Artykuł przeglądowy *Review*

Application and substitution of antibiotics in animal feeding*

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Application and substitution of antibiotics in animal feeding

Summary

The addition of subtherapeutic doses of antibiotics to feed as antibiotic growth promoters (AGP) can effectively inhibit or eliminate pathogenic microorganisms, reduce the incidence of livestock diseases, optimize feed conversion efficiency, and improve livestock growth performance. Antibiotics play a vital role in ensuring the supply of animal products and enhancing the economic benefits of animal husbandry. Nevertheless, global attention has been drawn to issues such as bacterial resistance, drug residue, and environmental pollution caused by antibiotic misuse – including excessive dosage, prolonged administration, and broad application scope. With the growing emphasis on food safety, an increasing number of countries have prohibited the addition of antibiotics to feed as growth promoters for livestock. To ensure a sustainable and healthy development of the feed industry, research on efficient and low-side-effect antibiotic substitutes has become a focal point. This paper provides a review and summary of both the advantages and disadvantages of adding antibiotics to feed, as well as the alternative research.

Keywords: application, substitution, antibiotics, feed

Antibiotics are a class of secondary metabolites produced by microorganisms, higher animals, and plants capable of resisting pathogens and interfering with the growth of other living cells. Incorporating subtherapeutic doses of antibiotics into feed as antibiotic growth promoters (AGPs) can effectively inhibit or eliminate pathogenic microorganisms, prevent and control animal diseases, promote animal growth, and offer a cost-effective solution. For over 70 years, the use of antibiotics in animal feed has facilitated intensive breeding practices while generating significant economic benefits for the animal husbandry industry. Driven by economic interests, the abuse of antibiotics, including over-dosage, continuous drug use during withdrawal period, beyond application range of drugs and superimposed drugs incorrectly, occurs from time to time. With the advancement of research and application, a series of issues stemming from the misuse of antibiotics have been gradually coming to light (40). The problems of antibiotic resistance, drug residue, and environmental pollution caused by the misuse of antibiotics have attracted global attention. With the advancement of society, people's demand for high quality food is continuously increasing. Consequently, more and more countries have established and enforced relevant laws and regulations to restrict or prohibit the use of antibiotics in animal feed. On January 28, 2022, the European Union's new laws came into force, banning routine feeding a diet of antibiotics to farmed animals – a move that World Animal Protection considers to be the most progressive in the world (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02019R0006-20220128

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&qid=1693301373272). As a result, it has become inevitable to develop efficient antibiotic substitutes with minimal side effects, which is currently a hot topic among scholars worldwide.

Advantages of adding antibiotics to feed

In 1928, Alexander Fleming' s discovery of penicillin marked a pivotal moment in the history of medicine, catalyzing the development of antibiotics and initiating what is now known as the "antibiotic revolution" (9). Since then, more than 200 antibiotics have been successfully developed worldwide, with over 60 being utilized as feed additives in the livestock production.

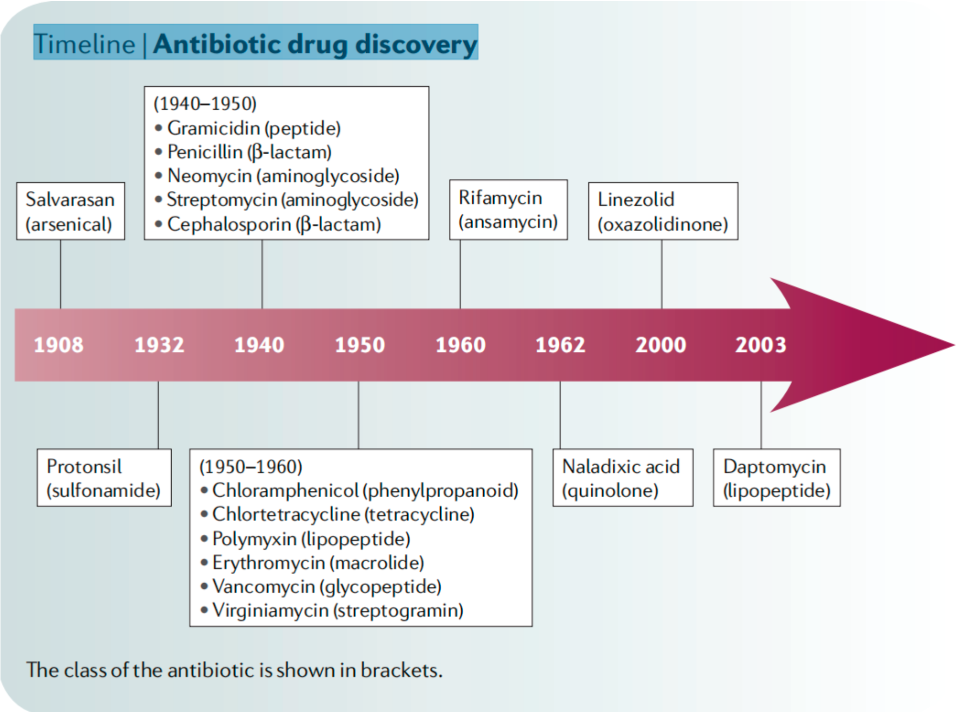

Fig. 1. The timeline of antibiotic drug discovery (38)

From the 1950s to the 1970s, scholars from various countries conducted numerous studies on the addition of antibiotics to animal feed. Moore et al. (24) were the first to report that adding an appropriate amount of streptomycin to broiler feed significantly improved daily weight gain. Stokstad et al. (30) found that metabolites produced by *Streptomyces aureofaciens* could promote the growth of poultry. Whitehill et al. (36) found that adding 25 mg/kg chlortetracycline hydrochloride to the diet could promote the growth of chicks. In 1951, the US Food and Drug Administration (FDA) officially sanctioned the use of antibiotics as feed additives without veterinary prescription (16), making the United States the first country in the world to approve antibiotic feed additives. Subsequently, scholars worldwide conducted numerous experiments on antibiotic feeding. Messersmith et al. (22) discovered that administering chlortetracycline throughout the entire reproductive cycle of sows post-weaning can effectively enhance sow live litter rates. Zimmerman (45) observed that the addition of antibiotic feed additives can improve finishing pig growth performance, resulting in a 15% increase in the early-stage growth rate and a 6% improvement in the feed conversion rate, as well as a 4% increase in the late-stage growth rate and a 2% enhancement in the feed conversion rate. Zimmerman (45) estimates that the economic benefits of adding antibiotic growth additives to the feed of broiler chickens, fattening pigs, and fattening cattle are \$ 0.11, \$ 2.64, and \$ 6.50, respectively.

The use of antibiotics as growth promoters in small quantities can effectively maintain animal health and enhance growth performance (13), disease resistance, feed conversion rate, growth rate, and product quality, while reducing morbidity and mortality rates. This practice also reduces feeding costs and brings economic benefits. Antibiotics can prevent and treat animal diseases, as well as improve animal performance through various mechanisms, such as causing fatal or subfatal damage to pathogenic bacteria, reducing the production of bacterial toxins, decreasing nutrient utilization by bacteria, increasing synthesis of vitamins and other growth factors, promoting nutrient absorption by thinning intestinal epithelium, and slowing down intestinal peristalsis. Due to their evident efficacy, subtherapeutic doses of antibiotics have been widely accepted as growth promotion additives in animal feed by manufacturers of both feed and veterinary drugs across various countries. Cromwell's estimates indicate that antibiotics are added to approximately 80-90% of nursery pig feed, 70-80% of growing pig feed, 50-60% of finishing pig feed, and 40-50% of sow feed (4). The use of antibiotics as growth promoters is prevalent in animal husbandry.

Problems caused by the addition of antibiotics to feed

The addition of antibiotics to animal feed can provide numerous benefits, but improper usage of those substances may result in various adverse consequences. Since the 1950s, subtherapeutic doses of antibiotics have been used as feed additives in animal husbandry to promote growth performance. While antibiotic growth promoters have laid the foundation for standardized animal husbandry production and played an important role, they have also brought hidden dangers to the long-term healthy development of animal husbandry, animal food safety, and human health. Due to various factors, such as interest-driven motives and inadequate supervision, irregular use of antibiotics has been observed, including, but not limited to, excessive scope, dosage and duration of treatment, indiscriminate combination therapy with multiple antibiotics, and even the utilization of prohibited agents. According to the World Health Organization (WHO), 90% of all antibiotics consumed worldwide each year are used in livestock, with 90% of that amount being utilized for feed conversion optimization. China produces approximately 210,000 tons of antibiotics annually, with 100,000 tons allocated towards poultry and livestock farming (40). The prolonged misuse of antibiotics can result in a range of severe consequences, including antibiotic residues, bacterial resistance, environmental pollution, compromised immune function, and adverse effects on human health.

Increased bacterial resistance. Bacterial resistance refers to the phenomenon where a certain bacterium becomes resistant to a drug it was previously susceptible to, and is no longer responsive to that particular drug (38). Bacterial drug resistance is an adaptive mechanism that bacteria use for survival. Once such resistance develops, the therapeutic efficacy of drugs will be significantly reduced. Since the initial use of antibiotic growth promoters in livestock production, there has been a growing prevalence of antibiotic-resistant bacteria isolated from livestock (1, 3, 15). For instance, multidrug-resistant *Salmonella typhimurium* was first identified in livestock (18), while drug-resistant *Campylobacter jejuni*, which causes human disease, was initially detected in poultry farms where antibiotics were utilized (42).

Bacterial resistance is an escalating global issue, and the development of novel drugs fails to counterbalance this challenge (27). Antibiotic residues in livestock products can enter the human body through the food chain or wastewater discharge into land, which may lead to an expansion of resistant strains or their mutation into more resilient microbes. Long-term use of antibiotics as feed additives can lead to the development of bacterial resistance to therapeutic antibiotics. If these resistant microbes are transmitted to humans, they may pose a potential risk. The utilization of antimicrobials in food animals is regarded as a public health concern by both the World Health Organization (1997) and the Economic and Social Committee of the European Union (1998) (5). Worldwide, 700,000 people die from drug-resistant bacterial infections, HIV, tuberculosis, and malaria, and antibiotic resistance causes nearly $ 20 billion in direct healthcare costs each year. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent antibiotic-resistant bacterium, responsible for over 80,000 infections and 11,000 fatalities annually in the United States alone (17).

Environmental pollution. A significant quantity of feed containing antibiotics is used in the breeding of livestock and poultry. The discharge of these antibiotics into the environment through animal excrement, either in their raw form or as metabolites, can directly impact environmental microecology and lead to water and soil pollution. Antibiotic residues in contaminated water and soil can be absorbed by animals and plants, subsequently entering the food chain and posing a threat to human health. In addition, the global annual production of livestock and poultry manure is substantial. However, the presence of antibiotic residues in feces diminishes its value as a fertilizer and impedes its reuse. Furthermore, waste feces exacerbate environmental pollution.

Decreased immunity. The administration of antibiotics at excessive doses and for prolonged periods can severely impair the immune system of livestock and poultry, reduce their ability to resist viral and external infections, and lead to frequent illnesses. Following administration in animals, antibiotics circulate through immune-related organs, such as the liver, spleen, thymus, lymph nodes, and bone marrow, via blood flow. Furthermore, they possess certain toxicities towards body tissues. Prolonged use of antibiotics can impair immune organ function and weaken the animal's innate immunity, thereby significantly increasing its susceptibility to infections. A prolonged and excessive use of antibiotics can result in significant immune morphological changes within the animal body, thereby affecting its immune processes.

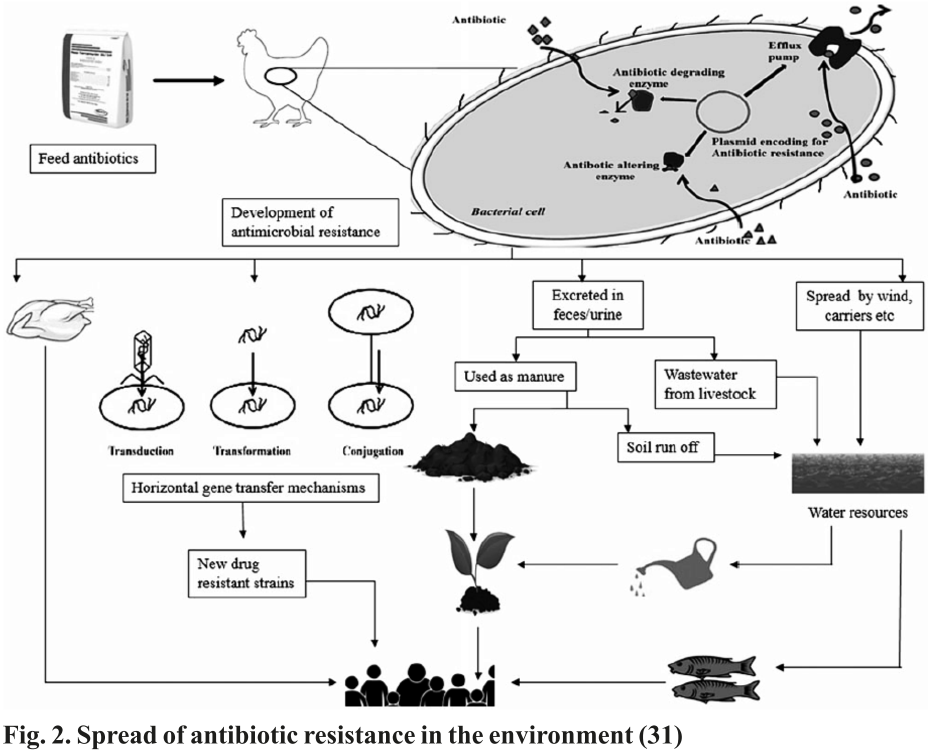

Fig. 2. Spread of antibiotic resistance in the environment (31)

Furthermore, certain drugs, such as Flufenicol and Kanamycin, have been found to inhibit the immunity of pigs, impede their response to vaccines, and adversely impact vaccination outcomes.

Disturbance of the microecological balance of the organism. The animal body harbors a diverse and abundant microbiota in the digestive, respiratory, and reproductive tracts and on the skin surface, comprising both pathogenic and non-pathogenic bacteria. In a state of homeostasis, the microecology is maintained by antagonistic interactions among microbial communities, which contributes to the host's health and disease resistance. However, the misuse of antibiotics can disrupt the composition and function of intestinal microbiota, disturb the microecological balance in animal bodies, reduce beneficial bacteria populations in intestines, facilitate colonization and proliferation of pathogens and enterotoxin-producing bacteria in intestines, leading to digestive disorders that compromise animal health and even cause mortality.

Restrictions and prohibitions of the use of antibiotics in feed

With the progress of research, it has become increasingly evident that the misuse of antibiotics can result in severe consequences. This has caused widespread concern and alarm among the public, prompting many countries to impose restrictions or even bans on antibiotic use in animal feed. The European Union (EU) first began restricting antibiotic use in 1973, with Sweden becoming the first country to implement a total ban on antibiotics in feed in 1986. In 2006, all EU member states officially banned the addition of antibiotics. In 2008 and 2011, Japan and South Korea, respectively, prohibited the use of antibiotics as growth promoters in animal feed. The Veterinary Feed Directive (VFD) was published by the United States in early 2015, mandating veterinary supervision for the use of important antimicrobials in feed and stipulating their usage only when necessary to ensure animal health. In late 2015, California passed Senate Bill No. 27, which strictly prohibits the use of important antimicrobial drugs in animal feed for growth promotion and disease prevention (8). China's Ministry of Agriculture and Rural Affairs has declared that as of July 1, 2020, the use of growth-promoting drugs other than traditional Chinese medicine in feed will be prohibited. This marks a significant milestone for China's feed industry as it enters an era free from antibiotics. To ensure a stable development of the breeding industry and secure a consistent supply of animal products, it is imperative for the feed industry to invest in research and development of effective alternatives to antibiotics.

Substitution of antibiotics in feed

The use of antibiotics in animal feed is associated with a range of issues, including drug residues and bacterial resistance, which pose significant threats to the long-term development of animal husbandry and human health. However, a complete ban on antibiotic use in feed could negatively impact animal growth performance, increase morbidity and mortality rates, and ultimately reduce the supply of animal products. To ensure a stable development of the breeding industry and secure a consistent supply of animal products, it is imperative for the feed industry to invest in research and development of effective alternatives to antibiotics (41). The pursuit of such alternatives has garnered global attention. World Organization for Animal Health (WOAH) hosted the second International Seminar on Antibiotic Alternatives in Paris, France from December 12 to 15, 2016 to deliberate on the development of antibiotic alternatives and related topics (20). In recent decades, research into antibiotic alternatives has centered, among others, on probiotics, prebiotics, synbiotics, organic acids, enzymes, and phytochemicals. In recent years, novel alternatives, such as hyperimmune egg antibodies, antimicrobial peptides, bacteriophages, clay, and metals, have emerged as substitutes for antibiotics (8). Apart from maintaining bodily health and promoting growth, these new antibiotic substitutes exhibit minimal toxic side effects and leave no residue.

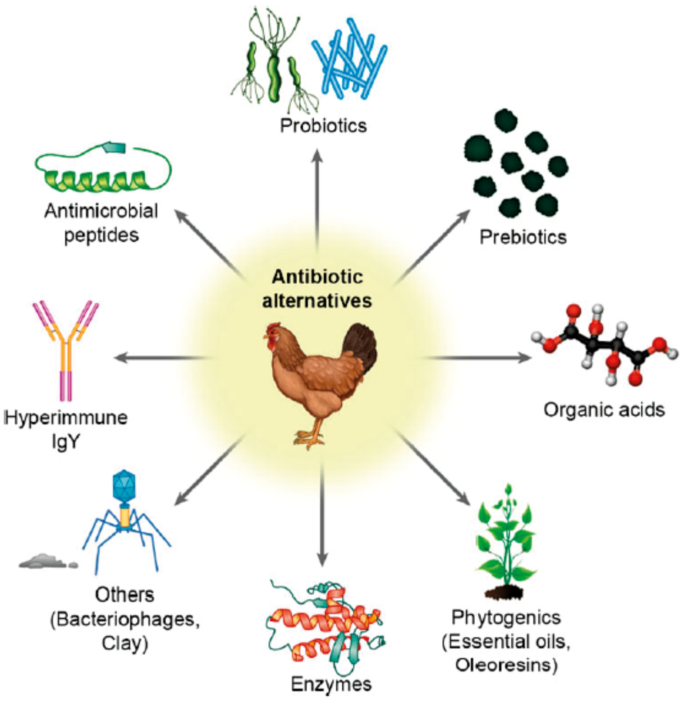

Fig. 3. Various classes of antibiotic alternatives (8)

Microecological preparations. Microecological preparations are a category of probiotics, probiotic metabolites, and biologics that can regulate the microecological balance of the intestines and improve the host's health according to principles of microecology (35). Probiotics are live microbial feed supplements that improve the host's intestinal microbial balance, thereby conferring health benefits (7). Probiotics can promote the proliferation of dominant bacterial communities while inhibiting inferior ones, thereby maintaining a balanced bacterial community structure. Adequate supplementation of probiotics (such as *Lactobacillus*, *Bifidobacterium*, *Bacillus subtilis*, etc.) in animal feed can enhance the host's digestion and absorption capacity, boost immunity, and stimulate growth. Prebiotics are substances that can be selectively utilized by microorganisms, such as undigested oligosaccharides and unsaturated fatty acids. Adding prebiotics to animal feed can enhance the activity of beneficial intestinal bacteria, indirectly affecting host animals and promoting growth. Biostime, a synergistic blend of probiotics and prebiotics, effectively modulates the intestinal microbiota and enhances host immunity. The addition of microecological preparations to feed supplements mainly beneficial microorganisms that inhibit harmful bacterial colonization while reducing the intestinal mucosal metabolic rate and energy consumption.

Phytogenic feed additives. Phytogenic feed additives (PFAs), also referred to as plant biologics or plant preparations, are natural bioactive compounds extracted from plants and utilized to enhance animal growth performance (37). Plant extracts have garnered significant attention in recent years because of their inherent natural properties, safety profile, and lack of drug residue. Flavonoids, alkaloids, saponins, polysaccharides, polyphenols, and volatile oils present in plant extracts serve as the fundamental basis for utilizing plant extracts as feed additives. In recent years, plant extracts have been increasingly utilized as natural growth promoters in the pig and poultry industries (6). A diverse array of herbs and spices, including thyme, oregano, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin seeds, coriander, and cinnamon, have been employed in farmed poultry production because of their potential as alternatives to antibiotic growth promoters. In addition to herbs and spices, various plant essential oils, such as thymol, carvacrol, and cinnamaldehyde, are commonly used alone or in combination to enhance the health status and growth performance of animals. The chemical composition and mechanism of action of plant extracts vary, with their efficacy being dependent on the active ingredients present. While the exact mechanisms are not fully understood, the beneficial effects of plant extracts are generally attributed to their antibacterial, antioxidant and immune-regulating properties. Studies have demonstrated that the inclusion of plant extracts in diets can modify and stabilize intestinal microbiota, reduce toxic metabolites produced by microorganisms in the gut, and produce direct antibacterial effects against various pathogenic bacteria. As a result, this approach alleviates intestinal challenges and immune stress while improving production performance (33, 44). Additionally, plant extracts can modulate immune function by enhancing immune cell proliferation, cytokine expression, antibody titers (28), pancreatic enzyme production and activity, as well as bile secretion (11, 21). PFAs also help maintain and improve the intestinal tissue structure and increase the villus height, thereby expanding intestinal absorption surfaces (25). At present, there are many commercial additives including plant extracts, such as *Macleaya cordata* extract, origanum oil, cinnamon oil, or allicin.

Tab. 1. The villus height, crypt depth, and thickness of muscularis mucosae (micrometer) of broilers supplemented with either an antibiotic or a phytogenic feed additive during the first 39 days of life* (25)

	Dietary treatments**			SEM	P value
	Control	AGP	PFA		
Duodenum					
Villus height	2549.1^b	3481.1^a	2903.4^{ab}	140.23	0.02
Crypt depth	45.3	42.7	32.8	2.55	0.10
Mucosa thickness	387.1^a	183.9^b	230.4^b	22.69	< 0.01
Jejunum					
Villus height	2583.6^c	2969.9^b	3290.1^a	280.51	< 0.01
Crypt depth	29.8^a	31.1^a	20.2^b	1.31	< 0.01
Mucosa thickness	206.8	215.6	212.9	6.85	0.88
Ileum					
Villus height	2050.1^b	2736.4^a	2839.9^a	94.03	< 0.01
Crypt depth	34.1	30.9	31.6	1.04	0.45
Mucosa thickness	320.3^a	233.9^b	211.8^b	14.31	< 0.01

Explanations: Means with dissimilar letters in a row varied significantly. * Means of 12 birds per treatment. Birds were randomly selected and euthanized at 39 days of age. ** Supplemented with either an antibiotic growth promoter (bacitracin methylene disalicylate) at 500 mg/kg (AGP) or a phytogenic feed additive (Digestarom® Poultry) at 150 mg/kg (PFA). The control group received the unsupplemented basal diet.

Enzyme preparations. An enzyme preparation is a substance obtained by extracting and processing enzymes from animals, plants, and microbial fermentation. It possesses the catalytic efficiency and specificity characteristic of enzymes. The primary active constituent of an enzyme preparation are dietary enzymes, which are biologically active proteins that facilitate the chemical breakdown of nutrients into smaller compounds for subsequent digestion and absorption (32). Enzyme preparations can supplement digestive enzymes in young animals, catalyze and hydrolyze nutrients, eliminate anti-nutrients in the diet, and accelerate nutrient digestion and absorption. The addition of enzyme preparations can compensate for the deficiency of animal endogenous enzymes, increase non-synthesizable enzymes in the animal body, eliminate anti-nutritional factors, improve intestinal flora composition, and increase beneficial bacterium, thereby improving the feed utilization rate and promotor growth performance. There are numerous factors that influence the efficacy of enzyme preparations, including, but not limited to, the quality of the preparation, animal species and health status, environmental conditions, etc. A wide range of enzyme preparations are utilized in feed production, with commonly used types including protease, amylase, xylanase, glucose oxidase, β-mannanase, phytase, and non-starch polysaccharide enzymes.

Acidifiers. Organic acids and/or salts thereof used in animal husbandry can be classified as either simple monocarboxylic acids (such as formic, acetic, propionic, and butyric acids) or carboxylic acids containing hydroxyl groups (such as lactic, malic, tartaric, and citric acids). These organic acids may be administered to livestock through feed or drinking water in the form of organic acids or their corresponding salts (sodium, potassium, or calcium), either alone or in combination with other organic acids or salts (14). Acidifiers are crucial feed additives that possess the attributes of non-residue, non-resistance, and non-toxicity. The optimal acidity environment is necessary for nutrient digestion and absorption in the gut. Acidifiers can compensate for gastric acid secretion deficiency, regulate gastrointestinal tract pH levels, and facilitate pepsinogen conversion into pepsin. They also maintain the intestinal flora balance, enhance intestinal morphology and structure, and promote reproduction of beneficial bacteria while suppressing harmful ones. The use of acidifiers can enhance nutrient digestibility by promoting protein and dry matter retention, augmenting mineral absorption and phosphorus utilization (26, 29), as well as fostering intestinal health through direct effects on epithelial cells. For instance, short-chain fatty acids serve as a direct energy source for the growth of epithelial cells.

Antimicrobial peptides. Antimicrobial peptides are genetically encoded small peptides that are widely distributed and exhibit activity against a variety of pathogens including Gram-negative and Gram-positive bacteria, fungi, enveloped viruses, and parasites (19). Typically composed of 12 to 100 amino acids, mature antimicrobial peptides possess an amphoteric structure rich in hydrophobic cationic residues which facilitates interaction with negatively charged membranes of microorganisms as well as other cellular targets (34, 39). To date, more than 2,600 endogenous antimicrobial peptides have been isolated, and numerous synthetic antimicrobial peptide compounds have been reported in various literature sources (8). Antimicrobial peptides exhibit broad-spectrum antibacterial activity as well

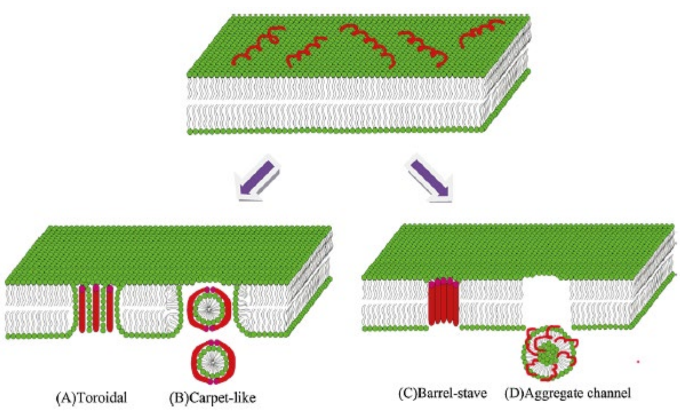

Fig. 4. Mode of action of AMPs (19)

as antiviral, antitumor, antifungal, and anti-parasitic properties while also stimulating immune responses. Additionally, they have strong environmental resistance, and it is difficult for microorganisms to develop drug resistance against them. Currently, it is widely accepted that antimicrobial peptides exert their action by forming transmembrane ion channels on the cell membrane, leading to microbial death (12).

This mechanism makes it difficult for microorganisms to develop resistance against them (10). The formation of these channels disrupts the integrity of the membrane and ultimately causes cell lysis. Moreover, there have been reports suggesting that antimicrobial peptides can also interfere with cellular metabolism in a specific manner (2). Antimicrobial peptides can significantly improve the health status and growth performance of livestock and poultry, and are an efficient, low toxic, and no-residue substitute for antibiotic growth promoters.

Microbial fermented feed. Microbial fermented feed are plant-based agricultural products with high levels of anti-nutrient factors, low digestibility, and poor palatability that can be used as primary raw materials under controlled conditions. Through microbial fermentation, macromolecular substances, such as cellulose, protein, and fat, are degraded into easily absorbable small molecular substances, such as sugars, soluble peptides, and organic acids. Consequently, a nutritionally rich feed with excellent palatability and a high content of beneficial microorganisms can be obtained (23). Microbial fermentation of feed not only eliminates or degrades most of anti-nutrient factors and toxic substances, but also enhances the content of digestible nutrients and improves feed utilization efficiency in comparison to traditional feed. It enriches feed resources while simultaneously reducing environmental pollution. Additionally, microbial fermented feed exhibits functional properties comparable to those of antibiotics. For instance, it can enhance the organic acid content within the intestinal tract, lower the pH value of the intestines, suppress the proliferation of detrimental bacteria, and augment the population of beneficial bacteria. Consequently, this regulates the equilibrium of intestinal flora and enhances intestinal health (43). The utilization of microbial fermented feed can enhance the body's ability to combat oxidative stress, augment the quantity and vitality of immune cells, and elevate the levels of immune active substances, such as cytokines, antibodies, and lysozyme, which leads to improvement in both antioxidant capacity and immune function. The use of fermented feed in livestock and poultry breeding can serve as a viable alternative to antibiotic drugs, without inducing resistance or causing significant pollution to the natural environment. By prioritizing the well-being of animals, it is possible to promote the healthy and sustainable development of the livestock breeding industry.

Conclusion

The incorporation of subtherapeutic doses of antibiotics into animal feed has been shown to enhance growth performance, optimize feed conversion efficiency, and increase revenue. A growing number of countries and regions are prohibiting the use of antibiotics in animal feed due to a series of adverse effects caused by their abuse. To ensure the healthy and sustainable development of animal husbandry and feed industry, it is scientifically important and promising to research and develop high-efficiency, low-residue, and low-toxicity substitutes for antibiotic growth promoters. From the perspective of animal nutritional efficiency, the selection order of antibiotic substitutes in feed can be categorized as follows: phytogenic feed additives, antimicrobial peptide, enzyme preparations, microecological preparations, microbial fermented feed, and acidifiers.

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