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# Content of crude protein in bread winter wheat grain depending on nitrogen fertilization and foliar application of effective microorganisms

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## Abstract

The aim of this study was to determine the grain protein content of wheat varieties grown under different application rates of effective microorganisms (EM) and nitrogen, and two sowing rates. Three-year research was conducted at the experimental filed of the Scientific Institute PKB Agroekonomik Padinska Skela, using four varieties of wheat (Ratarica, Pobeda, Nogal and Apache), two sowing densities of 400 and 500 seeds/m$^2$ and T1 fertilization (150 kg/ha N); T2 (150 kg/ha N + one foliar treatment, T3 (100 kg/ha N + two foliar treatments), and T4 (50 kg/ha N + three foliar treatments). The preparation EM Aktiv 7 l/ha, a mixture of multiple selected microorganisms, was used for foliar treatment with EM. The average protein content was 13.95% and was statistically significantly dependent on the variety, fertilization, and their interaction $p<0.01$. The Ratarica and Pobeda varieties had the highest grain protein content. T3 treatment had the greatest impact on grain protein content in all three years of research, by using effective microorganisms, the level of protein in the grain can be maintained when the amount of nitrogen is reduced.

*Key words:* wheat, effective microorganisms, sowing rate, protein content

## Introduction

Wheat is one of the most important crops for human consumption. In the sowing structure in the world and Europe areas under winter wheat are larger than maize. The main purpose of wheat production is for the production of flour and other products intended for the bakery industry. According to Peña (2007), in the developed part of the world 53% of the countries use wheat for human consumption, while this amounts to 85% in the underdeveloped part of the world. Wheat flour consists mainly of starch (70–75%), water (12–14%), and protein (8–16%) (Egesel and Kahriman, 2013). Wheat is unique among plant species because wheat flour contains a protein complex which has the ability to form a dough with rheological properties necessary for the production of bread and other bakery products (Simić and Žilić 2018).

Based on the classification scheme, total wheat proteins are divided into albumins, globulins, gliadins, and glutenins. The content of total proteins in wheat grain depends on the genotype, climatic conditions, and plant nutrition. It is known that the level of wheat yield and protein synthesis are significantly influenced by the nitrogen nutrition. In order to protect the environment and produce safe food, increasing preference is given to growing practices methods that eliminate mineral fertilizers. By applying effective microorganisms in plant production, it is possible to achieve economic and environmental effects. According to a research by Cvijanović et al. (2017), soil treatment and foliar application of effective groups of microorganisms can increase the yield of different wheat genotypes by 3.41%, as well as the total number of microorganisms, azotobacteria, and dehydrogenase enzyme activity in wheat root rhizosphere. Roljević et al. (2018) found that the use of a microbiological preparation in organic wheat production can increase the yield of different types of wheat by up to 46%.

According to previous research, protein content is variable and depends on genotype (Preston et. al. 2001), grain size (Evers, 2000; Wiersma et al., 2001), locality (Menkovska et. al. 2015; Đurić et al. 2019; Egesel and Kahriman 2013), and fertilization.

The aim of this study was to determine the effect of foliar application of effective microorganisms on grain protein content of bread winter wheat cultivars under different sowing and fertilization rate over three seasons.

## Materials and methods

Design of experimental research – a field trial was conducted in the 2016/2017 -2018/2019 period on the experimental field of the Institute of PKB Agroekonomik in Padinska Skela, Vojvodina Province (ΨN 440 56', λE 250 28'). The size of the experimental plot was 576 $m^2$, and the elementary plot 5 $m^2$. The plots were arranged according to the plan of divided plots in four replications. Large plots were sowing rate, sub plots were varieties and sub-subplots were different fertilization. Corn was a preceding crop. The sowing was: 22 November 2016; 07 October 2017; and 16 November 2018. The harvest was: 18 July 2017; 16 June 2018; and 24 July 2019.

Factor A means sowing rates of 400 seeds/m$^2$ and 500 seeds/m$^2$ were applied.

Factor B consisted of four varieties of bread wheat of favourable technological quality as follows: Ratarica, Pobeda, Nogal, and Apache. The wheat varieties were of different origin.

Factor C implies 400 kg/ha of NPK fertilizer 15:15:15 was used as the basic fertilizer applied in autumn. In the spring 46% of urea nitrogen fertilizer was applied, so that the total amounts of nitrogen were: 150, 100, and 50 kg/ha. During the growth period, foliar fertilization was performed with the preparation EM Aktiv in treatments T1 (150 kg/ha N); T2 150 kg/ha N + EM Aktiv, at tilling T3 100 kgN/ha + EM Aktiv, applied twice at the stage of the second node visible, and the stage of flag just visible; and T4 50 kgN/ha + EM Aktiv, applied three times (the second node visible, stage flag leaf just visible and heading). For each application with EM Aktiv the dose was 7 l/ha. EM Aktiv (commercial name) is a multiple microbiological preparation that contains a mixture of a large group of effective microorganisms with over 80 different species.

Meteorological and soil conditions for plant growth were very similar in 2016/2017 and 2018/2019. Mean air temperatures for these two growing seasons were 10.97 and 10.98ºC, the sum of precipitation amounted to 536.7 and 537.3 mm (Table 1). The second growing season of research in 2017/2018 had a lower air temperature of 9.73ºC and lower precipitation of 482.3 mm. The soil can be classified as marsh black. The agrochemical characteristics of the soil showed that the pH KCl was 7.40. The humus content was (3.43%), also in the category of medium secured soils. The content of inorganic nitrogen was 6.98 mg/kg. The content of phosphorus was 19.74 mg/100g and potassium was 16.58 mg/100g.

Total protein content in wheat grain was measured by the micro-Kjeldalh method (Bremner and Mulvaney, 1982). Data were processed by using a three-factor split plot experiment (plot division) using the DSAASTAT Statistics 2011 software. Significance of differences between means was tested by LSD test.

Tab. 1. Average monthly temperatures (ºC) and sum of precipitation (mm) for the growing period of wheat

| Years | Mean monthly air temperatures (°C) | | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | XI | XII | I | II | III | IV | V | VI | VII | |
| 2016/17 | 10.8 | 6.5 | 2.8 | 0.2 | 7.3 | 7.8 | 13.9 | 16.3 | 21.5 | 22.6 | 10.97 |
| 20/1718 | 9.6 | 5.9 | -0.6 | -5.1 | 3.3 | 9.9 | 11.1 | 17.2 | 22.5 | 23.5 | 9.73 |
| 2018/19 | 11.0 | 6.3 | 3.3 | 3.1 | 1.4 | 5.2 | 16.5 | 19.8 | 21.1 | 22.1 | 10.98 |
| | Sums of monthly precipitation (mm) | | | | | | | | | | Sum |
| 2016/17 | 70.6 | 50.8 | 10.8 | 46.5 | 46.4 | 78.8 | 34.4 | 74.4 | 89.2 | 34.8 | 536.7 |
| 20/1718 | 70.7 | 75.0 | 4.6 | 18.6 | 26.9 | 22.0 | 46.2 | 71.6 | 106.5 | 40.9 | 482.3 |
| 2018/19 | 57.0 | 48.1 | 40.6 | 39.2 | 47.2 | 58.2 | 29.4 | 80.1 | 70.1 | 66.7 | 537,3 |

*Source: Meteorological station of the PKB Agroekonomik Institute*

## Results and Discussion

Protein content as the main parameter of technological quality of wheat largely depends on temperature, water supply, and nitrogen nutrition (Đurić et al., 2019). Of all the macronutrients, nitrogen is the most important for the normal growth and development of wheat, and needs to be taken in certain amounts and ratios. (Sarić and Jocić, 1993). Đekić et al. (2016) stated that the fertilization system is one of the key factors influencing the amount of yield and its quality. Jaćimović et al. (2008) state that grain weight per spike significantly depended on the mineral nutrition of wheat, primarily on the applied doses. However, the use of preparations of microbiological or plant origin as an additive or substitute for mineral fertilizers are becoming increasingly popular today in crops production.

Analysis of variance showed that factors of variety and fertilization as well as their interaction had significant impact on wheat grain protein content in all growing seasons ($p>0.01$). Sowing rate did not have a significant effect on protein content over three growing seasons. In the year 2016/2017 the average protein content was 13.39% (Tab. 2).

Tab. 2. Crude protein content (%) of bread winter wheat varieties grown under different application rates of nitrogen and effective microorganisms, and sowing rates in the year 2016/2017.

| Sowing rate (seeds/m$^2$) (A) | Genotype (B) | Treatments (C) | | | | $\bar{X}$ AB | $\bar{X}$ A |
|---|---|---|---|---|---|---|---|
| | | T1 | T2 | T3 | T4 | | |
| 400 | Ratarica | 13.02 | 13.39 | 13.28 | 12.86 | 13.14 | 13.41 |
| | Pobeda | 13.19 | 13.26 | 14.06 | 14.03 | 13.64 | |
| | Nogal | 13.64 | 13.69 | 14.44 | 12.88 | 13.66 | |
| | Apache | 12.68 | 13.29 | 14.10 | 12.72 | 13.20 | |
| | $\bar{X}$ AC | 13.13 | 13.41 | 13.97 | 13.12 | | |
| 500 | Ratarica | 12.94 | 13.25 | 13.01 | 12.95 | 13.04 | 13.39 |
| | Pobeda | 12.84 | 13.36 | 13.79 | 13.76 | 13.44 | |
| | Nogal | 13.84 | 13.45 | 14.34 | 13.06 | 13.68 | |
| | Apache | 12.88 | 13.29 | 14.46 | 12.59 | 13.31 | |
| | $\bar{X}$ AC | 13.13 | 13.34 | 13.90 | 13.09 | $\bar{X}$B | |
| $\bar{X}$ BxC | Ratarica | 12.98 | 13.32 | 13.15 | 12.90 | 13.09 | |
| | Pobeda | 13.02 | 13.31 | 14.93 | 13.90 | 13.54 | |
| | Nogal | 13.74 | 13.57 | 14.39 | 12.97 | 13.67 | |
| | Apache | 12.78 | 13.29 | 14.28 | 12.66 | 13.25 | |
| | $\bar{X}$ C | 13.13 | 13.37 | 13.94 | 13.11 | | |
| AVERAGE 2017 | | | | | | 13.39 | |
| Variables and interactions | | A | B | C | AB | AC | BC |
| *Prob F* | | 0.42 | 0.00** | 0.00** | 0.47 | 0.96 | 0.00** |
| LSD (P<0.05) | | 0.19 | 0.22 | 0.13 | 0.31 | 0.18 | 0.26 |
| LSD (P<0.01 | | 0.13 | 0.30 | 0.17 | 0.43 | 0.25 | 0.35 |

T3-fertilization treatment with 100 kg/ha of nitrogen and two EM foliar treatments had significantly higher grain total protein content (13.94%) compared to other treatments. There was no significant difference in the protein content between treatments T4 (13.11%) and T1 (13.13%). The highest protein content was in the Pobeda cultivar in treatment T3 (14.93%). On average, for all treatments, the highest grain protein content was in the Nogal variety 13.67%, and the lowest in the Ratarica variety 13.09%. In Nogal the determined protein content, in comparison to Ratarica (13.09%) was higher by 4.43%, in relation to the Pobeda cultivar (13.54%) by 0.96% and the Apache variety (13.25%) by 3.16%. In year 2017/2018 the average protein content was 13.68% (Tab. 3).

Tab. 3. Crude protein content (%) of wheat varieties grown under different application rates of nitrogen and effective microorganisms, and sowing rates in the year 2017/2018

| Sowing rate (seeds/m$^2$) (A) | Genotype (B) | Treatments (C) | | | | X̄ AB | X̄ A |
|---|---|---|---|---|---|---|---|
| | | T1 | T2 | T3 | T4 | | |
| 400 | Ratarica | 13.79 | 14.19 | 13.47 | 13.98 | 13.86 | 13.68 |
| | Pobeda | 13.40 | 13.52 | 13.98 | 14.10 | 13.75 | |
| | Nogal | 13.72 | 14.09 | 14.00 | 12.80 | 13.65 | |
| | Apache | 12.78 | 13.32 | 14.15 | 13.52 | 13.44 | |
| | X̄ AC | 13.42 | 13.78 | 13.90 | 13.60 | | |
| 500 | Ratarica | 13.77 | 13.94 | 13.54 | 14.13 | 13.85 | 13.69 |
| | Pobeda | 13.29 | 13.35 | 14.13 | 13.94 | 13.68 | |
| | Nogal | 13.95 | 13.74 | 13.84 | 13.25 | 13.70 | |
| | Apache | 13.13 | 13.16 | 13.95 | 13.95 | 13.55 | |
| | X̄ AC | 13.54 | 13.55 | 13.86 | 13.82 | X̄B | |
| X̄ B | Ratarica | 13.78 | 14.07 | 13.50 | 14.06 | 13.85 | |
| | Pobeda | 13.35 | 13.44 | 14.05 | 14.02 | 13.71 | |
| | Nogal | 13.84 | 13.92 | 13.92 | 13.03 | 13.67 | |
| | Apache | 12.96 | 13.24 | 14.05 | 13.73 | 13.49 | |
| | X̄ C | 13.48 | 13.67 | 13.88 | 13.71 | | |
| AVERAGE 2018 | | | | | | 13.68 | |
| Variables and interactions | | A | B | C | AB | AC | BC |
| *Prob F* | | 0.33 | 0.01** | 0.00** | 0.76 | 0.07 | 0.00** |
| LSD (P<0.05) | | 0.05 | 0.18 | 0.17 | 0.26 | 0.25 | 0.35 |
| LSD (P<0.01 | | 0.04 | 0.26 | 0.23 | 0.36 | 0.33 | 0.47 |

T3 treatment (13.88%) led to significantly higher grain protein content in comparison to T1 (13.48%), while compared to T2 (13.67%) %) and T4 (13.71%) the differences were significant at the level of r<0.05. The interaction between cultivars and fertilization was statistically significant. The highest protein content was in the Ratarica cultivar (13.85%), which was higher than in the Pobeda cultivar (13.71%), at the level of p<0.05 of statistical significance, while in relation to the Nogal cultivar (13.67%) and the Apache variety (13.49%), the difference was at the level of p<0.01 significance.

In the year 2018/2019, the average protein content was 13.55% (Tab. 4). The highest protein content was found in T3 treatment (14.05%) which was significantly higher (p<0.01) compared to other treatments (T1-13.29%, T2-13.48%, and T4-13.39%). Differences in protein content between treatments T3 (13.39%) and T2 (13.48%) were not statistically significant, while treatment T1 (13.29%) had a statistically significantly lower protein content compared to other treatments.

Tab. 4. Crude protein content (%) of wheat varieties grown under different application rates of nitrogen and effective microorganisms, and sowing rates in the year 2018/2019

| Sowing rate (seeds/m$^2$) (A) | Genotype (B) | Treatments (C) T1 | T2 | T3 | T4 | $\bar{X}$ AB | $\bar{X}$ A |
|---|---|---|---|---|---|---|---|
| 400 | Ratarica | 13.40 | 13.79 | 13.38 | 13.42 | 13.50 | 13.54 |
| | Pobeda | 13.29 | 13.39 | 14.02 | 14.07 | 13.69 | |
| | Nogal | 13.68 | 13.89 | 14.22 | 12.84 | 13.66 | |
| | Apache | 12.73 | 13.31 | 14.13 | 13.12 | 13.32 | |
| | $\bar{X}$ AC | 13.28 | 13.60 | 13.93 | 13.36 | | |
| 500 | Ratarica | 13.35 | 13.60 | 13.27 | 13.54 | 13.44 | 13.53 |
| | Pobeda | 13.07 | 13.36 | 13.96 | 13.85 | 13.56 | |
| | Nogal | 13.90 | 13.60 | 14.09 | 13.16 | 13.69 | |
| | Apache | 13.01 | 13.23 | 14.21 | 13.27 | 13.43 | |
| | $\bar{X}$ AC | 13.33 | 13.44 | 13.88 | 13.45 | $\bar{X}$ B | |
| $\bar{X}$ BC | Ratarica | 13.18 | 13.37 | 13.99 | 13.96 | 13.63 | |
| | Pobeda | 13.79 | 13.74 | 14.16 | 13.00 | 13.67 | |
| | Nogal | 12.87 | 13.27 | 14.17 | 13.19 | 13.37 | |
| | Apache | 13.30 | 13.52 | 13.91 | 13.41 | 13.53 | |
| | $\bar{X}$ C | 13.29 | 13.48 | 14.05 | 13.39 | | |
| AVERAGE 2019 | | | | | | 13.55 | |
| Variables and interactions | | A | B | C | AB | AC | BC |
| *Prob F* | | 0.65 | 0.01** | 0.00** | 0.17 | 0.00 | 0.44** |
| LSD ( p <0.05) | | 2.80 | 0.30 | 0.17 | 0.42 | 0.24 | 0.34 |
| LSD ( p <0.01) | | 1.99 | 0.41 | 0.22 | 0.59 | 0.32 | 0.45 |

The Pobeda variety had the highest protein content (13.67%). There were no statistical differences in relation to the Ratarica cultivar (13.63%), while in relation to the Nogal (13.37%) and Apache (13.53%) cultivars the determined differences were at the level of r<0.01 of statistical significance

Sowing density is considered to be the beginning of the technological process of growing crops, which due to competition for nutrients and water affects the yield and quality of products (Bokan and Malešević, 2004). The difference in protein content between two sowing rates was not statistically significant in any year.

The results obtained are compatible with the research of many authors. According to Muhae-Ud-Din et al. (2018), in wheat production where the seed is inoculated with application of bacterial strain *Bacillus sp*. MN54, fungal strain *Trichoderma sp*. MN6, and their consortium (*Bacillus sp*. MN54 + *Trichoderma sp.* MN6), the effect of consortium application was more prominent. It has been determined that with consortium application of endophytic bacteria and fungus, there was an increase in the yield enhancement through the improvement of various yield attributes like number of spikelets, grains per spike, and grain yield per plant. Cvijanović et al. (2017) showed that application of effective groups of microorganisms in soil and foliar treatment can increase the yield of different wheat genotypes by 3.41%, as well as the total number of microorganisms, the number of azotobacteria, and the activity of dehydrogenase enzymes in wheat root rhizosphere. Roljević et al., (2018) found that the use of a microbiological preparation in organic wheat production can increase the yield of different types of wheat up to 46%. The same authors determined that the application of biofertilizers did not increase total proteins in grain, but increased the content of two high-quality gluten subunits. Wheat has a good relationship with bacteria such as *Azotobacter* and *Azospirillum*, which stimulate the development of lateral roots and root mass by hormonal exchange of auxin (Vejan et al., 2016; Zeffa et al., 2019). According to Del Cortivo et al. (2017), by applying foliar treatment with *Azospirillum+Azoarcus+ Azorhizobium*, an increase in root mass and nitrogen accumulation was determined. Pagnani et al. (2019) found that the application of plant growth bacteria twice during growth increases the yield and protein content and their effect depends on weather conditions.

Based on the presented results, it can be noticed that with the application of effective microorganisms and the amount of mineral nitrogen of 100 kg/ha, a significant protein content in wheat grain can be achieved. The importance of the protein content in wheat grain is evidenced by the fact that the EU has adopted the EC 742/2010 regulation, which prescribes the lowest value of protein content for wheat, durum wheat, barley, and corn used in public procurement. The quality of wheat grains with more than 12% protein is valued in the world market, and the price is 6-10 US dollars higher per ton.

## Conclusion

Wheat grain protein content was significantly influenced by the variety and treatments with mineral nitrogen soil application and foliar application of effective microorganisms. The Ratarica and Pobeda cultivars, which belong to the medium late to late cultivars with a high degree of adaptability, had the highest protein content on average over all treatments. The treatment with 100 kg/ha N and two foliar treatments with efficient microorganisms resulted in significantly higher protein content compared to other treatments. The obtained results imply that the adaptation of winter wheat cropping technology with partial replacement of mineral nitrogen with foliar treatment with EM can be beneficial for quality of winter wheat. The use of effective microorganisms can improve wheat production.

## Confirmation

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# Садржај сирових протеина у зрну озиме пшенице у зависности од ђубрења азотом и фолијарне примјене ефикасних микроорганизама

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## Сажетак

Циљ овог рада био је да се утврди садржај протеина у зрну код сорти пшенице гајених при различитим количинама ефективних микроорганизама и азота, као и сетвеним количинама. Трогодишња истраживања спроведена су на огледном пољу Научног института ПКБ Агроекономик Падинска Скела. Четири сорте пшенице (Ратарица, Победа, Ногал и Апача) од 400 и 500 зрна/м$^2$, и ђубрење са различитим количинама минералног азота Т1 (150 kg/ha); Т2 (kg/ha + једноструки фолијарни третман са вишеструким инокулумом, Т3 (100 kg/ha + двоструки фолијарни третман) и Т4 (50 kg/ha + троструки фолијарни третман). За фолијарно третирање коришћен је препарат (ЕМ Актив 7 l/ha), мешавина вишеструко одабраних микроорганизама. Просечан садржај протеина је 13,95% и статистички значајно зависи од сорте и ђубрења и њихове интеракције $p<0,01$. Сорте Ратарица и Победа су имале највећи садржај протеина у зрну. Третман Т3 је имао највећи утицај на садржај протеина у све три године истраживања, коришћењем ефективних микроорганизама ниво протеина у зрну се може одржати када се смањи количина азота.

*Кључне ријечи:* генотип пшенице, прихрана, микробиолошки препарат, минерални азот, протеини

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