

CHANGING QUALITY INDICATORS OF WHEAT CROPS FOLLOWING FERTILIZERS APPLICATION

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Abstract: Amino acid composition is an important feature in determining the nutritional value of wheat grain for human and animal diets. The main objective of the research is to track how the localized application with seeding, chemical fertilizers micro granulated, under the different type fertilizers, influence the variation in the raw protein and amino acid composition of the grain. The amino acids were assayed using ion-exchange chromatography after hydrolyzing with 6 M HCl for 24h at 110°C. Raw protein content from wheat grain was determined by Kjeldahl method, as Kjeldahl nitrogen multiplied with 6.25. Methionine and cystine were analyzed by using formic acid protection prior to acid hydrolysis. The research takes place in experimental field from U.S.A.M.V.B. Didactic Station from Timisoara and after that in the research lab of Soil Science and Plant Nutrition Department from Faculty of Agriculture. The experiments are stationary type, with wheat – maize rotation. Field experiences are stationary type, in which to practice wheat-corn rotation. Each plot is subdivided into four repetitions, four variants of fertilization placed linearly, one still other, with dimensions 10 x 3.5 m (35m²) The variety used for this experiment is Alex wheat (*Triticum aestivum* L.). The fertilizers used are: 15.15.15 complex mineral fertilizers, N 28 liquid foliar fertilizers, Nitrophoska 13.42.0 micro granulated fertilizers.

Key words: fertilization, micro granulated fertilizers ,wheat, raw protein, amino acids.

INTRODUCTION

In relation to the climatic conditions of our country, the same variety grown in different localities was a stroke of protein content between 9.85% and 19.12%. Amino acid composition is an important feature in determining the nutritional value of wheat grain for human and animal diets. From all agricultural crops, wheat represents the most important cultivated species.

Wheat contains a large quantity of starch (65-70%), the main component of grain, and also, some sugars (maltose, sucrose). All these have an important energetic role.

Biological value of the protein of wheat is given several essential amino acids that the body can not synthesize.

Nitrogen fertilizer whether applied to the soil 4, 6, 19 or to the plant 7, 9 can be used in to increase the protein content of wheat (*Triticum aestivum* L.) and changes in amino acid composition are associated with increased grain protein.

The grain protein content and amino acid composition of wheat also vary genotype and environment, including rate and time of nitrogen fertilization, water availability and temperature during grain filling 11, 15, 16.

MATERIAL AND METHOD

A series of wheat samples, fertilized with different fertilizers, was studied, in pedoclimatical conditions from USAMVB Timisoara. The samples were obtained from field plots treated with foliar fertilizers:

- Nitrophoska 13.42.0(NPK 13% N, 42% P₂ O₅) - 20kg/ha

- Granulated 15.15.15. (15% N, 15% P 2O₅, 15% K₂O) - 150kg/ha.
- N28 liquid (28% N) - 20L/ha

The wheat samples were finely ground and dried for 24 hours at 60°C. Raw protein content from wheat grain was determined by Kjeldahl method, as Kjeldahl nitrogen multiplied with 6.25.

The amino acids were determined using ion-exchange chromatography after hydrolyzing with 6 M HCl for 24h at 110°C. Methionine and cystine were analyzed by using formic acid protection prior to acid hydrolysis.

The chromatographic conditions are: DIONEX ICS-3000 Amino Analyzer, AMINOPAC PA10 Analytical Column (2x250 mm, P/N 055406), AMINOPAC PA10 Analytical Guard Column (2x50 mm, P/N 055407), Mobile phase: E1: water, E2: NaOH 250 mM, E3: NaAc 1 M, Reference electrode: pH/Ag/AgCl, Flush volume: 250 µL, Flow rate: 0,25 mL/min, Column temperature 30°C

The minimum detection levels of standard was 5 ng/L for each of the amino acids and have been established based on signal to noise ratios of 3:1. The linear dynamic range of the detector response was checked. The average correlation coefficient was between 0, 9886-0,8653.

The values obtained are expressed as percent of a given amino acid in the whole grain. All values are expressed based on the moisture free samples. The original samples contained 13 to 14% moisture.

RESULTS AND DISCUSSIONS

Of all nutrients, the increase of wheat production is mostly influenced by nitrogen, followed by phosphorus and potassium. Besides the direct effect, most cases show the positive effect of interaction between them, especially between nitrogen and phosphorus. Nitrogen is the nutrient involved in production components formation, having a favorable effect on plant roots. However, it increases grains number/ear, grain weight and improve their content in protein substances.

Mineral fertilization is most effective to the extent that it combines analytical and control agrochemical agrophytotechnical other measures which enhances the results of applying fertilizers.

Micro granulated fertilizer plant vegetative development influence the formation of vigorous plants, higher, better twin, large leaves and dark green, protein is the most important substances in nutritional aspect, the quantity and quality depending on flour quality.

In plant, nitrogen is involved in the formation of organic substances. It is well known that plants grown on a medium rich in nitrogen are dark green and develop twinning more abundant vegetation. The most vigorous nitrogen accumulation in wheat occurs during the taxidermy, and ear forming phase but starting to milk phase, nitrogen accumulation decreases.

The absence of change in the ratio of protein with application of N fertilization in the study² may have been due to the fact that they used only two rates of N (0 and 44 kg/ha).

In the first year of experimentation, total nitrogen content of wheat grain varies between 1.88% and 2.69% for the application of Nitrophoska (NPK) + 15.15.15. + N 28 liquid, growth was 58.35%. Raw protein has values between 11.57% and 16.82% in Nitrophoska(NPK) + 15.15.15. + N 28 liquid, while the starch content decreases from 60.78% in untreated version to 58.35% (table 1).

Table 1

Influence of chemical fertilizers on total nitrogen content in NT, raw protein and starch (%) of whole wheat, crop year 2010 to 2011

Variant	Nt%	PB%	A%
Control variant	1,88	11.57	60.78
Nitrophoska (NPK) 13.42.0	2.15	13.43	60.03
Nitrophoska(NPK) 13.42.0 + 15.15.15	2,38	14.88	59.12
Nitrophoska (NPK) 13.42.0 + 15.15.15+ N 28 liquid	2,69	16.82	58.35

It is considered that wheat is most sensitive to the inadequacy of cereal phosphorus, it affects primarily young plants with poorly developed root system yet. At the beginning of vegetation wheat seedlings absorb readily soluble phosphorus in fertilizers and only later have the ability to use phosphorus from soil reserves.

Table 2

Influence of chemical fertilizers on total nitrogen content in NT, raw protein and starch (%) of whole wheat, crop year 2011-2012

Variant	Nt%	PB%	A%
Control variant	1.91	11.94	60,55
Nitrophoska(NPK) 13.42.0	2.20	13.75	59,87
Nitrophoska(NPK) 13.42.0 + 15.15.15	2.42	15.13	58,79
Nitrophoska (NPK) 13.42.0 + 15.15.15+ N 28 liquid	2.73	17.06	58.55

In the second year of experimentation, total nitrogen content ranges between 1.91% and 2.73% in the unfertilized Nitrophoska (NPK) application 13.42.0 + 15.15.15 + N28 liquid .As in the first year of study, the highest values are determined in the case of the maximum dose of nitrogen fertilizer, phosphate fertilizer but increasing the dose of potassium influenced only slightly in grain nitrogen content.

Determined values of raw protein of whole wheat are higher than last year, maximum of 17,06% recorded in Nitrophoska (NPK) variant 13.42.0 + 15.15.15 + N28 liquid. Also, starch content values are higher in this experimental year. The maximum decrease from control variant, with 5.12 units, can be observed in the application of the maximum dose of nitrogen fertilizer on, the lowest content in phosphorus and potassium pre fertilizing trial. (Table2)

These researches indicated that fertilization, especially nitrogen one, has a strong influence on wheat grain protein content and determine significant amount of protein per area unit.

Phosphorus from fertilizers applied micro granulated located hub that it balances the nitrogen effect, improves resistance to winter, fall and diseases, promote root system development and twinning, improves crop quality and hastens maturity.

Results from some studies indicate that the proportions of certain amino acids in wheat protein may depend upon the total nitrogen content of the wheat. Lysine, in particular, has been found in greater concentration in wheat's of low protein content than in high protein samples study 14,17

Although phosphorus has no such a big influence as the nitrogen on protein content, it supports the effect of nitrogen, resulting in better assimilation and metabolisation of absorbed nitrogen forms. Application of nitrogen fertilizers gave the best results on the pre fertilizing trial with the lowest dose of phosphorus and potassium. Between nitrogen and potassium is an antagonistic action, application of high doses of potassium fertilizers can decrease potassium content of wheat grain assimilation and metabolism of nitrogen forms absorbed.

These years of experience in application of high doses of phosphorus and potassium indicated a decrease of raw protein content. This can be explained by the fact that while potassium and phosphorus are necessary for plant growth and protein biosynthesis, these intensely stimulate the synthesis and carbohydrate movement from the other organs to seeds, than nitrogen substances synthesis.

Each of macro participate in the formation of chemical compounds necessary for normal development of wheat plants, or helping in the formation of key compounds that do not contain himself. Amino acids - "bricks" that make up proteins are indispensable elements in any human or animal food. Lack of one of them will feel overall, the efficacy of others is greatly reduced. Amino acids, substances which in combination with nitrogen generate proteins, are not only their constituent units, but their by-products resulting from digestion. We now know a number of about twenty-two amino acids. Of these, a total of eight were essential amino acids. They cannot be synthesized by the body and must be taken from food or supplements with amino acids (proteins).

Foliar applications of nitrogen and fertilization late in the growing season tended to have a greater effect on increasing grain protein concentration than on increasing grain yield study 2.

Table 3

Influence of chemical fertilizers on amino acid content in wheat grain (%)
averages 2010 – 2012

Variant	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Tryptophan	Valine
Control variant	3.7	1.8	2.9	3.5	2.4	0.3	3.3	0.4	2.6
Nitrophoska (NPK) 13.42.0	3.7	2.1	3.3	3.9	2.7	0.2	3.6	0.3	2.9
Nitrophoska (NPK) 13.42.0 + 15.15.15	3.9	2.8	3.6	4.5	2.3	0.6	4.2	0.5	3.3
Nitrophoska (NPK) 13.42.0 + 15.15.15+ N 28 liquid	4	2.6	3.8	5.1	1.8	0.5	4.3	0.4	3.5

Histidine, another amino acid, is considered essential for children only. In order for human body to be able to synthesize and use in the most effective protein, is required to find that all the amino acids in proportions. Absence or insufficient presence of any amino acid will decrease in proportion to the effectiveness of others. The nine essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine.

Other known amino acids are alanine, arginine, aspartic acid, ornithine, cysteine, cystine, proline, glutamic acid, serine, glutamine, glycine. Wheat is containing ten essential amino acids. Protein substances found at the periphery of the grain, so the flour from center of the grain is low in protein. The wheat grain protein substances are formed by gliedina (40-50%), gluteina (30-40%), globulin (6-10%) and albumin (3-5%). Gluteina and gliedina form gluten, which imprint bread quality.

The lowest amounts of amino acids were determined for methionine and tryptophan, the highest being for arginine, leucine and phenylalanine. Of common fertilizers, the nitrogen fertilizers causes significant changes in protein content and composition changes. As shown in Table 3 amino acid content increases as the dose of nitrogen fertilizer increases, regardless of pre fertilization with phosphorus and potassium. The exception is lysine content of which is negatively influenced by increasing the dose of nitrogen fertilizer.

Conditions of nitrogen and potassium-phosphorus nutrition of plants have a substantial effect on the amino-acid composition of protein substances in wheat. With a nitrate source of nitrogen, the content of the aromatic amino acids tryptophan and tyrosine are substantially increased in wheat while given ammonia nutrition the cystine content is higher. When wheat is inadequately supplied with phosphorus a sharp rise in gluten yield and decrease in middling's content occurs. The tryptophan content in gluten protein with a phosphorus deficiency is substantially reduced. As a result, the total tryptophan content in the wheat grain drops in this case. Thus, normal phosphorus nutrition is vitally important for tryptophan synthesis in wheat ¹³.

Changes in amino acids composition of wheat grain protein that occurred as a result of nitrogen fertilization generally agree with other studies study^{1,10} in phenylalanine increased, lysine and valine decreased.

Phosphorus does not affect the same extent as the nitrogen the content of amino acids. However, phosphorus support the effect of nitrogen and especially reduces the negative influence of high doses of nitrogen on the amount of amino acid metabolism resulting in better forms of nitrogen absorbed. Application of potassium fertilizers increases the production of protein per unit area and improves its quality by increasing the content of essential amino acids.

The total amino acid composition of the grain was mainly dependent on N content of grain. Phosphorus and potassium affected the amino acid composition of wheat grain only indirectly through their effects on nitrogen concentration. In wheat increasing grain N% was accompanied by a decrease in the amount of lysine, threonine, methionine, cystine and an increase in glutamic acid, proline, phenylalanine, and serine contents study⁵. Changes in amino acid composition of wheat grain protein that occurred as a results of nitrogen fertilization generally agree with other studies^{8,19}.

The changes in total amino acids composition of the seed due to N application are consistent with the studies published for grain^{1,19}.

One study⁹ showed that increased levels of nitrate fertilizers resulted in changes in the amino acid composition of protein from wheat grain. Since the various soluble protein fractions possess different amino acids compositions, Kolderup proposed that changes in amino acid composition he observed might have been a reflection of shifts in the proportions of certain fractions.

Micro granulated fertilizers applied to allow a reduction of noticeable contribution in the field of chemicals that allow to build soil reserves, before turning retrograde phenomena and fixing the inevitable consequences of loss of digestible phosphorus. Can be considered, so even organic.

Some researchers analyzed the relationship between nitrogen supply, grain yield, and grain protein concentration for wheat. They found that a greater nitrogen supply increased grain protein concentration linearly while grain yield response to added nitrogen had a diminishing return relationship. They also found that when nitrogen was very limiting, small nitrogen additions resulted in greater grain yield with decreased protein concentration caused by dilution of the plant nitrogen. However, at higher levels of nitrogen, which are far more common, grain and protein yields usually increased while the grain protein concentration increased as well.

Wheat protein is high in glutamic acid and proline, whereas lysine, threonine, methionine, and cystine concentrations are lower than those recommended by the World Health Organization (WHO)^{17,18} determined that lysine was the most limiting amino acid for human nutrition in wheat protein. The composition of amino acids in wheat protein is affected by nitrogen fertilization. Some research^{12,20} found that nitrogen fertilization increased the proportion of glutamine, proline, and phenylalanine in wheat protein, while threonine, serine, glycine, alanine, valine, and sulphur amino acids decreased. The balance between nitrogen and sulphur nutrition of the wheat crop also had an effect on grain amino acid composition.

Existing in the body of essential nutrients plants have decisive roles in plant life. Although arable layer of soil nutrients can be found in larger quantities than they extract the

wheat crop during the growing season, however, these substances are in some cases able to plant less accessible and therefore used limited quantity. An example of this is the digestible N content of which is subject to large fluctuations.

Of the three amino acids shown in Figure 1, we observed that the increase rate of mineral fertilizers affect in the utmost levels of leucine, 99.8%, while the content is influenced to the extent histidine: the smallest, at the rate of 86.53%. All three response curves have an upward trend.

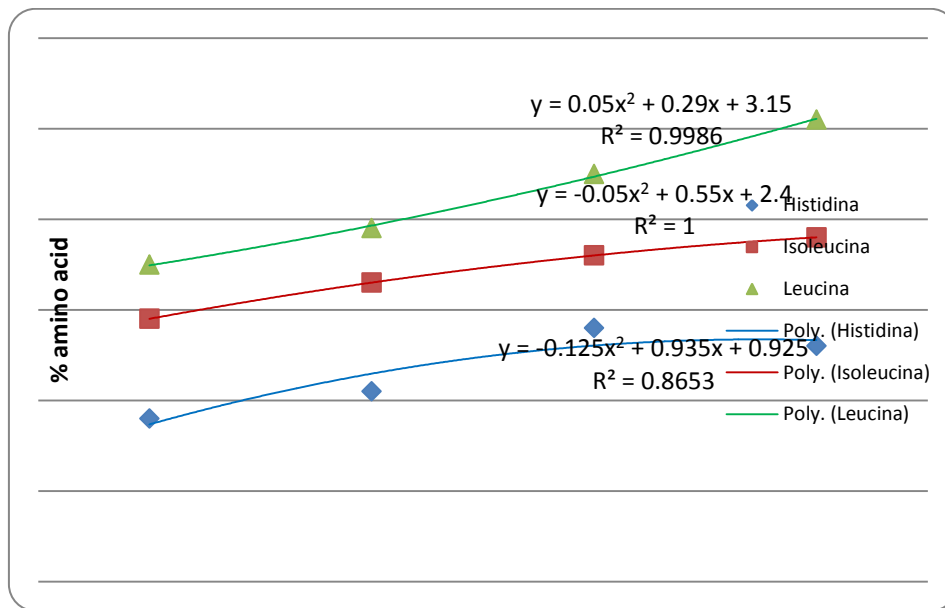


Figure 1. Response curve of the contents of histidine, isoleucine and leucine the application of mineral fertilizers

Figure 2 present the increasing trend of levels of lysine on the extent of increasing doses of fertilizers; they influence the amount of lysine in wheat grain at a rate of 95.0%. Response curve of the metionine content of the application of fertilizers is a downward trend, the correlation coefficient with the lowest namely 0.5. Arginine content of the wheat grain is low, application of fertilizers modifying it in a proportion of 95.71%.

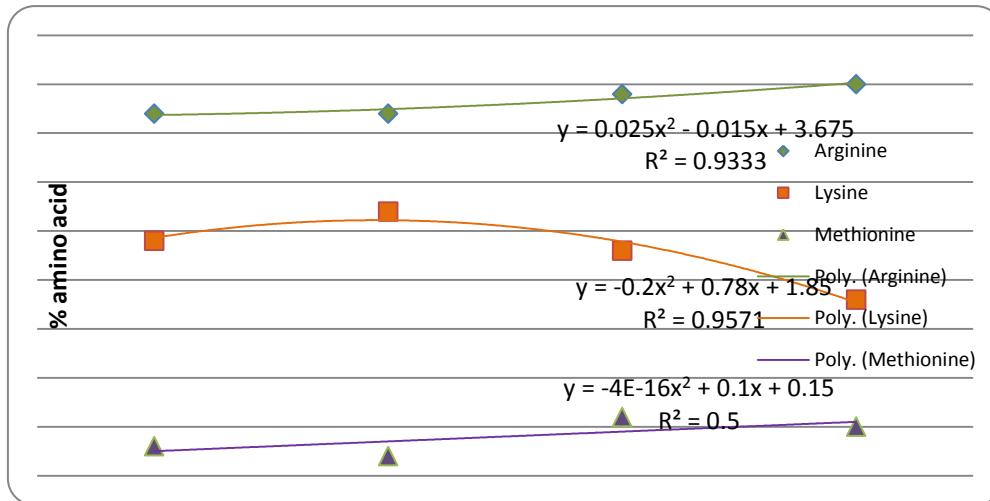


Figure 2. Response curve of the contents of arginine, lysine and methionine to apply fertilizers

Response curves of the contents of phenylalanine and valine have an upward trend, with very close values of correlation coefficients : 0.9536 for phenylalanine and 0.9908 for valine. Tryptophan is found in small quantities in wheat grain, its content is modified by fertilization with increasing doses of mineral fertilizers at a rate of 10%.

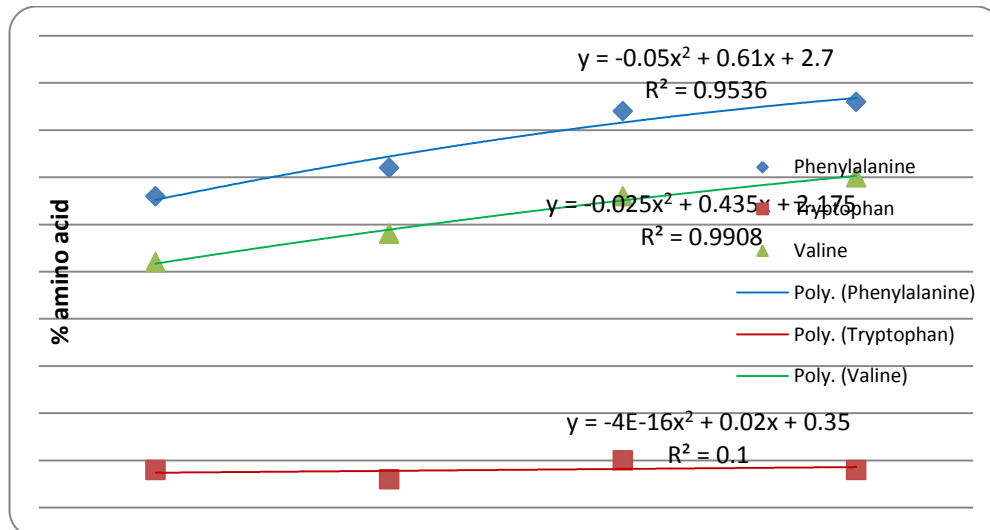


Figure 3. Response curve of the contents of phenylalanine, tryptophan and valine to apply fertilizers

CONCLUSIONS

1. The raw protein content from wheat grain is significant in the case of fertilized wheat; the lowest content in raw protein was found in the case of unfertilized wheat.
2. The higher values for raw protein were founded in the case of maximum dose of nitrogen fertilizers. The increase of phosphorus and potash fertilizers doses influence in an insignificant manner the nitrogen content of grains.
3. Effective complex mineral fertilization is significantly distinct from the first dose of fertilizer micro granulated and maximum yield the highest dose (with granular and foliar), proving that the soil responds positively applying mineral elements are made available from the first plant vegetation phenophases but optimal climate conditions.
4. The raw protein content from wheat grain is significant in the case of fertilized wheat; the lowest content in raw protein was found in the case of unfertilized wheat.
5. The increase of mineral fertilizers dose has a major role in the variation of histidine content. The content of isoleucine depend in a less important manner by the fertilizers doses.
6. The arginine content from wheat grain is found in connection with fertiliser dose. The metionine content from wheat grain is undersized; the use of mineral fertilizers has an insignificant impact.
7. Micro granulated fertilizers applied to allow a reduction of noticeable contribution in the field of chemicals that allow to build soil reserves, before turning retrograde phenomena and fixing the inevitable consequences of loss of digestible phosphorus. Can be considered, so even organic.

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