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SORGHUM STALK YIELD AND GRAIN NUTRITIONAL QUALITY IMPROVEMENT BY FOLIAR METAL CHELATES

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Abstract

Under the field conditions, the effectiveness of foliar application of metal-amino acid chelates including metal-glycine [$Zn(Gly)_2$], [$Fe(Gly)_2$], [$Cu(Gly)_2$] and metal-methionine [Zn(Met)], [Fe(Met)], [Cu(Met)] on yield of stalk and grain as well nutritional quality of sorghum was investigated. The foliar application increased stalk and grain yield 23.8-25.16% and 16.1-20.1% for the first and second year respectively. The grain zinc, iron, copper and protein concentration was also increased significantly as compared with non-chelated and control. On the average 26.2%, 23.3%, 45% and 25.3% increase in sorghum grain Zn, Fe, Cu, and protein respectively was noted for both the growing years sprayed with [$M(Gly)_2$] and [M(Met)] than those sprayed with non-chelated micronutrients. Significant correlation between the protein, Zn, Fe, and Cu concentration indicates that the genes affecting the grain accumulations of Zn, Fe, Cu and protein are probably closely linked. In the present study, the results obtained by foliar application of [$M(Gly)_2$] and [M(Met)] should consider as new micronutrient sources to improve the stalk and grain yield along the nutritional quality.

Key words: Glycine, Methionine, Protein, Trace metals.

Introduction

Micronutrients are important to plant growth and profitability as the main crop nutrients. Each nutrient is has its own importance but are used in different amounts and work collectively as activators of many plant functions. These micronutrients must provide the plants to complete their life cycle (Chaney *et al*., 1992). Copper needed for synthesis, functioning of chlorophyll and various enzymes which are involved in growth and reproduction of plants. Copper increases the utilization of ammonium nitrogen and acts as catalyst for auxins to improve the plant growth. It also regulates the many biochemical reactions in plants to promote the production of seeds. Frost damage, insect damage, diseases and herbicides injury can be protected by balance provision of copper as micronutrient (Baszynski *et al*., 1978; Droppa *et al*., 1987; Himelblau & Amasino, 2000). Iron involved in many physiological processes of plants such as activation of many enzymes which are used in photosynthesis and respiration along with synthesis of chlorophyll. Iron is associated with heme and non-heme protein such as ferrodoxin, main constituent in the chloroplast where it participates in sulphur and nitrogen metabolism and have important role in energy transfer within plants. Deficiency results in chlorosis in young leaves that is shown by the yellow and green strips along the length which is avoided by spraying iron complexes (Schmidt, 1999; Tagliavini & Rombola, 2001; Ashemad, 2001). Zinc is also micronutrient for plant also involved in the metabolism of carbohydrates, protein and auxin hormone which is responsible for the stem elongation. Zinc is a precursor to tryptophan and also responsible for resistance to infection of pathogens. Plants are stunted and the leaves become yellowish due to deficiency of zinc. Because zinc is trans-located very slowly in plants therefore its deficiency symptoms appear in the young leaves (Al Mustafa *et al*., 1994; Cakmak, 2008; Gao *et al*., 2009). In the present study, foliar copper (Cu), iron (Fe) and zinc (Zn) amino acid (AA) chelates has been established for yield enhancement and grain nutritional quality of sorghum. Sorghum (*Sorghum bicolor* (L.) Moench) is important food crop after barley, maize, rice and wheat which are consumed by large population of poor countries in Africa and Asia, although its processed seeds are being utilized even in developed countries as protein source (Anon., 2006). In Pakistan, it is used as silage crop which is advantageous over maize silage due to lower production cost. This silage crop is suitable for forage as palatable animal feed source. Optimum concentration of protein and trace metals as nutrient source is required by dairy animals to produce milk on sustainable basis (Iqbal & Iqbal, 2015). We already reported the chelated micronutrient (Qadir *et al*., 2014a, 2014b) for plant growth and animal nutrition.The amino acids are the organic molecules that bind and protect the micronutrients from outer environment until these are utilized by the plants. Since complex formation takes place at the proximal site of the carboxyl active site and alpha amino nitrogen promote the formation of five membered lactone rings. This ring is strong enough for the molecule to remain intact for higher absorption rate but not so strong to resist the break down for the metabolic usage of metal item. Amino acid chelated minerals can pass through the cuticle very easily by reduction of charges taking place either on the metal atom or molecule as a whole (Ho & Hidiroglou, 1977; Hernandez-Apaolaza *et al*., 1995).The aim of present field study was established to examine the effects of foliar application of amino acid chelated micronutrients on growth response as well seed nutrition quality of sorghum and made comparison with control and non-chelated micronutrients.

Materials and Methods

Synthesis and characterization of metal-AA chelates: The complexes of Cu, Fe and Zn were synthesized by reaction of glycine (Gly) and methionine (Met). The mole ratio between the metal and Gly [M(Gly)$_2$] was 1:2 while 1:1 metal-Met [M(Met)] complexes were established (Ghasemia *et al*., 2013; Tewari, 2013). Chlorides salts of respective metals were used for complexation. All the reagents were of AR grade (Sigma Aldrich, USA) used as such without further purification.The respective amounts of AA (10 mmol Gly and 5.0 mmol Met) and salts of metal (5.0 mmol) were charged into 100 mL round bottom flask containing 50 mL of water and refluxed for 3-4 hrs. In order to separate the complex, this refluxed solution was heated in china dish to reduce the volume to 15-20 mL. The dish was then cooled at room temperature and was then kept in 4-8ºC for crystallization of complexes. The crystals were then dissolved in small amount of water and recrystallized for several times after washing with cold ethanol.The non-chelated micronutrient was received from Al-Khalid Chemicals, Lahore-Pakistan, under the trade name Multimix (Zn 5.0%, Fe 2.0%, Cu 1.0%, Mn 1.0%, B 1.0%) and used by filling the consent form (Document number Int-001-13:14) for comparison studies. Alpha IR spectrometer (FTIR-ATR) and NMR spectrometer, Bruker, Germany were used to record the IR and ^{1}HNMR (500 MHz), ^{13}CNMR (125 MHz) spectra respectively. PG-990 atomic absorption spectrometer, UK, Flash HT Plus elemental analyzer, Thermo Scientific, UK were used for estimation of metals and concentration of carbon (C), hydrogen (H), sulfur (S) and nitrogen (N) of respective synthesized complexes respectively while the melting point was measured by Gallenkamp apparatus. Non-chelated formulation was also analyzed for the metal contents only.

Soil and water analysis of selected fields: 15 soil samples were collected from 15-25 cm, 15-30 cm and 60-100 cm depth to estimate the nutrient supplying power, the samples were collected randomly by following grid pattern using stainless steel soil auger and composite sample was prepared for final analysis. The soil samples were stored in plastic (HDPE) bags until analysis. 500 mL water sample from area located for plant cultivation was taken and stored in plastic bottles, which were soaked with concentrated hydrochloric acid overnight, then washed with distilled water. The water samples were preserved by adding 2 drops of concentrated nitric acid. All the soil samples were crushed and ground in wooden pestle mortar and sieved through 2 mm plastic sieve. A saturation paste of soil was made by taking 300 g dry mass in enameled cup and stayed overnight to ensure the saturation. Then the saturation extract was obtained. The pH (soil: 7.82-7.88 and water: 7.38-7.41) and electrical conductivity in mS cm^{-1} (soil: 1.41-1.48 and water: 0.85-0.88) by multi-meter (Model: Orion 5 Star multimeter-Thermo Scientific, UK), macronutrients in µg g^{-1} (Na in soil: 137-139, Na in water: 57-68, K in soil: 59-60, K in water: 40-41, Ca in soil: 211-121, Ca in water: 151-152, Mg in soil: 48-46, Mg in water: 38-41) by Flame photometer (Model: 410C-Sherwood, UK), micronutrients in µg g^{-1} (Zn in soil: 9-11, Zn in water: 1.5-1.7, Fe in soil: 20-22, Fe in water: 0.8-0.9, Cu in soil: 2.1-2.8, Cu in water: 0.8-0.9) by atomic absorption spectrometer (PG- Instruments, UK) while bicarbonates (soil: 111-116 µg g^{-1}, water: 53-54 µg g^{-1}), carbonates (soil: 18-19 µg g^{-1}, water: 1.7-1.8 µg g^{-1}) and chlorides(soil: 71-76 µg g^{-1}, water: 61-64 µg g^{-1}) were determined using saturated extract by titrimetric methods. The metals were determined by digesting the 1.0 g dried soil in 4 mL in aqua regia (Issam and Antoine 2007). The study was performed in two successive sorghum growing seasons 2012-2013 and 2013-2014 in Kasuria Agricultural Farm (31° 1'33.58"N, 74°17'15.39"E) Kasur-Pakistan. The mean temperature was 31.7ºC (max.) and 16.2ºC (min.) while the rainfall was 629 mm (max.) and 632 mm (max.) for first and second year respectively.

Field experiment: Cultivar J-S-6090 of sorghum was used in this study. 2.25 kg of seed were planted at depth of 2-5 cm in each plot in the 1st week of April in each growing season of 2012-2013 and 2013-2014 because the temperature conditions are appropriate for best growth (Kanemasu *et al*., 1975). The selected soil for sorghum cultivation was micronutrient deficient (Zn: 9 µg g^{-1}, Fe: 229 µg g^{-1} and Cu: 2.1 9 µg g^{-1}). Four plots each of 9000 ft^2 were selected for control (no micronutrient application), foliar applications of non-chelated micronutrients and metal-amino acids chelated micronutrients [M(Gly)$_2$] and [M(Met)] respectively. The amount of micronutrient sprayed in each plot in three different growth stages i.e. tillering, before emergence of main spike, during grain filling. Non-chelated, Multimix of 125 mL (Zn: 5.0%, Fe: 2.0%, Cu: 1.0%, Mn: 1.0%, B: 1.0%), [M(Gly)$_2$] of 250 mL (Zn(Gly)$_2$: 6.0 g, Fe(Gly)$_2$: 2.5 g, Cu(Gly)$_2$: 50 mg) and [M(Met)] of 250 mL (Zn(Met): 6.0 g, Fe(Met): 2.5 g, Cu(Met): 50 mg). These amounts were further diluted upto 250 litres and applied on each prescribed plot separately at afternoon in order to prevent the damage of leaf at day high temperature. 12.5 kg N plot^{-1} as urea, 15.0 kg P plot^{-1} as super phosphate and 12.5 kg K plot^{-1} as potassium sulphate were utilized into 0-20 cm layer of soil as per local agricultural practice before transplanting and 12.5 kg N plot^{-1} in the form of urea was top dressed (Panhwar, 2005).

Stalk and grain sampling and its contents measurements: Sorghum plants were counted from twenty random selected places of 1 ft^2 in each plot and weighed. A set of grab samples made composite sample after harvesting the sorghum plants (Skoog *et al*., 2004). The husks were removed to prevent the contamination and dried at 60ºC for 48 hrs and preserve in desiccator for its content analysis.The concentration of grain protein was measured using Kjaldahl apparatus (VELP Scientifica, Italy: Model, DKL 20) by Kjaldahl method multiplying 5.7 factor to convert nitrogen to protein on dry basis (Bremmer & Mulvancey, 1982). The micronutrients (Zn, Fe, Cu) were measured by atomic absorption spectrometry (PG- Instruments, UK) by digesting the grain samples in HNO_3–H_2O_2 by a microwave accelerated reaction system (CEM, Matthews, USA). NIST No. 1515 apple leaves as reference standard and reagent blank were used for quality control purposes.

Statistical analysis: SPSS version 15 for windows was used for statistical analysis; the main effects viz. fertilizer (fixed factor) and year (random factor) were evaluated by using analysis of variance (ANOVA). Means and interactions were determined by general linear models (GLM) and least significant differences (LSD) respectively at 95% level of significance ($p < 0.05$)

Results

Excellent yield of all the synthesized complexes were obtained, the confirmation of metal amino acids were done by elemental analysis (Table 1) and spectroscopic techniques (FTIR and NMR). The amino group stretching and bending vibration at 3300-3240 cm^{-1} and 1514-1500 cm^{-1} respectively confirm the involvement of amino group in metal complexation. The IR spectra at 563-550 cm^{-1} attributed to M-O bond while 530-450 cm^{-1} frequencies support the coordination of N-atom of amino group to metal ion (Silverstein and Webster, 1998). ^{1}HNMR and ^{13}CNMR spectra of all the complexes were recorded in $CDCl_3$ and the chemical shift (δ = 10-12 ppm) of hydroxyl proton is absent in all complexes confirm the deprotonation of ligands and its involvement in bond formation with metal ions, while the keto- group carbon chemical shift (δ = 172-175 ppm) in all the synthesized complexes further support their formation (Raman *et al.*, 2008). The accuracy and precision for determination of micronutrient in grains was between 95-105% and RSD < 4.2% respectively.

The foliar application of metal-amino acid chelates significantly affect the stalk and grain yield as compared with control (no fertilizer applied) and non-chelated foliar micro-nutrients. In year 1, the stalk yield was increased 40-41% and 23.8-25.16% (Fig. 1) as compared with control and non-chelated foliar micro-nutrients respectively. Although 20% increase in yield was noted by foliar application of non-chelated micro-nutrients as compared with control.

This increase in stalk yield was significant by the interaction of foliar application of [M(Gly)$_2$] and [M(Met)] (Table 2) but there is no significant difference between the yield of stalk among the [M(Gly)$_2$] and [M(Met)] foliar applications. In growing year 2, no significant difference in stalk yield was noted although it was slightly increased (1.3-2.4%). By the interaction of foliar application of [M(Gly)$_2$] and [M(Met)], the sorghum grain yield was significantly increased as compared with control and non-chelated foliar micro-nutrient application (Table 2). 33.3-36.4% and 16.1-20.2% incremented in grain yield was seen by foliar application of [M(Gly)$_2$] and [M(Met)] as compared with control and non-chelated foliar application of micro-nutrient respectively (Fig. 1). Both application of foliar [M(Gly)$_2$] and [M(Met)] have no significant effect among each other for increase of grain yield. Between the years no significant increase in grain yield was noted by applying foliar application of [M(Gly)$_2$] and [M(Met)], only 1.6-2.1% increment in yield was noted. Foliar application of [M(Gly)$_2$] and [M(Met)] significantly effect the grain Zn-concentration (Table 2).

The Sorghum grain Zn-concentration was increased by 46.6-49.8% and 27.1-31.4% as compared to control and non-chelated nutrient foliar application respectively (Fig. 2). Between the years the foliar application of [M(Gly)$_2$] and [M(Met)] has no significantly effect on increase in grain Zn-concentration. Among the foliar application the [M(Met)] is more effective than [M(Gly)$_2$] for increase in Zn-concentration. This increment effect was significant at $p<0.05$ using LSD for multiple comparisons.

The Sorghum grain Fe-concentration was increased 42.6-42.9% and 23.1-23.5% as compared with control and non-chelated micro-nutrient application. This increase in Fe-concentration was significant than control and non-chelated foliar application (Table 2) and in year 2 no significant increase in Fe-concentration was seen (Fig. 2). Both foliar [M(Gly)$_2$] and [M(Met)] have almost same effect on Fe-concentration of grain. The grain Cu-concentration was significantly increase by the foliar application of M(Gly)$_2$] and [M(Met)] and increase of 45-46% in Cu-concentration (Fig. 2) was noted as compared with control (no foliar application). In growing year 2, no significant increase was observed (Table 2). An increase of 29.5-29.9% and 25% protein was noted by the foliar application of [M(Gly)$_2$] and [M(Met)] as compared with control and non-chelated micro-nutrient foliar application (Fig. 3). The interaction of [M(Gly)$_2$] and [M(Met)] significantly increase the grain protein concentration (Table 2). This significant increase in protein concentration was also observed in growing year 2. Also significant difference was noted between the [M(Gly)$_2$] and [M(Met)], [M(Met)] effect was stronger than [M(Gly)$_2$] in increment of protein (Fig. 3).

Table 1. Analytical data of metal-amino acid complexes.

Metal-Glycine complexes	**% Found (Calc.)**				**% Yield**
	C	**H**	**N**	**Metal**	
$C_4H_8N_2O_4Zn$ [215.51]	22.41(22.50)	3.71(3.78)	13.17(13.12)	30.65(30.63)	83.22
$C_4H_8N_2O_4Cu$ [211.66]	22.65(22.70)	3.75(3.81)	13.19(13.23)	30.05(30.02)	81.55
$C_4H_8N_2O_4Fe$ [203.96]	23.61(23.55)	3.91(3.95)	13.68(13.73)	30.65(27.38)	84.13
Metal-Methionine complexes					
$C_5H_{10}ClNO_2SZn$ [249.05]	24.14(24.11)	4.11(4.05)	5.67(5.62)	26.21(26.26)	78.02
$C_5H_{10}ClNO_2SFe$ [239.50]	25.05(25.07)	4.25(4.21)	5.88(5.85)	23.35(23.32)	84.82
$C_5H_{10}ClNO_2SCu$ [247.20]	24.21(24.29)	3.99(4.08)	5.61(5.67)	25.69(25.71)	83.67

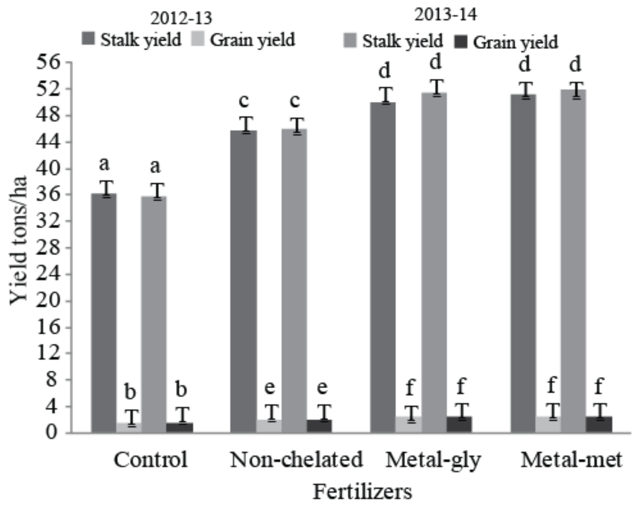

Fig. 1. Effects of different foliar fertilizers on stalk and grain yield comparison with control (no fertilizer) and non-chelated micro-nutrients. Significantly different values are represented by different letters (a, b, c, d, e, f) ($p<0.05$).

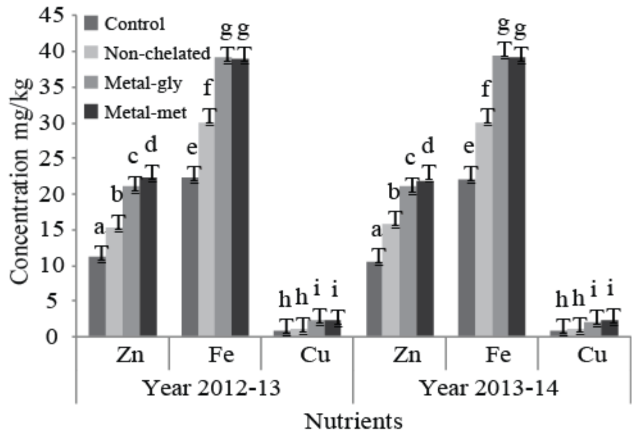

Fig. 2. Effects of foliar metal amino acid chelates and non-chelates on nutrient concentration in comparison with control (no fertilizer) and non-chelated micro-nutrients. Significantly different values are represented by different letters (a, b, c, d, e, f, g, h, i) ($p<0.05$).

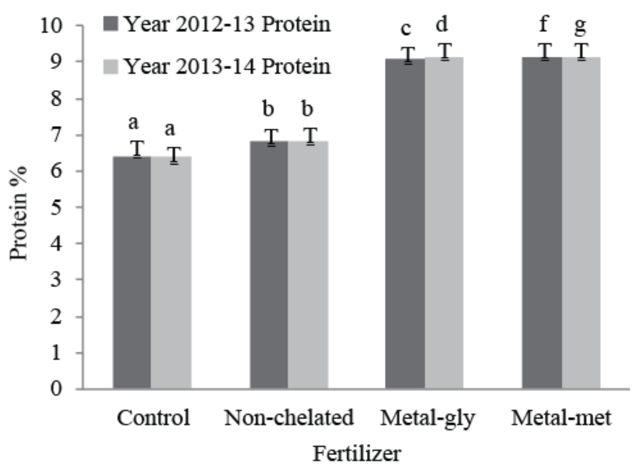

Fig. 3. Effects of foliar metal amino acid chelates and non-chelates on protein concentration in comparison with control (no fertilizer) and non-chelated micro-nutrients. Significantly different values are represented by different letters (a, b, c, d, e, f, g) ($p<0.05$).

Table 3. Correlation coefficients between grain Zn and Fe concentration with protein content.

Micronutrient	Protein	
	Year 1	Year 2
Zn	0.972*	0.954*
Fe	0.960*	0.957*

*Significant at $p<0.05$

Discussion

Foliar application of metal amino acid chelates in crop production is effective agronomical practice which influence substantially on both the yield and grain nutrition quality. Foliar substances penetrate the cuticle and cellulose wall via free diffusion and then these penetrated substances adsorbed to plasma membrane surface and finally adsorb substances are taken into cytoplasm. However, absorption by leaves stomata is also proved, foliar absorption is easier when stomatas are open (Eichert & Burkhardt, 2001; Khoshgoftarmanesh *et al*., 2010). In present study, foliar application of metal (Zn, Fe and Cu) chelated with amino acids (glycine, methionine) was established. The efficacy of synthesized chelated metal amino acid was investigated and their comparison was done with non-chelated foliar micronutrients and control (no fertilizer was added). The yield of stalk and grain along its nutritional quality (trace metals and protein) was increased significantly which indicated the effectiveness of foliar application of metal amino acid as compared with control and non-chelated foliar spray. The increase in stalk and grain yield due to involvement of amino acids in different biological processes such as cell growth, division, embryogenesis and seed development (El-Bassiouny *et al*., 2008). In both year of study, the climatic characteristics were almost same so the stalk and grain yield along with nutritional concentration was not significantly different. Only increase in protein concentration was observed which is might be associated with nitrogen (N) nutritional status improvement. The uptake and translocation of Zn, Fe and Cu in plant tissue is due to N nutritional measurement, amino acids provide the N source for plant nutrition. Nutritional uptake and plant growth is promoted by higher N application. Amino acids are absorbed directly by most of plants and use them in their physiological processes and structure (Kutman *et al*., 2011). The foliar applications are much more efficient for improvement of micronutrients deficiency rather to soil application which is proved in our study and reported earlier (Ghasemia *et al*., 2013). Foliar products containing micronutrient chelated amino acids are cost effective because these produce the better results than soil applications of fertilizers containing these micronutrients with same price. It is now become economic for farmers to spray the micronutrient chelated amino acids for improvement of stalk and grain yield because spraying machinery is easily available not specifically purchased for micronutrient chelated amino acids application. Such kind of spraying machinery is already being practiced for spraying the insecticides, pesticides, fungicides and herbicides. Also the foliar application of micronutrient chelated amino acids maintained the soil quality, agro ecosystem as well as human and animal health. In the present study, Zn and Fe concentration of grains were increased by the application of $[M(Gly)_2]$ and [M(Met)] as compared with non-chelated foliar application which in turn increase the protein concentration. So there is direct or positive correlation between the Zn, Fe and protein. This correlation is significant at $p<0.05$ (Table 3).

Since the objective of present study was to enhance the stalk and grain yield of sorghum to use it as silage crop to fulfill the feed requirement of live stocks in Pakistan which is continuously increasing 6-7% every year. Pakistan is sixth most populous and third largest milk producing country in world, 64% is produced by buffalos and 34% by cows. Unfortunately 0.2 million tons feed is produced against the total feed requirement (40 million tons). So this study was established to get cheaper source of silage for dairy live stocks. This approach is now become practicable to replace the maize with Sorghum for silage because crude protein level (mean = 9.17%) achieved almost equal after foliar application of micronutrient chelated amino acids in two consecutive years.

Table 2. Analysis of variance of yield and grain nutritional quality of Sorghum.

Source	df	Mean square					
		Stalk yield	Grain yield	Zn	Fe	Cu	Protein
Year (Y)	1	0.372 ^{NS}	0.000 ^{NS}	0.053^{NS}	0.041 ^{NS}	0.020 ^{NS}	0.001
Fertilizer (F)	3	102.057	.341	54.71	133.697	.855	4.271
Y x F	3	0.246	0.001	.123	0.035	0.002	5.00E-005

ns = No significant difference at $p<0.05$

Conclusion

The results obtained in present study by the foliar application of metal amino acid chelates indicated that these are the effective source to increase the grain protein, zinc and iron concentration as compared with non-chelated micronutrient foliar. The obtained results presented that the zinc and iron concentration in grain not only increased and also a direct correlation between them and protein concentration. In regard of high consumption of Sorghum as silage crop for forage production, foliar application of $[M(Gly)_2]$ and [M(Met)] can be considered as effective approach for nutrient source which is required by dairy animals to produce milk on sustainable basis.

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