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Potential of Ionophores as A Feed Additive for Sustainable Beef Cattle Production: Review article

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ABSTRACT

Ionophores are naturally occurring polyether antibiotics that disrupt ion balance in bacterial cells by selectively transporting metal ions across lipid membranes, primarily affecting Gram-positive bacteria in the rumen. The use of ionophores as a feed additive in sustainable beef cattle production offers a promising and innovative solution to key challenges in livestock farming, such as enhancing feed efficiency, lowering methane emissions, and promoting animal health. However, their use is surrounded by several misconceptions, which can lead to confusion among consumers, policymakers, and even within the agricultural industry. This review aims to assess the advantages, disadvantages, and safety aspects of ionophore use in beef cattle production. Published literature related to ionophores in cattle diets and their effects was collected from PubMed, ScientificGate, Google Scholar, ResearchGate and Academia. Ionophores have been reported to reduce rumen disorders like bloat and acidosis. Ionophores reduce methane emissions by altering ruminal fermentation to favor propionate production over acetate and butyrate. Ionophores may exert varying effects depending on the animal, diet, and type and dose of ionophore administered. Studies suggest limited cross-resistance to medically important antibiotics. As ionophores are metabolized and excreted rapidly, it is expected to have minimal adverse effects on human health. Overall, ionophores are promising feed additives that may play a significant role in sustainable beef cattle production, offering producers the opportunity to improve profitability while reducing environmental risks.

Keywords: Antimicrobial resistance, Environmental hazards, Ionophores, Methane reduction, Rumen fermentation.

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INTRODUCTION

Beef cattle production is an integral part of the food supply chain and economy worldwide. The beef industry faces challenges such as rising demand for beef, struggling for feed resources, antibiotic resistance, and environmental concerns. Therefore, exploring alternative strategies to enhance feed efficiency, animal growth, and productivity without any health risk is crucial. As a result, ionophores have gained attention for their potential uses and benefits, as well as their drawbacks, in beef cattle diets **(Marques and Cooke, 2021)**. The usage of ionophores as feed supplements in cattle has manifested the ability to modify rumen fermentation patterns, resulting in enhanced feed efficiency, increased average daily gain, and improved carcass quality in beef cattle **(Duffield *et al*., 2012)**. The compounds selectively target Gram-positive bacteria and ciliate protozoa, reducing competition for nutrients between beneficial and harmful microbial populations in the rumen **(Baba *et al*., 2020)**.

Ionophores have been reported to enhance the digestibility of dry matter and nitrogen, with monensin and lasalocid being the most commonly used and extensively studied in dairy cows due to their role in improving feed efficiency and rumen fermentation **(McGuffey *et al*., 2001)**. Since their introduction in the

1970s, ionophores have become an essential component of beef cattle diets, and numerous studies have demonstrated their effectiveness in animal performance **(Ipharraguerre and Clark, 2003)**. However, their use is most prevalent in feedlot cattle diets, with an estimated 90% of cattle on feed in the United States receiving ionophores (**FELIX, 2024**). Recently, concerns have grown about the environmental impact of livestock production, particularly the emissions of greenhouse gases such as methane. A potential strategy to address this issue involves the inclusion of ionophores in the diets of beef cattle. Ionophores have also been demonstrated to reduce methane emissions by adjusting the microbial populations within the rumen and increasing feed efficiency.

Incorporating ionophores in beef cattle diets could also have notable impacts on the sustainability of beef cattle farming and its role in mitigating climate change. The use of ionophores carries certain risks that need to be taken into consideration. Some studies have suggested that ionophores may harm poultry health, such as impairing immune function and increasing the risk of coccidiosis **(Adhikari *et al*., 2020)**. In addition, there are concerns about the development of antibiotic resistance and the potential impact of ionophores on non-target species in the environment (**Mooney *et al*., 2020**).

Despite these concerns, ionophores remain a promising tool for improving the efficiency and sustainability of beef cattle production. The reviewed publications mostly focused on specific features of ionophores, such as their modes of action in the rumen, toxicity, antibiotic resistance, and safety. We intended to integrate these topics into a comprehensive framework, creating a more unified and accessible resource. This approach offers a concise and systematic synthesis of the current knowledge on ionophore application in beef cattle, thereby serving as a valuable reference for researchers and stakeholders in the field. Although there is confusion about the uses of ionophores in livestock. So, this mini-review aims to provide a balanced overview to guide future research and policy decisions regarding the use of ionophores in beef cattle diets. It will discuss the opportunities and challenges associated with ionophore use in beef cattle production, including their environmental impact. Additionally, the review will explore the role of ionophores in reducing methane emissions, highlighting their potential benefits for sustainable livestock management.

MATERIALS AND METHODS

Published literature related to ionophores in cattle diets and their effects was collected from PubMed, ScientificGate, Google Scholar, ResearchGate and Academia. The identified literatures were meticulously examined and downloaded for detailed and critical analysis subsequently. This review exclusively includes publications that were written in the English language and contained original research data and review articles. The preliminary assessment of the entire content was carried out by selecting abstracts of the published articles. All authors carried on an independent screening of titles and abstracts. The full-text articles are subsequently assessed to determine their eligibility. A total of 52 studies were ultimately selected for detailed analysis. Subsequently, ionophore dosages, mechanisms of action, and applications in livestock, particularly in beef cattle, as well as their toxicity and environmental hazards, were reviewed. Numerous studies have demonstrated that ionophores exert beneficial effects on cattle; however, their use requires careful monitoring due to their classification as a type of antibiotic. Nevertheless, there is a limited body of literature addressing the potential toxicity of ionophores within cattle herds.

RESULTS AND DISCUSSION

Discovery and development of ionophore

In the early days, scientists discovered certain compounds that had the ability to alter the bacterial population in rumen, which aids in break down of feed in rumen. In 1960s monensin was first discovered as an ionophore (**Novilla, 2018)**. The compound was found to significantly enhance feed efficiency in cattle by modulating the balance of microorganisms in the rumen and improving the animals' ability to digest and assimilate nutrients *(***Ogunade *et al*., 2018).** The adoption of ionophores gained rapid popularity by 1970s, monensin had become a prevalent feed supplement for ruminants *(***Frederiksen *et al*., 2024).** During the 1980s, other ionophores such as lasalocid and laidlomycin were created and authorized for implementation in cattle *(***Russell and Houlihan, 2003).** In the 1970s, the U.S. Food and Drug Administration (FDA) approved ionophores as feed additives in cattle *(***Van Norman, 2016).**

General properties of ionophore

As shown in **Fig. 1,** ionophores are molecules that selectively bind and transport ions across biological membranes, including cellular or organelle membranes **(Su *et al*., 2020).** Ionophores are both synthetic and naturally occurring compounds that bind with cations and form complexes **(Li *et al*., 2022).** One of the major properties of ionophore is to disrupt transmembrane ion gradient and electric potentials. Inonophores can discriminate between different ions by their charge, size, and chemical properties. Another fascinating property of ionophore is their structural diversity, ranging from small organic molecules to large protein**.**

For this structural diversity, ionophores show multiple functions in various biological systems, such as signaling, metabolism, osmoregulation, or host defense. Ionophores can modify rumen microbial population. It has huge applications in biology and technology (**Riccardis *et al.*, 2013).** However, ionophores can selectively inhibit specific types of bacteria, which in turn enhances the production of rumen volatile fatty acids, the primary source of energy for ruminants (**Tedeschi *et al.*, 2011).** Moreover, ionophore has been shown to reduce certain diseases like coccidiosis in poultry and in case of cattle, they can be beneficial for controlling bloat and acidosis.

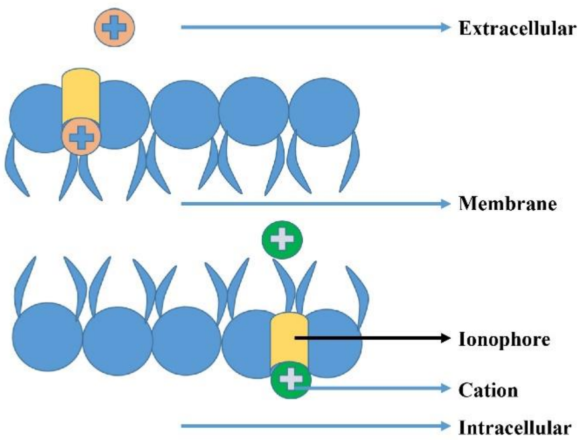

Fig. 1: General mode of action of ionophores by assisting the movement of ions through biological membranes.

Types of ionophore

Ionophores with different types and brands are commercially available worldwide. These include monensin, marketed as Rumensin®, lasalocid, known as Bovatec®, and laidlomycin propionate **(Antoszczak *et al.*, 2014)**. In veterinary medicine, ionophores such as monensin, narasin, salinomycin, lasalocid, laidlomycin propionate, maduramicin, and semduramicin are utilized. While over 120 polyether carboxylic ionophore antibiotics have been identified, only six have received official approval **(Ekinci *et al.*, 2023)**. Mostly used ionophores with their generic name and doses are illustrated in **Table 1**.

Table 1: Types and dosages of ionophores used in different animal species

Generic name	Dose	Indication	Species to be treated	Reference
Monensin	100-125 mg/kg	Improve growth and feed efficiency, coccidiosis control in poultry, bloat, acidosis in cattle.	Beef cattle feedlot, beef cattle pasture, beef calves (not veal), dairy cows, dairy heifers, confined goats, chickens, quail, and turkeys.	
Lasalocid	75-125 mg/kg	Improve growth and feed efficiency, bloat and acidosis control in cattle, coccidiosis control in poultry	Beef cattle feedlot, beef calves (not veal), beef cattle pasture, dairy heifers pasture, domestic rabbits, partridges, turkeys, and chickens.	**(Ekinci *et al.*, 2023; Roder, 2011)**
Salinomycin	50-70 mg/kg	Improve growth and feed efficiency, coccidiosis control for grower and finisher cattle.	Broiler chickens and quail.	
Narasin	60-70 mg/kg	Improve growth and feed efficiency, coccidiosis control in poultry.	Beef, dairy cattle, poultry.	

Mode of action of ionophore against pathogens

Ionophores represent a category of carboxylic polyether antibiotics that occur naturally in *Streptomyces* spp. (**Dembitsky, 2022**). Ionophores demonstrate a high degree of lipophilicity **(Marques and Cooke, 2021)** and the vulnerability of bacteria and protozoa in the gastrointestinal tract is regulated by how well ionophores can adhere to their membranes. The extent of adherence is influenced by the bacterial cell wall architecture **(Weimer *et al*., 2008; Schären *et al*., 2017)**. Ionophores exert a more significant effect on Gram-positive bacteria due to their absence of protective membranes. In contrast, Gram-negative bacteria possess an outer membrane that provides a protective barrier, making them less susceptible to the action of ionophores. However, this mechanism is not fully cracked. Ionophores possess the unique ability to interact with metal ions and act as carriers for their transport across lipid membranes **(Oliveri, 2020).**

Bacteria maintain a more alkaline pH environment by modulating elevated intracellular potassium and diminished intracellular sodium concentration in the rumen. But the rumen environment is the complete opposite of that. The high concentration of short-chain fatty acids (SCFAs) contributes to the slightly acidic pH of the rumen environment **(Russell and Houlihan, 2003)**. Furthermore, mucosal immune response can be supported by SCFAs in the gut and can be used as energy source to modulate gut motility as well as reduce local inflammation **(El-Sayed *et al*., 2022)**. Rumen bacteria depend on maintaining a precise equilibrium between sodium and potassium ion gradients to uphold optimal intracellular conditions. Ionophores function as antiporters of metal and protons, enabling the exchange of hydrogen ions for either sodium or potassium ions **(Russell and Houlihan, 2003; Azzaz *et al*., 2015)**.

As shown in **Fig. 2**, ionophores, when administered, incorporate themselves into the lipid membranes of bacteria in the rumen. The action of ionophores disrupts the ionic equilibrium of bacterial cells both intracellularly and extracellularly. This disruption leads to a decrease in intracellular potassium levels and pH, alongside an increase in intracellular sodium levels. These alterations in ion concentrations occur as a result of the ionophores' ability to influence the transmembrane flux of ions, ultimately leading to ion imbalances inside the bacterial cell. In response to the disruption caused by ionophores, rumen bacteria activate ATPase systems for sodium/potassium and hydrogen to remove excess protons from the cell. Although these ATPase systems facilitate the removal of excess protons, the antiporter activity may lead to a depletion of intracellular ATP during hydrogen ion removal. Thus, the cellular viability is decreased **(Azzaz *et al*., 2015).**

Ionophores possess a unique ability to selectively bind specific ions **(Huczyński, 2012)**. This selectivity is a hallmark characteristic of each ionophore and serves as a critical index of their ion-binding preferences **(Russell and Houlihan, 2003)**. Despite a shared mechanism of action, the variations in selectivity across ionophores can influence their effectiveness in achieving optimal concentrations in the rumen and modifying bacterial populations. Certain bacteria are capable of producing ionophores, which can disrupt the ion balance of other bacteria and inhibit their growth. Interestingly, these ionophore-producing bacteria themselves are naturally resistant to ionophores, but the mechanisms behind this resistance are not well understood **(Azzaz *et al*., 2015).** Though the initial belief was that ionophores could only permeate the cell membrane of Gram-positive bacteria, rendering them more vulnerable to inhibition by ionophores **(Weimer *et al*., 2008).**

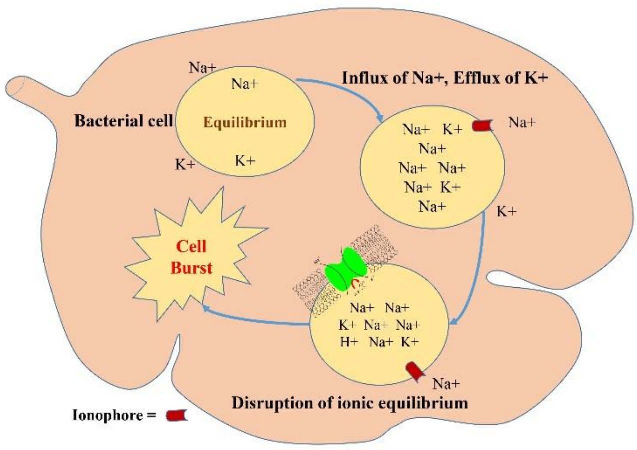

Fig. 2: Bacterial cell disruption in the rumen by ionophore.

Role of ionophore based diet in cattle production

The beef industry frequently utilizes ionophores as rumen modulators and coccidiostats. Numerous meta-analyses regarding the performance of beef cattle have been previously documented (**Table 2**). The application of monensin in feedlot cattle was associated with a 3.1% reduction in dry matter intake (DMI) and a 2.5% increase in average daily gain (ADG), leading to a 1.3% improvement in feed efficiency **(Duffield *et al.*, 2012)**. This decrease is thought to be a result of advancements in feedlot cattle management, nutrition, and health. Monensin is a widely used ionophore to enhance weight in ruminants, mostly in beef cattle (Table 2). The inclusion of narasin at a concentration of 13 ppm in a forage-based diet resulted in a 14.8% increase in average daily gain in Nellore bulls **(Limede *et al.*, 2021)**. As a result, the live body weight of the animals was higher at the end of the 140-day supplementation period. **Weiss *et al.*, (2020)** also observed that average daily gain by adding monensin and lasalocid to a corn-based supplement increased in grazing steers**. Duffield *et al.*, (2012)** demonstrated that including monensin in grain-based diets for cattle resulted in a linear effect. Higher doses of monensin led to enhanced efficiency but decreased both intake and average daily response. Additionally, in ruminants, ionophores exhibited the capacity to enhance feed efficiency by 10 to 20% in both feedlot and pastured cattle through their effect on the composition of the ruminal microflora **(Marques and Cooke, 2021)**.

Table 2: Effect of monensin and lasalocid supplemented diet on cattle performance

Animals type and diet	Ionophore supplement	Weight gain	Reference
Grazing steer, heifer	Monensin-155 mg/day	81.65 g/day	(**Kunkle *et al*., 2000**)
Growing calf	Monensin-200/400 mg/day	77.11/81.65 g/day	(**Hersom *et al*., 2014**)
Grazing cattle	Lasalocid-200 mg/day	99.79-208.65 g/day	(**Golder and Lean, 2016**)
Concentrate diet on beef cattle	Monensin-28 mg/kg	1.6-1.8%	(**Duffield *et al*., 2012**)

Fermentation role of ionophore in rumen

The addition of ionophores in the diet leads to alterations in the ruminal microbiota and fermentation pathways, which is responsible for the observed impacts on animal performance. Studies indicate that incorporating ionophores into ruminant diets results in enhanced animal performance and feed efficiency. This is achieved by modifying the rumen microbial ecology and fermentation processes **(Duffield *et al*., 2012; Azzaz *et al*., 2015)**. Ruminants derive a significant portion, around 60 to 75%, of their digestible energy from the fermentation of carbohydrates in the rumen. This process produces various compounds such as SCFA, methane, carbon dioxide, ammonia, and microbial cells **(Marques and Cooke, 2021)**. Acetate, propionate, and butyrate are the predominant SCFA in the rumen, and their proportions are influenced by the diet **(Wang *et al*., 2020)**.

Forage-based diets typically exhibit ruminal proportions of acetate, propionate, and butyrate at a ratio of 70:20:10, with an acetate to propionate ratio of 3:1. In contrast, grain-based diets show ruminal proportions of these SCFA at 50:40:10, with an acetate to propionate ratio of 2:1 **(Marques and Cooke, 2021)**. Propionate constitutes a substantial proportion, ranging from 27 to 54%, of the total glucose synthesized by the liver and is considered the most crucial SCFA produced through ruminal fermentation **(Marques and Cooke, 2021)**. In contrast, acetate and butyrate serve as hydrogen donors, with hydrogen being a primary substrate for methane synthesis (**Roder, 2011)**. The production of methane leads to an energy loss for the animal, accounting for 2 to 12% of the total gross energy intake **(Ellis *et al*., 2012)**. However, increasing propionate production while reducing acetate and butyrate production is linked to improved feed energy efficiency and enhanced animal performance.

Studies have demonstrated that the addition of ionophores in both forage-based **(Bell *et al*., 2017)** and grain-based diets **(Azzaz *et al*., 2015)** increases ruminal propionate concentrations and decreases acetate **levels.** A meta-analysis **(Golder and Lean, 2016)** indicated that beef cattle supplemented with over 200 ppm of lasalocid experienced a 4.6% increase in ruminal propionate levels and a 3.2% decrease in acetate levels. Similarly, other studies **(Polizel *et al.*, 2020; Limede *et al.*, 2021)** have reported increases in ruminal propionate concentration, along with reductions in acetate levels and the acetate-to-propionate ratio, in beef cattle fed forage-based diets supplemented with narasin.

Influence of ionophores in methane reduction

Human activities are responsible for generating approximately two-thirds of the total global methane emissions **(Saunois *et al*., 2016)**. The United Nations predicts a global population of 9.8 billion by 2050 and 11.2 billion by 2100, leading to an increase in demand for milk and meat products by 1.04 million tons and 465 million tons, respectively **(Tseten *et al*., 2022).**

However, the rising demand for ruminant

livestock could exacerbate the issue of methane production, further contributing to global warming. The most efficient route of methane production in ruminats has been explained in **Fig. 3**. The fermentation of acetate and butyrate is linked to the formation of methane. Ionophores encourage a change in the pattern of ruminal fermentation that favors propionate synthesis over butyrate and acetate. Propionate is a hydrogen sink, meaning that during its synthesis, hydrogen is consumed. Hydrogen is released during the process of methane production in the rumen. Ionophores limit the amount of hydrogen available for the creation of methane by promoting the production of propionate. The other two main SCFAs, butyrate and acetate, are suppliers of hydrogen **(Tseten *et al*., 2022)**. The generation of propionate by ionophores decreases the amount of hydrogen available for the synthesis of methane. Methane is a waste product generated by the ruminants due to microbial interactions in the rumen. Ionophores function as hydrogen sinks, which reduce the amount of methane produced.

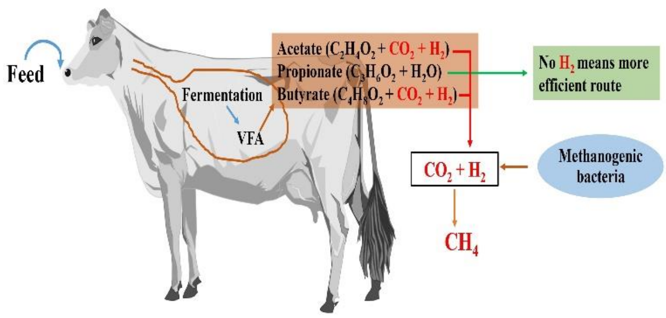

Fig. 3: Association of volatile fatty acids with methane production pathway in the rumen.

In the rumen, the fermentation process involves the cooperation and competition between different microbial communities, such as protozoa, fungi, bacteria, and methanogens. One important aspect of this process is the transfer of hydrogen between these communities. Methanogens consume hydrogen during methanogenesis, while other microbes produce hydrogen during the fermentation of carbohydrates **(Adeniji *et al*., 2020)**.

To prevent the buildup of hydrogen, which can inhibit carbohydrate oxidation, the hydrogen must be transferred between the different microbial communities. **Fig. 3** shows that the propionate production process is free from hydrogen, suggesting it as the best route for methane reduction. Ionophores mostly produce propionate (**Tedeschi *et al*., 2011)** which explains how ionophores help in reducing methane production. Studies reveal that the addition of monensin and lasalocid as feed additives decreases acetate but increases propionate production **(Table 3).** There are multiple strategies that can be used to reduce methane emissions from ruminants, each with its own benefits and drawbacks. These strategies include feed manipulation, supplementation of additives, and probiotics. The challenge is to find a balance between reducing emissions and maintaining the health and performance of the animal. Ionophores like monensin, lasalocid, salinomycin and laidlomycin are used in various countries, including the United States, Canada, Brazil, Argentina, Australia, New Zealand, and South Africa. Ionophores manipulate ruminal fermentation, leading to improved feed efficiency **(Tseten *et al*., 2022). Tedeschi *et al*., (2011)** and **Guan *et al*., (2006)** conceded that the application of ionophores has been found in a noteworthy result of reduction in methane production, specifically by 25 to 30%. **Appuhamy *et al*., (2013)** conducted research on methane mitigation and reported that monensin is more efficient to reduce methane in beef cattle (15%) compared to dairy (2%). Interestingly, feed intake decreased about 4% without showing any negative impact on animal performance.

Table 3: Effect of ionophore inclusion in diet on propionate and acetate production.

Ionophore	Diet type	Change in propionate (%)	Change in acetate (%)	Reference
Monensin	Feedlot	increased	decreased	(Bell **et al.,** ***2017)***
Lasalocid	Feedlot	4.6%	-3.2%	***(Limede** et al**., 2021)***
Monensin	Bermuda grass, hay	10.4%	-1.7%	(Bell **et al.,** ***2017)***

Prevention of metabolic disorders: Bloat, acidosis

The production of excessive stable foam in the rumen can cause bloat, where gas becomes trapped and causes acute abdominal distension. This disorder is often fatal within hours of ingestion. Research has demonstrated that bloat-susceptible animals have a higher viscosity of rumen fluid compared to non-susceptible animals on a feedlot diet. During a 30-day control feeding period, 86.3% of bloat-susceptible animals experienced bloat. However, the inclusion of monensin at a dosage of 40 mg/kg in their diet reduced the incidence of bloat to 4.2% over the subsequent 36 days. Conversely, the withdrawal of monensin from the diet resulted in an increase in bloat incidence to 24.3% over the following 36 days **(McGuffey *et al*., 2001)**. During monensin supplementation, the viscosity of rumen fluid in bloat-susceptible animals decreased to levels similar to those of normal animals. Ionophores can potentially improve feed efficiency by reducing energy wastage and minimizing the risk of bloat.

Ionophores have the potential to alleviate acidosis **(El-Waziry *et al*., 2022)**. Research suggests that ionophores such as lasalocid and monensin can suppress the growth of several predominant strains of lactic acid-producing bacteria, including *Streptococcus bovis*. In contrast, lactate-fermenting bacterial strains are found to be resistant to ionophores. Cattle treated with glucose and ionophore showed that viable cell counts of lactate-producing gram-positive bacteria (*S. bovis* and *Lactobacillus*) were reduced **(Azzaz *et al*., 2015)**. These findings indicate that ionophores may effectively reduce the growth of bacteria that produce lactic acid in the rumen, thereby aiding to alleviate acidosis.

Lethal dosage of ionophore in livestock

Ionophores have been shown to be effective in improving cattle performance on grain and forage-based diets, as reviewed **(Marques and Cooke, 2021)**. Overconsumption of ionophore may result in hazards for grazing animals. Researchers found that ionophores have a long-lasting effect on the amount of SCFA, methane production, and rumen microbes that are insensitive to ionophores. It is also reported that long-time use of ionophores may change ruminal microbes (**Roder, 2011)**. The administration of ionophores to cattle can result in persistent and consistent changes in ruminal fermentation for up to 240 days **(Marques and Cooke, 2021)**. Some studies have also shown that ionophores may suppress methane production, but the duration of suppression may depend on the type of diet animals receive **(Islam and Lee, 2019)**. Overall, further research is needed to confirm the long-term efficacy of its persistence on rumen fermentation dynamics. Compared to other species, cattle are less vulnerable to the harmful effects of ionophores, likely due to factors such as ruminal breakdown, reduced absorption, and differences in cell wall structure **(Ensley, 2020).** The estimated LD_{50} (mg/kg) for monensin, lasalocid, narasin and salinomycin for various species (**Table 4).** The LD_{50} value varies with species and types of ionophores. The highest LD_{50} value for monensin (200 mg/kg), narasin (67 mg/kg), and salinomycin (40-44.3 mg/kg) was observed in chicken. In contrast, the highest lethal dose for lasalocid was observed in cattle (50-100 mg/kg). Ionophore poisoning detected high levels of monensin in the skeletal muscle (25.5 µg/kg) and liver (209.4 µg/kg) of the affected animal. In this study, the clinical signs included muscle weakness, ataxia, recumbence, bilateral jugular distention, and death in cattle.

Table 4: Lethal doses of ionophores in various species.

Generic name	lethal dose (LD_{50}) mg/kg	Species	Reference
Monensin	2-3	Horse	**(Ekinci *et al.*, 2023; Roder, 2011)**
	11.9	Sheep	
	16.7	Swine	
	26.4	Goat	
	200	Chicken	
	20-80	Cattle	
Lasalocid	21.5	Horse	
	71.5	Chicken	
	58	Swine	
	50-100	Cattle	
Narasin	67	Chicken	
	0.8	Horse	
	8.9	Swine	
Salinomycin	40-44.3	Chicken	
	0.6	Horse	
	0.6	Turkey	

Escalating concerns of ionophore in antibiotic resistance

Antimicrobial resistance is a threat to human and animal health nowadays. Polyether ionophores can enhance feed conversion in ruminants due to their antibiotic effects **(Frederiksen *et al.*, 2024)**. Therefore, there is a huge concern regarding resistance due to the widespread usage of ionophores in animal feed, though there are few studies regarding ionophore resistance in cattle production. **Table 5** displays the antibiotic resistance profiles of ionophores. Administration of narasin may contribute to vancomycin resistance in Swedish broiler chickens due to a shared plasmid carrying both narasin and vancomycin resistance genes **(Nilsson et *al.*, 2016; Wong, 2019; Carresi *et al.*, 2024)**. Avoparcin, a drug similar to vancomycin, showed resistance with monensin contributing to cross-resistance likely due to bacterial cell wall thickening (**Carresi *et al.*, 2024**). According to recently published multiple review papers, the ionophore monensin may significantly contribute to the problem of antibiotic resistance **(Brito *et al.*, 2020; Warsi *et al.*, 2024)**. Monensin showed resistance against *Staphylococcus hyicus* and *Enterococcus* spp. in pigs **(Wong, 2019).** It is also reported that multiple mutations of *Staphylococcus aureus* result in resistance to monensin **(Warsi *et al.*, 2024)**. However, it is important to note that when assessing the resistance of mutants, the conventional minimum inhibitory concentration (MIC) test is limited due to the paradoxical growth pattern observed in certain mutants, a phenomenon known as the Eagle effect.

Table 5: Antibiotic resistance profile of ionophores

Species	Ionophore	Resistance	Reference
Pig	Monensin	Up to 6% in *Staphylococcus hyicus* and *Enterococcus* spp.	
Swedish Broiler Chicken	Narasin	Vancomycin resistance in *Enterococcus* spp.	**(Wong, 2019)**
Not specified	Monensin or Lasalocid	No significant cross-resistance to MIAs	
Not specified	Tetronasin	*P. ruminicola* increase in resistance to avoparcin	**(Frederiksen *et al.*, *2024)***
Cattle	Monensin	No significant effect on antibiotic resistance genes in gut	**(Thomas *et al.*, 2017)**
Poultry	Narasin	Possible interaction with vancomycin resistance	**(Wong, 2019; Frederiksen *et al.*, 2024)**

The hydrophobic nature and molecular size (exceeding 500 daltons) of ionophores make them resistant to gram-negative bacteria due to their protective outer membrane. Conversely, gram-positive bacteria, lacking this protective outer membrane, are typically sensitive to ionophores **(Azzaz *et al.*, 2015)**. A crown ether-based synthetic ionophore called 'hydraphiles' exhibited toxicity against bacteria due to selective transport of ions across bilayer membranes **(Patel *et al.*, 2019)**. Ionophores have been widely used in poultry farms as a coccidiostats and growth promoter safely for over 45 years **(Parker *et al.*, 2021)**. Adding ionophore to chicken feed appears to have mixed effects on antimicrobial resistance **(Wong, 2019)**, while a recent metagenomic study in cattle found no significant impact on the population of antibiotic resistance genes **(Thomas *et al.*, 2017)**. However, national, regional, and worldwide organizations, including the World Health Organization (WHO), do not recognize ionophores as medically important antibiotics (MIA) **(Parker *et al.*, 2021)**. Thus, it is logically hypothesized that their agricultural use is unlikely to affect human health. A systematic review sought to assess the potential for cross-resistance between ionophores and MIAs. The findings showed that among 16 drugs (most of which are MIAs) tested against monensin- or lasalocid-resistant cultures of *Clostridium aminophilum*, cross-resistance was observed only for bacitracin, suggesting that cross-resistance is not a significant concern **(Wong, 2019)**.

Navigating safety and environmental hazards in ionophore usage

Ionophores have been spotted in various environmental components, including surface water, groundwater, and sediment **(Hansen *et al*., 2009)**. Ionophores are only used in animals; therefore, they have no medical importance in human health **(Wong, 2019)**. Reports suggested that ionophores can interact with antioxidants such as dihydroquinolone and also antibiotics like tiamulin, macrolide, chloramphenicol, and sulfonamides, resulting in increased toxicity (**Roder, 2011)**. In addition, researchers have heightened concerns that polyether ionophores, such as lasalocid, maduramycin, monensin, narasin, salinomycin, and senduramycin, may have negative health impacts on humans. Such as they can enhance blood flow and coronary dilation due to muscle contractility which may lead to coronary heart disease **(Soares *et al*., 2022)**.

Fortunately, the liver swiftly absorbs and metabolizes ingested ionophores, which are subsequently excreted via bile and primarily eliminated through feces (**Fig. 4**).

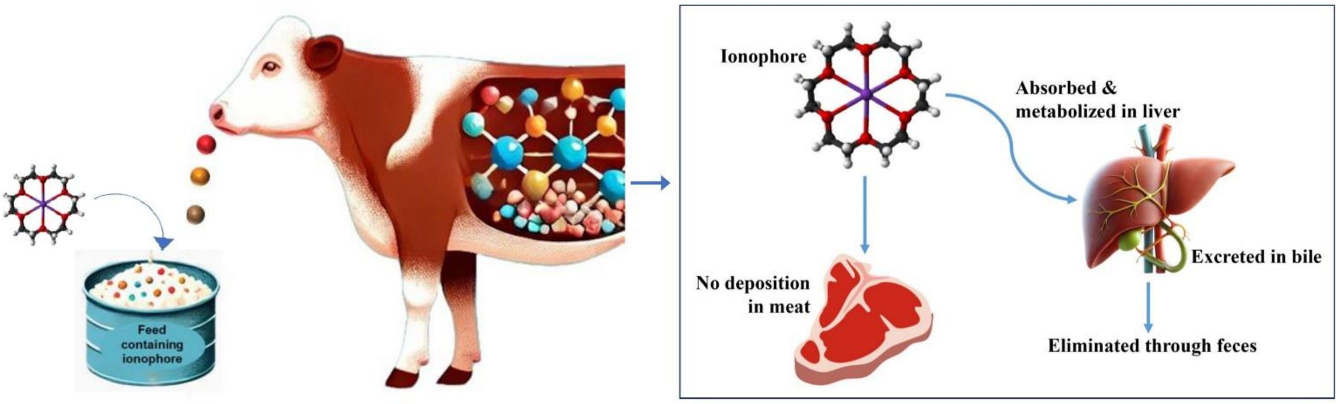

Fig. 4: Absorption, metabolism, and excretion of ionophores in beef cattle.

Consequently, administering ionophores as a feed additive in cattle and poultry poses no risk of tissue deposition (**Roder, 2011)**. Treatment with ionophore in beef production did not impact meat color, surface discoloration, or lipid oxidation in retail meat **(Wong, 2019)**. Determining the extent of ionophore resistance is challenging due to the absence of defined clinical breakpoints for assessing resistance levels. However, it is noteworthy that the use of ionophores for the enhancement of ruminal fermentation may be hampered due to long-term supplementation **(Prathap *et al*., 2021)**. Despite substantial studies on the application of ionophores in beef cattle, a considerable deficiency persists in comprehending their environmental residues. Although ionophores are commonly utilized, their usefulness and possible environmental consequences remain little characterized. Consequently, additional research is necessary to evaluate and quantify ionophore residues in the environment linked to beef cattle production.

CONCLUSION

In conclusion, extensive research has demonstrated that ionophores, when supplemented in beef cattle diets, consistently yield positive outcomes on the rumen microbiome, fermentation processes, digestive disorders, and the reduction of methane emissions. These beneficial effects may assist beef producers in formulating nutritional strategies that can improve productivity and profitability. Ionophores also reduces common digestive disorders in cattle through the disruption of bacterial cell walls and ion balance. Despite these advantages, there remains a lack of conclusive evidence regarding the overall safety and long-term effects of ionophore use in beef cattle, although they have been deemed safe for human health. However, considering the existing research gap, modeling is essential to leverage experimental data for enhancing our understanding of the effects of ionophores on ruminant metabolism and their implications for diet formulation. Based on this review, ionophores may be advised as feed supplements in beef cattle, ensuring appropriate doses. Ionophores are safe for cattle if administered with proper dose. It helps to improve rumen fermentation. Ionophores can be used for methane reduction in cattle. It has limited cross-resistance to medically important antibiotics. It does not have notable consequences on the environment and human health.

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Conflict of interests

There is no conflict of interest to be declared by the authors.

Authors' contribution

Sabrina Zaman Seema and Md. Morshedur Rahman concepted and designed the study. Data collection and interpretation were done by **Moin Uddin, Shamsun Nahar Tamanna and Kazi Md. Al-Noman**. **Sabrina Zaman Seema, Md. Morshedur Rahman and Khatun-A-Jannat Esha** took part in draft manuscript preparation and final reviewing of the manuscript. **Abu Sadeque Md. Selim and S. A. Masudul Hoque** were involved in critical reviewing and overall discussion. All authors reviewed the text and approved the final version of the manuscript.

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