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THE QUALITATIVE POTENTIAL OF SOME GRASS-LEGUME MIXTURES FOR FEED. CASE OF STUDY

Carmen Claudia DURĂU[1], Codruța CHIȘ[1], Nicolae Marinel HORABLAGA[1, 2], Christianna Maria ISTRATE-SCHILLER[1, 2], Casiana Doina MIHUȚ[1], Vlad Dragoslav MIRCOV[1], Anișoara Claudia DUMA COPCEA[1], Daniela SCEDEI[1]

[1]University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului Street, Timisoara, Romania
[2]Lovrin Agricultural Research and Development Station Lovrin,
200 Principala Street, Lovrin, Timiș, Romania

Corresponding author email: carmen_durau@usvt.ro

Abstract

The use of chemical fertilizers was one of the most common technological links for increasing feed production. The motivation for this measure was the need to increase the food requirement for the constantly growing population of humanity correlated with the consumption of animal products. One of the challenges of the last decade is to use chemical fertilizers in the smallest possible quantity, preserving the properties of the soil in order to obtain qualitative productions. One of the current trends of modern agriculture is to use mixtures (grasses and legumes) that are agro-economically efficient. In this sense, the purpose of this work is to test mixtures of annual grasses and legumes for fodder in order to evaluate their qualitative potential (CP%). Thus, mixtures of annual clover (T) and two oat varieties (O1, O2) were used. The proportions in which they were sown were: clover (T)/(O1, O2) 50%/50%, 33%/66%, 66%/33%. The mixtures were sown under the same conditions. From the statistically analyzed data, we can conclude that the best results in terms of CP% were T/O2 33%/66%, and fiber T/66%/33%.

Key words*: crud protein %, fiber %, mixture, qualitative potential.*

INTRODUCTION

In the last decade, one of the topics debated in the field of agriculture in Europe is the decline of feed quality and the use of legume mixtures with cereals with a potential to improve the agronomic performance of crop systems and to provide protein-rich foods and feed contributing to reducing European dependence on imported proteins (Watson et al., 2017; Krga et al., 2022). Reducing the use of nitrogen (N) fertilizers and their negative impact on agricultural economic activities on the environment shows that nitrogen fixing legumes cultivated in rotation or in intercalated cultures are considered a sustainable alternative to introduce nitrogen into agroecosystems (Fustec et al., 2010). Increasing the intensity of using land among small farmers is a global tendency that threatens the productive basis of farms in many developing countries.

From the perspective of environmental protection, the mixtures of legume and non-legume crops can offer more advantages than those in pure culture. Their benefits come mainly from their complex efficiency in the management of carbon and nitrogen (Kramberger et al., 2013).

Thus, the study of intercalated crops for feed and coverage crops has become a theme of interest for agronomists, biologists, and ecologists (Broker et al., 2014; Durău et al., 2024).

Annual fodder species can provide high yields and quality feed or conservation as hay or silo for subsequent feeding.

They can be cultivated as a single crop or mixed with other annual or perennial plants (Havilah, 2011).

Most research results suggest that very good performance in terms of feed quality are obtained when some annual legumes, such as *Trifolium incarnatum* L., are cultivated in mixtures (legumes - cereals) unless they are cultivated alone.

Legumes are the second most important family of agricultural crop species after grasses worldwide and are used for both cereals and feed, but are underrepresented in European crop systems, which are dominated by cereals and oil seeds.

Crimson/Italian clover grows well in mixtures with small cereals, herbs, and other species of clover.
An oat culture is a common companion, either as a coverage culture, or as a partner with high biomass and nutrients (SARE, 2012).
As Knight (1985) detailed, crimson/Italian clover can be cultivated with rye, annually ray grass, and various cereals.

MATERIALS AND METHODS

In Romania, there are few studies on the culture of crimson clover both in pure crop and in mixture for feed, maybe because of the difficulty of harvesting, keeping, and storing.
In this sense, the purpose of this paper is to highlight the qualitative potential from a fodder point of view of some crimson clover - oats mixtures.
The experimental field was located in the pedoclimatic conditions of the didactic station of the U.S.V.T. (Western Romania) in 2023 (Figure 1)

Figure 1. View from experimental field
(original foto: Carmen Claudia Durău)

Thus, the multiannual average of precipitations was, generally, between 500-650 mm, but the year 2022 was below the multiannual average (446 mm) and the year 2023 was above the multiannual average (698 mm).
The southwest of Romania, due to its geographical position in the temperate area, is exposed to a range of phenomena of meteorological and climate risk with the potential to take place throughout the year (Mircov et al., 2024).
Analysed data show that the soil is a cambic chernozem, medium clay clay/medium argyle clay with a weak alkaline pH. The concentration of humus is characteristic of this type of soil mentioned (Durău et al., 2024). The evaluation of agricultural land is one of the most important stages resulting in the increase of agricultural production and establishing a sustainable agricultural system. (Mihuț et al., 2024). The research was carried out on mixtures of crimson/(*Trifolium incarnatum* L). Kardinal (TiK) variety and two varieties of oats – (*Avena sativa* L.) Ovidiu variety (O.Ov) and Lovrin 1 variety (O.L1).
For sowing, the following mixtures were used: TIK+O.Ov 1: 1 (50%: 50%), 1: 2 (33%: 66%), 2: 1 (66%: 33%), Tik+O.L1 1: 1 (50%), 1: 2 (33%: 66%), 2: 1 (66%: 33%). Three variants were applied in three repetitions for each of the mixtures (Figure 2)

Figure 2. The experimental field
(original foto: Carmen Claudia Durău)

Each variant was 7 m^2. The mixtures were sown in the second decade of March 2023, when it was possible to enter the field.
The applied culture technology was under non-irrigation conditions and no fertilizers were applied.
According to the BBCH decimal unit for grasses and legumes (Meier, 2001), the harvesting was performed at the grass ear formation and at legume budding.
For the laboratory analyses, samples were collected from each variant on 1 m^2 and the results represent an average of the three variants. The determination of the amount of crud protein (CP%) was done by evaluating the nitrogen content using the Kjedahl method.
Statistical calculations were performed using SPSS Version 20, IBM and Microsoft Excel (2016, Microsoft Corporation, Redmond, WA, USA). Elements of descriptive statistics were determined mean, standard deviation, minimum and maximum values and 95% confidence

interval for the mean) and to determine significant differences, the Kruskal Wallis test and the Mann Whitney test were applied.

RESULTS AND DISCUSSIONS

Analysing the crud protein (CP%) depending on the combination of TiK - *Trifolium incarnatum* Kardinal and the two oat varieties in question (O.Ov and O.L1), statistically significant differences were determined in the case of the 1/2 percent (Tabel 1, Figure 3).

A similar result was obtained in an experiment carried out in the same period and in the same proportion 1: 2 (33%: 66%), but with peas and oats Lovrin 1 (Durău et al., 2024).

Table 1. Numerical characteristics associated with the mixtures according to the varieties analysed (O.Ov and O.L1) and the proportions in which the mixtures are found.

Ratio	Mixture	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	Mann-Whitney U(p)
				Lower Bound	Upper Bound			
Protein (CP%) 1/1	Tik+OOv	12.11	0.37	11.20	13.02	11.82	12.52	3(0.513)
	TiK+OL1	12.28	0.09	12.05	12.50	12.18	12.36	
Protein (CP%) 1/2	Tik+OOv	11.49	0.53	10.17	12.81	11.10	12.10	0(0.04<0.05)
	TiK+OL1	13.20	0.53	11.88	14.52	12.63	13.68	
Protein (CP%) 2/1	Tik+OOv	12.68	0.36	11.78	13.58	12.26	12.90	1(0.200)
	TiK+OL1	12.14	0.48	10.95	13.33	11.67	12.63	
Fiber(F%) 1/1	Tik+OO	19.64	2.15	14.30	24.98	17.87	22.03	3(0.513
	TiK+OL1	19.73	1.03	17.18	22.28	19.04	20.91	
Fiber(F%) 1/2	Tik+OO	20.32	1.02	17.79	22.84	19.61	21.48	1(0.127)
	TiK+OL1	22.24	1.97	17.33	27.14	20.16	24.09	
Fiber(F%) 2/1	Tik+OO	20.25	3.05	12.66	27.83	18.12	23.75	1(0.127)
	TiK+OL1	24.02	1.66	19.89	28.15	22.20	25.45	

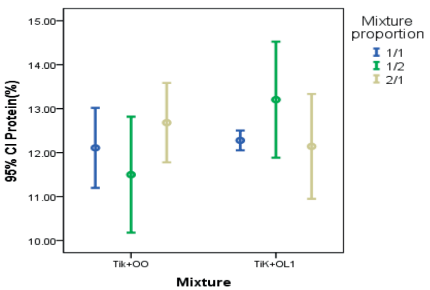

Figure 3. Mean and 95% CI for Protein (CP%)

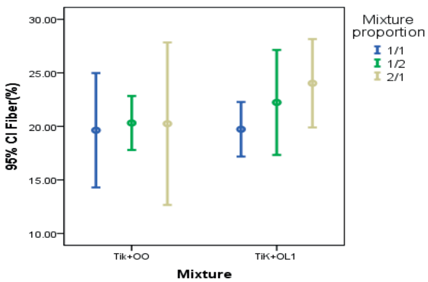

Figure 4. Mean and 95% CI for Fiber (F%)

In the case of fibre (F%), no statistically significant differences were determined between the Tik+OO and Tik+OL1 mixtures, regardless of the percentage considered (Table 1, Figure 4). Crimson clover feed is very nutritious, with over 25% gross protein that can be 80% digestible. They are harvested in early spring and can contain 12-14% raw protein and 60-65% digestible nutrients of dry substance even in full flowering (Young-Mathews, 2013; Ball et al., 2000).

Table 2. Numerical characteristics associated with the TiK+O.Ov mixture in protein (CP%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	12.11[ab]	0.36	11.19	13.01	11.82	12.52
1/2	11.49[a]	0.53	10.17	12.81	11.10	12.10
2/1	12.68[b]	0.36	11.77	13.58	12.26	12.90

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

Table 3. Numerical characteristics associated with the TiK+O.Ov mixture in fiber (F%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	19.63[a]	2.14	14.29	24.97	17.87	22.03
1/2	20.31[a]	1.01	17.79	22.83	19.61	21.48
2/1	20.25[a]	3.05	12.66	27.83	18.12	23.75

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

The analysis of the differences between the proportions in the case of crud protein (CP%) shows that there are significant differences between the proportions of 1/2 and 2/1 for the TiK+O.Ov mixture. (Table 2, Figure 5). Kramberger et al. (2013) concluded that, to obtain benefits, it is extremely important that the legumes in the mixtures of coverage or for feed be complementary to facilitate good growth under the specific conditions of the field.

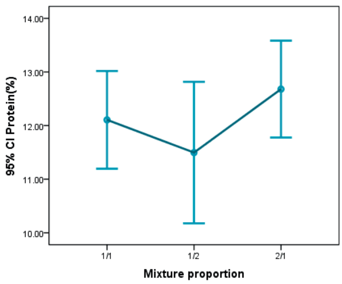

Figure 5. Mean and 95% CI for protein (CP%) TiK+O.Ov mixture depending on the proportion

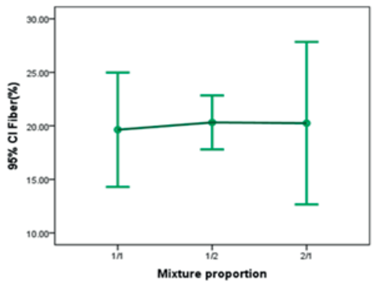

Figure 6. Mean and 95% CI for fiber (F%) TiK+O.Ov mixture depending on the proportion

For the fibre (F%) the analysis between the proportions 1/1, 1/2, 2/1 shows that there are no statistically significant differences in the case of the mixture TiK+O.Ov (Table 3, Figure 6). Analysing the percentages of crud protein (CP%) in the TiK+O.L1 mixture in relation to the proportions 1/1, 1/2, 2/1, statistically significant differences were determined between the proportion 1/1 and 1/2 (Table 4, Figure 7).

Table 4. Numerical characteristics associated with the TiK+OL1 mixture in crud protein (CP%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval or Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	12.2767[a]	0.09074	12.0513	12.5021	12.18	12.36
1/2	13.2033[b]	0.53163	11.8827	14.5240	12.63	13.68
2/1	12.1400[ab]	0.48031	10.9468	13.3332	11.67	12.63

Means followed by the same letter do not differ statistically(Mann-Whitney U test), *p<0.05.

For percentages of fiber (F %) the statistically significant differences for the TiK+OL1 mixture in relation to the percentages 1/1, 1/2, 2/1 were between the proportions 1/1 and 2/1 (Table 5, Figure 8)

Table 5. Numerical characteristics associated with the TiK+O.L1 mixture in fibre (F%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	19.73[a]	1.02	17.17	22.28	19.04	20.91
1/2	22.23[ab]	1.97	17.33	27.14	20.16	24.09
2/1	24.02[b]	1.66	19.89	28.15	22.20	25.45

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

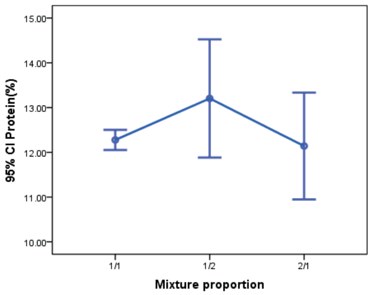

Figure 7. Mean and 95% CI or crud protein (CP%) TiK+O.L1 mixture depending on the proportion

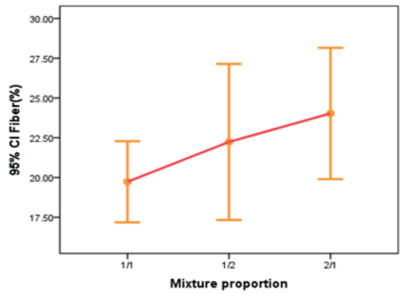

Figure 8. Mean and 95% CI for fiber (F%) TiK+O.L1 mixture depending on the proportion

As the feed matures, the fibre level increases and the protein level decreases, resulting in a general decrease in the nutritional value of the feed (Bracey et al., 2022). Akin et al. (1982) concluded, as a result of research carried out on crimson clover, that its digestibility decreases by 5% when it is harvested upon budding and 20% when it is harvested upon flowering compared to the young pre-budding stage.

CONCLUSIONS

The study of the ratios and species that fall into the composition of these fodder mixtures is very important because some species can complete each other to a certain level of participation, after which they become dominant and, implicitly, affect raw protein and fibre protein content. The interaction between the species and the moment of harvesting the mixture is very important for a maximum amount of raw protein (CP%) and fibre (F%). Data collected from the experimental field and processed with the help of Kruskal-Wallis and Mann Whitney tests have indicated some significant differences between certain parameters. Regarding raw protein (CP%), significant differences (*p <0.05) were between the two TiK+O.Ov and TiK+O.OL1 mixtures, but in the same proportions 1: 2 (33%: 66%) where oats O.OL1 also used the resources better. The largest amount of fibre (F%) was obtained with TiK+O.OL1 (F%-24) mixture but no significant differences were found compared to the TiK+O.Ov mixture, for the same ratio. Significant differences in the case of raw protein (CP%) were found for the same mixture but in different proportions 1: 2 (33%: 66%) and 2: 1 (66%: 33%) for TiK+O.OL1 1: 1 (50%) and 1: 2 (33%: 66%) TIK+O.Ov, respectively. This case study is for a short period of time and there were obviously other factors that contributed to the outcomes. The research will continue for a longer period to highlight the benefits and qualitative potential of the mixture of oats with crimson clover under the conditions of Timiș County (western Romania).

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REFERENCES

Akin, D. E., Robinso E. L. (1982). Structure of leaves and stems of arrow leaf and crimson clovers as related to *in vitro* digestibility. *Crop Sci.,* 22(1): 24–29.

Ball, D.M., and G.D. Lacefield. (2000). Crimson clover. Circular 00-1. Oregon Clover Commission, Salem. http://www.oregonclover.org/downloads/files/crimsonclover.pdf

Bracey, W.M., Sykes, V.R., Yin, X., Bates, G.E.; Butler, D.M., McIntosh, D.W., Willette, A.R. (2022). Forage Yield, Quality, and Impact on Subsequent Cash Crop of Cover Crops in an Integrated Forage/Row Crop System. *Agronomy,* 12, 1214.

Brooker, R.W., Bennett, A. E., Cong, W.-F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L, McKenzie, B.M., Pakeman, R.J., Paterson, E., Schob, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., White,P.J. (2014). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1).

Cupina B., Manojlović M., Krstić D., Čabilovski R., Mikić A., Ignjatović-Ćupina A., Erić P. (2011). Effect of winter cover crops on the dynamics of soil mineral nitrogen and yield and quality of Sudan grass [*Sorghum bicolor* (L.) Moench.]. *Australian Journal of Crop Science*, 7: 839–845.

Durău C.C., Chiș, C., Horablaga M.N., Istrate-Shiler C.M., Răchitean D., Mihuț, C.D., Scedei, D., Dicu, D. (2024). Productivity aspects of some annual forage mixtures in the conditions of Banat Plane. *Scientific Papers. Series A. Agronomy, Vol. LXVII*, No. 1, 380–385.

Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.B. (2010). Nitrogen rhizodeposition of legumes. A review *Agron. Sustain. Dev.* 30, 57–66.

Génarda, T., Etiennea, P., Laînéa,P., Yvind, J.C., Diquéloua, S. (2016). Nitrogen transfer from *Lupinus albus* L., *Trifolium incarnatum* L. and *Vicia sativa* L. contribute differently to rapeseed (*Brassica napus* L.) nitrogen nutrition. *Helyon*, 2(9).

Harper, C.A. (2004). Growing and managing successful food plots for wildlife in the mid-South. PB1743-7M 9/04 E12-4915-00-007-04 04-0269. *Univ. of Tennessee Agric*. Extension Serv.

Havilah E.J. (2011). Forages and pastures, Annual Forage and Pasture Crops – Species and Varieties *Encyclopedia of Dairy Sciences*, 552-562.

Knight, W.E. (1985). Crimson Clover. In Clover Science and Technology. *Agronomy Monograph*, No. 25. N.E. Taylor (ed.) 24:491-502. ASA/CSSA/SSSA, Madison, WI.

Kramberger, B., Gselman, A., Podvršnik, M., Kristl, J., Lešnik, M. (2013). Environmental advantages of binary mixtures of *Trifolium incarnatum* and *Lolium multiflorum* over individual pure stands. *Plant Soil Environ*. *59*(1), 22–28.

Krga, I., Simic, A., Dželetovic, Ž., Babi´ c, S., Katanski, S., Nikolic, S.R., Damnjanovic, J. (2021). Biomass and Protein Yields of Field Peas and Oats Intercrop Affected by Sowing Norms and Nitrogen Fertilizer at Two Different Stages of Growth. *Agriculture,* 11, 871.

Meier U. (2001). *Growth stages of mono-and dicotyledonous plants e BBCH monograph, Federal Biological Research Centre for Agriculture and Forestry,* 2001, 158 pp.

Mihut, C., Niță, L., Duma Copcea, A., Rinovetz, A. (2024). Assessment of the productive capacity of agricultural lands for their sustainable use. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 24*, Issue 1, 619–625.

Mircov, V.D., Mihuț, C., Cozma, A. (2024). Schimbările climatice și efectele aceste asupra solurilor și agriculturii în Vestul și Sud-Vestul României. *Scientific Papers. Series B, Horticulture. Vol. LXVIII,* No. 1, pg. 470–477.

Sustainable Agriculture Research and Education (SARE)(2012)

Vanek, S. J., Meza,K., Ccanto, R., Olivera, E., Scurrah, M., Steven J. Fonte, S. (2020). Participatory design of improved forage/fallow options across soil gradients with farmers of the Central Peruvian Andes. Agric. *Ecosyst. Environ.,* Vol. 300.

Watson, C.A., Reckling, M., Preissel, S., Bachinger, J.; Bergkvist, G.; Kuhlman, T.; Lindström, K.; Nemecek, T.; Topp, C.F.E.; Vanhatalo, A.; et al. (2017) Chapter Four Grain Legume Production and Use in European Agricultural Systems. *Adv. Agron.*, *144*, 235–303.

Young-Mathews, A. (2013). Plant guide for crimson clover (*Trifolium incarnatum*). *USDA-Natural Resources Conservation Service, Plant Materials Center*, Corvallis, OR.