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Possibility of Limiting Mineral Fertilization in Potato Cultivation by Using Bio-fertilizer and Its Influence on Protein Content in Potato Tubers

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Abstract: Potato protein is a valuable source of essential plant-derived amino acids, the composition of which is similar to that of chicken egg protein considering the amino acid reference. Many factors used in potato cultivation can modify its composition. The use of bio-fertilizers in potato growing offers a possibility of a better use of minerals from soil and organic sources and reducing the need for mineral fertilizers by activating minerals present in soil. The effect can be to improve not only the potato tuber yield but also the nutritional value. The aim of this study has been to determine the hanges in the content of crude protein and the composition of amino acids in potato tubers, depending on the application of the bio-fertilizer (UGmax), organic fertilizers (pea as a catch crop, straw, and farmyard manure (FYM)) as well as mineral fertilization (100% and 50% of the reference rate). The application of bio-fertilizer significantly increased the content of essential and non-essential amino acids in potato tuber protein. With the half-decreased mineral fertilization rate, bio-fertilizer most effectively increased the content of tyrosine, methionine, asparagine in potato tuber protein in the treatments with FYM or with a catch crop as well as without organic fertilization.

Keywords: UGmax; crude protein; essential amino acids; non-essential amino acids

1. Introduction

A modern intensive management system, based on the use of fertilizers acquired outside the farm, especially mineral fertilizers and pesticides, to achieve maximum profits, has caused environmental pollution (water pollution, intensification of soil degradation, and simplification of biodiversity). Monoculture, zero tillage, reduced organic fertilization, and the introduction of new cultivars accompanied by increased pesticides also pose a threat, which results in some deterioration of the physical, chemical and microbiological properties of the soil and, as a result, of the quantity and the quality of the crop [1,2]. This is counteracted by the implementation of the concept of sustainable agricultural production, which, however, requires a number of adjustments in terms of organization, especially the basic elements of technology, especially crop rotation, fertilization, and plant protection. In recent years there has been observed much interest in the use of various types of biostimulants, effective microorganisms, preparations, and fertilizers enriching the soil with humus, improving the health and resistance of plants to stress conditions, facilitating the intake of nutrients, and enhancing good-quality crops [3–6]. The use of composting flora can be one of the most important sources of soil regeneration, and such possibilities are provided by the use of UGmax soil fertilizer [7]. The UGmax bio-fertilizer is a microbiological preparation which consists of yeast, lactic acid bacteria-*Lactobacillus* and *Lactococcus; Pseudomonas; Actinobacteria* and *Azotobacter* as well as small amounts of micro-and macronutrients and, in favorable climate conditions, it can accelerate the decomposition of humus, crop residue and organic fertilizers, launch nutrients from minerals or insoluble compounds, restore a better soil structure, improve water relations and affect the growth and quality of potato crops [2,6,8–10].

Potato growing conditions affect the total nitrogen content in the dry matter of tuber, including the number of amino acids [11–13]. As demonstrated by the studies of various authors conducted for many years [13–16], most protein fractions building potato protein show a well-balanced amino acid composition and a high nutritional value, as evidenced by the relevant indicators: CS (Chemical score) in the range of 57–96 [13,17,18] or BV (Biological value) in the range of 45–88 [19]. Chemical indicators, such as CS, allow a simple and quick determination of the quality of the protein tested by comparing its amino acid composition with the composition of the protein adopted as a standard and an indication of a limiting amino acid. In addition to the standard protein amino acid composition modified in 1991 by the FAO/WHO (Food and Agriculture Organization/World Health Organization Expert Committee United Nations University), newer standards are proposed, e.g., developed by WHO/FAO/UNU (United Nations University) in 2007 [20], with fewer amino acids corresponding to current research on human demand for amino acids [21].

Depending on the variety, cultivation, and storage conditions, the amino acids which limit the nutritional value of potato protein can include leucine, isoleucine [17], methionine, cysteine, and less often threonine and lysine [17,22] and valine [12]. Potato protein is particularly rich in amino acids such as glutamic and aspartic acid accounting for 30-50% of all the amino acids in tubers. Leucine, valine, alanine, lysine, and arginine are present in smaller but significant amounts (4–8%), while methionine and histidine constitute, according to Danilchenko et al. [11], a low share in potato protein. According to Eppendorfer and Eggum [19], the content of exogenous amino acids in potato tubers is negatively correlated with the level of fertilization, and the changes in the share of exogenous amino acids in the sum of all the amino acids primarily due to changes in non-protein nitrogen fractions, especially free amino acids and amides, which make up about 40–60% of the total protein in tubers. According to Eppendorfer and Eggum [19], a sulphur deficiency in soil contributes to a decrease in the content of sulphur amino acids, cysteine and methionine in potato protein and it triggers deviations from the constant dependence between the content of nitrogen and amino acids, which can be due to the effect of this component on nitrogen metabolism and protein amino acid composition. Thus, a reduction in nitrogen fertilization for organic potato cultivation can contribute to an increase in the share of protein nitrogen and exogenous amino acids in the total protein.

2. Materials and Methods

2.1. Experimental Location and Treatment

Potato tubers of the medium-early potato (*Solanum tuberosum* L.) cultivar, 'Satina N', were obtained from the field experiment as a three-factor split-split-plot design at the Experiment Station of the UTP University of Science and Technology in Mochełek (53°13′ N, 17°51′ E). Cereals constituted the catch crop. Potatoes were mechanically planted at 0.75 × 0.35 m row spacing. The single plot size was 31.5 m^2. The experiment treatments are shown in Table 1. All the mineral fertilizers were applied in spring prior to potato planting (every research year), at the rates considering the soil richness (a high phosphors content and a medium low content of potassium) and the nutrition requirements of the plants. The condition of the soil was measured every year. The chemical composition of soil before the start of the field experiment in the years analyzed was similar; it demonstrated a very low richness in available forms of magnesium and also a low amount of available nitrogen. The soil with slightly acid reaction is presented in Table 2. During cultivation the following potato protection agents were applied; herbicide: linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, Agan Chemical Manufactures Ltd., Israel-Afalon 50WP; 2 dm^3 ha^{-1}); insecticide: chlorpyrifos and cypermethrin (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate and (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, respectively, Agriphar

S.A., Belgium-Nurelle D 550 EC; 0.6 dm^3 ha^{-1}) as well as fungicides: cymoksanil and mancozeb (2-cyano-*N*-[(ethylamino) carbonyl]-2 (methoxyimino) acetamide and zinc complex of ethylene-bis-dithiocarbamate manganese, Helm AG, Germany-Helm-Cymi 72,5 WP; 2 kg ha^{-1}), mefenoxan and mancozeb (methyl *N*-(methoxyacetyl)-*N*-2,6-xylyl-D-alaninate and zinc complex of ethylene-bis-dithiocarbamate manganese, Syngenta Crop Protection AG, Switzerland-Ridomil Gold MZ 67,8 WG; 2 dm^3 ha^{-1}). The UGmax bio-fertilizer is a microbiological preparation which consists of yeast, *Lactobacillus* and *Lactococcus*, also *Pseudomonas; Actinobacteria* and *Azotobacter* as well as potassium 3.5 g dm^{-3}, nitrogen 1.2 g dm^{-3}, sulphur 1.0 g dm^{-3}, phosphorus 0.5 g dm^{-3}, sodium 0.2 g dm^{-3}, magnesium 0.1 g dm^{-3}, zinc 0.02 g dm^{-3} and manganese 0.003 g dm^{-3}.

Table 1. Experimental treatments.

	Type of Organic Sources (1st Factor)
Control	without organic sources
Catch crop (fodder pea)	27 t ha^{-1}, in autumn, after post-harvest treatments and before pre-winter plough
Straw	4 t ha^{-1}, in autumn, after post-harvest treatments and before pre-winter plough
Farmyard manure (FYM)	25 t ha^{-1}, in autumn, before pre-winter plough
	Rate of Mineral Fertilization (2nd Factor) (Ammonium Nitrate-34%, Triple Superphosphate-46%, Potassium Sulphate-50%)
100% NPK	100 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}
50% NPK	50 kg N ha^{-1}, 50 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}
	applied in spring, prior to potato planting, at the rates adjusted to the soil richness and nutritive requirements of the plant
	Bio-Fertilizer Application (3rd Factor)
Control	Without treatment
Bio-fertilizer	UGmax fertilizer applied at three rates: 0.6 l ha^{-1} in autumn, on organic sources, prior to pre-winter plough; 0.3 l ha^{-1} in spring, prior to tuber planting, during soil tillage; 0.3 l ha^{-1}1 as foliar fertilization, at the plant height of 15–20 cm

Table 2. Chemical properties of soil at the experimental site before experiment.

Parameters	**Unit**	**Value**	**Categories**
pH H_2O	-	5.1–6.7	Slightly acid
pH KCl	-	5.7–6.1	
Organic carbon	g kg^{-1}	7.55–7.80	-
Total nitrogen	g kg^{-1}	0.69–0.75	Low richness
Phosphorus	mg kg^{-1}	190.0–210.0	High richness
Potassium	mg kg^{-1}	95.0–150.0	Medium richness
Magnesium	mg kg^{-1}	<20.0	Very low richness

As organic matter, each research year, under winter plough, FYM was applied at the rate of 25 t/ha, straw–4 t/ha and pea (stubble intercrop) at the rate of 27 t/ha. The bio-fertilizer was applied each year at two rates applied into soil: 0.6 l/ha in autumn–prior to the winter plough, onto organic matter and 0.3 l/ha in spring, prior to tuber planting. Additionally, 0.3 l/ha was provided as foliar application at the plant height of 15–20 cm (Table 1). The chemical composition of soil prior to the field experiment was analyzed in each research year and it was similar. It showed a very low content of available forms of potassium and magnesium as well as a low amount of available phosphorus in soil (Table 2). The soil reaction was slightly acid. The chemical composition of organic sources and the total weight of macroelements applied by organic sources is presented in Table 3.

Table 3. Chemical composition of organic sources and the total weight of macroelements applied by organic sources.

Component	Organic Source [kg·t^{-1}]			Organic Source [t·ha^{-1}]		
	Catch Crop	Straw	Farmyard Manure	Catch Crop	Straw	Farmyard Manure
N	35.10	5.62	5.12	488.7	151.74	138.24
P_2O_5	4.34	1.06	4.48	90.18	28.62	120.96
K_2O	25.15	10.21	6.85	247.05	275.67	184.95
CaO	2.74	0.84	4.42	46.98	22.68	119.34
Mg	7.25	2.25	1.82	141.75	33.75	49.14

To determine the periods of drought and semi-drought, the Sielianinov hydrothermal coefficient was used (Table 4). It was determined by dividing the total precipitation by the total temperature of a given month decreased ten-fold.

Table 4. Weather conditions during the plant growing season at the Mochełek Experiment Station.

Years	Rainfall						Total
	Month						
	April	May	June	July	August	September	
2009	0.4	12.4	53.4	118.0	17.6	34.4	236.1
2010	33.8	92.6	18.1	107.4	150.7	74.7	477.3
2011	13.5	35.4	100.8	132.5	67.7	37.0	386.9
Average	15.9	46.8	57.4	119.3	78.7	48.7	366.8
	Temperature						
2009	9.8	12.3	14.5	18.6	18.2	13.7	87.1
2010	7.8	11.5	16.7	21.6	18.4	12.2	88.2
2011	10.5	13.5	17.7	17.5	17.7	14.3	91.2
Average	9.4	12.4	16.3	19.2	18.1	13.4	88.8
	Hydrothermal Coefficient K						Average
2009	0.01	0.32	1.23	2.05	0.61	0.84	0.84
2010	1.44	2.60	0.36	1.60	2.64	2.04	1.78
2011	0.43	0.85	1.90	2.44	1.23	0.86	1.29
Average	0.63	1.25	1.16	2.03	1.50	1.25	1.30

K: 0–0.5 drought; 0.6–1.0 mild-drought; 1.1–2.0 moist, optimal conditions; 2.1-humid.

K–hydrothermal coefficient value,
P–total monthly precipitation,
t–total mean daily temperature in a given month.

Value K falling in the range from 0 to 0.5 stands for drought, from 0.6 to 1.0–semi-drought, and the value above 1.0–for humid conditions.

The meteorological data were collated from the records of the Weather Station at the Experiment Station of the UTP University of Science and Technology in Mochełek (53°13′ N, 17°51′ E). The 2009 season showed drought periods during the early plant growth, much moisture afterwards and a spell of semi-dry weather at the end of the growing season. During the 2010 and 2011 growing seasons, it was quite humid, thus optimal for potato development and growth.

2.2. Plant Sampling and Chemical Analysis

Right after harvest, potato tubers were transferred to the storage chamber at temperature of 10 °C and 80% relative air humidity, and then a representative sample was prepared for analysis. The tubers were cut and frozen in liquid nitrogen and stored at the temperature of at least-18 °C for further studies. The frozen material was lyophilized (model Alpha 1–4 LDplus, Donserv, Warszawa, Poland), and then it was ground until fine powder (the particles 0.3–0.5 mm in size) was produced using the ultracentrifuge Retsch mill ZM 100 (Retsch, Haan, Germany). The ground samples were stored in the dark, in the bags which were placed in desiccators for further analysis.

The laboratory analyses (nitrogen content, and free amino acids) were carried out at the Department of Quality Plant Products in the Georg August University in Göttingen. The content of crude protein was determined by multiplying the total nitrogen by factor 6.25, with the Kjeldahl method using Büchi Labortechnik B-324 apparatus, after mineralization in concentrated sulphuric acid (VI). The total nitrogen was analyzed following the Dumas combustion method by LECO CN-2000 [23]. Free amino acids were extracted from freeze-dried potato flour using hydrochloric acid. as described by Fischer et al. [24]. 0.5 g of freeze-dried potato flour was suspended in a centrifuge tube with 4 mL 1 N hydrochloric acid. The tube was horizontally shaken for one hour and centrifuged at 15,000 relative centrifugal force (RCF) for 30 min. The supernatant was collected in a 10 mL volumetric flask. The extraction was performed three times, with the last two extractions—with 3 mL of hydrochloric acid. Finally, the hydrochloric acid was added to the flask to adjust the final 10 mL. To get a clear solution, the supernatant was centrifuged again at 15,000 RFC for 30 min.

Free amino acids were assayed using HPLC. The HPLC system consists of degasser WellChrom K-5004 (Knauer, Berlin, Germany), multisolvent delivery system 600 E (Waters, Milford, MA, USA), autosampler 2157 (Parmacia LKB, Sweden), the temperature control module (Waters, USA), 5 µm column LiChroCart 250-3 and pre-column LiChroCart 4-4 (Merc, Darmstadt, Germany), fluorescens detector 474 (Waters, USA). The gradient eluents, which consisted of methanol (71/29 and 20/80, v/v) in 50 mM sodium acetate buffer (pH 7.0), were used to separate the amino acids at the flow rate of 0.6 mL min^{-1} and the temperature of 45 °C. The amino acids were standardized using the Perbio Amino Acid Standard H (USA). The HPLC separation of fluorescent o-phtaldialdehyde (OPA) derivatives was applied to the assay of free amino acids according to the method described by Fischer et al. [24]. The procedure was based on the reaction of reducing agent β-mercaptoethanol, to give a complex which can be measured by fluorescence. The OPA solution was prepared by dissolving 125 mg of OPA in 22 mL of methanol, then mixing it with 500 µL of β-mercaptoethanol and 2.5 mL 0.5 M borate buffer at pH 9.5. The extract of amino acids was derived with the fluorescens reagent (o-phtaldialdehyde) for two minutes. The microliters of the mixture were injected into the HPLC.

The Amino Acid Score (AAS) was calculated using the following equation:

$$\mathrm{AAS} = \frac{a_p}{a_s}$$

where a is an essential amino acid (EAA), p is the test protein, *s* is the reference protein. The reference protein used was the FAO/WHO amino acid scoring pattern from the 2007 WHO/FAO report [20].

2.3. Statistical Analysis

A three-way ANOVA analysis was performed to reveal the significance of established effects of mineral fertilization, organic fertilizers and bio-fertilizer and their interactions. The tables and Figure 1 show the mean values of crude protein (CP) and amino acids for three years of research and standard deviations of the mean of 3 replications. The results are presented as mean values for three years of study plus standard deviation. All the data were reported as the means with standard errors of the mean (s.e.m.). The significance of differences (LSD–Lowest Significance Difference) was evaluated using the Tukey multiple confidence intervals for the significance level of α = 0.05 and 0.01. The multifactorial ANOVA was computed using Statistica® 13.1 package.

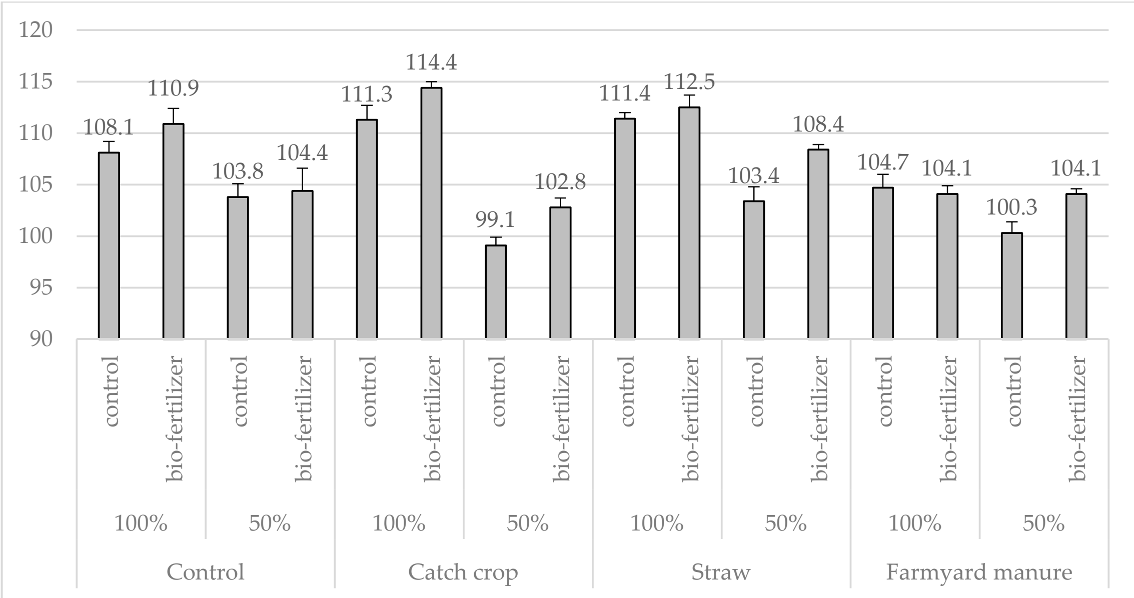

Figure 1. Content of crude protein in potato tubers as affected by organic, mineral, and bio fertilization, mean for three years of study (g·kg^{-1} of dry weight). 100%, 50%-rate of mineral fertilization with NPKLSD (Tukey-test at p $P \leq 0.05$) Organic sources = 3.595; mineral fertilizers = 1.282; Bio-fertilizers = 0.553.

3. Results

3.1. Crude Protein Content in Potato Tubers

Based on three years of research, the content of crude protein in the dry matter of edible potato tubers of the 'Satina' cultivar was determined. In our study the crude protein in tubers ranged between 99.1 and 114.4 g kg^{-1} DM (Figure 1). The effect of 100% mineral fertilization and the organic sources increased significantly the crude protein content in potato tubers. There are also positive interactions between all the factors. The highest content of crude protein in potato tubers was found after a combined application of green fertilizer (fodder pea) with a full rate of nitrogen, phosphorus and potassium (NPK) and bio-fertilizer used. A comparison of the content of crude protein in the tubers from the plots where half the rate of mineral fertilization was applied, the use of bio-fertilizer increased that component, especially after applying straw.

3.2. Essential and Non-Essential Amino Acids Content in Potato Tubers

The effect of combined organic matter, mineral fertilization, and UGmax bio-fertilizer applied on the content of essential and non-essential amino acids (Tables 5 and 6, respectively) was significant ($P \leq 0.01$), excluding mineral fertilization (significant $P \leq 0.05$) for non-essential amino acids and non-significant for essential amino acids. The content of eight essential and four non-essential amino acids identified in the potato tubers studied from the 3-year planting season are presented in Tables 7 and 8, respectively. Total free amino acids ranged between 18.00 and 64.65 g 100 g^{-1} of crude protein. The effect of mineral fertilization on methionine, valine, leucine, isoleucine, phenylalanine and glutamic acid was non-significant. The mineral fertilizer combined with organic matter with bio-fertilizer and the interaction between the three study factors affected significantly ($P \leq 0.01$) the content of tyrosine, valine, isoleucine, leucine, lysine, and phenyloalanine and significantly ($P \leq 0.05$) threonine and methionine in the crude protein content. The potato tubers derived from the experimental plot with the use of farmyard manure, 50% of the full rate of NPK and bio-fertilizer contained most tyrosine, with straw and 100% rate of NPK and bio-fertilizer; mostly threonine and lysine, in turn, 50% rate of NPK-mostly phenylalanine, with catch crop (pea) and 100% rate of NPK and bio-fertilizer–mostly valine, isoleucine, leucine. Organic fertilization increased the essential amino acids content, especially green manure (pea). A similar effect was produced after the use of bio-fertilizer. The results of the application of 100 and 50% rate of NPK were not significant for the content of methionine, valine, isoleucine, leucine, phenylalanine and a slightly significant full mineral rate increased the content of tyrosine, threonine and lysine in the protein content (Table 7). On average, for the other factors, the organic matter and bio-fertilizer resulted in a significant increase in the essential amino acid content. In turn, organic fertilization combined with bio-fertilizer had no significant influence on the aspartic acid content. Moreover, 100% rate of mineral fertilization combined with bio-fertilizer gave a non-significant result of glutamic acid content in potato protein (Table 6). The application of straw and bio-fertilizer and full rate of NPK increased aspartic acid and the alanine content (Table 8). A similar effect was found on the content of asparagine when no organic sources were applied. The highest asparagine content in crude protein of potato tubers was observed when 50% NPK rate was used, without organic fertilization and bio-fertilizer application. Green fertilizer (pea), 100% NPK mineral fertilization and bio-fertilizer increased the content of glutamic acid in potato protein (Table 8). On average for the other factors, organic fertilization and the application of bio-fertilizer resulted in the highest increase in the content of non-essential amino acids. The ASS of the potato tubers as affected by organic, mineral and bio fertilization studied. WHO/FAO adult maintenance pattern expressed as g amino acid/100 g protein shown in Figure 2. The content of leucine and valine was higher than recommended by the WHO/FAO, especially under the influence of green fertilizer and straw.

Table 5. Essential amino acids content. Significance of experimental factors and their interaction, mean for three years of study.

Essential Amino Acids	Organic Sources	Mineral Fertilizers	Bio-fertilizer	Organic Sources × Mineral Fertilizers	Organic Sources × Bio-Fertilizer	Mineral Fertilizers × Bio-Fertilizer	Organic Sources × Mineral Fertilizers × Bio-Fertilizer
Tyrosine	*	*	*	*	*	*	**
Threonine	*	*	*	**	**	*	*
Methionine	**	n.s.	**	*	*	**	*
Valine	**	n.s.	**	**	**	**	**
Isoleucine	**	n.s.	**	**	**	**	**
Leucine	*	n.s.	**	**	**	**	**
Lysine	**	**	**	**	**	**	**
Phenylalanine	**	n.s.	*	**	**	**	**
Sum	**	n.s.	**	**	**	**	**

The multifactorial ANOVA was used in order to test the single effects of organic sources, mineral fertilization, and bio-fertilizer as well as for the interaction. * significant difference $P \leq 0.05$; ** significant difference $P \leq 0.01$; n.s.-non-significant difference.

Table 6. Non-essential amino acids content. Significance of experimental factors and their interaction, the 2009-2011 mean.

Non-Essential Amino Acids	Organic Sources	Mineral Fertilizers	Bio-Fertilizer	Organic Sources × Mineral Fertilizers	Organic Sources × Bio-Fertilizer	Mineral Fertilizers × Bio-Fertilizer	Organic Sources × Mineral Fertilizers × Bio-Fertilizer
Aspartic acid	**	**	**	**	n.s.	**	**
Asparagine	**	**	**	**	**	**	**
Alanine	**	**	**	**	**	**	**
Glutamic acid	**	n.s.	**	*	*	n.s.	**
Sum	**	*	**	**	**	**	**

The multifactorial ANOVA was used to test the single effects of organic sources, mineral fertilization, and bio-fertilizer as well as the interaction. * significant difference $P \leq 0.05$; ** significant difference $P \leq 0.01$; n.s.–non-significant difference.

Table 7. Essential amino acids content (g 100 g^{-1} protein) in potato tubers. Mean for three years of study.

Organic Fertilization	Mineral NPK Fertilization	Bio-Fertilizer	Tyrosine	Threonine	Methionine	Valine	Isoleucine	Leucine	Lysine	Phenyl-Alanine	Sum
Control (without organic source)	100%	Control	0.64 ± 0.01	1.40 ± 0.08	2.19 ± 0.11	2.40 ± 0.14	1.84 ± 0.05	0.52 ± 0.04	0.17 ± 0.01	1.66 ± 0.04	10.82
		Bio-fertilizer	0.71 ± 0.02	1.32 ± 0.02	2.34 ± 0.05	2.54 ± 0.08	1.95 ± 0.11	1.72 ± 0.02	0.64 ± 0.0	1.39 ± 0.01	12.61
	50%	Control	0.83 ± 0.01	1.26 ± 0.01	2.09 ± 0.08	2.30 ± 0.01	1.57 ± 0.08	2.27 ± 0.02	0.09 ± 0.01	1.65 ± 0.01	12.06
		Bio-fertilizer	0.70 ± 0.01	1.28 ± 0.04	2.29 ± 0.03	2.31 ± 0.01	1.67 ± 0.05	2.31 ± 0.03	0.11 ± 0.03	1.26 ± 0.01	11.93
Catch crop (pea)	100%	Control	1.36 ± 0.02	2.49 ± 0.03	2.67 ± 0.07	3.89 ± 0.05	2.94 ± 0.04	4.67 ± 0.11	0.22 ± 0.02	2.39 ± 0.01	20.63
		Bio-fertilizer	1.06 ± 0.01	3.87 ± 0.11	3.05 ± 0.04	4.06 ± 0.04	3.41 ± 0.06	4.97 ± 0.08	0.89 ± 0.01	2.25 ± 0.02	23.56
	50%	Control	1.12 ± 0.05	2.21 ± 0.05	2.58 ± 0.05	3.72 ± 0.02	3.50 ± 0.07	3.92 ± 0.07	0.23 ± 0.01	2.25 ± 0.01	19.53
		Bio-fertilizer	1.35 ± 0.03	3.43 ± 0.08	2.96 ± 0.08	3.78 ± 0.01	3.57 ± 0.04	4.59 ± 0.02	0.44 ± 0.01	2.12 ± 0.01	22.24
Straw	100%	Control	1.18 ± 0.04	2.69 ± 0.02	2.34 ± 0.05	2.89 ± 0.03	3.03 ± 0.08	4.82 ± 0.03	0.31 ± 0.00	2.45 ± 0.01	19.71
		Bio-fertilizer	1.62 ± 0.01	4.52 ± 0.05	2.38 ± 0.03	2.94 ± 0.11	3.98 ± 0.11	3.45 ± 0.05	0.99 ± 0.01	2.51 ± 0.02	24.79
	50%	Control	0.91 ± 0.01	1.57 ± 0.02	2.28 ± 0.01	2.55 ± 0.08	2.15 ± 0.04	3.07 ± 0.02	0.28 ± 0.00	1.69 ± 0.01	14.50
		Bio-fertilizer	0.93 ± 0.01	3.06 ± 0.04	2.35 ± 0.05	2.98 ± 0.04	3.82 ± 0.03	3.97 ± 0.07	0.84 ± 0.00	1.78 ± 0.01	19.73
Farmyard manure (FYM)	100%	Control	1.60 ± 0.05	1.51 ± 0.02	1.98 ± 0.04	3.15 ± 0.09	2.67 ± 0.02	3.56 ± 0.01	0.14 ± 0.00	1.89 ± 0.00	16.50
		Bio-fertilizer	1.37 ± 0.04	1.16 ± 0.03	2.06 ± 0.06	3.51 ± 0.02	3.39 ± 0.05	3.30 ± 0.05	0.05 ± 0.00	2.34 ± 0.01	17.18
	50%	Control	1.04 ± 0.01	1.09 ± 0.02	1.86 ± 0.02	3.11 ± 0.03	2.39 ± 0.04	3.12 ± 0.04	0.02 ± 0.00	1.45 ± 0.00	14.08
		Bio-fertilizer	1.53 ± 0.04	2.57 ± 0.01	1.98 ± 0.03	3.47 ± 0.04	3.23 ± 0.03	3.24 ± 0.07	0.28 ± 0.00	2.11 ± 0.01	18.41
	Mean		1.12	2.21	2.34	3.10	2.82	3.34	0.36	1.94	17.24
Organic sources	Control		0.72 ^{c}	1.32 ^{b}	2.23 ^{c}	2.39 ^{d}	1.76 ^{c}	1.71 ^{d}	0.25 ^{c}	1.49 ^{c}	11.86 ^{d}
	Catch crop		1.22 ^{b}	3.00 ^{a}	2.82 ^{a}	3.86 ^{a}	3.36 ^{a}	4.54 ^{a}	0.44 ^{b}	2.25 ^{a}	21.49 ^{a}
	Straw		1.16 ^{ab}	2.96 ^{a}	2.34 ^{b}	2.84 ^{c}	3.25 ^{ab}	3.83 ^{b}	0.61 ^{a}	2.11 ^{ab}	19.08 ^{b}
	FYM		1.39 ^{a}	1.58 ^{bc}	1.97 ^{d}	3.31 ^{b}	2.92 ^{b}	3.31 ^{c}	0.12 ^{d}	1.95 ^{bc}	16.54 ^{c}
Mineral fertilization	100% NPK fertilization		1.13 ^{a}	2.26 ^{a}	2.36 ^{a}	3.09 ^{a}	2.78 ^{a}	3.06 ^{a}	0.40 ^{a}	2.05 ^{a}	17.14 ^{a}
	50% NPK fertilization		1.05 ^{b}	2.06 ^{b}	2.30 ^{a}	3.03 ^{a}	2.74 ^{a}	3.31 ^{a}	0.29 ^{b}	1.79 ^{b}	16.56 ^{b}
Bio-fertilizer (Bio-F)	Control		1.09 ^{b}	1.78 ^{b}	2.25 ^{b}	3.00 ^{b}	2.51 ^{b}	3.24 ^{b}	0.18 ^{b}	1.93 ^{b}	15.98 ^{b}
	Bio-fertilizer		1.16 ^{a}	2.65 ^{a}	2.43 ^{a}	3.20 ^{a}	3.13 ^{a}	3.44 ^{a}	0.53 ^{a}	1.97 ^{a}	18.51 ^{a}

Different letters following the mean values indicate significant differences with the Tukey test at $p \leq 0.05$ for a given feature for organic source, mineral fertilization and bio-fertilizer.

Table 8. Non-essential amino acids content (g 100 g^{-1} protein) in potato tubers. Mean for three years of study.

Organic Fertilization	Mineral NPK Fertilization	Bio-Fertilizer	Aspartic Acid	Asparagine	Alanine	Glutamic Acid	Sum
Control (without organic fertilizer)	100%	Control	6.37 ± 0.05	4.92 ± 0.04	0.22 ± 0.00	4.99 ± 0.02	16.50
		Bio-fertilizer	7.68 ± 0.07	5.83 ± 0.05	1.61 ± 0.04	6.72 ± 0.04	21.84
	50%	Control	4.47 ± 0.04	3.91 ± 0.02	0.22 ± 0.01	4.29 ± 0.05	12.89
		Bio-fertilizer	6.95 ± 0.08	7.07 ± 0.07	0.22 ± 0.00	5.95 ± 0.01	20.19
Catch crop (pea)	100%	Control	7.51 ± 0.11	4.56 ± 0.04	1.22 ± 0.01	7.56 ± 0.08	25.85
		Bio-fertilizer	8.95 ± 0.12	4.86 ± 0.05	1.24 ± 0.01	8.48 ± 0.11	28.53
	50%	Control	4.64 ± 0.04	2.24 ± 0.02	1.02 ± 0.01	7.10 ± 0.04	15.00
		Bio-fertilizer	6.62 ± 0.08	4.05 ± 0.03	1.15 ± 0.02	8.14 ± 0.03	19.96
Straw	100%	Control	6.06 ± 0.05	4.21 ± 0.05	0.27 ± 0.00	5.01 ± 0.05	15.55
		Bio-fertilizer	10.35 ± 0.14	4.97 ± 0.01	2.48 ± 0.02	8.40 ± 0.06	26.20
	50%	Control	5.86 ± 0.12	4.20 ± 0.02	0.31 ± 0.01	4.80 ± 0.05	15.17
		Bio-fertilizer	8.57 ± 0.09	1.76 ± 0.01	0.67 ± 0.01	5.81 ± 0.02	16.81
Farmyard manure (FYM)	100%	Control	4.71 ± 0.08	3.53 ± 0.02	0.22 ± 0.00	2.88 ± 0.01	11.34
		Bio-fertilizer	6.12 ± 0.11	3.37 ± 0.01	0.31 ± 0.00	5.31 ± 0.05	15.11
	50%	Control	4.12 ± 0.05	2.36 ± 0.04	0.21 ± 0.00	2.31 ± 0.04	9.00
		Bio-fertilizer	8.28 ± 0.06	1.89 ± 0.02	0.58 ± 0.01	7.50 ± 0.05	18.25
	Mean		6.70	3.98	0.75	5.95	17.39
Organic sources	Control		6.37 [c]	5.43 [a]	0.57 [c]	5.49 [c]	17.86 [c]
	Catch crop		6.93 [b]	3.93 [b]	1.16 [a]	7.82 [a]	19.84 [a]
	Straw		7.71 [a]	3.79 [b]	0.93 [b]	6.01 [b]	18.43 [b]
	FYM		5.81 [d]	2.79 [c]	0.33 [d]	4.50 [d]	13.43 [d]
Mineral fertilization	100% NPK fertilization		7.12 [a]	4.57 [a]	0.87 [a]	6.04 [a]	20.12 [a]
	50% NPK fertilization		6.19 [b]	3.43 [b]	0.55 [b]	5.74 [a]	17.66 [b]
Bio-fertilizer	Control		5.47 [b]	3.74 [b]	0.46 [b]	4.87 [b]	14.54 [b]
	Bio-fertilizer		7.94 [a]	4.23 [a]	1.03 [a]	7.08 [a]	20.24 [a]

Different letters following the mean values indicate significant differences with the Tukey test at $p \leq 0.05$ for a given feature for organic source, mineral fertilization and bio-fertilizer.

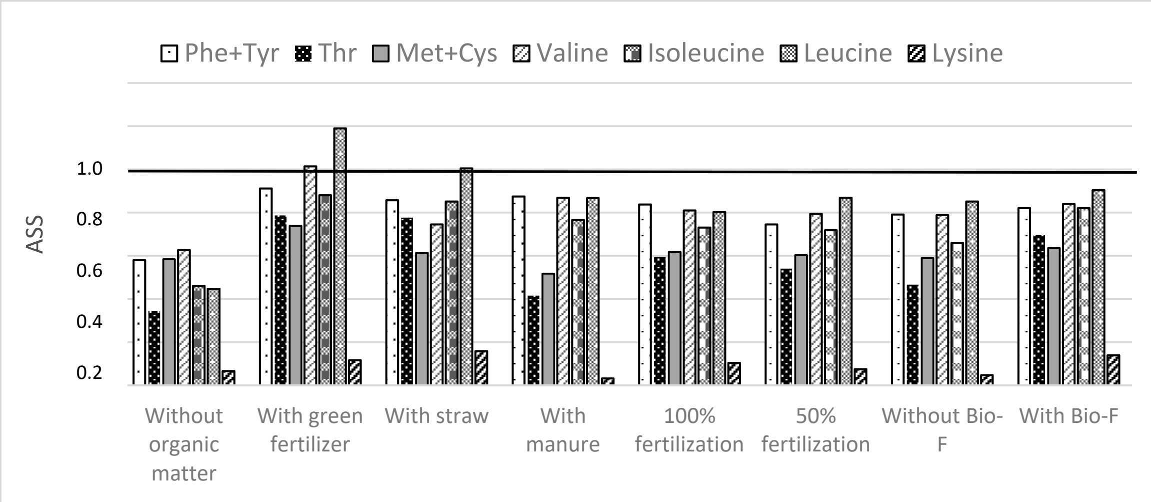

Figure 2. The ASS of the potato tubers as affected by organic, mineral and bio fertilization studied. WHO/FAO adult maintenance pattern expressed as g amino acid/100 g protein: Phe +Tyr, 3.8; Met + Cys, 2.7; Lys, 4.5; Thr, 2.3; Leu, 5.9; Val, 3.9; Ile, 3.0; His, 1.5 (WHO/FAO, 2007).

4. Discussion

The content of organic matter is the basic indicator of the soil quality and fertility and the key factor of the adequate growth, development, and efficiency of plants [25–27]. The UGmax bio-fertilizer, one of the products applied in crop cultivation, is a natural liquid concentrate, including beneficial microorganisms, including the bacteria of lactic acid, photosynthetic bacteria, Azotobacter, Pseudomonas, yeast, Actinobacteria, and some macro-and microelements [7]. However, the results of research on the effect of that product are sometimes ambiguous. A few authors did not confirm a positive effect of UGmax [28–30], while others confirmed a favorable effect of that conditioner [31,32]. Piotrowska et al. [33,34] found that the application of Ugmax increased the soil pH, the content of organic carbon (Corg), the total content of nitrogen, and the enzymatic activity of soil as compared with the control. Whereas, Dębska et al. [35] recorded an increase in the content of organic carbon in soil and in organic matter, including humins and humic acids. Many authors applied UGmax and noted a tendency of growing yields or a favorable effect on the chemical composition of the yield and an increased resistance of the plant to diseases. Sulewska et al. [31] report on the yield of maize grown for grain and silage increasing by 0.71 and 5.6 $t \cdot ha^{-1}$, respectively, as compared with the control. Kotwica et al. [36] noted an increase in the biomass of winter wheat and grain yield, while Górski et al. [37] point to an increased content of sugar in the sugar beet roots and a higher root efficiency. Kołodziejczyk et al. [38] report on an increase in the spring wheat grain yield. Zarzecka et al. [39,40] researching UGmax noted an increase in the amount of nitrogen and magnesium as well as in the content of starch and vitamin C in potato tubers, as compared to the control.

It is well known that protein, one of the most vital human food macronutrients, is involved in various physiological functions. The protein content in potato FM is low (2–2.5%). However, its nutritional value results from the beneficial composition of amino acids, especially the essential ones, which cannot be synthesized by the body [10,41] and, as such, they should be supplied with food. Potato protein has a high biological value; the highest one of all the plant proteins [42,43]. As one of the few plant proteins, it has a biological value corresponding to animal protein [44]. It is comparable with soybean protein and only slightly decreased in egg white [44–47]. Of the eight amino acids, people must ingest, seven are found in potato protein: leucine, lysine, isoleucine, and phenylalanine, threonine, methionine and valine [46,47]. Tyrosine does not need to be supplied with food if it contains enough phenylalanine it is made of [20]. It is included in the WHO recommendations as supplied externally (Protein standard WHO/FAO/UNU 2007). According to the American Association for Potato Research, potato has been recognized as food of excellent quality protein, gluten-free, with a number of possibilities of use. The main potato benefits are the lack of allergens, a good texture and a good nutrition value [47–50]. The daily protein requirements for an adult is 0.8 to 1.0 g per kilogram of the body weight, e.g., one with the body weight of 70 kg should consume 70 g of protein daily (including 35 g of animal and 35 g of plant protein).

In the present research, the average total protein content in the dry weight of potato tubers ranged from 99.1 to 114.4 g kg^{-1} of dry weight. In the study reported by Wierzbicka & Trawczyński [51], the protein content ranged from 75.4 to 113.7 g kg^{-1} of dry weight, whereas in the study by Mystkowska [52] it was higher, and it ranged from 129.8 g kg^{-1} to 138.2 g kg^{-1}. The differences in the protein content result from cultivar-specific properties; the capacity for accumulating that nutrient [51–57]. According to Baranowska et al. [58], biostimulants have a significant impact on the protein content, resulting in an increase in its content. A similar positive effect on crude protein content was recorded in the present research following the use of bio-fertilizer, increasing it in potato tubers. Stankiewicz et al. [12] found that changes in protein and essential amino acid contents as well as biological value of protein were mainly cultivar-specific and affected by nitrogen fertilization and weather conditions during potato growth. In the present research, both the full rate of mineral fertilization, i.e., 100 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}, and the bio-fertilizer applied significantly increased the content of crude protein in potato tubers of 'Satina' cultivar. The research reported by Stankiewicz et al. [12] and Mitrus et al. [17] demonstrated that the protein content in potato tubers increased significantly due to nitrogen fertilization. According to Różyło and Pałys [59], potato tubers collected from combined organic and mineral fertilization contained significantly more total protein than the tubers from the plots with mineral fertilization only. In the variant with mineral-organic fertilization, the amount of nitrogen was, on average, 14.9 g kg^{-1}, with organic fertilization only–14.1 g kg^{-1} and without fertilization–13.5 g kg^{-1}. The treatments with organic fertilization at the recommended mineral fertilization rates increased the content of nitrogen, and thus protein, by 20% on average. In the studies reported by other authors [59–61], each form of fertilization resulted in a significant increase in the content of nitrogen in tubers. In addition, according to Różyło and Pałys [59], the application of effective microorganisms has contributed to an increase in the content of nitrogen in tubers by an average of 1.2 g kg^{-1}. As demonstrated in our own research, the use of bio-fertilizer enhanced not only the protein content but also the composition of amino acids, increasing the content of essential amino acids, also with half the rate of mineral fertilization combined with FYM. The use of UGmax in crops can limit the application of mineral fertilizers by facilitating the launch of elements found in soil. The UGmax microelements, enriched with a starter medium, process (compost) crop residue, straw, manure, catch crops (organic fertilizers) and, together with soil minerals, they form humus, the natural habitat of soil life and the storage of nutrients [62]. Besides, according to Piotrowska-Długosz and Wilczewski [63–65], catch crops cultivated for green manure play an important role in improving soil quality by maintaining organic matter content in soil and returning nutrients to the soil. In this study, the most positive effect in terms of the quantity and quality of protein in potato tubers was recorded for the plots where green fertilizer (fodder pea) was involved.

The main free amino acids in potato research when considering the essential amino acids were methionine, phenylalanine, isoleucine, threonine, valine, leucine, tyrosine and lysine, while when it comes to non-essential amino acids–mostly glutamic acid and, sequentially, aspartic acid, asparagine, and alanine, respectively. Many authors [66,67] report on a high metabolic activity and a great importance of microorganisms for most processes occurring in the soil environment, with the decomposition and mineralization of organic matter (crop residue, manure and other natural fertilizers, composts, catch crops) being most important. Similar results were recorded in our research when the use of organic sources and bio-fertilizer resulted in a significant increase in essential and non-essential amino acids.

AAS for essential amino acids (Figure 2) exceeded 100% in potato tubers from plots with the use of green fertilizers and straw, especially leucine and valine, the content of that amino acids was higher than recommended by the WHO/FAO. Leucine and valine, in addition to their constituent function, also play a regulatory role in the effect on the secretion of hormones and catecholamines, the formation of neurotransmitters, and stimulation of protein synthesis. They can be a source of energy, influence wellbeing, reduce physical fatigue. They are a substrate for the synthesis of other amino acids and are an important factor in the treatment of certain diseases, as obesity [68].

5. Conclusions

This article has analyzed the content of CP and the profile of amino acids in 'Satina' potato tubers from a 3-year field experiment where a full and half-decreased mineral fertilization, organic sources (pea, straw, and FYM) and UGmax bio-fertilizer were applied. The application of the bio-fertilizer increased the crude protein, essential and non-essential amino acid contents in potato tubers. The highest content of crude protein was found following the use of catch crop (pea) as organic sources with a full rate of mineral fertilization (nitrogen, phosphorus, and potassium) and the bio-fertilizer. On average for the other factors, organic sources and bio-fertilizer significantly increased the essential-and non-essential amino acid content. The reduction in the NPK rate down to 50% revealed a significant negative effect on the total crude protein content, with no negative effect on the content of individual amino acids in potato tuber protein, especially after the application of organic sources and bio-fertilizer. The content of essential amino acids depending on the rate of mineral fertilizers was comparable. Based on the AAS value, the best results were recorded after the application of stubble catch crop (pea) and straw in terms of the content of essential amino acids in potato tubers, especially valine and leucine. The results of the research presented can be useful for selecting the most valuable fertilization combination, with a possibility of using the agricultural products, e.g., the effect of straw, green fertilizers or FYM on the nutrition value of potato tubers, especially the content of essential amino acids. The application of bio-fertilizer increases the effectiveness of organic fertilizers, it allows for limiting the application of mineral fertilizers, and thus provides a possibility of eco-friendly cultivation.

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