

Review

Nanozyme Based Strategies in Animal Nutrition and Reproductive Health: Opportunities and Challenges

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Abstract

Nanozymes, a class of nanomaterials with intrinsic enzyme-mimicking activities, have emerged as promising tools in animal science due to their high catalytic efficiency, stability, and cost-effectiveness. Nanozymes, having high broad-spectrum antimicrobial properties offer smart packaging systems for extending the shelf life of food, as well as enabling rapid and sensitive toxin sensing (such as mycotoxins, pathogens, etc.), and quality monitoring. In animal nutrition, nanozyme-based delivery systems upregulate nutrient bioavailability, real-time monitoring of metabolic disorders, and protect bioactive compounds. In animal reproduction, reproductive performance directly affects livestock productivity including milk and meat production, and overall profit of livestock. Therefore, to support optimal reproductive management, nanozymes provide innovative treatments such as targeted hormonal delivery, reproductive status monitoring, and early diagnosis of reproductive disorders. Nanozymes also play an important role in improving gamete cryopreservation by reducing oxidative stress, enhancing sperm viability, membrane integrity, and DNA stability. Despite these advantages, challenges such as biosafety, toxicity, and regulatory approval still limit their practical use. Overall, in this review, we highlight the recent advances in nanozyme development, classification, and catalytic mechanisms, along with their advanced applications in animal reproduction, nutrition, and food safety.

Keywords: artificial enzymes, nanozymes, food safety, sensing, reproductive status, hormone delivery, antibiotics, livestock

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1 INTRODUCTION

Over the years, animal husbandry and agriculture have been contributing significantly to human welfare in multiple ways. However, the rapid growth of the global population has led to an increasing demand for food. To meet this need, it is essential to enhance food production in sustainable ways and use novel strategies to boost the productivity of agriculture and animal goods. In the past few decades, variety of cutting-edge, interdisciplinary, and multidimensional concepts have evolved in response towards increasing public demand for safer, high-quality and nutrient-rich food products^[1]. Among these, enzyme-based food processing technologies are one of the important areas of progress. The global market for such technologies was anticipated to be worth 2.4 billion USD

in the year 2019, and it is expected to increase up to 3.2 billion USD by 2025^[2]. Despite their advantages, the proteinaceous nature of natural enzymes requires strict structural configurations, which restricts their industrial applicability. The developments in nanotechnology have provided exceptional possibilities to overcome these limitations through the design of highly effective artificial enzymes, including nanozymes for use in food processing. Nanozymes may be described as nanoparticles (NPs) possessing enzyme-mimicking catalytic activities, hence currently drawn a lot of interest for use in antibacterial treatments, therapeutics, and food handling applications^[3]. Yan and co-workers in 2007 demonstrated that iron oxide (Fe₃O₄) NPs exhibit inherent peroxidase (POD) mimetic enzymatic property, establishing the concept of inorganic nanozymes^[4]. Nanozymes offer remarkable advantages

over conventional enzymes in terms of ease of synthesis, cost-effectiveness, and enhanced stability. They are generally easier and more economical to produce, as they do not require complex biological synthesis processes. Moreover, they remain stable under unfavourable conditions (pH and temperature) showing enhanced stability, can perform numerous catalytic activities, etc., due to structurally robust non-proteinaceous nature of nanozymes^[5]. Nanozymes show enzyme-mimetic activity through material composition and surface chemistry rather than specific tertiary protein folding; hence the advantages significantly outperform the natural limitations present in natural enzymes for various applications. Additionally, although traditional enzymes are considered safe, they often face batch variability, allergic reactions during handling and immunogenicity, loss of function with increased time, microbial impurities, leading to inconsistent dosing. On the other hand, nanozymes show *in vitro/in vivo* biocompatibility, highlighting the need for standardised safety studies like long term accumulation, toxicity, species specific dosing before broad translation. A comparative study has shown that POD-mimetic activity of nanozyme can match or even surpass the performance of natural horseradish peroxidase (HRP) enzyme, highlighting the repeatability and robustness of nanozyme catalysis under varied conditions^[6]. In addition, nanozymes are highly suitable for real time monitoring applications; they are renowned for their durability, simple fabrication, and ease of surface modifications.

In food based applications, nanozymes composed of different nanomaterials such as carbon, metal/s and its oxides, metal-organic frameworks (MOFs), etc., provide various benefits than the conventional enzymes^[7]. The application of nanozymes in food sector can uplift the existing conventional technologies such as rapid detection of food spoilage, monitoring of hazardous contaminants, extending the shelf life of food items, preserving the nutritional quality of food, supporting the development of smart packaging materials with spoilage indicators. Ongoing research continues to explore their role in food processing and quality detection techniques, with the potential to significantly reduce food borne diseases and improve public health outcomes.

In animal farming, reproductive performance of the animals is closely correlated with animal productivity, profitability, and sustainability, which are connected to the production of milk and meat. Effective reproductive management, along with improvements on areas, such as feed & forage quality, disease management, animal husbandry practices, and overall farm management, plays a critical role in enhancing production (meat, milking, and eggs). Advanced reproductive technologies, such as artificial insemination, are widely employed to improve the genetic diversity and livestock quality. In this context, nanotechnology offers promising solutions such as non-invasive gametes bioimaging, nanopurification

techniques, and cryoprotection strategies, to improve sperm preservation, and artificial insemination^[8]. Various nanozymes such as cerium oxide (CeO₂) NPs^[9], nanobiopolymeric forms (BSA-AuNPs)^[10], silver-gold Apoferritin complex exhibit enzyme-like activity [e.g. catalase (CAT), POD and superoxide dismutase (SOD)]^[11] that help to reduce the freeze-thaw induced oxidative damage during sperm cryopreservation. Similarly, albumin coated copper cysteine (NA-Cu-Cys) nanozymes show antioxidant property by exhibiting SOD-like activity, thereby reducing the oxidative stress during sperm cryopreservation^[12]. Challenges in animal reproduction like estrus detection and hormone delivery, are also being addressed using nanotechnology. For example, nanotubes are being created as skin-implantable devices, which can monitor real-time blood estradiol in animals, improving estrus detection accuracy.

Furthermore nanotechnology enhances the delivery of drugs, nutrients, and biological molecules by improving bioavailability, mobility and cellular uptake, increased antibacterial effect, decreased toxicity. Overall, nanozyme based innovations represent a transformative approach in livestock production, offering new opportunities in nutrition, reproductive management, and overall farm efficiency. This review aims to provide a broad overview of these emerging advancements and their potential applications in the fields of nutrition and reproductive control.

2 NANOZYMES TYPES AND CATALYTIC MECHANISM

This section covers a systematic classification of nanozymes using multiple complementary perspectives to better understand their design, functionality, and applications.

2.1 Classification Based on Material Composition and Design Parameters

Nanozymes can be broadly classified based on their material composition, therapeutic functionality, structural integrity, and biointerface engineering strategies (Figure 1). These categories include metal-based (e.g., Au, Ag, Pt), metal oxide-based (e.g., Fe₃O₄, CeO₂), carbon-based (e.g., graphene, nanotubes), polymeric, and lipid-based nanomaterials, each possessing unique catalytic and physicochemical characteristics^[13,14]. The performance of nanozymes is strongly influenced by their core material properties, surface modifications, and interactions with biological systems. For instance, biointerface engineering (e.g. PEGylation, antibody conjugation) enhances stability, targeting ability, and biocompatibility, which are critical for biomedical and food-related applications^[15]. In addition to composition, the therapeutic modality of nanozyme systems plays a crucial role in determining their application potential. Their ability to combine catalytic activity with therapeutic functions enables simultaneous diagnosis and treatment, which is particularly valuable in precision

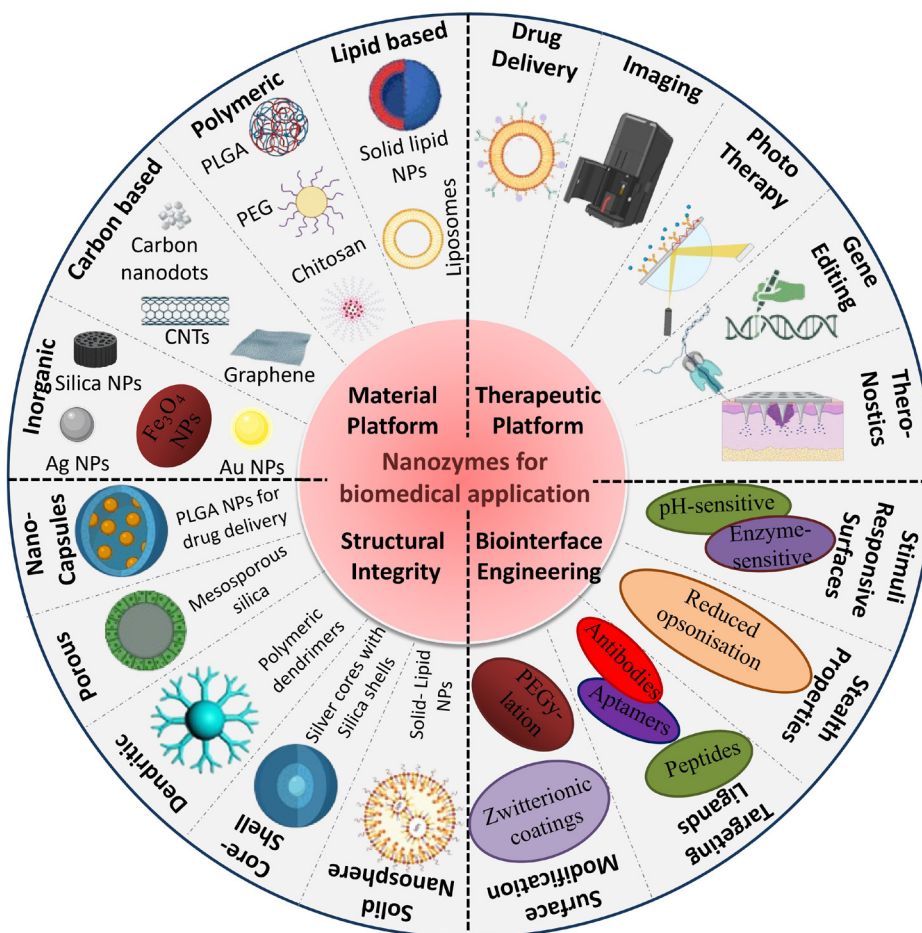


Figure 1. Schematic Illustration of Major Considerations in Nanozyme-based Systems for Biomedical Use. The central core presents the four fundamental sections stating nanozymes performance: material platform, therapeutic modality, structural integrity, and biointerface engineering. The outer ring illustrates representative examples within each category.

medicine. Structural integrity is another important factor influencing nanozymes performance^[15]. Parameters such as particle size, shape, porosity, and core-shell architecture directly affect catalytic efficiency and stability. For example, mesoporous and core-shell nanostructures provide a higher surface area and improved substrate accessibility, leading to enhanced catalytic activity and controlled release behavior. Furthermore, the interaction of nanozymes with the biological environment is governed by their surface chemistry and physicochemical properties. Surface charge, hydrophilicity, and functional groups determine protein adsorption, cellular uptake, and circulation time in biological systems. Proper surface engineering can reduce non-specific interactions, minimize immune recognition, and improve overall system performance^[16]. The integration of these four aspects allows for the rational design of nanozyme systems with tailored properties. Such an integrated approach is essential for optimizing their efficiency, specificity, and safety in diverse applications, including biomedical diagnostics, food safety monitoring, and environmental sensing^[5].

2.2 Classification Based on Catalytic Mechanism

Nanozymes are also classified based on their catalytic mechanisms, which mimic natural enzyme activities

through redox reactions, electron transfer processes, and reactive oxygen species (ROS) generation or scavenging (Figure 2)^[17,18]. These mechanisms include oxidation-reduction reactions, Fenton-like reactions, and surface-mediated catalytic pathways. Over the past decade, significant progress has been made in understanding how nanozymes replicate enzyme-like behavior such as POD-, oxidase (OXD-), CAT-, SOD-like activities^[19]. These advances have enabled their application in biosensing, environmental monitoring, and therapeutic interventions. Furthermore, the catalytic efficiency of nanozymes depends on factors such as particle size, surface area, valence state of metal ions, and environmental conditions, which can be tuned to optimize their activity. Recent studies have shown that the catalytic behavior of nanozymes is largely governed by their surface electronic structure and active sites, which determine their affinity toward substrates and intermediates^[20]. For e.g., transition metal based nanozymes often exhibit variable oxidation states that facilitate electron transfer reactions, enhancing their catalytic versatility^[21]. In addition, defect engineering and doping strategies have emerged as effective approaches to improve catalytic performance. Introducing vacancies or heteroatoms into nanomaterials can modify their electronic configuration, thereby

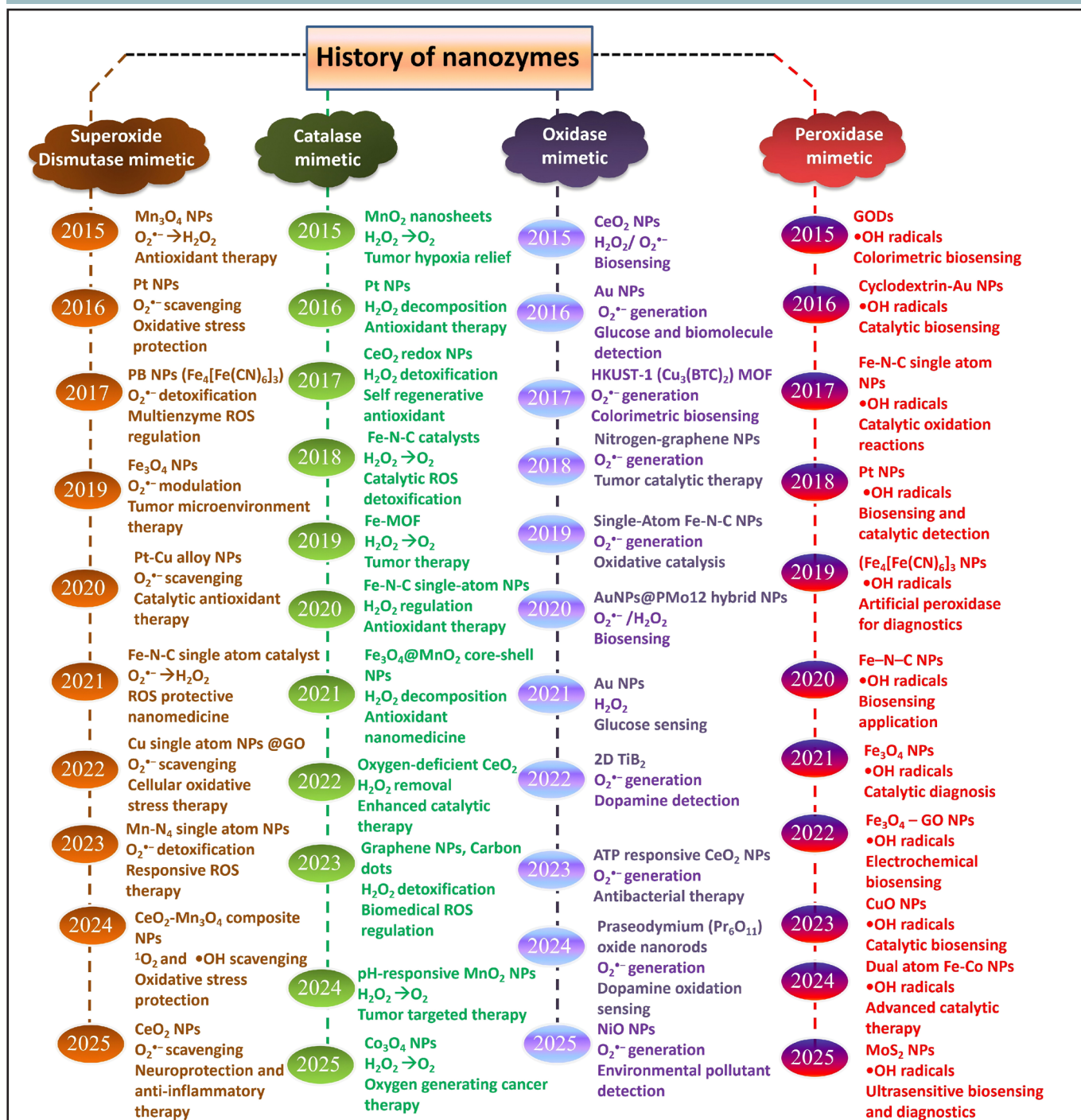


Figure 2. Timeline Highlighting Major Scientific Discoveries and Conceptual Advances that Shaped the Field of Catalytic Properties of Nanozymes Within Last 10 Years.

increasing active site density and improving reaction kinetics^[22]. The enzymatic pathways of nanozymes can also be regulated by external stimuli such as light, temperature, magnetic fields, and pH. This stimulus-responsive behavior allows precise control over catalytic activity, making nanozymes highly adaptable for targeted applications, particularly in smart sensing and controlled therapeutic systems^[23]. Moreover, the mechanistic understanding of nanozyme catalysis has evolved from simple enzyme mimicry to more complex multi-enzyme-like systems, over the years, where a single nanozyme can perform cascade reactions. Such multifunctional catalytic systems are particularly useful in biological

environments where sequential reactions are required.

2.3 Classification Based on Enzyme-mimicking Activities

In addition to composition and mechanism, nanozymes can be further classified according to their enzyme-like catalytic functions. These include POD-, OXD-, CAT-, and SOD-like activities, as well as enzyme-mimetic functions such as hydrolase and phosphatase activities (Figure 3). Each category plays a distinct role in biochemical reactions. For e.g., POD-like nanozymes catalyse substrate oxidation in the presence of hydrogen peroxide (H₂O₂), while CAT-like nanozymes decompose H₂O₂ into water (H₂O) and molecular

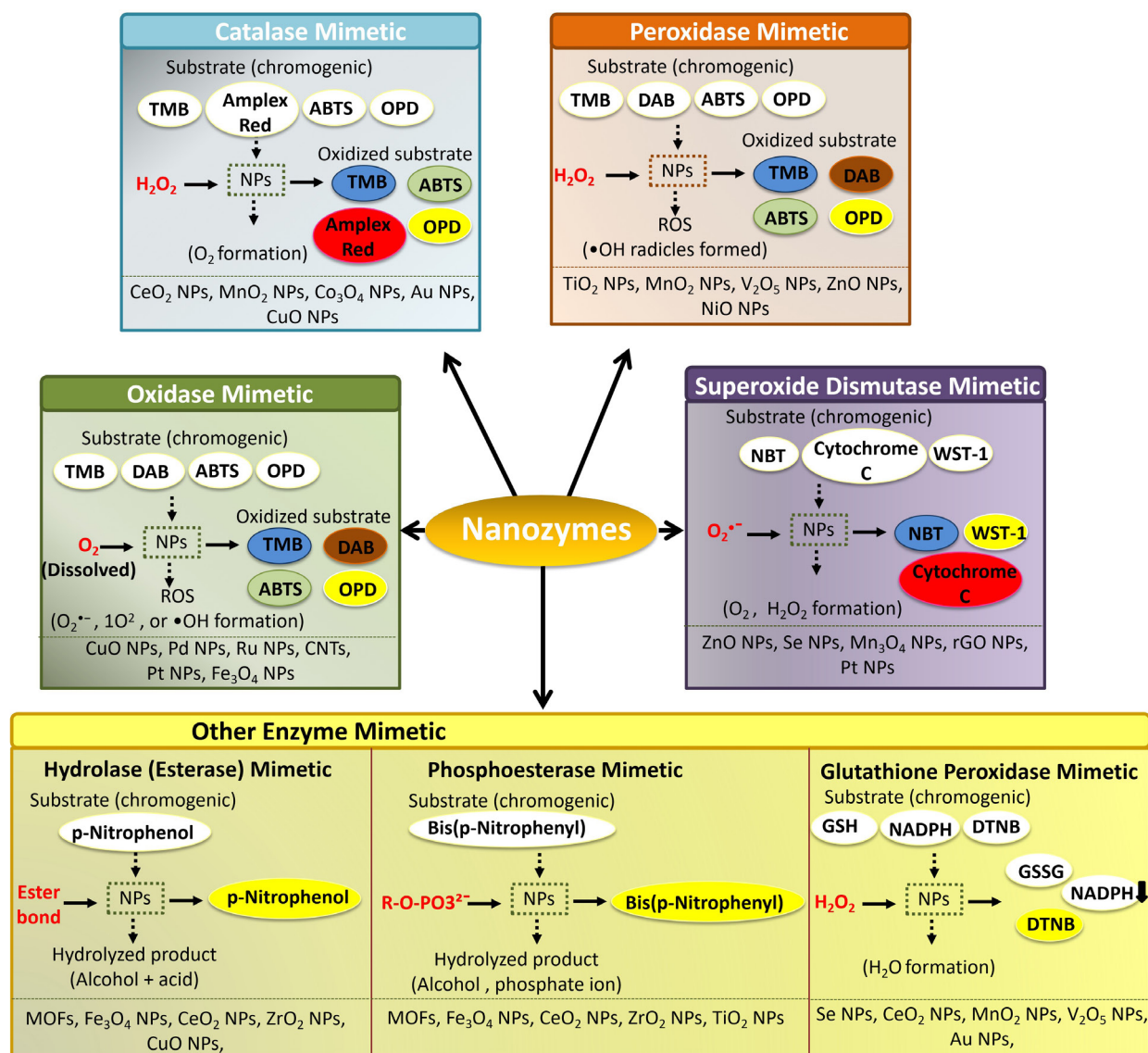


Figure 3. Schematic Representation of Major Nanozyme Categories Classified According to Their Enzyme-like Catalytic Functions.

oxygen (O_2), thereby reducing oxidative stress^[24]. Similarly, SOD-mimicking nanozymes convert superoxide radicals ($O_2^{\bullet-}$) into less harmful species, contributing to antioxidant defense mechanisms. This functional classification is particularly important for selecting appropriate nanozymes for specific applications, such as biosensing, disease diagnostics, food safety monitoring, and therapeutic antioxidant systems^[25]. In recent studies, it has been observed that the enzyme-like activity of nanozymes can be modulated by tuning their surface chemistry and structural features, allowing a single nanomaterial to exhibit multiple enzyme-mimicking functions under different conditions. For instance, certain metal oxide nanozymes such as Fe_3O_4 and CeO_2 demonstrate dual or even multi-enzyme activities (both POD-, CAT-like behavior), depending on environmental factors such as pH and substrate variability. This adaptability enhances their applicability in complex biological systems^[21,26]. OXD-like nanozymes are particularly useful in oxygen-rich environments, where they catalyse substrate oxidation without requiring H_2O_2 , making them advantageous for simplified biosensing systems^[27]. In contrast, POD-like

nanozymes are widely applied in colorimetric detection due to their ability to oxidize chromogenic substrates, producing measurable signals. Hydrolase- and phosphatase-mimetic nanozymes expand the functional scope beyond redox reactions by catalysing bond cleavage processes, such as ester or phosphate bond hydrolysis^[28]. These activities are valuable in environmental remediation and biochemical analysis. Moreover, the integration of multi-enzyme like activities into a single nanozyme system enables cascade reactions, where intermediate products generated by one catalytic function are utilised by another. This cascade behavior improves reaction efficiency and is particularly beneficial in biosensing and therapeutic applications. Overall, the classification of nanozymes based on enzyme-mimicking activity provides a functional framework for understanding their catalytic roles and facilitates the rational design of nanozymes tailored for specific industrial, biomedical, and food related applications. Additionally, the Table 1 summarises various nanomaterials, their catalytic mechanisms, and their practical applications, providing a clearer understanding of their multifunctional roles.

Table 1. The Catalytic Action of Nanomaterials and Their Applications as Nanozymes

Nanozymes	Catalytic reaction & mechanism	Applications	References
Graphene oxide quantum dots CAT-like nanozymes	CAT breaks down H_2O_2 into H_2O and O_2 . $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$	Reduce intracellular ROS and H_2O_2 in 1-methyl-4-phenyl pyridinium ion (MPP ⁺) induced rat pheochromocytoma (PC-12) cells which leads to reduction in oxidative stress and also prevents neurotoxicity.	[29]
Fe/N-doped porous carbon (Fe/NC-800) OXD-like nanozymes	Utilise O_2 as an electron acceptor to oxidise substrates, generating H_2O_2 . These nanozymes function without external H_2O_2 , making them suitable for simplified sensing systems.	Dopamine sensing in human serum samples.	[30]
Carbon nanodots POD-like nanozymes	Catalyse oxidation of substrates in presence of H_2O_2 . $\text{ROOR}' + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{ROH} + \text{R}'\text{OH}$ Widely used in colorimetric assays due to chromogenic substrate oxidation e.g. [tetramethylbenzidine (TMB), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)].	Glucose detection in serum samples.	[31]
Molybdenum quantum dots SOD-like nanozymes	Catalyse dismutation of $\text{O}_2^{\bullet -}$. $2\text{O}_2^{\bullet -} + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$ Provides antioxidant protection by converting toxic radicals into less harmful species.	Neuroprotection in Alzheimer's disease by SOD-mimicking property.	[32]
Multinuclear metal complex (DNase mimics) Hydrolase-like nanozymes	Nanozymes that uses water to break chemical bonds. $\text{A-B} + \text{H}_2\text{O} \rightarrow \text{A-OH} + \text{B-H}$ Involve cleavage of ester, amide, or phosphate bonds via nucleophilic attack.	Disruption of bacterial biofilms, hence antibacterial efficacy.	[33]
Silver NPs decorated on graphene Quantum dots (Ag NPs@GQDs) POD-like nanozymes	Composite nanozymes with enhanced electron transfer and surface reactivity enabling dual catalytic modes. $\text{H}_2\text{O}_2 + \text{TMB}(\text{reduced}) \rightarrow \text{TMB}(\text{oxidised, blue}) + 2\text{H}_2\text{O}$	Dual-mode colorimetric/fluorescence sensing.	[34]
Boron-functionalised polyethylene glycol-coated palladium nanozymes (Pd@PEG@Bor) SOD- and CAT-like nanozymes	Exhibit combined antioxidant activities enabling efficient ROS scavenging and cellular protection. $2\text{O}_2^{\bullet -} + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$; $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$	ROS scavenging; mitochondrial protection.	[35]
Polyvinylpyrrolidone-stabilized platinum-copper nanoclusters Multi-enzyme nanozymes	Simultaneously mimic multiple enzyme activities (SOD-, CAT-, and POD-like) through synergistic metal interactions.	Efficient ROS elimination and therapeutic applications (synergistic Pt-Cu).	[36]
Vanadium pentoxide (V_2O_5) nanowires POD-like nanozymes	Haloperoxidase-like and POD-like activity through redox cycling of vanadium ions.	Antibacterial and catalytic oxidation systems.	[37,38]
Cobalt oxide nanozymes (Co_3O_4) Multi-enzyme mimetic nanozymes	Exhibit redox cycling between $\text{Co}^{2+}/\text{Co}^{3+}$ enables multiple enzyme-mimicking activities (SOD, CAT, POD).	Biosensing and oxidative stress regulation.	[39]
Titanium dioxide nanotube arrays (TiO_2) POD-like nanozymes	Catalyse H_2O_2 dependent oxidation reactions via surface active sites. Photoactivation can further enhance catalytic performance.	Environmental sensing and biosensing.	[40]
Nickel oxide nanozymes (NiO) POD-like nanozymes	POD-like catalytic oxidation via surface redox reactions.	Glucose sensing (0.05–0.50 mM; LOD 2×10^{-5} M.)	[41].
Iron based materials institute Lavoisier-88 (amino functionalised) MOF (Fe-MIL-88NH ₂ MOF/ hemin@MOF) POD-like nanozymes	Catalytic metal centres and porous structures providing adsorption of substrate. MOFs provide H_2O_2 catalysed reactions. (TMB/ABTS)	Used for glucose sensing via colorimetric detection, and POD-mimetic activity respectively.	[42-44]
Co-Fe layered double hydroxides (Co-Fe LDH) POD-like nanozymes	Metal hydroxides and layered double hydroxides exhibit POD-like activity due to catalytic metal active sites and surface hydroxyl chemistry.	Used for chromogenic detection of glucose and H_2O_2 .	[45,46]

Copper II hydroxide Cu(OH) ₂ nanocages POD-like nanozymes	Provide highly active catalytic surface area structures enabling efficient H ₂ O ₂ dependent oxidation reactions.	Used for chromogenic sensing applications.	[47]
Cerium oxide nanozymes (CeO ₂) Multi-enzyme-mimetic nanozymes	Switchable SOD- and CAT-like activity via Ce ³⁺ /Ce ⁴⁺ redox cycling.	Antioxidant therapy and neuroprotection.	[48]
Single atom Iron-Nitrogen-Carbon nanozymes (Fe-N-C) POD-like nanozymes	Highly active isolated Fe sites enabling efficient POD-like catalysis.	Advanced biosensing and catalytic therapy.	[49]

3 NANOZYMES IN ANIMAL NUTRITION

In animals, the utilisation of enzymatic properties of nanozymes as processing aids in the food sector has not generated much exploration, because they are not as widely available compared to conventional enzyme classes, such as hydrolytic enzymes, transferases, and isomerases. In food processing, nanozymes are primarily applied to identify unwanted compounds produced or as antibacterial agents. Regarding the applications, nanozymes can detect either the endogenous food components (e.g., H₂O₂, food allergens, ascorbic acid, glucose, gallic acid, tannins, choline etc.), or non-desirable exogenous contaminants (e.g. mycotoxins, antibiotics, pesticides, pathogens such as bacteria and virus etc.)^[50-52]. The leverage of nanozyme functionalities provides sustainable, environmental friendly and cost-effective strategies for food preservation, food sensing and nutrient material delivery.

3.1 Applications in Food Safety

In this section, current improvements studied in food safety assessment are primarily categorised into four categories depending on contaminant type, comprising mycotoxins, biological hazards, pesticides, and veterinary drug residues. Representative nanozyme-based applications in food and feed analysis are summarised in Table 2.

3.1.1 Mycotoxins Detection

Mycotoxins are toxic secondary metabolites produced by fungi that can have an adverse effect on human and animal well-being, decrease livestock production, impair animal health, and influence the availability of food and nutrition. These toxins are commonly found almost everywhere in agricultural products like grains, cereals and oil seeds. Exposure to single or multiple mycotoxins can cause several toxicity issues that can lead to animal ailments like immune suppression, organ damage, and reproductive disorders in animals. Therefore, there is a strong need to make rapid, sensitive, and easy to carry biosensors that can detect these toxins. Wu et al.^[53] developed a double-signalling approach based ratiometric electrochemiluminescence (ECL) aptasensor for the detection of aflatoxin B1 (AFB1), which causes hepatotoxicity and reduced growth performance in

livestock. In this system, luminol as well as cadmium/zinc QDs (Cd/Zn) were used to generate dual ECL signals. Au nanorods functionalised with HRP acted as both signal quenchers and enhancers. They reduced the ECL signal intensity of QDs via energy conversion simultaneously catalyzing H₂O₂ to augment luminol emission (Figure 4A). Given the internal reference ability to self-calibrate, both the aptasensors provided precise as well as sensitive results for AFB1^[53]. Similarly, Xu and colleagues developed an MOF-based indirect competitive immunoassay using hydrothermal technique to identify AFB1 components. In this system, a secondary antibody (Ab2) was immobilized over the MOF surface through covalent binding technique, followed by conjugation with HRP. The resulting nanozyme displayed improved POD-like enzymatic activity and improved stability over natural HRP for detection of toxins by analysing steady state dynamics of the system (Figure 4B). This approach demonstrated improved stability for AFB1 detection, high accuracy and sensitivity (LOD, 0.009ng/mL)^[54]. In addition, Fe₃O₄ based POD-mimicking nanozymes have been used in colorimetric assays for AFB1 detection in feed, offering high sensitivity, rapid response, and improved stability compared to natural enzymes. These systems are particularly useful for on-site monitoring in animal production systems, where quick decision making is critical for preventing toxin exposure^[55]. Another toxin, zearalenone (ZEN), is an estrogen like compound that can interfere with fertilization in livestock notably piglets, cattle, as well as other bovine animals. Increased amounts of ZEN in feed have been linked to reproductive problems in animals. A colorimetric detection method using POD-mimicking AuNPs was developed based on the inhibition and restoration of nanozyme activity upon aptamer binding or release. This method demonstrated LOD as little as 10ng/mL in maize and corn oil specimens, which is equivalent to traditional ELISA techniques^[56]. In addition, Sun et al.^[57] presented a competitive immunoassay using paper-based lateral flow strips incorporated with AuNPs for concurrently detecting ochratoxin A (OTA) and ZEN (Figure 4C). The test strip consisted two test lines (T1 and T2 lines), formed by immobilizing two competing antigens (OTA-BSA and ZEN-BSA respectively) onto the nitrocellulose membrane while a control line was made of anti-mouse IgG (C line). The conjugation pad had two different types of AuNPs-Ab conjugates (AuNPs-OTA Ab & AuNPs-ZEN Ab), which can capture the contaminant in the samples by moving

Table 2. Types of Nanozymes and Their Applications in Food and Feed Systems

Topic area	Target contaminant	Nanozyme	Catalytic activity	Outcome	References
Mycotoxins sensing	AFB1 detection in food	Polydopamine-coated HKUST-1 (Cu based MOF)-based nanozyme LFIA	POD- like catalysis used for colorimetric signal amplification	Ultra-sensitive field screening for AFB1 to reduce exposure risk and improve quality control with LOD $\approx$ 0.0015 ng/mL	[71]
Mycotoxins sensing	AFM1 + OTA (multiplex) detection in milk.	Antibody- integrated bimetallic (Bi) Au nanozyme-based multiplex LFIA	POD-like activity for colorimetric based color amplification	One-strip multiplex screening of two high-impact mycotoxins enables rapid quality control in dairy supply chains with LODs of $\sim$ 0.06 ng/mL for AFM1 and $\sim$ 0.12 ng/mL for OTA	[72]
Antibiotic residues sensing	Enrofloxacin detection in milk	Polydopamine coated University of Oslo-66 MOF (UiO-66@PDA)-based nanozyme LFIA	Catalytic signal enhancement enabled by nanozymes, which improves detection sensitivity	Enhanced rapid antibiotic residue screening; supports compliance monitoring and minimizing antibiotic contamination into food with LOD $\sim$ 0.7 ng/mL	[73]
Adulterant/ drug-class screening	Sulfonylurea (adulteration) detection in milk	Polydopamine-coated porous coordination network-224 (PCN-224@PDA MOF)-based colorimetric nanozyme sensor	POD-like catalysis drives chromogenic detection	Sensitive detection of veterinary drug adulterants in milk matrices, with LOD values ranging from $\sim$ 0.85-1.13 ng/mL	[74]
Pesticide sensing	Glyphosate detection in water/ practical samples	N-doped carbon dots/ porphyrinic MOF composite (dual-mode system)	Nanozyme- assisted dual-mode sensing via catalytic/colorimetric and ratiometric fluorescence signals	Portable detection using smartphone- based analysis coupled with a fluorescence mode to enhance analytical robustness, with an LOD of $\sim$ 9.06 μ g/L	[75]
Spoilage detection (packaging indicators)	Trimethylamine (TMA) detection, key indicator of fish spoilage	Silver-doped PB nanozyme integrated hydrogel sensor	Strong POD-like activity generates a visible response, while hydrogel incorporation improved practical usability	Real-time TMA sensing for freshness monitoring and reducing spoilage-related waste, with LOD in the range of 0.21-0.54 ppm, and lowest detectable working concentration of 0.21 ppm	[76]
Food packaging/ preservation	Control of microbial growth through active packaging	Self-activating cascade chemodynamic antibacterial film for food packaging under NIR-regulation	Catalytic cascade-based antimicrobial action through reactive species generation	Stimulus- controlled active packaging strategy designed to extend shelf life and minimizing the use of chemical preservatives	[77]

through the pad with capillary action. Therefore, in the absence of any contaminant, all Ab conjugates had been acquired by the capturing materials, leading to the generation of three bright red lines (T1, T2, and C lines), as opposed to just one red line in the presence of both toxins, which was only visible on the C line due to the lack of accessible binding sites on either T line (Figure 4C). This approach provided rapid, multiplex, and field deployable detection.

3.1.2 Biological Hazards

Foods that have been contaminated with biological toxins, which often involve bacterial contamination, viral

contamination, or parasites, can lead to several diseases in the livestock and humans. While nosocomial microbes contaminated feed is often associated with transmission of pathogens to humans, it can also directly affect animal health. For example, the mastitis causing *Staphylococcus aureus* (*S. aureus*) can be transmitted from unpasteurised milk or colostrum from infected cows^[58]. Using AuNPs as signaling molecule, Mondal et al.^[59] created a robust biosensor to identify staphylococcal enterotoxin B (SEB). The technique was centred on aggregation/disaggregation mechanism of AuNPs in suspension which leads to changed color of AuNPs. Aptamers specific to SEB were attached to AuNPs to prevent aggregation under normal conditions.

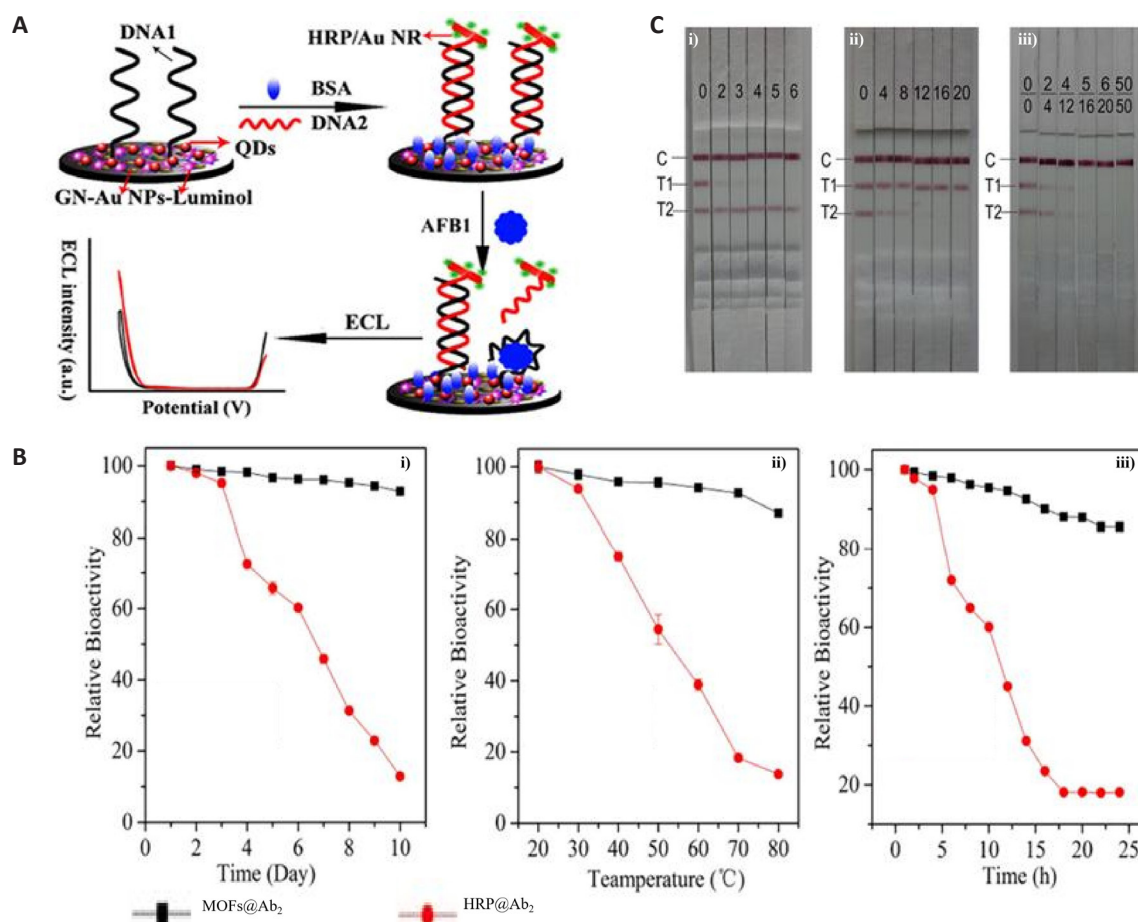


Figure 4. Schematic Representation of the Nanozymes Based Toxin Detection Strategies. (A) Illustration of a dual-signalling ECL aptasensor for AFB1 detection. (B) Stability comparison of bioactive substances in MOFs@Ab₂ (black) and HRP@Ab₂ (red) under diverse conditions: (i) storage for different time frames at ambient temperature, (ii) exposure to different temperatures for 60 min, and (iii) processing with trypsin (1mg/mL) for specific time intervals. (C) Lateral flow test strips for corn sample analysis: C, the control line; T1, the OTA test line; and T2, the ZEN test line. (i) OTA dosages of 0, 2, 3, 4, 5, and 6ng/mL. (ii) ZEN dosages of 0 to 20ng/mL. (iii) The combined dosages of OTA/ZEN are 0/0, 2/4, 3/8, 4/12, 5/16, 6/20, and 50/50ng/mL. Reprinted from Wu et al.^[53], Xu et al.^[54], Sun et al.^[57] with copyright permission for figure obtained from ACS Publications, Elsevier, Wiley.

The aptamers structural distortion in the proximity of SEB caused the clumping of AuNPs, which resulted in a shift from red to blue color. By using this method, SEB can be measured with intensity as low as 50ng/mL and 0.5ng/mL, respectively (Figure 5A and 5B)^[59]. Salmonellosis is another major concern in livestock animals and humans due to the presence of various *Salmonella* serotypes in contaminated feed^[60]. In one study, Pt nanozymes were deposited onto carboxyl-functionalized polystyrene nanospheres to create Ps-Pt nanozymes. Pt offered extremely high POD-like activity to Ps-Pt system. Moreover, streptavidin was attached to the carboxyl group present in Ps-Pt, which leads to higher stability and activity of the nanozymes. Further, biotin Ab was conjugated with streptavidin to obtain immune modified Ps-Pt (IPs-Pt). This system was able to detect *Salmonella typhimurium* (*in italics*) (*S. typhi*) colorimetrically with an LOD of as low as 100CFU/mL^[61]. In addition, Li et al.^[62] developed a Fe₃O₄ nanozyme based colorimetric immunoassay for detecting *Salmonella* in milk and animal derived samples. In this study, Fe₃O₄ nanozymes exhibiting intrinsic POD-like activity were

conjugated with specific antibodies, enabling catalytic oxidation of TMB in presence of H₂O₂ to generate a measurable color signal. The assay demonstrated high sensitivity (LOD $\approx 10^2$ - 10^3 CFU/mL), rapid response, and excellent performance in real milk samples, highlighting its applicability for monitoring *Salmonella* contamination in livestock production systems^[62]. Das et al.^[63] developed a rapid electrochemical aptamer based nanozyme detector to detect *E. coli*, achieving results within approximately 5 min, demonstrating its potential for rapid on-site pathogen detection. Similarly, Wang et al.^[64] introduced a nanozyme linked immunosorbent assay (NLISA) using bimetallic palladium-ruthenium (Pd-Ru) nanozymes for the detection of *E. coli* O157:H7. In this approach, the nanozymes replaced natural enzymes such as HRP, providing enhanced thermal and chemical stability. The nanozyme conjugates catalysed TMB oxidation, enabling sensitive colorimetric detection with limits as low as 8.7×10^2 CFU/mL and demonstrating strong potential for monitoring pathogens in food and animal related systems^[64]. In another approach, aptamer conjugated AuNPs with POD-mimicking enzymatic

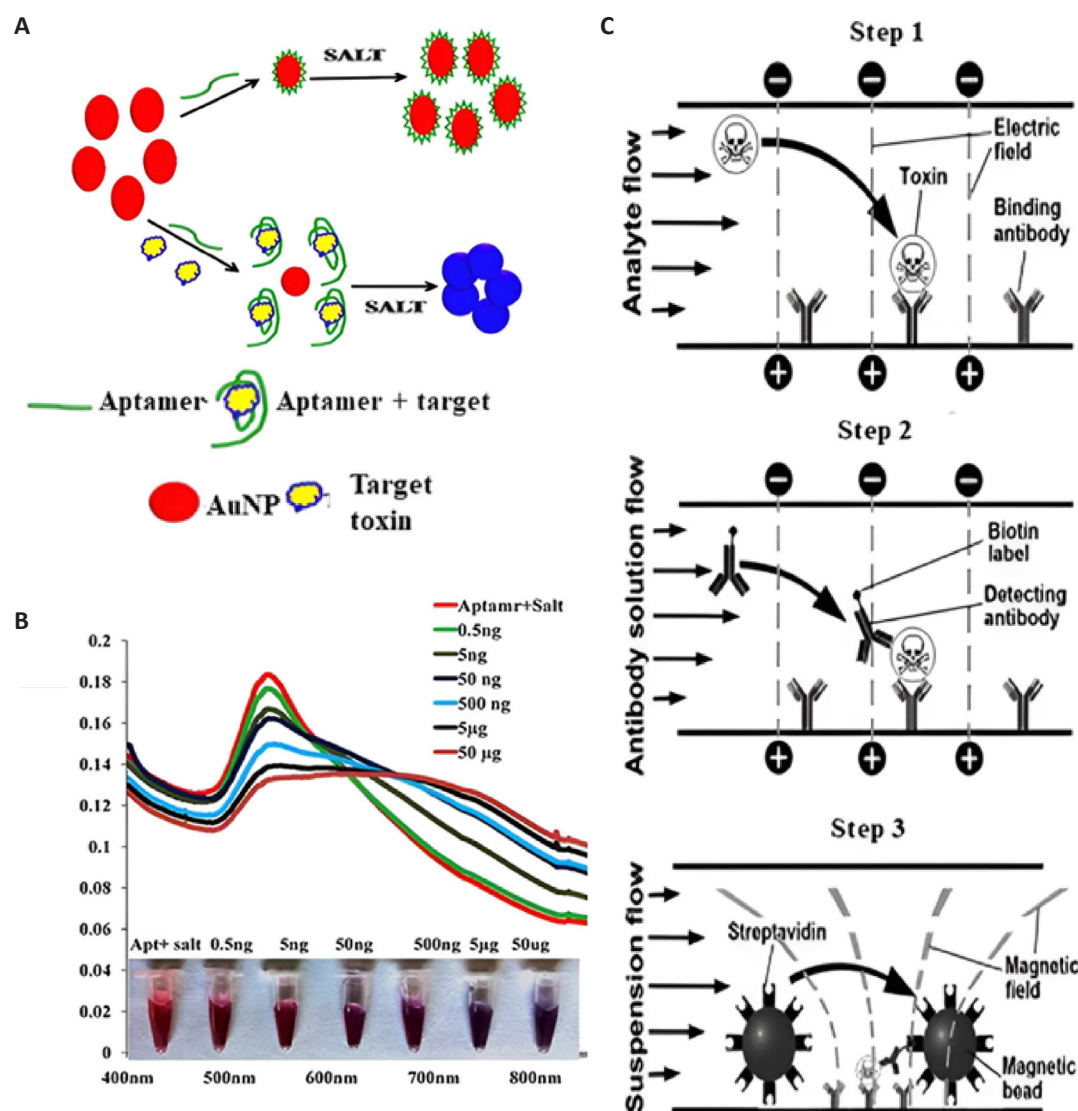


Figure 5. Colorimetric Detection Test Method Based on NPs. (A) SEB colorimetric detection using AuNPs. (B) The detection test relies on AuNPs sensitivity. Absorption spectra of NPs mixed solutions containing SEB in different concentrations. (C) The first step is the electrophoretic capture of contaminants on a microarray. Step 2: Dynamic electrophoretic tagging with biotin-linked secondary Abs of the collected analyte. Step 3 involves scanning the microarray interface with streptavidin-linked magnetic NPs to visualize the biotin labels attached to the microarray. Reprinted from Mondal et al.^[59], Shlyapnikov et al.^[65] with copyright permission for figure obtained from Frontiers (open access article distributed under the Creative Commons Attribution License), ACS Publications.

activity were used for pathogen detection. In this system, AuNPs catalyse the oxidation of TMB to produce a blue color. Binding of aptamers to AuNPs initially suppresses their catalytic activity. However, when the desired components (toxins) are present in the solution, the aptamers preferentially bind to the toxins rather than the NPs, leads to the oxidation of TMB due to the availability of unconjugated AuNPs. This method is very quick, incredibly sensitive, cost effective, and allows visual detection without sophisticated instrumentation. Shlyapnikov et al.^[65] developed a microarray based immunoassay to identify five distinct pathogenic toxins simultaneously. The test consists of three main phases (shown in Figure 5C): (1) toxins are initially captured across the Ab microarray, and then antigens are labelled with secondary biotinylated Abs, (2) magnetic beads coated with streptavidin are used to detect the biotin labels, (3) the microarray beads are detected optically

in a single flow cell when electromagnetic and electrical fields are applied. This detector was capable of detecting cholera toxin, *S. aureus* toxins, and the heat-labile *E. coli* toxin, with very high sensitivity. The reported LOD were 0.1-1pg/mL in water samples and approximately 1pg/mL in food matrices, indicating excellent analytical performance.

3.1.3 Pesticide Residues

The presence of pesticide residues in animal feed poses a significant risk to livestock health and can adversely affect the quality and safety of animal derived products consumed by humans. Although maximum residue levels for pesticide residues in feed have been established by the European Union and the World Health Organization under (EC) No. 396/2005, systematic monitoring of pesticide contamination in livestock feed materials remains inadequate. Pesticide contamination is

frequently seen in crops, livestock feed and animal origin food globally. Pesticide contaminants in the produced farm commodities can cause health issues to individuals (such as animals and humans) and result in financial losses for the livestock sector since pesticides are harmful and made to destroy or suppress target organisms. As a result, the detection and control of pesticides in animal feed is crucial since they may have an adverse effect on the health and productivity of animals and their commodities^[66]. There are several sensors platforms that were designed to detect pesticides; however, nanozyme based systems have gained considerable attention due to their enzyme-mimicking properties, high stability, and cost-effectiveness. In one approach, a heteroatom-doped graphene based nanozyme biosensor was developed for the detection of aromatic pesticide residues colorimetrically. The sensing mechanism relies on the inhibition of the POD-like catalytic properties of nanozymes, as pesticide molecules adsorb onto the graphene surface and block active sites. This system successfully differentiated multiple pesticides, including lactofen, fluoroxypry-meptyl, bensulfuron-methyl, fomesafen, and diafenthiuron, with a concentration of 5-500 μ M^[67]. Another study that employs an innovative biosensing assay for rapid pesticide (acetamiprid) detection based on colorimetric assay, which merges the intrinsic POD-mimicking property of Au NPs with the superior sensitivity and affinity of an acetamiprid-specific S-18 aptamer to detect the neurodegenerative pesticide quickly, precisely, and sensitively. The surface modification of Au NPs with pesticide oriented aptamer hindered the enzyme-mimicking activity of nanozyme. The aptamer particles vacate the surface of the Au NPs depending on the concentration of the target sample, restoring Au NPs POD-like activity. The results can be seen visually in the form of color change (oxidation of TMB) or by measuring the absorbance of the samples^[68]. Similarly, Pt NPs were used as the multichannel colorimetric sensor capable of distinguishing different pesticides, based on their unique inhibition or amplification effect on nanozyme activity. Distinct colorimetric patterns (fingerprint like motifs) were seen for every pesticide (Dursban, dimethoate, 3-ketocarbafuran, glyphosate, and malathion allowing simultaneous identification of multiple contaminants^[69]. In addition, Sun et al.^[70] have explored nanozyme based biosensor using bifunctional CeO₂ nanozyme based electrochemical sensor for detecting organophosphate pesticide methyl paraoxon. In this study CeO₂ exhibited POD-like activity to hydrolyse methyl paraoxon into p-nitrophenol, while the CeO₂ modified electrode simultaneously enhanced signal output. The sensor showed a wide linear range (0.1-100 μ mol/L) and a low detection limit of 0.06 μ mol/L, demonstrating the potential of CeO₂ nanozymes for pesticide monitoring in food and agricultural systems^[70]. These methods offer a simple yet affordable way to identify pesticides contamination and also significantly improve food hygiene and ecosystem.

3.1.4 Veterinary Drug Residues

Pharmacological food contaminants belong to a group of chemicals known as veterinary therapeutics which are regularly used to treat, detect, and prevent livestock ailments to deliberately regulate the biological mechanisms of livestock^[78]. However, feed made with vegetation supplemented using organic wastes, like livestock manure of treated animals, does have the potential to cause plants to absorb pharmaceuticals, which would then be blended into the feed. Small quantities of antibacterial in fermented materials, such as dry distiller grains containing soluble toxins, are another residues in livestock feed. Furthermore, those contaminants might end up in the food supply system and induce damage to individuals directly. Lateral flow immunoassays were extensively utilized for the diagnosis of pharmaceuticals due to the benefits of convenience, economic viability, and accessibility; nevertheless, low sensitivity remains a problem that has to be resolved^[7]. As a consequence, Wei et al.^[79] created an innovative non-lateral flow immunoassay which utilises POD-mimicking Au coupled Pt nanozymes like an optical marker and 3-amino-9-ethyl-carbazole like a colorimetric source. In comparison to conventional Au NPs based on LFIA (8ng/mL), the LOD of Au coupled Pt nanozyme based on NLFIA was reduced by 30%. Tang's team created a novel gaseous pressure-dependent aptasensing system combining a CAT-mimicking Pt nanozyme and a reduced graphene oxide (RGO) structure complexed with poly-aniline (PANI) nanowires to sense kanamycin electrochemically. The system has an LOD of 0.063pM, that is 5 times less than that of the control group, the kanamycin analytical method was very precise, accurate, and repeatable. Whenever the flexible PANI/RGO substrate being used at optimal circumstances in comparison to the control group (no nanozyme), the increase in kanamycin level could be investigated using a pressure-dependent signal ranging between 0.2-50pM^[80]. Other than microbial contamination, medicines used for livestock production, might build up within human tissues through consuming meat and leads to compromised physiological functions. For instance, common adrenoceptor antagonists like ractopamine (RAC) and clen-butanol (CLE) might enhance the proportion of meat and fish as well as stimulate the development of animals, through altering the route and distribution of micronutrients. The build-up of these antagonist remains in palatable portions leads to major negative consequences such as dizziness, muscular tremors, and heart palpitations. For the rapid screening of RAC and CLE in food samples, Liu et al.^[81] developed a multiplex lateral low immunoassay (MLFIA) based on PB nanozyme. The LOD of the PB- MLFIA for RAC & CLE was 0.1-0.2ng/mL, representing a ten-fold lesser threshold than that of the control. The optimum threshold was 3.5 & 6ng/mL respectively for RAC & CLE without TMB-H₂O₂, as well as the LODs both were 1.0ng/mL for RAC & CLE^[81]. Overuse of feed additives seems to have the capacity to result in

a negative influence on the ecosystem, human life, and livestock breeding. Consequently, it is vital for developing rapid and effective testing procedures to examine feed for pharmaceutical residues.

3.2 Food Preservation/Packaging

Food matrices are often thermally treated for seconds to minutes at temperatures between 60°C to 100°C to kill vegetative bacteria to preserve food. There is growing interest in non-traditional food preservation strategies aimed at controlling microbial activity and catalytic processes in order to ensure dietary safety while meeting consumer demands for maintaining nutritional value and quality attributes. Research on nanozymes for preserving food has focused on using persistent catalytic imitation to fight against microorganisms and biofilms^[82]. For instance, Huang et al.^[83] developed carboxymethyl cellulose nanofibers-supported cerium based MOF nanozyme (CNF@Ce-MOF) to create a bio-spraying layer for improved preservation of fruits. These nanozymes produce bactericidal $O_2^{\bullet-}$ and induce ATP depletion as potential reasons for bacterial destruction (Figure 6A). The nanofibers have shown efficient antibacterial activity towards different pathogens present in food, including *E. coli* O157:H7, *S. aureus*, and methicillin-resistant *S. aureus* (MRSA), with destruction rates as 90.46%, 86.91%, and 82.9% respectively (Figure 6B-6E), due to OXD-mimicking and apyrase-mimicking activities of the composite fibres (Figure 6F)^[83]. Furthermore, the development of sprayable or functional nanozyme-based films with antimicrobial properties has emerged as a promising strategy to extend the shelf life of perishable products such as meat. In this context, various metal-oxide based nanozymes, including ZnO, CuO, TiO₂, etc. as well as other inorganic nanozymes have been widely reported for their broad-spectrum antibacterial properties. Unlike conventional molecular antibacterial agents, inorganic nanozymes can be incorporated into polymeric matrices, thereby enhancing their mechanical strength, barrier performance, and antibacterial activity making them highly suitable for active food packaging applications. For instance, Priyadarshi et al.^[84] have developed multifunctional carboxymethyl cellulose-based films integrated with ZnO nanozymes (CAT- and POD-like activities), along with grape seed extracts serving as a natural antioxidant. This composite system was specifically designed for the preservation of high-fat meat products. Notably, ZnO nanozymes enhanced the thermoelectric, physical, and moisture barrier properties of the polymer matrix, while simultaneously imparting strong antibacterial activity against a broad-spectrum of foodborne microorganism, thereby contributing to prolonged freshness of packaged chicken products^[84]. In another study, melanin NPs were utilized to synthesize copper integrated nitrogen doped melanin NPs (Cu-NMPs) possessing SOD- and CAT-mimicking activities, which were subsequently embedded within carrageenan (Carr)

matrices to fabricate Carr/Cu-NMPs nanofilms. The fabricated nanofilms exhibited reduced moisture content, lower water absorption and moisture permeability, and significant free-radical scavenging capacity. Importantly, food preservation studies demonstrated that these nanofilms effectively extended the food storage capacity by approximately 3 days, highlighting its considerable potential in active food packaging applications^[85]. Beyond preservation, nanozyme integrated sensing systems have also shown substantial promise for real-time food quality monitoring. For instance, Sharifnezhad et al.^[86] developed an insulin-functionalized vanadium-iron PB analogue nanozyme ($V_{1/5}Fe(CN)_6$ PBA) as POD-mimicking colorimetric sensor to detect fish freshness. In this system, putrescine, a well-known spoilage biomarker, interacts with $V_{1/5}Fe(CN)_6$ PBA/INS, leading to suppression of its enzyme-like activity and a corresponding decrease in TMB oxidation. The sensor exhibited a highly sensitive response towards putrescine, with a detection limit of 9.0nM and a linear detection range between 30nM-12μM^[86]. Such nanozyme based biosensing platforms could be further integrated into smart packaging systems for the detection of spoilage indicators, pathogenic bacteria, contaminants, and pesticide residues, thereby offering a multifunctional approach to improve food safety, quality assurance, and shelf-life management.

3.3 Applications in Nutrient Delivery

Over the past decade, nanotechnology has emerged as a promising tool for improving livestock nutrition and feed efficiency. In particular, nanozyme-enabled delivery systems offer enhanced bioavailability, targeted delivery, and controlled release of nutrients and bioactive compounds. Conventional nutrient delivery is often limited by gastrointestinal barriers, poor solubility, limited epithelial transport, and enzymatic degradation. Nanozyme-assisted encapsulation systems can overcome these limitations by protecting bioactive compounds and enabling site-specific release through surface engineering, catalytic activity, and nano-scale interactions with biological membranes. These systems can be incorporated into peptide-based carriers, microcapsules, and other feed matrices, improving nutrient stability and absorption efficiency. The nanoscale size increases surface area and interaction with intestinal epithelium, while surface charge and functional groups facilitate mucoadhesion and trans-epithelial transport. From a mechanistic perspective, nanozyme-based systems can enhance nutrient delivery through multiple pathways. First, enzyme-mimicking catalytic activity can modulate local biochemical environments, such as reducing oxidative stress in gastrointestinal tract, thereby improving nutrient stability and absorption. Second, nanozymes can act as protective carriers, shielding bioactive compounds from enzymatic degradation under gastric conditions while enabling controlled release

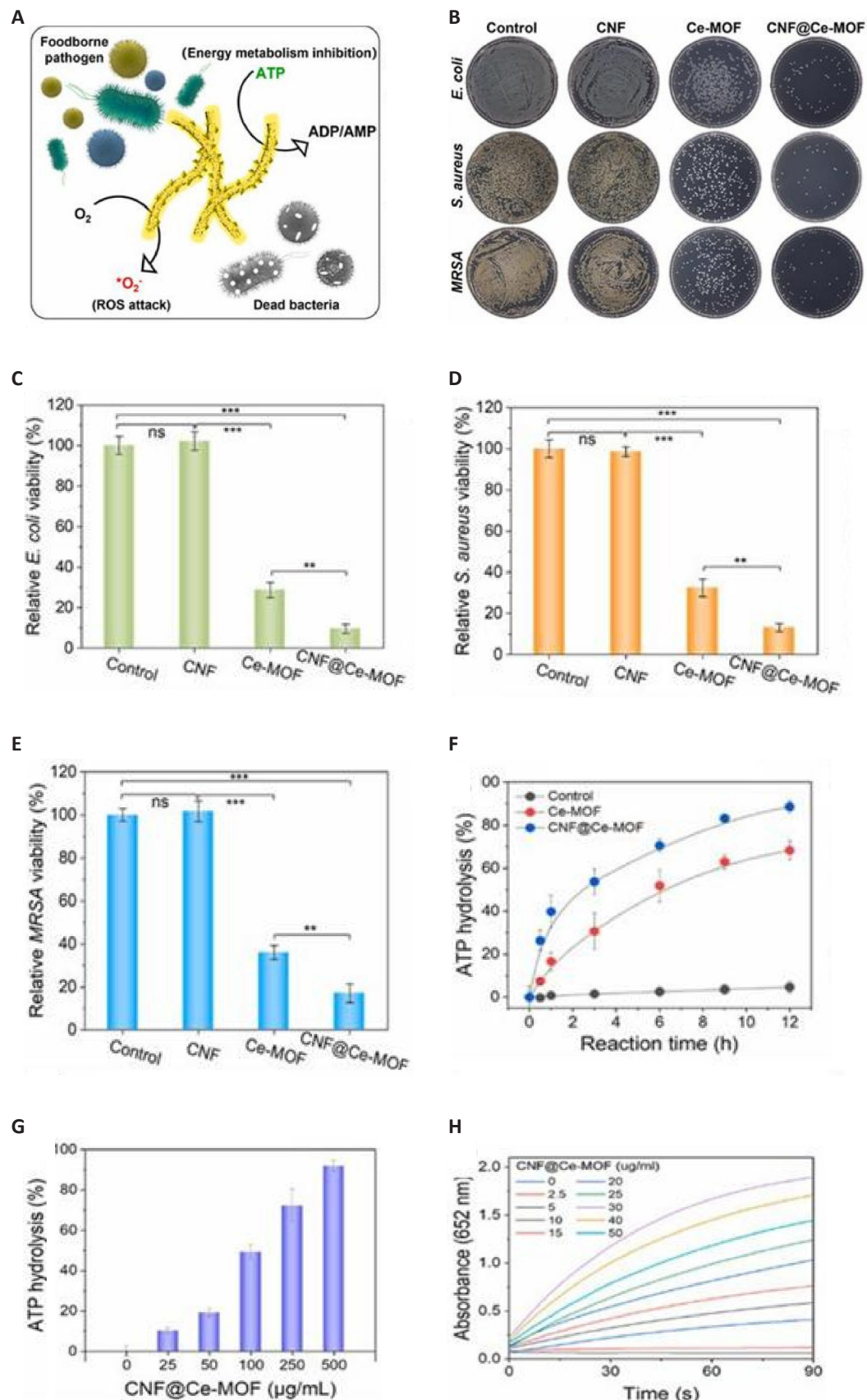


Figure 6. Antimicrobial Performance of CNF@Ce-MOF. (A) Representation of the MOFs based biocatalytic disinfectant method, (B) Antibacterial activity by plate count method using different antimicrobial nanozyme compositions against *E. coli* O157:H7, *S. aureus*, and MRSA bacteria, respectively, (C-E) %viability of *E. coli* O157:H7, *S. aureus*, and MRSA bacteria, respectively, (F) Time-dependent apyrase-mimicking activity of Ce-Uio-66 and CNF@Ce-MOF at a concentration of 100 $\mu\text{g/mL}$, (G) Concentration-dependent apyrase-mimicking activity of CNF@Ce-MOF, (H) Concentration-dependent OXD-mimicking activity of CNF@Ce-MOF. Data are presented as the mean $\pm$ SD. $n=3$, $*p<0.05$; $**p<0.01$; $***p<0.001$; ns, not significant. Reprinted from Huang et al. [83], which is an open access article distributed under the Creative Commons Attribution License.

under intestinal pH. Third, surface functionalization (e.g. polymer coating, ligand conjugation) improves interaction with epithelial cells and enhances cellular uptake through endocytosis or paracellular transport pathways. For instance, Almeldin et al.^[87] reported that dietary supplementation with nano-Fe (20-40mg/kg) significantly improved growth performance, feed efficiency, and tissue iron deposition in broiler chickens, confirming enhanced bioavailability compared to conventional Fe sources. Similarly, Basharat et al.^[88] reported improved intestinal morphology, including increased villus height, indicating enhanced nutrient absorption following Fe₃O₄ nanozyme supplementation. Further, Akbari et al.^[89] employed a cold gelation technique to fabricate calcium-induced nano-carrier systems using cruciferin, a major storage protein derived from canola. These nano-carriers were found biocompatible and did not exhibit any significant cytotoxicity towards Caco-2 cells at concentrations up to 2.5mg/mL. Release studies further demonstrated that the nano-carriers remained stable under simulated gastric conditions, including acidic pH and proteolytic enzymes (pepsin) exposure, while efficiently releasing the encapsulated bioactive compounds (β -carotene), under simulated intestinal conditions. Moreover, encapsulation significantly improved the thermal stability and retention of β -carotene following heat treatment (75°C for 30 min) compared with its unencapsulated control. These findings highlight the potential of cruciferin-based nano-carriers as efficient delivery systems for the stabilization, protection, and controlled release of bioactive food and feed ingredients^[89]. Similarly, Shah et al.^[90] developed zein-chitosan complex Pickering emulsions for vitamin D3 demonstrating improved physicochemical stability and protection of the encapsulated compound under gastrointestinal conditions. In another study, Cai et al.^[91] evaluated Se nanozyme-based (0.3–2.0mg/kg) supplementation in broiler chickens through controlled feeding trials. The experimental group receiving Se nanozymes did not show significant improvements in growth performance matrices. Biochemical analysis revealed increased GPx activity, reduced lipid peroxidation, and enhanced immune response markers, demonstrating improved nutrient assimilation and antioxidant capacity^[91]. Beyond general nutrient delivery, nano-enabled feed systems may also hold particular relevance for addressing production-related health challenges in livestock. For instance, leg abnormalities in broiler chickens represent a major concern in the poultry sector, as they are associated with impaired growth performance, reduced mobility, increased mortality, and consequent economic losses. Although leg weakness is known to have a substantial hereditary component, nutritional interventions remain important for supporting bone and muscle development. Nanotechnology-based delivery of dietary hemp and cannabinoids facilitates the absorption of these compounds as encapsulation within micro-scale, water-soluble carriers, bypasses the gastrointestinal tract's harsh enzymes

and thus significantly improves absorption rates and therapeutic effectiveness^[92]. In this regard, nutrient-loaded nanomaterials could offer a targeted strategy for improving the delivery and bioavailability of minerals and other functional nutrients required for skeletal and muscular development, thereby contributing to improved animal performance and welfare^[93].

3.4 Devices for Real-time Non-invasive Monitoring of Nutrition Related Metabolites

Proper nutritional status plays a crucial role in maintaining livestock health and productivity. Under-nutrition, over-nutrition, and deficiencies of essential vitamins and minerals can lead to several physiological disorders and reduced animal performance^[94]. In ruminants, important biological processes such as conception, ovulation, and the onset of lactation impose high metabolic demands on animal body and can affect health as well as productive efficiency^[95]. During postpartum period, alterations in insulin sensitivity help to redirect carbohydrates, proteins, and fats towards the mammary gland to support milk production^[96]. However, excessive insulin resistance during late pregnancy and early lactation may increase fat mobilization and raise the risk of metabolic and nutritional disorders^[97]. Therefore, monitoring metabolic biomarkers is important for evaluating nutritional status, physiological stress, immune response, and overall metabolic balance in livestock^[98]. Such biomarkers can also help in the diagnosis of metabolic disorders and support the development of more precise nutritional strategies^[92]. In this aspect, nanozyme-based sensing systems have gained attention as they offer rapid, sensitive, and less invasive methods for detecting important metabolites. For example, Hsu et al.^[99] have developed a non-invasive glucose-sensing microneedle patch (GBMP) for monitoring glycemic status. The patch was made using swellable methacrylate gelatin and incorporated MnO₂/graphene oxide-linked glucose oxidase (GOx) nanozymes (GOx-MnO₂@GO). Upon insertion into the skin, the patch collected interstitial fluid (ISF) and detected glucose through the catalytic action of GOx, which converted glucose into gluconic acid and H₂O₂. This reaction caused a visible color change that could be observed visually or through an RGB value using a smartphone. The GBMP can sense glucose within 3 min using only 5.1 μ L of ISF from skin and visible with the unaided eye or by measurement with a smartphone, showing its potential for rapid and user-friendly metabolic monitoring^[99]. In another study, Sun et al.^[97] and others have reported a portable visual glucose-sensing system that could be used with a smartphone. In this work, oxygen-sensitive polymer dots (Pdots) were prepared and implanted under the skin on the dorsal side of mice for *in-vivo* glucose monitoring^[97,100]. The sensing principle relied on the combination of Pdots and GOx, in which glucose oxidation consumed oxygen and altered the optical response of the sensor. The resulting

color changes were captured and analysed using a smartphone, allowing clear differentiation between normal glucose levels and hyperglycemia. The Pdot-based sensor exhibited a sensitivity of approximately 60% and a glucose detection range of 6-18mM, highlighting its potential for portable metabolic monitoring applications.

4 APPLICATIONS IN ANIMAL REPRODUCTION

Progress in farm animal reproduction depends not only on the understanding of reproductive physiology, but also on the use of advanced scientific technologies. Assisted reproductive techniques have made it possible to obtain offspring from valuable livestock animals that could not reproduce successfully through conventional breeding methods. In recent years, nanotechnology has attracted growing interest in the field of animal reproduction and fertility^[101,102]. NPs have been widely investigated for improving conception rates and protecting sperm cells. In addition, nanotechnology-based approaches such as non-invasive imaging of germ cells and nanopurification methods are being explored to improve assisted reproduction. These advances may help preserve and distribute desirable livestock traits more effectively. Research in this area mainly focuses on understanding the nanoscale features of germ cells^[103], developing nano- biosensors to detect physiological or abnormal reproductive status caused by pathogens and diseases^[104], designing metal NPs-based systems for fertility control^[105], and creating nanodevices for applications such as gamete and embryo cryopreservation^[106,107]. This section presents recent advances in the use of nanotechnology in animal reproduction, with a focus on reproductive hormone delivery, gamete cryopreservation, and the diagnosis and treatment of reproductive disorders. Overall, these developments show the ongoing efforts to improve existing reproductive techniques and to develop new strategies for enhancing the reproductive performance of livestock.

4.1 Cryopreservation of Gametes

Several strategies have been explored to improve sperm cryopreservation in different ruminant species. However, a considerable proportion of sperm cells still undergo severe cryodamage during the freeze-thaw process, which can negatively affect sperm quality and fertility^[108]. In recent years, advances in sperm cryobiology have improved the understanding of molecular and genetic changes associated with cryopreservation. One of the major causes of cryodamage is the excessive generation of ROS, which leads to oxidative stress, lipid peroxidation, membrane damage, and reduced sperm function. To minimize these effects, antioxidants are often added to freezing media to improve sperm survival after thawing^[109,110]. These protective effects are closely associated with antioxidant defense systems involving CAT,

glutathione reductase, SOD, GPx, and reduced glutathione (GSH)^[108,111]. Recently, nanozymes have been investigated as promising alternatives to conventional antioxidants, mainly because of their improved stability and prolonged activity under harsh cryopreservation conditions^[11]. For example, supplementation of bull semen extender with selenium nanozymes at concentrations of 0.5-1.0µg/mL significantly improved sperm vitality, membrane fluidity, and chromosomal integrity after thawing, leading to better *in vivo* reproductive performance compared with the control group. However, increasing the Se nanozymes concentration to 1.5µg/mL showed negative effects on post-thaw sperm quality^[112]. Similar, beneficial effects of Se nanozymes were also reported in cryopreserved ram sperm^[113]. These improvements are mainly attributed to the GPx-like antioxidant activity of Se nanozymes, which helps reduce oxidative stress, lipid peroxidation, and apoptosis during freezing and thawing. Likewise, the addition of Se nanozymes or ZnO nanozymes to the freezing medium improved the post-thaw qualities (motility, viability) of dromedary camel sperm. Compared with conventional additives such as salts and vitamins, these nanozymes increased sperm motility and viability by up to 12% and 20%, respectively while also reducing sperm abnormalities to nearly 5%^[114]. These effects are likely due to their ability to enhance GSH production, improve GPx- and SOD-like activities, and promote ROS scavenging. As a result, sperm survival, membrane integrity, and motility were improved, while apoptotic damage was reduced. Another interesting approach involved the use of an Ag-Au nanohybrid encapsulated within an apoferritin cage, designed to mimic the activities of SOD-, CAT-, and GPx-like^[11]. Enrichment of semen extender with 1 µg/mL of these apoferritin-containing Au-Ag nanozymes significantly improved sperm motility and viability after thawing up to 10% compared with the control. This improvement was mainly associated with reduced ROS generation and lower apoptosis during cryopreservation. Similarly, a nano-lecithin extender conjugated with GPx was shown to improve the plasma membrane integrity of cryopreserved bull sperm and reduce malondialdehyde levels by nearly 20%, although no significant improvement in sperm motility was observed compared with the control^[115]. In addition, the inclusion of CeO₂ nanozymes in semen extender improved sperm motility for up to 96 hours of incubation (Figure 7A). This effect was linked to the preservation of plasma membrane and DNA integrity, which is mainly attributed to the antioxidant properties of CeONPs (Figure 7B and 7C)^[116]. Beyond extender-based applications, dietary supplementation with Zn nanozymes at 50-100mg/kg was also reported to improve seminal plasma antioxidant status, epididymal sperm quality^[117]. Overall these findings suggest that nanozyme-like and antioxidant NPs can play an important role in protecting sperm against oxidative damage during cryopreservation, thereby improving post-thaw sperm quality and reproductive performance.

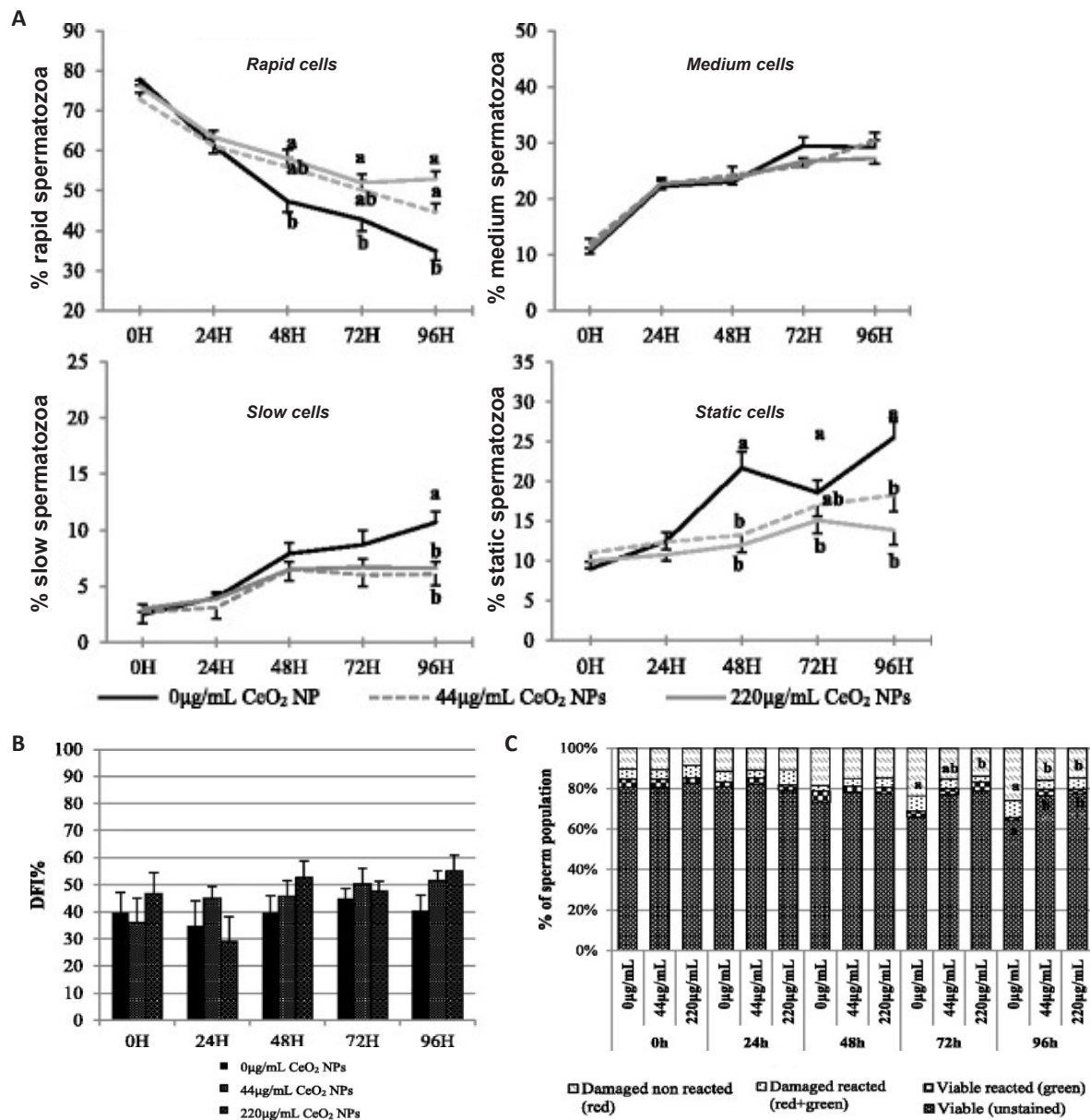


Figure 7. Effects of CeO NPs on Sperm Functional Parameters During Storage. (A) Velocity profile of ram spermatozoa stored at 4°C for 96 hours in the presence of increasing concentrations of CeONPs. (B) ROS generation in ram semen exposed to increasing concentrations of CeONPs. (C) Integrity of the plasma membrane and acrosomal membrane of spermatozoa after 96 hours of incubation at 4°C in the presence of CeONPs. Reprinted from Falchi et al.^[116], an open-access article distributed under the Creative Commons Attribution License.

4.2 Reproductive Hormone Delivery, Reproductive Interventions and Detection of Reproductive Status

The development of nano-based systems for targeted delivery and sustained release of bioactive compounds has emerged as a promising approach in animal reproduction. These advanced delivery platforms have the potential to improve reproductive efficiency by enabling controlled transport and site-specific delivery of functional molecules to reproductive tissues^[118]. Because of their favourable biological properties, nanostructured delivery systems are being increasingly explored for reproductive applications, including hormone delivery, reproductive monitoring, and disease diagnosis^[119]. Simultaneously, nanobiosensors have gained attention as highly sensitive and rapid tools for monitoring

reproductive status. These systems generally combine immobilized biological probes with functional NPs, allowing the detection of hormones, infections, oestrus cycle, and metabolic changes relevant to reproductive health. As a result, nanobiosensors are becoming valuable tools for reproductive management and early diagnosis in livestock. For example, Progesterone (P4), a key steroid hormone involved in maintaining reproductive function, has been detected in bovine serum using an immunosensor. In this system, anti-progesterone monoclonal antibodies were immobilized onto a modified gold disc electrode containing a cystamine self-assemble monolayer and AuNPs, forming the mAbP4-AuNP-cys-Au disc immunosensor^[109]. This type of sensor offers a sensitive and selective method for monitoring hormone levels, which may be useful for estrus tracking and reproductive management. Nanotechnology has

also shown strong potential in reproductive hormone delivery and intervention strategies. For instance, Hasham and Sallem evaluated gonadorelin, a GnRH analog, delivered either in free form or through chitosan-sodium tripolyphosphate (Ch-Na-TPP) NPs in goats. The nanoformulated gonadorelin improved reproductive performance by increasing ovulation rate, serum progesterone levels, and embryo yield. Importantly, the NPs-based formulation reduced the conventional gonadorelin dose by nearly 75% without negatively affecting reproductive or economic performance^[120]. This demonstrates the ability of nanocarrier systems to improve hormone efficiency while lowering dosage requirements^[120]. Similarly, Cerbu et al.^[121] developed chitosan NPs loaded with human chorionic gonadotropin (hCG) for ovulation induction in cattle. The synthesized NPs showed an entrapment efficiency of $54.1 \pm 12.9\%$ and an average particle size of $226.2 \pm 26.3\text{nm}$. These hCG-loaded NPs improved ovulation induction and showed potential as an alternative hormone delivery system in dairy cattle. This study further supports the usefulness of nano-enabled reproductive hormone delivery in livestock^[121]. A related animal study by Hashem et al.^[122] further demonstrated that GnRH-encapsulated chitosan NPs can be used as an alternative to eCG for oestrus induction and ovulation in sheep during the non-breeding season. The nanoformulation supported effective oestrus expression, ovulation, and reproductive performance, indicating its practical value in seasonal breeding management^[122]. Such findings suggest that nanoparticle-assisted hormone delivery may reduce dependence on conventional reproductive drugs while maintaining biological efficiency.

4.3 Diagnosis and Treatment of Reproductive Infections

In addition to hormone delivery, nano-enabled tools are also being applied for the diagnosis of reproductive infections, which are a major cause of infertility, abortion, and reduced productivity in livestock. One important example is porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped single-stranded RNA virus that causes reproductive failure in breeding pigs and severe losses in the swine industry. To detect PRRSV with high sensitivity, Shao et al.^[123] developed a near-infrared electrochemiluminescence immunoassay using PtAu-based branched nanotags. The sensing system relied on enzyme-mimicking catalytic amplification and host-guest molecular recognition, which improved signal stability and sensitivity. The developed sandwich immunosensor achieved a low detection limit of 10.8pg/mL and a broad linear detection range, demonstrating excellent analytical performance for PRRSV detection^[123]. This study highlights the usefulness of nanozyme-assisted sensing for early diagnosis of viral reproductive diseases in livestock. Another major reproductive infection in farm animals is brucellosis, particularly caused by *Brucella*

abortus in cattle. The disease is associated with abortion, infertility, inflammation of reproduction organs, and major economic losses. To improve diagnosis, Zhang et al.^[124] developed a rapid vertical flow technique (RVFT) using PB NPs as nanozyme labels. In this system, lipopolysaccharide, a key antigenic component of *Brucella*, was immobilized onto a nitrocellulose membrane, while the PB NPs provided POD-like catalytic activity to amplify the chromogenic signal. This nanozyme-assisted RVFT significantly improved visual signal intensity and lowered the detection limit for *Brucella* antibodies in both humans and farm animals, making it a promising tool for rapid field diagnosis^[124]. The use of nanozyme-mediated signal amplification in this assay clearly demonstrates its value in veterinary reproductive diagnostics. Another relevant example is porcine parvovirus (PPV), which is an important reproductive pathogen causing embryonic death, fetal loss, and reduced litter performance in pigs. To detect this virus, Lu et al.^[125] developed nanobody-based fusion probes by isolating five PPV-VP2-specific nanobodies from an immunized Bactrian camel. These nanobodies were then fused with either HRP or enhanced green fluorescent protein to create rapid diagnostic probes. Using these reagents, the authors developed both a sandwich ELISA-like assay and a direct fluorescence assay for detecting PPV in swine testicular cells. These assays reduced detection time, eliminated the need for secondary antibodies, and showed strong agreement with real-time PCR, demonstrating their value for rapid viral screening in pig herds^[125]. The assay can be utilised globally to detect the virus with 99.21% accuracy. Overall, these studies show that nano-based systems can support multiple aspects of animal reproduction, including hormone delivery, reproductive intervention, reproductive status monitoring, and diagnosis of infectious reproductive disorders. Together, they demonstrate the growing potential of nanotechnology, and in some cases nanozyme-assisted platforms, to improve reproductive efficiency, disease detection, and reproductive management in livestock. The different types of nanozyme-related and nano-enabled systems used in animal reproduction are summarized in Table 3.

4.4 Nanozymes for Advanced Reproductive Applications

Among several nanozymes platforms, the most promising systems for reproductive applications are mainly used for semen preservation and reproductive disease diagnosis. Their major benefits arise from either reducing oxidative stress during sperm storage and cryopreservation or improving diagnostic sensitivity for reproductive diseases and herd health monitoring. CeO_2 nanozymes are among the most widely studied reproductive nanozymes due to their reversible $\text{Ce}^{3+}/\text{Ce}^{4+}$ redox cycling, which enable CAT- and SOD-like activity. These properties make them useful for protecting sperm cells against oxidative stress. For example, Falchi et al.^[116] evaluated the effects of

Table 3. A Summary of Nanozyme Applications in Animal Reproduction

Nanozyme/Nanoparticle	Application Area	Mode of Treatment/ Exposure period	Reproductive Outcome	Refereces
Albumin coated Cu-Cysteine nanozyme (NACu-Cys)	Cryopreservation	Added to the semen freezing medium during freeze-thaw cycle	SOD-mimetic activity enhanced antioxidant defense, improve post-thaw sperm motility & viability, and reduced apoptosis through ROS suppression	[12]
GO/MnO ₂ nanocomposite electrochemical sensor	Hormone monitoring	On-site progesterone detection using an electrochemical differential pulse voltammetry (DPV) assay, with LOD of 0.001 μmol/L	High recovery in serum and milk samples, supporting oestrus & pregnancy monitoring	[126]
DNAzyme based colorimetric biosensor	Infection diagnostics	Single diagnostic run for <i>Leptospira</i> via colorimetric molecular assay	Rapid detection aiding early reproductive herd management	[127]
CeO ₂ nanozyme	Cryopreservation/ oxidative stress control	Tested during the sperm cryopreservation and evaluated post-thaw in human sperm samples	Improved sperm motility, membrane integrity, and DNA stability	[9]
PBNPs in rapid vertical flow immunoassay (RVFT) assay	Reproductive infection detection	Rapid detection of <i>Brucella</i> antibodies within a few minutes using RVFT	Lower detection limit, improved early Brucellosis diagnosis	[124]
Fe ₃ O ₄ nanozyme	Pathogen/ biomarker biosensing platform	Colorimetric biosensor/ immunoassay platform showing rapid detection of reproductive pathogens and hormones	Enhanced signal amplification for the diagnosis of fertility-related disorders	[128]

CeO₂ nanozymes on ram sperm stored at 4°C for 96h. The study examined ROS generation, sperm velocity, and structural integrity, and showed that CeO₂ nanozymes reduced oxidative stress while preserving sperm quality for long-term cryopreservation and transport^[116]. Se-based nanozymes have also shown strong reproductive potential because selenium is an essential trace element for sperm function and antioxidant defense, particularly through GPx-related pathways. In bull semen cryopreservation, Se nanozymes added to semen extender improved post-thaw sperm quality by enhancing antioxidant status, preserving sperm ultrastructure, and improving fertility-related parameters after freezing and thawing^[112]. In another study, Se conjugated chitosan nanozymes were used as a cryoprotectant during bull sperm cryopreservation. This nanoformulation improved plasma membrane integrity, increased post-thaw progressive motility, enhanced antioxidant defense, and reduced lipid peroxidation, thereby minimizing cryo-oxidative injury^[129]. Recent studies have further strengthened the role of Se-based nanoformulation in reproductive preservation. For instance, Li et al.^[130] showed that co-supplementation of glutathione with Se based nanozymes significantly improved sperm motility, membrane integrity, antioxidant balance, and mitochondrial activity after thawing, indicating improved cryo-tolerance and post-thaw sperm function. Similarly, Sindi et al.^[129] conducting a study in buffalo bull semen reported that nanozyme-conjugated chitosan nanozymes enhanced antioxidant enzyme activity, improved

mitochondrial function and significantly preserved semen quality after cryopreservation, further extending the application of nanozyme-assisted reproductive preservation to agriculturally important livestock. A further important approach involves multifunctional hybrid metal nanozymes, which are designed to mimic multiple natural antioxidant enzymes like SOD-, CAT-, and POD-like. These multifunctional systems are particularly valuable because they can provide broader protection against cryo-induced oxidative injury and helps to improve diagnostic sensitivity for reproductive infections like embryonic loss, infertility, or abortion. For example, Ag-Au apoferritin nanozyme was shown to reduce oxidative stress-associated apoptosis and improved sperm quality post cryopreservation^[11]. Such multi-enzyme-mimicking nanozymes are highly relevant in reproductive preservation because sperm cells are especially vulnerable to oxidative stress, membrane destabilization, and mitochondrial damage during freezing and thawing. In addition to semen preservation, nanozymes are also being increasingly explored for reproductive disease diagnosis. In this context, POD-like nanozymes are useful as they can amplify catalytic or chromogenic signals, thereby improving diagnostic sensitivity. For example, PBNPs known for their stable POD-mimicking activity were applied in a rapid vertical flow assay for the diagnosis of brucellosis. These nanozymes enhanced antibody detection signals, lowered the detection limit; and improved test sensitivity. Since, brucellosis is highly associated with abortion, infertility, and reproductive

failure in livestock, such early diagnostic platforms may help improve herd reproductive health while reducing economic losses. In another recent study, Au@Mn₃O₄ nanozymes were integrated into a dual-signal vertical flow immunofiltration assay for brucellosis detection. The nanozyme provided catalytic signal amplification and dual readout capability, resulting in improved sensitivity and better field applicability for livestock diagnosis^[131]. This study further highlights the practical potential of nanozyme-based reproductive disease screening in animal production systems. Likewise, Fe₃O₄ NPs forms the basis of many biosensor platforms due to their intrinsic POD-like activity. These nanozyme-based biosensors have been explored for pathogen detection and biomarker monitoring, helpful for the management of fertility disorders, reproductive diseases, and associated metabolic disturbances^[124]. Their catalytic amplification properties can make reproductive diagnostics faster, more sensitive, and more suitable for field-based animal health monitoring. Collectively, these findings show that nanozymes can improve reproductive outcomes during cryopreservation through two major pathways. First, antioxidant nanozymes help in protecting sperm cells during storage and cryopreservation by preserving DNA integrity, membrane stability, mitochondrial function, and post-thaw sperm motility showing enhanced fertility potential. Second, POD-like nanozymes improve reproductive health monitoring by enabling sensitive and rapid detection of reproductive infections and related disorders, thereby helping to reduce production loss, infertility, and related abortions. Future developments require dose optimisation, long term toxicology evaluation, and species specific validation to ensure that nanozymes are effective and safe for reproductive applications^[128].

Despite their promising benefits, the *in vivo* biosafety of nanozymes must be carefully evaluated before their wider use in reproductive applications. Because of their persistent catalytic activity and nanoscale size, these particles may accumulate in sensitive organs such as the gonads, liver, spleen, or endocrine tissues depending on their physicochemical properties, surface coating, dose, and route of administration. Such accumulation may influence reproductive physiology and endocrine balance if not properly controlled. For instance, one study revealed that prolonged oral exposure of CeO₂ nanozymes for 32 days resulted in nanozyme accumulation in testes and caused DNA damage, histopathological changes in sperm-producing tissues, reduced sperm motility, and decreased testosterone levels. The mentioned study indicates that enzyme-mimetic NPs may exhibit dose-dependent reproductive toxicity when exposure is prolonged or excessive^[132]. Similarly, another study highlighted bio-distribution concerns associated with PB nanozymes. Following intravenous administration in mice, these nanozymes accumulated mainly in liver and spleen, and induced temporary immune-related alterations. The accumulated NPs were gradually cleared within 60 days, suggesting organ-level distribution and recovery

kinetics should be carefully considered, especially when repeated or chronic dosing is intended^[133]. Importantly, not all nanozyme formulations produce harmful effects. In contrast, some studies have shown clear reproductive protection when nanozymes are carefully designed and used at appropriate doses. For instance, Se nanozymes administered in a chemically induced rodent testicular injury model enhanced antioxidant defense, restored testosterone levels, improved sperm motility, and helped normalize testicular histology^[134]. These benefits suggest that nanozymes may provide both protective and therapeutic benefits in reproductive biology when their dose and biological compatibility are properly optimized. Overall, the above cited studies suggest that nanozymes hold considerable promise for advanced reproductive applications, particularly in semen preservation and reproductive diagnostics. However, their successful future application will depend on proper dose standardization, long-term biosafety evaluation, organ bio-distribution studies, and species-specific reproductive validation to ensure both safety and efficacy.

5 CHALLENGES AND LIMITATIONS

The successful translation of nanozyme-based therapies from laboratory to practical applications in food and animal production requires comprehensive evaluation of biosafety, environmental impact, regulatory compliance, and scalability. Despite their promising catalytic efficiency and stability, nanozymes present several challenges that currently limit their widespread adoption. One of the primary concerns is biosafety and toxicity, as the same physicochemical properties that enhance nanozyme performance such as high catalytic activity, nanoscale size, and prolonged persistence may also contribute to cytotoxicity, organ accumulation, and unintended biological interactions. The toxicity profile of nanozymes is highly dependent on material composition, particle dosage, surface charge, coating and dosage, making it essential to evaluate each formulation individually. In livestock systems, exposure routes are diverse and include direct ingestion through feed and water, administration via reproductive technologies (e.g., semen extenders), and on-farm diagnostic applications, thereby increasing the complexity of risk assessment. For e.g., intravenous (I.V.) dosing of PB nanozymes showed high accumulation in liver and lungs leads to increased intracellular oxidative stress and inflammatory response^[135]. In contrast, surface modification of the PB nanozymes with PEG did not show accumulation in organs while Polyvinylpyrrolidone-coated PB nanozymes leads to acute toxicity in murine models, emphasizing the critical role of surface functionalization in determining biocompatibility^[135]. These reports emphasize on the requirement of surface functionalization, dose standardisation, and long-term toxicological evaluation prior to field application. Another major limitation is the environmental impact and ecotoxicity associated with

nanozyme use. Following application in livestock systems, nanozymes may enter the environment through manure, slurry, soil runoff, and waterways, where they can undergo physicochemical transformations such as aggregation, dissolution, and redox cycling. These transformations may alter their reactivity and persistence, potentially affecting soil microbiota, aquatic organisms, and nutrient cycling process^[136]. Furthermore, the long-term fate and bioaccumulation of nanozymes in environmental matrices remain insufficiently understood, raising concerns about chronic ecological effects and unintended trophic transfer. In addition to safety and environmental concerns, regulatory and ethical challenges represent significant barriers to commercialization. Currently, there is a lack of standardized regulatory frameworks specifically tailored for nanozyme based materials, particularly in the context of food and livestock applications. Existing guidelines for nanomaterials in the food chain (e.g., EFSA, FDA) require rigorous evaluation of toxicity, bioaccumulation, and exposure pathways, but do not fully address the catalytic and dynamic behavior unique to nanozymes. Moreover, ethical considerations related to animal welfare, long-term exposure risks, and consumer acceptance must be carefully addressed to ensure transparency and public trust. Nanozyme-based interventions should therefore demonstrate clear benefits without compromising animal health or introducing hidden long-term risks. Moreover, most nanozymes studies remain at the proof-of-concept or laboratory scale, with limited progress toward large-scale production and commercialization. Challenges include cost-effective synthesis, reproducibility, batch-to-batch consistency, and integration into existing agricultural systems. For instance, PBNPs based detection of Brucellosis via rapid vertical flow test show visible results within 2–3 minutes, considered a promising advancement for veterinary, stuck only as a diagnostic prototype with no commercial rollout^[124]. At present, only a limited number of nanozyme enabled systems have progressed towards pilot scale validation, primarily in biosensing applications, while therapeutic and nutritional delivery systems largely remain in preclinical or experimental stages. For e.g., Cheng et al.^[137] developed a Si@D(AuPt) bimetallic nanozyme-based LFA platform that was validated using real clinical urine samples for the detection of urinary tract infection biomarkers and antibiotics (LOD 11.64 cells/mL for *Pseudomonas aeruginosa* and 3.69pg/mL for cephalexin). Importantly, this platform exhibited excellent reproducibility indicating its readiness for point-of-care and pilot scale diagnostic deployment^[137]. Further, a Pt nanozyme based lateral flow device has been successfully applied for antioxidant detection in both food matrices (fruit juice) and biological fluids (saliva), demonstrating its applicability as a prototype quality control tool^[138]. These studies indicate that nanozyme technologies, particularly in biosensing are approaching field level implementation, although large-scale commercialisation remains limited. This gap highlights the need for standardized manufacturing

protocols and industry-ready platforms. In this context, the above-mentioned evidences reflect strong nanozyme potential in animal nutrition and reproduction, while cytotoxicity, bioaccumulation, and environmental persistence are found to be the major bottlenecks. The major decisive factor for the translation of nanozymes from bench to commercial use is based on regulatory compliance under food chain nanomaterial guidance^[139].

6 CONCLUSION AND FUTURE PERSPECTIVES

Nanozymes termed as new class of synthetic enzyme mimics which can complement or in some cases are projected to replace the natural enzymes due to their high stability, tunable catalytic activity, cost-effectiveness, and structural versatility. Traditional biological enzymes have been found to catalyse a wide range of processes namely translocase, hydrolase, oxido-reductase, ligase etc. Nanozymes, however, can currently only catalyse a limited number of enzymatic processes, primarily those with an oxido-reductase like functions. Nevertheless, continuous advancements in material engineering and catalytic design are expanding the functional diversity of nanozymes. This review highlights current advancements of nanozyme development, classification, and catalytic mechanisms along with their emerging applications in food safety, animal nutrition, and reproductive biology. In food systems nanozyme-dependent sensing platform enable rapid detection of feed contaminants such as pesticides and veterinary drugs, and pathogens, also enhancing preservation of food through antimicrobial property of nanozymes, upregulating nutrient or bioactive delivery, as well as in treatments, diagnostics, and livestock production. In animal nutrition, nanozyme enabled delivery systems improve nutrient bioavailability, controlled release, and metabolic monitoring, thereby enhancing feed efficiency and livestock productivity. In veterinary reproductive biology, nanozymes show enhanced semen motility, improved post thaw recovery by reducing oxidative stress, maintaining sperm membrane and DNA integrity, enabling early detection of reproductive infections to control infertility, and abortion.

Despite nanozyme based health and safety assessment has made considerable advances, still several progress is required to translate the therapies from laboratory research to field compatible technologies. These advances include the dose dependent accuracy, safer design, and specificity of the surface charge of the nanozyme for controlled catalytic behaviour. Increased *in vivo* evaluation, especially in livestock models to access toxicity, bioaccumulation, multi-generation reproductive health, environmental eco-accumulation, modified endocrine pathways needs to be considered. Furthermore, the lack of standardized regulatory frameworks and limited scalability of nanozyme synthesis currently restrict their commercialization.

Future research should focus on expanding the catalytic repertoire of nanozymes beyond oxidoreductase-like activities, enabling the development of multifunctional systems capable of mimicking complex enzymatic pathways. Advances in biointerface engineering, defect modulation, and hybrid nanozyme design may further enhance catalytic efficiency and specificity. Moreover, interdisciplinary collaboration between material scientist, veterinarians, toxicologists, and regulatory agencies will be essential to bridge the gap between laboratory innovation and field level implementation. Overall nanozymes have promising scope to advance targeted nutrient delivery, reproductive efficiency, and food safety analysis in livestock.

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Conflicts of Interest

The authors declared no conflict of interest.

Data Availability

Data sharing is not applicable to this review as no datasets were generated or analyzed during the current study.

Author Contribution

Singh S contributed to the conceptualization, materials and resources, supervision, manuscript review and editing, funding acquisition. Arora P contributed to the literature review survey, Formal analysis, Methodology, Writing – original draft. Mehta D contributed to the literature review survey, writing – original draft.

Abbreviation List

Ab2, Secondary antibody
AFB1, Aflatoxin B1
BSA, Bovine serum albumin
Carr, Carrageenan
CAT, Catalase
CLE, Clen-buterol
DNA, Deoxyribonucleic acid
ECL, Electrochemiluminescence
GBMP, Glucose-sensing microneedle patch
GOx, Glucose oxidase
GSH, Reduced glutathione
hCG, Human chorionic gonadotropin
ISF, Interstitial fluid

NPs, Nanoparticles
OTA, Ochratoxin A
OXD, Oxidase
P4, Progesterone
PANI, Poly-aniline
PB, Prussian blue
Pdots, Polymer dots
POD, Peroxidase
PPV, Porcine parvovirus
PRRSV, Porcine reproductive and respiratory syndrome virus
RAC, Ractopamine
RGO, Reduced graphene oxide
ROS, Reactive oxygen species
RVFT, Rapid vertical flow technique
SEB, Staphylococcal enterotoxin B
SOD, Superoxide dismutase
TMB, Tetramethylbenzidine
ZEN, Zearalenone

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