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AGRONOMIC AND GENETIC STRATEGIES FOR ZINC BIOFORTIFICATION OF MAIZE FODDER: IMPLICATIONS FOR DAIRY COW NUTRITION AND HEALTH

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ABSTRACT

Maize (Zea mays L) is a multi-purpose cereal used for both food and fodder purposes. Its fodder has many beneficial characteristics, such as palatability, silage quality, digestibility, and high energy density, making it a suitable option for dairy cows, but it has one major issue: low Zn content. Zn is one of the most essential micronutrients nutritionally for dairy animals as it is required for proper growth, development, and enzymatic activities. Current maize cultivars have very low Zn content; lower Zn content negatively affects dairy animals, causing diseases like parakeratosis and lameness, which also affect the animal's everyday life. To uplift Zn contents in maize fodder, biofortification is the most feasible option. The efficiency of Zn fortification is influenced by some factors, such as the technique used, genotype responses, soil properties, geographical parameters, and the requirements of farmers. Biofortification techniques, such as genetic and agronomic fortification, can be very helpful in enhancing the nutritional value of the fodder. Through these techniques, the Zn concentration in maize can be elevated according to the daily requirement of dairy cows. Future work should focus on the development of superior maize fodder varieties with increased Zn contents, along with their integration with proper agronomic practices specific to agro-ecological conditions. This review deals with the dietary importance of zinc in dairy cows, diseases caused by Zn deficiency in dairy cows, biofortification of maize with Zn, and methods used for biofortification of maize.

Key words Maize, Dairy cows, Zinc, metabolic diseases, Biofortification, genetic biofortification.

1. INTRODUCTION

Maize (Zea mays L) is one of the most essential cereal crop around the world and has the highest genetic yield potential among all the cereals (Salman et al., 2022; Purwanto et al., 2025; Sharif et al., 2026). More than 130 countries cultivate the maize crop, as it can be easily grown in a wide range of environmental conditions. (Hossain et al., 2021; Mukhtiar et al., 2024; Ali et al., 2025). Maize is widely used as animal feed, as it has the best nutritional profile among the non-legume fodders when it cut in optimal size (Salman et al., 2022; Syamsu et al., 2025). Milk is a nutritious diet for human beings, and milk buffalo and cow nutrition balance is very important for its production (Raza et al., 2025). Maize forage, being high in energy and low in protein, can be given as a combination with alfalfa as a protein source and used as feed for dairy cattle (Blume et al., 2021; Karnatam et al., 2023). Maize fodder is prioritized for silage, as it has properties, like greater starch contents, softness, and suitable soluble sugars for optimal forage preservation (Selim et al., 2024; Bashir and Mahmud, 2025; Wróbel et al., 2025). The worldwide maize silage market is expected to expand by 7.84% from 2021 to 2030. Maize contains many vital micronutrients, such as zinc, selenium, magnesium, and iron, which are required in small amounts (mg/kg), but are vital for the proper growth and development of farm animals, including maintaining health, productivity, and reproduction rates (Bonou et al., 2024; Farooq et al., 2025). These are mainly enzyme activators, components of proteins, and regulate the physiological activities, i.e., digestion, lactation, reproduction, and metabolic efficiency (Fadlalla, 2022; Karnatam et al., 2023).

Zn is a key enzyme activator, as more than 300 enzymes are catalyzed by it, including enzymes like RNA polymerase and carbonic anhydrase (Solanki, 2021). It is important for muscle development as it activates satellite cells, which further promote proliferation and differentiation via ERK, and PI3K (Phosphoinositide 3-kinase)/Akt signaling, which results in myofiber and myotube formation (Hernández-Camacho et al., 2020). Proper development of the placenta and fetus is also associated with Zn concentration (Messersmith et al., 2021; Kumar et al., 2025). Optimum zinc levels have a beneficial effect on udder health, while Zn deficiency can ultimately lead to mastitis (Libera et al., 2021). Mastitis and general health problems form major indications of liver diseases that cause reduced milk production in dairy cows, which lowers productivity drastically (Muhammad and Ali, 2024; Kamal et al., 2025). There are a lot of anti-microbial drugs used against mastitis, but their resistance is also a big issue (Widiastuti et al., 2025). One health approach to cure mastitis by management is essential (Hossain et al 2025). Healthy puberty, oestrus onset, management, and fertility rate are also affected by zinc content. Zn also has an integral role in the movement and protection of vitamins A and E; Vitamin A (retinol) requires a carrier named Retinol-Binding Protein (RBP) as it cannot move alone in blood serum. In the liver, Zn is required for the development of RBPs. Moreover, free radicals are removed or reduced by Zn-Superoxide dismutase (Zn-SOD); this activity is also Zn-dependent and protects vitamin E from depletion. It takes part in cell division and cell repair, also sustains the function of deoxythymidine kinase by maintaining perfect amounts of adenosine (5′) tetraphosphate (5′)-adenosine for proper DNA synthesis in the cell (Keshri et al., 2021). Different enzymes like Alcohol dehydrogenase, Carbonic anhydrase, Cu/Zn Superoxide dismutase (SOD1), and Alkaline phosphatase are all catalyzed or influenced by Zn (Wang et al., 2022)

The amount of zinc required for dairy cows depends on the physiological stage of the cow. Based on milk production, a dairy cow requires 6mg Zn per 100g of dry matter (Oconitrillo et al., 2024). Maize fodder contains 0.46mg of zinc per 100g, which is drastically low compared to the required amount. Moreover, only around 15% of Zn in maize fodder is bioavailable for dairy cows. (Baseggio et al., 2021; Bakr et al., 2023). Global climate change is also a big issue for livestock (Naqvi et al., 2025; Qamar et al., 2025; Sarwar et al., 2025). Both hot and cold climate produce negative effect on all crops including maize (Chang, 2025). Feed rationing can be done to counter this issue, but it is not suitable for completing the zinc requirements of dairy cows due to its availability issue throughout the year (Daniel and Martín-Tereso, 2025). To elevate Zn contents, fortification of maize fodder with Zn can be done. Fortification can be defined as the process by which we enhance the nutritional value of a food by adding nutrients like vitamins or minerals, nutrients that are important to overcome dietary deficiencies and are required for the proper growth and development of the animal body. Several methods can be used for fortification of maize fodder, like Genetic (conventional breeding) biofortification, Transgenic biofortification (genetic engineering), Nano-fortification, Chemical fortification, Agronomic biofortification, Food fortification (post-harvest fortification), Microbial-assisted biofortification, and Metabolic engineering (Ammar et al., 2024; Azeem et al., 2025; Murtaza et al., 2025). Genetic biofortification is the type of fortification in which we use conventional breeding techniques to elevate the Zn content in the crop (Avnee et al., 2023; Imtaiz, 2025). Transgenic biofortification uses genetic engineering and modern biotechnology for the same purpose, while in agronomic biofortification, fertilizers or nutrient-rich amendments are mostly used to increase Zn contents (Hina et al., 2023). Due to the vast gap between requirement and supply, there is an urgent need for biofortification in maize through genetic improvement using conventional breeding or through biotechnology. In this regard, Guatemala was the first country to introduce biofortified maize varieties named ICTA HB-18 and ICTA B-15 with enhanced Zn content, developed by the International Maize and Wheat Improvement Center (CIMMYT) through the conventional breeding method. These varieties are highly renowned among local farmers due to their traits, including high protein content, early maturity, high zinc content, and larger kernel size. For example, tortillas made from ICTA B0-15 contain 60% more zinc compared to other tortillas made from other commercial varieties, and kernels of ICTA HB-18 have 15% more zinc than other commercial varieties (Boddupalli et al., 2025). Along with Guatemala, many other countries are also working on the development of biofortified maize cultivars. Artificial Intelligence (AI) also play its role in sustainable crop management through genetic interventions (Irfan, 2024; Ali et al., 2025; Muhammad Ahad, 2025; Tariq, 2025). Molecular techniques like CRIPRs Cas and other are also used for molecular evaluation of gene study in plants including maize (Fatima, 2025). This review discusses the dietary importance of zinc, its daily requirement in dairy cows, low Zn concentration in maize fodder, metabolic diseases caused by the deficiency of Zn in dairy cows, biofortification of maize with Zn to resolve this issue, and methods used for biofortification of maize.

2. Dietary importance of zinc in dairy cows

In dairy cows, Zn is also crucial due to its role as a major enzymatic catalyst; enzymes like Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) are Zn-dependent for their activation (Solanki, 2021). It improves resistance against diseases as it is required for the production and activation of necessary defensive cells like neutrophils and T-lymphocytes. Zinc aids these cells in identifying and eliminating harmful disease-causing agents like bacteria. After vaccination, it helps T-lymphocytes in the production of antibodies, strengthens wound healing, and promotes overall health. T cells require thymulin hormone for their differentiation and adaptive responses; thymulin is also regulated by zinc (Majumdar et al., 2025). For the protection of intestinal mucosa from oxidative damage, the enzyme Cu/Zn-SOD promotes the disproportionation reaction of superoxide anions through the copper-zinc active center, which inhibits the peroxidation of lipids and retains the intestinal mucosal barrier (Yousif et al., 2024; Yang and Lui, 2025). With enough zinc, the antioxidant enzymes work properly and effectively remove harmful free radicals that further decrease the oxidative stress in cows during transition and early lactation, which is elevated during this time (Abuelo et al., 2019). Enhanced zinc content or zinc supplementation improves pregnancy rates and strengthens ovarian operations by improving early embryonic survival in dairy cows (Anam et al., 2021; Vallejos-Fernández et al., 2025). Normal zinc levels improve wound healing due to the high expression of proteins responsible for healing, like Vascular Endothelial Growth Factor (VEGF) and Fms-like tyrosine kinase 1 (FLT1) (Kamrani et al., 2024). Two more important enzymes catalyzed by Zn are carboxypeptidase and Carbonic anhydrase. Carbonic anhydrase regulates the carbon dioxide level in the blood, the conversion of CO_2 into bicarbonates, and the bicarbonates back to CO_2, while the carboxypeptidase enzyme is responsible for breaking down proteins in the animal body. In the Carboxypeptidase enzyme, the carbonyl group (C=O) binds with Zn, and by creating a coordinate covalent bond with the peptide terminus, it gives a positive charge to carbon. The protein's polar portion is attracted to the hydrophobic pocket of the enzyme during digestion, which is present near the zinc site (Keshri et al., 2021). Feed quality enormously affects the productivity of dairy cow's notable percentage occupied by essential macro and micronutrients like Zn (Silvi et al., 2024)

3. Metabolic Consequences of Zinc Augmentation in Cattle

Experimental evidence has shown that elevated Zn levels have multiple positive outcomes on the health, growth, productivity, and metabolism of dairy cows. For example, during study, when pre-ruminant calves were supplemented with zinc (up to 80 mg/kg) of dry matter in the form of zinc hydroxychloride or zinc sulfate, these supplementations resulted in significant improvements in the feed intake, body weight, average daily body mass gain, insulin-like growth factor, and thyroxin concentrations in supplemented calves after 90 days. Plasma zinc, as well as zinc retention, also increased upon supplementation, which validated the enhanced metabolic status (Pal et al., 2021; Oconitrillo et al., 2024). Dietary zinc (54 to 174 mg/kg DM) caused a linear improvement in plasma zinc, and a quicker recovery of the dry matter intake after transport stress (by day 2) in beef steers. The post-transit average daily gain (ADG) and muscle energy were better in supplemented steers (Heiderscheit & Hansen, 2022). Increasing the dietary zinc-methionine levels of the high-producing dairy cow by 76-97 mg/kg DM enhanced post-peak milk and milk protein yields and decreased the somatic cell count (SCC), indicating improved dairy cows' udder health and metabolic performance. This was linked to better antioxidant signs and a reduction in oxidative stress (Hidalgo, 2021; Mesgaran et al., 2022; Oconitrillo et al., 2024). In ruminant research, zinc supplementation always enhanced average daily gain (17.4 g/day), dry matter intake, and feed ratio (by 1.56 g/ g). On the other hand, the effect of the organic sources of Zn, like zinc proteinate and zinc-methionine, depends on the dose size (Ángeles-Hernández et al., 2021; Kusuma et al., 2024; Darabi et al., 2025). ZIP transporters are responsible for Zn influx into the cytoplasm from the extracellular space in the form of Zn^{+2}, from the cytosol. Excess Zn is controlled by metallothioneins, which buffer and release. Zn^{+2} when needed. Zn is then distributed to organelles, where it acts as an enzyme cofactor, supports the DNA process, and regulates signaling pathways involved in metabolism and cell growth. This whole process has been discussed in Figure 1

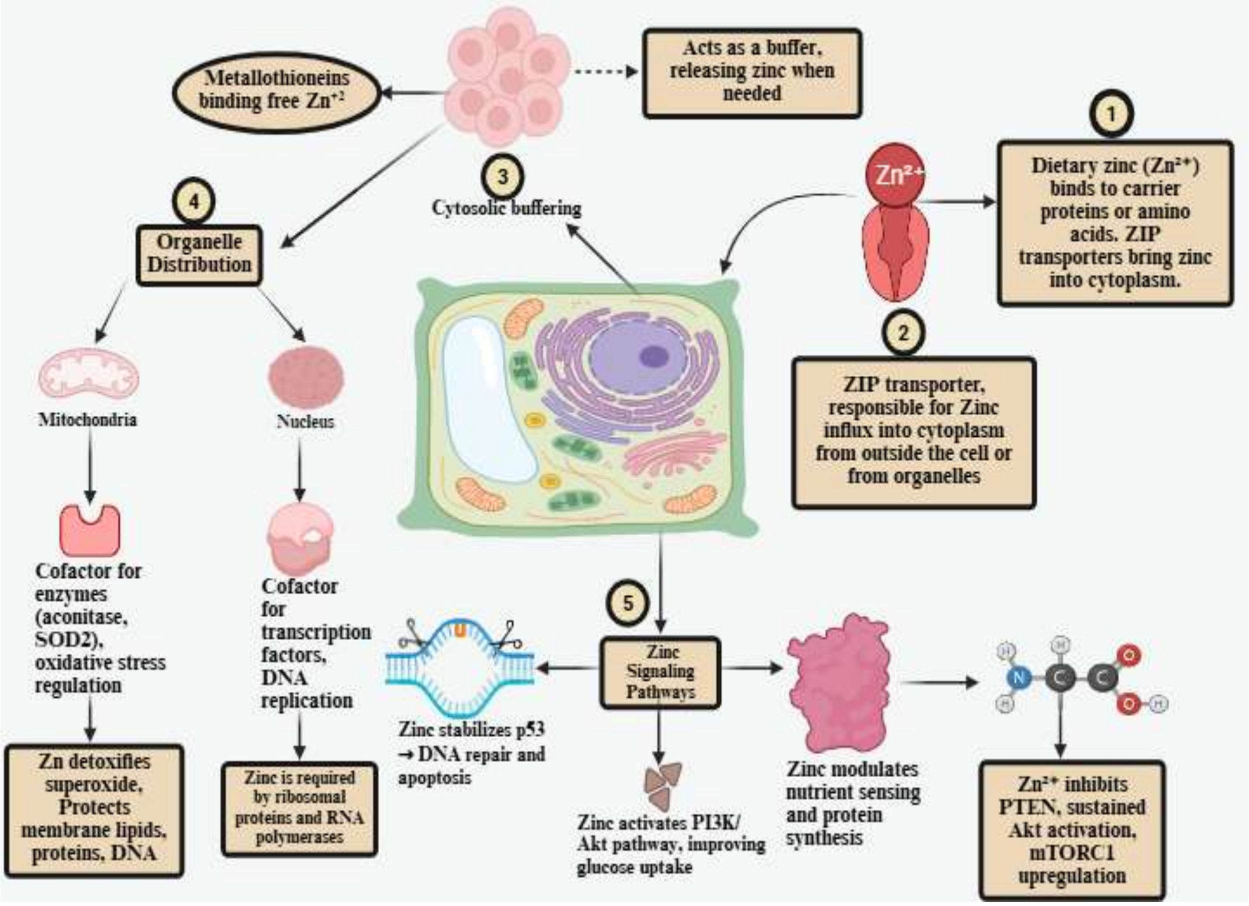


Figure 1: cellular zinc metabolism and signaling pathway

4. Disease caused by zinc deficiency in dairy cows

Zinc shortage can lead to a range of different diseases, such as parakeratosis, lameness, and hoof horn deformation. Parakeratosis is a skin disease characterized by symptoms such as harsh, dense, and crusty skin, deformed hoof horn, joint swelling and malformation, and reduced reproductive rates (Nikulin et al., 2021; Abed et al., 2024). Zn is crucial for antioxidant defense as it is vital for maintaining enzymes like superoxide dismutase and metallothionein. Lower Zn contents impair spermatogenesis in dairy animals primarily by disrupting antioxidant defense (Sun et al.,2023; Zeng et al., 2023). This disruption results in reduced reproductive capacity and maturity, sperm density, testis size, and mating interest in bulls (Nair et al., 2025).

Zn deficiency in dairy cows can directly or indirectly cause a number of issues, like small litter size, reduced fertility rates, ovulatory issues, delayed puberty, and embryo implantation failure (Kumar et al., 2025). It disturbs mitochondrial function, which alters the development of ovarian follicles. This causes autophagy inhibition, oxidative stress, and elevated apoptosis in ovarian cells, ultimately decreasing the number of mature follicles and reducing anti-Mullerian hormone (AMH) levels, which are essential for follicle maturation (Liu et al., 2024). Truthful egg activation post-fertilization is also influenced by Zn, which also helps in maintaining meiotic arrest of the oocyte. Lower Zn contents can negatively affect these stages by lowering fertilization efficiency (Garner et al.,2021). Zn deficiency before fertilization lowers placental growth and trophoblast differentiation, resulting in high pregnancy failure rates, stunted embryo, and poor embryo implantation (Liu et al., 2024). Synthesis of collagen, a crucial component of the extracellular supportive matrix of the dermis, is also regulated by Zn contents. Induction of type I collagen gene expression and matrix deposition are both controlled by Zn; on the other hand, low Zn levels can decrease the level of procollagen I and extracellular collagen. Zn also authorities prolyl and lysyl hydroxylases enzyme important for collagen maturation (Molenda and Kolmas, 2023; Kiouri et al., 2024).

Zn also plays a key role in wound healing by initiating the formation of new blood vessels at the affected (wounded) site (Hassan et al., 2021). Moreover, it is also involved in cellular migration to the injury site for further assistance in wound healing. Cells like fibroblasts, epithelial cells, and immune cells proliferate and migrate under the influence of Zn in collaboration with enzymes such as metalloproteinases. For example, the fibroblast movement and growth are facilitated by matrix metalloproteinases (MMPs), which degrade the extracellular matrix barriers and liberate growth factors bound to the matrix, which not only creates a physical route to move but also activates the proliferation process (Chen et al., 2023). It also participates in the process of breaking down, remolding injured tissue, and developing new healthy tissue to attain delayed wound healing by low Zn contents (Shi et al., 2024; Ding et al., 2025). Zn deficiency also affects the immune system by compromising the cell-mediated and humoral immune system, making animals more susceptible to disease-causing agents (Yao et al., 2025). It acts as a building block of the organic extracellular matrix of the bone called the ossein. Moreover, it acts as a catalyst for many enzymes that produce other components of ossein, and it also maintains the process of bone catabolism. Extreme Zn deficiency can cause abnormalities in bone development and growth, and can lead to weak bones, swollen joints, stiffness, and higher fracture risk (Ciosek et al., 2023; Sadri et al., 2025).

Dietary enzymes influenced by Zn

The importance of Zn as an enzymatic activator is mentioned above; it not only has a very pivotal role in activation, but also in proper working, structural stabilization, and protection of enzymes. These enzymes are involved in metabolic and physiological processes in dairy animals. Some of these enzymes that play important dietary roles in dairy cows and are affected by Zn have been mentioned in Table 1, with their class, specific role in the cows, physiological importance, mode of action, and location.

Table 1 Dietary Enzymes influenced by Zinc in dairy cows

Enzyme name	Class	Location	Mode of action	Role of zinc	Function in dairy cows	Physiological importance	References
Zn superoxide dismutase (Sod1)	Oxidoreductase	Mammary gland, live	Through the intercellular antioxidant defense system	Serves as a cofactor	Plays an important role in the Regulation of GCs apoptosis under heat stress, Regulates cell cycle progression and plays an important role in hormonal regulation.	Udder protection and resistance against mastitis	(Khan et al., 2021; Ateya et al., 2022; Saxena et al., 2022; Lokesha et al., 2025; Purwantiningsih et al., 2025; Wang et al., 2025)
MMP2 (Matrix Metalloproteinase 2), MMP9 (Matrix Metalloproteinase 9)	Hydrolase	Blood serum, ovarian tissue	Releases matrix-bound growth factors through ECM (extracellular matrix) degradation, involved in exposing cryptic binding sites, which further help in angiogenesis, cell signaling, and migration	Catalyst	Helps in the structural remolding of the uterus during uterine involution	Degrades structural scaffolds like collagen and other proteins.	(Cabral-Pacheco et al., 2020; Ghosh et al., 2024; Grötter et al., 2025)

ALP **(**Alkaline Phosphatase)	Hydrolase	Cell surface and matrix vesicles	Hydrolyses phosphate ester and releases inorganic phosphate at an acidic pH.	Cofactor	Bone mineralization, liver function, and nutrient absorption	Related to osteoblastic activities, the level of ALP represents the rate of bone calcification.	(Lowe et al., 2023; Dhal et al., 2025; Phanrungsuwa et al., 2025; Swetha et al., 2025)
Lactate Dehydrogenase (LDH)	Oxidoreductase	Present in almost all tissues, but abundant in muscle tissue, liver, and kidney	Interconversion of pyruvic acid into lactic acid	It modulates the activity of LDH.	Cellular energy production, especially under low oxygen levels	An important biomarker to detect mastitis	(Camacho et al., 2020; Farhana and Lappin., 2023; Adnan & Al-Abady, 2024; Barrak et al., 2024; Hernández-Novac et al., 2025)
Carbonic Anhydrase	Lyase	Mammary glands, epithelial cells	Catalyzes the reversible reaction of the conversion of carbon dioxide and bicarbonates	Catalyst	Electrolyte transport, pH regulation, respiration, bone resorption, and strengthening	Plays a vital role in vascular functions	(Wong et al., 2020; Hirakawa et al., 2021; Yrjänäinen et al., 2022; Lionetto, 2023; García-Llorca et al., 2024)
Glutamate Dehydrogenase (GDH)	oxidoreductase	In neuroendocrine cells,	Relates the multifunctional amino acid glutamate, which is closely associated with glutamate metabolism, to the Krebs cycle.	Modulatory	Maintains the synthesis and uptake of glutamate	Plays a vital role in protein and carbohydrate metabolism	(Gazioglu et al., 2021; Zhou et al., 2025)
DNA polymerase	Polymerase	Nucleus and mitochondria	Reads existing DNA strand and adds complementary nucleotides to the template strand in the 5 to 3 direction	Stabilizes the structure of the enzyme	Adds nucleotides to the template strand for DNA synthesis during DNA replication	Important for DNA replication, it transfers genetic information from the parent to the offspring.	(Nikoomanzar et al., 2020; Wu & Wu, 2023; Tian et al., 2024)

RNA polymerase	Polymerase	Nucleus and mitochondria	Transcribes DNA into tRNA, mRNA, and rRNA	Stabilizes the enzyme and can regulate degradation and recycling	Initiates gene expression	Transcribes DNA into RNA	(Kornienko et al., 2024; Wang et al., 2026)
Fructose-1,6-Bisphosphatase	Hydrolase	Liver and kidney	Converts Fructose-1,6-Bisphosphate into Fructose-1,6-phosphate	Acts as a cofactor	Key enzyme in gluconeogenesis, Glucose formation in early lactation	Helps maintain blood glucose levels during starvation	(Liu et al., 2025; Chang & wang, 2026; Kumar et al., 2026)
Alcohol Dehydrogenase (ADH)	Oxidoreductase	Cytoplasm	Catalyzes the oxidation of alcohols to aldehydes/ketones	Acts as a catalyst	Through metabolism, Detoxifies ethanol and other aldehydes from animal feed	Helps in the metabolism of alcohols and aldehydes	(Habte & Beyene, 2020; Gyaneshwari et al., 2023; Zhang et al.,2023; Alberto et al., 2026)
Carboxypeptidase A	Hydrolase	Pancreas/ small intestine	Acts by hydrolyzing peptide bonds at the carboxy terminal	Acts as a catalyst	Releases C-terminal aromatic amino acids from peptides to catalyze protein digestion	Helps in protein digestion	(Auld, 2025; Kumari and Sharma, 2025; Li et al., 2025)
Carboxypeptidase B	Hydrolase	Pancreas/ small intestine	Hydrolyzes peptide bonds at the C-terminal of Arg/Lys	Stabilizes the enzyme	Releases C-terminal basic amino acids from peptides	Helps in protein digestion	(Adeluola et al., 2025; Li et al., 2025; Shukla, 2025)
Δ5-Desaturase	Oxidoreductase	Endoplasmic Reticulum (ER) membrane	Introduces double bonds into fatty acids	Zn stabilizes enzyme structure and activity.	Convert fatty acids to polyunsaturated fatty acids for milk fat	PUFA(Polyunsaturated Fatty Acid) synthesis	(Banaszak & Górna, 2025; Mansilla et al., 2025)
Δ6-Desaturase	Oxidoreductase	ER membrane	Catalyzes the insertion of double bonds into fatty acids	Zn require for proper enzyme activity	Converts linoleic and linolenic acids to GLA and SDA for milk fat	PUFA synthesis	(Du et al., 2025; Shetty & Shetty, 2025)

Histone Deacetylase (HDAC)	Transferase	Nucleus	Deacetylation of histones, which suppresses transcription.	stabilizes the catalytic domain	Alters the gene expression in mammary and liver cells.	Regulation of Gene Expression	(Kumar et al., 2022; Bozdemir & Uysal, 2023; Aybek et al., 2025)
Matrix Metalloproteinase 1 (MMP-1)	Hydrolase (metalloprotease)	Extracellular/Secreted	Cleaves collagen and ECM proteins	for proteolytic activity	ECM turnover in the udder and connective tissue	Tissue remodeling	(Dalton, 2023; Fu et al., 2023; Dewangan et al., 2025)
Thymidine Kinase	Transferase	Cytoplasm/nucleus	Phosphorylates thymidine to TMP for DNA replication	stabilizes enzyme structure	Supports the proliferation of immune and mammary gland cells	DNA synthesis and repair	(Costa Syedman et al., 2022; Lee et al., 2022)
Carboxyl Esterase	Hydrolase	Cytoplasm/Liver	Hydrolyzes ester bonds in lipids	Acts as a catalyst	Hydrolysis of esterified lipids and detoxification of compounds	Lipid metabolism	(Rafeeq et al., 2022; Sachet-Fernandez et al., 2024)

5. Zn biofortification in maize

Currently, there is a huge gap between the requirement and availability of Zn levels in the maize plant in accordance to dairy cows. To overcome this issue, fortification practices, involves improving the nutritional value of foods by adding essential vitamins and minerals, are considered the most effective way of enhancing Zn levels in maize (ÇÖVEN, 2024). This practice is typically implemented to combat widespread deficiencies of these nutrients. Fortification can be achieved through various methods, such as coating with zinc (Yadav et al., 2023; Prajapati & Sharma, 2024; Ahmad et al., 2025). The simplest method of biofortification is genetic biofortification, as many Biofortified Varieties with enhanced Zn levels have been developed, such as BIO-MZN01 in Colombia, created by CIMMYT in collaboration with Harvest Plus, International Center for Tropical Agriculture (CIAT), and the Consultative Group on International Agricultural Research (CGIAR) through their maize research program (Komolafe et al., 2025). BIO-MZN01 contains 36% more it in the kernel compared to other varieties, along with improved Zn content, it also possesses many other desirable traits, such as high yield potential (up to 8 tons per hectare), resistance to major maize diseases (rust, gray leaf spot, and northern leaf blight), and consistent yields across various farming systems and even at high altitudes from sea level, making it a comprehensive package for farmers (Gashu et al., 2021; Nazli and Zahra, 2024; Ali, 2025). CIMMYT also released varieties such as ICTA HB-18 and ICTA B-15 in Guatemala, which have enhanced Zn content; both were developed through conventional breeding (Boddupalli et al., 2025).

Along with this, in countries such as India, Pakistan, and Bangladesh, work on zinc-fortified varieties is ongoing, with many fortified lines developed by research institutes in collaboration with CIMMYT (Tanveer et al., 2024). Biofortified maize usually attains 30 to 34mg Zn concentrations in kernels, whereas in traditional maize varieties, the concentration is 20 to 22mg (Goredema-Matongera et al., 2021). This is accomplished by using conventional plant breeding techniques. There is greater bioavailability of organic sources of Zn, like zinc-amino acid chelates, zinc-methionine, compared to inorganic sources. The organic Zn is used to increase levels of Zn in the blood and milk of dairy cows for better immune and antioxidant responses, along with increased digestibility of Zn and other nutrients (Hidalgo, 2021; Dresler et al., 2023; Klein et al., 2025; Li et al., 2025). Organic Zn supplementation is a significant way to increase the Zn concentration in the milk and blood serum, implying better absorption and transfer of Zn in body tissues and milk (Cai et al., 2021; Xu et al., 2021; Kusuma et al., 2024). Zn oxide in the nano-size also demonstrates great bioavailability and remains at approximately the same or even higher concentrations of Zn in plasma and milk than organic zinc, and has no detrimental effects on health or milk production (Bakhshizadeh et al., 2019; Al-Gheffari et al., 2024; Iqbal et al., 2024).

Variety	Origin	Year of release	Targeted nutrient	Technique used	Developed by	Key features	References
ICTA HB-18	Guatemala	2018	Zinc	Conventional breeding	CIMMYT	High Zn concentration, 15% more kernel Zn than other commercial varieties	(Goredema et al., 2021; Botoman et al., 2022)
ICTA B-15	Guatemala	2019	Zn	Conventional breeding	CIMMYT	Enhanced zinc, along with good agronomic traits, 36% more kernel zinc	(Goredema et al., 2021; Botoman et al., 2022; Khan et al., 2023)
BIO-MZN01	Columbia	2018	Zn	Pedigree selection	CIMMYT, Harvest Plus, CIAT, and CIGAR	Elevated Zn contents, high-yielding, and disease resistance	(Goredema et al., 2021)
Obatanpa	Ghana	1992	Quality protein, high lysine & tryptophan	Recurrent selection and backcross breeding	Crop Research Institute (CRI), Ghana	Biofortified, Higher yield than other local maize, good productivity under scarce input conditions	(Tandzi et al., 2017; Adu et al., 2021; Abdul Rahman et al., 2022; Pimpong, 2022; Ummer et al., 2024)
GV662A	Zambia	2012	Pro-vitamin	Conventional Hybrid breeding	CIMMYT	Pest resistance, biofortified	(Johnmark et al., 2022; Mingramm et al., 2024)
HQPM-5	India	2007	QPM (Quality protein in maize)	Hybrid breeding	Indian Agricultural Research Institute (IARI)	High-yielding, biofortified for quality protein in maize	(Zunjare et al., 2018; Goredema et al., 2021) (Mingramm et al., 2024)
ZS261	Zimbabwe	2006	QPM (Quality protein in maize)	Conventional Hybrid breeding	CIMMYT	Biofortified for QPM, high-yielding, and yield stability under a different set of environments	(Goredema-Matongera et al., 2021; Mebratu et al., 2019; Engida et al., 2024; Mebratu et al., 2024; Xiao et al., 2024)
Sammaz 52(PVA SYN 13)	Nigeria	2017	Pro-vitamins A (provitamin-A carotenoids like β-carotene)	Conventional breeding along with MAS	IITA, in partnership with the Institute of Agricultural Research	Orange maize, maturity in 110 to 120 days, and resistant to major maize diseases	(Iseghohi et al., 2020; Are et al., 2023; Sirajo et al., 2024; Akintunde et al., 2025)
HKZM-1	Sub-saharan Africa	Under trials	Zinc	Conventional breeding	IITA and CIMMYT	High kernel zn	(Taleon et al., 2023; Taleon et al., 2024)

6. Biofortification of maize through conventional breeding

There are several strategies used for fortification, and among these, breeding maize to develop Zn-fortified varieties is one of the most innovative techniques to achieve this goal (Iqbal et al., 2024; Matumba et al., 2025). For example, in conventional breeding programs, the first step is to acquire desired germplasm, which is evaluated to discover genetic diversity that helps identify parent plants for crossing (Ebert et al., 2023). Genetic diversity across the germplasm aids in developing a fortified variety with superior agronomic traits that appeal to farmers (Gupta and Lowe., 2025). Maize shows high genetic divergence for Zn accumulation. CIMMYT screened 1,400 genotypes and 400 landraces, revealing significant genetic variation in kernel Zn content (Shariatipour et al., 2020; Djalovic et al., 2024). Some inbred lines from the International Institute of Tropical Agriculture (IITA, Nigeria) displayed remarkably high Zn levels, ranging from 14 to 180% above the trial average (Gupta and Lowe., 2025). Modern maize genotypes tend to have lower Zn contents compared to landraces (Cetiner et al., 2023). Currently, plant breeding focuses on increasing grain yield, and an increase in yield often correlates with a decline in quality (Boddupalli et al., 2025).

Landraces generally have larger grains and better soil adaptation, resulting in higher grain quality. Conversely, fortified varieties need to have high yield potential to be attractive to farmers (Vázquez-Carrillo et al., 2024; Azeem et al., 2025). Therefore, selecting the most suitable germplasm for the breeding program is crucial for parent selection (Mujtaba and Firdous, 2025). In addition to evaluating variation, understanding the molecular and genetic basis of Zn accumulation in maize aids in selecting parents and improving the chances of developing superior plants. After parent selection, germplasm is crossed, and the F1 generation is grown to verify successful hybridization. Subsequently, in the F2 generation, superior plants are selected, focusing on the best fortified lines. Superior lines are selected and are subjected to further generational advancements. To check the Zn levels, early selection techniques like Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) can be used to obtain high-throughput analysis, as they use chemical or microwave-assisted sample digestion (Rawat et al., 2024).

7.Biofortified varieties approved around the globe

Development of biofortified varieties is one of the primary objectives for breeders around the world to tackle malnutrition, hidden hunger, and attain global food security. In this regard many biofortified varieties are developed and approved throughout the world. These varieties have enhanced micronutrient contents along with improved agronomic traits. These varieties are mentioned in Table 2 along with their origin, year of release, targeted nutrient, breeding method used, and other key features of the varieties.

Table 2 Biofortified Varieties developed around the world

8.Biotech, Omics, MAS, and mutation-based Maize fortification

If the required diversity is not found, then we can move towards biotechnology or mutation breeding, and more advanced concepts like omics. In biotechnology, the desired gene can be injected into maize by using tools like CRISPR-CAS without any species barrier. CRISPR works in a very systematic way to perform genome editing using two domains of Cas-9 endonucleases, the HNH domain incises one strand of DNA, while the RuvC-like domain is responsible for cutting the other strand of DNA, causing a double-stranded break. Following these DNA injuries, the internal repair system of the plant, by default, repairs DSBs in vivo, using error-prone non-homologous end-joining. As a result, huge insertions or fragment replacements can be made (Kumar et al., 2024), and in mutation breeding, seed or plants are exposed to mutagens like Ethyl Methane sulfonate (EMS) to cause mutation (Shamshad et al., 2023; Rupasinghe, 2024). Then, after securing the desired mutant, the plant with the mutant is propagated and multiplied (Spinoso-Castillo et al., 2025).

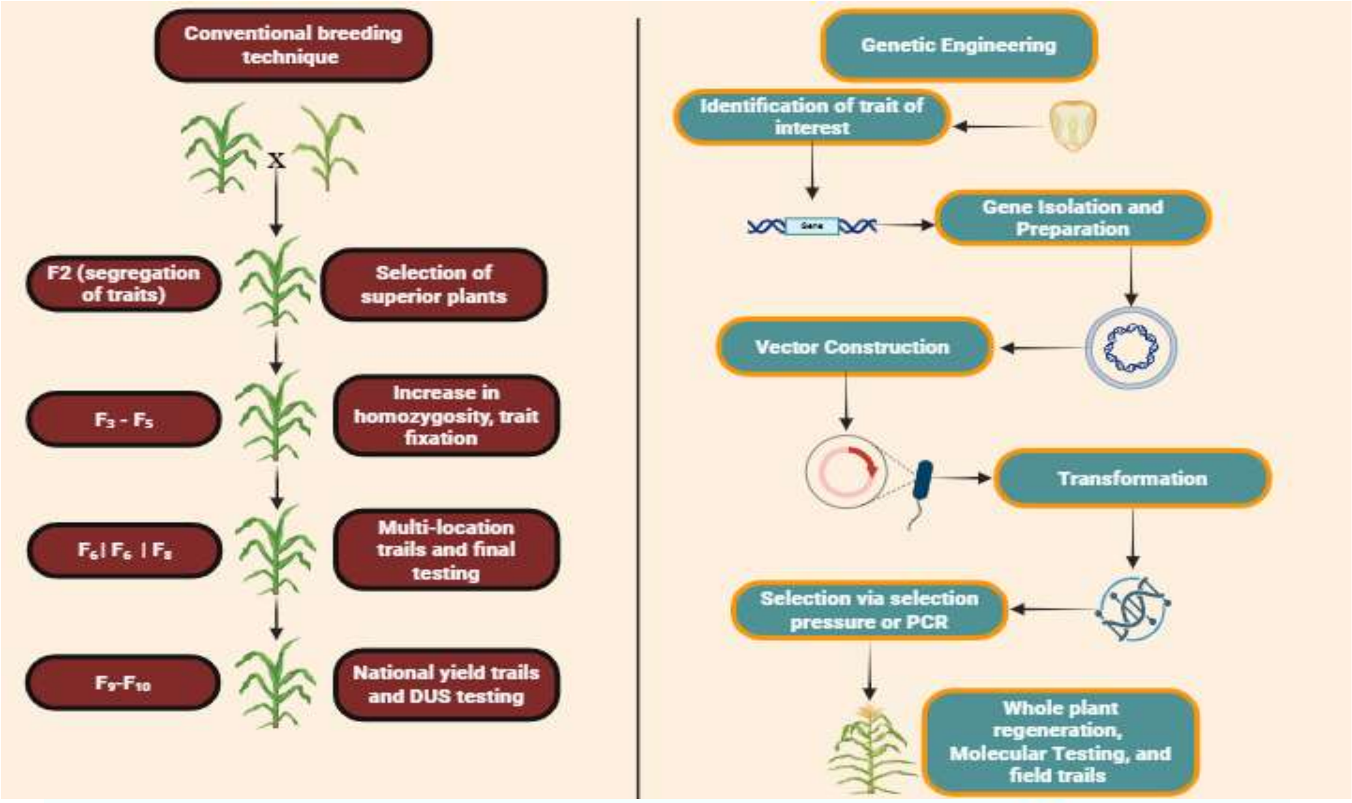
Advanced techniques, such as Omics, can be employed by conducting large-scale studies at the molecular level by integrating genomics, phenomics, transcriptomics, proteomics, metabolomics, and epigenomics (Azeem et al., 2025). Marker-assisted selection (MAS) can also be beneficial for maize biofortification; there are approximately 17 QTLs, and 10 meta-QTLs are associated with Zn concentration in maize. Along with these, 17 genes across contrasting families are responsible for endosperm Zn-Fe accumulation, 26 SNPs, and 12 SNPs are present on 9 different chromosomes, which play a vital role in Zn contents of the parental population of maize. The effectiveness of MAS heavily relies on the germplasm, the distance between the marker and the gene of interest, selection based on the marker in each generation, and gene-environment interaction, which still need to be studied thoroughly to manage Zn fortification more efficiently (Basnet et al., 2022). Both conventional and biotech-driven varietal development techniques, along with their complete pathway and key features, are demonstrated in Figure 2

9. Agronomic fortification of maize

The genetic range of Zn concentration in maize is very low, due to limiting breeding-based fortification, the other option for biofortification is agronomic fortification, which improves the nutritional profile through targeted agronomic practices (Xue et al., 2023). These practices include the incorporation of fertilizers, such as Zn sulphate ($ZnSO_4 \cdot 7H_2O$), into the soil before sowing, as well as the foliar application of $ZnSO_4$ on the standing crop (Kumar et al., 2024), but $ZnSO_4$ has low efficiency as plants only absorb 4-8% of applied zinc (Farooq et al., 2025). Foliar application up to 2000 mg $ZnSO_4.7H_2O$ per kg or 425 mg of actual Zn per kg of spray solution is believed to be beneficial and safe for maize leaves. However, maize is not markedly receptive to the use of foliar fertilization and, therefore, foliar spray can be observed as incomplete in addressing the crop's Zn requirement (Xue et al., 2023).

When Zn is applied in soils, it can easily be chemically transformed into sparingly soluble and unavailable forms depending on the soil type, chemical, and physical traits of the soil (Sethi et al., 2025). Nevertheless, these immobilized Zn forms can be transformed back into plant-available forms by bioaugmentation with zinc-solubilizing bacteria, which release Zn because of a biological reaction (Yadav et al., 2021). Similarly, vesicular-arbuscular mycorrhizal (VAM) biofertilizers improve the availability and uptake of Zn by acidifying the rhizosphere and allowing external mycelium access to tightly bound zinc, making it available to plants. (Singh et al., 2024; Mohan et al., 2025). Soil application of Zn sulphate, Foliar application through using ZnSO4-7H2O, seed priming with Zn sulphate, and vesicular arbuscular mycorrhizal biofertilizers, all these techniques are shown and explained in Figure 3

Figure 2: conventional and transgenic variety development program



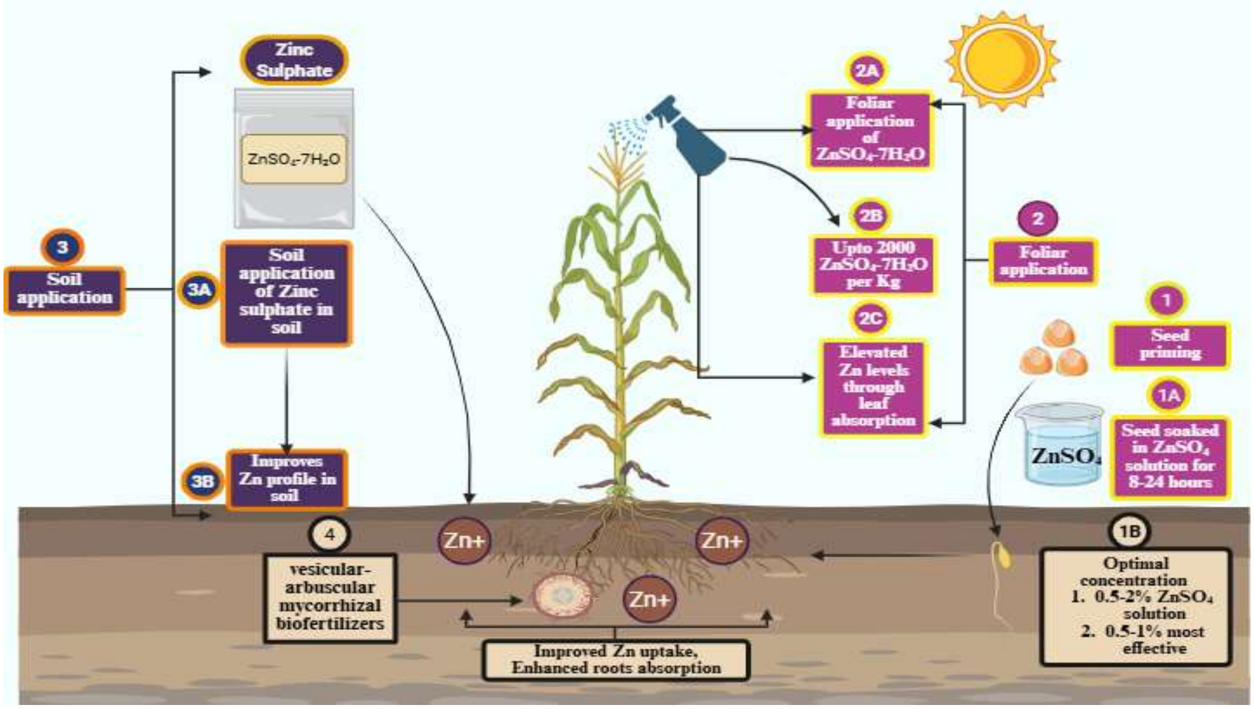


Figure 3 Methods for agronomic biofortification of Maize

10. Effect of biofortified maize fodder on the productivity of dairy cows

Agronomic Biofortified maize grown on adequately fertile soil can partially meet the dietary needs of dairy cows. Milking cows require 63mg/kg DM Zn, on the other hand, heifers require 31mg/kg DM Zn, and the value for dry cows is 22.8 mg/kg DM as per these values, agronomic biofortified maize can meet the dietary requirements of dry cows and heifers, as average maize shoot DM Zn lies between 7 and 24 mg/kg. (Sinclair et al., 2015; Grujcic et al., 2021; Mthi et al., 2021). To meet the Zn requirements of milking cows, the genetic potential for Zn concentration in maize can be enhanced through genetic biofortification (Kaya, 2025). As Zn is associated with keratin production, enhanced Zn in fodder can result in improved hoof and skin health. If Zn levels are low in fodder, it can lead to growth retardation, compromised immunity, and decreased milk production (Oconitrillo et al., 2024). Milk Zn concentration is greatly influenced by forage quality. Biofortified maize with enriched Zn increases milk production in cows, improves reproductive efficiency, and reduces susceptibility to infectious diseases such as metritis and mastitis (Kumar and Ram, 2021; Altaf et al., 2025; Sadi et al., 2025).

Zn-enriched maize fodder can minimize the chances of mastitis incidences, as metabolic diseases like ketosis and hypercalcemia indirectly increase the risk of mastitis; these are maximized by compromised immunity and physical injuries. Adequate Zn results in lower somatic cell count and declined milk amyloid A levels. Elevated Zn in fodder improves the stability of mammary epithelium, enhances immunity, and protects intestinal mucosa from oxidative harm (Khan et al., 2024; Majumdar et al., 2025; Yang and Lui, 2025). Proper Zn in fodder improves efficiency, maximizing productivity (Angeles-Hernandez et al., 2021; Ogbuewu et al., 2023; Zarghami et al., 2025). Zn fortified feed has a good influence on immunity, antioxidant defense, and biochemicals associated with inflammatory reactions. An improved Zn profile positively correlates with feed efficiency. Dietary Zn profile was also enhanced, leading to a significant improvement in the efficiency of the diet. Animal milk that contained organic concentrations of Zn exhibited higher levels of zinc, which indicated a high bioavailability of the mineral. Besides, alterations in the milk fatty acidation that may help in human nutrition were also observed (Amriani et al., 2025; Klein et al., 2025). Organic forms of zinc, such as zinc-methionine, enhance antioxidant activity, improve immune markers, and reduce inflammation in cows by promoting leukocytes and antibodies (Mesgaran et al., 2022). The mechanistic association of zinc deficiency with epidermal diseases like parakeratosis, and the high predisposition to bacterial mastitis in dairy cows, along with the protective physiological functions of zinc in the maintenance of epithelial integrity, antioxidant protection, and immunity, and also the ability of zinc-biofortified maize to enhance zinc nutrition and the incidence of epidermal diseases, is discussed in Figure 4.

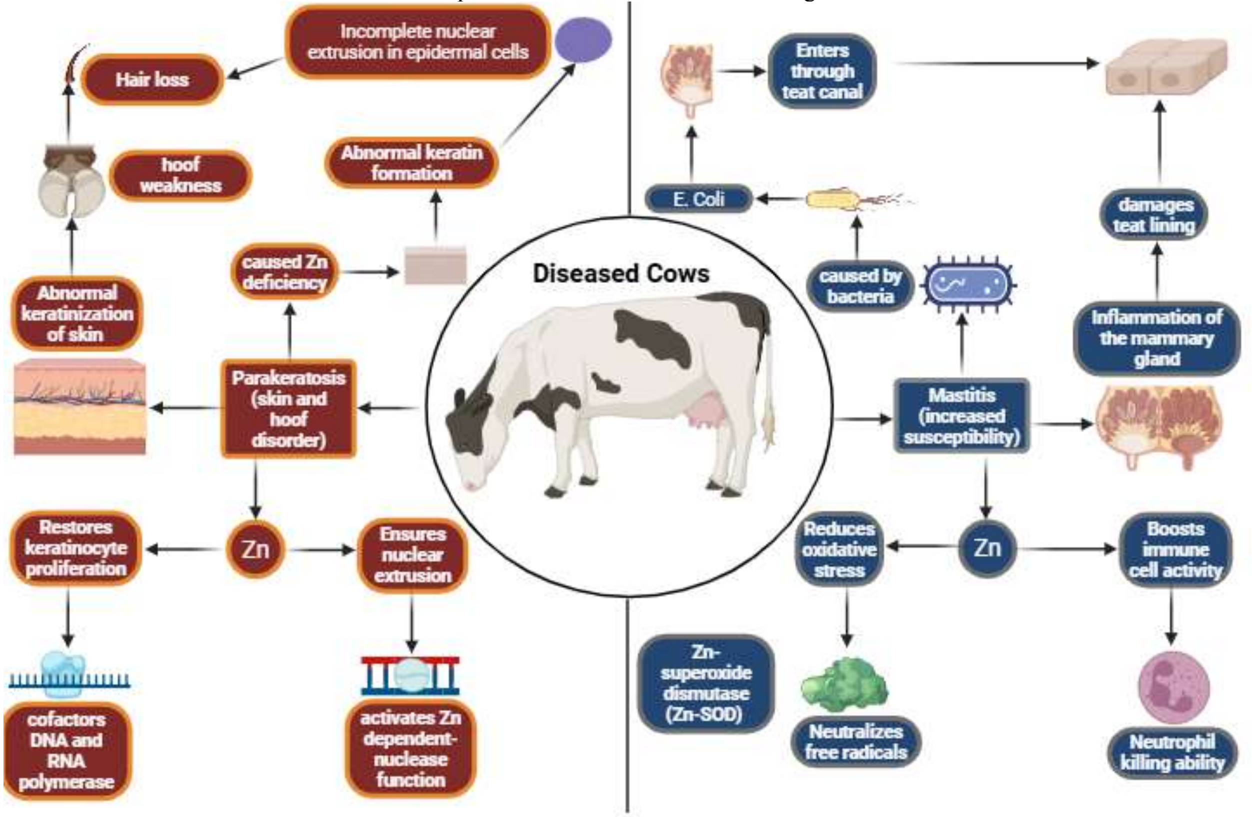


Figure 4 Effect of zinc deficiency on skin integrity, immune function, and mastitis susceptibility in dairy cows

11. Challenges and future prospects

Although maize is an essential crop, it faces different climate and nutritional deficiency challenges continuously (Tariq et al., 2022). Single-nutrient biofortification, such as Zn in maize, faces several major challenges that undermine its effectiveness. Agricultural soils worldwide, especially in arid and tropical areas, are extremely low in naturally available Zn, which limits the impact of Zn fortification efforts (Goredema-Matongera et al., 2021; Gedil et al., 2024). Mycotoxins also poison maize crops which ultimately compromises animal health (Khatoon et al., 2025). Moreover, Zn uptake by maize plant is also retarded by soil properties like High pH, low soil organic matter, and soil compaction and aeration, and presence of silicates, phosphatase, and oxides due to excess fertilization which reacts with Zn and make it unavailable for maize plant (Suganya et al., 2020; Jalal et al.,2022; Van Eynde et al.,2023). Most of the time, Zn fertilization does not affect maize yield; it only increases Zn concentration, which is a drawback from a farmer's perspective (Mutambu et al., 2023). Usually, maize varieties have very low Zn concentration, and breeding for Zn fortification in maize is a very complex process (Jalal et al., 2022). The grain yield has a negative relationship with the Zn concentration, the dilution effect: the higher the yield, the lower the Zn concentration in the grain can be, and thus it is hard to get both high yield and high Zn at the same time (Matongera et al., 2023; Mutambu et al., 2023). Drought stress and salinity are also big issues for maize production (Hussnain et al., 2025; Tariq et al., 2025; Tariq et al., 2026). So, farmers awareness for all crop production and nutritional balance is essential (Haider et al., 2025).

If somehow biofortification is achieved against all odds, then maintaining high nutrient composition along with high yielding and pest resistance to make it attractive to farmers is even more challenging and complex (Arif et al., 2025; Paul et al., 2024; Sen et al., 2024). Zn is needed by dairy cows to be healthy and produce milk, but when consumed in excess amounts, it causes toxicity, decreased feed consumption, and metabolic disorders. The majority of the studies demonstrate that supplementing at levels below 100 mg/kg dry matter (DM) has benefits, but supplementing beyond that range has the potential of reducing the intake of dry matter and causing a reduction in milk production in the short term. High Zn supplementation has a short-term effect of reducing feed intake and milk production, particularly during the initial stages of feeding, but long-term supplementation may increase milk production and udder health (Oconitrillo et al., 2024). When there is excess zinc, it may affect the absorption of other trace minerals, especially copper, and cause deficiencies unless it is corrected. Maize also has phytic acid that can bind Zn and make it less bioavailable to cows, but it is less important to ruminants because microbial phytases are present. Excessive supplementation can cause Zn to be expelled into manure, potentially causing environmental pollution if not used appropriately. The farmers may have to spend more money on feeds that have been fortified and also on zinc-specific sources (Kumar et al., 2021; Mesgaran et al., 2022).

Zn is a crucial micronutrient required by dairy cows at different stages of their life cycle, and present-day fodder does not fulfill the requirement of Zn for dairy cows. To tackle this problem of concern, efforts should be made in a direction to make fodders rich with Zn through biofortification. More varieties with high Zn concentration should be developed using modern techniques like QTL mapping, GWAS (Genome-Wide Association Studies), genome selection, speed breeding, phenomics, omics, MAS, and genetic engineering (Suthar, 2025). Breeding programs should be based on developing high-yielding, highly palatable Zn biofortified varieties that can be cultivated in a vast range of climatic conditions, having the potential to attract farmers. Along with biofortified varieties, soil degradation should be controlled, and degraded soils should be recovered. To enhance Zn contents, the best agronomic practices should be used. In this way, the metabolic disease caused by Zn deficiency in dairy cows can be controlled.

12. CONCLUSION

Maize is the most important cereal fodder, but it has some major shortcomings in its nutritional profile, as it is low in important micronutrients like Zn. It is one of the most influential enzymatic activators with more than 300 important enzymes dependent on it directly or indirectly. With low Zn contents, maize fails to provide a balanced diet to dairy cows, to maintain proper growth and high productivity, which is why there is a need of the time to enhance the nutritional profile of maize through biofortification. Genetic, agronomic, and other approaches can be used to achieve the goal of enhancing Zn contents in maize. Through genetic approaches, breeding programs can be designed to enhance Zn contents, and modern biotechnological tools like CRISPR-CAS9 can be used to achieve genetic biofortification. Another way to go is agronomic fortification, which can be done through better agronomic practices, like fertilization, along with fortification. These varieties must have good agronomic characteristics to attract farmers. Some varieties have been developed that have high Zn contents, but those varieties are area-specific. To tackle this issue effectively, a widely adoptable maize variety that can be cultivated in different sets of environmental conditions should be developed. Research institutes like CIMMYT are working on this topic, which can lead to a suitable solution for this problem.

Declarations

Conflict of interest: The authors declare no competing interests.
Ethical approval: Not applicable. Informed
Consent: Not applicable.
Data availability: All the data present within the manuscript.
Author Contribution: M.B.H. and H,H,. wrote the Breeding section of the manuscript; N.T. completed the Veterinary portion; H.B., A.S. and J.A. wrote the nutritional point of view of the manuscript, M.T. and N.B. covered the health portion and reviewed the manuscript; M.B.H. and N.T. also prepared Figures of their respective portions.

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