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RESPONSE OF AGRONOMIC BIOFORTIFICATION OF ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* L.) WITH ZINC ON YIELD, NUTRIENT AND QUALITY PARAMETERS IN NORTHEASTERN REGION OF INDIA

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ABSTRACT

A field experiment was carried out at Assam Agricultural University, Jorhat during the *rabi* season of 2021 - 2022 to evaluate the effect of agronomic biofortification of annual ryegrass variety 'Makhan grass' with zinc. The experiment was laid out in randomized block design with 12 treatments and 3 replications. Zinc sulphate ($ZnSO_4$) was applied as basal (5 kg ha^{-1}, 10 kg ha^{-1} and 15 kg ha^{-1}), foliar (0.5%) and in combination of basal and foliar along with the recommended dose of fertilizer (RDF) for annual ryegrass. The multi-cut fodder species was harvested at 60, 90 and 120 days after sowing (DAS) for subsequent analysis. The experiment resulted a significant improvement in plant growth and yield parameters *viz.* height, number of tillers per meter square, periodic dry matter accumulation and green fodder yield with combined application of zinc sulphate as basal and foliar at the rate of 15 kg ha^{-1} and 0.5% at 45 DAS and 15 days after the first cut, respectively along with the RDF. Furthermore, the same treatment recorded the maximum nutrient (N, P, K, Zn) as well as crude protein content and also achieved the highest economic returns. An improvement of zinc content by 14.73 – 19.32% over control under the aforementioned treatment indicated successful biofortification of zinc. From the experiment, combined application of zinc as soil and foliar can be a recommended among the farmers to alleviate micronutrient crisis in the region.

Key words: Economics, Growth, Multi cut fodder, Quality, Yield, Zinc fertilization

Introduction

Livestock sector plays a pivotal role in shaping Indian economy. India accounts for 15% of global livestock population showing an impressive increase at the rate of 4.6%, from 512.06 million in 2012 to 536.76 million in 2019. However, fodder cultivation accounts for only 4% of gross cropped area due to greater emphasis being laid on foodgrain production creating fodder crisis nationwide. According to ICAR – NIANP, there is shortage of green fodder, dry fodder and protein at the rate 36%, 23% and 36%, respectively, which is anticipated to increase to 40%, 23% and 38%, respectively, by 2025 (Bhatta and Kumar, 2020). Inadequate access to high quality feed limits animal from expressing their genetic potential affecting Indian economy. The primary challenge is the limited land availability for green fodder, forcing farmers to rely on crop residues, weeds, and grasses from marginal lands, often overlooking toxicity issues. Additionally, fodder shortages become critical during extreme weather events such as floods, droughts, and temperature stress, exacerbated by climate change often referred as lean period. The rising fodder demand due to a growing livestock population, coupled with stagnant supply driven by increased foodgrain production, population growth, and climate change, threatens animal productivity and subsequently food, nutritional and economic security of the nation. Addressing this gap necessitates innovative strategies, with a key focus on cultivating high-yielding and nutritionally rich fodder species such as annual ryegrass/Italian rye (*Lolium multiflorum* L.) to improve animal productivity.

Originating in Europe, annual ryegrass is a fast-growing, nutrient-rich fodder species with high digestible dry matter, crude protein content, and high adaptability to wide range of soil (Ertekin *et al*., 2022). Its resilience to overgrazing and trampling makes it excellent forage, providing highly palatable green feed. A well-drained fertile soil with a pH between 5.0 and 7.8 is conducive for rye grass cultivation. Beyond pasture use, it can be preserved as hay, silage, or haylage, ensuring a steady feed supply during lean periods (Worku *et al*., 2021).

Fodder production must prioritize both quantity and nutritional quality. Micronutrient deficiency, also known as hidden hunger" is one of the major abiotic stresses that restricts normal metabolism in plants and animals resulting in decline yields. Zinc, in particular, plays a crucial role in auxin metabolism, chlorophyll synthesis, drought resistance, disease defense, and enzyme activation in plants (Rudani *et al*., 2018; Das *et al*., 2019). Zinc deficiency in animals leads to parakeratosis, which is manifested by hard skin lesions. Zinc deficiency with less than 0.6 ppm in Indian soil accounts for 46%, especially in northeastern regions due to leaching as a result of high rainfall and forming precipitates with oxides and hydroxides present in abundance (Behera *et al.*, 2011). Nutrient deficiencies in one trophic level, however is aggravated in the next due to their interconnectedness in the form soil-plant-animal-human continuum, necessitating enrichment of zinc. Therefore, this study aims to enhance the yield and nutritional quality of annual ryegrass through agronomic biofortification with zinc.

Materials and Methods

Crop growing conditions

The experiment was carried out at the Instructional-cum-Research (ICR) Farm of Assam Agricultural University, Jorhat during the *rabi* season of 2021 – 2022. The site is located at 26°45'N latitude and 94°12'E longitude, at an elevation of 87 m above MSL. The soil was uniformly fertile, well-drained, and sandy loam in texture, with a pH of 5.5 (1:2.5, soil-to-water ratio). The chemical investigation of soil reported that the experimental field was low in organic carbon (0.45%), available phosphorus (21.66 kg ha^{-1}), available potassium (150.95 kg ha^{-1}) and DTPA extractable zinc (0.58 mg kg^{-1}), while medium available nitrogen content (306.92 kg ha^{-1}). During the cropping season, weekly maximum and minimum temperatures ranged from 21.2°C to 33.3°C and 8.2°C to 18.9°C, respectively. There were a total of 10 rainy days during the crop growing season with the overall precipitation of 110 mm, being highest (33.7 mm) during the last week of March. The amount of bright sunshine hours ranged from 1.0 to 8.6 hours day^{-1}. The weekly mean evaporation varied from 1.2 mm to 2.9 mm.

Experimental design and crop management

The experiment was laid out in randomized block design (RBD) with 12 treatments and 3 replications. The treatments are: T_1: Control (RDF: N: P_2O_5: K_2O at the rate 60-30-30 kg ha^{-1}), T_2: RDF + 5 kg $ZnSO_4$ ha^{-1}, T_3: RDF + 10 kg $ZnSO_4$ ha^{-1}, T_4: RDF + 15 kg $ZnSO_4$ ha^{-1}, T_5: T_2 + one foliar application of 0.5% $ZnSO_4$ (at 45 DAS), T_6: T_3 + one foliar application of 0.5% $ZnSO_4$ (at 45 DAS), T_7: T_4 + one foliar application of 0.5% $ZnSO_4$ (at 45 DAS), T_8: T_2 + two foliar application of 0.5% $ZnSO_4$ [(1st at 45 DAS and 2nd at 15 days after the first cut (DAFC)], T_9: T_3 + two foliar application of 0.5% $ZnSO_4$ (1st at 45 DAS and 2nd at 15 DAFC), T_{10}: T_4 + two foliar application of 0.5% $ZnSO_4$ (1st at 45 DAS and 2nd at 15 DAFC), T_{11}: RDF + Water spray at 45 DAS and T_{12}: RDF+ Water spray (1st at 45 DAS and 2nd at 15 DAFC). The dimension of each plot was 4m x 3m with spacing of 0.5 m between each plot. The sowing of annual ryegrass variety 'Makhan grass' was performed on 30th November, 2021 by adopting line sowing method at the rate of 20 kg ha^{-1} with a row spacing of 30 cm. The intercultural operations, including irrigation, plant protection and fertiliser management, were carried out in accordance with established protocols (Chandrasekaran *et al.*, 2010). The recommended dose of fertilizer (RDF) @ 60-30-30 kg N, P_2O_5 and K_2O per hectare were applied as urea, SSP and MOP. One third of urea was applied along with full dose of SSP, MOP and $ZnSO_4$ (5, 10 and 15 kg ha^{-1}) at the time of final land preparation. Of the remaining urea, two split applications as one third was applied after the first and second cut. Foliar spray of $ZnSO_4$ at the rate of 0.5% was applied at 45 days after sowing (DAS) and 15 days after first cut (DAFC).

Data collection and laboratory analysis

Observations for plant height were measured from the base of the plant to the tip of the longest leaf and were expressed in centimeters (cm). The number of tillers was counted in running meters at each cut by randomly selecting rows of a particular plot. For periodic dry matter accumulation, five plants were randomly selected from each plot, excluding the boundary rows. The samples were shade dried for 3-4 days and subsequent oven drying at 65 ± 5°C until a constant weight is achieved. After

Table 1: Growth parameters of annual rye grass as influenced by agronomic biofortification with zinc.

Treatments	Plant height (cm)			Number of tillers per meter square			Periodic dry matter accumulation (g plant^{-1})		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
T_1	39.21	55.88	49.62	175.66	196.75	178.62	3.62	4.73	4.30
T_2	41.87	58.63	51.61	187.56	206.43	185.80	3.95	5.03	4.53
T_3	42.38	58.87	52.64	189.86	207.28	189.51	4.02	5.06	4.65
T_4	44.43	61.04	55.96	199.05	214.90	201.46	4.28	5.29	5.02
T_5	44.56	63.17	51.89	199.63	222.42	186.82	4.29	5.52	4.56
T_6	47.35	63.41	53.34	212.11	223.25	192.03	4.65	5.55	4.73
T_7	49.66	65.67	56.39	222.48	231.21	203.02	4.93	5.79	5.07
T_8	45.17	67.84	52.39	202.36	238.86	188.62	4.37	6.03	4.62
T_9	47.58	68.58	54.01	213.14	241.45	194.43	4.67	6.11	4.80
T_{10}	49.68	70.72	56.93	222.54	249.00	204.95	4.94	6.34	5.13
T_{11}	39.84	56.47	49.79	178.48	198.83	179.25	3.70	4.80	4.32
T_{12}	38.98	55.51	49.32	174.63	195.45	177.57	3.59	4.69	4.27
SEM	0.688	0.672	0.548	3.081	2.367	1.971	0.087	0.073	0.062
LSD(P<0.05)	2.017	1.972	1.606	9.035	6.942	5.781	0.255	0.214	0.182

that, their average dry weights were converted to gram per plant. 'Makhan grass' is a multicut variety and hence, was harvested at 60, 90 and 120 DAS. Crop was harvested at 5 cm above to ground allowing sufficient regrowth to occur. Fresh weight of the harvested plants in net plot was recorded for green fodder yield (GFY) and expressed in q ha^{-1}. The representative plant samples collected from each plot during harvest was studied for its nutrient and quality parameters post oven drying, grinding and passing through a 0.5 mm sieve. The total nitrogen content was analyzed by following the micro Kjeldahl method (Jackson, 1973). For the analysis of phosphorus content, the samples were digested in HNO_3 and $HClO_4$ prior to determining by ammonium molybdate method (Olsen and Sommers, 1982). The flame photometry method was adopted for estimating the potassium content in the samples (Jackson, 1973). Finally, the nutrient of interest *i.e.*, zinc content was analyzed with the help of atomic absorption spectroscopy (AAS) after extracting it with DTPA (diethylenetriaminepentaacetic acid) solution (0.005MDTPA + 0.01M$CaCl_2$ + 0.1M triethanolamine, pH 7.3) (Lindsay and Norvell, 1978). The crude protein content was calculated from the product of nitrogen content and a factor of 6.25. The analysis for crude fibre and crude fat was performed according to the AOAC (1975).

Analysis of economics

The cost of cultivation and farm return were based on the variable costs. The input cost included rental values of land, seed, fertilizers, manures, labour, machinery, irrigation, plant protection, harvesting etc. Further, the net returns were computed on per hectare basis by subtracting the cost of cultivation from gross returns based on the prevailing market price of green fodder. Benefit-cost ratio was calculated for each treatment by dividing gross return with the total cost of cultivation.

Statistical analysis

The data collected on various parameters were evaluated statistically following the analysis of variance for randomized block design as suggested by Gomez and Gomez (1984). The "F" value was used to test for statistical significance at the 5% level of probability. Wherever the "F" values were found significant, critical difference was worked out at 5% level of probability and the values are furnished. Non-significant treatment differences were represented as NS.

Results and Discussion

Growth parameters

The results for the growth parameters *viz.*, plant height, number of tillers per meter square and periodic dry matter accumulation showed a significant increase with the combined application of $ZnSO_4$ along with RDF

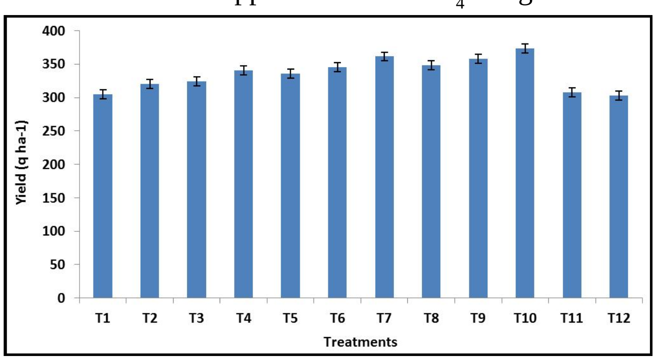

Fig. 1: Total green fodder yield of annual ryegrass as influenced by agronomic biofortification with zinc.

Table 2: Green fodder yield of annual rye grass as influenced by agronomic biofortification with zinc.

Treatments	Green fodder yield (q ha^{-1})		
	First cut	Second cut	Third cut
T_1	78.42	116.42	110.26
T_2	83.73	122.15	114.69
T_3	84.76	122.65	116.98
T_4	88.86	127.16	124.36
T_5	89.12	131.61	115.32
T_6	94.69	132.1	118.54
T_7	99.32	136.81	125.32
T_8	90.34	141.34	116.43
T_9	95.15	142.87	120.02
T_{10}	99.35	147.34	126.51
T_{11}	79.68	117.65	110.65
T_{12}	77.96	115.65	109.61
SEM	1.375	1.400	1.217
LSD(P<0.05)	4.034	4.107	3.569

as basal and foliar at the rate of 15kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}) in all the cuts (Table 1). This result reported due to the enhanced synthesis of auxin and tryptophane under the influence of zinc that is responsible for cell enlargement, stem elongation and photosynthetic activity (Bhoya *et al.,* 2013). Moreover, greater number of tillers per meter square was observed with each cut due to the promotion of development of new shoots. The significant increase in the growth parameter with zinc application over control was also reported by Mahato *et al*., (2020) and Sher *et al.,* (2022).

Green fodder yield

The response of yield under zinc fertilization on annual ryegrass at all cuts indicated that green fodder yield (99.35 q ha^{-1}, 147.34 q ha^{-1} and 126.51 q ha^{-1} at 60, 90, and 120 DAS, respectively) were significantly highest with the combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15 kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}) (Table 2). Likewise, the total green fodder yield (373.20 q ha^{-1}) was also significantly highest under the same treatment (Fig. 1). According to Ahmad *et al.,* (2018) application of zinc resulted 7.15 – 7.41% increase in green fodder yield. The exogenous application of zinc has promoted the growth parameters, auxin synthesis and photosynthetic efficiency which might be the reason for this effect (Augustine and Kalyanasundaram, 2021). Zinc application has improved the availability of nutrients in the rhizosphere, allowing for enhanced crop uptake and utilisation, which has improved photosynthesis (Porwal *et al.*, 2024). Improvements in photosynthetic efficiency and translocation of photosynthates have enhanced yield attributing characters that may have augmented the production of green fodder. According to Dhaliwal *et al.*, (2020), there was a significant improvement in the herbage biomass yield when oat was fertilized with zinc at 60 and 90 DAS.

Nutrient content

The laboratory analysis for nutrient content at the first, second and third cut revealed that nitrogen (2.114%, 2.310 and 1.923% respectively), potassium (1.192%, 1.158% and 1.001%, respectively) and zinc content (32.962 mg kg^{-1}, 31.960 mg kg^{-1}and 30.362 mg kg^{-1}, respectively) in the plant sample were significantly highest with combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15 kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}) (Table 3). The improved cation exchange capacity of roots and enhanced absorption and

Table 3: Nutrient content in annual rye grass as influenced by agronomic biofortification with zinc.

Treatments	N content (%)			P content (%)			K content (%)			Zn content (mg kg^{-1})		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
T_1	1.669	1.825	1.676	0.271	0.277	0.259	0.941	0.915	0.872	27.879	26.783	26.462
T_2	1.782	1.915	1.743	0.272	0.278	0.262	1.005	0.960	0.907	29.296	27.759	27.526
T_3	1.804	1.923	1.778	0.273	0.279	0.267	1.017	0.964	0.925	29.335	27.854	28.075
T_4	1.891	1.994	1.890	0.276	0.283	0.271	1.066	0.999	0.984	30.336	28.655	29.846
T_5	1.896	2.064	1.753	0.279	0.281	0.264	1.069	1.034	0.912	30.410	29.457	27.677
T_6	2.015	2.071	1.802	0.284	0.285	0.269	1.136	1.038	0.938	31.871	29.550	28.450
T_7	2.113	2.145	1.905	0.287	0.289	0.271	1.191	1.075	0.991	32.942	30.347	30.077
T_8	1.922	2.216	1.770	0.281	0.293	0.266	1.084	1.111	0.921	30.812	31.143	27.943
T_9	2.025	2.240	1.824	0.285	0.296	0.270	1.142	1.123	0.949	31.933	31.168	28.805
T_{10}	2.114	2.310	1.923	0.289	0.298	0.276	1.192	1.158	1.001	32.962	31.960	30.362
T_{11}	1.696	1.845	1.682	0.271	0.277	0.260	0.956	0.925	0.875	28.294	26.903	26.556
T_{12}	1.659	1.813	1.666	0.269	0.276	0.258	0.936	0.909	0.867	27.727	26.722	26.306
SEM	0.029	0.022	0.018	0.008	0.008	0.007	0.016	0.011	0.010	0.317	0.257	0.292
LSD(P<0.05)	0.086	0.064	0.054	NS	NS	NS	0.048	0.032	0.028	0.929	0.755	0.856

Table 4: Quality parameters of annual rye grass as influenced by agronomic biofortification with zinc.

Treatments	Crude proteincontent (%)			Crude fibre content (%)			Crude fat content (%)		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
T_1	10.430	11.409	10.475	13.47	13.74	14.26	3.48	3.66	3.78
T_2	11.136	11.971	10.896	12.01	12.65	14.07	3.53	3.68	3.80
T_3	11.273	12.020	11.113	11.92	12.57	14.05	3.52	3.69	3.80
T_4	11.818	12.462	11.814	11.89	12.50	14.02	3.55	3.71	3.84
T_5	11.853	12.898	10.955	11.90	12.56	14.03	3.55	3.73	3.80
T_6	12.594	12.946	11.261	11.81	12.42	14.00	3.25	3.73	3.81
T_7	13.210	13.407	11.905	11.66	12.33	13.93	3.62	3.75	3.85
T_8	12.015	13.851	11.061	11.78	12.41	13.99	3.56	3.78	3.80
T_9	12.655	14.001	11.402	11.70	12.35	13.93	3.59	3.78	3.82
T_{10}	13.214	14.439	12.018	11.32	12.31	13.92	3.62	3.80	3.85
T_{11}	10.597	11.530	10.512	13.36	13.65	14.24	3.49	3.67	3.77
T_{12}	10.369	11.334	10.413	13.08	13.81	14.31	3.48	3.66	3.77
SEM	0.183	0.137	0.116	0.296	0.032	0.053	0.132	0.244	0.093
LSD(P<0.05)	0.536	0.403	0.339	0.868	0.094	0.157	NS	NS	NS

translocation of nutrients on the administration of zinc might be the reason for such an effect (Chand *et al.*, 2018). Notably, it can be mentioned that the synergism that exist between nitrogen and zinc, and potassium and zinc has increased the nutrient content in the fodder. These results are in conformity with Anwar *et al.* (2021) and Chaudhary *et al.*, (2021). In case of phosphorus, zinc application did not show any significant effect. However, it was highest (0**.**289% at first cut, 0.298% at second cut and 0.276% at third cut**)** with the treatment T_{10} since synergism between phosphorus and zinc occur only at their lower concentrations. When the concentration of either of the nutrient is high, the synergism no longer exists and antagonistic relationship sets in (Paramesh *et al.*, 2014).

Quality parameters

Analysis for quality parameters were presented in Table 4. Experimental data revealed that crude protein content was significantly highest at first (13.21%), second (14.44%) and third cut (12.02%) with combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}) due to the direct involvement of zinc in enhancing the nitrogen content within the plant that has led to subsequent increase in the crude protein content (Kumar *et al.*, 2015). This effect might be due to the conspicuous contribution of zinc to plant nitrogen metabolism, which in turn elevated the crude protein content (Kumawat *et al.*, 2017). Due to synergistic effect of zinc with nitrogen, the application of zinc increased the nitrogen absorption by the plant from the rhizosphere which in turn improved the crude protein content in plant (Sharifi *et al.*, 2016).

The crude fibre content was significantly lowest at all the cuts (13.47% at first cut, 13.81% at second cut and 14.31% at third cut) with the combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}). However, the crude fat content was found non-significant in all the three cuts. Zinc is responsible for increasing nitrogen metabolism that maintains the succulency of the crop by lowering the content of pectin, cellulose and hemicellulose and hence results depicted that zinc administration reduced the crude fibre content (Almodares *et al.*, 2009). Forage materials with lower crude fiber content and higher crude protein content are actually highly palatable and digestible. As reported, the

Table 5: Comparative economics of different treatment combinations in the experiment.

Treatments	Cost of cultivation (Rs./ha)	Gross return (Rs./ha)	Net return (Rs./ha)	B-C ratio
T_1	25359	61020	35661	2.41
T_2	26228	64114	37886	2.44
T_3	26628	64878	38250	2.44
T_4	27028	68076	41048	2.52
T_5	27056	67210	40154	2.48
T_6	27456	69066	41610	2.52
T_7	27856	71929	44073	2.58
T_8	27884	69622	41738	2.50
T_9	28284	71608	43324	2.53
T_{10}	28684	75386	46702	2.63
T_{11}	25828	61596	35768	2.38
T_{12}	26297	60644	34347	2.31
SEM	-	352	352	0.013
LSD(P<0.05)	-	1033	1033	0.038

crude fiber content was found to increase with age (Anwar *et al.*, 2021), and thus was found to be more in 120 DAS as compared to 60 and 90 DAS.

Economics

A perusal of data indicated that the highest cost of cultivation (Rs. 28,684/ha) was obtained under combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}) and the lowest (Rs. 25,359/ha) was obtained in control (T_{10}) (Table 5). This might be due to higher application doses of $ZnSO_4$ under T_{10}. The significantly highest gross return (Rs. 75,386/ha) and net return (Rs. 46,702/ha) were recorded under treatment T_{10} and the lowest gross (Rs. 60,644/ha) and net return (Rs. 34,347/ ha) were observed under treatment T_{12} (RDF + water spray at 45 DAS and 15 DAFC). Similarly, the significantly highest benefit-cost ratio (2.63) was achieved under combined application of $ZnSO_4$ along with RDF as basal and foliar at the rate of 15kg ha^{-1} and 0.5% at 45 DAS and 15 DAFC (T_{10}), whereas the lowest (2.31) was recorded under T_{12}. The economic superiority observed under the treatment T_{10} was due to increased green fodder yield integrated with improved monetary returns and benefit-cost ratio. Similar results were also reported by Mahato *et al*., (2020) and Sher *et al*., (2022).

Conclusion

From the present experiment, it is clear that soil and foliar application of zinc, has a tremendous effect not only in improving the growth parameters and yield but also in enhancing the nutrient content and quality traits of annual ryegrass. In regions where zinc deficiency is acute, its application can improve the crop yield and productivity and play a critical role in bringing the concept of nutritional and feed security into reality. In annual ryegrass production, combined application of 15 kg ha^{-1} of zinc sulphate as basal along with two foliar sprays at the rate 0.5% at 45 DAS and 15 days after the first cut, in addition to RDF, can be recommended for producing highly nutritious quality fodder with higher economic return. Thus, it can be concluded that zinc application successfully biofortified annual ryegrass under zinc deficient soils of northeastern region.

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