed and reported separately for each year. Comparison of the treatments across years was performed on mean values of the 4 years of study with one-way ANOVA.

3. Results

3.1. Botanical composition

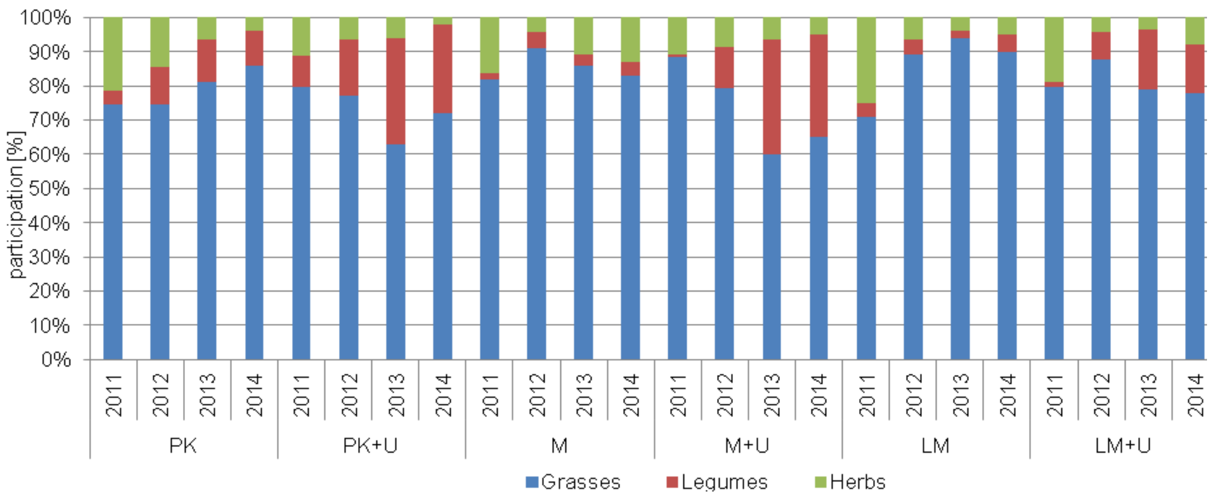
On plots not undersown with red clover, an increased share of grasses and to a lesser extent legume plants has been observed; it was the result to take place at the expense of dicotyledons and weeds. Expansion of grasses was particularly determined by the fertilisation with liquid manure. In the plot fertilized in this manner, the share of grasses in the sward increased from 71% (in 2011) to 90% (in 2014); at the same time, participation of legumes increased only from 4% to 5%.

Manure fertilisation had the most stabilising effect on the sward composition. An increase in the participation of grasses from 82% to 91% (in 2012) has been recorded. Contribution of legumes increased only from 2 to 4%.

Under the effect of PK fertilisation, the share of the two plant groups (grasses and legumes) increased by 12% and 6%, respectively. Dominating grass species on these plots were: *Poa pratensis* L., and *Alopecurus pratensis* L., and *Lolium perenne* L. and *Dactylis glomerata* L. on the plot fertilised with the liquid manure.

Undersowing with red clover changed botanical composition of the sward. This species was competitive, mainly in relation to the grasses. In the year of undersowing, the share of red clover in plant yield was the smallest. Along with time the share increased to reach maximum in the second year after undersowing and to decrease in the next year.

Participation of red clover in the sward depended mainly on the type of fertilisation. Manure fertilisation was the most favourable method for the stimulating growth of red clover, then PK. The weakest effect was observable when liquid manure application has been used for fertilisation. In the 3rd year after undersowing, the share of red clover in the swords accounted nearly 33.7%, it should be noted, that swords were fertilised with manure. In the case of sward fertilized with liquid manure significantly lower level - 14% has been demonstrated. Insertion of red clover to the meadow sward significantly reduced the participation of other dicotyledon plants on all plots (Fig. 2).



Source: own study / Źródło: opracowanie własne

Fig. 2. The participation of main plant groups in the meadow sward at the four consecutive years

Rys. 2. Udział głównych grup roślin w runi łąkowej w ciągu czterech kolejnych lat badań

3.2. Yields of meadow sward

Fertilisation with mineral phosphorus and potassium (PK), despite the lack of nitrogen fertilisation, enabled obtaining a relatively high annual yield, ranging from 6.44 to 7.85 $t \cdot ha^{-1}$. Dry matter yield from manure fertilised (M) plot was similar (mean 7.26 $t \cdot ha^{-1}$). Fertilisation with liquid manure was found to be more effective solution. Significantly higher yield compared to those obtained for PK and M plots in the years 2012 and 2014 has been recorded (Table 2).

Regardless of the type of fertilisation, undersowing red clover resulted in a progressive increase in meadow sward yields, compared with those plots which were not undersown. Undersowing the sward fertilised with PK increased plant yield from 7.09 $t \cdot ha^{-1}$ (in 2011) to 11.75 $t \cdot ha^{-1}$ (in 2014). Undersowing the manure fertilised plot contributed to considerable increase in yields. The highest yield increase (5.33 $t \cdot ha^{-1}$) compared with not undersown plot was obtained in 2014 year, in the 3rd year after action. Comparable level of yield increase (from 8.85 to 10.87 $t \cdot ha^{-1}$) due to red clover undersowing were also observed on plot fertilised with liquid manure (Table 2).

3.3. The content of nutritive components

The content of total protein in the sward not undersown with red clover was the highest on the manure fertilised plot (163.2 $g \cdot kg^{-1}$ of dry mass) and the lowest on plot fertilised with the liquid manure (144.0 $g \cdot kg^{-1}$ dry mass) (Table 3). Undersowing resulted in an increase of the total protein content on all plots. On PK+U and M+U plots the increase of protein content was on the lowest level in the year of undersowing. However, in subsequent years progressive increase has been demonstrated. Undersowing with red clover proved to be the most effective on manure fertilised plot, where the content of protein exceeded 180 $g \cdot kg^{-1}$ of dry mass in the 2nd year after action. A reverse phenomenon – a decreasing in the content of total protein in the 4 consecutive years on plots fertilised with the liquid manure (LM and LM+U) has been noted (Table 3).

The type of fertilisation and undersowing did not affect the content of crude fibre and its NDF and ADF fractions. The exception was manure fertilised meadow sward (M+U), which were harvested in 2013, in respect of which the study has shown significantly lower concentration of NDF fraction compared to sward from other plots.

An important component of green fodder particularly that intended for ensilage are soluble carbohydrates. They provide a food source for lactic acid bacteria, which are responsible for the proper conduct of ensiling. The lowest content of soluble carbohydrates (96.9 $g \cdot kg^{-1}$ of dry mass) was found in sward fertilised with manure and the highest – in that fertilised with liquid manure (146.0 $g \cdot kg^{-1}$ of dry mass). Studies have shown that, sward undersowing with red clover had no significant effect on the development of this component (Table 3).

The ratio of sugars to total protein is an important parameter used to estimate whether meadow sward is suitable for ensilage. This ratio was significantly different among experimental variants (Table 3). The highest ratio (1.58) was found in the sward from plots fertilised with liquid manure, the lowest (0.79) – on manure fertilised plots, which would indicate that the sward from these plots was less suitable for ensilage. An increased share of red clover in swards fertilised with mineral fertilisers and liquid manure significantly decreased the ratio, while the same treatment on plots fertilised with manure had no significant effect. The ratios were at their lowest level in 2013, when the participation of red clover in the sward was the highest among all study years (Fig. 2).

3.4. Protein yield

The yield of total protein from hectare of particular plots were quite differentiated (Table 4). The yield from plots not undersown with the red clover was on the lowest level in 2011 and increased in consecutive years to achieve the maximum in 2013 - this tendency was only applied plots where fertilisation with manure and mineral fertilisers has been introduced. Studies have shown that, the level of protein yield from plot fertilised with liquid manure was more uniform and amounted slightly more than 1000 kg per ha. Undersowing resulted in significant increase in the yield of total protein from all experimental plots. However, the opposite trend has been recorded in the 2nd and 3rd year after application of the treatment (Table 4).

Among both plots and four consecutive years, study have shown an increase in total protein production per hectare, it was so-called "protein effect" (Fig. 3). The increment was on the lowest level on PK+U plot in the year of undersowing, but progressively increased to achieve the highest value of 834 $kg \cdot ha^{-1}$ in 2014. Similar tendency has been observed on M+U plots, However, on plots fertilised with liquid manure the increment of total protein content compared with plots devoid of undersowing was at the similar level during the whole experiment and ranging from 294 to 388 $kg \cdot ha^{-1}$. From among compared plots, undersowing with red clover was the most efficient on the manure fertilised plot. Protein effect on this plot in the 3rd year after application of the treatment amounted 1047 $kg \cdot ha^{-1}$.

Table 2. Dry matter yields ($t\ ha^{-1}$) of meadow sward in the following years of study
Tab. 2. Plony suchej masy ($t\ ha^{-1}$) runi łąkowej w kolejnych latach badań

Year of the study	Experimental treatments						Significance of the effect		
	PK		Manure		Liquid manure		Fertilisation	Undersowing	Interaction
	PK	PK+U	M	M+U	LM	LM+U	(F)	(U)	Fx U
2011	6.44	7.09	6.72	6.54	7.05	8.85	**	*	*
2012	6.46	8.58	6.58	8.01	8.28	9.63	**	**	NS
2013	7.85	9.31	7.92	9.88	7.67	9.76	NS	**	NS
2014	7.42	11.75	7.80	13.13	9.64	10.87	NS	**	**
Mean from years	**7.04**	**9.18**	**7.26**	**9.39**	**8.16**	**9.78**	*	**	*
Significance of year effect	**	**	*	**	**	*	-	-	-

*, ** significant at the level P<0.05 or P<0.01, respectively, NS - not significant

Source: own study / Źródło: opracowanie własne

Table 3. Content of nutritive components in meadow sward (g kg^{-1} of dry mass) in the following years of study
Tab. 3. Zawartość składników pokarmowych w runi łąkowej (g kg^{-1} suchej masy) w kolejnych latach badań

Examined parameter	Year	Experimental treatments						Significance of effect		
		PK		Manure		Slurry		Fertilisation (F)	Undersowing (U)	Interaction FxU
		PK	PK+U	M	M+U	LM	LM+U			
Total protein [g·kg^{-1} DM]	2011	147.5	140.5	150.6	155.9	161.4	171.3	**	NS	NS
	2012	154.5	158.9	166.3	167.5	146.6	155.9	**	NS	NS
	2013	168.1	174.1	173.9	180.8	144.7	155.2	**	**	NS
	2014	148.1	164.5	162.1	176.5	123.4	142.8	**	**	*
	Mean	**154.6**	**159.5**	**163.2**	**170.2**	**144.0**	**154.6**	**	**	*
	Significance of year effect	**	**	*	**NS**	**	*	-	-	-
Crude fibre [g·kg^{-1} DM]	2013	275.9	284.2	286.8	267.1	291.8	281.1	NS	NS	NS
	2014	286.3	284.1	286.1	288.5	281.6	286.1	NS	NS	NS
	Mean	**280.7**	**284.1**	**286.5**	**276.8**	**287.1**	**283.4**	NS	NS	NS
	Significance of year effect	NS	NS	NS	**	NS	NS	-	-	-
NDF [g·kg^{-1} DM]	2013	479.9	473.4	482.6	432.5	522.4	496.2	*	**	*
	2014	515.4	496.8	502.3	493.7	515.2	514.3	NS	NS	NS
	Mean	**496.0**	**484.0**	**491.5**	**460.3**	**519.2**	**504.4b**	**	**	*
	Significance of year effect	**	**	NS	**	NS	NS	-	-	-
ADF [g·kg^{-1} DM]	2013	333.0	334.1	347.0	324.7	346.0	338.7	NS	**	NS
	2014	339.4	337.6	345.9	338.3	329.1	334.8	NS	NS	NS
	Mean	**335.9**	**335.7**	**346.5**	**330.9**	**338.3**	**336.9**	NS	NS	NS
	Significance of year effect	NS	NS	NS	NS	NS	NS	-	-	-
Crude ash [g·kg^{-1} DM]	2013	88.8	88.9	95.3	94.5	86.3	88.0	*	NS	NS
	2014	91.9	89.8	98.2	90.5	83.2	88.1	*	NS	*
	Mean	**90.2**	**89.3**	**96.6**	**92.7**	**84.9**	**88.1**	**	NS	*
	Significance of year effect	NS	NS	NS	NS	NS	NS	-	-	-
Sugars [g·kg^{-1} DM]	2013	130.3	112.2	91.9	104.0	133.6	128.5	**	NS	*
	2014	135.5	128.7	102.8	113.1	160.9	136.3	**	NS	*
	Mean	**132.6**	**119.7**	**96.9**	**108.2**	**146.0**	**132.0**	**	NS	*
	Significance of year effect	NS	NS	NS	NS	**	NS	-	-	-
Sugars/crude protein ratio	2013	1.16	0.81	0.73	0.68	1.37	1.16	**	**	**
	2014	1.32	1.10	0.86	0.86	1.82	1.29	**	**	*
	Mean	**1.23**	**0.94**	**0.79**	**0.76**	**1.58**	**1.22**	**	**	**
	Significance of year effect	NS	**	NS	**	**	NS	-	-	-

NDF – neutral detergent fiber; ADF – acid detergent fiber
*, ** significant at the level P<0.05 or P<0.01, respectively
NS – not significant

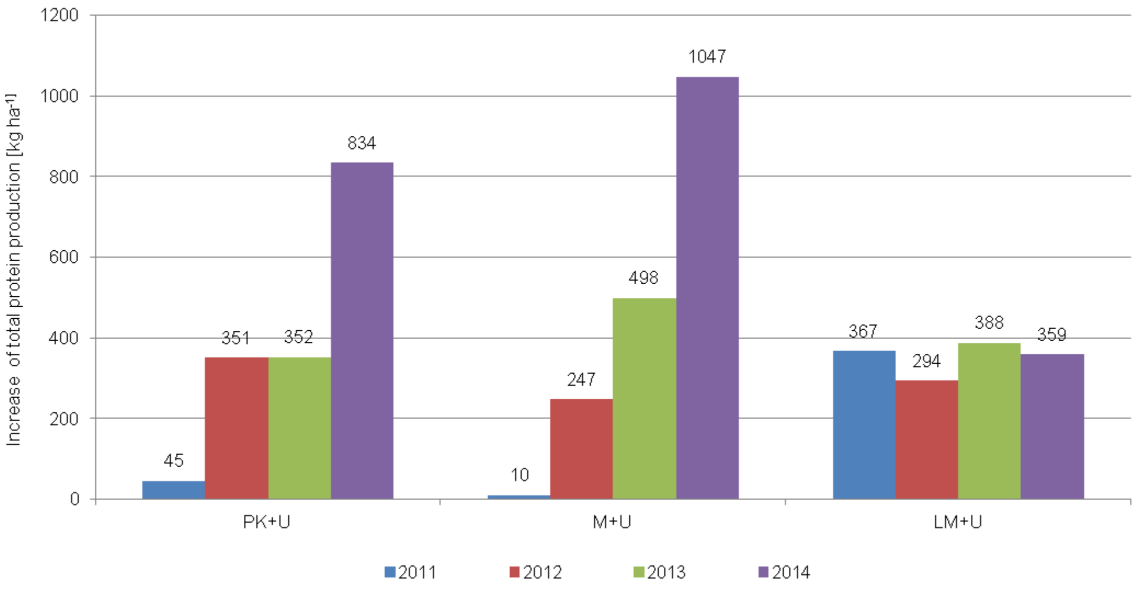
Source: own study / Źródło: opracowanie własne

Table 4. Protein yields (kg·ha^{-1}) in the following years of study
Tab. 4. Plon białka (kg·ha^{-1}) w kolejnych latach badań

Year of study	Experimental treatments						Significance of effect		
	PK		Manure		Slurry		Fertilisation (F)	Undersowing (U)	Interaction FxU
	PK	PK+U	M	M+U	LM	LM+U			
2011	950	995	1011	1021	1139	1506	NS	NS	NS
2012	998	1349	1095	1342	1210	1504	*	**	NS
2013	1319	1671	1372	1870	1110	1498	**	**	NS
2014	1101	1935	1265	2312	1192	1551	**	**	**
Mean from years	1092	1487	1186	1636	1163	1515	NS	**	**
Significance of year effect	**	**	**	**	NS	NS	-	-	-

*, ** significant at the level P<0.05 or P<0.01, respectively
NS – not significant

Source: own study / Źródło: opracowanie własne



ource: own study / Źródło: opracowanie własne

Fig. 3. The increase of total protein production (kg·ha^{-1}) in the following years of study
Rys. 3. Wzrost produkcji białka (kg·ha^{-1}) w kolejnych latach badań

4. Discussion

Application of grassland undersowing with red clover in adopted amount efficiently increased its share in meadow sward to 30%, and thus confirmed its rapid growth, competitiveness and suitability for undersowing [10]. Undersowing with red clover significantly affected dry mass and protein yielding which was also found in the studies by Barszczewski et al. [2, 3] and Terlikowski [23]. The effect of undersowing expressed in dry mass and protein yield was especially pronounced in combination with manure fertilisation.

Higher yields from undersown meadows, an increase in protein content and yields demonstrated the usefulness of this procedure on permanent grasslands and confirm the results obtained by Goliński [9], Mikołajczak et al. [19] and Barszczewski [2, 3]. Undersowing is also advised due to: reduced costs of forage production and limited consumption of fertilisers and finally – to possibility of limiting the emission of greenhouse gasses during production of fodder.

Most effective way of utilizing meadow sward – apart from direct feeding – is its conservation through ensilage [25]. Too high percent (>30%) of red clover in meadow sward, especially at its high moisture, makes the process of ensiling difficult. Performed chemical assessment indicates that meadow sward undersown with red clover, particularly from PK+C and M+C plots, had a high content of total protein but lower concentration of sugars compared with the sward from other plots. Unfavourable sugar to protein ratio, particularly from manure fertilised plot, indicates a poor suitability of this plant material for ensilage, opposite to plants from liquid manure fertilised plots. A precondition for full utilization of nutritive value of such fodder in cattle breeding is its good quality resulting from appropriate conservation [13; 26], which may be greatly improved using additives to stimulate the process of lactic acid fermentation.

5. Conclusions

Undersowing with red clover was most effective on plot fertilised with manure and the least efficient – on those which have been fertilised with slurry. The contribution of red clover on the latter was 2-fold lower compared to the other plots.

Undersowing meadow with red clover is a highly efficient procedure resulting in increasing dry weight (by 20%) and protein yields, particularly on plots fertilised with phosphorus and potassium and on those fertilised with manure.

The presence of undersown red clover in the meadow sward increased protein content, improved nutritive value of obtained green fodder but on the other hand detoriorating the usefulness of plant material for ensilage.

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