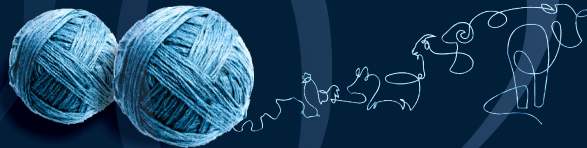


13th
INTERNATIONAL
SYMPOSIUM

MODERN
TRENDS
IN LIVESTOCK
PRODUCTION



P R O C E E D I N G S

6 - 8 October 2021, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade - Zemun, SERBIA

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BIOFORTIFICATION AS A WAY OF NUTRIENT DENSE FEED PRODUCTION

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Invited paper

Abstract: Malnutrition is a global problem, hitting both, people and animals. Due to the many factors, such as climate change, soil depletion, anthropogenic impact, including irresponsible soil management, high fertilization rates with macro-nutrients, crops lack in essential nutrients, particularly minerals, Mg, Fe, Zn, etc., as well as important vitamins, like carotenoids, vitamins from B group. Also, some arable soils have naturally low fertility. All of that negatively affect production of domestic animal, including animal health and quality of livestock products. To mitigate malnutrition, a bio-fortification strategy was developed. It is based on increase in the concentration of essential nutrients in food and feed, and also to promote further bio-availability from digestive organs. Bio-fortification implies various practices. Two main types of bio-fortification were developed: genetic bio-fortification, and agronomic bio-fortification. The first one uses standard breeding techniques, marker assistant selection, transgenic approaches, genome editing, etc., to develop highly-efficient genotypes, that are able to absorb and accumulate higher concentrations of essential nutrients in biomass and grains. Agronomic bio-fortification uses different practices, such is special fertilizers, enriched with essential minerals, foliar fertilizers, bio-fertilizers, growth hormones and enhancers, and some lesser known sustainable practices, like inter-cropping, cover cropping, in order to increase crop yields, as well as the concentration of essential nutrients. The bio-fortification approach, based on the development and commercialisation of highly efficient genotypes, as well as agricultural practices that enable and support better absorption and accumulation of essential nutrients option that is safer for both, people and domestic animals. Mutual increase in concentration of essential minerals, vitamins, and other promoting substances is of a particular importance for bio-fortification programs, increasing efficiency and success of applied practices, thus positively reflecting on animal health and wellbeing.

Key words: breeding, cropping practices, mineral nutrients, antinutrients, promoters.

Introduction

Humans and animals require variety of mineral elements, but in relatively small amounts, 1-2500 mg day⁻¹. They present important body constituents. For example, Ca is an important part of bones, nerves, and muscle structures; P is a part of ATP and nucleic acids molecules, as well as bones and is important for maintaining of pH balance; K, Na, and Cl maintain osmotic balance at the cellular and inter-cellular level; Fe is an important part of haemoglobin and cytochrome molecules involved in cell respiration; Mg, Fe, Zn, Cu, Mn, Se, and Mo are important co-factors of various enzymes, and they are also involved in numerous biochemical pathways; Se and Zn are important for the proper immune system function; I is an important part of thyroid hormones; vitamin A is important for optimal vision, function of the immune and reproductive system; vitamins from B group are important for metabolism, cell reproduction, blood, skin, and health of many other organs in human and animal bodies, etc. Excessive intake, as well as deficiency of essential minerals and vitamins disrupts homeostasis of mineral balance, metabolism, and hormone balance in humans and animals, causing as a consequence various diseases in humans and animals.

An inadequacy in the intake of minerals and vitamins is referred as malnutrition. The malnutrition is a worldwide problem, affecting not just people, but also animals. Nowadays, when sufficient food/feed amount is mainly enabled, malnutrition is predominantly connected to the lack of particular components, such as protein, minerals, and vitamins, thereby affecting human and animal health and welfare. *Welch (2008)* and *Keding et al. (2013)* pointed that the goal of the farming systems is the maximization of the production, with minimal costs, rather than maximization of nutrient output to produce food/feed for nutrient dense and balanced diet. The industrialization of an agricultural sector, particularly a crop production is based on a yield increase, including breeding of high yield genotypes, applying of agricultural practices that support high yields of biomass and grain, based on high inputs of agro-chemicals, such as pesticides and mineral fertilizers (mainly N and NPK fertilizers). All mentioned contributed to the sufficient production of food and feed, reflected through increased amounts of carbohydrates, i.e. calories. The “Green revolution” paradigm changed face of the agriculture, with multiply higher crop and livestock production, CO₂ emission, as well as an increased share of processed foods in nutrition (*Gomez et al., 2013; Zeng et al., 2014*). *Fardet and Rock (2020)* indicated that the livestock production occupy globally 26% of areas aimed for rangelands and 33% of the agricultural areas

aimed for fodder production, whereas the intensive livestock and the production of other animal species use less land, higher inputs, and high concentration of animals, thus contributing to nutrient pollution, increased greenhouse gas emission (GHGE), habitat destruction and diversity reduction. It was accentuated that among 6300 domestic animal breeds, about 1350 are close to extinction or already disappeared over against breeds that have high growth rate and altered body structure and composition (*Hocquette, 2010; Hayes et al., 2013*).

As a consequence of all mentioned, an environmental depletion and pollution rose, with altered soil pH (to low or high values), mineral imbalance, erosion, increased greenhouse gasses emission, etc. Even more, climate change, recognized through meteorological extremes additionally worse the situation. In such conditions, agricultural plants are unable to provide basic elements for growth and development, whereas deficit of essential elements and vitamins, particularly Fe, Mg, Zn, Se, I, carotenoids and other vitamins in food and feed is present worldwide (*Welch, 2005; Clemens, 2014*). One of the most important aspects of food security, irrespective that food and/or feed are considered is to provide optimal amounts/concentration of the essential elements in edible parts of plants. Insufficient concentrations of essential elements and vitamins could be reduced by an introduction of a food diversity (for humans), as well as supplementation and/or increased concentration of highly available nutrients in food and feed, by application of various bio-fortification practices. In regard to supplementation, advantage of bio-fortification is reflecting through improved concentration of mineral and other nutrients, with reduced possibility of overdose or toxicity, due to the fact that nutrients in plant tissues are mainly in highly available forms, what in combination to the other important plant constituents, such as carotenoids, vitamin C, some amino acids, soluble fibre, etc. additionally enhance their bio-availability during digestion.

It is also important to underline that concentration of mineral nutrients in animal tissues does not follow increased concentration of mineral elements in biomass and/or grains used for feed, since bio-availability depends on feed composition, where so called anti-nutrients, such as phytic acid, certain fiber (cellulose, hemicelluloses, lignin, suberin etc.), lignans and other polyphenolics, hemagglutinins, goitrogens, heavy metals restrain bio-availability and absorption of mineral nutrients. Though, so called promoters: organic acids (e.g. ascorbate, fumarate, malate, citrate), hemoglobin, certain amino acids (methionine, cysteine, histidine, lysine), long-chain fatty acids, fats and lipids, Se, Fe, Zn, β -carotene, inulin and other non-digestible carbohydrates increase the absorption of nutrients, diminishing negative effects of anti-nutrients (*Welch and Graham, 2005*). Anti-nutrients and promoters are normal plant metabolites, thus variation in their concentrations could seriously affect bioavailability of mineral nutrients for humans and non-ruminants. Nevertheless, ruminants, due to the presence of specific microorganisms in their guts are able to digest most of the mentioned anti-

nutrients, having better conversion of mineral elements from feed into their bodies. Some strategies, like addition of enzyme preparations into feed (either they are active prior or after feed consumption) could also contribute to increased nutrient bio-availability (*Pariza and Cook, 2010*).

As an answer to a globally rising problem of the malnutrition in people and animals, bio-fortification, as a sustainable practice was developed. It combines various techniques and practices, aimed to increase concentration of important nutrients, as well as their further bio-availability, in regard that absorption and accumulation of mineral nutrients in plants, particularly in grains is under the control of various physiological processes. Several important strategies as a parts of bio-fortification programs were developed. Some of them use diverse genetic resources and breeding of genotypes with improved absorption and accumulation of mineral elements, as well as factors that contribute their further bio-availability from digestive system. Other strategies combine various cropping practices, particularly fertilizers with desirable composition, also the bioactive stimulants that enhance absorption and accumulation of mineral elements. Many practices, commonly used in sustainable and organic agriculture, which improve soil fertility, diversity and quality, at the same time contribute to the better absorption and accumulation of mineral nutrients, thus enhancing the nutritional quality of feed crops.

Abiotic and Biotic Factors Affecting Utilization of Mineral Nutrients for Feed Production

An agricultural production is highly dependable on the environment, particularly variations in meteorological conditions. Extreme fluctuations of meteorological conditions are able not just to reduce, but also to destroy crop yields. Besides that, climate change affects socio-economic aspects of agricultural production, livestock production, transport, demography, altering production and food security (*Tirado et al., 2010*). Climate change is closely tied to the increased atmospheric concentration of CO₂ and other GHGE-s, thus increasing C portion in plant tissues, which is not in parallel, followed by the increased absorption from the soil and accumulation of mineral elements (*Loladze, 2002*), thus reducing minerals:C ratio. Increased CO₂ reduces transpiration intensity, accelerate plant growth, affect flowering and grain filling and reduce absorption and accumulation of Ca and Si, contributing to the decreased resistance to pathogens attack, as well as lower concentrations of protein and lipids in grains (*Fernando et al., 2012*). Fabaceous and gramineous are C₃ plants, which are mainly used for forage production, accumulate lesser Zn and Fe concentrations, lesser water usage and increased accumulation of carbohydrates in green parts, when they were grown in atmosphere with high CO₂ concentration, while C₄ plants were mainly unaffected

(Myers *et al.*, 2014). According to Bornman *et al.* (2019) changes in climate caused by ozone depletion and thus increased exposure to UV radiation is connected to increases and reductions in the growth, survival and reproduction of plants and animals. What is more, the fluctuations in meteorological conditions, like drought, could also affect nutrient uptake, distribution, and accumulation in plants, thus altering its chemical composition and nutritional quality (Hart *et al.*, 1998; Rouphael *et al.*, 2012).

The primary source of mineral nutrients for plants is the soil. Beside the amount of mineral nutrients in the soil, its characteristics and condition are mainly responsible for availability, absorption, and accumulation of mineral nutrients in plants. Mineral nutrients are present in the form of free ions, salts, adsorbed by various minerals and organic components, parts of soil solution, as well as parts of soil living and decomposing organisms (microbiota, worms and other living systems) (White and Broadley, 2009). An optimal mineral nutrition, provided by fertilizer inputs enables normal plant growth and development, particularly when they are grown on poorer soils with low fertility. Accordingly, connection between the Zn deficiency in humans and animals and low Zn concentration in soil is well known (Cakmak and Kutman, 2018; Dhaliwal *et al.*, 2020). Chemical reactions in a soil, such as redox reactions, could affect an availability of mineral nutrients. Besides, Fe- and Mn-oxides, organic matter, including humic substances, various microorganisms, and products of their decompositions presents active sorbents of various mineral elements, influencing their availability to plant roots (Violante *et al.*, 2010; Lin *et al.*, 2019; Hacquardet *et al.*, 2015). Physical characteristic, such as high soil hardness, low penetration or high drainage degree, high or low pH, high salinity, toxicity of some ions and heavy metals (like Na, Cl, Al, Fe, Mn, Cd, Pb etc.) affect not just availability of important mineral elements, but also could induce abiotic stress to plants grown on these soils. Mineral elements with similar characteristics, including association to group in Periodic System of Elements, atom mass, charge and other, could compete each other during absorption by roots. Thus, Ca and Cd could compete with Zn, contributing additionally to oxidative stress increase (Rose *et al.*, 2013; Slamet-Loedin *et al.*, 2015). It is important to underline that deficiency symptoms will be observable on older leaves, if plants are grown on soils with poor availability of particular mineral elements that have better mobility, and *vice versa*, (Soetan *et al.*, 2010).

The important factor that contributes to imbalance in mineral nutrients in a soil, through degradation, erosion, desertification, acidification, increasing salinization, high irrigation rates, and inadequate fertilization is an anthropogenic factor. From this viewpoint, high P fertilization rates conducts to the low Zn availability. Some, commonly used amelioration practices, such as increased fertilization rates with macro-elements (N, P, K, S, Ca, and Mg), including practices that affect soil fertility and pH balance, incorporation of Ca-carbonate and -oxide, gypsum, high amount of organic fertilizers could negatively affect the

availability of some mineral elements, particularly, micro-elements. When plants were grown on soils where long-term mineral fertilization is present, stoichiometric balance between N and P in plant leaves that is closely tied to the yield losses is actually consequence of unbalanced N and P inputs through fertilization (*McKenzie and Williams, 2015*). On the other hand, the positive influence of the anthropogenic factor is reflected through inputs of organic matter, bio-fertilizers (containing mycorrhizal fungi, N-fixing bacteria, microorganisms that accelerate crop residues decomposition) which could positively affect, absorption of mineral elements (particularly of P, Fe, Mn, Zn, and Cu), thus contributing to the enhanced stress tolerance, photosynthesis and increased crop yield (*El-Sirafy et al., 2006; Bhardwaj et al., 2014; Lehmann and Rillig, 2015*).

Other than soil characteristics, there are genotypes that have high efficiency in nutrient elements absorption from soil, even from soils with low level or deficient in a particular nutrient(s), such as soils with poor fertility, acid, saline or similar soils. According to *Eckhard et al. (2012)*, high efficient genotypes are able to achieve high yields on soils deficient in one or several mineral nutrients, due to the genes that could synthesize transporters with high affinity to particular mineral elements, even in conditions of deficiency or poor availability. For instance, some rice genotypes, with high efficiency for Zn absorption have genes that encode Zn absorption and translocation from older to physiologically more active, younger leaves (*Impa et al., 2013; Tiong et al., 2015*), even more these plants easier translocate metabolites from older leaves to the grains.

Breeding and Genetic Engineering as an Integral Part of the Bio-fortification

Modern genotypes must be able to produce high yields of biomass and grains, and to be able to grow in conditions with low inputs of water, fertilizers, and other agro-chemicals. What is more important, their nutritional status has to meet requirements of animals for all necessary elements. Nevertheless, nutritionally quality and yield potential are reversely correlated. Thus, new wheat genotypes with high yield potential are also low in Zn, Fe, and Se in regard to older genotypes (*Garvin et al., 2006*). The necessity for crops that are able to have high yields, with improved efficiency to use resources is an important objective of modern agricultural production (*Raboy, 2013*).

Highly-efficient genotypes could achieve by different methods, such as standard breeding techniques, marker assistant selection (MAS), transgenic approaches, genome editing, etc. Environmental impacts are important obstacle for breeding. Genotype × environment interaction direct Fe and Zn accumulation in maize grain two-fold more than each factor individually (*Oikeh et al., 2004a*). Accordingly, conventional breeding is a long-term strategy, due to the variable

agro-ecological factors, primary soil and climate, while MAS is a better solution, when low heritability traits are considered, due to the fact that genotypes without desirable traits were excluded from further tests at the early stages. For MAS application, high variability of desirable traits (efficiency of mineral nutrient absorption) must be present, and an important part of this method is results proofing in a field conditions. Both mentioned strategies are important for selection and development of genotypes which possess high variability in efficiency to utilize and accumulate mineral elements, as well as factors that restrain or promote bio-availability of absorbed minerals. From this viewpoint, maize, as one of the most important crops used for feed production, either biomass, or grain are used, has high variability in concentration of carotenoids, phenolics, phytic acid, Fe, Mn, Zn, and other minerals (Dragičević *et al.*, 2013; Gupta *et al.*, 2015), what is particularly referred to the genotypes with relative low phytic acid concentration in grain (Mladenović Drinić *et al.*, 2009; Dragičević *et al.*, 2010; Dragičević *et al.*, 2013; Dragičević *et al.*, 2016). This was supported by results present in Table 1, done on 78 maize lines, where Mn and Zn concentration in the grain correlated significantly on the phytate concentration, from which Zn correlated negatively, thus indicating its better accumulation in grain of genotypes lower in phytic acid, as well as potentially enhanced further bio-availability. Based on conventional breeding techniques, it was established that heterotic background presents an important source of highly-efficient genotypes, when concentration of mineral nutrients and promoters was considered. Hence, based on results obtained on 51 maize lines (Figure 1), it was established that in grain of genotypes from Lancaster heterotic group relative low concentration of phytic acid was present, together with enhanced Zn concentration while in lines from Independent source variations in Mg, Fe, and Mn concentration were independent on phytic acid status, indicating that they could serve as an advantageous source for increased Mg, Fe, and Mn concentration and improved bio-availability (Dragičević *et al.*, 2016). In parallel, combination of conventional breeding and MAS could be successfully used for mapping and selection of maize lines with desirable traits, such as genotypes high in mineral elements and low phytic acid (Šimić *et al.*, 2012).

Table 1. Correlation between examined traits: phytic (P_{phy}), inorganic (P_i) and total (P_{tot}) phosphorus, and β -carotene, Fe, Mn and Zn in grain of 78 maize lines (Dragičević *et al.* 2013).

	P_{phy}	P_i	P_{tot}	β -carotene	Fe	Mn
P_i	0.05					
P_{tot}	0.43*	0.31*				
β -carotene	0.12	-0.11	-0.01			
Fe	0.01	0.23	0.15	0.06		
Mn	0.36*	0.17	0.30*	0.21	0.20	
Zn	-0.25*	0.17	0.06	0.05	0.34*	-0.04

*The significant values at the level of significance of 0.05.

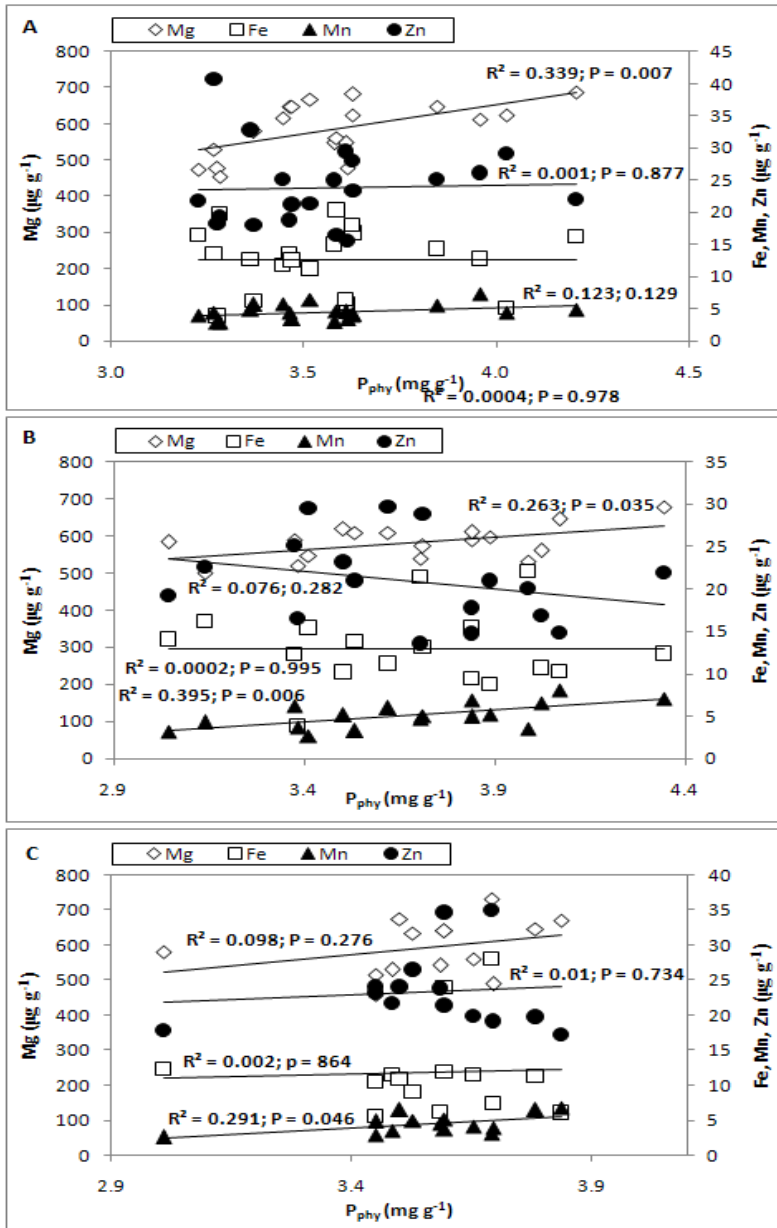


Figure 1. Interdependence between phytic P (P_{phy}) and mineral elements in grain of maize lines belong to (A) *BSSS* heterotic group, (B) *Lancaster* heterotic group, and (C) Independent source (*Dragicevic et al., 2016*).

The application of transgenic techniques depends, not only on an identification and introduction of genes responsible for the improved absorption and accumulation of mineral nutrients into crop biomass and/or grain, but also on acceptance of transformed genotypes by the market, including consumers (for human nutrition) as well as farmers engaged to the livestock production. Criterion for the commercialisation of bio-fortified genotypes lies in their ability to achieve same or even higher yield, reached concentration of nutrients must be stable in various environments, including positive effects on human or animal health, by the definition (Welch and Graham, 2005). Bio-availability and absorption into animal organisms must be tested in practice, prior to releasing of modified genotypes to the market.

There are many examples of bio-fortified transgenic crops, where strategic role play chelators that enhance mobility and accumulation of mineral elements, including nicotinamine, of which three genes that encode nicotinaminesynthetase and genes that encode regulatory proteins involved into Fe accumulation were successfully used for rice transformation with six-fold higher Fe concentration (Clemens, 2014). Even more, the combination of genes for nicotinamine synthetase and ferritin increased 1.5-fold Zn concentration. From the viewpoint of increased Fe accumulation, soybean ferritin genes in combination with genes that encode mugineic acid synthesis resulted in four-fold higher Fe concentrations in the grain of polished rice that was grown in conditions of the Fe deficiency (Masuda et al., 2013). One of the important strategies, when transgenic approach is considered, is isolation of genes from hyper-accumulators involved into metabolism and accumulation of particular mineral elements, such as ZIP-transporters from *Thlaspi caerulescens* and *Arabidopsis* genome involved into Zn transport, as well as genes from *Astragalus* species, known as a Se accumulators (Guerinot and Salt, 2001). Transformations of crops in bio-fortification programs are not based only on improvements in accumulation of mineral elements, but also on the increased accumulation of promoters, such as carotenoids, vitamin C, and folate. There are many genes involved in synthesis of various carotenoids that have been used in improvements of genome of different plant species (Suwarno et al., 2015). Introduction of some of these genes resulted in maize grain with 169-folds higher β -carotene concentration + 6-folds higher vitamin C level + 2-folds higher folate concentration (Naqvi et al., 2009). Due to the fact that P is mainly present in the plant tissues in the form of phytic acid, its utilization efficiency in the livestock production is below 40%. Kebreab et al. (2012) summarized that usage of low phytate transgenic plants and transgenic animals increased P availability by 14% and 52–99%, respectively, whereas combination of phase feeding and enzymes that decompose phytic acid increased P availability from 42 to 95%, having the greatest importance in practice.

Irrespective that breeding and genetic method are promising bio-fortification tools, their combination with other techniques could give better results in enrichment of forage crops with mineral elements and vitamins, particularly when it was taken into consideration that even highly efficient genotypes are unable to absorb elements that lack in soil.

The Agronomic Bio-fortification – Cropping Practices for Enhanced Feed Quality

The most important factor that supports high availability, confident and stable absorption, and accumulation of mineral elements in crops is the soil fertility. The application of various fertilizers could contribute to the maintaining, increase, or depletion of the soil fertility. The usage of various supplements as feed additives with intention to enable optimal and balanced livestock nutrition have potential risks, reflected through overdosing and increased potential toxicity, as well as increased excretion, what is environmentally unacceptable (*Anderson et al., 2012; Titcomb and Tanumihardjo, 2019*). The same authors pointed that the introduction of bio-fortified crops as feed is safer for animals and environmentally rational, since nutrients are mostly in the highly available forms, what with promoters from feed enable enhanced absorption and usage on the cellular level.

When the bio-fortification was considered in general, it is important to underline that the agronomic bio-fortification and genetic bio-fortification should be jointly used, to provide high efficiency in nutrient usage and delivery to the human and animal organisms. For instance, Zn enriched NPK fertilizers could present complementary amendment of breeding programs (*Cakmak, 2008*). It is well known that macro-elements from fertilizers affect availability and further absorption of micro-elements, such as N, which positively affect the absorption of micro-elements by rice plants (*Hao et al., 2007*). Also, an optimization of Zn-enriched urea for the agricultural production was developed as a part of the bio-fortification (*Shivay and Prasad, 2012; Cakmak and Kutman, 2018*). Some of the bio-fortification techniques include utilization of plants originating from locations under fitoremediation programs, such as soils with extreme high Se or Zn content, as a green manure, as well as part of feed mixtures (*Wu et al., 2015; Schiavonand Pilon-Smits, 2017; Wang et al., 2021*).

One of the most efficient agronomic bio-fortification practices is an application of foliar fertilizers, since unfavourable soil conditions that could affect availability and absorption of mineral nutrients were avoided and metabolizing of mineral nutrients by plants was assured. Therefore, the method for increase in Zn, Se, and Fe concentration in rice by the foliar fertilization was developed (*Fang et al., 2008*). Many other agro-chemicals, such as bio-stimulants, plant, and algae

extracts, phytohormone preparations, herbicides, etc. could be successfully used to alleviate status of mineral elements and factors that positive or negative affect their further bio-availability, such asphytate, phenolics carotenoids, glutathione, and some other vitamins in the plant tissue (*Dragičević et al., 2015a; Dragičević et al., 2016b; Mesarović et al., 2019; Đurović et al., 2019; Brankov et al., 2020*). It is important to underline that efficiency of foliar fertilizers is highly dependable on agro-meteorological factors, particularly when crops were grown in dry farming conditions (*Dragičević et al., 2016b*), where low precipitation level and particularly drought could severely decrease grain yield, but in parallel, it could positively affect accumulation of essential mineral elements in barley grain (Figure 2), what additionally, in combination with reduced concentration of phytic acid, could be positively reflected on further bio-availability during digestion.

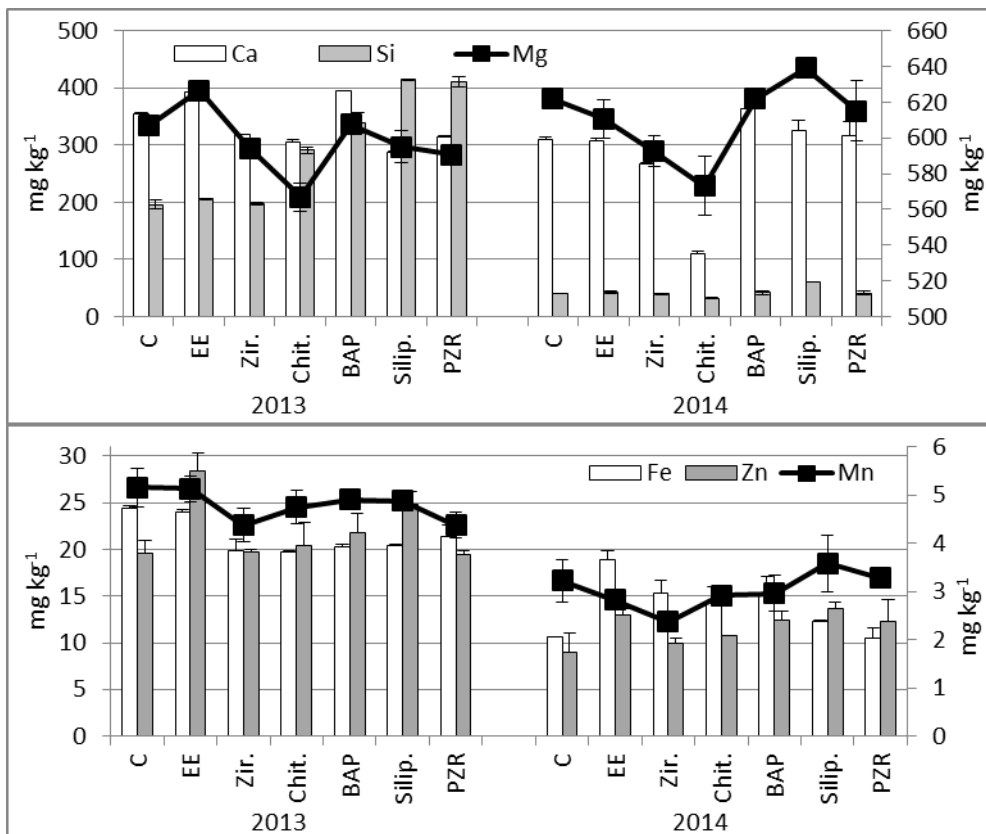


Figure 2. The effect of different foliar fertilizers on Ca, Mg Si, Fe, Zn and Mn concentration in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP –

Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean \pm SD (standard deviation) (Dragicevic et al., 2016b).

Some other practices, included in sustainable agricultural systems could play an important role in the availability and absorption of mineral elements into crop biomass and grains, due to the fact that sustainable systems basically tend to maintain or even increase soil fertility, increase biodiversity, including crop yields and their quality. From this standpoint bio-fertilizers are very important: N fixing bacteria, symbiotic bacteria, fungi, and other microorganisms have pivotal role in metal homeostasis in soils (González-Guerrero et al., 2014; Lehmann and Rillig, 2015), what could present a great potential for bio-fortification programs. Bio-fertilizers are able to stimulate crop growth, productivity, stress tolerance mechanisms, synthesis of antioxidants and photosynthetic pigments, to enhance of absorption and accumulation of P, Fe, Mn, Zn and Cu (El-Sirafy et al., 2006; Bhardwaj et al., 2014). Bio-fertilizers are also able to increase a protein level, parallel with reduction of phytic acid concentration in soybean grains (Zarei et al., 2012; Hussain et al., 2020). It is interesting that microbiota that are present in plant roots and animal gut, and are highly responsible for acquisition of mineral elements and some vitamins, evolved in different directions, possible due to the differences in environments, such as oxygen levels, temperature, pH, and organic carbon availability, but there are some similar taxa - overlapping, present in both kingdoms (Hacquard et al., 2015), emphasizing importance of further research in microbiota role for plants and animals/humans.

Furthermore, growing of inter- and cover- crops presents unavoidable part of sustainable agricultural systems. By combining of different crops on the same field at the same time, support of different crop species in acquisition of mineral elements was enabled. Phytosiderophores, excreted by maize root help other crops that were grown in combination, like peanut or soybean to absorb and accumulate greater amounts of Mg, Fe, and Zn in grains (Xiong et al., 2013; Dragicevic et al., 2015b). In parallel, crop combining could positive reflect on increased synthesis and accumulation of promoters, such as β -carotene (Dragicevic et al., 2015b). In experiment with soybean and proso millet, grown in different intercrop combinations it was shown that combinations, particularly with two rows of soybean and four rows of millet (SS-MMMM combination, Figure 3) was responsible for greater Fe accumulation in biomass of both crops, while Zn was greater in soybean biomass (one row of soybean and one row of millet, S-M combination), what was not the case for proso millet, with lower accumulation ability present in all combinations. This refers of potential competitiveness between crops, what is an important part, when intercropping design is establishing.

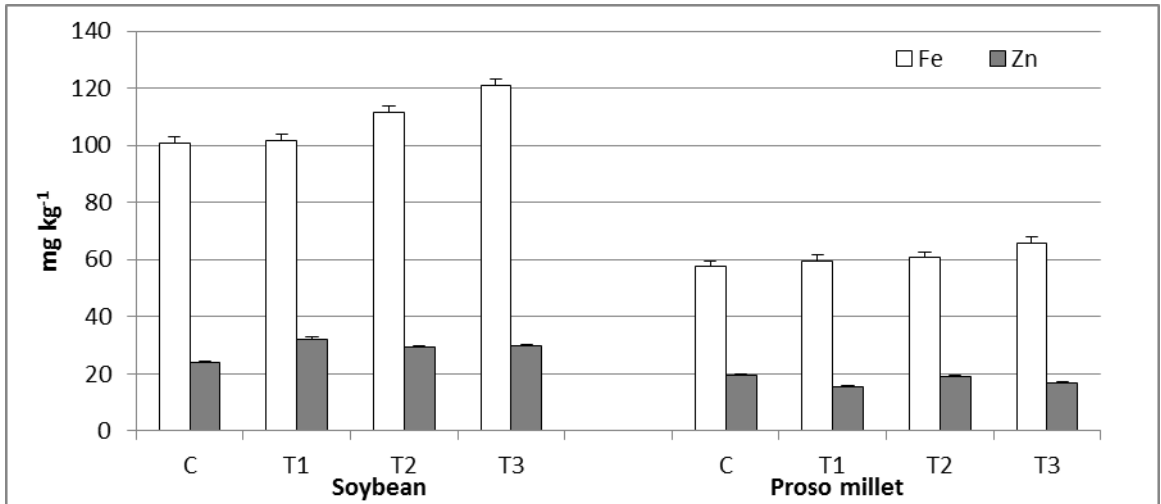


Figure 3. The effect of different soybean and proso millet intercrop combinations on P, S, Fe and Zn accumulation in biomass (C - sole crop, control; T1 - S-M combination, T2 - SS-MM combination and T3 - SS-MMMM combination); S - soybean, M - proso millet: Results present mean \pm SD (standard deviation)

Cover crops, that are commonly used as sustainable practice for protection from erosion, leaching of mineral nutrients into soil depth, weed suppression, could be also successfully used for soil enrichment with N (particularly leguminous covers), as well as improved absorption and accumulation of mineral elements and other bioactive compounds, which could serve as promoters (Baligar *et al.* 2006; Janošević *et al.*, 2017; Dragicevic *et al.*, 2021)

Conclusion

Past and still on-going changes and fluctuations in the world, including climate, soil devastation, biodiversity reduction, etc., affected severely agricultural sector, including livestock. From this standpoint, malnutrition is recurring problem, which does not affect human health, but also health and wellbeing of domestic animals. When essential elements, including minerals and vitamins lack from animal diets, i.e. feed, they suffer from various diseases, production is reduced and further livestock products have low quality, and are also unable to meet human requirements for vital nutrients, that could be provided only by animal products. This problem is especially important for domestic animals, since their diets are specific, in regard to humans. Supplementation is not always the best solution, due

to the poor bio-availability or even toxicity, since overdosing of ingested amount of some nutrients is hard to control.

Thus, it is important to increase concentration of essential nutrients in crops used for feed, in which nutrients are in highly available forms, in combination with lot of other important nutrients, which could support or suppress bio-availability from animal intestines. The bio-fortification approach, based on development and commercialisation of highly efficient genotypes, as well as agricultural practices that enable and support better absorption and accumulation of essential nutrients is safer option. Mutual increase in concentration of essential minerals, vitamins, and other promoters is of the particular importance for bio-fortification programs, increasing efficiency and success of applied practices, thus positively reflecting on animal health and wellbeing.

Biofortifikacija, kao način proizvodnje nutritivno bogate hrane za domaće životinje

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Rezime

Neishranjenost predstavlja globalni problem, pogađajući i ljude i domaće životinje, paralelno. Zahvaljujući broujnim faktorima, kao što su promena klime, ispoščavanje zemljišta, uticaj antropogenog faktora preko neodgovornog upravljanja zemljištem, visokih unosa mineralnih đubriva baziranih na makroelementima, u usevima se javlja se nedostatak esencijalnih hraniva, kao što su Mg, Fe, Zn, itd., kao i važnih vitamina, kao što su karotenoidi i vitamini iz B grupe. Takođe, neke obradive površine imaju prirodno nisku plodnost. Sve navedeno se negativno odražava na proizvodnju domaćih životinja, uključujući zdravlje životinja, kao i kvalitet životinjskih proizvoda. Kao odgovor u borbi protiv neishranjenosti, razvijena je strategija bio-fortifikacije, koja se bazira na povećanju koncentracije esencijalnih hraniva u hrani i hranivima, kao i većoj pristupačnosti iz organa za varenje. Bio-fortifikacija koristi različite mere. Dva osnovna tipa bio-fortifikacije su razvijena: genetička i agronomska bio-fortifikacija. Prva koristi standardne tehnike selekcije, marker asastiranu selekciju, transgene pristupe, editovanje genoma, i dr. u dobijanju vioko-efikasnih genotipova koji su sposobni da apsorbuju i akumuliraju esencijalna hraniva u većim koncentracijama u biomasi i zrnu. Agronomska bio-fortifikacija koristi različite tehnike, kao što je primena specijalnih đubriva obogaćenih sa esencijalnim mineralima, folijarnih đubriva,

hormona i poboljšivača rasta, kao i nekih manje poznatih mera koje se koriste u održivoj poljoprivredi, kao što su kombinovani i pokrovni usevi, koji su prvenstveno namenjeni povećanju prinosa, kao i koncentracije esencijalnih hraniva. Strategija bio-fortifikacije, bazirana na razvijanju i komercijalizaciji visoko efikasnih genotipova, kao i agronomskih tehnika, koje omogućavaju i podržavaju bolje usvajanje i akumulaciju esencijalnih hraniva je bezbednija opcija i za ljude i za domaće životinje. Paralelno povećanje koncentracije esencijalnih minerala, vitamina i drugih supstanci koje pomažu usvajanje je od posebnog značaja za programe bio-fortifikacije, povećavajući efikasnost i uspešnost primenjenih mera, i odražavajući se pozitivna zdravlje i blagostanje domaćih životinja.

Ključne reči: uzgoj, prakse u ratarstvu, mineralni nutrijenti, antinutrijenti, promotori

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