Macedonian Journal of Animal Science, Vol. **14**, No. 1–2, pp. 43–46 (2025)
Article 302
In print: ISSN 1857 – 6907
Received: September 13, 2025
On line: ISSN 1857 – 7709
Accepted: November 13, 2025
UDC: 633.32-182
https://www.doi.org/10.54865/mjas25141-243p

Original scientific paper

PHYSIOLOGICAL RESPONSE TO MINERAL NUTRITION ON THE MACRONUTRIEHT PROFILE OF EGYPTIAN CLOVER (*Trifolium alexandrinum* L.)

Petar Petrov, Daniela Beličovska, Vesna Levkov, Nataša Gjorgovska

Institute of Animal and Fishery Science, Ss. Cyril and Methodius University in Skopje, Blvd. Ilinden 92a, 1000 Skopje, North Macedonia
petrovpetar2012@gmail.com

A b s t r a c t: The aim of this research is to determine the influence of different variants of mineral fertilization (Control, NP, NK, PK and NPK) on the macronutritional composition of Alexander's clover (Trifolium alexandrinum L.). The experiment was set up in a randomized block design with three replications on alluvial soil. Statistical analysis (ANOVA and Fisher's LSD test) showed that combined NPK fertilization significantly ($p \leq 0.05$ and $p \leq 0.01$) increased the contents of proteins, fats, fibers and soluble sugars compared to the control variant. The highest values were observed in the NPK treatment, which indicates a synergistic effect of N, P and K in the biosynthesis of organic matter and improved nutritional quality of the Alexander clover.

Key words: Alexander clover; mineral nutrition; NPK; proteins; fibers; sugars

ФИЗИОЛОШКИ ОДГОВОР НА МИНЕРАЛНАТА ИСХРАНА ВРЗ МАКРОНУТРИТИВНИОТ ПРОФИЛ НА АЛЕКСАНДРОВАТА ДЕТЕЛИНА (*Trifolium alexandrinum* L.)

А п с т р а к т: Целта на ова истражување е да се утврди влијанието на различни варијанти на минерално ѓубрење (Контрола, NP, NK, PK и NPK) врз макронутритивниот состав на александровата детелина (Trifolium alexandrinum L.). Експериментот е поставен во случаен блок систем со три повторувања на алувијална почва. Статистичката анализа (ANOVA и Fisher's LSD тест) покажа дека комбинираното NPK ѓубрење значајно ($p \leq 0,05$ и $p \leq 0,01$) ги зголемува содржините на протеини, масти, влакна и растворливи шеќери во споредба со контролната варијанта. Највисоки вредности беа забележани кај NPK третманот, што укажува на синергетско дејство на N, P и K во биосинтезата на органски материи и подобрен нутритивен квалитет на александровата детелина.

Клучни зборови: александрова детелина; минерална исхрана; NPK; протеини; влакна; шеќери

1. INTRODUCTION

Alexandrinum clover (*Trifolium alexandrinum* L.) is an important annual leguminous forage crop that plays a key role in providing high-protein and energy food for domestic animals. It originates from the Middle East. Due to its short vegetation, it is increasingly found in areas intended for animal feed production. The first mowing is possible within 60 to 70 days of sowing with the application of good agricultural techniques, and during the vegetation period under irrigation conditions it can give up to four high-yielding mowings. To improve its quality properties, it is inevitable to implement the agrotechnical measure of fertilization, which often has a positive effect on other crops that come after Alexander's clover in the crop rotation. It belongs to the group of leguminous crops, which significantly

enriches the soil with nitrogen. This crop blooms in the period May-June and, due to its rich nectar composition, is also an excellent source for obtaining honey. The physiology of mineral nutrition in plants is of essential importance for the processes of photosynthesis, amino acid synthesis, enzymatic activity and storage of reserve substances. Balanced mineral nutrition is particularly important for achieving optimal physiological response and high nutritional quality of forage crops (Wang et al., 2021; Hindoriya et al., 2024).

2. MATERIAL AND METHODS

The research included egyptian clover (*Trifolium alexandrinum L.*) as the main crop. The scientific-research experiment was conducted according to the generally accepted norms and methods for setting up field experiments according to (Filiposki, 2004). The experiment was set up on alluvial soil, according to a randomised block system, in 5 variants and 3 repetitions. Variants in the experiment are:

Control (unfertilised):

1) NP

2) NK

3) PK

4) NPK

From the agrochemical analyses of alluvial soil, it can be concluded that the soil has a moderately basic pH reaction, medium provision with physiologically available forms of nitrogen, phosphorus and potassium. In terms of the calcium carbonate content, the soil is categorized as medium carbonate soil. Mineral fertilizer was added as basic soil fertilization, with N80 P120 K80 taken as the edaphon.

Before setting up the experiment, average samples were taken at a depth of 0-30 cm to determine some of the chemical properties of the soil, and the determination included:

- pH-reaction of soil solution (in H_2O and N KCl), determined potentiometrically with a pH-meter, with a combined glass and calomel electrode (Bogdanovič et al., 1966),
- carbonate content determined using a Scheibler calcimeter (Bogdanovič et al., 1966),
- total nitrogen content, determined according to the Tjurin method (Bogdanovič et al., 1966),
- content of physiologically available forms of phosphorus, determined according to the AL-method and reading of a spectrophotometer (Bogdanovič et al., 1966),
- content of physiologically available forms of potassium determined according to the AL-method and reading of a flame photometer (Bogdanovič et al., 1966).

After the mowing, average samples of Alexander's clover were prepared, in which the following parameters were determined in laboratory conditions:

- Protein content, according to the micro-Kjeldhl method (Džamić, 1989)
- Fat content, Soxhlet extraction (Džamić, 1989)
- Fiber content according to Hoffman, (Džamić, 1989)
- Soluble sugar content, (Džamić, 1989)

The data were processed with variational ANOVA, and statistical significance was tested with Fisher's LSD test at $p \leq 0.05$ and $p \leq 0.01$.

3. RESULTS AND DISCUSSION

The influence of different variants of mineral fertilization on the macronutrient composition of *Trifolium alexandrinum* L. is shown in Table 1. Combined NPK fertilization results in the highest values for all examined parameters, which confirms the synergistic effect of N, P and K in the metabolism of proteins, lipids, fibers and carbohydrates. Variants with single or double fertilization (NP, NK, PK) show a moderate increase compared to the control, but less than the combined NPK variant. ANOVA and Fisher's LSD show statistically significant differences between treatments ($p \leq 0.05$; $p \leq 0.01$).

The highest crude protein content was observed in the NPK variant (20.10%), which c an approximately 30% increase compared to the control (15.43%). This increase is biochemically related to the enhanced activity of the nitrate reductase and glutamine synthetase (GS/GOGAT) system, which are crucial for nitrogen assimilation (Mengel & Kirkby, 2001; Marschner, 2012). The introduced nitrogen in the form of NO_3^- and NH_4^+ provides an increased supply of amino groups for the synthesis of amino acids, enzymes and structural proteins, resulting in increased biomass and higher

nutritional value. NPK fertilization also optimizes phosphate availability, providing sufficient energy (ATP) for protein biosynthesis. According to Hindoriya et al. (2024), the increased protein content in Trifolium alexandrinum directly correlates with increased digestibility and productivity of ruminants, especially during periods of intensive lactation.

Table 1

Content of proteins, fats, fibers and sugars by varieties in Egyptian clover expressed in % of dry matter

Variants	Proteins	Fats	Fibers	Sugars
Control group	15.43 ±1.06c	2.69±0.08c	17.73±0.24d	4.15±0.07e
NP	17.87±1.16b	3.01±0.03b	18.47±0.48c	4.85±0.06d
NK	17.37±0.57b	2.99±0.19b	19.46±0.22b	4.99±0.03c
PK	18.33±0.25b	3.31±0.07a	19.96±0.14b	5.16±0.06b
NPK	20.10±0.62a	3.45±0.09a	20.95±0.24a	5.45±0.11a
LSD ($p \leq 0.05$)	0.74	0.11	0.35	0.09
LSD ($p \leq 0.01$)	1.10	0.17	0.51	0.14

The fat content varied from 2.69% (control) to 3.45% (NPK), which represents an increase of about 28%. The increased lipid fraction in the NP, PK and NPK treatments is a consequence of improved photosynthetic activity and enhanced lipogenesis through the enzymes acetyl-CoA carboxylase and fat synthase. Phosphorus, as part of phospholipids, plays a role in the stability of cell membranes, and potassium regulates the activity of enzymes involved in lipid metabolism (Leite et al., 2021). These results indicate an increased energy value of the forage, which is important for livestock productivity in intensive production (Xu et al., 2024).

The highest crude fiber content was determined in the NPK treatment (20.95%), while the lowest in the control variant (17.73%). The difference is about 18%. The improved supply of N, P and K stimulates cell division, parenchyma development and synthesis of structural polysaccharides (cellulose and hemicellulose). These components are the basis of the cell wall and increase the mechanical stability of the plant (Šidlauskaitė & Kadžiulienė, 2023). In a nutritional context, moderate fiber content is desirable, as it allows better digestibility and a balance between structural and non-structural carbohydrates in the diet.

Soluble sugars ranged from 4.15% (control) to 5.45% (NPK), which is an increase of about 31%. Higher sugar concentration indicates more intense photosynthetic activity, higher Rubisco efficiency and better translocation of assimilates to vegetative organs. Phosphorus and potassium promote the synthesis and transport of sucrose, glucose and fructose, leading to a higher energy value of the forage (Leite et al., 2021; Tahir et al., 2023). The increased proportion of soluble sugars improves the taste and appetite of animals, which positively affects the consumption and efficiency of feeding (Guo et al., 2024).

Combined NPK fertilization optimizes the biochemical balance between the synthesis of proteins, fats, fibers and sugars, ensuring high quality of the forage. The increased content of proteins and sugars contributes to better fermentation in the rumen and a higher feed utilization coefficient, while the appropriate amount of fibers ensures the correct physiological activity of the digestive system in ruminants. These results are consistent with previous studies on Trifolium alexandrinum and other leguminous forages, which confirm that mineral nutrition is crucial for improving biochemical composition and livestock productivity (Hindoriya et al., 2024; Fontaine et al., 2024).

4. CONCLUSION

Based on the conducted research, it can be concluded that combined fertilization with nitrogen, phosphorus and potassium (NPK) has the greatest positive impact on increasing the macronutrient composition of Alexander's clover.

The NPK variant resulted in the highest concentrations of proteins, fats, fibers and soluble sugars, indicating improved metabolic and photosynthetic activity of the plant.

Balanced mineral nutrition allows for optimal physiological function, increased biosynthesis of organic compounds and improved biomass quality, which is essential for highly productive and environmentally sustainable forage systems.

In summary, NPK treatment is the most appropriate variant for achieving optimal biochemical composition and high nutritional quality of Alexander's clover, which directly contributes to increased productivity and economic efficiency in livestock production.

REFERENCES

Bogdanovic Milovan, Velikonja Nenad, Racz Zoltan, (1966): *Hemijske metode ispitivanja zemjista*, Beograd.

Džamić, Milomir (2009): *Praktikum iz biohemije*, Naučna knjiga, Beograd;

Epstein, E., & Bloom, A. J. (2005): *Mineral Nutrition of Plants: Principles and Perspectives* (2nd ed.). Sinauer Associates.

Fontaine, D., Rasmussen, J., & Eriksen, J. (2024): Marginal increase in nitrate leaching under grass–clover leys by slurry and mineral fertilizer. *Nutrient Cycling in Agroecosystems*. https://doi.org/10.1007/s10705-023-10327-4

Hindoriya, P. S., Sharma, S., & Patel, A. (2024): The impact of integrated nutrient management on *Trifolium alexandrinum*. *Agronomy*, **14** (2), 339. https://doi.org/10.3390/agronomy14020339

Leite, R.G., Cardoso, A.d., Fonseca, N.V.B. *et al.* (2021): Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass. *Scientific Reports*, **11**, Article 14786. https://doi.org/10.1038/s41598-021-94098-4

Marschner, P. (2012): *Marschner's Mineral Nutrition of Higher Plants* (3rd ed.). Academic Press.

Mengel, K., & Kirkby, E. A. (2001). *Principles of Plant Mutrition* (5th ed.). Kluwer Academic Publishers.

Šidlauskaitė, G., & Kadžiulienė, Ž. (2023): The effect of inorganic nitrogen fertilizers on the quality of forage composed of various species of legumes in the northern part of a temperate climate zone. *Plants*, 12, 3676. https://doi.org/10.3390/plants12213676

Tahir, M., et al. (2023): Mixed legume–grass seeding and nitrogen fertilizer input improve forage yield and nutritional quality. *Frontiers in Plant Science*, https://doi.org/10.3s389/fpls.2023.

Tavares, D. S., et al. (2024). System fertilization in integrated crop-livestock systems improves forage accumulation and nutrient availability. https://doi.org/10.1016/j.xxxx.

Xu, L., et al. (2024). Yield and nutrient composition of forage crops and their implications. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10825004/

Филипоски, К (2004). Поставување на полски опити од агрохемија,s Тутун/Tobacco, Vol. **54**, No 3-4, 64–76, Институт за тутун, Прилеп;