



Enhancing Animal Nutritional Security Through Biofortification in Forage Crops: A Comprehensive Review

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ABSTRACT

Livestock nutrition is crucial for sustaining the health and productivity of farm animals, which are a cornerstone of the global food supply. Proper nutrition ensures that animals receive the necessary nutrients for essential bodily functions, growth, reproduction and lactation. Tailored, balanced diets that cater to the specific nutritional needs of different livestock species and their developmental stages also boost reproductive performance, leading to higher birth rates and healthier offspring. Conversely, inadequate nutrition can lead to stunted growth, diminished productivity and increased vulnerability to diseases, ultimately resulting in significant economic losses. Nutritional imbalances among animals, especially in dairy goats and cattle, pose a significant challenge in livestock management. These disorders result from inadequate or imbalanced nutrient intake, leading to a range of metabolic and health issues. Fodders are essential to livestock nutrition, providing a balanced diet that supports overall animal health, growth and productivity. They are primary sources of energy, with grasses and cereals supplying carbohydrates needed for maintenance and production activities. Leguminous fodders like alfalfa and clover are rich in protein and crucial for muscle development, milk production and growth. Additionally, green fodders offer vital vitamins (A, D, E, K) and minerals such as calcium and phosphorus, which are necessary for various metabolic functions. The high fibre content in fodders aids in proper digestion and prevents digestive disorders. Overall, the review highlights the impact of various nutrient deficiencies on livestock, including the effects of anti-nutritional factors and the mechanisms of nitrate, oxalate, and prussic acid toxicity in animals. It underscores the importance of agronomic bio fortification as a promising strategy to enhance the nutritional quality of fodder crops, thereby improving animal health and welfare, while also contributing to food security and sustainable agriculture.

Key words: Anti-nutritional factors, Bio fortification, Forage quality, Livestock health and productivity, Livestock nutrition.

Livestock is crucial in India's agricultural landscape, significantly contributing to millions of farmers' rural economy and livelihoods. According to the 20th Livestock Census, the total Livestock population is 535.78 million in the country showing an increase of 4.6% over the Livestock Census 2012. The total number of cattle in the country was 192.49 million in 2019, a rise of 0.8% over the previous Census (Statistics, 2019). India is the largest milk producer in the world, the livestock sector accounts for about 4.11% of the national GDP and 25.6% of the agricultural GDP, underscoring its economic importance. Dairy farming is particularly prominent with India being the largest milk producer in the world, producing around 188 million tonnes annually. This sector not only provides nutritional security but also generates substantial employment, particularly for women and smallholder farmers. In addition to dairy, the country is a significant producer of meat, eggs and wool, catering to both domestic consumption and export markets.

Fodders are essential to livestock nutrition, providing a balanced diet that supports overall animal health, growth and productivity. They are primary sources of energy, with grasses and cereals supplying carbohydrates needed for maintenance and production activities. Leguminous fodders like alfalfa and clover are rich in protein and crucial for muscle development, milk production and growth.

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Additionally, green fodders offer vital vitamins (A, D, E, K) and minerals such as calcium and phosphorus, which are necessary for various metabolic functions. The high fibre content in fodders aids in proper digestion and prevents digestive disorders. Different types of fodders, including green fodder, silage, hay and crop residues, provide versatility in feed, ensuring year-round availability and nutritional adequacy (Kumar *et al.*, 2016). Green fodder is highly palatable and nutrient-rich, while silage and hay, preserved through fermentation and drying respectively, are critical during off-seasons. Crop residues like straw are efficiently utilized, reducing waste and promoting sustainability. Proper nutrition from fodder enhances growth rate, reproductive health, milk yield and disease resistance, contributing to the overall productivity and profitability of livestock farming (Bouis *et al.*, 2011). Cultivation and utilization of fodder are cost-effective, reducing dependence on expensive commercial feeds and promoting local agriculture (Karthikeyan *et al.*, 2024). Sustainable practices in fodder production improve soil health through crop rotations and nitrogen fixation by legumes, enhancing farm biodiversity and resilience to climate variability. Efficient fodder management, including quality control and preservation techniques, ensures a continuous supply of high-quality feed, essential for animal welfare and optimal production (Ravi *et al.*, 2024). Encouraging farmers to utilize locally grown fodders can help them minimize their carbon footprint and cultivate sustainable farming communities, thereby bolstering the economic viability and environmental sustainability of livestock farming.

Despite being the world's largest milk producer, the animal productivity of India (1538 kg/year) is lower than the global average (2238 kg/year), which may be related to malnutrition because of the massive feed shortage. The country is experiencing a net deficit of 30.65% of green fodder, 24.6% of crude protein and 19.87% of total digestible nutrients (Statistics, 2019). The excessive application of chemical fertilizers devoid of micronutrients to meet the increased demand for forage has negatively impacted soil

health and decreased crop yield (Ravi *et al.*, 2023). As population pressure rises, more land is allocated to food and cash crop cultivation, leaving limited arable land for fodder production. This highlights the importance of focusing on fodder production in modern agriculture. To meet the high demand for fodder, excessive and continuous application of large quantities of high-analysis chemical fertilizers has had detrimental effects, resulting in decreased productivity due to nutrient limitations. Consequently, it faces major difficulties in optimizing the use of available land to produce adequate and high-quality animal feed. Hence, to address the critical gap in crude protein and total digestible nutrients (TDN) availability in India as given in Fig 1, it is essential to prioritize the biofortification of fodder crops. Enhancing the nutritional quality of these crops through biofortification can significantly improve livestock productivity and contribute to the sustainability of the agricultural sector. This approach is crucial for meeting the growing nutritional demands of livestock and ensuring food security. Thus, the main objective of this review is to explore the different methods used for biofortifying fodder crops in agronomical approaches. The expected scenario for the availability of total digestible nutrients (TDN) and crude protein (CP) as well as their potential deficiencies are given in Fig 1.

Importance of livestock nutrition

The health and productivity of farm animals, which underpin the world's food supply are deeply reliant on proper livestock nutrition (Liu *et al.*, 2021). Adequate nourishment ensures that animals receive the essential nutrients needed for critical physiological functions, growth, reproduction, and lactation. Balanced and species-specific diets, tailored to the varying developmental stages of livestock, further enhance reproductive outcomes, leading to higher birth rates and healthier offspring (Ajaykumar *et al.*, 2024). Conversely, poor nutrition can result in stunted growth, reduced productivity and increased vulnerability to diseases, all of which can lead to substantial financial

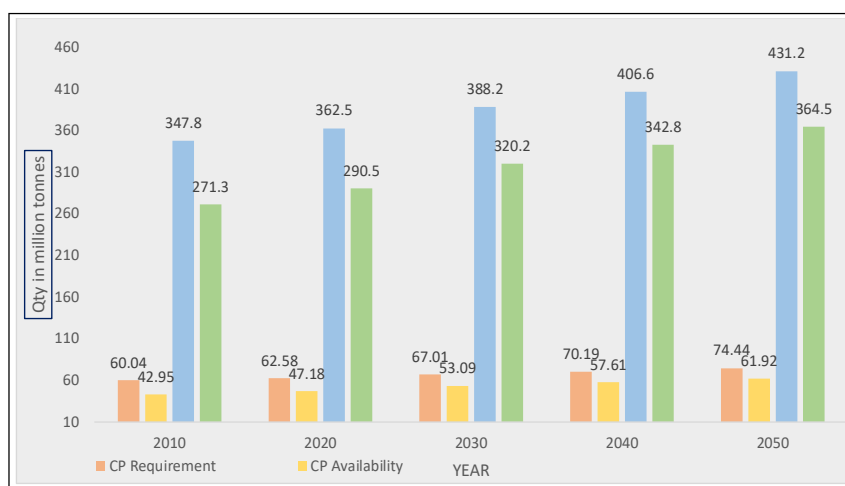


Fig 1: Projected requirement, availability and deficit of CP and TDN (million tonnes).

Table 1: Impact of deficiency of different nutrients on livestock.

Nutrient	Symptoms
Nitrogen	Stunted growth and poor weight gain, reduced milk yield and lower milk protein content (Liu <i>et al.</i> , 2021).
Phosphorous	Lack of phosphorus can lead to "pica," which includes symptoms such as decreased appetite, stunted growth, lower milk production, and reduced fertility (Jain and Mudgal, 2021).
Potassium	Lower reproductive efficiency in livestock (Talukdar <i>et al.</i> , 2016).
Calcium	The primary cause of milk fever, a metabolic disorder that occurs shortly after calving, can lead to decreased milk production, impaired fertility and a higher risk of other health issues. Furthermore, a lack of calcium can weaken the immune system, potentially raising the risk of mastitis (Fekata, 2021). When calcium levels are low, calcium from bones enters the blood and new bone forms to replace the loss. Without sufficient calcium, bones aren't properly replenished, leading to weakened structure. Calcitonin helps reduce blood calcium by promoting its deposition in bones. Factors like sex hormones, calcitonin, vitamin D and calcium-rich foods support bone density. Inadequate calcium intake can cause osteoporosis, making bones thin and fragile. Osteoporosis is a progressive bone disease primarily driven by genetic factors and a prolonged deficiency of dietary calcium. This condition causes bones to become extremely porous, fragile and increasingly susceptible to fractures, with a slower healing process. It can also lead to a decrease in height due to the collapse of spinal vertebrae, often resulting in a hunched posture. Chronic low calcium levels weaken bones, diminishing their strength and density, which ultimately leads to the onset of osteoporosis (Pandey, 2011).
Magnesium	Hypomagnesemia can result in disorders like grass tetany and milk fever and in severe instances, it may be fatal (Pinotti <i>et al.</i> , 2021).
Zinc	A lack of zinc reduced growth rates, hindered reproductive performance and increases susceptibility to diseases (Duffy <i>et al.</i> , 2023).
Iron	Iron deficiency in dairy cattle can cause anemia, impacting hematological parameters and growth rates (Allan <i>et al.</i> , 2020).
Copper	Copper is needed for proper immune development including the formation of antibodies and white blood cells in addition to antioxidant enzyme production. Copper deficient cattle are more susceptible to infections and do not respond as well to vaccinations. In addition, they tend to be less resistant to parasitic challenge (Spears, 2000). Studies have shown that cattle receiving proper copper nutrition tend to be less susceptible to infections and have less severe infections when disease does occur. Cows with copper deficiency showed reduced hematological parameters, such as lower erythrocyte counts, hemoglobin levels and leukocyte numbers (Abramowicz <i>et al.</i> , 2019).
Selenium	Lacking these antioxidants can result in oxidative stress, weakened immune function and reproductive issues (Dhara <i>et al.</i> , 2022).

losses (Table 1). Moreover, the quality of animal products, such as meat and dairy, is directly influenced by the diet of the livestock for instance, specific nutrients can affect milk composition and meat marbling.

A significant challenge in livestock management is addressing nutritional imbalances, particularly in dairy goats and cattle. These imbalances, often resulting from inadequate or unbalanced diets, can lead to a range of metabolic and physiological disorders (Simoes and Gutierrez, 2017). For example, deficiencies in vitamins and minerals among grazing sheep can increase their susceptibility to oxidative stress, metabolic disorders and other health issues (Masters, 2018). The cumulative effects of these nutritional deficiencies can have serious repercussions for both animal and human health. Implementing a well-balanced diet and nutrition plan, designed to avoid nutrient toxicities, is crucial for preventing and managing these diseases (Awuchi, 2020).

Fodder and forage alternatives play a crucial role in cattle nutrition, providing essential nutrients needed for

growth, reproduction and overall health (Saikanth *et al.*, 2023). Nutrient deficiencies in fodder can significantly impact its growth and development, leading to reduced yield and quality (Bhaumik *et al.*, 2024). This, in turn, directly affects the productivity and well-being of cattle, as well as the nutritional value of the animal products consumed by humans (Reddy *et al.*, 2023). Addressing nutrient deficiencies in fodder through appropriate management practices and agronomic interventions is vital for ensuring the efficiency and health of both livestock and people. Additionally, micronutrient deficits in agricultural soils can diminish the nutritional quality of crops, potentially contributing to malnutrition in humans (Bouis *et al.*, 2011).

Biofortification of fodder crops

Biofortification involves enhancing the content and/or bioavailability of essential nutrients in crops during their growth phase by utilizing genetic and agronomic methods (Bouis *et al.*, 2011). Agronomic biofortification in fodder crops is crucial as it enhances nutrient content through sustainable

farming practices, improving livestock nutrition without the need for genetic modification. This method can be quickly implemented and adapted to various environmental conditions, making it a cost-effective and accessible solution. Additionally, it promotes soil health and reduces the reliance on chemical fertilizers, contributing to overall agricultural sustainability (Jadhav *et al.*, 2020).

Techniques of Agronomic bio fortification

Agronomic biofortification, a key component of biofortification, involves enhancing the nutrient content of crops through soil and foliar application of fertilizers, soil inoculation with beneficial microorganisms and other techniques.

Fertilization in fodder crop biofortification

One of the most significant factors that directly affect the quantity and quality of fodder is the application of fertilizer. The response of dry matter production in maize to the fertilizer rate was linear (Kumar *et al.*, 2016).

Nitrogen

Nitrogen is a critical nutrient that significantly enhances both crop improvement and fodder crop production. Nitrogen is essential for the formation of several structural elements, including molecules, proteins, amino acids, chlorophyll and other components.

Nitrogen fertilization is essential for improving the quality of forage, especially in terms of dry matter production and the concentration of crude protein (Maheswari *et al.*, 2017). However, the impact of nitrogen on forage quality can vary depending on the specific crop and the presence of other nutrients such as phosphorus (Aydin and Uzun, 2005). For example, in cluster bean varieties, nitrogen application significantly increased forage yield and quality, with the variety BR-99 showing the highest yield and protein content. Likewise, applying nitrogen fertilizer to native pasture overseeded with ryegrass enhanced forage qualities and the performance of beef calves (Brambilla *et al.*, 2012). These results emphasize the significance of nitrogen fertilization in improving forage quality, while also highlighting the importance of a balanced approach that considers the particular crop and the presence of other essential nutrients.

Soil application

When 25 t/ha FYM was applied, the number of micronutrients such as zinc, copper, manganese and iron in fodder maize was improved by 15.3%, 7.5%, 28.4% and 15.6% respectively relative to the control, but declined when the amount of nitrogen increased. They have also reported that the application of FYM 25 t/ha and nitrogen 120 kg/ha results in increased crude protein yield (kg/ha) by 33.78% and 36.56% respectively (Knez and Graham, 2012).

Vennila *et al.* (2017) stated that the application of both organic and inorganic sources of nutrients to bajra Napier hybrid grass increases yield, yield attributes and nutrient uptake. It was found that the application of nitrogen at the rate of 180 kg/ha results in maximum crude protein contents

(10.52%) which might be due to enhanced production of amino acid resulting from nitrogen application and the highest percentage of crude protein (11.54%) and lowest percentage (7.84%) were observed at 45 and 65 days after sowing, respectively (Swathi *et al.*, 2015).

Livestock nutritional response

Nitrogen enhancement in forage for livestock has been shown to have a positive impact on forage production and livestock performance. Brambilla *et al.* (2012) found that nitrogen fertilization increased forage accumulation rate and production, leading to improved livestock performance. Delevatti *et al.* (2019) similarly reported that increasing nitrogen levels in Marandu grass improved herbage mass forage quality and animal production. However, Jacobs and Ward, (2011) noted that the effect of nitrogen application on forage crops was limited by available moisture, with variable dry matter yield responses. Rouquette and Smith, (2010) highlighted the role of biological nitrogen fixation in reducing input costs for forage production, particularly in cow-calf and stocker programs.

Zinc

Zinc plays a crucial role in the growth and development of fodder crops, particularly maize and cowpea, by improving their yield and quality (Rathore *et al.*, 2015). Furthermore, it has been noted that zinc is essential for various vital processes in plants, such as protein synthesis and gene expression (Ahmad and Tahir, 2019). Therefore, the inclusion of zinc in the fertilization and management of fodder crops is crucial for their optimal growth and nutritional value.

Importance of dietary zinc in livestock

The insufficient presence of zinc in forage can lead to substantial effects on livestock, including decreased growth and reproduction, as well as impaired health of bone and skin tissues. The easiest and latest practice to deal with micronutrient deficiency in crops is the application of micronutrient fertilizers (Gupta *et al.*, 2008). Enhancing the micronutrient content of fodder crops through foliar application is a promising and economical method of agronomic biofortification, which will improve animal health and productivity Singh *et al.* (2023). The agro-qualitative characteristics of fodder are affected by the micronutrient content thereby directly impacting animal productivity. Therefore, fortifying plants with zinc and copper presents a superior approach to addressing their deficiency in both green and dry fodder (Kumar *et al.*, 2016).

Soil application

The effect of varying doses of zinc fertilizer on plant growth is different among the crops because the response of fodder crops depends on the application of zinc fertilizers (Mohan *et al.*, 2015). Applying 20 kg/ha of ZnSO₄ heptahydrate as a basal dose result in 31.3% higher zinc content and 50.9% greater zinc uptake compared to the control, along with the highest levels of crude protein, ether extract and ash content in fodder (Kumar *et al.*, 2017).

Application of zinc at a rate of 10 kg/ha to fodder maize significantly improved plant height, leaf area index and green fodder yield (Table 2-3). Additionally, it increased the content and uptake of nitrogen and zinc, while calcium and crude protein content remained unchanged (Sheraz *et al.*, 2001). Higher crude protein yield which is due to the combined effect of more crude protein content in plants, dry-matter yield, and leafless plants and higher digestible dry-matter yield because of higher digestibility% and higher dry-matter yield was recorded in fodder sorghum with the application of 5 kg Zn/ha (Verma *et al.*, 2005).

Agronomic management of anti-nutritional factors

Anti-nutritional factors are substances that, either on their own or through their metabolic by products, hinder feed utilization, negatively impacting animal health and production. These factors reduce nutrient intake, digestion, absorption and utilization, potentially causing other adverse effects (Kumar *et al.*, 2017).

Antinutritional factors in forages

Nitrate toxicity

Nitrate toxicity in livestock can have severe consequences on their health and productivity. Even though nitrate by itself is not harmful to animals, rumen microorganisms in ruminants convert nitrate to nitrite, which they then use as a source of nitrogen by turning it into ammonia (Lee and Beauchemin, 2014).

The excess uptake of nitrate by plants beyond the limit leads to the accumulation of nitrate at toxic concentrations. Plant density/seed rate, genotypes (variety) and nitrogen management are the agronomic practices that influence nitrate toxicity (Oberoi and Kaur, 2019). When nitrogen fertilizers are used on sorghum and oats, the unabsorbed nitrates remaining on the plant surface become a substantial source of nitrates in the diet of animals (Kamra *et al.*, 2015).

Brassica rapa has the lowest nitrate concentration, while *Sorghum bicolor*, *Avena sativa* and *Brassica*

campestris had the highest. Compared to stem parts, young plants, and plant samples taken in the morning, the concentrations of nitrate were lower in the afternoon in the leaves and mature crops (Rashid *et al.*, 2019). The highest concentration of nitrate is found in stalks, with leaves and grain following in descending order. The nitrate content of sorghum Sudan grass, piper sudan grass and pearl millet is three times higher in the lower six inches of their stems than in the upper part of the plant (Kumar *et al.*, 2018).

The effect of irrigation and nitrogen on bajra proved that the nitrate level was significantly lower in irrigated conditions and at an application of 100kg N/ha when compared to the toxicity level (Table 4). However, the amount of nitrate in the unirrigated condition was more than in the irrigated condition and in both cases rise in nitrate as nitrogen levels increased (Rashid *et al.*, 2019).

Mechanism of Nitrate toxicity in animals

When forages have an unusually high concentration of nitrate, the animal cannot complete the conversion and nitrite accumulates. As nitrite accumulates and enters the bloodstream, it combines with a ferrous ion (Fe⁺²) of haemoglobin (Hb) to create methemoglobin (met-Hb) which is incapable of carrying oxygen (Sidhu *et al.*, 2011). As a result, the blood turns a chocolate brown colour instead of the typical bright red. An animal succumbing to nitrate (nitrite) poisoning actually dies from asphyxiation or a lack of oxygen (Kumar *et al.*, 2018).

Oxalate toxicity

Frequent ingestion of an excessive amount of oxalic acid can lead to serious intestinal lining irritation and nutritional deficits in animals (Oberoi and Kaur, 2019). A few nutrients such as N, K, Na and Ca have a role in the buildup of oxalate in forage. Oxalic acid forms strong bonds with various minerals, including calcium, magnesium, sodium and potassium. This chemical interaction leads to the creation of oxalate salts (Kumar *et al.*, 2018). When dietary

Table 2: Effect of fertilizer spraying on the quality of forage maize.

	Leaf chlorophyll (SPAD units)	Total dry biomass (kg/ha)	Crude protein (kg/ha)	Soluble carbohydrates (kg/ha)	Crude fiber (kg/ha)
Nano-Fe	44.4±0.1	6351±10	604.1±8.2	1045±10	1302±8
Nano-Zn	43.6±0.1	6276±14	562.5±0.9	1045±10	1330±20
Chemical Fe	47.1±0.1	6260±27	578.3±1.0	990±3	1486±6
Chemical Zn	42.8±0.1	6176±14	553.4±6.1	958±11	1514±18

Source: Sharifi, (2016).

Table 3: Effect of seed priming on quality of forage maize.

	Leaf chlorophyll (SPAD units)	Total dry biomass(kg/ha)	Crude protein (kg/ha)	Soluble carbohydrates (kg/ha)	Crude fiber (kg/ha)
Nano-Fe	42.7±0.2	6081±4	524.3±15.3	968±15	1405±20
Nano-Zn	42.7±0.2	6076±21	540.6±14.4	970±21	1406±14
Chemical Fe	42.8±0.3	6071±30	537.2±8.6	970±10	1404±10
Chemical Zn	42.7±0.2	6072±21	521.5±9.3	971±6	1401±8

Source: Sharifi, (2016).

Table 4: Level of nitrate in forage (dry matter basis) and potential effects on animals.

Nitrate content (ppm)	Effect on animals
0-1000	This level is regarded as safe to feed under all conditions.
1000-1500	This level should be safe to feed to non-pregnant animals under all conditions. It may be best to limit its use to pregnant animals to 50 per cent of the total ration on a dry basis.
1500-2000	Feeds are safely provided when limited to 50 per cent of the total dry matter in the ration.
2000-3500	Feeds should be restricted to 35-40 per cent of the total dry matter in the ration. Feeds with more than 2000 ppm nitrate nitrogen should be avoided for pregnant animals.
3500-4000	Feeds should be limited to 25 per cent of total dry matter in the ration. Do not use it for pregnant animals
>4000	Feeds with over 4000 ppm are potentially toxic and should not be fed.

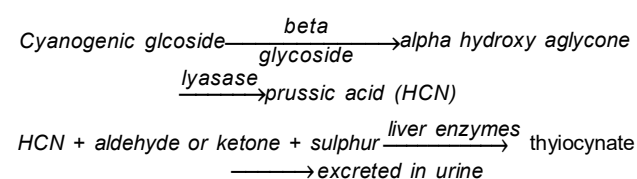
Source: Kumar *et al.*, (2018).

calcium (Ca) and ingested oxalate combined insoluble Ca oxalate is formed. This causes abnormalities in the metabolism of calcium (Ca) and phosphorus (P), which results in an excessive mobilization of bone material in animals (Rahman and Kawamura, 2011).

Oxalate toxicity in fodder crops can vary significantly depending on factors such as plant species, nitrogen fertilization and harvesting interval. Napiergrass experiments revealed a strong correlation between the K concentration and the soluble oxalate level (Rahman *et al.*, 2008).

Prussic acid toxicity

Prussic acid is typically absent in plants, but certain conditions can cause several common plants to accumulate large amounts of cyanogenic glycosides, which can then convert to prussic acid. The risk of prussic acid poisoning in livestock increases during drought periods and becomes even higher when drought conditions end and stressed, stunted plants start growing again (Robson, 2007). Ruminant animals, such as cattle and sheep are more prone to prussic acid poisoning compared to monogastric animals like horses and pigs. This is because the lower pH in the stomachs of monogastric animals helps to break down the enzymes that convert cyanogenic glycosides into prussic acid (Kumar *et al.*, 2018).



Certain conditions can lead to dangerous levels of cyanogenic glycosides in plants. These conditions include periods of rapid regrowth following stunting, such as after a drought breaks, when a crop is eaten back and then allowed to regrow, or when a crop is harvested for hay and then regrows, with levels being highest in young plants with green, growing shoots. Additionally, frosted or wilted plants, herbicide-treated plants, high nitrogen and low phosphorus levels in the soil and specific plant species like sorghum, which can contain more prussic acid than sudan grass, contribute to increased glycoside levels. Varieties within these species can vary in their prussic acid

potential. Plants that are wet with dew or light rain also exhibit higher glycoside levels (Robson, 2007).

CONCLUSION

It was observed that several methods are available for the agronomic biofortification of forage crops with nitrogen (N) and zinc (Zn) including soil application, foliar application, a combination of soil and foliar application and seed priming. Among these, soil application is the most preferred due to its ease of use. However, foliar application is used in emergencies to address nutrient deficiencies quickly.

In conclusion, agronomic biofortification stands as a promising avenue for enhancing the nutritional quality of fodder crops, thereby bolstering livestock health and productivity. Through the application of various agronomic interventions such as soil management practices, and crop selection it becomes feasible to elevate the levels of essential nutrients crucial for animal nutrition, including vitamins, minerals and antioxidants. This strategy not only addresses potential deficiencies in animal diets but also holds the potential to improve the nutritional status of humans consuming animal products derived from biofortified livestock.

However, while the concept of agronomic biofortification shows immense potential, its widespread adoption and efficacy in diverse agricultural systems necessitate further research and development. Challenges such as ensuring nutrient bioavailability, minimizing environmental impacts, and optimizing cost-effectiveness must be addressed to realize the full benefits of biofortified fodder crops. Collaboration among multidisciplinary teams comprising agronomists, breeders, nutritionists and policymakers is essential to overcome these challenges and drive innovation in the field. Additionally, long-term studies assessing the impact of biofortified fodder on animal health, reproduction and performance are crucial for establishing its efficacy and value proposition. By harnessing the power of agronomic biofortification, we can not only enhance livestock welfare and productivity but also contribute to broader goals of sustainable agriculture and food security in a changing world.

Conflict of interest

Authors declare that there is no conflict of interest.

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